Species Biological Report for Southern Edwards Aquifer Springs and Associated Aquatic Ecosystems

Fountain darter (*Etheostoma fonticola*), Peck's cave amphipod (*Stygobromus pecki*), Comal Springs riffle beetle (*Heterelmis comalensis*), Texas wild-rice (*Zizania texana*), San Marcos salamander (*Eurycea nana*), Texas blind salamander (*Eurycea rathbuni*), and Comal Springs dryopid beetle (*Stygoparnus comalensis*)

U.S. Fish and Wildlife Service
Southwest Region
Albuquerque, NM

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Summary

This Species Biological Report informs the Draft Recovery Plan for the Southern Edwards Aquifer Springs and Associated Aquatic Ecosystems (2024a, entire). The Species Biological Report is a comprehensive biological status review by the U.S. Fish and Wildlife Service (USFWS) for seven species and provides an account of species overall viability. A Recovery Implementation Strategy, which provides the expanded narrative for the recovery activities and the implementation schedule will later be available at the USFWS Environmental Conservation Online System website within each species profile page. The Recovery Implementation Strategy and Species Biological Report are finalized separately from the Recovery Plan and will be updated on a routine basis.

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Prepared by Donelle Robinson, Ph.D. (USFWS, Headquarters Office) and Amelia Hunter (USFWS, Southwest Regional Office). We dedicate a special thanks to former USFWS biologist, Pat Connor (Austin Ecological Services Field Office) for gathering source material used to inform this Species Biological Report.

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Notes

Critical habitat definitions previously defined Primary Constituent Elements (PCEs) as those specific elements of the physical and biological features that provide for a species' life history processes and are essential to the conservation of the species (50 CFR 424.12(b)). The new critical habitat regulations (81 FR 7414) discontinue use of the PCE term or reference to essential habitat features and rely exclusively on use of the term "physical or biological features" (PBFs) for that purpose. To be consistent with that shift in terminology and in recognition that the terms PBFs, PCEs, and essential habitat features are synonymous in meaning, we are only referring to PBFs herein.

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Executive Summary

This biological report provides a scientific review by the U.S. Fish and Wildlife Service (USFWS) for seven aquatic species (Table 1) that occur in the hydraulically connected subsurface aquifer habitats and spring-fed headwater habitats in the southern segment of the Edwards Aquifer in Comal and Hays Counties in Texas. This report summarizes our evaluation of their life history, ecology, threats, and viability, in terms of the conservation biology principles of resiliency, redundancy, and representation (i.e., the 3Rs). We will use the information presented in this biological report to provide the best available scientific information on which to base recovery planning and implementation. It is a living document, so it can be updated as needed, and may be used to help support any other future decisions for these species under the Endangered Species Act (ESA), if necessary.

The primary threat to these species is the loss of springflows and decreases in subsurface habitat due to drawdown of the Edwards Aquifer. Climate change may increase pressure to withdraw water from the aquifer. Additional threats include factors that decrease water quality, direct or indirect habitat destruction, alterations of natural flow regimes, disturbance, or modification by humans (e.g., recreational activities, dam building, concrete filling, excavation, bank stabilization, and control of aquatic vegetation), and nonnative species. The area is experiencing rapid development that is expected to increase the extent of threats to water quality and quantity.

In terms of viability, all species are narrow endemics and rely on springflow or groundwater from the southern Edwards (Balcones Fault Zone) Aquifer. Each species needs resilient populations across its range to maintain its persistence into the future and to avoid extinction. Three species (i.e., Texas wild-rice, San Marcos salamander, and Texas blind salamander) have only one extant population and effectively lack redundancy compared to species with more than one population, making it more difficult for these species to withstand and recover from stochastic or catastrophic events. All species are highly susceptible to extinction from perturbations that would affect water quality and quantity in the aquifer, and management is needed to maintain the resiliency of each species. Future potential losses from habitat disturbances associated with examined threats may reduce the number of resilient populations (redundancy), and the genetic diversity of the species (representation), affecting their ability to adapt to changes in the environment.

Table 1. Listing action and critical habitat designation summary table for the seven aquatic Edwards Aquifer species, Comal Springs dryopid beetle (*Stygoparnus comalensis*), Comal Springs riffle beetle (*Heterelmis comalensis*), Fountain darter (*Etheostoma fonticola*), Peck's cave amphipod (*Stygobromus pecki*), San Marcos salamander (*Eurycea nana*), Texas blind salamander (*Eurycea rathbuni*), and Texas wild-rice (*Zizania texana*). "FR" stands for Federal Register.

Species	**Listing FR**	**Listing Date**	**Critical Habitat FR**	**Critical Habitat Date**	**Critical Habitat**
Comal Springs dryopid beetle	62 FR 66295	1997	72 FR 39248, 78 FR 63100	2007, 2013	15.56 hectares (38.45 acres) surface habitat and 56 hectares (138 acres) subsurface habitat at Fern Bank and Comal springs
Comal Springs riffle beetle	62 FR 66295	1997	72 FR 39248, 78 FR 63100	2007, 2013	22 hectares (55 acres) surface habitat at San Marcos and Comal springs
Fountain darter	35 FR 16047, 40 FR 44412	1967	45 FR 47362	1980	San Marcos River from Spring Lake to 0.8 kilometers (0.5 mile) downstream of I-35
Peck's cave amphipod	62 FR 66295	1997	72 FR 39248, 78 FR 63100	2007, 2013	15.16 hectares (37.46 acres) surface habitat and 56 hectares (138 acres) subsurface habitat at Comal and Hueco springs
San Marcos salamander	45 FR 47355	1980	45 FR 47362	1980	San Marcos River from Spring Lake to 50 meters (164 feet) downstream of the Spring Lake dam
Texas blind salamander	32 FR 4001, 35 FR 16047, 40 FR 44412	1967			Critical habitat is not designated
Texas wild-rice	43 FR 17910	1978	45 FR 47355	1980	San Marcos River from Spring Lake to the confluence with the Blanco River

1.0 Species Information and Ecosystems

This section consists of background information on the distribution, status, habitat requirements, biology, ecology, and habitat of the seven species included in this recovery plan. This information provides the basis for assessing the current status, threats to persistence, and the most effective recovery and conservation strategies for these species.

1.1 Ecosystem Hydrology

The Edwards (Balcones Fault Zone) Aquifer (herein referred to as the "Edwards Aquifer") is an extensive karst aquifer system in central Texas. It is an important natural resource which provides unique habitat for federally listed species and supports a high degree of endemism (62 FR 66295; Hutchins 2018, p. 490; Devitt et al. 2019, p. 2,630). The species covered are dependent upon the southern segment of the Edwards Aquifer and its associated aquatic habitats. The southern segment (Figure 1) is the most geographically extensive segment, comprised of all or parts of Kinney, Uvalde, Medina, Bexar, Frio, Comal, and Hays Counties, approximately 290 kilometers (km) (180 miles [mi]) in length (Lindgren et al. 2004, p. 2).

There are three hydrogeological zones (Figure 1): the contributing zone (drainage area), the recharge zone, and the artesian (confined) zone (Schindel 2019, pp. 12-13). Rainfall and surface water accumulate and flow downgradient through the watershed over generally impermeable limestone of the contributing zone. As these streams cross the recharge zone, streamflow enters the ground through open and interconnected fractures and filters through sinkholes and caves (Clark 2000, pp. 6–8; Ferrill et al. 2019, p. 173). Geologic features receive water, nutrients, and contaminants that percolate and circulate underground (Schindel 2019, pp. 11-16). The water flows underground downgradient to the artesian zone and is pressurized by subsurface geologic formations (Hanson and Small 1995, pp. 5-7; Ferrill et al. 2019, pp. 176-184). The hydraulic pressure of groundwater between these rock layers forces groundwater to the surface through cavities, faults, and fissures where it resurfaces at springs (Lindgren et al. 2004, pp. 35, 39-40).

The important spring and riverine ecosystems for these seven listed endemic species are in Comal and Hays counties, Texas. The high degree of endemism of the San Marcos and Comal fauna may be a result of the relatively constant, isolated spring habitat and water quality.

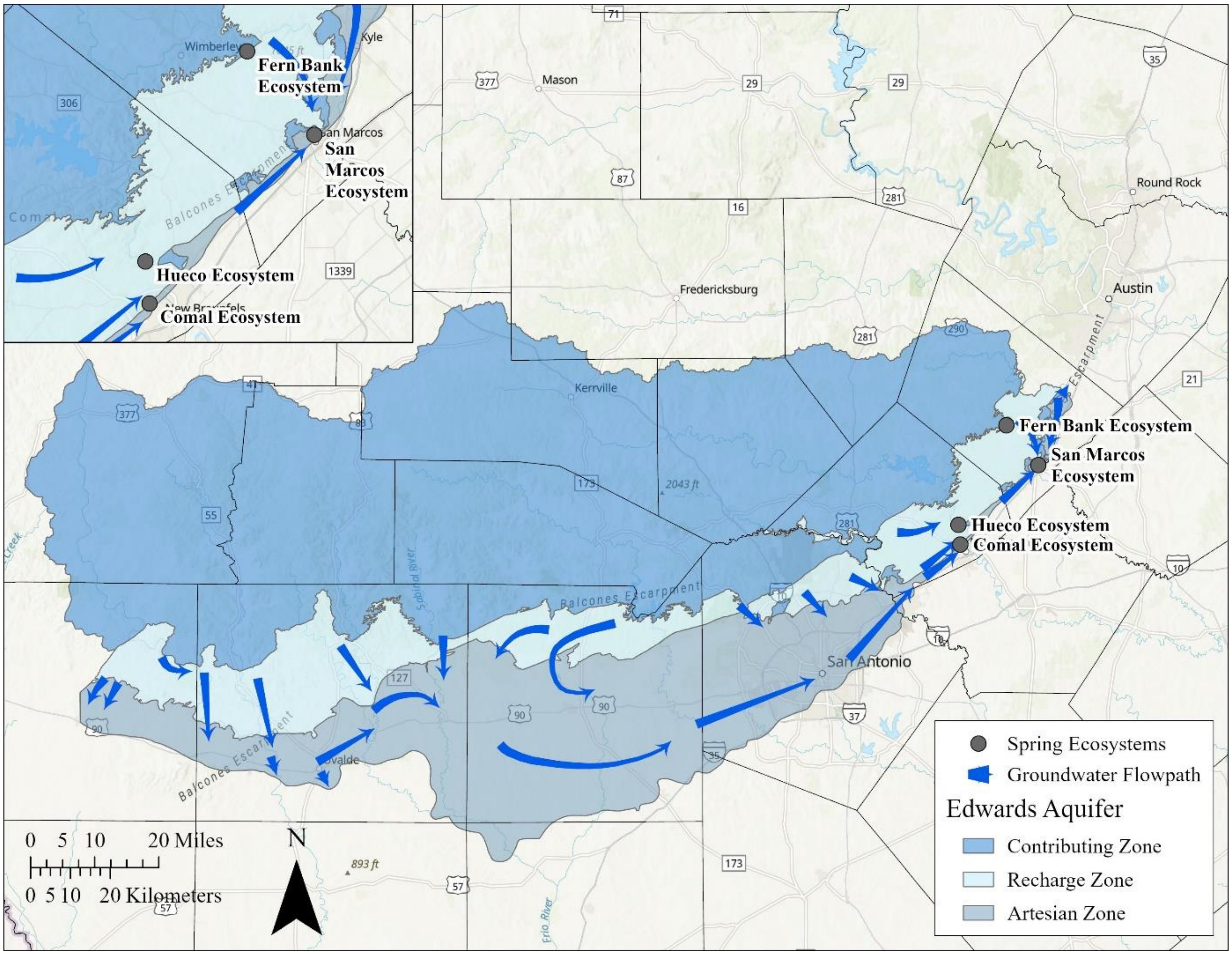

Figure 1. General conceptual flowpath of the Edwards Aquifer's southern segment and locations of the four spring ecosystems of this report. The drainage area is also referred to as the contributing zone, as applied in this report. The groundwater flowpaths on this map are simplified for informational purposes only. Adapted from Edwards Aquifer Authority (EAA) 2023, unpaginated.

1.1.1 Comal Ecosystem

Comal Springs, the largest spring system in Texas and the headwaters to the Comal River (Figure 2), is situated in the recharge and artesian zones of the Edward Aquifer. It primarily receives its flow from the deep, regional flowpath, with minimal local contributions (George et al. 1952, p. 50; Puente 1976, p. 22; Rothermel and Ogden, 1987, pp. 68–69; Musgrove and Crow 2012, pp. 60, 68). The main recharge for this ecosystem comes from major basins to the southwest, including West Nueces/Nueces, Frio/Dry Frio, Sabinal, and Medina basins, while the Guadalupe River basin has a negligible impact (Guyton and Associates 1979, p. 71; Slattery and Choi 2021, unpaginated). During low flows, the regional flowpath may bypass Comal Springs, continuing toward San Marcos Springs (Figure 1) (LBG-Guyton Associates et al. 2004, p. B-42).

The hydrological separation of Comal Springs is underscored by faulting, crucial in delineating flowpaths to the springs where the species are found. The hydrogeology of the Comal Springs ecosystem exhibits distinct patterns between the three primary spring runs 1 through 3 (historically J, K, and L, respectively) and spring runs 4, 5, and 7, as well as Landa Lake (Brune 1981, p. 130; LBG-Guyton Associates et al. 2004, p. B-18; Hutchins et al. 2016, p. 1539). These spring runs empty into Blieder's Creek and into the western end of Landa Lake, which was originally dammed in 1847 (U.S. Army Corps of Engineers 1964, p. 36; Linam et al. 1993, p. 342).

Spring runs 1 through 3 receive groundwater above the Comal Springs Fault, while runs 4, 5, and 7, along with Landa Lake, discharge deeper groundwater below the fault. This hydrological separation is underscored by variations in water chemistry, such as water temperatures (Rothermel and Ogden 1987, p. 129; LBG-Guyton Associates et al. 2004, p. B-24–B-25; Lucas et al. 2016, p. 2). The fault-induced separation not only affects groundwater depths but also influences the physical connectivity of spring runs 1-3, as they are not entirely interconnected due to faulting. This additional hydrological intricacy further enhances the distinctiveness of the springs' responses during storm events (Brune 1981, p. 130; LBG-Guyton Associates et al. 2004, p. B-18; Hutchins et al. 2016, p. 1539). These observations underscore the multifaceted nature of the hydrological dynamics within the Comal Springs ecosystem and may elucidate the absence of certain species between spring runs, implying potential movement restrictions due to the observed hydrological disparities.

Spring runs 1 through 3 near Panther Canyon have gravel substrate with some bedrock, while spring run 4, near Blieder's Creek, is slower with muddy substrate and vegetation (Crowe 1994, p. 41). Blieder's Creek also provides water to Landa Lake at Comal Springs and is subject to high surface water flow in the watershed (HMT Engineering and Surveying 2011, pp. 1-2). Landa Lake is deeper, due in part to dams, and has low water velocity with a muddy bottom and aquatic vegetation (Crowe 1994, pp. 41-42). Two land masses, Pecan Island to the southwest and Spring Island (located within the Comal County Water Recreation District #1) to the northeast, are positioned in the center of Landa Lake.

Water flows from Landa Lake to the old and new channels of the Comal River, merges downstream, then flows generally southward 2.5 km (1.6 mi) to the confluence of the Guadalupe River (Crowe 1994, p. 44; Ockerman and Slattery 2008, p. 2). The old channel is largely unaltered, with some flows diverted to Schlitterbahn water park (Crowe 1994, p. 44). The new channel is man-made with some areas of bedrock riverbed (Crowe 1994, p. 44). At the confluence with the Guadalupe, a backwater effect is created due to the river depth and reduces velocity into the Guadalupe River (Crowe 1994, p. 57). This river responds with higher discharge during spring storms and receives storm run-off, and flows are reduced during fall and winter months (Crowe 1994, p. 46).

Flows at Comal Springs can fluctuate significantly between wet and dry years, and severe droughts can occur. Spring run 1 (of spring runs 1 through 3) is the first to go dry during drought conditions when springflows drop below 2.8 m^3/s (100 cfs) (Spangler and Barr 1995, p. 304). Comal Springs is perennial but ceased flowing for five months in 1956, during the most severe drought on record, and portions have dried for several months at a time during many subsequent droughts (U.S. Army Corps of Engineers 1964, p. 59; Wanakule 1990, p. 2). This occurred because recharge into the aquifer was reduced due to low rainfall in the recharge and contributing zones and because groundwater withdrawal was not curtailed enough to prevent springs from dewatering (Loáiciga et al. 2000, pp. 184, 192; Johnson and Schindel 2008, p. 60; Musgrove and Crow 2012, pp. 82, 88).

Water Quality

The median water temperature remains relatively stable at both the spring runs and Landa Lake at Comal Springs, measuring a mean daily average of 20.7°C (69.3°F) and 23.4°C (74.1°F), respectively (BIO-WEST, Inc. 2021b, p. 18; EAA 2024a, unpaginated). The spring runs maintain on average a specific conductivity (585 micro siemens/centimeter) and dissolved oxygen (5.25 milligram per liter [mg/L]), with few detections of contaminants, such as personal care products and pharmaceuticals (BIO-WEST, Inc. 2021b, p. 18; EAA 2021a, pp. 27-36, 45-47; EAA 2024a, unpaginated).

Despite the generally good groundwater quality at Comal Springs, there has been a noticeable trend since the 1970s. While total dissolved solids and conductivity have been on the rise, they are currently stabilizing. Conversely, nitrates have doubled, with a median concentration of 2 mg/L, since the 1970s (Musgrove et al. 2016, pp. 462, 465, 467; EPA 2023, unpaginated). These shifts in water quality within both streams and groundwater align with the escalation of impervious cover across the watershed (Kaushal et al. 2005, p. 13,518; Baker et al. 2019, pp. 6,494–6,495; Castaño-Sánchez et al. 2020, p. 6). These alterations in water quality parameters may serve as a long-term indicator of the urbanization that has already transpired in the recharge zone. More discussion about this threat is described in Section 2.1.2.

The Comal River, spring-fed with a mean water temperature of 23.6°C (74.5°F) at the Old Channel, exhibits fluctuations in mean daily conductivity in the Old Channel (573 micro siemens/centimeter) and dissolved oxygen averaging 7.02 mg/L, impacted by rainfall occurrences that result in reduced conductivity values relative to springwater due to surface water runoff (EAA 2022a, p. 35; MCWE 2023, pp. 20, 24; EAA 2024b, unpaginated). Water temperature fluctuates downstream from the springs due to increased exposure time to ambient temperatures and runoff from rain events (BIO-WEST 2019a, p. 20).

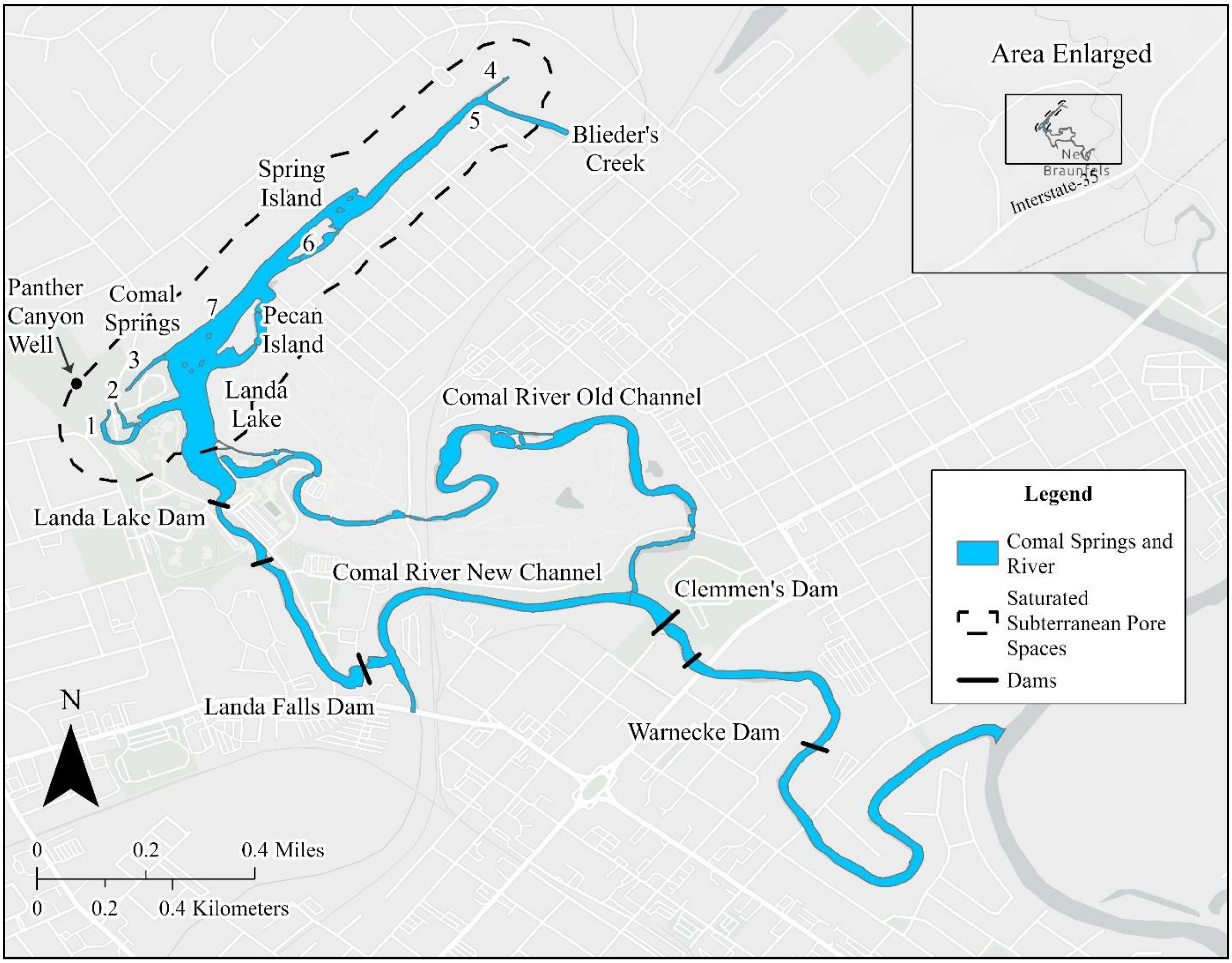

Figure 2. Comal ecosystem in Comal County, Texas. Numbers on map indicate spring run locations referenced in this report. The saturated subterranean pore spaces perimeter is equivalent to the subsurface critical habitat designation of 110 meters (360 feet) within spring outlets where invertebrates are known to range (78 FR 63100).

1.1.2 Hueco Ecosystem

Hueco Springs (Figure 3) features numerous spring upwellings at the head of the spring run located on private property, while across a road, a private campground hosts two major springs with several upwellings (Guyton and Associates 1979, p. 55; Krejca and Sprouse 2003, p. 1; Fries et al 2004, p. 21). While the primary spring outlet is situated on undeveloped land, additional satellite springs are found within a privately owned campground (78 FR 63109). Hueco Springs is situated adjacent to River Road, a popular route for recreational activities along the Guadalupe River. Due to its high recreational traffic, there is a potential vulnerability to road runoff and spills associated with the frequent passage of vehicles (62 FR 66295).

These springs consists of a mixture of local and recent surface-water recharge that discharges from alluvium west of the Guadalupe River and flows southward into the river (Guyton and Associates 1979, p. 21; Musgrove and Crow 2012, p. 86). During wet periods, flows at Hueco Springs are highly responsive to storm events that increase and dilute water with higher proportions of local recharge (Ogden et al. 1986, pp. 118, 125, 127; Musgrove and Crow 2012, pp. 53, 56-57). Tracer tests and a contamination event (i.e., diesel spill) suggests a regional groundwater connection to Comal Springs, though further testing is needed (Ogden et al. 1986, pp. 122-126; Gibson et al. 2008, p. 75). During dry periods, the springs may receive water from the Trinity Aquifer (Otero 2007, pp. 18, 21). Of the two major spring orifices, the large spring on the west side stops flowing during severe drought events, and the spring on the east side of River Road typically stops flowing during the driest months each year (Puente 1976, pp. 25-27; Guyton and Associates 1979, p. 46; Ogden et al. 1986, p. 122; Barr 1993, p. 36).

The average annual discharge from 2002 to 2022 (USGS station 08168000) was 1.2 m^3/s (43 ft^3/s) (U.S. Geological Survey 2023, unpaginated). However, due to the location of these springs on private property, access for researchers is rarely granted, leading to relatively little information about other habitat conditions. Consequently, the best available data on water quality in these springs remains incomplete. The shallow spring waters are reported to maintain relatively constant conditions, with near neutral pH (6.8-7.0), temperatures ranging between 20.7-21.5 °C (69.3-70.7 °F), and oxygen levels that are supersaturated (5.0-6.8 mg/L; over 100 percent saturation). Few detections of contaminants, such as personal care products and pharmaceuticals, have been reported (Fries et al. 2004, pp. 4, 13; EAA 2015, pp. 56-58; EAA 2018, p. 5). Notably, historical data indicated relatively high nitrate levels of 2.1 mg/L in the water from Hueco Springs, suggesting a higher susceptibility to surface pollution compared to San Marcos and Comal Springs. The smaller and more localized recharge area of Hueco Springs supports this observation, although it is currently the least developed by human activities (Guyton and Associates 1979, pp. 77, 79). However, the lack of comprehensive data due to restricted access poses challenges in fully understanding and managing the water quality of these springs. More discussion about this threat is described in Section 2.

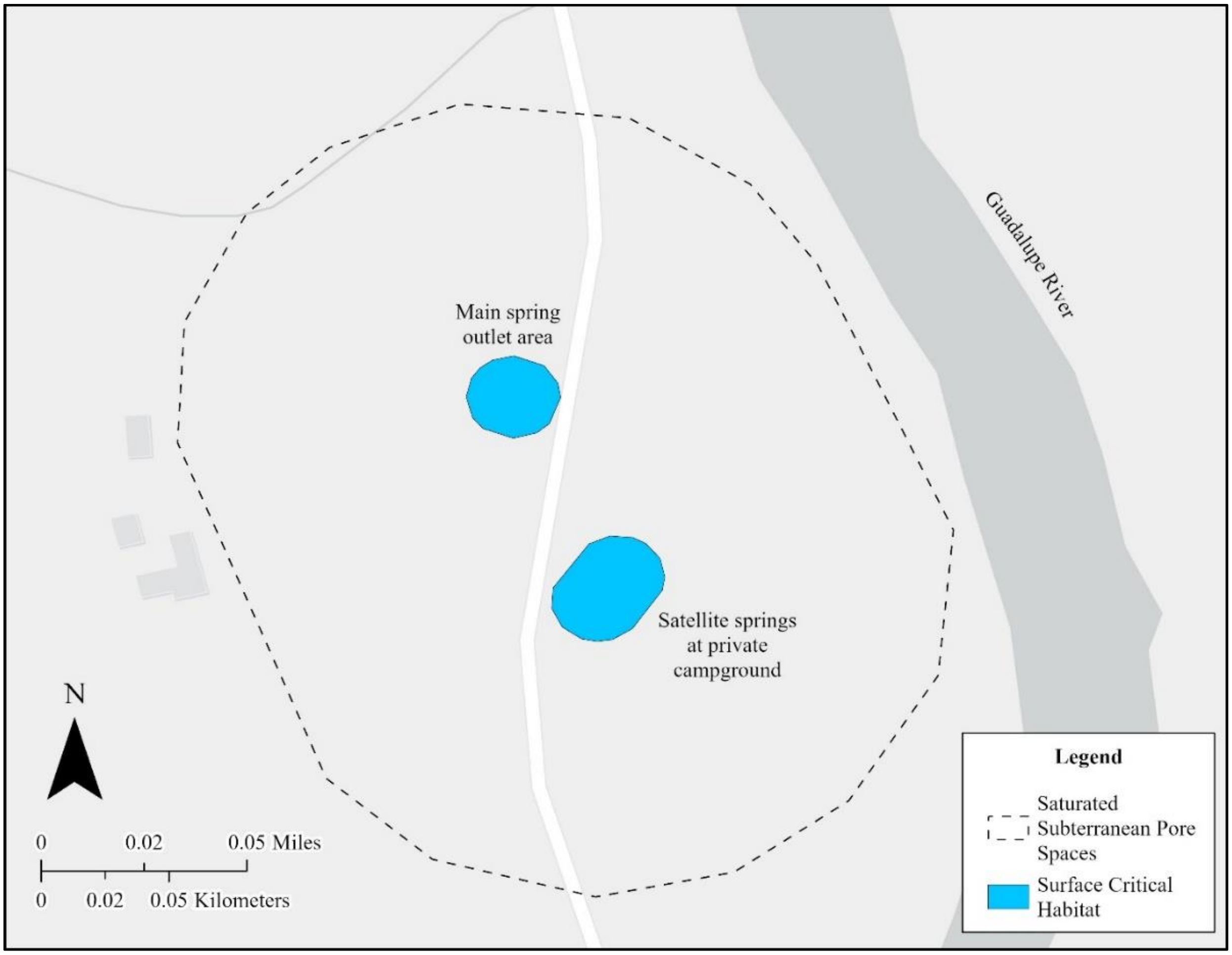

Figure 3. Hueco Springs ecosystem adjacent to the Guadalupe River in Comal County, Texas on private property. The saturated subterranean pore spaces perimeter is equivalent to the subsurface critical habitat designation of 110 meters (360 feet) within spring outlets where invertebrates are known to range (78 FR 63100).

1.1.3 San Marcos Ecosystem

San Marcos Springs is the second largest spring system in Texas and is the headwaters of the San Marcos River (Figure 4). San Marcos Springs is located in the artesian zone of the aquifer and receives water from the regional flowpath northeast from Comal Springs parallel to the Balcones Fault Zone (DeCook 1963, pp. 48, 53; Puente 1976, pp. 25–26; Guyton and Associates 1979, p. 71). Groundwater traveling northeast from Comal Springs becomes increasingly diluted with recharge upon reaching San Marcos Springs along the regional flowpath, cooling one degree to approximately 22°C (72°F) once it reaches San Marcos Springs (Ogden et al. 1986, p. 121; Johnson and Schindel 2008, pp. 45–47). The water discharging from San Marcos Springs represents the lowest altitude spring of the southern segment of the Edwards Aquifer, which is more narrow and closer to the surface (Guyton and Associates 1979, pp. 57, 61, 79; Longley 1981, p. 125; Lindgren et al. 2004, p. 40). Historically, this spring ecosystem exhibited the greatest flow dependability and environmental stability among all spring ecosystems in the southwestern United States (Brune 1981, p. 220). In addition to sustaining surface habitat in the ecosystem through springflows, Edwards Aquifer groundwater also provides subsurface habitat for species in wells and caves in the San Marcos area (see Sections 1.2.3, 1.3.3, 1.4.3, 1.6.3, 1.7.3).

San Marcos Springs receives primarily regional recharge but can be influenced by minor amounts of local recharge sources and/or saline groundwater for short periods, with water quality representative of shallow groundwater and no seasonality (Ogden et al. 1986, p. 120; LBG-Guyton Associates et al. 2004, p. B-43; Johnson and Schindel 2008, p. 60; Musgrove and Crow 2012, pp. 47, 80, 89; Nowlin and Schwartz, 2012 p. 56). Additional local recharge sources to San Marcos ecosystem might include the Blanco River, Sink, Purgatory, York, and Alligator Creek basins (Guyton and Associates 1979, p. 71; Ogden et al. 1985, p. 118). Transitioning from dry to wet conditions, San Marcos Springs might receive water an additional smaller, and more variable amount influence from local flow sources such as Sink Creek, but the percentages of local sources reported in early studies (Guyton and Associates 1979, p. 71; Ogden et al. 1985, p. 118) should be interpreted with caution due to conflicting findings in subsequent research (Johnson and Schindel 2008, p. 60; Johnson et al. 2012, pp. 49, 86–87; Musgrove and Crow 2012, pp. 65, 89).

Downstream of the San Marcos Springs headwaters, a dam was constructed in 1849 to create Spring Lake with a maximum depth of 6.7 m [22 ft]) (Bousman and Nickels 2003 p. 1; ZARA Environmental, LLC. 2005, p. 1). Groundwater discharges from the bottom of Spring Lake through sand boils (75 percent) and discrete orifices (25 percent) (Quick and Ogden 1985 p. 507; Ogden et al. 1986, p. 121; LBG-Guyton and Associates et al. 2004, p. B-49; Musgrove and Crow 2012 pp. 86–89). The spring water originates primarily from dilute regional discharge and limited local surface recharge (less than 30 percent), and a subset of springs discharge a small contribution from a saline water source (less than 1 percent), which tends to increase from dry to wet conditions (Johnson and Schindel 2008, p. 44; Musgrove and Crow 2012, pp. 57, 65, 80, 86–87). Local recharge is primarily sourced from the Blanco River and supplemented by ephemeral streams, as well as other potential contributions from groundwater, either originating from the Trinity Aquifer or the saline water zone (Guyton and Associates 1979, p. 71; Musgrove and Crow 2012, pp. 80, 88).

The upper San Marcos River is primarily fed from San Marcos Springs and extends from Spring Lake to the confluence with the Blanco River, a distance of approximately 11 km (7 mi). This portion of river is 5-15 m (16-49 ft) wide and up to 4 m (13 ft) deep with predominantly gravel and gravel/sand substrate. It contains numerous shallow riffles interspersed with deep pools (Crowe 1994, p. 110). Willow Springs and Purgatory creeks are normally dry except during periods of high rainfall (Brune 1981, pp. 224-225; Quick and Ogden 1985, p. 502). The lower San Marcos River, the area beyond the confluence with the Blanco River, is not exclusively spring-fed, has reduced water quality, increased stormwater runoff, and increased wastewater treatment discharges. Consequently, it does not offer suitable habitat for the majority of the species under consideration in this report. More discussion on river water quality is described in Section 2.1.2.

Sessom Springs, located 0.8 kilometers [km] (0.5 mile [mi]) from San Marcos Springs, emerges from a fault between the recharge and artesian zones of the Edwards Aquifer and Sessom Creek (Schwartz et al. 2020, p. 10). It exhibits greater water quality variability compared to the San

Marcos River due to responses from rain events (EAA 2022a, p. 24). Hydrologically interconnected with San Marcos Springs, Sessom Springs supports listed species such as the Texas blind salamander (Section 1.7) and Comal Springs dryopid beetle (Section 1.3) (Krejca 2007, p. 3; Kosnicki and Julius 2019b, p. 3).

Flow at San Marcos Springs has been monitored intermittently since 1894, with more reliable measurements beginning in 1956 (USGS gage 08170000) (Puente 1976, p. 27; U.S. Geological Survey 2022, unpaginated). The mean annual discharge (USGS station 08178710) from 1956 to 2022 was 4.9 m^3/s (175 ft^3/s) (EAA 2022a, p. 14). Temperatures are stable in areas closest to springs, like Spring Lake, and increase in variability downstream in the river (BIO-WEST, Inc. 2021c, pp. vii, 24; EAA 2022a, p. 16). Continuous monitoring of dissolved oxygen shows that dissolved oxygen varies at different springs in Spring Lake, and also with springflows, and varied from approximately 1.5-5.5 mg/L at different springs during 2010-2012 (MCWE 2012, p. 66).

Similar to Comal Springs, flows can fluctuate significantly between wet and dry years, such that severe droughts are a threat to the listed species (Section 2.1). The lowest recorded monthly flow from the San Marcos River was 1.53 m^3/s (54 ft^3/s) during 1956 (Guyton and Associates 1979, p. 71). The lowest measured daily flow rate occurred on August 15th and 16th, 1956 when the San Marcos River flowed at only 1.29 m^3/s (45.5 ft^3/s). San Marcos Springs has never ceased flow in recorded history, even during the drought of record and during many subsequent droughts (Nace and Pluhowski 1965, pp. 81–87; Ogden et al. 1986, pp. 117–118; LBG-Guyton Associates et al. 2004, p. B45).

Water Quality

Continuous groundwater flows in San Marcos Springs result in nearly constant temperatures (2021 average: 22°C [72°F]) (Musgrove and Crow 2012, p. 47; EAA 2022a, pp. 15–16). The flowing spring waters at San Marcos Springs at Spring Lake are clear with varying levels of dissolved oxygen, dependent on amount and source of discharge (less than 40 to 63 percent saturated, 2-7 mg/L) and few detections of contaminants, such as personal care products and pharmaceuticals (Tupa and Davis 1976, p. 182; Groeger et al. 1997, pp. 285-286; Nowlin and Schwartz 2012, pp. 65-67; EAA 2015, pp. 58-59). San Marcos Springs receives primarily regional recharge but can be influenced by minor amounts of local recharge sources and/or saline groundwater for short periods, with water quality representative of shallow groundwater and no seasonality (Ogden et al. 1986, p. 120; LBG-Guyton Associates et al. 2004, p. B-43; Johnson and Schindel 2008, p. 60; Musgrove and Crow 2012, pp. 47, 80, 89; Nowlin and Schwartz 2012, p. 56).

The San Marcos River is spring-fed, with a mean water temperature of 22°C (72°F) at the headwaters above the intersection of Aquarena Springs Drive and originates from the Edwards and other karst limestone aquifers, resulting in relatively high total alkalinity (200–300 mg/L as calcium carbonate), conductivity mean daily average of 612 microsiemens per centimeter, and 8 mg/L dissolved oxygen (Benavides et al. 2023, pp. 199, 221; EAA 2024c, unpaginated). Water temperature fluctuates downstream from the springs due to increased exposure time to ambient temperatures and runoff from rain events (BIO-WEST 2019b, p. 16). The upper San Marcos River is known for its rapid flow and remarkable clarity. However, adverse impacts on water quality due to urbanization and development within the upper San Marcos River Watershed have been observed (Arismendez 2020, entire). Water quality impairments are further discussed in Section 2.1.2.

Water quality has not been documented at the shallow spring waters at Sessom Springs. While the springs are likely fed by regional groundwater flow, the possibility of contributions from surface water flows, possibly comprising Sessom Creek water with known higher non-point source contaminant levels upstream of the springs, adds an additional layer of uncertainty (Loiácomo 2019, p. 42; EAA 2022, pp. 24, 52-53).

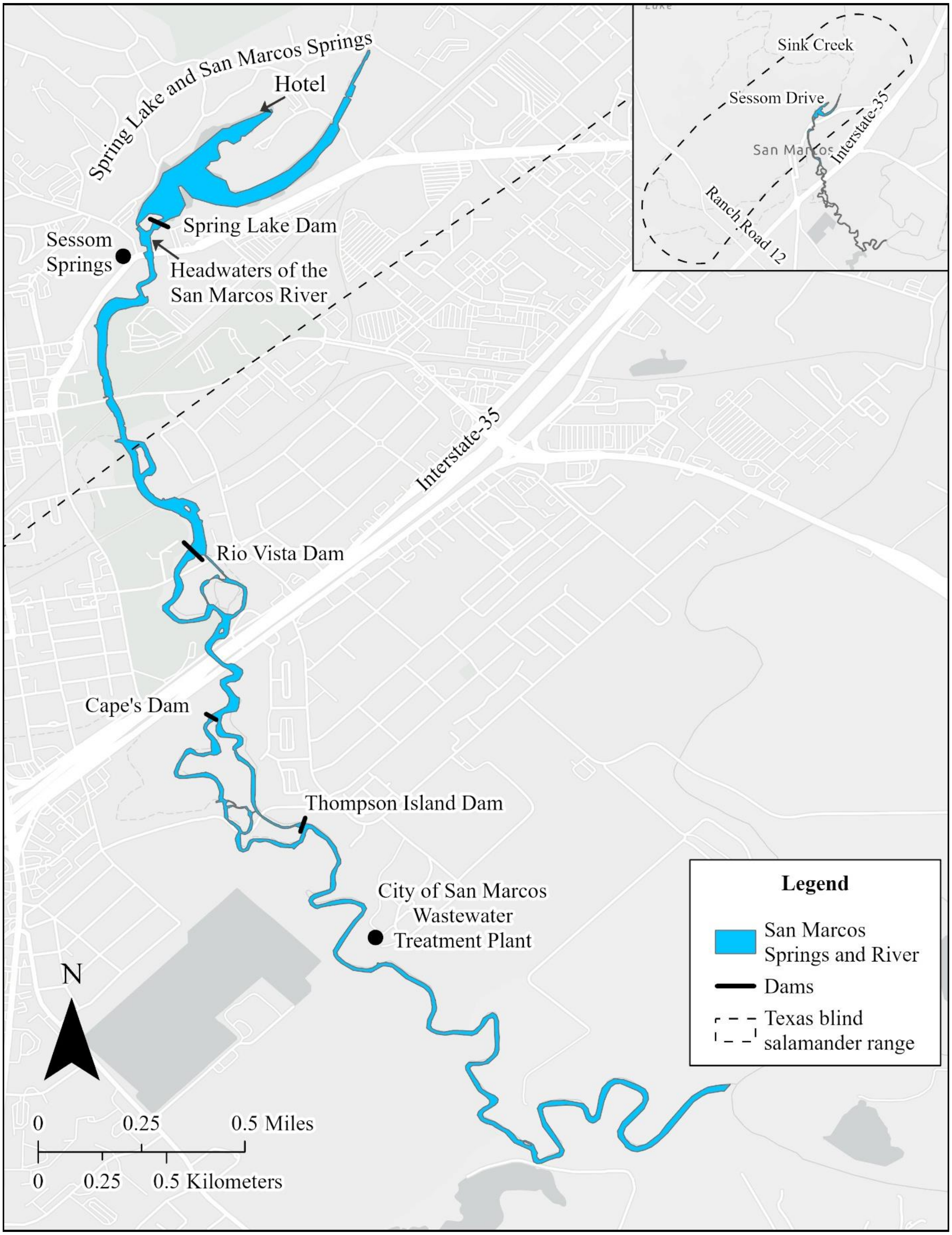

Figure 4. San Marcos ecosystem in Hays County, Texas. The dotted outline encompasses subsurface habitat for the Texas blind salamander, including private caves and wells that intersect the aquifer.

