Effects of Altered Water Quality on Populations of Smalleye Shiner and Sharpnose Shiner in the Upper Brazos River

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Acronyms

Acronym	Definition
ANOSIM	analysis of similarity
BLM	biotic ligand model
LCRA-ELS	Lower Colorado River Authority – Environmental Services Lab
PRIMER	Plymouth Routines in Multivariate Ecological Research
RO	reverse osmosis
TCEQ	Texas Commission on Environmental Quality
TDS	total dissolved solids
TPDES	Texas Pollution Discharge Elimination System
TSWQS	Texas Surface Water Quality Standards
TWDB	Texas Water Development Board
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
WER	water effects ratio
YSI	Yellow Springs Instruments

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Introduction

Smalleye Shiner *Notropis buccula* and Sharpnose Shiner *N. oxyrhynchus* were federally listed as endangered species in 2014 by the U.S. Fish and Wildlife Service (USFWS 2014a). These cyprinids are endemic to the Brazos River Basin, historically occurring throughout the Brazos River mainstem (Moss and Mayes 1993). Populations of these species are now restricted to the upper Brazos River drainage upstream of Possum Kingdom Lake, an area designated as critical habitat in 2014 (USFWS 2014b).

Threats to Smalleye Shiner and Sharpnose Shiner include fragmentation of the river (e.g., Wilde and Urbanczyk 2011), alteration of natural flow regimes, and degradation of water quality including the discharge of treated effluent (USFWS 2014a, b, c, 2015). There are eleven facilities currently permitted to discharge treated domestic and industrial wastewater effluent through the Texas Pollution Discharge Elimination System (TPDES) into the upper Brazos River Texas Commission on Environmental Quality (TCEQ) stream segments 1208, 1238, and 1241, all of which are in the shiner's designated critical habitat. Some municipalities in the basin use reverse osmosis (RO) to treat brackish groundwater for use as a public drinking water source. The process of treating groundwater with reverse osmosis forces out various impurities and pollutants, leaving a less saline form of water suitable for use as drinking water. However, the wastewater concentrate that is left behind can contain very high concentrations of total dissolved solids (TDS) and other constituents, such as heavy metals and nitrates, that can be harmful to wildlife, especially aquatic organisms. One heavy metal of concern that is common to this part of the state, selenium, is a micronutrient that is necessary in very small amounts but can bioaccumulate and become toxic at amounts only slightly higher than those required to meet dietary needs. Elevated selenium can cause lethal or sub-lethal effects such as developmental deformities, decreased reproduction, or direct mortality in aquatic organisms (Lemly 1997). The sensitivity of smalleye and sharpnose shiners to most pollutants is largely unknown. Ostrand and Wilde (2009) reported the tolerance levels for adult Sharpnose and Smalleye Shiners for temperature (37°C to 41°C), salinity (14 ppt), and dissolved oxygen (2.1 mg/L); however, little is known of the tolerances of early life stages. The effects of high concentrations of selenium on the reproductive capabilities of Smalleye Shiner and Sharpnose Shiner are unknown and the effects of TDS and other physical and chemical stressors on the early life history stages of the smalleye and sharpnose shiners is also unknown. This study will provide data that can be used to assess the level of threat posed by water quality degradation in the shiners' designated critical habitat.

The objectives were to examine the effects that sources of point and non-point pollution are having on Smalleye Shiner and Sharpnose Shiner by collecting and analyzing water quality samples and trace metal residues in fish tissue from surrogate fish (Table 3) at Segments 1208, 1238, and 1241 of the Brazos River, and comparing the results to the current Texas Water Quality Standards and toxicity values referenced in the scientific literature.

Materials and Methods

Sample Locations

Water quality samples and fish tissue were collected quarterly at two sites (11871 and 13641) on the Brazos River above Possum Kingdom Lake in Segment 1208, one site (12022) on the Salt Fork Brazos River in Segment 1238, and one site (12029) on the Double Mountain Fork Brazos River in Segment 1241 (Figure 1. Surface water quality monitoring sites where water quality and fish tissues samples were collected in the Brazos River above Possum Kingdom Lake (1208), Salt Fork Brazos River (1238), and Double Mountain Fork Brazos River (1241). Site numbers from Texas Commission on Environmental Quality Surface Water Quality Monitoring Program.). The four sample sites are established water quality sites routinely monitored by the Surface Water Quality Monitoring Program (SWQM) at TCEQ (Table 1).

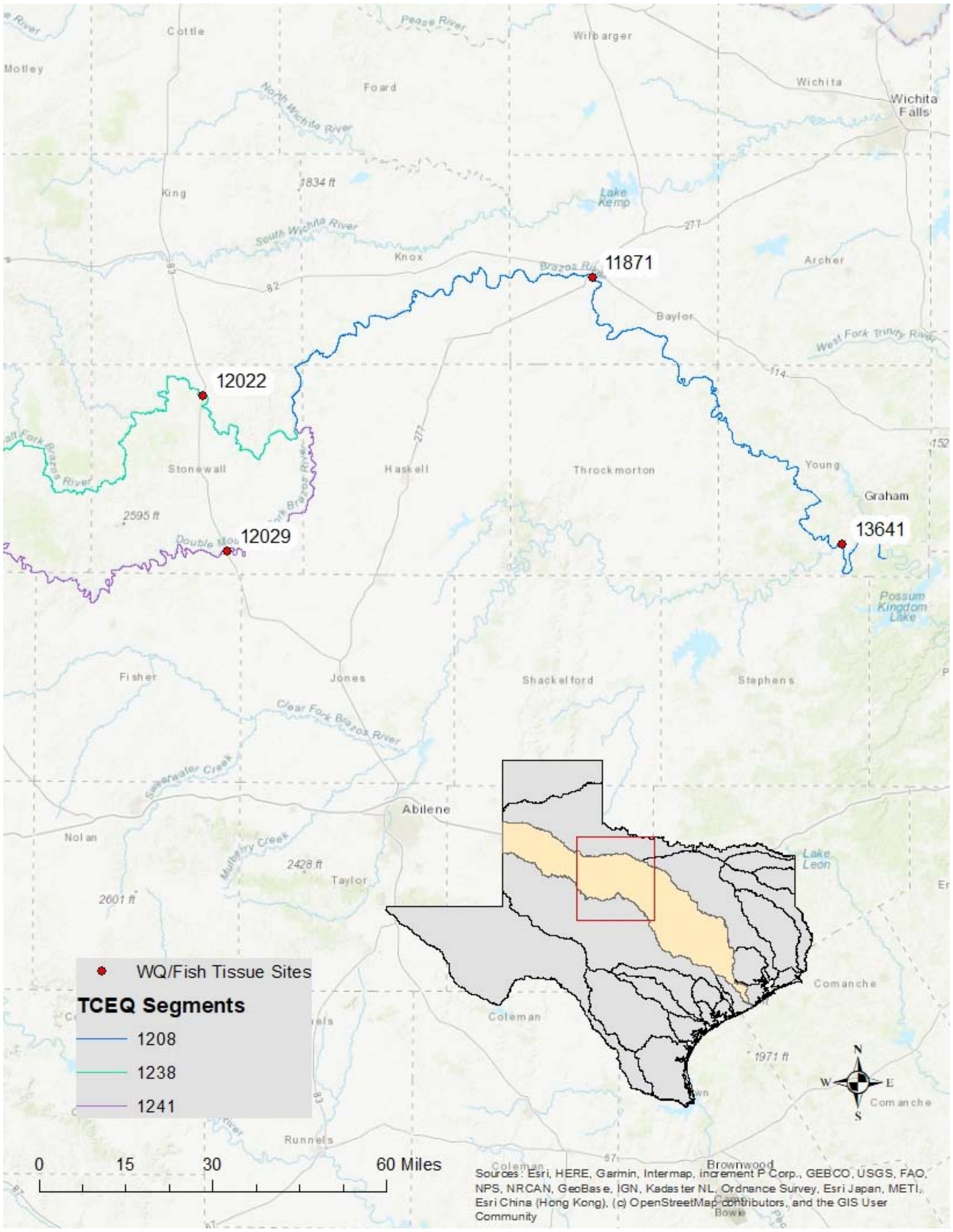

Figure 1. Surface water quality monitoring sites where water quality and fish tissues samples were collected in the Brazos River above Possum Kingdom Lake (1208), Salt Fork Brazos River (1238), and Double Mountain Fork Brazos River (1241). Site numbers from Texas Commission on Environmental Quality Surface Water Quality Monitoring Program.

Table 1. TCEQ Surface Water Quality Monitoring Stations and number of samples collected.

Site	Description	No. Water Samples	No. Fish Tissue Samples	Latitude	Longitude
11871	Brazos River at US 183 near Seymour, Texas	8	2	33.580772	-99.267662
13641	Brazos River at SH 67, south of Graham, Texas	8	2	33.023941	-98.644341
12029	Double Mountain Fork Brazos River at US 183, south of Aspermont, Texas	8	2	33.008686	-100.180275
12022	Salt Fork Brazos River at US 183, north of Aspermont, Texas	8	2	33.333889	-100.239998

Physicochemical In-situ Measurements (basic water chemistry)

An EXO1 YSI (Yellow Springs Instruments) multiparameter datasonde was used to measure water temperature, pH, specific conductivity, and dissolved oxygen at all four sample sites. Physicochemical data was collected at each site before any other field work was done to ensure that measurements were not affected. All measurements were taken in accordance with the TCEQ SWQM Procedures Manual Volume I (TCEQ 2014).

Water Quality

Water quality samples were collected in accordance with the TCEQ SWQM Procedures Manual Volume I (TCEQ 2014). Samples were collected from the centroid of flow in containers provided by the Lower Colorado River Authority–Environmental Lab Services (LCRA–ELS) and preserved on ice. Within 24 hours of collection, the water quality samples were delivered by hand to the LCRA-ELS for routine water chemistry, anions, nutrients, and total and dissolved metals analysis. Analyses followed U.S. Environmental Protection Agency (USEPA) methods identified in Table 2. Samples were collected in November 2016, February 2017, May 2017, August 2017, November 2017, February 2018, May 2018, and August 2018. Water quality sample results from May 2017 were not returned from the LCRA–ELS due to lab error. Water quality data were compared to established Federal and State water quality standards and screening criteria. Water quality criteria for some constituents are calculated based on ambient water hardness. Formulae for these criteria are presented in Appendix I. Many of the water quality values reported were below the lab's minimum detection limits. These non-detects in the water quality data were halved. Water quality data were further analyzed using PRIMER 7 (Plymouth Routines in Multivariate Ecological Research) statistical software. PRIMER 7 was used to conduct non-metric multidimensional scaling (n-MDS) of the data by ranking similarities between samples and then drafting a "map" of the data where samples that are more similar are placed closer than those they are dissimilar too. An ANOSIM (Analysis of Similarity) was used to compare water quality results between waterbodies as well. Total metals in fish tissue data were compared to the 85th percentile of the same constituents in samples from a national level study conducted by the United States Geology Survey (USGS; Hinck et al 2009).

Table 2. Water quality parameters analyzed by the Lower Colorado River Authority–Environmental Lab Services, U.S. Environmental Protection Agency methods used, and the limit of quantitation and limit of detection reported by the lab.

Analyte	USEPA Method	Limit of Quantitation (mg/L)	Limit of Detection (mg/L)
Total Alkalinity	SM3230B	20.0	20.0
Total Inorganic Carbon	SM5310C	0.500	0.200
Total Dissolved Solids	SM2540C	2500	2500
Total Suspended Solids	SM2540D	1.00	1.00
Volatile Suspended Solids	E160.4	1.00	1.00
Chloride	E300.0	1000	400
Sulfate	E300.0	5.00	2.00
Fluoride	E300.0	1000	400
Hardness	E2340B	1.32	NA
Ammonia-Nitrogen	E350.1	0.0200	0.0080
Nitrate - Nitrite	SM4500-NO3-H	0.0400	0.0160
Total Kjeldahl Nitrogen	E351.2	0.800	0.320
Total Phosphorus	E365.4	0.0200	0.00800
Chlorophyll-a	E445.0	0.500	0.200
Pheophytin-a	E445.0	0.500	0.200
Total Aluminum	E.200.8	0.250	0.200
Total Arsenic	E.200.8	0.0500	0.0350
Total Cadmium	E.200.8	0.0500	0.0200
Total Calcium	E200.7	2.00	0.700
Total Chromium	E.200.8	0.0500	0.0200
Total Copper	E.200.8	0.0500	0.0350
Total Iron	E200.7	0.500	0.200
Total Lead	E.200.8	0.0500	0.0200
Total Magnesium	E.200.7	2.00	0.700
Total Manganese	E.200.8	0.0500	0.0200
Total Nickel	E.200.8	0.0500	0.0350
Total Potassium	E200.7	2.00	0.700
Total Selenium	E.200.8	0.250	0.0750
Total Silver	E.200.8	0.0500	0.0200
Total Sodium	E200.7	20.0	7.00
Total Zinc	E.200.8	0.250	0.0850
Dissolved Aluminum	E200.8	0.0500	0.0400
Dissolved Arsenic	E200.8	0.0100	0.00700
Dissolved Cadmium	E200.8	0.00100	0.00400
Dissolved Calcium	E200.7	1.00	0.350
Dissolved Chromium	E200.8	0.0100	0.00700
Dissolved Copper	E200.8	0.0100	0.00400
Dissolved Iron	E200.7	0.250	0.100

Analyte	USEPA Method	Limit of Quantitation (mg/L)	Limit of Detection (mg/L)
Dissolved Lead	E200.7	0.0100	0.00400
Dissolved Magnesium	E200.7	1.00	0.350
Dissolved Manganese	E200.8	0.0100	0.00400
Dissolved Nickel	E200.8	0.0100	0.00700
Dissolved Potassium	E200.7	1.00	0.350
Dissolved Selenium	E200.8	0.0500	0.0150
Dissolved Silver	E200.8	0.0100	0.00400
Dissolved Sodium	E200.7	1.00	0.350
Dissolved Zinc	E200.8	0.0500	0.0170

Fish Tissue

Fish tissue was collected at the same sites as water quality in May of each year of the study (Table 3). Fish were collected with a 10 ft (3.05 m) seine with ¼-inch (6.35 mm) mesh. Individuals from surrogate species with similar diets and life histories to Smalleye Shiner and Sharpnose Shiner were collected for tissue analysis (Table 3). In order to minimize impacts to Sharpnose Shiner and Smalleye Shiner, short seine hauls were conducted, and the fish were worked down into the center of the net which was kept in the water while the surrogate species were culled out for preservation. Any shiners collected in seine hauls were immediately returned to the river. Seine hauls were conducted until at least 300 g of the tissue was collected except for one sample due to lack of fish in the stream (Table 3). Fish samples were wrapped in aluminum foil (shiny side out) and then placed in a zip-lock bag and preserved on ice. The samples were then transported the next day to the LCRA-ELS and whole-body composite tissue samples from each site were analyzed for total arsenic, cadmium, chromium, copper, lead, mercury, and selenium following the USEPA methods identified in Table 4. Fish tissue results were compared to results from a national level study conducted by the United States Geological Survey (Hinck et al. 2009). The LCRA-ELS analyzed the water samples in-house but subcontracted the whole-body tissue samples to North Water District Laboratory Services, Inc. (Woodlands, Texas).

Table 3. Surrogate fish species and total composite tissue weights collected in 2017 and 2018 at four sites on upper Brazos River drainages for whole-body tissue analysis.

