



Appendix E:

Biological Assessment



Valley Link Rail Project

**Dublin/Pleasanton to Mountain House Community
Alameda and San Joaquin Counties, California**

Biological Assessment

November 2024

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Executive Summary

The Federal Transit Administration, as lead federal agency, and the Tri-Valley – San Joaquin Valley Regional Rail Authority, as project sponsor, are proposing the Valley Link Rail Project (Proposed Project), which would establish a new passenger rail service along a 22-mile corridor between the existing Dublin/Pleasanton Bay Area Rapid Transit District Station in Alameda County and the proposed Mountain House Community Station in San Joaquin County, California (Appendix A, Figure 1). The rail alignment would be constructed within a combination of existing corridors, including the Interstate 580 (I-580) freeway median, the transportation corridor owned by Alameda County (formerly Southern Pacific Transcontinental Railroad alignment), the California Department of Transportation right-of-way adjacent to the westbound I-580 freeway, and new right-of-way to be acquired for the Proposed Project. The Proposed Project would include four new stations, as well as a maintenance-of-way staging area, a layover facility (LF), and an Operations and Maintenance Facility/Operations Support Site (OMF/OSS) in areas at the east end of the alignment (Appendix A, Figure 1).

The Proposed Project Footprint is approximately 848 acres in total—659 acres for the alignment (inclusive of track, stations, and the LF) and 189 acres for the OMF/OSS on Schulte Road in San Joaquin County, California. The Action Area encompasses approximately 4,108 acres around the Proposed Project Footprint with a 500-foot-wide buffer (Appendix A, Figure 2).

Federally Listed, Proposed, and Candidate Species and Critical Habitat

Based on the U.S. Fish and Wildlife Service’s Information for Planning and Consultation species list, the species in Table ES-1 were evaluated for the potential to occur in the Action Area and the potential to be affected by the Proposed Project. Critical habitat for three listed species was also evaluated (Table ES-2). The evaluation consisted of desktop research, field reconnaissance, habitat-based modeling, and focused sampling for aquatic species (environmental DNA sequencing), and species and habitat effects were analyzed based on this information.

Table ES-1: Federally Listed, Proposed, and Candidate Species, Listing Status, and Effects Determination

Common Name	Scientific Name	Federal Status	Effect Determination
Invertebrates			
Conservancy fairy shrimp	<i>Branchinecta conservatio</i>	Endangered	No Effect^a
Longhorn fairy shrimp	<i>Branchinecta longiantenna</i>	Endangered	No Effect^a
Vernal pool fairy shrimp	<i>Branchinecta lynchi</i>	Threatened	May Affect – Likely to Adversely Affect
Vernal pool tadpole shrimp	<i>Lepidurus packardii</i>	Endangered	No Effect^a
Valley elderberry longhorn beetle	<i>Desmocerus californicus dimorphus</i>	Threatened	No Effect^a

Common Name	Scientific Name	Federal Status	Effect Determination
Monarch butterfly	<i>Danaus plexippus</i>	Candidate	May Affect – Not Likely to Jeopardize the Species
Fish			
Delta smelt	<i>Hypomesus transpacificus</i>	Threatened	No Effect^a
Amphibians and Reptiles			
California tiger salamander – Central California DPS	<i>Ambystoma californiense</i>	Threatened	May Affect – Likely to Adversely Affect
Foothill yellow-legged frog – Central Coast DPS (population 4)	<i>Rana boylei</i>	Threatened	No Effect^a
California red-legged frog	<i>Rana draytonii</i>	Threatened	May Affect – Likely to Adversely Affect
Western spadefoot	<i>Spea hammondi</i>	Proposed Threatened	May Affect – Not Likely to Jeopardize the Species
Alameda whipsnake	<i>Masticophis lateralis euryxanthus</i>	Threatened	No Effect^a
Northwestern pond turtle	<i>Actinemys marmorata</i>	Proposed Threatened	May Affect – Not Likely to Jeopardize the Species
Birds			
California condor	<i>Gymnogyps californianus</i>	Endangered	No Effect^a
California least tern	<i>Sterna antillarum browni</i>	Endangered	No Effect^a
Mammals			
San Joaquin kit fox	<i>Vulpes macrotis mutica</i>	Threatened	May Affect – Likely to Adversely Affect
Plants			
Large-flowered fiddleneck	<i>Amsinckia grandiflora</i>	Endangered	No Effect^a
Lassics lupine	<i>Lupinus constancei</i>	Endangered	No Effect^a
Palmate-bracted bird's-beak	<i>Chloropyron palmatum</i> (syn. <i>Cordylanthus palmatus</i>)	Endangered	May Affect – Likely to Adversely Affect

DPS = distinct population segment

^a Listed species with an effect determination of “No Effect” are presented in this Biological Assessment (BA) but not discussed in detail due to no habitat being present within the Action Area.

Table ES-2: Critical Habitat Effects Determination

Common Name	Scientific Name	Federal Status	Effect Determination
Vernal pool fairy shrimp	<i>Branchinecta lynchi</i>	Threatened	No Modification
Delta smelt	<i>Hypomesus transpacificus</i>	Threatened	No Modification
California red-legged frog	<i>Rana draytonii</i>	Threatened	No Adverse Modification

Determinations

Based on the analyses in this BA, the Proposed Project **May Affect and is Likely to Adversely Affect** the following federally listed species:

- Vernal pool fairy shrimp (no modification to the critical habitat for this species)
- California tiger salamander
- California red-legged frog (no adverse modification to the critical habitat for this species)
- San Joaquin kit fox
- Palmate-bracted bird's-beak

The Proposed Project will have **No Effect** on the following federally listed species:

- Conservancy fairy shrimp
- Longhorn fairy shrimp
- Vernal pool tadpole shrimp
- Valley elderberry longhorn beetle
- Delta smelt (including no modification to the critical habitat for this species)
- Foothill yellow-legged frog
- Alameda whipsnake
- California condor
- California least tern
- Large-flowered fiddleneck
- Lassics lupine

The Proposed Project **May Affect but is Not Likely to Jeopardize** the following proposed and candidate species:

- Western spadefoot
- Northwestern pond turtle
- Monarch butterfly

Conservation Measures

General and species-specific conservation measures will be implemented to reduce potential effects to listed, proposed, and candidate species. General avoidance measures include, for example, conducting environmental education for construction personnel, delineating work areas and environmentally sensitive areas with fencing, avoiding wildlife entrapment by covering trenches or providing escape routes, and other standard construction practices. General conservation measures include surveys to be conducted

during final design to identify and avoid key habitats when possible; exclusion fencing; and reducing disturbance due to nighttime lighting, noise, vibration, and dust resulting from construction, operations, and maintenance activities associated with the Proposed Project.

Species-specific conservation measures will be implemented to offset potential effects of the Proposed Project. These conservation measures include, for example, conducting pre-construction surveys within suitable habitat, relocating individuals to areas outside the Proposed Project Footprint, and compensatory mitigation for habitat loss.

Compensatory Mitigation

If required, compensation for permanent and temporary effects to listed species and critical habitats will be implemented as specified by the U.S. Fish and Wildlife Service. Compensatory mitigation may follow local guidelines as stated in the East Alameda County Conservation Strategy (EACCS) (EACCS 2024) and the San Joaquin County Multi-Species Habitat Conservation and Open Space Plan (SJMSCP 2000).

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Acronyms and Abbreviations

ACU	adaptive capacity unit
AECOM	AECOM Technical Services, Inc.
Authority	Tri-Valley – San Joaquin Valley Regional Rail Authority
BA	Biological Assessment
BAAQMD	Bay Area Air Quality Management District
BART	Bay Area Rapid Transit
BIOS	Biogeographic Information and Observation System
BMP	best management practice
BO	Biological Opinion
Caltrans	California Department of Transportation
CARI	California Aquatic Resources Inventory
CDFW	California Department of Fish and Wildlife
CFR	Code of Federal Regulations
CM	Conservation Measure
CNDDB	California Natural Diversity Database
CNPS	California Native Plant Society
CWHR	California Wildlife Habitat Relationships
dBA	decibels, A-weighted
DPS	distinct population segment
EACCS	East Alameda County Conservation Strategy
eDNA	environmental DNA
ESA	Endangered Species Act
FR	Federal Register
FTA	Federal Transit Administration
I-205	Interstate 205
I-580	Interstate 580
IPaC	Information for Planning and Consultation
LF	layover facility
MCV	<i>Manual of California Vegetation</i>
MOW	maintenance-of-way
mph	miles per hour
NOAA	National Oceanic and Atmospheric Administration
NRCS	National Resources Conservation Service
OMF	Operations and Maintenance Facility

OSS	Operations Support Site
PBF	physical and biological feature
ppm	part per million
Proposed Project	Valley Link Rail Project
SJMSCP	San Joaquin County Multi-Species Habitat Conservation and Open Space Plan
UPRR	Union Pacific Railroad
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
USFS	U.S. Forest Service
USGS	U.S. Geological Survey
WAFWA	Western Association of Fish and Wildlife Agencies

1 Introduction

The Federal Transit Administration (FTA), as lead federal agency, and the Tri-Valley – San Joaquin Valley Regional Rail Authority (Authority), as project sponsor, are proposing the Valley Link Rail Project (Proposed Project), which would establish a new passenger rail service along a 22-mile corridor between the existing Bay Area Rapid Transit (BART) Dublin/Pleasanton Station in Alameda County and the proposed Mountain House Community Station in San Joaquin County, California (Appendix A, Figure 1). The rail alignment would be constructed within a combination of existing corridors, including Interstate 580 (I-580) freeway median, transportation corridor owned by Alameda County (formerly Southern Pacific Transcontinental Railroad alignment), California Department of Transportation (Caltrans) right-of-way adjacent to the westbound I-580 freeway, and new right-of-way to be acquired for the Proposed Project. The Proposed Project includes four new stations, as well as a maintenance-of-way (MOW) staging area, a layover facility (LF) and an Operations and Maintenance Facility/Operations Support Site (OMF/OSS) in areas at the east end of the alignment (Appendix A, Figure 1).

1.1 Purpose of this Biological Assessment

The purpose of this Biological Assessment (BA) is to support Section 7 consultation under the Endangered Species Act of 1973 (ESA) between FTA and the U.S. Fish and Wildlife Service (USFWS). It provides technical information and analyzes potential effects of the Proposed Project on federally listed, proposed, and candidate species and critical habitat. This BA has been prepared in accordance with 50 Code of Federal Regulations (CFR) Section 402, legal requirements found in Section 7(a)(2) of the ESA (16 U.S. Code 1536(c)), and FTA guidance for preparing BAs.

1.2 Consultation History

To identify species to consider in this BA, the Authority obtained a species list through the USFWS' Information for Planning and Consultation (IPaC) website (Appendix B). The Authority and FTA had preliminary discussions with USFWS on July 18, 26, and 29, 2024, to present and discuss the approach for the BA, including the Action Area, data collection methodologies, and species determinations and appropriate mitigation measures. FTA also shared a draft of this BA with USFWS before initiating formal consultation.

1.3 Description of the Proposed Project

1.3.1 Project Purpose and Need

The purpose of the Proposed Project is to:

- Provide a frequent and reliable transit option in the I-580 corridor while connecting housing, people, and jobs and promoting transit-oriented development.

- Connect the Tri-Valley Hub, a transit hub located at the Dublin/Pleasanton BART Station offering connections to intercity and local buses and the Proposed Project, to the state rail system to support megaregional mobility, furthering the vision of the 2023 *California State Rail Plan* (Caltrans 2023a), the Association of Bay Area Governments (ABAG)/Metropolitan Transportation Commission's (MTC) *Plan Bay Area 2050* (ABAG/MTC 2021), and the San Joaquin Council of Governments (SJCOG) *Regional Transportation Plan and Sustainable Communities Strategy* (SJCOG 2022).
- Enhance safe mobility and accessibility options for all communities within the Northern California Megaregion.
- Provide a sustainable transportation option and support local, state (California Climate Initiative), and federal goals to promote sustainability, reduce greenhouse gas emissions, and enhance environmental quality.

The Proposed Project would respond to the following needs:

- **Access:** Inadequate mode choice limits regional connectivity and access to employment, housing, education, healthcare, and recreational opportunities. Better connectivity and access between major activity and employment centers is needed between the San Joaquin Valley and the Bay Area, while supporting local land use plans that foster compact development in Livermore and the San Joaquin Valley.
- **Mobility:** The existing transportation system is not adequate to serve current and future transportation conditions. Expanded transit options between the Bay Area and San Joaquin Valley are needed that seamlessly connect with the existing and future Northern California transit system and offer a competitive, reliable transportation solution that will reduce travel time.
- **Safety:** Increased safety through decreased vehicle miles traveled is needed along this congested section of I-580, which is a major truck freight route and commuter corridor. Safe travel options are needed for commuters and travelers through the corridor.
- **Equity:** Disadvantaged populations are overburdened by a lack of jobs, education, and healthcare options in the San Joaquin Valley and a lack of affordable housing in the Bay Area, requiring these populations to commute into the Tri-Valley and larger Bay Area. These communities are also subject to the negative effects of greenhouse gas emissions. A zero-emission travel option for commuters would provide air quality benefits to disadvantaged communities in the region.
- **Sustainability:** Regional, state (California Climate Initiative), and federal (2021 Global Climate Ambition Initiative) initiatives have been established to decarbonize and reduce greenhouse gas emissions. A sustainable zero-emission transportation choice is needed to support these initiatives.

1.3.2 Project Overview

The Proposed Project would establish a new passenger rail service along a 22-mile corridor between the existing Dublin/Pleasanton BART Station in Alameda County and the proposed Mountain House Community Station in San Joaquin County, with all-day bi-directional service at frequent intervals using zero-emission multiple-unit vehicles. The Action Area map (Appendix A, Figure 2) identifies the project alignment, stations, and operations and maintenance facilities, as well as the Action Area. The following facilities would be constructed:

- **Train tracks:** 22 miles of single and double track between Dublin/Pleasanton and the Mountain House Community along I-580, Altamont Pass Road, and Interstate 205 (I-205) with associated structures, such as viaducts and retaining walls where needed. There would be no at-grade crossings of public roads; the tracks would be viaducted over such crossings. The tracks would be installed over a gravel bed using concrete ties, with culverts installed where needed to allow stormwater to drain properly (Appendix A, Figure 3 depicts a typical rail bed). No fencing would be installed along the tracks other than barbed wire where needed to keep livestock off the tracks.
- **Dublin/Pleasanton Station:** A station platform south of the eastbound I-580 freeway across the highway from the existing Dublin/Pleasanton BART Station with stairs, escalators, and elevators for vertical circulation in the station. This facility would be fenced for security purposes.
- **Isabel Station:** A station platform in the I-580 median near the Isabel Avenue I-580/interchange East Airway Boulevard with a pedestrian overcrossing, elevators, and stairs; a 24-acre parking lot along East Airway Boulevard; and associated road improvements for access to the lot, including driveways and restriping of East Airway Boulevard. This facility would be fenced for security purposes.
- **Southfront Road Station:** A station platform in the I-580 median near Southfront Road in Livermore with a pedestrian overcrossing, elevators, and stairs; a 7-acre parking lot off the road; and associated highway and road improvements to accommodate the station and access to the lot. This facility would be fenced for security purposes.
- **Mountain House Community Station:** A station platform north of I-205 near Mountain House Parkway with pedestrian overcrossings, a 54-acre parking lot off the road, and associated road improvements for access to the lot. This facility would be fenced for security purposes.
- **Altamont MOW staging area:** A staging area on a portion of a 24-acre property along Altamont Pass Road near Dyer Road to provide maintenance vehicle staging and for use during construction. This facility would be fenced for security purposes.
- **Mountain House LF:** A facility for train layovers, storage, and light maintenance east of the Mountain House Community Station on an 86-acre property with

administrative offices and other supporting facilities. This facility would be fenced for security purposes.

- **Tracy OMF/OSS:** A support facility on an approximately 200-acre property along West Schulte Road west of Tracy for heavy maintenance activities and to support the control center and warehouse storage. This facility would be fenced for security purposes.

Retaining walls would be constructed in several locations to minimize right-of-way impacts, avoid impacts to existing interchange overcrossing structures, and support the ramp approaches and roadway embankments. Noise barriers (permanent and temporary) would be constructed where appropriate. Concrete barriers would be installed in the center median on both sides of the proposed rail alignment, and Caltrans standard concrete barriers would be constructed as part of the freeway realignment between local roadways and I-580.

The Proposed Project includes design features to help avoid permanent effects to sensitive areas, including aquatic features and critical habitat. The Proposed Project deviates from I-580 and travels through the Altamont Pass for approximately 6.25 miles (Appendix A, Figure 2). This stretch of the Proposed Project passes through extensive tracts of sensitive habitat, and 1.15 miles of the Proposed Project through this area (18.4 percent) have been designed to travel on viaducts and bridges to avoid and minimize effects to the sensitive habitat and listed species that may be present. An additional 2.16 miles of the rail are elevated along the sections of the Proposed Project that are parallel to I-580 (approximately 16.13 miles in total). This elevation would aid in efforts to avoid and minimize effects to listed species. In addition, to the extent feasible, Proposed Project infrastructure would be located within or adjacent to existing transportation infrastructure or corridors, utility corridors, and other development to minimize effects to sensitive habitats. Additionally, culverts would be designed and installed to minimize impacts to aquatic features and not restrict connections or flow between potential aquatic habitats. Permanent fencing would be located at the MOW staging and the LF area for security purposes. The remainder of the Proposed Project would not be fenced to minimize barrier impacts.

1.3.3 Construction Methods, Schedule, and Duration

Generally, construction of components of the Proposed Project would require the realignment of portions of I-580, including freeway ramps; replacement of bridges to include the removal of existing structures (i.e., culverts, barriers); clearing/grubbing of existing vegetation; relocation of utilities; embankment construction; earthwork excavation; grading and compaction; structural work; and installation of lighting. Modifications to existing overhead structures would require clearing, grubbing, and rough grading for the installation of pier protection along the existing piers that support the overhead roadway structure and retaining walls along the length of existing abutment slopes. Construction of stations and supporting facilities would include associated mechanical, electrical, and plumbing infrastructure.

Additional construction site preparation activities would include installation of environmentally sensitive area fencing and installation of water quality best management practices (BMPs), such as silt fences, fiber rolls, and drainage inlet protection systems.

Large construction equipment would be utilized for various phases of the Proposed Project, including but not limited to clearing/grubbing, demolition, and excavation. In addition, on-track equipment would be required to unload ballast onto the track and compact and make adjustments to the track alignment. Typical construction equipment would include graders, water trucks, bull dozers, compactors, dump trucks, heavy vehicle cranes, impact pile drivers, and concrete mixers and pumps. Where utility lines conflict with the Proposed Project, the lines would be relocated in coordination with the utility company.

Artificial nighttime lighting would be required for nighttime construction but would comply with conservation measures described in Section 1.6 to limit disturbance.

Special haul roads would not be required for the proposed improvements to I-580. Temporary concrete railing with other traffic control devices would be used to separate the work area from moving traffic and to close travel lanes, sidewalks, and other areas as needed to provide construction staging areas.

Three haul roads would be constructed along Altamont Pass Road for access to the construction site. These roads would be constructed on privately owned parcels to be acquired for the Proposed Project and would be used for maintenance access during operation.

The contractor(s) would be responsible for obtaining environmental clearance for additional temporary staging areas (if needed) that would be outside of the identified construction staging area for the Proposed Project.

Construction-related materials, including the environmentally sensitive area fencing, would be removed after construction activities are complete. The temporarily disturbed areas and staging areas would be cleaned up, recontoured to original grade, and revegetated with appropriate native species, as necessary. Permanent erosion control, including soil stabilization measures such as hydroseeding and coir netting, would be applied to temporarily affected areas of the Proposed Project to minimize erosion after construction.

The Authority would retain a construction contractor for the Proposed Project. In general, in areas of construction, the sequence of construction would be:

- Clearing and grubbing
- Topsoil removal
- Grading and compaction
- Excavation and/or structure removal
- Foundation preparation

- Structure placement
- Restoration of impacted areas

Table 1 identifies the duration of construction for each Proposed Project improvement. The construction durations are not sequential; construction could occur simultaneously at several locations. Logical work improvements are organized by section; the Tri-Valley Section refers to the west portions of the Proposed Project in the I-580 median, and the Altamont Section refers to improvements in the east portion of the Proposed Project through the Altamont Pass to Mountain House and Tracy. Proposed Project improvements would require permitting, contractor selection, and final design prior to construction; therefore, the total duration could be longer than the construction duration. However, the approach will be in compliance with the recommendations and conservation measures in this BA.

Table 1: Construction Duration

Improvement	Construction Duration (Months)	Section
Track Work	36	Tri-Valley Section
I-580 Modifications	42	Tri-Valley Section
Dublin/Pleasanton Station	24	Tri-Valley Section
Isabel Station	18	Tri-Valley Section
Southfront Road Station	16/18	Tri-Valley Section
Track Work including Altamont MOW	30	Altamont Section
Mountain House Community Station	12/16	Altamont Section
Mountain House LF	36	Altamont Section
Tracy OMF/OSS	36	Altamont Section

Based on similar projects, construction associated with modified overhead structure under crossings would last approximately 30 to 120 working days, with an average of 60 working days; construction of station platforms would last approximately 3 months, with more complex station facilities (i.e., pedestrian overcrossing access structures) extending to 6 to 9 months; and track work would last approximately 36 months.

1.3.4 Operations and Maintenance

The Authority's trains would operate 7 days a week between the Mountain House Community Station and the Dublin/Pleasanton Station, with all trains stopping at the Isabel and Southfront Road Stations. The first weekday train to depart the Mountain House Community Station would be timed to arrive at the Dublin/Pleasanton Station 11 minutes prior to the first BART departure at 5:06 a.m. On weekdays, trains would operate at 15-minute headways during peak periods and 45-minute headways during non-peak periods. The last westbound weekday train would depart the Mountain House Community Station at 7:45 p.m., and the last eastbound weekday train would depart the Dublin/Pleasanton Station at 8:30 p.m. Weekend and holiday headways would be 45 minutes, with trains operating from 8:00 a.m. until 8:00 p.m. Typical travel speeds between the stations situated in the I-580 median (Dublin/Pleasanton, Isabel, and

Southfront Road) may reach up to 79 miles per hour (mph). In the Altamont Pass area, between the Southfront Road Station and Mountain House Community Station, average travel speeds may range between 79 mph and 25 mph, with higher speeds on straighter segments and slower speeds along curves.

Maintenance activities would include periodic inspections, scheduled maintenance of the buildings, rail structures, drainage structures, and access roads; scheduled overhaul and unexpected repairs; and maintenance of the vegetation within the corridor. Maintenance of vegetation and disturbance of established areas would be limited to what is necessary for the safe operation of the trains. Maintenance activities would comply with mitigation and conservation measures specific to vegetation removal, restoring disturbed areas, and preventing the introduction or spread of invasive plants.

1.4 Action Area

The Action Area (Appendix A, Figure 2) is defined as the area where potential direct or indirect effects may occur to federally listed species from construction, operations, and maintenance of the Proposed Project. This section defines the terms used in reference to the areas potentially affected by the Proposed Project.

Two distinct terms are used in this BA to describe the areas evaluated and potentially affected by the Proposed Project: Proposed Project Footprint and Action Area (Appendix A, Figure 2).

The **Proposed Project Footprint** is the area that would be directly affected (both permanent and temporary effects) by construction activities related to the Proposed Project, as well as permanent Proposed Project components. This includes staging areas, access roads used for Proposed Project activities, and any areas used for operating, storing, and fueling construction equipment. The Proposed Project Footprint is approximately 848 acres in total—659 acres for the track, stations, and the LF and 189 acres for the OMF/OSS on Schulte Road in San Joaquin County, California.

The **Action Area** is the area that would be directly or indirectly affected by the Proposed Project and includes a 500-foot buffer extending out from the edge of the Proposed Project Footprint (for a total of 4,108 acres). Direct effects would occur as a result of the Proposed Project and have an immediate effect on a species or its habitat, while indirect effects are those that would be caused by, or result from, the Proposed Project at a later time but would be reasonably certain to occur. The 500-foot distance was used to capture the farthest extent of indirect effects generated by Proposed Project activities, including noise, vibration, dust, water quality, and/or geomorphological effects relevant for federally listed species.

1.5 Compensatory Mitigation Framework

Portions of the Proposed Project would traverse areas covered by the San Joaquin County Multi-Species Habitat Conservation and Open Space Plan (SJMSCP) (SJMSCP 2000), which is an adopted Habitat Conservation Plan that covers all of San Joaquin County, and the East Alameda County Conservation Strategy (EACCS) (EACCS 2024),

which is a conservation framework for eastern Alameda County. The EACCS was developed to be consistent with the conservation goals of the SJMSCP so that applicants operating in more than one regional conservation planning jurisdiction would obtain consistent permit requirements. Federally listed species jointly covered by these conservation planning documents that are the subject of this BA include vernal pool fairy shrimp, California tiger salamander, California red-legged frog, and San Joaquin kit fox. The FTA is not a signatory to the SJMSCP or the EACCS, and the Proposed Project and the FTA are not subject to the requirements within. However, these conservation planning documents include standardized avoidance and minimization measures and compensatory mitigation strategies that have been previously approved by USFWS.

The mitigation framework prescribed in the EACCS and SJMSCP will be used as a basis for development of the compensatory mitigation package for the portion of the Proposed Project alignment within these areas. For effects on listed species that occur outside of the EACCS or SJMSCP areas, or on species that are not covered by these conservation planning documents, the Authority will provide compensatory mitigation for the loss of occupied habitat at a ratio agreed upon with USFWS.

The mitigation framework in the EACCS and SJMSCP have been used to establish mitigation ratios for Proposed Project-related permanent and temporary effects on species habitat. To determine the mitigation ratio for a species within the EACCS area, mitigation tables (Tables 3-4, 3-7, 3-8, and 3-11 of the EACCS; Appendix C of the Programmatic Biological Opinion [BO]) are used in conjunction with the standardized reference map (Figures 3-6, 3-9, 3-10, and 3-13 of the EACCS). While mitigation ratios are standardized in the EACCS, impact and mitigation sites may be evaluated using score sheets provided in Appendix E of the EACCS to determine whether the habitat value is equivalent at each site. If the score sheets determine that the sites are not equivalent, adjustments may be made to the standardized mitigation ratio accordingly. Compensatory mitigation must be located within the study area of the EACCS.

Compensatory mitigation within the SJMSCP portion of the alignment is based on the area of project effects for four habitat types: agricultural habitat lands, natural lands (non-wetlands), natural lands (vernal pools), and natural lands (wetlands other than vernal pools). The SJMSCP provides standardized mitigation ratios for conversion of these habitat types from open space use (Table 5.3-1 of the SJMSCP). SJMSCP Index Zones, which include the Vernal Pool Zone, Southwest Zone, Central Zone, Primary Zone of the Delta, and Central/Southwest Transition Zone (Table 5.1-1 of the SJMSCP), are used to determine the location of compensatory mitigation; impacts within a given zone must be offset with compensatory mitigation in that same zone except as noted in the plan.

Species-specific compensatory mitigation ratios based on temporary and permanent Proposed Project effects on suitable habitat within the Action Area are provided in Chapter 4 of this BA. Refinement of the ratios is ongoing based on advancement of the Proposed Project design and resulting impact assessment, selection of mitigation sites, and feedback provided by USFWS.

1.6 General Conservation Measures

The Authority will implement a series of avoidance, minimization, and conservation measures (CMs) prior to and during construction activities in accordance with the construction management plan for the Proposed Project. The general conservation measures listed below (CM-1 through CM-10) will be implemented as part of construction, operations, and maintenance, as applicable, to minimize and/or avoid effects to federally listed species and habitat as well as to common biological resources. General minimization measures from the EACCS (Table 3-2, measures 1 through 17) (EACCS 2024) and the SJMSCP (Section 5.2) (SJMSCP 2000) were considered and incorporated as appropriate. Where relevant, the EACCS measure from Table 3-2 is referenced after each of the following measures.

CM-1 – Worker Environmental Awareness Training: Before any equipment staging, grading, or vegetation removal in areas supporting or potentially supporting federally listed species habitat, the Authority will prepare and implement a worker environmental awareness training program. The training program will be provided to construction personnel (contractors and subcontractors) to brief them on the need to avoid effects on sensitive biological resources and the penalties for not complying with applicable state and federal laws and permit requirements. The training program will be delivered by a biologist and will include information on the life history and habitat requirements of federally listed species that could occur in the Action Area, the importance of protecting habitat, and the terms and conditions of the BO and applicable permits. The training program will also cover general restrictions and guidelines that must be followed by construction personnel to reduce or avoid effects on sensitive biological resources during construction [*EACCS AMM Gen-0, Gen-02, and Gen-03*].

CM-2 – Protect Sensitive Natural Communities, Including Riparian Habitat, During Construction: The Authority will ensure that before site preparation a resource specialist (i.e., biologist, botanist, ecologist, or soil scientist) will clearly identify, using high-visibility construction fencing or markers (e.g., lathe or pin flags), any sensitive natural communities to be preserved, including riparian habitat, abutting the Proposed Project Footprint. Construction will not encroach on sensitive natural communities that the resource specialist identifies to be preserved. The resource specialist will use the verified wetland delineation, soils data, and land cover data to confirm the location of sensitive natural community boundaries, based on existing conditions at the time of the avoidance marking. Exclusion fencing or markers will be installed before construction activities are initiated, and the fencing will be maintained in the section throughout the construction period. Vehicles and equipment will be parked on pavement, existing roads, and previously disturbed areas to the extent practicable. No construction activity, traffic, equipment, or materials will be permitted in fenced sensitive natural community areas to be preserved. Exclusion fencing and markers will be removed following completion of construction activities [*EACCS AMM Gen-05, Gen-14 and Gen-15*].

CM-3 – Vegetation Removal and Restoration: Vegetation removal will be limited to the designated work areas needed for access and workspace. Where possible, vegetation removal in temporary work areas will be cut above soil level to promote regrowth of established plants following construction. The Authority will restore temporarily disturbed areas to the pre-construction contours and functions to the maximum extent practicable.

Exposed slopes and bare ground will be reseeded with native local grasses and shrubs to stabilize and prevent erosion. Where disturbance includes the removal of trees and woody shrubs, coordination with the appropriate permitting agency will be warranted, and planting may be required. A native hydroseed mix will be identified in the plans and specifications.

Seeding of areas that have been disturbed by the Proposed Project will be conducted prior to the beginning of each wet season (November 1). Seed will be applied in late fall to improve germination. If more than 30 days will elapse before the application of the seed mix, a temporary soil stabilizer will be applied to disturbed soils except in wetlands. Establishment of vegetation cover in disturbed areas will be monitored at the end of the first growing season. The seed mix will be reapplied in the fall if adequate cover (total vegetation cover at least 30 percent) is not achieved by the end of the first growing season. Reseeded areas will be covered with a nontoxic stabilizing (i.e., tackified) mulch or seed-free hay material [EACCS AMM Gen-10].

CM-4 – Prevent Introduction or Spread of Invasive Plant Species: To reduce the spread of invasive non-native plant species and minimize the potential decrease of palatable vegetation for wildlife species, the Authority will comply with Executive Order 13112, Invasive Species (1999), which includes the following actions to avoid and minimize the spread or introduction of invasive plant species:

- Construction equipment and vehicles will be cleaned in a designated wash area before entering and exiting the construction site [EACCS AMM Gen-09].
- Construction supervisors and managers will be educated about invasive plant identification and the importance of controlling and preventing the spread of invasive plant infestations.
- Small, isolated infestations will be treated with eradication.
- Surface disturbances will be minimized to complete the work.
- Native, noninvasive species or nonpersistent hybrids will be used in erosion control plantings to stabilize site conditions and prevent invasive plant species from colonizing.
- Weed-free imported erosion control materials (or rice straw) will be used in upland areas.

One year after construction, the Authority will conduct a monitoring visit to each active or previously active (within 1 year) improvement footprint to ensure that no new occurrences of invasive plant species not previously present have become established. Any newly established invasive species not previously present will be eradicated [EACCS AMM Gen-09 and Gen-10].

CM-5 – Implement Avoidance of Nighttime Light Disturbance: Prior to construction requiring nighttime lighting, a lighting plan will be prepared verifying how the nighttime construction lighting will be shielded and directed downward toward active construction areas to avoid exposing nocturnal wildlife to excessive glare. The lighting plan will be submitted to FTA and the Authority for review and approval prior to any work requiring

nighttime lighting. The lighting plan will describe the type of lighting that will be used and maximum level of lumens to be emitted and include a schematic showing where lighting equipment will be stationed and which cardinal direction(s) the lighting equipment will face. Any nighttime lighting used for nighttime construction will be evaluated for its ability to safely light the construction work area while reducing light spill and glare. At a minimum, the construction contractor shall minimize Proposed Project-related light and glare to the maximum extent feasible, given safety considerations, for all viewer groups. Color-corrected halide lights or balloon lights, if suitable for construction of the Proposed Project, will be used. Portable lights will be operated at the lowest allowable wattage and height; they will be raised to a height no greater than 20 feet, except for pedestrian bridge and flyover work. Lights will be screened and directed downward toward work activities and away from the night sky and sensitive biological areas (e.g., wetlands, ponds) to the maximum extent possible. The number of nighttime lights used will be minimized to the greatest extent possible. Directional lighting and shields will be used when night construction is necessary to prevent light intrusion into adjacent areas. Permanent lighting will be equipped with shields to focus light downward onto the appropriate subject area and to avoid having light escape beyond the Proposed Project Footprint.

CM-6 – Implement Fugitive Dust Controls During Construction: The Authority will require the following fugitive dust control requirements to be included in construction contract specifications.

The construction contractor shall implement basic and enhanced control measures at construction and staging areas to reduce construction-related fugitive dust. The following measures are based on Bay Area Air Quality Management District's (BAAQMD) California Environmental Quality Act guidelines (BAAQMD 2022) and are in conformance with San Joaquin Valley Air Pollution Control District fugitive dust control requirements (Regulation VIII).

- **Basic Fugitive Dust Control Measures**
 - Exposed surfaces (e.g., parking areas, staging areas, soil piles, graded areas, and unpaved access roads) will be watered two times per day.
 - Haul trucks transporting soil, sand, or other loose material off-site will be covered.
 - Visible mud or dirt track-out onto adjacent public roads will be removed using wet power vacuum street sweepers at least once per day. The use of dry power sweeping is prohibited.
 - Roadways, driveways, and sidewalks to be paved will be completed as soon as possible. Building pads will be laid as soon as possible after grading unless seeding or soil binders are used.
 - A publicly visible sign with the telephone number and the name of the person to contact at the lead agency regarding dust complaints will be posted. This person will respond and take corrective action within 48 hours. The phone number of the district will also be visible to ensure compliance.

- Enhanced Fugitive Dust Control Measures for Land Disturbance
 - Exposed surfaces will be watered at a frequency adequate to maintain minimum soil moisture of 12 percent. Moisture content can be verified by laboratory samples or moisture probe.
 - Excavation, grading, and/or demolition activities will be suspended when average wind speeds exceed 20 mph.
 - Wind breaks (e.g., trees, fences) will be installed on the windward side(s) of actively disturbed areas of construction. Wind breaks should have at maximum 50 percent air porosity.
 - Vegetative ground cover (e.g., fast-germinating native grass seed) will be planted in disturbed areas as soon as possible and watered appropriately until vegetation is established.
 - The simultaneous occurrence of excavation, grading, and ground-disturbing construction activities on the same area at any one time will be limited. Activities will be phased to reduce the amount of disturbed surfaces at any one time.
- Measures for Entrained Road Dust
 - Trucks and equipment, including their tires, will be washed off prior to leaving the site.
 - Site accesses to 100 feet from the paved road will be treated with a 6- to 12-inch compacted layer of wood chips, mulch, or gravel.
 - Sandbags or other erosion control measures will be installed to prevent silt runoff to public roadways from sites with a slope greater than 1 percent.
 - Vehicle speeds on unpaved roads will be limited to 15 mph [EACCS AMM Gen-07].
 - Unpaved roads will be watered twice daily.

CM-7 – Implementation of Water Quality/Erosion Control BMPs: The Authority will prepare a stormwater pollution prevention plan that identifies appropriate erosion and sediment control BMPs to minimize any wind- or water-related erosion, in compliance with the General Permit for Storm Water Discharges Associated with Construction and Land Disturbance Activities (CA Order No. 2022-0057-DWQ). These measures will include, at a minimum, the following:

- Significant earth-moving activities will not be conducted in riparian areas within 24 hours of predicted storms or after major storms (defined as 1 inch of rain or more) [EACCS AMM Gen-16].
- Stockpiling of material in riparian areas will occur outside of the top of bank, and preferably outside of the outer riparian dripline and will not exceed 30 days [EACCS AMM Gen-13].

- No debris, soil, silt, sand, bark, slash, sawdust, cement, concrete, washings, petroleum products, or other organic or earthen material will be allowed to enter or be placed where it may be washed by rainfall or runoff into waters of the U.S. or drainages. No discharge of excessively turbid water will be allowed, and all equipment will be well-maintained and free of leaks.
- No discharge of pollutants from vehicle and equipment cleaning will be allowed into any storm drains or watercourses.
- Vehicle and equipment fueling and maintenance operations will be kept at least 100 feet away from watercourses and sensitive habitats (e.g., wetlands), except at established commercial gas stations or established vehicle maintenance facilities [EACCS AMM Gen-08].
- Concrete waste will be collected in washouts, and water from curing operations will be collected and disposed of. Neither will be allowed into watercourses.
- Spill containment kits will be maintained on-site during construction operations and/or staging or fueling of equipment.
- Dust control measures will include use of water trucks and dust palliatives to control dust in excavation-and-fill areas; covering temporary access road entrances and exits with rock (rocking); and covering temporary stockpiles when weather conditions require.
- Graded areas will be protected from erosion using a combination of silt fences and fiber rolls along toes of slopes or along edges of designated staging areas, and erosion control netting (e.g., jute or coir) will be used as appropriate on sloped areas. Erosion control materials that use plastic or synthetic monofilament netting will not be used in the Proposed Project Footprint. This will include products that use photodegradable or biodegradable synthetic netting, which can take several months to decompose. Acceptable materials will include natural fibers, such as jute, coconut, twine, or other similar fibers [EACCS AMM Gen-12].

CM-8 – Develop and Implement a Construction Noise and Vibration-Reduction

Plan: Prior to the issuance of any demolition or construction permit for each phase of project construction, the Authority will develop a construction noise and vibration-reduction plan to minimize daytime and nighttime construction noise at nearby noise-sensitive and vibration-sensitive receptors. The plan will be developed in coordination with an acoustical consultant and the project construction contractor and shall be approved by applicable agencies. The plan shall include the following elements:

- A sound barrier plan that includes the design, implementation, and construction schedule of the temporary sound barriers for the construction phase of the Proposed Project. At a minimum, these barriers shall be designed to meet the applicable impact criteria (e.g., 80 decibels, A-weighted [dBA], 8-hour time-average sound level [Leq]) at all noise-sensitive receptors.
- Buffer distances and types of equipment shall be selected to minimize noise impacts.

- Construction contractors shall utilize equipment and trucks equipped with the best available noise control techniques, such as improved mufflers, equipment redesign, use of intake silencers, ducts, engine enclosures, and acoustically attenuating shields or shrouds, wherever feasible and practicable.
- Impact tools (e.g., jackhammers, pavement breakers) used for project construction shall be hydraulically or electrically powered wherever possible to avoid noise associated with compressed air exhaust from pneumatically powered tools. Where use of pneumatic tools is unavoidable, an exhaust muffler on the compressed air exhaust and external jackets shall be used where feasible to lower noise levels. Quiet procedures shall be used, such as drills rather than impact equipment, whenever practicable.
- Stationary noise sources (e.g., generators) shall be muffled and sited within distances from noise-sensitive receptors that will not exceed noise standards. When equipment must be sited within said distances, stationary noise sources shall be wholly or partially shielded from adjacent noise-sensitive receptors such that noise levels at all noise-sensitive properties are below guidance thresholds. Pole power shall be utilized at the earliest feasible point in time and to the maximum extent feasible in lieu of generators.
- “Quiet” pile-driving technology (such as auger displacement installation) shall be used where feasible in consideration of geotechnical and structural requirements and conditions.
- A community affairs liaison shall be designated, and a telephone hotline and email address created to reach this person, with contact information conspicuously posted around the Proposed Project site, in adjacent public spaces, and in construction notifications. If the community affairs liaison hotline is not staffed 24 hours per day, the hotline shall provide an automatic answering feature, with date and time stamp recording, to answer calls when the phone is unattended.
- The community affairs liaison shall be responsible for responding to any local complaints about construction activities associated with the Proposed Project. The community affairs liaison shall investigate, evaluate, and attempt to resolve noise complaints related to construction activities of the Proposed Project. The community affairs liaison shall coordinate with a designated construction contractor representative to implement the following:
 - Document and respond to each noise complaint.
 - Attempt to contact the person(s) making the noise complaint as soon as feasible and no later than one construction day.
 - Conduct a prompt investigation to determine whether construction activities related to the Proposed Project contribute a substantial amount of noise related to the complaint.
- If it is reasonably determined by the community affairs liaison that construction-related noise described in the complaint exceeds ambient exterior noise levels by 5 dBA or more at a noise-sensitive use, then the community affairs liaison shall

identify and implement feasible reasonable measures within the vicinity of the Proposed Project Footprint to address the noise complaint.

- Examples of reasonable measures that may be implemented within the Proposed Project Footprint include but are not limited to:
 - Confirming construction equipment and related noise suppression devices are maintained per manufacturers' specifications
 - Ensuring construction equipment is not idled for extended periods of time
 - Evaluating feasible relocations of equipment, alternatives to specific types of equipment, or resequencing of construction activities, as appropriate, while maintaining the project schedule and safety

CM-9 – Avoid Entrapment of Federally Listed Species: To prevent inadvertent entrapment of wildlife during construction, excavated holes or trenches more than 1 foot deep with walls steeper than 30 degrees will be covered by plywood or similar materials at the close of each working day. Alternatively, an additional 4-foot-high vertical barrier will be used to further prevent the inadvertent entrapment of wildlife. If it is not feasible to cover an excavation or provide an additional 4-foot-high vertical barrier, independent of exclusionary fences, one or more escape ramps constructed of earth fill or wooden planks will be installed. Before such holes or trenches are filled, they will be thoroughly inspected for trapped animals. Culverts or similar enclosed structures with a diameter of 4 inches or greater that are stored at a construction site will be inspected for federally listed wildlife species before the pipe is subsequently used or moved [*EACCS AMM Gen-11 and Gen-17*].

CM-10 – Construction Requirements for Work Areas: The Authority will impose the following site restrictions to avoid or minimize effects on federally listed species and their habitats [*EACCS AMM Gen-04*]:

- Routes and boundaries of roadwork will be clearly marked before the start of construction or grading.
- Food and food-related trash items will be enclosed in sealed trash containers and will be properly disposed of off-site.
- No pets belonging to construction personnel will be allowed anywhere in the work area during construction.
- No firearms will be allowed in the work area except for those carried by authorized security personnel or local, state, or federal law enforcement officials.
- A spill response plan will be prepared. Hazardous materials (e.g., fuels, oils, or solvents) will be stored in sealable containers in a designated location that is at least 50 feet from any hydrologic features.

1.7 Study Methods

This section describes the methods utilized to collect data on federal species and critical habitat and assess the potential effects of the Proposed Project.

1.7.1 Preliminary Field Reconnaissance

Two preliminary biological reconnaissance visits to the Action Area were conducted by AECOM Technical Services, Inc. (AECOM) biologists: one team of two biologists on September 26, 2022, and a second team of different biologists on June 21, 2023. These visits were conducted by biologists local to the area and familiar with the special-status species with potential to occur. These visits were conducted in order to collect preliminary information on plant communities, special-status species potential, and hydrologic features. AECOM biologists drove the publicly accessible portions because permission to access the proposed right-of-way had not been granted by the time of the surveys.

To the extent feasible, inaccessible areas were assessed using binoculars from public access points or were evaluated using aerial imagery. No focused or protocol-level species surveys were conducted. In addition, large portions of the Action Area were not surveyed due to private property restrictions. Photographs taken during the surveys are available in Appendix C.

1.7.2 Desktop Review

AECOM biologists conducted desktop database searches and background literature reviews to collect information on federal species with potential to occur in the vicinity of the Action Area and critical habitat that overlaps the Action Area. The best scientific and commercial data available to the AECOM team were reviewed to assess the habitats and potential for species to occur in the Action Area.

The following sources were consulted prior to, during, and after field studies to obtain information on the species:

- Official species list from the Sacramento Office of USFWS, generated using the IPaC online tool (USFWS 2024b) (Appendix B)
- USFWS-designated Critical Habitat Mapper (USFWS 2024c)
- California Natural Diversity Database (CNDDDB) RareFind 5 occurrence records from 10-mile search (California Department of Fish and Wildlife [CDFW] 2023)
- California Native Plant Society (CNPS) Inventory of Rare and Endangered Plants Database search of the U.S. Geological Survey (USGS) Proposed Project quads and surrounding quads (23 quads total) (CNPS 2023b)
- National Wetlands Inventory and California Aquatic Resources Inventory (CARI) for wetland habitat features (to assess potential habitat for listed aquatic species) (U.S. Department of Agriculture [USDA] National Resources Conservation Service [NRCS] 2023; San Francisco Estuary Institute 2023)
- Aerial imagery for land cover and potentially unmapped aquatic features (Esri 2024)
- Ground-based photographs

- Existing commercial and regulatory agency resources (e.g., CDFW Wildlife Habitat Relationships System [CDFW 2024a], agency notices and species documents and recovery plans for selected species)
- Environmental setting chapters and spatial data from existing conservation planning documents (e.g., SJMSCP, EACCS)

1.7.2.1 Vegetation Classification and Land Cover

Vegetation and land cover classification mapping for the Action Area was conducted by AECOM using aerial imagery during the desktop analysis. The land cover types identified broad land cover categories that can be confidently identified via aerial imagery, such as built environments (e.g., roads and buildings), ruderal areas (e.g., disturbed areas adjacent to built environments), and open water (e.g., ponds and the California Aqueduct). Additionally, aerial imagery was used to identify potential wetlands to support future focused field survey efforts.

1.7.2.2 Species Occurrence Potential

Database and literature reviews were conducted to evaluate the potential for federal species to occur in the Action Area. This investigation included review of aerial imagery, CNDDDB searches, National Wetlands Inventory and CARI databases to delineate potential habitat for aquatic species, and the USFWS species list to characterize the potential for distribution and relative abundance of wildlife and associated habitats. Conclusions were based on the existence of known occurrences and dates of those occurrences, habitat quality, and proximity to development and highways.

ArcGIS was used to determine the amount of habitat supporting federal species in the Action Area or that would be affected by the Proposed Project.

1.7.2.3 Critical Habitat

Database and literature reviews were conducted to evaluate the potential for physical and biological features (PBFs) within designated critical habitat units overlapping the Action Area. PBFs include features laid out in the appropriate quantity and arrangement, exhibit necessary parameters (salinity, temperature, size, etc.) essential to providing life cycle needs of the species, and may require special management considerations or protection if present within proposed modification areas. Habitat-based modeling, discussed in Section 1.7.3, was utilized to aid in the identification of potential PBFs within designated critical habitat units overlapping the Action Area.

1.7.3 Habitat-Based Modeling

Since detailed wetland, waters, and habitat surveys have not yet been conducted for the Proposed Project, a habitat-based modeling approach was used to map habitat types in the Action Area and estimate the amount of habitat that could be affected by the Proposed Project for specific species determined to potentially occur within the Action Area.

Specific to this modeling approach, taxon-specific buffers were added to the Proposed Project Footprint to capture the farthest possible extent of indirect effects for the species. Within the Proposed Project Footprint, landcover area was delineated using aerial imagery, and beyond the Proposed Project Footprint it was delineated using CalVeg (U.S. Forest Service [USFS] 2024), CARI, and aerial imagery confirmed by AECOM GIS and biology staff. CalVeg categories were visually assessed using current aerial imagery to ensure they were still accurate, and in cases where the mapped habitat category was out of date (e.g., had been converted to developed land), it was manually recategorized.

As described in Section 1.4, a 500-foot buffer was applied to the Proposed Project Footprint to establish the extent of the Action Area. The maximum extent for the potentially suitable habitat for each species was set based on USFWS-designated critical habitat (if present), Predicted Habitat Models (Biogeographic Information and Observation System [BIOS] and USGS; available for a subset of species), and CNDDDB records (if present). The Predicted Habitat Model data used CalVeg as an input and resulted in 100-foot square cells. Cells of this resolution often resulted in the omission of appropriate habitat in areas where suitable habitat was near developed habitat. A smoothing function (smoothed at a 200-foot tolerance) was applied to the polygon edges to fill in gaps between cells. Developed and urban areas were removed from the acreage calculations.

CARI streams and wetlands were visually evaluated by biologists using current aerial imagery and removed if no longer present. The remaining streams and rivers were buffered 4 feet on both sides of available line data (average channel width based on aerial interpretation), and CARI attributes were retained and stamped into the habitat layer with wetlands. Species-specific modeling parameters are discussed below.

1.7.3.1 Modeling for Vernal Pool Invertebrates

Habitat-based modeling was used to calculate the potentially affected areas for vernal pool fairy shrimp, which occur in ephemeral/vernal pools. A 250-foot buffer was applied to the Proposed Project Footprint to capture the extent of potential Proposed Project indirect effects for fairy shrimp. This distance was used because effects to the water table or clay soils may extend beyond the Proposed Project Footprint, and this distance is consistent with guidance in the 1996 Programmatic BO (USFWS 1996a). For this modeling approach, wetland categories that could potentially include appropriate fairy shrimp habitat were merged with critical habitat areas (if present within the 250-foot buffer of the Proposed Project Footprint) and any CNDDDB records within, or adjacent to, the buffer. Any developed or urban habitat present within the merged areas was removed, and the potential affected areas were then calculated. There were no Predicted Habitat Models (e.g., via BIOS and USGS) for these species.

1.7.3.2 Modeling for Amphibians and Reptiles

Habitat-based modeling was used to calculate the potentially affected areas for California tiger salamander, foothill yellow-legged frog, California red-legged frog, western spadefoot, and northwestern pond turtle. The approach used for these species differed from that used for the vernal pool invertebrates because amphibians and reptiles utilize

both aquatic and associated upland habitats to varying degrees; therefore, a multi-tiered assessment was used. Species-specific modeling approaches were utilized as follows:

- **California tiger salamander:** Breeding habitat was first estimated including all waterways, ponds, and wetlands, excluding perennial waterways, canals, and ditches that are concrete-lined or otherwise engineered to remove any pools of water that would not be suitable for breeding. A 0.7-mile buffer was applied to any potentially suitable breeding waterbodies to capture dispersal habitat as well as upland refugia. This buffer size is the same as that used by USFWS in its critical habitat modeling and should capture 99 percent of all adult California tiger salamanders. Any urban or developed habitat within this buffer was excluded. Finally, any areas outside of the California Wildlife Habitat Relationships (CWHR) modeled suitable habitat for California tiger salamander (CDFW 2024b) were excluded.
- **California red-legged frog:** Breeding habitat was first estimated including all waterways, ponds, and wetlands, excluding canals and ditches that are concrete-lined or otherwise engineered to remove any pools of water that would be suitable for breeding. A 1.0-mile buffer was applied to any potentially suitable breeding waterbodies to capture dispersal habitat as well as upland refugia. This buffer size should capture the vast majority of dispersing and estivating individuals. Any urban or developed habitat within this buffer was excluded. Finally, any areas outside of the CWHR modeled suitable habitat for California red-legged frog (CDFW 2024b) were excluded.
- **Western spadefoot:** Breeding habitat was first estimated including all waterways, ponds, and wetlands, excluding perennial waterways, canals, and ditches that are concrete-lined or otherwise engineered to remove any pools of water that would be suitable for breeding. A 2,000-foot buffer was applied to any potentially suitable breeding waterbodies to capture dispersal habitat as well as upland refugia. Any urban or developed habitat within this buffer was excluded. Finally, any areas outside of the CWHR modeled suitable habitat for western spadefoot (CDFW 2024b) were excluded.
- **Northwestern pond turtle:** Potentially suitable water habitat was first estimated, and this included all waterways, ponds, wetlands, and ditches. A 1,300-foot buffer was applied to any potentially suitable waterbodies to capture dispersal habitat, upland refugia, and nest sites. Any urban or developed habitat within this buffer was excluded. Finally, any areas outside of the CWHR modeled suitable habitat for northwestern pond turtle (CDFW 2024b) were excluded.

1.7.3.3 Habitat-Based Modeling

Habitat-based modeling was not completed for monarch butterfly, San Joaquin kit fox, and palmate-bracted bird's-beak. No habitat-based modeling was completed for the monarch butterfly based on its candidate status and the potential widespread presence of native and non-native milkweed (*Asclepias* spp.) host plants within the Action Area. Habitat-based modeling was not completed for San Joaquin kit fox due to the extensive area that could be suitable habitat and the lack of records of occurrence within the Action

Area within the past 20 years. Habitat-based modeling was not completed for the palmate-bracted bird's-beak since this species has not been documented within the Action Area and is only known to occur outside of the Action Area in an isolated alkali sink. Additionally, this species can occur in grasslands and wetlands in alkaline soils, which represents most of the undeveloped land within the Action Area, and thus, modeling would result in an overestimation of potentially occupied habitat.

1.7.4 Aquatic Habitat Environmental DNA Sampling and Visual Encounter Surveys

To better inform the potential for aquatic species to occur in the Action Area, environmental DNA (eDNA) sampling and visual encounter surveys were performed at 6 publicly accessible aquatic features where a total of 21 samples were collected to further evaluate the potential presence of California red-legged frog (*Rana draytonii*), foothill yellow-legged frog (*Rana boylei*), California tiger salamander (*Ambystoma californiense*), and northwestern pond turtle (*Actinemys marmorata*) in waterbodies located in the Upper- and Lower Arroyo Las Positas watersheds throughout the Proposed Project Footprint in Alameda County, California. Appendix D contains a map of sampling locations and other details regarding sampling methods. A primary goal of this effort was to further inform the potential for foothill yellow-legged frog to occur in the Action Area, since there are many presumed extant occurrences of the other species located within the Action Area. AECOM biologists collected water samples in four waterbodies: Tassajara Creek, Arroyo Las Positas Creek, an unnamed tributary to Arroyo Las Positas Creek, and in one unnamed roadside drainage ditch along Dyer Road in the vicinity of the South Bay Aqueduct and Altamont Creek. Appendix D provides details on the methodology of this eDNA sampling effort.

In advance of site sampling, a brief survey plan for potential water features based on a desktop review of aerial photos, wetland data, permission-to-enter, and prior survey data to refine and prioritize the sampling plan was outlined. The plan identified the number of sampling locations along each creek based on a combination of the availability of suitable habitat and the overall length of creek accessible for sampling.

Biologists conducted eDNA sampling and visual encounter surveys as described above. eDNA sampling was performed after an initial visual encounter survey, and before coming in contact with the water, to prevent contaminating the eDNA field samples. Biologists collected replicate (paired) samples from a minimum of two, and as many as eight, locations (sites) per creek. In addition, two field blanks consisting of bottled water were collected on-site, and two positive control samples were collected at the end of the sampling event from a watershed where foothill yellow-legged frogs are known to breed. After the eDNA sampling component was completed, biologists took general notes on the habitat characteristics present, including potential predators and invasive species, and generalized observations regarding the available prey base were documented.

Samples were preserved and stored in a freezer and delivered same-day or overnight to a laboratory where they were evaluated for evidence of target species eDNA by isolating total DNA from each water sample and analyzing them in triplicate using qualitative polymerase chain reaction. Appendix D contains the results of this sampling.

1.7.5 Effects Analysis

Federally listed wildlife and plant species and candidate and proposed to be listed wildlife species were evaluated for their potential to occur in the Action Area primarily using the above-described preliminary reconnaissance site visits, desktop-level analysis, habitat modeling, and eDNA sampling. This evaluation for wildlife species considered whether:

- The Action Area occurs within the known range of these species
- There is suitable habitat in the Action Area
- There is suitable habitat in or near the Action Area that is contiguous with known occupied habitat
- There are multiple CNDDDB occurrences near the Action Area
- The Action Area is potentially accessible to these species

The evaluation for plant species considered the presence of potentially suitable habitat, the range of the species, and occurrences of the species within 10 miles of the Proposed Project Footprint.

Results of eDNA sampling were positive or negative.

Following this evaluation, an effects analysis was performed to identify potential effects to species as a result of the Proposed Project. Effects were categorized as direct (permanent or temporary) or indirect.

For critical habitat, an analysis was performed to identify the potential for adverse modification to the habitat as a result of the Proposed Project. These determinations were based on an evaluation of identified PBFs and proximity of PBFs to direct Proposed Project activities.

1.8 Limitations That May Influence Results

Many portions of the Proposed Project were inaccessible during the field reconnaissance visits. Heavy traffic on I-580 and a lack of safe parking prevented access to some areas adjacent to the highway, which were accessible only in certain off-ramp intersections. This portion of the Proposed Project is largely characterized by existing development. Furthermore, due to varying restrictions on property access, many of these parcels had to be assessed from aerial photography and representative access points along roads. Inaccessible areas were closely examined on Google Earth and Google Maps to determine whether they may provide habitat for federally listed species and other sensitive natural resources. Although reconnaissance surveys were conducted where possible, focused or protocol-level surveys for federally listed plant and animal species have not been conducted for the Proposed Project.

The habitat-based modeling approach described in Section 1.7.3 was used to generate estimates of potential affected habitat, but the results are likely to be an overestimate of

suitable habitat for many of these species due to presumed occupancy without focused survey results.

2 Environmental Setting

Topography in the Action Area varies from relatively flat areas to rolling hills and steep slopes at Altamont Summit. The Action Area is characterized by a transitional zone that encompasses the Inner Coast Range separating the San Francisco Bay Area from the Central Valley. Most of the region east of Livermore is used for cattle rangeland and wind resources for power generation. The area is still used for agriculture; however, increased urbanization is reducing agricultural areas in Livermore and Mountain House.

2.1 Physical Conditions

The existing physical conditions of the Action Area are typical of the San Francisco East Bay Area Inner Coast Range and western San Joaquin Valley environments. The climate in the Action Area is Mediterranean, which has moist, mild winters and dry summers. The average annual rainfall is approximately 18 inches, most of which occurs between November and March (National Oceanic and Atmospheric Administration [NOAA] 2023). The Action Area elevation ranges from approximately 108 to 875 feet above mean sea level. The Action Area is mostly surrounded by developed land in the western half, with open grasslands on rolling hills in the eastern half of the Proposed Project.

Soils in the Action Area consist of loam, clay, and silt textures typically found in landscapes including terraces, foothills, alluvial fans, and flood basins. Additionally, the majority of the soil series mapped throughout the Action Area are slightly to strongly alkaline (USDA Soil Survey Staff 2023). These soils range in drainage and permeability from poorly drained to well drained and are commonly used for farmland, including grain farming, row crops, pasture, and rangeland. Vegetation supported by these soils includes annual grassland; forbs; scattered trees, including oaks; and scrub.

2.2 Vegetation Communities and Land Cover Types

The Proposed Project spans the Inner Coast Range and the western San Joaquin Valley from the cities of Pleasanton and Dublin at the western end to Mountain House Community in the east. Natural communities are an assemblage of species that co-occur in the same habitat or area. Vegetation communities are defined by an assemblage of plant species that collectively provide habitat for wildlife. Land cover types are defined by the dominant character of the land surface, as determined by vegetation, water, or human uses.

Vegetation communities in the Action Area include a variety of upland and wetland types that are common to this region of California. In the Action Area, vegetation ranges from developed/landscaped and ruderal in the western portion to open grassland and wetlands in the eastern portion. Vegetation communities and land cover types are typically classified according to the *Preliminary Descriptions of the Terrestrial Natural Communities of California* (Holland 1986) and *A Manual of California Vegetation*

(MCV), which CDFW considers superseding the Holland descriptions (CNPS 2023a). While vegetation communities were classified as best possible from publicly accessible areas, some refinement may be necessary if landowner permission is granted. Furthermore, land cover types were characterized to the finest level possible from analysis of aerial imagery due to the lack of access to private property. The lack of access prevented classification to the level of alliance or association as defined in the MCV, so broader, more qualitative descriptions were used.

Vegetation communities and land cover types in the Action Area are depicted on Figure 4 in Appendix A and listed in Table 2. Wildlife associations for each vegetation type are described in the following subsections.

Table 2: Vegetation Communities and Land Cover Types in the Action Area

General Vegetation Community/Land Cover Type	Includes Subcategories	Acreage
Agricultural	None	663
Ruderal	None	225
Developed/Landscaped	Urban	1,511 (including 1,322 of Urban)
Non-native Grassland	None	1,572
Scrub	None	1
Aquatic (Open Water)	Riverine Depressional (Ponds)	28
Riparian	Mixed Riparian Woodland Mixed Willow Riparian Scrub	5
Wetland (Potential)	Alkali Seasonal Wetland Freshwater Marsh Seasonal Wetland/ Riverine Seasonal Wetland Vernal Pool	103
Total		4,108

2.2.1 Agriculture

Cropland includes both currently cultivated lands (e.g., row crops, orchards) and fallow fields. Most agricultural lands occur in the eastern portion of the Action Area in the western San Joaquin Valley, west of Mountain House Community. Row crops in the Action Area include but are not limited to alfalfa (*Medicago sativa*), strawberries (*Fragaria* spp.), watermelon (*Citrullus lanatus*), cantaloupe (*Cucumis melo*), tomato (*Lycopersicon* spp.), and pumpkin (*Cucurbita pepo*). Orchards consist of monocultures of evenly spaced, low, bushy trees similar in canopy size and height. Canopy cover ranges from open to dense, depending on the age of the trees, with saplings and young trees having relatively open canopies and older trees providing more closed canopy cover. Depending on management practices, the understory is either devoid of vegetation or composed of various weedy annual grasses and forbs. Where herbaceous vegetation is present, it is often mowed, sprayed, or tilled to facilitate harvesting and conserve water. Fallow fields are agricultural lands that are not currently used for cultivation. These areas are generally

devoid of vegetation and left unseeded for several seasons so that the soil can regain fertility prior to being cultivated again for row or orchard crops.

2.2.2 Ruderal

Ruderal cover occurs in areas where natural vegetation has been removed or is significantly degraded by past or current human activity. Ruderal vegetation is often associated with areas alongside railroad tracks, vacant lots, roadsides, and other highly disturbed areas. Ruderal vegetation is typified by the dominance of non-native forbs that thrive in disturbed conditions, including bristly ox-tongue (*Helminthotheca echioides*), bull thistle (*Cirsium vulgare*), Italian thistle (*Carduus pycnocephalus*), prickly lettuce (*Lactuca serriola*), shortpod mustard (*Hirschfeldia incana*), stinkwort (*Dittrichia graveolens*), yellow star-thistle (*Centaurea solstitialis*), English plantain (*Plantago lanceolata*), jimson weed (*Datura* sp.), and Russian thistle (*Salsola* sp.). Because of the highly variable nature of ruderal habitats, this type was not classified according to CNPS (2023a) or Holland (1986). Ruderal areas may be like California annual grassland areas but are characterized by a greater level of disturbance.

2.2.3 Developed/Landscaped

Developed/landscaped areas include all types of residential, commercial, industrial, transportation, landscaping, and recreational development (e.g., sites with structures, paved surfaces, horticultural plantings, golf courses, or irrigated lawns). Vegetation in developed/landscaped areas is highly variable, ranging from nonexistent in paved areas (classified as urban on the vegetation exhibits) to maintained lawns and ornamental shade trees. Common ornamental species include California fan palm (*Washingtonia filifera*), Canary Island palm (*Phoenix canariensis*), eucalyptus (*Eucalyptus* spp.), olive (*Olea europaea*), oleander (*Nerium oleander*), and pepper tree (*Schinus molle*), among others. Ground cover generally consists of ruderal vegetation. The developed/landscaped land cover type is one of the most expansive land cover types in the Action Area.

2.2.4 Non-native Grassland

California annual grassland is an herbaceous plant community that is dominated by non-native annual grasses (Holland 1986; CNPS 2023a). In the Action Area, California annual grassland was mapped where grasses dominate the land cover and trees, and shrubs provide minimal cover. Dominant species are non-native annual grasses such as wild oats (*Avena barbata*, *A. fatua*), ripgut grass (*Bromus diandrus*), soft chess (*Bromus hordeaceus*), rye grass (*Festuca perennis*), and wall barley (*Hordeum murinum*). Herbaceous cover includes primarily non-native forbs such as bristly ox-tongue, bull thistle, Italian thistle, prickly lettuce, field mustard, stinkwort, yellow star-thistle, and native lupine (*Lupinus* spp.).

Salt grass flats are an herbaceous plant community that is dominated by salt grass (*Distichlis spicata*) in uplands, as opposed to wetlands. Salt grass flats are a natural community of special concern in undisturbed situations (CDFW 2018). In the Action Area, salt grass flats contain more than a 30 percent relative cover of salt grass in the herbaceous layer (CNPS 2023a). California annual grassland species are found scattered throughout the salt grass flat community.

Grasslands are generally found in the central portion of the Action Area from the Altamont Hills to the western portion of Mountain House Community.

2.2.5 Scrub

Alkali scrub is typically dominated by a salt-tolerant shrub known as four-winged saltbush (*Atriplex lentiformis*), with salt grass often present in the understory. Alkali scrub occurs in abandoned agricultural fields and is often dominated by monocultures of saltbush.

2.2.6 Aquatic

2.2.6.1 Riverine

Riverine features include intermittent and ephemeral watercourses characterized by a defined bed and bank. Intermittent streams carry water through most of the wet season (November through April), then are dry through most or all the dry season (June through October) in a normal rainfall year. Ephemeral streams carry water only during or immediately following a rainfall event. Riverine features are closely associated with riparian plant communities when sufficient hydrology supports riparian vegetation. The riparian plant composition, as well as the width of the riparian corridor, vary, depending on channel slope, magnitude, the frequency of channel and overbank flows, and the frequency and duration of flooding that inundates the broader floodplain. Several intermittent and ephemeral watercourses occur in the Action Area, including streams, creeks, canals, and ditches. Intermittent streams include Tassajara Creek, Arroyo Mocho, Arroyo Las Positas, Cottonwood Creek, Collier Canyon Creek, Cayetano Creek, and Mountain House Creek. Canals and ditches are included in the riverine land cover type because their functions are like those of degraded streams. In addition, the Delta-Mendota Canal and the California Aqueduct convey water to southern California. Because of the nature of these built structures, canals and ditches are often managed with minimal vegetation to enhance the flow of water through the channels. Federally listed wildlife species known to use riverine habitat include California red-legged frog, foothill yellow-legged frog, and northwestern pond turtle.

2.2.6.2 Depressional (Ponds)

Ponds are small (smaller than 0.5 acre in surface area) perennial or seasonal waterbodies that support little or no vegetation. If vegetation is present, it is typically submerged or floating. Ponds in the Action Area are limited to stock ponds, settlement ponds, and constructed ponds. Federally listed wildlife species known to use ponded freshwater aquatic habitat include California red-legged frog, California tiger salamander, western spadefoot, and northwestern pond turtle.

2.2.7 Riparian

2.2.7.1 Mixed Riparian Woodland

Mixed riparian woodland is a natural community of special concern in undisturbed situations. This land cover type occurs along the margins of an active channel. Generally, no single species dominates the canopy, and composition varies with elevation, aspect, and hydrology. The dominant canopy species are California sycamore (*Platanus racemosa*), valley oak (*Quercus lobata*), coast live oak (*Quercus agrifolia*), willow (*Salix*

spp.), California bay (*Umbellularia californica*), and Fremont cottonwood (*Populus fremontii*).

2.2.7.2 Mixed Willow Riparian Scrub

Typically, mixed willow riparian scrub consists of willow stands that may or may not be dominated by a single species. Willow stands occur in environmental conditions like those for alder (*Alnus* spp.), cottonwood (*Populus* spp.), and other willow series (Holland 1986). In the Action Area, the mixed willow riparian scrub community typically consists of scattered willows and fast-growing shrubs and vines. In the Action Area, mixed willow riparian scrub is found in patches associated with the mixed riparian forest and woodland surrounding some of the intermittent creeks.

2.2.8 Wetland

2.2.8.1 Alkali Seasonal Wetland

The dominant species throughout the Action Area in alkali seasonal wetlands is salt grass, but this community represents seasonal wetlands as opposed to uplands. The majority of alkali seasonal wetland in the Action Area is generally north and east of Livermore or in Altamont Hills. Alkali seasonal wetland is a natural community of special concern in undisturbed situations. Associated species include alkali heath (*Frankenia salina*), alkali Russian thistle (*Salsola soda*), annual beard grass (*Polypogon monspeliensis*), common gumplant (*Grindelia camporum*), perennial pepperweed (*Lepidium latifolium*), and fat-hen (*Atriplex prostrata*). Ripgut grass, soft chess, and wall barley are also present in small amounts.

2.2.8.2 Freshwater Marsh

Freshwater marshes in the Action Area are dominated by emergent herbaceous wetland plants in areas that are either intermittently flooded or perennially saturated. Typically, cattails (*Typha* spp.) and bulrushes (*Schoenoplectus* spp.) are the dominant plant species in freshwater marshes. Freshwater marshes can be found in numerous locations in the Action Area.

2.2.8.3 Seasonal Wetland/Riverine Seasonal Wetland

Seasonal wetlands in the Action Area are freshwater wetlands that support inundation or saturated soil conditions, typically during the wet season. These features are often inundated by direct rainfall and runoff from adjacent uplands (seasonal wetlands) or runoff from adjacent streams (riverine seasonal wetlands). Seasonal wetlands do not inundate for as long as vernal pools, ultimately resulting in a hydroperiod that is too short to support vernal pool branchiopods (e.g., fairy shrimp). Consequently, plants that are often found on the high margins of vernal pools (where the pools first dry down) make up the floristic species composition of seasonal wetlands. These floristic species include smooth-rayed goldfields (*Lasthenia glaberrima*), Fitch's spikeweed (*Centromadia fitchii*), peppergrass (*Lepidium nitidum*), cowbag clover (*Trifolium depauperatum*), and Greene's popcornflower (*Plagiobothrys greenei*). Seasonal wetlands can be found in multiple locations in the Action Area. Seasonal wetlands provide aquatic breeding habitat for western spadefoot and California tiger salamander.

2.2.8.4 Vernal Pool

Vernal pools are seasonal wetlands in which water ponds on the surface for extended durations in winter and spring, then dries completely in late spring and summer. Areas with high concentrations of vernal pools in the Action Area are generally north and east of Livermore and in Altamont Hills. These areas support flora that is largely made up of native wetland plant species such as Fremont's goldfields (*Lasthenia fremontii*), yellow-rayed goldfields (*Lasthenia glabrata*), common spikerush (*Eleocharis macrostachya*), vernal pool buttercup (*Ranunculus bonariensis*), coyotethistle (*Eryngium vaseyi*), doublehorn calicoflower (*Downingia bicornuta*), toothed calicoflower (*Downingia cuspidata*), flatface downingia (*Downingia pulchella*), and blow wives (*Achyraea mollis*). Vernal pools generally have longer periods of inundation than alkali seasonal wetlands or seasonal wetlands, and they provide suitable habitat for federally listed species, including vernal pool fairy shrimp, western spadefoot, and California tiger salamander.

3 Species and Critical Habitats Assessed

This section provides background information for each of the federally listed, candidate, and proposed to be listed species, including their status, the presence of critical habitat in the Action Area, details on the physical characteristics of the species and their life cycle, geographic distribution, habitat associations, results of CNDDDB searches within 10 miles of the Proposed Project Footprint, and any species-specific parameters used in the habitat-based modeling approach. Federally listed, proposed, and candidate species and critical habitat considered in this BA include:

- Species listed or proposed for listing as threatened or endangered under the ESA (50 CFR 17.12 for listed plants, 50 CFR 17.11 for listed animals, and various notices in the *Federal Register* [FR] for species proposed for listing)
- Species that are candidates for possible future listing as threatened or endangered under the ESA
- Critical habitat for listed species

This section includes those federally listed, proposed, and candidate species and/or critical habitats identified on the federal species list from USFWS or via the CNDDDB search. Table 3 provides a summary of those species and the likelihood of their occurrence in the Action Area, and Table 4 provides a summary of critical habitat that overlaps the Action Area.

Table 3: Federally Listed, Proposed, and Candidate Species with Potential to Occur in Action Area

Common Name (Scientific Name)	Federal Status	Habitat Description	Potential to Occur in Action Area
Wildlife			
Conservancy fairy shrimp (<i>Branchinecta conservation</i>)	Endangered	Conservancy fairy shrimp are extremely rare and only found in California's Central Valley. They are found in a variety of vernal pool habitats ranging from the transverse regions in Ventura County to the Vina Plains in Tehama County. They are mostly found in large, turbid vernal pools with neutral pH.	No. The CNDDDB has no records of this species within 10 miles of the Proposed Project Footprint (CDFW 2023). The range of this species is restricted to the Central Valley, with the exception of an outlier population in Ventura County (USFWS 2024d). Where the Action Area overlaps with the Central Valley near Mountain House, there are no vernal pool features present.
Longhorn fairy shrimp (<i>Branchinecta longiantenna</i>)	Endangered	Longhorn fairy shrimp live in clear to turbid freshwater vernal pools, as well as water-filled depressions in sandstone, in grasslands and agricultural lands near the City of Tracy, grass-bottomed pools in Merced County, or claypan pools around Soda Lake in San Luis Obispo County. It is only known from five locations, including vernal pools in the Brushy Peak Preserve in Alameda County; claypan pools in and adjacent to the Carrizo Plain National Monument in San Luis Obispo County; vernal pools and grass-bottomed pools in the San Luis National Wildlife Refuge Complex in Merced County; vernal pools in the Vasco Caves Preserve near the town of Byron in Contra Costa County; and vernal pools in the Alkali Sink Conservation Bank east of the City of Mendota in Fresno County.	No. Species is extremely rare and only known from five locations (USFWS 2021a), the closest of which is the Brush Peak Preserve located approximately 3 miles north of the Action Area. All known populations in the East Bay Hills are associated with pools in large rock outcroppings, which are not present in the Action Area.
Vernal pool fairy shrimp (<i>Branchinecta lynchi</i>)	Threatened	Vernal pool fairy shrimp habitat includes vernal pools in grasslands and rural/agriculture land.	Yes. Vernal pool fairy shrimp may occur in depressional vernal pool habitats within the grasslands and agricultural lands in the Action Area.

Common Name (Scientific Name)	Federal Status	Habitat Description	Potential to Occur in Action Area
Vernal pool tadpole shrimp (<i>Lepidurus packardii</i>)	Endangered	Vernal pool tadpole shrimp are found in the ephemeral wetlands from Kings County to Tehama County. They live in clear to turbid freshwater vernal pools throughout their range.	No. Species is principally found in the Central Valley, and requires vernal pools that are sufficiently large enough or hold water for approximately 2 months so that the species can complete its life cycle (USFWS 2024d). The nearest CNDDDB record for vernal pool tadpole shrimp is approximately 15 miles southwest of the Proposed Project Footprint in Bayfront Wetlands near the City of Fremont (CDFW 2023). Due to the lack of large vernal pools and records in the Proposed Project vicinity, this species is not expected to occur.
Valley elderberry longhorn beetle (<i>Desmocerus californicus dimorphus</i>)	Threatened	The valley elderberry longhorn beetle lives throughout California's Central Valley from Shasta County in the north through Madera County in the south. The species is dependent on its host plant, the elderberry, a shrub that grows in riparian areas and foothill oak woodlands in California. Although these shrubs are widely distributed, the valley elderberry longhorn beetle is only found on the valley floor and low foothills.	No. The species range is limited to the Central Valley and adjacent low foothills (USFWS 2024e), and not known to occur in the Livermore area. There are two records of valley elderberry longhorn beetle within 5 miles of the proposed Action Area, south of Mountain House (CDFW 2023). However, there are no riparian corridors or other areas likely to support elderberry host plants where the Action Area overlaps with the range of the species.
Monarch butterfly (<i>Danaus plexippus</i>)	Candidate (California-overwintering population)	Breeding and migratory habitat is primarily defined by the presence of native milkweeds to host eggs and larvae, as well as nectar sources and trees and shrubs for shading and roosting for adults. Overwintering habitat consists of tree groves that provide suitable microclimates for winter diapause.	Yes. The monarch butterfly is likely to be found throughout the Action Area.
California tiger salamander – Central California DPS (<i>Ambystoma californiense</i>)	Threatened	California tiger salamanders require access to both aquatic and upland habitat throughout their life cycle. They use standing bodies of fresh water, like ponds, vernal pools, and other ephemeral or permanent waterbodies for breeding.	Yes. California tiger salamander may occur within wetlands and water features identified within the Action Area that are likely to have a seasonal wet/dry cycle that can support the species.

Common Name (Scientific Name)	Federal Status	Habitat Description	Potential to Occur in Action Area
Foothill yellow-legged frog – Central Coast DPS, population 4 (<i>Rana boylei</i>)	Threatened	The foothill yellow-legged frog lives in foothill and mountain streams from the Pacific Coast to the western slopes of the Sierra Nevada and Cascades Mountains, up to approximately 5,000 feet in elevation. The foothill yellow-legged frog occurs in a variety of vegetation types including valley-foothill hardwood, valley-foothill hardwood-conifer, valley-foothill riparian, ponderosa pine, mixed conifer, mixed chaparral, and wet meadows. The frog is closely associated with streams and is rarely observed far from the water's edge. Breeding stream habitat is typically shallow, rocky, and at least partially exposed to direct sunlight.	No. Based on the lack of CNDDDB records, eDNA sampling results, and unsuitable habitat characteristics of the waterways (e.g., Tassajara Creek, Arroyo Las Positas Creek, Cottonwood Creek, Cayetano Creek, and Arroyo Seco) within the Action Area, the foothill yellow-legged frog is not expected to be present. eDNA sampling conducted in July 2024 did not detect this species in Tassajara or Arroyo Las Positas Creek, which are the largest waterways that intersect the Action Area. Habitats within these waterways, and the other unnamed tributaries within the Action Area, generally lack rocky or cobbly substrate, have sparse or are lacking riparian corridors, and are dominated by muddy, silty bottoms. These waterways also typically dry into isolated pools during the summer months, and many of them have been channelized, constrained by urban development, or are impacted by grazing.
California red-legged frog (<i>Rana draytonii</i>)	Threatened	The California red-legged frog spends the bulk of its life in or near water sources like streams or stock ponds, which the species uses for breeding. The frog moves into neighboring upland areas to feed and shelter when stream flow levels are high. In the summer, they seek relief from the heat by hiding under rocks or boulders, leaf litter, small stream channels, or animal burrows.	Yes. eDNA sampling in July 2024 detected this species in an unnamed tributary of Arroyo Las Positas. California red-legged frog may occur in aquatic breeding and/or nonbreeding habitat in any of the named and unnamed streams and their tributaries, flood control channels, aqueducts (excluding the Delta-Mendota Canal and Governor Edmund G Brown California Aqueduct), and natural and unnatural ponds in the Action Area.

Common Name (Scientific Name)	Federal Status	Habitat Description	Potential to Occur in Action Area
Western spadefoot (<i>Spea hammondi</i>)	Proposed Threatened	The western spadefoot is a near endemic species to California, ranging from the Central Valley, bordering foothills, and Coast Ranges south of Monterey Bay into northwestern Baja California, Mexico. The western spadefoot is primarily terrestrial over the course of the annual cycle, spending most of its life aestivating underground in upland habitat that is generally within 0.5 mile of aquatic breeding habitat. Vernal pools are considered the primary aquatic breeding habitat; however, western spadefoots have been observed within a variety of permanent and temporary breeding habitat, both natural and altered, including rivers, creeks, artificial ponds, livestock ponds, sedimentation and flood control ponds, irrigation and roadside ditches, roadside puddles, tire ruts, and borrow pits.	Yes. Western spadefoot may occur primarily in seasonal wetlands, vernal pools, ephemeral drainages, and associated undeveloped upland land cover.
Alameda whipsnake (<i>Masticophis lateralis euryxanthus</i>)	Threatened	Alameda whipsnake is endemic to the northern Diablo Range, primarily in Alameda and Contra Costa Counties. They rely on chaparral and scrub habitats, but will use adjacent grasslands, woodlands, and riparian areas for foraging and movement.	No. There are 60 records of Alameda whipsnake within 10 miles of the Proposed Project Footprint. The closest record is approximately 1.5 miles from the Proposed Project Footprint from 1991 in an area that has since been developed (CDFW 2023). However, the Action Area does not contain nor is adjacent to chaparral or scrub habitats that may support the species, and the species is not expected to be present.
Northwestern pond turtle (<i>Actinemys marmorata</i>)	Proposed Threatened	The northwestern pond turtle is found in portions of Washington, Oregon, Nevada, and northern and central California. Within California, it is found in areas of the Coast Range from the Oregon border to northern Monterey County, the foothills and lower elevation. The northwestern pond turtle inhabits various waterbodies, including ponds, marshes, rivers, streams, and irrigation canals. It can tolerate saltwater for short periods of time, but is typically found in freshwater habitats.	Yes. The northwestern pond turtle is known to occur or may occur in portions of Altamont Creek, Arroyo Las Positas Creek, Cayetano Creek, Arroyo Mocho Canal, as well as perennial wetlands, stock ponds, ditches, and adjacent upland habitats within the Action Area. Additionally, this species was visually detected in Arroyo Las Positas, its unnamed tributary, and was detected in Tassajara Creek using eDNA sampling in July 2024.

Common Name (Scientific Name)	Federal Status	Habitat Description	Potential to Occur in Action Area
California condor (<i>Gymnogyps californianus</i>)	Endangered	Condors typically roost in steep terrain on ridgelines, rocky outcrops, steep canyons, and tall trees because they have difficulty taking flight from flat ground due to their heavy weight. The active range of the California condor in the state of California has previously been restricted to the central California Coast, Transverse Ranges, and southern Sierra Nevada Ranges, although USFWS now identifies the Diablo Range as part of the range for this species.	No. The nearest California condor record is approximately 90 miles south of the Proposed Project Footprint (CDFW 2023). Although condors could pass through the Action Area to forage, based on lack of CNDDDB records of California condor occurrence in proximity and the lack of potential roost sites (i.e., steep terrain on ridgelines, rocky outcrops, steep canyons, tall trees), this species is not anticipated to occur in the Action Area.
California least tern (<i>Sterna antillarum browni</i>)	Endangered	The California least tern breeds along the Pacific Coast from San Pablo Bay, California, to San Jose del Cabo, Baja California, Mexico. California least terns nest on 23 sites, some of which are natural, and others are human-made and include beaches close to river mouths, estuaries, and coastal embayments. The nest sites require active management activities, including protection from disturbance and predators, and/or vegetation management to retain habitat suitability.	No. The nearest CNDDDB record for California least tern is approximately 13.5 miles southwest of the Proposed Project Footprint along the eastern shore of San Francisco Bay (CDFW 2023). The Action Area is not along the coastline and does not contain potential habitat for the species.
San Joaquin kit fox (<i>Vulpes macrotis mutica</i>)	Threatened	The San Joaquin kit fox lives in the desert and grasslands of California's San Joaquin Valley. They prefer areas with minimal shrubs and grasses. The kit fox's range in the San Joaquin Valley extends from southern Kern County north to Contra Costa, Alameda, and San Joaquin Counties on the western side of the valley; and to the La Grange area of Stanislaus County on the eastern side of the valley. The kit fox's range also includes valleys along the Coast Range, including the Panoche and Cuyama Valleys and the Carrizo Plain in San Luis Obispo County.	Yes. The San Joaquin kit fox could be found in grassland, ruderal and agricultural land habitats in the Action Area.

Common Name (Scientific Name)	Federal Status	Habitat Description	Potential to Occur in Action Area
Plants			
Large-flowered fiddleneck (<i>Amsinckia grandiflora</i>)	Endangered	Large-flowered fiddleneck grows on hilly grasslands at lower elevations in clay-rich soil. It can be found on steep, north-facing slopes that are in shaded terrain and remain moist for longer into the day than surrounding areas. It prefers a moderate climate with average seasonal lows in the upper 30 degrees Fahrenheit and highs in the lower 90 degrees Fahrenheit.	No. Species is only known to occupy 11 sites in Contra Costa and San Joaquin Counties (USFWS 2021b). CNDDDB has six records for large-flowered fiddleneck within 10 miles of the Proposed Project Footprint (CDFW 2023). Of these, three are presumed to be extant. The nearest CNDDDB record that is presumed extant is 6 miles south of the Proposed Project Footprint and is the “Drop Tower” experimental reintroduced population at Lawrence Livermore National Laboratory (CDFW 2023).
Lassics lupine (<i>Lupinus constancei</i>)	Endangered	The Lassics Lupine is found near the summits of the Lassics, a remote mountain range in northern California (USFWS 2024a). These mountains are located in Humboldt and Trinity Counties within the Six Rivers National Forest. They grow on serpentine barrens and high elevations of 5,000 to 6,000 feet.	No. The nearest CNDDDB record is approximately 200 miles from the Proposed Project Footprint (CDFW 2023). This species has a very limited distribution and potential habitat (serpentine barrens at high elevations) is not present within the Action Area.
Palmate-bracted bird's- beak (<i>Chloropyron palmatum</i> (syn. <i>Cordylanthus palmatus</i>))	Endangered	Palmate-bracted bird's-beak is a hemi-parasitic annual herb in the broomrape family (<i>Orobanchaceae</i>). This species has been documented in the Central Valley, from Fresno County in the south to Yolo County in the north, at elevations from 16 to 509 feet (CDFW 2023). Palmate-bracted bird's-beak occurs in chenopod scrub and valley and foothill grasslands in alkaline soils.	Yes. The palmate-bracted bird's-beak has a potential to occur in alkaline grasslands and wetlands in the Action Area, specifically alkaline grasslands that are seasonally flooded.