1.1.4 Fern Bank Ecosystem

Fern Bank Springs (Figure 5), also known as Little Arkansas Spring, is located in the recharge zone of the aquifer (Barr 1993, p. 53; Johnson et al. 2012, p. 80). Fern Bank Springs discharges from a cave, and the stream cascades into a manmade pool and continues down the bluff into the Blanco River just upstream of the Edwards Aquifer recharge zone (Fries et al. 2004, p. 8; Gibson et al. 2008, p. 76; Johnson et al. 2012, pp. 79-80). These springs are located on private property, and researchers are rarely granted access to this site.

Hydrogeologists have not fully resolved the source(s) that provide springflow to Fern Bank Springs, but it is most likely sourced from the Edwards Aquifer (Gary 2023, pers. comm.). Fern Bank springflow has been hypothesized to originate from one or a combination of the additional sources: the upper member of the Glen Rose formation (upper Trinity Aquifer), and/or waters associated with the Blanco River (Heitmuller and Reece 2006, zipped file; Veni 2006, pers. comm.; Johnson et al. 2012, pp. 80-81). However, the upper Trinity Aquifer is unlikely to contribute much artesian flow, because the water levels of this aquifer around Wimberley, Texas are lower than the elevation, and other nearby springs are draining from the unsaturated Edwards Aquifer in this area (Gary 2023, pers. comm.).

Fern Bank Springs discharge is not gaged, is only intermittently estimated, and is considered perennial (Barr 1993, p. 39). Early reports estimate springflow discharges of 0.14 m^3/s (4.9 ft^3/s) in 1975, and 0.008 m^3/s (0.3 ft^3/s) in 1978 (Brune 1981, p. 225). The shallow spring waters at Fern Bank Springs are neutral (pH 7.2), the temperature averages 21°C (70°F) (higher on average than Comal Springs during low flows), waters are supersaturated with oxygen (6.8 to 7.4 mg/ L; 98 to 100 percent saturation), and flows are relatively constant (Barr 1993, p. 40; Fries et al. 2004, pp. 4, 13). Information for other habitat conditions at Fern Bank Springs are incomplete due to lack of access.

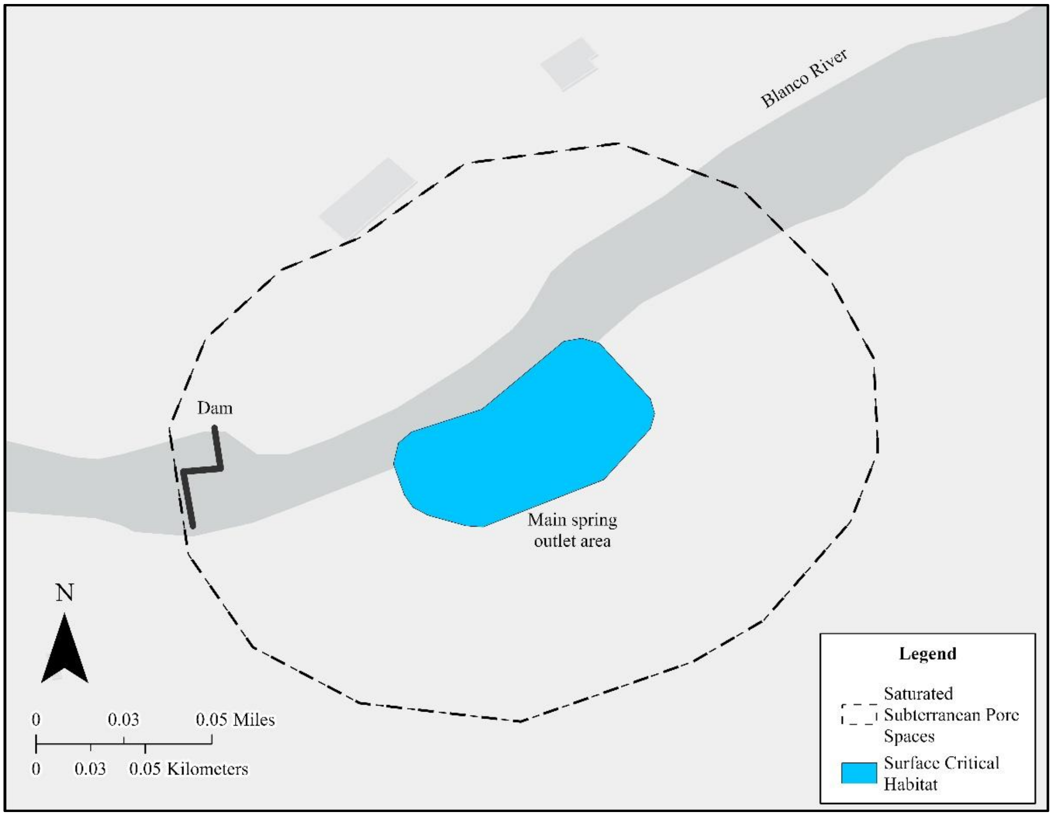

Figure 5. Fern Bank Springs ecosystem adjacent to the Blanco River in Hays County, Texas on private property. The saturated subterranean pore spaces perimeter is equivalent to the subsurface critical habitat designation of 110 meters (360 feet) within spring outlets where invertebrates are known to range (78 FR 63100).

1.2 Peck's Cave Amphipod

1.2.1 Description and Taxonomy

The amphipod family Crangonyctidae (Insecta: Amphipoda) is a group with freshwater origins (Holsinger and Longley 1980, p. 5). This Holarctic and Laurasian family includes 12 genera found in North America, Africa, and Asia (Lowry and Myers 2013, p. 47-48; Horton et al. 2022, entire). *Stygobromus* (Cope 1872, entire) is a large genus with 140 described species and four subspecies (Horton et al. 2022, entire). Species in this genus are all aquatic subterranean crustaceans, and *Stygobromus* is the most speciose genus of subterranean amphipods known from Texas, with 13 recognized species (Gibson et al. 2021, entire).

The Peck's cave amphipod (*Stygobromus pecki*) was described from two female specimens collected at Comal Springs by Stewart B. Peck in 1964 (Holsinger 1967, p. 119). The original description placed this medium-sized species in the genus *Stygonectes* (Holsinger 1967, entire), which was later synonymized into the genus *Stygobromus* and placed into the *flagellatus* group (Holsinger 1967, entire). This species is also referred to in some references as the "Peck stygobromid" or "Peck's cave scud" (40 FR 18477; McLaughlin et al. 2005, p.145). The Peck's cave amphipod can be identified through microscopic examination of adult specimens. The species has five pairs of legs, and the body is flattened laterally with two pairs of antennae and claws (Arsuffi 1993, p. 13).

1.2.2 Historical and Current Distribution

Peck's cave amphipods are groundwater obligate invertebrates that inhabit subterranean areas and gravel near springs, have restricted ranges, and can potentially occupy deep groundwater niches (Holsinger 1967, p. 119; Arsuffi 1993, p. 14; Krejca and Sprouse 2003, p. 9). Mature and immature life stages have been collected only near spring outlets, from seeps along the spring runs, and from a shallow groundwater well (Gibson et al. 2008, p. 76). This species is known from the headwaters of the Comal Springs complex at spring runs 1 through 4 and 7, the western shoreline (the impounded portion of the Comal Springs system) and Spring Island of Landa Lake, and Panther Canyon well (a shallow well located 110 m (360 ft) upslope of Comal Springs) in Comal County, Texas (Holsinger 1967, p. 119; Fries et al. 2004, pp. 5, 14; Gibson et al. 2008, pp.76-81). It also occurs in Hueco Springs at all four primary spring runs in Hays County, Texas (7 km [4 mi] north of Comal Springs) (Barr 1993, pp. 56-57; Fries et al. 2004, pp. 5, 14; Gibson et al. 2008, pp.76-81).

Panther Canyon well is a cased borehole approximately 15 cm (6 in) in diameter, 18 m [59 ft] deep, and located about 110 m (361 ft) upslope from Comal Springs. The well head is situated inside of a small well house and currently is not pumped. The collection of Peck's cave amphipods at Panther Canyon well lends support to early characterizations of the *flagellatus* group, suggesting Peck's cave amphipods inhabit deeper groundwater niches compared to other amphipods found in hyporheic habitat (i.e., saturated sediments near a streambed gravel or river) or inhabiting surface waters (Holsinger 1967, pp. 143, 159).

Genetic analysis using mitochondrial DNA sequences indicated high levels of differentiation within and among Peck's cave amphipod localities, but the localities were found to contain sequences from two distinct haplotype groups with deep divergence (Ethridge et al. 2013, p. 233, 235). The two haplotypes were not geographically separated, and they co-occurred often in similar proportions. This observation, in addition to the hydrogeology of the Comal ecosystem, suggests the Peck's cave amphipod species is composed of two sub-populations that at one time were separated and now converge between surface habitats at Comal Springs, and that migration is present within the Comal ecosystem (Nice and Lucas 2015, pp. 18, 22; Lucas et al. 2016, pp. 8, 12).

This distinction in partitioned niche habitat zones was also exhibited at Hueco Springs, with Peck's cave amphipods found more prevalent at deeper sites than others in the genus, *S. russelli* (Gibson et al. 2008, p. 80). The extent of subterranean occupancy by Peck's cave amphipods along the hydrological linkage between Hueco and Comal springs remains uncertain (72 FR 39255).

Despite the geographical separation of these springs, the subsurface hydrological interconnectivity may explain the distributional dynamics of these sub-populations. Both springs receive contributions from local and regional groundwater sources, with Comal Springs exhibiting a more phreatic and aged origin in comparison to Hueco Springs (Ogden et al. 1986, pp. 80, 124; Rothermel and Ogden 1987, p. 76). During periods of heightened aquifer levels, increased mixing of differing flowpaths between both spring ecosystems facilitates an interconnected subterranean network that potentially facilitates the migration of amphipods. Conversely, during severe droughts, the segregation of these sources may influence observed distribution patterns (Gibson et al. 2008, p. 75). In essence, the hydrological interconnectivity between Hueco and Comal Springs emerges as a pivotal determinant influencing the habitat and distributional dynamics of Peck's cave amphipods.

1.2.3 Habitat Requirements

The Peck's cave amphipod inhabits the shallow, saturated subterranean spaces associated with thermally stable spring orifices issuing from the Edwards Aquifer (Holsinger 1967, p. 119; Ogden et al. 1986, p. 123). In captivity and in the wild, these amphipods typically inhabit the space beneath leaf substrate or interstitial spaces between rocks, displaying a preference for shelter rather than swimming freely or exposed at the surface. (Fries et al. 2004, p. 8; USFWS 2020b, pp. 17, 25-26). It is unknown if this species has the ability to re-enter the subterranean aquifer once it has emerged or discharged through the springs (Barr 1993, p. 52). Specific springflow requirements and how much subterranean habitat this species uses is unknown; management relies on assuring historical conditions are maintained within the natural habitat for the species (LBG-Guyton and Associates et al. 2004, pp. C-4–C-5).

This cave amphipod is likely an omnivore and upon reaching the surface, consumes terrestrial-derived organic matter sources from riparian vegetation (78 FR 63100; Nair et al. 2021, p. 3). In the Comal Springs ecosystem, this species occupies a higher trophic level as a predator consuming other surface aquatic crustaceans (Nowlin et al. 2017, pp. 15-16). Therefore, riparian areas adjacent to the spring ecosystem provide a necessary role in the nutrient cycle for the food web of this invertebrate and influences its habitat distribution.

For additional details on the habitat and water quality conditions of the spring ecosystems at Comal and Hueco springs, refer to Sections 1.1.1 and 1.1.2, respectively.

1.2.4 Life History and Ecology

The majority of the information for this section refers to life history observations under in-situ manmade conditions and not from the species' natural habitat. Where specific life history aspects of the Peck's cave amphipod have not been well studied or supported, references to characteristics known from other species within the family Crangonyctidae are described herein.

As obligate subterranean residents, these amphipods have evolved adaptations enabling them to live in aquifer conditions like other aquatic subterranean species in the United States (Culver et al. 2000, p. 387). These amphipods cannot swim against strong currents and are not known to actively disperse downstream (Arsuffi 1993, p. 14). In the absence of eyes, this amphipod can detect and avoid light, but does not have mechanoreceptors to detect prey beyond direct interaction (Nowlin and Worsham 2015, pp. 49-50; Nowlin et al. 2016, p. 30; Kosnicki and Julius 2019a, p. 21). Like other subterranean invertebrates, the Peck's cave amphipod has a slower metabolism than surface species, despite having a deeper range within groundwater and a larger body size (Nair et al. 2020, pp.9-10).

Available evidence suggests the Peck's cave amphipod is a shallow phreatic zone specialist serving as a top invertebrate predator within the food web at both spring run 3 and Spring Island food webs within the Comal Springs ecosystem, consuming other invertebrates in addition to both photosynthetic (e.g., periphyton and wood-based biofilms) and chemolithoautotrophic (i.e., in situ derived food biofilms created underground in karst environments) organic matter sources (Hutchins et al. 2016, p. 1536; Nowlin and Worsham 2015, pp. 43-45; Kosnicki and Julius 2019a, pp. 20-21; Nair et al. 2020, p. 10; Nair et al. 2021, p. 239, 242). Photosynthetic, terrestrial-derived food sources may include detritus (i.e., decomposed materials), leaf litter, bacterial biofilms (i.e., bacteria and fungi associated with decaying plant materials), decaying roots, or woody debris (Nair et al. 2021, p. 241). Peck's cave amphipods have a more plastic feeding strategy than the other listed invertebrates in the Comal ecosystem (Section 1.3 and 1.4) and may be able to switch to alternative food sources spatially (i.e., a wood biofilm-based food chain at spring run 3 vs. the periphyton-based food chain at Spring Island) or when environmental conditions are reduced or altered (Nowlin and Worsham 2015, pp. 45, 49, 51).

Stygobromus sp. collected from Comal Springs can be shades of orange in contrast to colorless specimens at Hueco Springs (Fries et al. 2004, p. 5). This is likely due to a carotenoid present in the food resources (e.g., leaf litter) found in Comal Springs (Gibson et al. 2008, p. 77). Peck's cave amphipods collected at Spring Island had higher periphyton organic matter present in their guts compared to individuals collected at Comal Springs spring run 3 (Nair et al. 2021, p. 239). This difference of 28 percent can be attributed to canopy cover and riparian vegetation densities and proximity to the edge water between the two locations (Nair et al 2021, p. 242).

The Peck's cave amphipod in a laboratory study exhibited lower basal metabolic rates, greater bulk carbohydrate reserves, and slower declines in lipid reserve uptake when starved compared to a surface amphipod species, *Sicifera* (*Synurella*) sp. (Nair et al. 2020, p. 11). These lowered energetic requirements match metabolic strategies of deep phreatic organisms of low or infrequent food accessible systems, despite the species' association with shallower groundwater habitat. This suggests that earlier in the evolutionary history of this amphipod, the species may have occurred at deeper depths (Nair et al. 2020, p. 11).

Brooding females are capable of cannibalizing their young or dropping eggs with agitation or motility, with those eggs unable to survive outside of the marsupium (i.e., a type of brooding pouch of a female crustacean) (Nowlin et al. 2016, p. 31; Kosnicki and Julius 2019a, p. 12; USFWS 2019b, p. 57). Live prey crustacean species (*Daphnia* sp., *Lirceolus* sp., and *Hyalella* sp.) have been fed as supplemental feed in the refugia but are not actively sought out for consumption; this hunting behavior has not been observed in the wild (USFWS 2020b, p. 20).

Subterranean amphipods can live for four to 10 years (Wellborn et al. 2015, p. 788). Wild-caught Peck's cave amphipods have survived in captivity for three years, and F2 generations have been accomplished (BIO-WEST, Inc. 2007b, p. 40). No mating behavior or cues have been identified for this amphipod, but larger females have been observed cannibalizing smaller males (Nowlin et al. 2016, p. 31). Wild adult females kept in captive environments were able to produce eggs, with an average of 10 eggs per female, 24 percent hatching success and average brooding incubation times of 49.7 ± 12.4 days (Fries et al. 2004, p. 9; Kosnicki and Julius 2019a, p. 11). It was observed that eggs need more than 32 days to survive their first molting event to the next life stage as neonates (i.e., newborns), having an average of 50 days between several molts (up to eight instars in captivity) to final adult life stage under stressful captive conditions (Kosnicki and Julius 2019a, pp. 11-12, 20). The number of instars to reach maturity likely depends on food resources available and water temperature (Kosnicki and Julius 2019a, p. 19).

1.2.5 Abundance and Trends

Little is known about limiting factors that may impact the abundance and distribution of the Peck's cave amphipod, because subterranean habitats they inhabit are largely inaccessible to humans aside from wells and springs. Current abundance estimates include samples collected only at the surface.

The species' reclusive nature and life history adds complexity to determining abundance, as individuals spend most of their lives underground. Thus, no population estimates are available for the Peck's cave amphipod. Various researchers have examined amphipod assemblages from springs, caves, and wells in Comal, and neighboring Hays and Bexar counties, without finding the Peck's cave amphipod other than at Comal Springs and Hueco Springs (Holsinger 1967, entire; Holsinger and Longley 1980, entire; Barr 1993, entire; Gibson et al. 2008, entire). Measurements of genetic diversity across populations of *Stygobromus* species show Peck's cave amphipod to be comparable to populations of congeneric species from central Texas. Future sampling would be beneficial for estimation of population size (Nice and Lucas 2015, pp. 42-44).

The first recorded specimen of the amphipod was collected at Comal Springs (spring run 1) in June 1964 and again at the same place in 1965, in addition to collections by other researchers in 1977, 1981, 1984, 1985, 1987, and 1991, expanding the range to spring run 3 at Comal Springs (Holsinger 1967, p. 117; Barr 1993, p. 6). At this point, 32 specimens had been collected and had not been caught at spring run 2 or 4 (Barr 1993, p. 57).

A 1992 study indicates the amphipod was abundant in Comal Springs (spring runs 1, 2, 3, and 4) driftnet samples, with 271 individuals, and one specimen at a new locality, Hueco Springs, over 96 hours of combined drift time (Barr 1993, pp. 37, 56-57). The species was abundant at all spring runs but spring run 4 (spring run 1: 78; spring run 2: 62; spring run 3: 130; spring run 4: 1) (Barr 1993, p. 56).

Surveys in 2003 collected an average of 9.2/day at Comal Springs (spring runs 1, 2, and 3) and an average of 1.2/day at Hueco Springs (Fries et al. 2004, pp. 6-7; Gibson et al. 2008, p. 79). Individual amphipods were more abundant and easily accessible via hand collection or driftnetting at Comal Springs compared to Hueco Springs, suggesting Peck's cave amphipods inhabit a deeper section at Hueco Springs compared to the Comal Springs sampling locations (Krejca and Sprouse 2003, p. 9; Fries et al. 2004, p. 7).

Biomonitoring for all benthic macroinvertebrates in the Comal Springs system was established in 2000 and occurs every spring and fall using driftnets (BIO-WEST, Inc. 2003, pp. 37-41). This amphipod was discovered at the western shoreline and upwellings in Landa Lake (BIO-WEST, Inc. 2004b, p. 37). Between 2017 and 2021, with 29 sampling events, the long-term median number of Peck's cave amphipods collected per cubic meter (m^3) of water is $0.25/m^3$ (8.8 per cubic foot [ft^3]) (BIO-WEST, Inc. 2021b, pp. 39-40).

1.2.6 Critical Habitat

Critical habitat for Peck's cave amphipod was revised on November 22, 2013, in areas of occupied, spring-related aquatic habitat (78 FR 63101). Critical habitat includes a total of 15.16 hectares (ha) (38.4 acres [ac]) of surface habitat and 56 ha (138 ac) of subsurface habitat between two units. The Comal Springs unit (Unit 1) consists of 50 ha (124 ac) of subsurface and 15 ha (38 ac) of surface critical habitat for the Peck's cave amphipod. The Hueco Springs unit (Unit 2) consists of 6 ha (14 ac) of subsurface and 0.16 ha (0.4 ac) of surface critical habitat for the Peck's cave amphipod. The original critical habitat designation in 2007 included 15.6 ha (38.5 ac) of surface habitat without subsurface (72 FR 39248). Springs, associated streams, and underground spaces immediately inside of or adjacent to springs, seeps, and upwellings are the physical or biological features essential to the conservation of this species (50 CFR 17.95).

In designating critical habitat, the USFWS identifies physical and biological features, referred to PBFs, that are essential to conservation of the species and may require special management considerations or protection (50 CFR 424.12). Two PBFs were identified in the final rule designating revised critical habitat for Peck's cave amphipod, Comal Springs (Unit 1) and Hueco Springs (Unit 2) (78 FR 63109). At both Comal Springs and Hueco Springs, designated critical habitat includes only those areas where PBFs required by the species exist, and does not include areas where these features do not occur, such as buildings, lawns, or paved areas (78 FR 63116).

The critical habitat rule defined the PBFs as:

i. High-quality water with no or minimal pollutant levels of soaps, detergents, heavy metals, pesticides, fertilizer nutrients, petroleum hydrocarbons, and semi-volatile compounds such as industrial cleaning agents; and
ii. Hydrologic regimes similar to the historical pattern of the specific sites, with continuous surface flow from the spring sites and in the subterranean aquifer.
    a. Spring system water temperatures that range from approximately 20-24°C (68-75°F); and
    b. Food supply that includes, but is not limited to, detritus (decomposed materials), leaf litter, living plant material, algae, fungi, bacteria, other microorganisms, and decaying roots.

The final rule designating critical habitat describes those actions that would adversely modify designated critical habitat. Such activities could include, but are not limited to:

i. Change existing flow regimes:
    a. Impound water,
    b. Water withdrawal, or
    c. Water diversions.
ii. Introduce, spread, or augment nonnative species into critical habitat:
    a. Stocking or transporting nonnative species.
iii. Alter current habitat conditions:
    a. Release of chemical or biological pollutants into the surface water or connected groundwater at a point source or by dispersed release (nonpoint source).
iv. Physically remove or alter the habitat used by the amphipod:
    a. Channelization,

b. Impoundment,
c. Road and bridge construction,
d. Deprivation of substrate resource,
e. Destruction and alteration of riparian vegetation,
f. Excessive sedimentation from road construction,
g. Vegetation removal,
h. Recreational facility development, or
i. Other watershed disturbances.

1.3 Comal Springs Dryopid Beetle

1.3.1 Description and Taxonomy

Dryopidae (Insecta: Coleoptera) is a family of long-toed water beetles distributed worldwide, with the exception of Australia and Antarctica, with approximately 300 species (Yee and Kehl 2015, p. 1029). In North America, there are 5 genera and 13 species of dryopids described (Shepard 2002, p. 122). The Comal Springs dryopid beetle is the first and only subterranean adapted member of this family and is easy to distinguish from others in the genera (Barr and Spangler 1992, pp. 40-41). The cuticle (skin) is thin compared to other dryopid beetles and is reddish-brown and translucent (Barr and Spangler 1992, pp. 43, 47).

Unique morphological distinctions include vestigial (i.e., poorly developed and non-functioning) eyes and wings, and eight-segmented antennae (Barr and Spangler 1992, p. 47). Adult beetles have a slender body with a length of 3-3.7 millimeters (mm) (0.12-0.16 inches [in]) and are unable to swim (Barr and Spangler 1992, p. 47; Nowlin et al. 2022, p. 20). Setae (i.e., bristle-like structures along the body) length varied between wild individuals and grooming behaviors were observed (Barr and Spangler 1992, p. 51). Adults respire through a plastron (i.e., small, hydrophilic hairs that diffuse oxygen from the water into the body) limiting them to habitats with high dissolved oxygen (Brown 1987, p. 260; Barr and Spangler 1992, pp. 43-49; Yee and Kehl 2015, p. 1011). Internal sexual dimorphism can be assessed non-lethally using lateral lighting (Kosnicki 2019, pp. 3-5). One historical survey noted some males measured slightly larger than females, but more recent investigations did not corroborate these findings and additionally did not see differences in body morphology between sites (Barr and Spangler 1992, pp. 47, 49; Nowlin et al. 2022, p. 20).

Larvae lack eyes, are elongate, cylindrical, and yellowish-brown in color, with wedge-shaped teeth (tridentate) with a fusiform (round) head (Barr and Spangler 1992, pp. 44, 49). Larvae develop a terrestrial breathing apparatus called spiracles to breathe air, unlike other Coleopteran larvae that use anal gills to breathe in water (Barr and Spangler 1992, p. 50). Mature larvae are approximately 6-8 mm (0.24-0.31 in) long (Barr and Spangler 1992, p. 49). The pupal stage for this species has not been observed or described, and questions remain at which points in the life cycle this species exists in the terrestrial and aquatic stages.

1.3.2 Historical and Current Distribution

Comal Springs dryopid beetles are groundwater obligate invertebrates and spring endemics that have not been observed outside of springs, are likely restricted to these locations once at the surface and are not distributed throughout the aquifer (62 FR 66295). The species occurs within the aquifer at distances up to 110 m (360 ft) from spring outlets, somewhere within the groundwater-surface water interface (78 FR 63103).

The first Comal Springs dryopid beetles were discovered in 1987 in Comal County, Texas, specifically at spring run 2 in Comal Springs (Barr and Spangler 1992, p. 41). Since then, specimens have been identified at various locations within Comal Springs, including spring runs 1, 3, 4, 5, and 7 at Comal Springs, the western shoreline and Spring Island areas of Landa Lake (impounded section of the Comal Springs system), and Panther Canyon well (a shallow well 110 m [360 ft] upslope of Comal Springs) (Barr 1993, pp. 31, 53-55; BIO-WEST, Inc. 2004b, p. 34; Fries et al. 2004, pp. 9, 14-15; Gibson et al. 2008, pp. 76-77).

Additionally, two locations in Hays County, Texas have been identified: Sessom Springs and Fern Bank Springs (32 km [20 mi] northeast of Comal Springs), specifically at the easternmost orifice ("hill 3") and Cove Spring (Barr 1993, pp. 31, 53-55; BIO-WEST, Inc. 2004b, p. 34; Fries et al. 2004, pp. 9, 14-15; Gibson et al. 2008, pp. 76-77; Kosnicki and Julius 2019b, p. 3). This species has not been found at other spring localities and could be difficult to sample due to its coloration and cryptic behavior (Barr 1993, pp. 53-54).

Population structure suggests genetic differentiation between Comal, Sessom, and Fern Bank Springs locations, and there has not been gene flow or admixture between these sites (Nowlin et al. 2022, pp. 12-13, 23-24). More research and collection efforts between these spring systems are needed to better understand the species' range and patterns of genetic diversity, as well as for husbandry and management purposes within the refugia population (Nowlin et al. 2022, p. 24).

1.3.3 Habitat Requirements

This groundwater obligate invertebrate, known to be in the aquifer at distances up to 110 m (360 ft) from spring outlets, likely occupies saturated subterranean pore spaces associated with springs issuing from the Edwards Aquifer (78 FR 63103). However, their specific habitat requirements remain unknown due to the difficulty in accessing their subsurface habitat (Gibson et al. 2008, p. 77), and their association with the surface can only be hypothesized (Barr and Spangler 1992, p. 52). . It is also unknown if this species has the ability to re-enter the subterranean aquifer once it has emerged or been discharged through the springs (Barr 1993, p. 52). Specific springflow requirements and the breadth of subterranean habitat this species uses are unknown; habitat management relies on assuring historical conditions are maintained within the natural habitat for the species (LBG-Guyton and Associates et al. 2004, pp. C-4–C-5).

Comal Springs dryopid beetles are collected from the clear headwater spring orifices consisting of coarse sand and angular cobbles or along seeps of the terrestrial margin where soil, fallen leaves, and rocks line the surface 5-31 centimeters (cm) (2-12 in) deep (Barr and Spangler 1992, p. 41). Roots and organic debris associated with the aquifer and spring outlets may act as substrate for growth of microorganisms for food and may provide shelter (Gibson et al. 2008, p. 77; 77 FR 64274). The beetles are attracted to flowing water sources in captive settings, working against the flow to stay near a food source (Kosnicki and Julius 2019b, pp. 11, 19).

Individuals have not been observed feeding on leaf litter fragments, but greater than 75 percent of their diet is derived from terrestrial organic matter (Barr and Spangler 1992, p. 51; Nair et al. 2021, pp. 240, 242; Nowlin et al. 2022, pp. 16-19). Comal Springs dryopid beetle adults feed on photosynthetic (terrestrial) organic matter energy sources (e.g., biofilms) scraped from surfaces, such as rocks, wood, and vegetation, and not on periphyton-based organic matter, such as detritus, leaf litter, and decaying roots (Simon et al. 2003, p. 2404; Hutchins et al. 2016, pp. 1536, 1538; Nowlin et al. 2017, pp. 16-18; Nair et al. 2021, pp. 240, 242). A co-occurring species, the Comal Springs riffle beetle (*Heterelmis comalensis*), derives most of its food from the same organic matter sources, but has a less than or equal to 1 percent niche overlap with the dryopid beetle at Comal Springs (Nair et al. 2021, p. 244).

Larvae are presumed to occupy moist soils at the terrestrial margins and are presumed to have the capacity to live in microhabitats in the air-filled pockets within the ceiling of the spring orifice where organic debris may serve as shelter and act as substrate for growth of microorganisms on which it feeds (Barr and Spangler 1992, pp. 41, 51-52). Larvae can get caught in the surface tension and float, but when submerged they do not survive (Kosnicki and Julius 2019b, pp. 3, 15). A single larva was captured using poly-cotton cloth lures placed in a shallow upwelling near the shoreline and captured in submerged driftnets over springs, alluding to the semi-aquatic nature of this life stage (Barr 1993, pp. 55-56; Fries et al. 2004, p. 10; BIO-WEST, Inc. 2008, p. 37). No other larvae were captured using any method, highlighting the rarity of this find.

Sessom Springs, a Comal Springs dryopid beetle site, presents a multifaceted challenge due to uncertainties in understanding the specific habitat conditions for this dryopid beetle population. The surface of this site is covered with concrete along a road, and access to the water emerging from the aquifer is facilitated through PVC piping hammered into the spring openings for sampling. This unique infrastructure, coupled with the concrete overlay, not only raises questions about the beetles' potential habitat, as they have been observed to also reside at the surface in other spring environments, but also means that there is a notable reduction of terrestrial organic matter compared to a more natural riparian area. According to another report, Sessom Springs adults occupy a more limited area than the other populations, and the presence of dryopid beetles strongly suggests that these populations utilize terrestrial organic matter as a significant component of their diet, as surface inputs of terrestrial organic matter can support subterranean and spring orifice invertebrates of the Edwards Aquifer (Nair et al. 2021, p. 246; Nowlin et al. 2022, pp. 4, 16). This highlights the need for more investigation due to a lack of detailed information about the habitat conditions for the dryopid beetle population at Sessom Springs. Understanding these conditions is crucial for effective conservation efforts.

For additional details on the habitat and water quality conditions of the spring ecosystems at Comal, Fern Bank, and Sessom springs, refer to Sections 1.1.1, 1.1.3, and 1.1.4, respectively.

1.3.4 Life History and Ecology

The majority of the information for this section refers to life history observations under in-situ manmade conditions and not from their natural habitat. Where specific life history aspects of the Comal Springs dryopid beetle have not been well studied or supported, references to characteristics known from other species within the family Dryopidae are described herein.

Pomatinus substriatus has been observed ovipositing eggs into submerged decaying wood and adults inhabiting spaces within the grooves of the wood, coexisting with other coleopteran species and likely competing for living space (Novaković et al. 2014, pp. 37-38, 40). Females produce several clutches of eggs over many months with a maximum capacity of 10-14 eggs, not dependent on body size (Kosnicki and Julius 2019b, pp. 12-13). The most productive female was estimated to potentially produce up to 130 eggs in her lifetime, but fecundity estimates could not be ascertained (Kosnicki and Julius 2019b, p. 13).

Eggs need two to three months to incubate above water before hatching under captive conditions, with a 22 percent success of hatching where disturbances are frequent (Kosnicki and Julius 2019b, p. 13). It is unknown if eggs can hatch underwater or if humid conditions are necessary for development (Kosnicki and Julius 2019b, p. 20). It is uncertain how eggs laid in subterranean voids are able to access air spaces to reach the next life stage and if those spaces are plentiful in their habitat. It is also uncertain how many and what motivations females may have to lay their eggs underground or at the surface.

A study of Comal Springs dryopid beetle larvae observed early instar individuals burrowing into conditioned poplar wood dowels and sycamore leaves to hide, and later instar larvae were seen excavating trenches into the dowel, which served as both a food source and shelter (Kosnicki and Julius 2019b, pp. 7, 15-18). Collections of larvae in the wild float because of surface tension at the water surface, indicating this life stage may need terrestrial or semiaquatic habitat conditions (Kosnicki and Julius 2019b, p. 3). Estimations of larval survival rates cannot be reliant due to this evasive sheltering behavior. A single Comal Springs dryopid beetle larva was produced and grew from approximately 2-10 mm (0.08-0.40 in) in length over nine months, suggesting development of larvae may only take one year (Fries et al. 2004, p. 10; Kosnicki and Julius 2019b, p. 4). Larvae are estimated to have six instars, with an average of 22.4 days per instar, to an estimate of 134 days needed to develop (Kosnicki and Julius 2019b, p. 16).

Helichus species inhabit moist soil along stream banks up to 5 m (16 ft) from stream edge, presumably feeding on roots and decaying vegetation, and may require two to five years before pupating in the soil (Ulrich 1986, pp. 326, 331; Brown 1987, p. 257). Comal Springs dryopid beetle pupae and eclosion (hatching) and their associated environmental cues (if they exist) have not been researched.

Some wild caught adult Comal Springs dryopid beetles have survived in captivity 11-21 months, but lifespan of this species is unknown (Barr and Spangler 1992, p. 51; Fries et al. 2004, p. 10). There is no research on survival rates of wild beetles in the surface or subterranean aquatic locations of this species and whether they differ (Barr 1993, p. 52). A beetle that lived for a year in captivity experienced a decrease in plastron surface area to the time of death, hypothesizing abrasion of the setae occurred or death was due to aging (Fries et al. 2004, p. 10).

1.3.5 Abundance and Trends

Little is known about limiting factors that may impact the abundance and distribution of the Comal Springs dryopid beetle. Current abundance estimates include samples collected only at the surface.

Fluctuations in the numbers of dryopid beetles and larvae have been observed by researchers for reasons that remain unknown. However, it is established that droughts can lead to a reduction in springflow, prompting the species to seek shelter and preferred water quality further down in the aquifer. Conversely, during periods of record-high springflows, the beetles may be dislodged into surface waters downstream due to their slow and fragile nature (Barr 1993, p. 54). The non-swimming, flightless aquatic beetle faces limited opportunities for expanding its range. This species is rarely collected, likely because its preferred habitat is challenging to sample (BIO-WEST, Inc. 2007, p. 39; Gibson et al. 2008, p. 77).

Drift and manual (kick) netting surveys in the 1990s resulted with at most 10 adults captured during a month sampling period and as many as four larvae at a subset of the sites sampled (Barr and Spangler 1992, pp. 41, 51; Barr 1993, pp. 54, 41). Fewer dryopid beetle individuals were captured when flows and aquifer levels increased (San Antonio reference well: J-17); with the caveat that comparisons were of small sample sizes (Barr 1993, p. 55). Most of the individuals were collected at low-volume springs (spring runs 2 and 4 at Comal Springs and Fern Bank Springs), compared to the high-volume spring run 1 and 3 (Barr 1993, p. 55).

Surveys in 2003 collected an average of 0.3/day at spring runs 1-3 of Comal Springs (Fries et al. 2004, pp. 6-7). At Fern Bank Springs, no subterranean species were caught at the pool or hillside, and driftnets elsewhere contained so few species, researchers had to extend the period of sampling (Gibson et al. 2008, p. 76). The species was confirmed at Fern Bank Springs in 2003, when a single larva was collected after 398 hours of sampling spring orifices with drift nets (Gibson et al. 2008, p. 77). A more recent sampling effort from a spring emanating from the bluff of the Blanco River adjacent to the spring property suggested dryopid beetles at this site are productive, with 31 adults and 8 larvae collected (Nowlin et al. 2022, pp. 8, 15, 24). Additionally, the species was also captured at Sessom Springs in Hays County; however, collections there are rare, and the species has not been detected there since 2017 (USFWS 2017, pp. 20-21; Clough 2022, p. 1).

This species does not have a targeted monitoring program (Kosnicki 2022a, pers. comm.). Monitoring for Comal Springs macroinvertebrates, in which the dryopid beetle is caught incidentally, occurs every spring and fall using driftnets at the major spring orifices (established 2000) and with poly-cotton cloth lures over spring orifices (established 2003) (BIO-WEST, Inc. 2003, pp. 37-41; BIO-WEST, Inc. 2004b, p. 38). There are not enough data for the monitoring program to compile a temporal trend and these data are not presented in annual reports.

1.3.6 Critical Habitat

Critical habitat for Comal Springs dryopid beetle was revised on November 22, 2013, in areas of occupied, spring-related aquatic habitat (78 FR 63100). Critical habitat includes a total of 15.56 ha (39.4 ac) of surface critical habitat and 56 ha (139 ac) of subsurface critical habitat between two units. The Comal Springs unit (Unit 1) consists of 50 ha (124 ac) of subsurface and 15 ha (38 ac) of surface critical habitat for the Comal Springs dryopid beetle. The Fern Bank Springs unit (Unit 2) consists of 6 ha (14 ac) of subsurface and 0.56 ha (1.4 ac) of surface critical habitat for the Comal Springs dryopid beetle. The original designation was surface critical habitat of 16 hectares (39.5 acres) of surface habitat without subsurface (72 FR 39248). Springs, associated streams, and underground spaces immediately inside of or adjacent to springs, seeps, and upwellings are the physical or biological features essential to the conservation of this species (50 CFR 17.95; 78 FR 63120).

In designating critical habitat, the USFWS identifies physical and biological features, referred to PBFs, that are essential to conservation of the species and may require special management considerations or protection (50 CFR 424.12). Two PBFs were identified in the final rule designating revised critical habitat for Comal Springs dryopid beetle, Comal Springs (Unit 1) and Fern Bank Springs (Unit 2) (78 FR 63109). At both Comal Springs and Fern Bank Springs, designated critical habitat includes only those areas where PBFs required by the species exist, and does not include areas where these features do not occur, such as buildings, lawns, or paved areas (78 FR 63120).

The critical habitat rule defined the PBFs as:

i. High-quality water with no or minimal pollutant levels of soaps, detergents, heavy metals, pesticides, fertilizer nutrients, petroleum hydrocarbons, and semi-volatile compounds such as industrial cleaning agents; and
ii. Hydrologic regimes similar to the historical pattern of the specific sites, with continuous surface flow from the spring sites and in the subterranean aquifer:
    a. Spring system water temperatures that range from approximately 20-24°C (68-75°F); and
    b. Food supply that includes, but is not limited to, detritus (decomposed materials), leaf litter, living plant material, algae, fungi, bacteria, other microorganisms, and decaying roots.

The final rule designating critical habitat describes those actions that would adversely modify designated critical habitat. Such activities could include, but are not limited to:

i. Change existing flow regimes:
    a. Impound water,
    b. Water withdrawal, or
    c. Water diversions.
ii. Introduce, spread, or augment nonnative species into critical habitat:
    a. Stocking or transporting nonnative species.
iii. Alter current habitat conditions
    a. Release of chemical or biological pollutants into the surface water or connected groundwater at a point source or by dispersed release (nonpoint source).
iv. Physically remove or alter the habitat used by the dryopid beetle:
    a. Channelization,
    b. Impoundment,
    c. Road and bridge construction,
    d. Deprivation of substrate resource,
    e. Destruction and alteration of riparian vegetation,
    f. Excessive sedimentation from road construction,
    g. Vegetation removal,
    h. Recreational facility development, or
    i. Other watershed disturbances.

1.4 Comal Springs Riffle Beetle

1.4.1 Description and Taxonomy

Elmidae (Insecta: Coleoptera) is a family of true aquatic beetles distributed worldwide except for Antarctica with approximately 146 genera (Yee and Kehl 2015, p. 1030). There are 35 riffle beetle species in Texas, with four species in the genus *Heterelmis* (Nair et al. 2019, p. 1076; Barr 2021, p. 93).

