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## MEMORANDUM

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TO: Scott Storment, Chad Furl, Kristina Tolman, Olivia Ybarra

FROM: BIO-WEST

DATE: December 28, 2023

SUBJECT: **ITEM M NET DISTURBANCE AND INCIDENTAL TAKE  
ASSESSMENT FOR 2023 EARIP ITP ANNUAL REPORT**

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### EXECUTIVE SUMMARY

The Edwards Aquifer Habitat Conservation Plan (HCP) Incidental Take Permit (ITP) requires a Net Disturbance and Incidental Take assessment to be conducted at the conclusion of each year for incorporation into the ITP Annual Report. Requirement M (1a and 2a) of the ITP specifically addresses minimization and mitigation activities associated with the HCP. This requirement stipulates that over the course of any given year no more than 10% of a covered species occupied habitat can be affected by HCP mitigation and restoration activities. Following quantification of net disturbance specific to these activities, incidental take was calculated for the disturbed areas. However, that is only part of the overall incidental take assessment. Incidental take associated with implementation of all other applicable HCP covered activities was then characterized and quantified to the degree practical. For a more detailed description of methodologies and species-specific results please refer to the Item M Net Disturbance (**SECTION 1**) and Incidental Take (**SECTION 2**) assessments of this technical memorandum. As in previous years, all 2023 assessments were performed in accordance with ITP requirements.

Table ES provides an overview of net disturbance percentages and a summary of incidental take for 2023. As shown in Table ES, only the Fountain Darter in the Comal System had a net disturbance when considering the project footprint for HCP mitigation and restoration activities overlaid on occupied habitat. The net disturbance was < 0.5% of the total occupied habitat for the Fountain Darter in the Comal system. In the San Marcos system, the Fountain Darter and San Marcos Salamander had net disturbance per this assessment with < 0.5% of their total occupied habitat disturbed, respectfully. In summary, the ITP 10% disturbance rule (Item M [a]) was in compliance for 2023.

The incidental take assessment for the Comal system resulted in calculated incidental take for all four monitored species (Table ES) which is only typical in drought years. As expected with the extreme drought, incidental take calculations for the Comal system exceeded those observed last year and in 2014 for all four species. The primary cause for this increase was low total system discharge which resulted in expanded amounts of exposed surface habitat characterized as Comal invertebrate occupied habitat. Over half of the incidental take calculated for the Fountain Darter was due to elevated water temperatures in the Upper Spring Run Section. For the San Marcos system, incidental take calculations were elevated in 2023 because the system experienced the lowest discharge conditions observed in the past two decades. These very low flow levels and resulting loss of aquatic vegetation / habitat and elevated water temperatures led to these larger calculations. It is important to emphasize that the San Marcos River in 2023 experienced the lowest total system discharge since Edwards Aquifer Authority (EAA) biological monitoring plan implementation in 2000. Not surprisingly, the 2023 spring to fall reductions in aquatic vegetation were considerably greater than other “drought” years characterized over the past two decades.

**Table ES. Summary of Impacted Habitat (m<sup>2</sup>) and Net Disturbance and Incidental Take for HCP Covered Species compared against ITP Maximum Permit Amounts.**

COVERED SPECIES PER SYSTEM	HCP Mitigation / Restoration		HCP Measures / Drought	Combined Impacted Habitat 2023 TOTAL (m <sup>2</sup> )	INCIDENTAL TAKE		2023 INCIDENTAL TAKE TOTAL	ITP Maximum Permit Amount	ITP Permit Maximum minus (combined first eleven years)
	IMPACTED HABITAT (m <sup>2</sup> )	NET Disturbance % OF TOTAL Occupied Habitat	IMPACTED HABITAT (m <sup>2</sup> )		HCP Mitigation / Restoration	HCP Measures / Drought			
COMAL SYSTEM									
Fountain Darter	358.0	<0.5%	5,273.1	5,631.1	537	72,093	72,630	797,000	652,719
Comal Springs Riffle Beetle	0	0%	379.1	379.1	0	2,502	2,502	11,179	5,642
Comal Springs Dryopid Beetle	0	0%	119.7	119.7	0	12	12	1,543	1,510
Peck's Cave Amphipod	0	0%	553.6	553.6	0	925	925	18,224	17,017
SAN MARCOS SYSTEM									
Fountain Darter	762.0	<0.5%	13,279.3	14,041.3	2,667.0	46,477.6	49,145	549,129	352,249
San Marcos Salamander	4.3	<0.5%	252.0	256.3	12.9	756.0	769	263,857	256,134
Texas Blind Salamander	0	0%	0	0	0	0	0	10	10
Comal Springs Riffle Beetle	0	0%	0	0	0	0	0	n/a	n/a
Comal Springs Dryopid Beetle	0	0%	0	0	0	0	0	n/a	n/a

When examining 2023 impacts from the extreme drought and covered activities, incidental take is being documented at some of the highest amounts over the course of the 11 year implementation to date. This is not unexpected with the severe drought, but yet remains considerably less than those characterized in the Biological Opinion Drought of Record (DOR)-like scenario. We are confident the incidental take numbers summarized in Table ES and documented in this memorandum continue to justify the data sets used and methodologies employed in 2023 relative to performing an incidental take assessment within the context of the Biological Opinion. It is understood that adjustments to data sets and/or methodologies may be employed based on feedback from the USFWS, HCP Science Committee, HCP participants, or others as deemed appropriate by the EARIP.

## SECTION 1: ITEM M NET DISTURBANCE ASSESSMENT

Requirement M (1a and 2a) of EAA’s USFWS threatened and endangered species permit (#TE63663A-1) addresses minimization and mitigation activities associated with the HCP. The requirements for Item M (1a and 2a) are stated below directly from the permit:

- 1 Comal Springs, Landa Lake, and the Comal River
  - a. The Permittees will limit disturbance of the (a) substrate, (b) water quality, (c) plants, and (d) animals of the Comal Springs, Landa Lake, and Comal River to no more than 10% of the occupied habitat on an annual basis when implementing HCP measures such as habitat and riparian restoration efforts that may directly or indirectly affect species considered here;
- 2 San Marcos Springs, Spring Lake, and the San Marcos River
  - a. The Permittees will limit disturbance of the (a) substrate, (b) water quality, (c) plants, and (d) animals of the San Marcos Springs, Spring Lake, and the San Marcos River to no more than 10% of the occupied habitat on an annual basis when implementing HCP measures such as habitat and riparian restoration efforts that may directly or indirectly affect species considered here;

All activities described in this memorandum pertain to the HCP covered species that are actively authorized (Item H: 1-6) in 2023 for incidental take via EAA’s ITP permit.

**Documentation of baseline habitat conditions:** For the actively covered HCP species, maps of occupied habitat for the Comal and San Marcos Springs/River systems were prepared in GIS, based on EAA biological monitoring data (BIO-WEST 2002 – 2013a, b; BIO-WEST 2014 - 2024a, b) and other existing sources for the HCP covered species. Table 1 summarizes the occupied habitat in meters squared (m<sup>2</sup>) for each of the covered species pertinent to the Item M assessment with associated figures presented in Appendix A. As per the ITP and USFWS Austin Ecological Services (ES) guidance, the 2023 assessment is representative of conditions for calendar year 2023 including any mitigation / restoration measures that resulted in a change in occupied habitat for any of the covered species.

**Documentation of HCP mitigation areal extent per project:** Descriptions of the HCP minimization and mitigation measures for the City of New Braunfels, City of San Marcos, and Texas State University are presented in the ITP Annual Report and will not be duplicated in this memorandum. For 2023 activities, pertinent to these projects, the areal extent of the project footprint has been quantified in Table 2 and depicted in subsequent figures per project. The project footprints were then overlaid on the occupied habitat maps in GIS and calculations of “Impact” area were performed. The results for each project and covered species are presented in Table 2.

**TABLE 1. COVERED SPECIES OCCUPIED HABITAT (Figures depicting occupied habitat included in Appendix A)**

ITEM M - SPECIES	OCCUPIED HABITAT (m <sup>2</sup> )	NOTES AND ASSUMPTIONS
<b>COMAL SPRINGS / RIVER</b>		
Fountain Darter	109,685	Based on collections and known occurrence in aquatic vegetation types sampled over the course of the HCP biological monitoring. Sampling included drop netting, dip netting, snorkel, SCUBA, and seining throughout the Comal system. Although Fountain Darters have been collected on bare substrate on occasion, no bare areas were included in this assessment.
Comal Springs Riffle Beetle	2,066	Based on collection of individuals via cotton lure, drift net, or quadrat sampling over the years. An area of 1 m <sup>2</sup> around each collection point was included but did not include any overlap between collection points.
Peck's Cave Amphipod	1,881	This species is considered subterranean and thus subsurface habitat is the more appropriate calculation. The total area of subsurface habitat for this species is presently unknown. Surface habitat was based on collection of individuals via cotton lure and drift net sampling. An area of 0.5 m <sup>2</sup> around each collection point was included but did not include any overlap between collection points.
Comal Springs Dryopid Beetle	363	This species is considered subterranean and thus subsurface habitat is the more appropriate calculation. The total area of subsurface habitat for this species is presently unknown. Surface habitat was based on collection of individuals via cotton lure and drift net sampling. An area of 0.5 m <sup>2</sup> around each collection point was included but did not include any overlap between collection points.
<b>SAN MARCOS SPRINGS / RIVER</b>		
Fountain Darter	95,169	Based on collections and known occurrence in aquatic vegetation types (including Texas wild-rice) sampled over the course of HCP biological monitoring. Sampling included drop netting, dip netting, snorkel, SCUBA, and seining throughout the San Marcos system. Although Fountain Darters have been collected on bare substrate in the river on occasion, no bare river areas were included in this baseline assessment. In contrast, bare substrate areas in Spring Lake were included for this assessment as Fountain Darters have frequently been observed inhabiting these areas within Spring Lake. Finally, although Fountain Darters have been collected further upstream in the slough arm of Spring Lake, those collections are considered seasonal at this time and thus were not included in the overall area calculated.
San Marcos Salamander	2,520	Based on observation or collection of individuals via snorkel / SCUBA over the course of HCP biological monitoring. Also, based on collections conducted by the USFWS San Marcos Aquatic Resources Center.
Texas Blind Salamander	n/a	This species is considered subterranean and thus subsurface habitat is the appropriate calculation. As such, no surface habitat was calculated as "occupied habitat" for this species.
Comal Springs Riffle Beetle	11	Based on collection of individuals via cotton lure and drift net sampling. An area of 1 m <sup>2</sup> around each collection point was included but did not include any overlap between collection points.
Comal Springs Dryopid Beetle	0.5	This species is considered subterranean and thus subsurface habitat is the more appropriate calculation. The total area of subsurface habitat for this species is presently unknown. Surface habitat was based on collection of individuals via drift net sampling. An area of 0.5 m <sup>2</sup> around each collection point was included but did not include any overlap between collection points.

**TABLE 2. Mitigation and Restoration Project Areas and Calculated Impact Area per Covered Species in 2023**

HCP ACTIVITY	Project Footprint Area (m <sup>2</sup> )	“Impact Area” Overlap with Occupied Habitat for Covered Species (m <sup>2</sup> )					
		Fountain Darter	Comal Springs riffle beetle	Comal Springs dryopid beetle	Peck’s Cave amphipod	San Marcos salamander	Texas blind salamander
CITY OF NEW BRAUNFELS							
Flow-split management	--	--	--	--	--		
Restoration and maintenance of native aquatic vegetation	748	358	0	0	0		
Decaying vegetation removal	A	--	--	--	--		
Aeration program	Discontinued in 2018						
Gill parasite	0	0	0	0	0		
Riparian restoration	3,495	--	--	--	--		
Bank Stabilization	Completed in 2016						
Riffle beetle restoration	1,725	--	0	0	0		
Non-native animal species removal	A	--	--	--	--		
Sediment Island removal	Completed in 2013						
TOTAL	5,968	358	0	0	0		
CITY OF SAN MARCOS / TEXAS STATE UNIVERSITY							
Enhancement and restoration of Texas wild-rice	B	--	--	--		--	--
Management of recreation specific to Protection zones (only)	6,000	22.9	--	--		4.3	--
Non-native animal species removal	A	--	--	--		--	--
Restoration and maintenance of native aquatic vegetation	1,405	739	0	0		0	0
Texas State Pump Intake Project	Completed in 2022						
Sediment removal	C	--	--	--		--	--
Access Points and Bank Stabilization	C	--	--	--		0	0
Riparian restoration	2,373	0	0	0		0	0
TOTAL	9,778	762	0	0		4.3	0

A Throughout system – described in qualitative impacts discussion

B Project footprint is accounted for in Native Aquatic Vegetation restoration project

C No EAHCP activities conducted in 2023.