Notes:

DPS = distinct population segment

Table 4: Federally Designated Critical Habitat Considered

Species and Critical Habitat Unit	Description	Presence of PBFs Within Proposed Project Footprint
Delta smelt (<i>Hypomesus transpacificus</i>) Critical Habitat	The delta smelt requires open surface waters of the San Francisco Bay Delta and Suisun Bay and shallow edge waters in the upper delta, which is used for spawning. PBFs of critical habitat include physical habitat, water, river flow, and salinity concentrations necessary for spawning, larval and juvenile transport, rearing, and adult migration. The San Francisco Bay Delta is broadly designated as critical habitat for this species with the legal delta boundary being used for the designation.	No. The easternmost portions of the Action Area overlap critical habitat for the smelt. However, current land cover in these areas is annual grassland, development, and intensive agriculture, which is not suitable for the species. Based on aerial review and desktop evaluation, the PBFs required for this species are not within the portion of critical habitat that overlaps with the Action Area.
Vernal pool fairy shrimp (<i>Branchinecta lynchi</i>) Critical Habitat Unit 19C	PBFs of critical habitat include components that include mounds, swales, and depressions within a matrix of surrounding uplands that exhibit continuous or intermittent flowing surface waters. Essential habitat includes connecting habitat features such as isolated vernal pools with underlying restrictive layers that continuously hold water for a minimum of 18 days to provide adequate water for incubation, maturation, and reproduction. Structure within the vernal pools consists of organic and inorganic materials providing shelter as well as food consisting of detritus contributed by overland flow or biological processes within the pools themselves.	No. Although critical habitat for this species overlaps with the Action Area, north of I-580, north of Northfront Road, and west of Altamont Pass Road (USFWS 2006), it does not overlap the Proposed Project Footprint. No impacts to nearby PBFs outside the Proposed Project Footprint would occur with implementation of various conservation measures.
California red-legged frog (<i>Rana draytonii</i>) Critical Habitat Unit ALA-2	PBFs of critical habitat include aquatic breeding habitat essential for all aquatic life stages of the California red-legged frog, including ponds, backwaters within streams and creeks, marshes, lagoons, and dune ponds with the capability to hold water for a minimum of 20 weeks. Water quality within these features requires low salinity (below 4.5 parts per million [ppm] up to 7 ppm) and temperatures below 73 degrees Fahrenheit. Nonbreeding aquatic and riparian habitat essential for the species includes shallow freshwater features such as streams, small seeps, and ponds that dry too quickly to support breeding. Upland adjacent habitat is essential to providing food and shelter sites for the species allow for dispersal between breeding and nonbreeding habitat.	Yes. Critical habitat for this species overlaps with the Action Area north of I-580 between Fallon Road and Collier Canyon Road, and the majority of the Action Area throughout the Altamont Pass. PBFs, including potentially suitable breeding habitat and adjacent upland habitat, are likely present within critical habitat within the Action Area.

For species with the potential to occur in the Action Area, brief species accounts are provided below. The species determined to have no potential to occur in the Action Area and critical habitat with no PBFs in the Proposed Project Footprint are not carried forward in the BA.

3.1 Vernal Pool Fairy Shrimp

3.1.1 Status and Critical Habitat

The vernal pool fairy shrimp is federally threatened (USFWS 2023h). Designated critical habitat for this species overlaps with the Action Area, north of I-580, and north of Northfront Road in the eastern portion (USFWS 2006) (Appendix A, Figure 5). The Proposed Project Footprint does not overlap with the critical habitat; therefore, critical habitat for the vernal pool fairy shrimp is not discussed further in this BA.

3.1.2 Physical Characteristics

The vernal pool fairy shrimp is a small, freshwater crustacean. They have slender bodies; large, stalked compound eyes; and 11 pairs of swimming legs that also function as gills. They glide gracefully through the water upside down, swimming by beating their legs in a complex, wavelike movement that passes from front to back. The vernal pool fairy shrimp can be differentiated from other fairy shrimp by the shape of the male's second antenna and the female's third thoracic segment, on the middle part of its body. The vernal pool fairy shrimp does not have a hard outer shell (USFWS 2023h).

3.1.3 Life Cycle

The average lifespan of the vernal pool fairy shrimp is 91 days. Vernal pool fairy shrimp can be found in vernal pools starting in November most years and complete their entire life cycle by early May. On average, vernal pool fairy shrimp take 18 days to mature after hatching and 40 days to reproduce. Female vernal pool fairy shrimp carry fertilized eggs in a brood sac on the underside of their bodies. The eggs are either dropped to the pool bottom or remain in the brood sac until the mother dies and sinks to the bottom of the pool. Multiple cohorts of eggs may hatch in a single vernal pool throughout the wet season, given the right conditions. Hatched vernal pool fairy shrimp die before the vernal pools dry. Resting fairy shrimp eggs are known as cysts, and they may remain viable for multiple years due to their protective coverings that help them withstand extreme environmental conditions and even digestion by other animals. The cysts remain in the dry pool bed until hatching begins in response to rains and the return of water in the vernal pools (USFWS 2023h).

Vernal pool fairy shrimp are opportunistic filter feeders. They eat algae, bacteria, protozoa, rotifers, and bits of waste from plants and other animals present in their environments. They face competition with these food sources from other fairy shrimp species (USFWS 2023h).

Vernal pool fairy shrimp are nonmigratory and have little ability to disperse on their own. Aquatic birds are the most likely agents of dispersal of vernal pool fairy shrimp. Large mammals are also known to act as distributors by wallowing in dirt, getting cysts caught in

their fur, and transporting the cysts to another wallow. Additionally, cysts can be ingested, passed through the digestive tract, and then deposited in new habitats when the animal urinates (USFWS 2023h).

3.1.4 Distribution and Habitat Associations

At the time of listing in 1994, the vernal pool fairy shrimp was known from 32 populations from Shasta County, California, south through the Central Valley to Tulare County, and along the central Coast Range from northern Solano County to San Benito County, California, as well as four additional populations in southern California (USFWS 2023h). Vernal pool fairy shrimp habitat includes vernal pools in grasslands and rural/agriculture land. Vernal pool fairy shrimp are opportunistic filter feeders. They eat algae, bacteria, protozoa, rotifers, and bits of waste from plants and other animals present in their environments. They face competition with these food sources from other fairy shrimp species (USFWS 2023h).

Since the vernal pool fairy shrimp's listing, surveys of vernal pools and other temporary waters throughout the western U.S. have resulted in an increase in the shrimp's known range. In addition to the 32 counties in California, the vernal pool fairy shrimp have been observed in Jackson County in southern Oregon. The species lives in a variety of vernal pool habitats and occurs in 13 of the 17 vernal pool regions and 45 of the 85 core recovery areas identified in California and Oregon (USFWS 2023h).

Thirteen records of this species have been documented within 10 miles of the Proposed Project Footprint, with the nearest record approximately 0.3 mile north of I-580, and north of Northfront Road in the eastern portion of the City of Livermore (CDFW 2023). There is a cluster of records between Altamont Creek and Altamont Pass Road, north of Greenville Road and I-580. None of these occurrences overlap with the Proposed Project Footprint.

Based on the resources within the Action Area, vernal pool fairy shrimp may occur in depressional vernal habitats within the grasslands and agricultural lands in the Action Area, although the overall potential is considered low. Habitat-based modeling resulted in 16 acres of habitat that exhibits characteristics that could provide essential resources for the vernal pool fairy shrimp to complete life cycle functions. These potentially suitable habitat areas are located within the area north of I-580 between Fallon and Dolan Roads, north of I-580 between Portola Avenue and Springtown Boulevard, and east of Greenville Road through the Altamont Pass to the California Aqueduct. Potential habitat within the Action Area is depicted on Figure 6 in Appendix A. All suitable habitat is assumed to be potentially occupied by the species for purposes of this BA.

3.2 Monarch Butterfly

3.2.1 Status and Critical Habitat

The monarch butterfly is a candidate for listing under the ESA (USFWS 2023e). Critical habitat has not yet been designated or proposed for this species.

3.2.2 Physical Characteristics

Adult monarch butterflies are characterized by their large (up to 4 inches) bright orange wings with black border and vein coloration. Caterpillars have black, white, and yellow transverse bands. Coloration is thought to be a warning to predators, because caterpillars accumulate cardenolide toxins through feeding on milkweed, making both larva and adult butterflies unpalatable to predators.

3.2.3 Life Cycle

Monarch butterflies typically overwinter in coastal California and Baja California, Mexico. When spring conditions begin, adult monarchs break diapause and begin migrating north and east. Although overwintering adults have a life span of 6 to 9 months, breeding adults only live 2 to 5 weeks. Migration occurs over multiple generations over the spring and summer, with reproduction following the typical emergence of milkweeds (*Asclepias* spp.) in the early spring (Western Association of Fish and Wildlife Agencies [WAFWA] 2019).

3.2.4 Distribution and Habitat Associations

Monarch butterflies are widely distributed from Australasia and portions of Europe to the Americas. Populations are delineated into eight geographical units based on genetic, morphological, behavioral, and ecological patterns that influence each population's ability to adapt to changes in the environment. These units are called adaptive capacity units (ACUs) (USFWS 2020). The western North American ACU engages in long-distance seasonal migration, although areas where non-native milkweeds (i.e., *Asclepias curassavica*) which emerge earlier than native species and can bloom during winter months in milder climates, may be encouraging monarch butterflies to become resident in areas where it previously only overwintered (WAFWA 2019).

Monarch breeding typically follows the emergence of milkweed in the early spring, and breeding and migratory habitat is primarily defined by the presence of native milkweeds to host eggs and larvae, as well as nectar sources and trees and shrubs for shading and roosting for adults. Often, breeding and migratory habitats are near water sources, but the relationship between distance to water and vertical structure for roosting and shading is not well understood for habitat suitability for the western North American ACU. Overwintering habitat consists of tree groves that provide suitable microclimates for winter diapause. For the western North American ACU, these are predominantly locations within 1.5 miles of the Pacific Ocean or San Francisco Bay in California and Baja California, Mexico (WAFWA 2019).

The nearest monarch butterfly occurrence in the CNDDDB is approximately 14 miles southwest from the Proposed Project Footprint. However, the Western Monarch and Milkweed Occurrence Database has records for both milkweed and monarch butterflies within 2 miles of the Proposed Project Footprint, and Calflora has a narrow-leaf milkweed (*Asclepias fascicularis*) observation recorded in the Action Area on Altamont Pass Road (Western Monarch and Milkweed Occurrence Database 2018; Calflora 2023). Based on the suitability of soils present in the Action Area, host plants for monarch butterflies are most likely to be observed in the hillsides of the Altamont Pass.

This migratory species breeds and overwinters in California, although the presence of non-native milkweed host plants may encourage the monarch to remain for longer periods. The monarch therefore may be present in the Action Area throughout the year, although most likely from October through February. While host plants for monarch butterflies are most likely to occur within the non-native grassland and scrub habitats within the eastern portion of the Proposed Project, host plants may also be present in developed areas and other ruderal areas within the Action Area. Natural vegetation communities (non-native grassland and scrub) within the Action Area that are most likely to support monarch butterfly host plants total approximately 1,572 acres (1,571 acres of grassland and 1 acre of scrub). Roosts have not been previously mapped and are unlikely in the Action Area because monarch butterflies typically congregate closer to the coast for winter roosting.

3.3 California Tiger Salamander – Central California Distinct Population Segment

The California tiger salamander is categorized into six populations, including:

- Sonoma County
- Santa Barbara County
- San Francisco Bay Area
- Central Valley
- Southern San Joaquin Valley
- Central Coast Range (USFWS 2023b)

These six populations are treated as three distinct population segments (DPS) that are genetically differentiated and geographically isolated from one another, including:

- Sonoma County DPS (federally listed as endangered)
- Santa Barbara County DPS (federally listed as endangered)
- Central California DPS (federally listed as threatened) (USFWS 2023b)

3.3.1 Status and Critical Habitat

The Central California DPS of the California tiger salamander was federally listed as threatened on August 4, 2004 (USFWS 2023b). Critical habitat was designated for the Central California DPS of California tiger salamander in 2005, but it does not overlap the Action Area.

3.3.2 Physical Characteristics

The California tiger salamander is a large, stocky salamander with a broad, rounded snout. Adults average 6 to 9.5 inches in length and have random white or yellowish spots or bars against a black body. Their small eyes, which have black irises, protrude from their heads. Males are typically larger than females (USFWS 2023b).

3.3.3 Life Cycle

California tiger salamanders can live for about 10 to 15 years. Adult California tiger salamanders leave their underground burrows and engage in mass migrations as they return to breeding ponds to mate during a few rainy nights per year. This mass migration is primarily from November through April. Males typically arrive before the females and stay in the ponds longer than females. Males stay in ponds an average of 45 days, while females stay an average of 12 days. The male deposits a spermatophore on the bottom of the pond, which the female picks up and uses to fertilize her eggs internally. Females then attach their eggs to twigs, grass stems, or other vegetation or debris. The eggs hatch in 10 to 28 days into larvae. The larvae remain in the pond, usually between 3 and 6 months, until they complete metamorphosis into juveniles. Once metamorphosis occurs, juveniles typically depart their birth ponds at night, primarily between May and July, and enter upland habitat in search of underground burrows (USFWS 2023b).

The California tiger salamander is an opportunistic hunter throughout all stages of its life. As larvae, the California tiger salamander feeds on zooplankton, small crustaceans, and aquatic insects, moving toward larger prey such as the tadpoles of the Sierran tree frog (*Pseudacris sierra*), western spadefoot, and California red-legged frogs as the larvae grow. Adult salamanders eat various invertebrate prey, including insects, arachnids, and terrestrial worms (USFWS 2023b).

3.3.4 Distribution and Habitat Associations

The known distribution of the California tiger salamander is near sea level up to approximately 3,900 feet in the Coast Range, and up to approximately 1,600 feet in the foothills of the Sierra Nevada. The species historically inhabited low-elevation grassland and oak savanna plant communities and adjacent foothills in the Central Valley and the Inner Coast Range in California (Shaffer, Fisher, and Stanley 1993; USFWS 2005, 2017). Along the Coast Range, the species occurs in the Santa Rosa area of Sonoma County; southern San Mateo County south to central San Luis Obispo County; and the vicinity of northwestern Santa Barbara County (USFWS 2004). Alameda and Contra Costa Counties support the greatest concentrations of California tiger salamander in the species' range (Shaffer, Fisher, and Stanley 1993).

Federally designated critical habitat for the California tiger salamander consists of three PBFs that are essential to the conservation of the species: (1) aquatic habitats, (2) upland (refugia) habitat for refuge and foraging, and (3) dispersal habitat (USFWS 2005).

California tiger salamanders require two different habitats to complete their life cycle. For much of their subadult and adult lives, they spend the dry summer and fall months in upland burrows originally excavated by small mammals. The burrows provide food sources, such as camel crickets (*Ceuthophilus* spp.) and other invertebrates, as well as protection from drying. An active population of burrowing mammals is necessary to provide underground refugia in California tiger salamander habitat (USFWS 2004). California tiger salamanders emerge from burrows on rainy nights during fall and winter to feed and migrate to breeding ponds, which may be vernal pools, stock ponds, or other ponded water.

Aquatic habitat provides the space, food, and cover necessary to support reproduction and to sustain early life history stages of larval and juvenile California tiger salamanders. Aquatic habitat consists of freshwater bodies, including natural and artificially made (e.g., stock) ponds, vernal pools, and vernal pool complexes. Aquatic and breeding habitats must be able to hold water for a minimum of 12 weeks in the winter or spring in a year of average rainfall, which is the amount of time needed for salamander larvae to metamorphose into juveniles capable of surviving in upland habitats.

Larval California tiger salamanders are aquatic and can metamorphose as soon as 10 weeks after hatching, although they typically take longer depending on environmental conditions. Dispersal habitat provides connectivity among California tiger salamander aquatic and upland habitats. The distance California tiger salamanders will travel between aquatic and upland refugia habitats varies greatly between sites. Adults have been observed migrating up to 0.7 mile (1.1 kilometer) from refugia sites to breeding ponds (USFWS 2004), whereas juvenile salamanders have been detected as far as 1.24 miles (2 kilometers) from breeding sites (USFWS 2003). In defining critical habitat for the California tiger salamander, USFWS used a distance of 0.7 mile (1.1 kilometer) from known occurrences that are not isolated by barriers that California tiger salamanders cannot cross. This distance would likely include 99 percent of the inter-pond movement of breeding adults.

Dispersal barriers such as roads, railroads, and croplands fragment the grassland and pond habitats of the California tiger salamander and can interrupt the life cycle. Additionally, dispersal barriers that isolate breeding sites reduce gene flow through the larger population and seriously threaten California tiger salamander populations. Dispersal barriers also cause direct mortality to California tiger salamander attempting to migrate between grassland burrows and breeding ponds (Andrews, Gibbons, and Jochimsen 2008). Significant numbers are killed by vehicle traffic while crossing roads (USFWS 2005). Road-kill mortality in the vicinity of breeding sites has been reported to be 25 to 72 percent of the observed salamanders crossing roads (Launer and Fee 1996). The low reproductive rate of California tiger salamander, in combination with loss of habitat, habitat fragmentation, hybridization with non-native eastern tiger salamander (*Ambystoma tigrinum*), and introduction of non-native predators, has imperiled populations throughout the range.

The CNDDDB has 330 records of this species within 10 miles of the Proposed Project Footprint, including 6 records within the Proposed Project Footprint (CDFW 2023). Records that overlap the Proposed Project Footprint include aquatic features at the intersection of Altamont Pass Road and Dyer Road, Cayetano Creek north of I-580, and between Dublin Road and I-580 in the western portion of the Proposed Project Footprint. This species was not visually observed or detected in July 2024 via eDNA at any of the sampled locations, including Tassajara Creek, Arroyo Los Positas or its unnamed tributary. For more information on the eDNA sampling results, see Appendix D.

Potentially suitable aquatic breeding habitat for California tiger salamander includes any natural and unnatural ponded areas in the Action Area, including ponded areas along seasonal drainages. Perennial waterways, such as Tassajara Creek and Arroyo Las

Positas, are not expected to be potential breeding sites for this species. These features are most likely to be located north of I-580 between Fallon Road and Springtown Boulevard, and from Lauglin Road through the Altamont Pass area west of the California Aqueduct. Suitable upland habitat in the Action Area for California tiger salamander includes all non-native grassland, scrub, riparian habitat that falls within the CWHR Predicted Habitat layer for California tiger salamander, and select ruderal habitat that is adjacent to suitable aquatic/upland habitat.

Therefore, California tiger salamander also could occur in grassland, riparian woodland, and wetland in the Action Area. Potential habitat for California tiger salamander within the Action Area is depicted on Figure 7 in Appendix A. Habitat-based modeling resulted in approximately 56 acres of potentially suitable breeding habitat and approximately 1,500 acres of upland habitat within the Action Area.

3.4 California Red-Legged Frog

3.4.1 Status and Critical Habitat

The California red-legged frog was listed as federally threatened on May 23, 1996 (USFWS 1996b; 2023a). Critical habitat was designated for the California red-legged frog in 2010 (USFWS 2010) and overlaps the Proposed Project Footprint to the north and south of I-580 east of Livermore (Appendix A, Figure 8).

The PBFs essential to the conservation of the species, these features originate from the primary constituent elements required by the species as described in the listing document of the species (USFWS 1996b). The habitats required by the species, as described in 75 FR 12816, include:

- Aquatic Breeding Habitat consisting of ponds and low-gradient freshwater creeks, which are essential to breeding as the waterbody must hold water for a minimum of 20 weeks for egg to tadpole development
- Nonbreeding Aquatic and Riparian Habitat consisting of shallow freshwater features that dry too quickly for breeding, that are essential for space, food, and cover
- Upland Habitat consisting of occupied wetland habitats often containing blackberry, poison oak, coyote bush, oaks, grasses, and other species, serving as foraging and shelter areas for the species
- Dispersal Habitat consisting of areas within 1 mile of occupied sites to support movement between breeding locations and other aquatic habitats that are free of barriers, this habitat is essential for breeding and nonbreeding behaviors

3.4.2 Physical Characteristics

The California red-legged frog is the largest native frog in the western U.S. Adult females can reach 5.4 inches in length and are larger than males, which can reach 4.5 inches in length. This species is a colorful amphibian. The abdomen and hind legs of adults are often red or salmon pink. The back of this species is characterized by small black flecks

and larger irregular dark blotches, with indistinct outlines on a brown, gray, olive, or reddish-brown background color (USFWS 2023a).

3.4.3 Life Cycle

Most male California red-legged frogs reach sexual maturity at 2 years and females at 3 years of age and are often prolific breeders. They lay their eggs during or shortly after large rainfall events in late winter and early spring between November through May. Each egg mass contains about 300 to 4,000 eggs, but fewer than 1 percent of eggs laid survive the tadpole phase. California red-legged frogs live up to 5 years in the wild, but few survive beyond 2 years (USFWS 2023a).

Adult California red-legged frogs are largely nocturnal, while juveniles are active both day and night. These frogs are inactive during cold and hot periods in the winter and late summer months, respectively. They may be active year-round in coastal areas, where temperatures do not fluctuate as much throughout the year (USFWS 2023a).

The California red-legged frog's diet is highly variable. Tadpoles feed on algae by grazing on the surface of rocks and vegetation. Juvenile and adult frogs mostly eat insects and other invertebrates. As they get larger, they also eat small vertebrates, including Pacific tree frogs (*Pseudacris regilla*) and California mice (*Peromyscus californicus*) (USFWS 2023a).

3.4.4 Distribution and Habitat Associations

The California red-legged frog occurs in California and Baja California, Mexico from sea level to 5,000 feet. In California, the California red-legged frog has been recorded from Riverside County to Mendocino County along the Coast Ranges, and from Calaveras County to Butte County in the Sierra Nevada. The species is common along parts of the central coast, but has been extirpated from most of the Sierra Nevada, northern Coast, and northern Transverse Ranges. The species is believed to have been extirpated from the southern Transverse and Peninsular Ranges, but is still present in Baja California, Mexico (USFWS 2010).

PBFs of California red-legged frog critical habitat include aquatic breeding habitat, nonbreeding aquatic habitat, upland habitat, and dispersal habitat. Aquatic breeding habitat is essential for all aquatic life stages of the California red-legged frog and includes ponds, backwaters within streams and creeks, marshes, lagoons, and dune ponds with the capability to hold water for a minimum of 20 weeks. Water quality within these features requires low salinity (below 4.5 parts per million [ppm] up to 7 ppm) and temperatures below 73 degrees Fahrenheit. Nonbreeding aquatic and riparian habitat essential for the species includes shallow freshwater features such as streams, small seeps, and ponds that dry too quickly to support breeding. Upland adjacent habitat is essential to providing food and shelter sites for the species to allow for dispersal between breeding and nonbreeding habitat (USFWS 2006).

California red-legged frogs have been found breeding in ponds and slow-moving or still sections of streams. Ideal ponds have a mix of deep sections for escaping from predators

and shallow sections that warm quickly and help the rearing of tadpoles and juveniles. Some emergent vegetation or shoreline such as cattails, bulrushes, or willows is also required for attachment of egg masses (USFWS 2002). Often, adults will stay in the breeding habitat year-round if sufficient water is present, but some will move into adjacent uplands or another nonbreeding aquatic habitat. Migrating individuals will disperse from breeding sites in straight-line movements, without regard to vegetation or topography (Bulger, Scott Jr., and Seymour 2003). Radio-tagged individuals have been found as far away as 2 miles from suitable aquatic breeding habitat (USFWS 2002), but nonmigrating individuals typically stay within 200 feet (60 meters) of aquatic habitat 90 percent of the time and have been found to be closely associated with dense cover (e.g., California blackberry [*Rubus ursinus*], poison oak [*Toxicodendron diversilobum*], and coyote bush [*Baccharis pilularis*]) (Bulger, Scott Jr., and Seymour 2003).

Aquatic breeding habitat consists of standing bodies of freshwater, including natural and human-made ponds, slow-moving streams or pools in streams, and other ephemeral or permanent waterbodies that typically become inundated during winter rains, and hold water for a minimum of 20 weeks in all but the driest years. Introduced species such as bullfrogs (*Lithobates catesbeianus*), largemouth bass (*Micropterus salmoides*), common carp (*Cyprinus carpio*), and mosquitofish may prey on one or more life stages (eggs, tadpoles, or adults) of California red-legged frogs.

Nonbreeding aquatic habitat consists of freshwater habitats that may or may not hold water long enough to be suitable for breeding, but that do provide shelter, foraging, predator avoidance, and aquatic dispersal. In addition to aquatic breeding habitat, nonbreeding habitat may include plunge pools in intermittent creeks, seeps, quiet water refugia during high-water flows, and springs of sufficient flow to withstand the summer dry period.

Upland areas are those areas adjacent to or surrounding breeding and nonbreeding aquatic and riparian habitat, in most cases up to a distance of 1 mile (1.6 kilometers), depending on surrounding landscape and dispersal barriers. These include various vegetation types such as grassland, woodland, forest, wetland, or riparian areas that provide shelter, forage, and predator avoidance for the California red-legged frog. Upland features are also essential in that they are needed to maintain the hydrologic, geographic, topographic, ecological, and edaphic features that support and surround the aquatic, wetland, or riparian habitat. These upland features contribute to (1) filling of aquatic, wetland, or riparian habitats; (2) maintaining suitable periods of pool inundation for larval frogs and their food sources; and (3) providing nonbreeding, feeding, and sheltering habitat for juvenile and adult frogs (e.g., shelter, shade, moisture, cooler temperatures, a prey base, foraging opportunities, and areas for predator avoidance). Upland habitat should include structural features such as boulders, rocks and organic debris (e.g., downed trees, logs), small mammal burrows, or moist leaf litter.

Dispersal habitat consists of accessible upland or riparian habitat within and between occupied or previously occupied sites that are located within 1 mile (1.6 kilometers) of each other, and that support movement between such sites. Dispersal habitat includes various natural habitats and altered habitats such as agricultural fields that do not contain

barriers to dispersal (e.g., heavily traveled roads without bridges or culverts). Dispersal habitat does not include moderate- to high-density urban or industrial developments with large expanses of asphalt or concrete, nor does it include large lakes or reservoirs over 50 acres (20 hectares) in size or other areas that do not contain those features identified as essential to the conservation of the species.

The CNDDDB has 338 records of this species within 10 miles of the Proposed Project Footprint, including 8 records within the Proposed Project Footprint (CDFW 2023). Records that overlap the Proposed Project Footprint include aquatic features at the intersection of Altamont Pass Road and Dyer Road, Cayetano Creek north of I-580, and between Dublin Road and I-580 in the western portion of the Proposed Project Footprint. This species was not visually observed at any of the sampled locations in July 2024, but was detected via eDNA sampling in an unnamed tributary to Arroyo Las Positas. The species was not detected in Tassajara Creek or the mainstem of Arroyo Las Positas. For more information on the eDNA sampling results, see Appendix D.

Based on desktop evaluation and habitat modeling (see Section 1.7), aquatic and wetland habitats may provide suitable aquatic breeding and nonbreeding habitat, while non-native grassland, scrub, riparian, and select ruderal habitat (specifically, those not entirely encircled by developed/landscaped habitat, and which are adjacent to natural land cover types) may provide upland aestivation and dispersal habitat. Based on the resources within the Action Area, suitable aquatic breeding and/or nonbreeding habitat for California red-legged frog includes any of the named and unnamed streams and their tributaries, flood control channels, aqueducts (excluding the Delta-Mendota Canal and Governor Edmund G Brown California Aqueduct), and natural and unnatural ponds in the Action Area, including Arroyo Las Positas, Arroyo Seco, Cayetano Creek, Cottonwood Creek, Mountain House Creek, One Hundred and Fifty-five Canal, and South Bay Aqueduct. In general, those aquatic features that are inundated by winter rains and hold water for a minimum of 20 weeks are likely to support suitable aquatic breeding habitat, while aquatic features that are intermittent or hold water for less than 20 weeks would be considered aquatic nonbreeding habitat. A waterbody's designation as breeding or nonbreeding habitat may vary depending on its position in the watershed, whether the segment is natural or channelized, whether it is managed for flood control purposes, and which PBFs are present at that specific location. Suitable upland habitat in the Action Area for California red-legged frog includes all non-native grassland, scrub, and riparian habitat that falls within the CWHR Predicted Habitat layer for California red-legged frog and select ruderal habitat that is adjacent to suitable aquatic/upland habitat.

Therefore, California red-legged frogs also have the potential to occur in grassland, riparian woodland, and wetland in the Action Area. Potential habitat for California red-legged frog within the Action Area is depicted on Figure 9 in Appendix A. Habitat-based modeling resulted in approximately 45 acres of potentially suitable breeding habitat and approximately 1,531 acres of upland habitat within the Action Area.

3.5 Western Spadefoot

3.5.1 Status and Critical Habitat

The northern and southern DPSs of the western spadefoot were proposed for listing as threatened DPSs under the ESA on December 5, 2023 (USFWS 2023d). There is currently no designated critical habitat for the western spadefoot.

3.5.2 Physical Characteristics

The western spadefoot is a small toad that lives underground during the dry season, and inhabits seasonal wetlands, ephemeral drainages, and vernal pools during the rainy season. Adult western spadefoots can reach up to 2.5 inches in length (snout to vent), have warty skin, vertical pupils, and a black spade used for burrowing on each hind foot. Tadpoles can grow to 3 inches in length, but usually metamorphose at smaller sizes.

3.5.3 Life Cycle

Western spadefoots are primarily terrestrial, living in underground burrows most of the year. They emerge from their upland aestivation sites during the start of the rainy season (approximately late October through late December), when the onset of rain prompts adults to disperse toward aquatic breeding habitat (Baumberger et al. 2019). Breeding occurs typically from late January through May; the exact timing is dependent on the occurrence of rain in a given year's winter/early spring rainy season (Baumberger et al. 2019).

Egg masses (generally clusters of 10 to 42 eggs) are attached to plant material or the upper surfaces of submerged rocks, and typically hatch within 3 to 4 days (Stebbins 1985; Morey 2005). Larval metamorphosis may be completed in as little 3 weeks or can take up to 11 weeks, but the average is approximately 8 weeks (Morey and Reznik 2001). Male western spadefoots likely reach sexual maturity 1 to 2 years after metamorphosis, and females likely reach sexual maturity 2 years after metamorphosis (Nafis 2023).

After the breeding season (approximately May through August), adults and juveniles disperse to nearby upland habitat. The post-breeding/post-metamorphosis dispersal distances traveled away from breeding ponds appear to be related to the amount of precipitation, with individuals traveling farther in wetter years (Baumberger et al. 2020). One study found the average dispersal distance to be 131 feet (Baumberger 2013), but that was during a dry year, and subsequent work indicated that individuals can travel approximately 2,000 feet from breeding habitat (Baumberger et al. 2020).

Adult and juvenile western spadefoots consume a variety of invertebrates including grasshoppers, true bugs, beetles, moths, butterfly and moth larvae, ants, termites, and worms. Larval western spadefoots consume planktonic organisms, algae, aquatic invertebrates, and other amphibian larvae (Zeiner et al. 1988-1990; USFWS 2023d).

3.5.4 Distribution and Habitat Associations

The western spadefoot is a near endemic species to California, ranging from the Central Valley, bordering foothills, and Coast Ranges south of Monterey Bay into northwestern

Baja California, Mexico (Roberts 2020; FR 2023). The western spadefoot is primarily terrestrial over the course of the annual cycle, spending most of its life aestivating underground in upland habitat that is generally within 0.5 mile of aquatic breeding habitat. Vernal pools are considered the primary aquatic breeding habitat; however, western spadefoots have been observed within a variety of permanent and temporary breeding habitat, both natural and altered, including off-channel pools along rivers, intermittent creeks, artificial ponds, livestock ponds, sedimentation and flood control ponds, irrigation and roadside ditches, roadside puddles, tire ruts, and borrow pits (USFWS 2023c). Upland habitat typically consists of grasslands, scrub, or mixed woodlands where western spadefoots forage, burrow during the breeding season, and aestivate during the nonbreeding season. At a microsite scale, western spadefoots appear to have a strong preference for burrow/aestivation sites with duff as the vegetative cover (i.e., leaves, twigs, decomposing vegetative matter) within a predominantly grassland habitat (Baumberger et al. 2019).

The CNDDDB has 13 records for western spadefoot within 10 miles of the Proposed Project Footprint, the closest of which is approximately 2.18 miles from the Proposed Project Footprint, at the southwest corner of 3rd Street and A Street, Sandia National Laboratories, Livermore, California. This species was not visually observed or detected in July 2024 via eDNA at any of the sampled locations, including Tassajara Creek, Arroyo Los Positas or its unnamed tributary. For more information on the eDNA sampling results, see Appendix D.

Based on the resources within the Action Area, the western spadefoot may occur primarily in seasonal wetlands, vernal pools, ephemeral drainages, and associated undeveloped upland land cover. These features are most likely to be located north of I-580 between Fallon Road and Springtown Boulevard, and from Laughlin Road through the Altamont Pass area west of the California Aqueduct. Perennial waterways, such as Tassajara Creek and Arroyo Las Positas, are not expected to be potential breeding sites for this species. Additionally, adults and juveniles originating from these wetlands, pools, and drainages may potentially use the habitat in the Action Area for upland refuge or dispersal.

Therefore, western spadefoot toads have the potential to occur in grassland, riparian woodland, and wetland habitats in the Action Area. Potential habitat for western spadefoot within the Action Area is depicted on Figure 10 in Appendix A. Habitat-based modeling resulted in approximately 56 acres of potentially suitable breeding habitat and approximately 1,492 acres of upland habitat within the Action Area.

3.6 Northwestern Pond Turtle

3.6.1 Status and Critical Habitat

The northwestern pond turtle was proposed for listing as threatened under the ESA on October 3, 2023 (USFWS 2023c). There is currently no designated critical habitat for the northwestern pond turtle.

3.6.2 Physical Characteristics

The northwestern pond turtle is a medium-sized turtle (typically 4 to 7 inches in length), with drab olive/brown coloration. The plastron (underside) is typically yellowish with dark blotches, although it may also be unmarked (McGinnis and Stebbins 2018).

3.6.3 Life Cycle

Northwestern pond turtles spend most of their lives in water, but they require upland habitat for nesting, for dispersal (when individual ponds are isolated from other waterbodies), and for aestivating. Northwestern pond turtles may enter aestivation in both the summer and winter. If their pond is drying down, they may leave the pond to aestivate for the warmer months. Additionally, they may leave during the cooler winter months to aestivate in upland habitats.

During the nesting period (April through August), females move away from their waterbodies into surrounding uplands, where they construct underground nests and lay eggs. The incubation period can take from 73 to 134 days. Female northwestern pond turtles are thought to achieve sexual maturity between 10 and 15 years of age, while males may reach sexual maturity between 8 and 12 years.

3.6.4 Distribution and Habitat Associations

The northwestern pond turtle is found in portions of Washington, Oregon, Nevada, and northern and central California. Within California, it is found in areas of the Coast Range from the Oregon border to northern Monterey County, the foothills and lower elevation areas of the Sierra Nevada and southern Cascade Mountains, and areas within Sacramento and San Joaquin Valleys (USFWS 2023c).

The northwestern pond turtle inhabits a variety of waterbodies, including ponds, marshes, rivers, streams, and irrigation canals. It can tolerate brackish water for short periods of time but is typically found in freshwater habitats. The northwestern pond turtle can often be found basking on rocks or logs, or the shores of its aquatic habitat. It requires terrestrial or upland features close to the aquatic habitat for nesting and aestivating or overwintering (USFWS 2023c). Nesting sites are generally in proximity to (10 feet to 1,300 feet) suitable aquatic habitat and are characterized by having sparse vegetation with short grasses and forbs, minimal canopy cover, hard-packed clay or silt soils, and along south- or west-facing slopes (Rathbun et al. 1992; Holland 1994; Reese and Welsh 1997). Upland overwintering/aestivating habitat is typically above the high-water elevation of the aquatic habitat, and outside of any riparian zone (USFWS 2023c).

The CNDDDB has 58 records for this species within 10 miles of the Proposed Project Footprint, 2 of which intersect with the Proposed Project Footprint between Isabel Station and Southfront Road Station. During eDNA sampling surveys in July 2024, three adult western pond turtles were observed in Arroyo Las Positas west of North Livermore Avenue, and a subadult western pond turtle was observed in a channelized, natural-bottomed unnamed tributary to Arroyo Las Positas in the vicinity of North Vasco Road. In addition, this species was detected via eDNA samples collected at Tassajara Creek,

Arroyo Las Positas, and an unnamed tributary to Arroyo Las Positas. For more information on the eDNA sampling results, see Appendix D.

Based on desktop evaluation and habitat modeling, aquatic and wetland habitats may provide suitable aquatic habitat, while non-native grassland, scrub, and select ruderal habitat (specifically, those not entirely encircled by developed/landscaped habitat, and which are adjacent to aquatic features) may provide upland nesting and dispersal habitat.

Based on the resources within the Action Area, the northwestern pond turtle may occur in any of the named and unnamed streams, tributaries, flood control channels, aqueducts (excluding the Delta-Mendota Canal and Governor Edmund G Brown California Aqueduct), and natural and unnatural ponds in the Action Area, including Arroyo Las Positas, Arroyo Seco, Cayetano Creek, Cottonwood Creek, Mountain House Creek, One Hundred and Fifty-five Canal, and South Bay Aqueduct. Suitable upland nesting and dispersal habitats within the Action Area includes all grasslands, scrub, and ruderal habitat between 10 and 1,300 feet from an aquatic and wetland habitat in areas with sparse vegetation of short grasses or forbs, in hard-packed clay or silt soils, and along south- or west-facing slopes.

Potential habitat for northwestern pond turtle within the Action Area is depicted on Figure 11 in Appendix A. Habitat-based modeling resulted in approximately 58 acres of potentially suitable aquatic habitat and approximately 1,447 acres of upland habitat that may contain suitable nesting sites within the Action Area.

3.7 San Joaquin Kit Fox

3.7.1 Status and Critical Habitat

The San Joaquin kit fox was federally listed as an endangered species on March 11, 1967 (USFWS 2023g). Critical habitat has not been designated for the San Joaquin kit fox.

3.7.2 Physical Characteristics

The San Joaquin kit fox is a small, tan fox with a bushy, black-tipped tail, and weighs about 5 pounds when fully grown. This species reaches a height of about 12 inches. It has a narrow nose and a small, slim body. The San Joaquin kit fox is specially adapted for its desert habitat. Its large, close-set ears help dissipate heat, keeping it cool in the hot desert (USFWS 2023g).

3.7.3 Life Cycle

The lifespan of the San Joaquin kit fox is approximately 7 years, and the species starts breeding when they are around 1 year old. In the fall, females begin to clean and enlarge their pupping dens. The foxes mate between December and March, with females giving birth to two to six pups. Pups stay inside the den for the first month of their lives. Both males and females care for the pups, and adult pairs stay together for the entire year (USFWS 2023g).

3.7.4 Distribution and Habitat Associations

San Joaquin kit foxes occur in a variety of land cover types, including grasslands, scrublands, vernal pool areas, alkali meadows and playas, and an agricultural matrix of row crops, irrigated pastures, orchards, vineyards, and grazed annual grasslands (USFWS 1998). San Joaquin kit foxes occur in some areas of suitable habitat on the floor of the San Joaquin Valley and in the surrounding foothills of the Coast Ranges, Sierra Nevada, and Tehachapi Mountains, from Kern County north to Contra Costa, Alameda, and San Joaquin Counties (USFWS 1998). Within these habitats, the San Joaquin kit fox is an opportunistic hunter. Its primary food is kangaroo rats, but it also actively hunts white-footed mice, pocket mice, ground squirrels, rabbits, and ground-nesting birds. During certain times of the year, kit foxes will also eat insects (USFWS 2023g).

The San Joaquin kit fox is nocturnal, hunting at night and resting most of the day in their dens. The dens help the fox escape the desert heat and provide shelter and cover from predators. Kit foxes either dig their own dens, use those constructed by other animals, or use human-made structures such as culverts, abandoned pipelines, or sumps in banks or roadbeds (USFWS 2023g).

The CNDDDB has 49 records for this species within 10 miles of the Action Area; however, none of them overlap with the Proposed Project Footprint and only 2 overlap with the Action Area (CDFW 2023). Of all 49 records, the most recent was observed in 2002, approximately 3 miles north of the Action Area, northeast of Livermore and east of North Vasco Road. The most proximal record of occurrence (observation year 1975) for this species is within 100 feet of the Proposed Project Footprint between the MOW facility and the Mountain House Community Station, just west of the California Aqueduct. Of the 49 records for this species within 10 miles of the Proposed Project Footprint, 26 mention the presence of dens; however, the most recent record of denning within 10 miles is from 2000. The nearest record for a den is a 1989 occurrence 1.5 miles southeast of the Action Area.

Based on the best available data, there are no recent occurrences of San Joaquin kit fox in the vicinity of the Action Area and no known dens within the Proposed Project Footprint. As described above, active dens have not been documented within 10 miles of the Proposed Project Footprint since 2000. However, approximately 487 acres of grasslands, ruderal, and agricultural land throughout the Action Area may provide suitable foraging and denning habitat for the species. This potentially suitable habitat is concentrated in the eastern portion of the Action Area, as the western portion of the Action Area is heavily developed. Although the species can move large distances across the landscape, the limited number of recent occurrences in the region indicates that there is a low potential for the San Joaquin kit fox to be present within the Action Area.

3.8 Palmate-Bracted Bird's-Beak

3.8.1 Status and Critical Habitat

Palmate-bracted bird's-beak is federally endangered (USFWS 2023f). Critical habitat has not been designated for palmate-bracted bird's-beak.

3.8.2 Distribution and Habitat Associations

Palmate-bracted bird's-beak is a hemi-parasitic annual herb in the broomrape family (*Orobanchaceae*). This species has been documented primarily in the Sacramento and San Joaquin Valleys, from Fresno County in the south to Yolo County in the north, at elevations from 16 to 509 feet (CDFW 2023). Palmate-bracted bird's-beak occurs in chenopod scrub and valley and foothill grasslands in alkaline soils (CNPS 2023a). More specifically, it grows in seasonally flooded lowland plains and basins with alkaline/saline soils (USFWS 2023f). It is known to bloom from May through October (CNPS 2023a), although most collection records of this species are from June to August, its flowering period reported in the Jepson eFlora (Jepson Flora Project 2024). Palmate-bracted bird's-beak is primarily threatened by agriculture, development, altered hydrology, and grazing (CNPS 2023b). Being an annual plant, palmate-bracted bird's beak populations can fluctuate dramatically from year to year, possibly in response to pollination success, rainfall patterns, freshwater influence, and marsh pollution (USFWS 2023f).

Nearly all occurrences of palmate-bracted bird's beak are known from either the Sacramento or San Joaquin Valley of California. The one CNDDDB occurrence located outside of these valleys was reported within 10 miles of the Proposed Project Footprint, consisting of a large population of palmate-bracted bird's-beak approximately 0.5 mile north of the Proposed Project Footprint. This occurrence was last updated in 2022 and is located within an isolated alkali sink in Springtown Wetland Reserve north of Livermore and I-580 and west of North Vasco Road (CDFW 2023). This occurrence has greatly declined in population size between 1990 and 2022, particularly in the eastern portion of the occupied area (CDFW 2023).

Based on the best available data, the palmate-bracted bird's-beak has a potential to occur in alkaline grasslands and wetlands in the Action Area, and more specifically, in alkaline grasslands that are seasonally flooded. Alkaline grasslands and wetlands are present within the Action Area north of I-580 between Isabella Avenue and Springtown Boulevard, Laughlin Road and Altamont Pass Road, north and south of Altamont Pass Road from Greenville Road east to the Proposed Project's realignment with I-580, and north and south of the alignment from the Proposed Project's realignment with I-580 east to the California Aqueduct. However, based on CNDDDB records of occurrence, there are no records of this species occurring within the Action Area (CDFW 2023).

4 Effects of Proposed Project

This section analyzes effects of the Proposed Project on the species that have potential to occur in the Action Area and describes conservation measures and compensatory mitigation to reduce or offset impacts.

4.1 Vernal Pool Fairy Shrimp

4.1.1 Project Effects

The Proposed Project could affect vernal pool fairy shrimp through habitat loss or degradation, impacts on individuals if present in vernal pool features, and habitat fragmentation over the long term. Standard and species-specific conservation measures will be implemented to reduce potential adverse effects on the fairy shrimp. Routine maintenance activities and operation of a new transit service would not affect vernal pool fairy shrimp because these activities would take place in areas disturbed and developed as part of the construction phase. For purposes of this analysis, the vernal pool features in the Action Area are assumed to be occupied until future surveys have determined them to be unoccupied.

Direct and indirect effects to these species and their potential habitat would be reduced through the implementation of general conservation measures consisting of protecting habitat during construction activities (CM-2), vegetation removal and restoration (CM-3), preventing invasive species entry into native habitat (CM-4), controlling dust emissions (CM-6), BMPs (CM-7), and implementing a noise and vibration-reduction plan (CM-8) as outlined in Section 1.6. Impacts to potential habitat for vernal pool fairy shrimp are depicted on Figure 6 in Appendix A.

4.1.1.1 Harm of Individuals

Soil or ground disturbance in the features could incidentally remove cysts, which could be present year-round, or individual shrimp, which are active during the rainy season (typically between November and early May). Soil compaction associated with access or staging in vernal pool features could prevent cysts from hatching if they become buried too deeply in the soil or are displaced to areas without suitable aquatic habitat by being transported in the wheels of construction vehicles and equipment. Construction activities within 250 feet of vernal pool features are not expected to alter habitat conditions in a way that makes them unsuitable for cysts or individuals to survive. The loss of cysts or individuals could decrease reproductive success and reduce the size of the local population of vernal pool fairy shrimp in the Action Area, which would adversely affect the species.

Impacts to cysts and individuals cannot be quantified due to their difficulty of being detected and fluctuation of local populations, but it is assumed that a small quantity of individuals and cysts would be subject to take based on the estimated amount of habitat impacts (3 acres). Conservation measures identified for habitat impacts would help reduce potential impacts on cysts or individuals in adjacent habitat, but unavoidable, adverse effects are expected in the directly disturbed vernal pool features.

4.1.1.2 Habitat Loss or Degradation

Construction activities would involve vegetation removal and soil disturbance in and near vernal pool features, which could remove vernal pool habitat and degrade adjacent habitat. Based on habitat mapping, an estimated 3 acres could be directly disturbed out of the potential 16 acres of vernal pool habitat in the Action Area. Most impacts would take

place along the proposed tracks (about 2 acres), with some impacts associated with the Altamont MOW staging area and Mountain House LF (about 1 acre). Temporary impacts could occur from access and staging activities, which could temporarily modify the function and quality of the vernal pools through alteration of runoff and increased sedimentation or pollutants in the features. Permanent impacts or a loss of vernal pool habitat would result from excavation, grading, and placement of fill in the features, which would eliminate functional habitat capable of supporting vernal pool fairy shrimp. Indirect impacts may also occur to vernal pool features within 250 feet of the Proposed Project Footprint, such as through introduction or spread of invasive plants, changes in the soil characteristics due to alteration of hydrology and sedimentation of aquatic habitat features, changes in the available water due to diversion of surface runoff, changes in soil compaction, and exposure to construction and urban contaminants and dust.

Temporary impacts to vernal pool features will be minimized by limiting work in and near the features to the dry season (typically May to October) and requiring wetland mats or similar materials to be used when staging in or driving across the features at any time year-round. Once vernal pool features are verified in the field, they would be avoided and fenced outside of the Proposed Project Footprint when possible (CM-2). With implementation of these measures, the soil would not become compacted or altered in a way that makes the features unsuitable for vernal pool fairy shrimp over the long term, and temporarily affected vernal pool features would continue to provide potential habitat for the species after construction. Temporary impacts are considered insignificant.

Based on the current design, the proposed tracks and a small portion of the Altamont MOW staging area and Mountain House LF would result in a permanent loss of 2 acres of vernal pool features, potentially occupied by vernal pool fairy shrimp. This estimate is based on the overlap of the Proposed Project Footprint with mapped habitat. The actual amount of loss would be confirmed through surveys during final design, with efforts made to minimize the loss through adjustment of design elements and protection of vernal pool features during construction (CM-11). This loss would remove potential habitat for vernal pool fairy shrimp, reducing available breeding habitat and preventing the shrimp from occupying the habitat in the future (if it is not actually occupied and is suitable). To offset this loss, compensatory mitigation is proposed to preserve or enhance similar habitat through a conservation bank (CM-12).

Standard construction practices and BMPs implemented throughout the construction phase (CM-7) and stormwater treatment measures incorporated into the design would help reduce the potential for indirect effects in adjacent vernal pool features. Temporary dust or pollutants in stormwater runoff would not have a measurable effect on features further from the Proposed Project Footprint, and closer features would be exposed to negligible concentrations of dust and pollutants with the measures in place. Restoration of temporarily disturbed areas with native vegetation (CM-3) would help prevent the spread of invasive plants into nearby vernal pool features. These indirect effects are considered insignificant. Alteration of soil and hydrologic conditions in the Proposed Project Footprint could affect adjacent vernal pool features, as discussed in Section 4.1.1.3.

4.1.1.3 Habitat Fragmentation

Based on the current design of the Proposed Project, the segments of the track at ground level and two facilities could fragment vernal pool habitat by removing portions of individual vernal pools or altering hydrologic patterns through soil compaction and converting native soil to track or developed areas that degrades adjacent features and disrupts vernal pool complex functions. Most of the proposed track would be along existing roads (72 percent) or on an elevated viaduct or bridge (15 percent), with the remaining 13 percent of the track at the ground level. Based on the few expected vernal pool features or complexes in and near the Proposed Project Footprint, long-term effects from habitat fragmentation would be minor and would not substantially reduce future available habitat or distribution of the vernal pool fairy shrimp.

4.1.2 Species-Specific Conservation Measures

In addition to general conservation measures discussed in Section 1.6, the following species-specific conservation measures will be implemented.

CM-11 – Vernal Pool-Endemic Species Surveys:

Identification and Avoidance of Vernal Pool Habitat: During the development of final project designs, a biologist will survey the Proposed Project Footprint and a 250-foot buffer (where access is granted) to map suitable habitat for vernal pool-endemic species, including vernal pool fairy shrimp, and assess whether the habitat is occupied. One wet season (October 15 through May 31) survey and one dry season (June 1 through October 14) survey will be completed within 3 years of each other in areas with the potential to have vernal pools or other suitable habitat for hosting vernal pool fairy shrimp species. Where possible, the Authority will refine the design of the Proposed Project to avoid direct impacts to vernal pool features and the area within 250 feet of them.

CM-12 – Protect Vernal Pool-Endemic Species During Construction:

Seasonal Restrictions and Exclusion: Where vernal pools or their 250-foot buffers cannot be avoided, the following measures will be implemented to minimize disturbance to the features and surrounding area:

- All ground-disturbing activities in or within 250 feet of vernal pool habitat will either be scheduled to take place entirely during the dry season (April 15 to October 14, depending on seasonal inundation conditions and guidance from the biologist) or will be scheduled to begin in the dry season with work during the wet season avoiding impacts to vernal pool habitat (i.e., removal of vernal pool features in the Proposed Project Footprint must take place during the dry season). Outside of the dry season, a USFWS-approved biological monitor will be present when work is occurring within 250 feet of a vernal pool feature to ensure protection of the feature.
- To ensure vernal pool habitat outside the Proposed Project Footprint is protected, the Authority or its contractor shall install exclusion fencing and erosion control measures along the construction limits in areas where vernal pool features will be

avoided prior to any ground disturbance. A biologist will supervise installation of the exclusion fencing. If vernal pools and their associated buffer cannot be fully avoided, a biologist may recommend a reduced buffer in consideration of site-specific conditions or the nature of nearby activities. The biologist will coordinate with the Authority and FTA on the appropriate buffer reduction and may contact USFWS for input.

- When dry-season temporary access is needed across vernal pool features, wetland mats or similar temporary structures will be used year-round to minimize ground and vegetation disturbance. This restriction will not apply to existing paved or unpaved access routes, including railroad grades.

CM-13 – Compensate for Loss of Vernal Pool Habitat: The Authority will compensate for the loss of occupied vernal pool habitat in the form of conservation credits purchased at an appropriate USFWS-approved conservation bank in an amount consistent with either the EACCS or SJMSCP before construction activities begin for the Proposed Project. Compensatory mitigation credits will be purchased at an 11:1 ratio (mitigation area to effect area) in accordance with the EACCS and SJMSCP. The conservation bank, specific amount, and type of credits will be coordinated with USFWS and confirmed following the final design plans.

4.1.3 Determination

The Proposed Project would result in temporary and permanent impacts to vernal pool features and **May Affect and is Likely to Adversely Affect** the vernal pool fairy shrimp. Implementation of conservation measures outlined in Section 1.6 and Section 4.1.2 would reduce or offset potential adverse impacts to the vernal pool fairy shrimp and its habitat.

4.2 Monarch Butterfly

4.2.1 Project Effects

As described below, construction of the Proposed Project has the potential to impact monarch butterfly that may be present in the Action Area through harm of individuals, the removal, or degradation, of habitat that has potential to support monarch butterfly's host plant.

Direct and indirect impacts to the species and their potential habitat would be reduced through the implementation of conservation measures outlined in Section 1.6 and Section 4.2.2.

4.2.1.1 Harm of Individuals

The Proposed Project may result in direct effects to monarch butterfly, including mortality of eggs and larvae occurring on milkweed (*Asclepias californica*) removed during construction activities. Adult butterflies could be struck by vehicles driving within the Proposed Project Footprint during construction or the train during operation; or if roosting on vegetation in the construction area, could be injured during vegetation removal. Locating the Proposed Project infrastructure adjacent to existing transportation infrastructure, utility corridors, and other development would minimize effects to the

monarch butterfly. Flagging host plants for avoidance described in CM-14 will minimize potential direct impacts. Additionally, general conservation measures including protecting habitat during construction activities (CM-2), vegetation removal and restoration (CM-3), preventing invasive species entry into native habitat (CM-4), implementing fugitive dust control (CM-6), and general construction BMPs (CM-7) will be implemented as outlined in Section 1.6.

4.2.1.2 Habitat Loss or Degradation

The Proposed Project is expected to result in permanent modification to approximately 103 acres of habitat (non-native grassland and scrub) that has potential to support the monarch butterfly's host plant. Direct permanent modification of potential habitat would result from construction of the rail trackway (34 acres); stations, MOW facilities, and other (48 acres); retaining walls (19 acres); and roadway widening (2 acres). The Proposed Project is expected to result in temporary impacts to approximately 48 acres of potential habitat that has potential to support host plant due to temporary staging and clearing.

Potential indirect effects to monarch butterfly include habitat degradation for host plants from invasive plants, contaminant exposure, and dust. Also, operational maintenance activities could result as continued disturbance of host plants during vegetation maintenance. Temporarily affected areas would be restored through restoration of natural habitat, including reseedling of native milkweed as described in CM-3 in Section 1.6 and CM-14 discussed in Section 4.2.2. Because milkweed is a fast-growing perennial plant, suitable habitat for monarch butterfly would likely be restored within a few growing seasons following seeding.

4.2.2 Species-Specific Conservation Measures

In addition to general conservation measures discussed in Section 1.6, the following species-specific conservation measures will be implemented. Compensatory mitigation is not proposed for the monarch butterfly.

CM-14 – Protect Host Plants for Monarch Butterfly: In order to reduce effects to Monarch butterfly, the following measures would be implemented:

Surveys and Host Plant Avoidance During Construction: If vegetation clearing is proposed to occur between March 1 and September 30, a biologist will conduct pre-construction surveys for milkweed in the Proposed Project Footprint and within 20 feet of the Proposed Project Footprint within 14 days before work is initiated in each area, with multiple surveys anticipated based on the construction phasing schedule. Any host plants within the Proposed Project Footprint would be inspected for eggs or larvae, and if they are present, they would be relocated by a biologist to suitable host plants outside of the Proposed Project Footprint. The biologist will flag any milkweed outside of the Proposed Project Footprint but within 20 feet of the Proposed Project Footprint for avoidance.

Milkweed Seeding: Native milkweed seeds will be included in the native seed mix as detailed in CM-3 in Section 1.6.

4.2.3 Determination

Due to the anticipated removal of host plants, and possibly eggs and larvae of the monarch butterfly, within the Action Area, the Proposed Project is anticipated to potentially impact monarch butterfly. However, very few records of monarch butterfly have been recorded near the Action Area. Implementation of conservation measures outlined in Section 1.6 and Section 4.2.2 would reduce potential impacts to the monarch butterfly, the Proposed Project **May Affect but is Not Likely to Jeopardize Species** due to the anticipated removal of host plants, and possibly eggs and larvae of the monarch butterfly, within the Action Area.

4.3 Amphibians

4.3.1 Project Effects

As described below, construction of the Proposed Project has the potential to affect California tiger salamander, California red-legged frog, and western spadefoot, which may be present in the Action Area, through direct injury or mortality or through behavioral changes due to construction activities. Operation of the Proposed Project has the potential to effect California tiger salamander, California red-legged frog, and western spadefoot by permanently altering habitat within the Proposed Project Footprint, also discussed below.

Direct and indirect effects to these species and their potential habitat would be reduced through the implementation of general conservation measures consisting of protecting habitat during construction activities (CM-2), preventing invasive species entry into native habitat (CM-4), controlling dust emissions (CM-6), BMPs (CM-7), implementing a noise and vibration-reduction plan (CM-8), and avoiding entrapment of listed species (CM-9) outlined in Section 1.6. Impacts to potential habitat for California tiger salamander, California red-legged frog, and western spadefoot within the Action Area is depicted on Figure 9, 10, and 11, respectively, in Appendix A.

4.3.1.1 Harm of Individuals

If occupied aquatic breeding habitat or upland aestivation habitat are present in the Proposed Project Footprint, individuals could be injured or killed if the aquatic or terrestrial habitats are removed through excavation and other ground disturbance, or if occupied burrows or refugia are collapsed on the occupants due to the movement of heavy equipment. Due to the cryptic nature of these species, which hide in underground refugia during the dry season, the potential take of individuals is difficult to quantify and would be difficult to observe during construction. As a result, the permanent loss of habitat is used as a surrogate for take of these species. Habitat loss resulting from the Proposed Project is discussed in Section 4.3.1.2. Implementation of conservation measures outlined in Section 1.6 and Section 4.3.2 would reduce the potential for take of these amphibian species as a result of construction and operation of the Proposed Project.

Aside from the potential for injury, indirect effects of construction noise and vibration (e.g., equipment involved in site preparation, grading, and earthwork and the installation of the rail tracks and infrastructure) and nighttime lighting on the California tiger salamander,

California red-legged frog, and western spadefoot could occur in the immediate area of construction. While there are currently no criteria for assessment of impacts from vibration on wildlife, during construction some activities may cause perceptible ground-borne vibration, most notably pile driving for structures and vibratory compaction for ground improvements. Potential vibration impacts are expected to be limited to annoyance effects, or may temporarily reduce foraging by masking the noise or vibrations produced by prey. Nighttime lighting associated with construction of the Proposed Project could also disturb individuals or could increase predation by predators that are visually oriented if the lighting is in proximity to suitable aquatic resources. These effects would be minimized through the implementation of the conservation measures described in Section 1.6.

There is also potential for indirect effects to individuals through water quality effects during construction due to sedimentation, as well as other potential indirect effects from contaminants and dust. Dust settling on the skin of amphibians could affect osmotic regulation, although this has not been well-studied. Implementation of the BMPs and conservation measures (CM-6 and CM-7) (Section 1.6) would prevent adverse effects from sedimentation and dust.

Equipment that was recently used in wetlands or waterbodies could also harbor amphibian pathogens if they were not properly cleaned. Disease-causing agents for amphibians (pathogens and parasites) may be transmitted between habitats on the hands, footwear, or equipment of construction/fieldworkers. One example of a highly pathogenic disease that can be transmitted by people from habitat to habitat is chytridiomycosis, which is an infectious disease affecting amphibians worldwide. The causative agent is the chytrid fungus (*Batrachochytrium dendrobatoides*), which is a fungal pathogen capable of causing 100 percent mortality in some amphibian populations. Implementation of CM-17 would reduce the potential for the Proposed Project to spread amphibian disease to a discountable level.

Implementation of conservation measures outlined in Section 1.6 and Section 4.3.2 would reduce the potential for take as a result of construction and operation of the Proposed Project, for example, by preventing the use of erosion control netting that could entangle amphibians.

4.3.1.2 Habitat Loss or Degradation from Construction

The Proposed Project would result in permanent modification and temporary effects to grassland, ruderal, and agricultural land cover that may provide upland estivation, burrowing, and/or dispersal habitat.

- **California tiger salamanders:** The Proposed Project is anticipated to result in permanent modification to approximately 5 acres of potential aquatic breeding habitat and approximately 108 acres of upland dispersal/burrowing habitat. Direct permanent modification of potential aquatic breeding habitat would result from construction of the rail trackway (3.3 acres); stations, MOW facilities, and other (0.8 acre); retaining walls (0.4 acre); and roadway widening/access road (0.5 acre). Direct permanent modification of potential upland dispersal/burrowing

habitat would result from construction of the rail trackway (38.1 acres); stations, MOW facilities, and other (28.5 acres); retaining walls (26 acres); and roadway widening/access road (15.4 acres). The Proposed Project is anticipated to result in temporary impacts to approximately 4 acres of potential aquatic breeding habitat and approximately 71 acres of upland dispersal/burrowing habitat due to temporary staging and clearing.

- **California red-legged frog:** The Proposed Project is anticipated to result in permanent modification to approximately 5 acres of potential aquatic breeding habitat and approximately 96 acres of upland dispersal/estivation habitat. Direct permanent modification of potential aquatic breeding habitat would result from construction of the rail trackway (3.2 acres); stations, MOW facilities, and other (1 acre); retaining walls (0.3 acre); and roadway widening/access road (0.5 acre). Direct permanent modification of potential upland dispersal/estivation habitat would result from construction of the rail trackway (29.9 acres); stations, MOW facilities, and other (24.5 acres); retaining walls (26.4 acres); and roadway widening/access road (15.2 acres). The Proposed Project is anticipated to result in temporary impacts to approximately 4 acres of potential aquatic breeding habitat and approximately 64 acres of upland dispersal/estivation habitat due to temporary staging and clearing.
- **Western spadefoot:** The Proposed Project is anticipated to result in permanent modification to approximately 5 acres of potential aquatic breeding habitat and approximately 189 acres of upland dispersal/burrowing habitat. Direct permanent modification of potential aquatic breeding habitat would result from construction of the rail trackway (3.3 acres); stations, MOW facilities, and other (0.9 acre); retaining walls (0.3 acre), and roadway widening/access road (0.5 acre). Direct permanent modification of potential upland dispersal/burrowing habitat would result from construction of the rail trackway (38.5 acres); stations, MOW facilities, and other (111.5 acres); retaining walls (25.8 acres); and roadway widening/access road (12.7 acres). The Proposed Project is anticipated to result in temporary impacts to approximately 4 acres of potential aquatic breeding habitat and approximately 67 acres of upland dispersal/burrowing habitat due to temporary staging and clearing.

4.3.1.3 Movement and Habitat Fragmentation

Based on the preliminary design, approximately 72 percent of the Proposed Project aligns with the existing freeway, which already presents a barrier to these species. Additionally, 15 percent of the Proposed Project would be constructed on viaduct and/or bridge structures, allowing for unimpeded movement beneath the tracks in these areas. As shown on Figure 2 in Appendix A, the viaducts are concentrated where the Proposed Project swings away from the I-580 corridor through the Altamont Pass area. However, the Proposed Project may still contribute to creating a partial impediment to dispersal and movement between aquatic bodies by the installation of retaining walls, impeding movement of the California tiger salamander, California red-legged frog, and western spadefoot across portions of the Action Area. However, retaining walls would be installed along steep hill slopes, not valley bottoms or drainages that are more likely to be

movement corridors for these species (the location of many of the viaducts). Where the track is installed at grade, it is anticipated that there will be sufficient gaps between the rail and gravel bed to permit amphibians to pass underneath the rail. It is also anticipated that California red-legged frogs and western spadefoot toads would be capable of jumping over the rail, if needed. The sections of the Proposed Project that include viaducts will also allow for unimpeded movement of amphibians. Due to these considerations, the Proposed Project's effect on landscape permeability for these amphibian species would be minimal.

4.3.1.4 Habitat Degradation from Operations

The indirect effects of nighttime lighting on the California tiger salamander, California red-legged frog, and western spadefoot would be limited to the immediate area of the Proposed Project Footprint. Nighttime lighting associated with operations or maintenance of the Proposed Project could disturb individuals or could increase predation by predators that are visually oriented if the lighting is in proximity to suitable aquatic resources. This potential effect would be minimized through implementation of CM-5 as outlined in Section 1.6.

While habituation to transportation noise, such as at airports, highways, and urban centers, is commonly seen in some species, the effect of trainset noise and vibration on amphibians is unclear as it has not been thoroughly studied. The Proposed Project would operate an electric train, which would generate less noise and vibration compared to diesel trains. For the majority of the Proposed Project's alignment, the Valley Link train would be operated in close proximity to I-580 and/or the Union Pacific Railroad (UPRR) freight corridor, both of which are significant existing sources of noise and vibration. As a result of these factors, the Proposed Project's contribution to noise and vibration is expected to be minimal and not affect aquatic habitats in the Action Area.

Additionally, operational maintenance activities could result in habitat modification associated with vegetation maintenance and the maintenance of drainage structures (such as the clearing of clogged culverts). This effect would be minimized by timing such activities to occur outside of the wet season (CM-16) when amphibians may be dispersing across the landscape surface.

There is potential for indirect effects to water quality during operation as a result of sedimentation as well as other potential indirect effects from contaminants and dust that may enter aquatic habitats. The Proposed Project would operate an electric train, which does not produce any exhaust or particulates, aside from potential brake dust. However, the train would utilize regenerative braking systems, using the trains motors to facilitate braking. As a result, the amount of brake dust or other particulates generated is expected to be minimal.

4.3.2 Species-Specific Conservation Measures

In addition to general conservation measures discussed in Section 1.6, the following species-specific conservation measures will be implemented.

CM-15 – California Tiger Salamander, Western Spadefoot, and California Red-Legged Frog Surveys:

Screening Surveys and Buffers: During final project design, a biologist will conduct wet season surveys for suitable aquatic habitat for California tiger salamander, western spadefoot, and California red-legged frog to determine where these species may be present in or within 250 feet of the Proposed Project Footprint (where access is granted). The biologist will identify, evaluate, and flag (pin flags or 4-foot lath) suitable aquatic habitat to be preserved outside of the Proposed Project Footprint and up to 250 feet from the edge of the Proposed Project Footprint and flag small mammal burrows that may be used by the species within 10 feet of the Proposed Project Footprint. Where possible, the Authority will refine the design of the Proposed Project to avoid direct impacts to suitable aquatic habitat, the area within 250 feet of the habitat, and small mammal burrows.

CM-16 – Protect California Tiger Salamander, Western Spadefoot, and California Red-Legged Frog During Construction:

Seasonal Restrictions: If the construction schedule can accommodate seasonal restrictions, vegetation removal (including mowing) and ground-disturbing activities that occur within 250 feet of suitable aquatic habitat will be conducted during the dry season (June 1 to October 14). California tiger salamander, western spadefoot, and California red-legged frog are less likely to be present in the waterbodies or moving across the upland habitats during this period.

Burrow Avoidance and Exclusion: Small mammal burrows outside of the Proposed Project Footprint will be avoided, and a 10-foot no-disturbance buffer around the burrows will be marked with fencing at the edge of the work area. For large concentrations of burrows within the Proposed Project Footprint that cannot be avoided, exclusion fencing may be installed around such areas prior to the wet season (October 15 to May 31) so that amphibians do not relocate to them as their aquatic habitat dries.

Flagging and Fencing: The Authority or its contractor shall protect adjacent habitat areas by installing both ESA high-visibility construction fencing and wildlife exclusion fencing (when feasible) as well as erosion control fencing at the maximum practicable distance from the work site, or if feasible, at least 250 feet from the aquatic habitat edge, wet or dry, to make it easily visible by construction crews.

Construction Monitoring and Relocation: For vegetation removal and ground-disturbing activities that must take place during the wet season (October 15 to May 31), a biologist will conduct daily surveys for federally listed amphibians in aquatic habitat each morning prior to the start of construction activities within 250 feet of suitable habitat to determine whether individuals are present. If individual amphibians are observed, a permitted biologist will relocate them to suitable habitat at least 300 feet from the construction boundary in accordance with a relocation plan approved by FTA, the Authority, and USFWS prior to the start of construction occurring within 250 feet of suitable habitat. Relocation sites will be confirmed in coordination with USFWS.

CM-17 – Implement Measures to Prevent Diseases: The contractor shall comply with the Declining Amphibian Populations Task Force Fieldwork Code of Practice (Declining Amphibian Populations Task Force 1998) to prevent the introduction and spread of amphibian diseases and parasites. Specific measures to be implemented include:

- Remove mud, snails, algae, and other debris from nets, traps, boots, vehicle tires and all other surfaces. Rinse cleaned items with sterilized (e.g., boiled or treated) water before leaving each study site.
- Boots, nets, traps, etc., should then be scrubbed with 70 percent ethanol solution (or sodium hypochlorite 3 to 6 percent) and rinsed clean with sterilized water between study sites. Avoid cleaning equipment in the immediate vicinity of a pond or wetland.
- When working at sites with known or suspected disease problems, or when sampling populations of rare or isolated species, wear disposable gloves and change them between handling each animal. Dedicate sets of nets, boots, traps, and other equipment to each site being visited. Clean and store them separately and the end of each field day.
- When amphibians are collected, ensure the separation of animals from different sites and take great care to avoid indirect contact between them (e.g., via handling, reuse of containers) or with other captive animals. Isolation from un-sterilized plants or soils which have been taken from other sites is also essential. Always use disinfected/disposable husbandry equipment.

CM-18 – Compensate for the Loss of Aquatic Habitat for Amphibians: The Authority will compensate for the loss of aquatic breeding habitat for California tiger salamander and California red-legged frog in the form of conservation credits purchased at an appropriate USFWS-approved conservation bank in an amount consistent with either the EACCS or SJMSCP before construction activities begin for the Proposed Project. The proposed mitigation ratio is 2.5:1 (mitigation area to effect area) based on the locations of the effects and mitigation sites as defined in the EACCS or SJMSCP. The conservation bank, specific amount, and type of compensation will be coordinated with USFWS, and confirmed following the final design plans. This compensation would also benefit western spadefoot by protecting aquatic habitat it may use.

4.3.3 Determination

Due to the anticipated impacts to potentially occupied aquatic breeding and upland aestivation and dispersal habitat within the Action Area, the Proposed Project is anticipated to adversely affect California red-legged frog, California tiger salamander, and western spadefoot, both through habitat modification and the potential for individuals to be killed by construction while estivating in burrows and other subterranean features. As a result, the Proposed Project was determined to **May Affect, and is Likely to Adversely Affect**, California tiger salamander and California red-legged frog, and **May Affect, Not Likely to Jeopardize Species** for the western spadefoot.

4.4 California Red-Legged Frog Critical Habitat

4.4.1 Project Effects

As described in Section 3.4.1, PBFs of critical habitat for California red-legged frog include aquatic breeding habitat, nonbreeding aquatic and riparian habitat, and upland habitat used for foraging, shelter, and dispersal. The Proposed Project would result in temporary and permanent effects in areas containing one or more of these PBFs during construction and operations. Within critical habitat for California red-legged frog, the Proposed Project is anticipated to result in permanent loss or modification to approximately 4 acres of potential aquatic breeding and nonbreeding habitat and approximately 36 acres of upland dispersal/estivation habitat (Appendix A, Figure 8). Direct permanent loss or modification of potential aquatic breeding or nonbreeding habitat would result from construction of the rail trackway (3 acres); stations, MOW facilities, and other structures (0.6 acre); retaining walls (0.3 acre); and roadway widening/access road (0.1 acre). Direct permanent loss or modification of potential upland dispersal/estivation habitat would result from construction of the rail trackway (12.8 acres); stations, MOW facilities, and other (8 acres); retaining walls (11.3 acres); and roadway widening/access road (3.9 acres). The Proposed Project is anticipated to result in temporary impacts to approximately 2 acres of potential aquatic breeding or nonbreeding habitat and approximately 22 acres of upland dispersal/estivation habitat due to temporary staging and clearing. Any aquatic habitat areas impacted would be fully evaluated during screening surveys (CM-15) in order to determine whether they may support breeding or are nonbreeding aquatic habitat. All temporarily impacted upland areas would be restored as described in CM-3. Temporarily impacted aquatic habitats would be restored by restoring the area to pre-construction grade.

The manner in which construction and operation may affect designated critical habitat would be similar to that described for amphibians in Section 4.3.1. During construction, such temporary effects would result from the collapse of potential aestivation burrows or other refugia, noise and vibration, construction lighting, water quality impacts, dust, and the spread of amphibian disease. During operations, effects would result from facility lighting, noise and vibration, culvert maintenance, and vegetation management.

As described in Section 4.3.1, approximately 72 percent of the Proposed Project aligns with the existing freeway, and 15 percent of the Proposed Project would be constructed on viaduct and/or bridge structures, allowing for unimpeded movement beneath the tracks in these areas. As shown on Figure 2 in Appendix A, the viaducts are concentrated where the Proposed Project swings away from the I-580 corridor through the Altamont Pass area, where the majority of the impacts to critical habitat for California red-legged frog would occur. However, the Proposed Project may still contribute to creating a partial impediment to dispersal and movement in some areas by the installation of retaining walls. Where the track is installed at grade, it is anticipated that California red-legged frogs would be capable of jumping over the rail, if needed. Due to these considerations, the Proposed Project's effect on landscape permeability for the species within California red-legged frog critical habitat would be minimal.

Conservation measures identified for California red-legged frog (CM-15 through CM-18) would reduce and offset impacts to PBFs in designated critical habitat.

4.4.2 Determination

While implementation of conservation measures outlined in Section 1.6 and Section 4.3.2 would reduce impacts to the critical habitat for California red-legged frog, temporary and permanent impacts to areas containing PBFs would still occur. The impacts would result in some incremental reduction in the ability of critical habitat unit CCS-2B to support California red-legged frog, but the impacts would occur to small areas in a non-contiguous chain along the Proposed Project Footprint, which is located near the edge of the critical habitat unit. Further, the Proposed Project would not create a movement barrier for the species, as discussed in Section 4.3.1. With the restoration of temporarily impacted areas and implementation of compensatory mitigation, the Proposed Project **May Affect but would not Adversely Modify** California red-legged frog critical habitat.

4.5 Northwestern Pond Turtle

4.5.1 Project Effects

As described below, construction of the Proposed Project has the potential to effect northwestern pond turtle that may be present in the Action Area through direct injury or mortality, or through behavioral changes due to construction activities. Operation of the Proposed Project has the potential to effect northwestern pond turtle by permanently altering habitat within the Proposed Project Footprint, also discussed below.

Direct and indirect effects to the species' potential habitat would be reduced through the implementation of conservation measures outlined in Section 1.6 and Section 4.5.2.

4.5.1.1 Harm of Individuals

If occupied aquatic breeding habitat or upland nesting habitat are present in the Proposed Project Footprint, individuals could be injured or killed if the occupied habitats are removed while individuals are present, or if active nests are collapsed. Adults and juveniles occupying aquatic habitat are relatively easy to spot and relocate, which will greatly reduce the potential for injury or death of individuals in areas where aquatic habitat may be directly impacted. Northwestern pond turtle nests are difficult to spot, and undetected nests containing eggs or hatchlings could be accidentally destroyed if they are present in the Proposed Project Footprint.

The indirect effects of construction noise and vibration (e.g., equipment involved in site preparation, grading, and earthwork and the installation of the rail tracks and infrastructure) and nighttime lighting on the western pond turtle would be limited to the immediate area of the construction site. While there are currently no criteria for assessment of impacts from vibration on wildlife, during construction, some activities may cause perceptible ground-borne vibration, most notably pile driving for structures and vibratory compaction for ground improvements. Nighttime lighting associated with construction of the Proposed Project could disturb individuals or could increase predation by predators that are visually oriented if the lighting is in proximity to suitable aquatic

resources. Implementation of CM-5 would reduce the severity of effects associated with lighting, and CM-8 would reduce the effects of noise and vibration.

There is potential for indirect effects to water quality during construction due to sedimentation as well as other potential indirect effects from contaminants and dust. Implementation of general conservation measures including protecting habitat during construction activities (CM-2), preventing invasive species entry into native habitat (CM-4), controlling dust emissions (CM-6), and general construction BMPs (CM-7) (as outlined in Section 1.6) would prevent adverse effects from sedimentation and dust.

Implementation of conservation measures outlined in Section 1.6 and Section 4.3.2 would reduce but not eliminate the potential for take as a result of construction of the Proposed Project.

4.5.1.2 Habitat Loss or Degradation from Construction

The Proposed Project is anticipated to result in permanent modification of approximately 5 acres of potential aquatic breeding habitat and approximately 99 acres of upland dispersal habitat (Appendix A, Figure 11). Direct permanent modification of potential aquatic breeding habitat would result from construction of the rail trackway (3.3 acres); stations, MOW facilities, and other (0.7 acre); retaining walls (0.5 acre); and roadway widening/access road (0.5 acre). Direct permanent modification of potential upland dispersal habitat would result from construction of the rail trackway (36.1 acres); stations, MOW facilities, and other (25.9 acres); retaining walls (23.6 acres); and roadway widening/access road (13.4 acres). The Proposed Project is anticipated to result in temporary impacts to approximately 5 acres of potential aquatic breeding habitat and approximately 68 acres of upland dispersal habitat due to temporary staging and clearing.

4.5.1.3 Movement and Habitat Fragmentation

The Proposed Project may contribute to creating a barrier between aquatic bodies and upland habitat for the species where the track bed is installed at grade, thereby impeding movement of the northwestern pond turtle across the landscape. Based on the preliminary design, approximately 72 percent of the Proposed Project aligns with the existing freeway, which already presents a barrier to these species. Additionally, 15 percent of the Proposed Project would be constructed on viaduct and/or bridge structures, allowing for unimpeded movement beneath the tracks in these areas. As shown on Figure 2 in Appendix A, the viaducts are concentrated where the Proposed Project swings away from the I-580 corridor through the Altamont Pass area, and many are located over valley bottoms that the species is more likely to use for dispersal. Due to these considerations, the Proposed Project's effect on landscape permeability for northwestern pond turtle would be minimal.

Effects would be reduced or avoided with the implementation of conservation measures including protecting habitat during construction activities (CM-3), preventing invasive species entry into native habitat (CM-4), controlling dust emissions (CM-6), managing water quality and runoff from construction operations and construction BMPs (CM-7); and species-specific conservation measures (CM-20), as described in Section 1.6, Section

4.5.2, and Section 4.5.3, respectively. Implementation of conservation measures outlined in Section 1.6 and Section 4.3.2 would reduce but not eliminate the potential for take as a result of operation of the Proposed Project.

4.5.1.4 Habitat Degradation from Operations

While habituation to transportation noise, such as at airports, highways, and urban centers, is commonly seen in some species, the effect of trainset noise and vibration on wildlife is unclear as it has not been thoroughly studied. The Proposed Project would operate an electric train, which would generate less noise and vibration compared to diesel trains. For the majority of the Proposed Project's alignment, the Valley Link train would be operated in close proximity to I-580 and/or the UPRR freight corridor, both of which are significant sources of noise and vibration. As a result of these factors, the Proposed Project's contribution to noise and vibration is expected to be minimal.

The indirect effects of nighttime lighting on the northwestern pond turtle would be limited to the immediate area of the Proposed Project Footprint. Nighttime lighting associated with operations or maintenance of the Proposed Project could disturb individuals or could increase predation by predators that are visually oriented if the lighting is in proximity to suitable aquatic resources, but this would be minimized through implementation of CM-9.

The Proposed Project would operate an electric train, which does not produce any exhaust or particulates, aside from potential brake dust. However, the train would utilize regenerative braking systems, using the trains motors to facilitate braking. As a result, the amount of brake dust or other particulates generated is expected to be minimal and not affect aquatic habitats in the Action Area.

While the passage of a trainset may not cause degradation in adjacent habitat, northwestern pond turtle may respond to this type of disturbance during operation. Additionally, operational maintenance activities could result in direct mortality as well as continued disturbance of habitat with the use of heavy equipment and vegetation maintenance.

4.5.2 Species-Specific Conservation Measures

In addition to general conservation measures discussed in Section 1.6, the following species-specific conservation measures will be implemented. Compensatory mitigation for the northwestern pond turtle is not proposed. In addition, general conservation measures discussed in Section 1.6, the following species-specific conservation measures will be implemented.

CM-19 –Northwestern Pond Turtle Screening:

Nesting Area Screening Surveys: During final project design, survey of potentially occupied habitat will be conducted (where access is granted). During the nesting season (roughly May through July), screening surveys to detect northwestern pond turtle nesting activity would be concentrated within 402 meters (1,319 feet) of suitable aquatic habitat and should focus on areas along south- or west-facing slopes with bare hard-packed clay or silt soils or a sparse vegetation of short grasses or forbs. Areas where nesting is

observed will be recorded and buffered by a 25-foot radius, and when possible, the buffered area will be avoided through refinements to the design of the Proposed Project or as described below in Seasonal Work Restrictions.

CM-20 – Protect Northwestern Pond Turtle During Construction:

Seasonal Work Restrictions: Work within known or suspected nesting locations should be limited to the period between October 1 and April 30 to avoid the nesting season (roughly May through July) and the 90-day incubation period (through September 30) when active nests and hatchlings may be present in the nest chamber.

Preconstruction Surveys: Where potentially occupied aquatic habitat is present or nesting areas identified by the nest screening surveys cannot be seasonally avoided, a biologist shall survey the work site no more than 48 hours before the onset of activities for signs of northwestern pond turtles and/or northwestern pond turtle nesting activity (i.e., recently excavated nests, nest plugs) or nest depredation (partially to fully excavated nest chambers, nest plugs, scattered eggshell remains, eggshell fragments). Construction monitoring of aquatic habitats should focus on suitable aerial and aquatic basking habitat such as logs, branches, rootwads, and riprap, as well as the shoreline and adjacent warm, shallow waters where pond turtles may be present below the water surface beneath algal mats or other surface vegetation. In areas found to or suspected to be occupied by northwestern pond turtle, the following measures will be implemented:

Work Site Exclusion: If preconstruction surveys identify occupied nests at or adjacent to work areas that cannot be seasonally avoided, wildlife exclusion fencing should be placed around the perimeter of the work area (with an appropriate buffer, if necessary, to allow construction activities up to the exclusion fencing) between October 1 and April 30 of the preceding year to avoid the nesting season (roughly May through July) and the 90-day incubation period (through September 30) when active nests and hatchlings may be present in the nest chamber. The fencing should be equipped with one-way escape features to allow any wildlife within the future work site to evacuate the area before work begins. Prior to the commencement of work, the excluded work area should be cleared by a biologist before ground-disturbing activities take place. Additionally, in areas that will not be directly impacted, exclusion buffers and nest enclosures will be installed to prevent hatchlings from entering construction areas.

Relocation: In the event that construction will fully remove an occupied aquatic habitat, a relocation plan will be developed including suitable relocation sites approved by USFWS occurring within occupied habitat. The biologist will relocate individuals to the nearest predetermined relocation site identified within the relocation plan. If USFWS approves of moving the animal, the biologist shall be allowed sufficient time to move the northwestern pond turtle(s) from the work site before work activities begin.

4.5.3 Determination

This species is known to occur and was observed in the Action Area in July 2024, and therefore, the Proposed Project may impact the northwestern pond turtle through permanent and temporary impacts to habitat, although implementation of the measures

outlined in Section 1.6 and Section 4.5.2 would reduce the impacts to the northwestern pond turtle. FTA finds that the Proposed Project is not expected to meaningfully contribute to the specific threats contributing to the species decline as described in the October 3, 2023, Proposed Rule (USFWS 2023c). However, FTA anticipates that the Proposed Project may affect the species through the permanent alteration of habitats discussed in Chapter 4. Through implementing the conservation measures described in Section 1.6 and Section 4.5.2, any adverse effects are expected to be reduced but not entirely avoided. As such, FTA has determined that the Proposed Project **May Affect but is Not Likely to Jeopardize Species** the ESA-proposed threatened northwestern pond turtle.

4.6 San Joaquin Kit Fox

4.6.1 Project Effects

As described below, construction of the Proposed Project has the potential to effect San Joaquin kit fox that may be present in the Action Area through direct injury or mortality, or through behavioral changes due to construction activities. Operation of the Proposed Project has the potential to effect San Joaquin kit fox by permanently altering habitat within the Proposed Project Footprint, also discussed below.