The adult Comal Springs riffle beetles, which were described in 1988, exhibit a reddish-brown coloration, possess eyes, and vary in length from 1.7-2.48 millimeters (0.07-0.09 inch) (Bosse et al. 1988, pp. 199, 202; Worsham and Julius 2017, p. 28). They respire through a plastron, facilitated by small, hydrophilic hairs that diffuse oxygen from the water into the body (Bosse et al. 1988, p. 199; Yee and Kehl 2015, pp. 1011, 1030). The hind wings of Comal Springs riffle beetles are short and non-functional, a subterranean characteristic that renders this species incapable of flight (Bosse et al. 1988, p. 201; Bowles et al. 2003, p. 379). Unlike other animals adapted to subterranean environments, Comal Springs riffle beetles do not possess additional features such as reduced or lack of eyes and pigmentation (Cooke et al. 2015, p. 117).

The larvae of Comal Springs riffle beetles are characterized by their elongated bodies and retractable heads and feature dorsal spines and a more flattened head capsule shape. These aquatic larvae develop anal gills used to retrieve oxygen from water (Brown 1987, p. 261). The pupae of Comal Springs riffle beetles are pale in color and possess setae that facilitate oxygen intake into the body. It is unknown whether the hydrophobic setae play a role in facilitating respiration underwater, possibly similar to the plastron observed in adult beetles (Huston and Gibson 2015, pp. 522-523).

Although Comal Springs riffle beetle is a genetically distinct species, it is most closely related to but significantly divergent from *H. glabra,* a species capable of flight associated with rivers and streams (Gonzales 2008, pp. 24-25). Bosse et al. (1988, p. 202) speculated that the Comal Springs riffle beetle likely evolved from an isolated population of *H. glabra*, which was substantiated by Gonzales (2008, p. 38).

Sexual dimorphism is present in this species and can be determined non-lethally (Kosnicki 2019, pp. 3-5; Nair et al. 2019, p. 1079). Adult male Comal Springs riffle beetles can be distinguished from other species of *Heterelmis*. Another riffle beetle, *Microcylloepus pusillus*, co-occurs at Comal, Fern Bank, and San Marcos springs and can be distinguished by the deep “Y” shaped indention on the prothorax (i.e., middle segment of the body). Too, M. *pusillus* larvae have dorsal tubercles used for respiration, with a highly visible head shape and dark-colored eyes (LeConte 1852, p. 42). Another species known to occur in Texas, *Heterelmis vulnerata*, lacks an inner fringe of setae on the male genitalia (Bosse et al. 1988, p. 202). *H. vulnerata* has been found in the upper portions of the Comal and San Marcos rivers in channels flowing out of the dams impounding Landa Lake and Springs Lake, but this species has not been found in the spring habitats where *H. comalensis* is known to occur (Cooke et al. 2015, p. 119). Male genitalia of the Comal Springs riffle beetle are smaller, paler, and slenderer than those of another riffle beetle species, *H. glabra* (Bosse et al. 1988, p. 202).

1.4.2 Historical and Current Distribution

The Comal Springs riffle beetle is an epigean (surface-dwelling), groundwater obligate invertebrate known from two major spring systems: Comal Springs at the spring outlets and Landa Lake (Comal County, Texas) and San Marcos Springs at a few headwater springs of Spring Lake (Hays County, Texas) (Bosse et al. 1988, entire; Barr 1993, pp. 31, 44; Gibson et al. 2008, p. 79).

Due to their flightless nature, these beetles have low dispersal abilities, limiting them to crawling or drifting downstream to habitats with adequate food resources and within their preferred physicochemical range. Their highest abundance is within 20 cm (8 in) from a spring outlet, and they are absent at a 1-meter (3 feet) distance when sampling the surface with cotton cloth traps (Cooke et al. 2015, pp. 114, 117-118; Huston et al. 2015, p. 797; Worsham and Julius 2017, p. 6). Specific springflow requirements and the extent of subterranean habitat usage by this species remain unknown; therefore, habitat management relies on maintaining historical conditions within the natural habitat for the species (LBG-Guyton and Associates et al. 2004, pp. C-4–C-5).

Not confined to spring openings, the Comal Springs riffle beetle is equally found in deeper habitats where diffuse springflows are present (BIO-WEST, Inc. 2005, p. 51; 2006, p. 39). Within these more lentic habitats, the beetles exhibit higher movement rates compared to a site at spring run 3, suggesting their ability to seek more suitable microhabitat conditions despite their inability to disperse via flight (BIO-WEST, Inc. 2006, p. 39).

Previously, it was believed that the existence of this species at spring run 4 was unlikely due to the lentic conditions and the dominance of a silt substrate (Bowles et al., 2003, p. 376). No specimens were identified in multiple surveys until 2020, when a few were collected by Texas State University (Nowlin and Worsham, 2015, p. 12; Nowlin 2022, pers. comm.). Subsequent surveys of spring run 4 did not reveal any further instances of this species, indicating that the finding in 2020 may be a one-time occurrence (Gibson, unpublished data).

Within the species' seven sampled populations (Figure 2), three populations of the Comal Springs riffle beetle had high between-population genetic variation: two at Comal Springs (Spring Island and western shoreline of Landa Lake) and San Marcos Springs (Gonzales 2008, p. 32). This isolation is due to the lack of recent gene flow, but historically they had a common ancestral population (Gonzales 2008, p. 32). Recent genetics suggests an even greater degree of isolation among populations (Coleman 2021, unpaginated). The spring runs and backwater spring populations have dried up during drought periods, and genetic bottlenecks were apparent (Gonzales 2008, p. 34). Dye tracing studies show a different water source for each of the three high-variance populations and informs the bottlenecks experienced during extensive drought periods (LBG-Guyton and Associates et al. 2004, pp. B-24, B-30; Johnson and Schindel 2008, pp. 12, 49, 59; Musgrove and Crow 2012, pp. 80, 86-87).

1.4.3 Habitat Requirements

Comal Springs riffle beetles inhabit gravel and cobble-dominated substrates with aquatic vegetation and submerged wood present at Comal Springs and San Marcos Springs (Brown 1972, p. 57; Bowles et al. 2003, p. 372). They are best captured within or around spring orifices, even at shallow water depths (Bowles et al. 2003, pp. 367, 373; Gibson et al. 2008, p. 77; Cooke et al. 2015, p. 117). Comal Springs riffle beetles, being ectothermic, exhibit a stenothermal adaption, preferring temperatures between 22.5-25.5°C (72.5-78°F) (Nair et al. 2023, p. 6). They avoid low concentrations of carbon dioxide and prefer dark spaces (Cooke et al. 2015, p. 115).

These beetles have low dispersal abilities because they are flightless, which limits them to crawling or drifting downstream to habitats that have adequate food resources and are within their preferred physicochemical range. Their highest abundance is within 20 cm (8 in) from a spring outlet, and they are absent at a 1-m (3 ft) distance when sampling surface with cotton cloth traps (Cooke et al. 2015, pp. 114, 117-118; Huston et al. 2015, p. 797; Worsham and Julius 2017, p. 6). Specific springflow requirements and how much subterranean habitat this species uses is unknown; habitat management relies on assuring historical conditions are maintained within the natural habitat for the species (LBG-Guyton and Associates et al. 2004, pp. C-4-C-5).

This species may prefer areas free of silt near spring outflows and are not limited to high-velocity spring orifices as previously believed (BIO-WEST, Inc. 2007b, p. 23). Laboratory studies showed adults move against flow (sometimes with flow towards food) and may prefer low flow conditions, like under rocks or leaves, or within interstitial spaces of submerged wood (BIO-WEST, Inc. 2002b, pp. 15, 23; BIO-WEST, Inc. 2004b, p. 38; Cooke et al. 2015, pp. 115, 119; Worsham and Julius 2017, p. 33; Kosnicki and Julius 2019b, p. 18). Other researchers hypothesize these behaviors indicate how the species might have survived the drought of record in 1956 and subsequent drought periods by locating refuge in deeper, watered substrates escaping evaporation and hydraulic stagnation (Bowles et al. 2003, p. 380; LBG-Guyton and Associates et al. 2004, p. C-4). In captivity, adults are found underneath substrate close to the source of groundwater (BIO-WEST, Inc. 2007b, p. 25). This burrowing behavior has been recorded in the wild, and beetles are likely to seek out sources of water when springflows decrease (BIO-WEST, Inc. 2007b, pp. 23, 25).

Larvae are caught in lower numbers during biomonitoring using the poly-cotton cloth method, suggesting they either occupy different habitats or a sampling bias where the biofilms produced on the cloth are not desired by this life stage (BIO-WEST, Inc. 2005, p. 65). Assembling leaves in a similar fashion to the cloth method did not result in a statistically significant preference for the leaves over cloth lures (Kosnicki 2021, p. 14). Some elmid larvae use gills to respire and are less susceptible to low flow conditions compared to adults, which respire with a plastron and prefer to stay in habitats with abundant dissolved oxygen (Walters and Post 2011, p. 168; Elliott 2008, p. 710).

Comal Springs riffle beetles are detritivores, feeding on organic matter sourced from terrestrial coarse and particulate materials scraped off substrates of microbial origin, including fungi and bacteria, as well as periphyton. This feeding behavior remains consistent irrespective of the canopy cover (Brown 1987, p. 262; Nowlin et al. 2017, pp. 16-18, 21, 27). A co-occurring species, the Comal Springs dryopid beetle (*Stygoparnus comalensis*), derives most of its food from the same organic matter sources but has a niche overlap of less than or equal to 1 percent with the riffle beetle at Comal Springs (Nair et al. 2021, p. 244).

For additional details on the habitat and water quality conditions of the spring ecosystems at Comal and San Marcos springs, refer to Sections 1.1.1 and 1.1.3, respectively.

1.4.4 Life History and Ecology

The majority of the information for this section refers to life history observations under in-situ manmade conditions and not from the natural habitat of the Comal Springs riffle beetle. This species' life cycle has been fully documented. Where specific life history aspects of Comal Springs riffle beetle have not been well studied or supported, characteristics known from other species within the family Elmidae are described herein.

Comal Springs riffle beetle surveys indicate the species has asynchronous generations, likely due to the consistent water quality at the springs (Bowles et al. 2003, p. 376; BIO-WEST, Inc. 2006, p. 39). Other elmids with stable environmental conditions can affect emergence timings and oviposition based on changes in water velocity or temperature and food availability (Passos et al. 2003, p. 34). There are no indicators or mechanisms for emergence of the Comal Springs riffle beetle.

Female adult Comal Springs riffle beetles reproduce multiple times in a year (iteroparous) with up to 121 larvae in their lifetime (Kosnicki 2022b, p. 2). In an egg deposition study, treatments with biofilm poly-cotton cloth as the substrate contributed to the most eggs hatching into viable larvae, suggesting the biofilms produced on the cotton cloth may be an important nutritional requirement for egg development (Worsham and Julius 2017, p. 12). Additionally, hatching success depended on the nutritional quality females received in captivity (Worsham and Julius 2017, p. 14).

Eggs have translucent shells, 150 micrometers in diameter, facilitating damage-free observation of development (Worsham and Julius 2017, p. 11). Egg development and incubation occur for 21-25 days until hatching, which is longer than other riffle beetle species (5-15 days) (Brown 1987, p. 254; Worsham and Julius 2017, p. 16). There is no evidence of diapause (i.e., period when development is delayed during unfavorable environmental conditions) during the incubation period either in captivity or in the wild (Bowles et al. 2003, p. 37; Worsham and Julius 2018, p. 3). Egg development starts with globular bodies like early cells of a zygote (3 days), to more cell division and smaller cells developing (7 days), to tissue differentiation with an embryo visible and budding appendage (14-18 days), to a fully developed larva observable inside the egg with a faint red eye (21 days) and hatching from the egg after 25 days (Worsham and Julius 2017, p. 15).

Larvae undergo six molts for a total of seven instars, reaching the final instar at 12 weeks (Cooke 2012, p. 28; Worsham and Julius 2017, p. 17). Similar to the adults, Comal Springs riffle beetle larvae feed on allochthonous material and acquire nutrients from associated microbial communities, particularly bacteria (Nair et al. 2021, p. 245). Larval instar determination is based on measuring the head capsule width of a larva (Worsham and Julius 2017, pp. 17-18). In captivity, larvae have been observed to persist in the final instar phase for over four months before pupating, possibly to assimilate nutrients necessary for the pupation process, due to inadequate habitat conditions, or because of food quality issues (Worsham and Julius 2017, pp. 17, 24). Notably, temperature variations within the range of 19-25°C (66-77°F) were not found to significantly affect larval survival (Worsham and Julius 2017, p. 20).

The Comal Springs riffle beetle has an extended period of larval development highlighting the complexity of the metamorphic process. The transition from larvae to pupae and the subsequent eclosion (i.e., hatching) into adults play crucial roles. The process of eclosion is completed in approximately one month (Worsham and Julius 2017, p. 24). A more recent study provides detailed insights into the larval development into pupae, indicating that pupation occurs 38 weeks (8.77 months) post-hatching, with more than half of that duration spent in the 7th instar (Worsham and Julius 2018, p. 5).

Pupae for this species, capable of eclosing both underwater and at the surface of the water, have delicate setae, possibly allowing for a plastron-like bubble (Huston and Gibson 2015, p. 523). Unfortunately, they are susceptible to damage, causing them to lose their hydrophobic qualities (Huston and Gibson 2015, p. 522). Following eclosion, adult individuals are initially light yellow in color (teneral) and gradually darken to an orange-brown, typical of mature adults. During this early stage of adulthood, the internal abdominal structure for determining sex is challenging to discern.

Adults in captivity have been reported to live up to a year and are estimated to have a long lifespan, with an average generation time of two years, although further research is needed (Bowles 2003, p. 376; Worsham and Julius 2017, p. 24). The gut microbiome of captive adults, which is influenced by various factors, including a different and more diverse bacterial community than that of their wild counterparts, may be attributed to human contact, varying sources of water with differing geochemical concentrations within the aquifer, or locations within the aquifer between the two source counties. These factors could potentially alter the microbial community. Additionally, biofilm shedding from well water pipes at the refugia may play a role (Mays et al. 2021, pp. 3, 9).

1.4.5 Abundance and Trends

Little is known about limiting factors that may impact the abundance and distribution of the Comal Springs riffle beetle.

Abundance of Comal Springs riffle beetles did not correlate significantly with water depth, current velocity, or distance downstream from the primary spring outlets (Bowles et al. 2003, pp. 370-371). Typical water depth in occupied habitat is 2-10 cm (1-4 in), but the beetle has been found in slightly deeper areas within the spring runs and around the spring upwellings at the impoundments (Bosse et al. 1988, p. 202; BIO-WEST, Inc. 2007b, p. 23). A mark-recapture study retrieved < 1 percent of the 100 beetles marked, suggesting the population in the sampling area (western shoreline of Landa Lake) is large (Huston et al. 2015, p. 797).

Larval and adult Comal Springs riffle beetle populations at Comal Springs may reach their greatest densities (about five per square meter) in late fall through winter, but all life stages can be found throughout the year, suggesting multiple broods in a season with overlapping generations (Bowles et al. 2003, p. 396). Biomonitoring of all benthic macroinvertebrates in the Comal Springs system occurs biannually, during spring and fall, and was initially established in 2000 using driftnets. Targeted sampling of spring orifices, employing poly-cotton cloth traps, commenced in 2003 (BIO-WEST, Inc. 2003, pp. 37-41; BIO-WEST, Inc. 2004b, p. 38). This particular species has been consistently collected within the Comal Springs system since 2003 (BIO-WEST, Inc. 2007b, p. 39).

Notably, larvae of this species are captured in lower numbers during biomonitoring using the poly-cotton cloth method, suggesting potential differences in habitat preference or a sampling bias where the biofilms produced on the cloth are not preferred by this life stage (BIO-WEST, Inc. 2005, p. 65). It is important to consider these nuances in sampling methods, especially when interpreting biomonitoring data for different life stages. Additionally, there are no population estimates available for this species, and caution is advised against utilizing the numbers of beetles retrieved with this cloth method to estimate population trends due to the associated high error rate and large natural variability of the Comal Springs population (Huston et al. 2015, p. 796-797; EARIP HCP 2020, p. 4-108–4-109).

1.4.6 Critical Habitat

Critical habitat for Comal Springs riffle beetle was revised on November 22, 2013, in areas of occupied, spring-related aquatic habitat (78 FR 63101). Critical habitat includes a total of 22 ha (54 ac) of surface habitat between two units were designated without additional subsurface designation because this species is restricted to surface waters (78 FR 63107). This species has not been caught at Panther Canyon well like the other two listed Edwards Aquifer invertebrates (Section 1.2 and 1.3) which included subsurface critical habitat designations. The Comal Springs unit (Unit 1) consists of 15 ha (38 ac) of surface critical habitat for the Comal Springs riffle beetle. The San Marcos Springs unit (Unit 2) consists of 6 ha (16 ac) of surface critical habitat for the Comal Springs riffle beetle. The original designation was surface critical habitat of 12.3 hectares (30.3 acres) of surface habitat without subsurface (72 FR 39248). Springs, associated streams, and underground spaces immediately inside of or adjacent to springs, seeps, and upwellings are the physical or biological features essential to the conservation of this species (50 CFR 17.95; 78 FR 63123).

In designating critical habitat, the USFWS identifies physical and biological features, referred to PBFs, that are essential to conservation of the species and may require special management considerations or protection (50 CFR 424.12). Two PBFs were identified in the final rule designating revised critical habitat for Comal Springs riffle beetle, Comal Springs (Unit 1) and San Marcos Springs (Unit 4) (78 FR 63109). At both Comal Springs and San Marcos Springs, designated critical habitat includes only those areas where PBFs required by the species exist, and does not include areas where these features do not occur, such as buildings, lawns, or paved areas (78 FR 63124).

The critical habitat rule defined the PBFs as:

i. High-quality water with no or minimal pollutant levels of soaps, detergents, heavy metals, pesticides, fertilizer nutrients, petroleum hydrocarbons, and semi volatile compounds such as industrial cleaning agents; and
ii. Hydrologic regimes similar to the historical pattern of the specific sites, with continuous surface flow from the spring sites and in the subterranean aquifer.
    a. Spring system water temperatures that range from approximately 20-24°C (68-75°F); and
    b. Food supply that includes, but is not limited to, detritus (decomposed materials), leaf litter, living plant material, algae, fungi, bacteria, other microorganisms, and decaying roots.

The final rule designating critical habitat describes those actions that would adversely modify designated critical habitat. Such activities could include, but are not limited to:

i. Change existing flow regimes:
   a. Impound water,
   b. Water withdrawal, or
   c. Water diversions.
ii. Introduce, spread, or augment nonnative species into critical habitat:
   a. Stocking or transporting nonnative species
iii. Alter current habitat conditions
   a. Release of chemical or biological pollutants into the surface water or connected groundwater at a point source or by dispersed release (nonpoint source).
iv. Physically remove or alter the habitat used by the riffle beetle:
   a. Channelization,
   b. Impoundment,
   c. Road and bridge construction,
   d. Deprivation of substrate resource,
   e. Destruction and alteration of riparian vegetation,
   f. Excessive sedimentation from road construction,
   g. Vegetation removal,
   h. Recreational facility development, or
   i. Other watershed disturbances.

1.5 Fountain Darter

1.5.1 Description and Taxonomy

Darters are in the family Percidae (ray-finned fishes) and subfamily Etheostomatinae. Recent studies reaffirm the placement of *E. fonticola* in the subgenus *Microperca*, along with *E. microperca* and *E. proeliare* (Ayache and Near 2009, entire; Near et al. 2011, entire; Smith et al. 2014, p. 263). The fountain darter was described as *Alvarius fonticola* from specimens collected from the San Marcos River just below the confluence of the Blanco River in 1884 (Jordan and Gilbert 1886, p. 23). The authors noted at that time that the species was abundant in the river.

Evermann and Kendall (1894, Plate XXXVI) included an illustration of the species by E. Copeland which was designated the lectotype by Jordan and Evermann (1900, p. 3271). Because the “type” referred to by Jordan and Evermann was a sample containing four specimens, Collette and Knapp (1966, p. 75) selected a lectotype from the U.S. National Museum collections of *E. fonticola* originally referenced by Gilbert (1887, p. 63-64). The remaining three specimens included in this collection are now paralectotypes (Burr 1978, p. 19). The fountain darter is the smallest species of darter (Page and Burr 1979, entire), usually less than 25 mm (1 in) standard length and is mostly reddish brown. The scales on the sides are broadly margined behind with dusky pigment. The dorsal region is dusted with fine specks and has about eight indistinct, dusky cross-blotches. A series of horizontal stitch-like dark lines occur along the middle of the sides, forming an interrupted lateral streak. Three small dark spots are present on the base of the tail and there is a dark spot on the opercle. Dark bars appear in front of, below, and behind the eye.

In males, the lower half of the spinous dorsal fin is jet-black; above this appears a broad red band, and above this band the fin is narrowly edged with black. Male fountain darters differ from females in four morphological characters: banding pattern, spinous dorsal fin coloration, genital papillae, and pelvic and anal fin nuptial tubercles (Jordan and Gilbert 1886, p. 23; Gilbert 1887, p. 64; Jordan and Evermann 1896, pp. 1104-1105; Collette 1965, pp. 605-606; Schenck and Whiteside 1977, p. 372-373; Burr 1978, pp. 20-22).

1.5.2 Historical and Current Distribution

The historical range of the fountain darter includes the San Marcos and Comal rivers in central Texas (Jordan and Gilbert 1886, pp. 21-23; Hubbs and Strawn 1957, p. 38). In 1884, Jordan and Gilbert (1886, pp. 21-23) collected the type specimens of the fountain darter in the San Marcos River from immediately below the confluence of the Blanco River. Fountain darters have been found intermittently between downstream of Cumming's Dam and Martindale. Hubbs and Strawn (1957, p. 38) collected this species from the Comal River in 1954, the last collection record for that locality of the original population, before its apparent extirpation and subsequent reintroduction into the Comal system (Schenck and Whiteside 1976, pp. 700-702). There are several hypotheses why the fountain darter was extirpated from the Comal River ecosystem, but the most likely cause is the drought of record that caused Comal Springs to stop flowing from June to November 1956. The Comal River was successfully repopulated with fountain darters from the San Marcos River in 1975-1976 (Schenck and Whiteside 1976, pp. 700-702).

A report of fountain darters in the Washita River, Arkansas, (Jordan and Gilbert 1886, p. 13) is the only record of fountain darters outside of central Texas. These specimens, now lost from the Smithsonian collections, are presumed to be *E. proeliare*, which were misidentified due to the early confusion in the taxonomy and systematics of the subgenus *Microperca* to which both *E. proeliare* and *E. fonticola* belong.

The present distribution of fountain darters in the San Marcos River is from Spring Lake (inclusive) downstream to the Martindale area. The presence of fountain darters in the San Marcos River in Martindale was confirmed in 2022 and 2023 (Chappell et al. 2024, entire). The distribution between the San Marcos wastewater treatment plant outfall and Martindale may not be continuous and may be associated with areas of submerged aquatic vegetation. The distribution in the Comal River is throughout the Comal River to its confluence with the Guadalupe River. Although regular sampling of fountain darters does not extend throughout these entire areas, dipnet sampling of these rivers found fountain darters in each area surveyed (BIO-WEST, Inc. 2021a, supplemental data).

1.5.3 Habitat Requirements

The fountain darter requires adequate springflows, clear and clean water of constant temperatures within the natural and normal range for the San Marcos and Comal Rivers, a food supply of living, small invertebrates, undisturbed stream floor habitats (including runs, riffles, and pools), and a mix of submergent vegetation (i.e., algae, mosses, and vascular plants) (Edwards and Bonner 2022, entire), in part for cover and egg deposition (Dowden 1968, pp.19-20; Phillips et al. 2011, entire).

Fountain darters are more abundant in areas containing vegetation (BIO-WEST, Inc. 2021b, p30; BIO-WEST, Inc. 2021c, p. 31). Fountain darter density is highly variable across vegetation types. In the Comal River, long-term fountain darter densities are highest in bryophytes (*Riccia* sp., *Amblystegium* sp.), *Cabomba*, and filamentous algae *Rhizoclonium* sp. (BIO-WEST, Inc. 2021b, p. 30). Long-term fountain darter densities in the Comal River are lower in *Ludwigia*, *Hygrophila*, and *Vallisneria* and lowest in *Sagittaria* and open water (BIO-WEST, Inc. 2021b, p. 30). The Comal River generally has higher fountain darter densities than the San Marcos River (BIO-WEST, Inc. 2021b, p.30; BIO-WEST, Inc. 2021c, p. 31). In the San Marcos River, long-term fountain darter densities are highest in *Cabomba*, followed by *Hydrilla*, *Hygrophila*, and *Hydrocotyle* (BIO-WEST, Inc. 2021c, p. 31). Long-term fountain darter densities in the San Marcos River are lower in *Ludwigia*, *Sagittaria*, and *Potamogeton*, and lowest in Texas wild-rice and open water (BIO-WEST, Inc. 2021c, p. 31). Fountain darters show a strong negative association with Texas wild-rice (Edwards and Bonner 2022, p. 8). Although fountain darter densities in Texas wild-rice have been sampled less than other vegetation types, conclusions of recent studies are similar to that found by Linam (1993, p. 11). The type of vegetation may affect fountain darter detection, which could affect these results. Habitat suitability models that incorporate the vegetation community composition and vegetation coverage accurately predict fountain darter occurrence (BIO-WEST, Inc. 2021c, Appendix H).

Cool, constant water temperature is also important to fountain darters. Several laboratory studies have investigated the impacts of water temperature on darter reproduction (Brandt et al. 1993, entire; Bonner et al. 1998, entire; McDonald et al. 2007, entire). The most recent work determined that reproduction is negatively impacted above 24°C (75.2°F), with almost no reproduction above 26°C (78.8°F) (McDonald et al. 2007, pp. 311, 314-316). For additional details on the habitat and water quality conditions of the spring ecosystems at Comal and San Marcos springs, refer to Sections 1.1.1 and 1.1.3, respectively.

1.5.4 Life History and Ecology

Fountain darter eggs attach to aquatic vegetation, including bryophytes, filamentous algae, and native and non-native submergent macrophytes (Dowden 1968, pp.19-20; Phillips et al. 2011, entire). After hatching, fry are not free swimming, in part due to the lack of swim bladders (Morgan 1936, pp. 20-21). Fountain darters spawn year-round (Schenck and Whiteside 1977, p. 367). Year-round reproduction can occur in areas of high-quality habitat in both the Comal and San Marcos systems (e.g., Spring Lake, Landa Lake), with a strong spring peak in reproduction (with limited reproduction in summer and fall of most years) in areas of lower quality habitat farther downstream (BIO-WEST, Inc. 2007a pp. 41, 43; BIO-WEST, Inc. 2007b pp. 43, 45). In a study examining egg deposition in the San Marcos River (Phillips et al. 2011, entire), significantly more fountain darter eggs were found on the filamentous algae, *Rhizoclonium*, with *L. repens*, *S. platyphylla*, *and Z. texana* also supporting eggs. More eggs were deposited on plants growing in sand than silt substrate (Phillips et al. 2011, pp. 1392, 1394, 1396).

While fountain darters can move between patches of vegetation, research indicates they are highly resident fish and were documented moving a maximum of 95 m [312 feet (ft)] over a 26-day period in the Comal River, and only 7 percent of fish were observed moving greater than 35 m (115 ft, Danmeyer et al. 2013, pp. 1049, 1052, 1055). Site fidelity was higher in areas of low-growing aquatic vegetation, such as algae or *Riccia fluitans* (a bryophyte, Danmeyer et al. 2013, pp. 1,052, 1,054). Fish were also more likely to move upstream (Danmeyer et al. 2013, pp. 1,053, 1,054) and toward areas of low growing aquatic vegetation (Danmeyer et al. 2013, pp. 1,052, 1,054).

The macroinvertebrate diet of fountain darters was examined by dissecting fish from five areas of the Comal River (Bergin 1996, entire). The taxa found in more than 10 percent of gut contents included Copepoda, Cladocera, Amphipoda, Diptera, Ephemeroptera, and Ostracoda (Bergin 1996, p. 9). Diet also varied with fountain darter size; Copepoda and Cladocera were more consumed by small darters, Ephemeroptera and Diptera by intermediate size darters, and Amphipoda by large darters (Bergin 1996, pp. 8, 10).

1.5.5 Abundance and Trends

Fountain darter abundance varies with dominant vegetation type, site location, and water depth (Perkin et al. 2018, pp. 58-59, 63-64). Headwaters of both the San Marcos and Comal rivers typically have more vegetation, which decreases downstream and is associated with lower fountain darter densities (e.g., BIO-WEST, Inc. 2018a, Appendix B; BIO-WEST, Inc. 2018b, Appendix B). The earliest comprehensive information about the amount of vegetation in fountain darter habitat is data from 1990 (Linam 1993, p. 12; Linam et al. 1993, p. 345). Tables 2 and 3 show the vegetation surveyed in the Comal River.

Table 2. Vegetation types found in the Comal River in 1990 (Linam et al. 1993, p. 345).

Vegetation	**Areal Coverage (m^2)**
Amblystegium	28
Bryophyta	1,027
Cabomba	20,602
Ceratopteris	431
Chara sp.	1,565
Egeria	9
Filamentous Algae	16,389
Hydrilla verticillata	19
Hydrocotyle	9
Justicia americana	2,258
Ludwigia sp.	33,636
Ludwigia sp./filamentous algae	2,171
Nuphar luteum	1,257
Potamogeton	2,800
Riccia	38
Sagittaria	18
Typha latifolia	95
Utricularia	25
Vallisneria	25,328
No vegetation	53,573
Total area surveyed	**161,322**

Table 3. Dominant vegetation types found in the San Marcos River in 1990 (Linam 1993, p. 12).

Vegetation	**Number of Transects**	**Percentage of Transects**
Egeria	282	15.6
Hydrilla verticillata	457	25.2
Ludwigia	94	5.2
Potamogeton	219	12.1
Rhizoclonium	5	0.3
Sagittaria	20	1.1
Vallisneria	62	3.4
Zizania	37	2.0
Other*	16	0.9
Debris	59	3.3
No vegetation	561	31.0
Total transects surveyed	**1,812**	

* Includes *Colocasia*, *Ceratophyllum*, *Cabomba*, and other species.

Table 4. Vegetation types found in the Comal and San Marcos Rivers in 2023. (BIO-WEST, Inc. 2023a, pp. 33-34; 2023b, pp. 20

Vegetation	**Comal Areal Coverage (m^2)**	**San Marcos Areal Coverage (m^2)**
Bryophyta	9,385	1,284
Cabomba	10,338	5,080
Filamentous Algae	Present but not estimated	Present but not estimated
Hydrilla verticillata		6,045
Hygrophila	22,424	4,720
Ludwigia sp.	2,505	415
Nuphar luteum	1,463	
Potamogeton		118
Sagittaria	14,186	1,948
Vallisneria	29,013	
Zizania		15,317
Other species	5,497	3,802
Total Coverage	85,426	38,347
Total area surveyed		

Over 90 percent of fountain darters are observed in vegetated habitat; they are generally positively associated with vegetated habitat for all locations, with the exception of the first reach of the San Marcos River where vegetation is abundant (Edwards and Bonner 2022, pp. 5-6). The number of fountain darters is also positively correlated with the amount of vegetation cover

(Edwards and Bonner 2022, p. 9). Fountain darters are not evenly distributed across vegetation types (Table 4). Fountain darter associations with vegetation can vary by location (wade-able versus non-wadeable habitat) and also by ecosystem (Edwards and Bonner 2022, entire); the composition of the aquatic vegetation community at each location may also affect these associations. Fountain darter associations with vegetation result in different fountain darter densities in different vegetation types (e.g., BIO-WEST, Inc. 2023a, pp. 45-46, 48-50; BIO-WEST, Inc. 2023b, pp. 30-32, 36-38).

Table 4. Fountain darter associations with vegetation type between San Marcos and Comal springs ecosystems (Edwards and Bonner 2022, p. 8).

Vegetation	**San Marcos Wadeable Habitat**	**San Marcos Non-wadeable Habitat**	**Comal Wadeable Habitat**	**Comal Non-wadeable Habitat**
Algae-Filamentous	Negative	Strong positive	Strong negative	Weak positive
Algae-Detrital	Neutral	Strong positive	Weak negative	Negative
Algae-Epiphytic	Neutral	Weak positive	Neutral	Negative
Bryophytes	Weak positive	Weak positive	Strong positive	Strong positive
Cabomba caroliniana	Weak positive	Strong positive	Positive	Strong negative
Cereratophylum demersum	Strong positive	Strong positive	Not reported	Not reported
Chara	Neutral	Weak positive	Weak positive	Neutral
Colocasia	Neutral	Weak negative	Strong negative	Neutral
Hydrilla verticillata	Strong positive	Strong negative	Weak positive	Weak negative
Hydrocotyle verticillata	Weak negative	Weak negative	Not reported	Not reported
Hygrophila polysperma	Strong positive	Negative	Strong positive	Weak positive
Justicia americana	Strong negative	Weak negative	Not reported	Not reported
Ludwigia repens	Positive	Negative	Strong negative	Weak positive
Myriophylum	Positive	Strong positive	Neutral	Weak positive
Nasturtium	Weak positive	Neutral	Not reported	Not reported
Nuphar	Not reported	Not reported	Weak positive	Neutral
Pistia stratiotes	Not reported	Not reported	Weak positive	Neutral
Potamogeton	Weak negative	Negative	Strong negative	Neutral
Sagittaria platyphylla	Positive	Strong positive	Weak negative	Weak positive
Terrestrial vegetation	Strong negative	Neutral	Weak negative	Neutral
Vallisneria	Positive	Weak negative	Strong negative	Negative
Zizania texana	Strong negative	Strong negative	Not present	Not present

Current sampling for fountain darters targets habitat type in specific locations of the river. The sampling data are useful to compare the fountain darter densities within vegetation types for a sampling location over time, but they cannot be used to estimate the total fountain darter population size.

In 2023, low flows from drought affected fountain darter habitat in both rivers, with some areas experiencing drying of surface habitat, increased sedimentation, and algal accumulation (BIO-WEST, Inc., 2023a, pp. 26, 33; 2023b, p. 15; EAA 2023, Appendix J, pp. 1-2). In the Comal River, fountain darter densities sampled were similar to long-term trends in spring 2023, and lower than long-term trends in fall 2023. There was a median of 7.0 darters per m^2 in spring 2023 and 0.5 darter per m^2 in fall 2023 (BIO-WEST, Inc. 2023a, p. 40), versus the long-term trend of 6.0 darters per m^2 in spring and 3.5 darters per m^2 in fall (BIO-WEST, Inc. 2021b, p. 24). These densities also vary by location in the river (BIO-WEST, Inc. 2023a, pp. 42-43). Indices of habitat suitability for fountain darters were lower than the long-term mean for Landa Lake, similar to the long-term mean for Upper Spring Run and Old Channel and were highly variable in the New Channel based on time of year (BIO-WEST, Inc. 2023a, pp. 50-52). Fountain darter densities from fish community surveys also varied greatly by season at most sampling locations (BIO-WEST, Inc. 2023a, pp. 53, 56).

In the San Marcos River, fountain darter densities sampled from drop nets in 2023 were similar to long-term trends for spring and fall, and higher in summer. The median density was 2.25 darters per m^2 in spring 2023 and 3.0 darters per m^2 in fall 2023 (BIO-WEST, Inc. 2023b, pp. 30-31). These densities also vary by location in the river (BIO-WEST, Inc. 2023b, pp. 32-33). Fountain darter densities from fish community surveys varied by season at the Middle River, while densities continued to be low for the Upper River and Lower River and became lower than the long-term median at Spring Lake (BIO-WEST, Inc. 2023b, pp. 41-42, 45).

Indices of habitat suitability in 2023 were similar to the long-term mean for Spring Lake Dam (BIO-WEST, Inc. 2023b, pp. 39-40). Habitat suitability decreased in City Park below the long-term median to its lowest level in five years (BIO-WEST, Inc. 2023b, pp. 39-40). At this location, Texas wild-rice has replaced non-native vegetation, and heavy recreation during low flows also likely contributed. Habitat suitability declined throughout the year at the I-35 reach to below the long-term median due to drought conditions affecting vegetation (BIO-WEST, Inc. 2023b, pp. 39-40). Texas wild-rice increases contributed to lowered darter habitat suitability because of its dominance in the river. In general, habitat suitability is lower in the San Marcos River than the Comal River.

No recent population estimates for fountain darters have been calculated by studies. Recent survey data from 2019 found that the spring and fall 2019 normalized population estimates for fountain darters for both Comal and San Marcos springs was within one standard deviation from the mean of the normalized population estimate for 2000-2019 (BIO-WEST, Inc. 2019a, p. 33; BIO-WEST, Inc. 2019b, p. 35). For the San Marcos River, the normalized population estimate incorporated an increase in Texas wild-rice, which typically has a low density of fountain darters (BIO-WEST, Inc. 2019b, pp. 34-35). The average of the normalized population estimate was lower than the long-term average in the San Marcos ecosystem (BIO-WEST, Inc. 2019b, p. 35), and higher than the long-term average in the Comal ecosystem (BIO-WEST, Inc. 2019a, p. 33).

The number of darters from 2002-2010 was estimated to be 58,562 to 471,315 in the San Marcos River, excluding Spring Lake (EARIP HCP 2020, p. 4-130). Population estimates including

Spring Lake would likely be much higher (EARIP HCP 2020, p. 3-58). The population estimates of fountain darters in the Comal River in representative reaches were 32,829-147,358 (EARIP HCP 2020, pp. 4-80-4-81). Population estimates for the entire Comal River would be higher but were not calculated. Estimating the population size of fountain darters is difficult and often results in large confidence intervals (EARIP HCP 2020, pp. 3-56-3-58). Different sampling methods have also been used in different studies that prevent direct comparison of population estimates.

Historical population estimates have large confidence intervals, indicating the difficulty in assessing the total population size. For the San Marcos River, the number of fountain darters was estimated at 103,000 by Schenck and Whiteside (1976). Linam (1993, p. 4) estimated the San Marcos River fountain darter population (excluding Spring Lake) to be 45,900, with a confidence interval (90 percent) ranging from -15,900 to 107,700. This could indicate a real decrease in fountain darter numbers in the San Marcos, or the difference in the population estimates may just reflect differences in the methods used to estimate population size. In 1991, Nelson conducted SCUBA-aided underwater surveys in Spring Lake and estimated at least 16,000 fountain darters at the spring openings and another 15,000 in the green algae habitat (Longley 1991, cited in USFWS 1996, p. 34). Linam et al. (1993, pp. 343, 345) sampled 7 transects in Landa Lake and the Comal River in 1990 and reported a population estimate of about 168,078 darters above Torrey Mill Dam, with a 95 percent confidence interval ranging from 114,178-254,110.

1.5.6 Critical Habitat

The final rule designating fountain darter critical habitat (45 FR 47362) includes the upper San Marcos River from Spring Lake downstream to approximately 0.8 km (0.5 mi) below the I-35 Bridge. Fountain darter critical habitat encompasses about 20 ha (49 acres) of the upper San Marcos River. This area is where fountain darters are found at higher densities in the San Marcos ecosystem. Critical habitat was not designated in the Comal River.

The critical habitat designation for the fountain darter predates the rulemaking that identifies PBFs (81 FR 7414). Based on the best available scientific and commercial data available, the PBFs could generally be defined as:

i. Undisturbed stream floor habitats (including runs, riffles, and pools),
ii. A mix of submergent vegetation (algae, mosses, and vascular plants),
iii. Clear and clean water,
iv. A food supply of small, living invertebrates,
v. Constant water temperatures within the natural and normal river gradients, and
vi. Adequate springflows to maintain the conditions above.

The final rule designating critical habitat describes those actions that would adversely modify designated critical habitat, including any actions that would:

i. Destroy or significantly reduce aquatic vegetation,
ii. Disturb the river bottom,
iii. Impound water,
iv. Excessively withdraw water,

v. Reduce the water level or flow, or
vi. Pollute the water.

Since the final rule did not include PBFs, there are additional actions that may affect these PBFs and may also result in adverse modification. Specifically, these actions include those that change natural substrate and submergent vegetation, such as the use of rip rap, grout, or other materials in the riverbed.

While the water in Spring Lake is exceptionally clear, turbidity increases downstream (Groeger et al. 1997, p. 286). Turbidity can increase from activities that contribute sediment loads, such as non-point source erosion from the urbanizing areas surrounding the upper San Marcos River or activities that suspend existing sediments, such as lake bottom disturbances by SCUBA divers in Spring Lake or swimmers in the San Marcos River. Such turbidity in Spring Lake and the San Marcos River usually dissipates as the suspended particulates flow downstream or settle out of the water column in relation to flow rate. Continual or repeated re-suspension of particulates can markedly reduce water clarity. This can be observed downstream of popular recreation sites in the river during periods of intense use, including weekends and holidays, and is more noticeable when springflows are low.

1.6 San Marcos Salamander

1.6.1 Description and Taxonomy

The San Marcos salamander (*Eurycea nana)* is a member of the family Plethodontidae (lungless salamanders). It is most closely related to *E. latitans*, *E. sosorum*, *E. neotenes*, *E. pterophila,* and an undescribed species from the Pedernales subbasin (Devitt et al. 2019, pp. 2627-2628). *Eurycea nana* is neotenic; they retain external gills and remain aquatic throughout their life cycle. The species was described by Bishop (1941, pp. 6-9) from specimens collected in 1938.