## Comal System

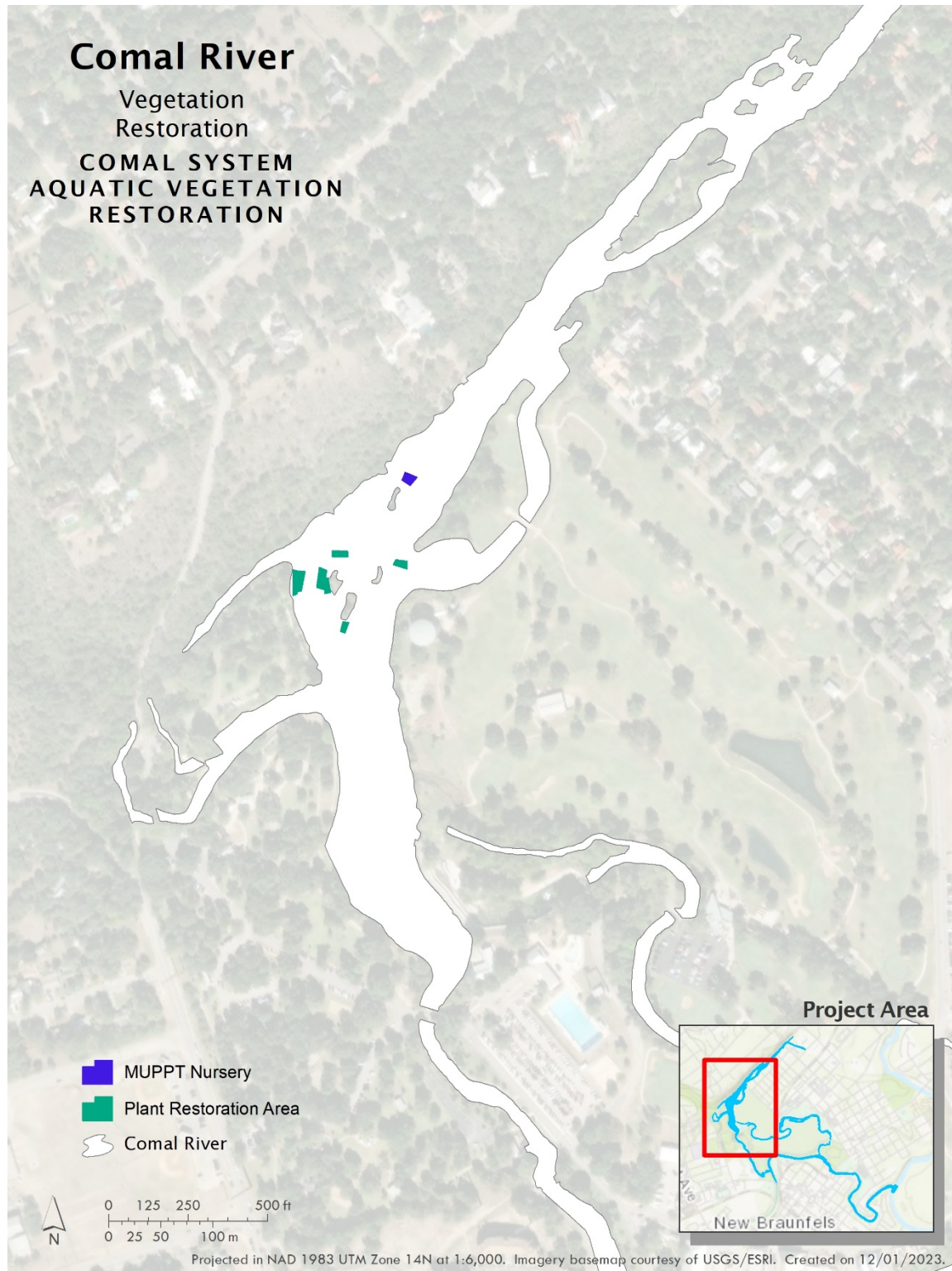
The **Old Channel bank stabilization** project construction was completed during 2016 and thus no calculations were included in the 2023 evaluation for that finished project. Similarly, the **Flow-split management** project was completed in spring 2014 and involved portions of Landa Lake and the Old Channel. Activities conducted in 2023 involved routine operation and maintenance that did not extend out beyond the existing renovated structure. As such, there was no additional footprint for this project in 2023.

The **restoration and maintenance of native aquatic vegetation** project involved restoration activities in the Comal system as shown in Figure 1. These activities included routine aquatic gardening and restoration plantings of native aquatic vegetation for portions of the start and finish of the year. However, only essential gardening and maintenance activities were conducted when the Comal system fell below 130 cfs (Provision M). The 2023 project footprint for native vegetation restoration activities are quantified in Table 2. Additionally, the MUPPT nursery area used to propagate native aquatic vegetation for restoration activities is also considered part of the project footprint (Figure 1). As noted in Table 2, the project footprint of the Native Aquatic Vegetation restoration effort in the Comal system encompassed 748 m<sup>2</sup> of which 358 m<sup>2</sup> overlaps with occupied Fountain Darter habitat. There was not any overlap with occupied habitat for the endangered Comal invertebrates. Although not quantified for this assessment, disturbance from foot traffic to and from these locations and from slightly elevated turbidity during non-native vegetation removal did temporarily occur.

The **Sediment Island removal** project in the Old Channel was completed in 2013 and thus no calculations were included in the 2023 evaluation for that finished project. Activities associated with supplemental planting of native aquatic vegetation in that section of the Old Channel were covered under the native aquatic restoration project. As presented in previous years, there is no project footprint map for the **Decaying Vegetation Removal** project as it was conducted throughout the main portion of Landa Lake and the New Channel on an as needed basis when floating mats of aquatic vegetation had built up. As such, no quantified area of impact was designated in 2023 for this activity. Temporary disturbance resulting from occasional foot traffic within Fountain Darter occupied habitat did occur as well as slightly elevated turbidity downstream from immediate work zone. The **Aeration** project in Landa Lake was discontinued in 2018 and thus no calculations were included in the 2023 evaluation.

The **Gill parasite** project involved one-time water sampling at designated cross sections in 2023 via kayak and thus no impacts were noted for this activity. The **Riffle beetle restoration** project was enhanced to include Spring Run 2 but as in previous years, only involved on shore activities (Figure 2). The **Non-native animal species removal** project had a change of contractors in 2019 to Atlas Environmental who continued these duties in 2023. There is no project footprint map per their methodologies as it is now conducted throughout Landa Lake and the Comal River without permanent or temporary installation of equipment. Most all work was conducted via snorkel or SCUBA in areas of high fish density with non-native fish being speared. **Riparian restoration** was continued in 2023 and involved a project footprint of 3,495 m<sup>2</sup>. The riparian treatment areas are depicted on Figure 3 and quantified in Table 2. All activities were conducted on the banks and water's edge and did not overlap with any occupied habitat for the covered species.