Direct and indirect effects to the species' potential habitat would be reduced through the implementation of conservation measures outlined in Section 1.6 and Section 4.6.2.

4.6.1.1 Harm of Individuals

If occupied dens are present in the Proposed Project Footprint, individuals could be injured or killed if the den is collapsed on the occupants. There is a low but not entirely discountable potential for occupied dens to be present in the Action Area. With implementation of CM-21 as described in Section 4.6.2, pre-construction surveys would be completed to ensure that occupied dens are not impacted by construction activities. As a result, direct injury or mortality of San Joaquin kit fox is not expected to occur.

San Joaquin kit fox within the Action Area may have behavioral reactions to construction noise and the presence of heavy equipment and personnel during construction. However, resident kit foxes have been documented to continue utilizing construction sites and other disturbed areas despite the intensive construction activities causing noise and vibration on the site (Caltrans 2023b). Therefore, indirect effects caused by construction noise and vibration (e.g., equipment involved in site preparation, grading, and earthwork and the installation of the rail tracks and infrastructure) are expected to be limited and not result in the permanent displacement of individuals, if they are present.

In addition, the use of pesticides to control rodents and other pests may impact the species directly through poisoning or indirectly through reduction of prey abundance. However, available data (CDFW 2023) indicate that San Joaquin kit fox do not regularly use the Action Area, and aside from the areas within the Proposed Project Footprint, potential dens within the portion of the Action Area outside of the Proposed Project Footprint would not be disturbed. The use of second-generation anticoagulant

rodenticides would be avoided in potential habitat as stated in CM-22, preventing the potential for secondary poisoning of kit fox that may be present.

4.6.1.2 Habitat Loss or Degradation from Construction

The Proposed Project would result in permanent modification to approximately 353 acres of grassland, ruderal, and agricultural land cover that may provide foraging habitat and denning opportunities. Direct permanent modification of potential habitat would result from construction of the rail trackway (41.2 acres); stations, MOW facilities, and other (265 acres); retaining walls (27.1 acres); and access roads and roadway widening (19.7 acres). Habitat loss due to the Proposed Project would occur along a narrow corridor and not appreciably alter habitat availability or suitability at the regional level.

The Proposed Project is anticipated to result in temporary effects to approximately 175 acres of potential foraging and denning habitat due to construction staging and clearing. All temporarily effected areas would be restored through reseeded (CM-3) and would be suitable habitat for San Joaquin kit fox within one growing season of restoration. Additional general conservation measures to reduce or avoid effects include protecting habitat during construction activities (CM-2), reducing lighting effects (CM-5), controlling dust emissions (CM-6), general construction BMPs (CM-7), implementing a noise and vibration-reduction plan (CM-8), and avoiding entrapment of listed species (CM-9), as outlined in Section 1.6.

During construction, the Proposed Project may directly impact potential den sites, limiting den availability in the Action Area. Although kit foxes are able to dig new dens, the creation of a network of dens required to successfully occupy an area may take months or even years. Lower den availability can decrease the use of an area by individuals, which in turn further reduces the distribution of the species.

4.6.1.3 Movement and Habitat Fragmentation

The grassland, ruderal, and agricultural land cover types within the Action Area support prey populations as well as a means for the species to move and disperse across the landscape. Despite the lack of recent documented occurrences within the Action Area, the ongoing, incremental loss and fragmentation of potential habitat for the species presents a risk to the San Joaquin kit fox, and the potential habitat within the Action Area may be important to the species to maintain the connectivity of occupied areas. The majority of the rail track would be unfenced and present a minimal obstacle to the species. Fragmentation would be minimal as the at-grade tracks are not expected to present a movement barrier, and approximately 15 percent of the Proposed Project would be on viaduct and/or bridge structure allowing for movement without crossing the tracks. As shown on Figure 2 in Appendix A, the viaducts are concentrated where the Proposed Project swings away from the I-580 corridor through the Altamont Pass area. In addition to movement under viaducted areas, San Joaquin kit fox would also be able to cross over at-grade tracks unimpeded, so the Proposed Project is not expected to affect landscape permeability for the species.

Based on the preliminary design, approximately 72 percent of the Proposed Project aligns with the existing freeway, which already presents a barrier to these species. This is particularly true for the approximately half of the Proposed Project Footprint that is west of Greenville Road, where heavy development has already occurred along the I-580 corridor. Past and continuing habitat fragmentation and degradation within the range of the species can result in lower suitability of the remaining habitat for the species. Similarly, reducing the occupancy of habitat reduces routine maintenance of existing dens, which causes them to begin to deteriorate and eventually disappear due to lack of use, resulting in lowered den availability.

4.6.1.4 Habitat Degradation from Operations

Additionally, nighttime lighting associated with operations or maintenance of the Proposed Project could disturb individuals or could increase predation by predators that are visually oriented if the lighting is in proximity to suitable aquatic resources. This potential effect would be minimized through implementation of CM-5 as outlined in Section 1.6.

The Proposed Project would permanently alter 353 acres of habitat potentially supporting occasional foraging and denning opportunities for the species. However, prey species such as ground squirrels, gophers, and mice are often seen occupying areas directly adjacent to railways and other developed areas, indicating that operation of the Proposed Project would not decrease the regional availability of potential prey for the species.

4.6.2 Species-Specific Conservation Measures

In addition to general conservation measures discussed in Section 1.6, the following species-specific conservation measures will be implemented.

CM-21 – San Joaquin Kit Fox Surveys: In areas that have the potential to support kit fox dens, which will include all areas of annual grassland and all ruderal areas adjacent to grasslands and agricultural areas, the following will be implemented.

Initial Den Surveys: During final design of the project, a biologist will conduct surveys to identify potential San Joaquin kit fox dens in the Proposed Project Footprint and surrounding 200 feet (where access is granted), in accordance with the Standardized Recommendations for Protection of the Endangered San Joaquin Kit Fox Prior to or During Ground Disturbance (2011 USFWS Standard Recommendations) (USFWS 2011). Different San Joaquin kit fox den types will be defined in accordance with the 2011 USFWS guidance. For dens that are located within the proposed work area and cannot be avoided, a biologist will determine whether the dens are natal dens, and are or were recently occupied using USFWS guidelines (USFWS 2011). Occupied or recently occupied natal dens will be documented and reported to FTA and USFWS in accordance with USFWS procedures. If unoccupied dens are located in the Proposed Project Footprint, the biologists will collapse the den by and in accordance with USFWS procedures. The biologists will prepare a report summarizing the survey observations and results, including maps depicting the locations of potential kit fox dens—and if possible—occupancy. Where possible, the Authority will refine the design of the Proposed Project to avoid direct impacts to active natal dens and the area within 200 feet of them.

Pre-construction Den Surveys: Pre-construction surveys will be conducted in any areas identified as containing occupied or recently occupied dens during the initial den surveys. In accordance with the 2011 USFWS Standard Recommendations, pre-construction surveys are to be conducted no less than 14 days and no more than 30 days before the initiation of construction at each environmental footprint (e.g., 1 week ahead of the construction crew for linear components). Construction activities will not take place within 100 feet of a potential den during the natal period (February 1 through September 30). If a known den or natal or pupping den is present 100 feet outside of the permanent Proposed Project Footprint, then a 200-foot no-disturbance exclusion zone during the natal period (100-foot buffer during the non-natal period) will be established around the den, with orange construction fencing at the edge of the disturbance limits nearest the den. If a known den or natal or pupping den is present in the permanent effects zone of the Proposed Project Footprint or within 200 feet of the Proposed Project Footprint during the natal period (100-foot buffer during the non-natal period), the foxes will be excluded outside of the natal period (from November 1 through January 31). A summary report will be prepared by the biologists following completion of all fox avoidance and exclusion activities.

CM-22 – Protect San Joaquin Kit Fox During Construction and Operations:

Site Inspections and Entrapment Avoidance: In areas where occupied or recently occupied dens are identified during pre-construction surveys (CM-21), site inspections prior to the initiation of work each day will be completed to ensure that foxes are not present and that new dens are not being created. Any new dens will be removed using USFWS-approved methods described above. Discouraging the denning within materials stored on-site will be done by installing fencing or elevating materials off of the ground. Pipes will be capped, and trenches will contain exit ramps to avoid direct entrapment during construction activities.

Restrictions and the Use of Rodenticide: During construction and operations, the use of second-generation anticoagulant rodenticides, such as brodifacoum, bromadiolone, difenacoum, and difethialone, will be avoided in San Joaquin kit fox habitat areas. Other pesticides and herbicides may be used in accordance with U.S. Environmental Protection Agency guidelines.

CM-23 – Compensate for the Loss of San Joaquin Kit Fox Denning Habitat: The Authority will compensate for the loss of denning habitat for San Joaquin kit fox in the form of conservation credits purchased at an appropriate USFWS-approved conservation bank in an amount consistent with the EACCS (zones North, East, and Central-West) before construction activities begin for the Proposed Project. The proposed mitigation ratio is 3:1 (mitigation area to effect area) based on the habitat effects and guidance in the EACCS. The conservation bank, type, and amount of compensation credit will be coordinated with USFWS and will be confirmed following initial den surveys and final design plans.

4.6.3 Determination

Based on the lack of recent documented occurrences within 10 miles of the Action Area and the measures to be implemented for avoidance of natal dens, and the measures implemented to collapse dens only when they are unoccupied, injury or mortality of San Joaquin kit fox is not expected to occur. Since the at-grade portions of the rail track would not be fenced, and much of the rail would be built adjacent to existing barriers such as I-580, fragmentation of potential San Joaquin kit fox habitat would have an insignificant effect on any population, if it is present. The Proposed Project would permanently alter 353 acres of habitat potentially supporting occasional foraging and denning opportunities for the species. As a result, the Proposed Project was determined to **May Affect, Likely to Adversely Affect**, the San Joaquin kit fox. This habitat loss would occur along a narrow corridor and not appreciably alter habitat availability or suitability at the regional level.

4.7 Palmate-Bracted Bird's-Beak

4.7.1 Project Effects

The Proposed Project could result in direct removal of individuals, permanent and temporary effects to alkaline grassland and wetland habitats that are suitable for palmate-bracted bird's-beak, and habitat degradation. Locating the Proposed Project infrastructure adjacent to existing transportation infrastructure, utility corridors, and other development would minimize potential impacts to the palmate-bracted bird's-beak.

4.7.1.1 Harm to Individuals

If present within the Proposed Project Footprint, removal of individual plants and or seeds would result in direct effects. Implementation of CM-24 would identify locations where this plant and/or seed occurs within the Proposed Project Footprint. Surveys will be conducted during final project design, and if present, avoidance of removal of this species will be considered (CM-25). If unavoidable, impacts will be minimized to the extent feasible, and CM-26 (implementation of propagation and monitoring plan) will be implemented.

4.7.1.2 Habitat Loss or Degradation

If this species occurs within the Action Area, potential indirect effects may include habitat degradation from invasive plants, sedimentation of wetland habitat features, grading or other ground disturbance that results in changes in surface runoff patterns, habitat fragmentation, and exposure to urban contaminants and dust. Due to the potential for indirect effects, any ground-disturbing work occurring within 50 feet of palmate-bracted bird's beak is assumed to potentially affect palmate-bracted bird's beak. However, indirect effects of the Proposed Project, particularly invasive species and changes in surface runoff patterns, could potentially be realized up to 250 feet from ground disturbance.

There is potential for indirect effects to water quality during construction due to sedimentation as well as other potential indirect effects from contaminants and dust. Implementation of general conservation measures (Section 1.6) would prevent adverse effects from sedimentation and dust including protecting habitat during construction

activities (CM-2), preventing invasive species entry into native habitat (CM-4), controlling dust emissions (CM-6), and construction BMPs (CM-7).

The Proposed Project is anticipated to result in permanent modification to approximately 2 acres of potential vernal pool habitat that may support palmate-bracted bird's beak. Direct permanent modification of potential habitat would result from construction of the rail trackway (1 acre); stations, MOW facilities, and other (0.8 acre); and retaining walls (0.2 acre). However, it is anticipated direct and indirect effects to potential habitat would be reduced through the implementation of conservation measures outlined in Section 1.6 and Section 4.7.2. Permanent changes to ground topography or permeability within the drainage areas of vernal features may result in changes to the extent and duration of flooding within that feature, which may affect habitat suitability for this species.

There is potential for indirect effects to water quality during operation as a result of sedimentation as well as other potential indirect effects from contaminants and dust that may enter aquatic habitats. The Proposed Project would operate an electric train, which does not produce any exhaust or particulates, aside from potential brake dust. However, the train would utilize regenerative braking systems, using the train's motors to facilitate braking. As a result, the amount of brake dust or other particulates generated is expected to be insignificant.

Within the Action Area but outside of direct habitat modification, potential indirect effects would include habitat degradation from the introduction or spread of invasive plants; changes in the soil regime due to alteration of hydrology and sedimentation of vernal pool features; changes in the available water due to diversion of surface runoff; and soil compaction. These effects would likely result in the decrease of suitability and functionality of vernal pool habitat over time and impair or eliminate them as a functional habitat that may support the species. Effects would be reduced or avoided with the implementation of conservation measures including protecting habitat during construction activities (CM-2), preventing invasive species entry into native habitat (CM-4), controlling dust emissions (CM-6), managing water quality and runoff from construction operations, and construction BMPs (CM-7) and species-specific conservation measures (CM-25 and CM-26) as described in Section 1.6 and Section 4.7.2, respectively.

4.7.2 Species-Specific Conservation Measures

In addition to general conservation measures discussed in Section 1.6, the following species-specific conservation measures will be implemented. Compensatory mitigation for palmate-bracted bird's-beak is not proposed.

CM-24 – Palmate-Bracted Bird's Beak Surveys: During final design of the Proposed Project, a biologist will conduct surveys for palmate-bracted bird's-beak in suitable habitat (all areas of wetland and grassland habitat with alkaline soils) within the Proposed Project Footprint and a 250-foot buffer (where access is granted) of the Proposed Project Footprint. Suitable habitat will be delineated based on desktop review, although general field surveys will be conducted in advance of focused plant surveys to refine the area of potential habitat for palmate-bracted bird's beak. The focused surveys will be conducted during an appropriate identification period in accordance with CDFW protocols (CDFW

2018). The identification period of palmate-bracted bird's beak is anticipated to be during its reported flowering period between June and August (Jepson Flora Project 2024); a known reference population within 5 miles of the Action Area or less will be checked within 1 week of conducting surveys to confirm that it is identifiable at the time of survey. The results of the surveys will be documented in brief reports or technical memoranda. If feasible, the project design would be modified to avoid impacts to palmate-bracted bird's beak, if they are discovered in the Proposed Project Footprint.

CM-25 – Protect Palmate-Bracted Bird's Beak During Construction:

Avoidance of Palmate-bracted Bird's Beak: If palmate-bracted bird's-beak plants are found in the Proposed Project Footprint and can be avoided, a 50-foot no-disturbance buffer will be installed around the plants. If palmate-bracted bird's-beak plants are present in the area and cannot be avoided (work within 50 feet), then a propagation and monitoring plan will be prepared by the Authority and reviewed by FTA prior to coordination with USFWS, as described below. Directly affected areas containing palmate-bracted bird's-beak will be documented by a biologist. Documentation will include density and percent cover; abundance; key habitat characteristics, including soil type, associated species, hydrology, and topography; and photographs of pre-construction conditions.

CM-26 – Develop Palmate-Bracted Bird's Beak Propagation and Monitoring Plan:

Propagation and Monitoring Plan: If palmate-bracted bird's-beak plants are present in the Proposed Project Footprint and cannot be avoided (work within 50 feet), then a salvage, propagation and monitoring plan will be prepared by the Authority and submitted to FTA and USFWS for review and approval before construction occurs in those areas. The plan may include provisions for performance that address survivability requirements, maintenance, monitoring, implementation, and annual reporting requirements.

Monitoring and success criteria applicable to federally listed plant salvage, relocation, or propagation will require the following:

- At least two surveys must be conducted per monitoring year.
- At least 80 percent of the planted area must support vegetation composition and density consistent with reference population conditions.
- At least 80 percent of the planted area must support target species amounts similar to reference feature conditions.
- A minimum of five consecutive years of monitoring must be conducted to ensure that success criteria are met.
- Remedial actions must be performed to restore the intended ecological function of planted areas that fail to meet the success criteria for three consecutive years.
- A worker environmental training program (CM-1) will be conducted to brief construction personnel on the need to avoid effects on sensitive biological resources.

4.7.3 Determination

While implementation of measures outlined in Section 1.6 and Section 4.7.2 would reduce the impacts to the palmate-bracted bird's-beak, focused rare plant surveys have not been performed due to inaccessibility as stated in Section 1.8; therefore, there is potential for this species to occur within the areas identified as potentially suitable habitat. As a result, the Proposed Project was determined to **May Affect, and is Likely to Adversely Affect**, the palmate-bracted bird's-beak.

5 Cumulative Effects

No specific non-federal projects are planned in the Action Area, but various development and road improvement projects may take place over the same timeframe as the Proposed Project. The City of Dublin established the Fallon Road Economic Development Zone on approximately 285 acres east of Fallon Road adjacent to I-580. This zone would accommodate development of industrial parks, general commercial/campus offices, and medium density residential (City of Dublin 2024), although specific projects have not been identified at this time. The City of Livermore established the Local Roadway Safety Plan & Vision Zero to reduce fatalities and severe injuries on city roadways and intersections. Individual projects have not currently been identified as part of this plan (City of Livermore 2024), but some may overlap with the Action Area. Private housing and businesses and infrastructure projects may also take place over the next several years.

These other projects may result in the incremental degradation of habitat for ESA-listed species, including both upland and wetland areas. Such degradation may include habitat fragmentation, increases in impervious surfaces resulting in altered hydrology of areas with wetlands, or the conversion of natural habitats to urban or developed areas. For projects that are entirely lacking a federal nexus, it is likely that compliance with the California Environmental Quality Act would require other projects in the Action Area to implement measures to reduce potentially significant impacts to ESA-listed species or offset habitat loss.

In the portions of the Action Area where future development is expected (the Dublin-Pleasanton, Livermore, and Mountain House areas), the Proposed Project Footprint is parallel to and in close proximity to I-580 and other developed areas, which would help reduce any cumulative contribution to habitat fragmentation and degradation in the region.

6 Determination and Conclusions

The total potential habitat within the Action Area, effects of the Proposed Project for each habitat type, and effects determination are summarized in Table 5. Additionally, total acres of critical habitat and effects of the Proposed Project on critical habitat areas are summarized in Table 6. Impacts on candidate and proposed threatened species are presented in Table 7.

With regards to the ESA-proposed threatened western spadefoot and northwestern pond turtle, FTA finds that the Proposed Project is not expected to meaningfully contribute to the specific threats contributing to the species decline as described in the December 5, 2023, and October 3, 2023, Proposed Rules, respectively (USFWS 2023c, 2023d). However, FTA anticipates that the Proposed Project may affect the species through the permanent alteration of habitats discussed in Chapter 4. Through implementing the conservation measures described in Section 4.3.2, any adverse effects are expected to be reduced but not entirely avoided. As such, FTA has determined that the ESA-proposed threatened western spadefoot and northwestern pond turtle are likely to be adversely affected by the Proposed Project, but the Proposed Project is not likely to jeopardize these species.

Monarch butterfly is a candidate species, and is not expected to be proposed in the near future. As a result, no conference for this species is requested.

Table 5: Federally Listed Species Effects Determinations

Species	Habitat Use	Habitat Type	Total Potential Habitat in the Action Area (acres)	Permanent Effects (acres)	Temporary Effects (acres)	Total Effects (acres)	Effects Determination
Vernal pool fairy shrimp (Threatened)	Breeding	Vernal Pools	16	2	1	3	May Affect, Likely to Adversely Affect
California tiger salamander (Threatened)	Breeding	Waters/Wetlands	56	5	4	9	May Affect, Likely to Adversely Affect
	Dispersal/Burrows	Grassland/Upland	1,500	108	71	180	
California red-legged frog (Threatened)	Breeding	Waters/Wetlands	45	5	4	9	May Affect, Likely to Adversely Affect
	Dispersal/Estivation	Riparian/Upland	1,351	96	64	160	
San Joaquin kit fox (Threatened) ^a	Foraging/Denning	Grassland Agricultural/Ruderal	487	353	175	528	May Affect, Likely to Adversely Affect
Palmate-bracted bird's-beak ^a	NA	NA	NA	NA	NA	NA	May Affect, Likely to Adversely Affect

^a Habitat-based modeling was not performed for these species.

Table 6: Federally Listed Species Critical Habitat

Species	Total Critical Habitat in the Action Area (acres)	Permanent Effects (acres)	Temporary Effects (acres)	Total Effects (acres)	Determination
California red-legged frog (Threatened)	837	58	31	89	No Adverse Modification

Table 7: Proposed and Candidate Species Conclusions

Species	Habitat Use	Habitat Type	Total Potential Habitat in the Action Area (acres)	Permanent Impacts (acres)	Temporary Impacts (acres)	Total Impacts (acres)	Effects Conclusion
Monarch butterfly ^a (Candidate)	Breeding	Wherever host plant occurs, non-native grassland and scrub	1,572	103	48	151	May Affect, Not Likely to Jeopardize Species
Western spadefoot (Proposed Threatened)	Breeding	Waters/Wetlands	56	5	4	9	May Affect, Not Likely to Jeopardize Species
	Dispersal/Burrows	Riparian/Upland	1,492	189	67	255	
Northwestern pond turtle (Proposed Threatened)	Aquatic	Waters/Wetlands	58	5	5	10	May Affect, Not Likely to Jeopardize Species
	Upland	Riparian/Upland	1,447	99	68	167	

^a Habitat-based modeling was not performed for this species.

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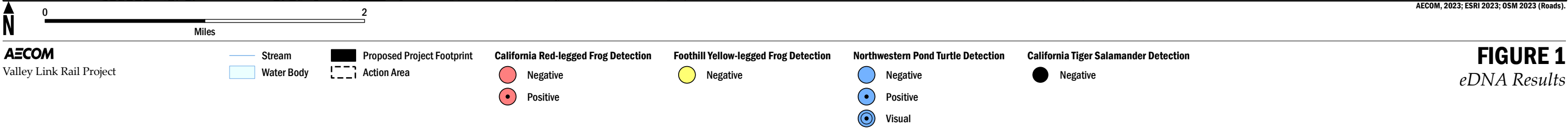
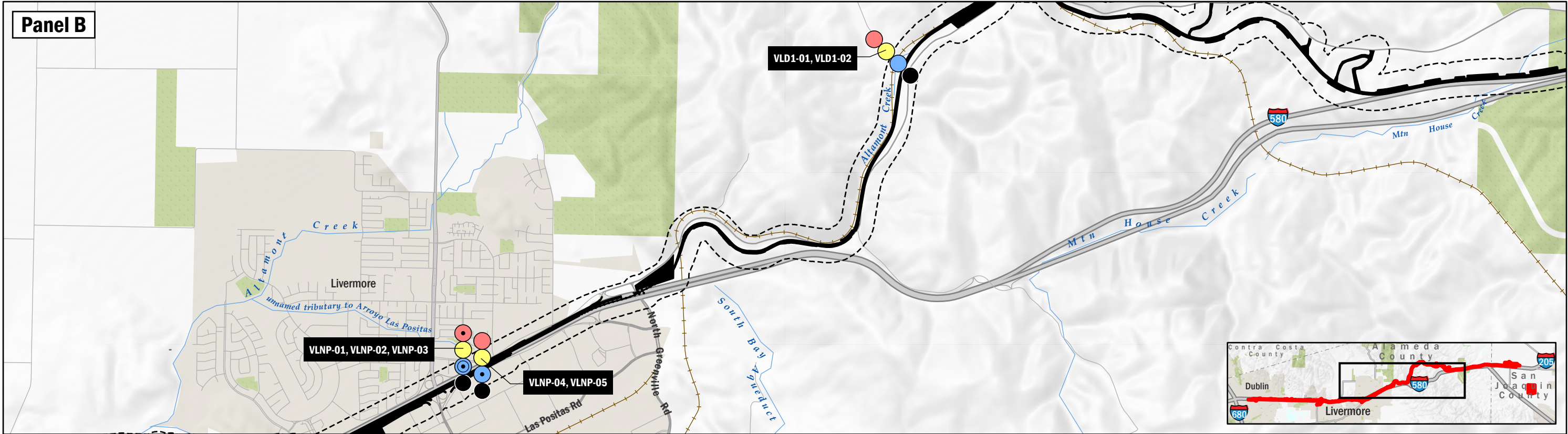
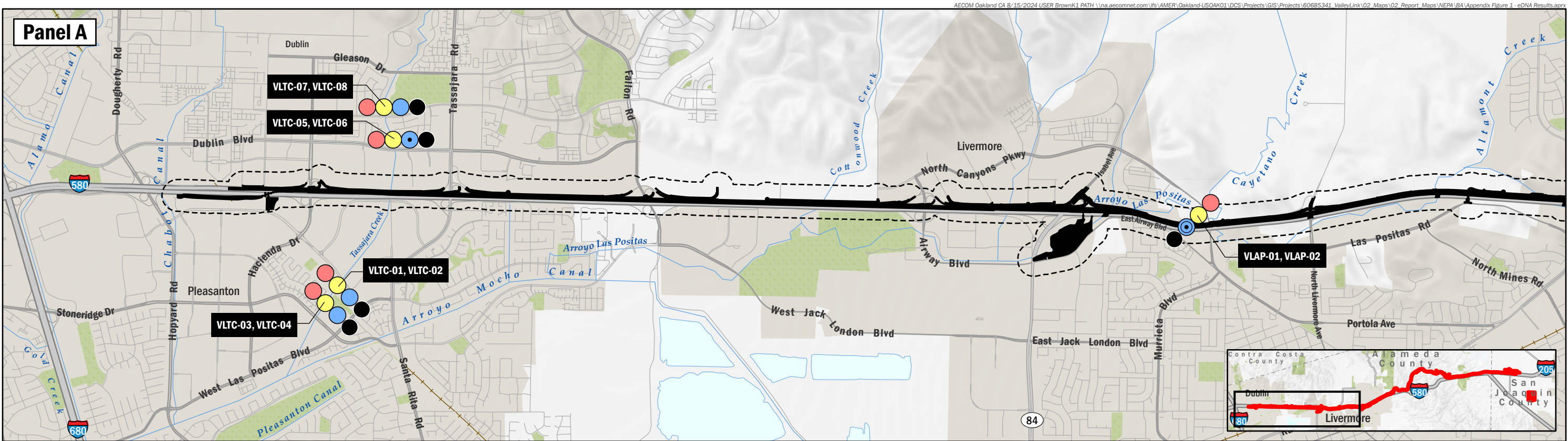
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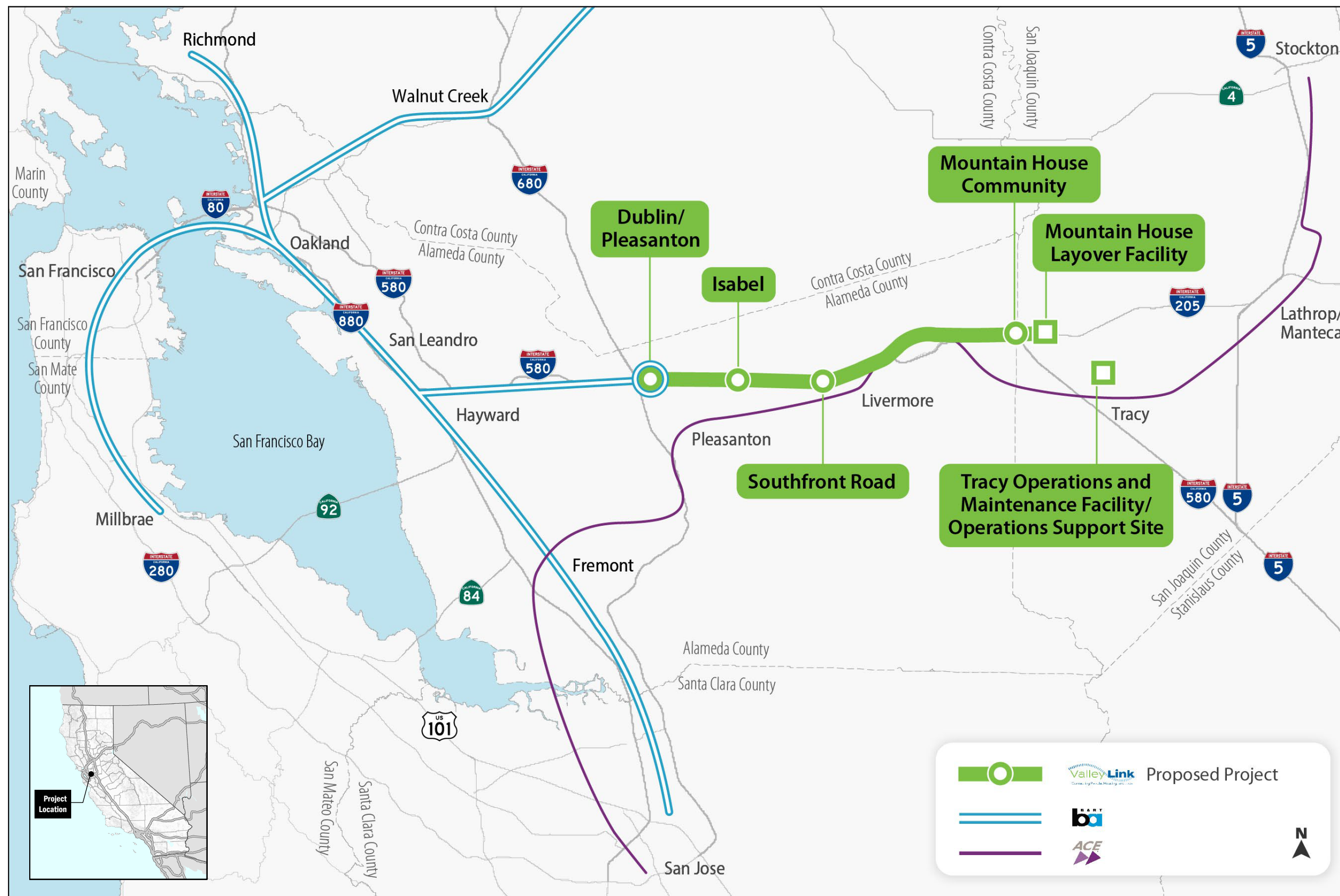
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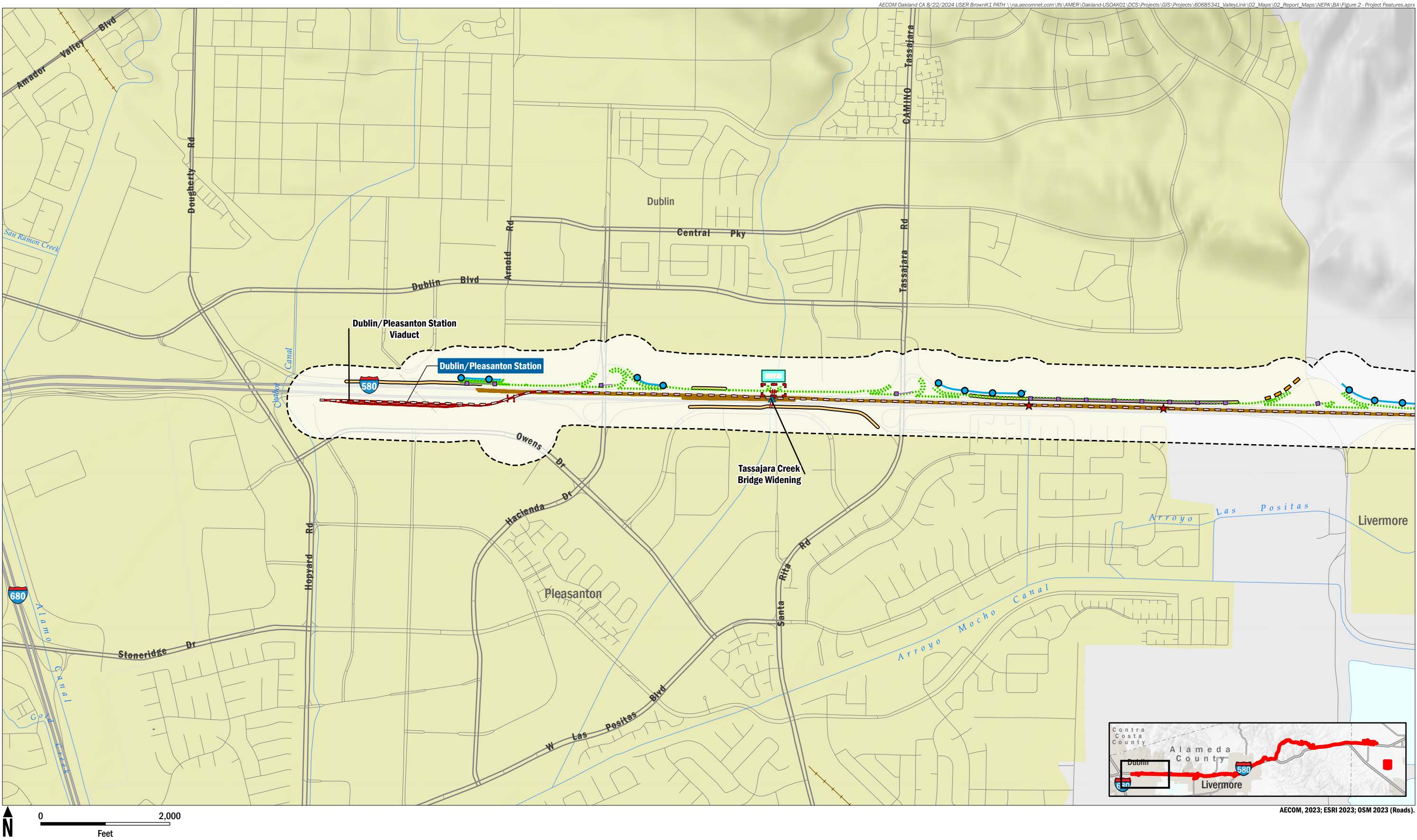
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Appendix A Figures



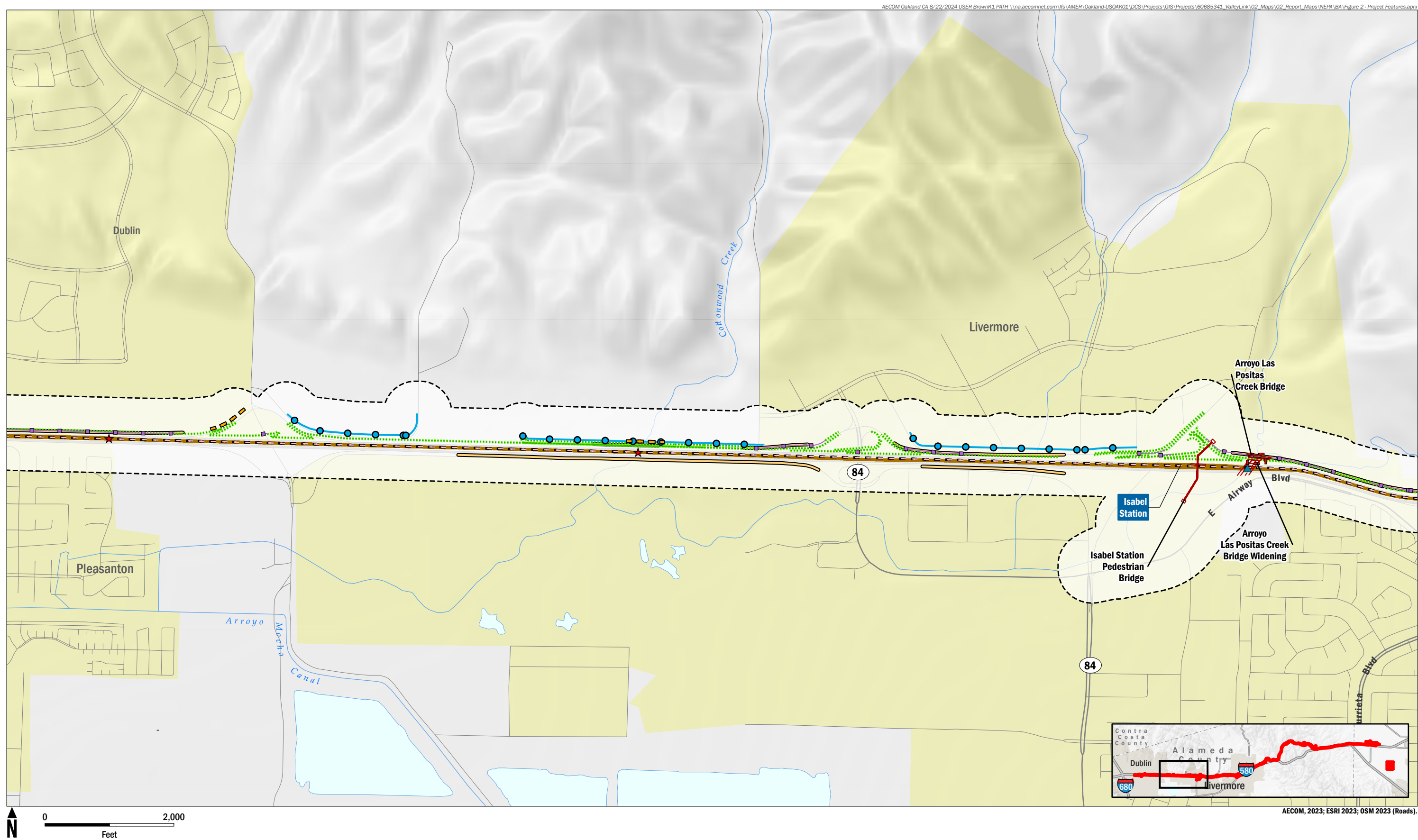


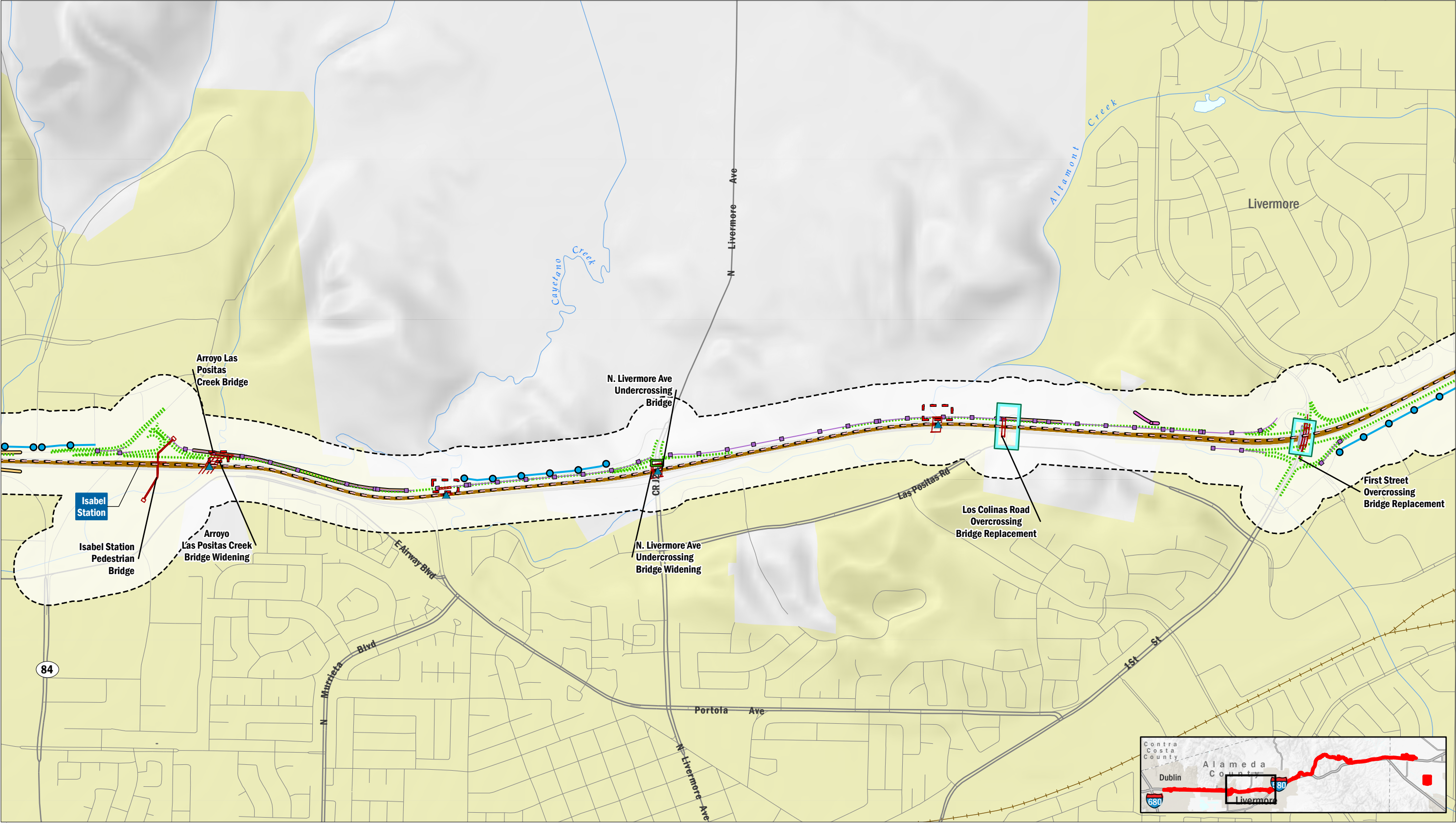


AECOM
Valley Link Rail Project

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|---------------------------------------|----------------------|-------------------------------|----------------------------------|---------------------|
| ▲ Replace Portion of Bridge in Median | — Structure | ▬ Culvert Extension | — Proposed Traffic Noise Barrier | --- Action Area |
| ★ New Median Bridge | — Median Widening | ● Frontage Road Realignment | ▬ Bridge Widening | — Existing Railroad |
| ▬ Proposed Project Rail Line | — New Retaining Wall | ... On / Off Ramp Realignment | ▬ Bridge Replacement | ■ City Limits |

FIGURE 2
Action Area and Project Features
Sheet 1 of 9



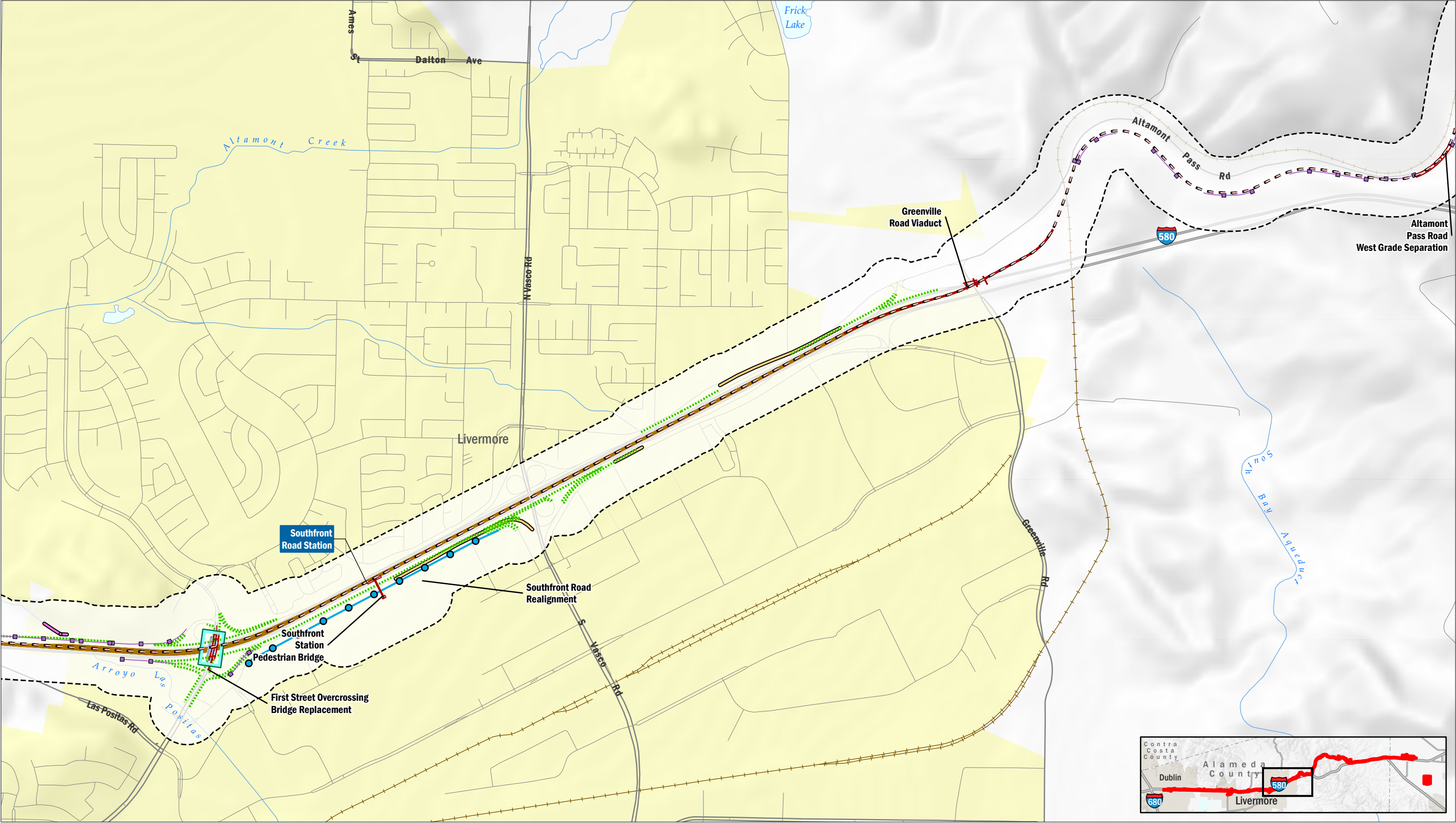


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

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|---------------------------------------|----------------------|-----------------------------------|----------------------------------|----------------------|---------------|
| ▲ Replace Portion of Bridge in Median | — Structure | ● Frontage Road Realignment | — New Bridge | ▭ Bridge Replacement | ■ City Limits |
| — Proposed Project Rail Line | — Median Widening | ... On / Off Ramp Realignment | — Proposed Traffic Noise Barrier | --- Action Area | |
| ▭ Overcrossing Removal | — New Retaining Wall | — Reinforced Concrete Box Culvert | — Bridge Widening | — Existing Railroad | |

FIGURE 2
Action Area and Project Features
Sheet 3 of 9

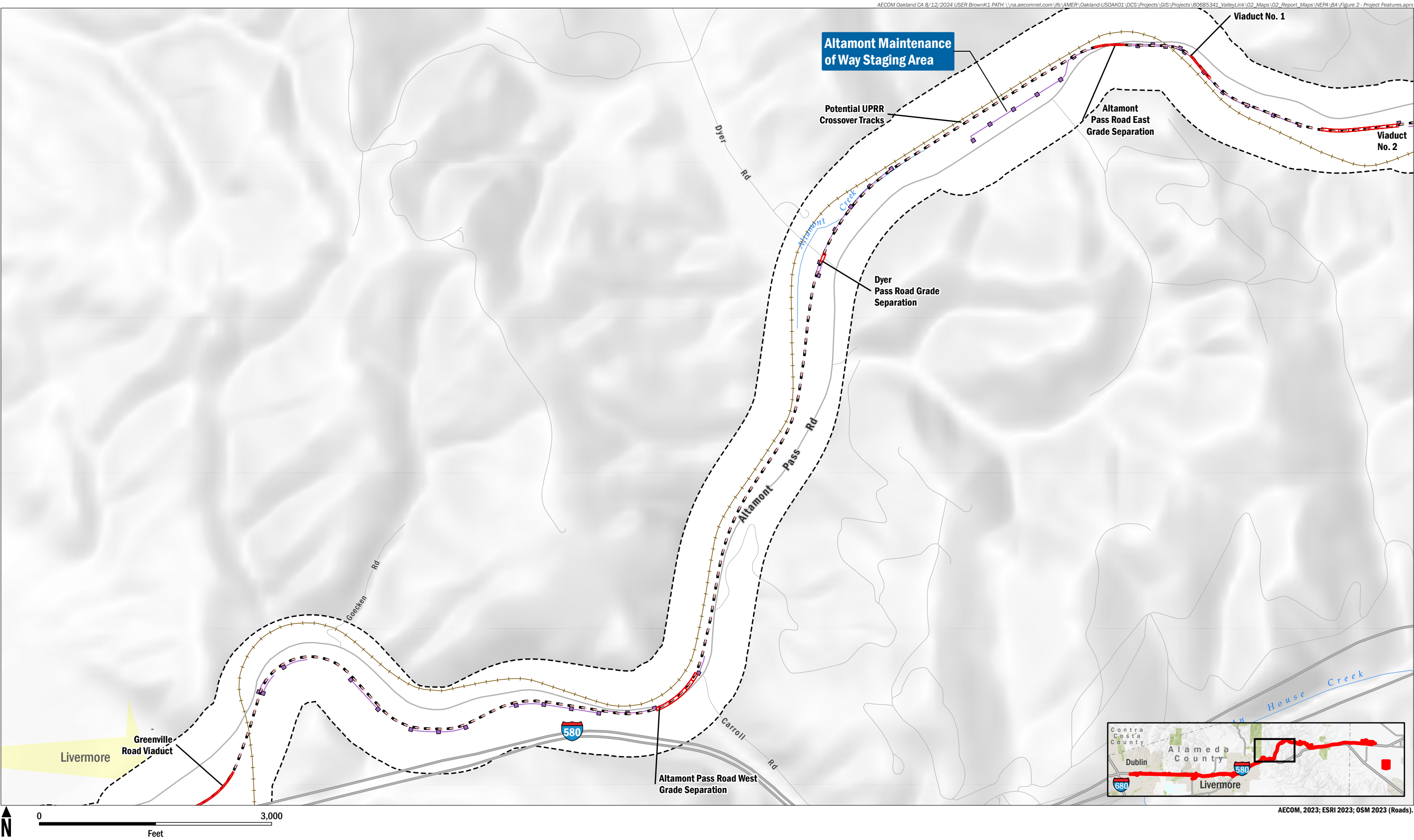


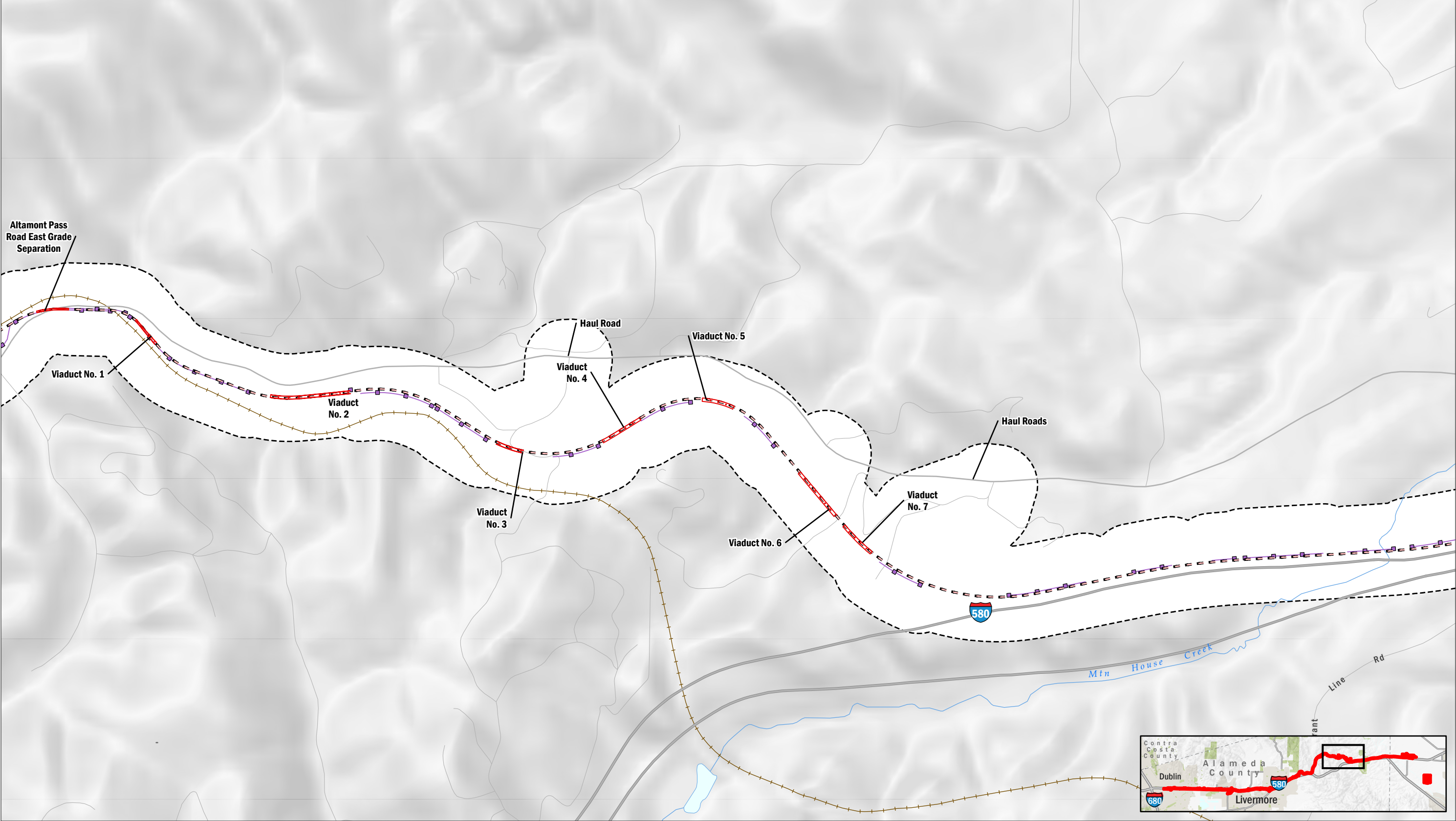
AECOM
Valley Link Rail Project

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|--------------------------------|-------------------------------|-----------------------------------|----------------------|---------------|
| — — Proposed Project Rail Line | — New Retaining Wall | — Reinforced Concrete Box Culvert | — Bridge Replacement | — City Limits |
| — Structure | ● Frontage Road Realignment | — New Bridge | — Action Area | |
| — Median Widening | ... On / Off Ramp Realignment | — Proposed Traffic Noise Barrier | — Existing Railroad | |

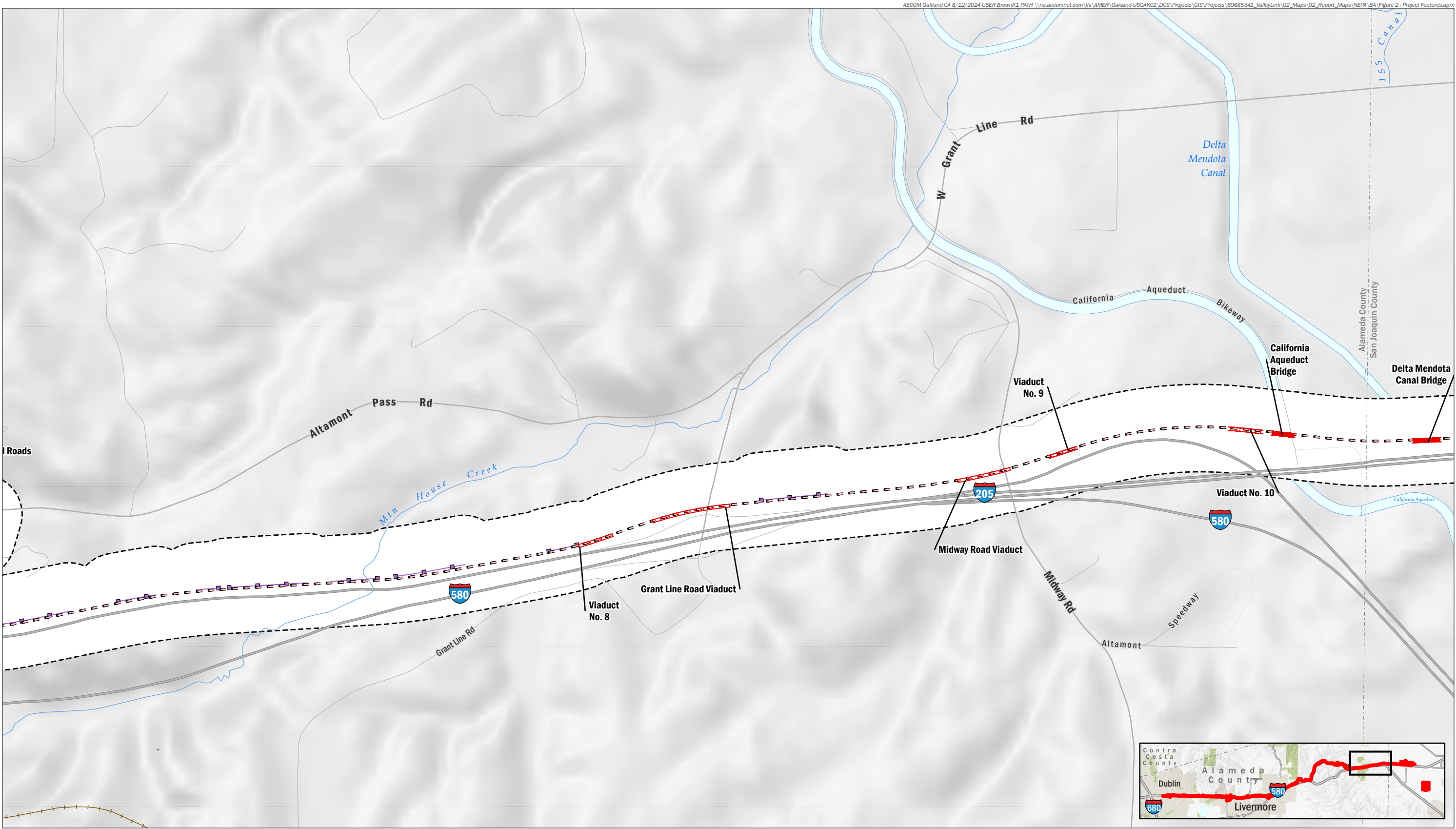
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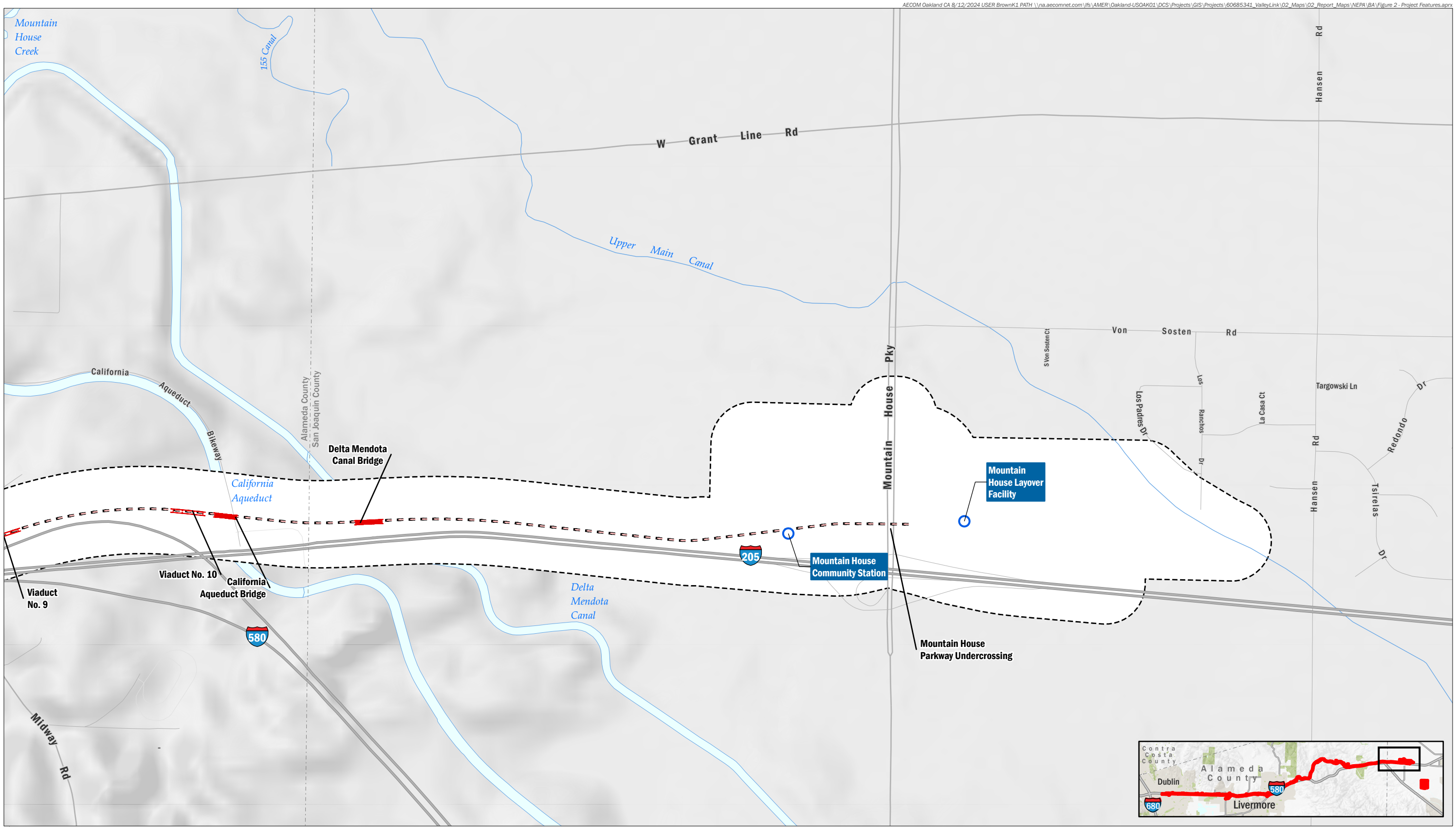
FIGURE 2
Action Area and Project Features
Sheet 4 of 9





AECOM, 2023; ESRI 2023; OSM 2023 (Roads).





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 Valley Link Rail Project

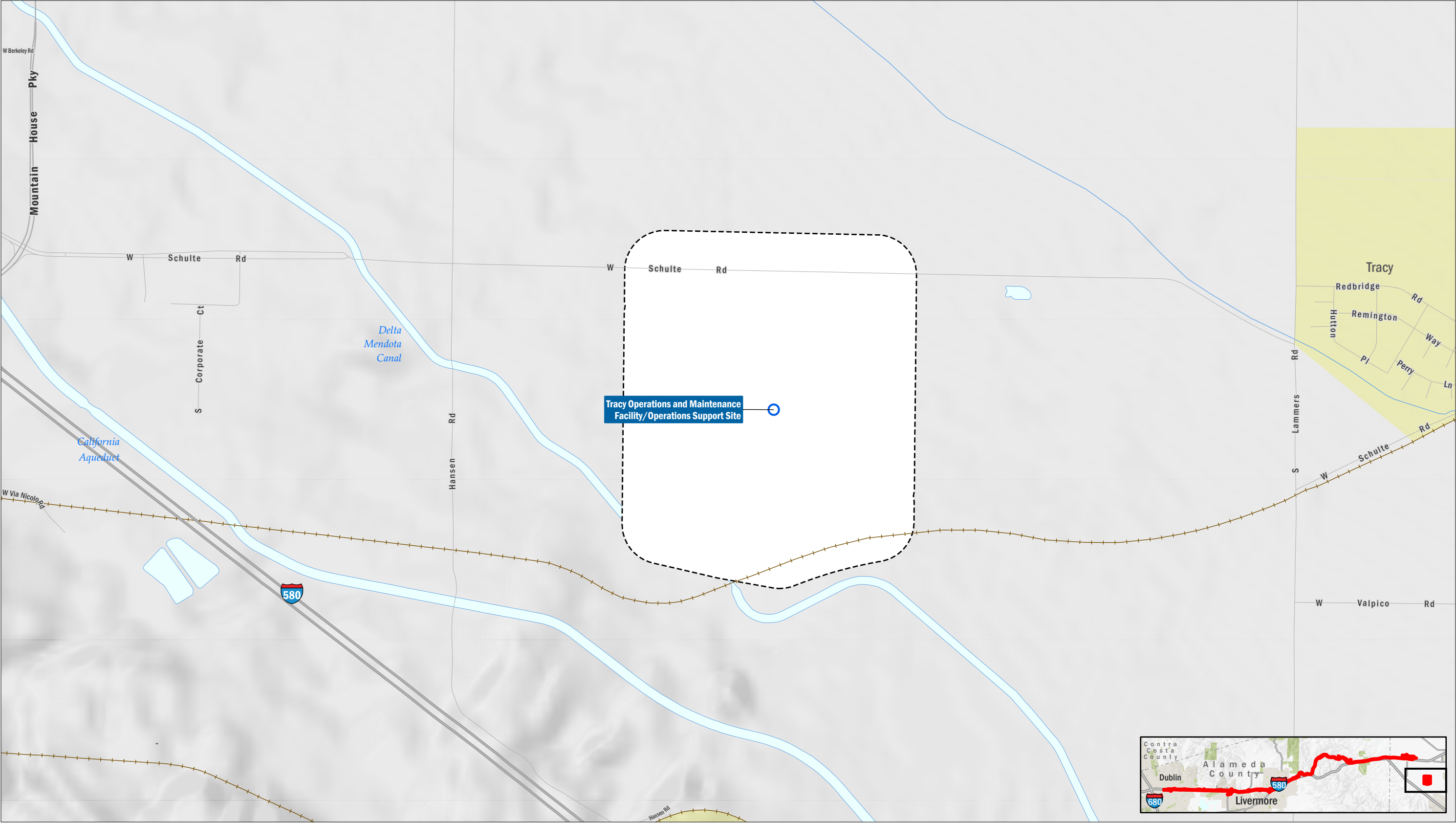
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| Proposed Project Station and Support Facility | Structure |
| Proposed Project Rail Line | Action Area |

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

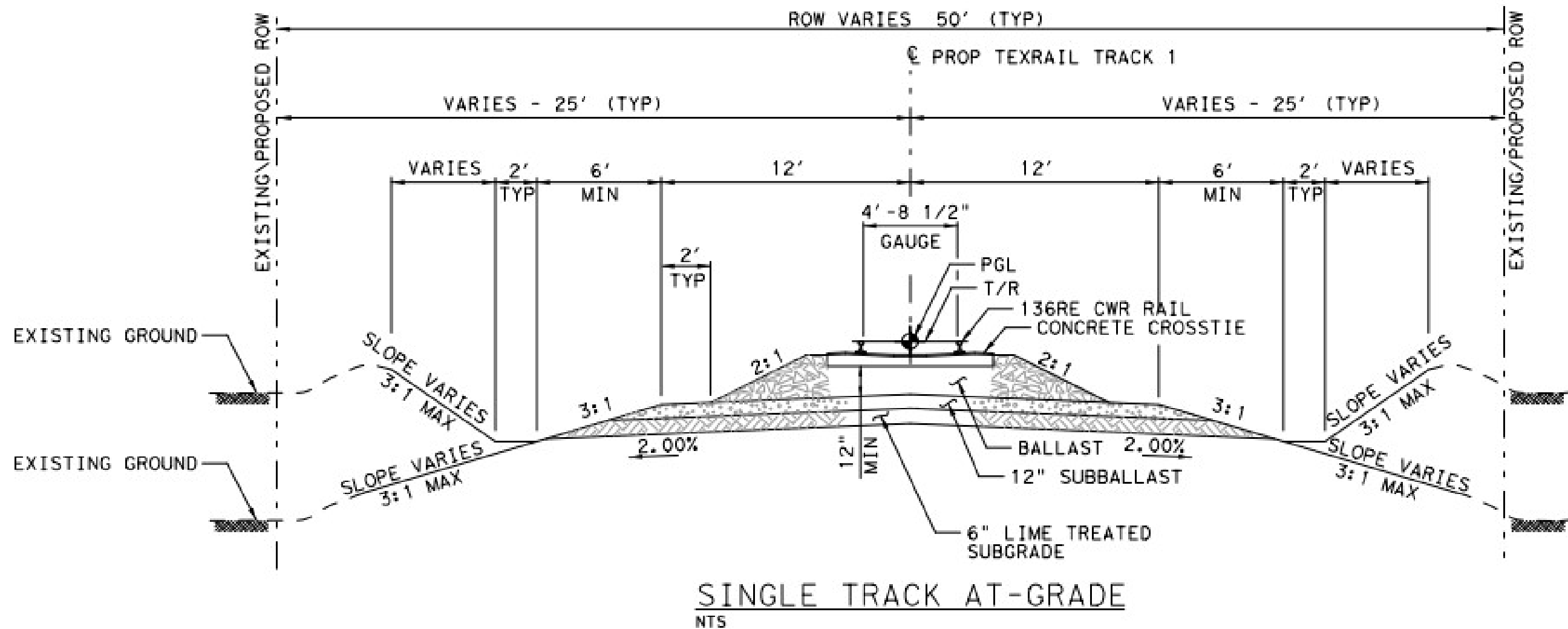
FIGURE 2

Action Area and Project Features

 Sheet 8 of 9



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



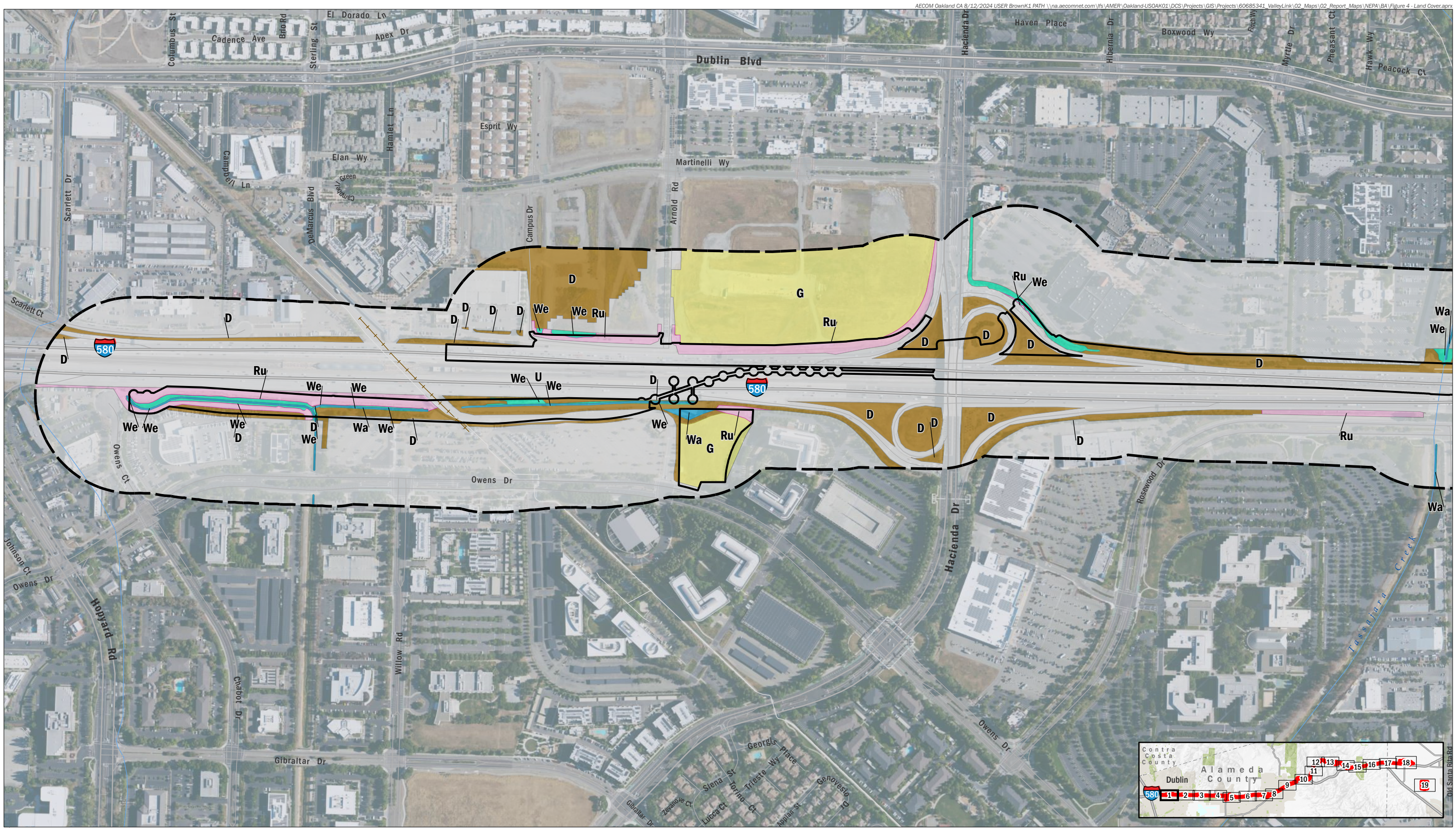


FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 1 of 19

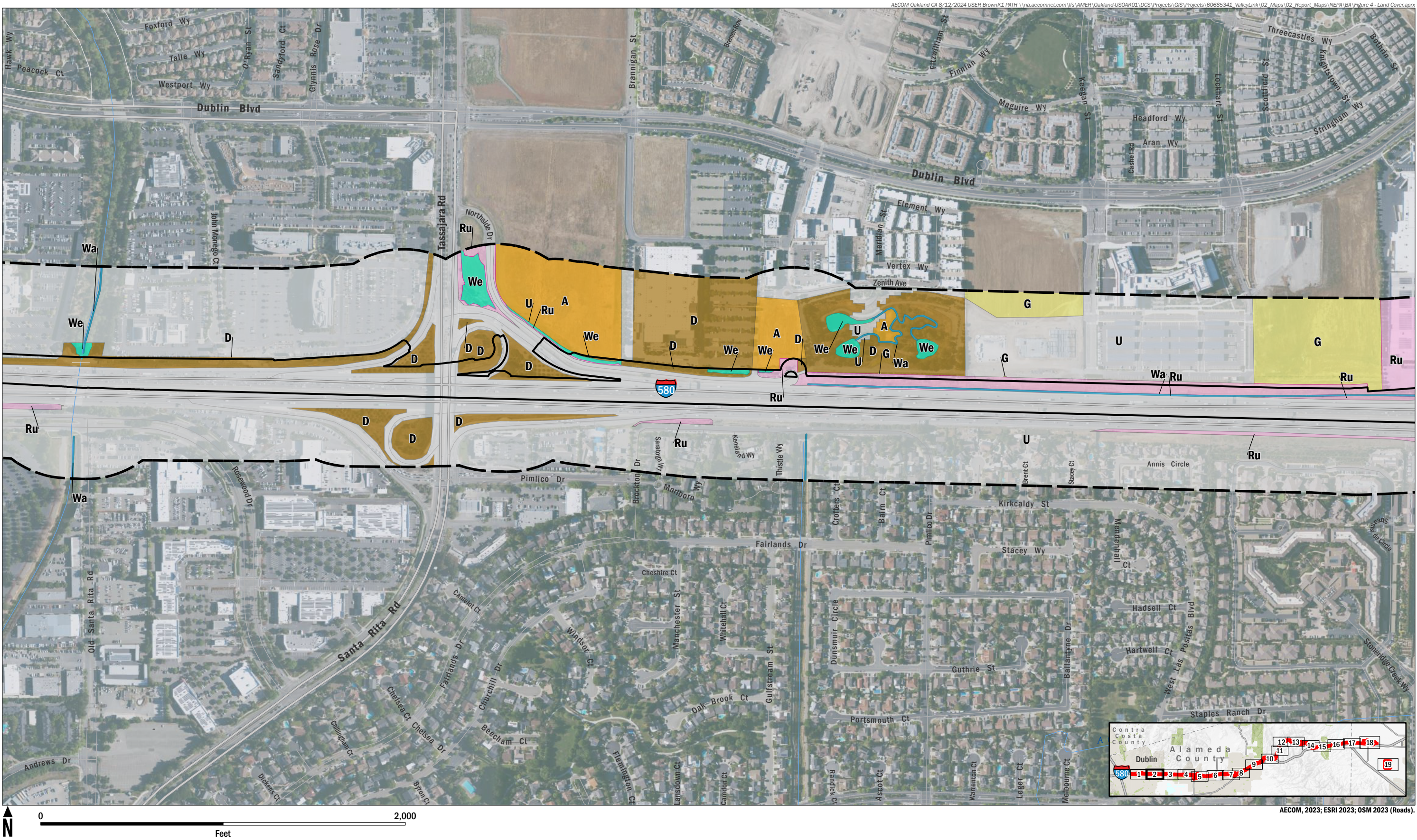
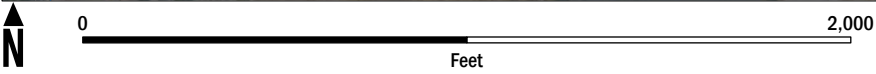
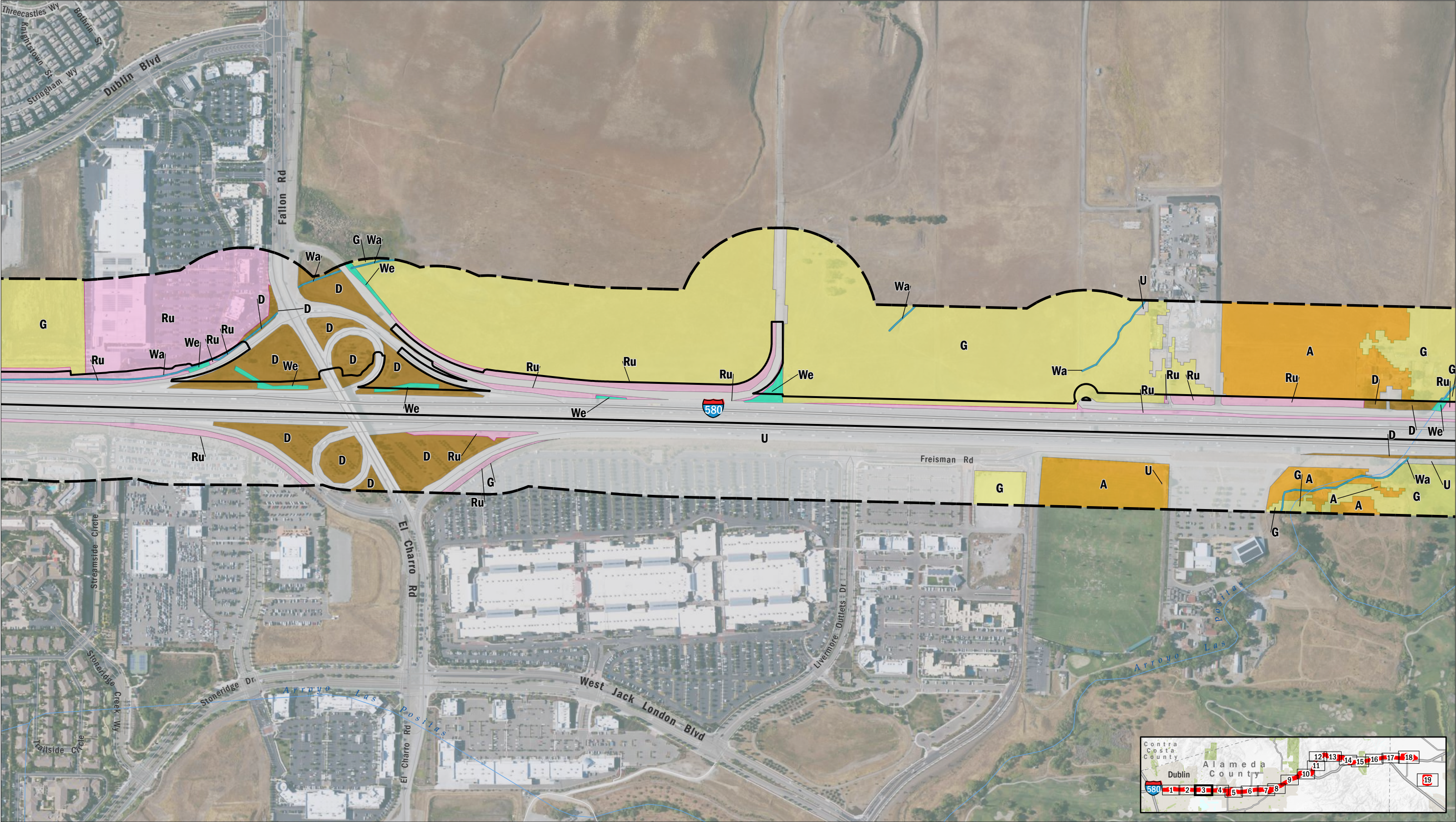


FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 2 of 19



AECOM
Valley Link Rail Project

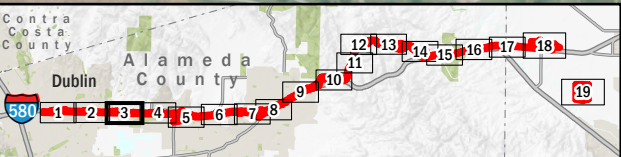
Proposed Project Footprint
 Action Area

Land Cover and Vegetation
 A : Agricultural
 G: Non-native grassland

D : Developed/landscaped
 Ru : Ruderal

We : Potential wetland
 Wa : Water

U : Urban



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 3 of 19







AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

 Proposed Project Footprint
 Action Area

Land Cover and Vegetation
 A : Agricultural
 G: Non-native grassland

 D : Developed/landscaped
 Ru : Ruderal

 We : Potential wetland
 U : Urban


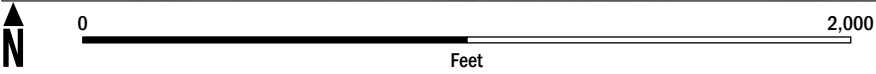
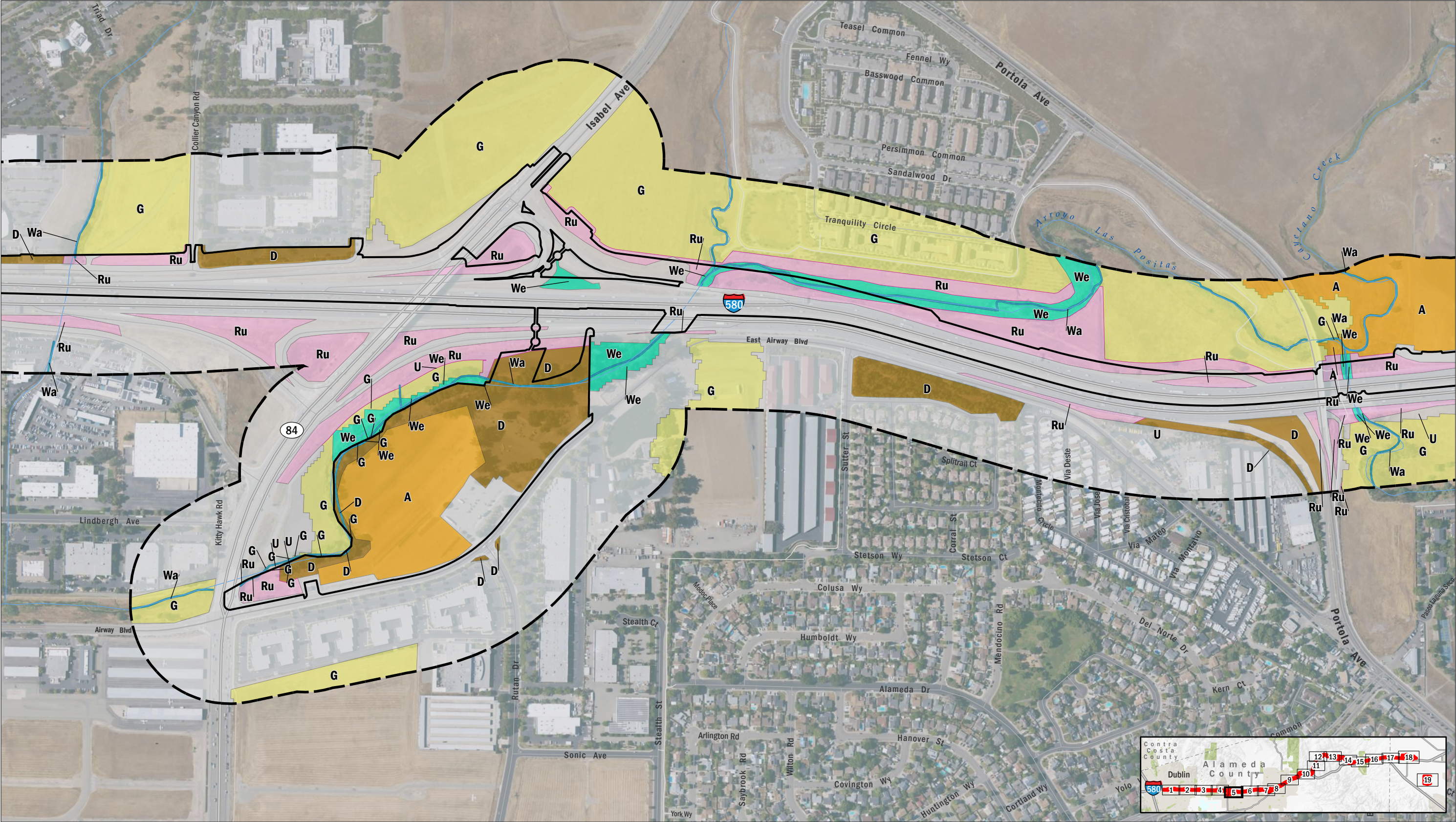
 Wa : Water