This dark, reddish-brown, slender salamander reaches lengths of one to two inches in the wild (2.5-5.1 cm [0.9-2 in]) and has moderately large eyes with a dark ring around the lens. The species has well developed and highly pigmented gills and relatively short, slender limbs with four toes on the fore feet and five toes on the hind feet. San Marcos salamanders have a slender tail with a well-developed dorsal fin. Males and females are not sexually dimorphic. Detailed morphological descriptions of this species are found in Bishop (1941, pp. 7-9; 1943, pp. 439-440), Baker (1957, entire; 1961, entire), Mitchell and Reddell (1965, pp. 16-22), Schwetman (1967, entire) and Tupa and Davis (1976, p. 186).

1.6.2 Historical and Current Distribution

This species is found only in Spring Lake and downstream in the San Marcos River to approximately 152 m (500 ft) below the Spring Lake Dam (Tupa and Davis 1976, p. 191; Nelson 1993, pp. 19-20), and mainly occurs in the remnant river channel (Diaz et al. 2015, p. 317). A population genetic study did not find evidence of genetic differences among three different sampling sites in the San Marcos River and Spring Lake (Lucas et al. 2009, p. 223). While salamanders at some springs north of San Marcos have mitochondrial haplotypes similar to San Marcos salamanders (Bendik et al. 2013, p. 14), further analyses determined that those salamanders were Barton Springs salamanders, *E. sosorum* (Chippindale 2012, entire; Devitt et al. 2019, p. 2,627-2,628). The San Marcos salamander was also collected in 2015 and 2022 at the Texas State University Artesian well (Longley et al. 2015, Attachment 1; Castillo 2023, pers. comm.), indicating some subsurface habitat.

1.6.3 Habitat Requirements

San Marcos salamanders live only near springs in the San Marcos River, resulting in stable habitat conditions consistent with the San Marcos Springs (see Section 1.1.3). Laboratory studies of water quality have found some of the water quality thresholds for this species. Laboratory studies of dissolved oxygen levels found 5 percent mortality of San Marcos salamanders at 4.5 mg/L, 10 percent mortality at 4.2 mg/L, 25 percent mortality at 3.7 mg/L, and 50 percent mortality at 3.4 mg/L (Woods et al. 2010, p. 549). Salamander activity and metabolic rate also decreased under low dissolved oxygen (Woods et al. 2010, p. 544). For additional details on the habitat and water quality conditions of the spring ecosystems at San Marcos Springs, refer to Section 1.1.3.

In captivity, these salamanders appear to become stressed at temperatures above 30°C (86ºF). Oxygen consumption in San Marcos salamanders was greatest at water temperatures of 25°C (77°F) as compared with 20 or 30°C (68 or 86°F) (Norris et al. 1963, p. 527). The critical thermal maximum was 35.8°C (96.4°F) for juveniles and 37.2°C (99°F) for adults (Berkhouse and Fries 1995, p. 431). In closely related Barton Springs salamander, the optimal temperature for growth was estimated to be 18.3-18.7°C (65.0-65.7°F, Crow et al. 2016, pp. 328, 330-331). The rate of growth was lower in salamanders in the 27.0°C (80.6°F) treatment than in treatments that ranged from 15.0°C-4.0°C (59.0°F-75.2°F; Crow et al. 2016, p. 331). San Marcos salamanders are likely to experience similar impacts of water temperature and dissolved oxygen because they are closely related to these species and inhabit the same aquifer with its associated environmental conditions.

At the surface, San Marcos salamanders are found in mesohabitats that contain cobble, gravel, and boulder substrates, and may have coverage from *Amblystegium* moss or filamentous algae (Diaz et al. 2015, pp. 307, 316). Moss was more abundant in the upper headwaters region (in 18 percent of the areas sample), while it was less abundant in the riverine area (in 2 percent of the areas sampled) (Diaz et al. 2015, p. 316). Salamanders are less likely to be found in areas that have mud or silt substrates and rooted macrophytes, which embed the substrate that they use as cover objects (Diaz et al. 2015, p. 316). Salamanders prefer water velocities of approximately 1 cm s^{-1} (Fries 2002, pp. 113, 115). High velocities can wash away potential habitat, while low velocities allow sediment to settle into the interstitial spaces that the salamanders use as habitat. It seems likely that the species reproduces underground, based on the lack of eggs found at the surface and documentation of other closely related *Eurycea* salamanders that use surface and subterranean habitat during their lifetime (e.g., Barton Springs salamander, McDermid et al. 2015, p. 556, Bendik et al. 2021, entire). Therefore, connection between surface and subsurface habitats at the springs is likely important for this species. The restricted points of access to subterranean areas limits our ability to sufficiently survey potential subterranean habitat.

1.6.4 Life History and Ecology

Males are sexually mature at a snout-vent length of 1.9 cm (0.74 in) (Tupa and Davis 1976, p. 186), but both sexes continue to grow after sexual maturity. Courtship and eggs have not been observed in the wild. Because gravid females and juveniles are found throughout the year, reproduction likely occurs throughout the year (Tupa and Davis, 1976 p. 190). In captivity, eggs were found on aquatic moss and on marbles (Najvar et al. 2007, p. 146). Clutch size ranged from 2-73 eggs, with a mean of 34.7 eggs (Najvar et al. 2007, p. 146). Eggs hatched 16-24 days after oviposition (Najvar et al. 2007, p. 146). It is possible that the conditions in captivity, such as abundant food resources and lack of potential predation, allow captive salamanders to allocate more resources to egg production than is possible in the wild (Najvar et al. 2007, p. 146).

San Marcos salamanders are generalist predators and prey on a variety of macroinvertebrates (Diaz 2010, p. 18). In a study of gut contents, they consumed the most common taxa, including amphipods, ostracods, chironomids, caddisflies, and snails (Diaz 2010, pp. 14-15).

Given the abundance of predators (primarily larger fish, but also crayfish, turtles, and aquatic birds) in the immediate vicinity of spring orifices, protective cover, such as vegetation and rocks, is essential to the survival of the salamander.

1.6.5 Abundance and Trends

San Marcos salamander density estimates are based on spring and fall sampling events since 2000 for specific areas that have active habitat management. The long-term average number of salamanders per m^2 is approximately 15 for Hotel Spring, 15 for Riverbed and 5 for Spring Lake Dam in the spring season and 26 for Hotel Spring, 13 for Riverbed, and 6 for Spring Lake Dam in the fall (BIO-WEST, Inc., 2023b, p. 46). Salamander densities are typically higher during low-flow years than during high-flow years (BIO-WEST, Inc., 2023b, p. 46). In 2023, the density of salamanders was lower in in the fall than spring (BIO-WEST, Inc. 2023b, p. 46). Salamander density was consistently lower at Spring Lake Dam and Hotel in 2023 than the low-flow average, but higher at Riverbed (BIO-WEST, Inc. 2023, p. 46). The flows in 2023 were lower than at any other point during the monitoring since 2000, and likely had more severe effects to habitat than previous low-flow years, including decreased springflow to habitat in the Spring Lake Dam area (D. Robinson personal observation). Spring Lake also had relatively increased sediment and algae, though areas with salamander habitat management showed decreased effects. Salamander densities outside of these areas are not monitored.

No recent population estimates from surveys have occurred. Estimating San Marcos salamander populations is intensive because juveniles are small, and salamanders can move undetected into interstitial spaces in the substrate. If they spend part of their lives underground, then a portion of the population is also likely missed during surveys. The population estimates could be resolved using mark-recapture methods or population genetics. Mark-recapture methods would focus on salamanders at the surface. Population genetics would provide a total population estimate but would not distinguish between the surface and subsurface. Previous population estimates for the San Marcos salamander have ranged from 17,000 to 21,000 individuals in the floating algal mats at the uppermost portion of Spring Lake (Tupa and Davis 1976, pp. 92-93), to as many as 53,200 salamanders from Spring Lake and the rocky substrates within about 150 ft (46 m) downstream of the Spring Lake Dam (Nelson 1993, pp. 19-20).

1.6.6 Critical Habitat

Critical habitat for the San Marcos salamander was designated on July 14, 1980, and is described as: Spring Lake and its outflow, the San Marcos River, downstream approximately 164 ft (50 meters) below Spring Lake Dam (45 FR 47362).

The critical habitat designation for the San Marcos salamander predates the rulemaking that identifies PBFs (81 FR 7414). Based on the best available scientific and commercial data available, the PBFs could generally be defined as:

i. Thermally constant waters,
ii. Flowing water,
iii. Clean and clear water,
iv. A food supply of small, living invertebrates,
v. Sand, gravel, and rock substrates with little mud or detritus, and
vi. Vegetation or rocks for cover.

The final rule designating critical habitat describes those actions that would adversely modify designated critical habitat, including any actions that would:

i. Lower the water table, such that Spring Lake could become either dry or intermittent, thus exposing algal mats, or
ii. Disturb algal mats or the bottom of Spring Lake, such as from SCUBA divers.

The water in Spring Lake and the San Marcos River is usually clear. Activities that contribute sediment loads, such as non-point source erosion from the urbanizing areas surrounding the upper San Marcos River, or activities that suspend existing sediments such as lake bottom disturbances by SCUBA divers in Spring Lake or swimmers in the San Marcos River, can increase turbidity and impact this element. Such turbidity in Spring Lake and the San Marcos River usually dissipates as the suspended particulates flow downstream or settle out of the water column in relation to flow rate. Continual or repeated re-suspension of particulates can markedly reduce water clarity. This can be observed downstream of popular recreation sites in the river during periods of intense use, such as during weekends and holidays (Bradsby 1994, pp. 48-49, 54-55; Breslin 1997, pp. 43, 47). Sediment can also fill the interstitial spaces in the substrate.

1.7 Texas Blind Salamander

1.7.1 Description and Taxonomy

The Texas blind salamander is a smooth, unpigmented, stygobitic (obligate aquatic cave-adapted) neotenic species. Adults attain an average length of about 4.7 inches (12 cm) with a large, broad head, and reduced eyes. The limbs are slender and long with four toes on the fore feet and five toes on the hind feet (Longley 1978, p. 9). The external appearance changes very little as the species matures (Vieira et al. 2020, pp. 7-8). One difference is small, pigmented melanocytes observed on juveniles, while adults have little to no dark pigmentation (Mitchell and Reddell 1965, p.14; Vieira et al. 2020, p. 7). Adults lack sexual dimorphism in Texas blind salamanders (Longley 1978, p. 26; Vieira et al. 2020, p.7).

The Texas blind salamander (*Eurycea rathbuni*) is a member of the family Plethodontidae (lungless salamanders). The type specimens of the Texas blind salamander were collected in 1895 at the Federal Fish Hatchery in San Marcos, Texas, where they were expelled from an artesian well drilled to supply water to the hatchery (Longley 1978, p. 2). The Texas blind salamander was first described by Stejneger (1896, entire), after the type specimen No. 22686, USNM (U.S. National Museum). The species was previously assigned to the genus *Typhlomolge*, but genetic studies indicate placement of this species within *Eurycea* (Chippindale 1995, entire; Chippindale et al. 2000, pp. 20, 23-24; Devitt et al. 2019, p. 2628). A technical correction published in 2021 officially recognized the genus change from *Typhlomolge* to *Eurycea* (86 FR 67352). *Typhlomolge* is still sometimes used as a subgenus within *Eurycea*. The subgenus *Typhlomolge* is most closely related to the other central Texas *Eurycea* species that occur south of the Colorado River (Devitt et al. 2019, pp. 2627-2628). The Texas blind salamander is most closely related to the Austin blind salamander, *E. waterlooensis* (Hillis et al. 2001, pp. 266, 274; Devitt et al. 2019, pp. 2627-2628). There are several morphological differences between these species (Hillis et al. 2001, entire). While other troglobitic *Eurycea* occur nearby in Comal County, they group genetically with *E. latitans* (Devitt et al. 2019, p. 2629).

1.7.2 Historical and Current Distribution

The Texas blind salamander is currently known from eight sites in Hays County, Texas, that include wells, fissures, caves and high and low flow springs: Diversion Spring, Sessom Springs, Rattlesnake cave, Rattlesnake well, Primer's fissure, Johnson's well, Texas State University artesian well, and Spring Lake outflow well (Russell 1976, pp. 1-4; Longley 1978, pp. 12-18; Chippindale 2009, pp. 8-11). Historically, the species also was collected at Wonder Cave (also known as Beaver or Beavers Cave) in 1917 (Uhlenhuth 1921, p. 87), but the cave has been modified, and searches in 1977 and since have not found any salamanders (Longley 1978, p. 17). A genetic assessment of population structure in wild Texas blind salamanders was conducted; preliminary results suggest there is not substantial genetic structure in the wild (Chippindale 2009, entire; Chippindale and Gluesenkamp, 2011, entire).

Hydrogeologic studies suggest significant connectivity among these groundwater collection sites exists (Krejca 2007, p. 3). The Blanco blind salamander, *Eurycea robusta*, was found from drilling along the Blanco River and was suggested to either be extinct or could possibly be the Texas blind salamander (87 FR 14227), although no analyses have been done to consider this latter possibility. Groundwater connectivity would facilitate subterranean movement between San Marcos and this area on the Blanco River (87 FR 14227).

1.7.3 Habitat Requirements

The Texas blind salamander is adapted to the water-filled subterranean caverns of the Edwards Aquifer in the San Marcos area (Longley 1978, p. 21). Observations indicate that this salamander moves through the aquifer by traveling along submerged ledges and may swim short distances before spreading its legs and settling to the bottom of the pool (Longley 1978, p. 21). For additional details on the habitat and water quality conditions of the spring ecosystem at San Marcos Springs, refer to Section 1.1.3.

Due to the relatively constant 21°C (69.8°F) temperature of subterranean waters in the Edwards Aquifer, the Texas blind salamander may be sensitive to changes in water temperatures. Limited exposure to higher temperatures has been documented as being tolerated but is not optimal for the species (Vieira et al 2020, pp. 4-5, 13). As water temperature increases for other species of *Eurycea* salamanders, metabolic rate increases and salamander activity decreases (Woods et al. 2010, pp. 544, 549). Tolerated temperature and dissolved oxygen thresholds under laboratory conditions of closely related *Eurycea* (Section 1.6.3) are likely to be similar for the Texas blind salamander because they occupy similar habitats and because of their phylogenetic similarity.

1.7.4 Life History and Ecology

The Texas blind salamander is an active predator. Prey items include amphipods, blind shrimp (*Palaemon antrorum*), daphnia, small snails, and other invertebrates (Longley 1978, p. 24; Hutchins et al. 2016, p. 1537). Observations of captive individuals indicate that Texas blind salamanders feed indiscriminately on small aquatic organisms and do not appear to exhibit an appreciable degree of food selectivity (Longley 1978, pp. 24, 26).

Subterranean fauna, such as the Texas blind salamander, are not strictly reliant on a food chain based on photosynthetically-derived organic matter, as chemolithoautotrophy can serve as basal food resources in cave ecosystems absent of sunlight (Hutchins et al. 2016, entire). Stable isotope analyses for nitrogen indicate this species is a predator in the subterranean ecosystem (Hutchins et al. 2016, p. 1536). This analysis also detected differences in diet across individuals (Hutchins et al. 2016, p. 1536).

In captivity, Texas blind salamanders are classified as juveniles from hatching to two years old (10-35 mm [0.4-1.4 in] snout to vent length); subadults from two to three years old (35-50 mm [1.4-2.0 in] snout to vent length; eggs and testes may be visible), and sexually mature adults from three years of age and up (50 mm [2.0 in] and greater snout to vent length, eggs and testes visible) (Vieira et al. 2020, p. 7). Captive female Texas blind salamanders become gravid at 1.5 to 2 years of age, but the presence of eggs does not necessarily result in the production of offspring (Vieira et al. 2020, pp. 7-8).

Individuals continue to grow throughout their lifespan, which is estimated to be 10 years in the wild based on size (Petranka 1998, p. 274; Chippindale and Price 2005, p. 761). Captive animals are known to experience greater longevity; one captive female is estimated to be 20 years old and measured 146.5 mm (5.8 in) (Vieira et al. 2020, p. 7). Growth rate throughout the life of the species does not remain constant. Texas blind salamanders exhibit the fastest growth rate as juveniles, then growth rate progressively decreases with age (Vieria et al. 2020, p. 7). These growth rates may vary in wild salamanders due to variations in food resource availability and temperature, which can vary widely and affect amphibian growth (Vieira et al. 2020, pp. 7-8).

Records from captive Texas blind salamanders from 2008-2020 indicate an average clutch size of 23.8 eggs per female (n= 81 clutches) (Vieira et al. 2020, pp. 7-8). Wild Texas blind salamander larvae are found throughout the year; thus, breeding is hypothesized to be seasonally unrestricted (Longley 1978, p. 26). Larval abundance in the wild fluctuates greatly, and reproductive cues are unknown (Vieira et al. 2020, p. 7).

Distinct from other salamander species, the Texas blind salamander female initiates courtship, and clutch sizes are small. Chemical cues play an important role in social interactions of Texas blind salamanders, indicating water quality may be an important component to the behavior of this aquifer species (Gabor et al. 2010, p. 297).

1.7.5 Abundance and Trends

The population size remains unknown for this species, largely due to the inaccessibility of the subterranean habitat (Vieira et al. 2020, p. 13). Population estimates using traditional mark-capture-release, survey, count, or other methods are difficult based on the inaccessibility of the subterranean habitat of the Texas blind salamander. Most of the population is assumed to reside in the aquifer itself, with only a subset of individuals accessing data collection sites near the surface. Mark-recapture at specific surface locations has been attempted (Krejca 2007, entire), but extrapolating population estimates from a single site to the entire subsurface population is problematic. Environmental factors such as nutrient concentration, distance to the surface, energy input from leaf litter and scat from trogloxenes (cave crickets, bats), sediment size and type, groundwater flow, and slope of the surrounding terrain are all important habitat characteristics shown to affect Texas blind salamander abundance (Krejca 2007, pp. 6-7).

Ezell's cave, in Hays County, Texas was historically known as one of the most abundant data collection sites for Texas blind salamanders. Since the 1940s, Ezell's cave has experienced salamander population declines; although this decline has not been quantified, it is attributed to over-collecting in the 1940s-1960s, heavy visitor traffic, lack of management, and the extirpation of the resident, cave myotis bat (*Myotic velifer*) in 1962, which historically played a key role in transporting nutrients into the cave (Krejca 2007, p. 3). The Nature Conservancy acquired Ezell's Cave in 2004 and it is currently protected by the Texas Cave Management Association (Kennedy 2015, p. 87). A gate that allows bats to enter the cave was installed in 2015 (Kennedy 2015, entire). To a lesser degree, Rattlesnake cave also suffered from illegal collecting of Texas blind salamanders (Krejca 2007, p. 13), but now is a protected site that has controlled access through Texas State University (Lee et al. 2021, p. 568).

1.7.6 Critical Habitat

Critical habitat has not been designated for the Texas blind salamander.

1.8 Texas Wild-Rice

1.8.1 Description and Taxonomy

Texas wild-rice is an aquatic, monoecious (pistillate and staminate flowers are on the same stem), perennial grass, which can grow to over 2 m long in the swift-flowing water of the San Marcos River (USFWS 1996, p. 42; Poole and Bowles 1999, p. 292). The reproductive stems (culms) are erect and emergent. Asexual reproduction occurs by development of basal tillers and stolons. The leaves are linear, elongate, green, and 5-25 mm (0.2-1.0 in) wide. The female inflorescence is a narrow panicle, 16-31 cm (6.3-12.2 in) long, and 1-10 cm (0.4-3.9 in) wide.

Although flowering and seed production occur year-round, peak flowering and production of viable seed occurs from May through July. The spreading staminate branches occur below the appressed pistillate branches. Spikelets consist of a single naked floret and lack glumes. The staminate spikelets are 6-11 mm (0.24-0.43 in) long, 1.2-2 mm (0.05-0.08 in.) wide, with white stamens, and hang down when mature. The pistillate spikelets are 8-12 mm (0.32-0.4 in) long by 1.2-1.8 mm (0.05-0.07 in) wide. Each spikelet has a 10 to 35-mm-long (0.39 to 1.38-inch) hair-like awn at its tip. The awns are scabrous with scattered prickle hairs, and 10-35mm (0.39- 1.38 in) long. The seeds (as obtained from cultivation) are cylindrical, 4.3-7.6 mm (0.17-0.30 in) long, 1-1.5 mm (0.04 -0.06 in) wide, 1/2 to 3/4 as long as the lemma and palea, and black, brown, or greenish. The chromosome number is n= 15. (Silveus 1933, pp. 473-475; Hitchcock 1950, pp. 561-563; Correll and Correll 1975, pp. 277-279; Terrell et al. 1978, pp. 53-55; USFWS 1996, p. 42).

The plant was formally described and named as *Z. texana* by Hitchcock (1933, p. 454) after originally being incorrectly identified as *Z. aquatica* in 1892 (U.S. National Herbarium sheet 979361). It is one of three *Zizania* species in North America; phylogenetic analysis indicates that *Z. texana* is more closely related to *Z. palustris* than to *Z. aquatica* (Xu et al. 2010, entire).

1.8.2 Historical and Current Distribution

Texas wild-rice is endemic to the upper San Marcos River. The current distribution is from the Spring Lake to approximately 4.3 km (2.7 mi) downstream. The designated critical habitat extends to the confluence with the Blanco River (ca. 8.1 river-km [5 river-mi]). Low water velocity, water depth, turbidity, sediment, and shading may limit the establishment of Texas wild-rice in the lower San Marcos River (Poole et al. 2022, p. 7). However, depth may be limiting primarily due to effects of water clarity, since Texas wild-rice has been planted successfully in depths greater than 2 m (Crawford-Reynolds 2018, p. 10). Over 80 percent of the Texas wild-rice population is located from the Spring Lake dam to the Rio Vista railroad bridge, and less than 5 percent occurs downstream of I-35 (USFWS 2019c, entire, BIO-WEST, Inc. 2023b, p. 27).

1.8.3 Habitat Requirements

Texas wild-rice forms large stands at depths from 0.23-1 m (0.76-3.3 ft) and requires clear, relatively cool, thermally constant (about 22.2°C [72°F]) flowing water (Poole and Bowles 1999, entire). Springflow and San Marcos River discharge are critically important for growth and survival of Texas wild-rice (Saunders et al. 2001, pp. 28, 30). Texas wild-rice relies on carbon dioxide as its carbon source for photosynthesis rather than the more commonly available bicarbonate used by most other aquatic plants (Rose and Power 2001, pp. 59-65). Edwards Aquifer water contains relatively high levels of carbonic acid, formed by the combination of carbon dioxide and water through karstification of carbonate rocks and microbial processes (BIO-WEST, Inc. 2004a, p. 10; Birdwell and Engel 2009, p. 147; Gray and Engel 2013, p. 335). Carbon dioxide in the water is readily available near spring openings and in relatively fast-moving waters that transport the dissolved gas downstream. Low springflows can be carbon-limiting for carbon dioxide-using obligates including Texas wild-rice. Texas wild-rice occurs primarily on gravel and sand substrates overlaying Crawford black silt and clay (Poole and Bowles 1999, entire; Saunders et al. 2001, p. 24; Vaughan 1986, p. 17).

1.8.4 Life History and Ecology

Reproduction of Texas wild-rice occurs either asexually (clonally) through stolons or sexually via seeds. Asexual reproduction occurs primarily by tillering at the base of the plant and from adventitious roots at the nodes of stolons. Clonal reproduction appears to be the primary mechanism for expansion of established stands (Emery 1967, p. 204; Emery 1977, p. 394; Power 2002, p. 573), but does not appear to be an efficient mechanism for dispersal and colonization of new areas. Texas wild-rice segments have, however, been observed floating downstream and some of these may become established plants; but plants would only establish if they were lodged into suitable substrate and habitat.

During sexual reproduction, Texas wild-rice flowers above the water surface and wind pollinated florets produce seed (Power 1997, p. 435). This typically occurs from May to July, although it can occur year-round. The triggers for flowering are not well understood but are probably related to increased photoperiod and sufficient access to sunlight by individual plants in relatively shallow water.

1.8.5 Abundance and Trends

When Texas wild-rice was described in 1933, it was reported to be abundant in the San Marcos River, including Spring Lake. By 1967, only one plant was found in Spring Lake, scattered plants were found in the last 1.5 mi of the upper San Marcos, and no plants were found in the lower San Marcos River (Emery 1967, p. 204). The decline was due to several reasons: bottom plowing to keep the lake and river clean for tourists, floating debris from mowing (which damages the emergent part of Texas wild-rice, preventing it from reproducing), plant collection, and pollution (Emery 1967, p. 204). Texas wild-rice coverage was estimated at 1,130 m^2 in the upper San Marcos River in 1977 (Emery 1977, p. 394) and 454 m^2 in 1986 (Vaughan 1986, p. 14).

Texas Parks and Wildlife Department (TPWD) has surveyed Texas wild-rice areal coverage since 1989 (Poole 2022, p. 2). TPWD used the same survey methodology from 1993 through 2012, and Texas wild rice areal coverage has ranged from 1,456-4,995 m^2 (0.4-1.2 ac). Since 2000, BIO-WEST, Inc., a consultant to the Edwards Aquifer Authority (EAA), has monitored Texas wild-rice. Since 2013, the USFWS and our conservation partners have also conducted annual monitoring of Texas wild-rice coverage. Implementation of the Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan (EARIP HCP, see Section 1.9 for additional information) has increased planting and management for Texas wild-rice (EARIP HCP 2020, pp. 5-20, 5-21). Texas wild-rice coverage peaked during 2020, when recreation in the San Marcos River was restricted due to park closures. Texas wild-rice coverage was estimated at 13,965-17,236 m^2 (BIO-WEST, Inc. 2021c, p. 21; USFWS 2020c, entire; USFWS 2021c, entire). In 2022, Texas wild-rice coverage was estimated at 13,272 m^2 (BIO-WEST, Inc. 2022, p. 24). By fall 2023, Texas wild-rice coverage decreased to 8,211 m^2 (BIO-WEST, Inc. 2023b, p. 25). The decline is most likely due to a combination of recreation resuming in 2021 and decreasing flows in 2022 and 2023 that reduced water depth and, in some areas, dewatered habitat containing Texas wild-rice. Hotter than average temperatures in 2022 and 2023 also may have increased recreational intensity. In general, factors contributing to differences in the amount of Texas wild-rice across seasons include periods of intense recreation, periods without recreation, seasonal differences in growth, springflows, runoff, observer error and possible methodological differences.

1.8.6 Critical Habitat

Critical habitat was designated for Texas wild-rice on July 14, 1980, and consists of the upper San Marcos River from Spring Lake downstream to the confluence with the Blanco River (45 FR 47355). This critical habitat designation encompasses the entire range of Texas wild-rice and is approximately 253,000 m^2 (about 25 ha or 62 acres).

The critical habitat designation for Texas wild-rice predates the rulemaking that identifies PBFs (81 FR 7414). Based on the best available scientific and commercial data available, the PBFs could generally be defined as:

i. Clear high-quality water,
ii. Unaltered San Marcos River flow,
iii. Constant year-round temperature, and
iv. Maintenance of natural substrate.

The final rule designating critical habitat describes those actions that would adversely modify designated critical habitat, including any actions that would:

i. Significantly alter the flow or water quality in the San Marcos River;
ii. Physically alter Spring Lake or the San Marcos River, such as dredging, bulldozing, or bottom plowing; or
iii. Physically disturb the plants, such as harrowing, cutting, or intensive collecting. Texas wild-rice habitat also may be adversely affected by invasive non-native plants and animals.

1.9 Conservation Efforts

There are several laws and regulations to protect water quality that apply to the Edwards Aquifer. The Federal Safe Drinking Water Act of 1974, as amended, regulates pollution and sedimentation of public drinking water sources, including the Edwards Aquifer. This legislation mandates enforcement of drinking water standards established by the Environmental Protection Agency. The Texas Commission on Environmental Quality (TCEQ) is responsible for enforcement of these standards in Texas. Under the authority of the Texas Administrative Code (30 TAC § 213), the TCEQ regulates activities having the potential for polluting the Edwards Aquifer and hydrologically connected surface streams through the Edwards Aquifer Protection Program, or "Edwards Rules." The Edwards Rules require water-quality protection measures for new development occurring in the recharge zone and portions of the contributing zone of the Edwards Aquifer. The TCEQ also prohibits facilities such as municipal solid waste landfills and waste disposal wells from being built in the recharge or transition zones.

Discharge from non-point residential or agricultural sources is one of the primary sources of pollution in the Edwards Aquifer. Texas has an extensive program for the management and protection of water that operates under State statutes and the Federal Clean Water Act. The Program includes regulatory programs such as the following: Texas Pollutant Discharge

Elimination System, Texas Surface Water Quality Standards, and Total Maximum Daily Load Program (under Section 303(d) of the Clean Water Act).

The TCEQ's Texas Pollutant Discharge Elimination System program regulates discharges of pollutants to Texas surface water. Through the Pollutant Discharge Elimination System program, the TCEQ authorizes the discharge of stormwater and non-stormwater to surface waters in Texas associated with storm sewer systems and construction sites, which must meet the requirements of the Edwards Rules.

A watershed protection plan (WPP) was accepted in 2018 by TCEQ for the Dry Comal Creek and Comal River Watershed by the City of New Braunfels. Dry Comal Creek has not met state water quality standard for bacteria, and the WPP is intended to address and reduce the elevated bacteria levels through management (TCEQ 2020, p.1). Another WPP for the upper San Marcos River was approved in 2018 by TCEQ. This WPP addresses the impairment of the upper San Marcos River due to elevated total dissolved solids, and proactively addresses bacteria, nutrients, sediment, and future growth scenarios for the watershed (TCEQ 2018, p. 1).

The EAA has additional regulations (EAA rule 713) that apply to the recharge zone and five miles upgradient of the recharge zone. Much of the contributing zone occurs outside of the EAA jurisdiction (EARIP HCP 2020, pp. 1-4, 1-5) and is not subject to these regulations. New development in the Edwards Aquifer recharge, transition, or contributing zones is reviewed by the TCEQ Edwards Aquifer Protection Program (30 TAC § 213.1). For the contributing zone, the rule covers activities that disturb more than two ha (five ac) in Medina, Bexar, Comal, Kinney, Uvalde, Hays, Travis, and Williamson counties (30 TAC § 213.20). The contributing zone in Bandera, Kerr, and Kendall counties does not have additional protections under either program.

In addition to these state and federal regulations, a significant number of local regulations to protect water quality were implemented by the City of San Marcos, City of New Braunfels, EAA, and Texas State University as part of the EARIP HCP. Texas Water Code (Chapter 36) allows groundwater districts, but not cities, to regulate groundwater, including groundwater quality. However, cities can regulate pollution at the surface that ultimately impacts groundwater quality.

During the 1980s to 1990s, the EAA tested for industrial pollution in their water quality program, finding that the aquifer maintained high water quality standards. The testing for industrial pollution was discontinued to focus on a growing uncertainty on personal care products and accumulation of contaminants (e.g., pesticides, herbicides, and metals) in fish tissues and sediment (EAA 2021b entire; EAA 2022a, p. 8-9, 13;). Continuous water quality data (i.e., water temperature and dissolved oxygen) is monitored at three locations in Comal and San Marcos Springs (EAA 2022a, pp. 11, 14-36).

The EARIP HCP was finalized in 2013, amended in 2020, and covers incidental take of these species for groundwater withdrawal, recreation, and other activities through 2028 (EARIP HCP 2020, entire). Permittees to the plan include the Edwards Aquifer Authority, City of San Antonio acting through the San Antonio Water System, City of New Braunfels, City of San Marcos, and Texas State University. This HCP includes measures to minimize and mitigate impacts of these actions and contribute to the recovery of these species and addresses a variety of aquifer management issues, including ensuring springflow during a repeat of the drought of record (EARIP HCP 2020, pp. 4-52-4-55, 4-58-4-62). Conservation measures in the EARIP HCP should ensure that Comal Springs maintains at least 0.85 m^3/s (30 cfs) daily average minimum during a drought of record (EARIP HCP 2020, p. iv). Long-term commitments to protect listed species in the Edwards Aquifer beyond the HCP and the term of its associated section 10(a)(1)(b) permit are not currently in place. However, a new HCP is expected in 2028.

The EARIP HCP (2020, pp. 5-15-5-17, 5-26, 5-38, 5-43-5-45) includes measures to monitor water quality and to minimize and mitigate threats to water quality. Since 1968, the EAA and its precursor, the Edwards Underground Water District, have collaborated with the U.S. Geological Survey and the Texas Water Development Board to conduct a water quality sampling initiative (Reeves and Blakey 1970, p. 4). The EAA's pre-existing program underwent expansion following the adoption of the EARIP HCP (EAA 2016, p. 3). This expansion aimed to identify and evaluate potential impairments to water quality within the Comal River and headwaters of the San Marcos River systems. Additionally, the EAA has implemented a water quality protection program, and the City of San Marcos has added regulations to protect water quality in the recharge zone (EARIP HCP 2020, p. 3-39).

Significantly, coal tar sealants, which contribute to polycyclic aromatic hydrocarbons (PAHs) in stormwater runoff (Mahler et al. 2012, entire), were banned by the EAA in 2012 in a portion of the recharge zone in Comal and Hays counties (EAA rule §713.703). The City of San Marcos also passed a coal tar ban in 2016, which protects additional fountain darter habitat in the San Marcos River from surface water runoff with high levels of PAHs. Downstream habitat outside of boundaries of the EAA-regulated area in the Comal River could still be impacted by coal tar sealants, although the EAA intends to work with local government to encourage bans on coal tar sealants (EARIP HCP 2020, pp. 5-44-5-45).

As part of the expanded water quality monitoring program, fish tissues were analyzed from the Comal and San Marcos rivers to detect the presence of several pollutants that could bioaccumulate (EAA 2019, p. 2, 5). No PAHs were detected in the fish tissues (EAA 2019, p. 27). The following chemicals exceeded the 12 meals/month Environmental Protection Agency cancer health endpoint fish consumption value: arsenic in largemouth bass from Spring Lake and lower San Marcos River, arsenic in *Gambusia* from Spring Lake (EAA 2019, p. 28), polychlorinated biphenyl in largemouth bass from Spring Lake and the San Marcos River between Rio Vista Dam and the I-35 bridge (Figure 4), and *Gambusia* from the San Marcos River (EAA 2019, p. 27).

Both the City of San Marcos and City of New Braunfels are Permittees for the EARIP HCP and have developed stormwater management plans. The City of New Braunfels established a stormwater management plan in 2014 (Lockwood, Andrews & Newman, Inc. 2014, entire) and is also developing a Watershed Protection Plan along with the EAA, the Guadalupe Blanco River Authority, and stakeholders, to decrease bacteria in the Comal River. The City of San Marcos completed its Stormwater Master Plan in 2018 (Lockwood, Andrews & Newman, Inc., and Halff Associates 2018, entire). Both cities also have hotlines to report spills or other water quality concerns.

Management of nonnative organisms in the Comal and San Marcos rivers is part of the EARIP HCP. For the Comal River, this includes management of harmful nonnative animals (EARIP HCP 2020, pp. 5-15–5-17, 5-18–5-19) and monitoring and management of the trematode *Centrocestus formosanus* (EARIP HCP 2020, p. 5-17). For the San Marcos River, this includes management of nonnative plants (EARIP HCP 2020, pp. 5-28–5-29, 5-38), and harmful nonnative and predator species (EARIP HCP 2020, pp. 5-28–5-29, 5-38). The plan also has measures to reduce nonnative species introductions (EARIP HCP 2020, pp. 5-18, 5-26, 5-38) and to restore native riparian vegetation (EARIP HCP 2020, pp. 5-18, 5-42) and native aquatic vegetation (EARIP HCP 2020, pp. 5-12–5-14).

The EARIP HCP includes several measures to minimize impacts of recreation by the City of New Braunfels (EARIP HCP 2020, pp. 5-14–5-15), City of San Marcos (EARIP HCP 2020, pp. 5-23–5-28), and Texas State University (formerly Southwest Texas State University; EARIP HCP 2020, pp. 5-30–5-33, 5-36–5-37). These measures limit recreation in some of the higher quality habitat in Spring Lake and Landa Lake, create access points, improve bank stabilization and decrease bank erosion, manage aquatic vegetation, reduce turbidity and sedimentation, and seek to educate river users.

To promote conservation of listed species and minimize the impacts of recreational activities on such species and their habitats, TPWD designated a State Scientific Area encompassing a 3.2 km (2 mi) segment of the San Marcos River effective July 8, 2012 (31 TAC § 57.910). This designation includes habitat utilized by the fountain darter. This regulation may provide some conservation value to the species by limiting impacts from recreation during periods of low flow in the San Marcos River.

Modeling of conservation measures in the EARIP HCP indicates that springflows will continue at San Marcos and Comal springs during the Drought of Record (EARIP 2020, pp. 4-57–4-60, 4-63–4-67). However, a drought worse than the Drought of Record is included as an unforeseen circumstance in the EARIP HCP (2020, pp. 8-7–8-8). The EAA, Oklahoma State University, the University of Texas at San Antonio, and the U.S. Geological Survey’s South-Central Climate Adaptation Science Center are currently pursuing a cooperative regional project assessing effects of climate change on future groundwater levels, springflow, and aquifer recharge through artificial intelligence modeling and physics-based modeling approaches (Başağaoğlu 2021, entire). The results of this project will be incorporated into the EARIP’s incidental take permit renewal in 2028 in an effort to reduce potential climate-change driven impacts to the Edwards Aquifer and the EARIP HCP’s covered species.

A captive refugia (operation and maintenance) and associated research is funded by the EARIP HCP through a contract (Contract # 16-822-HCP) with the USFWS at facilities in San Marcos and Uvalde, Texas (EARIP HCP 2020, p. 5-3). The contract was established to protect species left vulnerable to extirpation throughout a significant portion of their range due to a limited geographic distribution of the population, and it will preserve the capacity for these species to be re-established in the event of the loss of population due to a catastrophic event, such as the unexpected loss of springflow or a chemical spill.

Refugia research activities expand knowledge on habitat requirements, biology, life histories, and effective reintroduction techniques for the species. The Department of Defense has also funded Texas wild-rice research in the refugia. Researchers at local universities have also been very involved in research for these listed species, which has also contributed information on the life history and status of these species. Texas State University is also a Permittee with the EARIP HCP. In one instance, the USFWS provided funding under the Fish and Aquatic Conservation Program's 1311 system-wide funding source to improve aquaculture practices for the Comal Springs riffle beetle.

Another important conservation measure is implementation of the City of San Antonio's Edwards Aquifer Protection Program (Stone and Schindel 2002, pp. 38-39; City of San Antonio 2023, pp. 3, 6). In 2000, the voters of San Antonio passed Proposition 3, a $65 million sales tax initiative, to fund the acquisition (i.e., fee-simple and conservation easements) of open space to protect the contributing and recharge zones of the aquifer in Bexar County (Romero 2018, p. 2). Protection of open space has the potential to reduce the impacts of development (e.g., run-off from impervious cover, fertilizer applications, and wastewater) or maintain aquifer recharge (Reilly and Carter 2018, pp. 3-2, 3-6; Romero 2018, pp. 5-6). That program was re-approved in 2005, 2010, and 2015 with additional funds to acquire open space (Reilly and Carter 2018, pp. 1-3–1-5). The effort was later expanded to acquire lands in Medina and Uvalde counties that contain larger portions of the contributing and recharge zones (Romero 2018, pp. 5-6, 8). The dedicated sales tax expired in 2021 with 97,124 ha (240,000 ac) acquired under the Edwards Aquifer Protection Program (Siglo Group 2022, pp. 51-52). The City of San Antonio recently approved an alternative funding stream to support land acquisitions through the commitment of $100 million over ten years (City of San Antonio 2023, pp. 3, 6).

Several other entities also have measures to protect groundwater from contamination, including the EAA's Aboveground Storage Tank Program, Agricultural Secondary Containment Assistance Program, and Abandoned Well Program, among others (EAA 2022b, unpaginated). The San Antonio Water System implements several water quality protection measures, including development regulations (i.e., Aquifer Quality Protection Ordinance No. 81491) for properties over the contributing and recharge zones, review of building permits and master development plans, regulation of underground storage tanks, commercial/industrial compliance, and an abandoned well program (San Antonio Water System 2022, unpaginated).

1.10 Biological Constraints and Needs

These species occur in a limited range at a small number of localities, with little or no ability to disperse between or beyond these localities. These characteristics make them susceptible to local extirpation and extinction due to stochastic events (McKinney 1997, p. 499; O'Grady et al. 2004, p. 514). Having a high number of individuals at a site provides no protection against extinction from these threats. Dispersal beyond their extant range is unlikely, given the isolated nature of the spring headwater system dynamics and aquifer hydraulic connectivity that limit movement of individuals.

It is speculated that the Comal Springs riffle beetle may be able to retreat into spring openings or burrow down to the hyporheos (i.e., groundwater zone below the stream channel) during times of drought (Bowles et al. 2003 p. 359). However, a severe drought or water contamination event could eliminate many or all of the existing populations (Bowles et al. 2003 p. 380).

The areas inhabited by individuals of each species can be protected through localized conservation measures (e.g., intact riparian zones, springflow protection measures); however, the groundwater that provides water quality and quantity for the species can originate a significant distance from these habitats, and efforts that protect or conserve groundwater may be variable in their success and implementation. Even with the most effective management and recovery plans in place, the species remain vulnerable to devastating stochastic events such as floods or droughts that could eliminate the species.