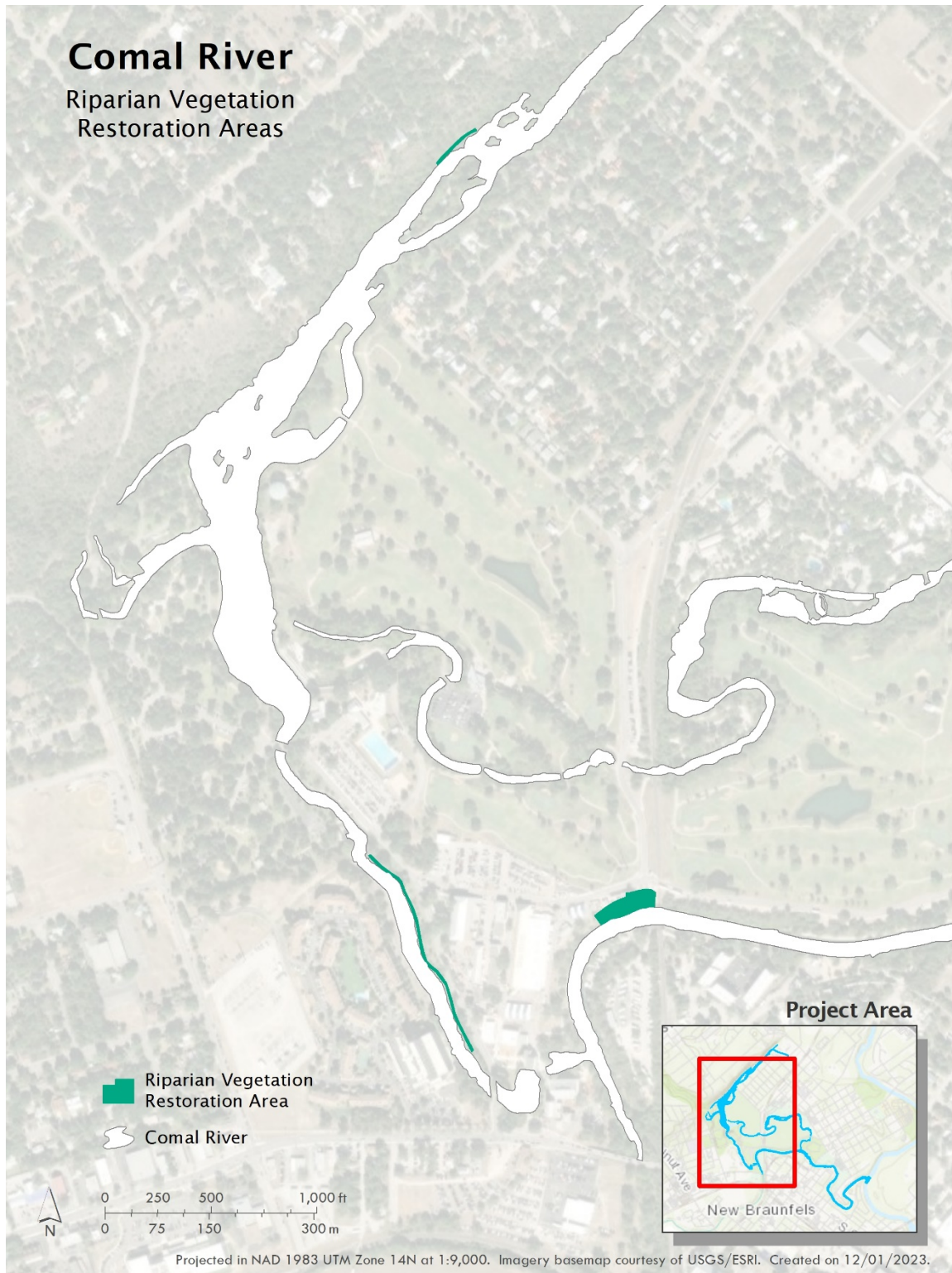


**Figure 1.** Restoration and Maintenance of Native Aquatic Vegetation project in the Comal system.



**Figure 2.** Comal Springs Riffle Beetle Restoration project – Comal System.





**Figure 3.** 2023 Riparian Vegetation Restoration Areas – Comal System.

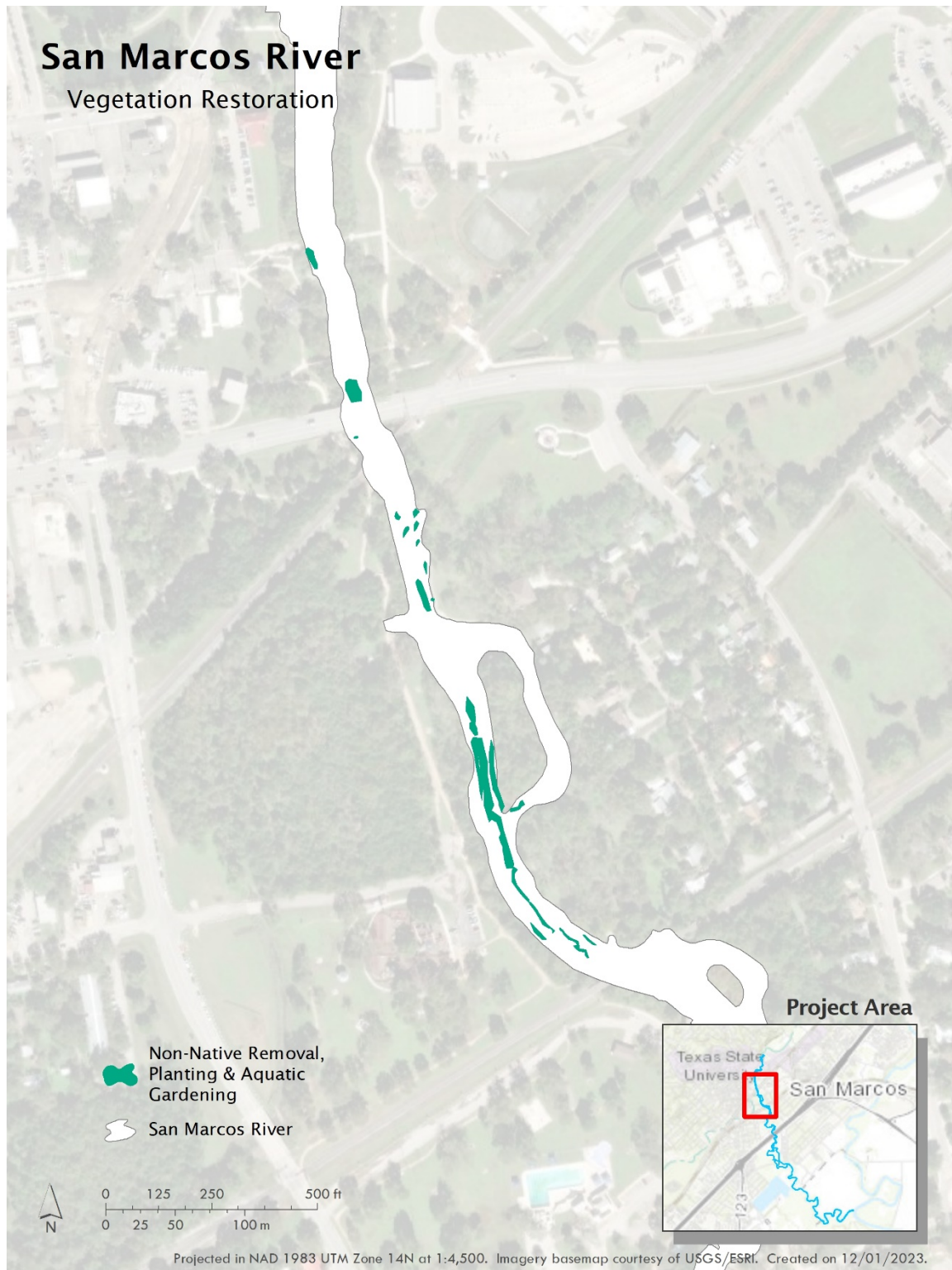
## San Marcos System

The **Enhancement and restoration of Texas wild-rice** and **Restoration and maintenance of native aquatic vegetation** project areas are depicted in Figure 4. As described in the ITP Annual Report, select non-native aquatic vegetation was removed from these areas allowing native vegetation (including Texas wild-rice) to expand over 2023. Native aquatic vegetation was also planted in cleared areas within these sections to promote restoration activities where practical and appropriate. These activities included routine aquatic gardening and restoration plantings of native aquatic vegetation for the start of the year. However, only essential gardening and maintenance activities were conducted after the San Marcos system fell below 120 cfs (Provision M). As evident in Table 2, the working project area supports a footprint of 1,405 m<sup>2</sup> of which 739 m<sup>2</sup> overlaps with Fountain Darter occupied habitat. Although not quantified for this assessment, disturbance from foot traffic to and from these locations and from slightly elevated turbidity during non-native vegetation and sediment island removal did temporarily occur.

Total system discharge in the San Marcos River declined below 120 cfs during the spring and thus, four Texas wild-rice **Protection Zones** were incorporated in 2023 (Figure 5). These areas included Clear Springs, river left; immediately below Sewell Park, river right; directly across from the Veramendi access point, river left; and Bicentennial Park, river right. The total footprint of these areas resulted in the protection of approximately 6,000 m<sup>2</sup>. The upstream protection zone in the eastern spillway below Spring Lake Dam was strategically placed over Fountain Darter and San Marcos salamander occupied habitat as well as Texas wild-rice. Although this area overlaps each of these covered species occupied habitats, the majority of the project footprint is a net benefit from the protection of recreation in these areas. The impact areas listed in Table 2 represent a 0.5 m swath across the floating buoy installation path of the protection zones to account for the placement of the floating buoys (45.85 total linear feet) used to deter recreators. As such, the total disturbance area for the four protection zones was 22.9 m<sup>2</sup> for the Fountain Darter and 4.3 m<sup>2</sup> for the San Marcos salamander which is only impacted by the upper portion of the protection zone just below Spring Lake dam.

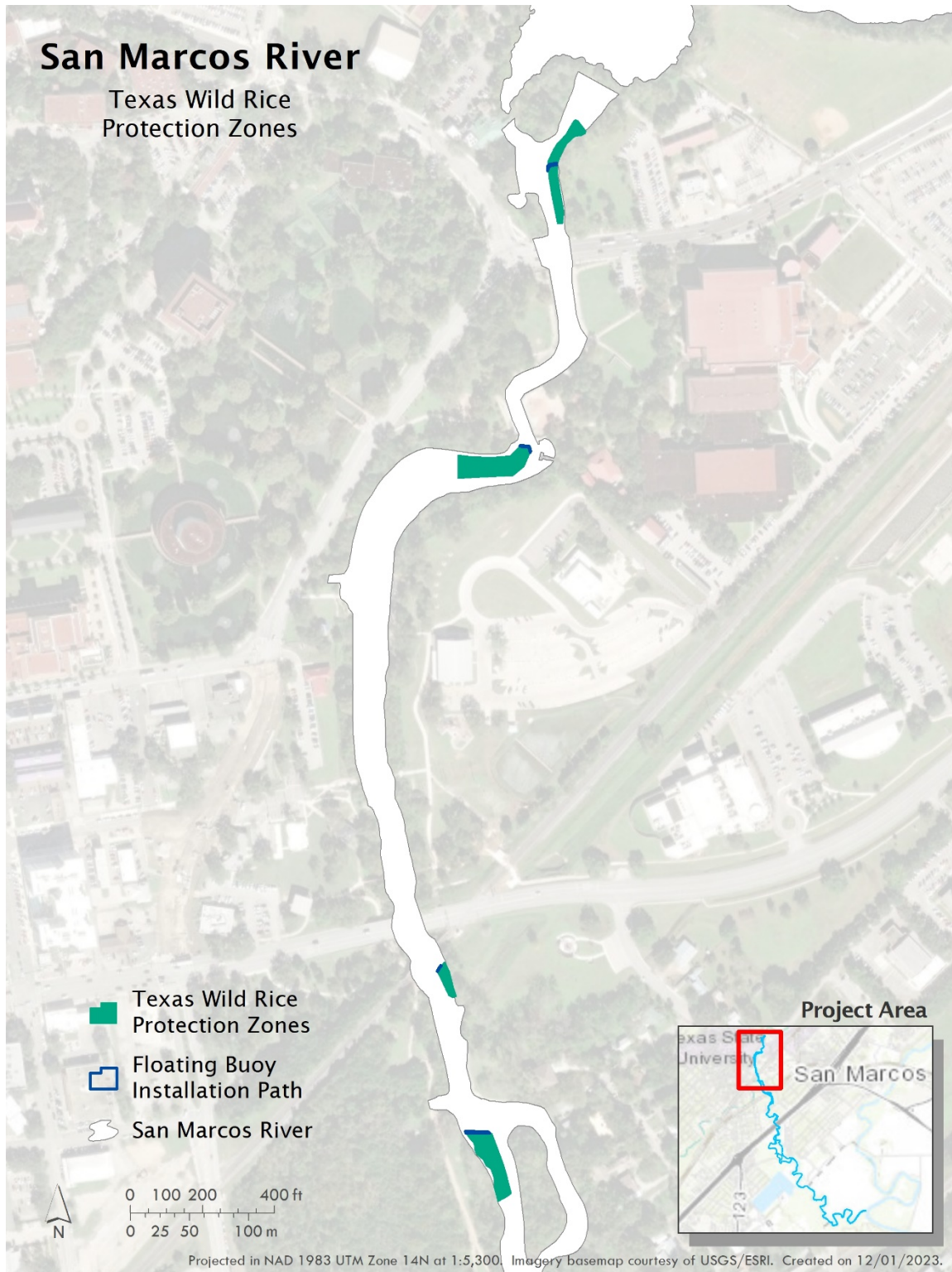
As in years past, there is no project footprint map for the **Non-native animal species removal** project as it was conducted throughout Spring Lake and the San Marcos River without permanent or temporary installation of equipment. Most work was conducted via snorkel or SCUBA in areas of high fish density with non-native fish being speared.

There was no new EAHCP work in 2023 with respect to **Access Points** or **Bank Stabilization** in the San Marcos system and thus, no calculations were included in the 2023 evaluation. The **Riparian restoration** project along the San Marcos River in 2023 involved a project footprint of approximately 2,373 m<sup>2</sup>. The active riparian treatment areas are depicted on Figure 6 and quantified in Table 2. As in years past, the riparian restoration project took place on the banks and water's edge and did not overlap with any occupied habitat for the covered species.