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 4 of 19



AECOM
Valley Link Rail Project

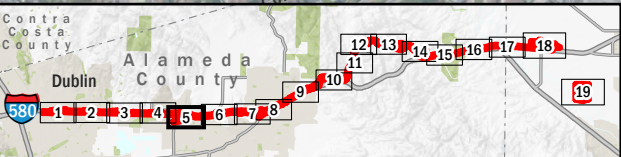
Proposed Project Footprint
 Action Area

Land Cover and Vegetation
 A : Agricultural
 G: Non-native grassland

D : Developed/landscaped
 Ru : Ruderal

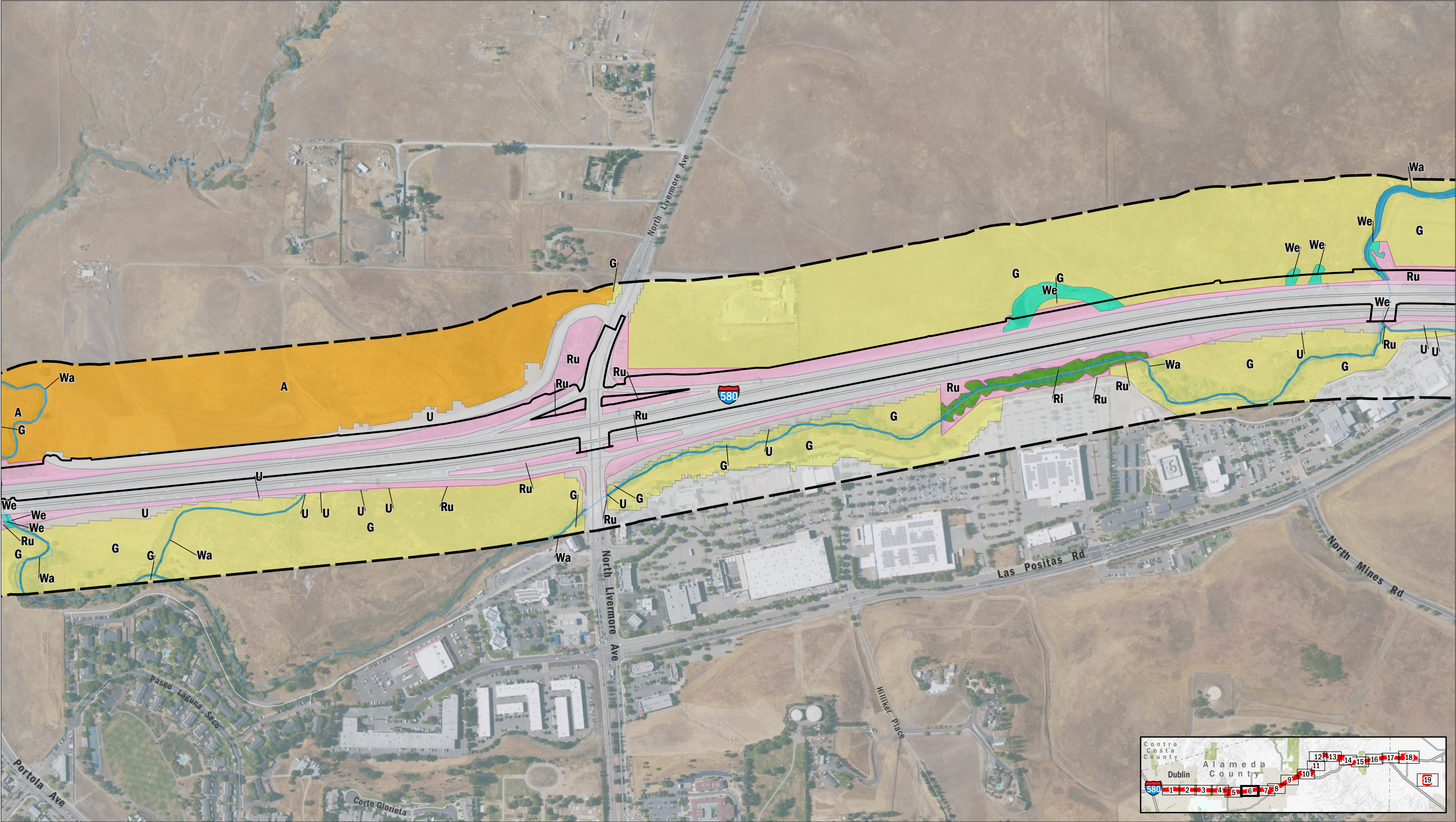
We : Potential wetland
 Wa : Water

U : Urban



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 5 of 19

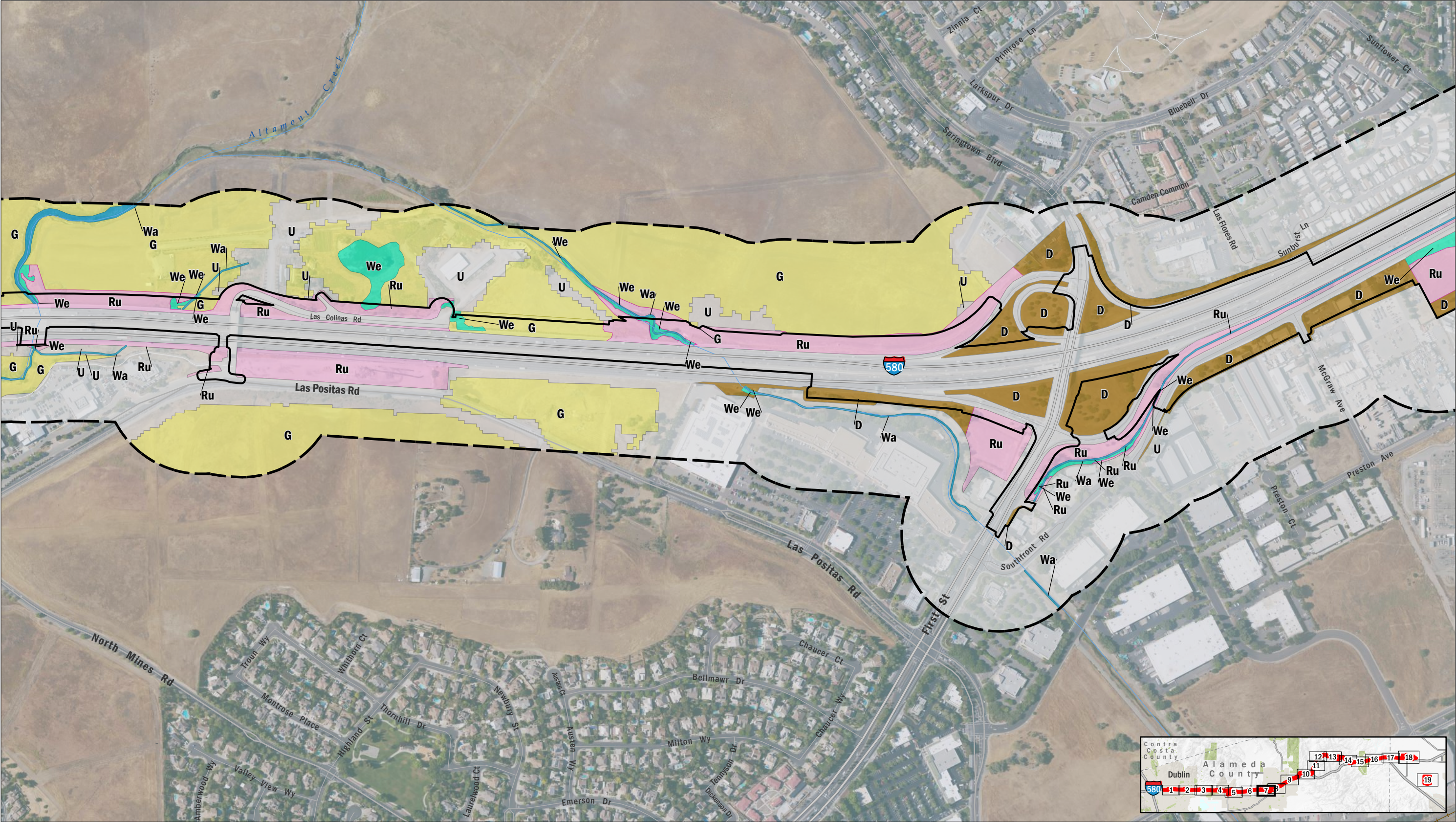


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

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|----------------------------|----------------------------------|-------------------------|--------------|-----------|
| Proposed Project Footprint | Land Cover and Vegetation | G: Non-native grassland | Ri: Riparian | U: Urban |
| Action Area | A: Agricultural | We: Potential wetland | Ru: Ruderal | Wa: Water |

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 6 of 19

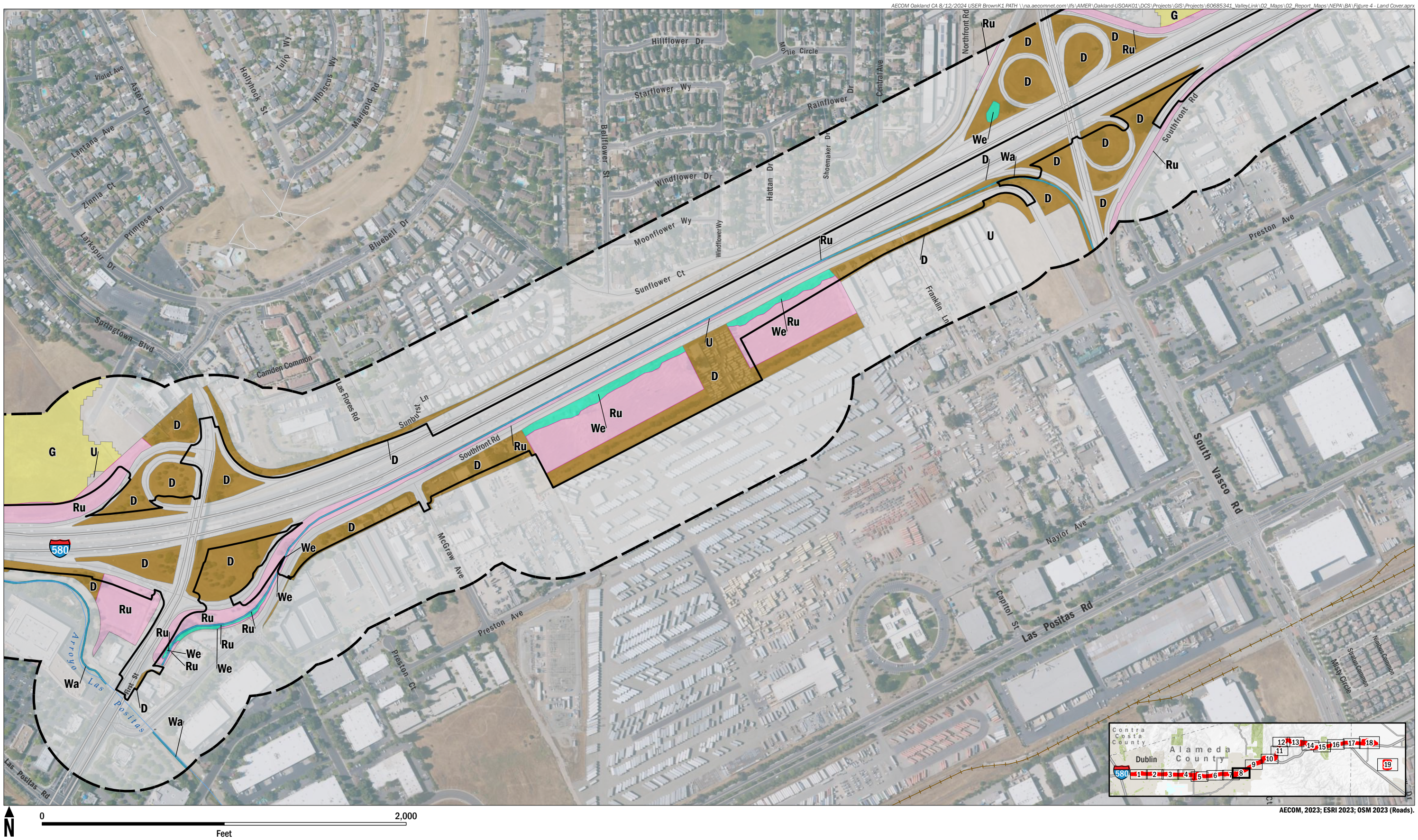


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AECOM
Valley Link Rail Project

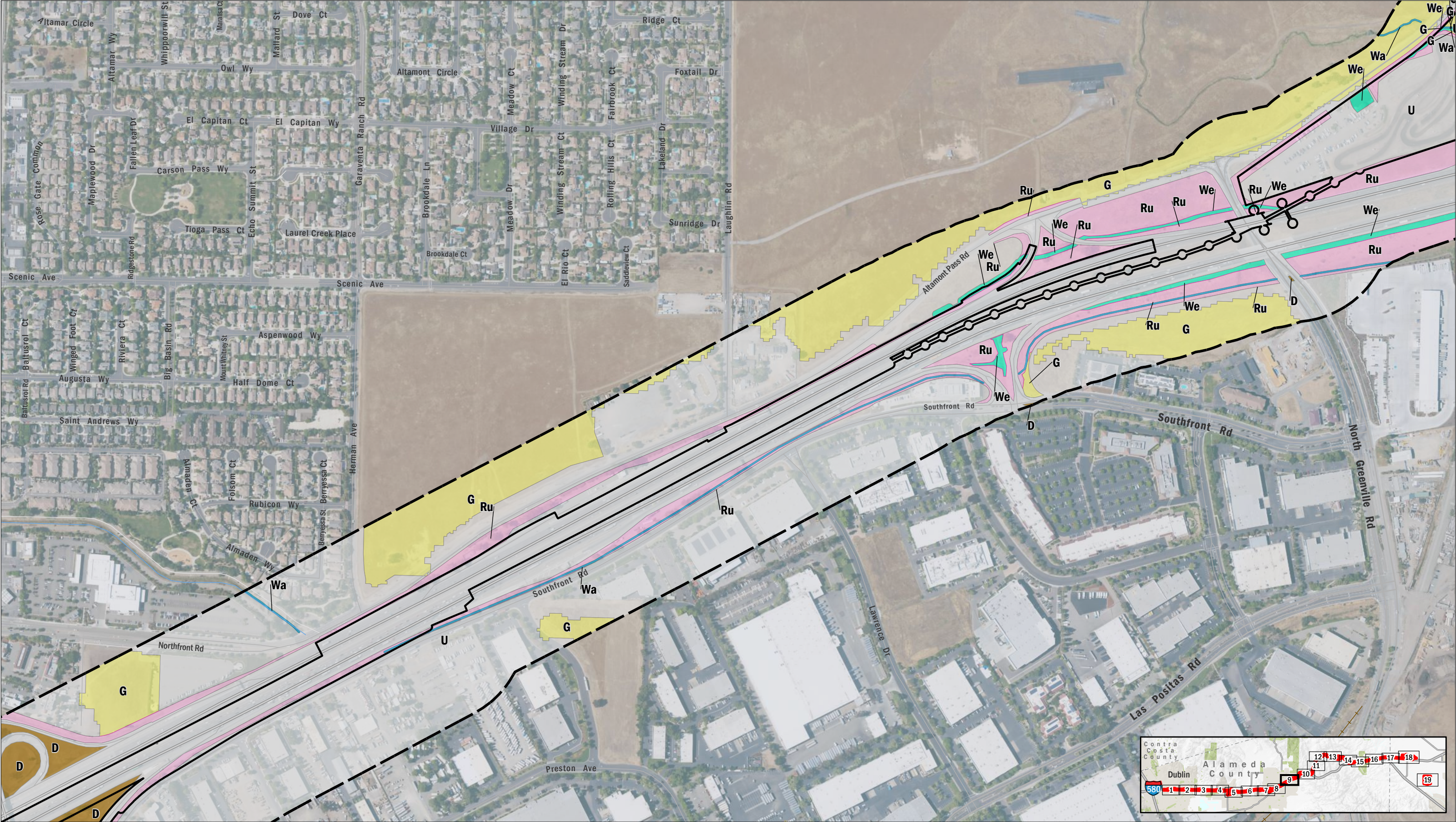
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|----------------------------|----------------------------------|-------------------------|-------------|-----------|
| Proposed Project Footprint | Land Cover and Vegetation | G: Non-native grassland | Ru: Ruderal | Wa: Water |
| Action Area | D: Developed/landscaped | We: Potential wetland | U: Urban | |

FIGURE 4
Land Cover and Vegetation in the Action Area
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AECOM
Valley Link Rail Project

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 8 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

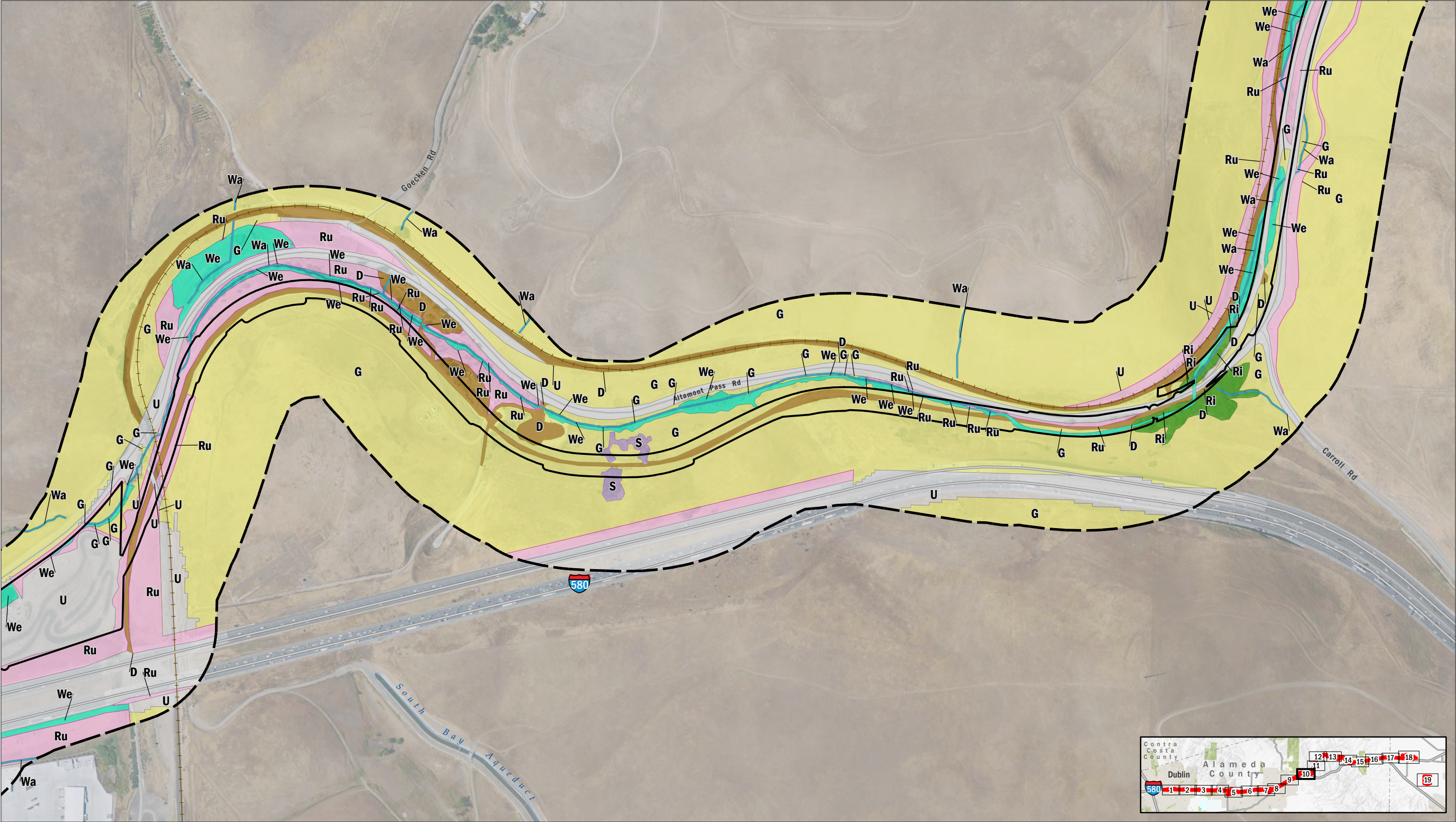
Land Cover and Vegetation
 D : Developed/landscaped

G: Non-native grassland
 We : Potential wetland

Ru : Ruderal
 U : Urban

Wa : Water

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 9 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

Land Cover and Vegetation

D : Developed/landscaped

G: Non-native grassland

We : Potential wetland

Ri : Riparian

Ru : Ruderal

S : Scrub

U : Urban

Wa : Water

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 10 of 19





AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

 Proposed Project Footprint
 Action Area

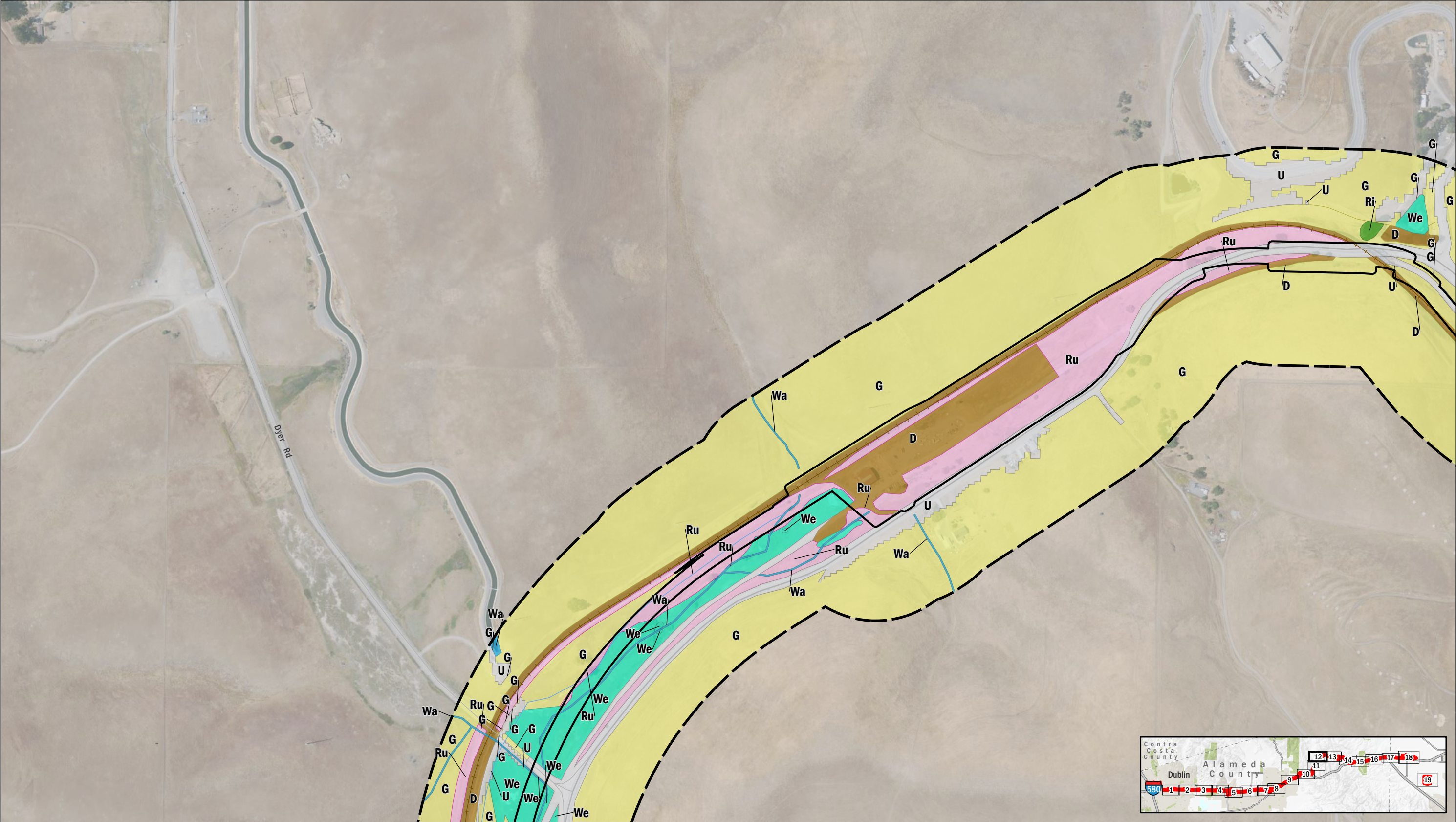
Land Cover and Vegetation
 D : Developed/landscaped

 G: Non-native grassland
 We : Potential wetland

 Ru : Ruderal
 U : Urban

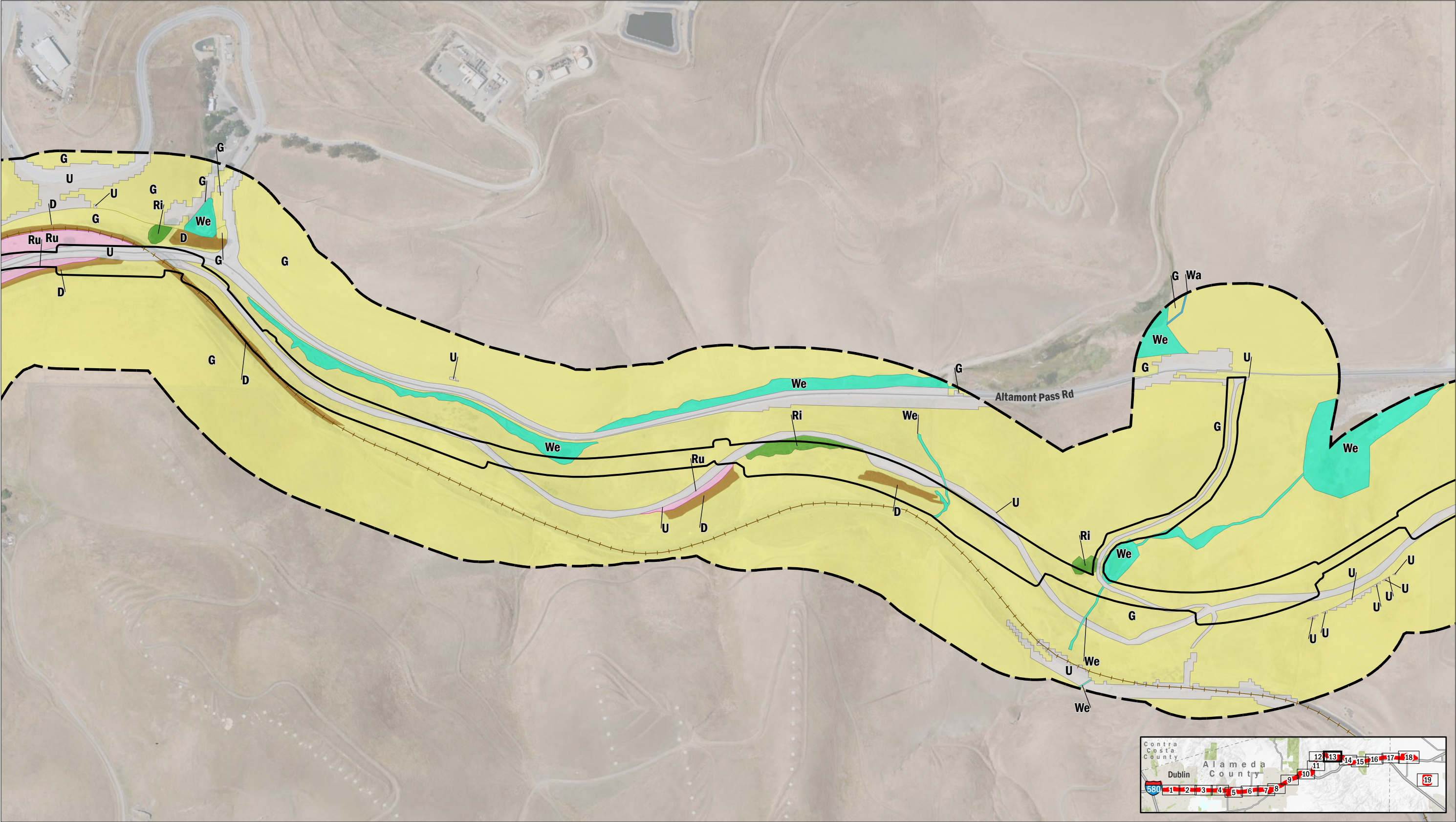
 Wa : Water

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 11 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 12 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

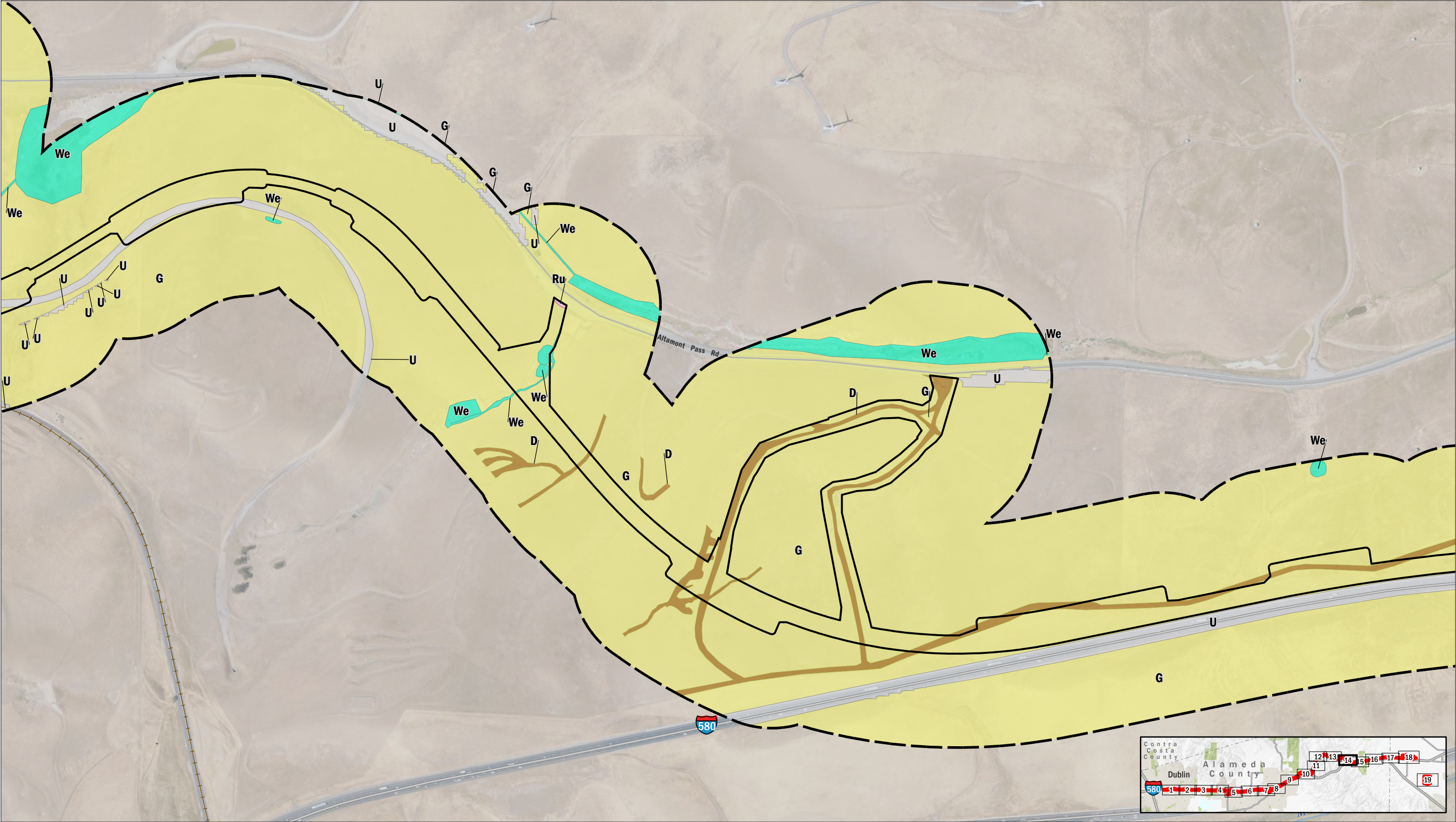
Land Cover and Vegetation
 D : Developed/landscaped

G: Non-native grassland
 We : Potential wetland

Ri : Riparian
 Ru : Ruderal

U : Urban
 Wa : Water

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 13 of 19





AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

 Proposed Project Footprint
 Action Area

Land Cover and Vegetation
 D : Developed/landscaped

 G: Non-native grassland
 We : Potential wetland



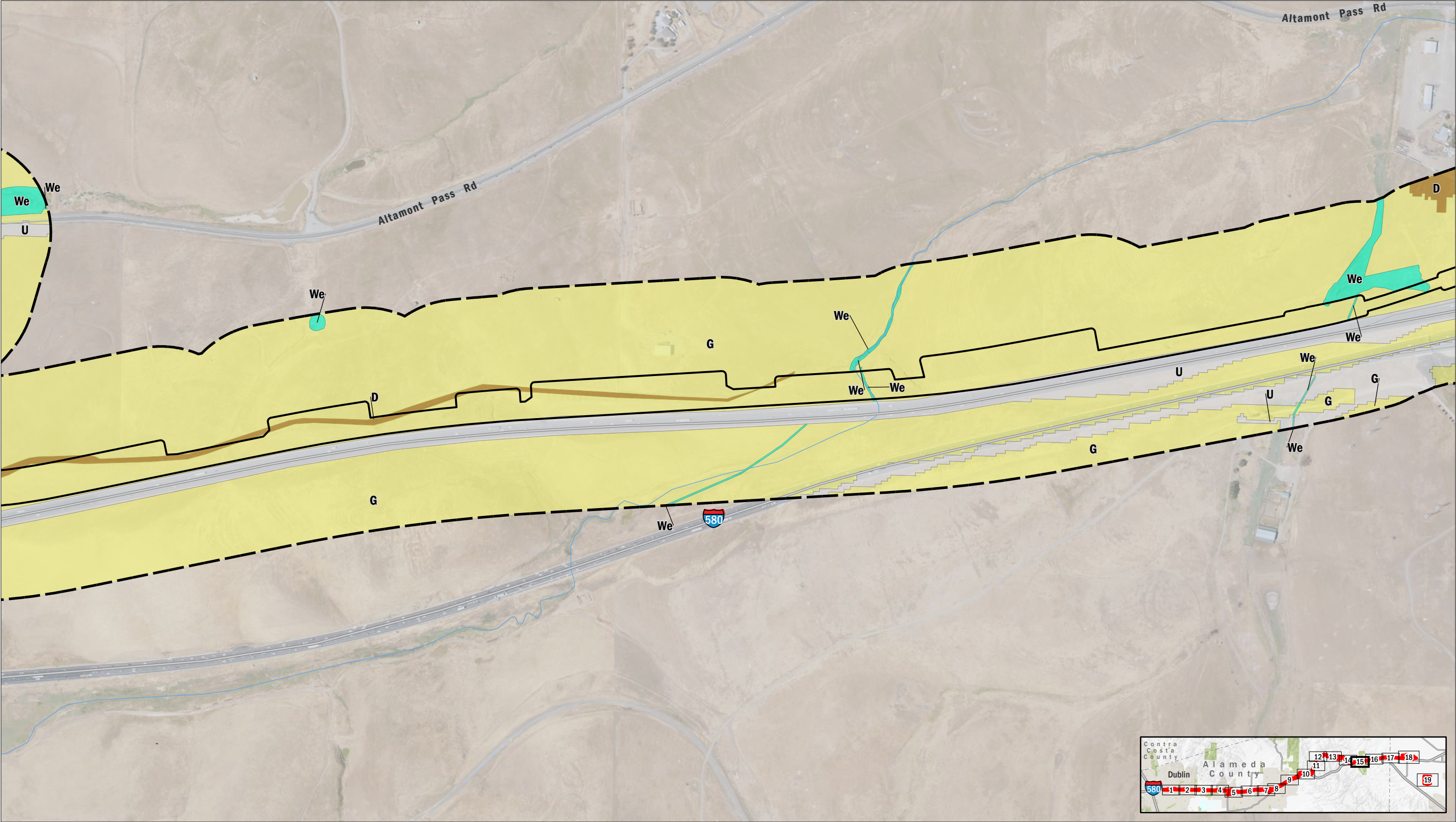
 Ru : Ruderal
 U : Urban

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 14 of 19





AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

 Proposed Project Footprint
 Action Area

Land Cover and Vegetation
 D : Developed/landscaped

 G: Non-native grassland
 We : Potential wetland


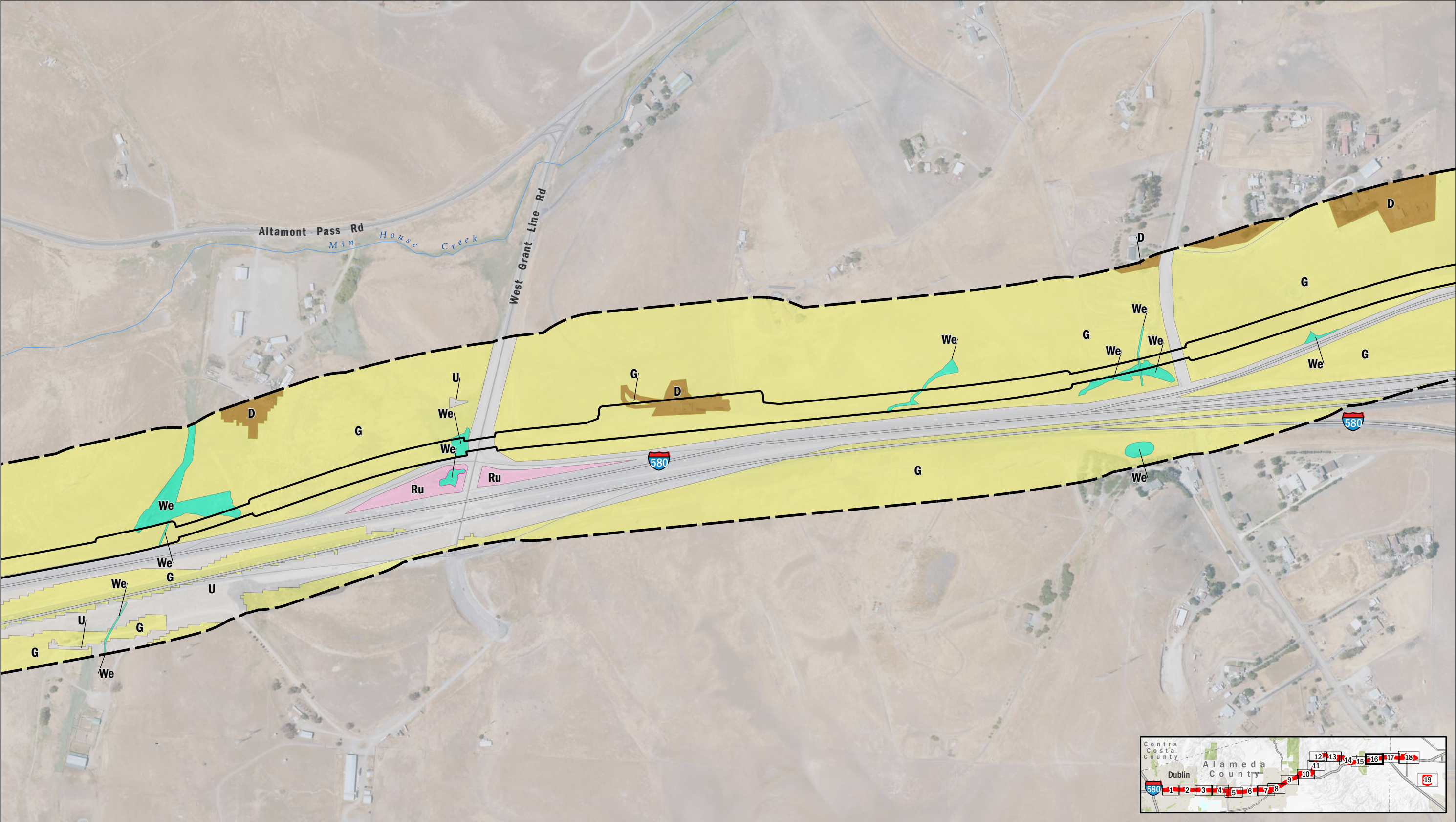
 U : Urban

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 15 of 19



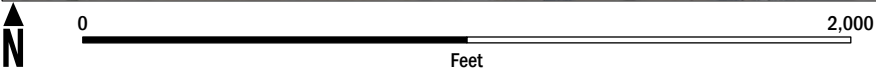
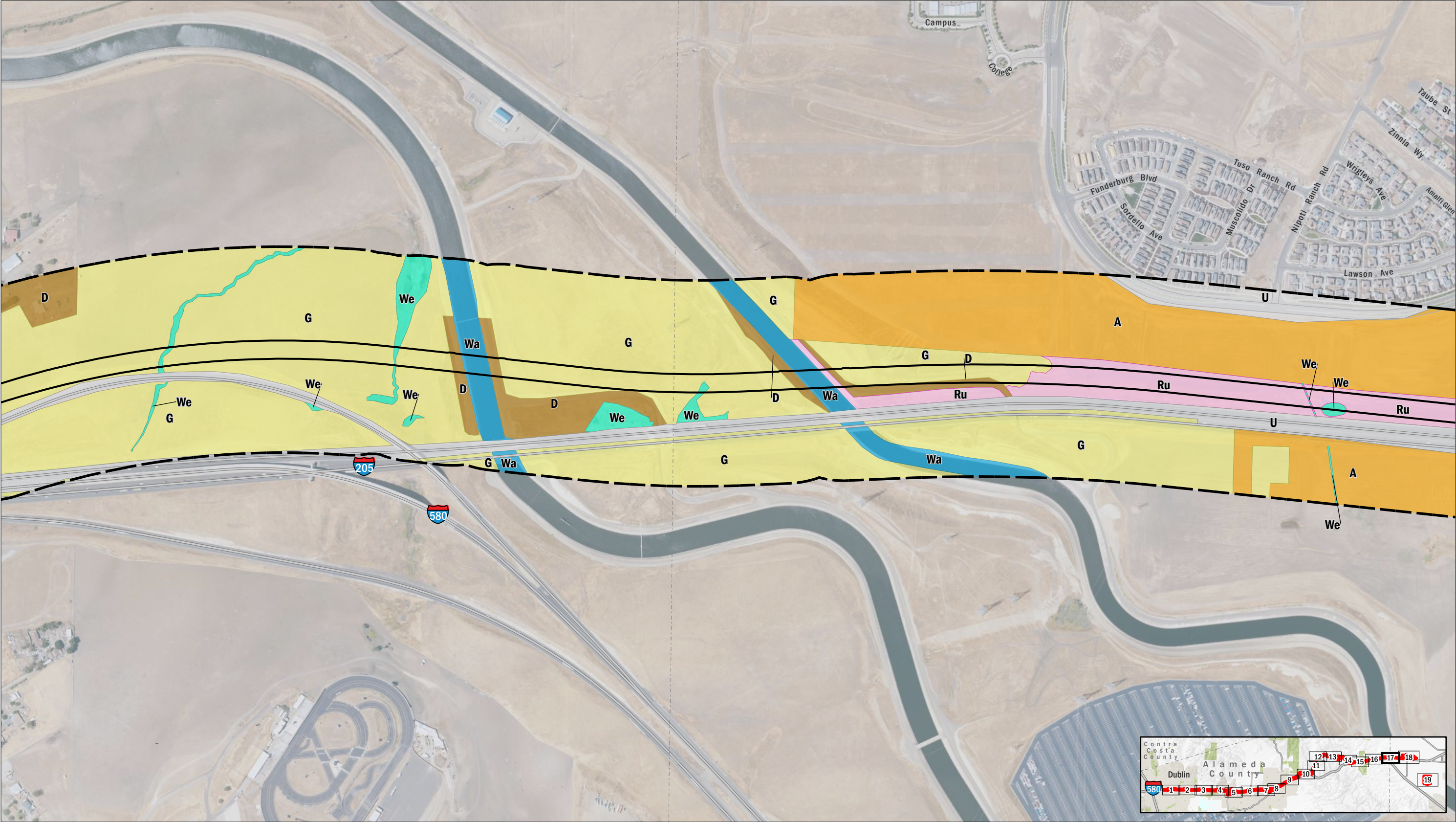
AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

Land Cover and Vegetation
 D : Developed/landscaped
 Ru : Ruderal
 G: Non-native grassland
 We : Potential wetland
 U : Urban

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 16 of 19

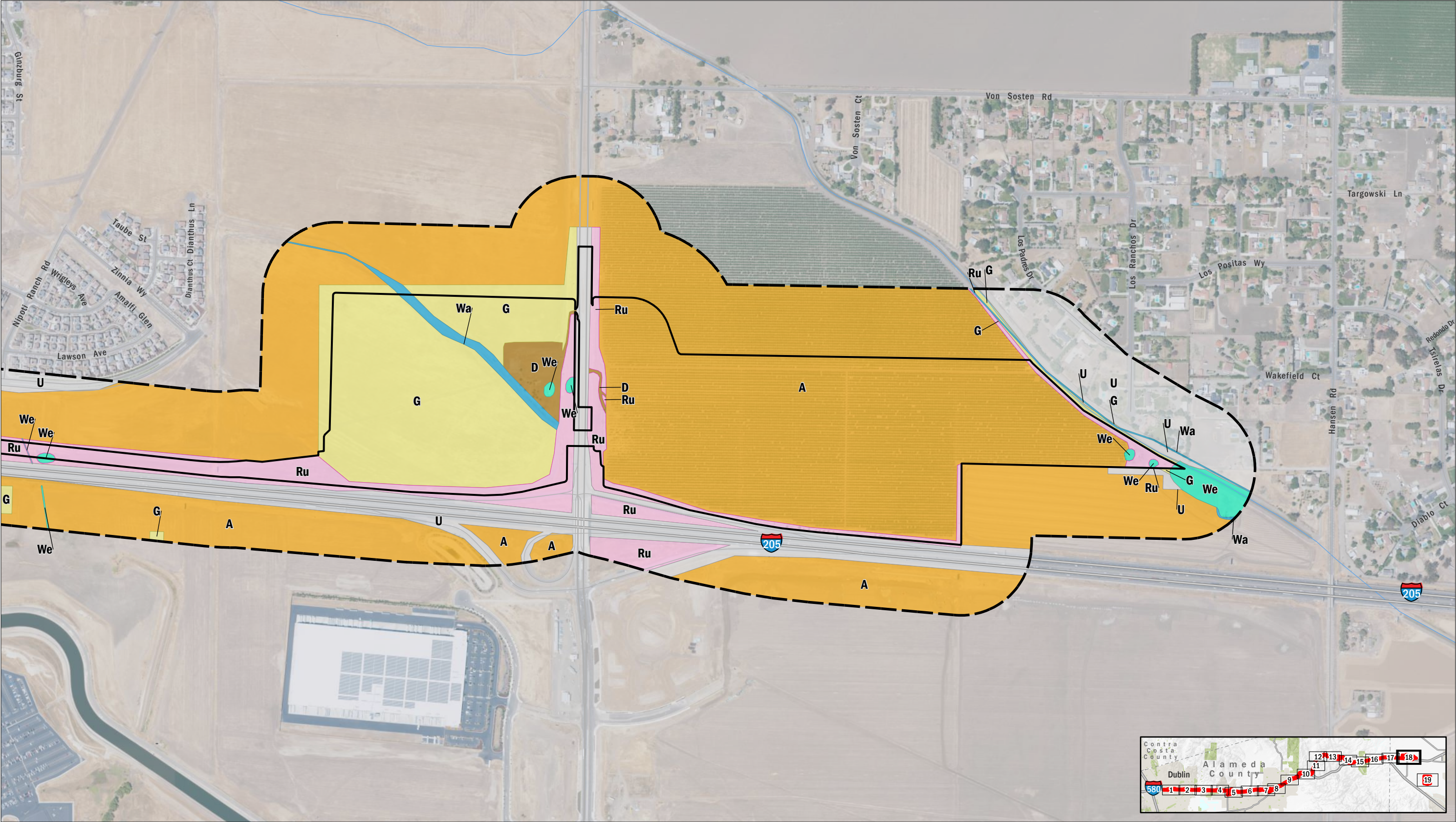


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

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|----------------------------|----------------------------------|--------------------------|------------------------|------------|
| Proposed Project Footprint | Land Cover and Vegetation | D : Developed/landscaped | We : Potential wetland | U : Urban |
| Action Area | A : Agricultural | G : Non-native grassland | Ru : Ruderal | Wa : Water |


FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 17 of 19







AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

 Proposed Project Footprint
 Action Area

Land Cover and Vegetation
 A : Agricultural

 D : Developed/landscaped
 G: Non-native grassland

 Ru : Ruderal
 We : Potential wetland


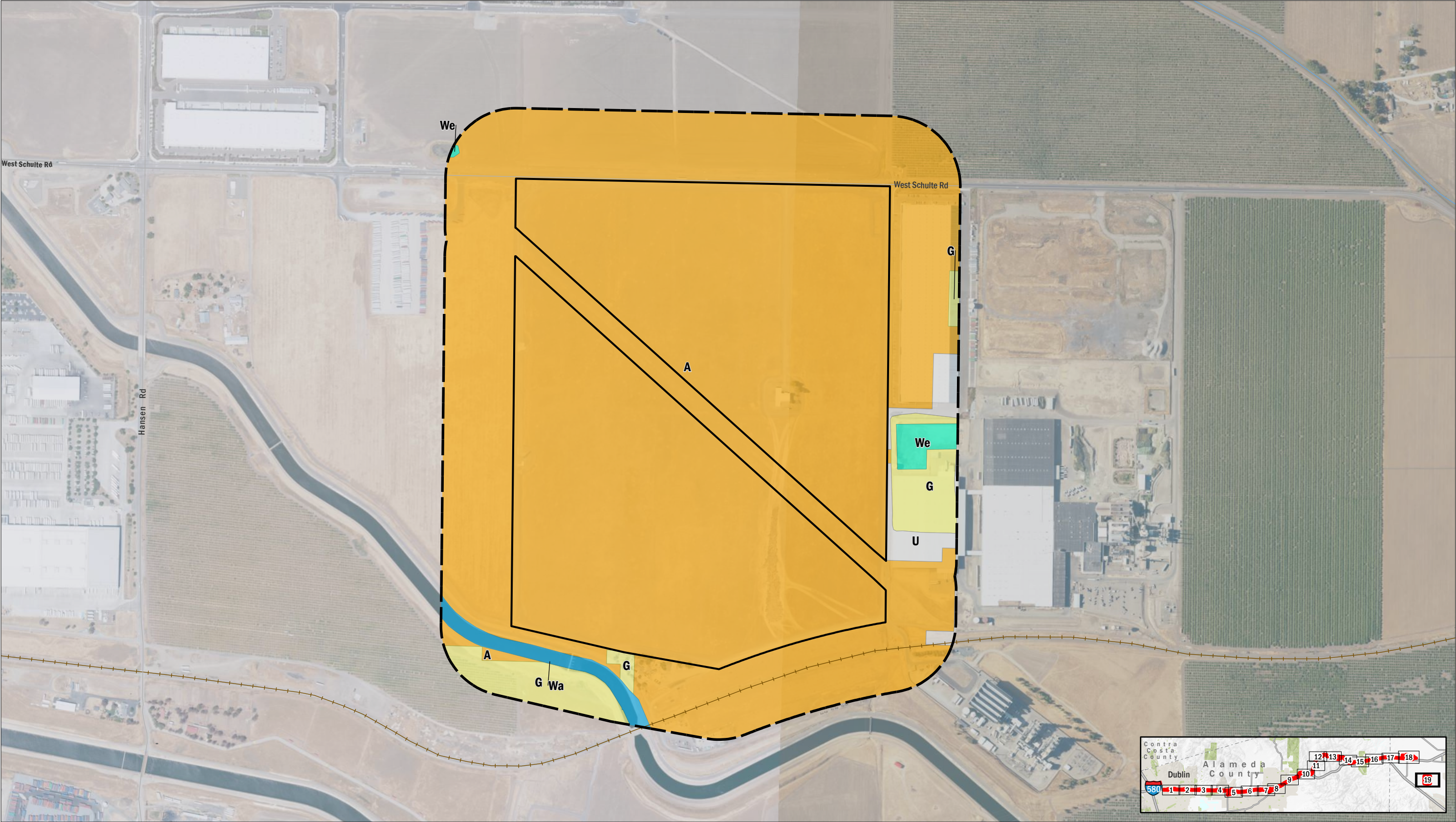
 U : Urban
 Wa : Water

FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 18 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

 Proposed Project Footprint
 Action Area






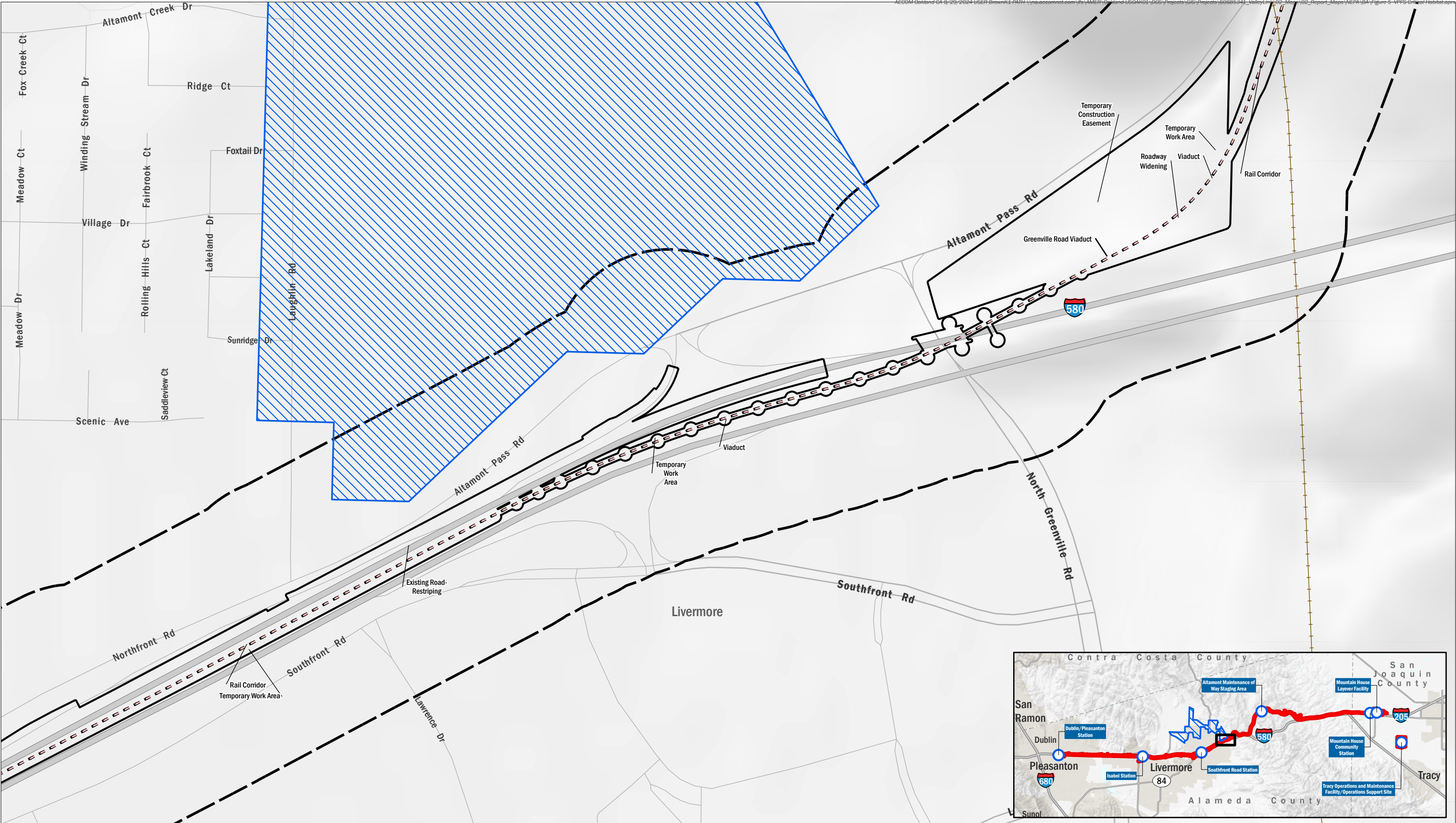
Land Cover and Vegetation
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 We : Potential wetland
 U : Urban
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FIGURE 4
Land Cover and Vegetation in the Action Area
Sheet 19 of 19



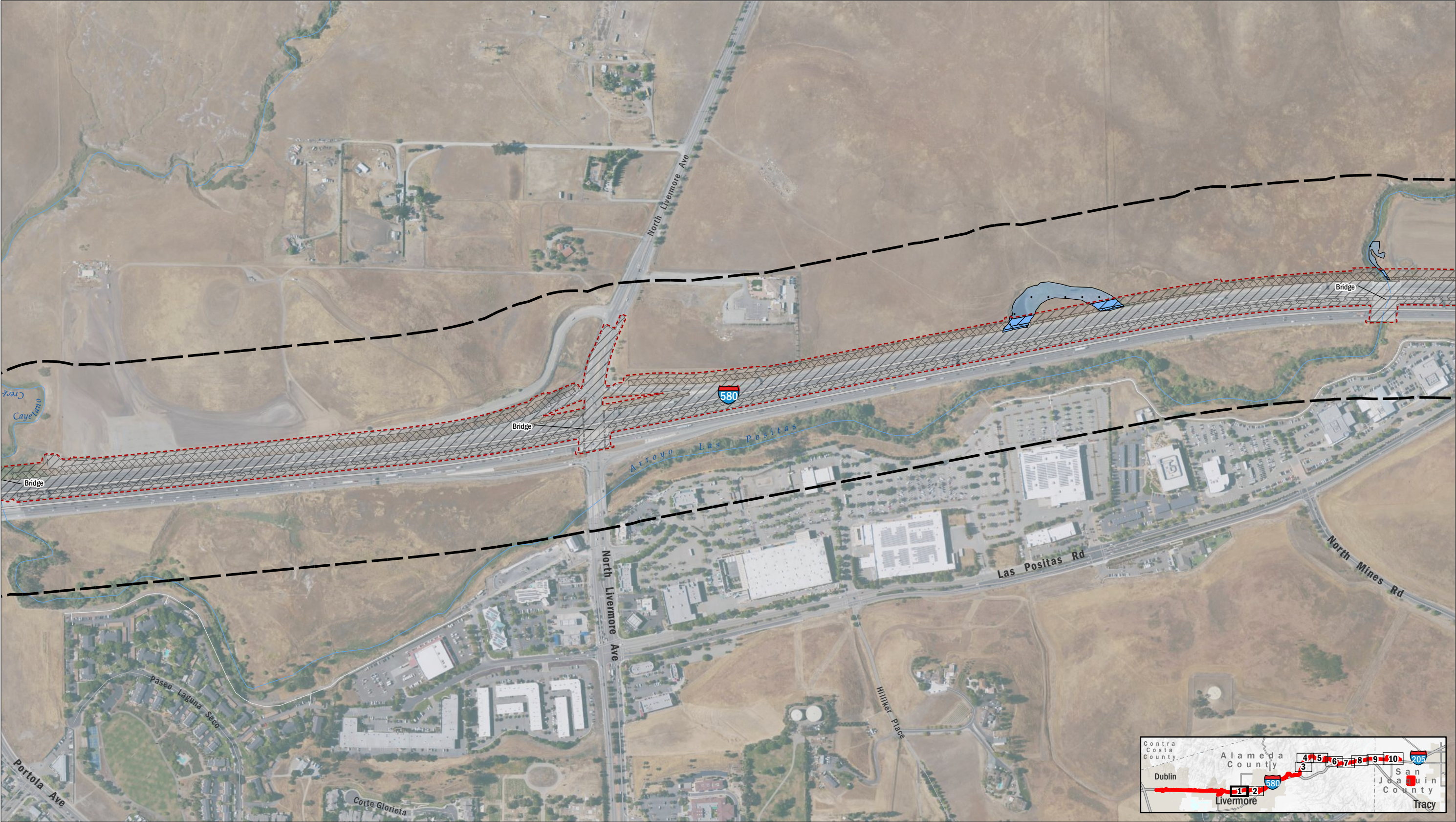
AECOM
Valley Link Rail Project

○ Proposed Valley Link Stations and Facilities
- - Proposed Project Rail Line

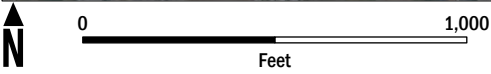
▭ Proposed Project Footprint
▭ Action Area

Critical Habitat
▨ Vernal pool fairy shrimp

FIGURE 5
Vernal Pool Fairy Shrimp Critical Habitat



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 6
*Vernal Pool Fairy Shrimp (*Branchinecta lynchi*) Potential Habitat*
Sheet 1 of 10



FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 2 of 10



Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

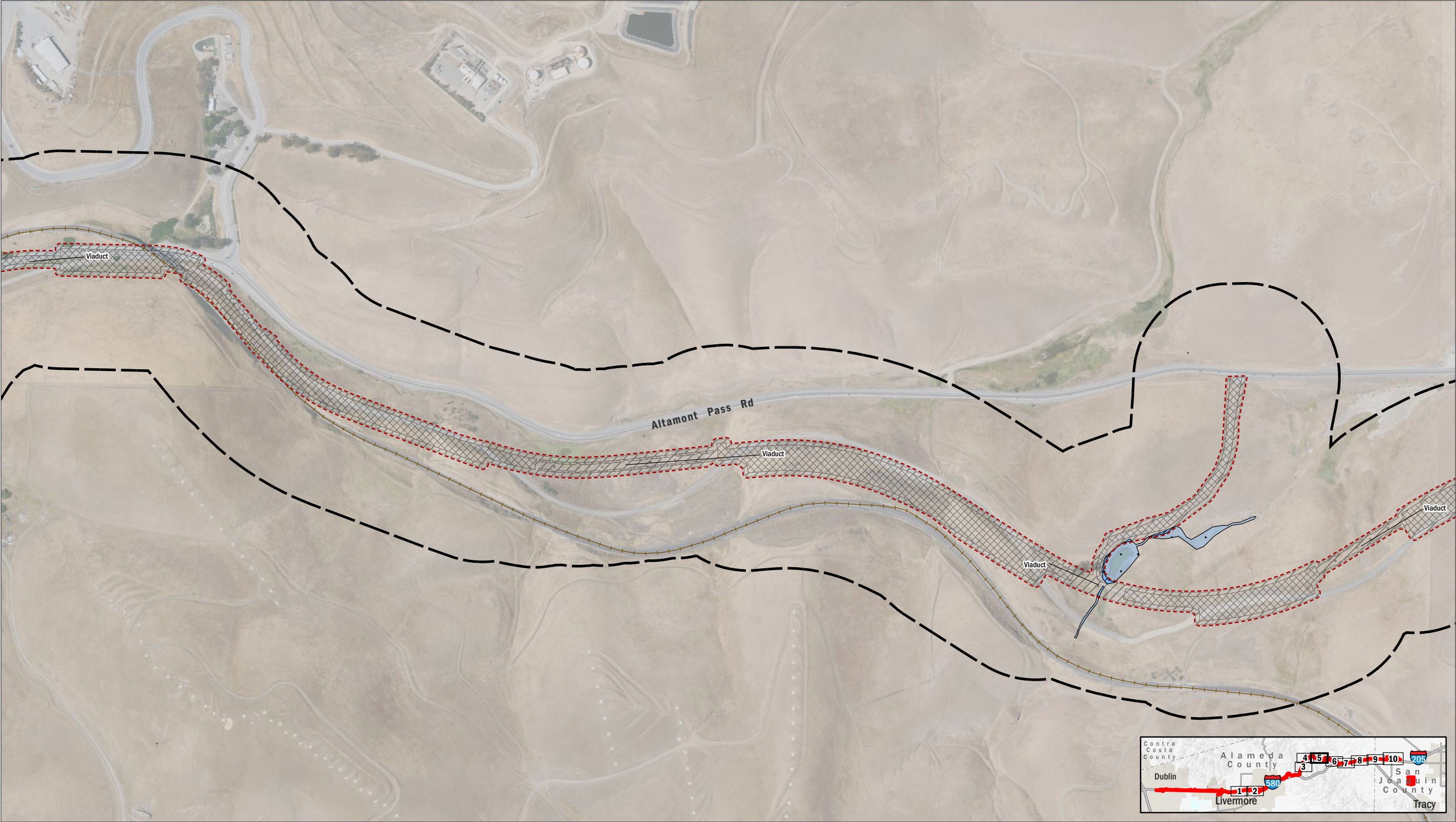
AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 6
*Vernal Pool Fairy Shrimp (*Branchinecta lynchi*) Potential Habitat*
Sheet 3 of 10



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 4 of 10



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

- Proposed Project Footprint

Action Area
- Habitat Type (Direct Effects)**

Wetlands and Waters (Breeding)

Upland
- Habitat Type (Indirect Effects)**

Wetlands and Waters (Breeding)

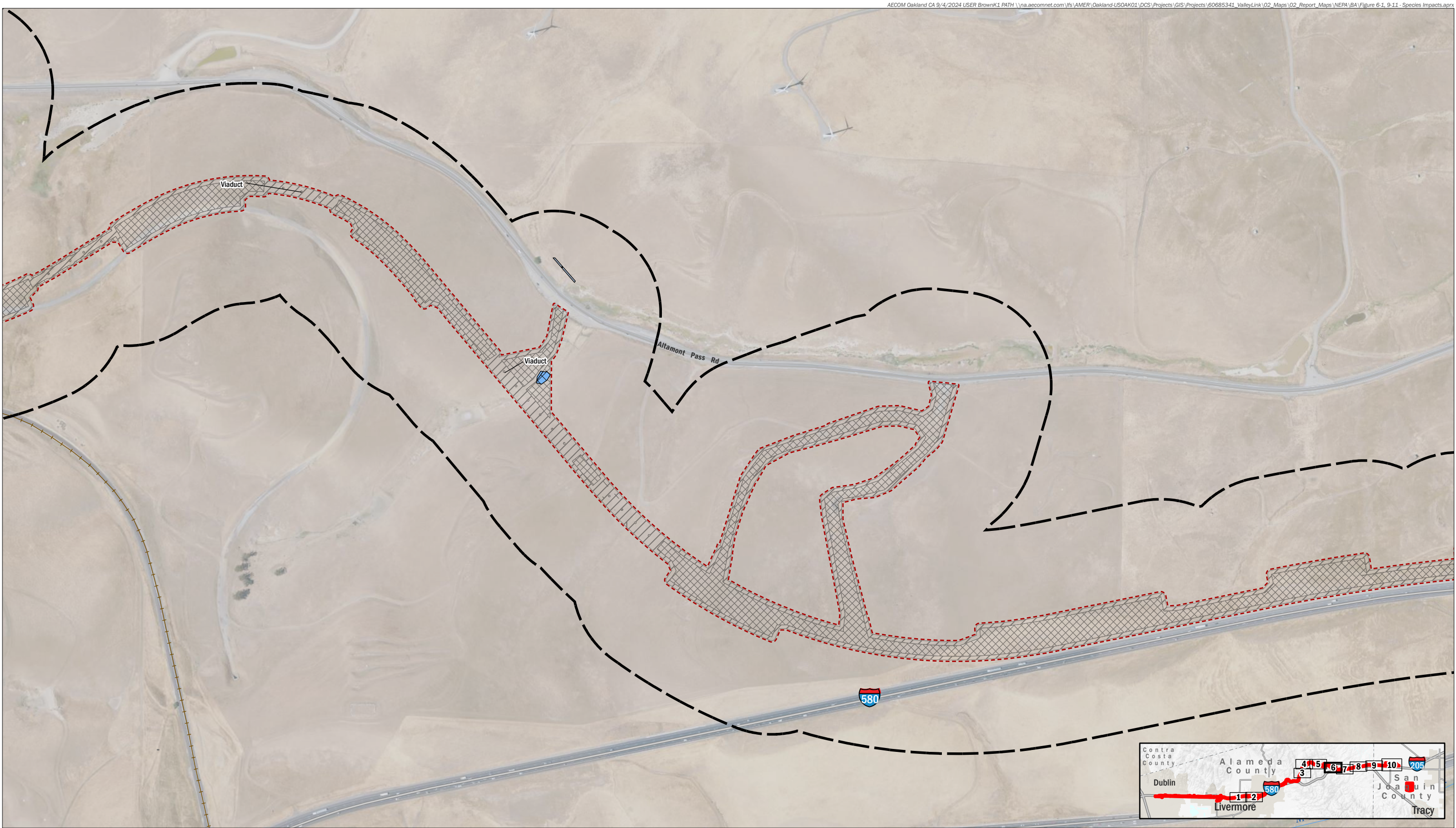
Upland
- Effects**

Direct Permanent Effect

Direct Temporary Effect

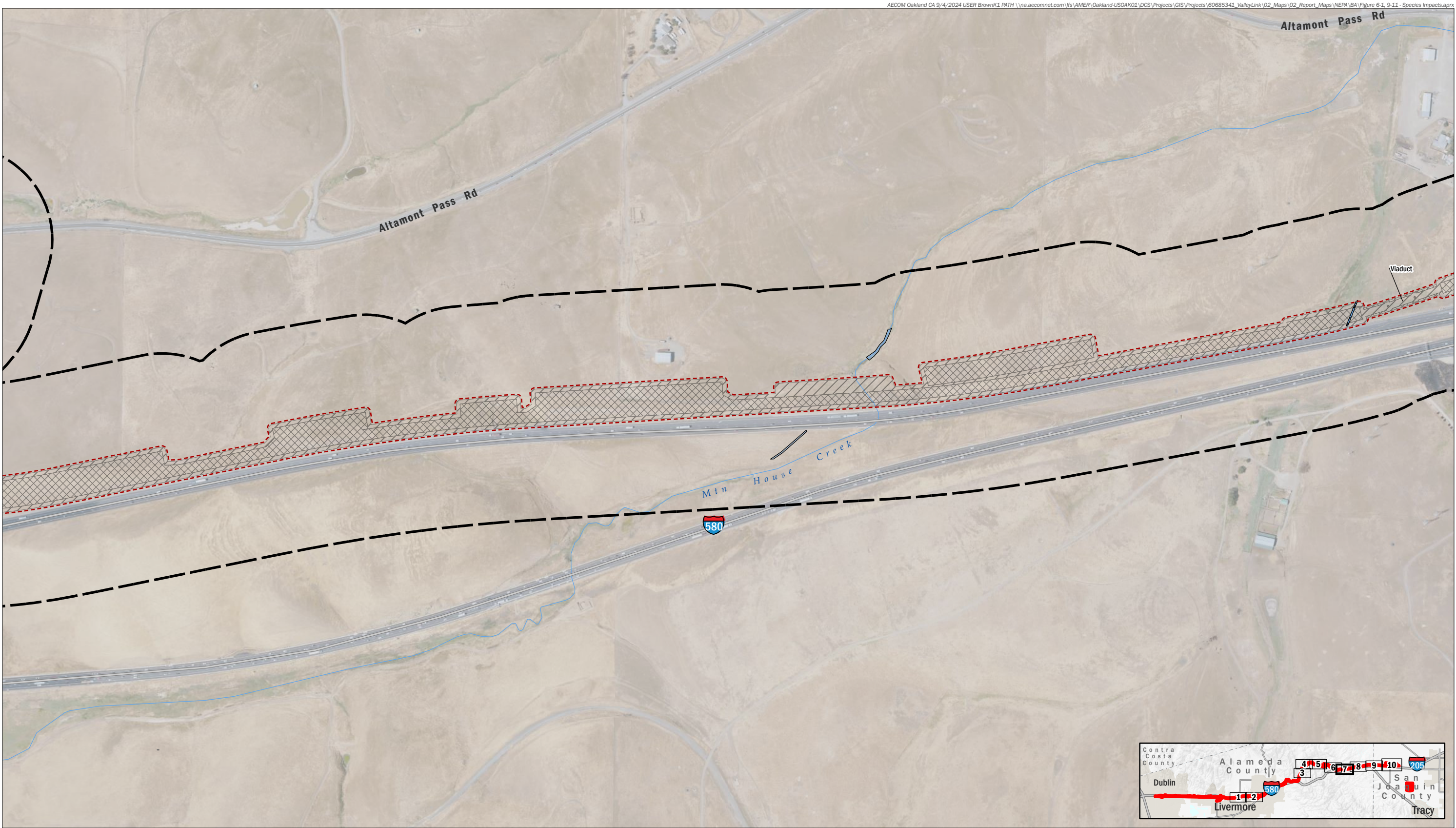
Indirect Effect

FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 5 of 10



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 6 of 10



AECOM
Valley Link Rail Project

Proposed Project Footprint
Action Area

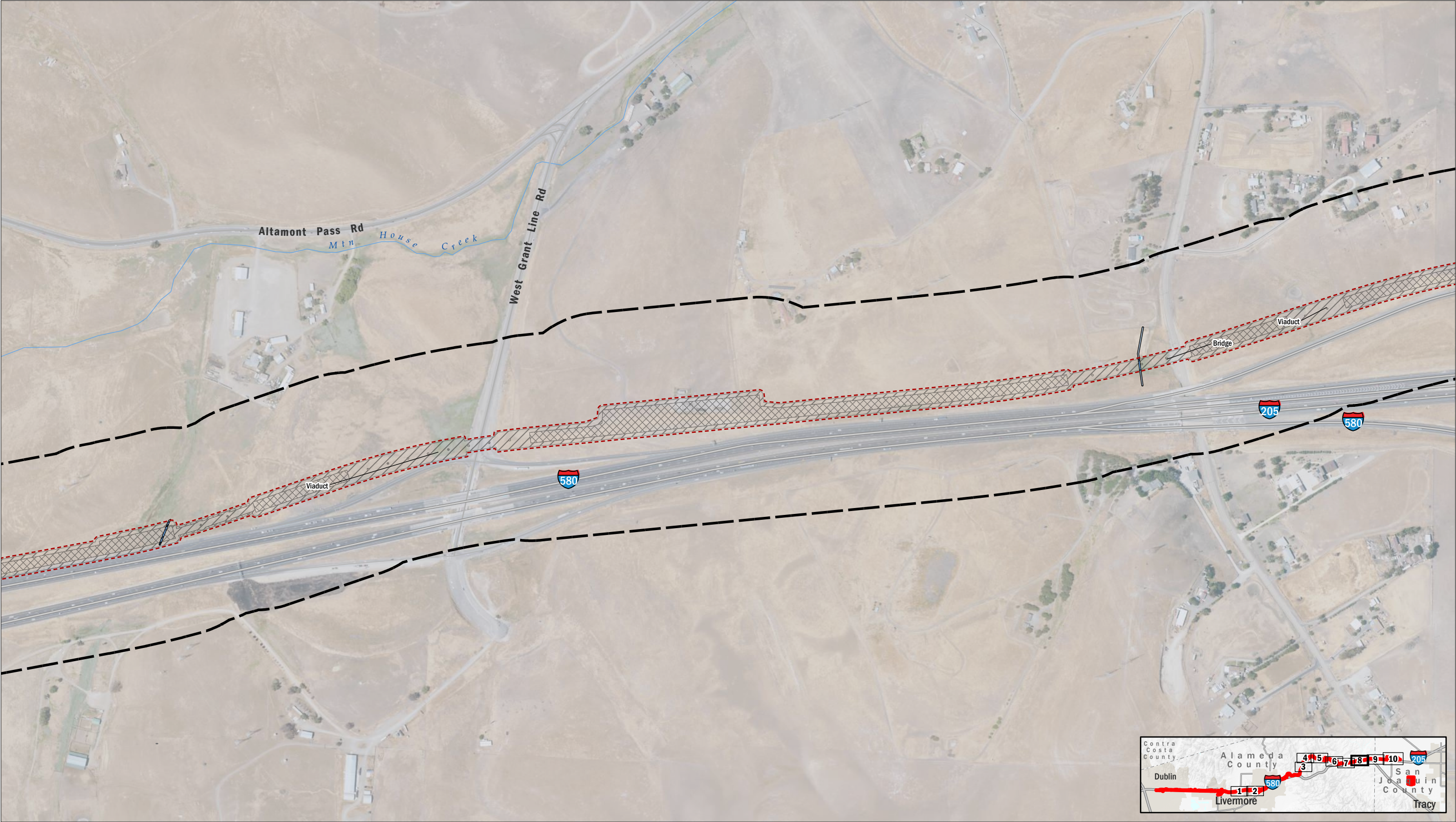
Habitat Type (Direct Effects)
Wetlands and Waters (Breeding)
Upland

Habitat Type (Indirect Effects)
Wetlands and Waters (Breeding)
Upland

Effects
Direct Permanent Effect
Direct Temporary Effect
Indirect Effect

FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 7 of 10

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

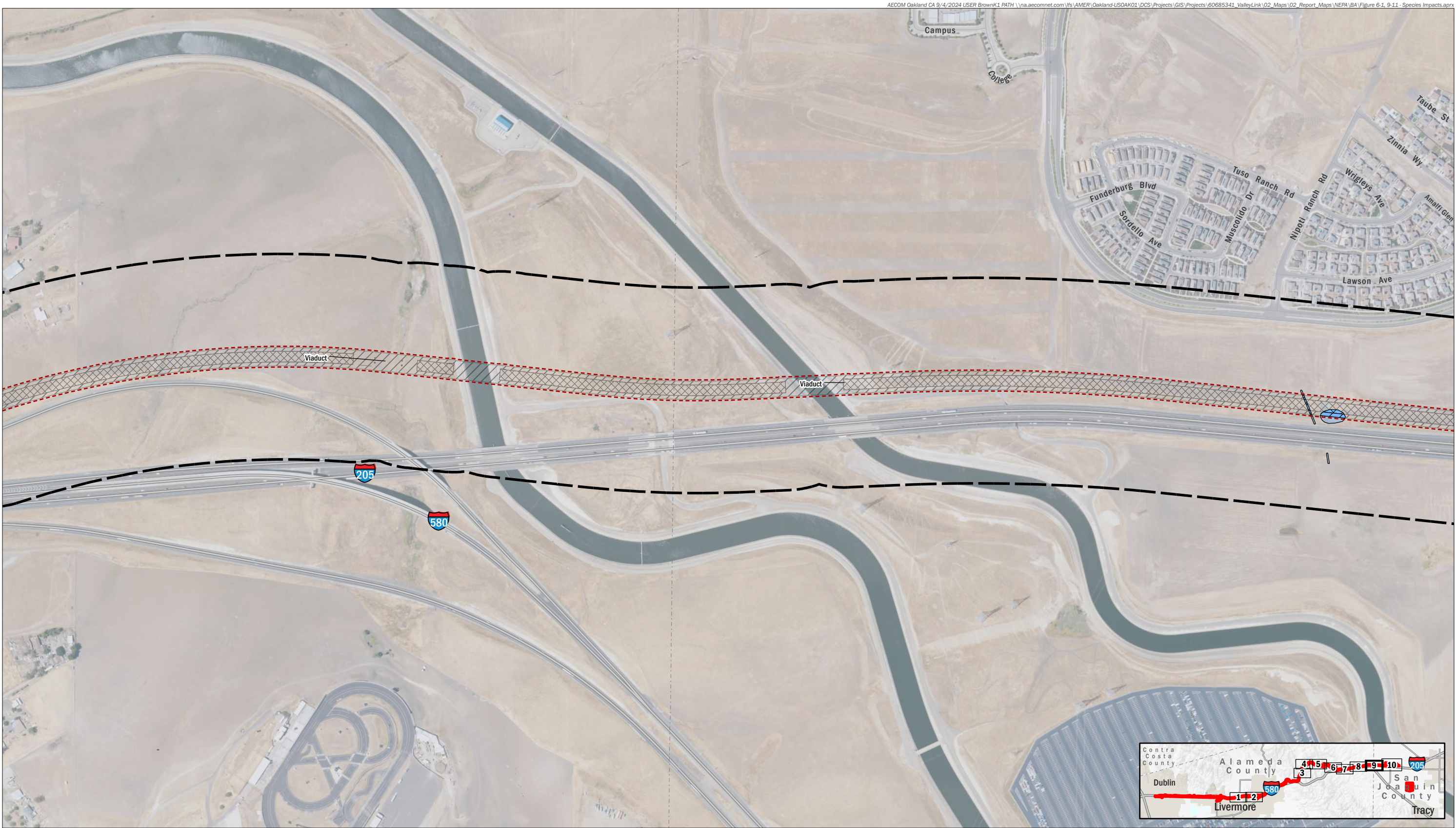
Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

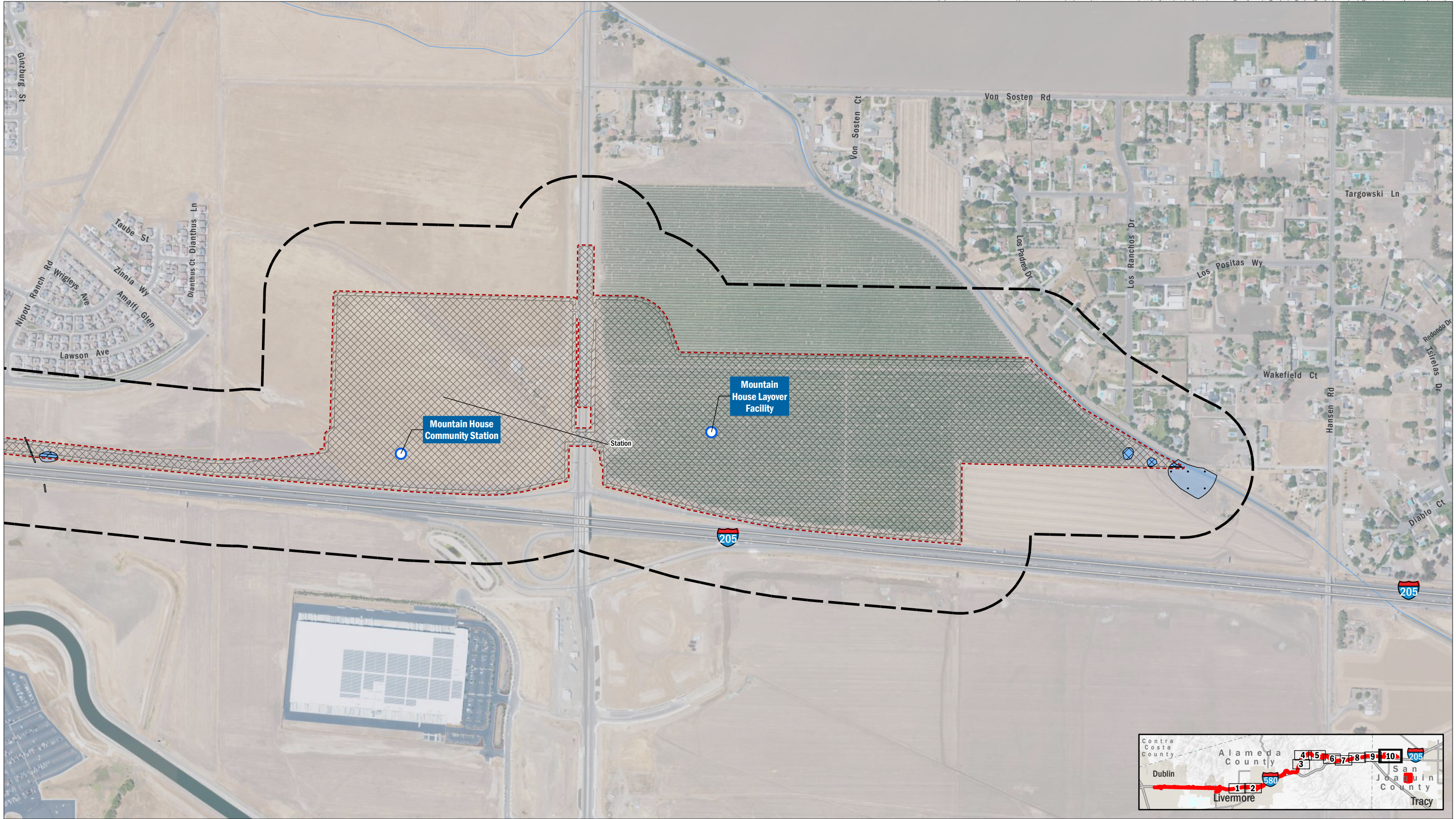
AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 8 of 10