2.0 Threats

The five listing factors, along with all the identified threats to the species and their habitats related to each factor, are listed below, and remain relevant to each of the species based on best available information. Not all of the threats are equally significant.

2.1 Listing Factor A: Habitat Loss and Degradation

2.1.1 Water Quantity

A primary threat to the species and their respective ecosystems is the potential loss of springflows. Springflows at San Marcos Springs, Comal Springs, and other locations where these species occur are tied inseparably to groundwater pumping in the southern segment of the Edwards Aquifer. Groundwater pumping to meet municipal, industrial, and irrigation uses is a widely recognized threat to the persistence of subsurface and surface groundwater-dependent ecosystems (Danielopol et al. 2003, pp. 109-112; Eamus et al. 2016, pp. 317, 333-335; Mammola et al. 2019, pp. 645-646). Removal of groundwater from an aquifer leads to water level decline, especially if discharge of groundwater significantly exceeds recharge (Theis 1940, pp. 278-280; Alley et al. 2002, pp. 1,986; Foster and Chilton 2003, pp. 1961-1962). Declining aquifer levels can result in springflow decline or failure, loss of stream and creek base-flow, and/or drying of water-filled caverns (Springer and Stevens 2009, pp. 9-10; Eamus et al. 2016, pp. 316-318, 333-335).

If not replenished through recharge, groundwater discharged through wells and springs is removed from aquifer storage (i.e., total amount of water in aquifer) [Lindgren et al. 2004, p. 41]. With absent or much reduced recharge, persistent groundwater removal would initially lead to decline and/or cessation in springflows (Lindgren et al. 2004, p. 41). Like other karst aquifers, water levels of the Edwards Aquifer fluctuate with recharge (i.e., distribution, amount, and intensity of rainfall) and discharge (i.e., wells or springs) (Petitt and George 1956, p. 49; Buszka 1987, pp. 24-27; Maclay 1995, pp. 48, 52; Worthington 2003, p. 4; Lindgren et al. 2004, pp. 40-41, 45). Prolonged dry periods result in declines in aquifer water levels, but levels rebound rapidly with return of precipitation (Petitt and George 1956, p. 49). Groundwater pumping has exceeded recharge multiple times, with water levels rebounding with increased rainfall (Petitt and George 1956, p. 49). The longest such period was the drought of record from the late 1940s to mid-1950s (Arnow 1959, pp. 27-29). During the drought of record, San Marcos Springs flow declined to 1/3 m^3/s (47 cfs) (Votteler 1999, pp. 10, 34; EARIP HCP 2020, p. 3-21), and Comal Springs stopped flowing for four months, likely resulting in the extirpation of the fountain darter at the Comal River (Schenck and Whiteside 1976, pp. 700-702).

In the early 1990s, federal litigation (i.e., *Sierra Club v. Secretary of the Interior* [No. MO-91-CA-069] United States District Court for the Western District of Texas) resulted in the creation of the EAA in 1993 by the State of Texas to manage groundwater withdrawals (i.e., by nonexempt wells) from the southern segment (National Research Council 2015, pp. 24-26; Hardberger 2019, pp. 193-194; Payne et al. 2019, p. 199). The regulatory area of the EAA includes most of the artesian and recharge zones of the southern segment, and a small portion of the contributing zone. The Texas Legislature directed the EAA in 1993 to limit Edwards Aquifer pumping authorized by permits to a maximum of 555,067 megaliters (450,000 acre-feet) per year, and to reduce that total to 493,392 megaliters (400,000 acre-feet) per year by December 31,

2007. During the 2007 legislative session, the Texas Legislature increased the annual maximum amount of pumping that could be authorized by permits to 705,551 megaliters (572,000 acre feet) and directed the EAA to adopt and enforce a "Critical Period Management" (CPM) plan establishing targeted withdrawal reductions during times of drought to achieve the water, species, and species habitat conservation goals established in the agency's enabling legislation (80th Texas Legislature, 2007, Senate Bill 3). Aquifer management since these rules were implemented has been successful at reducing groundwater withdrawals, but currently the management does not account for future droughts worse than the drought of record. The Stage V CPM that currently exists is also tied to the EAHCP but could be subject to change after species recovery.

Springflow has been protected at San Marcos and Comal springs during recent droughts in the 2000s and 2010s because of groundwater pumping restrictions from the EAA during periods of drought. During the 2008-2009 drought, springflows remained at sufficient levels to maintain resiliency for species (above 2.3 m^3/s [80 cfs]) in the San Marcos River (USGS stations 08169000 and 08170500), whereas flows decreased to near the drought of record level at a lowest monthly average of 0.4 m^3/s (14.9 cfs) for a different portion of the Edwards Aquifer in the Barton Spring segment (USGS station 08155500). By EAA estimates, Comal Springs likely would have gone dry during the 2014 drought without the enforcement of CPM (EAA 2015, pp. 1, 62).

Groundwater will continue to be a source of water in the future as city populations increase. For the four counties within the San Antonio pool (i.e., Hays, Comal, Bexar, Medina), predicted water demands increase by 48 percent in the year 2070, for which existing water supplies would be insufficient (Texas Water Development Board 2021, p. A-2–A-3). The State of Texas and Groundwater Conservation Districts for these counties have identified surface and groundwater management supply strategies that could supplement the forecasted needs of each county, but these are contingent on funding and infrastructure availability.

While a repeat drought of record has not occurred since the 1950s, modeling indicates that the CPM plan during Phase II of the EARIP HCP will maintain springflows above 0.85 m^3/s (30 cfs) at Comal Springs and above 1.3 m^3/s (45 cfs) at San Marcos Springs during a drought of record (EARIP HCP 2020, p. 4-63). However, the plan is currently unable to return springflows at either spring system to 2.3 m^3/s (80 cfs) within six months (EARIP HCP 2020, pp. 4-58, 4-66). Future droughts may also be more severe than the drought of record, and current aquifer management does not account for this (see Section 2.5 for more discussion of climate change).

Springflows needed to sustain resilient populations are species-specific and contingent on habitat use and species needs. The biological opinion (USFWS 2013, p. 129) associated with the EARIP HCP concluded that the issuance of the Incidental Take Permit for the EARIP HCP is not likely to jeopardize the continued existence of the species included in this recovery plan or destroy or adversely modify their designated critical habitat. Modeled springflows during Phase II projected Comal Spring flows to remain at approximately 1.4 m^3/s (50 cfs) during a repeat drought of record (USFWS 2013, pp. 32, 91, 100). The 0.8 m^3/s (27 cfs) at Comal Springs is greater than the springflows during the drought of record, when springflows ceased for four months in 1956.

When the 1996 recovery plan was amended to include quantitative delisting criteria in 2019 for the fountain darter, Texas blind salamander, and Texas wild-rice, the springflows necessary for recovery were included. Recovery of the fountain darter was possible in the Comal River if average springflow exceeds 6.4 m^3/s (225 cfs) for 50 years, and minimum springflows were 0.9 m^3/s (30 cfs) for no more than six months, followed by three months of 2.3 m^3/s (80 cfs) or greater. For the San Marcos River, recovery of the three species was possible if average springflow exceeds 4.0 m^3/s (140 cfs) for 50 years, and minimum flow were 1.3 m^3/s (45 cfs) for no more than six months, followed by three months of 2.3 m^3/s (80 cfs) or greater. While the San Marcos salamander was not included, the flow threshold would be the same as the other species included in the amendment. Threats to population viability are reduced by limiting the period of low flows, preventing reduced recruitment over longer periods of drought. However, it may not be feasible to reliably increase springflows from the minimum flows. Drought conditions may persist beyond six months, preventing a return of adequate springflows.

Springflows for the invertebrates were not included in the 1995 recovery plan or quantitative delisting criteria. The springflows that affect the invertebrates and their habitats may differ from those for other species. For example, at 0.9 m^3/s (30 cfs) at Comal Springs, runs 2 and 3 do not provide surface habitat for invertebrates (EARIP HCP 2020, pp. 4-97–4-98). Water from Panther Canyon well, from seeps along the western shoreline of Landa Lake, and within upwellings near Spring Island is expected to continue to provide habitat during low flow conditions. Invertebrates may be able to use subterranean habitat, but it is possible that genetic diversity at some subpopulations may be lost (USFWS 2013, pp. 100, 104, 110; Lucas et al. 2016, pp. 6, 12). The USFWS determined that 0.9 m^3/s (30 cfs) during a repeat drought of record is not likely to jeopardize the invertebrate species (USFWS 2013, p. 129).

2.1.2 Water Quality

The water quality at all management units is affected by both groundwater and surface water. It relies on the groundwater flow originating from the southern segment of the Edwards Aquifer. Numerous stream systems contribute to this segment of the aquifer through recharge features.

Groundwater Quality

The Edwards Aquifer is vulnerable to contamination because the limestone and carbonate rocks are highly permeable and exposed at the surface in the recharge zone (Clark 2000, pp. 1-2, 8-9; Burri et al. 2019, p. 150). Some of the sources of water quality degradation include impervious cover and stormwater runoff, construction activities, recharge from irrigation return flow (i.e., water that is not lost from evapotranspiration on lawns or to stream runoff), wastewater discharge, transportation infrastructure, and hazardous materials spills resulting from development within the watersheds, all of which may enter groundwater and affect the water quality in species' habitat (Passarello et al. 2012, pp. 29–34; Lapworth et al. 2012, entire).

Although water quality in the Edwards Aquifer is generally good, several studies have detected contaminants in groundwater from the southern segment including nitrates, herbicides, pesticides, polycyclic aromatic hydrocarbons, and many others (Fahlquist and Ardis 2004, pp. 7-8, 10; Johnson et al. 2009, pp. 10-13, 23-26, 31-35; Musgrove et al. 2014, pp. 67, 69-71; Opsahl et al. 2018, p. 58; Opsahl et al. 2020, pp. 17-30). Contaminants, commonly linked to urban and suburban activities such as residential and commercial development, industrial operations, transportation infrastructure, and waste disposal, tend to accumulate in higher concentrations within the shallow areas of recharge zones, especially in regions characterized by urban land uses (Wilson 2011, pp. 1-2; Lin and Gong 2016, pp. 384-385; Opsahl et al. 2018, p. 58). Preliminary studies suggest that groundwater contaminants are most prevalent at relatively shallow aquifer depths in the southern segment, like Hueco Springs (Ogden et al. 1986, p. 126; Guyton and Associates 1979, p. 21; Musgrove and Crow 2012, p. 86).

Groundwater contamination has not been shown to be widespread or with large numbers of substances present in concentrations that exceed the U.S. Environmental Protection Agency's (EPA) drinking water standards (Bush et al. 2000, pp. 1-2, 14-21; Fahlquist and Ardis 2004, pp. 7-8, 10; EPA 2009, entire; Johnson et al. 2009, 44, 47; Opsahl et al. 2018, p. 58; Opsahl et al. 2020, pp. 17-30; EARIP HCP 2020, pp. 3-40-3-42; EPA 2022 unpaginated). Twenty-seven contaminants exceeded public drinking water standards, including 19 contaminants in groundwater, four contaminants in springwater, and 12 contaminants in surface water recharging the aquifer (Johnson et al. 2009, p. 45). Many contaminants currently dilute to levels that meet drinking water standards by the time they reach the springs.

There are currently no established groundwater quality standards for subterranean ecosystems, making harmful impacts to the species from existing pollutant concentrations unclear (Hinsby et al. 2008, p. 10; Manenti et al. 2021, p. 2). However, subterranean fauna are likely to exhibit greater vulnerability to contaminants and a longer recovery period from stochastic events compared to surface fauna because of their inherent limitations, including a lack of adaptations to pollutants, isolation within their habitat, and restricted dispersal abilities, all of which render them sensitive to environmental disturbances (Hose 2005, p. 961; Di Lorenzo et al. 2019, pp. 293–294, 300; Hose et al. 2022, p. 2206).

Nitrogen is highly soluble and a threat to groundwater quality and a stressor to subterranean taxa (Castaño-Sánchez et al. 2020, pp. 6, 11; Banerjee et al. 2023, pp. 3–6). Land-use changes, particularly increases in impervious cover, are known stressors to aquatic systems and are difficult to predict, model, and remediate (Sharp 2010, p. 3; Coles et al. 2012, p. 65). Additionally, nitrate runoff from surface water recharge leads to increased nitrate concentrations in the aquifer, and concentrations over 1 mg/L are indicative of anthropogenic inputs, which have been recorded historically at Comal Springs and have doubled over the last 70 years (median concentration 2 mg/L) (Dubrovsky et al. 2010, p. 79; Musgrove et al. 2016, pp. 462, 465, 467; Castaño-Sánchez et al. 2020, p. 6). While safe for humans at these concentrations, it is unknown what effect these elevated nutrients will have over time within the aquifer food web and if conditions would become more favorable for surface species to colonize further underground (Notenboom et al. 1994, pp. 482–484, 490; Opsahl et al. 2018, p. 3). However, generally across the aquifer the available evidence does not indicate that contamination is widespread at high levels, regulations exist, and the subterranean community does not show signs of degradation (Hutchins 2018, pp. 481–482).

Forested land with limited human disturbances contributes to high-quality recharge (Dudley and Stolten, 2003 pp. 11, 58; Shah et al. 2022, p. 120396), while rural and exurban land uses contribute to groundwater contamination from leaking sewage, refuse dumping, and dead livestock (Sui et al. 2015, p. 21; Katz 2019, p. 565; EARIP HCP 2020, pp. 5-43). Septic systems are a likely source of nutrients (EARIP HCP 2020, p. 5-43; Sui et al. 2015, p. 21). Water tests from Rattlesnake Cave near San Marcos Springs once detected significant *Escherichia coli* and elevated phosphorus and ammonia, indicative of sewage contamination from surrounding rural septic systems (Furl and Bertetti 2017, pers. comm.). Once a source of pollution enters groundwater, it can be difficult if not impossible to track, intercept, and remediate because of karst conduit complexity (Humphreys 2011, p. 297). Since water quality in the Edwards Aquifer is generally good, this indicates that local sources of water pollution can disproportionately affect water quality in portions of the aquifer.

Oil and gas transmission pipelines are another potential source of hazardous material spills on the contributing and recharge zones of the aquifer. The "development and production of oil, gas, or a geothermal resource within the jurisdiction of the Texas Railroad Commission" are not considered regulated activities "having the potential for polluting the Edwards Aquifer and hydrologically connected surface water in order to protect existing and potential uses of groundwater and maintain Texas Surface Water Quality Standards" (Texas Natural Resource Conservation Commission 1996, p. 1). Consequently, the construction and maintenance of these pipelines are not subject to guidance mitigating impacts to karst features such as voids and are not subject to the Edwards Aquifer rules (Texas Natural Resource Conservation Commission 1996, p. 1; Burri et al. 2019, p. 141).

Abandoned groundwater wells are another source of potential contamination from shallow groundwater into subsurface habitat. Shallow wells (< 300 m [< 984 ft]) are less likely than deep wells to intercept older groundwater that received cumulative, diluted inputs of pollutants across the aquifer, and therefore shallow wells are more likely to intercept anthropogenic contaminants coming directly from the surface than are deep wells (Musgrove et al. 2014, pp. 69, 73). The EAA funds a needs-based abandoned well closure assistance program to assist well owners with proper well plugging in cooperation with San Antonio Water System (EAA 2021c, pp. 50-53). Likewise, former oil wells require maintenance decades after plugging (cement plugs in a steel pipe) and can blowout underground and break free under artesian pressure if not properly maintained (Gold 2022, entire).

Surface Water Quality

Despite the clear and stable conditions typically associated with the upper San Marcos and Comal rivers, pollution stemming from urbanization and development within its watershed has been documented (MCWE 2018, entire; Arismendez 2020, entire). Creeks, storm sewers, and one wastewater treatment plant discharge into the upper San Marcos River downstream of the main springs. Sink Creek discharges large quantities of storm runoff from the north into Spring Lake (Ogden et al. 1986, p. 119; Barr 1993, p. 47).

The lower San Marcos River experiences fluctuations in temperature and turbidity. These variations become more pronounced with the influx of tributaries, exacerbated by urban development, stormwater runoff, and wastewater treatment discharges. Consequently, the downstream trajectory witnesses a discernible shift in water quality, reflecting the cumulative impact of human activities and natural processes along its course (Benavides et al. 2023, p. 221).

Sediment samples from the San Marcos ecosystem indicate contaminants throughout the river (EAA 2022a, p. 52). These contaminants are commonly linked to either combustion engine byproducts or are components found in dyes, insecticides, and preservatives. Notably, City Park, Spring Lake, Sessom Creek, and Rio Vista sites displayed the highest levels of detectable contaminants. Specifically, the results from Sessom Creek samples revealed particularly elevated levels of contaminants compared to Sink Creek and I-35, where lower levels of contaminants were detected.

For the Comal Springs ecosystem, volatile organic compounds have been detected at Comal Springs but are rare (Johnson and Schindel 2014, p. 21). There is one documented diesel spill (i.e., naphthalene) that occurred in 2000 at spring run 7 at Comal Springs (Gibson et al. 2008, p. 75). It is unknown what effect this had on the subterranean or surface communities. However, it's worth noting that spring runs 1 through 3 receive groundwater above the Comal Springs Fault, while spring runs 4, 5, and 7, along with Landa Lake, discharge deeper groundwater below the fault line. This suggests a hydrological separation between these springs, further confirmed by variations in water chemistry (Rothermel and Ogden 1987 p. 129; LBG-Guyton Associates et al. 2004 p. B-24–B-25; Lucas et al. 2016 p. 2). Thus, this spill may have been confined to affecting only a portion of the Comal ecosystem.

Additionally, instances of elevated levels of fecal coliform (*E. coli* bacteria) have been observed, indicating contamination of the Comal River. In 2010, the Dry Comal Creek was identified by the State of Texas as having poor water quality due to fecal coliform. The concentration of fecal coliform in the creek exceeded the acceptable limit for surface water designated for primary contact recreation. The Comal River (Segment ID 1811) has held a status of impairment due to elevated levels of waterborne bacteria, specifically *E. coli*, as outlined in the TCEQ 303(d) List since 2016. This bacterial contamination is believed to originate primarily from recreational activities, as indicated in TCEQ reports (TCEQ 2022a, p. 190; TCEQ 2022b, p. 27). In addressing this issue, further data and information will be collected and evaluated, and a suitable management strategy will be selected to mitigate the bacterial impairments in the Comal River (MCWE 2023, p. 12). Additionally in 2010, the upper San Marcos River (Segment ID 1814) failed to meet water quality standards for total dissolved solids and was added to TCEQ’s 303(d) List (TCEQ 2012, p. 79). However, as of 2023, it now meets TCEQ's water quality standards (MCWE 2018, p. 9).

Water Quality Future Threat Analysis

Land use across the southern segment plays a major role in groundwater quality and surface water quality. Contaminants in groundwater increase with agriculture, residential and commercial developments, industrial facilities, military installations, and transportation infrastructure (Bush et al. 2000, pp. 6-9; Fahlquist and Ardis 2004, p. 7; Johnson et al. 2009, p. 46; Wilson 2011, pp. 1-2; Musgrove et al. 2014, pp. 69-71; Opsahl et al. 2018, p. 58; Opsahl et al. 2020, pp. 17-30). Urban and agricultural land uses dominate the artesian zone in the southern segment (Figure 6).

For projected land-use changes, we used the EPA’s (2019, unpaginated) Integrated Climate and Land-Use Scenarios which we used to project out to 2050 (Figure 6), which has also been used in Species Status Assessment for other species in the Edwards Aquifer, including the Texas troglobitic water slater and Edwards Aquifer diving beetle (USFWS 2023, pp. 73-85; USFWS 2024b, Appendix B.5). These outputs produce spatially explicit projections of population and land-use that are based on the Intergovernmental Panel on Climate Change’s Special Report on Emissions Scenarios (2023). The combination of SSP5-RCP8.5 illustrates a higher population growth and higher emissions, and a faster rate of human population growth is consistent with population projections from the Texas Demographic Center for the Bexar County and the San Antonio-New Braunfels Metropolitan Area (EPA 2017, pp. 34-35, 46; Texas Demographic

Center 2022, unpaginated). Within the Edwards Aquifer artesian, recharge, and contributing zones (543,498 ha^2 [134,3014 ac^2]), developed land-use classes are projected to grow from 21 percent in 2020 to 27 percent developed by 2050. When examining delineated areas at a finer scale around Comal and San Marcos springs, both areas are also projected to increase in development: Comal Springs area of influence from 66-82 percent and the San Marcos Springs area of influence from 44-65 percent developed. These areas thus may be important to assess more immediate impacts from groundwater contamination.

Based on the EPA's Integrated Climate and Land-Use Scenarios results, land development and population growth will continue to expand outward outside of the major metropolitan areas, San Antonio and Austin, Texas. Over time, these alterations have the potential to affect recharge rates, leading to deteriorating groundwater quality because of contaminated runoff from impervious surfaces in suburban and urban areas and septic systems that are poorly managed and prone to leakage in exurban areas (Berube et al. 2006, pp. 10, 38; Barkfield 2022, p. 2).

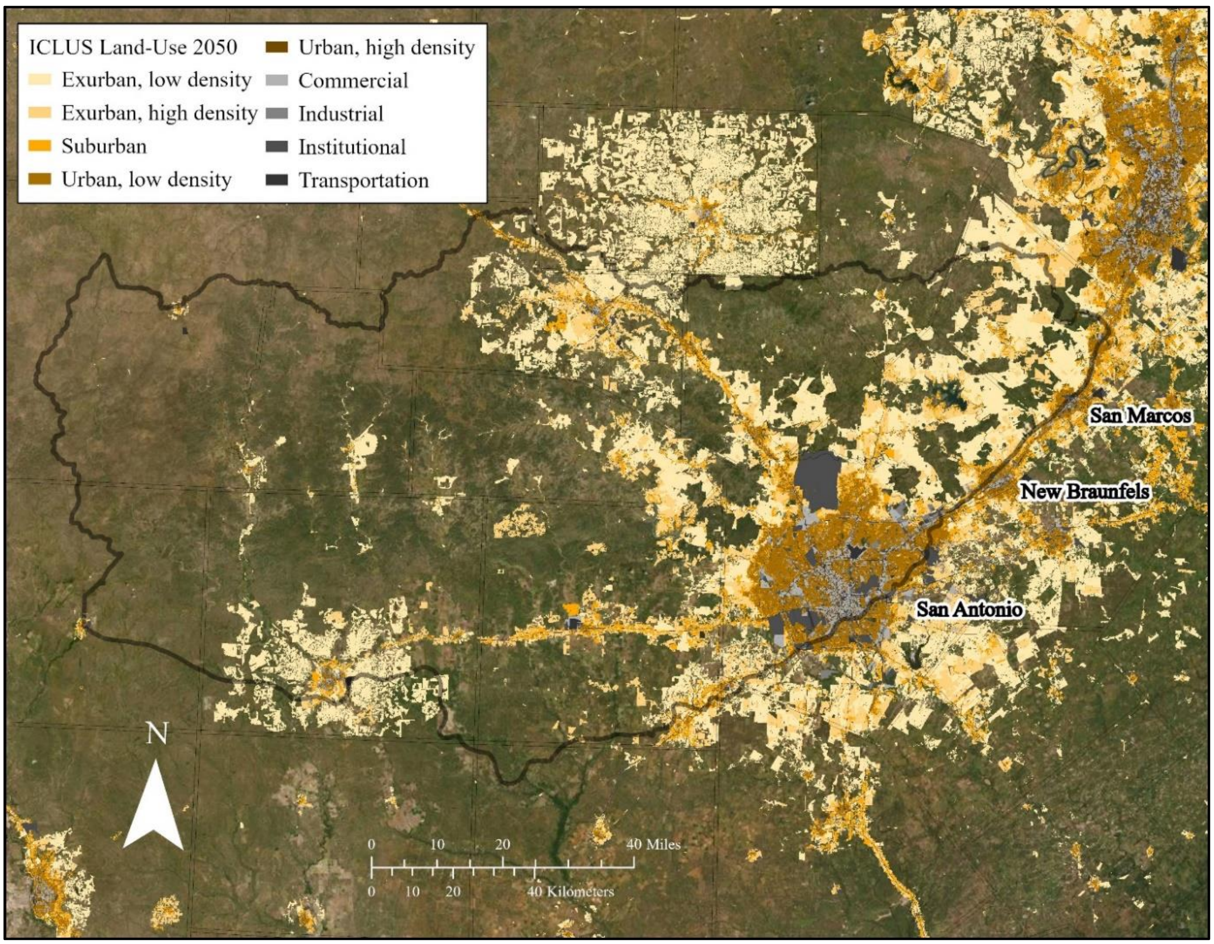

Figure 6. Projected urbanized land-use classes in 2050 across the southern segment of the Edwards Aquifer (black outline) using the Integrated Climate and Land-Use Scenarios, version 2.1.1 (EPA 2019, unpaginated).

The U.S. Census Bureau (2020, unpaginated) ranked several of the counties in the recharge and contributing zones of Comal and San Marcos springs among the fastest growing in the United States from April 2010 to July 2019: Hays County was the second fasting growing county with a 46.5 percent population increase, Comal County the fourth fastest growing county with a 43.9 percent population increase, and Kendall County the fifth fastest growing county with a 42.1 percent population increase. Since 2000, these three counties have doubled in population and have seen substantial associated development. Projections indicate that the human population of Bexar, Comal, Hays, and Kendall counties will continue to increase substantially over the next three decades. The San Antonio-New Braunfels Metropolitan Area is projected to increase from 2,741,008 in 2022 to 4,467,980 in 2050 (Texas Demographic Center 2022, unpaginated).

Conversion of natural habitat to urban, suburban, and exurban development is likely to accompany this population growth. Under a high human population growth scenario, land use projections suggest that large areas west and north of Bexar County will be converted to increasingly more urbanized land-use classes by 2100 (Figure 6; EPA 2019, unpaginated). Much of the exurban and suburban development is postulated to occur outside of municipal boundaries in unincorporated areas of counties where land use regulations (e.g., restrictions on impervious cover) are currently non-existent (Siglo Group 2022, pp. 13-14). Run-off from existing and expanded impervious cover in sensitive areas of the aquifer could affect groundwater quality over time. New contaminant sources are expected to be added to the region as increased human populations and expanded development continue; many existing contaminant sources will persist.

Future development in the recharge and contributing zones is likely to decrease water quality because of the increased risk of contamination entering the aquifer. A chief indicator of urbanization is the establishment of impervious cover (Arnold and Gibbons 1996, pp. 244-245; Brabec et al. 2002, pp. 501-503). A review of research found that impacts to aquatic species are seen with impervious cover of 10 percent or more (Center for Watershed Protection 2003, p. 97). Although the studies were focused on stream systems, we assume that shallow groundwater habitats would experience similar impacts because shallow groundwater ultimately flows into streams through discharge features. While physical parameters may be different (e.g., higher oxygen, lower temperatures, higher conductivity) in the shallow groundwater, pollutants entering both systems would be the same.

The EAA does not have explicit impervious cover limits in the recharge zone, with the intent that structural best management practices will protect water quality (Greater Edwards Aquifer Alliance 2010, p. 3). The TCEQ limits impervious cover through a construction permit review process for development proposals of more than 20 percent impervious cover and includes structural best management practices (30 TAC § 213).

Hays County limits impervious cover to 15 percent on properties within conservation lands on the recharge zone and 20 percent outside of the recharge zone (Hays County 2017, p. 204). Additionally, commercial property within the recharge zone shall not exceed 35 percent, or 65 percent if outside of the recharge zone (Hays County 2017, p. 207). Additionally, Comal County has goals to minimize impervious cover within the city of New Braunfels to limits of 26 percent per parcel (Design Workshop, Inc. 2012, pp. 4–5). While the efforts to implement such limits are intended to help ameliorate at least some water quality impacts, these percentages are nonetheless higher than 10 percent where aquatic impacts start to be seen. Likewise, lands over the contributing zone are not managed with land use regulations (e.g., impervious cover restrictions) (Siglo Group 2022, pp. 13–14).

2.1.3 Habitat Disturbance

Sediment

Excessive deposition of sediment can physically reduce the amount of available habitat and protective cover for these species. Activities that may increase sediment deposition include, but are not limited to, channelization, impoundment, road and bridge construction, deprivation of substrate source, destruction and alternation of riparian vegetation, vegetation removal, recreational facility development, and other watershed disturbances. Once deposited in large volumes, sediment can become anoxic (devoid of oxygen) and cease to provide suitable habitat or food resources. Silt and sediment can embed substrate, reduce or eliminate the interstitial spaces of the substrates that offer protective cover, and can influence total biomass of invertebrates (Reynolds and Benke 2012, pp. 174-175; John Gleason LLC 2017, p. 44; McCready Wright 2022, pp. 16, 25-26). Comal Springs riffle beetles prefer gravel and cobble-dominated substrates with aquatic vegetation and submerged wood present, free of silt (Brown 1972, p. 57; Bowles et al. 2003, p. 372; BIO-WEST, Inc. 2007b, p. 23). Because it affects invertebrate habitat, sediment also reduces prey available for salamanders. In a mesohabitat study, San Marcos salamanders were found in areas dominated by cobble, gravel, and boulder substrates, and had a negative association with areas dominated by silt and mud substrates (Diaz et al. 2015, entire). Sediment may also affect the type of vegetation that grows in areas, affecting fountain darter habitat. Sediment settling over aquatic plants and increased suspended particles can affect Texas wild-rice, since it requires clean and clear water with low turbidity (Poole and Bowles 1999, entire). Turbidity also has been shown to decrease prey items consumed by fountain darters in lab experiments (Swanbrow Becker et al. 2016, entire) and impairs the ability of fountain darters to detect and respond to predators in lab experiments (Swanbrow Becker and Gabor 2012, p. 117).

Recreation

The Comal River and San Marcos areas are high recreational use areas, and the severity of recreational use effects on the habitat are seasonal and temporal (Bradsby 1994, pp. 28, 35-49; Breslin 1997, pp. 44, 55-56). These activities and their associated support facilities may directly or indirectly impact the ecosystems and their species (Breslin 1997, p. 58; Owens et al. 2001, entire; Hardy and Raphelt 2015, pp. 18, 21). Habitat alteration due to recreation occurs from direct impacts such as substrate and vegetation disturbance, or indirectly due to introduction of nonnative bait fish and streamside influences such as increased compaction, erosion, litter, pollution, and runoff from parking areas and support facilities. Comal Springs historically was a recreation site, with negative effects in spring run 2 where prohibited-use signs were ignored and not enforced (Barr 1993, p. 62). The area was heavily trafficked with people swimming and wading, and by 1992, Comal Springs dryopid beetles were caught in low numbers or not at all (Barr 1993, pp. 30, 62; Arsuffi 1993, p. 22). Subterranean invertebrate diversity was also lowest between spring runs 1 through 3 during this time (Arsuffi 1993, p. 21). Currently, Comal Springs rules are enforced by park rangers, and no one is permitted to step within the spring runs without prior authorization by the park manager to access these areas for activities such as research and habitat restoration projects. Documentation must be provided on-site prior to any work conducted at the springs. On June 1, 2024, a portion of the Comal River was designated a National Water Trail (Department of the Interior 2024, unpaginated). It is currently unclear whether associated changes to river access and potential increases in recreation will affect the fountain darter.

The San Marcos River downstream of Spring Lake is especially susceptible to effects of recreation because it is shallow enough to be wadable in many areas, resulting in direct habitat disturbance. The effects of recreation to fountain darters were documented by monitoring that occurred in 2020 when the river was closed to recreation due to coronavirus restrictions and then reopened in 2021. In 2020, native vegetation that fountain darters use for habitat expanded when recreation was not occurring (BIO-WEST 2021b, p. 20). However, habitat loss occurred in 2021 after public access was restored and affected 12,843 m^2 (134,244 ft^2) of fountain darter habitat, resulting in the calculated take of approximately 44,951 fountain darters (BIO-WEST 2021b, p. 43). The City Park reach was most affected and had the lowest amount of vegetation in the 5-year period (BIO-WEST 2021b, p. 20). The City Park reach had 12,607 m^2 (135,700 ft^2) of habitat affected, or 45.65 percent of the vegetation present in that reach (EARIP HCP 2021, Appendix J, p. 30).

Habitat for San Marcos salamanders may also be disturbed by recreation downstream of Spring Lake Dam due to wading recreation. Because San Marcos salamanders hide under cobble, it is difficult to calculate these impacts. Vegetation disturbance in the Spring Lake Dam reach was used as a surrogate for disturbance of San Marcos salamander habitat (EARIP HCP 2020, Appendix J, p. 31), which resulted in calculated take of 708 San Marcos salamanders for the EARIP HCP (EARIP HCP 2020, Appendix J, p. 37). The most likely effect of recreation beyond vegetation disturbance that can decrease salamander habitat is the crushing of salamanders hiding underneath rock substrates that were moved or walked on. Signs exist to notify the public of habitat downstream of the dam that indicate disturbance is prohibited, though disturbance may still occur. Disturbance was not calculated for the listed aquatic invertebrates or the Texas blind salamander because they only occur in Spring Lake, where recreation is well regulated by the EARIP HCP and Texas State University.

Texas wild-rice is mapped annually by the EARIP HCP and USFWS. While Texas wild-rice coverage decreased in 2021 from the record in 2020, it remained at the second highest coverage level observed for the species (BIO-WEST 2021b, p. 21). There are other effects of recreation on Texas wild-rice. Texas wild-rice plants may be physically damaged by water activity, or its inflorescences may be prevented from emerging so that the plants cannot successfully produce seed (Vaughan 1986, pp. 20-22, Bradsby 1994, pp. 70-71).

Channel Modifications

The San Marcos and Comal systems have been modified by activities such as bank stabilization, dam construction, and removal of riparian areas. These alterations have changed the historical magnitude and occurrence of episodic events such as flooding. Indirect impacts from surrounding development and urbanization have also changed these systems. Understanding these changes and their impacts is important to the conservation of the listed species in the Comal and San Marcos systems and the ecosystems upon which they depend.

Dams occur at several locations in the San Marcos and Comal River ecosystems. Dams at Landa Lake and Spring Lake create larger and deeper areas for recreation. Dams downstream are also present for water control structures. Five flood-retardation structures built by the Soil Conservation Service (now known as the Natural Resource Conservation Service) on tributary creeks into the San Marcos River were expected to decrease the severity of flooding in the watershed and to slightly increase the recharge into the aquifer (U.S. Department of Agriculture 1978, pp. P-3–P-4). The effect of these structures on flushing flows and silt accumulation has not been evaluated. Flooding still occurs and may flush silt and other soft materials from the river bottom but may not be frequent enough to prevent sediment accumulation.

Additional habitat modifications have occurred since the last revision to the recovery plan for the fountain darter, San Marcos and Texas blind salamanders, and Texas wild-rice (USFWS 1996, entire). On the San Marcos River, Cape's Dam, Rio Vista Dam, and Spring Lake Dam have been modified (USFWS 2006, entire; USFWS 2018a, entire; USFWS 2022, pp. 4-5, 14; USFWS 2021a, entire). Cape's Dam, located downstream of I-35, partially failed in 1999, and repair work was conducted to secure the structure. Rio Vista Dam, located north of Cheatham Street, underwent a complete renovation in 2006 (USFWS 2006, entire). On the Comal System, habitat modifications have included the reconstruction a low-water dam and installation of culverts to maintain water levels in Landa Lake and control water entering the old channel (City of New Braunfels 2017, p. 35; USFWS 2001, entire).

The effects of dams to aquatic habitat are well documented (e.g., Baxter 1997, entire; McCartney et al. 2001, entire; Alla and Liu 2021, entire). Dams modify habitat by increasing water depth, resulting in overall changes to the aquatic community, which can have many effects, including habitat fragmentation and elimination of riverine species. Dams can also fragment populations by restricting the ability of species to move upstream. Predatory fish are more likely to occur in deeper water, which can increase predation. Dams also reduce water movement, resulting in reduced flows at the substrate and increasing sediment deposition that affects habitat.

The importance of existing modifications, the more-recent noted modifications, and continued operating practices all have an effect on the aquatic environment in these systems. The fishing pier in Landa Lake displaced a small area of aquatic vegetation due to shading, and it catches floating debris, which expands the area of shading. The partial failure of Cape's Dam and subsequent dewatering of the channel left some Texas wild-rice stands stranded. The upgrade and operation of the culvert system in the old channel of the Comal River led to several quantifiable negative effects, as documented by the loss of native vegetation and subsequent reduction of high-quality fountain darter habitat (BIO-WEST, Inc. 2007b, pp. 28, 44). A study investigating whether barriers like low-head dams impede gene flow in fountain darters was inconclusive (Olsen et al. 2016, pp. 1393, 1398-1399). Population effects may also occur with San Marcos salamanders, since they occur above and below Spring Lake dam. Texas wild-rice is highly managed, so it is possible that dams are less likely to affect its population structure.

Flooding

Surface habitat modification can occur as the result of flooding. Flash flooding is common throughout the Edwards Plateau (Woodruff and Wilding 2008, pp. 614-616). However, channel modification and the elimination of riparian zones can increase the severity of flooding (Schoof 1980, p. 697). Depending on the severity of floods, they can either deposit or increase suspended sediment loads over species' habitat or scour substrate and vegetation from species' habitat under high velocities (Griffin 2006, pp. 57-58, 61, 64; BIO-WEST, Inc. 2016b, p. 26; BIO-WEST, Inc. 2019b, pp. 14, 17; Schwartz et al. 2020, pp. 12). It is possible that individuals of species may also be washed away in floods, though this has not been studied for the species in this plan.

Record flooding occurred in the San Marcos River in 2015 and scoured large amounts of aquatic vegetation (BIO-WEST, Inc. 2016b, p. vi, 48). Fountain darter surveys in spring 2016 were lower than average, which may have been due to a delayed impact to the population from the 2015 floods (BIO-WEST, Inc. 2016a, p. 40; BIO-WEST, Inc. 2016b, p. 37). Floods have deposited finer sediments (e.g., silt) over invertebrate surface habitat at Comal and Sessom springs, reducing springflow and quality of habitat (BIO-WEST, Inc. 2002a, p. 11; Gibson 2022, pers. comm.). Habitat disturbance due to floods can uproot Texas wild-rice (BIO-WEST, Inc. 2016b, p. 26) and can also uproot important vegetation for fountain darters, negatively affecting the darter population (BIO-WEST, Inc, 2019b, pp. 14, 17). Scouring from floods could also remove the substrate that San Marcos salamanders use as habitat.

2.1.4 Nonnative Species

Introduced species are one of the primary threats contributing to extinctions (Pimentel et al. 2000, p. 53) and are one of the most serious threats to native aquatic species (Williams et al. 1989, p. 3; Lodge et al. 2000, p. 8; Lydeard et al. 2004, p. 324), especially in the Southwest (Miller et al. 1989, p. 34; Minckley and Douglas 1991, p. 17). It is estimated that approximately 50,000 non-native species have been introduced into the United States (Pimentel et al. 2000, p. 53). Although a few of these introductions have been beneficial, many have caused dramatic declines in populations of native plants and animals (Pimentel et al. 2000, p. 58).

Plants

There have been high numbers of invasive plants growing in the Comal and San Marcos rivers. These species include (listed in alphabetical order): *Alternanthera philoxeroides* (alligatorweed) (USFWS unpublished data); *Arundo donax* (giant reed); *Ceratopteris thalichtroides* (floating fern); *Colocasia esculenta* (elephant ear); *Cryptocoryne beckettii* (Beckett's water trumpet); *Egeria densa* (leafy elodea); *Eichornia crassipes* (water-hyacinth); *Hydrilla verticillata* (hydrilla); *Hygrophila polysperma* (Indian hygrophila); *Limnophila sessiliflora* (limnophila); *Myriophyllum aquaticum* (parrot's feather); *Myriophyllum spicatum* (Eurasian water-milfoil); *Pistia stratiotes* (water lettuce); *Potamogeton crispus* (curly pondweed); *Ranunculus sceleratus* (cursed buttercup), *Rorippa nasturtium-aquticum* (watercress); *Vallisneria spiralis* (water celery); and *Xanthosoma sagittifolium* (elephant ear) (Bowles and Bowles 2001, pp. 2-6). These nonnative plants can displace native plant species, including Texas wild-rice, through direct competition for space, light, and nutrients. Native vegetation for fountain darters is often displaced by nonnative species, though fountain darters are documented to use some nonnative plants such as *Hygrophila* (EARIP HCP 2020, p. 4-11). One of the measures in the EAHCP manages nonnative plant species in the Comal and San Marcos rivers.

Fish

Many fish species have been introduced into the San Marcos and Comal ecosystems (e.g., *Oreochromis aureus* (tilapia), *Cyprinus carpio* (common carp), *Ambloplites rupestris* (rock bass), *Lepomis auritus* (redbreast sunfish), *Herichthys cyanoguttatus* (Texas cichlid), *Poecilia latipinna* (sailfin mollies), *Hypostomus plecostomus* (armored catfish)). Some of these species may consume fountain darters and listed salamanders or compete for needed resources (e.g., food, breeding habitat). Introduced fish may cause changes in habitat characteristics (for example, by removal of vegetation or substrate disturbance) or introduce diseases and parasites (Taylor et al. 1984, pp. 327, 332-340). The South American armored catfish (*Hypostomus* and *Pterygoplichthys*) maintain dense populations in the San Marcos and Comal rivers and may have a large impact on the food web because they graze on algae (Hoover et al. 2004, p. 7). They may also consume fountain darter eggs, which can be laid on the algae that they consume (EARIP HCP 2020, p. 5-28). Nonnative fish are abundant enough in the San Marcos River that annual polespear tournaments and bounty hunts are held to suppress nonnative populations (Warner 2018, pp. 37, 42-43; Hay et al. 2022, pp. 3123, 3125).