Site	Date	Description	Species	Weight (g)
11871	5/15/2017	Brazos River at US 183/US 277	Plains Killifish *Fundulus zebrinus*	340
13641	5/15/2017	Brazos River Downstream of SH 67	Red Shiner *Cyprinella lutrensis*	300
12029	5/15/2017	Double Mountain Fork Brazos River at US 183	Red Shiner *Cyprinella lutrensis* Plains Minnow *Hybognathus placitus*	327/355
12022	5/16/2017	Salt Fork Brazos River at US 183	Red River Pupfish *Cyprinodon rubrofluviatilis*	495
11871	5/15/2018	Brazos River at US 183/US 277	River Carpsucker *Carpiodes carpio* Plains Killifish *Fundulus zebrinus*	370/261
13641	5/15/2018	Brazos River Downstream of SH 67	Red Shiner *Cyprinella lutrensis*	319
12029	5/15/2018	Double Mountain Fork Brazos River at US 183	Plains Minnow *Hybognathus placitus*	381
12022	5/14/2018	Salt Fork Brazos River at US 183	Red River Pupfish *Cyprinodon rubrofluviatilis*	185

Table 4. Fish tissue parameters analyzed by the North Water District Laboratory Services, Inc. The table includes USEPA methods used and the limit of quantitation and limit of detection reported by the lab.

Analyte	USEPA Method	Limit of Quantitation (mg/kg)	Limit of Detection (mg/kg)
Total Arsenic	SW846 3050B	1.00	1.00
Total Cadmium	SW846 3050B	0.100	0.100
Total Chromium	SW846 3050B	0.500	0.500
Total Copper	SW846 3050B	1.00	1.00
Total Lead	SW846 3050B	0.500	0.500
Total Mercury	SW846 7471B	0.020	0.020
Total Selenium	SW846 3050B	2.00	2.00

Results

Physicochemical Parameters

Instantaneous physicochemical field parameters were measured at each station during each sampling event using a hand-held multi-probe, including water temperature (°C), pH (standard units), dissolved oxygen (mg/L), and specific conductivity (µmhos/cm). Most of the field parameters sampled were within ranges that support aquatic life and met the established 2018 Texas Surface Water Quality Standards (TSWQS; TCEQ 2018) for segments 1208, 1238, and 1241 (Table 5).

Table 5. Texas Commission on Environmental Quality Surface Water Quality Standards for the Brazos River (1208), Salt Fork Brazos River (1238), and Double Mountain Fork Brazos River (1241) (PCR1 – Primary Contact Recreation).

Segment	1208	1238	1241
Segment Description	Brazos River Above Possum Kingdom Lake	Salt Fork Brazos River	Double Mountain Fork Brazos River
Recreation Use	PCR1	PCR1	PCR1
Aquatic Life Use	High	High	High
Domestic Water Supply Use	N/A	N/A	N/A
Chloride (mg/L)	5,000	28,060	2,630
Sulfate (mg/L)	2,000	3,470	2,400
Total Dissolved Solids (mg/L)	12,000	54,350	5,500
Dissolved Oxygen (mg/L)	5.0	5.0	5.0
pH Range (s.u.)	6.5 – 9.0	6.5 – 9.0	6.5 – 9.0
Indicator Bacteria (#/100 mL)	33	33	33
Temperature (°C)	35.0	33.8	35.0

Water temperature fluctuated seasonally and varied by site with temperature exceeding the TSWQS in the Brazos River at US 183 near Seymour, Texas on August 29, 2018 by approximately 2.1 °C (Figure 2). All pH values for all sites were within the TSWQS range of 6.5 to 9.0 °C. Dissolved oxygen values were greater than the TSWQS for high aquatic life use (5.0 mg/L) except for one measurement (3.3 mg/L) from the Brazos River at US 183 near Seymour, Texas (Figure 3). Dissolved oxygen also reached supersaturation in the Brazos River at SH 67 reached supersaturation in the winter of 2017, in the Brazos River at US 183 in both the winter and summer of 2018, and in the Double Mountain Fork Brazos River at US 183 in the winter of 2018 (Figure 3). Specific conductivity varied among sites but was several orders of magnitude greater in the Salt Fork Brazos River (Figure 4).

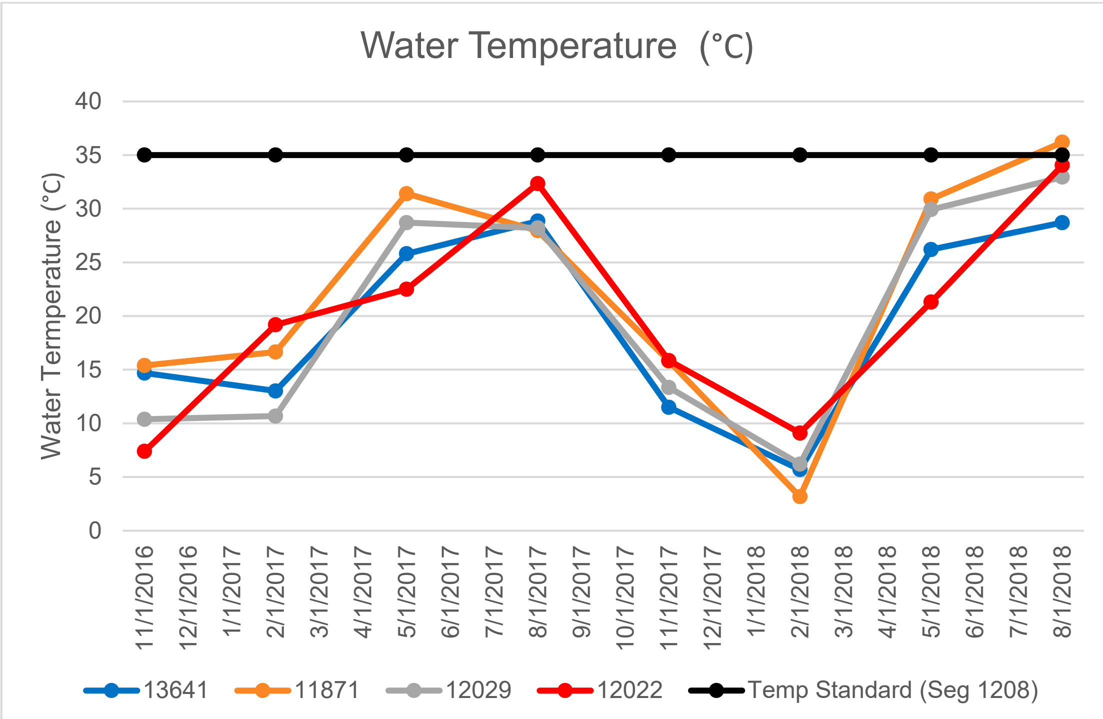

Figure 2. Water temperature measurements from Brazos River at SH 67 near Graham, Texas (13641), Brazos River at US 183 near Seymour, Texas (11871), Salt Fork of the Brazos River at US 183 north of Aspermont, Texas (12022), and Double Mountain Fork of the Brazos River at US 183 south of Aspermont, Texas (12029).

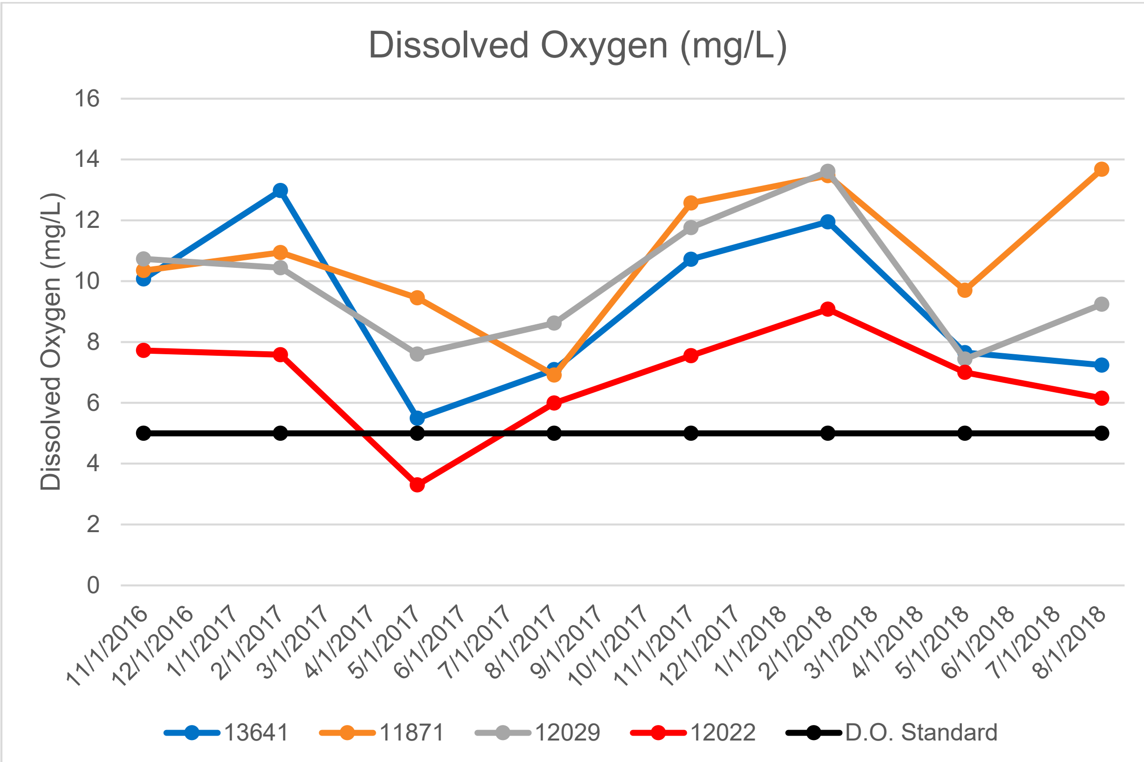

Figure 3. Dissolved oxygen measurements from Brazos River at SH 67 near Graham, Texas (13641), Brazos River at US 183 near Seymour, Texas (11871), Salt Fork of the Brazos River at US 183 north of Aspermont, Texas (12022), and Double Mountain Fork of the Brazos River at US 183 south of Aspermont, Texas (12029).

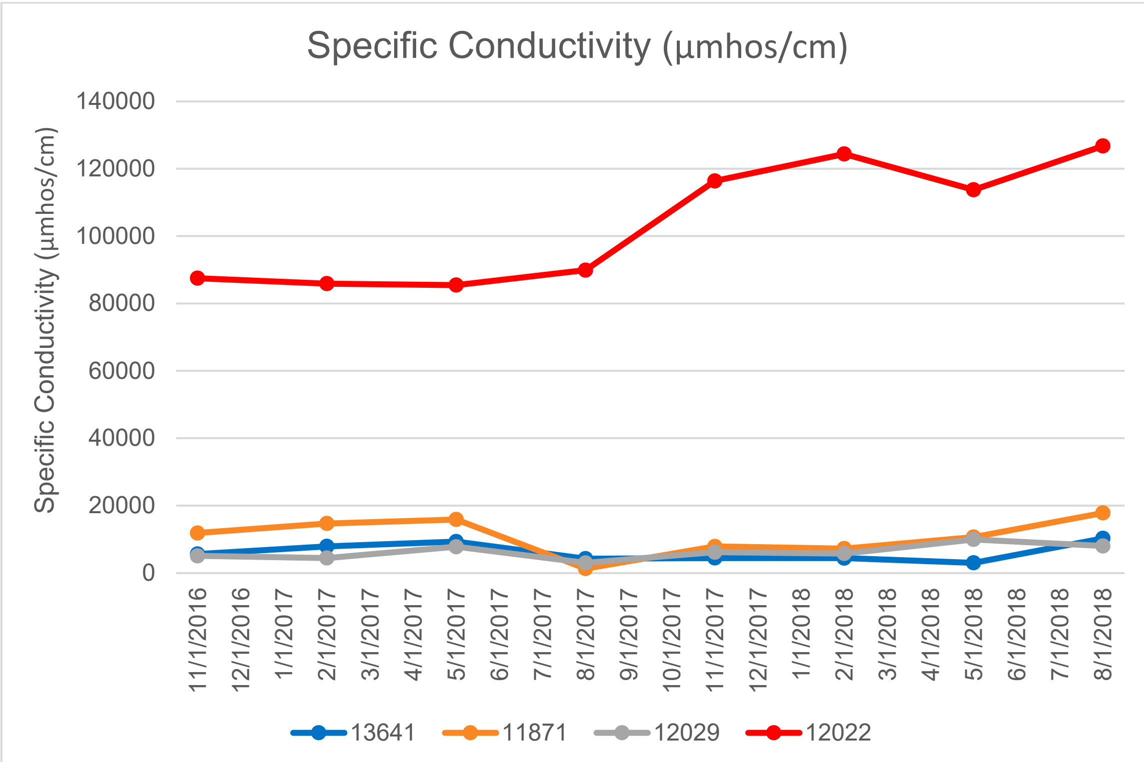

Figure 4. Specific conductivity measurements from Brazos River at SH 67 near Graham, Texas (13641), Brazos River at US 183 near Seymour, Texas (11871), Salt Fork Brazos River at US 183 north of Aspermont, Texas (12022), and Double Mountain Fork Brazos River at US 183 south of Aspermont, Texas (12029).

Water Chemistry

Routine Chemistry

Routine water chemistry parameters were collected quarterly for two years and included: total dissolved solids, total suspended solids, volatile suspended solids, chloride, sulfate, fluoride, total alkalinity, hardness, ammonia nitrogen, nitrate/nitrite, total Kjeldahl nitrogen, chlorophyll-a, and pheophytin-a. The TCEQ has established "general use" water quality standards for surface waters in the State of Texas and uses chloride, sulfate, and total dissolved solids (TDS) to assess if this use is being met (Table 5). Chloride, sulfate, and TDS were all less than the established TSWQS for Segment 1208 in the water quality samples collected from the Brazos River at SH 67 (13641). One chloride sample from the Brazos River at US 183 (11871) collected on August 29, 2018 exceeded the TSWQS for Segment 1208 and all sulfate and TDS samples were within TSWQS. For the Salt Fork Brazos River at US 183 (12022) six of the seven chloride results exceeded the TSWQS for Segment 1238, four of the seven sulfate samples exceeded the TSWQS, and all seven TDS results were greater than TSWQS. The Double Mountain Fork Brazos River (12029; Segment 1241) water quality samples met the "general use" requirements except for one TDS sample on May 14, 2018 with a result of 7,360 mg/L.

Nutrients

Ammonia nitrogen, nitrate/nitrite, total Kjeldahl nitrogen, chlorophyll-a, and pheophytin-a were sampled at each site during the study. The TCEQ does not have established surface water quality standards for nutrients in streams but has developed screening criteria for ammonia nitrogen, nitrite, total phosphorus and chlorophyll-a (Table 6).

Table 6. Texas Commission on Environmental Quality nutrient screening level criteria for ammonia nitrogen, nitrate, and chlorophyll-a for segments 1208, 1238, and 1241 of the Brazos River and mean exceedance values for the assessment units containing the sample sites (TCEQ 2020).

Parameter	Screening Level Criteria	Segment 1208 Mean Exceedance Values	Segment 1238 Mean Exceedance Values	Segment 1241 Mean Exceedance Values
Ammonia nitrogen	0.33 mg/L	0.41 mg/L	0.85 mg/L	N/A
Nitrate	1.95 mg/L	2.77 mg/L	N/A	N/A
Total Phosphorus	0.69 mg/L	1.72 mg/L	2.00 mg/L	4.14 mg/L
Chlorophyll-a	14.1 µg/L	70.20 µg/L	17.03 µg/L	35.35 µg/L

The TCEQ Draft 2020 Texas Integrated Report of Surface Water Quality (IR; TCEQ 2020) lists segments 1208 (Brazos River Above Possum Kingdom Lake) and 1241 (Double Mountain Fork Brazos River) as having screening level concerns for chlorophyll-a meaning that chlorophyll-a levels routinely exceed the screening level of 14.10 µg/L. One chlorophyll-a sample at 19.8 µg/L exceeded the screening criteria of 14.10 µg/L in the Brazos River at US 183 on August 29, 2018 and two exceeded the criteria in the Brazos River at SH 67 with results of 37.1 µg/L and 51.7 µg/l on August 16, 2017 and August 29, 2018, respectively. Two chlorophyll-a results exceeded the screening criteria in the Double Mountain Fork Brazos River at US 183 on August 15, 2017 and August 29, 2018 with results of 42.5 µg/L and 27.3 µg/L, respectively. Total phosphorus exceeded the screening criteria once in the Brazos River at US 183 near Seymour (11871). Ammonia nitrogen and nitrate/nitrite values for all sites were below the established screening level criteria listed in Table 6.