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 9 of 10



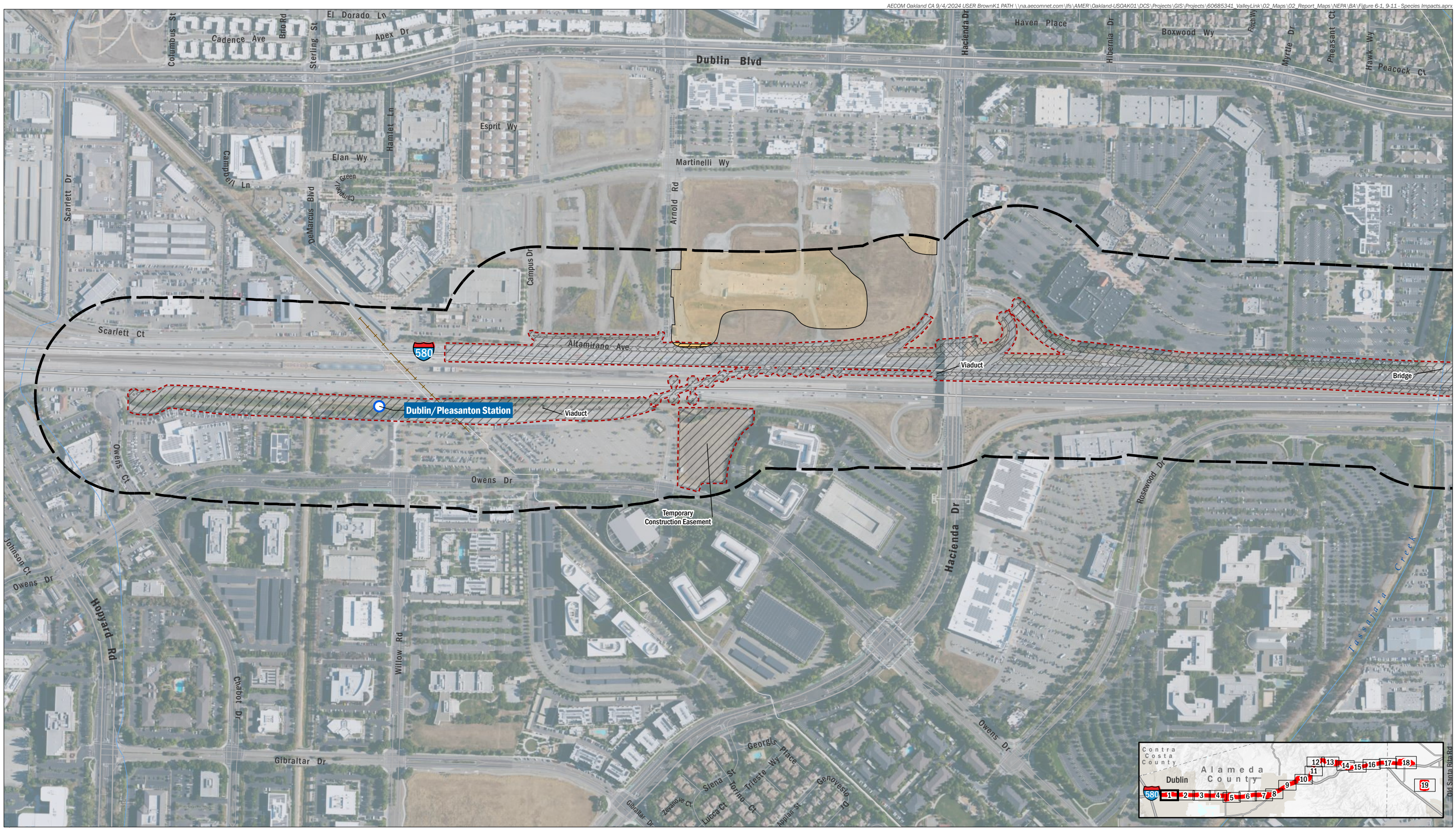
- Proposed Valley Link Stations and Facilities
- Proposed Project Footprint
- Action Area

- Habitat Type (Direct Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Habitat Type (Indirect Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Effects**
- Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

FIGURE 6
Vernal Pool Fairy Shrimp (Branchinecta lynchi) Potential Habitat
Sheet 10 of 10



AECOM
Valley Link Rail Project

0 1,000
Feet

Proposed Valley Link Stations and Facilities
Proposed Project Footprint
Action Area

Habitat Type (Direct Effects)
Wetlands and Waters (Breeding)
Upland

Habitat Type (Indirect Effects)
Wetlands and Waters (Breeding)
Upland

Effects
Direct Permanent Effect
Direct Temporary Effect
Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 1 of 19

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



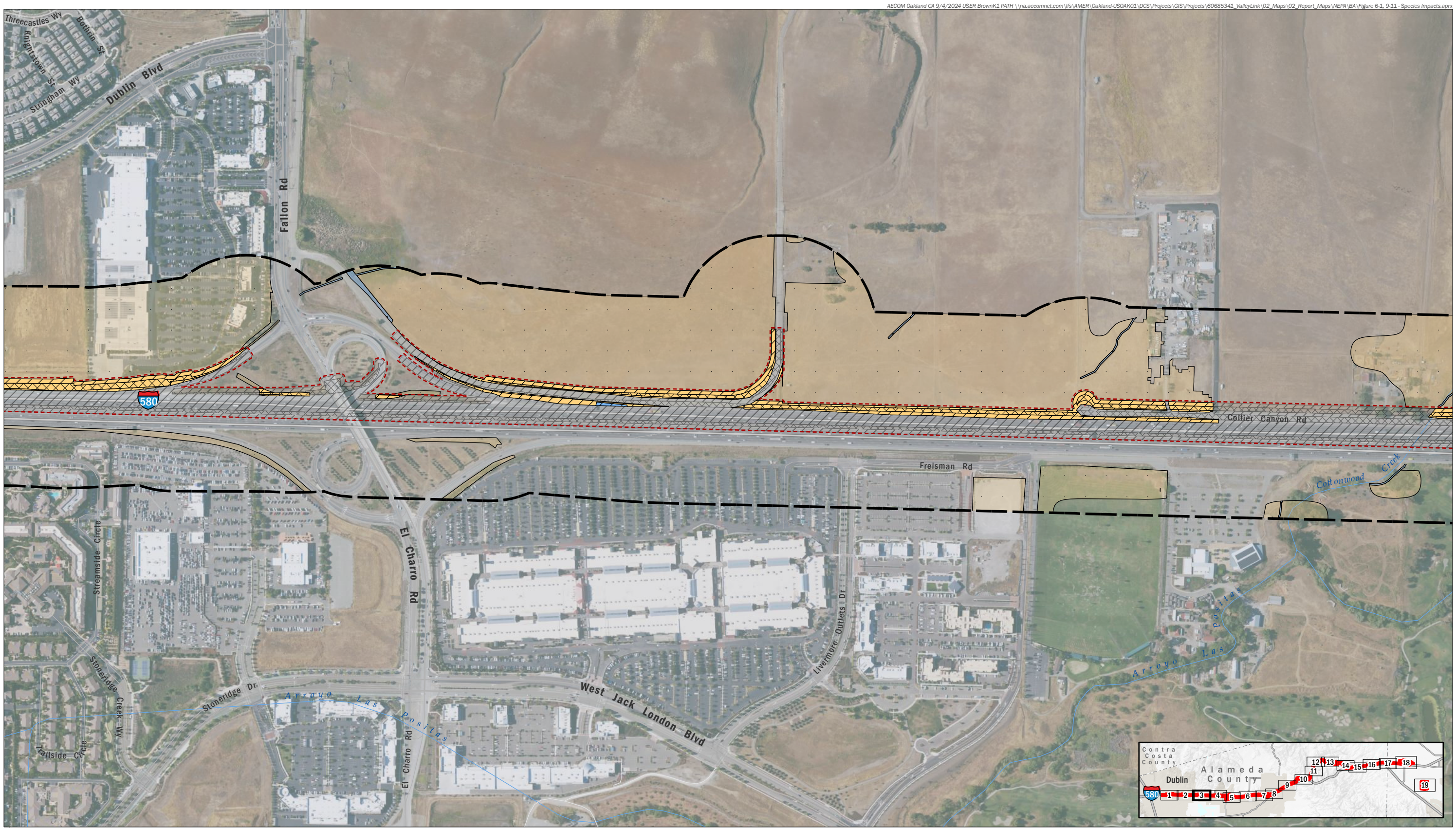
AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

Habitat Type (Direct Effects)**Habitat Type (Indirect Effects)**

Effects

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 2 of 19



AECOM
Valley Link Rail Project

Habitat Type (Direct Effects)

- Wetlands and Waters (Breeding)
- Upland

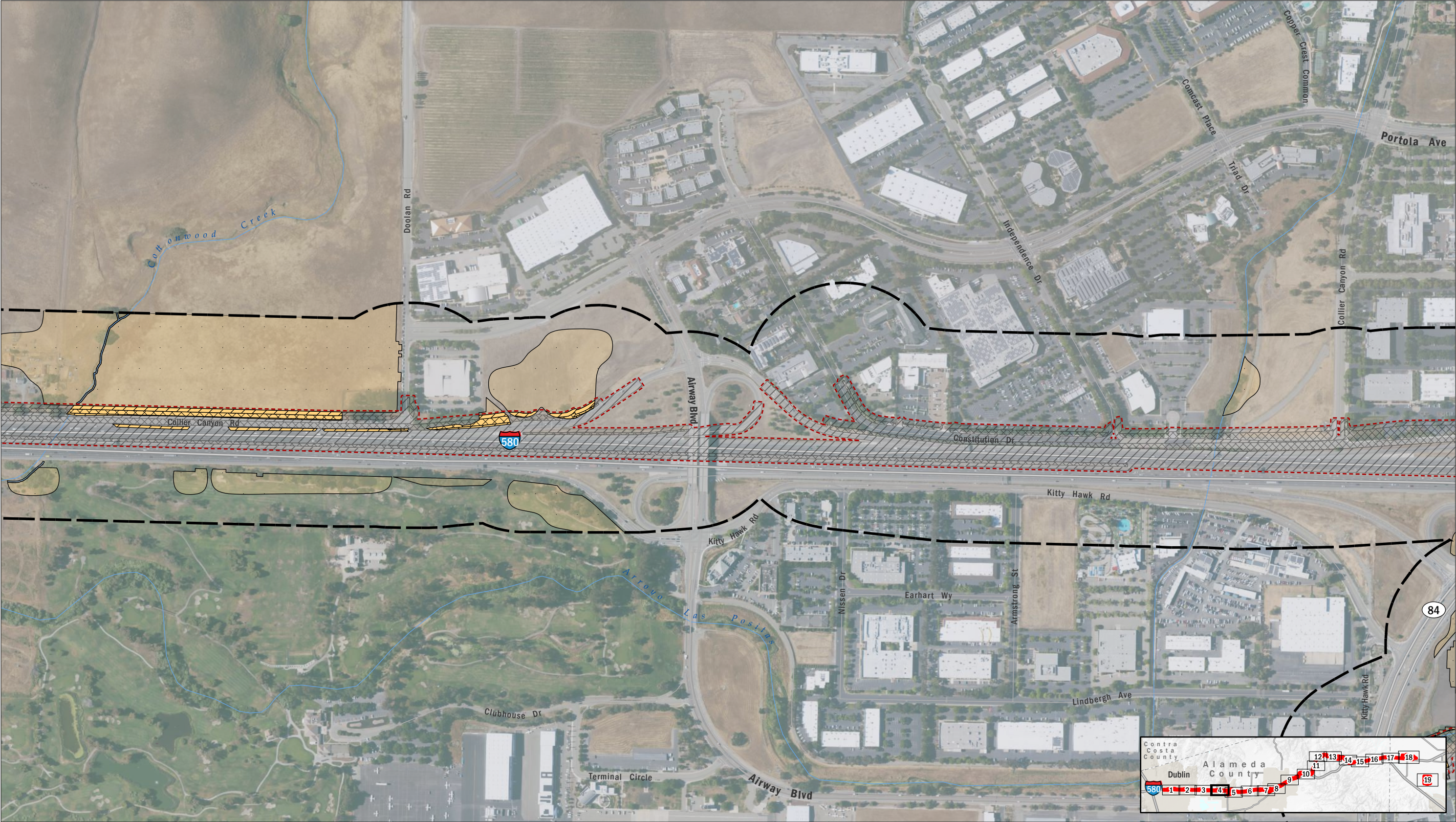
Habitat Type (Indirect Effects)

- Wetlands and Waters (Breeding)
- Upland

Effects

- Direct Permanent Effect
- Direct Temporary Effect
- Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 3 of 19



AECOM
Valley Link Rail Project

0 1,000
Feet

Proposed Project Footprint
 Proposed Project Footprint

Action Area
 Action Area

Habitat Type (Direct Effects)

- Wetlands and Waters (Breeding)
- Upland

Habitat Type (Indirect Effects)

- Wetlands and Waters (Breeding)
- Upland

Effects

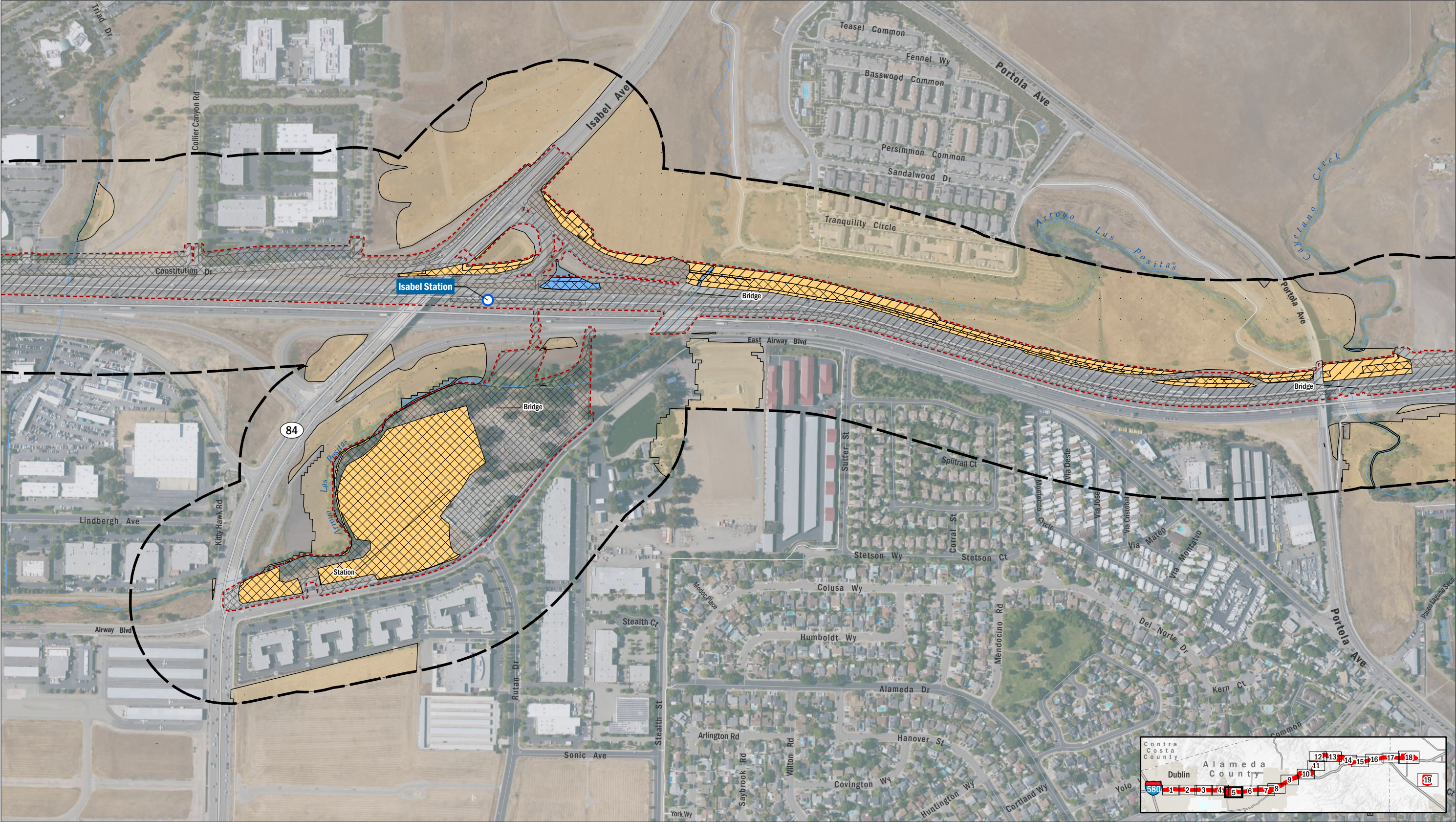
- Direct Permanent Effect
- Direct Temporary Effect
- Indirect Effect

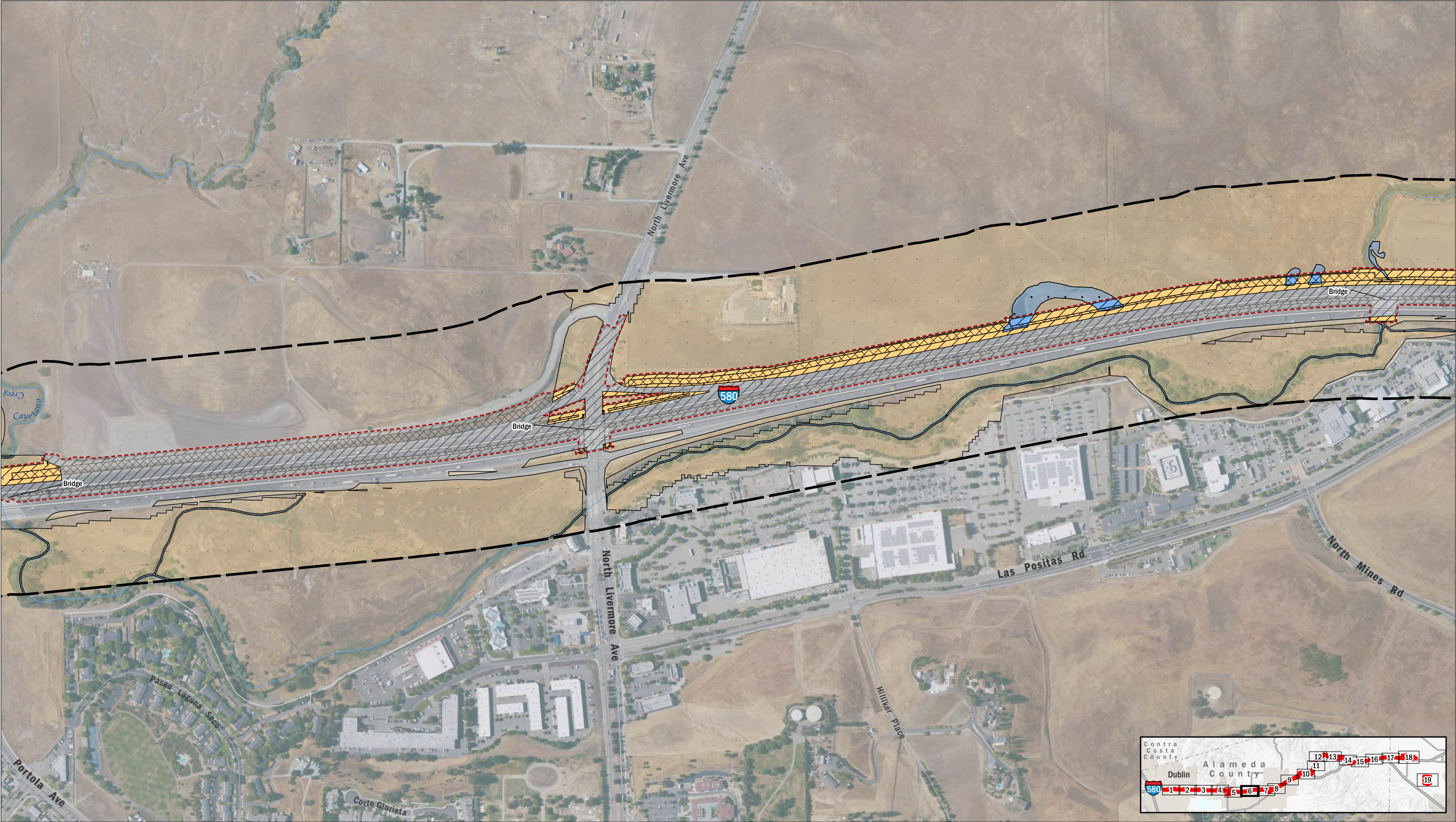
Contra Costa County
Dublin
Alameda County

12 13 14 15 16 17 18
11
1 2 3 4 5 6 7 8 9 10 19

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 4 of 19





AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

Habitat Type (Direct Effects)

- Wetlands and Waters (Breeding)
- Upland

Habitat Type (Indirect Effects)

- Wetlands and Waters (Breeding)
- Upland

Effects

- Direct Permanent Effect
- Direct Temporary Effect
- Indirect Effect

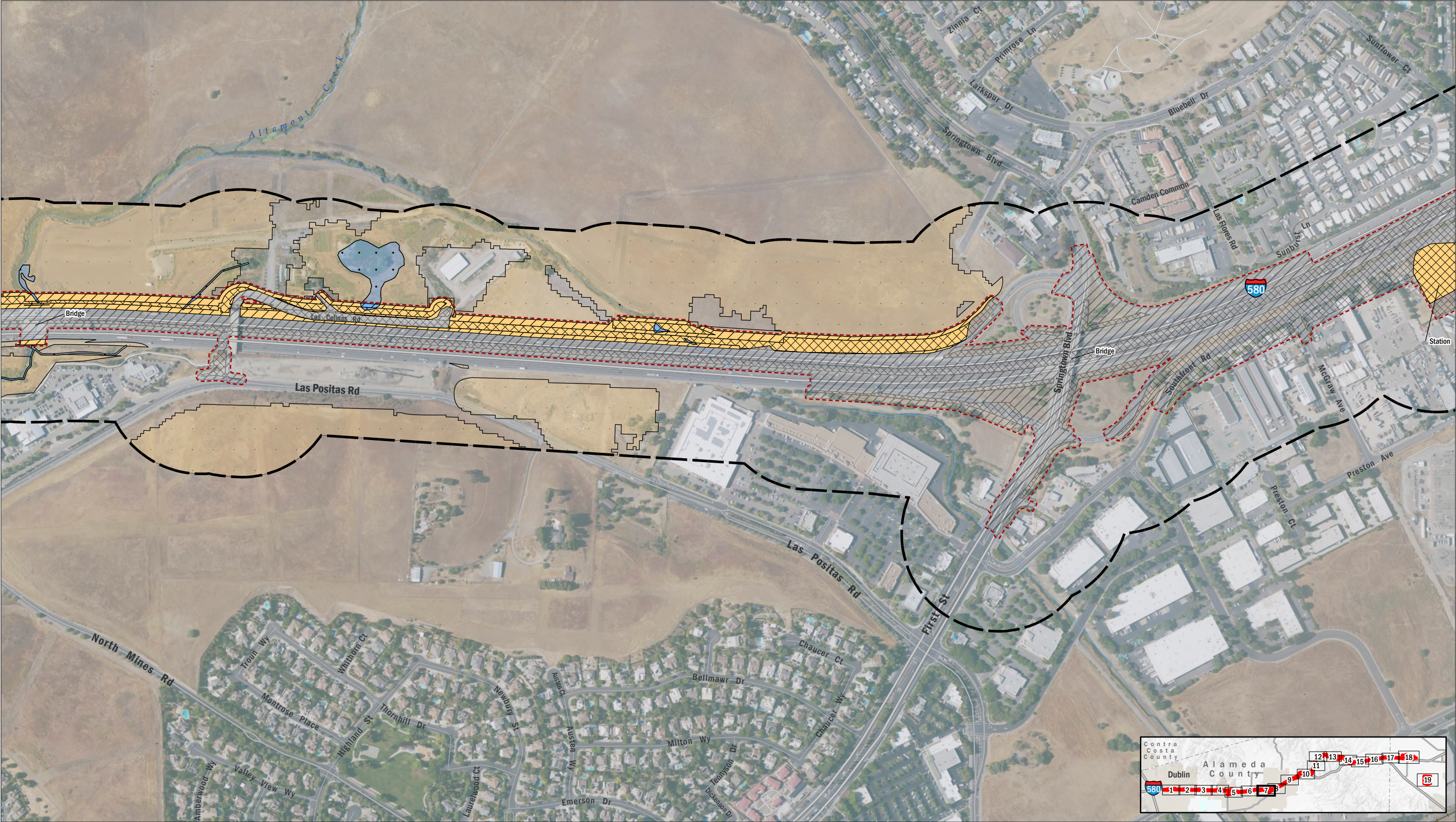
Proposed Project Footprint

-

Action Area

-

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 6 of 19



0 1,000
Feet

AECOM
Valley Link Rail Project

Proposed Project Footprint
Action Area

Habitat Type (Direct Effects)

Wetlands and Waters (Breeding)
Upland

Habitat Type (Indirect Effects)

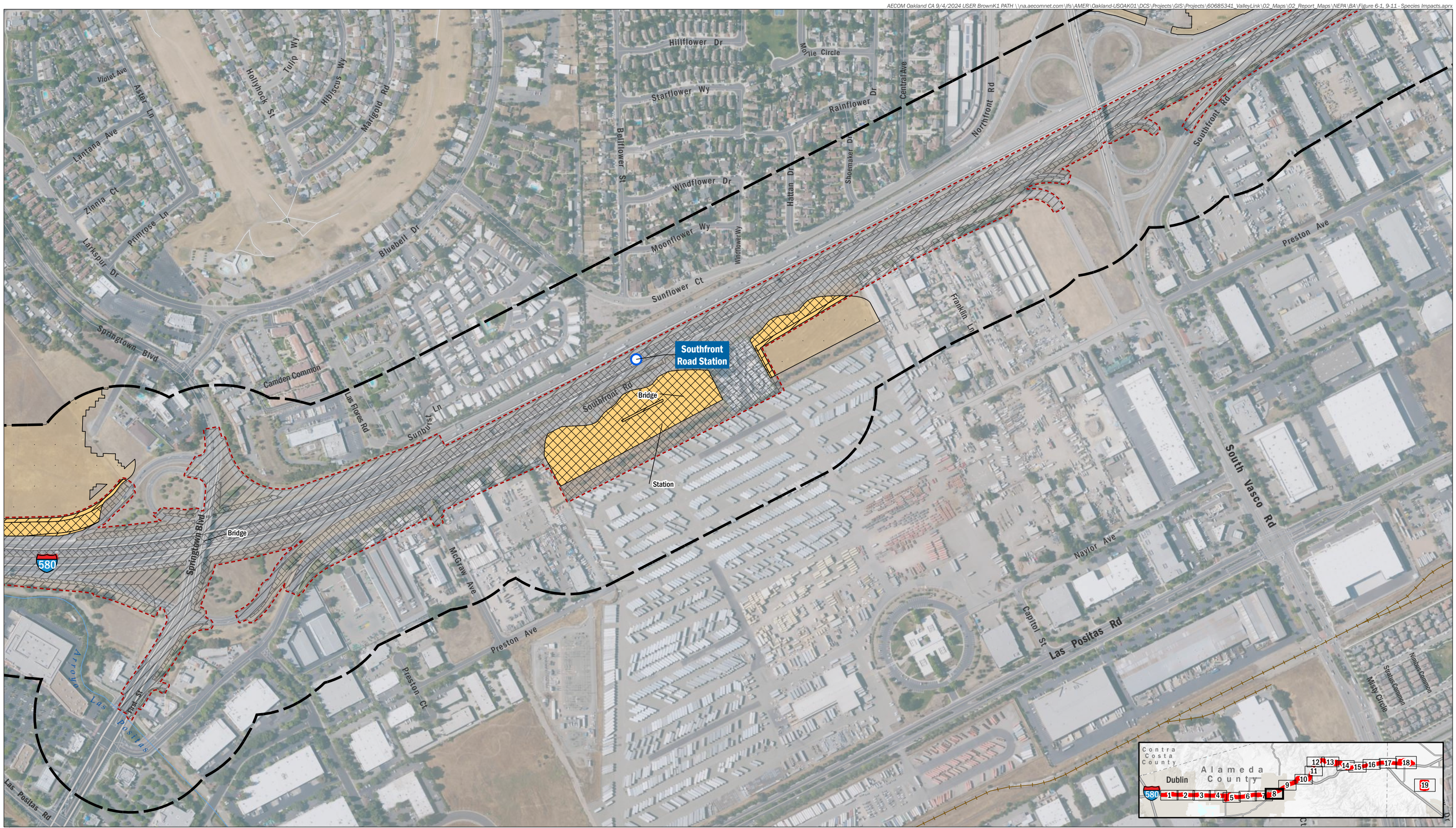
Wetlands and Waters (Breeding)
Upland

Effects

Direct Permanent Effect
Direct Temporary Effect
Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 7 of 19

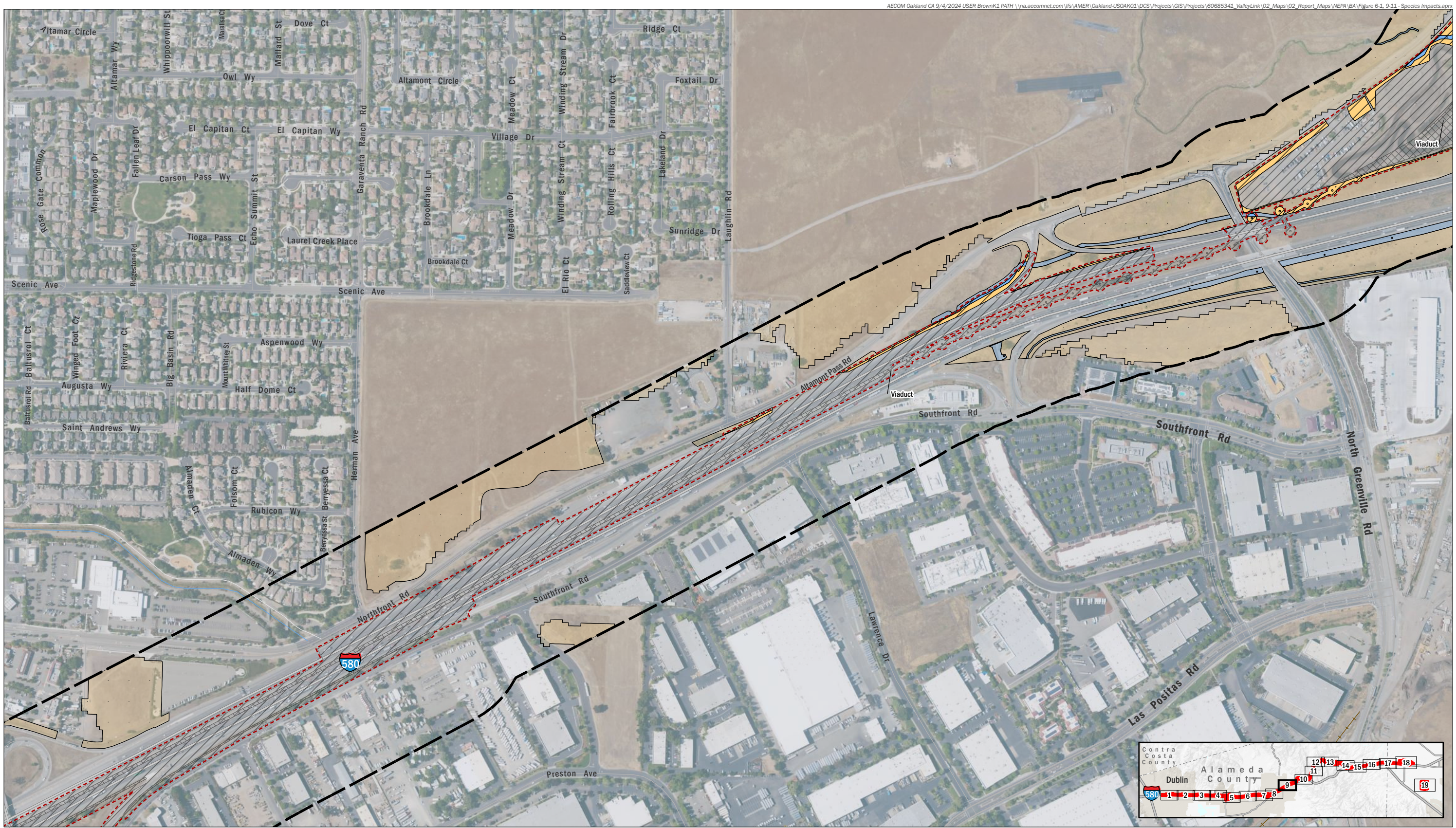


AECOM
Valley Link Rail Project

Legend:

- Proposed Valley Link Stations and Facilities
- Proposed Project Footprint
- Action Area
- Habitat Type (Direct Effects)**
 - Wetlands and Waters (Breeding)
 - Upland
- Habitat Type (Indirect Effects)**
 - Wetlands and Waters (Breeding)
 - Upland
- Effects**
 - Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

Figure 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 8 of 19



AECOM
Valley Link Rail Project

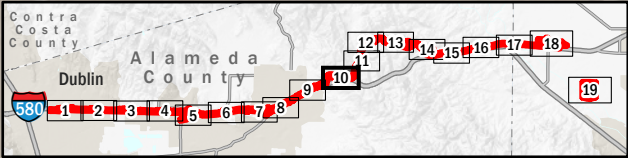
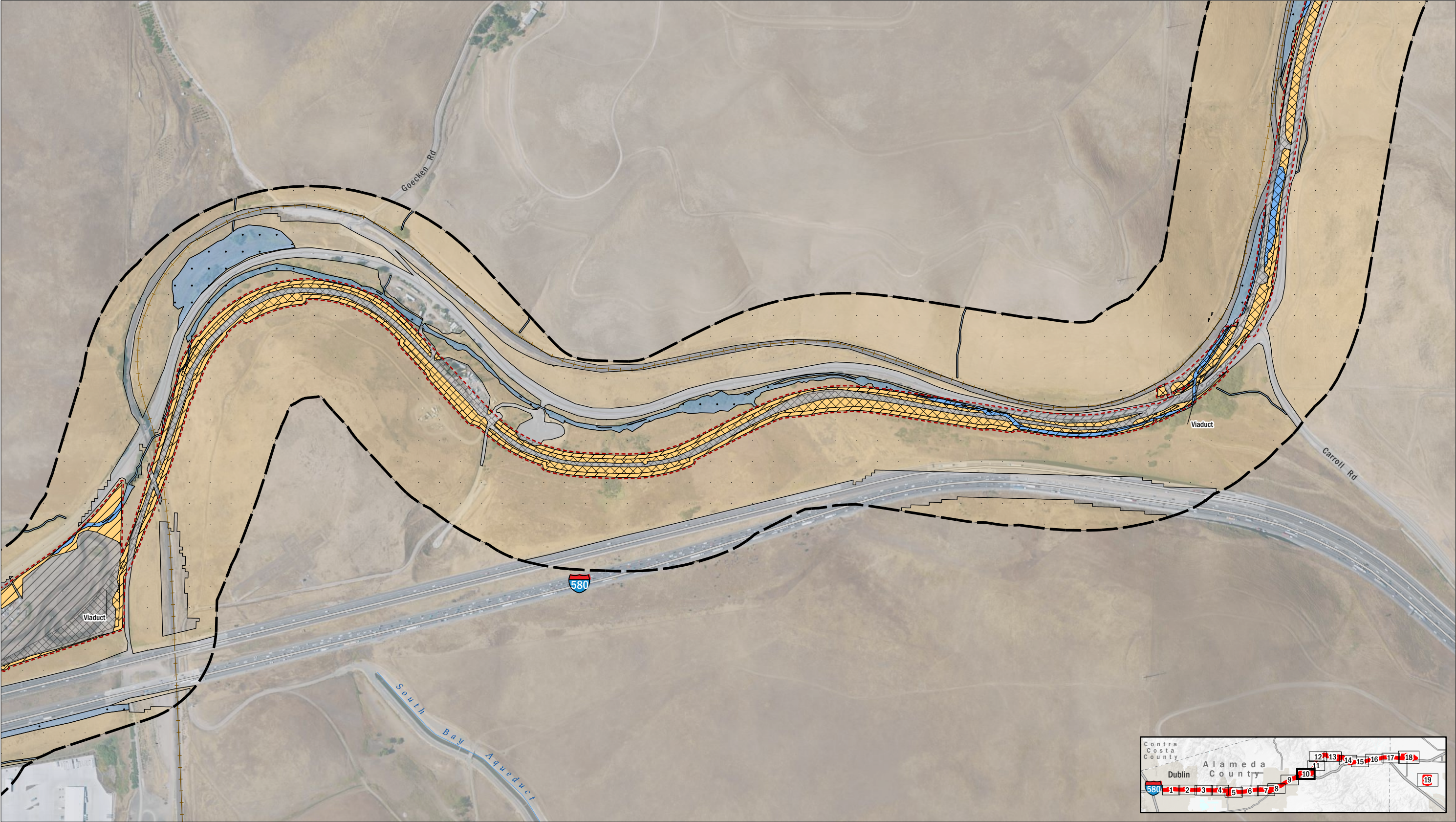
Habitat Type (Direct Effects)
Wetlands and Waters (Breeding)
Upland

Habitat Type (Indirect Effects)
Wetlands and Waters (Breeding)
Upland

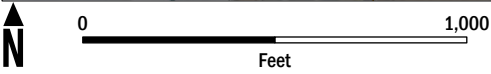
Effects
Direct Permanent Effect
Direct Temporary Effect
Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
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AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 10 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

0 1,000
Feet

Proposed Project Footprint
Action Area

Habitat Type (Direct Effects)	Habitat Type (Indirect Effects)	Effects
Wetlands and Waters (Breeding)	Wetlands and Waters (Breeding)	Direct Permanent Effect
Upland	Upland	Direct Temporary Effect
		Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
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AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

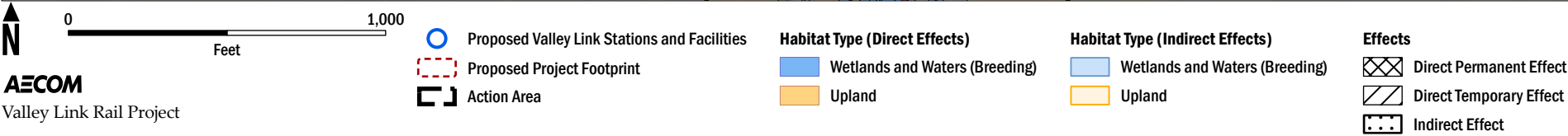
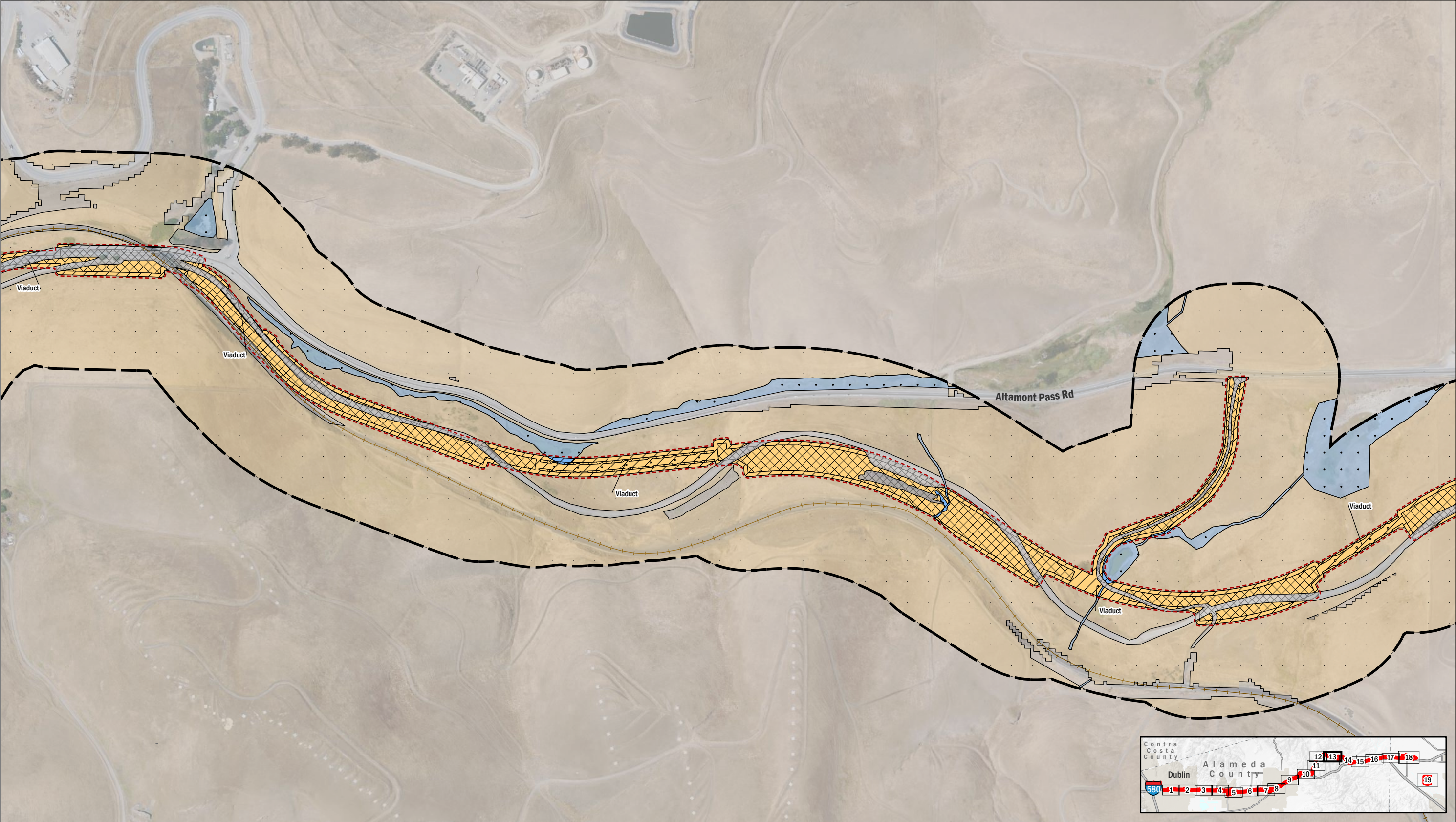


FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
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AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

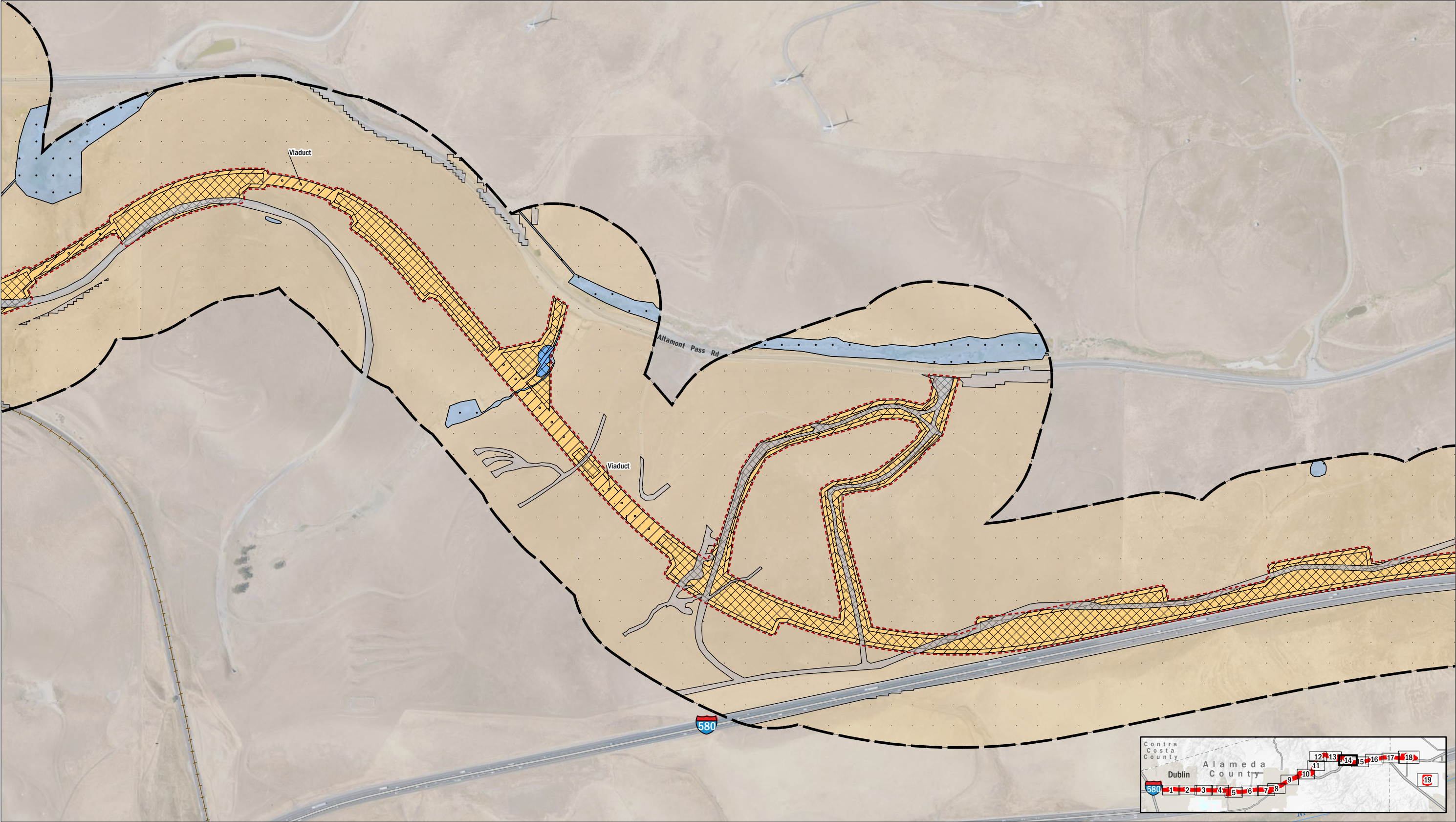
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 13 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)

Wetlands and Waters (Breeding)
 Upland

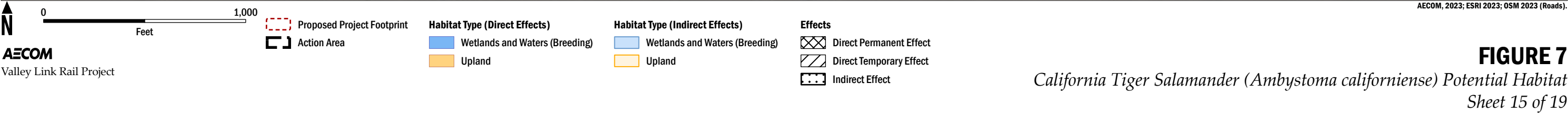
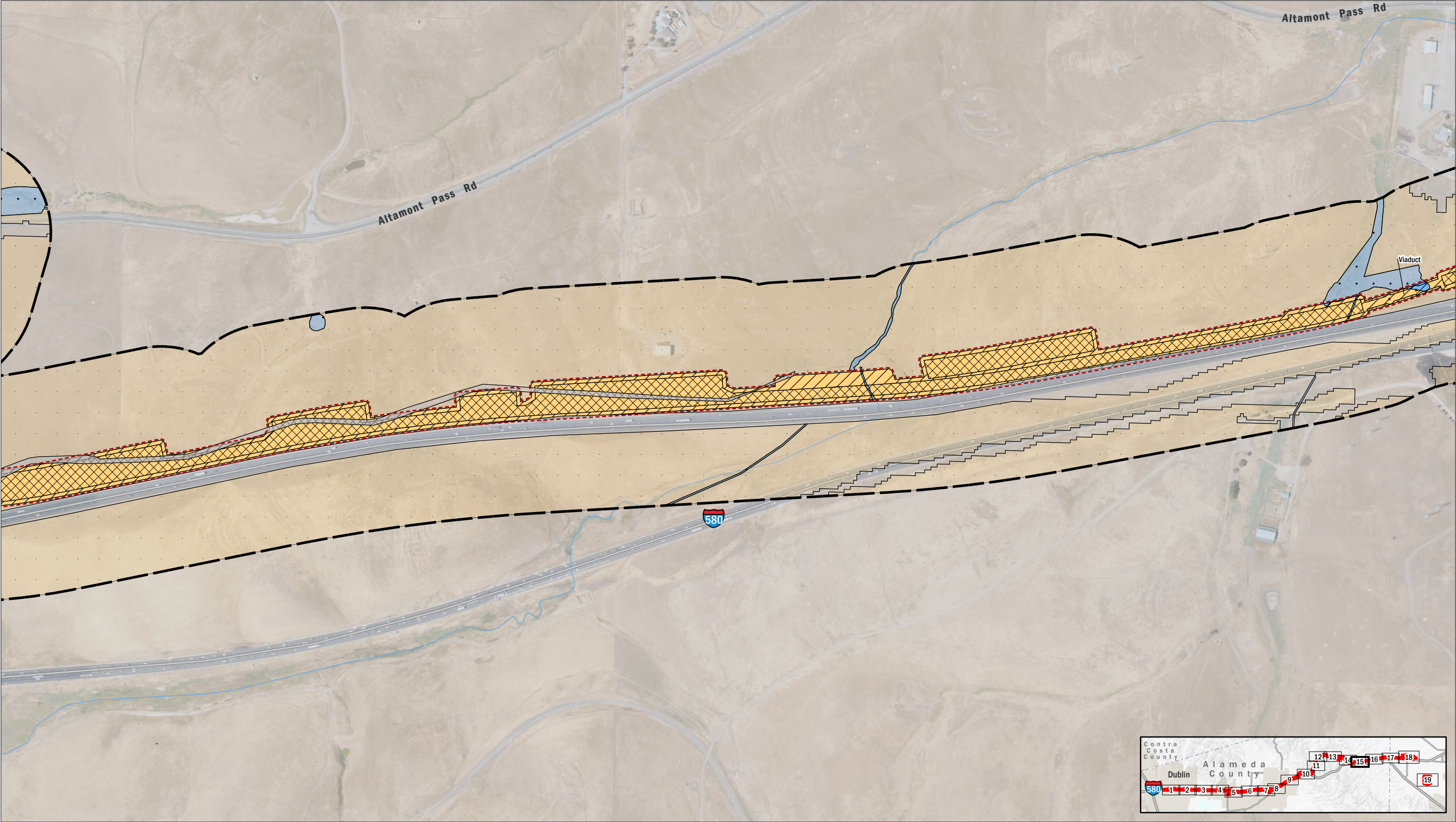
Habitat Type (Indirect Effects)

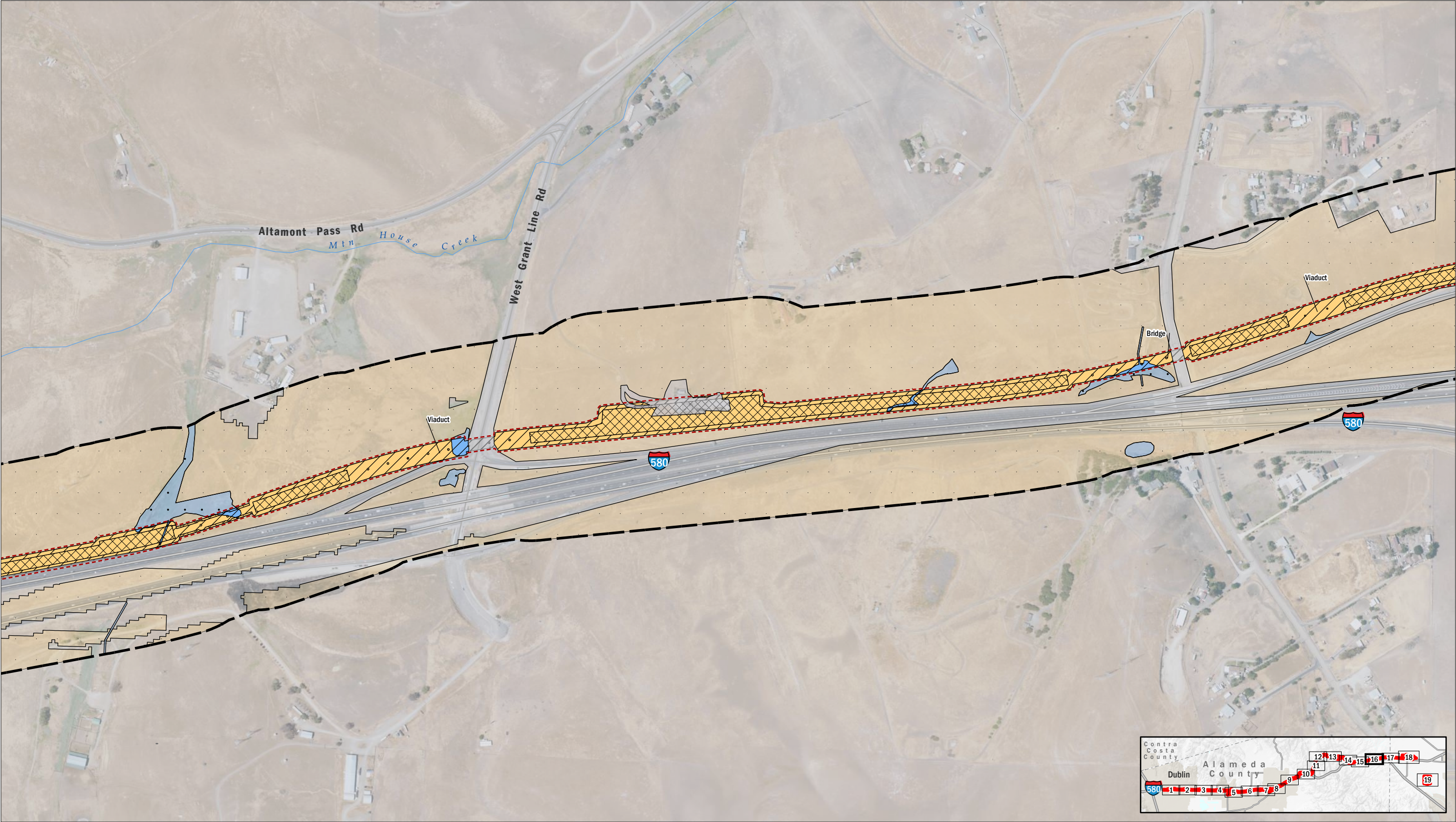
Wetlands and Waters (Breeding)
 Upland

Effects

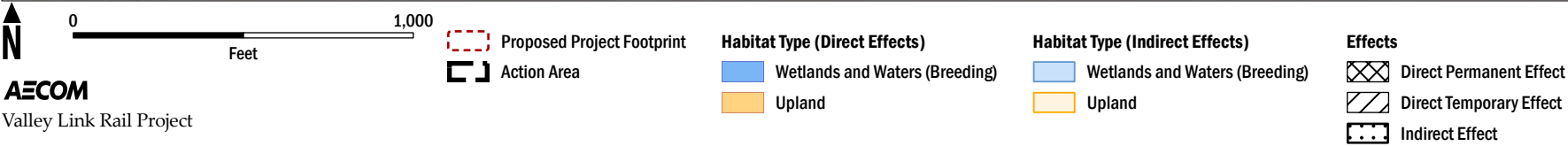
Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
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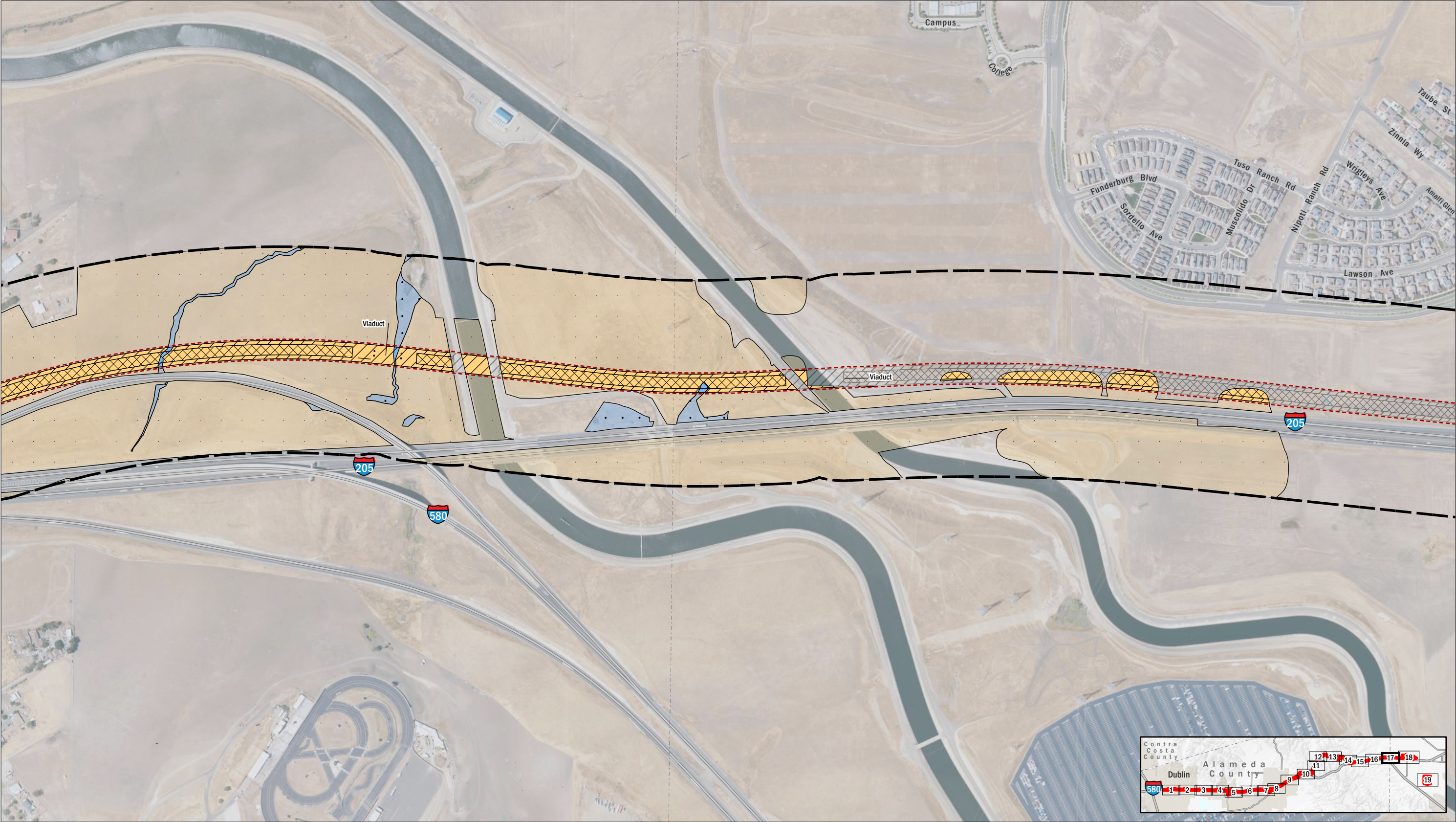


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM
Valley Link Rail Project

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
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AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

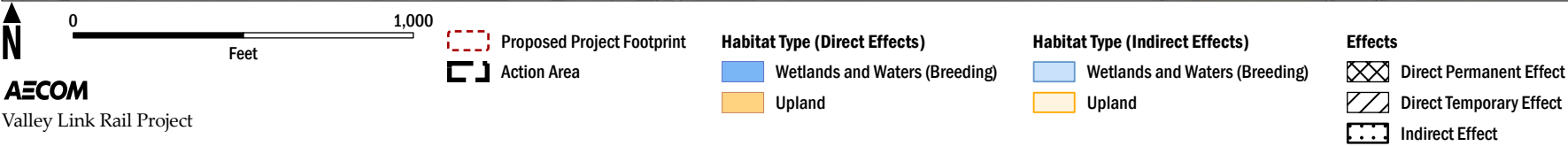
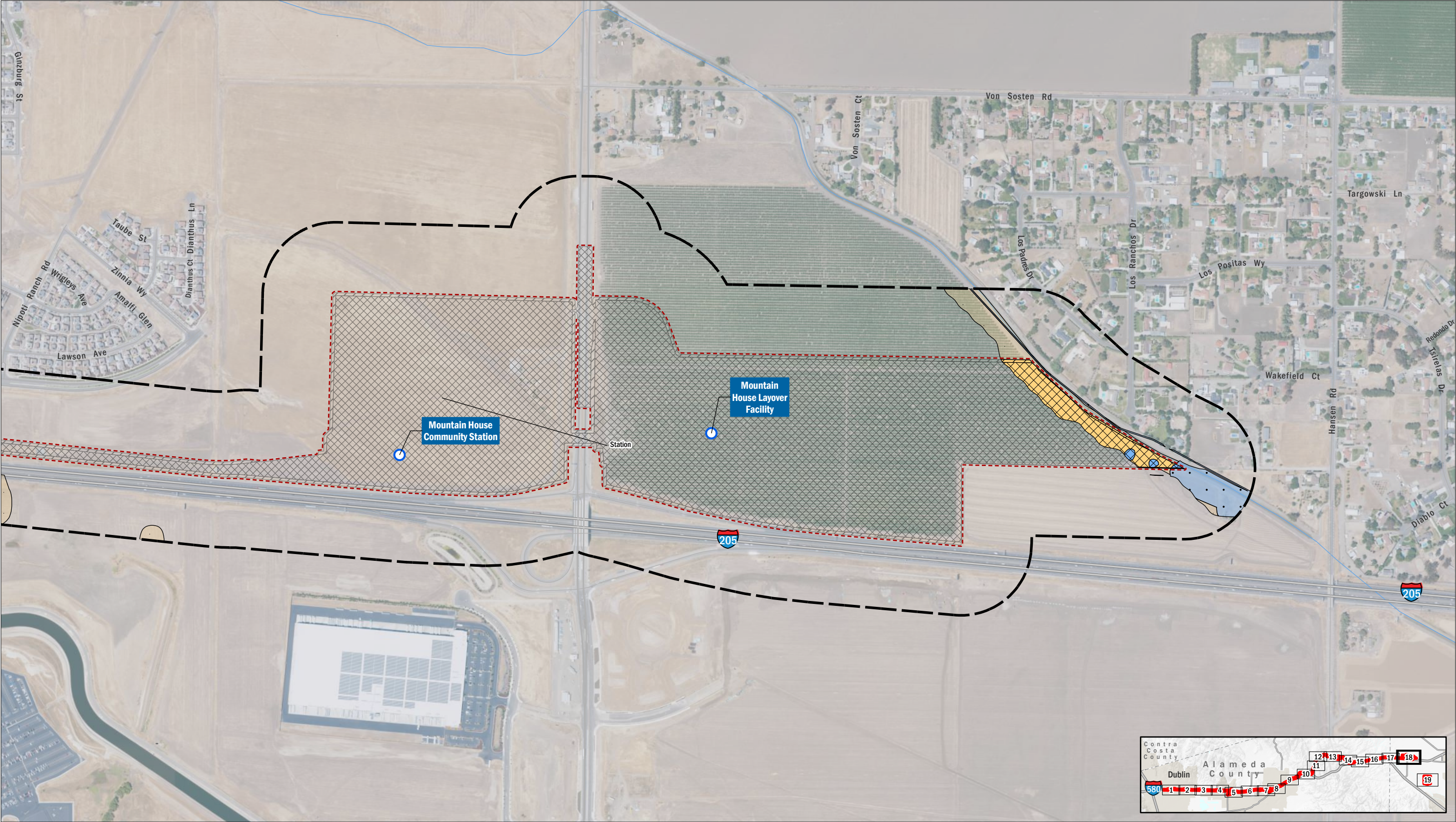


FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 17 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

0 1,000
Feet

○ Proposed Valley Link Stations and Facilities

--- Proposed Project Footprint

--- Action Area

Habitat Type (Direct Effects)

Wetlands and Waters (Breeding)

Upland

Habitat Type (Indirect Effects)

Wetlands and Waters (Breeding)

Upland

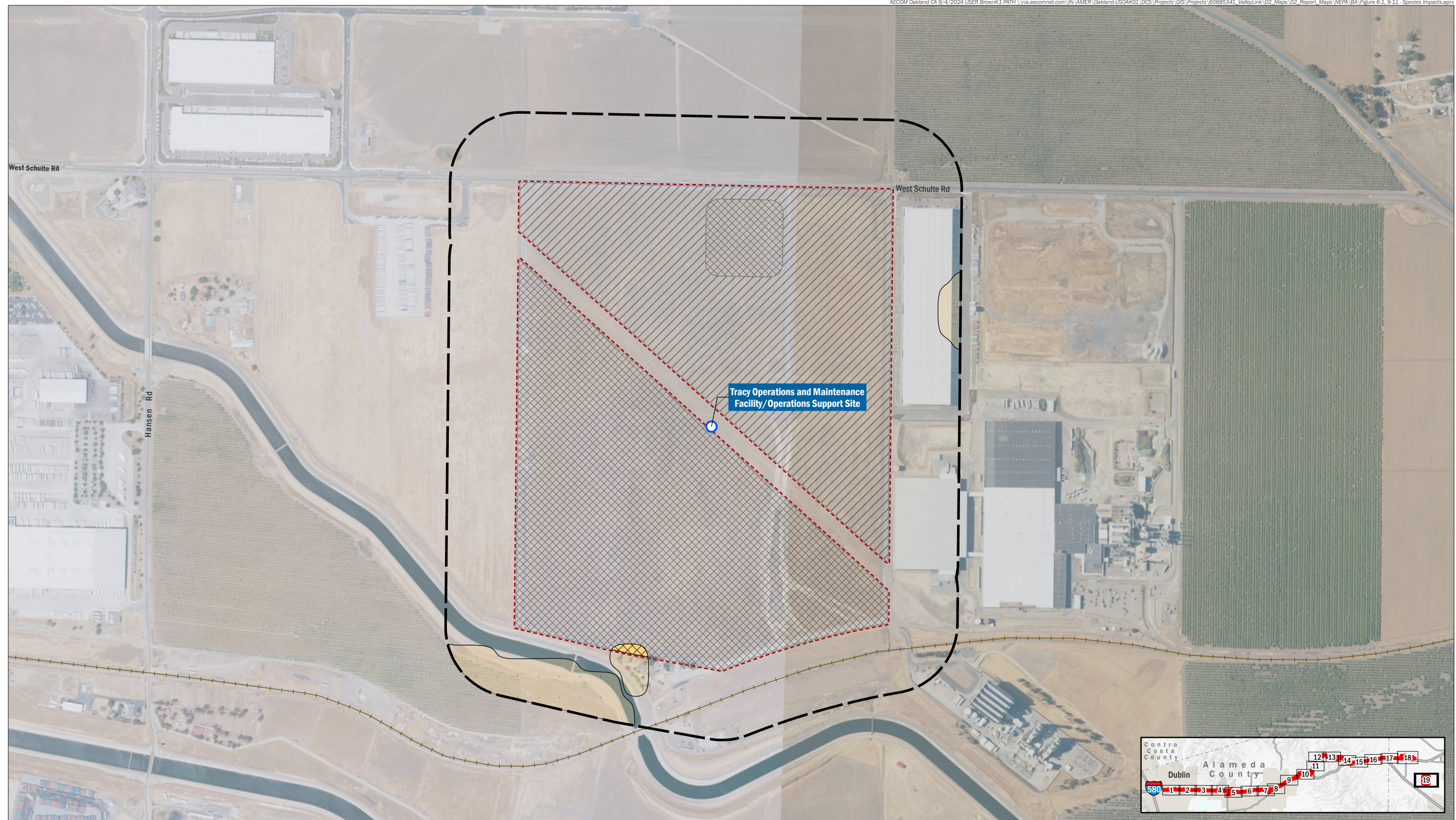
Effects

Direct Permanent Effect

Direct Temporary Effect

Indirect Effect

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 18 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 7
California Tiger Salamander (Ambystoma californiense) Potential Habitat
Sheet 19 of 19

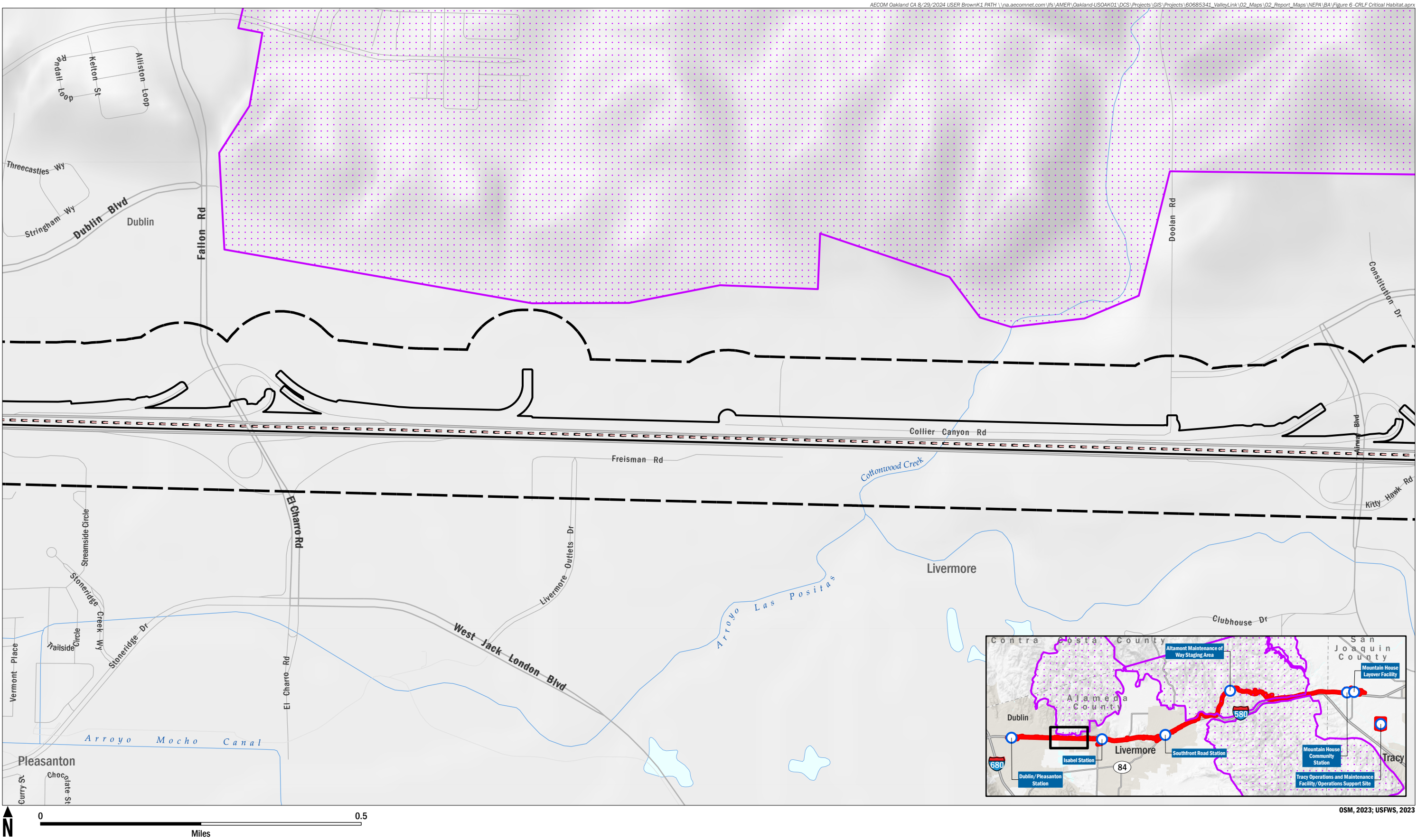
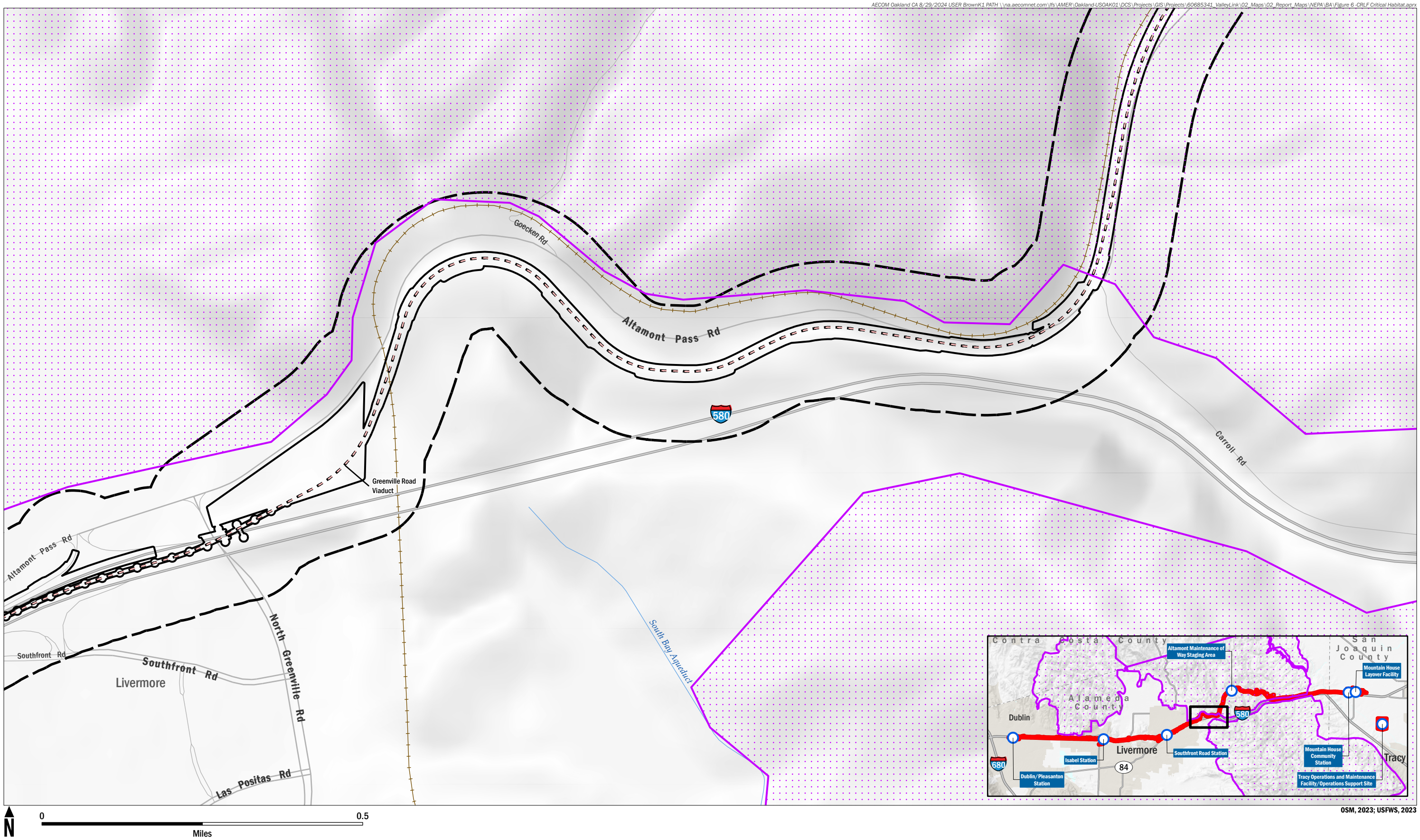
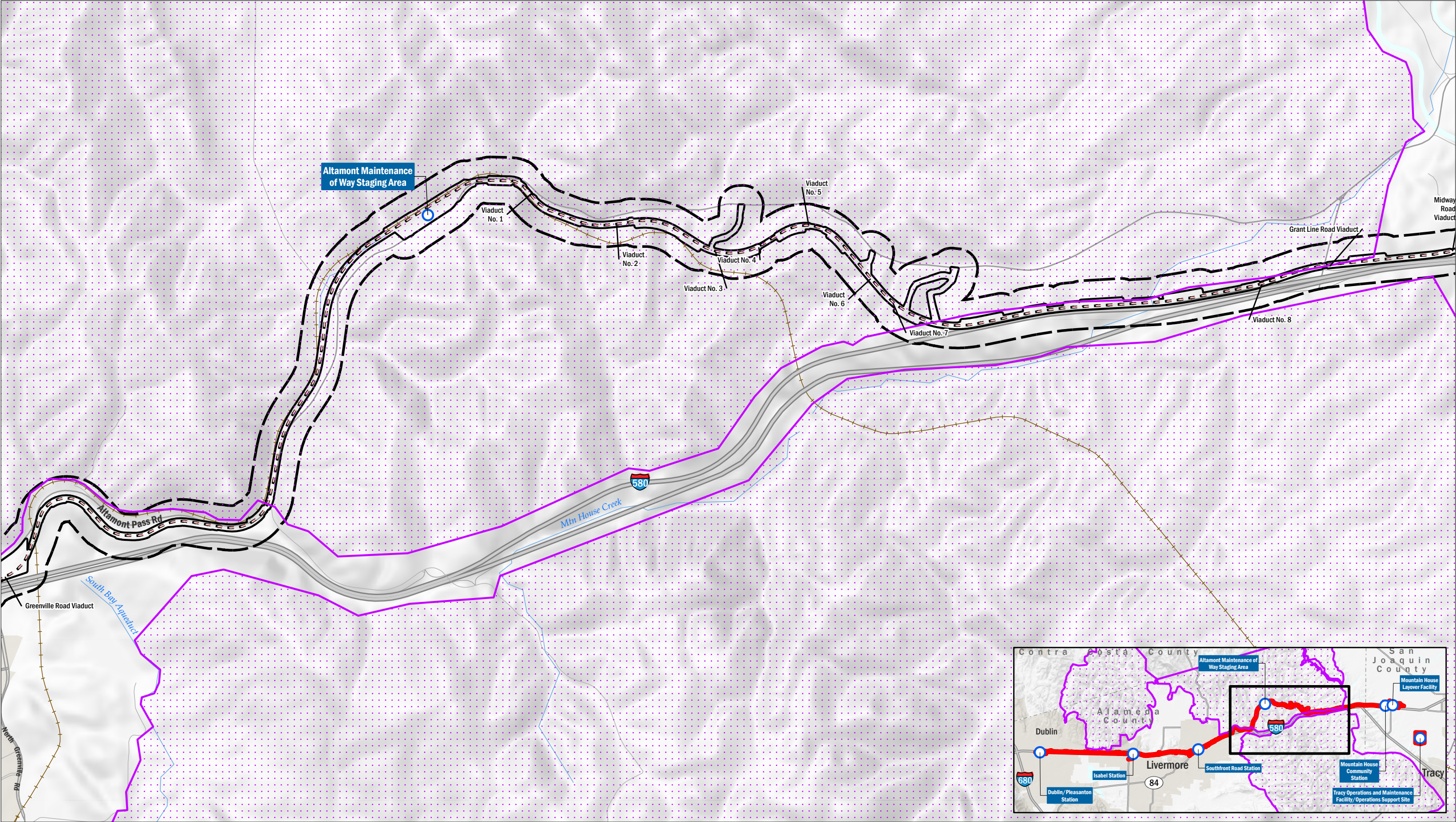
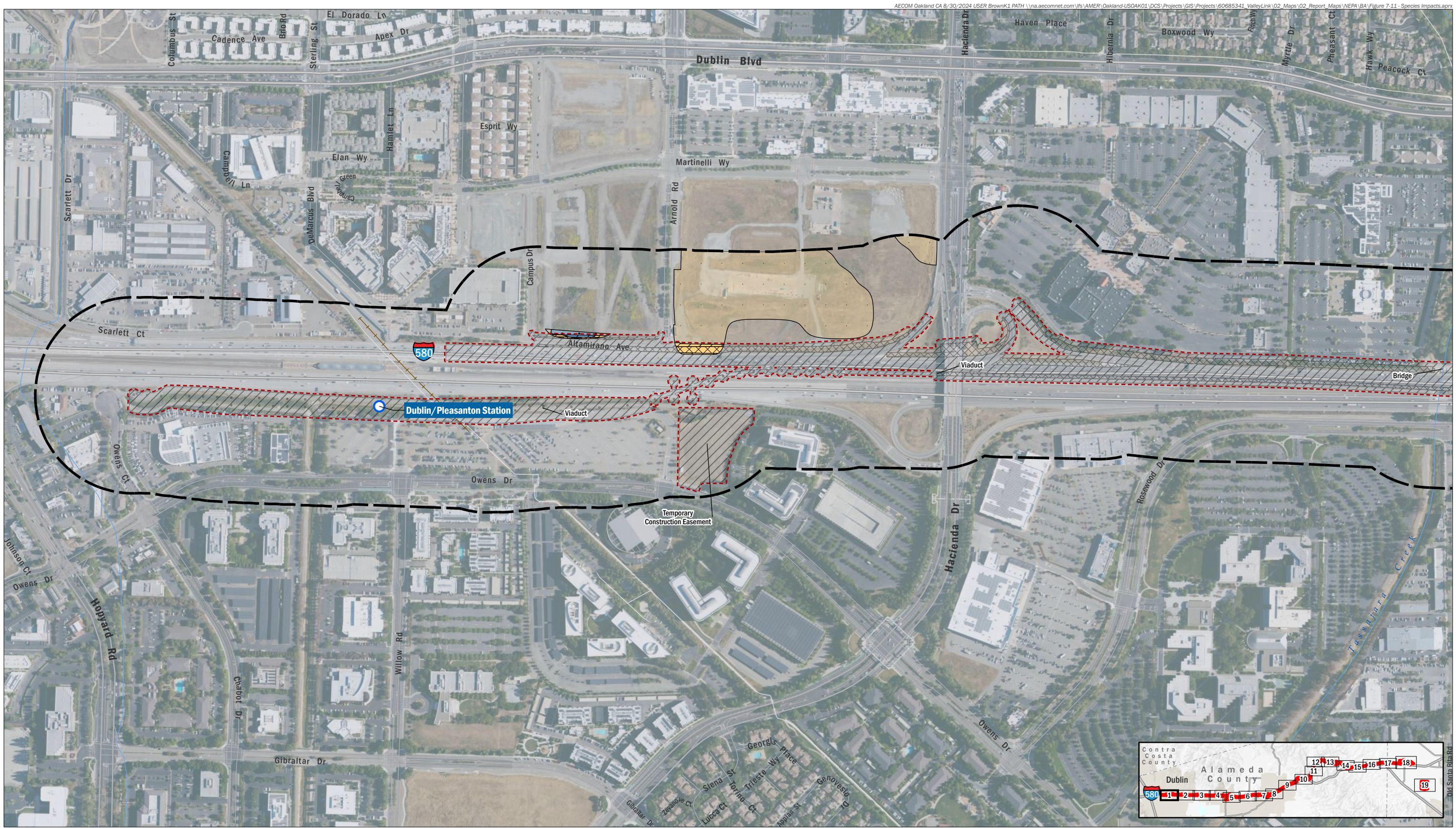


FIGURE 8
California Red-Legged Frog Critical Habitat
Sheet 1 of 3





OSM, 2023; USFWS, 2023



AECOM
Valley Link Rail Project

0 1000
Feet

○ Proposed Valley Link Stations and Facilities
- - - Proposed Project Footprint
- - - Action Area

Habitat Type (Direct Effects)
Wetlands and Waters (Breeding)
Upland

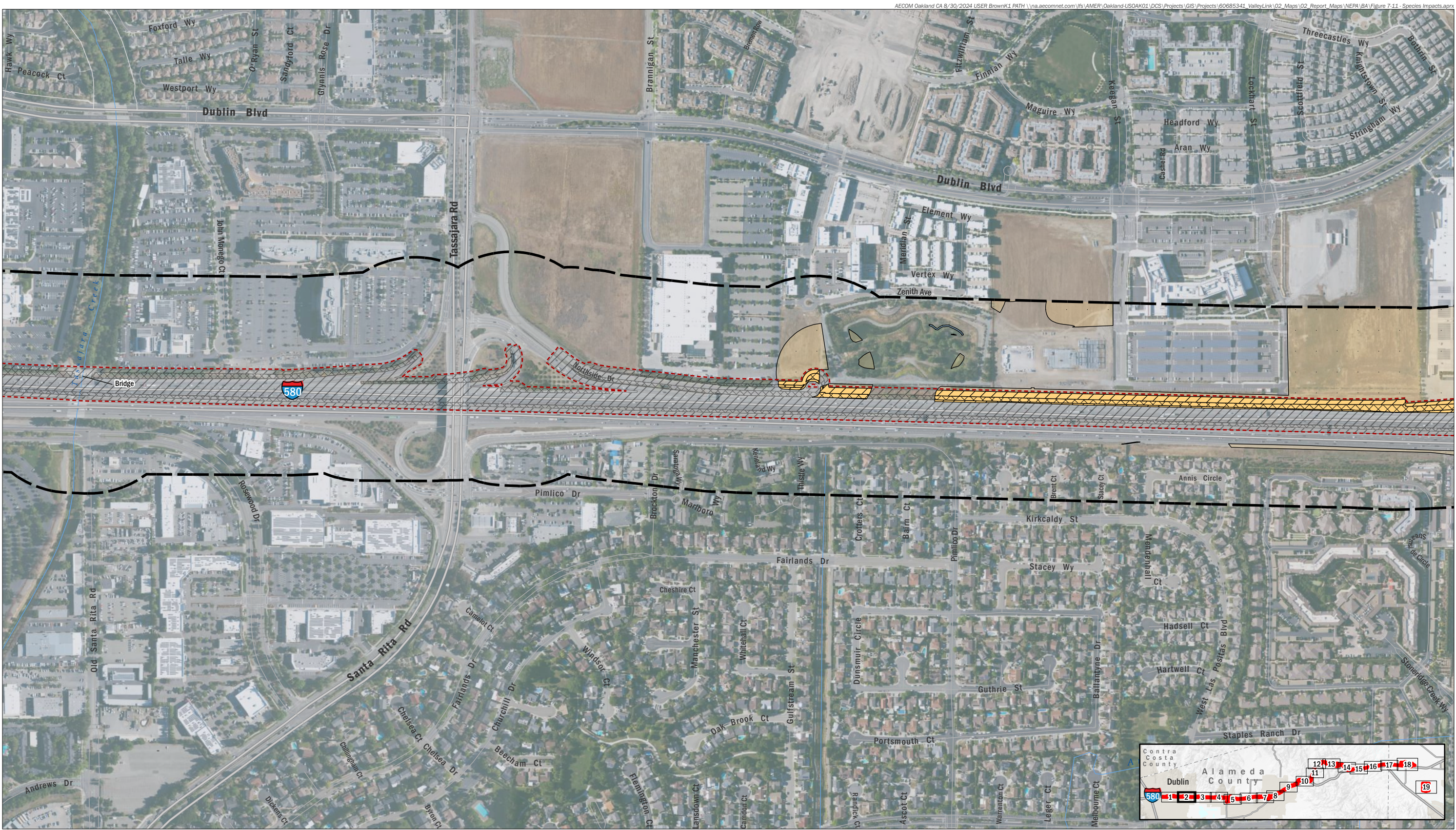
Habitat Type (Indirect Effects)
Wetlands and Waters (Breeding)
Upland