Mollusks

Several species of nonnative mollusks have been introduced into spring ecosystems of the Edwards Aquifer. They include *Corbicula fluminea* (Asian clam), *Marisa cornuarietis* (L.) (giant rams-horn snail), *Melanoides tuberculatus* (red-rimmed melania), and *Tarebia granifera* (quilted melania). Some of these species consume vegetation that fountain darters use and previously reduced biomass in Landa Lake.

Nonnative mollusks are considerably more abundant than the native mollusks at both Comal and San Marcos springs. At San Marcos Springs, nonnative mollusks in this system account for over 90 percent of the total population in some samples, of which approximately 60 percent were quilted melania (Fries and Bowles 2002, p. 263). Similarly, nonnative species had the highest population density of mollusks in a study completed at the Comal River (Tolley-Jordan and Owen 2008, pp. 33). In some areas of the spring runs, shells, especially those of Asian clam and quilted melania, represent a main component of the surface substrate. Additionally, red-rimmed melania serves as an intermediate host in the life cycle of a fluke (Trematoda) that parasitizes the gills of certain fish, including the fountain darter (Mitchell et al. 2000, pp. 285-286). Parasites are further discussed in Section 2.3.

Additional mollusk species may enter the spring ecosystems and pose a risk in the future. Channeled apple snail (*Pomacea canaliculata*), zebra mussel (*Dreissena polymorpha*), and quagga mussel (*Dreissena rostriformis bugensis*) have been introduced into North America (Rawlings et al. 2007, pp. 4-6, Grigorovich et al. 2008, pp. 431-433).

Turtles

The nonnative Florida red-bellied turtle (*Pseudemys nelsoni*) is native to Florida and southeastern Georgia (Ernst et al. 1994, pp. 345-347). The first reported occurrence of the Florida red-bellied turtle in the San Marcos River was by Rose et al. (1998, entire), who reported that it probably was established there based on the occurrence of large fecund females and hatchlings in Spring Lake. The impacts caused by this species on listed species, or their habitats, are not known.

Birds

Seven species of nonnative waterfowl have been introduced to the San Marcos and Comal springs ecosystems. These species include the Egyptian goose (*Alopochen aegyptiacus* (L.)), mute swan (*Cygnus olor* (Gmelin)), black swan (*Cygnus atratus* (Latham)), Chinese or African goose (*Anser cygnoides* (L.)), and domestic goose (*Anser anser* (L.)). These birds occur at the springs frequently and appear to have reproducing populations based on the occurrence of juveniles. The impacts these species have on the native species and their habitats in the springs are generally unknown, but they will feed on and among aquatic vegetation causing physical damage to plants. Additionally, fecal material is deposited directly in the water or in the riparian zone where it can be washed into the water as storm run-off. It is possible that native birds also have some of these effects. Long (1981, entire) provides an exhaustive review of exotic birds, including the species presented here, and notes the types of damage they are known to cause.

Mammals

Nutria, *Myocaster coypus* (Molina), occur at both San Marcos and Comal springs. The primary impacts to aquatic systems attributed to the nutria include destabilization of stream banks associated with burrowing and eating aquatic vegetation (Atwood 1950, p. 250). Relatively little is known about the specific impact's nutria may have on springs and native vegetation.

2.2 Listing Factor B: Overutilization for Commercial, Recreational, Scientific, or Educational Purposes

Most of these species are collected as specimens for scientific study and for two refugia populations. Collections have not been documented to negatively impact total population numbers. These species are currently not known to be of commercial value, and overutilization has not been documented at this time and is not considered a significant threat. Although scientific collecting is not presently identified as a threat, unregulated collecting by private and institutional collectors could pose a threat to these locally restricted populations.

Due to the small number of localities for these species, they are vulnerable to unrestricted collection, vandalism, or other disturbance. Some fish and salamanders are prized by collectors, so it is possible that these species' rarity and some unique characteristics may make them a target in the future. In 2016, the Texas blind and San Marcos salamanders went missing from a captive refugia population, possibly for the black market (LeBlanc 2016, entire). However, there is no evidence that unauthorized collections from the wild have occurred.

Texas wild-rice may present a unique challenge because collection of this species and other plants in areas that are not under Federal jurisdiction or prohibited by State law is not explicitly prohibited by the ESA (16 U.S.C .1529 et seq. section 9(a)(2)), although the collection of Texas wild-rice could also affect other listed species. Thus, collections may occur without knowledge from the USFWS, increasing complexity to track and assess the effects of collections of this species. However, the high numbers of Texas wild-rice currently present (Section 1.8.5) indicates that overcollection is not currently harming the population size.

2.3 Listing Factor C: Disease or Predation

2.3.1 Comal Springs riffle beetle, Peck's cave amphipod, and Comal Springs dryopid beetle

The amount of predation that occurs in the wild has not been examined for the Comal Springs macroinvertebrates. It is assumed that predation is not a major threat to riffle beetles, but blind, fragile subterranean-adapted species such as the Peck's cave amphipod and Comal Springs dryopid beetle may be more susceptible to predation once they enter surface waters (Brown 1987, p. 263; Barr 1993, pp. 63-64). Fishes compete for prey expelled from the aquifer at discharge features (e.g., spring openings). Researchers have seen Mexican tetras (*Astyanax mexicanus*), sunfish (*Lepomis* sp.), and mosquitofish (*Gambusia* sp.) congregating at spring openings waiting for the driftnet to be removed and consuming the bycatch, including subterranean invertebrates (BIO-WEST, Inc. 2003, p. 42). Macroinvertebrates are a part of the food chain, and it is assumed that any number of individuals removed from the listed macroinvertebrate populations through typical levels of predation are likely to be negligible.

Fungal bodies have been observed growing outside of live riffle beetle joints, but not in Comal Springs riffle beetles (Gibson 2022, pers. comm.). Fungi have not been observed on living Comal Springs dryopid beetles, but benign fungal parasites on *Dryops* species have been documented (Brown 1987, p. 266). Filamentous fungi have been documented on deceased wild and captive Comal Springs riffle and dryopid beetle larvae and adults, but whether the fungi were the cause of the mortality or occurred post-mortem is uncertain (Worsham and Gibson 2022, pers. comm.).

Obligate ectoprotozoans are found around the mouths and faces of wild Comal Springs riffle beetles. The ectoprotozoans decrease in number over time in captivity where access to wild, living food resources are not provided. It is uncertain to what extent parasitism has an effect on this species, but the protozoans are likely receiving shredded food from the beetles and are benign (Brown 1987, pp. 266, 269).

Disease and parasites are rarely observed for the Peck's cave amphipod. A nematode (*Amphibiocapillaria texensis*) and an acanthocephalan (*Dendronucleata americana*) parasite have been observed in Texas blind and San Marcos salamanders and a *Hyalella* amphipod species (likely as an intermediate host); other *Stygobromus* taxa may serve as a possible intermediate host within the parasites' life cycle (Moravec and Huffman 2000, entire; Worsham and Gibson 2022, pers. comm.).

A facultative ectoparasite (e.g., rotifers, Phylum Rotifera) can be found on the gills of other amphipod taxa of this aquifer ecosystem but has not been observed in Peck's cave amphipods and needs further investigation (Worsham and Gibson, 2022 pers. comm.).

Seen in other members of the subphylum Crustacea (e.g., prawn, crab, and lobster juveniles and adults), a rickettsia-like bacterium causes milky haemolymph disease and can be treated (Nunan et al. 2010, p. 105, 111). This syndrome has been identified in other *Stygobromus* species, softening exoskeletons, and killing the individual (Worsham and Gibson 2022, pers. comm.). It is unknown if the Peck's cave amphipod is affected by this disease and to what extent contact with other infected freshwater crustaceans at the surface has on this species.

2.3.2 Fountain darters

Nonnative trematodes can parasitize fountain darters. *Centrocestus formosanus* transmitted by nonnative snails infect fountain darters and are more likely to kill larval and juvenile fountain darters than adults in the laboratory (McDonald et al. 2006, entire). This could indicate that infected fountain darters are less likely to reach reproductive age. While a laboratory study did not find differences in fountain darter reproduction due to *C. formosanus* or temperature (McDonald et al. 2007, pp. 312-314), the interaction between temperature and parasitic infection needs to be investigated because reproduction varied with temperature. In the Comal River, the cercariae (free-swimming larval stage) density of *C. formosanus* decreased from 2006-2010, which may be a natural decline after the initial invasion of this nonnative species (Johnson et al. 2012, pp. 111, 115).

Centrocestus formosanus is found in higher concentrations in areas of low velocity and may be diluted by the volume of water (Cantu 2003, pp. 17-19). Thus, these parasites may increase in the environment during low flows and, as a result, may also increase the infections in fountain darters during those times. A preliminary study found that removing host snails was an effective method of controlling the densities of gill parasites in the Comal River (USFWS and BIO-WEST, Inc. 2011, pp. 5-8).

The trematode, *Haplorchis pumilio*, also infects fountain darters (Huston et al. 2014, entire). All 10 fountain darters collected for a study were infected with *H. pumilio*, although infection intensity was considered low (Huston et al. 2014, p. 192). The authors speculated that the parasite was missed by other researchers due to its small size and atypical location for infection on the fin insertions and caudal peduncle (Huston et al. 2014, p. 189). The trematode may be controlled in the wild by targeting large snail removal of *Tarebia granifera* in all areas, and removal of *Melanoides tuberculata* in areas with high densities of detritus and vegetation (Tolley-Jordan and Chadwick 2019, p. 121).

Comal River fountain darters collected from the wild have had high mortality when brought into captivity since 2017 (USFWS 2019a, p. 40). Although the cause of the mortality is uncertain, this timeline corresponds with Comal fountain darters testing positive for largemouth bass virus (LMBV). Recently, survival of fountain darters collected from the Comal River was 90 percent for those that tested negative for LMBV versus 46.1 percent for those that tested positive (USFWS 2019a, p. 41). The symptoms exhibited by fish are not consistent with LMBV in other fish species. LMBV typically attacks the swim bladder, which is not present in fountain darters (USFWS 2018b, p. 27). While this has impacted collections for the refugia population, the normalized population estimate of Comal fountain darters in 2019 was not significantly different than the population average for 2000-2019, indicating a stable population in the wild (BIO-WEST, Inc. 2019a, p. 33). Additional work is occurring at the captive refugia to determine the nature of the disease problems (USFWS 2020a, p. 4). It is possible that the polymerase chain reaction (PCR) primers for LMBV may amplify other DNA. DNA sequencing would be useful to determine whether this pathogen is LMBV.

A novel virus belonging to the *Aquareovirus* genus was recently described in fountain darters (Iwanowicz et al. 2018, entire). The virus was found in healthy darters, and at this time it is unknown whether the virus is pathogenic and needs further investigation. In other fish species, aquareoviruses can be pathogens, and sometimes are pathogens circumstantially under stressful conditions.

Research on predation on fountain darters has mostly been in experimental settings and is unlikely to reflect the rates of predation in the wild. However, these studies are useful for comparing what would be expected to increase predation in the wild. Nonnative armored catfish in the genus *Hypostomus* decreased the survival rate of fountain darter eggs from 92 percent to 23 percent in experimental aquaria (Cook-Hildreth 2008, pp. 27-28), with survival being higher with algae than without any vegetation. Native and nonnative snails also consumed fountain darter eggs in lab studies, with the amount consumed varying substantially by species (Phillips et al. 2010, pp. 116-117). The nonnative freshwater snail, *Marisa cornuarietis*, consumed the most eggs (74.7 percent). Another study examined predation on adult fountain darters in outdoor raceways by red swamp crayfish (*Procambarus clarkii*) and largemouth bass at varying temperatures (Clark et al. 2017, entire). At 18°C (64.4°F) and 27°C (80.6°F), fountain darter mortality was lower when only exposed to red swamp crayfish and was higher in raceways that had either exclusively largemouth bass or a combination of largemouth bass and red swamp crayfish (Clark et al. 2017, p. 295). However, at 22°C (71.6°F), fountain darter mortality was higher in the raceways where both largemouth bass and red swamp crayfish were present, and lower in the raceways that only had one species of predator (Clark et al. 2017, p. 295). The 22°C (71.6°F), treatment is the closest to the temperature experience by fountain darters in the wild and thus may be the closest to what occurs in natural ecosystems. However, natural ecosystems have much more complex predator-prey interactions, with multiple prey types available and more potential mechanisms for predator avoidance by fountain darters. This study also did not detect an effect of vegetation on fountain darter mortality, though the authors suggested that changes to the experimental design in other studies may change this result (Clark et al. 2017, p. 295).

One study of predatory fish in the wild found that fountain darters had been consumed by other fish. Examination of 231 fish from the Comal system indicated that two fountain darters were consumed by largemouth bass (Perkin et al. 2018, p. 108-109). Examination of 200 fish from the San Marcos system indicated that one fountain darter was consumed by a warmouth bass (*Lepomis gulosus*) (Perkin et al. 2018, pp. 108, 110).

Antipredator behavior in captivity has been examined in response to visual and chemical cues of green sunfish, *Lepomis cyanellus* (Swanbrow Becker and Gabor 2012, entire). Fountain darters required both types of cues to exhibit an antipredator response (Swanbrow Becker and Gabor 2012, pp. 994, 998). Fountain darters also did not exhibit an antipredator response when vision was impaired by a semitransparent tint that prevented full visibility of green sunfish (Swanbrow Becker and Gabor 2012, pp. 994, 997, 998). This result could indicate that other visual impairments, such as turbid water, could reduce the antipredator response of fountain darters in the wild.

2.3.3 Texas blind and San Marcos salamanders

A pathogen present in the Edwards Aquifer since 2004, *Batrachochytrium dendrobatidis* (Bd), was found to have infected captive Texas blind and San Marcos salamanders, although all positive individuals were asymptomatic (USFWS 2021b, p. 83). *Batrachochytrium salamandrivorans* (Bsal, or salamander chytrid) is carried on the skin of various salamander species, which has caused major die-offs of salamanders in Europe and poses an imminent threat to native salamander populations in the United States (USFWS 2017, entire). To date, no cases of Bsal have been documented in the United States.

A study published in 2000 found Texas blind salamanders to be host to three new previously undescribed species of intestinal helminth species: *Brachycoelium longleyi* (Trematoda), *Dendronucleata americana* (Acanthocephala), and *Amphibiocapillaria texensis* (Nematoda) (Moravec and Huffman 2000, entire). The effect of these parasites on the fitness of Texas blind salamanders is not known at this time.

Microsporidian infections (microsporidiosis) were detected in two species of Plethodontidae salamanders through histopathological examination in sick and dead *Eurycea sosorum* (Barton Springs salamander) and *E. nana* (San Marcos salamander), housed at SMARC as part of the facility's captive breeding program (Yu et al. 2019, entire). Microsporidia are a group of parasites known to immunocompromise and infect many animals, including amphibians (Yu et al. 2019, pp. 8). This study presented molecular evidence that microsporidiosis was a major cause of the 10 percent mortality increase of these two *Eurycea* species at SMARC (Yu et al. 2019, p. 2). Although Texas blind salamander was not a species of concern from this study, these findings suggest that captive salamanders are more susceptible to disease and infection due to stress from captivity.

While the amount of predation that occurs in the wild has not been examined for San Marcos salamanders, predation is assumed not be a major threat to salamander species. Several studies have been performed on the response of San Marcos salamanders to the visual and chemical cues of predators in the laboratory (e.g., Epp and Gabor 2008, entire; Davis et al. 2012, entire; Epp 2013, entire). Davis et al. (2012, entire) found that San Marcos salamanders demonstrated a generalized response to predators that allowed them to avoid novel predators. Interestingly, antipredator behavior was reduced in turbid water in the closely related Barton Springs salamander (Zabierek and Gabor 2016, pp. 7-9), which indicates that the effects of predators may vary with environmental conditions.

Texas blind salamanders are adapted to their subterranean habitat, where they are unlikely to encounter larger predators in the subterranean food web. When they are ejected to surface habitats, they are vulnerable to consumption by predators, though the amount of predation that occurs has not been documented.

2.3.4 Texas wild-rice

Texas wild-rice can be consumed by nutria and giant rams-horn snail (McKinney and Sharp 1995, pp. 4.8, 4.10). Waterfowl that eat other vegetation may also consume it. However, recent surveys of Texas wild-rice indicate that herbivory does not have a large effect on the species (Poole et al. 2022, p. 7).

2.4 Listing Factor D: Inadequacy of Existing Regulatory Mechanisms

Under this factor, we examine the stressors identified within the other factors as ameliorated or exacerbated by any existing regulatory mechanisms or conservation efforts. Section 4(b)(1)(A) of the ESA requires that the USFWS consider "those efforts, if any, being made by any State or foreign nation, or any political subdivision of a State or foreign nation, to protect such species…". In relation to Factor D under the ESA, we interpret this language to require the USFWS to consider relevant Federal, State, and Tribal laws, regulations, and other such binding legal mechanisms that may ameliorate or exacerbate any of the threats we describe in threat analyses under the other four factors or otherwise enhance the species' conservation. Our consideration of these mechanisms is described in detail within each of the threats or stressors to the species (see discussion under the other Factors). Much of the information under Listing Factor A should also be considered as relevant here because it is often the inadequacy of existing regulations that contributes to habitat loss and degradation for these species.

The recharge and contributing zones to the Edwards Aquifer continue to experience rapid human population growth and conversion of natural habitat to developed land-use types, which continues to threaten water quality. Much of the contributing zone is not under the same regulations to protect water quality as the recharge zone, even though much of the water that recharges the aquifer originates in the contributing zone. Regulatory mechanisms that protect water in the Edwards Aquifer are crucial to the future survival of these species. Federal, State, and local laws and regulations have improved water quality and quantity protection but could be insufficient to prevent ongoing impacts to the species and their habitats from water quality degradation, reduction in water quantity, and surface disturbance of spring sites, and are unlikely to prevent further impacts to the species in the future. Knowledge of the source, accumulation, and transport of these compounds in the aquifer are lacking, and investigations into their effects on the habitat quality are necessary for the recovery of these species and for sustainable use of the aquifer (Danielopol et al. 2004, pp. 187-188; Opsahl et al. 2018, p. 2).

Under Texas Parks and Wildlife Code (Chapter 68) and TAC (31 TAC § 65.171-65.176), the Texas Parks and Wildlife Department is authorized to add species to the agency's List of State Threatened and Endangered Nongame Species and List of State Endangered, Threatened, and Protected Native Plants. The seven species in this plan are also state listed. The Texas Parks and Wildlife Department prohibits the taking, possession, transportation, or sale of any animal species that are state listed as threatened or endangered. State law prohibit commerce in threatened and endangered plants, and also prohibits collection of listed plant species from public land without a permit. However, prosecutions for these prohibited actions are rare, and the burden of proof to prosecute is high, which can result in unauthorized take of state listed species. In addition, it is likely that at the time of recovery they would no longer be state listed. Because these species are conservation reliant, the species would need other mechanisms to continue conservation after delisting and to prevent future threats to the species.

While the EAA was granted regulatory authority by the Texas Legislature, there have been several legal challenges to the EAA permitting program. For example, in court cases *EAA v. Day* (2012, Supreme Court of Texas No. 08-0964) and *EAA v. Bragg* (2013, Court of Appeals of

Texas No. 04-11-00018-CV), courts awarded landowners compensation for groundwater permits that were denied by the EAA due to lack of historical usage. The ruling for *EAA v. Day* by the Texas Supreme Court argued that there was no reason to treat groundwater differently than oil and gas and recognized groundwater as real property. In both cases, landowners owned the land prior to enactment of new groundwater pumping regulations. There remains a lack of clarity with Texas groundwater law that results in ongoing legal challenges regarding groundwater regulation, and these could impact the EAA's ability to regulate the aquifer in the future.

For the Edwards Aquifer, the EAA manages, and issues permits for groundwater withdrawals with conservation and drought management in place. The EAA jurisdiction is limited to the Edwards Aquifer in Uvalde, Medina, Bexar, and portions of Comal, Guadalupe, Hays, and Caldwell counties. The contributing zone in Bandera, Kerr, and Kendall counties do not have additional protections under either program. Thus, the EAA's water quality regulations do not protect most of the contributing zone, which may ultimately reduce the water quality of the Edwards Aquifer.

As described above, the TCEQ regulates activities that have the potential to pollute the Edwards Aquifer and hydrologically connected surface streams through the Edwards Aquifer Protection Program or "Edwards Rules" and for the same counties. This means portions of the contributing zone do not have additional protections that could affect the amount and quality of recharge that enters the Edwards Aquifer, resulting in lower water quality protection for the aquifer and for the spring associated ecosystems.

Likewise, this agency does not address development or other land use, impervious cover limitations, some nonpoint source pollution, or application of fertilizers and pesticides over the recharge zone (30 TAC § 213.31). Changes to how surface water and the Trinity Aquifer are managed are likely to change the amount that can be sustainably pumped from the Edwards Aquifer during drought conditions. For example, the Hays-Trinity Groundwater Conservation District also manages groundwater that influences the water at San Marcos Springs.

2.5 Listing Factor E: Other Natural or Manmade Factors Affecting the Species' Continued Existence

Global climate change is already affecting many regions' biodiversity, with stressors driven by increasing temperatures and extreme climatic events and will continue to in the near-term (Intergovernmental Panel on Climate Change 2023, pp. 5, 15). Over the last 115 years, the global averaged surface air temperature has increased by 1.0°C (1.8°F) with recent decades being the warmest in 1,500 years (Vose et al. 2017, pp. 186, 188). With the highly karstic permeability of the Edwards Aquifer, climate change and variability strongly influence this vulnerable aquifer that relies heavily on rainfall for recharge (Mace and Wade 2008, p. 659; Taylor et al. 2013, p. 312; Ding and McCarl 2019, p. 11; Nielsen-Gammon et al. 2020, p. 9). The Fourth U.S. National Climate Assessment (U.S. Global Change Research Program 2018, pp. 1,002-1,003) presents the Edwards Aquifer as a case study in vulnerability to climate change, citing the shallow karst aquifer as especially sensitive to climate change, and the regional human population growth and development as exacerbating the effects of decreased water supply during droughts. While average rainfall is not projected to change significantly in central Texas, the distribution of precipitation is anticipated to change with more extreme droughts and extreme rain events (Geos Institute 2016, pp. 14-15).

Warmer temperatures will also create drier conditions due to increased evapotranspiration (Loáiciga and Schofield 2019, p. 224). Extreme droughts in Texas are more likely than they were 40-50 years ago (Rupp et al. 2012, p. 1,054; Nielsen-Gammon et al. 2020, entire). A recent study predicts megadroughts in Texas, more severe than have been seen for the past thousand years, that will occur before 2100 (Nielsen-Gammon et al. 2020, entire). Droughts worse than the drought of record have occurred since the 1600s and are not uncommon in the region (Mauldin 2003, entire; Cleaveland et al. 2011, entire). It is not possible to ensure that there will be adequate flow to these springs without planning for more extreme droughts than the drought of record (Loáiciga and Schofield 2019 p. 236; Mace 2019, p. 212). The sustainable water yield for the Edwards Aquifer will decrease in a dry climate (EARIP HCP 2020, pp. 3-12, 3-31, 3-43; Loáiciga and Schofield 2019, pp. 223, 235-236) while human demand for groundwater will increase (EARIP HCP 2020, pp. 3-10-3-11), making it more challenging to balance groundwater use for human needs and ecosystem function. In 2010, Texas set a record for lowest rainfall, with similar conditions persisting until 2013 (Nielsen-Gammon 2012 p. 59; National Research Council 2015, p. 168). The effects of low springflows due to extreme drought on the species in this plan are anticipated to be similar to the effects of over pumping described above in section 2.1. Heavy rainfall leading to floods may also become more common from extreme precipitation events and may result in increased habitat disturbance due to movement of materials and scouring.

Average air temperature in Texas has risen 1.5°C (2.7°F) since the early 1900s (National Oceanic and Atmospheric Administration 2022 unpaginated). Future air temperature changes will depend on the amount of future greenhouse gas emissions (U.S. Global Change Research Program 2018, p. 995). Based on current projections of greenhouse gas emissions, air temperature is projected to increase 2.0-2.8°C (3.6-5.1°F) by 2050, and 2.4-4.7°C (4.4-8.4°F) by 2100 for the southern Great Plains (U.S. Global Change Research Program 2018, p. 995). Projections by Sharif (2018, p. 4) predict a greater rise in air temperature by 2100, 2.7-5.6°C (5-10°F). Studies have not explicitly addressed groundwater temperature increases for the Edwards Aquifer. Based on other research into changes in groundwater temperature, it is reasonable to expect that groundwater temperature will increase as air temperature increases, with a possible lag in groundwater temperature increase (Mahler and Bourgeais 2013, p. 295). Groundwater temperature also increases with urbanization and vegetation removal (Benz et al. 2017, entire). This could further increase groundwater temperatures as more development occurs. Groundwater temperature typically increases with depth due to geothermal heat flow, although this also varies locally with other variables such as vertical groundwater flow (Bense and Kurylyk 2017, pp. 1, 8). This suggests that deeper water would not provide a long-term buffer to increasing temperatures.

Some subterranean-adapted species would likely be incapable of adapting to modified temperatures in the medium- to long-term and less capable, due to restricted dispersal capabilities, to flee rising temperature conditions than surface-adapted species (Culver and Pipan 2009, pp. 207–208; Taylor et al. 2013, pp. 324–325; Mammola et al. 2019, p. 646). Subterranean adaptations in ectothermic animals allow tolerance of small fluctuations in temperature, but increased temperatures due to climate change can affect subterranean species diversity by altering mobilization of contaminants (i.e., change in recharge rates through the unsaturated zone) and disruption to biogeochemical processes (e.g., carbon and nitrogen cycle) (Kløve et al. 2014, p. 263; Castaño-Sánchez et al. 2020, p. 7). Water quality at the subsurface and surface is also likely to decrease with increased water temperature, for example, as dissolved oxygen decreases and microbial activity increases (Bates et al. 2008, p. 43). Therefore, the adaptive capacity that ectothermic animals have to environmental changes is presumed to be low.

Surface water temperature will also increase during warm months. Data from the EAA indicates greater temperature fluctuations downstream from the springs due to increased exposure time to ambient temperatures and runoff from rain events (BIO-WEST, Inc. 2019a, p. 20; BIO-WEST, Inc. 2019b, p. 16). Low spring discharge is also a mechanism that increases the water's exposure time to ambient temperature. Thus, both future droughts and increased ambient temperature are likely to increase the surface water temperature. Continuous temperature data for the springs began in 2000, and to date groundwater temperature at Spring Lake and Comal Springs is relatively constant (BIO-WEST, Inc. 2019a, p. 20; BIO-WEST, Inc. 2019b, p. 16). Continuous water temperature monitoring in the Comal and San Marcos rivers should indicate whether water temperatures rise in the future.

There is currently no information on whether increased temperatures can affect different life stages or reproduction of several of these species, or how quickly water temperature will change in their habitats into the future. As discussed previously, water temperature increases beyond 24.4°C (76°F) could impact fountain darter reproduction. For ectothermic animals (e.g., macroinvertebrates), overall vulnerability to climate change will depend on thermal sensitivity and how quickly their buffered environment changes (Pallarés et al. 2021 p. 487; Delić et al. 2022 p. 2). Comal Springs riffle beetles are considered spring specialists which are unable to tolerate larger and more variable environmental (e.g., temperature and dissolved oxygen) parameter ranges in their habitat (Nowlin and Worsham 2015, pp. 49-50; Nair 2019, pp. 21, 23, 26-27; Nair et al. 2023, p. 6). These species have no opportunity to migrate and are unlikely to successfully relocated due to their specific habitat requirements (Kløve et al. 2014, p. 263; Castaño-Sánchez et al. 2020, p. 7; Simčič and Sket 2021, entire; Becher et al. 2022, pp. 4–5).

An assessment by U.S. Geological Survey evaluated the projected future vulnerability through 2050 of the Texas blind salamander, fountain darter, Comal Springs riffle beetle, Peck's cave amphipod, and Comal Springs dryopid beetle, and rated them all as moderately vulnerable to climate change (Stamm et al. 2015, pp. 1, 40, 42, 47). This is defined as "abundance and/or range extent within geographical area assessed likely to decrease by 2050". Notably, this index is better suited for assessing vulnerability at larger geographic scales, such as the size of a wildlife refuge or a state (Young et al. 2016, p. 9). When applied to smaller scales, like a spring ecosystem, the climate change vulnerability index may not capture the fine-grained variability and local conditions that can affect species and ecosystems differently and thus may not be accurate and/or may be biased (see Young et al. 2016 for more on the limitations of the vulnerability index).

The assessment did not include rankings for the San Marcos salamander and Texas wild-rice. There is currently no information indicating whether increased temperatures would affect different life stages or reproduction of these species, or how quickly groundwater temperature will change in the Edwards Aquifer in response to climate change at the surface. Without more information, it is unknown to what extent these temporally delayed changes to the aquifer would affect the listed species and if the species would have sufficient time and appropriate traits to adapt. These are important factors that require more research globally to fully understand vulnerability of these aquifer ecosystems and their subterranean communities (Mammola et al. 2019, pp. 646–647; Hose et al. 2022, entire).

3.0 Species Viability

To evaluate a species' viability, or long-term persistence in the wild over time, we use the conservation principles of redundancy (the ability of a species to withstand catastrophic events; spreading risk among multiple populations to minimize the potential loss of the species from catastrophic events), representation (the ability of a species to adapt to changing environmental conditions over time, via the range of genetic and ecological variation found within the species), and resiliency (the ability of a population to withstand environmental and demographic stochasticity and disturbance).

3.1 Species Redundancy

The southern Edwards Aquifer species occupy a very restricted range. Texas wild-rice, San Marcos salamanders, and Texas blind salamanders consistent of a single extant population and effectively lack redundancy. Fountain darters, Peck's cave amphipods, and Comal Springs riffle beetles have two populations. Comal Springs dryopid beetles have three populations. The single-population species lack this crucial redundancy, making them particularly vulnerable to catastrophic events, including those that might arise from disturbances affecting water quality, quantity, and drought in the aquifer. In such cases, the likelihood of adaptation is low, and these species are at a higher risk of losing their only population and becoming extinct in the wild.

Although the overall water quality in the aquifer is generally good, there is a possibility of undocumented sublethal effects. It is essential to acknowledge the distinct ecological niches between the surface and spring or subterranean-adapted species. These differ significantly, as the latter rely on the consistent regional flow from the aquifer and face heightened vulnerability to disturbances, including drought.

Moreover, it is crucial to note that conservation measures and regulations are in place, and we anticipate that redundancy (in some cases via refugia populations) may be sustained, providing some level of protection for these species. Nevertheless, all seven species are susceptible to significant impacts from disturbances, including those potentially leading to a loss of redundancy, that would be influenced by changes in water quality and quantity in the aquifer.

3.2 Species Representation

All of these species are narrow endemics reliant on the spring systems of the southern Edwards Aquifer, thus reducing their ability to adapt to both near-term and long-term changes in their physical (e.g., climate and habitat structure, etc.) and biological (e.g., competitors, pathogens, and predators, etc.) environments.

3.3 Population Resiliency

To maintain population resiliency, these species rely upon (1) adequate water quantity, (2) adequate water quality, (3) intact undisturbed surface ecosystems, and (4) control of invasive competitors and predators. When each of these physical and biological needs is present and functioning, resilient populations are expected.

4.0 Literature Cited

Alla, Y.M.K. and L. Liu. 2021. Impacts of dams on the environment: a review. International Journal of Environment, Agriculture, and Biotechnology 6(1):64–74.

Alley, W.M., R.W. Healy, J.W. LaBaugh, and T.E. Reilly. 2002. Flow and storage in groundwater systems. Science 296:1,985–1,990.

Arismendez, S. 2020. Upper San Marcos River Watershed annual water quality data summary and analysis report FY20. Meadows Center for Water and the Environment prepared for Texas Commission on Environmental Quality, 37 pp.

Arnold, C.L. and C.J. Gibbons. 1996. Impervious surface coverage: The emergence of a key environmental indicator. Journal of the American Planning Association 62(2):243–258.

Arnow, T. 1959. Ground-water geology of Bexar County, Texas. Texas Board of Water Engineers Bulletin 5911, 52 pp.

Arsuffi, T. L. 1993. Status of the Comal Springs riffle beetle (*Heterelmis comalensis* Bosse, Tuff, and Brown), Peck’s cave amphipod (*Stygobromus pecki* Holsinger), and the Comal Springs dryopid beetle (*Stygoparnus comalensis* Barr and Spangler). Report prepared for US Fish and Wildlife Service, Ecological Services Field Office, Austin, TX, 36 pp.

Atwood, E. L. 1950. Life history studies of nutria, or coypu, in coastal Louisiana. Journal of Wildlife Management 14:249–265.

Ayache, N.C. and T.J. Near. 2009. The utility of morphological data in resolving phylogenetic relationships of darters as exemplified with *Etheostoma* (Teleostei: Percidae). Bulletin of the Peabody Museum of Natural History, 50(2):327-346.

Baker, J.K. 1957. *Eurycea troglodytes*: a new blind salamander from Texas. Texas J. Sci. 9(3): 328–336.

Baker, J.K. 1961. Distribution of and key to the neotenic *Eurycea* of Texas. S.W Nat. 6(1):27-32.

Baker, M.E., M.L. Schley, and J.O. Sexton. 2019. Impacts of expanding impervious surface on specific conductance in urbanizing streams. Water Resources Research 55: 6482–6498.

Banerjee, P., P. Garai, N. C. Saha, S. Saha, P. Sharma, and A. K. Maiti. 2023. A critical review on the effect of nitrate pollution in aquatic invertebrates and fish. Water, Air, & Soil Pollution 234(6):333.

Barkfield, R. F. 2022. Infrastructure consequences of exurb growth in Texas. Texas A&M University, The Bush School of Government and Public Service, Mosbacher Institute White Paper Spring 2022, 11 pp.

Barr, C.B. 1993. Survey for two Edwards Aquifer invertebrates: Comal Springs dryopid beetle *Stygoparnus comalensis* Barr and Spangler (Coleoptera: Dryopidae) and Peck's cave amphipod *Stygobromus pecki* Holsinger (Amphipoda: Crangonyctidae), 70 pp.

Barr, C. 2021. Revision of *Macrelmis* Motshulsky, 1860 in the Southwestern United States and Northern Mexico with description of four new species (Coleoptera: Elmidae: Elminae). The Coleopterist Bulletin 75(1): 93–120.

Barr, C., and P.J. Spangler. 1992. A new genus and species of stygobiontic dryopid beetle, *Stygoparnus comalensis* (Coleoptera: Dryopidae), from Comal Springs, Texas. Proceedings of the Biological Society of Washington 105(1):40–54.

Başağaoğlu, H. 2021, September 15. Using artificial intelligence-based methods to assess possible climate change impacts on the Edwards Aquifer system. Microsoft PowerPoint and Microsoft Teams call, Edwards Aquifer HCP Science Committee Meeting.

Bates, B.C., Z.W. Kundzewicz, S. Wu, and J.P. Palutikof, Editors. 2008. Climate change and water. Technical paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva, Switzerland. 210 pp.

Baxter, R.M. 1977. Environmental effects of dam and impoundments. Annual Review of Ecology and Systematics 8:255–283.

Becher, J., C. Englisch, C. Griebler, and P. Bayer. 2022. Groundwater fauna downtown – Drivers, impacts and implications for subsurface ecosystems in urban areas. Journal of Contaminant Hydrology 248:104021.

Benavides, J.A., J. Karges, K.B. Mayes, H.S. Rifai, and C.V. Castro. 2023. Gulf coast rivers of the Southwestern United States. In: A.C. Benke and C.E. Cushing [eds.], Rivers of North America, 2nd Edition. Elsevier. 1,088 pp.

Bendik, N.F., J.M. Meik, A.G. Gluesenkamp, C.E. Roelke, and P.T. Chippindale. 2013. Evolution of central Texas cave and spring salamanders. BMC Evolutionary Biology 13:201, 18 pp.

Bendik, N.F., D.A. Chamberlain, T.J. Devitt, S.E. Donelson, B. Nissen, J.D. Owen, D. Robinson, B.N. Sissel, and K. Sparks. 2021. Subterranean movement inferred by temporary emigration in Barton Springs salamanders (*Eurycea sosorum*). PeerJ 9:e11246.

Bense, V. and B.L. Kurylyk. 2017. Tracking the subsurface signal of decadal climate warming to quantify vertical groundwater flow rates. Geophysical Research Letters 44: 1-10.

Benz, S.A., P. Bayer, and P. Blum. 2017. Identifying anthropogenic anomalies in air, surface and groundwater temperatures in Germany. Science of the Total Environment 584–585:145–153.

Bergin, S.J. 1996. Diet of the fountain darter *Etheostoma fonticola* in the Comal River, Texas. Southwest Texas State University, San Marcos, TX. 45 pp.

Berkhouse, C.S. and J.N. Fries. 1995. Critical thermal maxima of juvenile and adult San Marcos salamanders (*Eurycea nana*). Southwestern Naturalist 40(4):430–434.

Berube, A., A. Singer, J.H. Wilson, and W.H. Frey. 2006. Finding exurbia: America's fast-growing communities at the metropolitan fringe. The Brookings Institution, On the Record, Washington, D.C. 47 pp.

BIO-WEST, Inc. 2002a. Comal Springs riffle beetle habitat and population evaluation. Project 802, Task 13. BIO-WEST, Inc. Prepared for the Edwards Aquifer Authority, Variable Flow Study, 11 pp.

BIO-WEST, Inc. 2002b. Comal Springs riffle beetle laboratory study: evaluation under variable flow conditions. Prepared for the Edwards Aquifer Authority, 27 pp.

BIO-WEST, Inc. 2003. Variable Flow Study: Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the Comal Springs/River aquatic ecosystem. Final 2002 Annual Report. Edwards Aquifer Authority, 45 pp.

BIO-WEST, Inc. 2004a. Aquatic vegetation laboratory study. Phase 1: Observations of water quality changes and plant growth under various flows. Phase 2: Effects of carbon dioxide level on aquatic plants found in the Comal and San Marcos Springs/River Ecosystems. Project 802, Task 27, 23 pp.

BIO-WEST, Inc. 2004b. Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the Comal Springs/River aquatic ecosystem. Final 2003 Annual Report. Edwards Aquifer Authority, 40 pp.

BIO-WEST, Inc. 2005. Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the Comal Springs/River aquatic ecosystem. Final 2004 Annual Report. Edwards Aquifer Authority, 70 pp.

BIO-WEST, Inc. 2006. Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the Comal Springs/River aquatic ecosystem. Final 2005 Annual Report. Edwards Aquifer Authority, 42 pp.

BIO-WEST, Inc. 2007a. Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the Comal Springs/River aquatic ecosystem. Final 2006 Annual Report. Edwards Aquifer Authority, 42 pp.

BIO-WEST, Inc. 2007b. Variable Flow Study: Seven years of monitoring and applied research. Prepared for Edwards Aquifer Authority. 70 pp.

BIO-WEST, Inc. 2008. Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the Comal Springs/River aquatic ecosystem. Final 2007 Annual Report. Edwards Aquifer Authority, 41 pp.

BIO-WEST, Inc. 2016a. Habitat Conservation Plan Biological Monitoring Program. Comal Springs/River Aquatic Ecosystem 2016 Annual Report. Prepared for Edwards Aquifer Authority, 64 pp.

BIO-WEST, Inc. 2016b. Habitat Conservation Plan Biological Monitoring Program. San Marcos Springs/River Aquatic Ecosystem 2016 Annual Report. Prepared for Edwards Aquifer Authority, 53 pp.

BIO-WEST, Inc. 2018a. Habitat Conservation Plan Biological Monitoring Program. Comal Springs/River Aquatic Ecosystem 2018 Annual Report. Prepared for Edwards Aquifer Authority, 59 pp.

BIO-WEST, Inc. 2018b. Habitat Conservation Plan Biological Monitoring Program. San Marcos Springs/River Aquatic Ecosystem 2018 Annual Report. Prepared for Edwards Aquifer Authority, 55 pp.

BIO-WEST, Inc. 2019a. Habitat Conservation Plan Biological Monitoring Program. Comal Springs/River Aquatic Ecosystem 2019 Annual Report. Prepared for Edwards Aquifer Authority, 53 pp.

BIO-WEST, Inc. 2019b. Habitat Conservation Plan Biological Monitoring Program. San Marcos Springs/River Aquatic Ecosystem 2019 Annual Report. Prepared for Edwards Aquifer Authority, 50 pp.

BIO-WEST, Inc. 2021a. Annual Report of 2021 sampling efforts related to Edwards Aquifer Habitat Conservation Plan Activities under USFWS permit number TE037155-0, 13 pp.

BIO-WEST, Inc. 2021b. Biological Monitoring Program Comal Springs/River Aquatic Ecosystem Annual Report. Prepared for Edwards Aquifer Authority, 52 pp.

BIO-WEST, Inc. 2021c. Biological Monitoring Program San Marcos Springs/River Aquatic Ecosystem Annual Report. Prepared for Edwards Aquifer Authority, 48 pp.