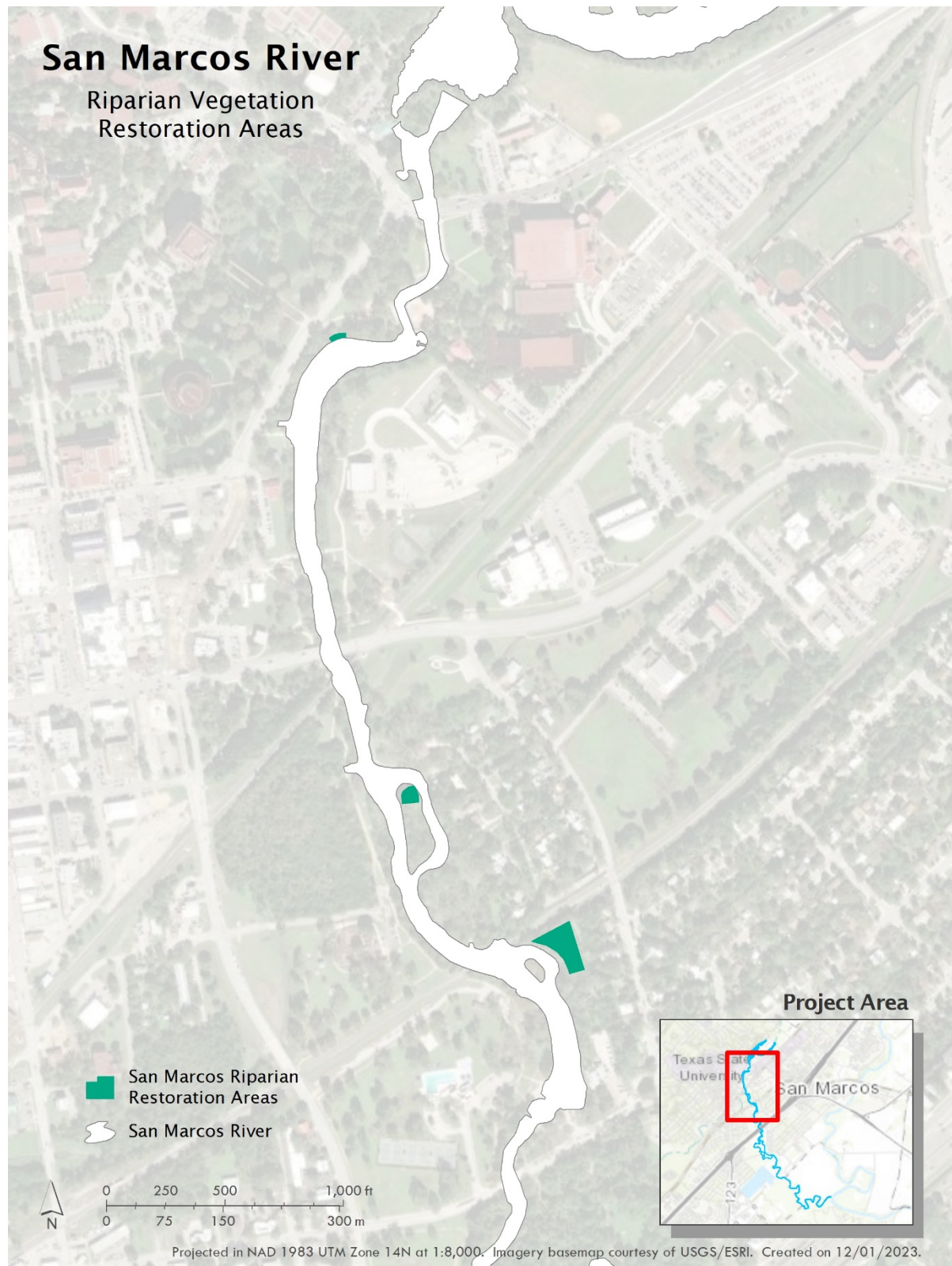


**Figure 4.** Restoration and Maintenance of Native Aquatic Vegetation and Enhancement of Texas wild-rice projects – San Marcos River.





**Figure 5.** Four Texas wild-rice Protection Zones installed during 2023 – San Marcos River.



**Figure 6.** 2023 Riparian Restoration areas – San Marcos River.



**Net Disturbance Assessment:**

As described above, the baseline maps of occupied habitat versus the HCP project footprint maps were examined to quantify the area of potential effects from mitigation and restoration activities as required in Item M (1a and 2a). This included a system-wide assessment of net disturbance. The focus was on quantifying the direct impacts via areal coverage of activity, but temporary disturbance from slightly elevated turbidity and increased foot traffic were also described. Table 3 shows the Net Disturbance calculation which is simply the sum of all project impact area that is overlaying baseline occupied habitat for a given covered species per system. As shown in Table 3, only the Fountain Darter in the Comal System had a net disturbance when considering the project footprints overlaid on occupied habitat. The Fountain Darter had < 0.5% of its total occupied habitat disturbed (Table 3) in the Comal system.

**TABLE 3. Net Disturbance Area and Percentage of Total per Species per System**

COVERED SPECIES	Total Occupied Habitat (m <sup>2</sup> )	Net Disturbance	
		Impact Area (m <sup>2</sup> )	% of Total
CITY OF NEW BRAUNFELS			
Fountain Darter	109,685	358	< 0.5%
Comal Springs riffle beetle	2,066	0	--
Comal Springs dryopid beetle	363 <sup>A</sup>	0	--
Peck’s Cave amphipod	1,881 <sup>A</sup>	0	--
CITY OF SAN MARCOS / TEXAS STATE UNIVERSITY			
Fountain Darter	95,169	762	< 0.5%
San Marcos salamander	2,520	4.3	< 0.5%
Texas blind salamander	<b>B</b>		
Comal Springs riffle beetle	11	0	0
Comal Springs dryopid beetle	0.5 <sup>A</sup>	0	0

<sup>A</sup> Although a minimal amount of surface habitat was documented for the baseline and comparison purposes, this species is subterranean and utilizes subsurface habitat.

<sup>B</sup> No surface habitat documented for this species.

In the San Marcos system, only the Fountain Darter and San Marcos Salamander had net disturbance per this assessment with both < 0.5% of their total occupied habitat disturbed, respectfully. For the Texas blind salamander, Comal Springs riffle beetle and Comal Springs dryopid beetle, there were no activities conducted in 2023 that directly impacted any of the locations or orifices where collections have routinely been made over the years. As such, no direct impacts to subterranean or aquifer habitat was experienced from 2023 HCP mitigation and restoration measures in the San Marcos system.

**In summary, the ITP 10% disturbance rule (Item M [a]) was in compliance for 2023.**

## SECTION 2 - INCIDENTAL TAKE

All discussions presented in this section relate back to the USFWS Biological and Conference Opinions for the Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan – Permit TE-63663A-1 (Consultation No. 21450-2010-F-0110), hereafter, Biological Opinion. The 2023 incidental take assessment described in this section was conducted in the same manner as previous years by first being broken down into two distinct categories to be carried forward in the assessment. The first category involves HCP mitigation and restoration activities specifically accomplished within the two springs ecosystems. These projects were the focus of the SECTION 1 - Item M net disturbance assessment. The second category pertains to covered activities that are foundational components (flow protection and springflow management measures) and on-going activities (water borne recreation, water diversions, existing water management infrastructure and operation, etc.). Each category is assessed independently below and then summed to represent the total amount of incidental take observed in 2023. Although calculated independently, a foundational first step to both assessments was the documentation of “occupied” habitat for the covered species as described in SECTION 1 (Table 1, Appendix A).

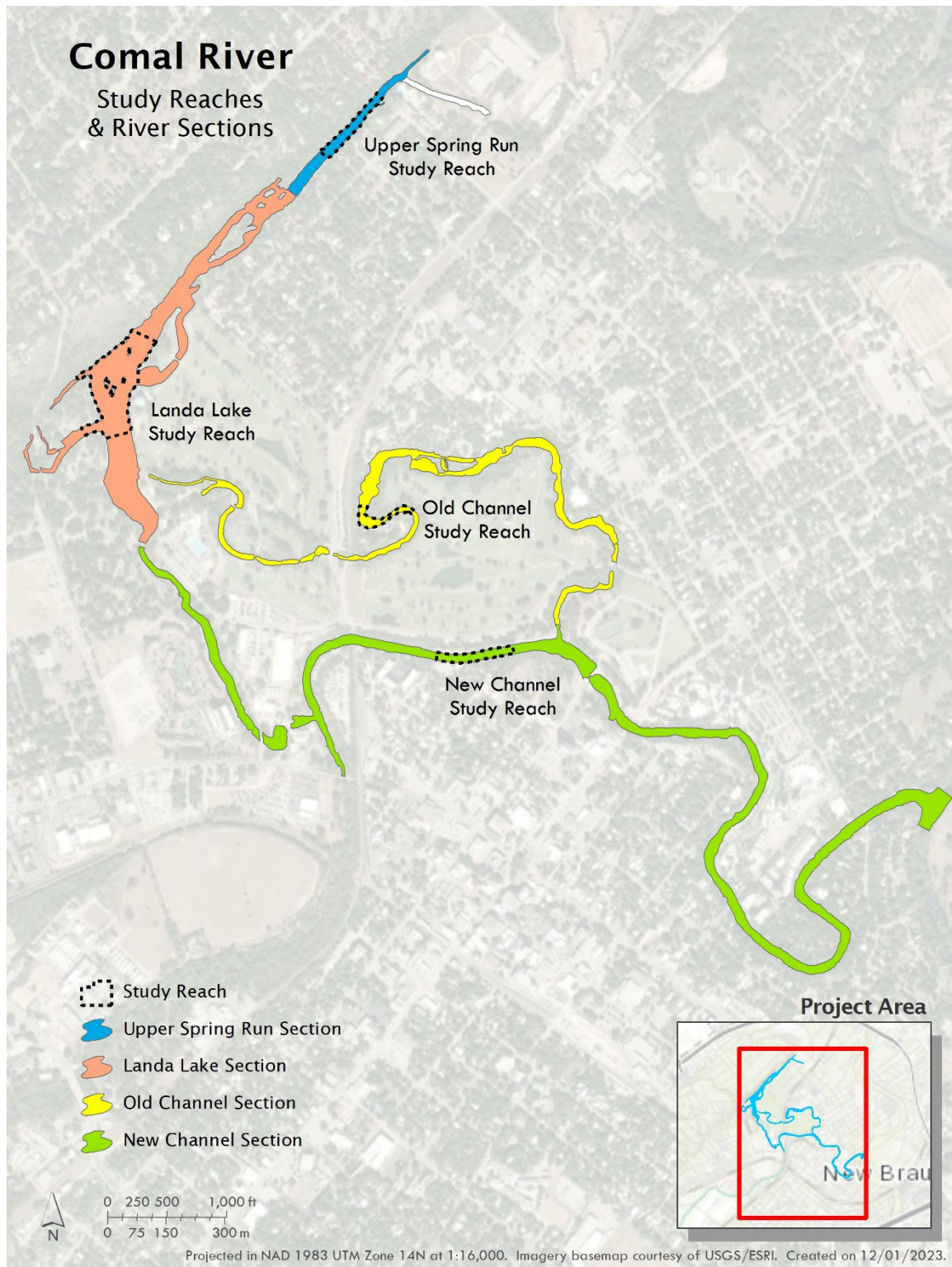
As described in SECTION 1, the baseline maps of occupied habitat versus the HCP project footprint maps were examined to quantify the area of potential effects from mitigation and restoration activities in Item M (1a and 2a) (Table 2). The focus was on quantifying the direct impacts (removal of non-native vegetation, removal of sediment, permanent placement of equipment, etc.) via areal coverage of activity, but temporary disturbance from slightly elevated turbidity and increased foot traffic were also qualitatively described. Table 3 in SECTION 1 shows the net disturbance calculation which is the sum of all project impact area that is overlaying baseline occupied habitat for a given covered species per system.

### **HCP MEASURES and DROUGHT: Documentation of impacted habitat for all other applicable HCP Covered Activities**

In addition to characterizing the impacted habitat from direct HCP mitigation measures and restoration activities as described SECTION 1, this assessment also addresses impacted habitat from all other applicable HCP Covered activities. As previously referenced, these other activities will be referred to as “HCP measures and drought” throughout the remainder of this assessment. As with the net disturbance assessment and Biological Opinion, this evaluation uses impacted habitat as the foundation for subsequent analysis. A discussion for each covered species is presented below.