Effects
Direct Permanent Effect
Direct Temporary Effect
Indirect Effect

Contra Costa County
Alameda County
Dublin
San Francisco Bay Area

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 1 of 19



0 1,000
Feet

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 2 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

0

1,000

Feet

Proposed Project Footprint

Action Area

Habitat Type (Direct Effects)

Wetlands and Waters (Breeding)

Upland

Habitat Type (Indirect Effects)

Wetlands and Waters (Breeding)

Upland

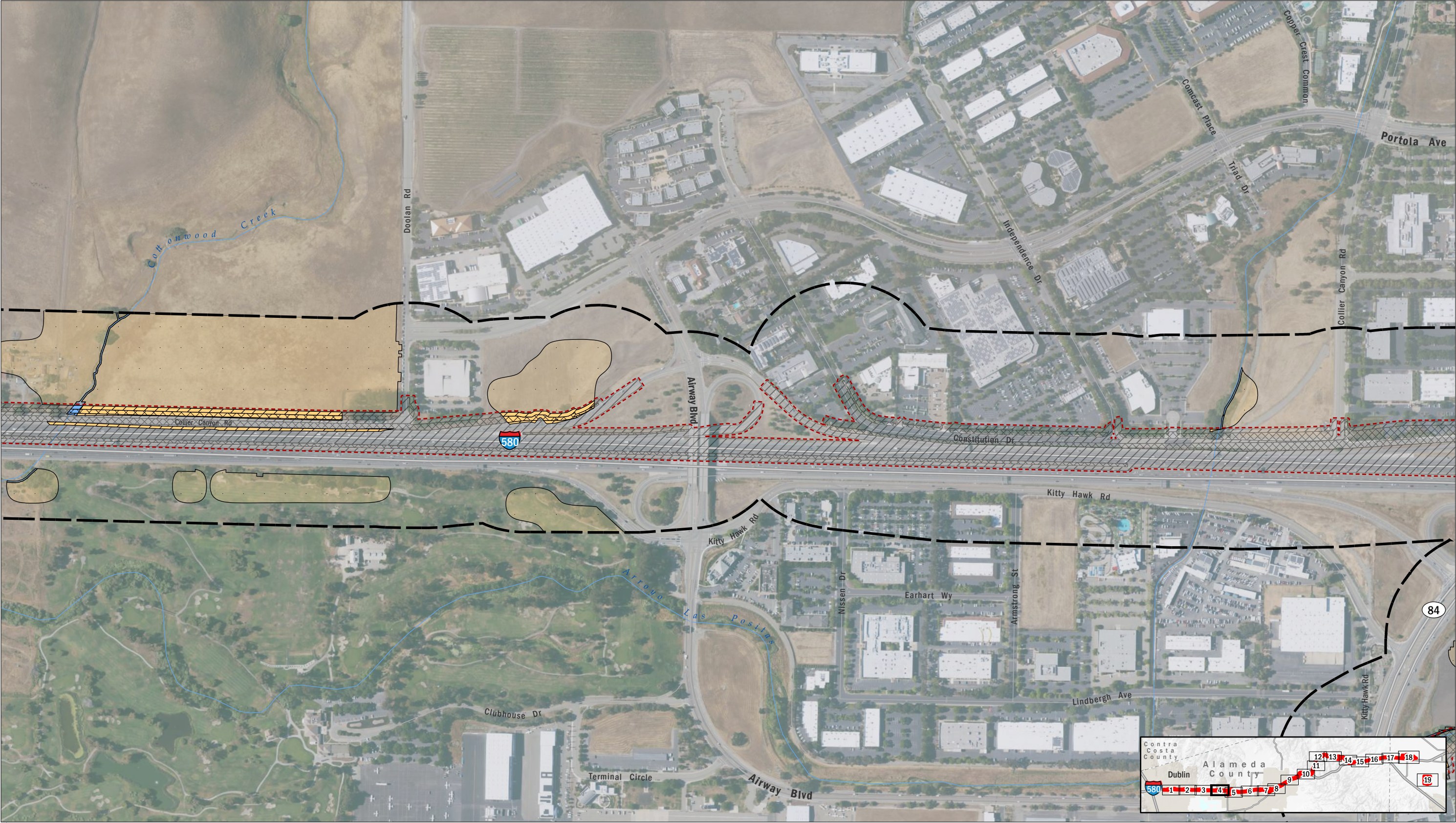
Effects

Direct Permanent Effect

Direct Temporary Effect

Indirect Effect

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 3 of 19



AECOM
Valley Link Rail Project

- Proposed Project Footprint
- Action Area

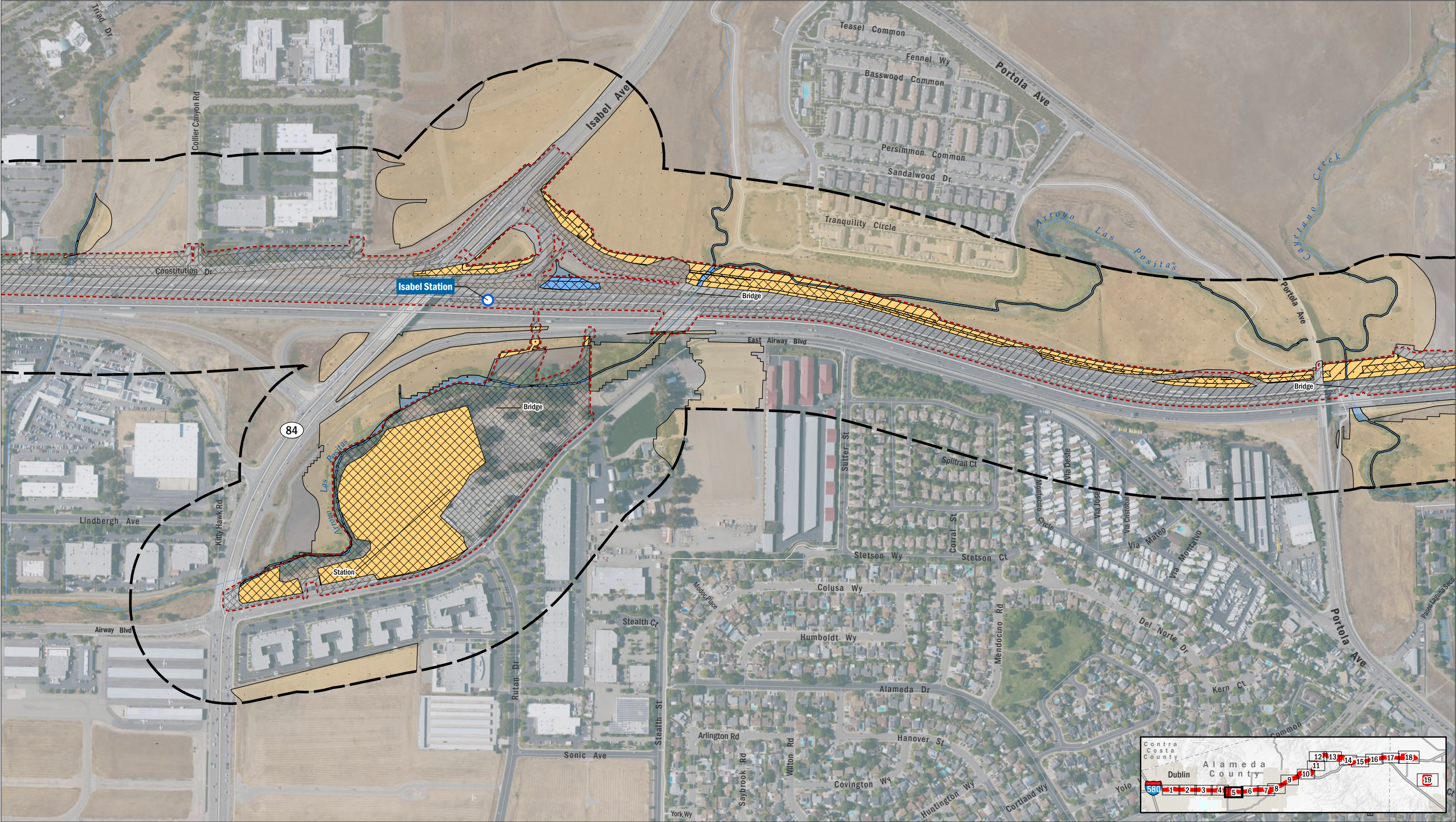
- Habitat Type (Direct Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Habitat Type (Indirect Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Effects**
- Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 4 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



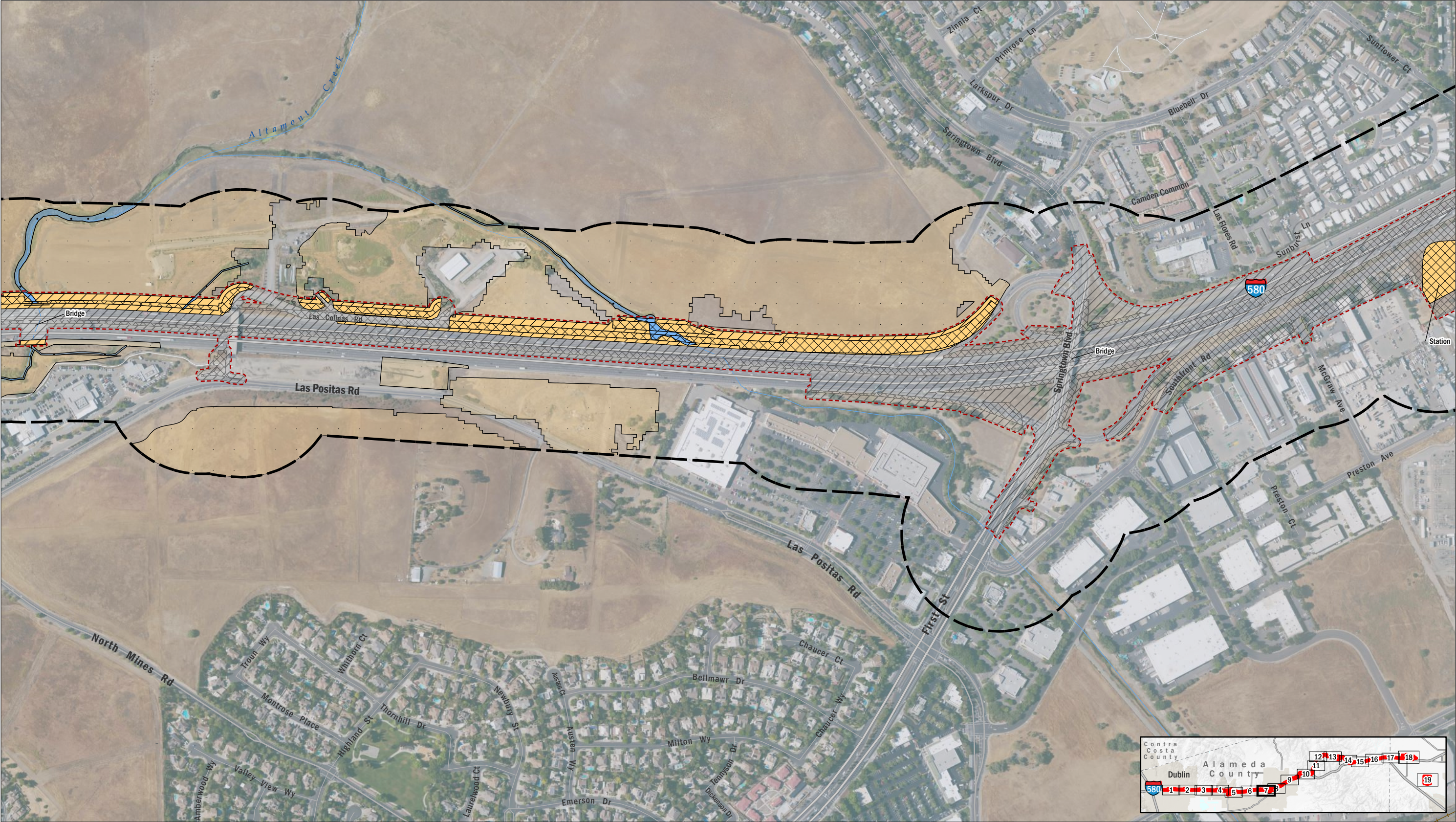
FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 5 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

- | | | | |
|----------------------------|--------------------------------------|--|-------------------------|
| Proposed Project Footprint | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Action Area | Wetlands and Waters (Breeding) | Wetlands and Waters (Breeding) | Direct Permanent Effect |
| | Upland | Upland | Direct Temporary Effect |
| | | | Indirect Effect |

FIGURE 9
*California Red-legged Frog (*Rana draytonii*) Potential Habitat*
Sheet 6 of 19

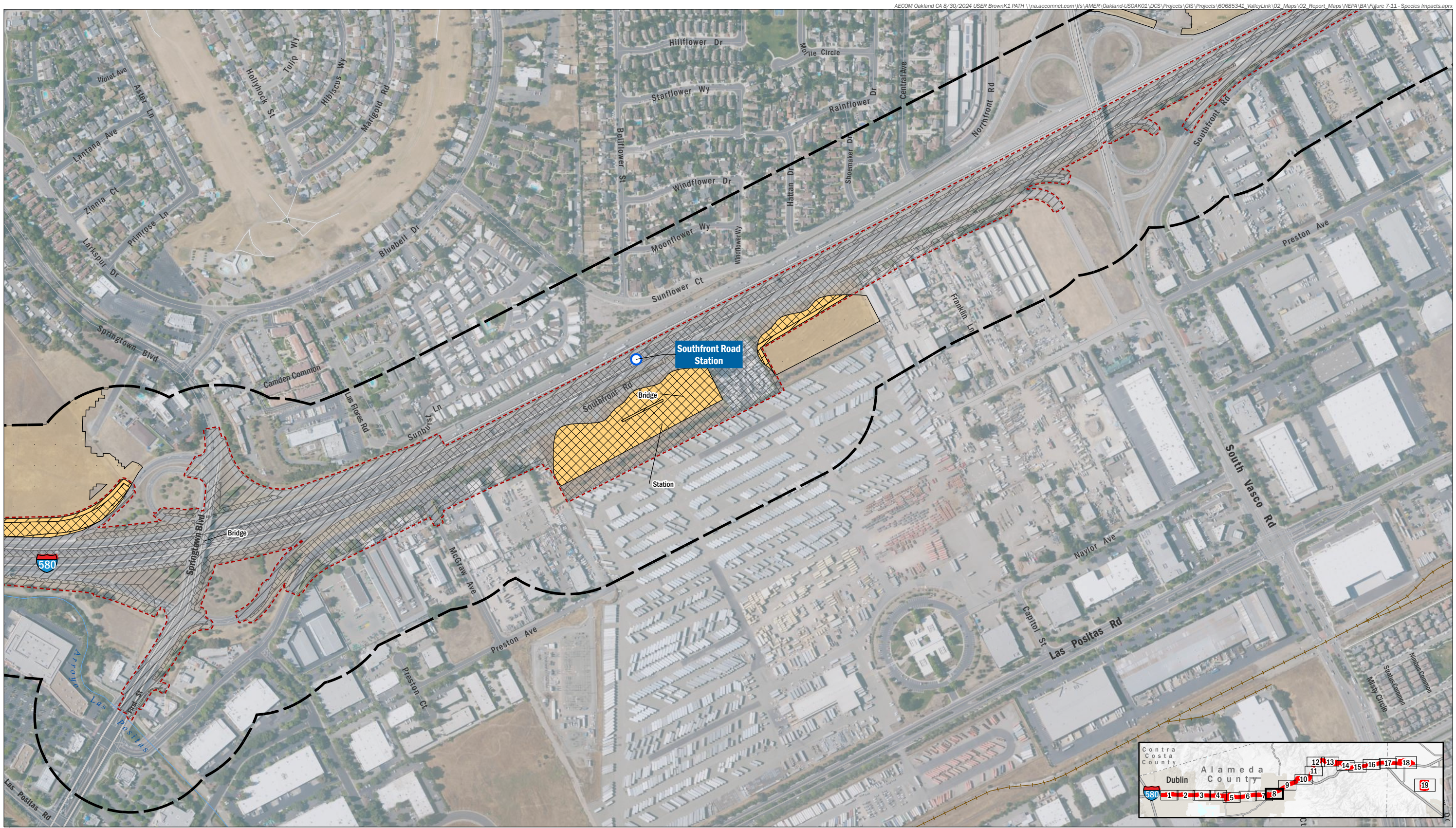


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

- | | | | |
|----------------------------|--------------------------------------|--|-------------------------|
| Proposed Project Footprint | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Action Area | Wetlands and Waters (Breeding) | Wetlands and Waters (Breeding) | Direct Permanent Effect |
| | Upland | Upland | Direct Temporary Effect |
| | | | Indirect Effect |

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 7 of 19



0 1,000
Feet

AECOM
Valley Link Rail Project

- Proposed Valley Link Stations and Facilities
- Proposed Project Footprint
- Action Area

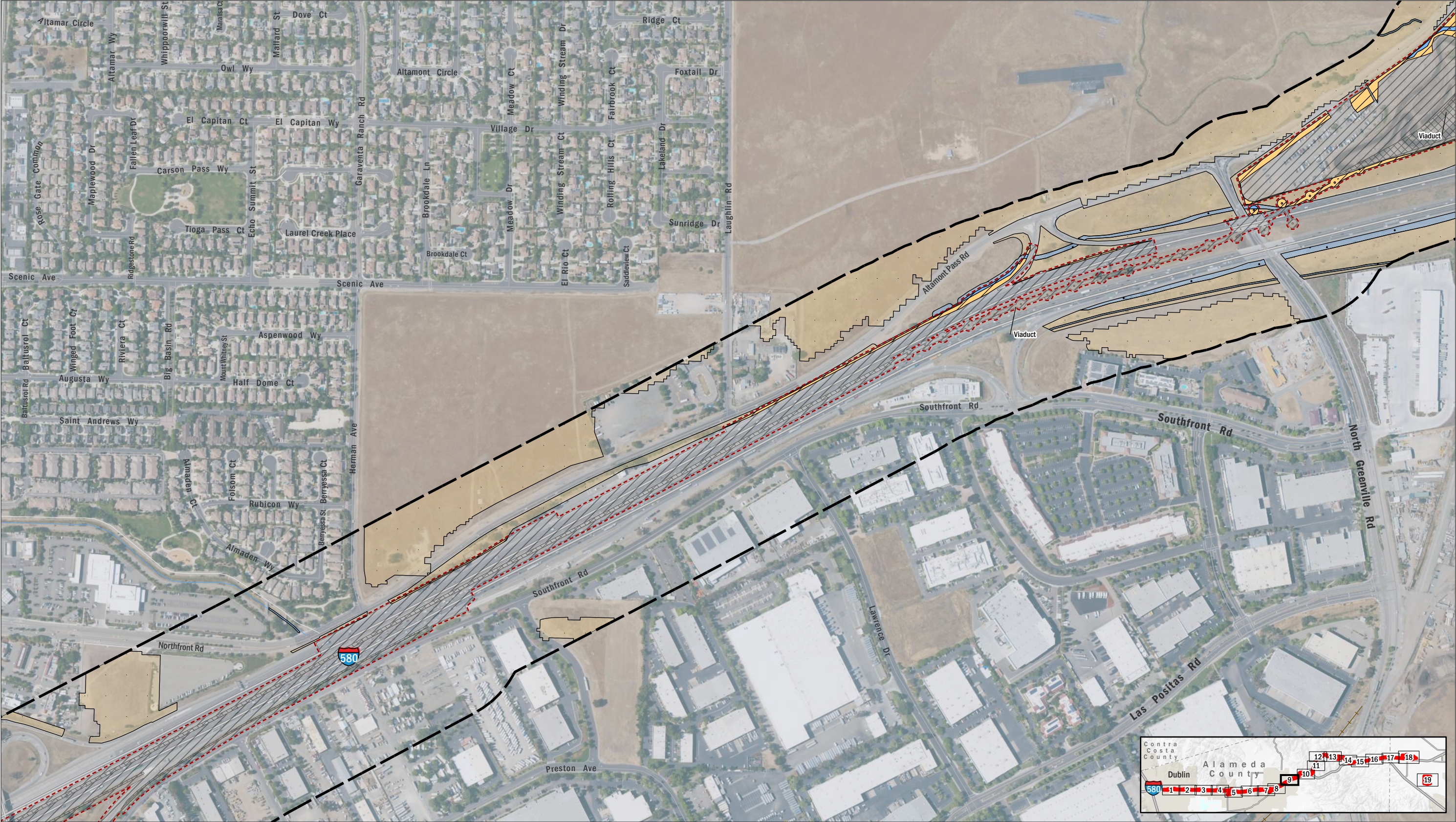
- Habitat Type (Direct Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Habitat Type (Indirect Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Effects**
- Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 8 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

N

01000

Feet

AECOM

Valley Link Rail Project

- Proposed Project Footprint

Action Area
- Habitat Type (Direct Effects)

Wetlands and Waters (Breeding)

Upland
- Habitat Type (Indirect Effects)

Wetlands and Waters (Breeding)

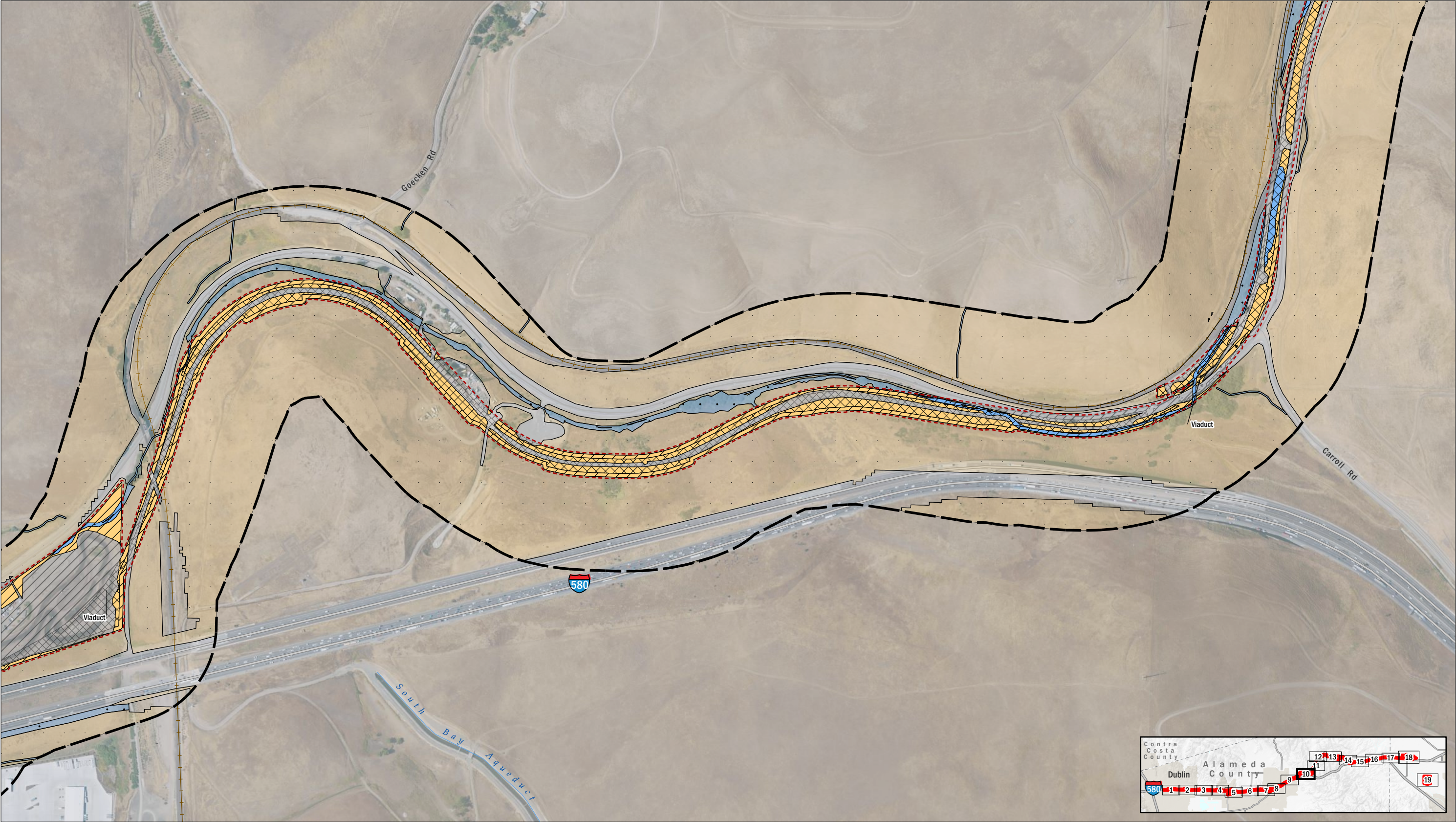
Upland
- Effects

Direct Permanent Effect

Direct Temporary Effect

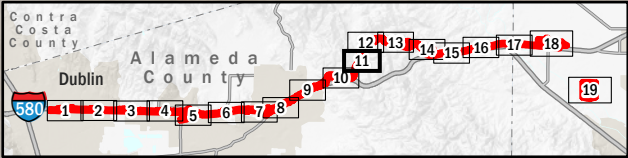
Indirect Effect

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
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AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 9
*California Red-legged Frog (*Rana draytonii*) Potential Habitat*
Sheet 10 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

N

0

1,000

Feet

AECOM

Valley Link Rail Project

- Proposed Project Footprint
- Action Area

- Habitat Type (Direct Effects)**

 - Wetlands and Waters (Breeding)
 - Upland
- Habitat Type (Indirect Effects)**

 - Wetlands and Waters (Breeding)
 - Upland

- Effects**

 - Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

FIGURE 9

California Red-legged Frog (*Rana draytonii*) Potential Habitat

Sheet 11 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

N

0

1,000

Feet

AECOM

Valley Link Rail Project

- Proposed Valley Link Stations and Facilities

Proposed Project Footprint

Action Area
- Habitat Type (Direct Effects)**

Wetlands and Waters (Breeding)

Upland
- Habitat Type (Indirect Effects)**

Wetlands and Waters (Breeding)

Upland
- Effects**

Direct Permanent Effect

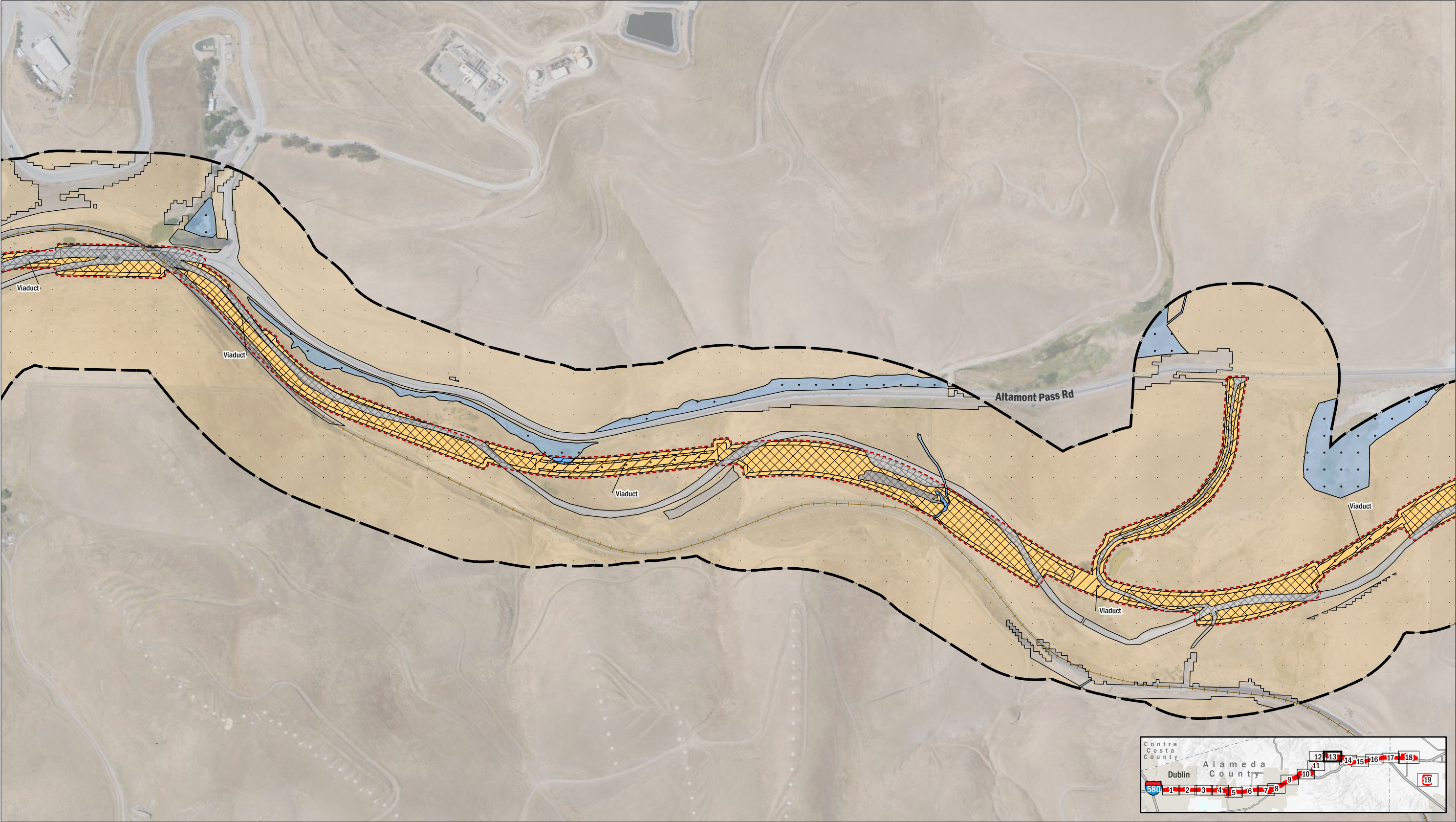
Direct Temporary Effect

Indirect Effect

FIGURE 9

California Red-legged Frog (*Rana draytonii*) Potential Habitat

Sheet 12 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

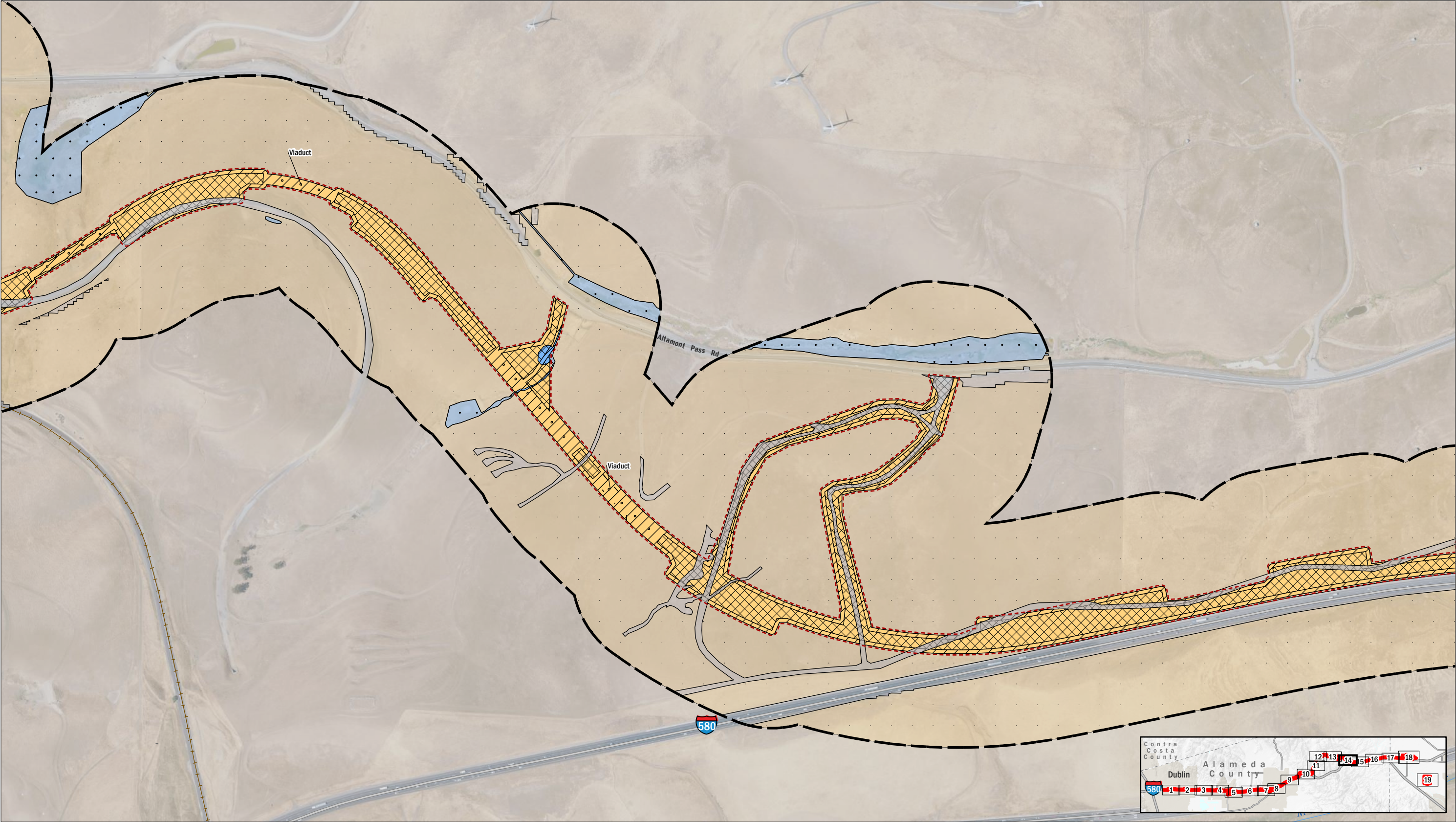
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 13 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

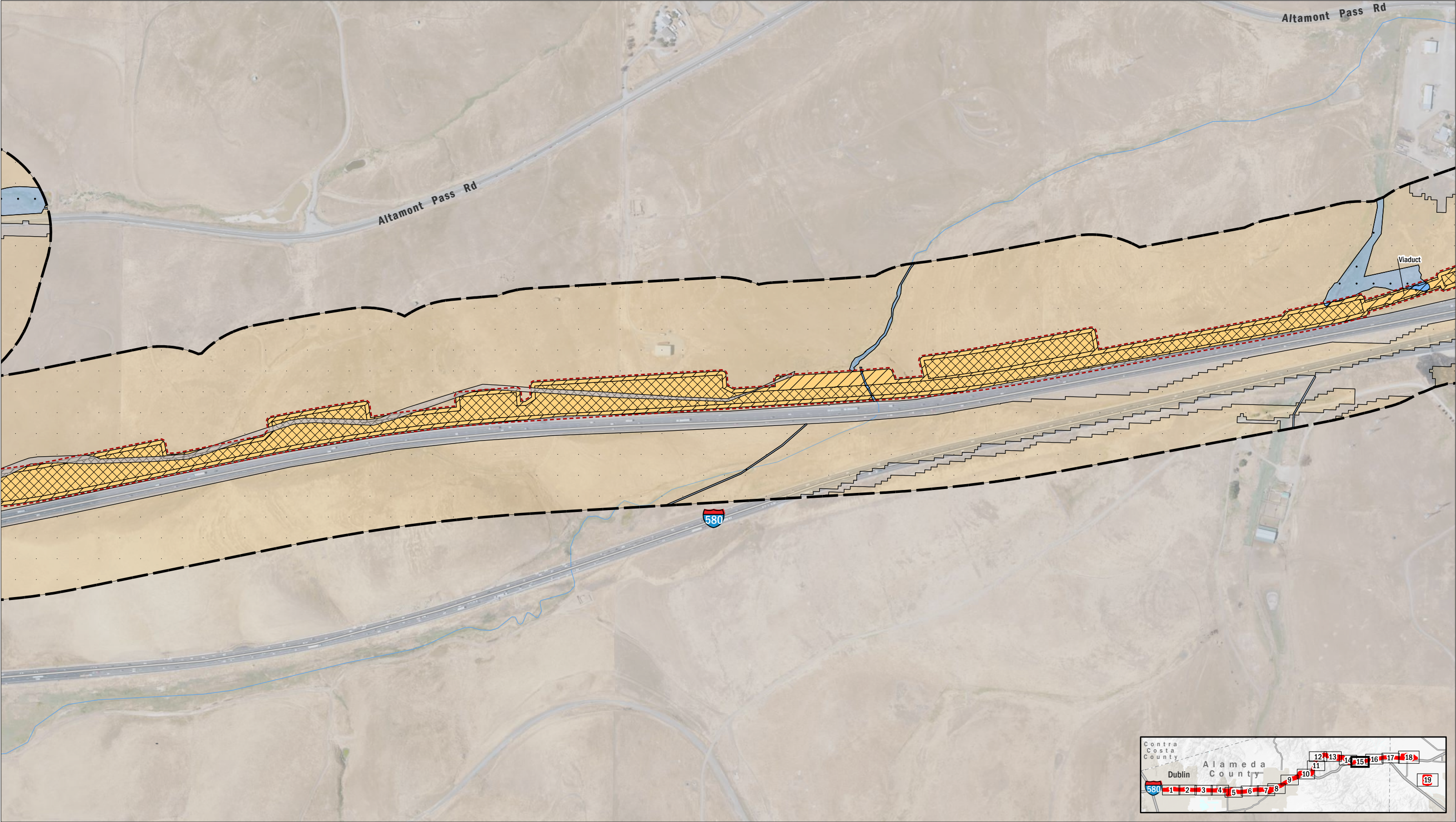
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 9
*California Red-legged Frog (*Rana draytonii*) Potential Habitat*
Sheet 14 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

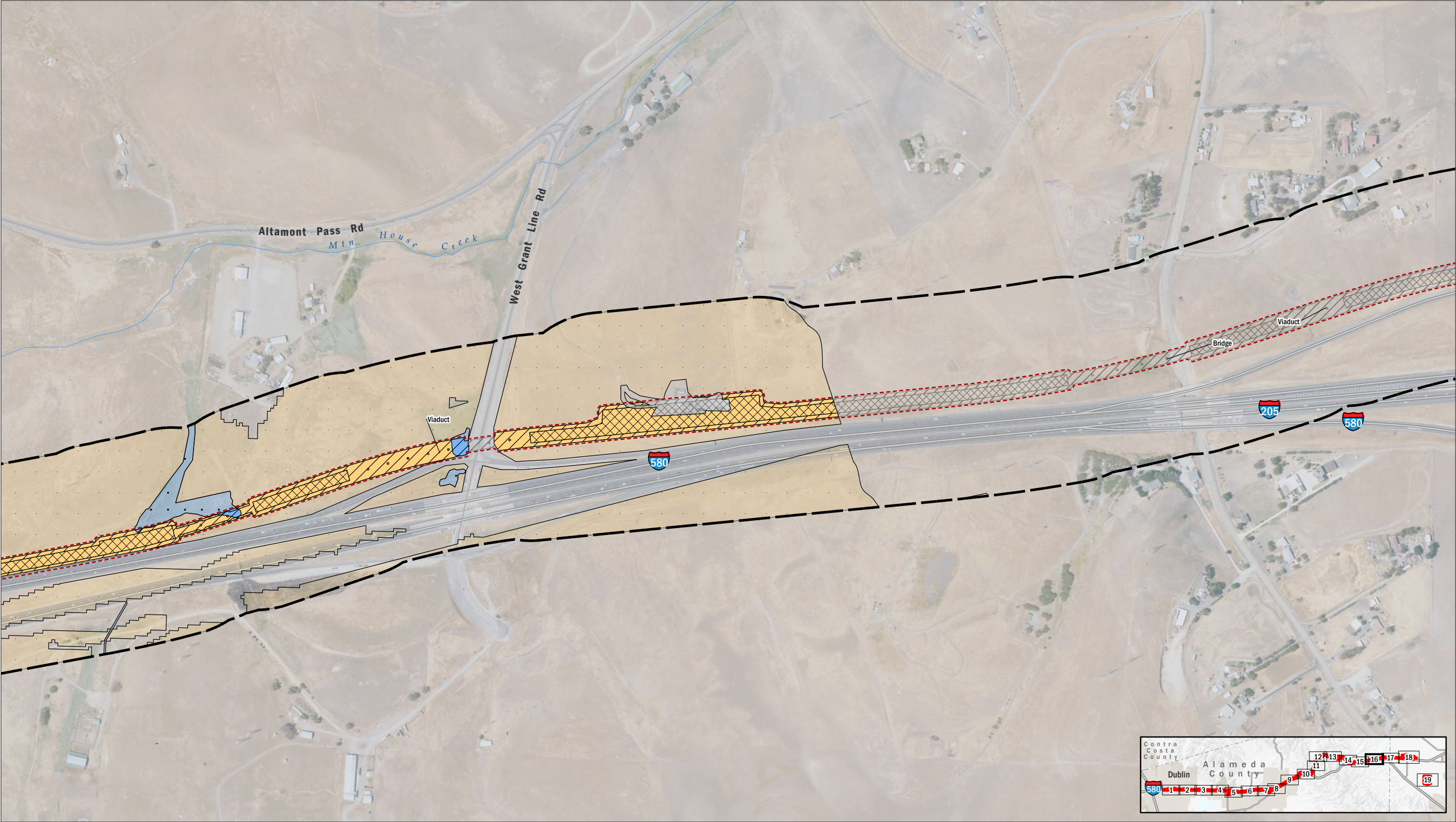
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 9
*California Red-legged Frog (*Rana draytonii*) Potential Habitat*
Sheet 15 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

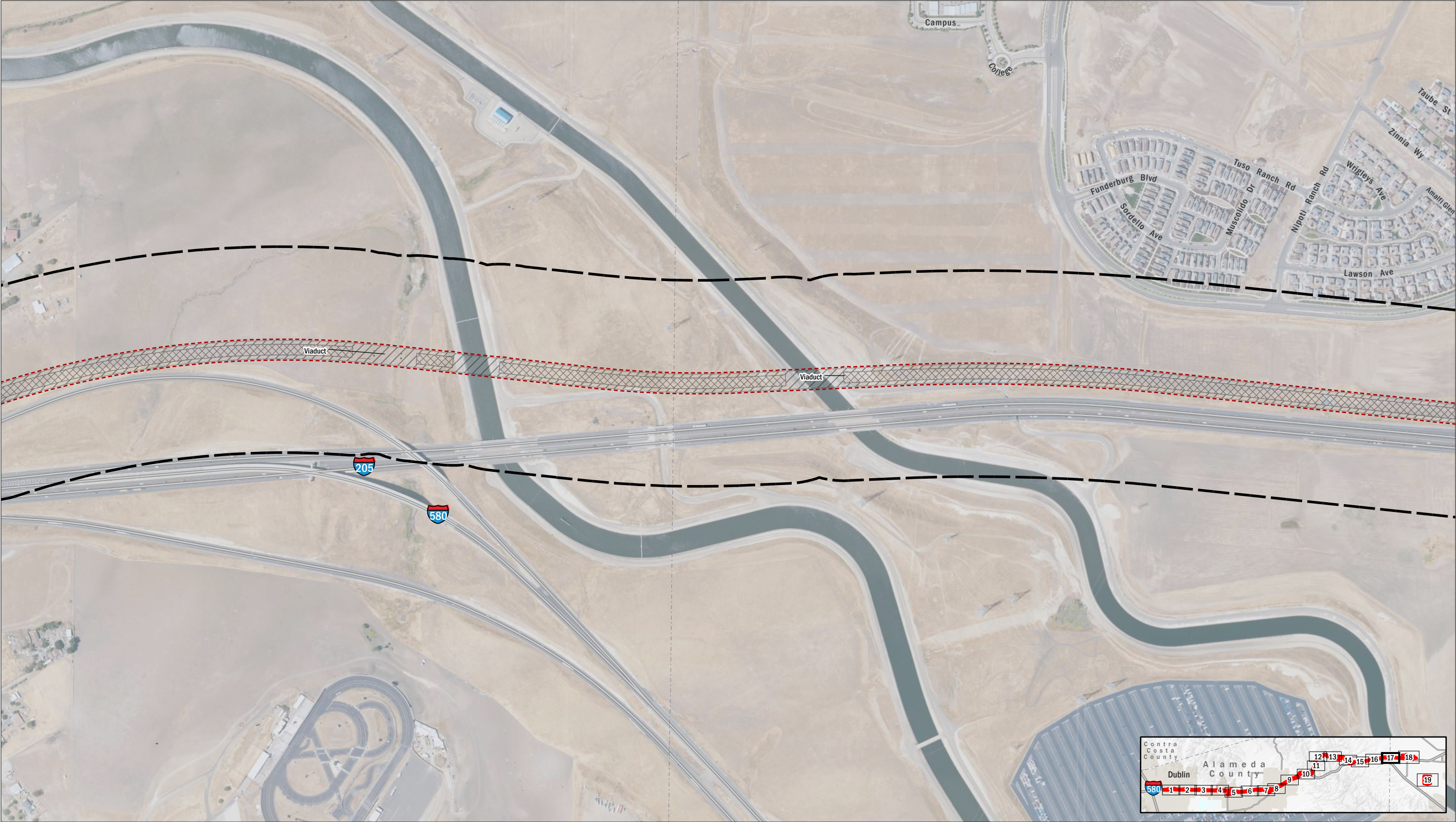
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 16 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

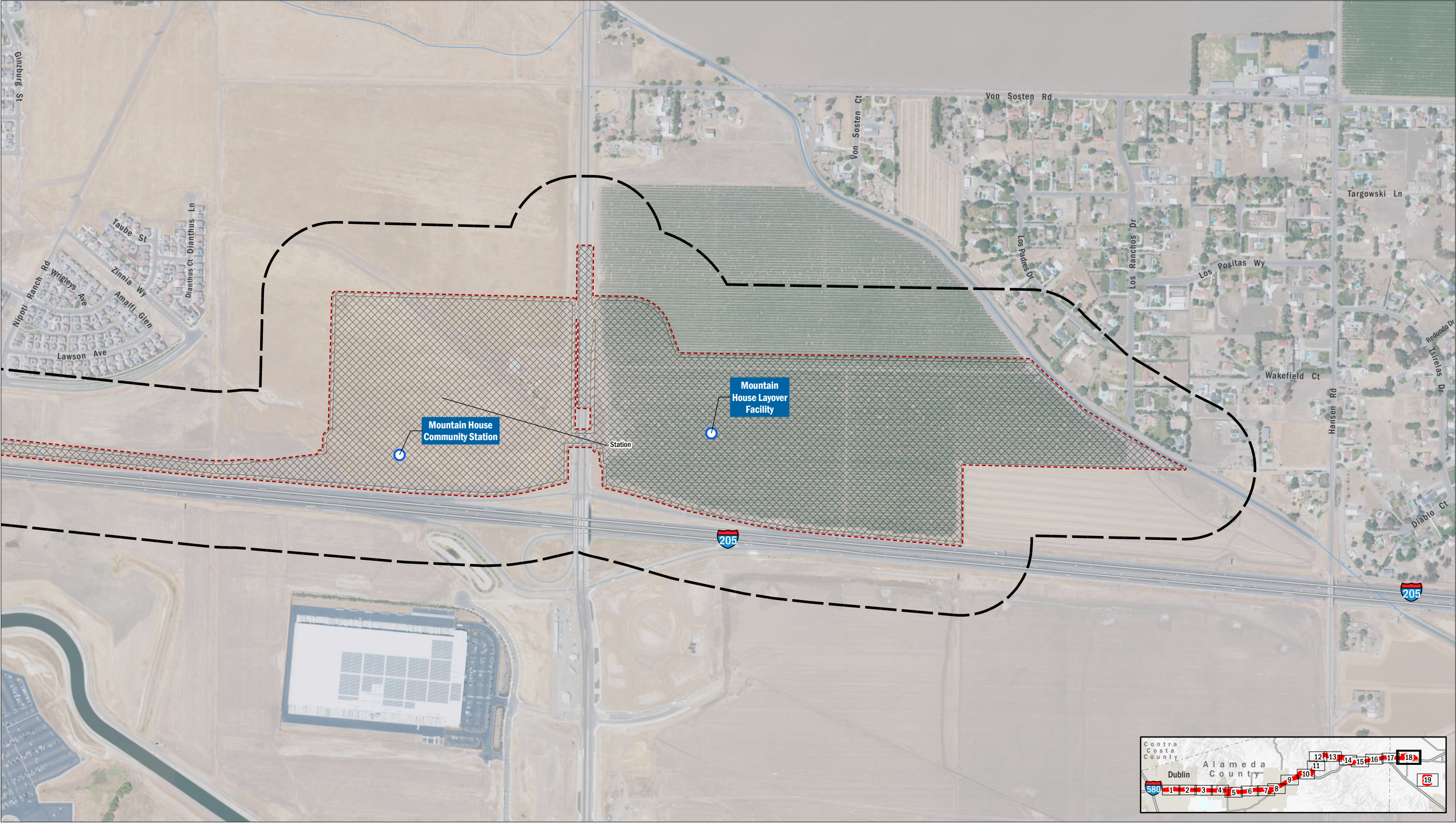
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 9
*California Red-legged Frog (*Rana draytonii*) Potential Habitat*
Sheet 17 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

0 1,000
Feet

AECOM
Valley Link Rail Project

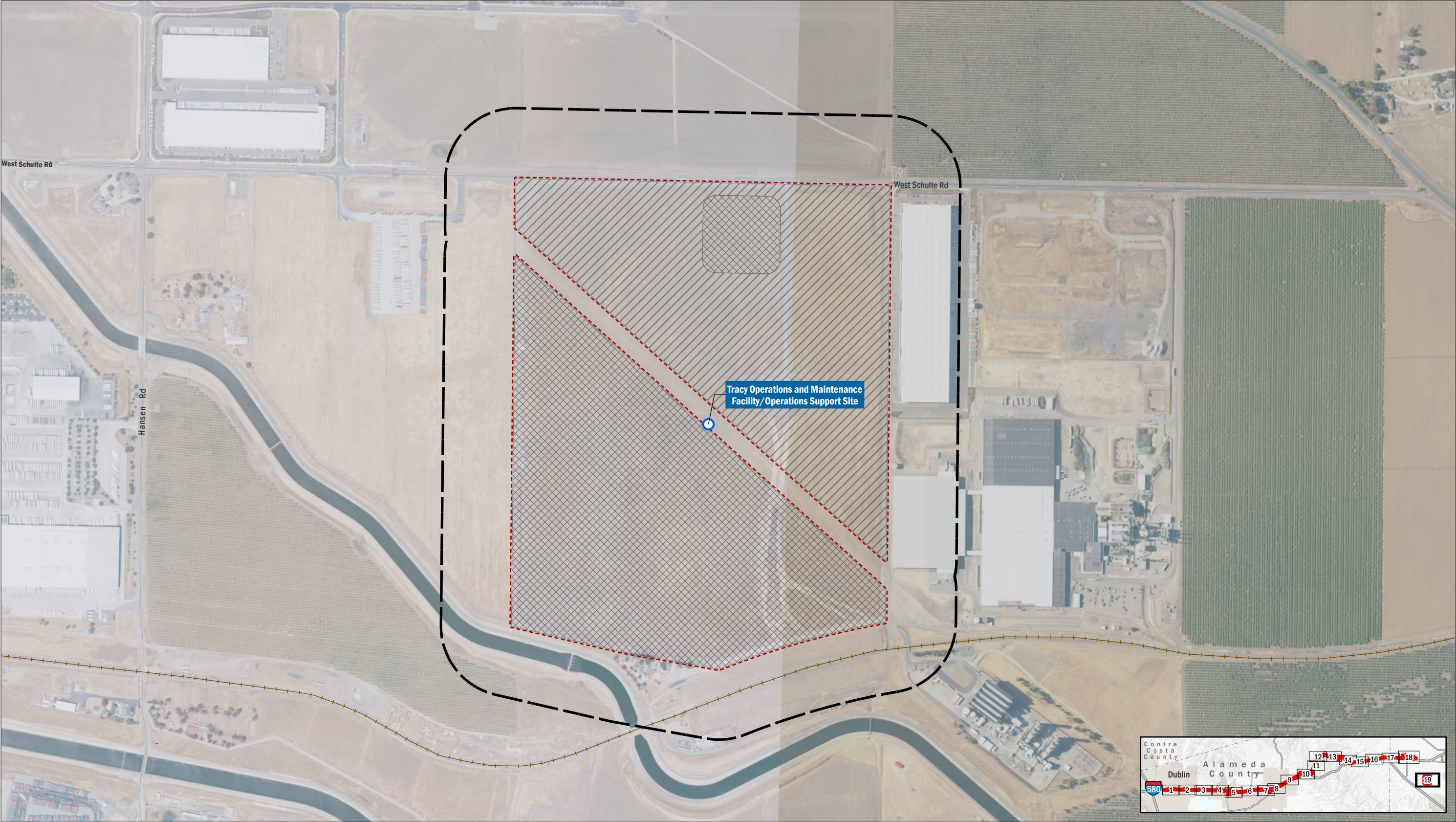
- Proposed Valley Link Stations and Facilities
- Proposed Project Footprint
- Action Area

- Habitat Type (Direct Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Habitat Type (Indirect Effects)**
- Wetlands and Waters (Breeding)
 - Upland

- Effects**
- Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 18 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

0 1,000
Feet

AECOM
Valley Link Rail Project

- Proposed Valley Link Stations and Facilities
- - - Proposed Project Footprint
- ▭ Action Area

Habitat Type (Direct Effects)

- Wetlands and Waters (Breeding)
- Upland

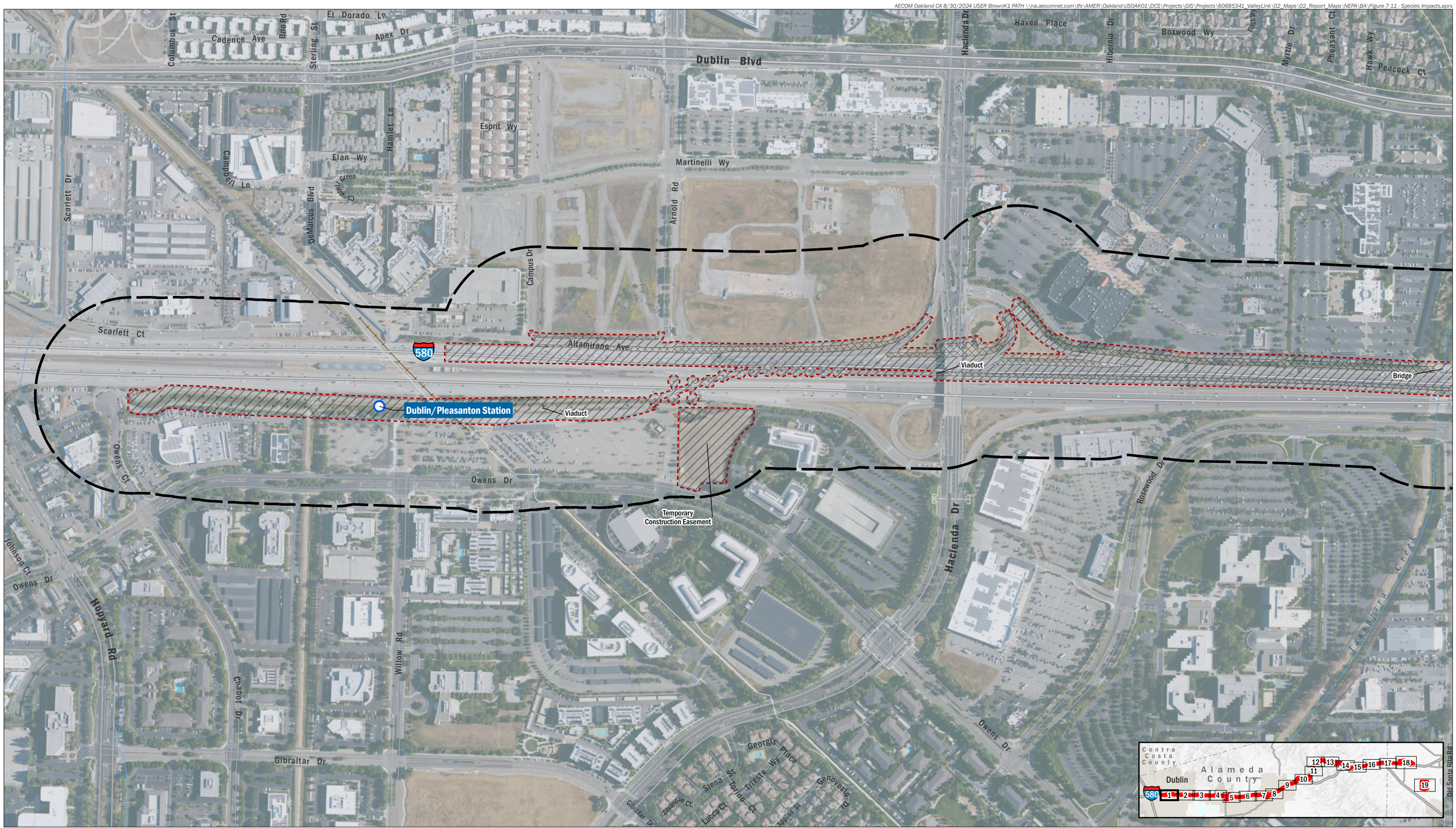
Habitat Type (Indirect Effects)

- Wetlands and Waters (Breeding)
- Upland

Effects

- ▨ Direct Permanent Effect
- ▨ Direct Temporary Effect
- ▨ Indirect Effect

FIGURE 9
California Red-legged Frog (Rana draytonii) Potential Habitat
Sheet 19 of 19



AECOM
Valley Link Rail Project

- | | | | |
|--|--------------------------------------|--|-------------------------|
| Proposed Valley Link Stations and Facilities | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Proposed Project Footprint | Wetlands and Waters (Breeding) | Wetlands and Waters (Breeding) | Direct Permanent Effect |
| Action Area | Upland | Upland | Direct Temporary Effect |
| | | | Indirect Effect |

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 1 of 19

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM

Valley Link Rail Project

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 2 of 19



0 1,000
Feet

AECOM
Valley Link Rail Project

Proposed Project Footprint

Action Area

Habitat Type (Direct Effects)

Wetlands and Waters (Breeding)

Upland

Habitat Type (Indirect Effects)

Wetlands and Waters (Breeding)

Upland

Effects

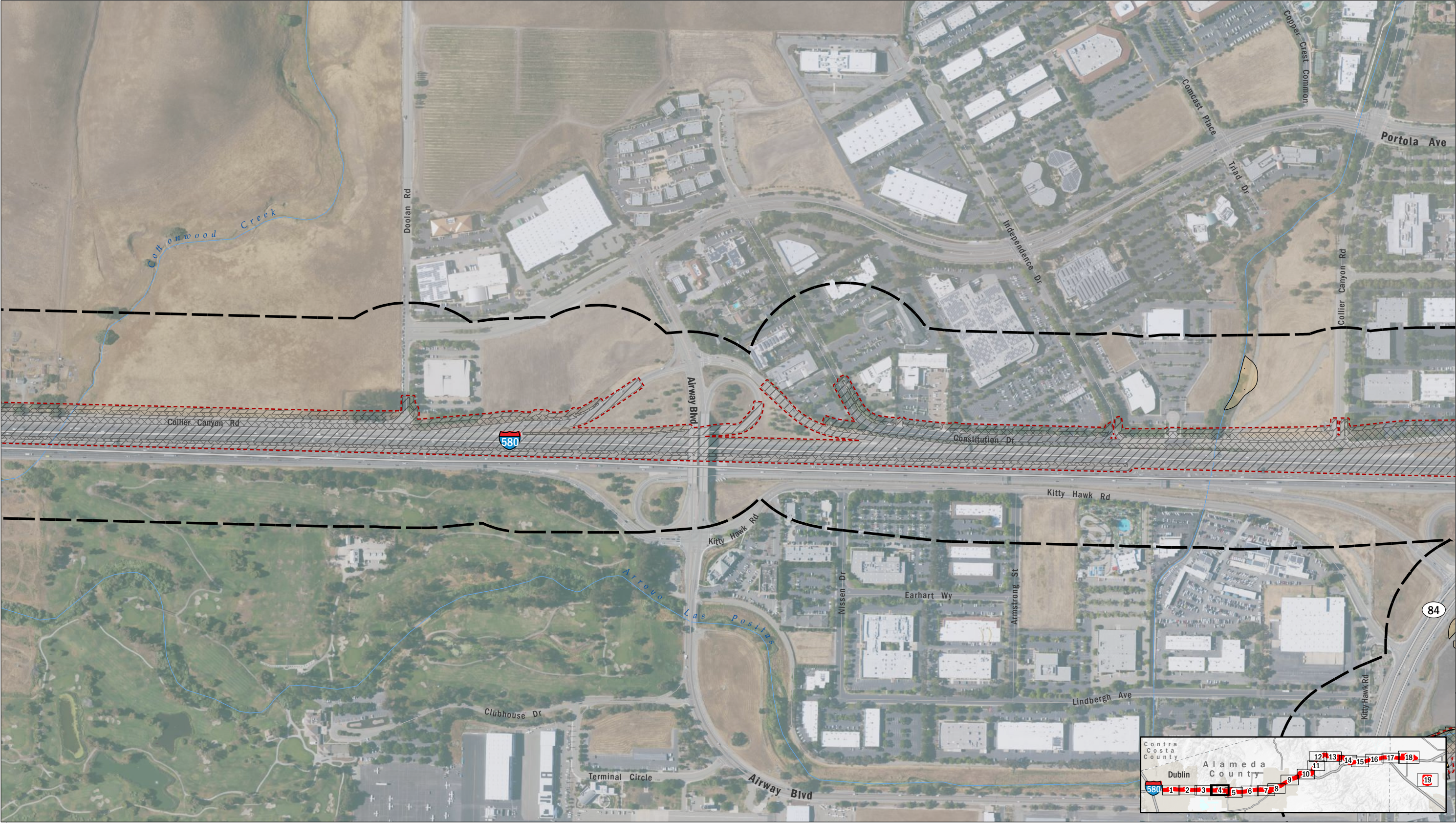
Direct Permanent Effect

Direct Temporary Effect

Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 3 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 4 of 19

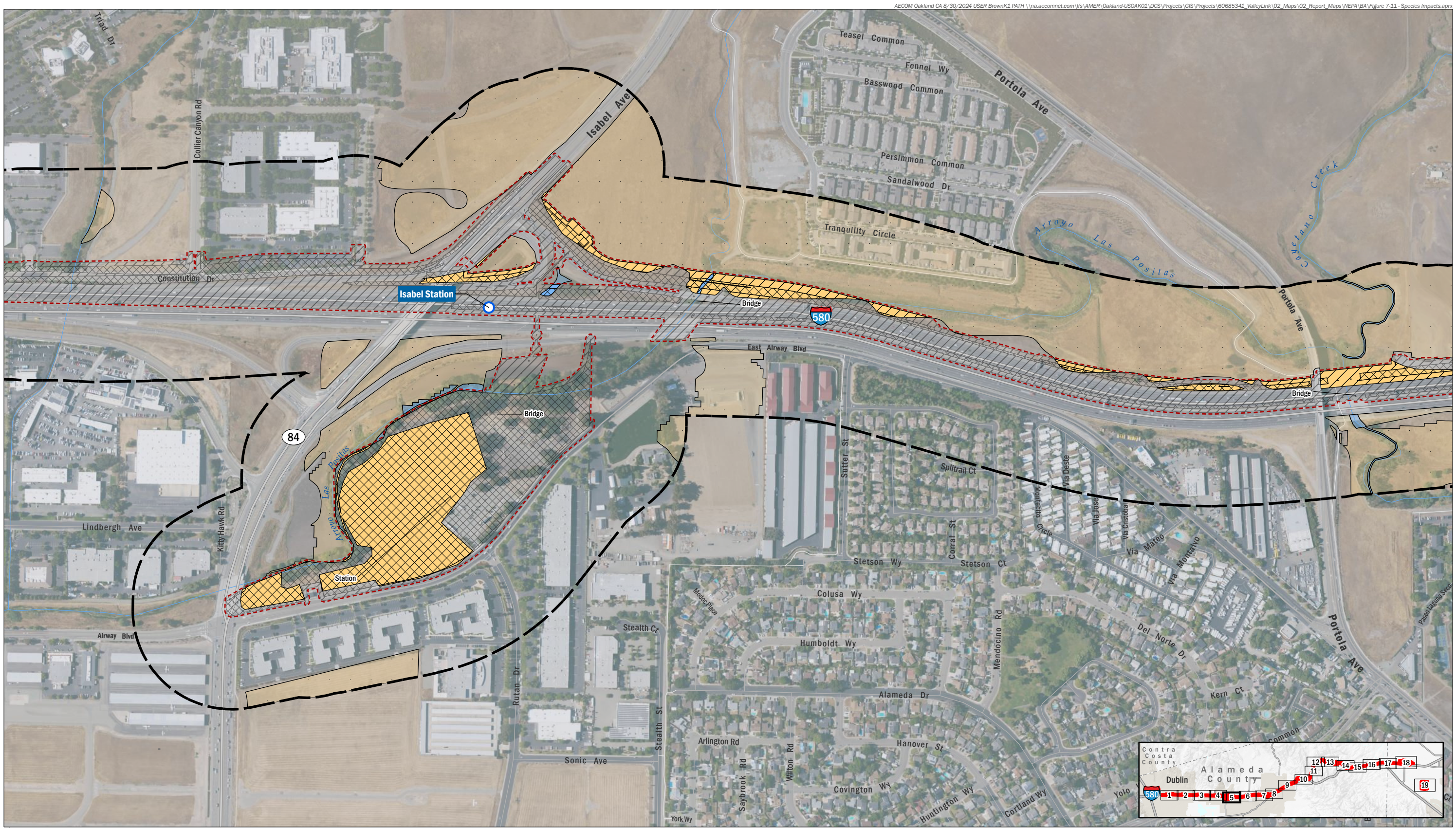


FIGURE 10
 Western Spadefoot (*Spea hammondi*) Potential Habitat
 Sheet 5 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 6 of 19

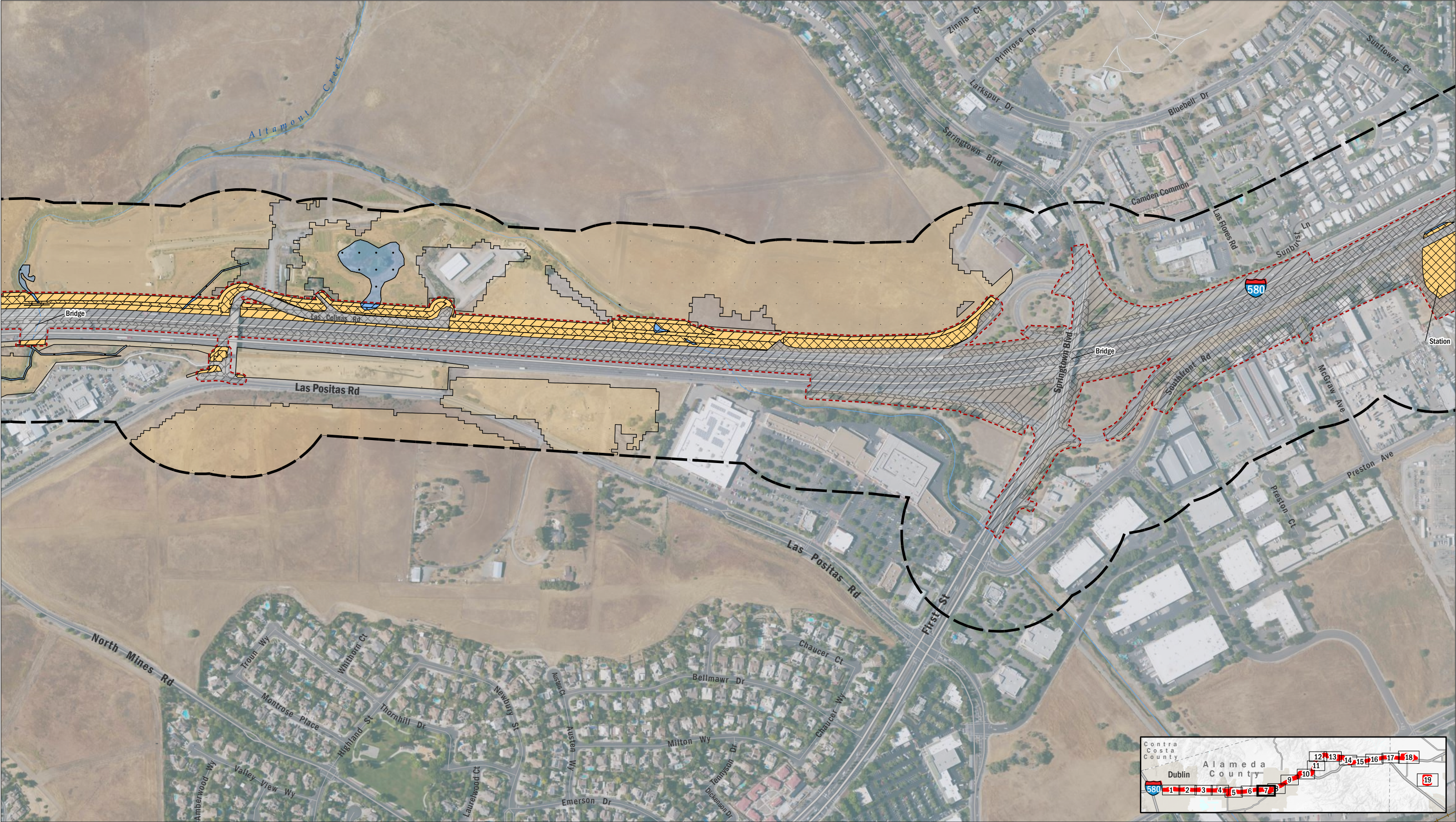
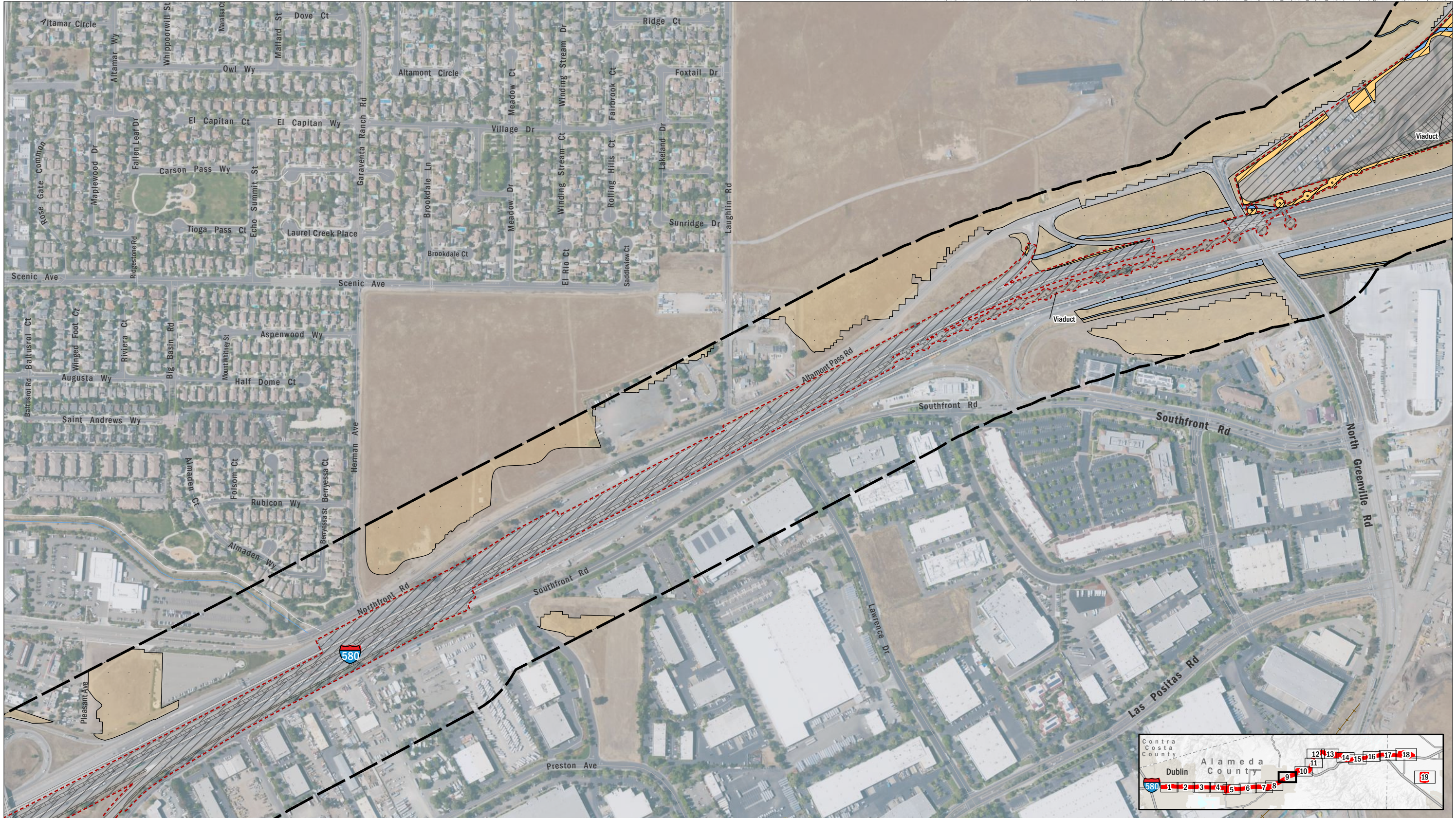


FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 7 of 19



FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 8 of 19



0 1,000
Feet

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

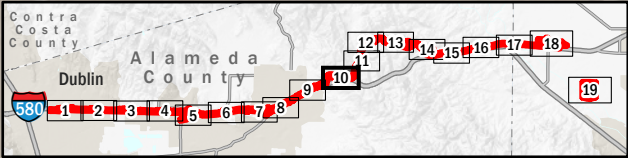
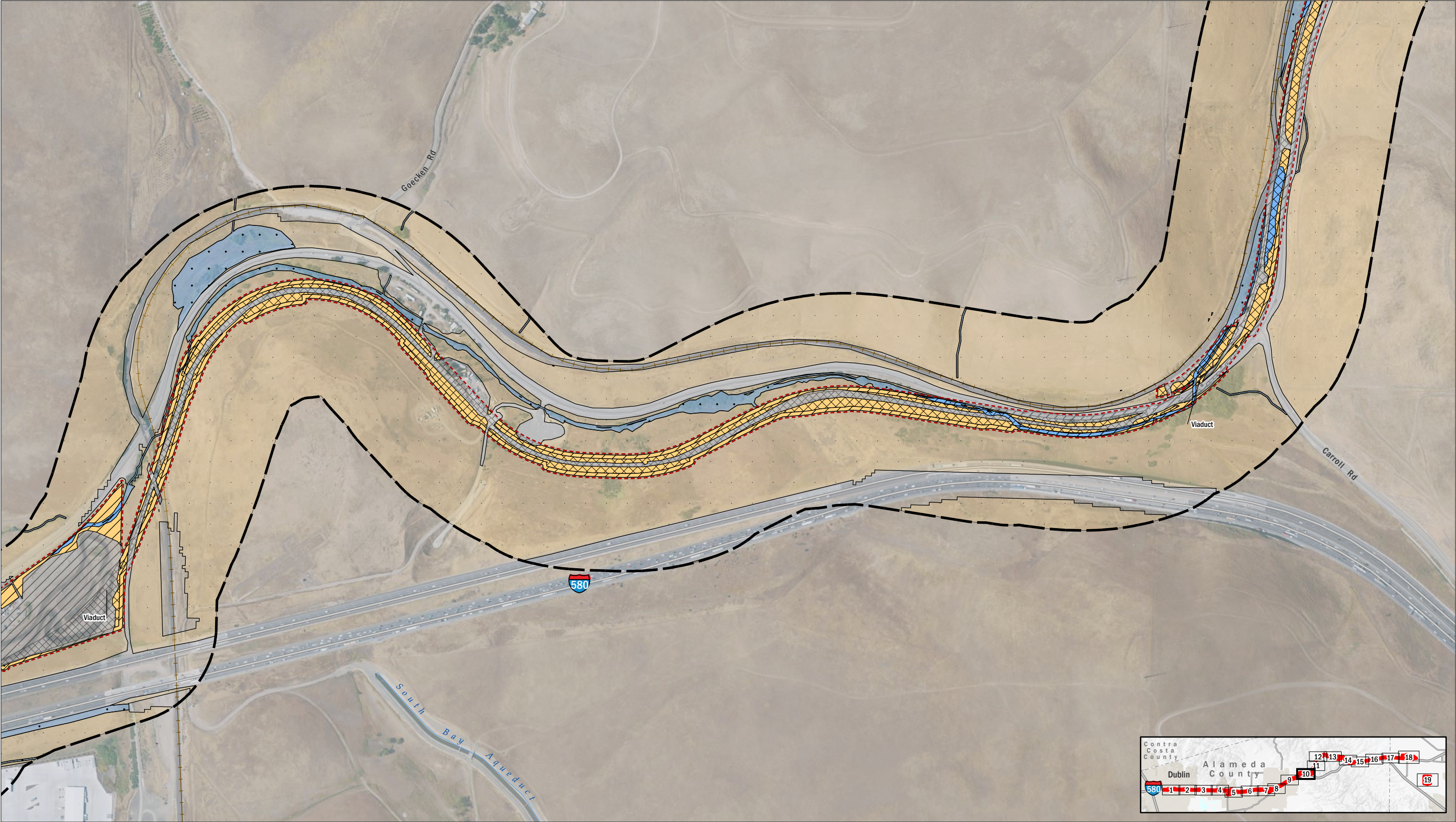
Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 9 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 10 of 19



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0 1,000
Feet
AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

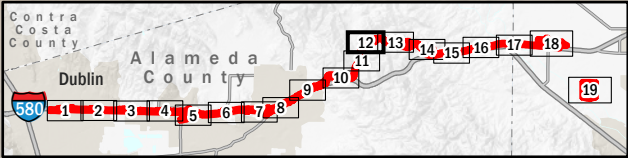
Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 11 of 19

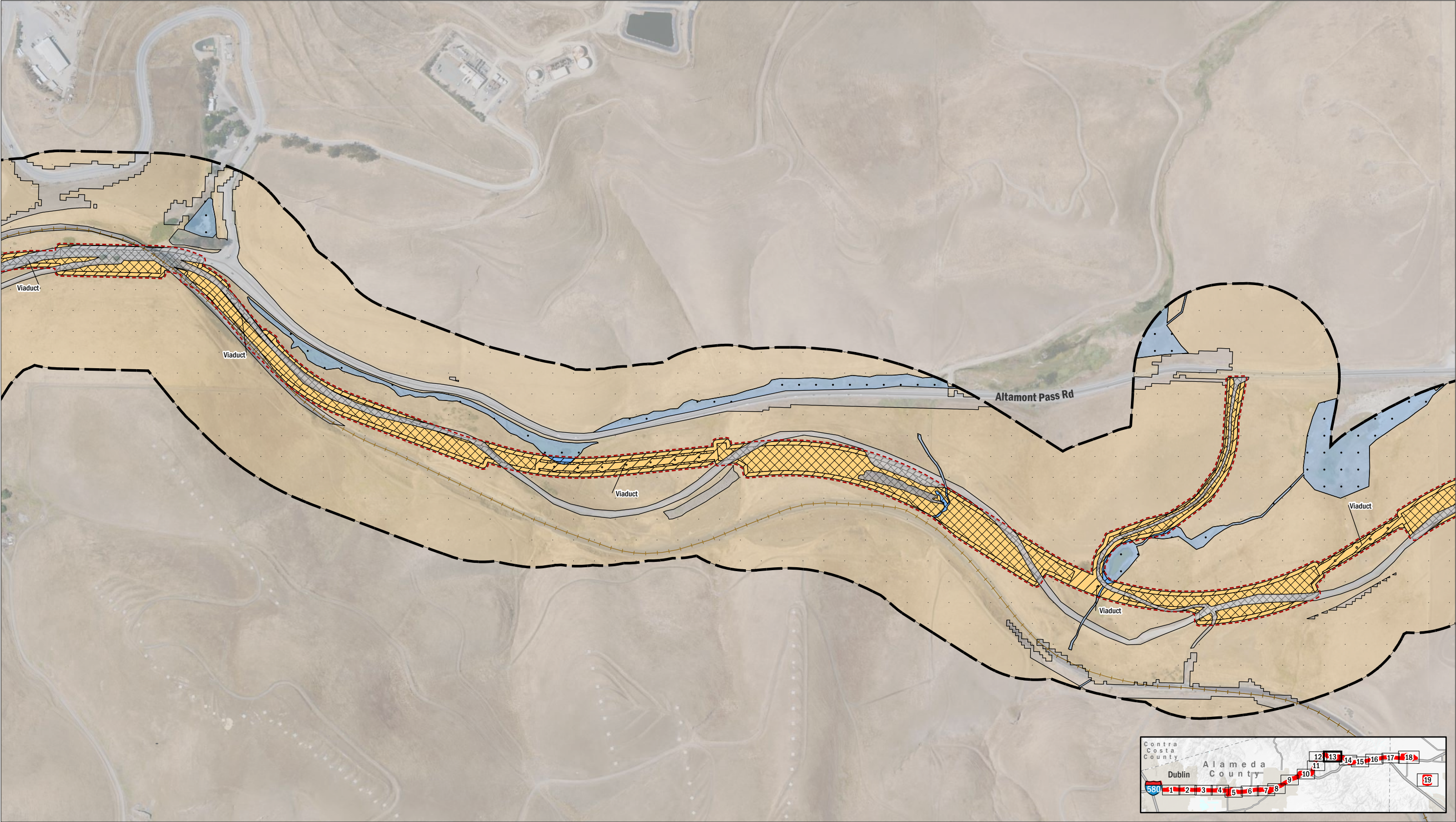


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

- Legend**
- Proposed Valley Link Stations and Facilities
 - Proposed Project Footprint
 - Action Area
 - Habitat Type (Direct Effects)**
 - Wetlands and Waters (Breeding)
 - Upland
 - Habitat Type (Indirect Effects)**
 - Wetlands and Waters (Breeding)
 - Upland
 - Effects**
 - Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 12 of 19



AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

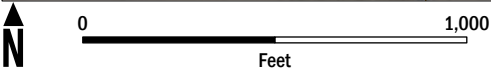
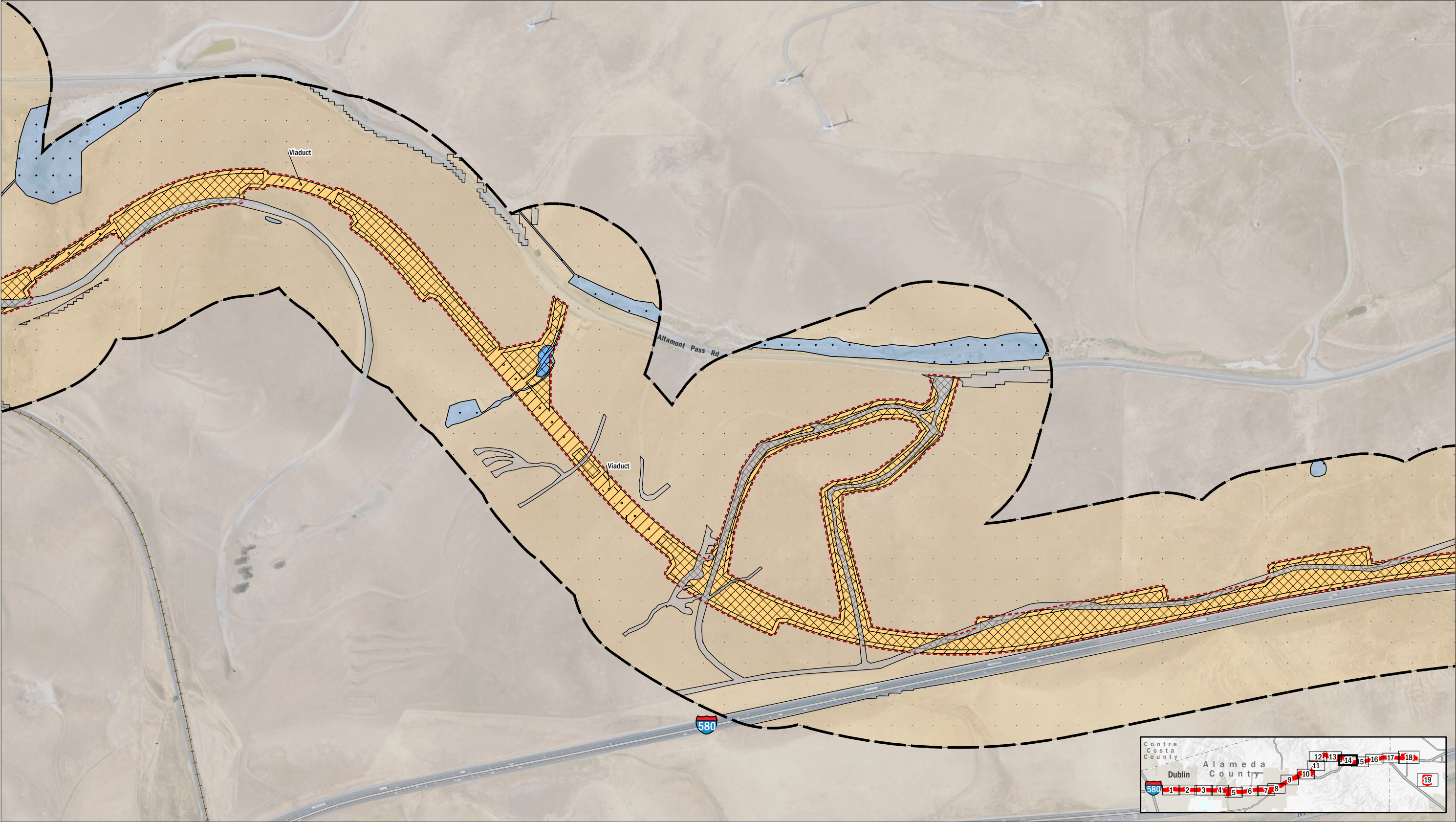
Habitat Type (Direct Effects)
 Wetlands and Waters (Breeding)
 Upland