Total and Dissolved Metals in Water and Fish Tissue

Water samples from each site were analyzed by LCRA-ELS for a suite of trace metals including total and dissolved forms of aluminum, arsenic, cadmium, calcium, chromium, copper, iron, lead, magnesium, manganese, nickel, potassium, selenium, silver, sodium, and zinc. Generally, the dissolved forms of these metals are used when assessing harm to aquatic life as they tend to be the more bioavailable form (TCEQ 2010). Total forms of arsenic, cadmium, chromium, copper, lead, mercury, and selenium were analyzed in fish tissue. Average values for total and dissolved metals in water are presented in Table 7 and actual values for fish tissue are reported in Table 8.

Table **7**. Average total and dissolved metals in water samples from four sites along the upper Brazos River drainage from November 2016 through August 2018 (N=7). Results are reported as micrograms per liter (µg/L). Total and dissolved results for cadmium and silver were below the LCRA-ELS minimum detection limits (MDL). Samples were collected from Brazos River at US 183 (11871), Brazos River at SH 67 (13641), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Metal	11871	13641	12029	12022
Total Aluminum (µg/L)	3211.4	1912.0	1829.5	437.6
Dissolved Aluminum (µg/L)	298.9	147.9	271.9	86.2
Total Arsenic (µg/L)	7.9	5.4	6.6	45.6
Dissolved Arsenic (µg/L)	4.6	4.8	5.7	18.3
Total Cadmium (µg/L)	< MDL	< MDL	< MDL	< MDL
Dissolved Cadmium (µg/L)	< MDL	< MDL	< MDL	< MDL
Total Calcium (mg/L)	344	205	355	1739
Dissolved Calcium (mg/L)	306	219	355	1494
Total Chromium (µg/L)	7.1	2.4	4.8	5.4
Dissolved Chromium (µg/L)	1.2	1.1	1.2	3.3
Total Copper (µg/L)	7.3	3.0	2.9	13.5
Dissolved Copper (µg/L)	3.1	3.7	2.7	25.0
Total Iron (µg/L)	1874.9	1054.3	1029.2	475.9
Dissolved Iron (µg/L)	415.0	512.0	240.3	248.0
Total Lead (µg/L)	11.9	1.3	2.3	2.7
Dissolved Lead (µg/L)	1.3	1.1	1.1	6.8
Total Magnesium (mg/L)	117	69	92	446
Total Manganese (mg/L)	105	114	62	463
Dissolved Manganese (mg/L)	42	26	46	452
Total Nickel (µg/L)	7.9	4.8	6.5	27.3
Dissolved Nickel(µg/L)	4.5	3.8	6.2	32.4
Total Potassium (mg/L)	17	12	15	125
Dissolved Potassium (mg/L)	15	13	15	102
Total Selenium (µg/L)	10.9	5.7	5.6	132.2
Dissolved Selenium (µg/L)	12.4	11.0	9.4	109.0
Total Silver	< MDL	< MDL	< MDL	< MDL
Dissolved Silver	< MDL	< MDL	< MDL	< MDL
Total Sodium (mg/L)	1823	1093	912	27700
Dissolved Sodium	1646	1032	874	24671
Total Zinc (µg/L)	15.6	11.3	12.1	33.6
Dissolved Zinc (µg/L)	16.4	14.8	10.5	223.0

Table 8. Whole-body composite fish tissue concentrations for total metals for the Brazos River at US 183 (11871), Brazos River at SH 67 (13641), Double Mountain Fork Brazos River at US 183 (12029), and Salt Fork Brazos River at US 183 (12022). All results are reported in mg/kg (wet weight).

Site	11871	13641	12029	12022	11871	13641	12029	12022
Date	5/15/17	5/15/17	5/15/17	5/16/17	5/14/18	5/14/18	5/14/18	5/15/18
Arsenic	<2.50	<2.50	<2.50	<2.50	<1.00	<1.00	<1.00	2.3
Cadmium	<0.500	<0.500	<0.500	<0.500	<0.100	<0.100	<0.100	<0.100
Chromium	<2.00	<2.00	<2.00	<2.00	0.731	<0.500	0.676	1.64
Copper	<2.50	<2.50	<2.50	3.9	1.18	<1.00	1.39	5.26
Lead	<2.50	<2.50	<2.50	<2.50	<0.500	<0.500	<0.500	0.725
Mercury	0.0647	<0.0435	0.0542	<0.0500	<0.020	0.022	0.026	0.021
Selenium	<4.50	<4.50	<4.50	<4.50	<2.00	<2.00	<2.00	<2.00

Aluminum

Aluminum is the most common metal in the Earth's crust and is commonly found in groundwater and surface water. Aluminum typically enters surface waters through natural weathering processes and can also be added to water through the discharge of industrial process water and stormwater. In higher concentrations, aluminum can cause harm to aquatic organisms by inhibiting respiratory processes (USEPA 2018). The USEPA (2018) published ambient water quality criteria for total aluminum for the protection of aquatic organisms in 2018 and recommended a range of 1 – 4,800 µg/L for acute effects and a range of 0.63 – 3,200 µg/L for chronic effects. The actual criteria are site specific and are based on pH, hardness, and dissolved organic carbon. Total and dissolved aluminum in water were sampled at each site quarterly for two years. Total aluminum concentrations were detected at all sites and concentrations across sites varied. Total aluminum in water concentrations were highest in the sample collected from the Brazos River at US 183 in August 2017 (3,211 ug/L) (Figure 5). Several samples were non-detect for dissolved aluminum in water, with all sites in the Salt Fork Brazos River at US 183 showing non-detect except for the sample collected in February 2017. Similarly, high concentrations were found in the sample collected in August of 2017 at the Brazos River at US 183 and in the Double Mountain Fork Brazos River at US 183 (Figure 6). The USEPA has not recommended criteria for dissolved aluminum, however, TCEQ has set a water quality standard of 991 µg/L for acute effects in freshwater (TCEQ 2018). Only one of the samples collected exceeded this standard for dissolved aluminum in water and was collected from the Brazos River at US 183 with a concentration of 1,300 µg/L. Total aluminum in fish tissue was not analyzed.

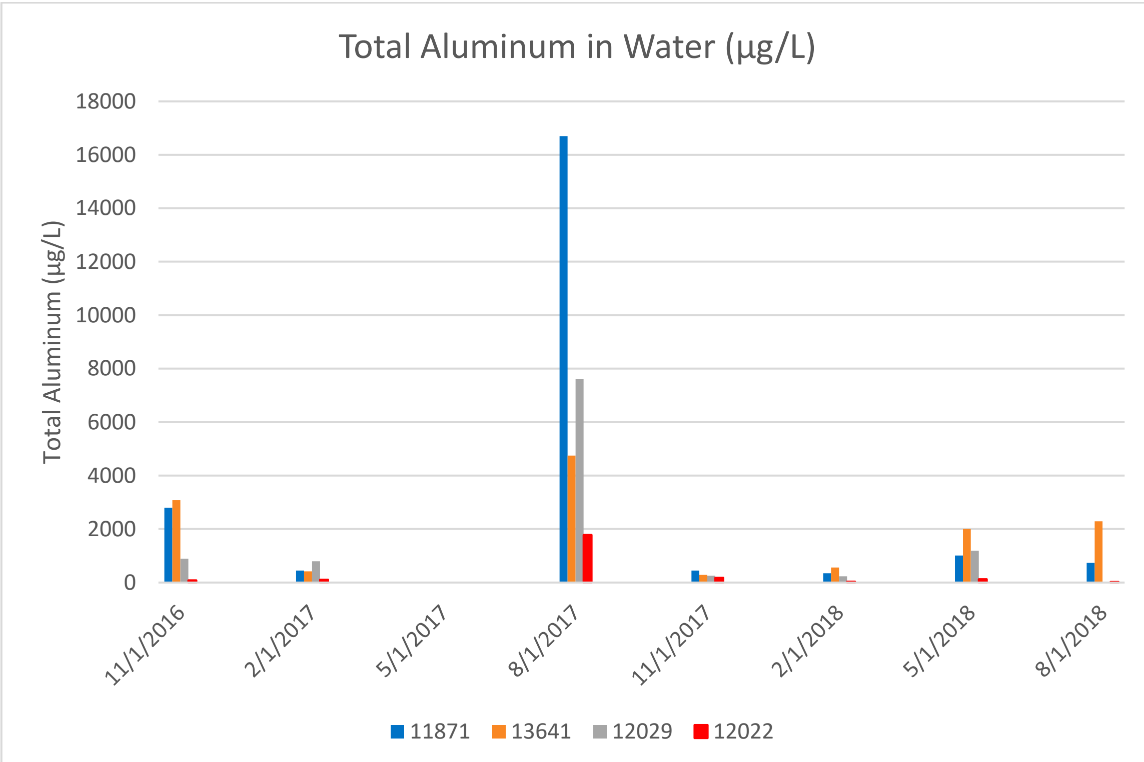

Figure 5. Total aluminum for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

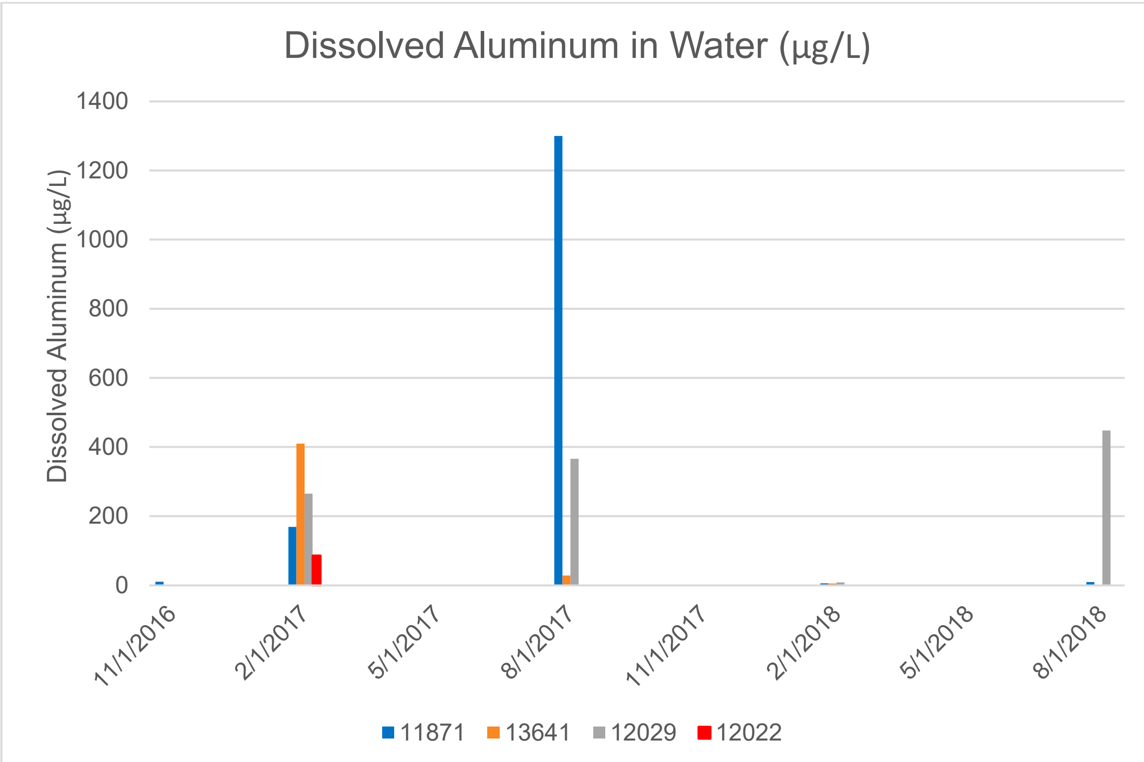

Figure 6. Dissolved aluminum for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Arsenic

Arsenic is a naturally occurring metal found in both ground and surface waters. Arsenic can be introduced to water by natural weathering processes and through anthropogenic sources such as pesticides and herbicides. Arsenic in water has been shown to cause osmoregulation issues and problems with growth and reproduction; its toxicity is dependent on the ambient water pH and the particular species of arsenic (Kumari et al. 2017). The TCEQ has established a surface water quality standard for dissolved arsenic of 340 µg/L for acute effects and 150 µg/L for chronic effects to aquatic life (TCEQ 2018).

Total arsenic in water was found at each site and in each sample during this study. Total arsenic in water the Brazos River at SH 67 averaged 5.4 µg/L, 7.9 µg/L in the Brazos River at US 183, 6.6 µg/L in the Double Mountain Fork Brazos River at US 183, and 4.5 µg/L in the Salt Fork Brazos River at 183. Concentrations of total arsenic in water remained relatively low with the exception of one sample from the Salt Fork Brazos River at US 183 in May 2018 with a concentration of 188 µg/L (Figure 7). Dissolved arsenic in water was collected during each site visit as well and was detected in each sample except for the sample collected in May 2018 which was below the LCRA-ELS minimum detection limit of 10 µg/L (Figure 8). Dissolved arsenic concentrations were low, with a slight increase occurring in August of 2017 and a larger increase in August 2018. All dissolved arsenic in water values from this study were less than the established water quality standards.

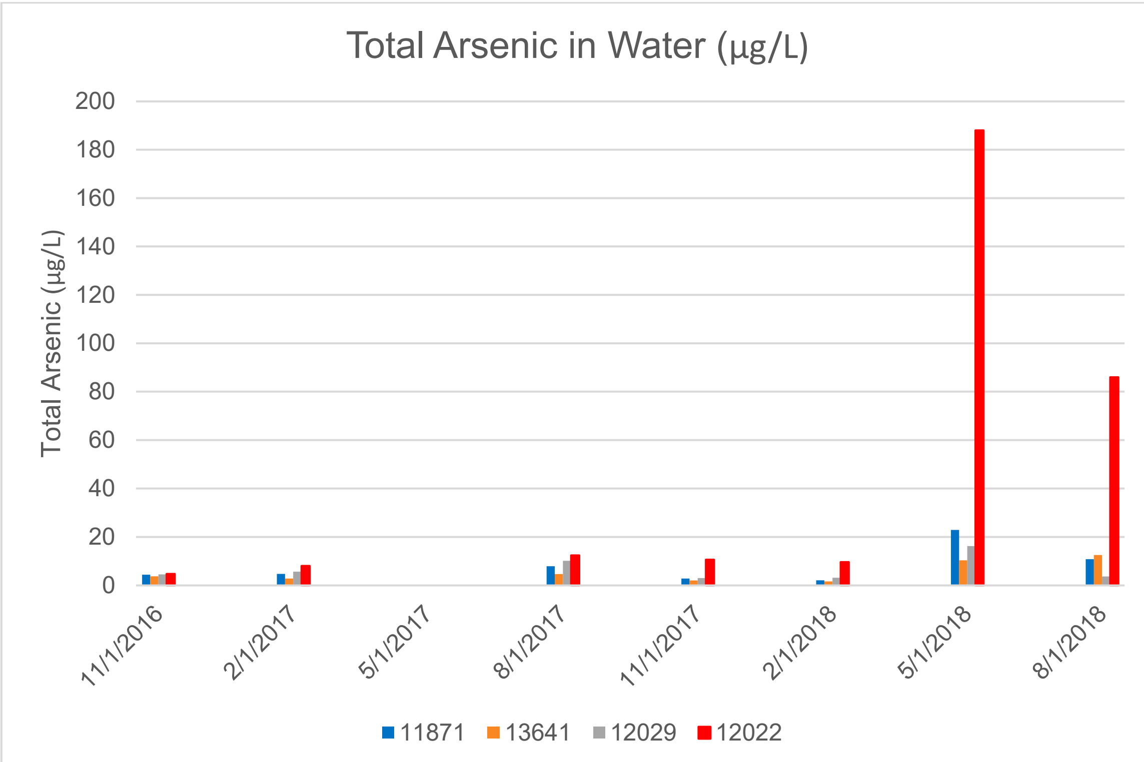

Figure **7**. Total arsenic for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