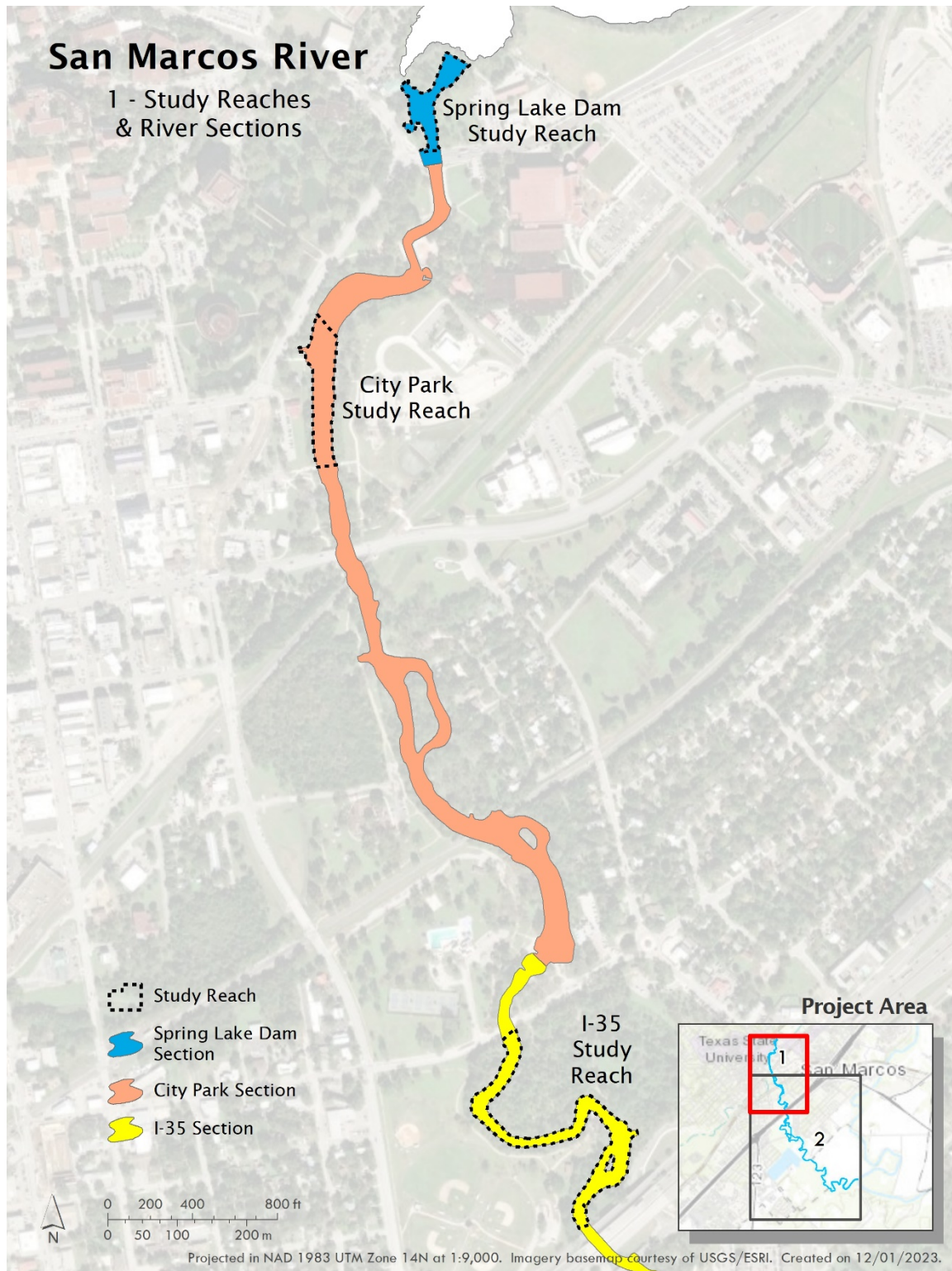
#### **Fountain Darter:**

A wealth of aquatic vegetation data over time is available per the long-term biological monitoring that has been conducted by EAA since 2000. The health and abundance of the Fountain Darter is strongly tied to the quantity and quality of aquatic vegetation present in both the San Marcos and Comal systems. As such, the determination was made to use the current aquatic vegetation data to characterize and quantify the amount of impacted habitat that occurred in 2023 relative to HCP measures and drought. Spring and fall sampling efforts for aquatic vegetation have been conducted in seven sample reaches (4 in Comal and 3 in San Marcos) since 2002. The sample reaches for the Comal System are shown in Figure 7 and include the Upper Spring Run sample reach, Landa Lake sample reach, New Channel sample reach, and Old Channel sample reach. The sample reaches for the San Marcos system are shown in Figure 8 and include the Spring Lake Dam sample reach, City Park sample reach, and the I35 sample reach. For both systems (Figures 8 and 8), the corresponding river section that corresponds to each sample reach is also shown.



**Figure 7.** Study Reaches (4) for the Comal System and Corresponding River Section.





**Figure 8.** Study Reaches (3) for the San Marcos System and Corresponding River Section.

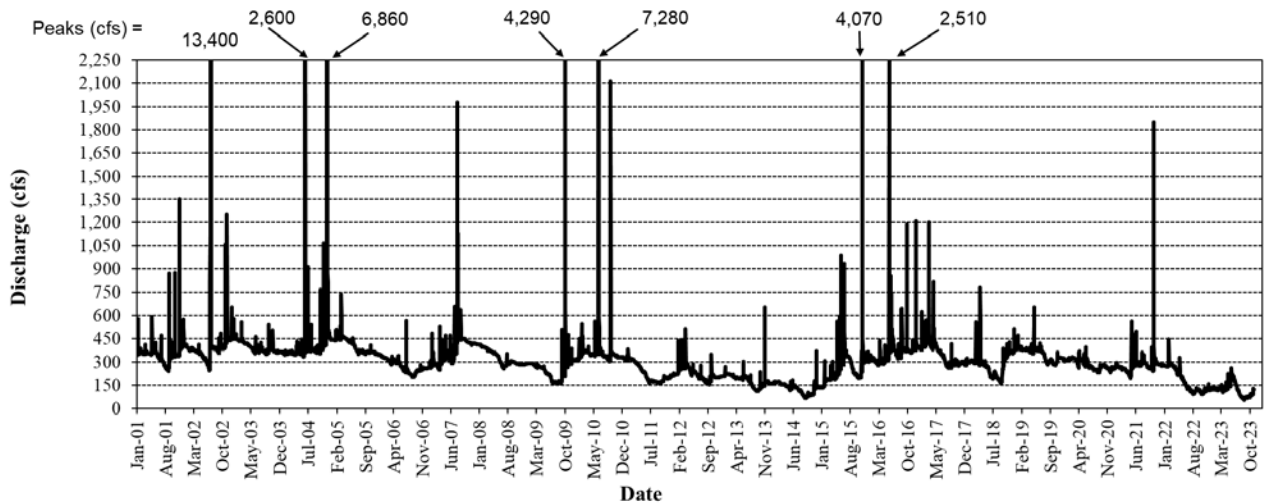


**Figure 8 cont.** I35 Study Reach and Corresponding Lower River Section in the San Marcos System.



The first step in this analysis was to compile all the spring and fall coverage of individual aquatic vegetation species from each of the seven sample reaches over time. All rooted aquatic vegetation per reach per event was combined into a total aquatic vegetation amount. Green algae were not included in the assessment because it is not rooted, is poor quality Fountain Darter habitat, and has a high level of variability from year to year. Although bryophytes are not rooted, they were included in the assessment for the slow-moving sample reaches of Landa Lake and the Upper Spring Run in the Comal system only. The main river sections that support a defined channel and greater velocities result in highly variable conditions for the non-rooted bryophytes in the New and Old Channels of the Comal River and all three reaches in the San Marcos River. However, in the Landa Lake and Upper Spring Run sample reaches, relationships between bryophytes and total system discharge are apparent, and bryophytes provide high quality Fountain Darter habitat in these reaches.

Table 4 (next page) shows the total aquatic vegetation ( $m^2$ ) present in each of the four study reaches in the Comal system over time. The color coding in Table 4 relates to “average” years [green], “flood event” years [blue], and “drought” years [orange]. Average years were determined as any year that exhibited over 225 cfs total system discharge throughout the majority of the year. The 225 cfs value was selected as it is the long-term average flow management objective specified in the HCP (EARIP 2011). In addition to being over 225 cfs, an average year for this assessment did not exhibit any flood events during the year or previous fall that substantially altered the aquatic vegetation within a given sample reach. If a flood event occurred in this manner and altered either the spring or fall aquatic vegetation amount, that year was discarded from the analysis. Finally, a drought year was determined as any year that exhibited total system discharge that went below 225 cfs for extended portions of the year. Concurrently, that drought year did not exhibit any flood events within the year that altered the aquatic vegetation in the sample reaches or it was discarded. As evident in Table 4, average and drought years were fairly consistent amongst reaches, but the Upper Spring Run and New Channel sample reaches were affected more frequently from flood-related high flow events. Figure 9 shows the Comal River hydrograph over the biological monitoring program time period with the larger daily average peak flows noted. Figure 9 also highlights that 2023 was a drought year as defined by this methodology.



**Figure 9.** Comal River hydrograph presented as daily discharge over the biological monitoring period.

**Table 4. Total Aquatic Vegetation in the Spring and Fall per reach on the Comal System over time.**

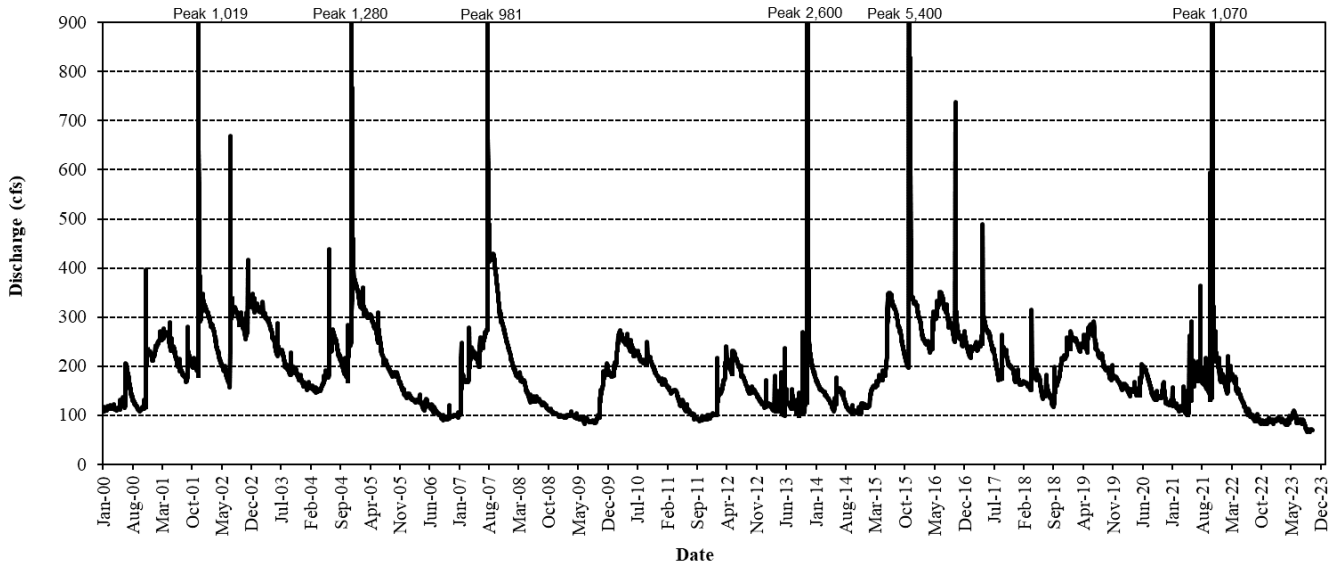
Season	Upper Spring Run Reach	Landa Lake Reach	Old Channel Reach	New Channel Reach
	Total Aquatic Vegetation (m <sup>2</sup> )	Total Aquatic Vegetation (m <sup>2</sup> )	Total Aquatic Vegetation (m <sup>2</sup> )	Total Aquatic Vegetation (m <sup>2</sup> )
Spring_02	1,569	19,497	509	3,304
Fall_02	2,701	19,033	486	2,555
Spring_03	3,909	19,351	554	3,259
Fall_03	2,743	17,946	872	3,588
Spring_04	2,744	17,241	1,226	3,576
Fall_04	1,584	16,102	1,173	623
Spring_05	2,376	18,431	1,291	18
Fall_05	2,968	16,754	1,752	220
Spring_06	3,108	17,617	1,843	325
Fall_06	2,574	16,870	1,760	869
Spring_07	3,668	18,954	1,774	1,223
Fall_07	3,907	19,083	1,769	1
Spring_08	4,218	19,908	1,587	1,566
Fall_08	2,470	17,310	1,647	2,895
Spring_09	3,278	19,640	1,731	2,695
Fall_09	1,819	16,330	1,823	173
Spring_10	2,949	19,010	1,842	230
Fall_10	548	15,967	1,495	363
Spring_11	1,345	17,703	1,814	538
Fall_11	789	16,049	1,954	1,484
Spring_12	2,792	19,349	1,942	1,999
Fall_12	1,348	19,735	1,939	2,569
Spring_13	2,143	23,092	1,527	2,596
Fall_13	1,020	21,595	1,402	2,893
Spring_14	1,511	19,233	1,319	3,249
Fall_14	861	17,759	1,502	3,400
Spring_15	1,381	16,396	1,778	2,898
Fall_15	1,436	17,431	1,210	3,541
Spring_16	1,963	17,566	794	2,377
Fall_16	1,610	18,945	543	2,045
Spring_17	2,914	19,631	1,011	1,223
Fall_17	2,047	18,714	821	2,224
Spring_18	2,409	19,019	877	1,637
Fall_18	1,603	17,499	1,053	2,579
Spring_19	2,088	18,925	302	2,104
Fall_19	1,922	18,496	535	2,221
Spring_2020	1,621	18,811	398	2,315
Fall_2020	1,434	17,344	560	3,008
Spring_2021	1,403	18,154	378	2,516
Fall_2021	1,785	18,144	779	2,511
Spring_2022	1,765	19,492	826	3,085
Fall_2022	1,909	18,316	895	2,798
<b>SPRING_2023</b>	1,853	17,540	255	3,027
<b>FALL_2023</b>	1,868	16,848	248	2,615

"AVERAGE YEAR" Total System discharge of >225 cfs throughout most of the year

"DROUGHT YEAR" Total System discharge of < 225 cfs discharge for most of the year

"FLOOD DISTURBANCE" Flood event affecting reach at some point between spring and fall or late fall previous year

Table 5 (next page) shows the total aquatic vegetation (m<sup>2</sup>) present in each of the three study reaches in the San Marcos system over time. Average years for the San Marcos River were determined as any year that exhibited over 140 cfs total system discharge throughout the majority of the year. The 140 cfs value was selected as it is the long-term average flow management objective specified in the HCP (EARIP 2011). Figure 10 depicts the San Marcos River hydrograph over the biological monitoring time period which also includes daily average peak flows and dates experienced. Figure 10 highlights that 2023 was defined as a drought year per this methodology and analyzed accordingly for this incidental take assessment.