Habitat Type (Indirect Effects)
 Wetlands and Waters (Breeding)
 Upland

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 13 of 19

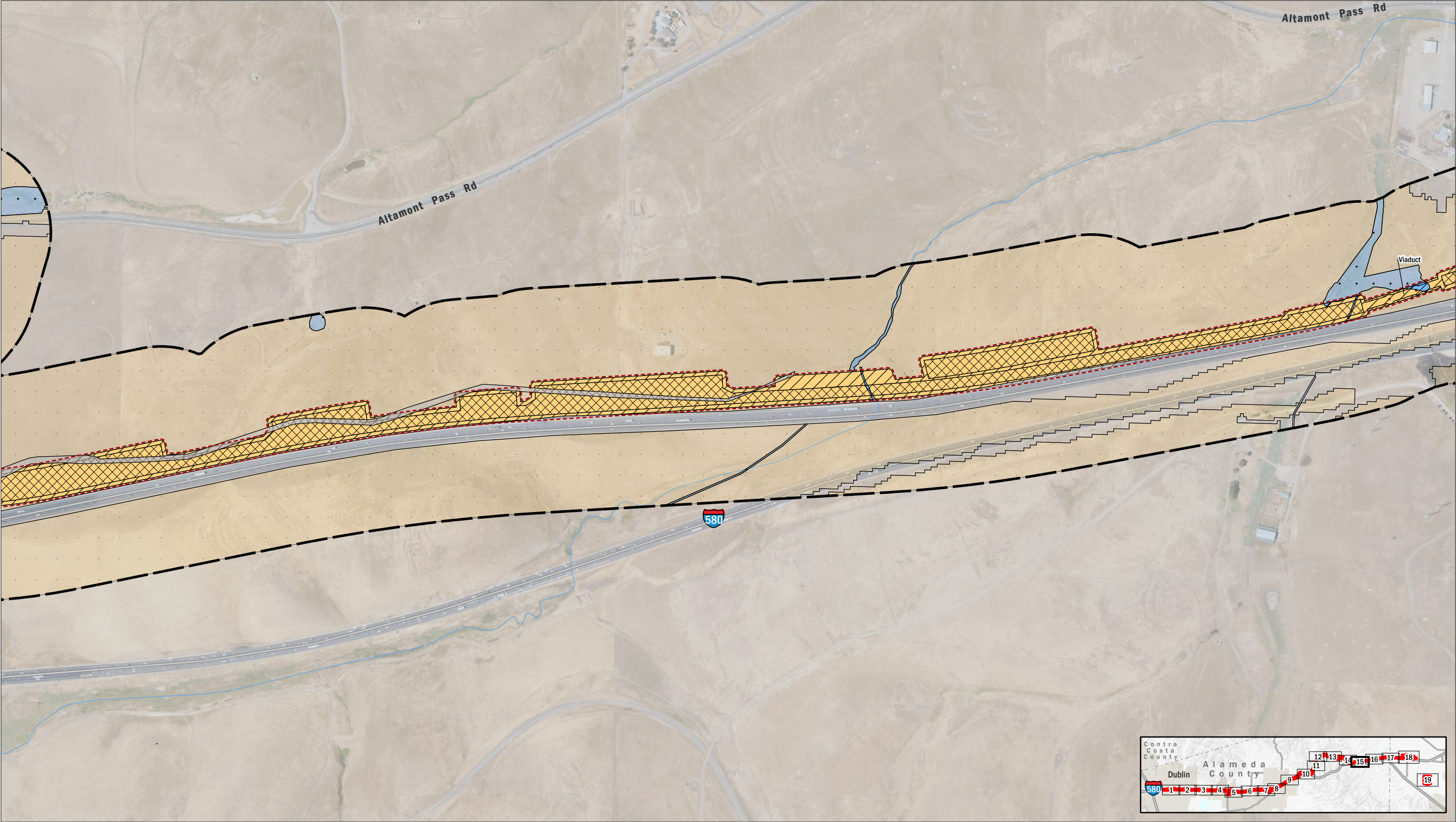


AECOM
Valley Link Rail Project

- | | | | |
|---|--|--|---|
| Proposed Project Footprint
Action Area | Habitat Type (Direct Effects)
Wetlands and Waters (Breeding)
Upland | Habitat Type (Indirect Effects)
Wetlands and Waters (Breeding)
Upland | Effects
Direct Permanent Effect
Direct Temporary Effect
Indirect Effect |
|---|--|--|---|

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 14 of 19



AECOM
Valley Link Rail Project

Proposed Project Footprint
[Red dashed line]
Action Area
[Black dashed line]

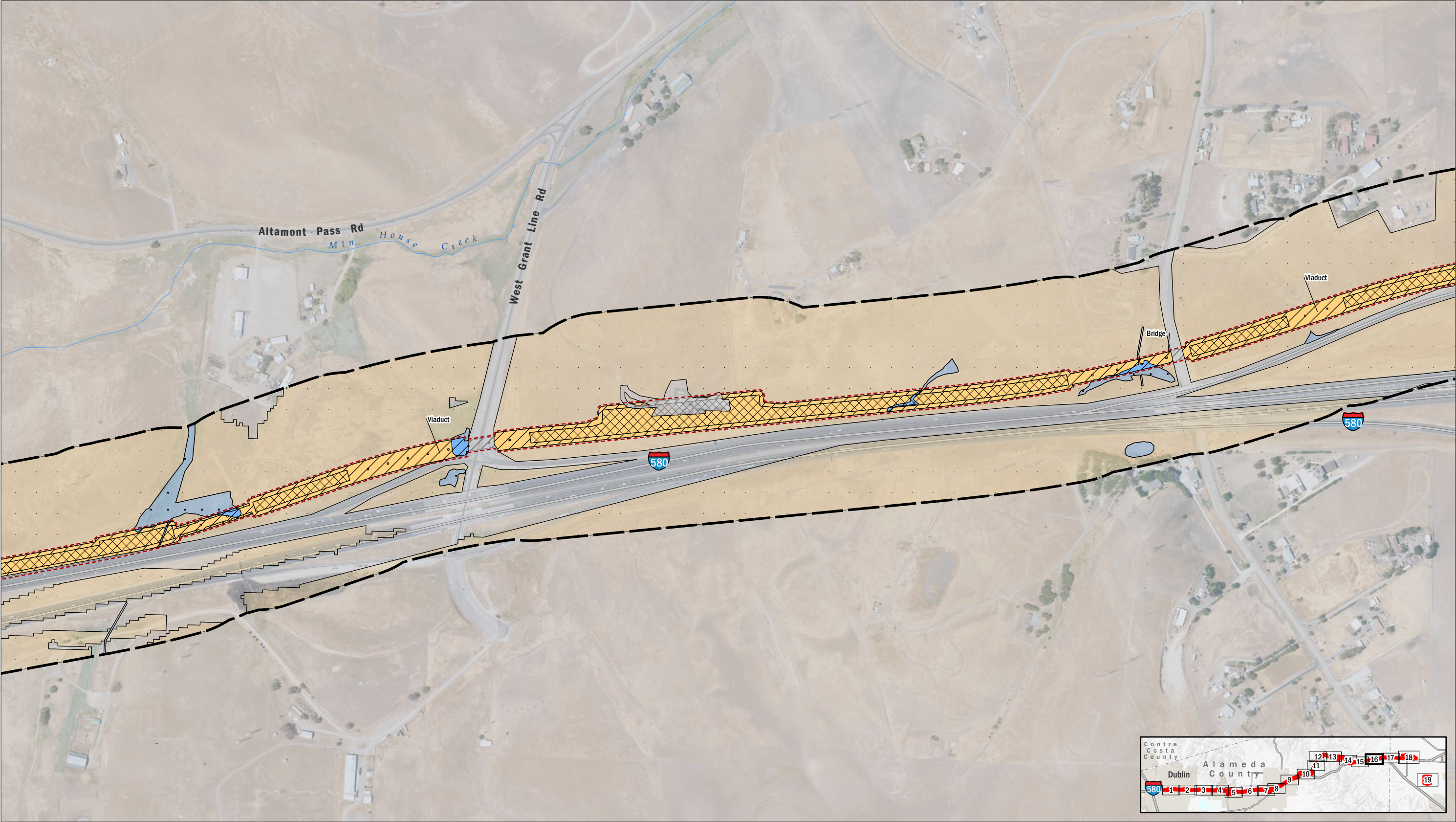
Habitat Type (Direct Effects)
[Blue box] Wetlands and Waters (Breeding)
[Yellow box] Upland

Habitat Type (Indirect Effects)
[Light blue box] Wetlands and Waters (Breeding)
[Light yellow box] Upland

Effects
[Yellow cross-hatch] Direct Permanent Effect
[Yellow diagonal lines] Direct Temporary Effect
[Yellow dotted lines] Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 15 of 19

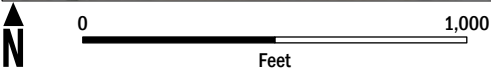
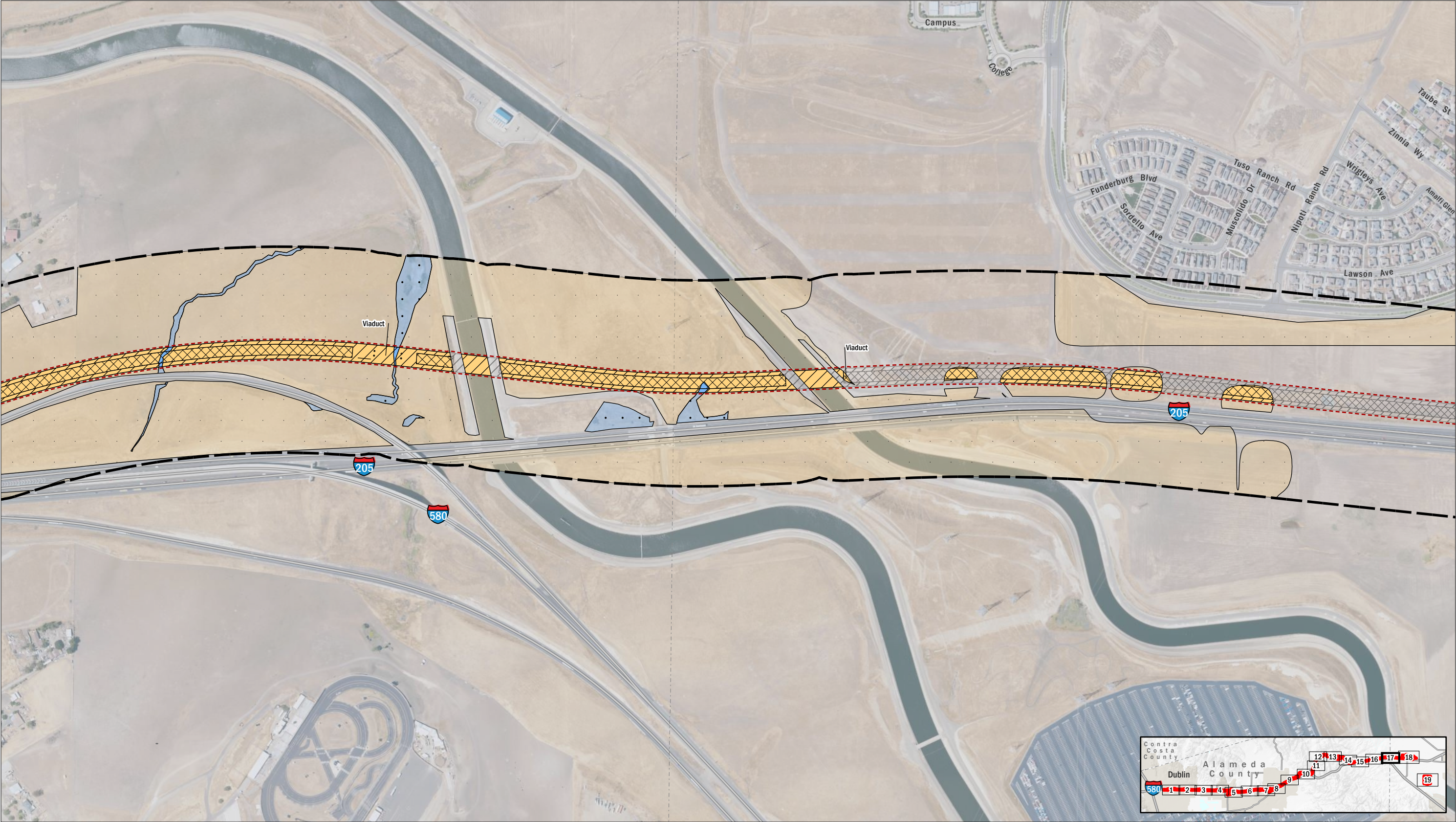


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

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|----------------------------|--------------------------------------|--|-------------------------|
| Proposed Project Footprint | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Action Area | Wetlands and Waters (Breeding) | Wetlands and Waters (Breeding) | Direct Permanent Effect |
| | Upland | Upland | Direct Temporary Effect |
| | | | Indirect Effect |

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 16 of 19

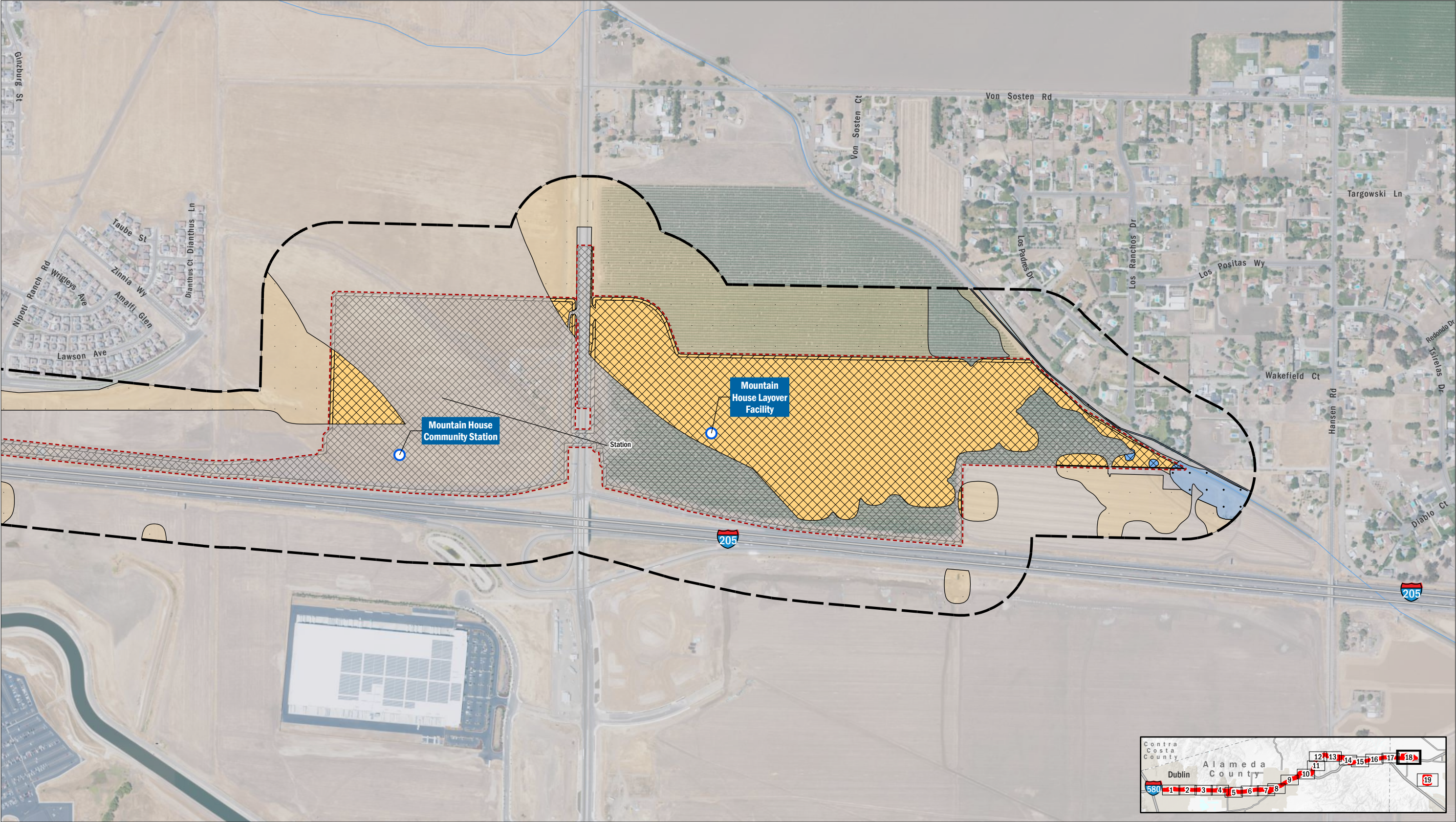


AECOM
Valley Link Rail Project

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|----------------------------|--------------------------------------|--|-------------------------|
| Proposed Project Footprint | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Action Area | Wetlands and Waters (Breeding) | Wetlands and Waters (Breeding) | Direct Permanent Effect |
| | Upland | Upland | Direct Temporary Effect |
| | | | Indirect Effect |

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

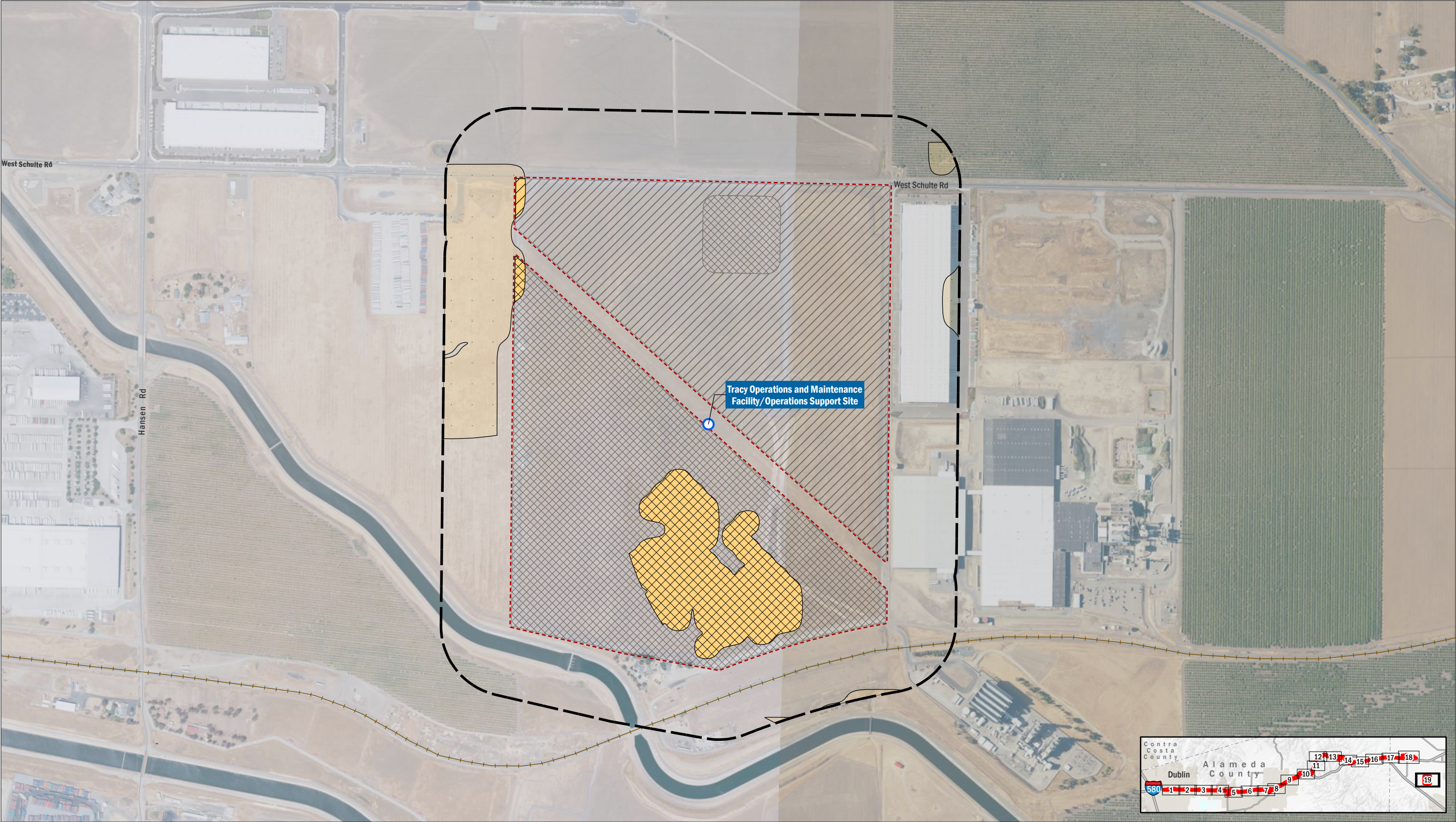
FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 17 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

- | | | | |
|--|--------------------------------------|--|-------------------------|
| Proposed Valley Link Stations and Facilities | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Proposed Project Footprint | Wetlands and Waters (Breeding) | Wetlands and Waters (Breeding) | Direct Permanent Effect |
| Action Area | Upland | Upland | Direct Temporary Effect |
| | | | Indirect Effect |

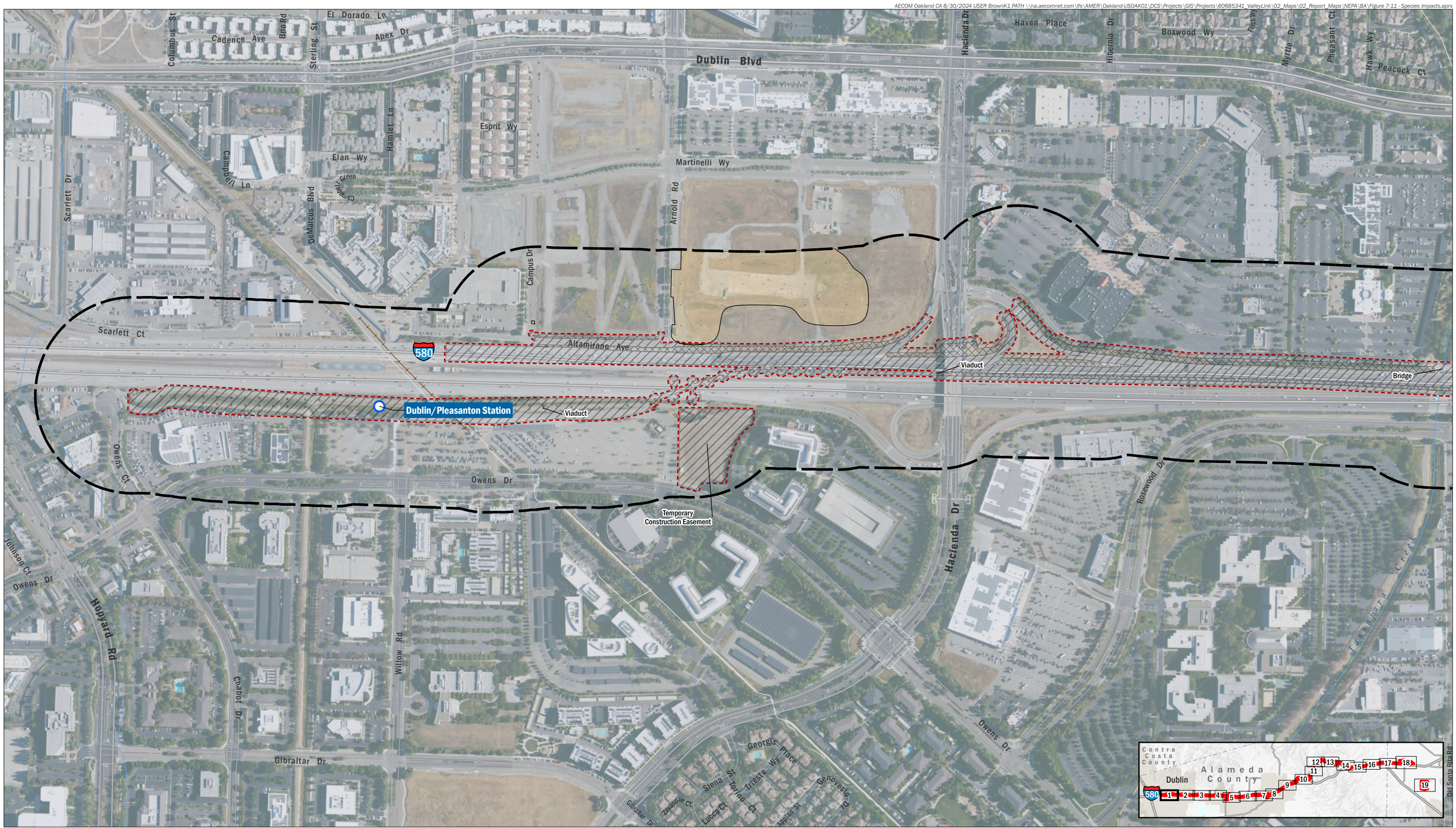
FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 18 of 19



AECOM
Valley Link Rail Project

- | | | | |
|--|--------------------------------------|--|-------------------------|
| Proposed Valley Link Stations and Facilities | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Proposed Project Footprint | Wetlands and Waters (Breeding) | Wetlands and Waters (Breeding) | Direct Permanent Effect |
| Action Area | Upland | Upland | Direct Temporary Effect |
| | | | Indirect Effect |

FIGURE 10
Western Spadefoot (Spea hammondi) Potential Habitat
Sheet 19 of 19



AECOM
Valley Link Rail Project

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Feet

○ Proposed Valley Link Stations and Facilities
- - - Proposed Project Footprint
- - - Action Area

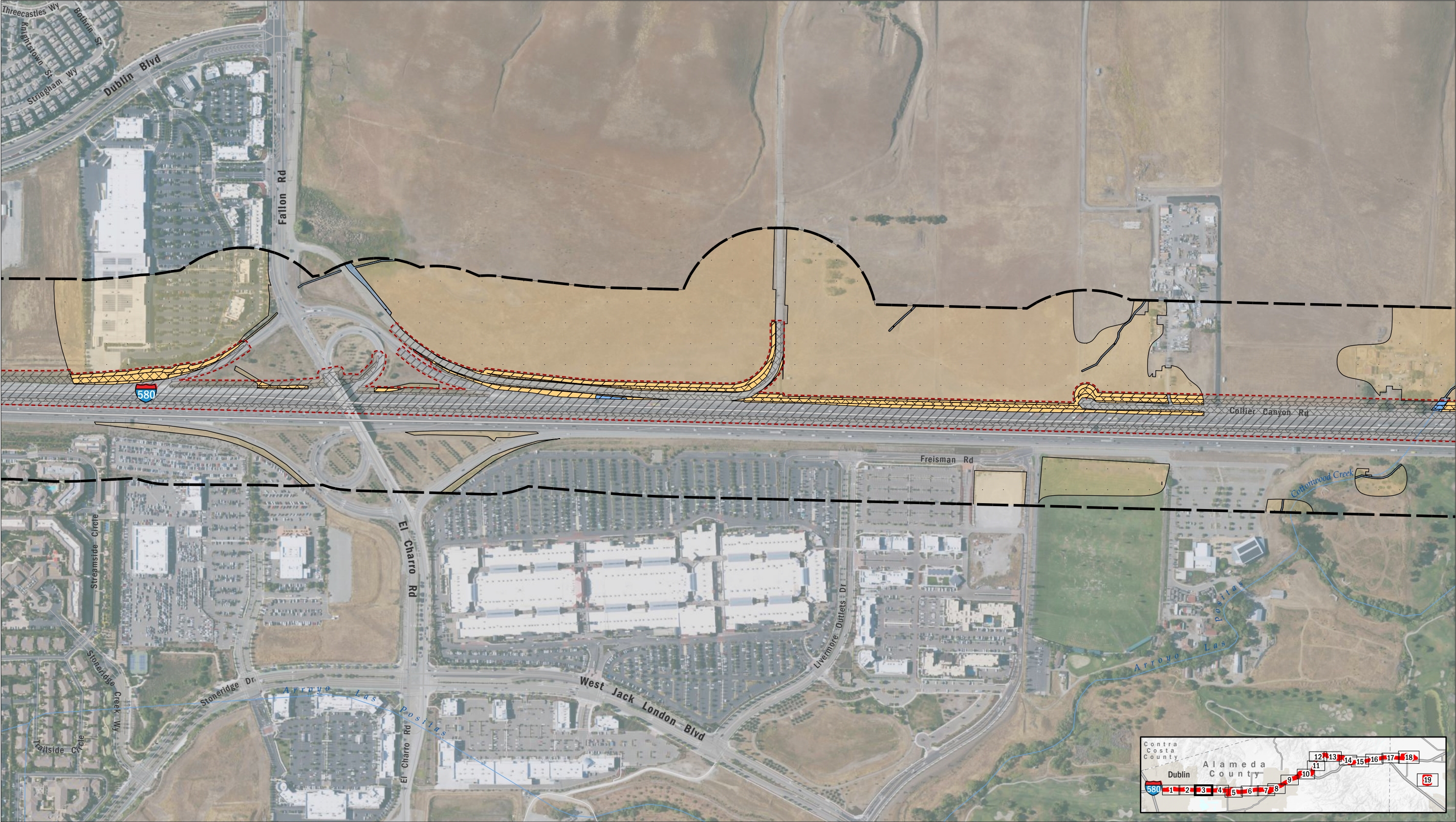
Habitat Type (Direct Effects)
Aquatic
Upland and Nesting

Habitat Type (Indirect Effects)
Aquatic
Upland and Nesting

Effects
Direct Permanent Effect
Direct Temporary Effect
Indirect Effect

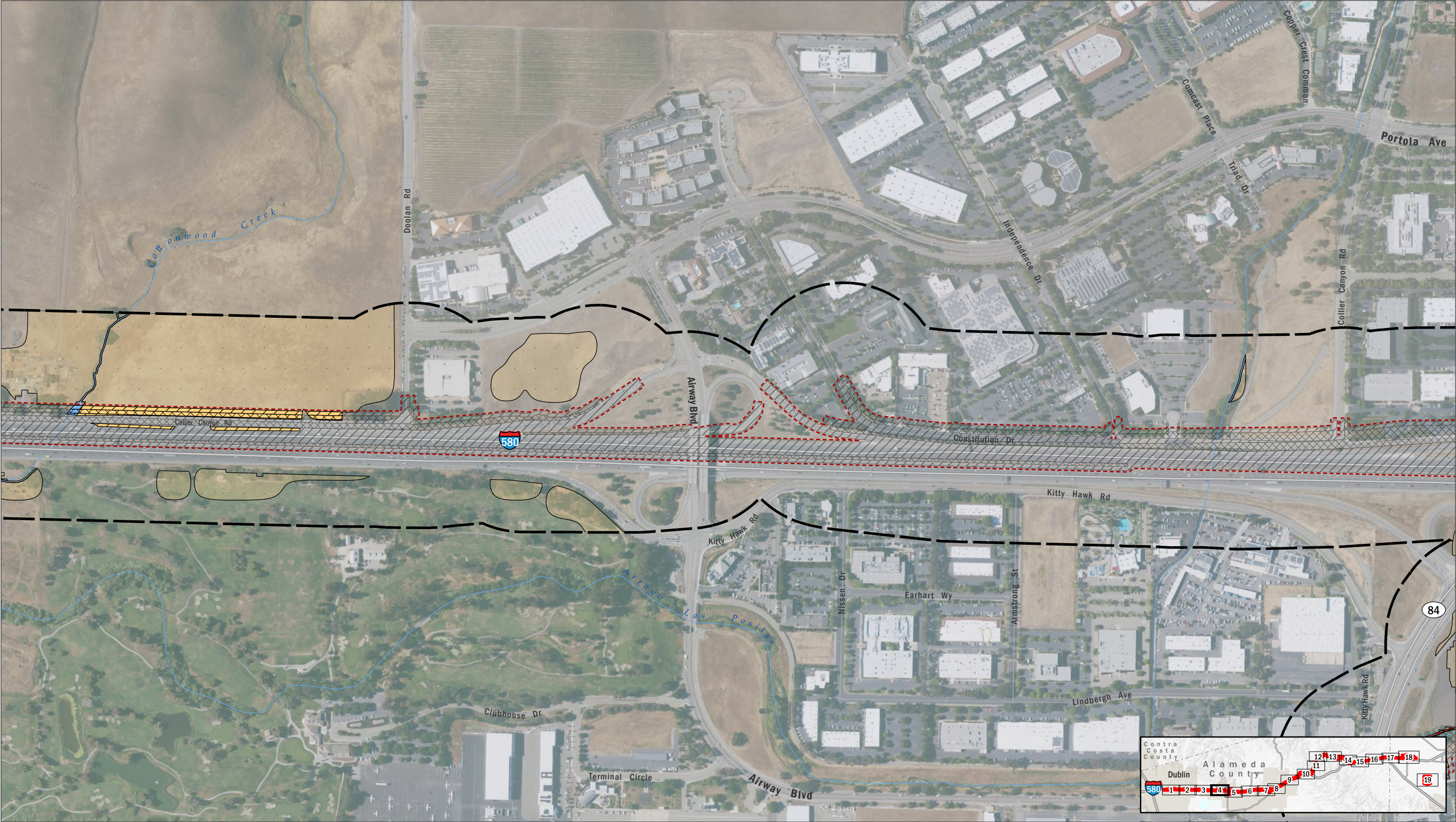
Contra Costa County
Alameda County
Dublin
San Francisco Bay Area

FIGURE 11
Northwest Pond Turtle (*Emys marmorata*) Potential Habitat
Sheet 1 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).





AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

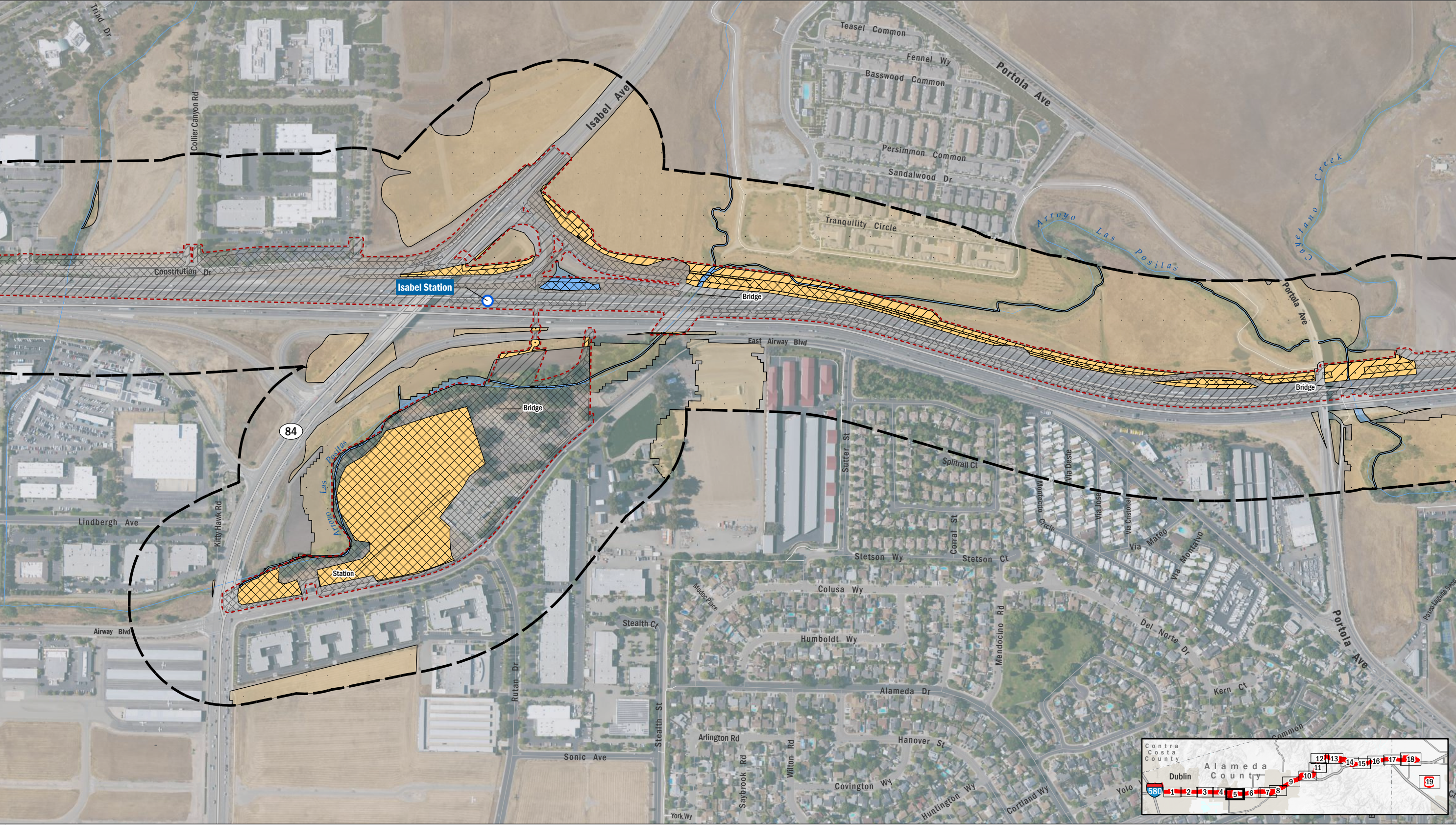
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)	Habitat Type (Indirect Effects)
Aquatic	Aquatic
Upland and Nesting	Upland and Nesting

Effects

	Direct Permanent Effect
	Direct Temporary Effect
	Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 4 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



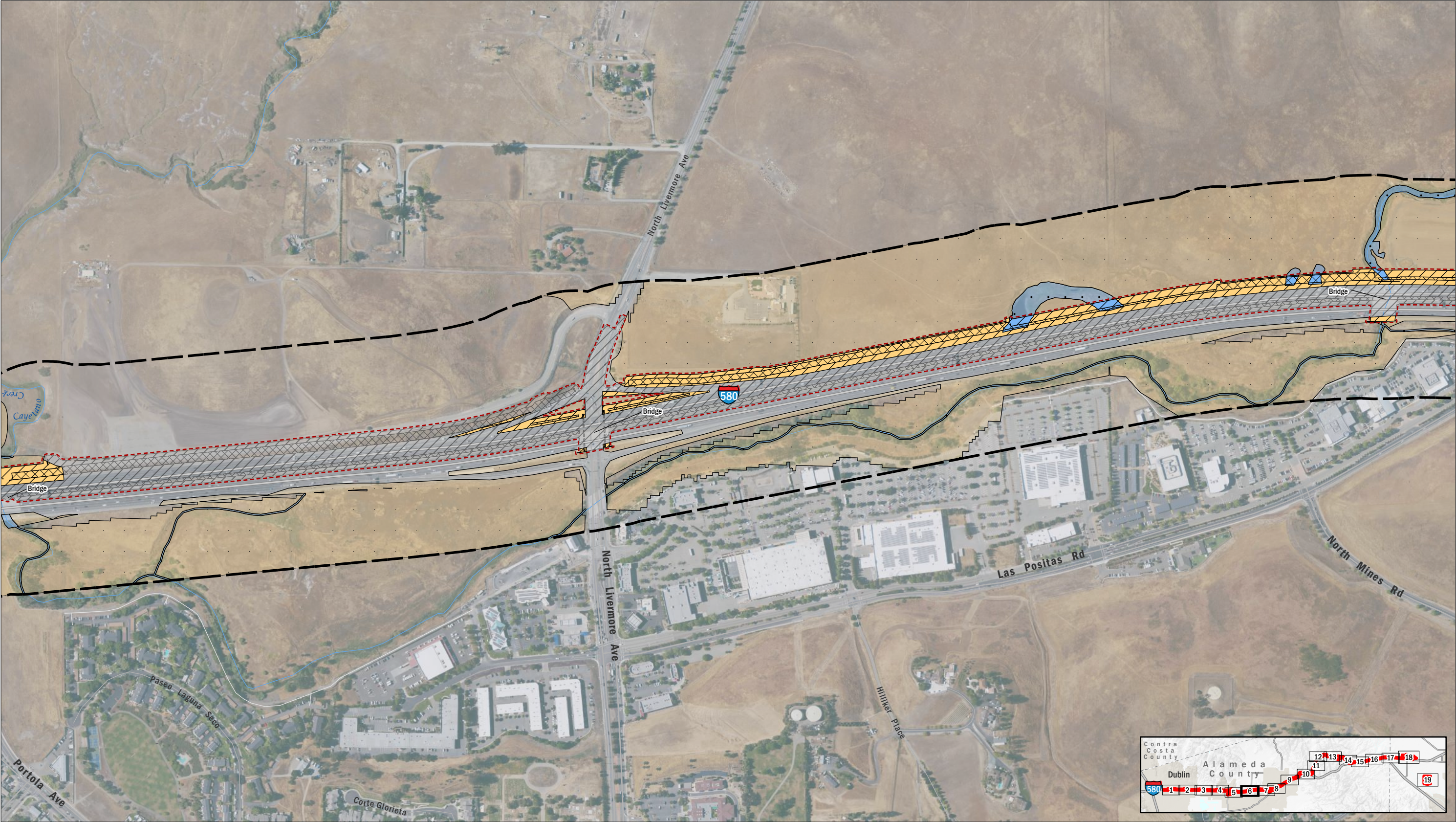
AECOM
Valley Link Rail Project

- Proposed Valley Link Stations and Facilities
- Proposed Project Footprint
- Action Area

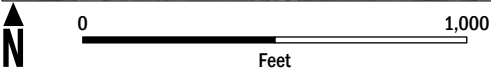
- | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) |
|-------------------------------|---------------------------------|
| Aquatic | Aquatic |
| Upland and Nesting | Upland and Nesting |

- Effects**
- Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 5 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM
Valley Link Rail Project

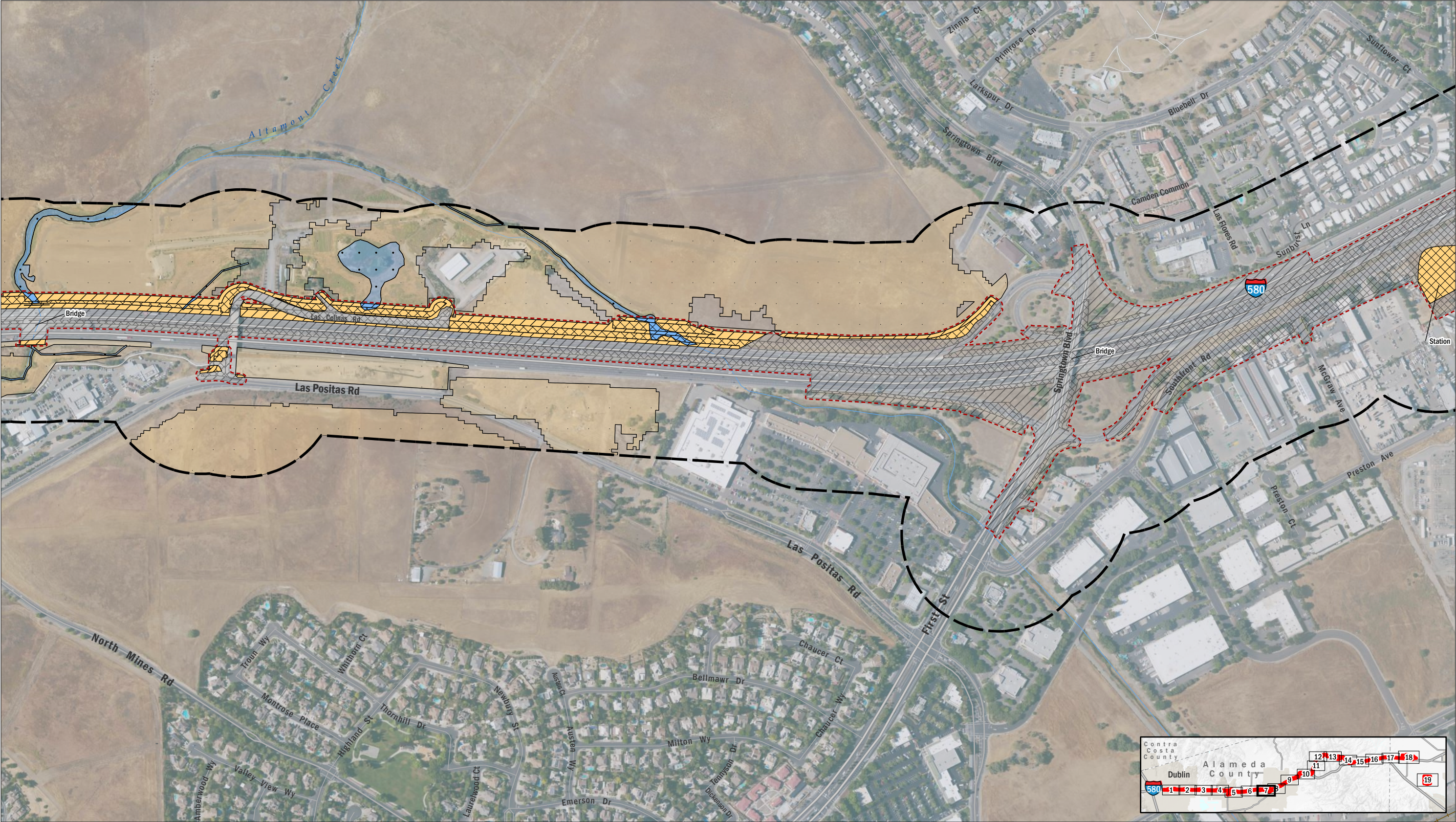
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Aquatic
 Upland and Nesting

Habitat Type (Indirect Effects)
 Aquatic
 Upland and Nesting

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 6 of 19

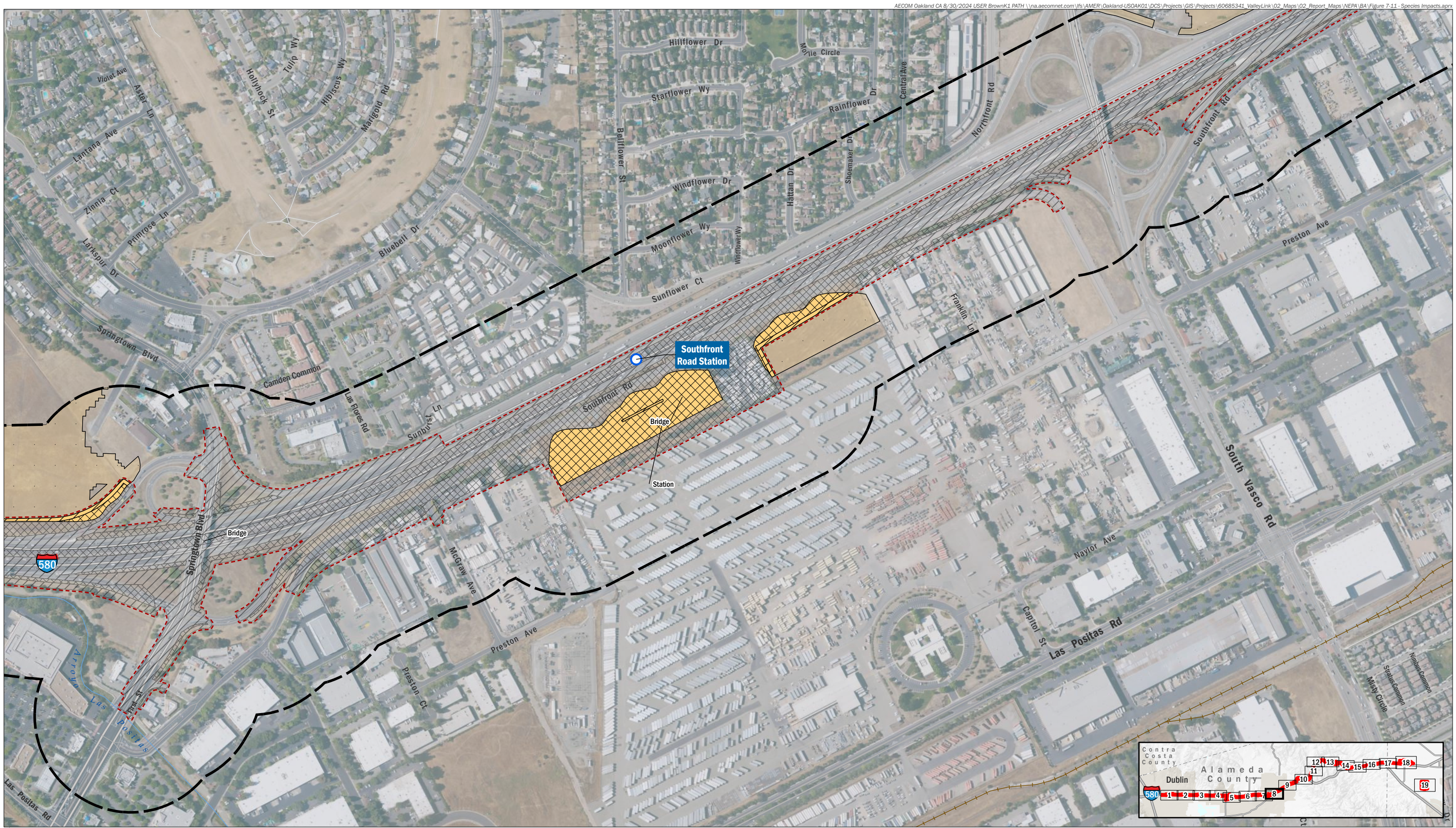


AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

- | | | | |
|----------------------------|--------------------------------------|--|-------------------------|
| Proposed Project Footprint | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Action Area | Aquatic | Aquatic | Direct Permanent Effect |
| | Upland and Nesting | Upland and Nesting | Direct Temporary Effect |
| | | | Indirect Effect |

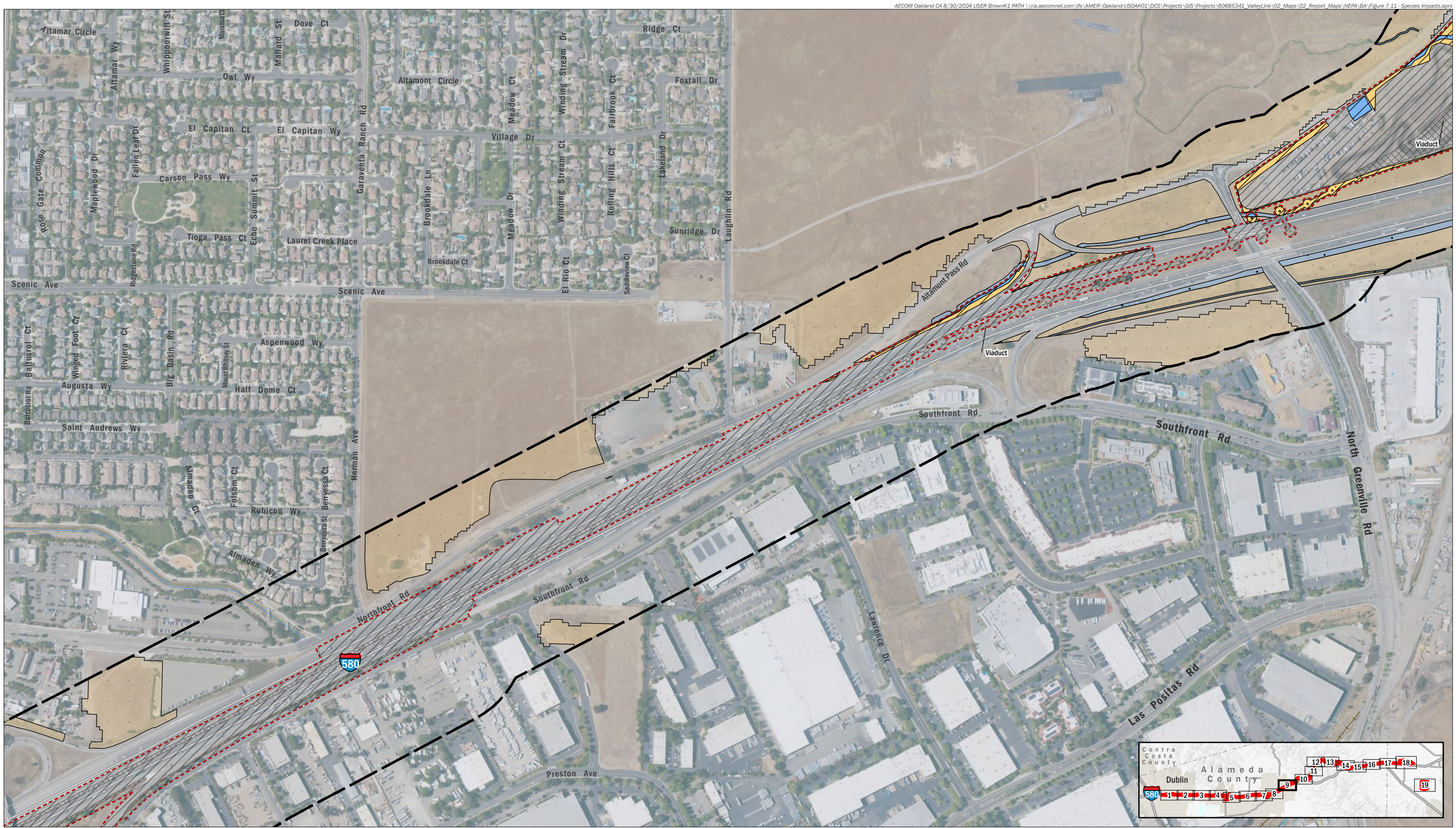
FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
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AECOM
Valley Link Rail Project

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|--|--------------------------------------|--|-------------------------|
| Proposed Valley Link Stations and Facilities | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) | Effects |
| Proposed Project Footprint | Aquatic | Aquatic | Direct Permanent Effect |
| Action Area | Upland and Nesting | Upland and Nesting | Direct Temporary Effect |
| | | | Indirect Effect |

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 8 of 19



0 1,000
Feet

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

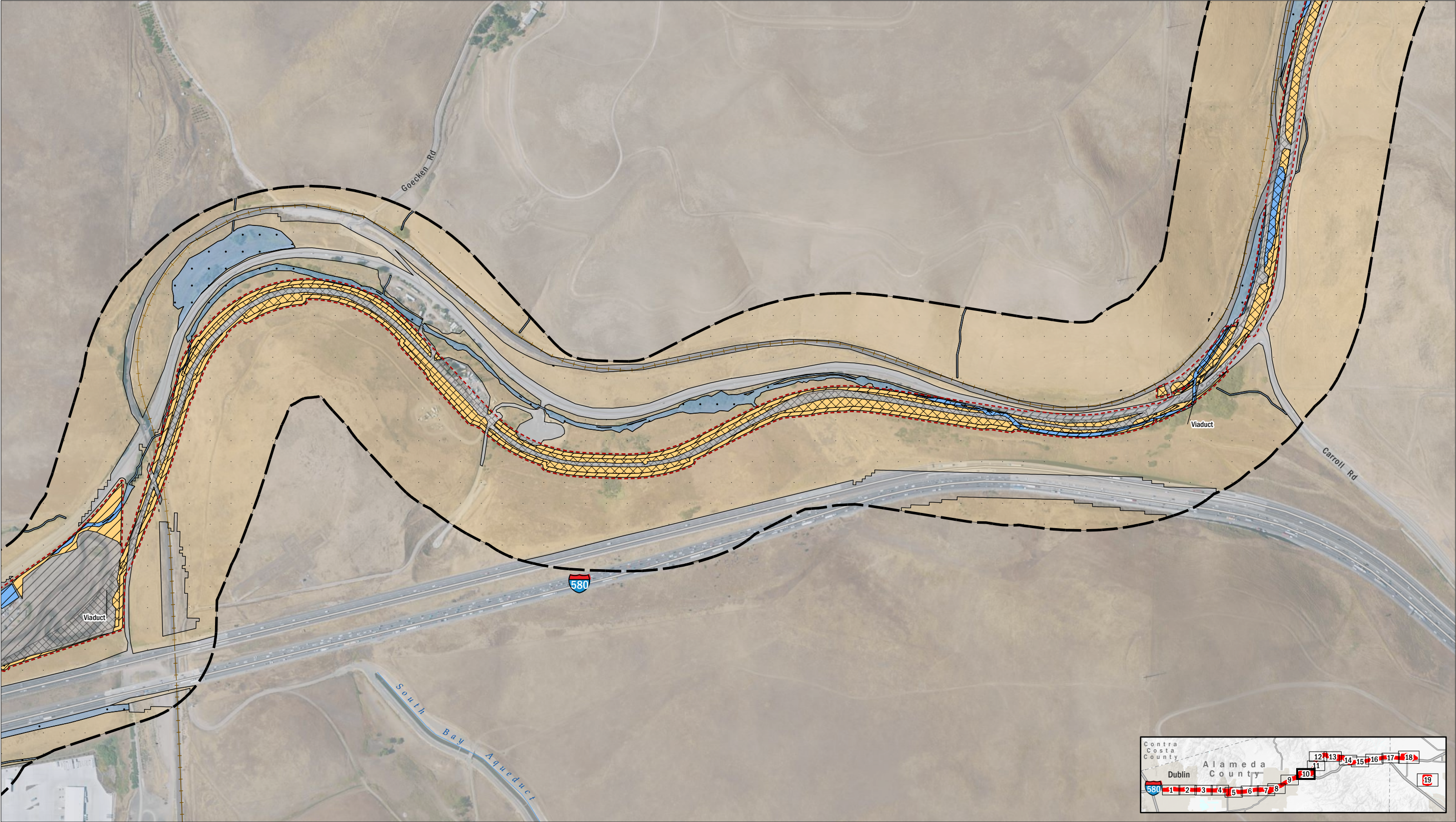
Habitat Type (Direct Effects)	Habitat Type (Indirect Effects)
Aquatic	Aquatic
Upland and Nesting	Upland and Nesting

Effects

	Direct Permanent Effect
	Direct Temporary Effect
	Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
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AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

0 1,000
Feet

Proposed Project Footprint
 Proposed Project Footprint

Action Area
 Action Area

Habitat Type (Direct Effects)

Aquatic

Upland and Nesting

Habitat Type (Indirect Effects)

Aquatic

Upland and Nesting

Effects

Direct Permanent Effect

Direct Temporary Effect

Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 10 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

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Feet

AECOM

Valley Link Rail Project

Proposed Project Footprint

Action Area

Aquatic

Upland and Nesting

Aquatic

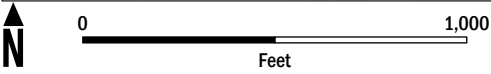
Upland and Nesting

Direct Permanent Effect

Direct Temporary Effect

Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 11 of 19



AECOM
Valley Link Rail Project

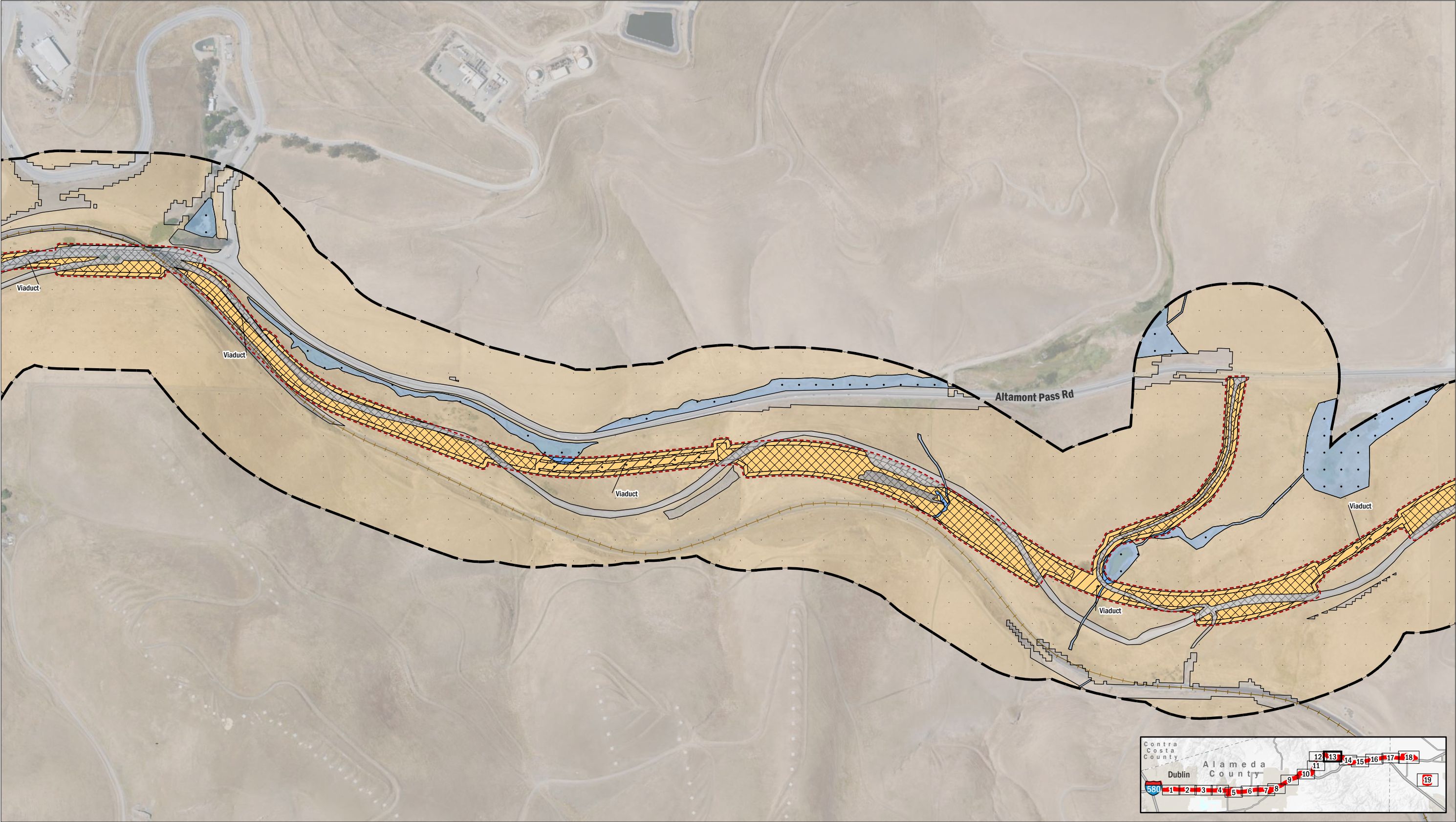
- Proposed Valley Link Stations and Facilities
- - - Proposed Project Footprint
- ▮ Action Area

- | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) |
|-------------------------------|---------------------------------|
| ■ Aquatic | ■ Aquatic |
| ■ Upland and Nesting | ■ Upland and Nesting |

- | Effects |
|---------------------------|
| ▨ Direct Permanent Effect |
| ▨ Direct Temporary Effect |
| ⋯ Indirect Effect |

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 12 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

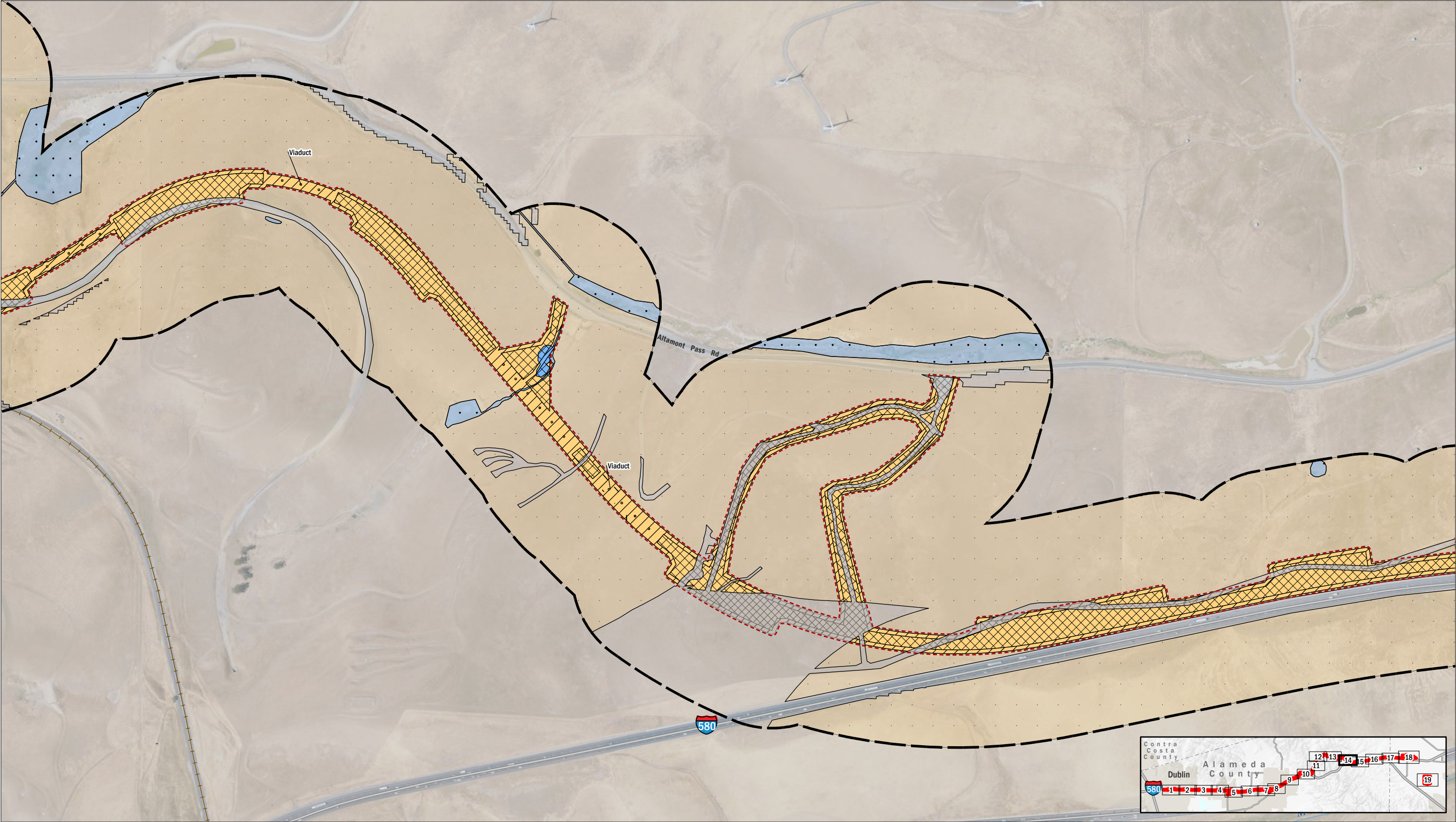
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Aquatic
 Upland and Nesting

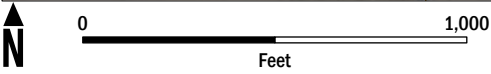
Habitat Type (Indirect Effects)
 Aquatic
 Upland and Nesting

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 13 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).



AECOM
Valley Link Rail Project

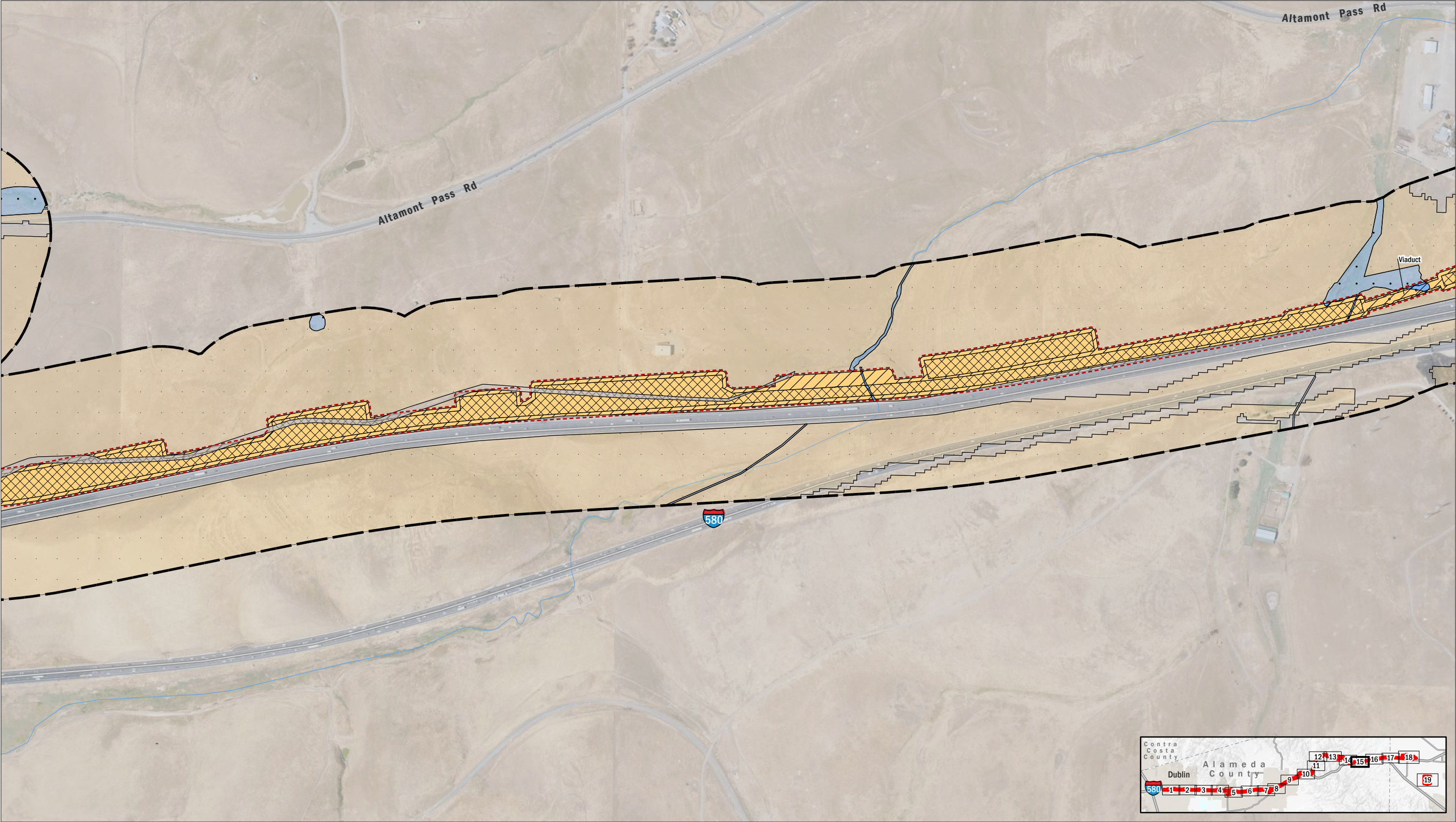
Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Aquatic
 Upland and Nesting

Habitat Type (Indirect Effects)
 Aquatic
 Upland and Nesting

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 14 of 19



AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

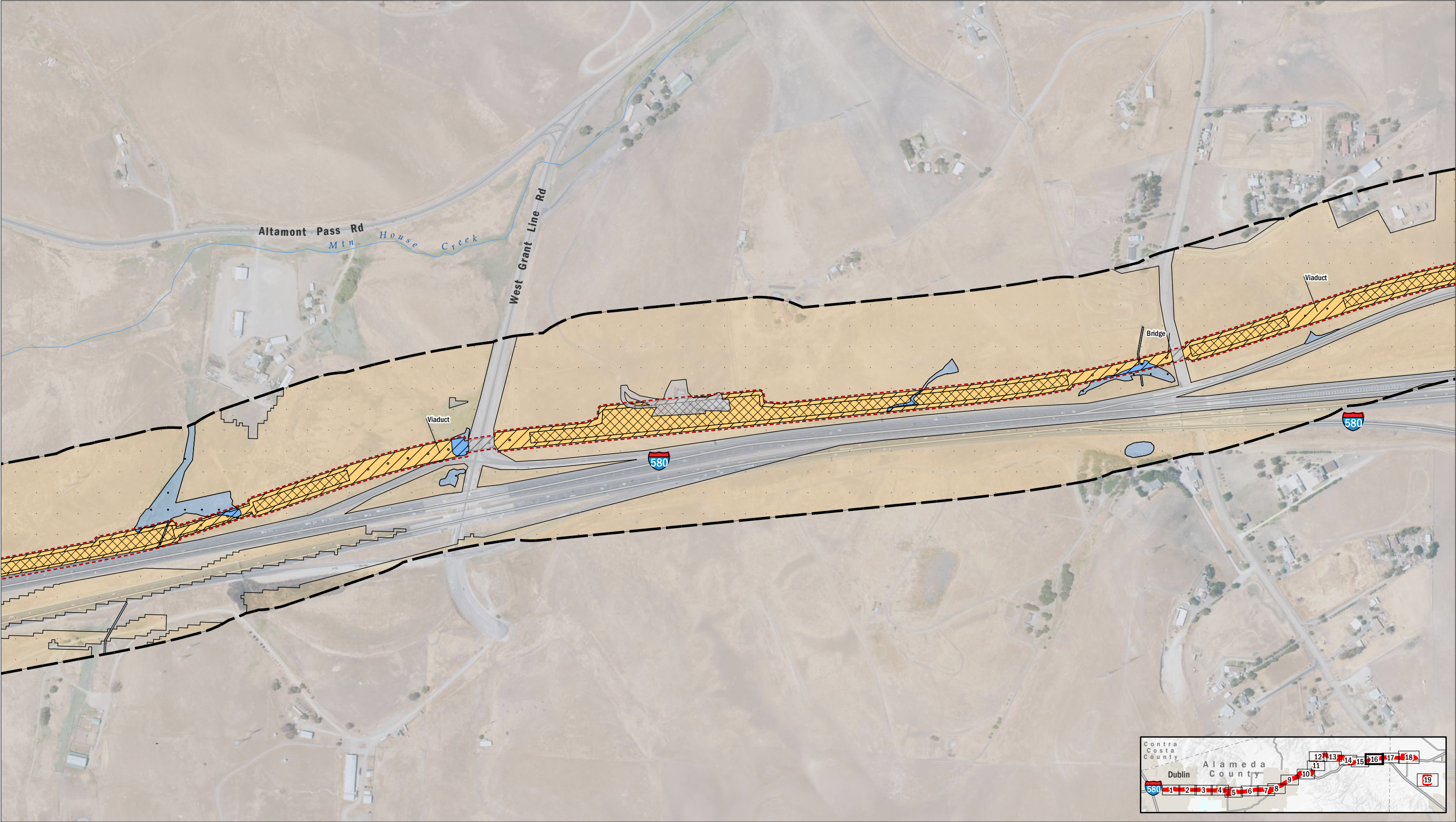
Habitat Type (Direct Effects)
 Aquatic
 Upland and Nesting

Habitat Type (Indirect Effects)
 Aquatic
 Upland and Nesting

Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 15 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

0 1,000
Feet

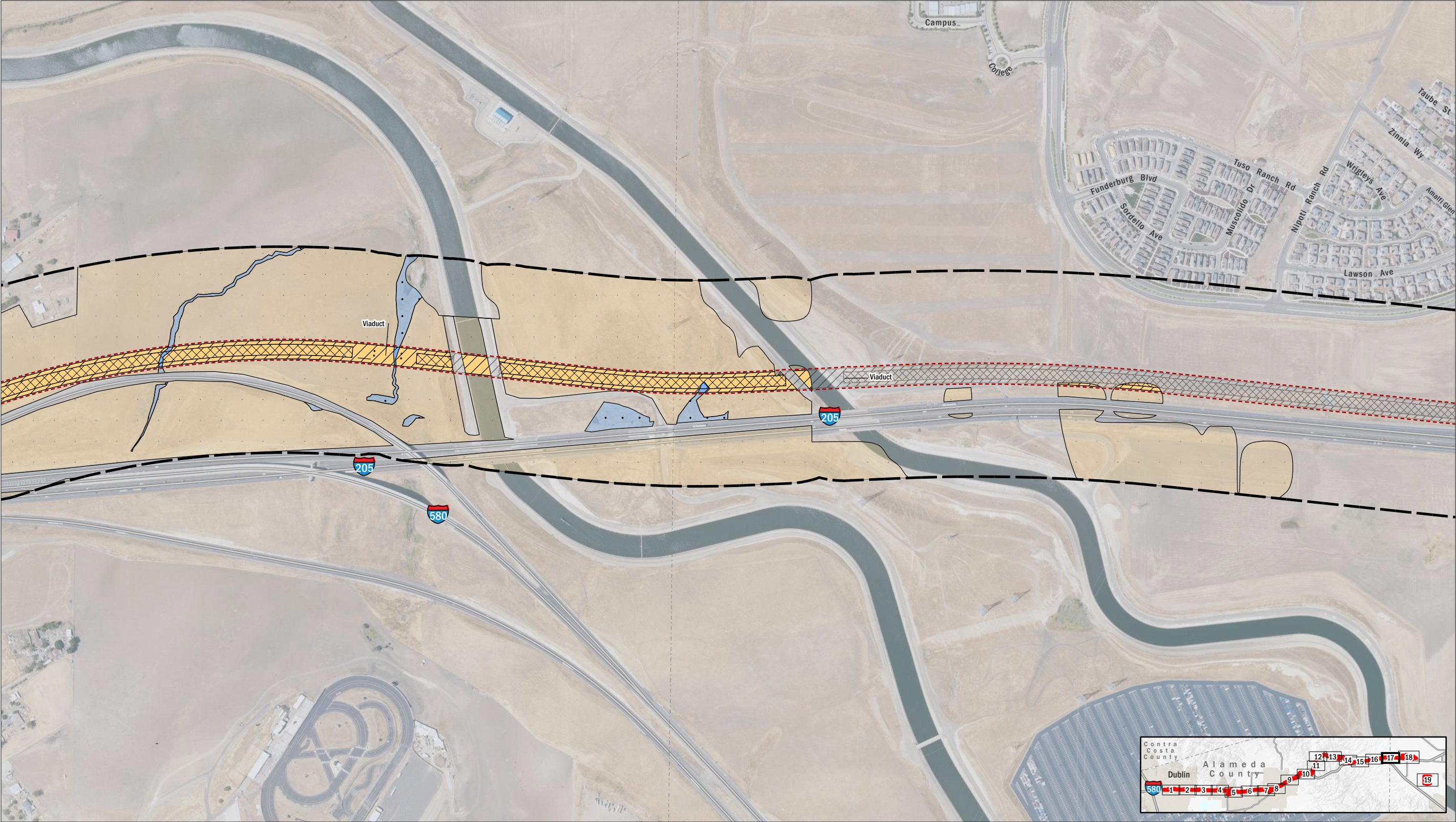
Proposed Project Footprint
Action Area

Habitat Type (Direct Effects)
Aquatic
Upland and Nesting

Habitat Type (Indirect Effects)
Aquatic
Upland and Nesting

Effects
Direct Permanent Effect
Direct Temporary Effect
Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 16 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

Proposed Project Footprint
 Action Area

Habitat Type (Direct Effects)
 Aquatic
 Upland and Nesting


Habitat Type (Indirect Effects)
 Aquatic
 Upland and Nesting


Effects
 Direct Permanent Effect
 Direct Temporary Effect
 Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 17 of 19


 Proposed Valley Link Stations and Facilities
 Proposed Project Footprint
 Action Area


Habitat Type (Direct Effects)

 Aquatic


 Upland and Nesting


Habitat Type (Indirect Effects)

 Aquatic

 Upland and Nesting

Effects

 Direct Permanent Effect

 Direct Temporary Effect


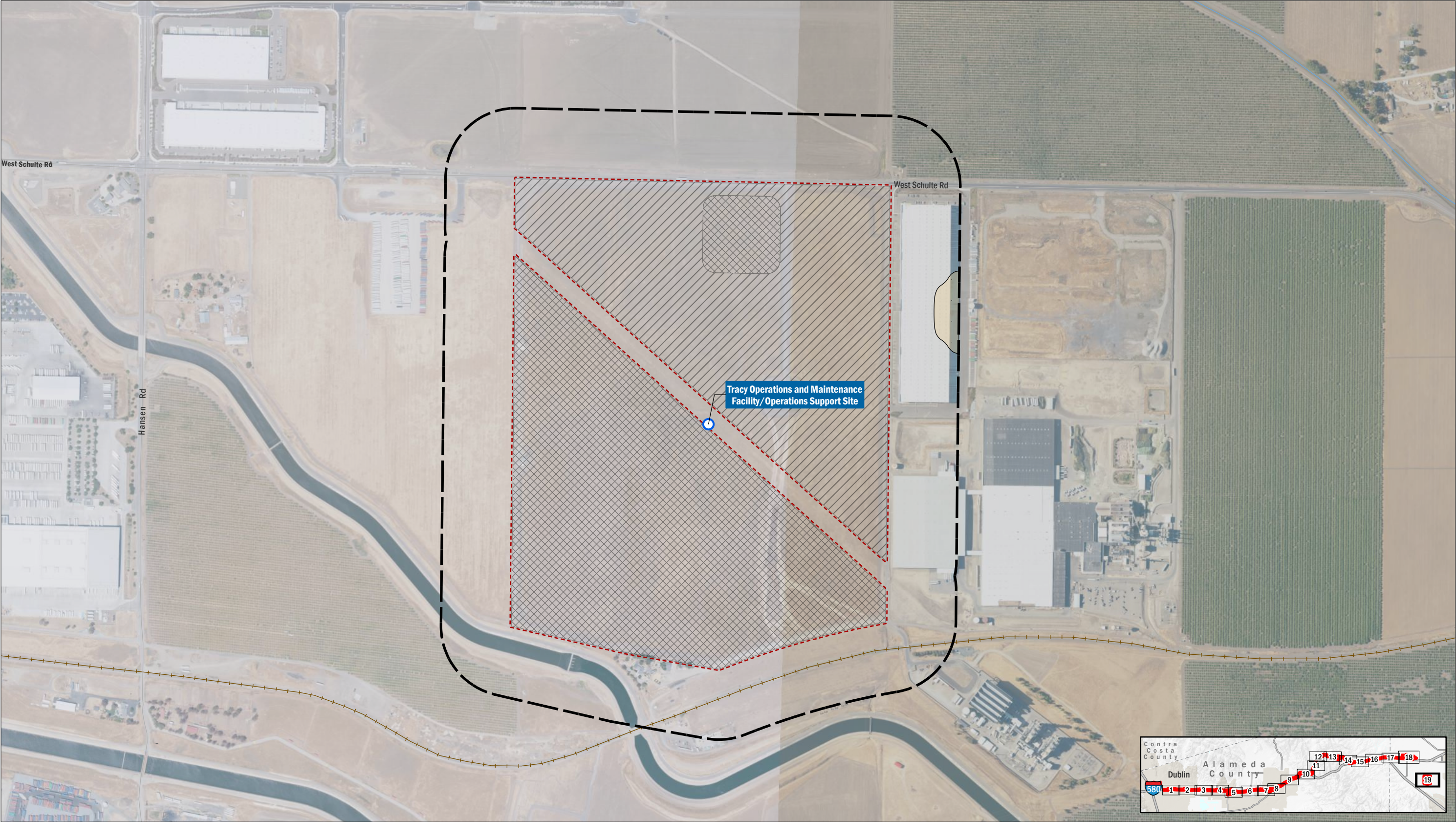
 Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 18 of 19



AECOM, 2023; ESRI 2023; OSM 2023 (Roads).