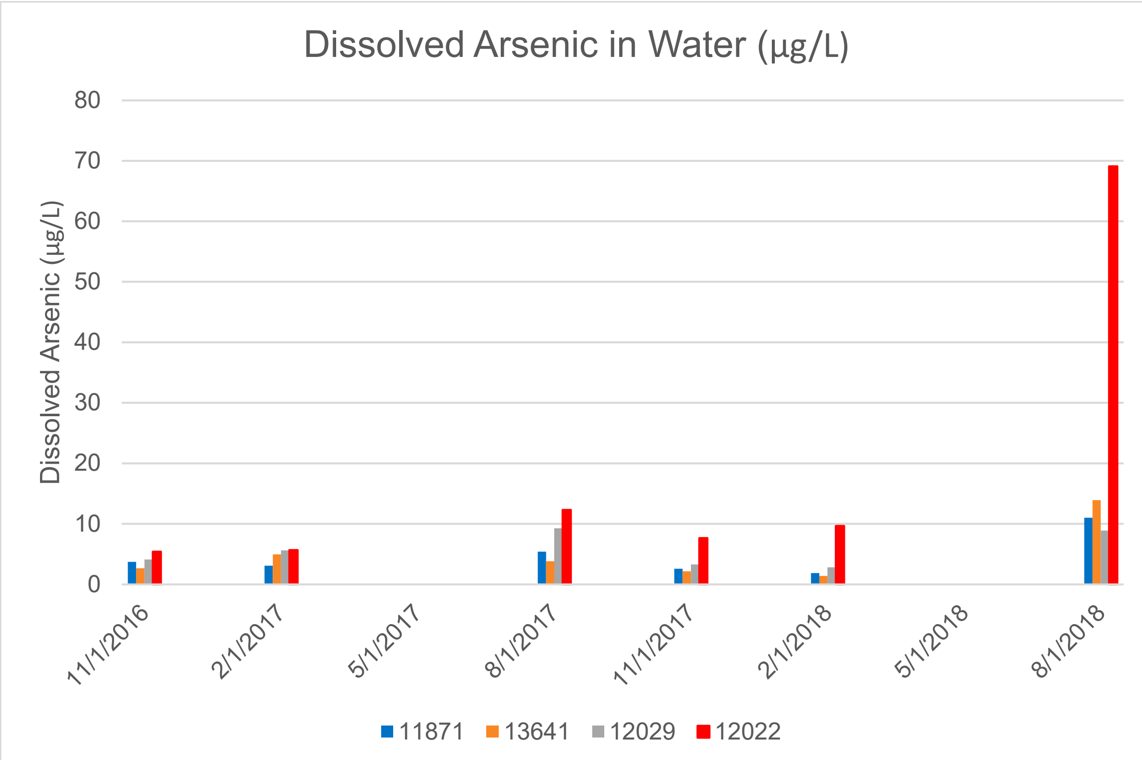

Figure 8. Dissolved arsenic for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Fish tissue samples were collected at each site during the spring (May) sample event. Total arsenic in tissue was analyzed and was below detection limits for all fish except for the Salt Fork Brazos River at US 183 in May 2018 where the concentration in Red River Pupfish *Cyprinodon rubrofluviatilis* whole-body composite tissue samples was 2.3 mg/kg (wet weight). This result is an order of magnitude higher than the 85th percentile for arsenic in whole-body tissue of 0.27 mg/kg (listed as ug/g) reported by Hinck et al. (2009) which reported total metals in fish tissue from larger bodied, longer lived fish from nine large river basins across the United States, including the Rio Grande in Texas.

Cadmium

Cadmium is a naturally occurring metal and can enter the water through natural processes but a large percentage of the cadmium in surface waters is from anthropogenic sources (USEPA 2016). Aquatic organisms are sensitive to cadmium with acute exposure causing mortality and chronic exposure affecting growth, reproduction, behavior, and immune and endocrine systems (USEPA 2016). The USEPA has recommended both acute and chronic water quality criteria for dissolved cadmium in water of 1.8 µg/L and 0.72 µg/L, respectively (based on ambient water hardness of 100 mg/L) (USEPA 2016). The TCEQ has established surface water quality standards to protect aquatic life from both acute and chronic effects of cadmium (TCEQ 2018). The acute and chronic criteria are both based on formulas which use ambient water hardness to calculate site specific standards. Based on the ambient water hardness measured during this study the average cadmium acute and chronic criteria would vary between sites (Table 9).

Table 9. Average acute and chronic water quality criteria for dissolved cadmium in water for four sites in upper Brazos River drainages. These criteria were calculated using the TCEQ's formulas outlined in the 2018 Texas Surface Water Quality Standards and ambient water hardness collected during the study.

Station	Acute Criteria (µg/L)	Chronic Criteria (µg/L)
11871	105.8	1.4
13641	64.1	1.0
12029	100	1.4
12022	463.4	4.2

Total cadmium and dissolved cadmium in water, and cadmium in whole-body fish tissue were analyzed and all results were below the LCRA-ELS minimum detection limits for all sites except for one total cadmium in water value of 25 µg/L from the Salt Fork Brazos River at US 183 on May 15, 2018.

Calcium

Calcium in a common alkaline metal that is abundant in both groundwater and surface water. Calcium is necessary for many biological processes and forms compounds such as calcium carbonate, which is important in aquatic systems to buffer pH levels appropriate for aquatic life. There are no established surface water quality standards for calcium in water, but calcium carbonate is frequently measured as a critical aspect of water quality in terms of water hardness and alkalinity, and a vast amount of information is available regarding the amount of calcium carbonate in surface and ground waters.

Total calcium in water was measured at each site. Concentrations of total calcium remained constant with levels increasing in 2018 likely due to lower flows in the watershed. Total calcium levels in the Salt Fork Brazos River were an order of magnitude higher than the Brazos River and Double Mountain Fork Brazos River (Figure 9). Dissolved calcium in water concentrations were similar to total calcium concentrations in that the concentrations increased in 2018 and were higher in the Salt Fork Brazos River (Figure 10).

Calcium was not analyzed in the fish tissue samples.

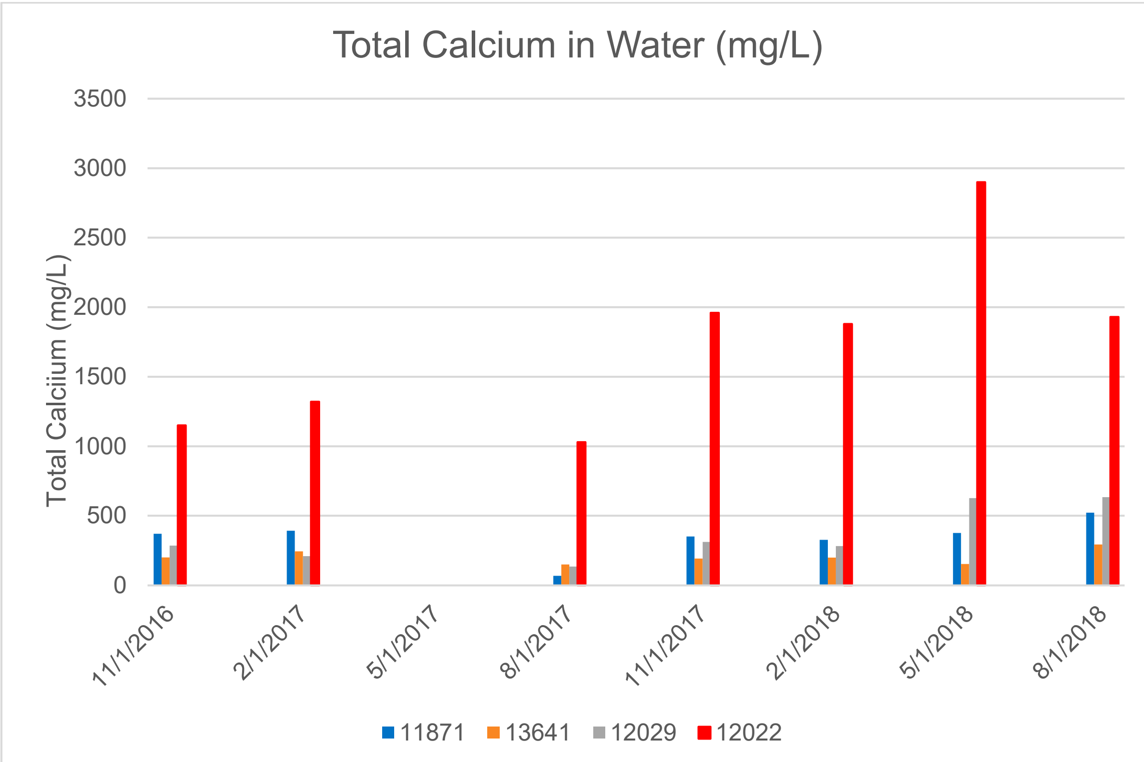

Figure 9. Total calcium for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

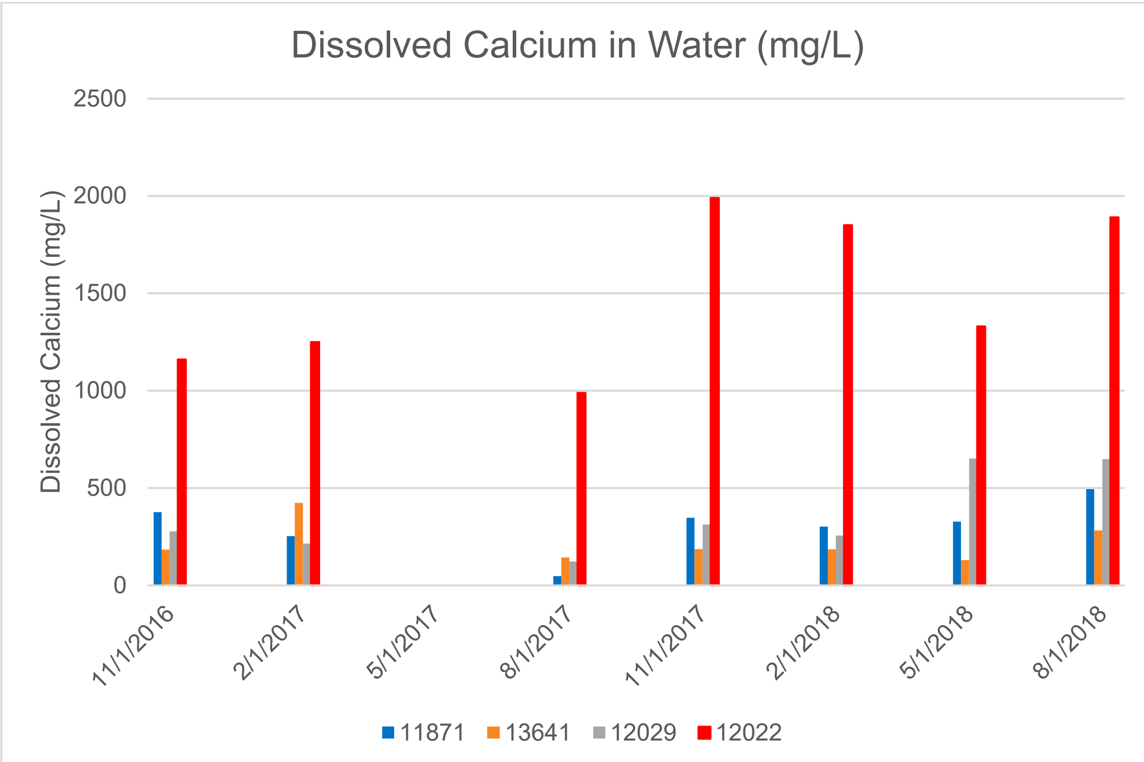

Figure 10. Dissolved calcium collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Chromium

Chromium is a metal that is present in the environment in trace amounts and, as a result of industrial processes, has become a pollutant of concern in certain parts of the world. Chromium exists naturally in several valent states. The trivalent and hexavalent forms of chromium can be toxic to aquatic life, causing mortality in fish at acute levels and issues with hematology and behavior at chronic levels (Aslam and Yousfzai 2017). The TCEQ has established TSWQS for both trivalent and hexavalent chromium based on the ambient water hardness (TCEQ 2018). Acute and chronic criteria for dissolved trivalent chromium in water were calculated using the hardness values measured at each site during the study (Table 10). The TCEQ has established screening criteria for trivalent chromium for the protection of aquatic life for the portion of the Brazos River containing station 11871, which are 3,263.55 µg/L (acute) and 364.62 µg/L (chronic) (TCEQ 2018).

Table 10. Average acute and chronic dissolved chromium in water criteria for the protection of aquatic life. Values were determined using the TCEQ's formula which uses ambient water hardness. Water hardness values collected during the study were used to calculate a criterion for each sample which were then averaged for each site.

Site	Average Acute Chromium Criteria (µg/L)	Average Chronic Chromium Criteria (µg/L)
11871	4,700.4	611.4
13641	3,103.0	403.6
12029	4,492.2	584.3
12022	16,576.3	2,156.2

Total chromium in water was measured at each site and twenty of the twenty-eight samples were below the LCRA-ELS minimum detection limit (Figure 11). The highest total chromium levels were from the Salt Fork Brazos River at US 183 with a value of 25 µg/L in May 2018. Dissolved chromium was measured with similar results. Twenty of the twenty-eight samples were below the LCRA-ELS minimum detection limit (Figure 12) and the highest levels were again from the Salt Fork of the Brazos River at US 183. All results for dissolved chromium in water were below the established water quality standards.

Total chromium was measured in whole-body fish tissue at all four sites in the spring (May) of each year of the study (Figure 13). Levels of total chromium in tissue were below the LCRA-ELS minimum detection limit of 0.5 mg/kg at each site in 2017, but were detected in fish at three sites in 2018; the Brazos River at US 183 (0.731 mg/kg), the Double Mountain Fork of the Brazos River (0.676 mg/kg), and the Salt Fork of the Brazos River (1.64 mg/kg). These values are all below the 85th percentile (2.38 mg/kg) reported by Hinck et al. (2009).