BIO-WEST, Inc. 2022. Biological Monitoring Program San Marcos Springs/River Aquatic Ecosystem Annual Report. Prepared for Edwards Aquifer Authority, 52 pp.

BIO-WEST, Inc. 2023a. Biological Monitoring Program Comal Springs/River Aquatic Ecosystem Annual Report. Prepared for Edwards Aquifer Authority, 74 pp.

BIO-WEST, Inc. 2023b. Biological Monitoring Program San Marcos Springs/River Aquatic Ecosystem Annual Report. Prepared for Edwards Aquifer Authority, 57 pp.

Birdwell, J.E., and A.S. Engel. 2009. Variability in terrestrial and microbial contributions to dissolved organic matter fluorescence in the Edwards Aquifer, Central Texas. Journal of Cave and Karst Studies 71: 144–156.

Bishop, S.C. 1941. Notes on the salamanders with descriptions of several new forms. Occasional Papers from the Museum of Zoology, University of Michigan 451:1–21.

Bishop. S.C. 1943. Handbook of salamanders. Comstock PubI. Co., Ithaca, New York. 555 pp.

Bonner, T.M., T.M. Brandt, J.N. Fries, and B.G. Whiteside. 1998. Effects of temperature on egg production and early life stages of the fountain darter. Transactions of the American Fisheries Society 127: 971–978.

Bosse, L.S., D.W. Tuff, and H.P. Brown. 1988. A new species of *Heterelmis* from Texas (Coleoptera: Elmidae). Southwestern Naturalist 33:199–203.

Bousman, C.B., and D. Nickels. 2003. Archaeological testing of the Burleson Homestead at 41HY37 Hays County, Texas. Index of Texas Archaeology: Open Access Gray Literature from the Lone Star State 2003(1):Article 17.

Bowles, D.E., and B.D. Bowles. 2001. A review of the exotic species inhabiting the upper San Marcos River, Texas, U.S.A. Texas Parks and Wildlife Department, Austin. 30 pp.

Bowles, D.E., C.B. Barr, and R. Stanford. 2003. Habitat and phenology of the endangered riffle beetle *Heterelmis comalensis* and a coexisting species, *Microcylloepus pusillus*, (Coleoptera: Elmidae) at Comal Springs, Texas, USA. Archiv für Hydrobiologie 156:361–384.

Brabec, E.A. Imperviousness and land-use policy: Toward an effective approach to watershed planning. Journal of Hydrologic Engineering 14(4):425–433.

Bradsby, D.D. 1994. A recreational use survey of the San Marcos River. M.S. thesis, Southwest Texas State University, San Marcos, Texas. 82 pp.

Brandt, T.M., K.G. Graves, C. Berkhouse, T. Simon, and B.G. Whiteside. 1993. Laboratory spawning and rearing of the endangered fountain darter. The Progressive Fish-Culturist 55: 149–156.

Breslin, S.L. 1997. The impact of recreation on Texas wild rice. M.S. Thesis, Southwest Texas State University, San Marcos, Texas. 69 pp.

Brown, H.P. 1972. Trials and tribulations of a riffle beetle buff (or why didn't I stick with the protozoa?). Bios 43(2):51–60.

Brown, H.P. 1987. Biology of riffle beetles. Annual Review of Entomology 32:253–73.

Brune, G. 1981. Springs of Texas. Volume 1 Part 2. Branch-Smith, Inc. (Gunnar Brune Private Publishing), Fort Worth, Texas. 115 pp.

Burr, B.M. 1978. Systematics of the percid fishes of the subgenus *Microperca*, genus *Etheostoma*. Bull. Alabama Mus. Nat. Hist. 4:1–53.

Burri, N.M., R. Weatherl, C. Moeck, and M. Schirmer. 2019. A review of threats to groundwater quality in the Anthropocene. Science of the Total Environment 684: 136–154.

Bush, P.W., A.F. Ardis, L. Fahlquist, and P.B. Ging. 2000, Water quality in south-central Texas 1996-98: U.S. Geological Survey Circular Report 1212, 32 pp.

Buszka, P.M. 1987. Relation of water chemistry of the Edwards Aquifer to hydrogeology and land use, San Antonio region, Texas. U.S. Geological Survey Water-Resources Investigations Report 87-4116, 100 pp.

Cantu, V. 2003. Spatial and temporal variation of *Centrocestus formosanus* in river water and endangered fountain darters (*Etheostoma fonticola*) in the Comal River, Texas. M.S. Thesis, Texas State University-San Marcos, 69 pp.

Castaño-Sánchez, A., G.C. Hose, and A.S.P.S. Reboleira. 2020. Ecotoxicological effects of anthropogenic stressors in subterranean organisms: A review. Chemosphere 244:125,422.

Center for Watershed Protection. 2003. Impacts of impervious cover on aquatic systems. Center for Watershed Protection, Ellicot City, MD. 135 pp.

Chappell, L.E., E. Leal, J.D. Tivin, and T.H. Bonner. 2024. New distributional records for *Etheostoma fonticola* (fountain darter) in the San Marcos River, Texas. Southeastern Naturalist 23(2): N6-N12.

Chippindale, P.T. 1995. Doctor of Philosophy Dissertation. Evolution, phylogeny, biogeography, and taxonomy of central Texas spring and cave salamanders, *Eurycea* and *Typhlomolge* (Plethonodtida: Hemidactyliini). The University of Texas at Austin, Austin, Texas. 233 pp.

Chippindale, P.T. 2009. Population genetic analysis of the Texas blind salamander, *Eurycea rathbuni*. Final report to the Texas Parks and Wildlife Department, Grant no. TX-E-78_R. 26 pp.

Chippindale, P.T. 2012. Status of newly discovered cave and spring salamanders (*Eurycea*) in southern Travis and northern Hays counties. Final Section 6 Report TX E-122-R to TPWD. 31 pp.

Chippindale, P.T. and A. Gluesenkamp. 2011. *Typhlomolge* tail tips tell tall tales: Population studies of *Eurycea rathbuni*. Presentation to the Texas Parks and Wildlife Department.

Chippindale, P.T., and A.H. Price. 2005. Conservation of Texas spring and cave salamanders (*Eurycea*). Pages 193–197 *In*: Lannoo, M., editor. Amphibian Declines: The Conservation Status of United States Species. Berkeley: University of California Press, 1,115 pp.

Chippindale, P.T., A.H. Price, J.J. Wiens, and D.M. Hillis. 2000. Phylogenetic relationships and systematic revision of Central Texas *Hemidactyliine* Plethodontid salamanders. Herpetological Monographs 14:1–80.

City of New Braunfels. 2017. Landa Park & Arboretum Master Plan, 185 pp.

City of San Antonio. 2023. Capital improvements program FY2023 FY2028, 241 pp.

Clark, A.K. 2000. Vulnerability of ground water to contamination, Edwards Aquifer recharge zone, Bexar County, Texas, 1998. Water-Resources Investigation Report 00-4149. U.S. Geological Survey, Austin, TX. 9 pp.

Clark, M.K., K.G. Ostrand, and T.H. Bonner. 2017. Implications of piscine predator control on the federally listed fountain darter. Fish Management and Ecology 24:292–297.

Cleaveland, M.K., T.H. Votteler, D.K. Stahle, R.C. Casteel, and J.L. Banner. 2011. Extended chronology of drought in South Central, Southeastern and West Texas. Texas Water Journal 2(1):54–96.

Clough, A. 2022. Comal Springs dryopid beetle refugia program. Edwards Aquifer Habitat Conservation Plan. Retrieved on June 2, 2022 from https://storymaps.arcgis.com/stories/c5c96b7d26814b7dad9e55cee55196e7/.

Coleman, W. 2021. Genetic Diversity of the Comal Springs Riffle Beetle (*Heterelmis comalensis*). 3rd Annual Texas Groundwater Invertebrate Forum presentation.

Coles, J.F., G. McMahon, A.H. Bell, L.R. Brown, F.A. Fitzpatrick, B.C. Scudder Eikenberry, M.D. Woodside, T.F. Cuffney, W.L. Bryant, K. Cappiella, L. Fraley-McNeal, and W.P. Stack. 2012. Effects of urban development on stream ecosystems in nine metropolitan study areas across the United States. U.S. Geological Survey, Circular 1373, 138 pp.

Collette, B.B. 1965. Systematic significance of breeding tubercles in fishes of the family Percidae. Proceedings of the U.S. National Museum. 117:567–614.

Collette, B.B., and L.W. Knapp. 1966. Catalog of type specimens of the darters (Pisces, Percidae, Etheostomatini). Proceedings of the U.S. National Museum. 119:1–88.

Cooke, M. 2012. Natural history studies on the Comal Springs riffle beetle (*Heterelmis comalensis*). Texas State University, San Marcos, Texas. 65 pp.

Cooke, M., G. Longley, and J.R. Gibson. 2015. Spring association and microhabitat preferences of the Comal Springs riffle beetle (*Heterelmis comalensis*). The Southwestern Naturalist 60(1):110–121.

Cook-Hildreth, S.L. 2008. Exotic armored catfishes in Texas: reproductive biology, and effects of foraging on egg survival of native fishes (*Etheostoma fonticola*, endangered and *Dionda diaboli*, threatened). M.S. Thesis, Texas State University-San Marcos, 50 pp.

Cope, E.D. (1872). On the Wyandotte Cave and its fauna. *American Naturalist.* 6:406–422.

Correll, D.S. and H.B. Correll. 1975. Aquatic and wetland plants of the southwestern United States. Stanford Press, California. 1,777 pp.

Crawford-Reynolds, M.L. 2018. An analysis of the effects of photosynthetically active radiation and recreation induced turbidity in the San Marcos River on the vegetative growth of Texas wild rice (*Zizania texana* Hitchc.). Ph.D. Dissertation, Texas State University-San Marcos, Texas. 85 pp.

Crow, J.C., M.R.J. Forstner, K.G. Ostrand, J.R. Tomasso. 2016. The role of survival and growth of the Barton Springs salamander (*Eurycea sosorum*). Herpetological Conservation and Biology 11(2):328–334.

Crowe, J.C. 1994. Detailed hydrogeologic maps of the Comal and San Marcos Rivers for Endangered species habitat definition, Texas, M.A Thesis, University of Texas at Austin, 154 pp.

Culver, D.C., and T. Pipan. 2009. The biology of caves and other subterranean habitats. Oxford University Press, New York. 276 pp.

Culver, D.C., L.L. Master, M.C. Christman, and H. H. Hobbs. 2000. Obligate cave fauna of the 48 contiguous United States. Conservation Biology 14(2):386–401.

Danielopol, D.L., C. Griebler, A. Gunatilaka, and J. Notenboom. 2003. Present state and future prospects for groundwater ecosystems. Environmental Conservation 30(2): 104–130.

Danielopol, D.L., J. Gibert, C. Griebler, A. Gunatilaka, H.J. Hahn, G. Messana, J. Notenboom, and B. Sket. 2004. Incorporating ecological perspectives in European groundwater management policy. Envir. Conserv. 31: 185–189.

Dammeyer, N.T., C.T. Phillips, and T.H. Bonner. 2013. Site fidelity and movement of *Etheostoma fonticola* with implications to endangered species management. Transactions of the American Fisheries Society 142: 1,049–1,057

Davis, D.R., K.J. Epp, and C.R. Gabor. 2012. Predator generalization decreases the effect of introduced predators in the San Marcos salamander, *Eurycea nana*. Ethology 118(12):1191–1197.

DeCook, K.J. 1963. Geology and ground-water resources of Hays County, Texas. U.S. Geological Survey Water-Supply Paper 1612, 72 pp.

Delić, T., P. Trontelj, V. Zakšek, A. Brancelj, T. Simčič, F. Stoch, and C. Fišer. 2022. Speciation of a subterranean amphipod on the glacier margins in South Eastern Alps, Europe. Journal of Biogeography 49(1):38–50.

Department of the Interior. 2024. Secretary Haaland designates four new National Recreation Trails. Press release. https://www.doi.gov/pressreleases/secretary-haaland-designates-four-new-national-recreation-trails.

Design Workshop, Inc. 2012. New Braunfels stormwater management strategy. Phase I Report, 48 pp.

Devitt, T.J., A.M. Wright, D.C. Cannatella, and D.M. Hillis. 2019. Species delimitation in endangered groundwater salamanders: Implications for aquifer management and biodiversity conservation. Proceedings of the National Academy of Sciences of the U.S.A, 116(7): 2624–2633.

Di Lorenzo, T., W.D. Di Marzio, B. Fiasca, D.M.P. Galassi, K. Korbel, S. Iepure, J.L. Pereira, A.S.P.S. Reboleira, S.I. Schmidt, and G.C. Hose. 2019. Recommendations for ecotoxicity testing with stygobiotic species in the framework of groundwater environmental risk assessment. Science of The Total Environment 681: 292–304.

Diaz, P.H. 2010. Diet and mesohabitat associations of the threatened San Marcos salamander (*Eurycea nana*). M.S. Thesis, Texas State University-San Marcos, 52 pp.

Diaz, P.H., J.N. Fries, T.H. Bonner, M.L. Alexander, and W.H. Nowlin. 2015. Mesohabitat associations of the threatened San Marcos salamander (*Eurycea nana*) across its geographic range. Aquatic Conservation: Marine and Freshwater Ecosystems 25:307–321.

Ding, J., and B. A. McCarl. 2019. Economic and ecological impacts of increased drought frequency in the Edwards Aquifer. Climate 8(1):2.

Dowden, D.L. 1968. Population dynamics of the San Marcos salamander, *Eurycea nana*. M.A. Thesis, Southwest Texas State University, 44 pp.

Dubrovsky, N.M., K.R. Burow, G.M. Clark, J.M. Gronberg, P.A. Hamilton, K.J. Hitt, D.K. Mueller, M.D. Munn, B.T. Nolan, L.J. Puckett, M.G. Rupert, T.M. Short, N.E. Spahr, L.A. Sprague, and W.G. Wilber. 2010. The quality of our nation’s waters: Nutrients in the nation’s streams and groundwater, 1992-2004. U.S. Geological Survey, Circular 1350, 174 pp.

Dudley, N., and S. Stolten. 2003. Running pure: The importance of forest protected areas to drinking water. A report to the World Bank and WWF Alliance for Forest Conservation and Sustainable Use, 112 pp.

EAA (Edwards Aquifer Authority). 2015. Hydrologic data report for 2014. Report No. 15-01, 69 pp.

EAA (Edwards Aquifer Authority). 2016. Report of the 2016 expanded Water Quality Monitoring Program Work Group and report of the 2016 Biological Monitoring Program Work Group, 11 pp.

EAA (Edwards Aquifer Authority). 2018. 2017 water quality summary, 6 pp.

EAA (Edwards Aquifer Authority). 2019. Edwards Aquifer Habitat Conservation Plan expanded water quality report 2019, 45 pp.

EAA (Edwards Aquifer Authority). 2021a. EARIP HCP annual expanded water quality monitoring report. Appendix F of 2021 Edwards Aquifer Authority Reports, 64 pp.

EAA (Edwards Aquifer Authority). 2021b. It all comes down to this: EARIP HCP water sampling program evolving to ensure continued high water quality. EAHCP Steward 2021 (September), 4 pp.

EAA (Edwards Aquifer Authority). 2021c. Plugging away at the EAA. Edwards Aquifer Authority News Drop (Summer 2021):56. Retrieved on April 14, 2022, from https://user-qzm76pf.cld.bz/NewsDrop-Summer-2021/.

EAA (Edwards Aquifer Authority). 2022a. 2022 EARIP HCP annual expanded water quality report, 56 pp.

EAA (Edwards Aquifer Authority). 2022b. Aquifer Protection. Retrieved on May 3, 2022 from https://www.edwardsaquifer.org/aquifer-protection/.

EAA (Edwards Aquifer Authority). 2023a. 2023 Annual Report Appendix J 2023 Item M Net Disturbance and Incidental Take Assessment for EARIP ITP. 34 pp.

EAA (Edwards Aquifer Authority). 2023c. Edwards Aquifer Maps and GIS. Retrieved on January 17, 2023 from https://www.edwardsaquifer.org/science-maps/maps/.

EAA (Edwards Aquifer Authority). 2024a. Open records request for Comal spring run 3 water quality data. Request received May 14, 2024 by E-Mail.

EAA (Edwards Aquifer Authority). 2024b. Open records request for Comal River old channel water quality data. Request received May 14, 2024 by E-Mail.

EAA (Edwards Aquifer Authority). 2024c. Open records request for San Maros River at Aquarena Springs Drive water quality data. Request received May 17, 2024 by E-Mail.

Eamus, D., B. Fu, A.E. Springer, and L.E. Stevens. 2016. Groundwater dependent ecosystems: classification, identification techniques and threats. Pages 313–346 *In*: Jakeman, A.J., O. Barreteau, R.J. Hunt, J. Rinaudo, and A. Ross, editors. Integrated groundwater management: concepts, approaches, and challenges. Springer Open, 762 pp.

EARIP HCP (Edwards Aquifer Recovery Implementation Program Habitat Conservation Program). 2020. Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan. Prepared by RECON, Environmental, Inc.; Hicks & Company; Zara Environmental, LLC; and BIO-WEST, Inc., 423 pp.

EARIP HCP (Edwards Aquifer Recovery Implementation Program Habitat Conservation Program). 2021. 2021 Annual Report. Prepared by ICF. 70 pp.

Edwards, C.R. and T.H. Bonner. 2022. Vegetation associations of the endangered fountain darter *Etheostoma fonticola*. Endangered Species Research 47:1–13.

Elliott, J.M. 2008. Ontogenetic shifts in drift periodicity and benthic dispersal in elmid beetles. Freshwater Biology 53(4):698–713.

Emery, W.H.P. 1967. The decline and threatened extinction of Texas wild rice (*Zizania texana* Hitchc.). Southwestern Naturalist 12:203-204.

Emery, W.H.P. 1977. Current status of Texas wild-rice. Southwestern Naturalist 22:393-394.

EPA (U.S. Environmental Protection Agency). 2009. National primary drinking water quality regulations. Report No. EPA 816-F-09-004, 7 pp.

EPA (U.S. Environmental Protection Agency). 2017. Updates to the demographic and spatial allocation models to produce Integrated Climate and Land Use Scenarios (ICLUS). Version 2. National Center for Environmental Assessment, Washington, DC; EPA/600/R-16/366F, 131 pp.

EPA (U.S. Environmental Protection Agency). 2019. Integrated Climate and Land-Use Scenarios (ICLUS) Version 2.1 projections. Retrieved on September 16, 2022, from https://www.epa.gov/gcx/iclus-downloads/.

EPA (U.S. Environmental Protection Agency). 2022. National recommended water quality criteria - Aquatic life criteria table. Retrieved on August 16, 2023, from https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table/.

EPA (U.S. Environmental Protection Agency). 2023. Comal Springs #1 (DX-68-23-301/ LR-68-23-301) monitoring report. Available at: https://mywaterway.epa.gov/monitoring-report/NWIS/USGS-TX/USGS-294300098080001/ (August 9, 2023).

Epp, K.J. 2013. Threat sensitivity in the San Marcos salamander: effects of predator diet and prey experience. Behaviour 150:617–634.

Epp, K.J., and C.R. Gabor. 2008. Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. Ethology 114:607–615.

Ernst, C.H., J.E. Lovich, and R. Barbour. 1994. Turtles of the United States and Canada. Smithsonian Institution Press, Washington, DC, 578 pp.

Ethridge, J.Z., J.R. Gibson, and C.C. Nice. 2013. Cryptic diversity within and amongst spring-associated *Stygobromus* amphipods (Amphipoda: Crangonyctidae): *Stygobromus* Amphipod Cryptic Diversity. Zoological Journal of the Linnean Society 167(2):227–242.

Evermann, B.W. and W.C. Kendall. 1894. The fishes of Texas and the Rio Grande Basin, considered chiefly with reference to their geographic distribution. Bull. U.S. Fish Comm. 1892, pp. 57-126, plates X to L.

Fahlquist, L., A.F. Ardis. 2004. Quality of water in the Trinity and Edwards Aquifers, south-central Texas, 1996-98: U.S. Geological Survey Scientific Investigations Report 2004-5201, 25 pp.

Ferrill, D.A., D.W. Sims, D.J. Waiting, A.P. Morris, N.M. Franklin, and A.L. Schultz. 2004. Structural framework of the Edwards Aquifer recharge zone in south-central Texas. Geological Society of America Bulletin 116: 407–418.

Foster, S.S.D. and P.J. Chilton. 2003. Groundwater: the processes and global significance of aquifer degradation. Philosophical Transactions of the Royal Society B 358: 1957–1972.

Fries J.N. 2002. Upwelling flow velocity preferences of captive adult San Marcos salamanders. North American Journal of Aquaculture 64: 113–116.

Fries, L.T., and D.E. Bowles. 2002. Water quality and macroinvertebrate community structure associated with a sportfish Hatchery outfall. North American Journal of Aquaculture 64(4):257–266.

Fries, J.N., J.R. Gibson, and T.L. Arsuffi. 2004. Edwards Aquifer spring invertebrate survey and captive maintenance of two species. San Marcos National Fish Hatchery and Technology Center and Texas State University. 23 pp.

Furl, C., and F.P. Bertetti. 2017. E-mail "Update on Rattlesnake Cave/well". December 21.

Gabor, C.R., R. Gonzales Jr., & K.J. Epp. 2010. The role of water-borne chemical cues in mediating social interactions of the Texas blind salamander, *Eurycea rathbuni*. Amphibia-Reptilia, 31(2), 294–298.

Gary, M.O. 2023. E-mail: "Time and Cost Estimate for Revised Recovery Plan Request". October 25.

George, W.O., S.D. Breeding, and W.H. Hastings. 1952. Geology and groundwater resources of Comal County, Texas. U.S. Geological Survey Water Supply Paper 1138, 126 pp.

Geos Institute. 2016. Hot enough yet? The future of extreme weather in Austin, Texas. Ashland, Oregon. 26 pp.

Gibson, J.R. 2022. Phone call on invertebrate threats for species biological report. November 29.

Gibson, J.R., S.J. Harden, and J. Fries. 2008. Survey and distribution of invertebrates from selected springs of the Edwards Aquifer in Comal and Hays Counties, Texas. Southwestern Naturalist 53:74–84.

Gibson, R., B.T. Hutchins, J.K. Krejca, P.H. Diaz, and P.S. Sprouse. 2021. *Stygobromus bakeri*, a new species of groundwater amphipod (Amphipoda, Crangonyctidae) associated with the Trinity and Edwards aquifers of central Texas, USA. Subterranean Biology 38:19–45.

Gilbert, C.H. 1887. Descriptions of new and little known etheostomatoids. Proceedings of the U.S. National Museum 10:47–64.

Gold, R. 2022, January 12. A forgotten oil well births a 100-foot geyser in West Texas. Texas Monthly. Retrieved on April 14, 2022, from https://www.texasmonthly.com/news-politics/west-texas-geyser-oil-well-chevron/.

Gonzales, T.K. 2008. Conservation genetics of the Comal Springs riffle beetle (*Heterelmis comalensis*) populations in Central Texas, with examination of molecular and morphological variation in *Heterelmis* Sp. throughout Texas. Master of Science, Texas State University, San Marcos, Texas. 67 pp.

Gray, C.J., and A.S. Engel. 2013. Microbial diversity and impact on carbonate geochemistry across a changing geochemical gradient in a karst aquifer. ISME J 7: 325–337.

Greater Edwards Aquifer Alliance. 2010. Permanent stormwater pollution prevention systems within the Edwards Aquifer Recharge Zone in Bexar County, Texas. An overview and assessment of current regulatory agency processes, 48 pp.

Griffin, K.L. 2006. An Analysis of changes in Texas wild rice distribution following the 1998 flood of the San Marcos River, Texas. M.A. Geo. Thesis, Texas State University, 68 pp.

Grigorovich, I.A., T.R. Angradi, and C.A. Stepien. 2008. Occurrence of the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*Dreissena polymorpha*) in the upper Mississippi River system. Journal of Freshwater Ecology 23:429–435.

Groeger, A.W., P.F. Brown, T.E. Tietjen, and T.C. Kelsey. 1997. Water quality of the San Marcos River. Texas Journal of Science 49(4):279–294.

Guyton, W.F., and Associates. 1979. Geohydrology of Comal, San Marcos, and Hueco Springs. Texas Department of Water Resources Report 234, 85 pp.

Hanson, J.A., and T.A. Small. 1995. Geologic framework and hydrogeologic characteristics of the Edwards Aquifer outcrop, Hays County, Texas. U.S. Geological Survey Branch of Information Services, Denver, CO. 10 pp.

Hardberger, A. 2019. Chapter 15. Texas groundwater law and the Edwards Aquifer. Pages 189–197 *In*: Sharp, J.M., Jr., R.T. Green, and G.M. Schindel, editors. The Edwards Aquifer: The Past, Present, and Future of a Vital Water Resource. Geological Society of America Memoir 215, 312 pp.

Hardy, T.B., and N. Raphelt. 2015. Effects of changing height of Cape's Dam on recreation, Texas wild rice and fountain darter habitat in the San Marcos River, Texas. San Marcos (Texas): City of San Marcos, 36 pp.

Hay, A., C. L. Riggins, T. Heard, C. Garoutte, Y. Rodriguez, F. Fillipone, K.K. Smith, N. Menchaca, J. Williamson, and J.S. Perkin. 2022. Movement and mortality of invasive suckermouth armored catfish during a spearfishing control experiment. Biological Invasions 24(10):3119–3131.

Hays County. 2017, February 21. Hays County development regulations. Retrieved on April 7, 2022, from https://hayscountytx.com/download/departments/development_services/regulations/2017-Hays-County-Development-Regulations.pdf/.

Heitmuller, F.T., and B.D. Reece. 2006. Spatial data for *Eurycea* salamander habitats associated with three aquifers in south-central Texas. Data Series 243. U.S. Geological Survey, Reston, Virginia. 3 pp.

Hillis, D.M., D.A. Chamberlain, T.P. Wilcox, and P.T. Chippindale. 2001. A new species of subterranean blind salamander (Plethodontidae: Hemidactyliini: *Eurycea*: *Typhlomolge*) from Austin, Texas, and a systematic revision of central Texas paedomorphic salamanders. *Herpetologica*, 57(3), 266–280.

Hinsby, K., M.T. Condesso De Melo, and M. Dahl. 2008. European case studies supporting the derivation of natural background levels and groundwater threshold values for the protection of dependent ecosystems and human health. Science of The Total Environment 401:1–20.

Hitchcock, A.S. 1933. New species and new names of grasses from Texas. Jour. Wash. Acad. Sci. 23:449–456.

Hitchcock, A.S. 1950. Manual of the grasses of the United States. Government Printing Office, Washington, DC. 1,051 pp.

HMT Engineering and Surveying. 2011. Blieders/German Creek Area preliminary engineering report. HMT Engineering and Surveying prepared for the City of New Braunfels, 8 pp.

Holsinger, J.R. 1967. Systematics, speciation, and distribution of the subterranean amphipod genus *Stygonectes* (Gammaridae). Bulletin of the United States National Museum, 259: 1–176.

Holsinger, J. R., and G. Longley. 1980. The subterranean amphipod crustacean fauna of an artesian well in Texas. Smithsonian Contributions to Zoology (308):1–62.

Hoover, J.J., K.J. Kilgore, and A.F. Cofrances. 2004. Suckermouth catfishes: Threats to aquatic ecosystems of the United States? Aquatic Nuisance Species Research Program Bulletin 4(1):1–9.

Horton, T., J. Lowry, C. De Broyer, D. Bellan-Santini, C.O. Coleman, C.O., L. Corbari, M.J. Costello, M. Daneliya, J.C. Dauvin, C. Fišer, R. Gasca, M. Grabowski, J.M. Guerra-García, E. Hendrycks, L. Hughes, D. Jaume, K. Jazdzewski, Y.H. Kim, R. King, T. Krapp-Schickel, S. LeCroy, A.N. Lörz, T. Mamos, A.R. Senna, C. Serejo, B. Sket, J.F. Souza-Filho, A.H. Tandberg, J.D. Thomas, M. Thurston, W. Vader, R. Väinölä, R. Vonk, K. White, and W. Zeidler. 2022. World Amphipoda Database. Retrieved on March 3, 2022, from https://www.marinespecies.org/amphipoda/.

Hose, G.C. 2005. Assessing the need for groundwater quality guidelines for pesticides using the Species Sensitivity Distribution Approach. Human and Ecological Risk Assessment: An International Journal 11: 951–966.

Hose, G.C., A.A. Chariton, M.A. Daam, T. Di Lorenzo, D.M.P. Galassi, S.A. Halse, A.S.P.S. Reboleira, A.L. Robertson, S.I. Schmidt, and K.L. Korbel. 2022. Invertebrate traits, diversity and the vulnerability of groundwater ecosystems. Functional Ecology 36(9):2200–2214.

Hubbs, C. and K. Strawn. 1957. Relative variability of hybrids between the darters *Etheostoma spectabile* and *Percina caprodes*. Evolution 11:1–10.

Humphreys, W.F. 2011. Management of groundwater species in karst environments. Pages 283–318 *In*: P.E. Van Beynen, editor, Karst management. Springer. Dordrecht, Netherlands.

Huston, D.C., and J.R. Gibson. 2015. Underwater pupation by the Comal Springs riffle beetle, *Heterelmis comalensis* Bosse, Tuff, and Brown, 1988 (Coleoptera: Elmidae), with an update on culture techniques. The Coleopterists Bulletin 69(3):521–524.

Huston, D.C., M.D. Worsham, D.G. Huffman, and K.G. Ostrand. 2014. Infection of fishes, including threatened and endangered species by the trematode parasite *Haplorchis pumilio* (Looss, 1896) (Trematoda: Heterophyidae). BioInvasions Record 3(3): 189-194.

Huston, D.C., J.R. Gibson, K.G. Ostrand, C.W. Norris, and P.H. Diaz. 2015. Monitoring and marking techniques for the endangered Comal Springs riffle beetle, *Heterelmis comalensis* Bosse, Tuff, and Brown, 1988 (Coleoptera: Elmidae). The Coleopterists Bulletin 69(4):793–798.

Hutchins, B.T. 2018. The conservation status of Texas groundwater invertebrates. Biodiversity and Conservation 27(2):475–501.

Hutchins, B.T., A.S. Engel, W.H. Nowlin, and B.F. Schwartz. 2016. Chemolithoautotrophy supports macroinvertebrate food webs and affects diversity and stability in groundwater communities. Ecology 97: 1530–1542.

Intergovernmental Panel on Climate Change. 2023. Summary for policymakers. Climate change 2023: Synthesis report, pp. 1–34. IPCC, Geneava, Switzerland.

Iwanowicz, L.R., D.D. Iwanowicz, C.R. Adams, T.D. Lewis, T.M. Brandt, L.R. Sanders, and R.S. Cornman. 2018. Isolation, characterization, and molecular identification of a novel aquareovirus that infects the endangered fountain darter *Etheostoma fonticola*. Diseases of Aquatic Organisms 130: 95–108.

John Gleason, LLC. 2017. Water Quality Protection Plan for the City of San Marcos and Texas State University. Prepared for the City of San Marcos. 239 pp.

Johnson, S.B., and G.M. Schindel. 2008. Evaluation of the option to designate a separate San Marcos pool for critical period management. 08-01. Edwards Aquifer Authority, San Marcos, Texas. 63 pp.

Johnson, S., G. Schindel, and J. Hoyt. 2009. Water quality trends analysis of the San Antonio segment, Balcones Fault Zone Edwards Aquifer, Texas. Edwards Aquifer Authority Report No. 09-03. 53 pp.

Johnson, S., G. Schindel, G. Veni, N. Hauwert, B. Hunt, B. Smith, and M. Gary. 2012. Tracing groundwater flowpaths in the vicinity of San Marcos Springs, Texas. 12–03. Edwards Aquifer Authority in cooperation with Barton Springs Edwards Aquifer Conservation District and City of Austin Watershed Protection. 147 pp.

Jordan, D.S. and B.W. Evermann. 1896. The fishes of North and Middle America: a descriptive catalogue of the species of fishlike vertebrates found in the waters of North America, north of the Isthmus of Panama. Part I. Bull. U.S. Nat. Mus. 47:1–1240.

Jordan, D.S. and B.W Evermann. 1900. The fishes of North and Middle America: a descriptive catalogue of the species of fishlike vertebrates found in the waters of North America, North of the Isthmus of Panama. Part IV. Bull. U.S. Nat. Mus. 47:3137–3313.

Jordan, D.S. and C.H. Gilbert. 1886. List of fishes collected in Arkansas, Indian Territory, and Texas, in September 1884, with notes and descriptions. Proc. U.S. Nat. Mus. 9:1–25.

Katz, B.G. 2019. Nitrate contamination in karst groundwater. Pages 756–760 *In*: Encyclopedia of Caves. 3rd edition. Academic Press. London, United Kingdom. 1,250 pp.

Kaushal, S.S., P.M. Groffman, G.E. Likens, K.T. Belt, W.P. Stack, V.R. Kelly, L.E. Band, and G.T. Fisher. 2005. Increased salinization of fresh water in the northeastern United States. Proc. Natl. Acad. Sci. U.S.A. 102: 13517–13520.

Kennedy, J. 2015. A modern gate for Ezells Cave. Programs and abstracts– 21st National Cave and Karst Management Symposium, pp. 86–89.

Kløve, B., P. Ala-Aho, G. Bertrand, J. J. Gurdak, H. Kupfersberger, J. Kværner, T. Muotka, H. Mykrä, E. Preda, P. Rossi, C. B. Uvo, E. Velasco, and M. Pulido-Velazquez. 2014. Climate change impacts on groundwater and dependent ecosystems. Journal of Hydrology 518:250–266.

Kosnicki, E. 2019. Determining sexual dimorphism of living aquatic beetles, *Stygoparnus comalensis* (Coleoptera: Dryopidae) and *Heterelmis comalensis* (Coleoptera: Elmidae), Using internal abdominal structures. Journal of Insect Science 19(4):9.

Kosnicki, E. 2021. Comal Springs riffle beetle cotton luring analysis. BIO-WEST, Inc., Texas Parks & Wildlife Department, and San Marcos Aquatic Resources Center prepared for the Edwards Aquifer Authority, 16 pp.

Kosnicki, E. 2022a. E-mail “Monitoring of the Comal Springs dryopid beetle”. June 6.

Kosnicki, E. 2022b. Fecundity of first-generation captively reared *Heterelmis comalensis* (Coleoptera: Elmidae) M. Chaudhury, editor. Journal of Insect Science 22: 5.

Kosnicki, E., and E.P. Julius. 2019a. Life-history aspects of *Stygobromus pecki*. BIO-WEST, Inc. and San Marcos Aquatic Resources Center Prepared for the Edwards Aquifer Authority. 24 pp.

Kosnicki, E., and E.P. Julius. 2019b. Life-history aspects of the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and notes on life-history aspects of the Comal Springs riffle beetle (*Heterelmis comalensis*). BIO-WEST, Inc. Prepared for the Edwards Aquifer Authority. 61 pp.

Krejca, J. K. 2007. Mark-recapture study of *Eurycea rathbuni* at three sites in San Marcos, Texas. Final Report for The Endangered Species Program. Texas Parks and Wildlife, 22 p.

Krejca, J.K. and P.S. Sprouse. 2003. Aquatic invertebrate fauna survey at Hueco Springs Creek, Comal County, Texas. Report prepared for Paul Price & Associates, 18 pp.

Lapworth, D. J., N. Baran, M. E. Stuart, and R. S. Ward. 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. Environmental Pollution 163:287–303.

LBG-Guyton Associates, BIO-WEST, Inc., Espey Consultants, Inc., and URS Corporation. 2004. Evaluation of augmentation methodologies in support of in-situ refugia at Comal and San Marcos Springs, Texas. 1,182 pp.

Leblanc, P. 2016. Were hundreds of endangered salamanders stolen from a San Marcos lab? Austin-American Statesman, published Dec 10, 2016. https://www.statesman.com/story/news/2016/12/11/were-hundreds-of-endangered-salamanders-stolen-from-a-san-marcos-lab/10126861007/

LeConte, J. L. 1852. Synopsis of the Parnidae of the United States. Proceedings of the Academy of Natural Sciences of Philadelphia 6:41–45.

Lee, M., A. Gluesenkamp, and B. Muscher-Hodges. 2021. *Eurycea rathbuni* (Texas blind salamander) cannibalism. Herpetoculture 52(3):567–568.

Lin, Y. and X. Gong. 2016. Risk assessment of water pollution exposure to hazardous waste sites: a case study in Bexar County, Texas. Papers in Applied Geography 2(4):383–394.

Linam, L.A. 1993. A reassessment of the distribution, habitat preference, and population size estimate of the fountain darter (*Etheostoma fonticola*) in the San Marcos River, Texas. In Conservation of the upper San Marcos and Comal Ecosystems, Section 6 report, Texas Parks and Wildlife Department, 13 pp.

Linam, G.W, K.B. Mayes, and K.S. Saunders. 1993. A habitat utilization and population site estimate of fountain darters (*Etheostoma fonticola*) in the Comal River, Texas. Texas Journal of Science, 45(5):341–348.

Lindgren, R.J., A.R. Dutton, S.D. Hovorka, S.R.H. Worthington, and S. Painter. 2004. Conceptualization and simulation of the Edwards aquifer, San Antonio region, Texas: U.S. Geological Survey Scientific Investigations Report 2004–5277, 143 pp.

Loáiciga, H. A., and M. Schofield. 2019. Climate variability, climate change, and Edwards Aquifer water fluxes. Pages 223–238 *In*: The Edwards Aquifer: The past, present, and future of a vital water resource. Abbott, P.L. and C.M. Woodruff, Jr., editors. Geological Society of America, 312 pp.

Loáiciga, H.A., D.R. Maidment, and J.B. Valdes. 2000. Climate-change impacts in a regional karst aquifer, Texas, USA. Journal of Hydrology 227:173–194.

Lockwood, Andrews & Newman, Inc. 2014. City of New Braunfels Stormwater Management Plan, Texas, 82 pp.

Lockwood, Andrews & Newman, Inc. and Halff Associates. 2018. San Marcos Stormwater Master Plan, Texas, 18 pp.

Lodge, D.M., C.A. Taylor, D.M. Holdich, and J. Skurdal. 2000. Nonindigenous crayfishes threaten North American freshwater diversity: lessons from Europe. Fisheries 25:7–19.

Loiácomo, D.J. 2019. Stormwater and non-point source pollutants in Sessom Creek, San Marcos, TX. Master of Science. Texas State University, 76 pp.

Long, J. L. 1981. Introduced birds of the world: the worldwide history, distribution, and influence of birds introduced to new environments. Universe Books, New York. 560 pp.

Longley, G. 1978. Status of the *Typhlomolge* (= *Eurycea*) *rathbuni*, Texas blind salamander. U.S. Fish and Wildlife Service Endangered Species Report, 52 pp.

Longley, G. 1981. The Edwards Aquifer: Earth's most diverse groundwater ecosystem? International Journal of Speleology 11(1/2):123–128.

Longley, G. 1991. Status and trends of the Edwards (Balcones Fault Zone) Aquifer in the San Antonio region. In: Proceedings of South Texas Irrigation Conference. Guy Fipps, editor, 146 pp

Longley, G., B. Schwartz, and V. Castillo III. 2015. Endangered Species Permit TE802211-0 Annual Report, 4 pp.

Lowry, J. K. and A.A. Myers. 2013. A Phylogeny and Classification of the Senticaudata subord. nov. (Crustacea: Amphipoda). *Zootaxa.* 3610(1):1–80.

Lucas, L., J.N. Fries, C.R. Gabor, and C.C. Nice. 2009. Genetic variation and structure in *Eurycea nana*, a federally threatened salamander endemic to the San Marcos springs. Journal of Herpetology 43(2):220–227.

Lucas, L.K., Z. Gompert, J.R. Gibson, K.L. Bell, C.A. Buerkle, and C.C. Nice. 2016. Pervasive gene flow across critical habitat for four narrowly endemic, sympatric taxa. Freshwater Biology 61(6):933–946.

Lydeard, C., R.H. Cowie, W.F. Ponder, A.E. Bogan, P. Bouchet, S.A. Clark, K.S. Cummings, T.J. Frest, O. Gargominy, D.G. Herbert, R. Hershler, K.E. Perez, B. Roth, M. Seddon, E.E. Strong, and F.G. Thompson. 2004. The global decline of nonmarine mollusks. BioScience 54(4):321.

Mace, R.E. 2019. The use of water from the Edwards Aquifers, Texas. Pages 207–212 *In*: The Edwards Aquifer: The past, present, and future of a vital water resource. Geological Society of America.

Mace, R.E., and S.C. Wade. 2008. In hot water? How climate change may (or may not) affect the groundwater resources of Texas. Gulf Coast Association of Geological Societies Transactions, 58: 655–668.

Maclay, R.W. 1995. Geology and hydrology of the Edwards Aquifer in the San Antonio area, Texas. Geological Survey Water-Resources Investigations Report 95-4186, 54 pp.

Mahler, B. J., and R. Bourgeais. 2013. Dissolved oxygen fluctuations in karst spring flow and implications for endemic species: Barton Springs, Edwards aquifer, Texas, USA. Journal of Hydrology 505:291–298.