AECOM
Valley Link Rail Project

- Proposed Valley Link Stations and Facilities
- - - Proposed Project Footprint
- Action Area

- | Habitat Type (Direct Effects) | Habitat Type (Indirect Effects) |
|-------------------------------|---------------------------------|
| ■ Aquatic | ■ Aquatic |
| ■ Upland and Nesting | ■ Upland and Nesting |

- Effects**
- Direct Permanent Effect
 - Direct Temporary Effect
 - Indirect Effect

FIGURE 11
Northwest Pond Turtle (Emys marmorata) Potential Habitat
Sheet 19 of 19

Appendix B USFWS Species List



United States Department of the Interior

FISH AND WILDLIFE SERVICE

Sacramento Fish And Wildlife Office

Federal Building

2800 Cottage Way, Room W-2605

Sacramento, CA 95825-1846

Phone: (916) 414-6600 Fax: (916) 414-6713



In Reply Refer To:

05/10/2024 00:21:17 UTC

Project Code: 2024-0035741

Project Name: Valley Link Rail Project

Subject: List of threatened and endangered species that may occur in your proposed project location or may be affected by your proposed project

To Whom It May Concern:

The enclosed species list identifies threatened, endangered, proposed and candidate species, as well as proposed and final designated critical habitat, that may occur within the boundary of your proposed project and/or may be affected by your proposed project. The species list fulfills the requirements of the U.S. Fish and Wildlife Service (Service) under section 7(c) of the Endangered Species Act (Act) of 1973, as amended (16 U.S.C. 1531 *et seq.*).

New information based on updated surveys, changes in the abundance and distribution of species, changed habitat conditions, or other factors could change this list. Please feel free to contact us if you need more current information or assistance regarding the potential impacts to federally proposed, listed, and candidate species and federally designated and proposed critical habitat. Please note that under 50 CFR 402.12(e) of the regulations implementing section 7 of the Act, the accuracy of this species list should be verified after 90 days. This verification can be completed formally or informally as desired. The Service recommends that verification be completed by visiting the IPaC website at regular intervals during project planning and implementation for updates to species lists and information. An updated list may be requested through the IPaC system by completing the same process used to receive the enclosed list.

The purpose of the Act is to provide a means whereby threatened and endangered species and the ecosystems upon which they depend may be conserved. Under sections 7(a)(1) and 7(a)(2) of the Act and its implementing regulations (50 CFR 402 *et seq.*), Federal agencies are required to utilize their authorities to carry out programs for the conservation of threatened and endangered species and to determine whether projects may affect threatened and endangered species and/or designated critical habitat.

A Biological Assessment is required for construction projects (or other undertakings having similar physical impacts) that are major Federal actions significantly affecting the quality of the human environment as defined in the National Environmental Policy Act (42 U.S.C. 4332(2)).

(c)). For projects other than major construction activities, the Service suggests that a biological evaluation similar to a Biological Assessment be prepared to determine whether the project may affect listed or proposed species and/or designated or proposed critical habitat. Recommended contents of a Biological Assessment are described at 50 CFR 402.12.

If a Federal agency determines, based on the Biological Assessment or biological evaluation, that listed species and/or designated critical habitat may be affected by the proposed project, the agency is required to consult with the Service pursuant to 50 CFR 402. In addition, the Service recommends that candidate species, proposed species and proposed critical habitat be addressed within the consultation. More information on the regulations and procedures for section 7 consultation, including the role of permit or license applicants, can be found in the "Endangered Species Consultation Handbook" at:

<https://www.fws.gov/sites/default/files/documents/endangered-species-consultation-handbook.pdf>

Migratory Birds: In addition to responsibilities to protect threatened and endangered species under the Endangered Species Act (ESA), there are additional responsibilities under the Migratory Bird Treaty Act (MBTA) and the Bald and Golden Eagle Protection Act (BGEPA) to protect native birds from project-related impacts. Any activity, intentional or unintentional, resulting in take of migratory birds, including eagles, is prohibited unless otherwise permitted by the U.S. Fish and Wildlife Service (50 C.F.R. Sec. 10.12 and 16 U.S.C. Sec. 668(a)). For more information regarding these Acts, see <https://www.fws.gov/program/migratory-bird-permit/what-we-do>.

The MBTA has no provision for allowing take of migratory birds that may be unintentionally killed or injured by otherwise lawful activities. It is the responsibility of the project proponent to comply with these Acts by identifying potential impacts to migratory birds and eagles within applicable NEPA documents (when there is a federal nexus) or a Bird/Eagle Conservation Plan (when there is no federal nexus). Proponents should implement conservation measures to avoid or minimize the production of project-related stressors or minimize the exposure of birds and their resources to the project-related stressors. For more information on avian stressors and recommended conservation measures, see <https://www.fws.gov/library/collections/threats-birds>.

In addition to MBTA and BGEPA, Executive Order 13186: *Responsibilities of Federal Agencies to Protect Migratory Birds*, obligates all Federal agencies that engage in or authorize activities that might affect migratory birds, to minimize those effects and encourage conservation measures that will improve bird populations. Executive Order 13186 provides for the protection of both migratory birds and migratory bird habitat. For information regarding the implementation of Executive Order 13186, please visit <https://www.fws.gov/partner/council-conservation-migratory-birds>.

We appreciate your concern for threatened and endangered species. The Service encourages Federal agencies to include conservation of threatened and endangered species into their project planning to further the purposes of the Act. Please include the Consultation Code in the header of this letter with any request for consultation or correspondence about your project that you submit to our office.

Attachment(s):

- Official Species List

OFFICIAL SPECIES LIST

This list is provided pursuant to Section 7 of the Endangered Species Act, and fulfills the requirement for Federal agencies to "request of the Secretary of the Interior information whether any species which is listed or proposed to be listed may be present in the area of a proposed action".

This species list is provided by:

Sacramento Fish And Wildlife Office

Federal Building

2800 Cottage Way, Room W-2605

Sacramento, CA 95825-1846

(916) 414-6600

PROJECT SUMMARY

Project Code: 2024-0035741

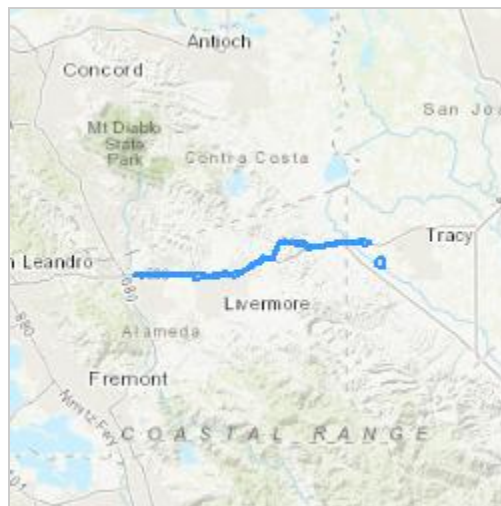
Project Name: Valley Link Rail Project

Project Type: Railroad - New Construction

Project Description: The Federal Transit Administration (FTA), as lead federal agency, and the Tri-Valley – San Joaquin Valley Regional Rail Authority (Authority), as project sponsor, are proposing the Valley Link Rail Project (Proposed Project), which would establish a new passenger rail service along a 22 mile corridor between the existing Dublin/Pleasanton Bay Area Rapid Transit District (BART) Station in Alameda County and the proposed Mountain House Community Station in San Joaquin County (Appendix A, Figure 1). The rail alignment would be constructed within a combination of existing corridors including Interstate 580 (I 580) freeway median, transportation corridor owned by Alameda County (formerly Southern Pacific Transcontinental Railroad alignment), and California Department of Transportation (Caltrans) right-of-way (ROW) adjacent to the westbound I 580 freeway; and new ROW to be acquired for the Proposed Project. The Proposed Project includes four new stations as well as a Maintenance of Way (MOW) staging area, a Layover Facility (LF) and an Operations and Maintenance Facility/Operations Support Site (OMF/OSS) in areas at the east end of the alignment.

Project Location:

The approximate location of the project can be viewed in Google Maps: <https://www.google.com/maps/@37.722107,-121.6840487890303,14z>



Counties: Alameda and San Joaquin counties, California

ENDANGERED SPECIES ACT SPECIES

There is a total of 17 threatened, endangered, or candidate species on this species list.

Species on this list should be considered in an effects analysis for your project and could include species that exist in another geographic area. For example, certain fish may appear on the species list because a project could affect downstream species.

IPaC does not display listed species or critical habitats under the sole jurisdiction of NOAA Fisheries¹, as USFWS does not have the authority to speak on behalf of NOAA and the Department of Commerce.

See the "Critical habitats" section below for those critical habitats that lie wholly or partially within your project area under this office's jurisdiction. Please contact the designated FWS office if you have questions.

-
1. [NOAA Fisheries](#), also known as the National Marine Fisheries Service (NMFS), is an office of the National Oceanic and Atmospheric Administration within the Department of Commerce.

MAMMALS

NAME	STATUS
San Joaquin Kit Fox <i>Vulpes macrotis mutica</i> No critical habitat has been designated for this species. Species profile: https://ecos.fws.gov/ecp/species/2873	Endangered

BIRDS

NAME	STATUS
California Condor <i>Gymnogyps californianus</i> Population: U.S.A. only, except where listed as an experimental population There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/8193	Endangered
California Least Tern <i>Sternula antillarum browni</i> No critical habitat has been designated for this species. Species profile: https://ecos.fws.gov/ecp/species/8104	Endangered

REPTILES

NAME	STATUS
Alameda Whipsnake (=striped Racer) <i>Masticophis lateralis euryxanthus</i> There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/5524	Threatened
Northwestern Pond Turtle <i>Actinemys marmorata</i> No critical habitat has been designated for this species. Species profile: https://ecos.fws.gov/ecp/species/1111	Proposed Threatened

AMPHIBIANS

NAME	STATUS
California Red-legged Frog <i>Rana draytonii</i> There is final critical habitat for this species. Your location overlaps the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/2891	Threatened
California Tiger Salamander <i>Ambystoma californiense</i> Population: U.S.A. (Central CA DPS) There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/2076	Threatened
Foothill Yellow-legged Frog <i>Rana boylei</i> Population: Central Coast Distinct Population Segment (Central Coast DPS) No critical habitat has been designated for this species. Species profile: https://ecos.fws.gov/ecp/species/5133	Threatened
Western Spadefoot <i>Spea hammondi</i> No critical habitat has been designated for this species. Species profile: https://ecos.fws.gov/ecp/species/5425	Proposed Threatened

INSECTS

NAME	STATUS
Monarch Butterfly <i>Danaus plexippus</i> No critical habitat has been designated for this species. Species profile: https://ecos.fws.gov/ecp/species/9743	Candidate
Valley Elderberry Longhorn Beetle <i>Desmocerus californicus dimorphus</i> There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/7850	Threatened

CRUSTACEANS

NAME	STATUS
Conservancy Fairy Shrimp <i>Branchinecta conservatio</i> There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/8246	Endangered
Vernal Pool Fairy Shrimp <i>Branchinecta lynchi</i> There is final critical habitat for this species. Your location overlaps the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/498	Threatened
Vernal Pool Tadpole Shrimp <i>Lepidurus packardii</i> There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/2246	Endangered

FLOWERING PLANTS

NAME	STATUS
Large-flowered Fiddleneck <i>Amsinckia grandiflora</i> There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/5558	Endangered
Lassics Lupine <i>Lupinus constancei</i> Population: There is final critical habitat for this species. Your location does not overlap the critical habitat. Species profile: https://ecos.fws.gov/ecp/species/7976	Endangered
Palmate-bracted Bird's Beak <i>Cordylanthus palmatus</i> No critical habitat has been designated for this species. Species profile: https://ecos.fws.gov/ecp/species/1616	Endangered

CRITICAL HABITATS

There are 3 critical habitats wholly or partially within your project area under this office's jurisdiction.

NAME	STATUS
California Red-legged Frog <i>Rana draytonii</i> https://ecos.fws.gov/ecp/species/2891#crithab	Final

NAME	STATUS
Delta Smelt <i>Hypomesus transpacificus</i> For information on why this critical habitat appears for your project, even though Delta Smelt is not on the list of potentially affected species at this location, contact the local field office. https://ecos.fws.gov/ecp/species/321#crithab	Final
Vernal Pool Fairy Shrimp <i>Branchinecta lynchi</i> https://ecos.fws.gov/ecp/species/498#crithab	Final

IPAC USER CONTACT INFORMATION

Agency: AECOM
Name: Loren Merrill
Address: 401 West A St
Address Line 2: Unit 1200
City: San Diego
State: CA
Zip: 92101
Email: loren.merrill@aecom.com
Phone: 8054501744

LEAD AGENCY CONTACT INFORMATION

Lead Agency: Federal Transit Administration

Appendix C Valley Link Rail Project Site Photographs



Photo 1. Canal 0.75mi east from Action Area on Shulte Rd in San Joaquin County, CA. This canal is the boundary for Delta Smelt (*Hypomesus transpacificus* - *Threatened*) critical habitat outside the Action Area. Photo Taken on June 21, 2023.



Photo 2. Orchard at eastern portion of Action Area on Mountain House Pkwy between I-580 and Von Sostan Rd in San Joaquin County, CA. This area is within designated Delta Smelt (*Hypomesus transpacificus* - *Threatened*) critical habitat. Photo taken on June 21, 2023.



Photo 3. Annual grassland looking southwest toward action area from vantage point at the intersection of South Central Park and East Funderburg Blvd in Mountain House, CA. Photo Taken on June 21, 2023.



Photo 4. Ephemeral wash appearing to be alkaline viewed from Altamont Pass Rd near mile marker 5.1 looking southwest toward action area. Wash is outside of the action area, but drains to the northeast from the action area. This area is within CNDDDB occurrence polygons for Longhorn Fairy Shrimp (*Branchinecta longiantenna* – *Endangered*) and California Tiger Salamander (*Ambystoma californiense* – *Threatened*) and is within designated critical habitat for California Red-legged Frog (*Rana draytonii* – *Threatened*). Photo taken on June 21, 2023.

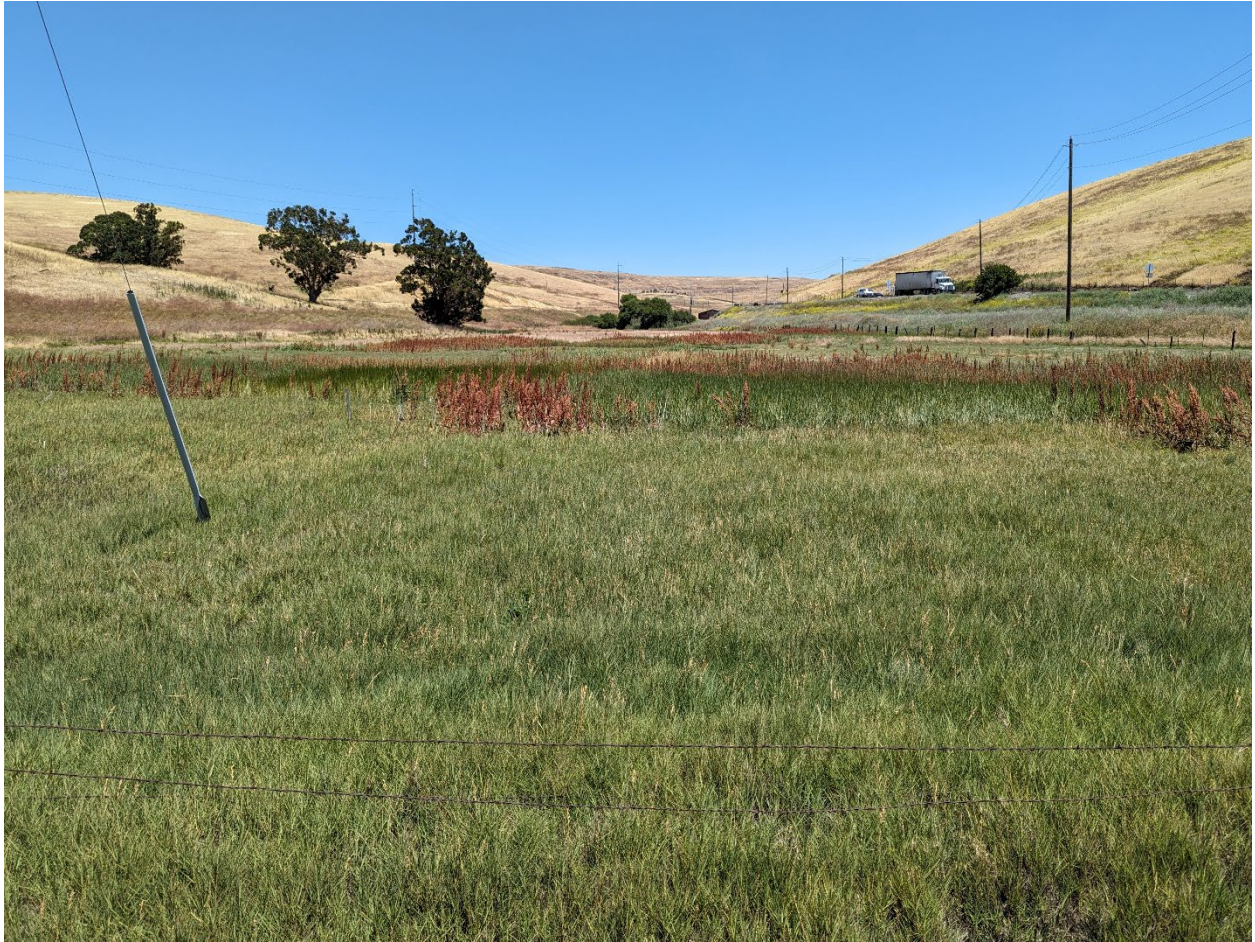


Photo 5. Salt grass flats and wetlands in unnamed seasonal stream channel within the action area at the intersection of Altamont Pass Rd and Dyer Rd in Alameda County, CA looking north east from Dyer Rd. The salt grass stands indicate alkaline influence. Surface water was observed during the site visit. This area is within CNDDDB occurrence polygons for Longhorn Fairy Shrimp (*Branchinecta longiantenna* – *Endangered*), California Tiger Salamander (*Ambystoma californiense* – *Threatened*), and California Red-legged Frog (*Rana draytonii* – *Threatened*) and is within designated critical habitat for California Red-legged Frog (*Rana draytonii* – *Threatened*). Photo taken on June 21, 2023.



Photo 6. Common three-square marsh south of the intersection of the intersection of Altamont Pass Rd and Dyer Rd in an unnamed seasonal stream channel within the action area in Alameda County, CA looking north east from Altamont Pass Rd. This area is within CNDDDB occurrence polygons for Longhorn Fairy Shrimp (*Branchinecta longiantenna* – *Endangered*) and California Tiger Salamander (*Ambystoma californiense* – *Threatened*) and is within designated critical habitat for California Red-legged Frog (*Rana draytonii* – *Threatened*). Photo taken on June 21, 2023.



Photo 7. Unnamed stream in Action Area on Altamont Pass Rd, 0.7mi south of intersection with Dyer Rd in Alameda county, CA looking north from turnout. This area is within CNDDDB occurrence polygons for Longhorn Fairy Shrimp (*Branchinecta longiantenna* – *Endangered*) and California Tiger Salamander (*Ambystoma californiense* – *Threatened*) and is within designated critical habitat for California Red-legged Frog (*Rana draytonii* – *Threatened*). Photo taken on June 21, 2023.



Photo 8. Annual grassland immediately outside of the Action Area to the north near the intersection of Northfront Rd and North Greenville Rd in Livermore CA. This area is mapped in the California Aquatic Resource Inventory (CARI) as a vernal pool complex. This area is within This area is within a CNDDDB occurrence polygon for Longhorn Fairy Shrimp (*Branchinecta longiantenna* – *Endangered*) and is within designated critical habitat for Vernal Pool Fairy Shrimp (*Branchinecta lynchi* – *Threatened*) and California Red-legged Frog (*Rana draytonii* – *Threatened*). Photo taken on June 21, 2023.

Appendix D Valley Link eDNA Sampling Results

August 14, 2024

Marianne Payne
Director of Policy & Planning
Tri-Valley – San Joaquin Valley Regional
Rail Authority
2600 Kitty Hawk Road, Suite 103
Livermore, CA 94551

Valley Link – eDNA Sampling Results for California red-legged frog (*Rana draytonii*), Foothill Yellow-legged Frog (*Rana boylei*), California Tiger Salamander (*Ambystoma californiense*), and Northwestern Pond Turtle (*Actinemys marmorata*)

The Federal Transit Administration (FTA), as lead federal agency, and the Tri-Valley – San Joaquin Valley Regional Rail Authority (Authority), as project sponsor, are proposing the Valley Link Rail Project (Proposed Project), which would establish a new passenger rail service along a 22-mile corridor between the existing Dublin/Pleasanton Bay Area Rapid Transit District (BART) Station in Alameda County and the proposed Mountain House Community Station in San Joaquin County, California. The Proposed Project is expected to be federally funded through the Fixed Guideway Capital Investment Grant. As lead federal agency, FTA is overseeing the preparation of a Draft Environmental Assessment (EA) and supporting technical reports, including a Biological Assessment (BA). AECOM staff has been preparing the BA report to support Section 7 consultation under the Endangered Species Act of 1973 (ESA) between FTA and the United States (U.S.) Fish and Wildlife Service (USFWS). One of the data gathering techniques employed in preparation of the BA is Environmental DNA (eDNA) sampling. This memorandum and its attachments are included with the BA as Appendix D.

AECOM biologists conducted eDNA sampling surveys for California red-legged frog (*Rana draytonii*), foothill yellow-legged frog (*Rana boylei*), California tiger salamander (*Ambystoma californiense*), and northwestern pond turtle (*Actinemys marmorata*) in waterbodies located in the Upper- and Lower Arroyo Las Positas HUC12 watersheds throughout the Valley Link Project Footprint in Alameda County, California. This technical memorandum provides an overview of the sampling methodology and the results of the surveys.

Methodology

On July 9, 2024, AECOM biologists Matthew Bettelheim and Hallie Daly visited waterbodies located in the Upper- and Lower Arroyo Las Positas HUC12 watersheds throughout the Valley Link project footprint to perform eDNA sampling surveys paired with visual encounter surveys for California red-legged frog, foothill yellow-legged frog, California tiger salamander, and northwestern pond turtle. The methodology for these surveys is outlined below.

Visual Encounter Survey

Informal visual encounter surveys were performed in loose accordance with the following published protocols in advance of each eDNA sampling survey; no protocol-level surveys were performed during this survey event:

- *Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog* (U.S. Fish and Wildlife Service 2005)

- *Visual Encounter Survey Protocol for Rana boylei in Lotic Environments* (Peek, Yarnell, and Lind 2017)
- *A Standardized Approach for Habitat Assessments and Visual Encounter Surveys for the Foothill Yellow-Legged Frog (Rana Boylei)* (Seldenrich and Pool 2002)
- *USGS Western Pond Turtle (Emys marmorata) Visual Survey Protocol for the Southcoast Ecoregion* (U.S. Geological Survey 2006)
- *Western Pond Turtle: Biology, Sampling Techniques, Inventory and Monitoring, Conservation and Management* (Bury et al. 2012)

All surveys were performed by two experienced biologists. Visual encounter surveys were performed prior to eDNA sampling to prevent contaminating the eDNA field samples. Biologists walked along the embankment, scanning the shoreline (several meters upland from the water's edge) and instream emergent substrates with binoculars as far as 10 meters upstream for all life stages (eggs, larvae, juveniles, adults) before walking the shoreline and shallow water habitats and inspecting exposed substrates and cover material for signs of species presence. Other herpetofauna, potential predators and invasive species, and generalized observations regarding the available prey base, predators, and habitat suitability were also documented (see Results).

eDNA Sampling

On July 9, 2024, AECOM biologists collected water samples in four waterbodies at eight discrete locations as follows: in Tassajara Creek (samples VLTC-01, VLTC-02, VLTC-03, VLTC-04, VLTC-05, VLTC-06, VLTC-07, and VLTC-08); in Arroyo Las Positas Creek (samples VLAP-01, VLAP-02); in an unnamed tributary to Arroyo Las Positas Creek (VLNP-01, VLNP-02, VLNP-03, VLNP-04, and VLNP-05); and in one unnamed roadside drainage ditch along Dyer Road in the vicinity of the South Bay Aqueduct and Altamont Creek (samples VLD1-01 and VLD1-02) (Figure 1). Altogether, AECOM biologists collected eight paired (e.g., two samples per location) 60-cc (cubic centimeter) or greater water samples at each discrete sampling location for a total of 16 samples. At the unnamed Dyer Road drainage ditch (VLD1-01 and VLD1-02), however, only roughly 30-ccs were collected per sample before the filter clogged due to the high levels of suspended matter observed in the ditch. In addition to the 16 samples, 1 additional unpaired sample (VLNP-03) was collected at Arroyo Las Positas Creek *immediately* downstream of a northwestern pond turtle, bringing the total number of field samples collected to 17 samples.

On July 9, 2024, AECOM biologists also collected two water samples (VLSW-01 and VLSW-02) from an off-site location where foothill yellow-legged frogs are known to occur. This location was in Alameda Creek (Upper Alameda Creek HUC12 Watershed), well outside of the Upper- and Lower Arroyo Las Positas HUC12 watersheds, in the East Bay Regional Park District's Sunol Wilderness Regional Preserve, Alameda County, California. This positive control sample was collected upstream of the Geary Road Bridge crossing from shallow waters where six foothill yellow-legged frog tadpoles were observed identified to species at the time the water sample was collected. Similarly, AECOM biologists also collect two water samples (VLWPT-01 and VLWPT-02) from a 28-gallon aquarium containing a captive western pond turtle (*Actinemys* spp.). The intent of these positive control samples was to validate the efficacy of the species-specific eDNA primer, demonstrating its ability to correctly confirm the presence of foothill yellow-legged frog and turtles in the western pond turtle complex (*Actinemys* spp.).

At the end of the day, two negative control samples (VLMB-01 and VLMB-02) were collected from bottled water to confirm that the sampling equipment wasn't contaminated with target species DNA and/or cross-contaminating samples from other locations.

eDNA water samples were collected using the Aquatic Environmental DNA Sampling Kit supplied by Jonah Ventures, the laboratory selected for these analyses. Samples were collected by drawing 60 milliliters (mL) of water into a sterile 60 mL syringe, and then expelling the water through a sterile 1 µm filter; this process was repeated at least five times per syringe for a total of 300 mL, unless the filter

became clogged with solid material suspended in the water column (e.g., suspended sediment). The filter was considered clogged when it became too difficult to pass additional water through the filter. Each filter was then injected with 5 mL of a preservative (Tris-EDTA [pH 8.0]) to prevent any eDNA present from being degraded or destroyed by high ambient temperatures. Samples were transported and stored on ice in a portable cooler, and then shipped overnight on ice in the cooler to the Jonah Ventures laboratory. Jonah Ventures conducted DNA analyses in adherence with laboratory protocols to identify the presence of eDNA from California red-legged frog, foothill yellow-legged frog, California tiger salamander, and northwestern pond turtle as described below. Photographs of sampling locations are presented in Attachment A.

Laboratory Protocol

A series of standard quantitative polymerase chain reaction (qPCR) methods were used to identify whether the DNA for each target species was present on each sample filter. The laboratory cycled samples through the qPCR instrument utilizing a species-specific primer, which is a short segment of DNA that has been synthesized to bind only to the target species' DNA; the primer will bind and initiate amplification if the target species' DNA is present. During the amplification process, the qPCR instrument quantified the amount of DNA present by evaluating the fluorescence generated during the procedure, which provides an absolute count of the number of copies of a DNA sequence in the sample. Three replicates were run for each target species and each sample. The samples were evaluated in adherence with the following standard protocols.

California Red-Legged Frog Assay

An amplicon from the cytochrome b (Cytb) gene was amplified via qPCR from genomic DNA samples using RedFrog FWD and REV primers, and RedFrog Probe. A standard curve was generated for each run to correspond to the targeted region of the California red-legged frog cytochrome b gene. Each qPCR reaction was run in triplicate and contained 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H₂O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 °C; followed by 50 cycles of 15 seconds at 95 °C and 1 minute at 60 °C.

Forward primer: 5' ATCGGCTCCGACCTAGTT 3'

Reverse primer: 5' AATGTGAAGAATCGGGTGAGA 3'

Probe: 5' /5Cy5/GACTGAAAA/TAO/GCCCCCTCAGATC/3IAbRQSp/ 3'

Primer/probe reference: Jonah Ventures (2019) (see Attachments B and C)

Foothill Yellow-Legged Frog Assay

An amplicon from the cytochrome b gene was amplified via qPCR from genomic DNA samples using YelFrog FWD and REV primers, and YelFrog Probe. A standard curve was generated for each run to correspond to targeted region of the yellow-legged frog cytochrome b gene. Each qPCR reaction was run in triplicate and contained 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H₂O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95°C; followed by 50 cycles of 15 seconds at 95°C and 1 minute at 60°C.

Forward primer: 5' ATCGGCTCCGACCTAGTC 3'

Reverse primer: 5' AAATGTAAAAAATCGGGTGAGG 3'

Probe: 5' /5Cy5/GAAGCCTCC/TAO/CCAGATTCATTGG/3IAbRQSp/ 3'

Primer/probe reference: Jonah Ventures (2019) (see Attachments B and C)

California Tiger Salamander Assay

An amplicon from the Cytochrome C oxidase subunit 1 gene was amplified via qPCR from genomic DNA samples using California Tiger Salamander FWD and REV primers and probe. A standard curve was generated for each run to correspond to the targeted region of the California Tiger Salamander, Cytochrome C oxidase subunit 1 gene. Each qPCR reaction was run in triplicate and contained 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H₂O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 °C; followed by 50 cycles of 15 seconds at 95 °C and 1 minute at 60 °C.

Forward primer: 5' AGATCAGTATTAATTACAGCAGTCCTTC 3'

Reverse primer: 5' GTTTCGATCCGTCAGCAGTAT 3'

Probe: 5' /56-FAM/TCTCTTCCGGTTTTAGCAGCG/3IABkFQ/ 3'

Primer/probe reference: Kieran et al. (2020) (see Attachments B and C)

Northwestern Pond Turtle Assay

An amplicon from the ND4 gene was amplified via qPCR from genomic DNA samples using ActiMarm01 FWD and REV primers and probe. A standard curve was generated for each run to correspond to the targeted ND4 region of *Actinemys marmorata*. Each qPCR reaction was run in triplicate and contained 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H₂O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 °C; followed by 50 cycles of 15 seconds at 95 °C and 1 minute at 60 °C.

Forward primer: 5' TATCCACAACACAATGAGGAGAGAC 3'

Reverse primer: 5' ATAGGGAGGATGTGAAGTATTATGAGG 3'

Probe: 5'- /56-FAM/TCATACATT/ZEN/AAAATAATCCCTCC/3IAbRQSp/ -3'

Primer/probe reference: Kamoroff et al. (2023) (see Attachments B and C)

Additional details regarding the laboratory protocol can be found in Attachments B and C.

Results

Visual Encounter Survey

During the July 9, 2024, survey, no California red-legged frog, foothill yellow-legged frog, or California tiger salamander were detected during the informal visual-encounter surveys in Tassajara Creek; Arroyo Las Positas Creek; the unnamed tributary to Arroyo Las Positas Creek; or other unnamed tributaries, channels, and drainage ditches. Northwestern pond turtles were observed in two discrete locations: along an unnamed tributary to Arroyo Las Positas Creek in the vicinity of Northfront Park (samples VLNP-03, VLNP-04, and VLNP-05) and in Arroyo Las Positas Creek in an in-channel pool downstream of the confluence of Cayetano Creek and Arroyo Las Positas (samples VLNP-01 and VLNP-02). In the unnamed

tributary, a single sub-adult northwestern pond turtle was observed in a shallow channel of water. In Arroyo Las Positas Creek, three adult northwestern pond turtles were observed basking and/or foraging in algal mats in a large pool in the Creek channel.

Potential predatory invasive species observed during the surveys include crayfish (either *Pacifastacus leniusculus* or *Procambarus clarkia*); other potential predatory native species include hitch (*Lavinia exilicauda*) and northwestern pond turtle, which could prey on egg and larval life stages of amphibians.

eDNA Sampling

The results of the eDNA sampling analysis (Attachments B and C) indicate that foothill yellow-legged frog and California tiger salamander are not present in the surveyed reaches of Tassajara Creek, Arroyo Las Positas Creek, the unnamed tributary to Arroyo Las Positas Creek, or the Drey Road drainage ditch (Table 1; Figure 1).

Foothill yellow-legged frog was detected in the positive control sample collected off-site at Alameda Creek (samples VLSW-01 and VLSW-02) where foothill yellow-legged frog tadpoles were visually confirmed at the time of sampling (Table 1; Figure 1; Attachment A: Photo 9). The positive results of the field positive control confirm the efficacy of the foothill yellow-legged frog primers (Table 1). No such field positive control for California tiger salamander was readily available in advance of this eDNA sampling survey.

California red-legged frog was detected in the samples collected along the unnamed tributary to Arroyo Las Positas Creek at two locations (samples VLNP-01 and VLNP-02, and sample VLNP-3) (Table 1; Figure 1).

Western pond turtle was detected in the samples collected along Arroyo Las Positas Creek (sample VLAP-02), along the unnamed tributary to Arroyo Las Positas Creek at two locations (samples VLNP-01, VLNP-02, VLNP-04, and VLNP-05), and at a single location along Tassajara Creek (sample VLTC-06) (Table 1; Figure 1; Attachment A: Photo 10-11). Western pond turtle was also detected in the positive control sample collected immediately downstream of a sub-adult northwestern pond turtle (sample VLNP-03) in Arroyo Las Positas Creek (see Photo 10), as well as two positive control samples collected from a captive western pond turtle (*Actinemys* sp.) (samples VLWPT-01 and VLWPT-02). Incidentally, northwestern pond turtle was also detected in the foothill yellow-legged frog positive control sample collected off-site at Alameda Creek (samples VLSW-01 and VLSW-02) where this species is known to occur. The positive results of the field positive controls confirm the efficacy of the northwestern pond turtle primer (Table 1).

The negative results of the field control (samples VLMB-01 and VLMB-02) confirm that there was no contamination in the field or laboratory sampling procedures.

Discussion

Based on the results of the eDNA sampling analysis (Attachments B and C) paired with species observations (visual encounter surveys) and an informal assessment of the existing conditions at each sampling location, the surveyed reaches of both Arroyo Las Positas Creek and the unnamed tributary to Arroyo Las Positas Creek provide suitable habitat for California red-legged frog and northwestern pond turtle. Northwestern pond turtle was visually confirmed at Arroyo Las Positas Creek, where three adult turtles were observed in an upstream pool, and along the unnamed tributary to Arroyo Las Positas Creek, where a single sub-adult turtle was observed in a shallow channel of water.

Generally speaking, at the time of the site visit, the surveyed reaches of the unnamed Arroyo Las Positas Creek conveyed a small amount of flowing water across what could be described as either a drainage ditch or a channelized Creek characterized by a muddy-to-rocky substrate, narrow reaches, intermittent pools, and algal mats. In comparison, the surveyed reaches of Arroyo Las Positas Creek conveyed a

significantly larger amount of flowing water along a meandering Creek characterized by wide reaches, deep pools, and well-developed wetland and riparian vegetation.

With the exception of extreme drought years, the presence of moderate-or-greater flows and the presence of intermittent or permanent pools during the summer months (July) indicates that the surveyed reaches of both Arroyo Las Positas Creek and the unnamed tributary to Arroyo Las Positas Creek provide suitable aquatic habitat and upland nesting/dispersal habitat for northwestern pond turtle (Table 2).

Table 1. Valley Link eDNA and Visual Survey Results

Sample Site	Sample Site Location	Lab Sample ID	GPS Coordinates	California Red-Legged Frog Detection (Replicates)	Foothill Yellow-Legged Frog Detection (Replicates)	California Tiger Salamander Detection (Replicates)	Northwestern Pond Turtle (Replicates)	Visual Confirmation in the Field
Project Footprint								
Tassajara Creek	VLTC-01	GB4ZKWYI	37.4133, -121.5307	No	No	No	No	No
Tassajara Creek	VLTC-02	BVRD4JOJ	37.4133, -121.5507	No	No	No	No	No
Tassajara Creek	VLTC-03	ZHT98444	37.4131, -121.5309	No	No	No	No	No
Tassajara Creek	VLTC-04	WF23N5AM	37.4131, -121.5309	No	No	No	No	No
Tassajara Creek	VLTC-05	E6U9M2DH	37.4223, -121.5243	No	No	No	No	No
Tassajara Creek	VLTC-06	6YKX2JD2	37.4223, -121.5243	No	No	No	Yes (3)	No
Tassajara Creek	VLTC-07	SY027KK9	37.4233, -121.5247	No	No	No	No	No
Tassajara Creek	VLTC-08	CZ9YYSRA	37.4233, -121.5247	No	No	No	No	No
Arroyo Las Positas	VLAP-01	38WGBCFB	37.4202, -121.4711	No	No	No	No	NWPT (07/09/2024)
Arroyo Las Positas	VLAP-02	7PYLRT5R	37.4202, -121.4711	No	No	No	Yes (2)	NWPT (07/09/2024)
Unnamed tributary to Arroyo Las Positas	VLNP-01	1X8JZ1VU	37.4245, -121.4312	Yes (1)	No	No	Yes (1)	NWPT (07/09/2024)
Unnamed tributary to Arroyo Las Positas	VLNP-02	H2N9C67R	37.4245, -121.4312	Yes (3)	No	No	Yes (3)	NWPT (07/09/2024)
Unnamed tributary to Arroyo Las Positas	VLNP-03	W9F50HXJ	37.4245, -121.4312	Yes (3)	No	No	Yes (3)	CONTROL
Unnamed tributary to Arroyo Las Positas	VLNP-04	SBI962N5	37.4243, -121.4306	No	No	No	Yes (1)	No
Unnamed tributary to Arroyo Las Positas	VLNP-05	H6C937UI	37.4243, -121.4306	No	No	No	No	No
Drey Road Drainage Ditch	VLD1-01	4744WVDQ	37.4421, -121.4017	No	No	No	No	No
Drey Road Drainage Ditch	VLD1-02	ENENYFCP	37.4421, -121.4017	No	No	No	No	No

Sample Site	Sample Site Location	Lab Sample ID	GPS Coordinates	California Red-Legged Frog Detection (Replicates)	Foothill Yellow-Legged Frog Detection (Replicates)	California Tiger Salamander Detection (Replicates)	Northwestern Pond Turtle (Replicates)	Visual Confirmation in the Field
Control Samples								
Alameda Creek (Off-Site)	VLSW-01	JEURXF26	37.50893, -121.82892	No	Yes (3)	No	Yes (1)	CONTROL
Alameda Creek (Off-Site)	VLSW-02	4GTK7IVI	37.50893, -121.82892	No	Yes (1)	No	Yes (3)	CONTROL
Captive <i>Actinemys</i> spp. Water Sample	VLWPT-01	COVH1G63	n/a	No	No	No	Yes (3)	CONTROL
Captive <i>Actinemys</i> spp. Water Sample	VLWPT-02	2QJIAD37	n/a	No	No	No	Yes (3)	CONTROL
AECOM Office	VLMB-01	V5JUHP6K	n/a	No	No	No	No	BLANK (BOTTLED WATER)
AECOM Office	VLMB-02	JZ597FZY	n/a	No	No	No	No	BLANK (BOTTLED WATER)

Likewise, the surveyed reaches of Arroyo Las Positas Creek provide suitable aquatic breeding, non-breeding habitat, and upland habitat for California red-legged frog, while the unnamed tributary to Arroyo Las Positas Creek provides, at the very least, suitable aquatic non-breeding habitat and upland habitat for California red-legged frog; given the smaller channel, certain locations along the unnamed tributary might also provide suitable aquatic breeding habitat under the right conditions if there are locations where pools persist long enough for California red-legged frogs to breed, deposit eggs, and successfully metamorphose (Table 2).

At the one (of four) locations where northwestern pond turtle was detected along Tassajara Creek, the surveyed reach of the Creek conveyed a moderate amount of slow-flowing water across what could be described as a channelized Creek characterized by banked, rip-rapped slopes in places, narrow-bottomed channels, intermittent pools, algal mats, and patches of wetland and riparian vegetation. Although there was only a single northwestern pond turtle detection along Tassajara Creek, the surveyed reaches provide suitable aquatic breeding habitat, non-aquatic breeding habitat, and upland habitat for California red-legged frog as well as aquatic habitat and upland nesting/dispersal habitat for northwestern pond turtle (Table 2).

The surveyed reaches of Tassajara Creek, Arroyo Las Positas Creek, the unnamed tributary to Arroyo Las Positas Creek, or the Drey Road drainage ditch described above do not provide suitable habitat for foothill yellow-legged frog or California tiger salamander (Table 2).

Likewise, at the time of the survey, the Drey Road drainage ditch did not appear to provide suitable habitat for northwestern pond turtle, and may – at best – provide poor intermittent aquatic nonbreeding habitat for California red-legged frog due to its warm, shallow water; high sedimentation; poor vegetative cover; unpredictable hydroperiod; and overall exposed setting within inches of an active paved roadway (Table 2). However, at least one California red-legged frog (CNDDDB Occurrence #616) has been reported within 350 feet of the drainage ditch in the vicinity of Altamont Creek, consisting of a single adult frog reported in December 2002.

Table 2. Survey Reach Habitat Suitability Assessment

Surveyed Reach Sampled	California Red-Legged Frog Habitat	Foothill Yellow-Legged Frog Habitat	California Tiger Salamander Habitat	Northwestern Pond Turtle Habitat
Tassajara Creek	Aquatic Breeding, Aquatic Non-Breeding, Upland	None	None	Aquatic, Upland Nesting/Dispersal
Arroyo Las Positas	Aquatic Breeding, Aquatic Non-Breeding, Upland	None	None	Aquatic, Upland Nesting/Dispersal
Unnamed Tributary to Arroyo Las Positas	Aquatic Breeding, Aquatic Non-Breeding, Upland	None	None	Aquatic, Upland Nesting/Dispersal
Drey Road drainage ditch	Aquatic Non-Breeding (poor)	None	None	None

The persistence of flowing water and/or deep in-channel and backwater pools lower in the watershed in early-to-mid-July in significant portions of Tassajara Creek, Arroyo Las Positas Creek, the unnamed tributary to Arroyo Las Positas Creek, and other unnamed tributaries, channels, and drainage ditches throughout the Upper- and Lower Arroyo Las Positas HUC12 watersheds indicates that, where water is

present, suitable aquatic breeding and non-breeding habitat and the surrounding uplands may be present for California red-legged frog and northwestern pond turtle; these same Creek and stream habitats are unlikely to support foothill yellow-legged frog or California tiger salamander. Higher in the watershed, especially in the vicinity of Altamont Creek, ephemeral pools and ponding water along intermittent Creeks and drainage ditches may support occasional-to-regular aquatic breeding habitat and/or nonbreeding habitat, depending on their individual hydroperiod and the availability of suitable upland dispersal habitat, upland refugia habitat, barriers to wildlife movement, purpose-built and incidental wildlife crossing structures, and known species occurrences in the region.

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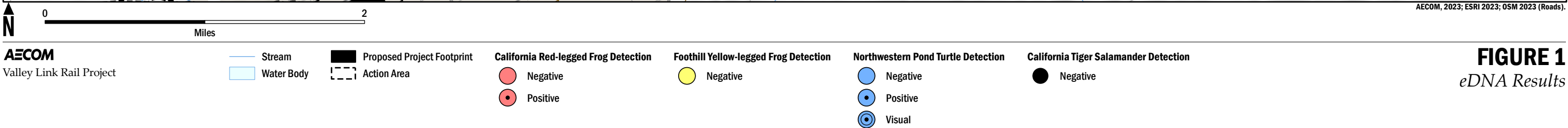
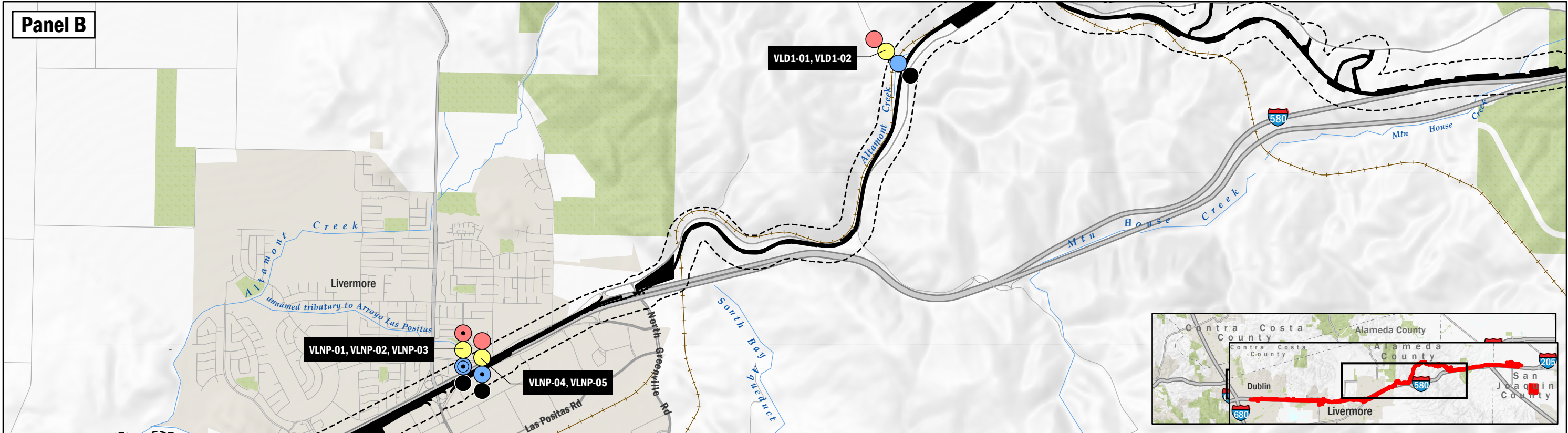
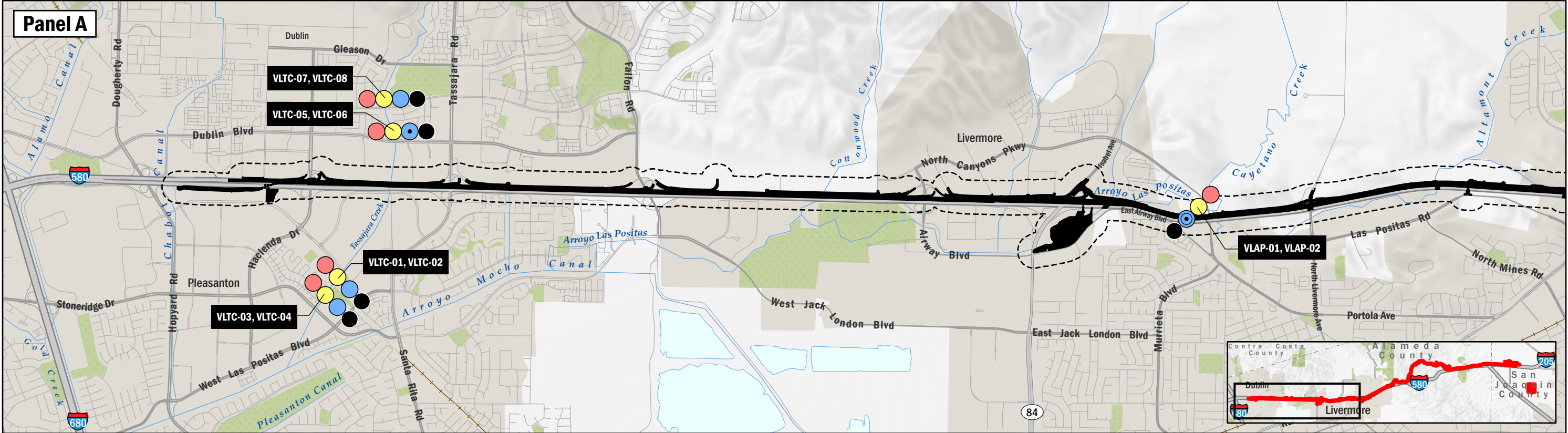
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https://sdmmp.com/upload/SDMMP_Repository/0/4fnpv18xm0sqtw29j7d3rz56bkychg.pdf



Attachment A: Photographs

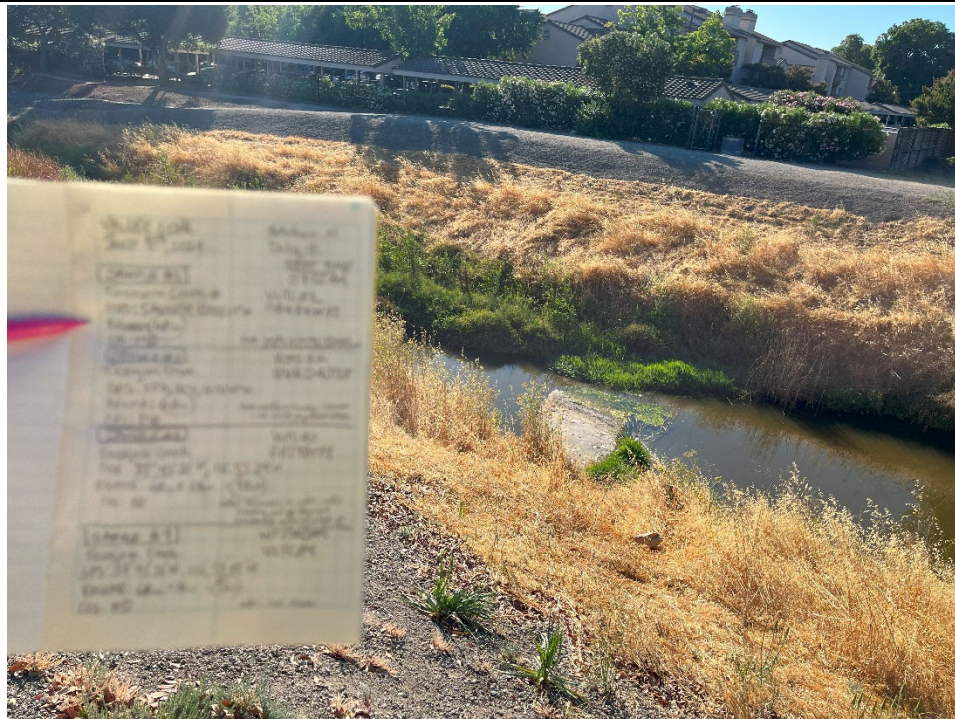


Photo 1. eDNA sampling location VLTC-01 and VLTC-02 (Tassajara Creek)

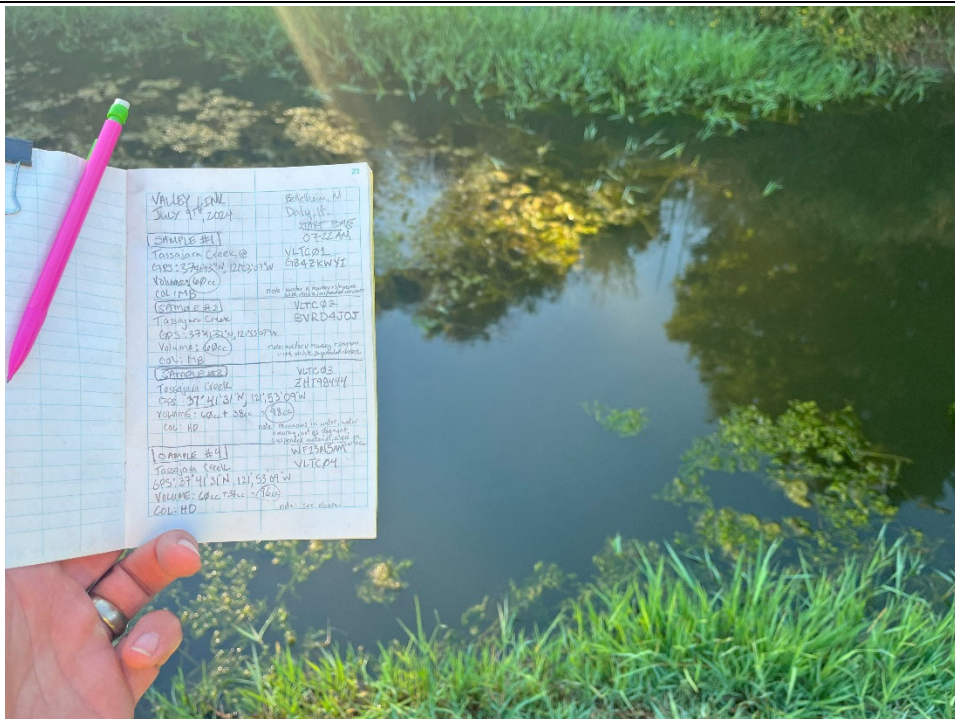


Photo 2. eDNA sampling location for VLTC-03 and VLTC-04 (Tassajara Creek)



Photo 3. eDNA sampling location VLTC-05 and VLTC-06 (Tassajara Creek)

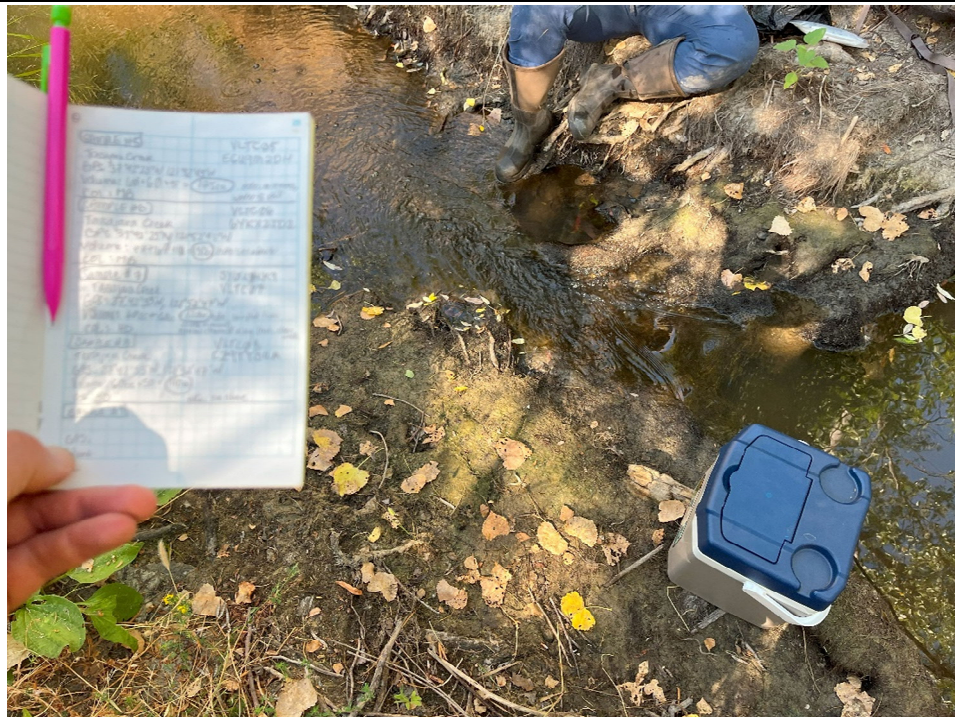


Photo 4. eDNA sampling location for VLTC-07 and VLTC-08 (Tassajara Creek)

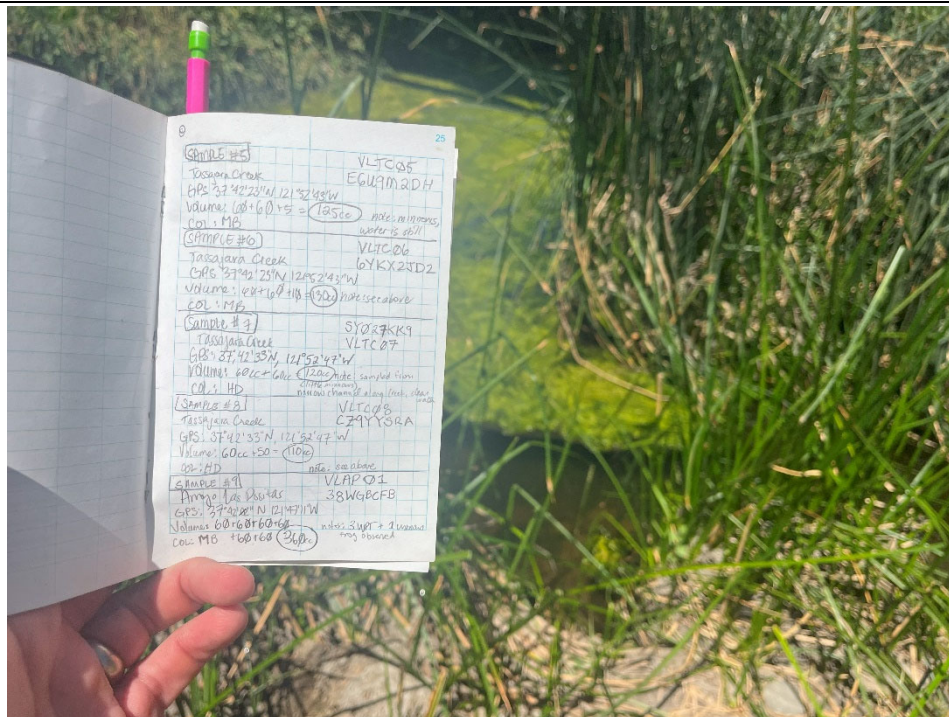


Photo 5. eDNA sampling location VLAP-01 and VLAP-02 (Arroyo Las Positas Creek)

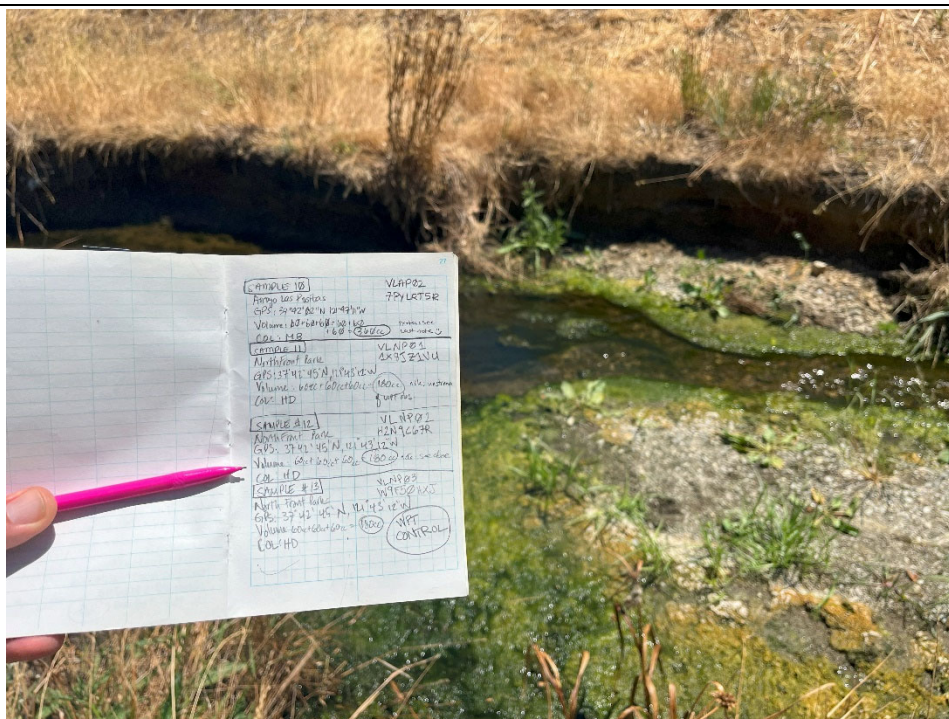
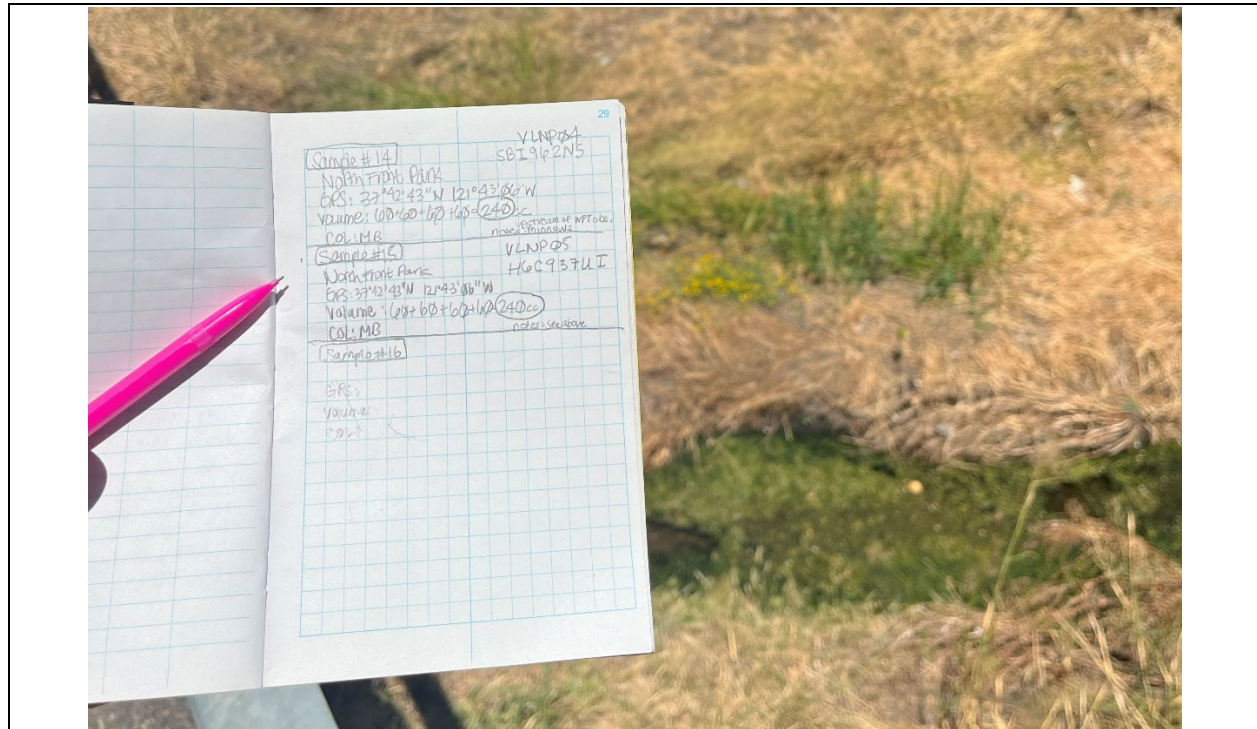


Photo 6. eDNA sampling location for VLNP-01, VLNP-02, and VLNP-03 (unnamed tributary to Arroyo Las Positas Creek)



**Photo 7. eDNA sampling location for VLNP-04 and VLNP-05
(unnamed tributary to Arroyo Las Positas Creek)**

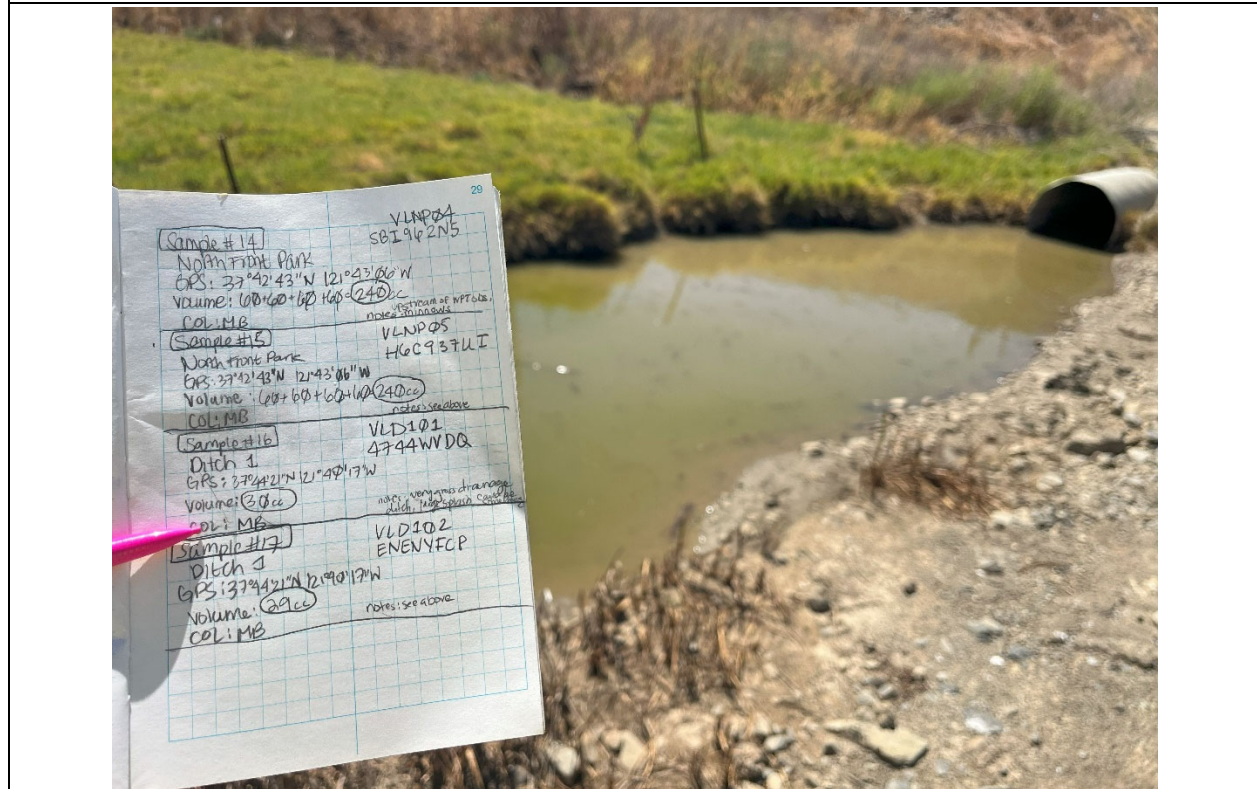


Photo 8. eDNA sampling location for VLD1-03 and VLD2-04 (drainage ditch)



**Photo 9. eDNA sampling location VLSW-01 and VLSW-02 (off-site location: Alameda Creek)
where foothill yellow-legged frog tadpole were present**



**Photo 10. eDNA sampling at location VLNP-03 (unnamed tributary to Arroyo Las Positas Creek)
immediately downstream of sub-adult northwestern pond turtle**



Photo 11. One of three adult northwestern pond turtles observed in Arroyo Las Positas Creek upstream of VLAP-01 and VLAP-02



Photo 12. Several fish, tentatively identified as hitch (*Lavinia exilicauda*) observed in Arroyo Las Positas Creek upstream of VLAP-01 and VLAP-02

Attachment B: Laboratory Results
(Foothill Yellow-Legged Frog, California Red-Legged Frog, California Tiger Salamander)



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KNOWLEDGE IN SEQUENCE

July 24 2024

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Report prepared for matthew.bettelheim@aecom.com

BatchId = JVB3654

Number of samples analyzed = 23

Average number of copies detected

This table shows the average number of copies detected for each target organism in each sample. Values represent the average number of copies / 100 mL when sample volume was provided. When sample volumes are not known, the values indicate the estimated number of copies in the sample.

SampleId	RedLeggedFrog01	YellowLeggedFrog01	CalTigSal01
1X8JZ1VU	13	0	0
38WGBCFB	0	0	0
4744WVDQ	0	0	0
4GTK7IVI	0	161	0
6YKX2JD2	0	0	0
7PYLRT5R	0	0	0
BVRD4JOJ	0	0	0
CZ9YYSRA	0	0	0
E6U9M2DH	0	0	0
ENENYFCP	0	0	0
GB4ZKWYI	0	0	0
H2N9C67R	84	0	0
H6C937UI	0	0	0
JEURXF26	0	17,218	0
JZ597FZY	0	0	0
SBI962N5	0	0	0
SY027KK9	0	0	0
V5JUHP6K	0	0	0
W9F50HXJ	317	0	0
WF23N5AM	0	0	0
ZHT98444	0	0	0

Percent of replicates above detection limit

This table provides data on what percentage of the replicates that were run were above the detection limit. The detection limit is as high as the lowest positive on the calibration curve, but can be up to an order of magnitude lower. For example, a calibration curve might generate a positive at 100 copies and no positive for 10 copies, but the actual detection limit would be 11 copies. See the next section for the range of copy numbers estimated for each assay.

SampleId	RedLeggedFrog01	YellowLeggedFrog01	CalTigSal01
1X8JZ1VU	33.3	0.0	0
38WGBCFB	0.0	0.0	0
4744WVDQ	0.0	0.0	0
4GTK7IVI	0.0	33.3	0
6YKX2JD2	0.0	0.0	0
7PYLRT5R	0.0	0.0	0
BVRD4JOJ	0.0	0.0	0
CZ9YYSRA	0.0	0.0	0
E6U9M2DH	0.0	0.0	0
ENENYFCP	0.0	0.0	0
GB4ZKWYI	0.0	0.0	0
H2N9C67R	100.0	0.0	0
H6C937UI	0.0	0.0	0
JEURXF26	0.0	100.0	0
JZ597FZY	0.0	0.0	0
SBI962N5	0.0	0.0	0
SY027KK9	0.0	0.0	0
V5JUHP6K	0.0	0.0	0
W9F50HXJ	100.0	0.0	0
WF23N5AM	0.0	0.0	0
ZHT98444	0.0	0.0	0

Detailed results

The following table provides the estimated copy number for individual technical replicates for each qPCR assay. Missing values indicate failed reactions or outliers that were removed from the analysis.

TestId	SampleId	Rep 1	Rep 2	Rep 3
CalTigSal01	1X8JZ1VU	0	0	0
	38WGBCFB	0	0	0
	4744WVDQ	0	0	0
	4GTK7IVI	0	0	0
	6YKX2JD2	0	0	0
	7PYLRT5R	0	0	0
	BVRD4JOJ	0	0	0
	CZ9YYSRA	0	0	0
	E6U9M2DH	0	0	0
	ENENYFCP	0	0	0
	GB4ZKWYI	0	0	0
	H2N9C67R	0	0	0
	H6C937UI	0	0	0
	JEURXF26	0	0	0
	JZ597FZY	0	0	0
	SBI962N5	0	0	0
	SY027KK9	0	0	0
	V5JUHP6K	0	0	0
	W9F50HXJ	0	0	0
	WF23N5AM	0	0	0
	ZHT98444	0	0	0
RedLeggedFrog01				
	1X8JZ1VU	0	0	38

TestId	SampleId	Rep 1	Rep 2	Rep 3
	38WGBCFB	0	0	0
	4744WVDQ	0	0	0
	4GTK7IVI	0	0	0
	6YKX2JD2	0	0	0
	7PYLRT5R	0	0	0
	BVRD4JOJ	0	0	0
	CZ9YYSRA	0	0	0
	E6U9M2DH	0	0	0
	ENENYFCP	0	0	0
	GB4ZKWYI	0	0	0
	H2N9C67R	68	88	95
	H6C937UI	0	0	0
	JEURXF26	0	0	0
	JZ597FZY	0	0	0
	SBI962N5	0	0	0
	SY027KK9	0	0	0
	V5JUHP6K	0	0	0
	W9F50HXJ	217	493	240
	WF23N5AM	0	0	0
	ZHT98444	0	0	0
YellowLeggedFrog01				
	1X8JZ1VU	0	0	0
	38WGBCFB	0	0	0
	4744WVDQ	0	0	0
	4GTK7IVI	0	482	0
	6YKX2JD2	0	0	0
	7PYLRT5R	0	0	0
	BVRD4JOJ	0	0	0
	CZ9YYSRA	0	0	0

TestId	SampleId	Rep 1	Rep 2	Rep 3
	E6U9M2DH	0	0	0
	ENENYFCP	0	0	0
	GB4ZKWYI	0	0	0
	H2N9C67R	0	0	0
	H6C937UI	0	0	0
	JEURXF26	17772	17503	16380
	JZ597FZY	0	0	0
	SBI962N5	0	0	0
	SY027KK9	0	0	0
	V5JUHP6K	0	0	0
	W9F50HXJ	0	0	0
	WF23N5AM	0	0	0
	ZHT98444	0	0	0

Sample metadata

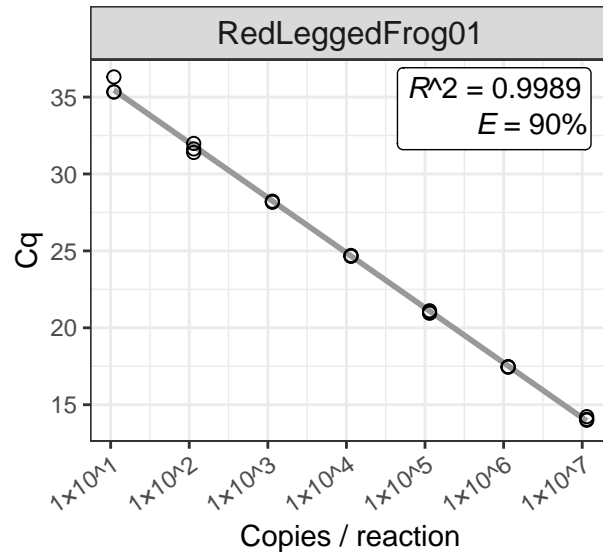
SampleId	Volume Water (ml)
1X8JZ1VU	180
2QJIADJ7	121
38WGBCFB	360
4744WVDQ	30
4GTK7IVI	120
6YKX2JD2	125
7PYLRT5R	360
BVRD4JOJ	60
COVH1G63	120
CZ9YYSRA	120
E6U9M2DH	125
ENENYFCP	29
GB4ZKWYI	60
H2N9C67R	180
H6C937UI	240
JEURXF26	120
JZ597FZY	240
SBI962N5	240
SY027KK9	130
V5JUHP6K	240
W9F50HXJ	180
WF23N5AM	96
ZHT98444	98

Methods and calibration curves

The following pages provide details of the methods used for each qPCR assay and the associated standard curves. Each assay in each run is associated with a calibration curve based typically on a series of 7, 10-fold dilutions of a standard with a known concentration. The calibration curves show the relationship between the \log_{10} -transformed standard concentration and the number of PCR cycles at which the detection threshold was reached (Cq). A linear regression is applied to this relationship and the r^2 intercept and slope extracted for further analyses.

- RunId = An internal identifier for the standard curve(s) used to calculate copy numbers in the submitted samples. Assays that share a RunId are multiplexed (i.e., multiple targets amplified in a single reaction).
- R^2 = The coefficient of determination, or goodness of fit for the linear relationship (should be > 0.98).
- (E) = The reaction efficiency, or how close to a doubling of product was achieved with each PCR cycle. For a 10-fold dilution, 100% efficiency is for ~ 3.3 cycles per 10-fold dilution. A range of values is acceptable here, but we try to keep efficiency between 85% - 110%.

RunID: JVQ0596



Forward primer: 5' ATCGGCTCCGACCTAGTT 3'

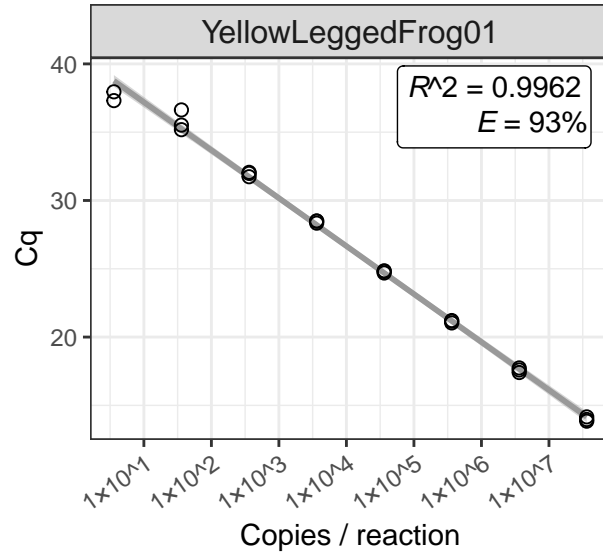
Reverse primer: 5' AATGTGAAGAATCGGGTGAGA 3'

Probe: 5' /5Cy5/GACTGAAAA/TAO/GCCCCCTCAGATC/3IAbRQSp/ 3'

Primer/probe reference: Jonah Ventures, 2019

An amplicon from the cytochrome b (Cytb) gene was amplified via qPCR from genomic DNA samples using RedFrog FWD and REV primers, and RedFrog Probe. A standard curve was generated for each run to correspond to targeted region of the California red legged frog cytochrome b gene. Each qPCR reaction is run in triplicate and contains 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H₂O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

RunID: JVQ0597



Forward primer: 5' ATCGGCTCCGACCTAGTC 3'

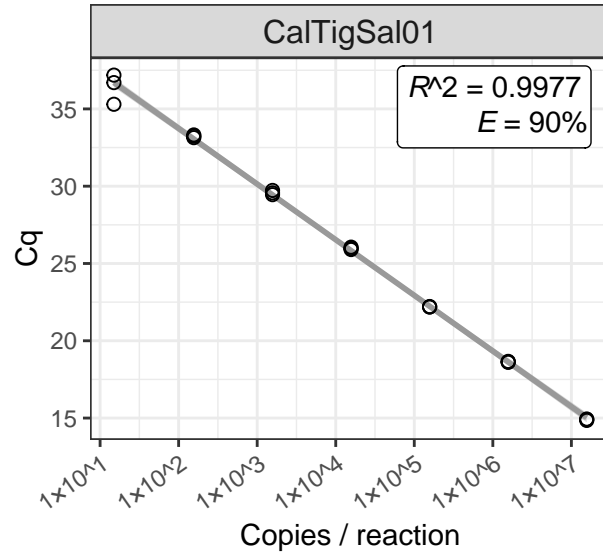
Reverse primer: 5' AAATGTAAAAAATCGGGTGAGG 3'

Probe: 5' /5Cy5/GAAGCCTCC/TAO/CCAGATTCATTGG/3IAbRQSp/ 3'

Primer/probe reference: Jonah Ventures, 2019

An amplicon from the cytochrome b (Cytb) gene was amplified via qPCR from genomic DNA samples using YelFrog FWD and REV primers, and YelFrog Probe. A standard curve was generated for each run to correspond to targeted region of the yellow legged frog cytochrome b gene. Each qPCR reaction is run in triplicate and contains 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H₂O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

RunID: JVQ0598



Forward primer: 5' AGATCAGTATTAATTACAGCAGTCCTTC 3'

Reverse primer: 5' GTTTCGATCCGTCAGCAGTAT 3'

Probe: 5' /56-FAM/TCTCTTCCGGTTTTAGCAGCG/3IABkFQ/ 3'

Primer/probe reference: Kieran et al., 2020

An amplicon from the Cytochrome C oxidase subunit 1 gene was amplified via qPCR from genomic DNA samples using California Tiger Salamander FWD and REV primers and probe. A standard curve was generated for each run to correspond to targeted region of the California Tiger Salamander, Cytochrome C oxidase subunit 1 gene. Each qPCR reaction is run in triplicate and contains 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H₂O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

**Attachment C: Laboratory Results
(Northwestern Pond Turtle)**



JONAH VENTURES

KNOWLEDGE IN SEQUENCE

August 07 2024

For more information go to www.jonahventures.com

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Report prepared for matthew.bettelheim@aecom.com

BatchId = JVB3795

Number of samples analyzed = 23

Average number of copies detected

This table shows the average number of copies detected for each target organism in each sample. Values represent the average number of copies / 100 mL when sample volume was provided. When sample volumes are not known, the values indicate the estimated number of copies in the sample.

SampleId	ActiMarm01
1X8JZ1VU_r2	43
2QJIADJ7_r2	192,297
38WGBCFB_r2	0
4744WVDQ_r2	0
4GTK7IVI_r2	135
6YKX2JD2_r2	383
7PYLRT5R_r2	28
BVRD4JOJ_r2	0
COVH1G63_r2	178,805
CZ9YYSRA_r2	0
E6U9M2DH_r2	0
ENENYFCP_r2	0
GB4ZKWYI_r2	0
H2N9C67R_r2	261
H6C937UI_r2	0
JEURXF26_r2	44
JZ597FZY_r2	0
SBI962N5_r2	22
SY027KK9_r2	0
V5JUHP6K_r2	0
W9F50HXJ_r2	32,451
WF23N5AM_r2	0
ZHT98444_r2	0

Percent of replicates above detection limit

This table provides data on what percentage of the replicates that were run were above the detection limit. The detection limit is as high as the lowest positive on the calibration curve, but can be up to an order of magnitude lower. For example, a calibration curve might generate a positive at 100 copies and no positive for 10 copies, but the actual detection limit would be 11 copies. See the next section for the range of copy numbers estimated for each assay.

SampleId	ActiMarm01
1X8JZ1VU_r2	33.3
2QJIADJ7_r2	100.0
38WGBCFB_r2	0.0
4744WVDQ_r2	0.0
4GTK7IVI_r2	100.0
6YKX2JD2_r2	100.0
7PYLRT5R_r2	66.7
BVRD4JOJ_r2	0.0
COVH1G63_r2	100.0
CZ9YYRA_r2	0.0
E6U9M2DH_r2	0.0
ENENYFCP_r2	0.0
GB4ZKWYI_r2	0.0
H2N9C67R_r2	100.0
H6C937UI_r2	0.0
JEURXF26_r2	33.3
JZ597FZY_r2	0.0
SBI962N5_r2	33.3
SY027KK9_r2	0.0
V5JUHP6K_r2	0.0
W9F50HXJ_r2	100.0
WF23N5AM_r2	0.0

SampleId	ActiMarm01
ZHT98444_r2	0.0

Detailed results

The following table provides the estimated copy number for individual technical replicates for each qPCR assay. Missing values indicate failed reactions or outliers that were removed from the analysis.

TestId	SampleId	Rep 1	Rep 2	Rep 3
ActiMarm01				
	1X8JZ1VU_r2	130	0	0
	2QJIADJ7_r2	193171	223634	160087
	38WGBCFB_r2	0	0	0
	4744WVDQ_r2	0	0	0
	4GTK7IVI_r2	136	136	132
	6YKX2JD2_r2	470	415	264
	7PYLRT5R_r2	44	40	0
	BVRD4JOJ_r2	0	0	0
	COVH1G63_r2	183001	181742	171673
	CZ9YYSRA_r2	0	0	0
	E6U9M2DH_r2	0	0	0
	ENENYFCP_r2	0	0	0
	GB4ZKWYI_r2	0	0	0
	H2N9C67R_r2	364	281	137
	H6C937UI_r2	0	0	0
	JEURXF26_r2	132	0	0
	JZ597FZY_r2	0	0	0
	SBI962N5_r2	66	0	0
	SY027KK9_r2	0	0	0
	V5JUHP6K_r2	0	0	0
	W9F50HXJ_r2	37992	31092	28270
	WF23N5AM_r2	0	0	0
	ZHT98444_r2	0	0	0

Sample metadata

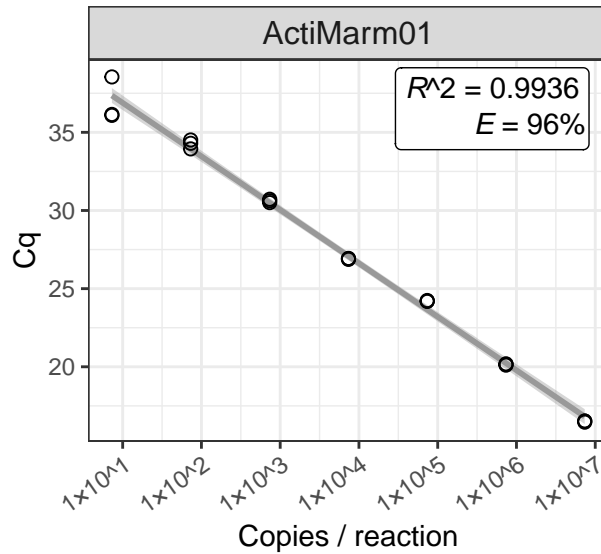
SampleId	Volume Water (ml)
1X8JZ1VU_r2	180
2QJIADJ7_r2	121
38WGBCFB_r2	360
4744WVDQ_r2	30
4GTK7IVI_r2	120
6YKX2JD2_r2	125
7PYLRT5R_r2	360
BVRD4JOJ_r2	60
COVH1G63_r2	120
CZ9YYSRA_r2	120
E6U9M2DH_r2	125
ENENYFCP_r2	29
GB4ZKWYI_r2	60
H2N9C67R_r2	180
H6C937UI_r2	240
JEURXF26_r2	120
JZ597FZY_r2	240
SBI962N5_r2	240
SY027KK9_r2	130
V5JUHP6K_r2	240
W9F50HXJ_r2	180
WF23N5AM_r2	96
ZHT98444_r2	98

Methods and calibration curves

The following pages provide details of the methods used for each qPCR assay and the associated standard curves. Each assay in each run is associated with a calibration curve based typically on a series of 7, 10-fold dilutions of a standard with a known concentration. The calibration curves show the relationship between the \log_{10} -transformed standard concentration and the number of PCR cycles at which the detection threshold was reached (Cq). A linear regression is applied to this relationship and the r^2 intercept and slope extracted for further analyses.

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- (E) = The reaction efficiency, or how close to a doubling of product was achieved with each PCR cycle. For a 10-fold dilution, 100% efficiency is for ~ 3.3 cycles per 10-fold dilution. A range of values is acceptable here, but we try to keep efficiency between 85% - 110%.

RunID: JVQ0608



“Forward primer: 5’ TATCCACAACACAATGAGGAGAGAC 3’

Reverse primer: 5’ ATAGGGAGGATGTGAAGTATTATGAGG 3’

Probe: 5’- /56-FAM/TCATACATT/ZEN/AAAATAATCCCTCC/3IAbRQSp/ -3’

Primer/probe reference: Kamoroff et al. (2023)

An amplicon from the ND4 gene was amplified via qPCR from genomic DNA samples using ActiMarm01 FWD and REV primers and probe. A standard curve was generated for each run to correspond to the targeted ND4 region of *Actinemys marmorata*. Each qPCR reaction is run in triplicate and contains 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.”