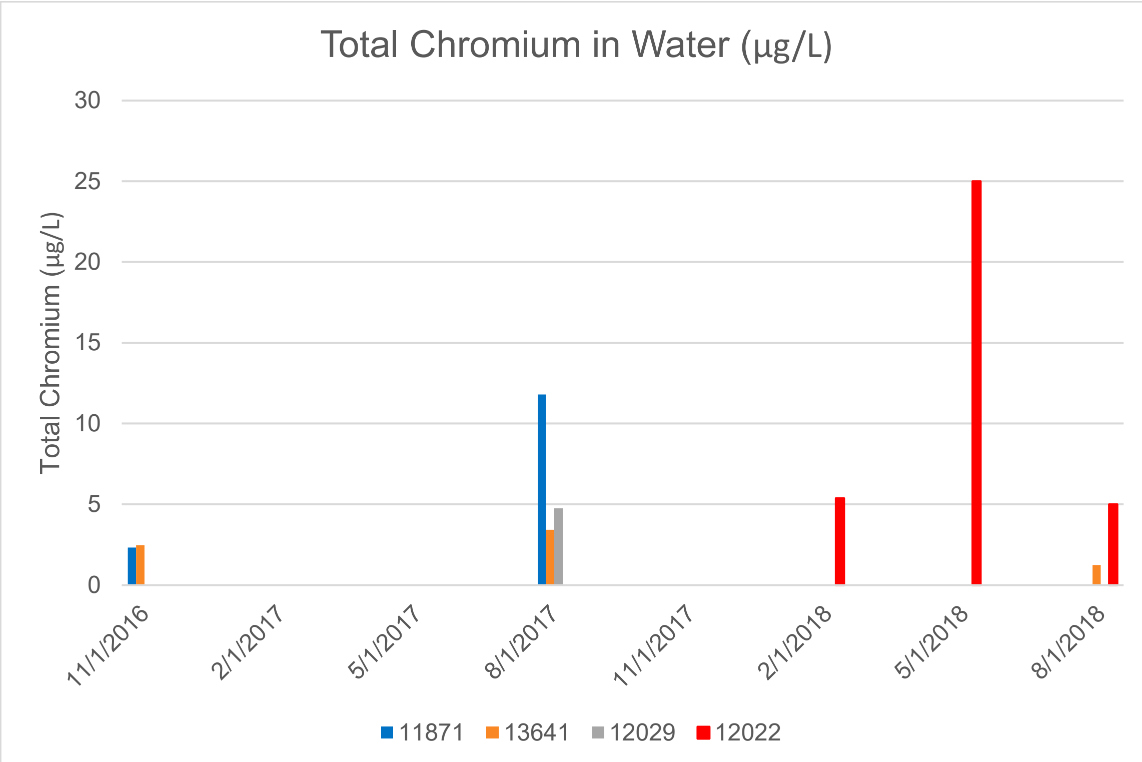

Figure 11. Total chromium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

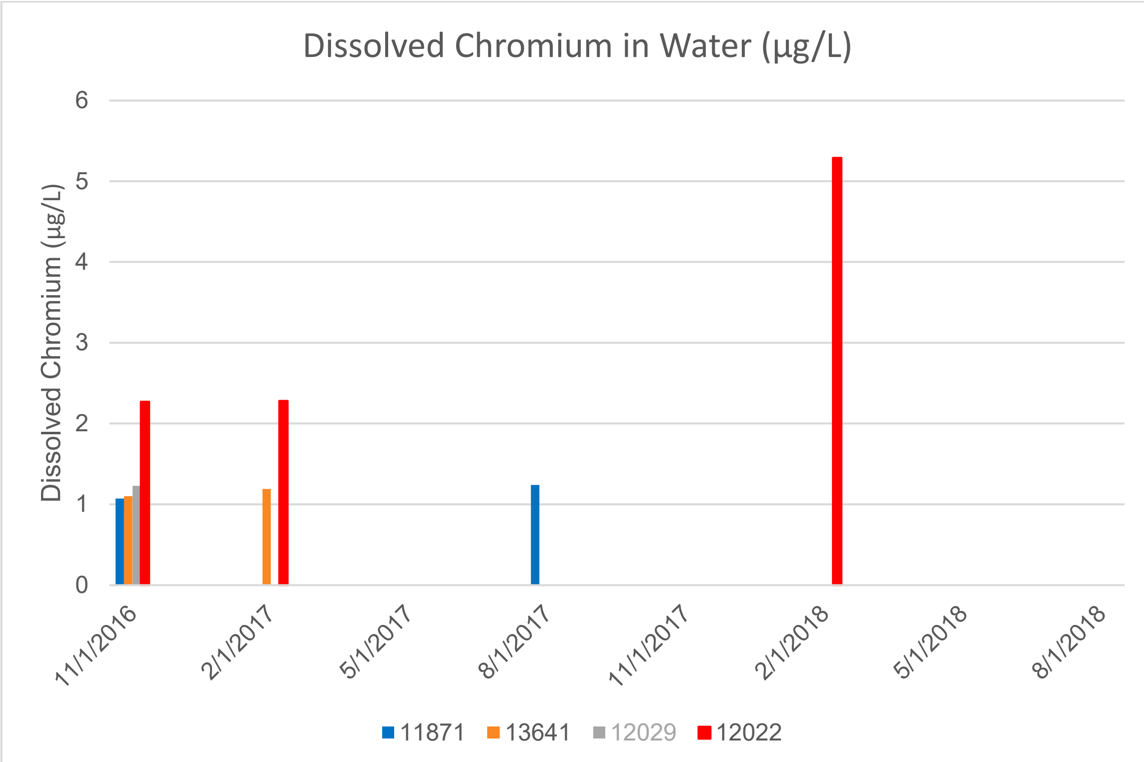

Figure 12. Dissolved chromium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

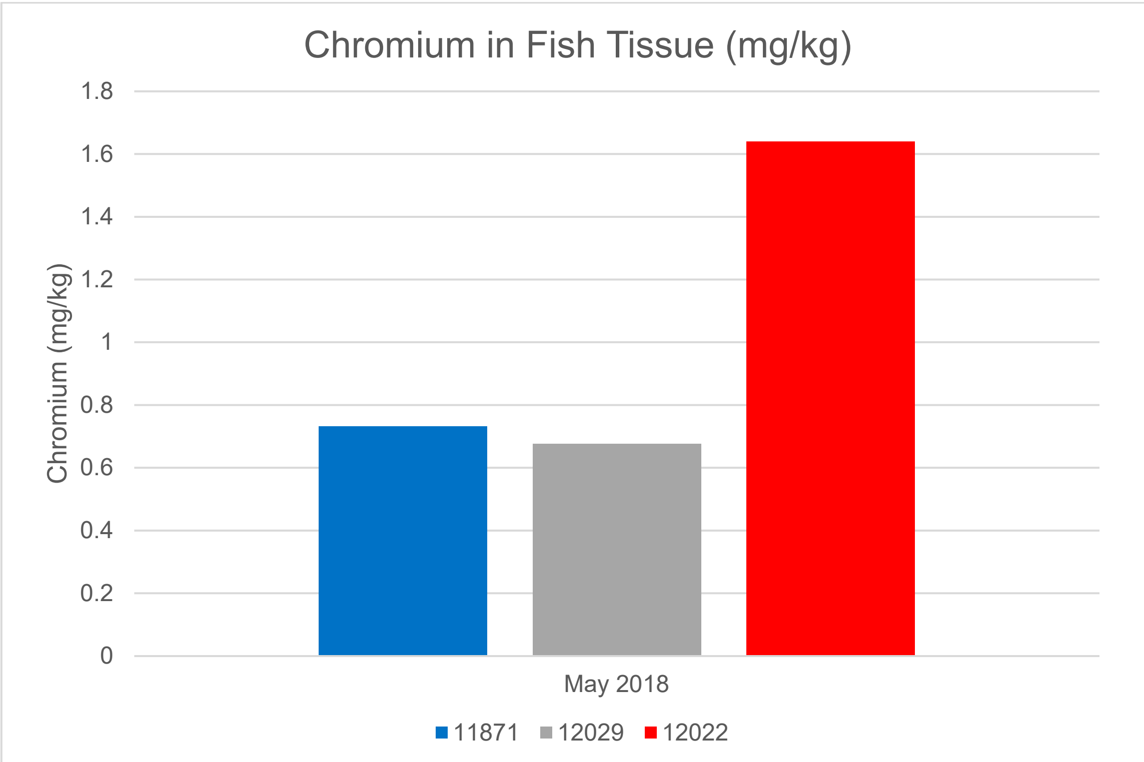

Figure 13. Chromium in whole-body composite fish tissue (mg/kg – wet weight) collected in May 2018 at the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Copper

Copper is a naturally occurring element and is often detected in both surface water and groundwater. Copper is an essential nutrient and necessary at low levels but can become toxic at higher levels. Copper can enter surface waters through natural weathering processes and through anthropogenic sources such as industrial discharges. The USEPA has established the Biotic Ligand Model (BLM) which sets dissolved copper criteria in surface waters to protect aquatic life based on ambient water characteristics such as hardness, pH, dissolved organic carbon, temperature, and the concentration of certain salts (USEPA 2007). The TCEQ uses a model based on ambient water hardness (TCEQ 2018) and criteria calculated based on the hardness values collected during this study are listed in Table 11. The TCEQ established criteria for the portion of the Brazos River containing sites 13641 and 11871. The acute and chronic screening criteria for 13641 for dissolved copper in water are 89.79 µg/L and 35.72 µg/L, respectively and the acute and chronic screening criteria for 11871 for dissolved copper are 105.77 µg/L and 35.72 µg/L (TCEQ 2018).

Table 11. Acute and chronic dissolved copper screening criteria based on the ambient water hardness collected during the study period (Nov 2016 through August 2018).

Site	Copper Acute Criteria (µg/L)	Copper Chronic Criteria (µg/L)
11871	162.8	85.9
13641	100.1	55.5
12022	154.5	81.9
12029	685.9	319.3

Total copper in water was collected at each site for the two years of the study. Copper was detected during each sample event except the May 2018 samples in both Brazos River sites and in the Double Mountain Fork Brazos River at US 183, and in the August 2018 sample at the Salt Fork Brazos River at US 183. The highest values for total copper were in the Salt Fork of the Brazos River (Figure 14). Dissolved copper in water was collected at the four sample sites and was found at each site with five of the 28 samples resulting in non-detects (Figure 15). No results for dissolved copper in water were above the established acute or chronic screening level criteria.

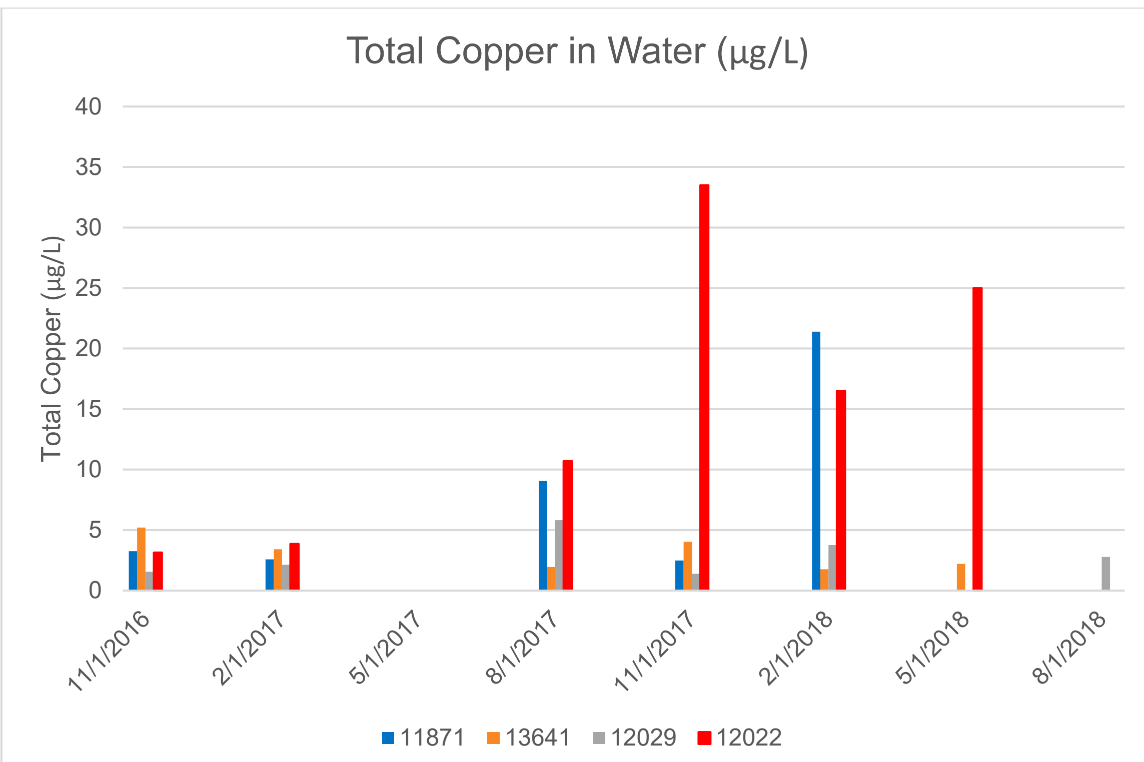

Figure 14. Total copper in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

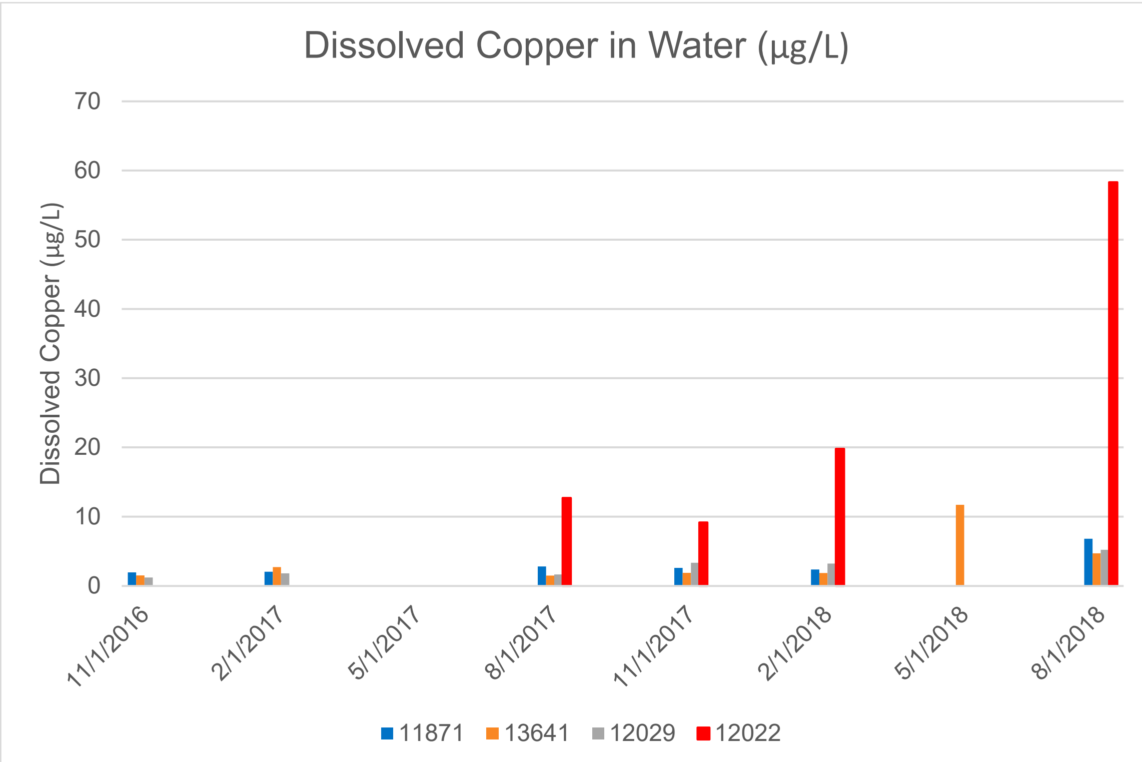

Figure 15. Dissolved copper in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Total copper in fish tissue was analyzed from whole-body composite samples collected in the spring (May) of each year of the study. Total copper was detected in fish tissue samples from the Salt Fork Brazos River at US 183 in 2017, and in the Brazos River at US 183, Double Mountain Fork Brazos River at US 183, and Salt Fork Brazos River at US 183 in May 2018 (Figure 16). Hinck et al. (2009) reported an 85^{th} percentile for copper in fish tissue of 1.26 mg/kg (wet weight) and a maximum of 3.92 mg/kg (wet weight). Total copper in fish tissue from the Salt Fork Brazos River at US 183 are well above the 85^{th} percentile and maximum concentrations from the Hinck study, with whole body composites for Red River Pupfish at 3.9 mg/kg in May 2017 and 5.26 mg/kg in May 2018. Total copper was also found in whole body composites of Plains Minnow *Hybognathus placitus* at 1.29 mg/kg from the Double Mountain Fork Brazos River at US 183 in May 2018.