Mahler, B.J., P.C.V. Metre, J.L. Crane, A.W. Watts, M. Scoggins, and E.S. Williams. 2012. Coal-Tar-Based pavement sealcoat and PAHs: Implications for the environment, human health, and stormwater management. Environmental Science & Technology 46(6):3039–3045.

Mammola, S., P. Cardoso, D. C. Culver, L. Deharveng, R. L. Ferreira, C. Fišer, D. M. P. Galassi, C. Griebler, S. Halse, W. F. Humphreys, M. Isaia, F. Malard, A. Martinez, O. T. Moldovan, M. L. Niemiller, M. Pavlek, A. S. P. S. Reboleira, M. Souza-Silva, E. C. Teeling, J. J. Wynne, and M. Zagmajster. 2019. Scientists' warning on the conservation of subterranean ecosystems. BioScience 69(8):641–650.

Manenti, R., B. Piazza, Y. Zhao, E. Padoa Schioppa, and E. Lunghi. 2021. Conservation studies on groundwaters' pollution: Challenges and perspectives for stygofauna communities. Sustainability 13:7030.

Mauldin, R. P. 2003. Exploring drought in the San Antonio Area between 1700 and 1979. The University of Texas at San Antonio Center for Archaeological Research.

Mays, Z., A. Hunter, L.G. Campbell, and C. Carlos-Shanley. 2021. The effects of captivity on the microbiome of the endangered Comal Springs riffle beetle (*Heterelmis comalensis*). FEMS Microbiology Letters 368(17):fnab121.

McCartney, M.P., C. Sullivan, and M.C. Acreman. 2001. Ecosystem impacts of large dams. Background Paper Nor. 2. Prepared for IUCN/UNEP/WCD, 82 pp.

McCready Wright, K.L. 2022. Spatial variation in macroinvertebrates in groundwater-dominated rivers: is community composition explained by discrete or continuous riverine models? M.S. Thesis, Texas State University, 52 pp.

McDermid K., P. Sprouse, and J. Krejca. 2015. Geographic distribution: *Eurycea sosorum* (Barton Springs salamander). Herpetological Review 46:556–5557.

McDonald, D.L., T.H. Bonner, T.M. Brandt, and G.H. Trevino. 2006. Size susceptibility to trematode-induced mortality in the endangered fountain darter (*Etheostoma fonticola*). Journal of Freshwater Ecology 21(2):293–299.

McDonald, D.L., T.H. Bonner, E.L. Oborny, Jr., and T.M. Brandt. 2007. Effects of fluctuating temperatures and gill parasites on reproduction of the fountain darter, *Etheostoma fonticola*. Journal of Freshwater Ecology 22(2):311–318.

McKinney, M.L. 1997. Extinction vulnerability and selectivity: combining ecological and paleontological views. Annual Review of Ecology and Systematics 28:495–516.

McKinney, D.C., and J.M. Sharp. 1995. Springflow augmentation of Comal Springs and San Marcos Springs, Texas: phase I-feasibility study. Center for Research in Water Resources Technical Report CRWR 247, Bureau of Engineering Research, University of Texas at Austin, Texas. 92 pp.

McLaughlin, P.A., D.K. Camp, M.V. Angel, E.L. Bousfield, P. Brunel, R.C. Brusca, A.C. Cohen, K. Conlan, L.G. Eldredge, D.L. Felder, J.W. Goy, T. Haney, B. Hann, R.W. Heard, E.A. Hendrycks, H.H. Hobbs III, J.R. Holsinger, B. Kensley, D.R. Laubitz, S.E. Le. 2005. Common and scientific names of aquatic invertebrates from the United States and Canada: Crustaceans. American Fisheries Society Special Publication 31, 545 pp.

MWCE (The Meadows Center for Water and the Environment). 2012. Spring Lake watershed characterization and management recommendations final report. Texas State University prepared for the Texas Commission on Environmental Quality and the U.S. Environmental Protection Agency Region VI, Nonpoint Source Protection Program CWA §319(h), 144 pp.

MWCE (The Meadows Center for Water and the Environment). 2018. Upper San Marcos River watershed protection plan. San Marcos Watershed Initiative Stakeholder Committee and The Meadows Center for Water and the Environment, San Marcos, Texas. 89 pp.

MWCE (The Meadows Center for Water and the Environment). 2023. Comal River watershed data report. The Meadows Center for Water prepared for the U.S. Environmental Protection Agency and Texas Commission on Environmental Quality, 26 pp.

Miller, R.R., J.D. Williams, and J.E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries 14:6, 22–38.

Minckley, W.L., and M.E. Douglas. 1991. Discovery and extinction of western fishes: a blink of the eye in geologic time. Pages 7–17 *In*: Battle against extinction, W.L. Minckley and J.E. Deacon, editors. University of Arizona, Tucson, Arizona. 538 pp.

Mitchell, R.W., and J.R. Reddell. 1965. *Eurycea tridentifera*, a new species of troglobitic salamander from Texas and a reclassification of *Typhlomolge rathbuni*. Texas Journal of Science 17(1):12–27.

Mitchell, A.J., M.J. Salmon, D.G. Huffman, A.E. Goodwin, and T.M. Brandt. 2000. Prevalence and pathogenicity of a heterphyid trematode infecting the gills of an endangered fish, the fountain darter, in two central Texas spring-fed rivers. Journal of Aquatic Animal Health 12:283–289.

Moravec, F., and D.G. Huffman. 2000. Three new helminth species from two endemic plethodontid salamanders, *Typhlomolge rathbuni* and *Eurycea nana*, in central Texas. Folia Parasitologica 47(3):186–194.

Morgan, G.D. 1936. Doctor of Philosophy Dissertation. A study of the vestigial air bladder in the darter (*Catonis* [sic] *flabellaris* Rafinesque). Ohio State University, Columbus, Ohio, 54 pp.

Musgrove, M., and C.L. Crow. 2012. Origin and Characteristics of Discharge at San Marcos Springs Based on Hydrologic and Geochemical Data (2008–10), Bexar, Comal, and Hays Counties, Texas. 2012–5126. U.S. Geological Survey in cooperation with San Antonio Water System, Report, Bexar County, Comal County, Hays County, 94 pp.

Musgrove, M., B.G. Katz, L.S. Fahlquist, C.A. Crandall, and R.J. Lindgren. 2014. Factors affecting public-supply well vulnerability in two karst aquifers. Groundwater–Focus 52: 63–75.

Musgrove, M., S. P. Opsahl, B. J. Mahler, C. Herrington, T. L. Sample, and J. R. Banta. 2016. Source, variability, and transformation of nitrate in a regional karst aquifer: Edwards aquifer, central Texas. Science of The Total Environment 568:457–469.

Nace, R.L., and E.J. Pluhowski. 1965. Drought of the 1950's with special reference to the Midcontinent. Water-Supply Paper 1804. U.S. Geological Survey, 88 pp.

Nair, P. 2019. Ecophysiology and food web dynamics of spring ecotone communities in the Edwards Aquifer, USA. PhD Dissertation. Texas State University, 114 pp.

Nair, P., A.H. Hunter, M.L.D. Worsham, M. Stehle, J.R. Gibson, and W.H. Nowlin. 2019. Sexual Dimorphism in Three Species of *Heterelmis* Sharp (Coleoptera: Elmidae). The Coleopterists Bulletin 73: 1075–1083.

Nair, P., M. Huertas, and W.H. Nowlin. 2020. Metabolic responses to long-term food deprivation in subterranean and surface amphipods. Subterranean Biology 33:1–15.

Nair, P., P.H. Diaz, and W.H. Nowlin. 2021. Interactions at surface–subterranean ecotones: structure and function of food webs within spring orifices. Oecologia 195:14.

Nair, P., J.R. Gibson, B.F. Schwartz, and W.H. Nowlin. 2023. Temperature responses vary between riffle beetles from contrasting aquatic environments. Journal of Thermal Biology 112:103485.

Najvar, P.A., J.N. Fries, and J.T. Baccus. 2007. Fecundity of San Marcos salamanders in captivity. The Southwestern Naturalist 52(1):145–147.

National Oceanic and Atmospheric Administration. 2022. NOAA National Centers for Environmental Information. State climate summaries 2022: Texas. 5 pp.

National Research Council. 2015. Review of the Edwards Aquifer Habitat Conservation Plan: Report 1. National Academies Press, Washington, D.C. 174 pp.

Near, T.J., C.M. Bossu, G.S. Bradburd, R.L. Carlson, R.C. Carrington, P.R. Hollingsworth, Jr., B.P. Keck, and D.A. Etnier. 2011. Phylogeny and Temporal Diversification of Darters (Percidae: Etheostomatinae). Systematic Biology 60(5):565–595.

Nelson, J. 1993. Population size, distribution, and life history of *Eurycea nana* in the San Marcos River. M.S. Thesis, Southwest Texas State University, 43 pp.

Nice, C., and L. Lucas. 2015. Genetic demography of endemic and endangered taxa in springs of the Edwards Plateau. Grant No. TX ET-148-R. Final Report for the Texas Parks and Wildlife Department, 49 pp.

Nielsen-Gammon, J. 2012. The 2011 Texas drought. Texas Water Journal 3(1):59–95.

Nielsen-Gammon, J., J.L. Banner, B.I. Cook, D.M. Tremaine, C.I. Wong, R.E. Mace, H. Gao, Z. Yang, M.F. Gonzalez, R. Hoffpauir, T. Gooch, and K. Kloesel. 2020. Unprecedented drought challenges for Texas water resources in a changing climate: What do researchers and stakeholders need to know? Earth’s Future 8:20.

Nielsen-Gammon, J., S. Holman, A. Buley, S. Jorgensen, J. Escobedo, C. Ott, and J. Dedrick. 2021. Assessment of historic and future trends of extreme weather in Texas, 1900-2036. OSC-202101. Texas A&M University, Document, Office of the State Climatologist, Texas A&M University, College Station, Texas.

Norris, W.E., Jr., P.A. Grandy. and W.K. Davis. 1963. Comparative studies on the oxygen consumption of three species of neotenic salamanders as influenced by temperature, body size, and oxygen tension. Biol. Bull. 125(3): 523–533.

Notenboom, J., S. Plénet, and M.-J. Turquin. 1994. Groundwater contamination and its impact on groundwater animals and ecosystems. Pages 477–500 *In*: J. Gibert, D.L. Danielopol, and J.A. Stanford, editors. Groundwater ecology. Academic Press. San Diego, California. 571 pp.

Novaković, B., M. Ilić, S. Anđus, J. Čanak Atlagić, N. Marinković, and J. Đuknić. 2014. Recent distribution and ecological notes on the dryopid beetle *Pomatinus substriatus* Müller, 1806 (Dryopidae: Coleoptera) in Serbia. Water Research and Management 4(2):37–41.

Nowlin, W.H. 2022. E-mail “Comal Springs spring run 4 and Comal Springs riffle beetle”. July 6.

Nowlin, W.H., and B. Schwartz. 2012. Spring Lake watershed characterization and management recommendations final report. Nonpoint Source Protection Program CWA §319(h). Texas State University, San Marcos, Texas. 130 pp.

Nowlin, W.H., and M.L.D. Worsham. 2015. Comal Springs riffle beetle habitat connectivity study. San Marcos, Texas. Texas State University and BIO-WEST, Inc. prepared for the Edwards Aquifer Authority, 76 pp.

Nowlin, W.H., B.F. Schwartz, M.L.D. Worsham, and J. R. Gibson. 2016. Refugia research: development of husbandry and captive propagation techniques for invertebrates covered under the Edwards Aquifer habitat conservation plan. Texas State University and San Marcos Aquatic Resources Center prepared for the Edwards Aquifer Authority, 36 pp.

Nowlin, W.H., D. Hahn, P. Nair, and F. Alfano. 2017. Evaluation of the trophic status and functional feeding group status of the Comal Springs riffle beetle. 148-15-HCP. Texas State University, 30 pp.

Nowlin, W.H., C. Nice, W. Coleman, and B.F. Schwartz. 2022. Trophic ecology and population genetics of the endangered Comal Springs dryopid beetle (*Stygoparnus comalensis*). Texas State University and Edwards Aquifer Research and Data Center prepared for Texas Parks and Wildlife Department, 27 pp.

Nunan, L., B. Poulos, S. Navarro, R. Redman, and D. Lightner. 2010. Milky hemolymph syndrome (MHS) in spiny lobsters, penaeid shrimp and crabs. Diseases of Aquatic Organisms 91(2):105–112.

O’Grady, J.J., D.H. Reed, B.W. Brook, and R. Frankham. 2004. What are the best correlates of predicted extinction risk? Biological Conservation 118:513–520.

Ockerman, D.J., and R.N. Slattery. 2008. Streamflow conditions in the Guadalupe River Basin, south-central Texas, water years 1987-2006- An assessment of streamflow gains and losses and relative contribution of major springs to streamflow. U.S. Geological Survey Scientific Investigations Report 2008-5165, 22 pp.

Ogden, A.E., A.J. Spinelli, and J. Horton. 1985. Hydrologic and hydrochemical data for the Edwards Aquifer in Hays and Comal Counties, October 1983 to June 1985. Edwards Aquifer Research and Data Center #R2-85. Southwest Texas State University, San Marcos, Texas. 83 pp.

Ogden, A.E., R.A. Quick, and S.R. Rothermel. 1986. Hydrochemistry of the Comal, Hueco, and San Marcos Springs, Edwards Aquifer, Texas. Pages 115–130 *In* The Balcones Escarpment: Geology, hydrology, ecology and social development in central Texas. Proceedings of the Geological Society of America. Geological Society of America. San Antonio, Texas. 204 pp.

Olsen, J.B., A.P. Kinziger, J.K. Wenburg, C.J. Lewis, C.T. Phillips, and K.G. Ostrand. 2016. Genetic diversity and divergence in the fountain darter (*Etheostoma fonticola*): implications for conservation of an endangered species. Conservation Genetics 17: 1,393–1,404.

Opsahl, S.P., M. Musgrove, B.J. Mahler, and R.B. Lambert. 2018. Water-quality observations of the San Antonio segment of the Edwards Aquifer, Texas, with an emphasis on processes influencing nutrient and pesticide geochemistry and factors affecting aquifer vulnerability. Scientific Investigations Report 2010-16. U.S. Geological Survey in cooperation with San Antonio Water System, 67 pp.

Opsahl, S.P., Musgrove, M., and Mecum, K.E. 2020. Temporal and spatial variability of water quality in the San Antonio segment of the Edwards aquifer recharge zone, Texas, with an emphasis on periods of groundwater recharge, September 2017–July 2019: U.S. Geological Survey Scientific Investigations Report 2020–5033, 37 pp.

Otero, C.L. 2007. Geologic, hydrologic, and geochemical identification of flow paths in the Edwards Aquifer, northeastern Bexar and southern Comal counties, Texas: U.S. Geological Survey Scientific Investigations Report 2007-5285, 48 pp.

Owens, C.S., J.D. Madsen, R.M. Smart, and R.M. Stewart. 2001. Dispersal of native and nonnative aquatic plant species in the San Marcos River, Texas. Journal of Aquatic Plant Management 39:75–79.

Page, L.M. and B.M. Burr. 1979. The smallest species of darter (Pisces; Percidae). American Midland Naturalist 101(2):452–453.

Pallarés, S., R. Colado, M. Botella-Cruz, A. Montes, P. Balart-García, D.T. Bilton, A. Millán, I. Ribera, and D. Sánchez-Fernández. 2021. Loss of heat acclimation capacity could leave subterranean specialists highly sensitive to climate change. Animal Conservation 24(3):482–490.

Passarello, M. C., J. M. Sharp, and S. A. Pierce. 2012. Estimating urban-induced artificial recharge: A case study for Austin, TX. Environmental & Engineering Geoscience 18(1):25–36.

Passos, M.I.S., Nessimian, J.L. and Dorvillé, L.F.M. 2003. Life strategies in an elmid (Insecta: Coleoptera: Elmidae) community from a first order stream in the Atlantic Forest, southeastern Brazil. Acta Limnologica Brasiliensia 15:29–36.

Payne, S., N. Pence, and C. Furhl. 2019. Chapter 16 The Edwards Aquifer Habitat Conservation Plan: its planning and implementation. Pages 109–206 *In*: Sharp, J.M., Jr., R.T. Green, and G.M. Schindel, editors. The Edwards Aquifer: The Past, Present, and Future of a Vital Water Resource. Geological Society of America Memoir 215, 312 pp.

Perkin, J.S., E. Kosnicki, and J. Jackson. 2018. Analysis of the Comal Springs and San Marcos Springs long-term monitoring dataset. Edwards Aquifer Authority, San Antonio, TX. 122 pp.

Petitt, B.M., Jr. and W.O. George. 1956. Ground-water resources of the San Antonio area, Texas: a progress report on current studies. Texas Board of Water Engineers Bulletin 5608. Volume 1, 80 pp.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Washington, DC: Smithsonian Institution Press. 592 pp.

Phillips, C. T., M. L. Alexander, and R. Howard. 2010. Consumption of eggs of the endangered fountain darter (*Etheostoma fonticola*) by native and nonnative snails. The Southwestern Naturalist 55:115–117.

Phillips, C.T., M.L. Alexander, and A.M. Gonzales. 2011. Use of macrophytes for egg deposition by the endangered fountain darter. Transactions of the American Fisheries Society 140(5):1,392–1,397.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience 50:53–65.

Poole, J.M. and D.E. Bowles. 1999. Habitat characterization of Texas wild-rice (*Zizania texana* Hitchcock), an endangered aquatic macrophyte from the San Marcos River, TX, USA. Aquatic Conservation: Marine Freshwater Ecosystems 9:291–301

Poole, J. J.T. Hutchinson, C.R. Hathcock, and D. Han. 2022. A thirty-year assessment of the endangered aquatic macrophyte, *Zizania texana*, endemic to the upper reach of the San Marcos River in Central Texas, USA. Aquatic Botany 177:103,482.

Power, P. 1997. Moisture, seeds, and reproductive failure in Texas wild-rice (*Zizania texana*). Southwestern Naturalist 42:435–440.

Power, P. 2002. Resource allocation patterns and phenotypic variation in the endangered Texas wild-rice (*Zizania texana,* Poaceae). SIDA, Contributions to Botany 20:571–582.

Puente, C. 1976. Statistical analysis of water-level springflow, and stream data for the Edwards aquifer in south-central Texas: Open-File Report 76-393. U.S. Geological Survey, San Antonio, TX. 59 pp.

Quick, R.A., and A.E. Ogden. 1985. Hydrochemistry as a means of delineating groundwater flow patterns in the Edwards Aquifer, San Marcos, Texas. Karst Water Resources (Proceedings of the Ankara-Antalya Symposium). IAHS Publication no. 161:12.

Rawlings, T.A., K.A. Hayes, R.H. Cowie, and T.M. Collins. 2007. The identity, distribution, and impacts of nonnative apple snails in the continental United States. BMC Evolutionary Biology 7:97.

Reeves, R.D., and J.F. Blakey. 1970. Geology and water quality at selected locations in the San Antonio area, Texas, Progress Report, 1969. Open-File Report 70-272. Prepared by the U.S. Geological Survey in cooperation with the Edwards Underground Water District and the Texas Water Development Board Metropolitan Water District, 17 pp.

Reilly, F.J., Jr. and K.A. Carter. 2018. Program study and analysis services for the Edwards Aquifer Protection Program. Report for the City of San Antonio, Texas' Parks and Recreation Department, 45 pp.

Reynolds Jr., S.K. and A.C. Benke, 2012. Chironomid production along a hyporheic gradient in contrasting stream types. Freshwater Science 31:167–181.

Romero, F.S. 2018. San Antonio's Edwards Aquifer Protection Program: Review and analysis. Texas Water Journal 9(1):1-15.

Rose, F.L. and P. Power. 2001. Project No. 38. Continued Maintenance, reintroduction, and research on *Zizania texana* (Texas wild-rice). Final report. Report submitted to Texas Parks and Wildlife Department, 160 pp.

Rose, F.L., R.W. Manning, T.R. Simpson, and S. Jenkins. 1998. A sustaining population of the Florida red-bellied turtle, *Pseudemys nelsoni* (Reptilia: Emydidae), in Spring Lake, Hays County, Texas. Texas Journal of Science 50(1):89–92.

Rothermel, S. R., and A. E. Ogden. 1987. Hydrochemical investigation of the Comal and Hueco Springs systems, Comal County, Texas. Report Number R1-87. Southwest Texas State University- Edwards Aquifer Research and Data Center, 182 pp.

Rupp, D. E., P. W. Mote, N. Massey, C. J. Rye, R. Jones, and M. R. Allen. 2012. Did human influence on climate made the 2011 Texas drought more probable? Explaining extreme events of 2011 from a climate perspective. Bulletin of the American Meteorological Society 93(7):1041–1067.

Russell, B. 1976. Distribution of troglobitic salamanders in the San Marcos area, Hays County, Texas. Texas Association for Biological Investigations of Troglobitic *Eurycea* (BITE) Report 7601, 35 pp.

San Antonio Water System. 2022. Aquifer protection and evaluation. Retrieved on January 12, 2023, from https://www.saws.org/protecting-our-environment/water-resource-compliance-protection/aquifer_protection/.

Saunders, K.S., K.B. Mayes, T.A. Jurgensen, J.F. Trungale, L.J. Kleinsasser, K. Aziz, J.R. Fields, and R.E. Moss. 2001. An evaluation of spring flows to support the upper San Marcos River spring ecosystem, Hays County, Texas. Texas Parks and Wildlife Department – River Studies Report No. 16, 33 pp.

Schenck, J.R. 1975. Ecology of the fountain darter, *Etheostoma fonticola* (Osteichthyes: Percidae). M.S. Thesis, Southwest Texas State University, 100 pp.

Schenck, J.R., and B.G. Whiteside. 1976. Distribution, habitat preference and population size estimate of *Etheostoma fonticola*. Copeia 1976(4): 697–703.

Schenck, J.R. and B.G. Whiteside. 1977. Reproduction, fecundity, sexual dimorphism and sex ratio of *Etheostoma fonticola* (Osteichthyes: Percidae). American Midland Naturalist 98(2):365–375.

Schindel, G.M. 2019. Genesis of the Edwards (Balcones Fault Zone) Aquifer. Pages 9–18 *In*: Sharp, J.M., Jr., R.T. Green, and G.M. Schindel, editors. The Edwards Aquifer: The past, present, and future of a vital water resource: Geological Society of America Memoir 215, 312 pp.

Schoof, R. 1980. Environmental impact of channel modification. Water Resources Bulletin 16(4):697–701.

Schwartz, B., W.H. Nowlin, T. Hardy, J. Jeong, and J. Wolfe, III. 2020. Sessom Creek sediment export study. Texas State University; Edwards Aquifer Research and Data Center; Texas A&M Agrilife Research, EAHCP proposal no. 160-17-TESS, San Marcos, TX. 7 pp.

Schwetman, N.H. 1967. A morphological study of the external features, viscera, integument, and skeletons of *Eurycea nana.* M.A. Thesis, Baylor University, 26 pp.

Shah, N.W., B.R. Baillie, K. Bishop, S. Ferraz, L. Högbom, and J. Nettles. 2022. The effects of forest management on water quality. Forest Ecology and Management 522:120,397.

Sharif, H. 2018. Climate projections for the City of San Antonio. University of Texas at San Antonio, San Antonio, TX. 17 pp.

Sharp, J.M. 2010. The impacts of urbanization on groundwater systems and recharge, 6 pp.

Siglo Group. 2022. State of the Hill Country: 8 key conservation and growth metrics for a region at a crossroads, 60 pp.

Shepard, W.D. 2002. Dryopidae Billberg 1820. Pages 121–122 *In*: R.H. Arnett, Jr., M.C. Thomas, P.E. Skelley, and J.H. Frank, editors. American Beetles, Volume II: Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, 880 pp.

Siglo Group. 2022. State of the Hill Country: 8 key conservation and growth metrics for a region at a crossroads. Report for the Texas Hill Country Conservation Network. 71 pp.

Silveus, W.A. 1933. Texas grasses. The Clegg Co., San Antonio, TX. 782 pp.

Simčič, T., and B. Sket. 2021. Ecophysiological responses of two closely related epigean and hypogean *Niphargus* species to hypoxia and increased temperature: Do they differ? International Journal of Speleology 50(2):111–120.

Simon, K.S., E.F. Benfield, and S.A. Macko. 2003. Food web structure and the role of epilithic biofilms in cave streams. Ecology 84(9):2395–2406.

Slattery, R.N., and N. Choi. 2021. Estimated annual recharge to the Edwards aquifer in the San Antonio area, by stream basin or ungaged area, 1934–2020. 4 pp.

Smith, T.A., P.J. Ciccotto, T.C. Mendelson, and L.M. Page. 2014. Dense taxon sampling using AFLPs leads to greater accuracy in phylogeny estimation and classification of darters (Percidae: Etheostomatinae). Copeia 2014(2):257–268.

Spangler, P.J., and C.B. Barr. 1995. A new genus and species of stygobiontic dytiscid beetle, *Comaldessus stygius* (Coleoptera: Dytiscidae: Bidessini) from Comal Springs, Texas. Insecta Mundi 9:301–308.

Springer, A.E. and L.E. Stevens. 2009. Spheres of discharge of springs. Hydrogeology Journal 17(1):83–93.

Stamm, J.F., M.F. Poteet, A.J. Symstad, M. Musgrove, A.J. Long, B.J. Mahler, and P.A. Norton. 2014. Historical and projected climate (1901–2050) and hydrologic response of karst aquifers, and species vulnerability in south-central Texas and western South Dakota. 2014–5089. U.S. Geological Survey in cooperation with the Department of the Interior South-Central Climate Science Center, Scientific Investigations Report, 59 pp.

Stejneger, L. 1896. Description of a new genus and species of blind, tailed batrachian from the subterranean waters of Texas. Proceedings of the National Museum. 18:619–621.

Stone, D. and G.M. Schindel. 2002. The application of GIS in support of land acquisition for the protection of sensitive groundwater recharge properties in the Edwards Aquifer of south-central Texas. Journal of Cave and Karst Studies, 64(1):38–44.

Sui, Q., X. Cao, S. Lu, W. Zhao, Z. Qiu, and G. Yu. 2015. Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: A review. Emerging Contaminants, 1(1):14–24.

Swanbrow Becker, L.J. and C.R. Gabor. 2012. Effects of turbidity and visual vs. chemical cues on anti-predator response in the endangered fountain darter (*Etheostoma fonticola*). Ethology 118:994–1,000.

Swanbrow Becker, L.J., E.M. Brooks, C.R. Gabor, and K.G. Ostrand. 2016. Effects of turbidity on foraging behavior in the endangered fountain darter (*Etheostoma fonticola*). American Midland Naturalist 175:55–63.

Taylor, J.N., W.R. Courtenay, Jr., and J.A. McCann. 1984. Known impacts of exotic fishes in the continental United States. Pages 322–373 *In*: W.R. Courtenay, Jr. and J.R. Stauffer, Jr., editors. Distribution, biology, and management of exotic fishes. Johns Hopkins University Press, Baltimore, Maryland. 448 pp.

Taylor, R. G., B. Scanlon, P. Döll, M. Rodell, R. van Beek, Y. Wada, L. Longuevergne, M. Leblanc, J. S. Famiglietti, M. Edmunds, L. Konikow, T. R. Green, J. Chen, M. Taniguchi, M. F. P. Bierkens, A. MacDonald, Y. Fan, R. M. Maxwell, Y. Yechieli, J. J. Gurdak, D. M. Allen, M. Shamsudduha, K. Hiscock, P. J.-F. Yeh, I. Holman, and H. Treidel. 2013. Ground water and climate change. Nature Climate Change 3(4):322–329.

TCEQ (Texas Commission on Environmental Quality). 2012. 2012 Texas Integrated Report - Texas 303(d) List (Category 5).

TCEQ (Texas Commission on Environmental Quality). 2018. Upper San Marcos River Watershed Protection Plan. TCEQ Nonpoint Source Program Fact sheet, 2 pp.

TCEQ (Texas Commission on Environmental Quality). 2020. Dry Comal Creek and Comal River Watershed Protection Plan Implementation. TCEQ Nonpoint Source Program Fact sheet, 1 pp.

TCEQ (Texas Commission on Environmental Quality). 2022a. 2022 Texas integrated report - Potential sources of impairments and concerns, 250 pp.

TCEQ (Texas Commission on Environmental Quality). 2022b. 2022 Texas integrated report - Texas 303(d) List (Category 5). 38 pp.

Terrell, E.E., W.H.P. Emery, and H.E. Beatty. 1978. Observations on *Zizania texana* (Texas wild-rice), an endangered species. Bulletin of the Torrey Botanical Club 105:50–57.

Texas Demographic Center 2022. Texas Population Projections Program. Retrieved on July 28, 2023, from: https://demographics.texas.gov/data/tpepp/projections/.

Texas Natural Resource Conservation Commission. 1996. Chapter 213 - Edwards Aquifer Rule Log No. 97105-213-WT. 406 pp.

Texas Water Development Board. 2021. 2022 State Water Plan, 167 pp.

Theis, C.V. 1940. The source of water derived from wells. Civil Engineering 10:277–280.

Tolley-Jordan, L.R. and M.A. Chadwick. 2019. Effects of parasite infection and host body size on habitat associations of invasive aquatic snails: implications for environmental monitoring. Journal of Aquatic Animal Health 31:121–128.

Tolley-Jordan, L.R. and Owen, J.M., 2008. Habitat influences snail community structure and trematode infection levels in a spring-fed river, Texas, USA. Hydrobiologia, 600(1):29–40.

Tupa, D.D. and W.K. Davis. 1976. Population dynamics of the San Marcos salamander, *Eurycea nana* Bishop. *Texas J. Sci.* 32:179–195.

U.S. Army Corps of Engineers. 1964. Survey report on Edwards Underground reservoir: Guadalupe, San Antonio and Nueces rivers and tributaries, Texas. Main Report. Fort Worth, TX. 198 pp.

U.S. Census Bureau. 2020. Resident population estimates for the 100 fastest-growing U.S. Counties with 10,000 or more population in 2010: April 1, 2010 to July 1, 2019 (COEST2019-CUMGR). 2 pp.

U.S. Department of Agriculture. 1978. Final watershed plan and environmental impact statement. Upper San Marcos River Watershed, Comal and Hays Counties, Texas. Soil Conservation Service. 136 pp.

USFWS (U.S. Fish and Wildlife Service). 1994. Endangered Species Permit Report. Austin, Texas. 16 pp.

USFWS (U.S. Fish and Wildlife Service). 1996. San Marcos & Comal Springs & associated aquatic ecosystems (revised) recovery plan. Southwest Region, Albuquerque, New Mexico, 93 pp.

USFWS (U.S. Fish and Wildlife Service). 2001. Biological opinion for the proposed repairs to: (1) retaining walls around Landa Lake, and the upper reach of the new channel of the Comal River and (2) Landa Lake Dam. Ecological Services Field Office, Austin, TX. 24 pp

USFWS (U.S. Fish and Wildlife Service). 2006. Biological opinion for the proposed dam stabilization and recreational facility construction at the Rio Vista Dam (Consultation # 21450-2006-F-0063). Ecological Services Field Office, Austin, TX. 30 pp.

USFWS (U.S. Fish and Wildlife Service). 2013. Biological and conference opinions for the Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan-Permit TE-63663A-0 (Consultation No. 21450-2010-F-0110). Ecological Services Field Office, Austin, TX. 169 pp.

USFWS (U.S. Fish and Wildlife Service). 2017. San Marcos Aquatic Resources Center annual station report, Fiscal year 2017. San Marcos Aquatic Resources Center, San Marcos, TX. 49 pp.

USFWS (U.S. Fish and Wildlife Service). 2018a. Biological Opinion for the proposed repairs to Spring Lake Dam. Ecological Services Field Office, Austin, TX. 33 pp.

USFWS (U.S. Fish and Wildlife Service). 2018b. Implementation of the aquifer refugia program under the Edwards Aquifer Habitat Conservation Plan annual report 2018. San Marcos Aquatic Resources Center, San Marcos, TX. 73 pp.

USFWS (U.S. Fish and Wildlife Service). 2019a. Amendment 1 - Recovery plan for *Zizania texana* (Texas wild-rice), fountain darter (*Etheostoma fonticola*) and Texas blind salamander (*Typhlomolge rathbuni*). Southwest Region, Albuquerque, New Mexico. 17 pp.

USFWS (U.S. Fish and Wildlife Service). 2019b. Implementation of the aquifer refugia program under the Edwards Aquifer Habitat Conservation Plan annual report 2019. San Marcos Aquatic Resources Center, San Marcos, Texas. 108 pp.

USFWS (U.S. Fish and Wildlife Service). 2019c. Summary report for 2019 Texas wild-rice survey, 3 pp.

USFWS (U.S. Fish and Wildlife Service). 2020a. 2020 EAHCP refugia work plan. Southwest Region, Albuquerque, New Mexico, 26 pp.

USFWS (U.S. Fish and Wildlife Service). 2020b, Standard operating procedures for the Peck's cave amphipod, *Stygobromus pecki*. San Marcos Aquatic Resources Center, San Marcos, Texas. 36 pp.

USFWS (U.S. Fish and Wildlife Service). 2020c. Summary report for 2020 Texas wild-rice survey, 3 pp.

USFWS (U.S. Fish and Wildlife Service). 2021a. Biological Opinion for the Rio Vista Falls Maintenance and Bank Stabilization Project, SWF-2005-00697, Hays County, Texas. Ecological Services Field Office, Austin, Texas. 28 pp

USFWS (U.S. Fish and Wildlife Service). 2021b. Implementation of the aquifer refugia program under the Edwards Aquifer Habitat Conservation Plan annual report 2021. San Marcos Aquatic Resources Center and Uvalde National Fish Hatchery, Texas, 73 pp.

USFWS (U.S. Fish and Wildlife Service). 2021c. Summary report for 2021 Texas wild-rice survey, 3 pp.

USFWS (U.S. Fish and Wildlife Service). 2022. Biological opinion for the dog beach maintenance project, SWF-2021-F-1884, Hays County, Texas. Ecological Services Field Office, Austin, Texas. 24 pp.

USFWS (U.S. Fish and Wildlife Service). 2023. Species status assessment report for the Texas troglobitic water slater (*Lirceolus smithii*). Version 1.1, January 2023. 119 pp. + Appendices.

USFWS (U.S. Fish and Wildlife Service). 2024a. Draft recovery plan for Comal and San Marcos Springs and associated aquatic ecosystems, second revision. Southwest Region, Albuquerque, New Mexico, 40 pp.

USFWS (U.S. Fish and Wildlife Service). 2024b. Species status assessment report for the Edwards Aquifer (Texas cave) diving beetle (*Haideoporus texanus*). Version 1.1, November 2023. 92 pp. + Appendices.

USFWS (U.S. Fish and Wildlife Service) and BIO-WEST, Inc. 2011. Effectiveness of host snail removal in the Comal River, Texas and its impact on densities of the gill parasite *Centrocestus formosanus* (Trematoda: Heterophyidae), 12 pp.

U.S. Geological Survey. 2022. USGS surface-water daily data for the nation. USGS 08170000 San Marcos Spgs at San Marcos, TX. Retrieved on August 2, 2022, from https://waterdata.usgs.gov/nwis/dv/?site_no=08170000/.

U.S. Geological Survey. 2023. USGS surface-water daily data for the nation. USGS 08168000 Hueco Spgs nr New Braunfels, TX. Retrieved on March 1, 2023, from https://waterdata.usgs.gov/monitoring-location/08168000/.

U.S. Global Change Research Program. 2018. Impacts, risks, and adaptation in the United States. Volume II. U.S. Global Change Research Program, Washington, D.C.

Uhlenhuth, E. 1921. Observations on the Distribution and habits of the blind Texan cave salamander, *Typhlomolge rathbuni*. Biological Bulletin, 40(2):73–104.

Ulrich, G.W. 1986. The larvae and pupae of *Helichus suturalis* Leconte and *Helichus productus* Leconte (Coleoptera: Dryopidae). Colepts Bull 40: 325–334.

Vaughan, Jr., J.E. 1986. Population and autecological assessment of *Zizania texana* Hitchc. (Poaceae) in the San Marcos River. M. S. Thesis, Southwest Texas State University- San Marcos, Texas. 52 pp.

Veni, G. 2006. E-mail "Sources of water to Fern Bank Springs". March 10.

Vieira, W.A., K. Anderson, L.G. Campbell, L.G., and C.D. McCusker. 2020. Characterizing the regenerative capacity and growth patterns of the Texas blind salamander (*Eurycea rathbuni*). Developmental Dynamics: 1–16.

Vose, R.S., D.R. Easterling, K.E. Kunkel, A.N. LeGrande, and M.F. Wehner. 2017. Temperature changes in the United States. Pages 185–206 *In*: D.J. Wuebbles, D.W. Fahey, K.A. Hibbard, D.J. Dokken, B.C. Stewart, and T.K. Maycock, editors, Climate science special report: Fourth National Climate Assessment. U.S. Global Change Research Program. Washington, D.C.

Votteler, T.H. 1999. The little fish that roared: The Endangered Species Act, state groundwater law, and private property rights collide over the Texas Edwards Aquifer. SSRN Electronic Journal:58.

Walters, A.W., and D.M. Post. 2011. How low can you go? Impacts of a low-flow disturbance on aquatic insect communities. Ecological Applications 21(1):163–174.

Wanakule, N. 1990. Stochastic drought analysis of the Edwards Aquifer. EARDC Number R1-90. 23 pp.

Warner, K. 2018. Instream habitat use and management of invasive armored catfishes in the Upper San Marcos River. M.S. Thesis, The University of Houston- Clear Lake. 79 pp.

Wellborn, G.A., J.D.S. Witt, and R.D. Cothran. 2015. Class Malacostraca, Superorders Peracardia and Syncardia. Pages 781–798 *In*: J.H. Thorp and D.C. Rogers, editors. Freshwater invertebrates Volume 1- Ecology and general biology, Fourth edition. Elsevier Academic Press, San Diego, California. 1,148 pp.

Williams, J.E., J.E. Johnson, D.A. Hendrickson, S. Contreras-Balderas, J.D. Williams, M. Navarro-Mendoza, D.E. McAllister, and J.E. Deacon. 1989. Fishes of North America endangered, threatened, or of special concern: 1989. Fisheries 14(6):2–20.

Wilson, J.T. 2011. Assessment of selected contaminants in streambed- and suspended-sediment samples collected in Bexar County, Texas, 2007–09: U.S. Geological Survey Scientific Investigations Report 2011-5097, 57 pp.

Woodruff, C.M. and L.P. Wilding. 2008. Bedrock, soils, and hillslope hydrology in the central Texas Hill Country, USA: implications on environmental management in a carbonate rock terrain. Environmental Geology 55:605–618.

Woods, H.A., M.F. Poteet, P.D. Hitchings, R.A. Brain, and B.W. Brooks. 2010. Conservation physiology of the plethodontid salamanders *Eurycea nana* and *E. sosorum*: Response to declining dissolved oxygen. Copeia 2010(4):540–553.

Worsham, M.L.D., and J.R. Gibson. 2022. E-mail "*Stygobromus* diseases". August 25.

Worsham, M.L.D and E. Julius. 2017. Comal Springs riffle beetle (*Heterelmis comalensis*): Life history and captive propagation techniques. BIO-WEST, Inc. prepared for the Edwards Aquifer Authority, 36 pp.

Worsham, M.L.D., and E. Julius. 2018. Evaluation of life history of the Comal Springs riffle beetle: Pupation! BIO-WEST, Inc., Texas State University, Desert Research Institute, and the San Marcos Aquatic Resources Center prepared for the Edwards Aquifer Authority. 10 pp.

Worthington, S.R.H. 2003. Conduits and turbulent flow in the Edwards Aquifer. Report prepared for the Edwards Aquifer Authority, 42 pp.

Xu, X., C. Walters, M.F. Antolin, M.L. Alexander, S. Lutz, S. Ge, and J. Wen. 2010. Phylogeny and biogeography of the eastern Asian–North American disjunct wild-rice genus (*Zizania* L., Poaceae). Molecular Phylogenetics and Evolution 55:1008–1017.

Yee, D.A. and S. Kehl. 2015. Order Coleoptera. 1003–1042 *In*: J.H. Thorp and A.P. Covich, Editors. Ecology and general biology, Fourth edition. Elsevier/AP, Academic Press is an imprint of Elsevier, Amsterdam and Boston, MA. 1,148 pp.

Young, B.E., E. Byers, G. Hammerson, A. Frances, L. Oliver, and A. Treher. 2016. Guidelines for using the NatureServe Climate Change Vulnerability Index. Release 3.02. 65 pp.

Yu, X., R.L. Hoyle, F. Guo, C.M. Ratliff, V. Cantu, J. Crow, L. Xiang, J.J. Heatley, and G. Zhu. 2019. A *Vavraia*-like microsporidium as the cause of deadly infection in threatened and endangered *Eurycea* salamanders in the United States. Parasites & Vectors 12:108.

Zabierek, K.C. and C.R. Gabor. 2016. Multimodal communication, mismatched messages and the effects of turbidity on the antipredator behavior of the Barton Springs salamander, *Eurycea sosorum*. Behavioural Processes 130:4–10.

ZARA Environmental, LLC. 2005. Diversion Spring Cave. Zara Environmental, LLC for the Edwards Aquifer Authority, San Marcos, TX. 12 pp.