**Figure 10.** San Marcos River hydrograph presented as daily discharge over the biological monitoring period.

**Table 5. Total Aquatic Vegetation in the Spring and Fall per reach on the San Marcos System over time**

Season	Spring Lake Dam reach	City Park reach	I35 reach
	Total Aquatic Vegetation (m <sup>2</sup> )	Total Aquatic Vegetation (m <sup>2</sup> )	Total Aquatic Vegetation (m <sup>2</sup> )
Spring_02	1,673	4,905	891
Fall_02	1,519	4,566	685
Spring_03	1,778	4,976	797
Fall_03	1,619	4,351	684
Spring_04	1,725	4,620	543
Fall_04	1,184	4,413	900
Spring_05	1,084	4,243	401
Fall_05	1,123	4,055	556
Spring_06	1,225	4,617	474
Fall_06	1,061	4,171	902
Spring_07	1,385	3,554	903
Fall_07	1,098	4,258	840
Spring_08	1,426	4,748	608
Fall_08	1,182	3,992	784
Spring_09	1,236	4,307	759
Fall_09	802	2,690	739
Spring_10	1,205	4,545	626
Fall_10	971	3,816	653
Spring_11	1,400	4,457	688
Fall_11	998	3,050	488
Spring_12	1,240	4,148	474
Fall_12	1,091	3,103	289
Spring_13	2,064	5,074	495
Fall_13	1,283	3,699	402
Spring_14	1,198	3,123	1,745
Fall_14	911	2,663	1,519
Spring_15	1,272	3,387	2,065
Fall_15	805	2,703	1,738
Spring_16	1,108	3,246	1,172
Fall_16	1,018	2,579	1,110
Spring_17	1,366	3,681	1,404
Fall_17	1,373	2,840	1,881
Spring_18	1,553	3,024	2,011
Fall_18	1,386	2,395	2,040
Spring_19	1,799	3,071	2,317
Fall_19	1,690	2,778	2,194
Spring_2020	1,817	3,723	2,651
Fall_2020	1,749	3,376	2,039
Spring_2021	2,060	4,139	2,170
Fall_2021	1,583	2,250	2,235
Spring_2022	2,077	4,135	2,519
Fall_2022	1,125	2,875	1,573
<b>SPRING_2023</b>	1,660	3,727	2,212
<b>FALL_2023</b>	1,241	2,145	1,554

"AVERAGE YEAR" Total System discharge of >140 cfs throughout most of the year

"DROUGHT YEAR" Total System discharge of < 140 cfs discharge for most of the year

"FLOOD DISTURBANCE" Flood event affecting reach after fall sampling period

Table 6 shows the percentage retention in aquatic vegetation observed from spring to fall for average and drought years as well as individually for 2023. As evident in Table 6, both the Old Channel and New Channel study reaches in the Comal system experienced a decline relative to average conditions in overall aquatic vegetation in 2023 from spring to fall. In the San Marcos system, all three study reaches experienced declines (relative to average conditions) in aquatic vegetation from spring to fall in 2023 (Table 6). It is also important to emphasize that 2023 experienced the lowest total system discharge since the biological monitoring plan implementation in 2000. As expected, the 2023 changes in aquatic vegetation were considerably greater in the City Park and I35 reaches than other “drought” years characterized over the course of the past two decades (Table 6).

**Table 6. Percentage Retention of aquatic vegetation from Spring to Fall per sample reach per system.**

Scenario	Percentage Retention in Aquatic Vegetation from Spring to Fall						
	Comal System Sample Reaches				San Marcos System Sample Reaches		
	Upper Spring Run	Landa Lake	Old Channel	New Channel	Spring Lake Dam	City Park	I35
Average Flow Condition Years	83.34%	95.98%	102.58%	122.40%	89.22%	91.74%	97.76%
Drought Years	51.58%	92.38%	103.43%	123.45%	72.97%	76.97%	101.37%
Spring 2023 coverage (m <sup>2</sup> )	1,852.95	17,540.49	255.46	3,026.97	1,659.78	3,726.66	2,212.23
Fall 2023 coverage (m <sup>2</sup> )	1,868.00	16,848.23	248.47	2,615.14	1,240.96	2,145.44	1,554.11
2023 Spring to Fall Retention	100.81%	96.05%	97.26%	86.39%	74.77%	57.57%	70.25%

Table 7 shows the conversion process from percentage retention between spring and fall aquatic vegetation during average years when compared directly to 2023. Using the Spring Lake Dam sample reach as an example, there is approximately an 89% retention during average years. This implies that under average conditions in the Spring Lake Dam reach there is a 11% decline in aquatic vegetation observed from spring to fall each year. This amount is considered a pre-HCP condition because 1) it is calculated based on routine conditions prior to the HCP, and 2) during average years, a lot of HCP measures would not be actively engaged. As such, the difference in retention ( $89.22 \text{ [average]} - 74.77\% \text{ [2023]} = 14.45\%$ ) is the value used to assess the overall loss of Fountain Darter occupied habitat within this river section. As shown in Table 7, the Old Channel reach and the New Channel reach in the Comal system showed a 2023 decline greater than average conditions resulting in 647 m<sup>2</sup> and 4,626 m<sup>2</sup> of impacted habitat, respectively. All three study reaches in the San Marcos River experienced declines greater than drought conditions during 2023. The total Fountain Darter occupied habitat designated for the Spring Lake Dam section is 1,746 m<sup>2</sup>; City Park section is 29,410 m<sup>2</sup>; and I35 section is 10,823 m<sup>2</sup> (Table 7). The percent difference from these reaches multiplied by the total m<sup>2</sup> from the entire section results in 252 m<sup>2</sup>; 10,050 m<sup>2</sup>; and 2,997 m<sup>2</sup>, respectively (Table 7). For this incidental take assessment, those values are considered the amount of habitat that was impacted by the HCP Measures and Drought category for those particular river sections.



**Table 7. Total Impacted Area (m<sup>2</sup>) for the Fountain Darter based on percentage retention of aquatic vegetation from Spring to Fall per sample reach per system.**

Scenario	Percentage Retention in Aquatic Vegetation from Spring to Fall						
	Comal System Sample Reaches				San Marcos System Sample Reaches		
	Upper Spring Run	Landa Lake	Old Channel	New Channel	Spring Lake Dam	City Park	I35
Average Flow Condition Years	83.34%	95.98%	100%	100%	89.22%	91.74%	97.76%
2023 Actual	100.81%	96.05%	97.26%	86.39%	74.77%	57.57%	70.25%
	HABITAT CALCULATIONS applied to river sections						
Difference between Average and 2023 (%)	0%	0%	2.74%	13.61%	14.45%	34.17%	27.51%
Total Fountain Darter Occupied Habitat (m <sup>2</sup> ) per entire river section	3,564	48,462	23,660	33,999	1,746	29,410	10,823
2023 Total Impacted Area (m <sup>2</sup> )	0	0	647	4,626	252	10,050	2,977

**Comal Springs Invertebrates:**

To calculate the impacted habitat area for the Comal Springs riffle beetle, Comal Springs dryopid beetle, and Peck's Cave amphipod, areas of disturbance in 2023 (not including the HCP mitigation and restoration measures assessed separately) were assessed and area of impact quantified by overlapping area of disturbance and occupied habitat. The occupied habitat maps for each of the Comal invertebrates are described in SECTION 1 and displayed in Appendix A. In 2023, disturbances pertaining to HCP measures and drought to the Comal invertebrate species were the drying of surface area in the spring runs, western shoreline, and Spring Island area in late summer/fall.

With HCP measures in place, the 2023 drought resulted specifically in the drying of surface habitat in Spring Run 1, Spring Run 2, Spring Run 4, Spring Run 5, along the fringe of the western shoreline of Landa Lake, and within the Spring Island area. This disturbance resulted in the amount of calculated impacted invertebrate habitat area displayed in Table 8. Please note that the overall area of exposed substrate in the system was greater than quantified in Table 8, as that value represents only the exposed surface substrate overlapping with occupied habitat for each covered species. This approach was used to stay consistent with the occupied habitat approach used for each covered species. Additionally, any impacted area calculated in 2022 that was not consistently re-wetted during 2023 was subtracted from the 2023 total per USFWS guidance. This was done to avoid duplicative counting of incidental take for areas that were already impacted and counted, but not yet recovered from the previous year.

**Table 8. Total Impacted Area (m<sup>2</sup>) for the Comal Springs Invertebrates.**

Covered Species	2023 Impacted Occupied Habitat Area (m <sup>2</sup> )			
	Main Spring Runs	Western Shoreline	Spring Island	TOTAL
Comal Springs riffle beetle	105.7	116.2	157.2	379.1
Comal Springs dryopid beetle	106.7	0.5	12.5	119.7
Peck's Cave amphipod	62.1	180.5	311.0	553.6

For the Comal invertebrates, no attempt was made to characterize subsurface habitat in this assessment. If a documented occupied habitat point had exposed substrate, it was included regardless of potential downward migration. When comparing against the occupied habitat maps, the greatest area of disturbance for the Comal Springs riffle beetles and Peck's Cave amphipods were around Spring Island and the Western Shoreline, while the main area of disturbance for the Comal Springs dryopid beetle was Spring Runs 1 and 2.

**San Marcos salamander:**

As San Marcos salamander habitat below Spring Lake Dam and in Spring Lake remains fairly consistent from spring to fall, there was no attempt to quantify direct habitat changes. Additionally, there was not any notable drying of surface habitat in the Spring Lake Dam section in 2023. As such, there was no quantification of disturbance using exposed surface area overlapping with occupied habitat. Therefore, the only known disturbance of occupied San Marcos salamander habitat in 2023 was from recreational activities below Spring Lake dam. As there is not a quantification of recreation in this sample reach, the percentage of retention of aquatic vegetation in the Spring Lake dam reach calculated for the Fountain Darter was used (as in each previous year) for the San Marcos salamander as a surrogate for disturbance. As shown in Table 7, there was a 252 m<sup>2</sup> impacted area calculated for the Spring Lake Dam reach.

**Texas blind salamander:**

There is no surface habitat documented in the Item M assessment (SECTION 1) for the Texas blind salamander. There were no aquifer impacts noted via HCP measures or drought in 2023, and thus, no impacted habitat is reported for the Texas blind salamander in this assessment.