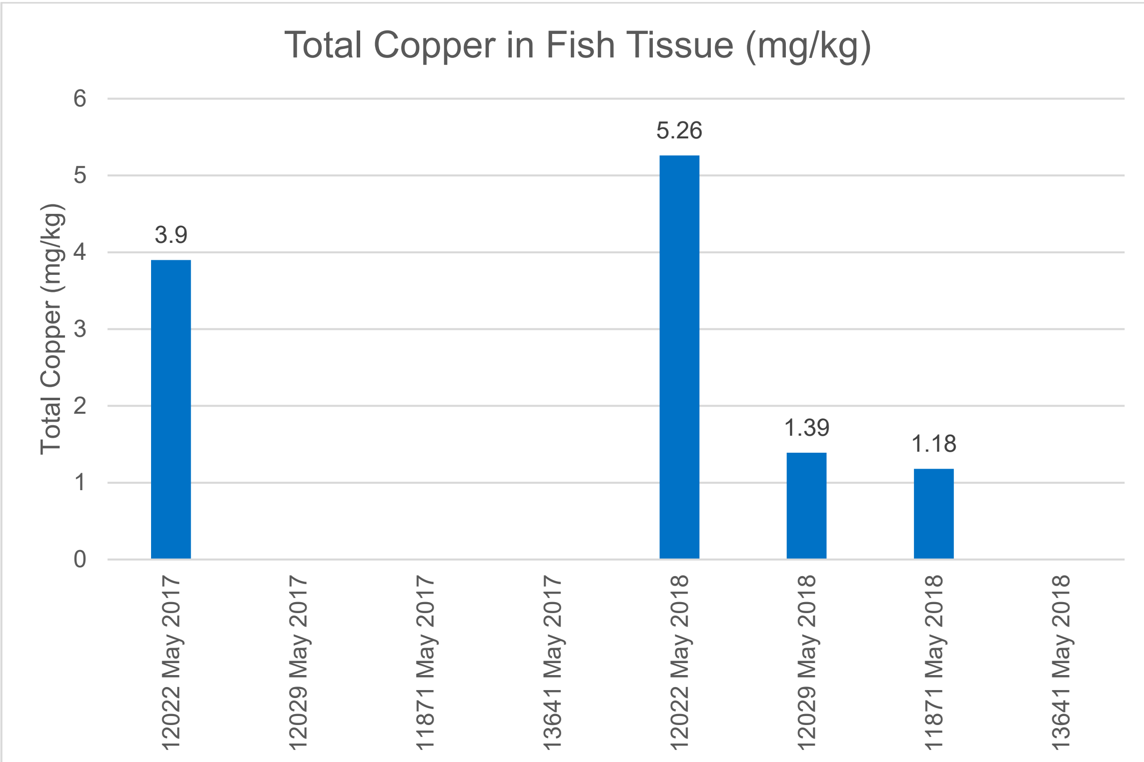

Figure 16. Total copper in whole-body fish tissue (mg/kg – wet weight) for four sites in the upper Brazos River drainage collected May 16 – 17, 2017 and May 14-15, 2018 at the Brazos River at SH 67 (13641), Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Iron

Iron is one of the most abundant elements on earth, exists in a number of different oxides and ores, and can be found in both groundwater and surface water. Iron is an important nutrient for living organisms and is generally not thought to be toxic to aquatic organisms. The USEPA's National Recommended Water Quality Criteria (USEPA 2004) suggests a level of 1,000 µg/L for dissolved iron in water to protect aquatic organisms from the chronic effects of iron. The TCEQ does not have screening criteria for total or dissolved iron.

Both total and dissolved iron samples were collected at each site for the two years of the study. Total iron was detected in most samples except for two samples from the Salt Fork Brazos River at US 183, which were below the LCRA-ELS detection limit for total iron (Figure 17). Levels of dissolved iron in water were mostly below the LCRS-ELS detection limits with only 7 results at measurable levels (Figure 18). None of the dissolved iron levels were above the 1,000 µg/L chronic criteria established by the USEPA.

Iron in fish tissue was not analyzed.

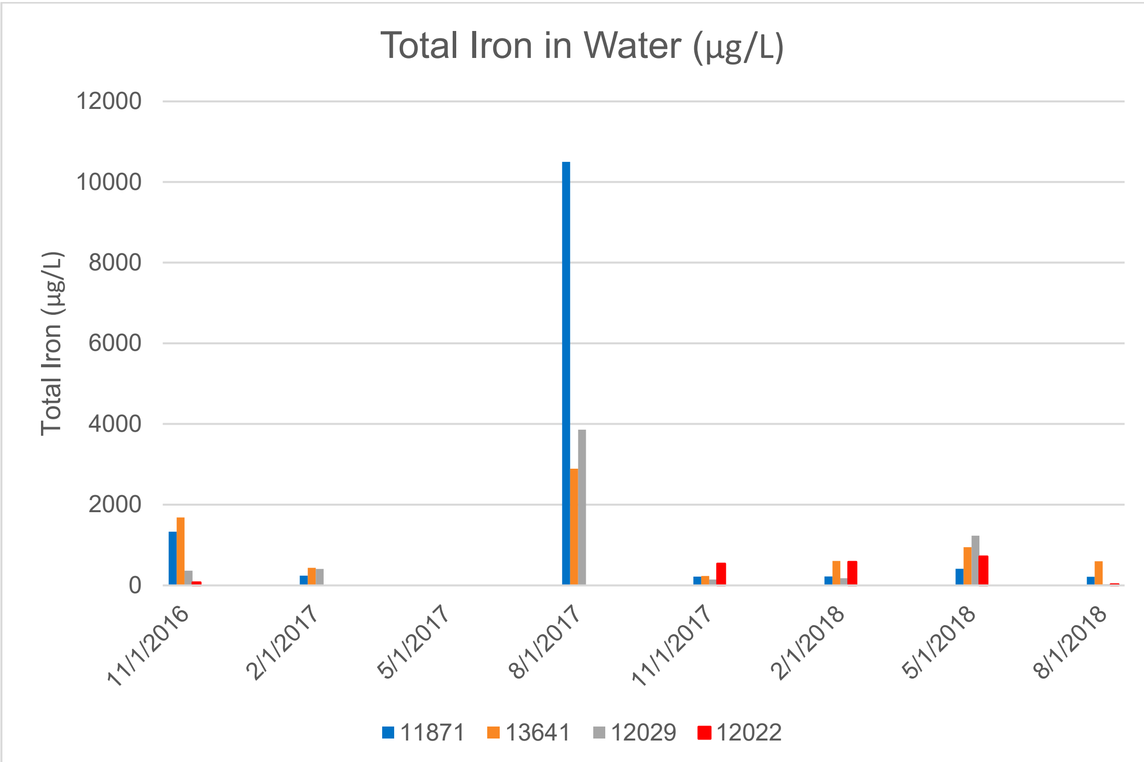

Figure 17. Total iron in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

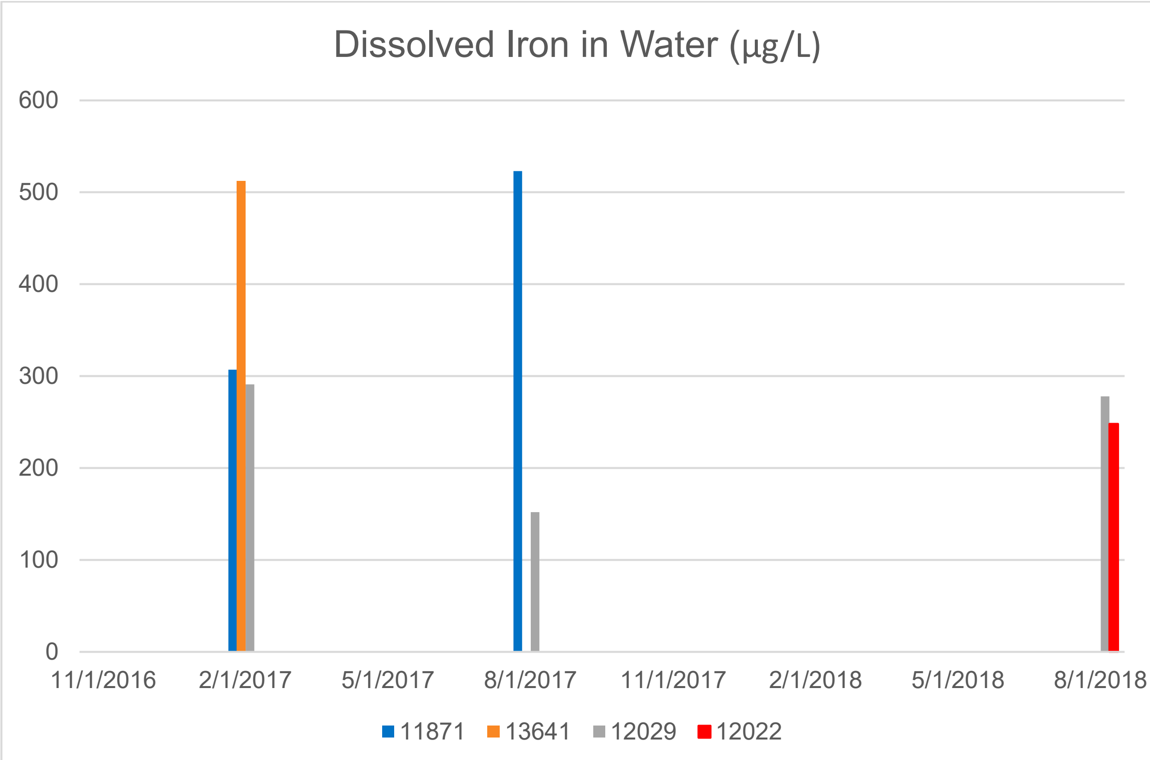

Figure 18. Dissolved iron in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Lead

Lead is a dense metal that is rarely found in its metallic form. Lead can be released into the environment through natural and industrial processes and can be very toxic to aquatic organisms. The USEPA recommended acute and chronic criteria are 82 µg/L and 3.2 µg/L (EPA 2004). The TCEQ uses a formula based on ambient water quality hardness and has established surface water quality screening criteria for dissolved lead for the portions of the Brazos River containing the two sites on the mainstem of the river (11871, 13641). The acute and chronic screening criteria for dissolved lead in water for the Brazos River near Seymour (11871) are 591.27 µg/L and 12.99 µg/L, respectively and the acute and chronic screening criteria for the Brazos River at SH 67 (13641) are 496.81 µg/L and 12.99 µg/L (TCEQ 2018).

Only six of the twenty-eight total lead in water samples were above the LCRA-ELS minimum detection limit (Figure 19) and only one dissolved lead in water sample was above the LCRA-ELS minimum detection limit in the Brazos River at US 183, with a dissolved lead concentration of 1.9 µg/L on August 15, 2017.

Total lead in fish tissues were analyzed at all four sites in the spring (May) of each year of the study; all were below the LCRA-ELS detection limit except for one sample (Red River Pupfish *Cyprinodon rubrofluviatilis)* from the Salt Fork Brazos River at US 183 on May 14, 2018 (0.725 mg/kg – wet weight).

This result is above the 85th percentile reported by Hinck et al. (2009) for lead of 0.27 mg/kg (wet weight), indicating this sample is high relative to concentrations in fish collected from across the U.S.

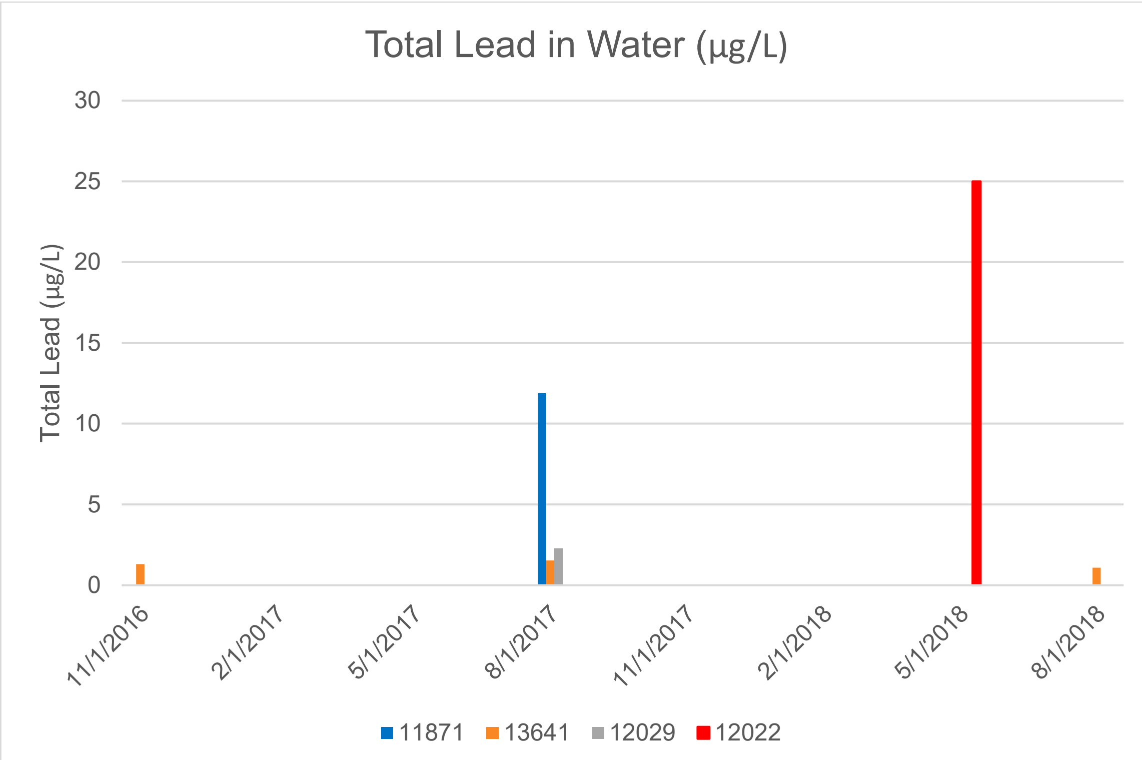

Figure 19. Total lead in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Magnesium

Magnesium is an alkaline metal that is reactive and not often found in its pure state in the environment. While magnesium itself is not thought to be toxic it does affect water hardness and alkalinity which can influence the toxicity of other contaminants. Neither the USEPA nor TCEQ have recommended criteria for magnesium in surface waters.

Total and dissolved magnesium in water were sampled at each site for the two years of the study. Both total and dissolved magnesium remained constant across all sites with an increase in concentration toward the end of the study in 2018, which is likely due to lower flows in the upper Brazos River. Magnesium concentrations were higher in the Salt Fork Brazos River samples than the other water bodies sampled (Figures 20 and 21).

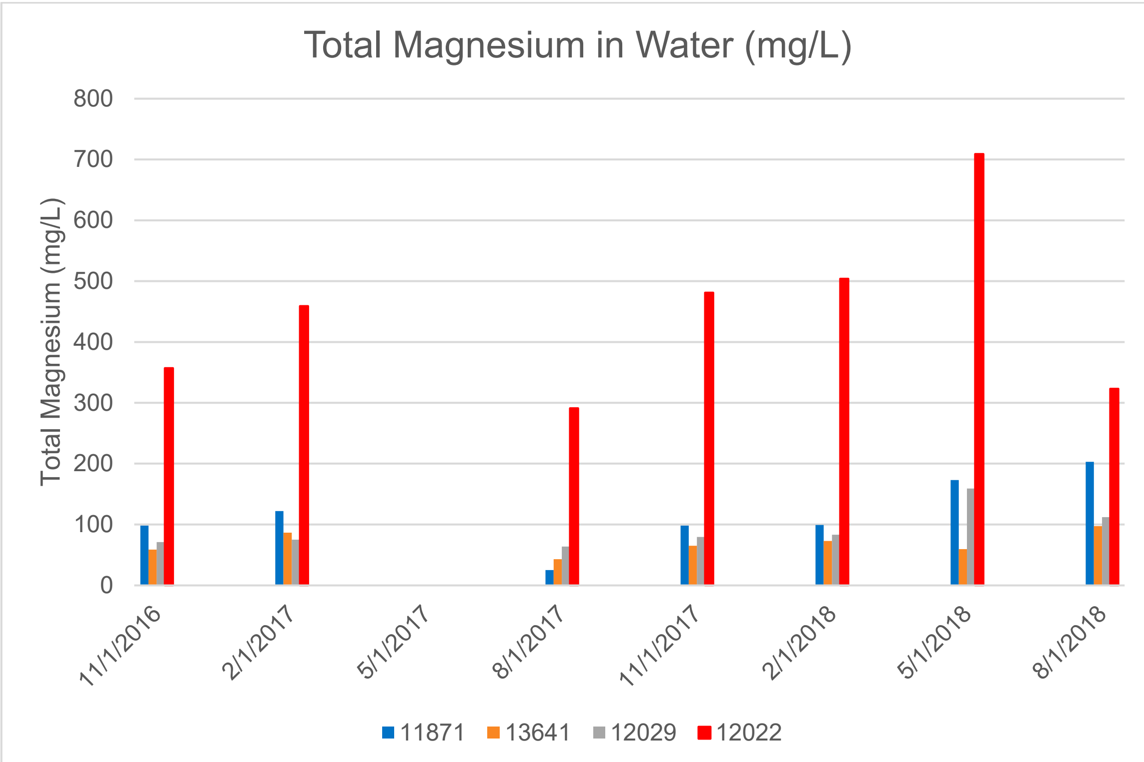

Figure 20. Total magnesium in water collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

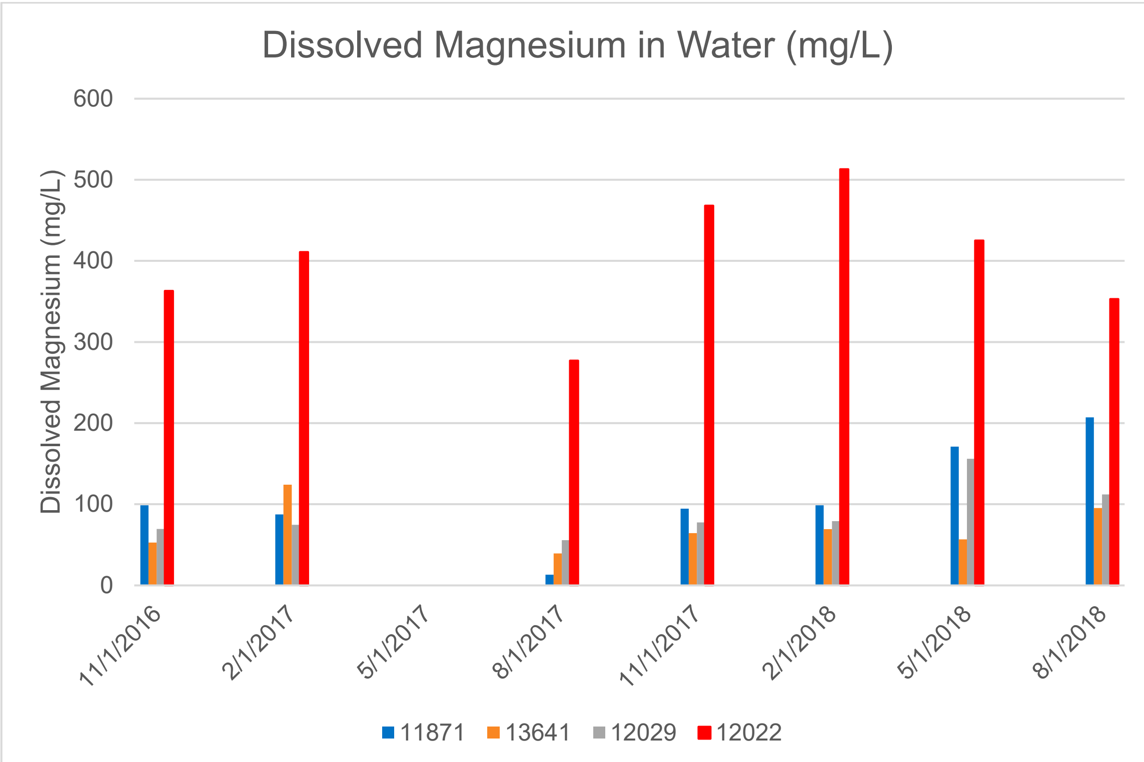

Figure 21. Dissolved magnesium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Manganese

Manganese is a metal that is found naturally and can enter ground and surface waters through natural weathering processes and through anthropogenic sources like domestic wastewater discharges. The USEPA and the TCEQ have not recommended screening criteria for total or dissolved manganese in surface waters.

Total manganese in water sample results from the four sites remained fairly constant with an increase in concentration in August 2017 and a substantial increase in concentration in the Salt Fork Brazos River at US 183 in 2018 (Figure 22). Dissolved manganese in water results were consistent through both years of the study for the sites in the main stem of the Brazos River and the Double Mountain Fork Brazos River; the Salt Fork Brazos River exhibited an increase in manganese concentration in 2018 (Figure 23), similar to what was seen for total manganese. Total and dissolved concentrations were nearly identical based on a site-by-site comparison, leading us to conclude that the majority of manganese present in water is in the dissolved form. Manganese in fish tissue was not analyzed.

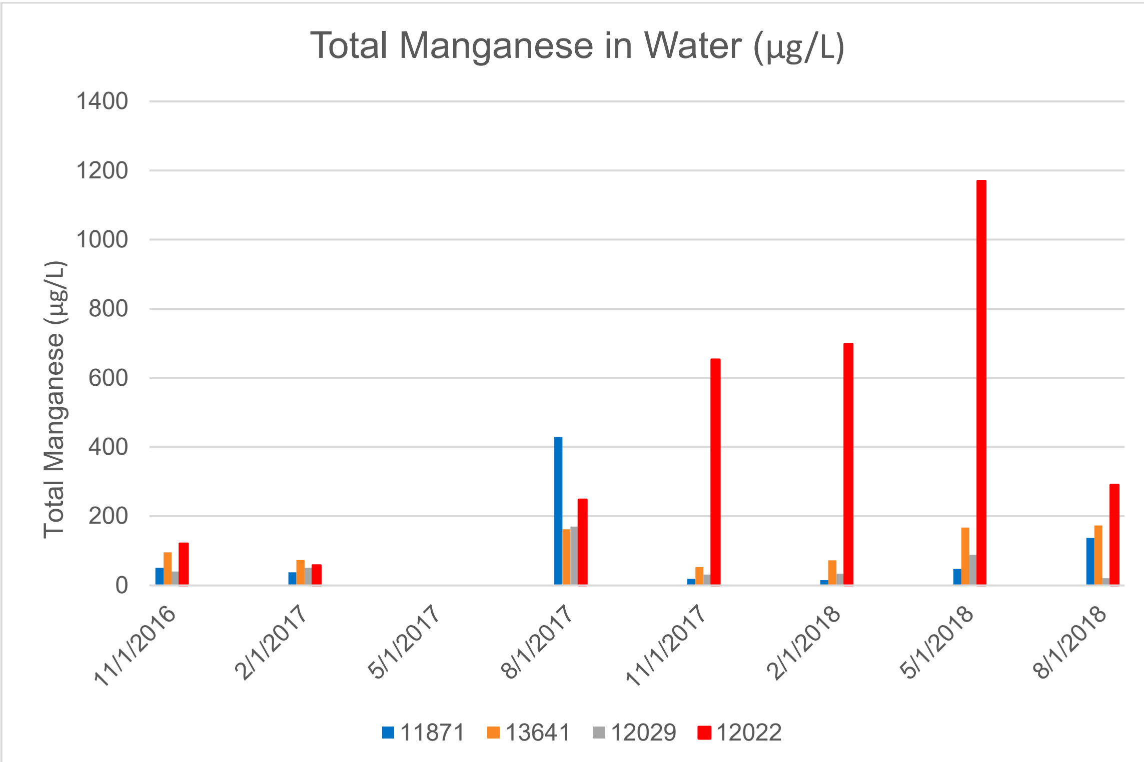

Figure 22. Total manganese in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

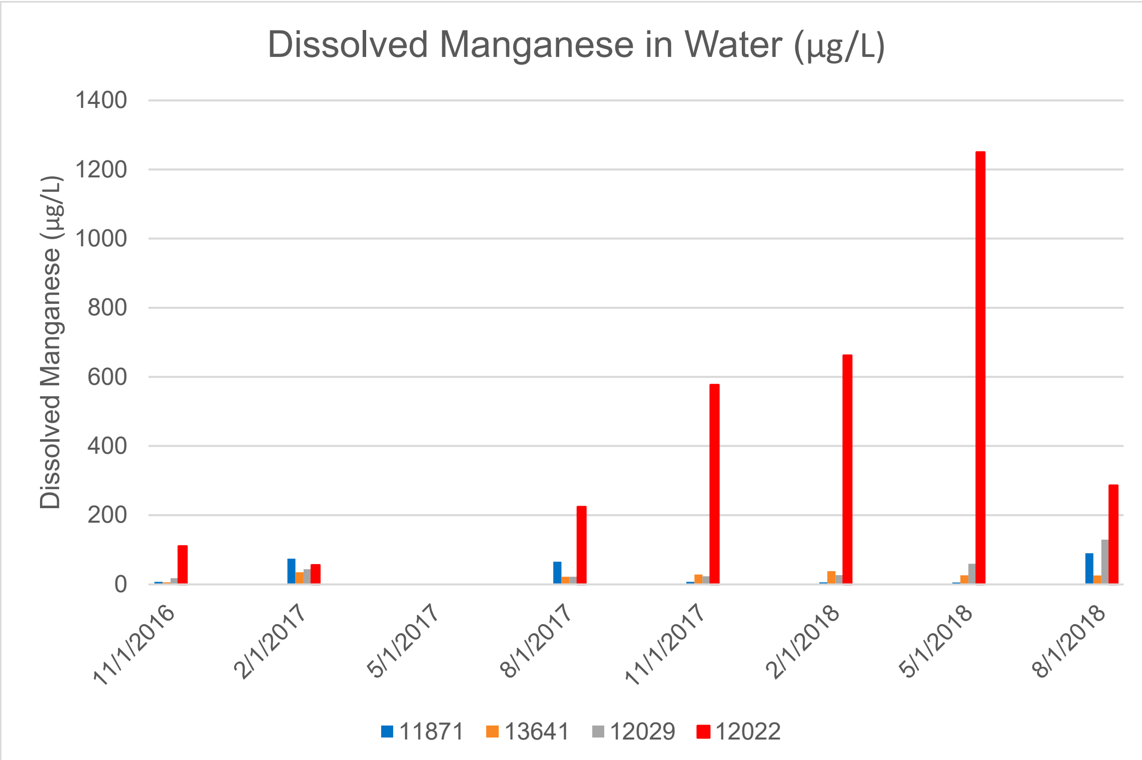

Figure 23. Dissolved manganese in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Mercury

Mercury is a heavy metal and is the only metallic element that exists in a liquid form at standard conditions for temperature and pressure. Mercury is most commonly found as mercuric sulfide. This element is introduced into the environment through natural processes like volcanic eruptions and anthropogenically through industrial processes such as coal-fired power plants and improper disposal of products containing mercury. Mercury is a powerful neurotoxin, is highly toxic to aquatic organisms, and can bioaccumulate and biomaginify up the food chain. Mercury persists in the environment for long periods, changing chemical forms and cycling back and forth between the air and soils, where it can persist for decades. The USEPA National Recommended Water Quality Criteria lists acute and chronic levels for total mercury in freshwater at 1.4 µg/L and 0.77 µg/L, respectively (USEPA 2004).

Mercury in water was not collected, however mercury in fish tissue was collected at all sites in the spring (May) of each year of the study (Figure 24). Mercury was detected at each site at least once. All total mercury concentrations in tissue were below the 85th percentile reported by Hinck et al. (2009) of 0.27 mg/kg (wet weight) and below the NOEL for toxicity in fish reported by Beckvar *et al.*, (2005) of 0.2 mg/kg (wet weight), indicating mercury is not a contaminant of concern to fish in the upper Brazos River at this time. Note that the short life cycle of sampled fish may limit their ability to appreciably accumulate certain contaminants of concern like mercury.

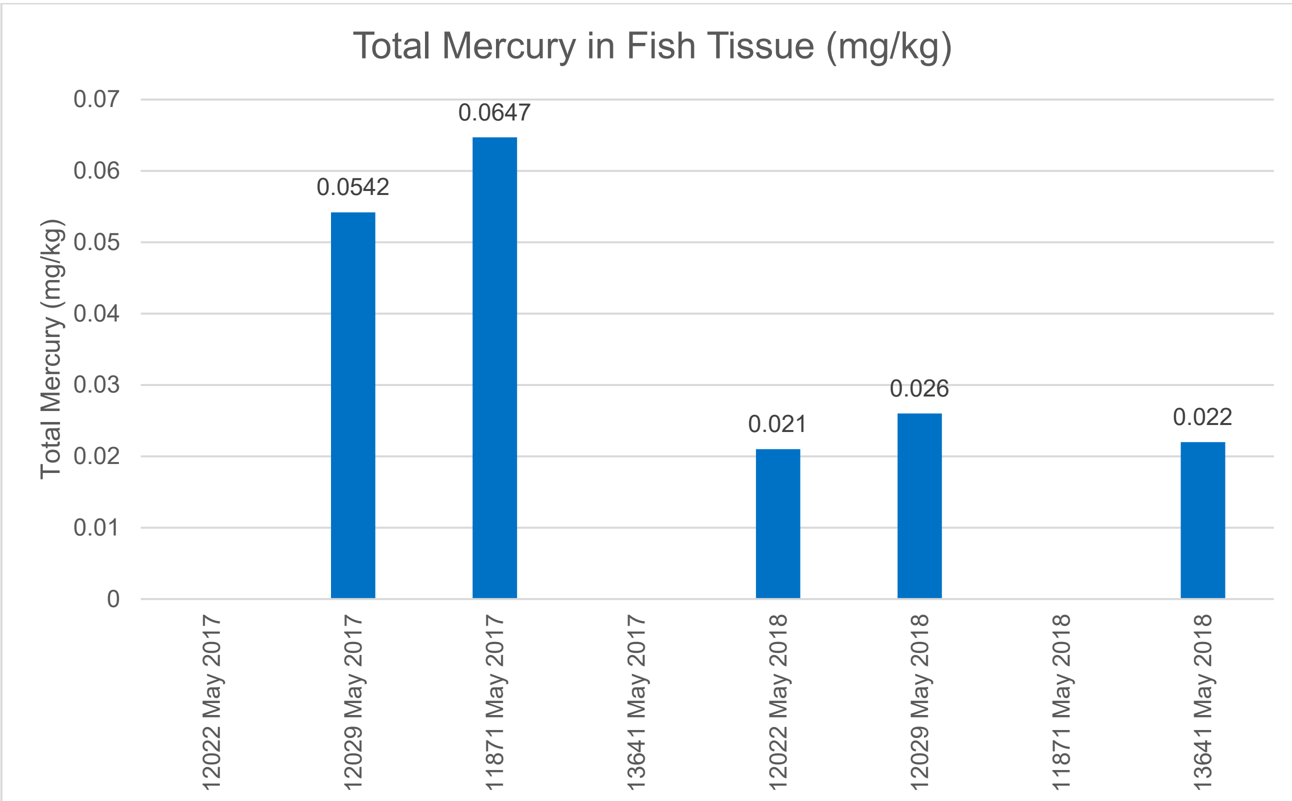

Figure 24. Total mercury in whole-body fish tissue (mg/kg – wet weight) for four sites in the upper Brazos River drainage collected May 2017 and May 2018 at the Brazos River at SH 67 (13641), Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Nickel

Nickel is a naturally occurring metal that is commonly found in surface water and groundwater. Nickel can enter these waters through natural weathering processes or through anthropogenic sources where nickel is used, such as plating facilities, the production of coins, and as a component of batteries. When elevated, nickel can cause hematological issues in aquatic organisms (USEPA 1986). Nickel toxicity is affected by ambient water hardness and the USEPA and TCEQ both base nickel criteria on hardness. The USEPA National Recommended Water Quality Criteria lists acute and chronic criteria for dissolved nickel in freshwater at 470 µg/L and 52 µg/L, respectively based on a hardness value of 100 mg/L (USEPA 2004). The TCEQ has established screening criteria for dissolved nickel in the portion of the Brazos River containing sites 11871 and 13641. The Brazos River at US 183 (11871) has screening criteria for dissolved nickel for acute and chronic effects (2,452.41 µg/L and 193.64 µg/L, respectively), as does the Brazos River at SH 67 (13641) (2,840.86 µg/L and 193.64 µg/L, respectively) (TCEQ 2018). Table 12 lists the acute and chronic screening criteria for all four sites based on ambient water hardness collected during the study.