**INCIDENTAL TAKE CALCULATIONS**

The next step in the analysis is converting the impacted habitat area to incidental take of individuals so that a comparison can be made to the ITP permit. In 2023, incidental take was again scaled in accordance with the condition of the system at that particular time. For instance, incidental take caused by a reduction of 10% of the occupied habitat in the system is not the same proportionally to a condition where 40%, 70%, or 90% of the occupied habitat is removed from the system. The rationale is that when only a small amount of habitat is removed, a large portion of quality habitat remains for the covered species to utilize. However, when larger portions of occupied habitat are reduced, the situation inherently becomes more stressful for the individuals. The word stressful is important in that take is more than just mortality as discussed at the start of this memorandum. In the Biological Opinion, the USFWS defines Take as "... to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Harass is further defined by the Service as an intentional or negligent act or omission which creates the likelihood of injury to a listed species by annoying it to such an extent as to significantly disrupt normal behavioral patterns, which include, but are not limited to, breeding, feeding and sheltering (50 CFS §17.3). Harm is also further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by impairing behavioral patterns, including breeding, feeding, and sheltering."

To explain the concept of non-proportional take as occupied habitat is reduced, it is important to start with mortality, but as described in the original HCP take assessment, incidental take goes beyond mortality. Habitat disturbances including physical (aquatic vegetation, silt-free substrate, etc.) and chemical (standard water quality parameters such as water temperature and dissolved oxygen) play a role in incidental take calculations as well. This is important in that one of the further definitions of "Harass" is that it annoys the individual or modifies its habitat to such an extent that behavior patterns (including breeding) are impaired. Of course, there are other behavioral components that may be disrupted either through direct annoyance of the individual or through habitat modifications, such as feeding and sheltering. During HCP measures and

drought, the loss or modification of habitat described in the previous section by definition clearly caused take beyond mortality. Considering that mortality represents a very small proportion of that number, characterizing the remaining amount becomes very important.

For this assessment, the densities of the covered species recorded over time via EAA biological monitoring in both systems prior to HCP implementation were used. The USFWS approach used the average density for covered species from the same biological monitoring program to make calculations in the biological opinion in many instances. For this assessment, the density statistics were broken down further to explore the component of scaling incidental take as habitat conditions get worse. Table 9 shows the density statistics chosen for each of the covered species. The 25<sup>th</sup>, 50<sup>th</sup> (median), 75<sup>th</sup>, and 90<sup>th</sup> percentile along with the mean density are included. Furthermore, only the spring and fall data sets were used for these density statistics. The rationale is that under drought or following high-flow events the densities within aquatic vegetation types may not be representative of average conditions with which to apply to incidental take. Additionally, as more and more critical period (low and high) events get added, it skews the data set towards those events.

**Table 9. Descriptive statistics of Covered Species density by System**

Covered Species	Density (individuals per m <sup>2</sup> ) Descriptive Statistics (Percentiles and Mean)				
	25	Median	Mean	75	90
<b>Fountain Darter</b>					
Comal system	1.50	6.00	11.35	15.50	29.30
San Marcos system	1.50	3.50	5.90	7.00	13.00
<b>Comal Springs riffle beetle</b>	6.60	9.10	10.71	12.40	19.38
<b>Comal Springs dryopid beetle<sup>A</sup></b>	-	-	0.10	-	-
<b>Peck's Cave amphipod</b>	1.04	1.67	2.05	2.33	4.33
<b>San Marcos salamander</b>					
San Marcos River	3.00	6.00	6.08	8.50	10.5
Spring Lake	10.00	12.00	13.17	16.25	19.00

<sup>A</sup> Too few collected to use full set of descriptive statistics

The same spring and fall sample sets were used for each covered species. Fountain Darter densities are presented by system and are comprised of drop net sampling in aquatic vegetation types used in the occupied habitat assessment. This approach deviates from the USFWS analysis in that only an average density calculated from both systems combined with all sample dates was included in the Biological Opinion. For this assessment, San Marcos salamander densities were developed from the quantitative snorkel/SCUBA sampling being conducted during biological monitoring in the San Marcos system. Densities within the San Marcos River and Spring Lake occupied habitat were broken out separately as done in the Biological Opinion.

Densities for the Comal Springs riffle beetles were generated from the cotton lure sampling at three locations (Spring Run 3, Western Shoreline, and Spring Island area). Densities for the Peck's Cave amphipod were generated from the drift net sampling conducted over the main orifices at Spring Run 1, Spring Run 3, and Spring Run 7. For the Comal Springs dryopid beetle, limited captures over time resulted in only using the mean presented in Table 9. The Biological Opinion estimated the total surface population of Comal springs dryopid beetles in the Comal Spring system to be 1,839 individuals (USFWS 2010). To

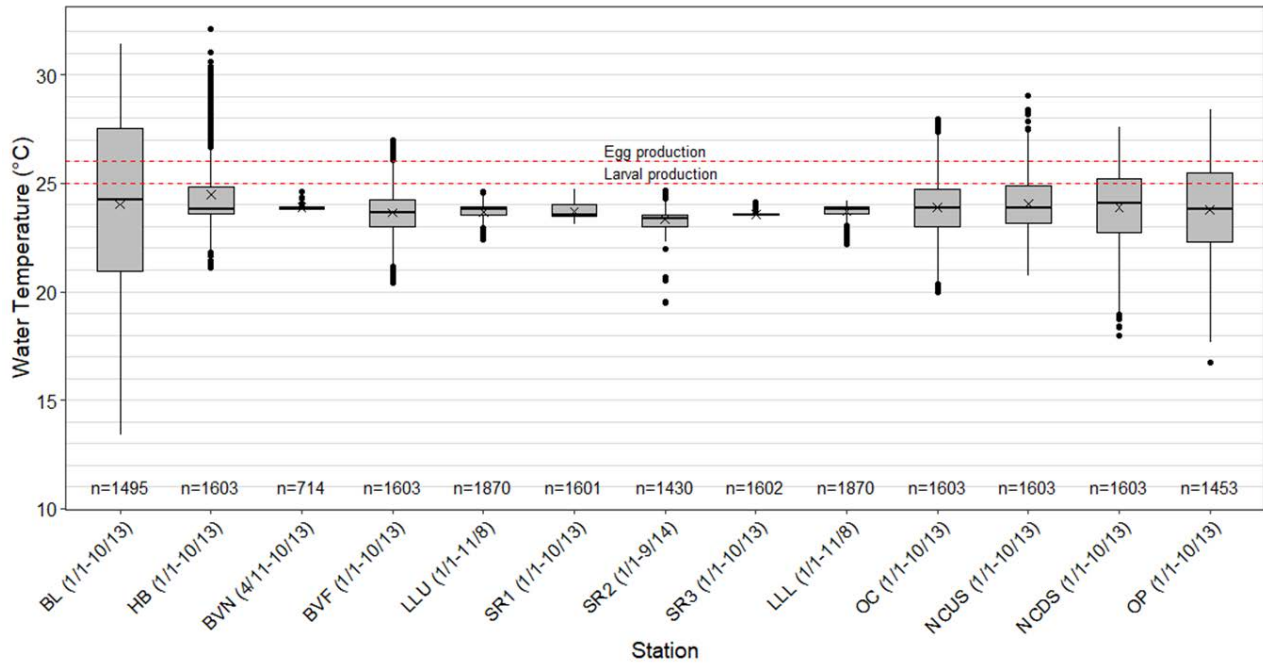
calculate their incidental take, they used a 5%, 10% rule based on an even distribution of individuals to come up with 9 individuals ( $1839 \times .05 \times .10 = 9.2$ ). In doing so, the underlying assumption forced was that the overall area was 1,839 square feet or 1 individual per square foot. One individual per square foot equals 0.09 per  $\text{m}^2$ . Although the biological monitoring data has limited Comal Springs dryopid beetle observations, the calculated mean density of 0.10 individuals per  $\text{m}^2$  is in line with the Biological Opinion estimate.

To account for a scaled approach for calculating incidental take (increased impacts with increased levels of habitat loss); the following schedule (Table 10) was used to determine which density statistic to multiply by impacted habitat area to generate the incidental take estimate. The schedule is based on remaining occupied habitat per covered species per system. For example, if 30% of the total occupied habitat was impacted for the Fountain Darter in the San Marcos system that would leave 70% of the occupied habitat for the Fountain Darter. For the incidental take calculation, the median density for the Fountain Darter (3.5 darters per  $\text{m}^2$ , Table 9) would be used to multiply against the total impacted area.

**Table 10. Density assignment schedule based on remaining occupied habitat**

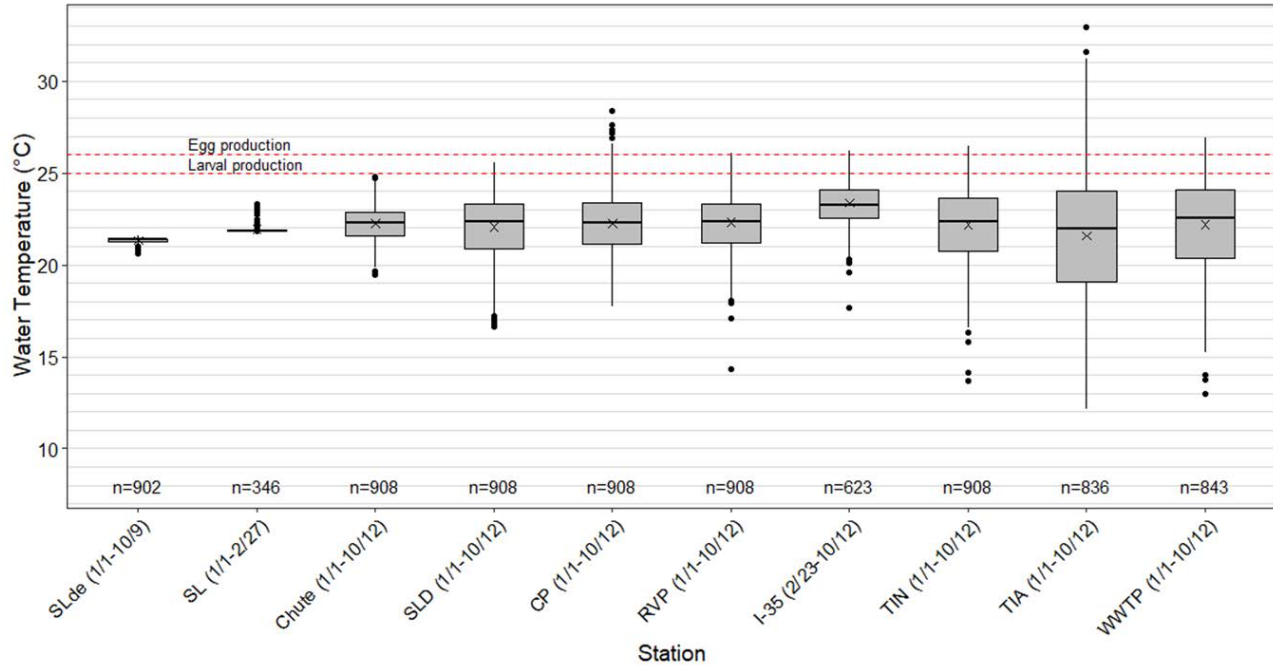
Remaining Occupied Habitat Percentage	Corresponding Density Statistic
100 to 75	25%
74 to 50	Median
49-25	Mean
24-10	75%
9-0	90%

In 2023, water temperature conditions within several reaches were elevated above the potential for impacts to Fountain Darter life stages and reproductive success based on literature (Brandt et al. 1993, Bonner et al. 1998, McDonald et al. 2007). Although spawning success and larval growth show declines in a laboratory setting at temperatures over 27 °C, it is a conservative temperature trigger; the lethal limit (50% mortality) for larval fountain darters is 31.9° C and approximately 3.0° C higher for adults (Brandt et al. 1993, Bonner et al. 1998, McDonald et al. 2007). Figures 11 and 12 show water temperature ranges observed in each system over the course of 2023. To account for potential additional impacts from elevated water temperatures, a density assignment scale was developed for water temperature specific to the Fountain Darter. This scale is presented in Table 11 with the corresponding density statistic increasing per elevated temperature ranges. This scale is to be used in combination with the density assignment schedule for remaining occupied habitat percentage (Table 10), with the higher of the two applied to that specific reach when making final calculations of incidental take. In the Comal System, the Upper Spring Run Reach exceeded 30 °C resulting in an adjustment to the Mean density statistic; while the Old Channel and New Channel reaches exhibited temperatures between 27 to 29 (Figure 11) requiring an adjustment to the Median density statistic for these respective reaches. In the San Marcos system, the City Park reach exhibited temperatures between 27 to 29 °C (Figure 12) and thus an adjustment to the Median density statistic was applied.



**Figure 11.** Boxplots displaying 2023 water temperatures at logger stations (data collection timeframe [Month/Day]). Data are based on measurements collected at 4-hour increments. Stations include Blieders Creek (BL), Heidelberg (HB), Boonville Near (BVN), Boonville Far (BVF), Landa Lake Upper (LLU), Spring Run 1 (SR1), Spring Run 2 (SR2), Spring Run 3 (SR3), Landa Lake Lower (LLL), New Channel Upstream (NCUS), New Channel Downstream (NCDS), and Other Place (OP). The thick horizontal line in each box is the median, x represents the mean, and the upper/lower bounds of each box represents the interquartile range. Whiskers represent minimum/maximum values up to 1.5 times the interquartile range, and outliers beyond this are designated with solid black circles. The “n” values along the x-axis represent the number of individual temperature measurements in each category. The red dashed lines indicate maximum optimal temperatures for Fountain Darter larval ( $\geq 25$  °C) and egg ( $\geq 26$  °C) production (McDonald et al. 2007).





**Figure 12.** Boxplots displaying 2023 water temperatures at logger stations (data collection timeframe [Month/Day]). Water temperature data are based on measurements collected at 4-hour increments. Stations include Spring Lake Deep (SLde), Spring Lake (SL), Chute, Spring Lake Dam (SLD), City Park (CP), Rio Vista Park (RVP), I-35, Thompson’s Island Natural Channel (TIN), Thompson’s Island Artificial Channel (TIA), and Wastewater Treatment Plant (WWTP). The thick horizontal line in each box is the median, x represents the mean, and the upper/lower bounds of each box represents the interquartile range. Whiskers represent minimum/maximum values up to 1.5 times the interquartile range, and outliers beyond this are designated with solid black circles. The “n” values along the x-axis represent the number of individual temperature measurements in each distribution. The red dashed lines indicate maximum optimal temperatures for Fountain Darter larval ( $\geq 25$  °C) and egg ( $\geq 26$  °C) production (McDonald et al. 2007).

**Table 11.** Density assignment schedule based on water temperature range within reach

Water Temperature range (°C)	Corresponding Density Statistic
< 27	25%
27 to 29	Median
29 to 31	Mean
31 to 33	75%
> 33	90%

Using the density schedules in Tables 10 and 11, impacted habitat areas calculated in Tables 3, 7, and 8, incidental take calculations were made for each covered species.

### Fountain Darter:

Table 12 shows the incidental take calculated for the Fountain Darter in the Comal system and San Marcos system (San Marcos River and Spring Lake) relative to HCP mitigation and restoration activities as well as the HCP measures and drought. It is important to keep the two categories (HCP mitigation / restoration and HCP measures / drought) separate in the analysis. The rationale is that HCP mitigation and restoration activities have a mandate to stay under 10% of the total occupied habitat or cease. Additionally, there is another clause in Provision M of the ITP that these activities should cease under certain low-flow triggers if undesirable impacts are encountered. As such, any impacts from the HCP measures or drought should be calculated independently for an accurate comparison in future drought years.

**Table 12. Calculated Incidental Take for the Fountain Darter per system based on impacted habitat.**

FOUNTAIN DARTER PARAMETERS	COMAL SYSTEM					SAN MARCOS SYSTEM				
	HCP Mitigation / Restoration	HCP Measures / Drought				San Marcos River			Spring Lake	
						HCP Mitigation / Restoration	HCP Measures / Drought		HCP Mitigation / Restoration	HCP Measures / Drought
	Landa Lake	Upper Spring Run*	Landa Lake	Old Channel	New Channel	All reaches	City Park	SLD and I35		
2023 Impacted Area (m <sup>2</sup> )	358	0	0	647	4,626	762	10,050	3,229	0	0
Total Occupied Habitat (m <sup>2</sup> )	109,685.08	3,564.25	48,461.82	23,660.20	33,998.81	41,978.30	29,409.81	12,568.49	53,190.59	53,190.59
% of Occupied Habitat Impacted	0.33%	0.00%	0.00%	2.74%	13.61%	1.82%	34.17%	25.69%	0.00%	0.00%
Corresponding Habitat Percentile Density (individual/m <sup>2</sup> )	1.50	1.50	1.50	1.50	1.50	1.50	3.50	3.50	--	--
Water Temperature Percentile Density adjustment	1.50	11.35	1.50	6.00	6.00	3.50	3.50	1.50	--	--
2023 Incidental Take Estimate	537.00	40,454.24	0.00	3,884.40	27,753.95	2,667.00	35,175.30	11,302.30	0	0
2023 TOTAL INCIDENTAL TAKE PER SYSTEM	72,630					49,145				

\* Although no spring to fall decrease in submerged aquatic vegetation was documented in the Upper Spring Run study reach, the extreme water temperatures resulted in the application of the Mean Fountain Darter Density to the full amount of occupied habitat in the Upper Spring Run Section.

### Comal Springs invertebrates:

Table 13 shows the incidental take calculated for the Comal Springs riffle beetle, Comal Springs dryopid beetle, and Peck's Cave amphipod relative to the HCP mitigation and restoration activities as well as the HCP measures and drought. For the Comal Springs riffle beetle, the percentage of impacted area was less than 25% so the 25<sup>th</sup> percentile density was applied (Table 9). For the Peck's Cave amphipod, the percentage of impacted areas was greater than 25% but less than 50% of the total occupied habitat and thus the median density (Table 9) was applied. As previously stated, only the mean is presently available for use in calculating incidental take for the Comal Springs dryopid beetle.

**Table 13. Calculated Incidental Take for the endangered Comal Springs invertebrates based on impacted habitat.**

COMAL INVERTEBRATES PARAMETERS	Comal Springs Riffle Beetle		Comal Springs Dryopid Beetle		Peck's Cave Amphipod	
	HCP Mitigation / Restoration	HCP Measures / Drought	HCP Mitigation / Restoration	HCP Measures / Drought	HCP Mitigation / Restoration	HCP Measures / Drought
2023 Impacted Area (m <sup>2</sup> )	0	379.1	0.0	119.7	0.0	553.6
Total Occupied Habitat (m <sup>2</sup> )	2,066	2,066	363	363	1,881	1,881
% of Occupied Habitat Impacted	0.00%	18.35%	0.00%	32.98%	0.00%	29.43%
Corresponding Percentile Density (individual/m <sup>2</sup> )	6.60	6.60	0.10	0.10	1.67	1.67
2023 Incidental Take Estimate	0	2,502	0	12	0	925
2023 TOTAL INCIDENTAL TAKE	2,502		12		925	

**San Marcos salamander:** Table 14 shows the incidental take calculated for the San Marcos salamander in the San Marcos system (San Marcos River and Spring Lake) relative to the HCP mitigation and restoration activities as well as the HCP measures and drought. In 2023, all calculated impacted area was below Spring Lake Dam so only the San Marcos River total occupied habitat area compared against. The percentage of impacted areas for HCP Measures / Drought was 17% of total occupied habitat and thus the 25<sup>th</sup> percentile density was applied for the San Marcos River (Table 9).

**Table 14. Calculated Incidental Take for the San Marcos salamander based on impacted habitat.**

SAN MARCOS SALAMANDER PARAMETERS	SAN MARCOS SYSTEM			
	San Marcos River		Spring Lake	
	HCP Mitigation / Restoration	HCP Measures / Drought	HCP Mitigation / Restoration	HCP Measures / Drought
2023 Impacted Area (m <sup>2</sup> )	4.3	252	0	0
Total Occupied Habitat (m <sup>2</sup> )	1,530	1,530	990	990
% of Occupied Habitat Impacted	0.3%	16.5%	0.0%	0.0%
Corresponding Percentile Density (individual/m <sup>2</sup> )	3.00	3.00	--	--
2023 Incidental Take Estimate	13	756	0	0
2023 TOTAL INCIDENTAL TAKE	769			

**Texas blind salamander:** There was no impacted habitat reported for the Texas blind salamander in 2023, thus no incidental take was calculated for the Texas blind salamander this year.

## COMPILATION OF RESULTS AND SUMMARY

Table 15 summarizes the 2023 impacted habitat area and incidental take attributed to the HCP relative to the ITP permit amount. Per the established methodologies, the Fountain Darter, San Marcos Salamander and Comal Invertebrates experienced incidental take during 2023.

**Table 15. Summary of Impacted Habitat (m<sup>2</sup>) and Incidental Take for HCP Covered Species compared against ITP Permit Amounts.**

COVERED SPECIES PER SYSTEM	IMPACTED HABITAT (m²)		HABITAT 2023 TOTAL (m²)	INCIDENTAL TAKE		2023 INCIDENTAL TAKE TOTAL	ITP Maximum Permit Amount	ITP Permit Maximum minus (combined first 11 years)
	HCP Mitigation / Restoration	HCP Measures / Drought		HCP Mitigation / Restoration	HCP Measures / Drought			
COMAL SYSTEM								
Fountain Darter	358.0	5,273.1	5,631.1	537.0	72,092.6	72,630	797,000	652,719
Comal Springs Riffle Beetle	0	379.1	379.1	0	2,502	2,502	11,179	5,642
Comal Springs Dryopid Beetle	0	119.7	119.7	0	12	12	1,543	1,510
Peck's Cave Amphipod	0	553.6	553.6	0	925	925	18,224	17,017
SAN MARCOS SYSTEM								
Fountain Darter	762.0	13,279.3	14,041.3	2,667.0	46,477.6	49,145	549,129	352,249
San Marcos Salamander	4.3	252.0	256.3	12.9	756.0	769	263,857	256,134
Texas Blind Salamander	0	0	0	0	0	0	10	10
Comal Springs Riffle Beetle	0	0	0	0	0	0	n/a	n/a
Comal Springs Dryopid Beetle	0	0	0	0	0	0	n/a	n/a

As shown in Table 15, only the Fountain Darter in the Comal System had a net disturbance when considering the project footprint for HCP mitigation and restoration activities overlaid on occupied habitat; while in the San Marcos system, the Fountain Darter and San Marcos Salamander had net disturbance per this assessment. In both systems, the ITP 10% disturbance rule (Item M [a]) was in compliance for 2023.

With the prolonged, extreme drought conditions experienced in 2023, incidental take was calculated for all four monitored species in the Comal system. As expected, incidental take calculation for the Comal system exceeded those observed in all previous drought years. The primary cause for this increase was low total system discharge which resulted in expanded amounts of exposed surface habitat characterized as Comal invertebrate occupied habitat. Over half of the incidental take calculated for the Fountain Darter was due to elevated water temperatures in the Upper Spring Run section. For the San Marcos system, incidental take calculations were also elevated above average conditions in 2023 because of the extreme drought. It is important to reemphasize that the San Marcos River in 2023 experienced the lowest total system discharge since the biological monitoring plan implementation in 2000. Not surprisingly, the 2023 spring to fall reductions in aquatic vegetation were considerably greater than other “drought” years characterized over the

past two decades. The resulting loss of aquatic vegetation / habitat and slightly elevated water temperatures recorded in 2023 led to these calculated conditions.

Finally, when examining 2023 results, habitat conditions in both systems were definitely impacted which was reflected by the larger than usual incidental take calculations. However, these larger numbers remain considerably below than those characterized in the Biological Opinion Drought of Record (DOR)-like scenario. As such, we are confident the incidental take numbers summarized in Table 15 and documented in this memorandum continue to justify the data sets used and methodologies employed relative to performing an incidental take assessment within the context of the Biological Opinion. It is understood that adjustments to data sets and/or methodologies may be employed based on feedback from the USFWS, HCP Science Committee, HCP participants, or others as deemed appropriate by the EARIP.

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