Table 12. Acute and chronic dissolved nickel in water screening criteria based on the ambient water hardness collected during the study period (Nov 2016 through August 2018) for the Brazos River at US 183 (11871), Brazos River at SH 67 (13641), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Site	Nickel Acute Criteria (µg/L)	Nickel Chronic Criteria (µg/L)
11871	4,151.1	461.1
13641	2,698.2	299.7
12029	3,690.6	439.9
12022	15,240.2	1,692.7

Samples for total and dissolved nickel analysis were collected at each site. Both total and dissolved nickel were present in all samples except for those collected on May 14, 2018 in the Brazos River and Double Fork Mountain Brazos River. Concentrations of total and dissolved nickel in water were higher in the Salt Fork Brazos River (Figure 25 and 26). All concentrations of dissolved nickel were well below the TCEQ established screening criteria for the mainstem of the Brazos River, as well as calculated screening criteria for the Double Mountain Fork Brazos River and Salt Fork Brazos River. Nickel in fish tissue was not analyzed for this study.

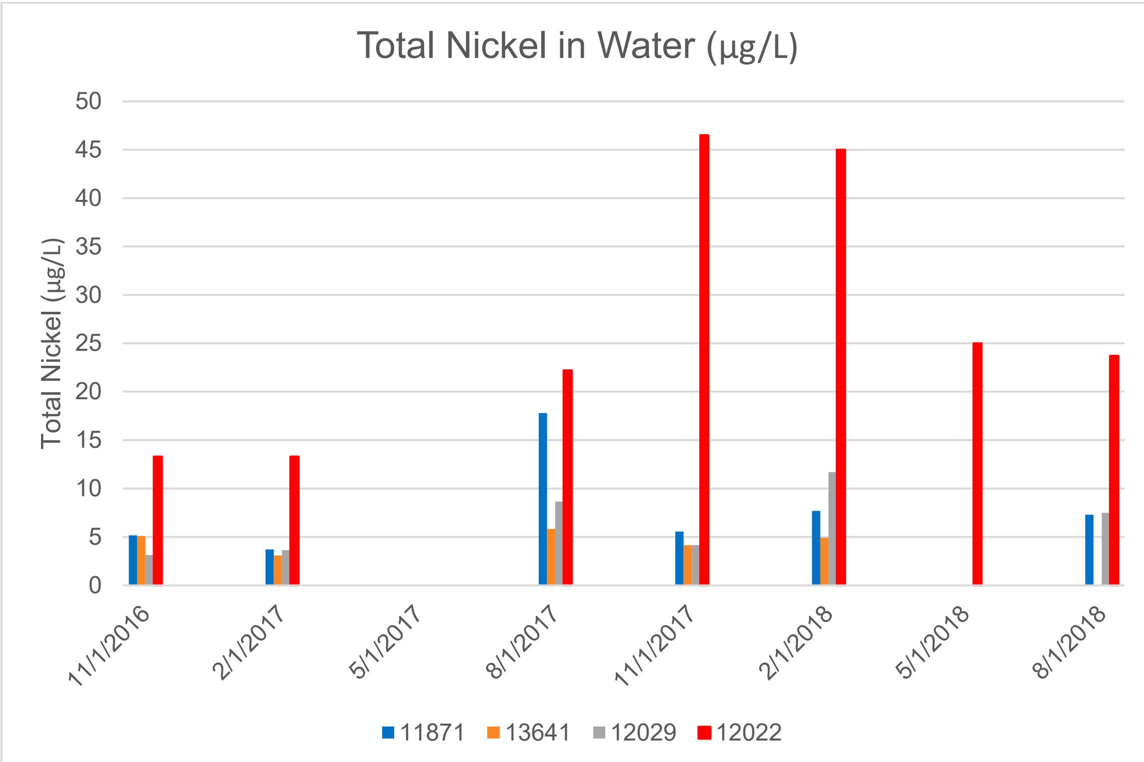

Figure 25. Total nickel in water for four sites collected November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

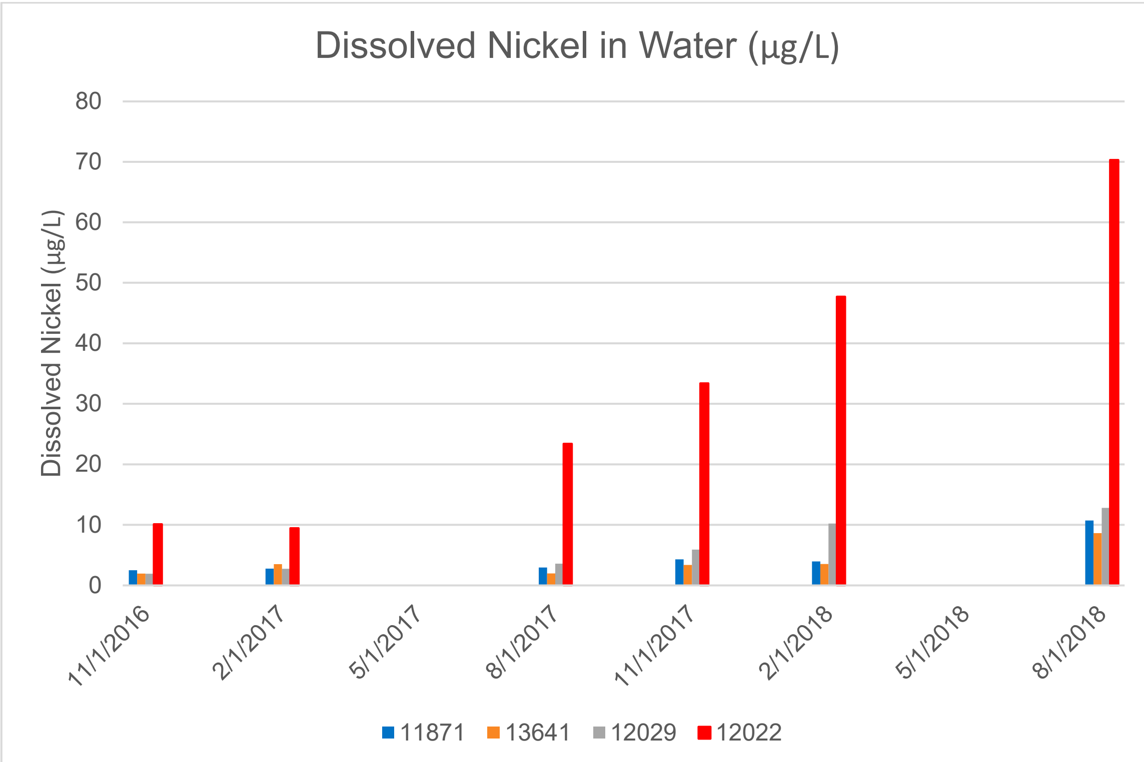

Figure 26. Dissolved nickel in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Potassium

Potassium is an alkaline metal that is common in ground and surface water. Potassium itself is not considered toxic but potassium salts can affect the toxic potential of certain pollutants by buffering aquatic systems. Neither the USEPA or TCEQ have established water quality standards or screening criteria for potassium.

Total and dissolved potassium in water were collected at each site during the study. Not surprisingly, concentrations were consistently higher in the Salt Fork of the Brazos River compared to the other three sites (Figures 27 and 28). Potassium was not analyzed in fish tissue.

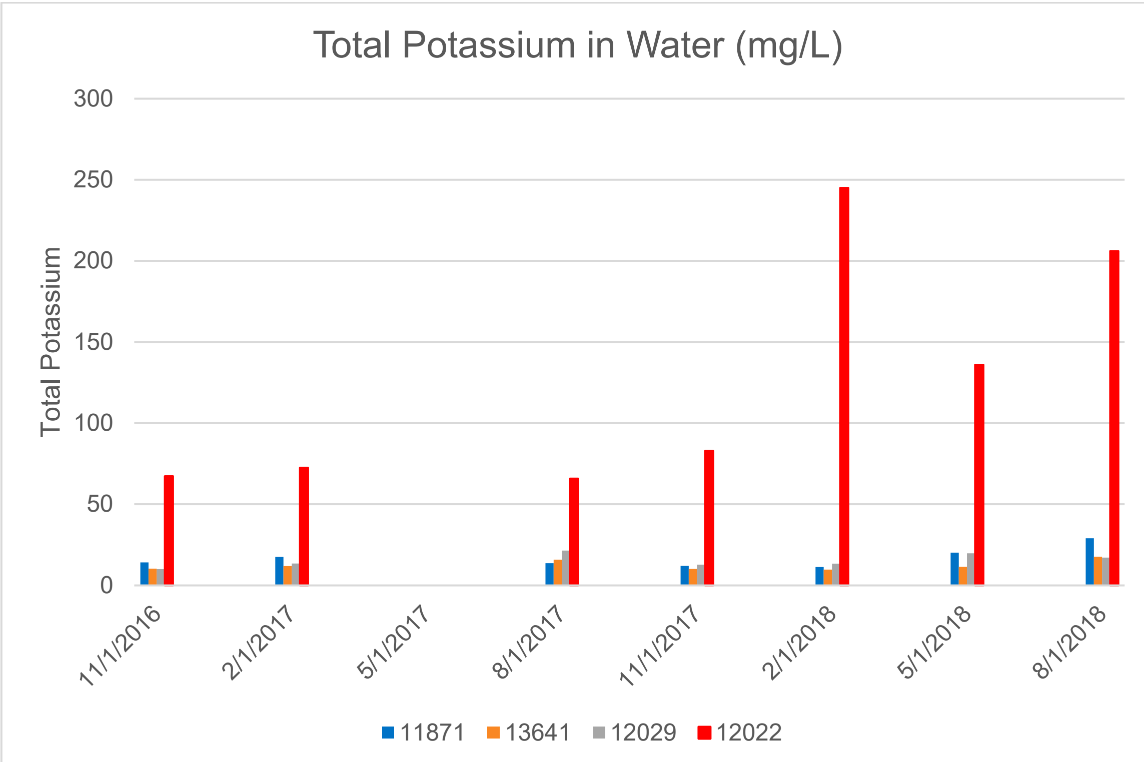

Figure 27. Total potassium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

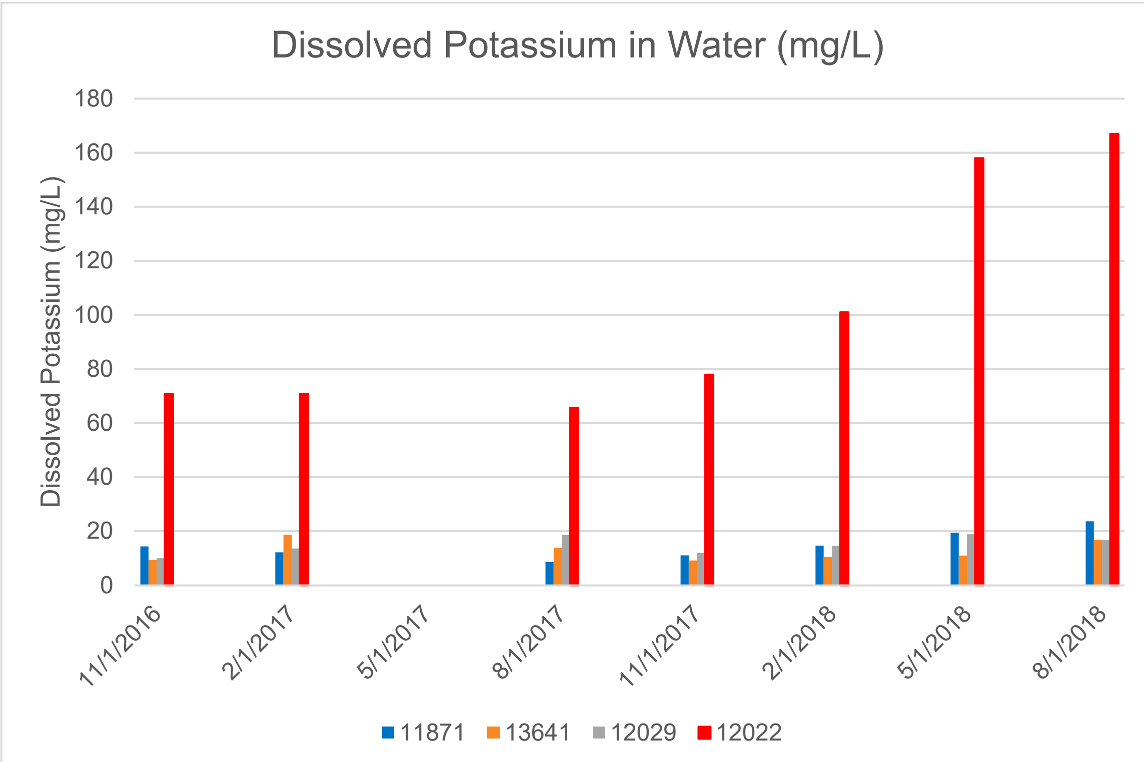

Figure 28. Dissolved potassium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Selenium

Selenium is a metalloid element that occurs naturally in a number of inorganic forms and can enter groundwater and surface water through natural geologic processes such as the weathering of rocks and soil, and through anthropogenic sources such as the burning of coal, mining, reject wastewater from reverse osmosis treatment, irrigation run-off from agriculture, and point source discharges. Selenium is an essential micronutrient at low levels, but can quickly become toxic at higher levels, which can lead to deformities, reproductive impairment, and death. Selenium toxicity in aquatic systems has been studied extensively and the USEPA developed recommended criteria for total selenium in water and fish tissue. For chronic effects, the USEPA recommends a criterion of 3.1 µg/L in lotic waters (based on a 30-day period) and a whole-body fish tissue concentration of 8.5 mg/kg (dry weight) (USEPA 2016). The TCEQ established water quality standards for total selenium in water for acute and chronic effects on aquatic organisms in freshwater (20 µg/L and 5 µg/L, respectively) (TCEQ 2018).

Total and dissolved selenium samples were collected at each site. Among the total selenium samples collected, only seven of the twenty-eight results were above the LCRA-ELS minimum detection limit (Figure 29). Total selenium in water was measured at all four sites and a number of exceedances for both acute and chronic criteria were observed (Table 13). The highest levels of total selenium in water were in samples from the Salt Fork Brazos River at US 183, with values well above both acute and chronic State criteria (and the EPA criterion). Concentrations detected at the other sites were all above the chronic criteria established by the USEPA and TCEQ as well. Dissolved selenium in water was collected during each site visit but was only found above detection limits on August 29, 2018 (Figure 30). Selenium in fish tissue was collected during both spring (May) sample events but all results were less than the laboratory's minimum detection limit.

Table 13. Total selenium exceedances of acute and chronic criteria (TCEQ 2018) and EPA Criterion for lotic waters (EPA 2016b) to protect aquatic life in the Brazos River at US 183 (11871), Brazos River at SH 67 (13641), Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Site	Date	Total Selenium (µg/l)	TCEQ Acute Criteria (20 µ/L)	TCEQ Chronic Criteria (5 µg/L)	EPA Criterion (3.1 ug/L)
11871	11/30/2017	13.4	-	Exceeds	Exceeds
11871	8/29/2018	8.42	-	Exceeds	Exceeds
13641	11/30/2017	5.32	-	Exceeds	Exceeds
13641	8/29/2018	6.16	-	Exceeds	Exceeds
12029	11/30/2017	5.58	-	Exceeds	Exceeds
12022	11/30/2017	170	Exceeds	Exceeds	Exceeds
12022	8/29/2018	94.4	Exceeds	Exceeds	Exceeds

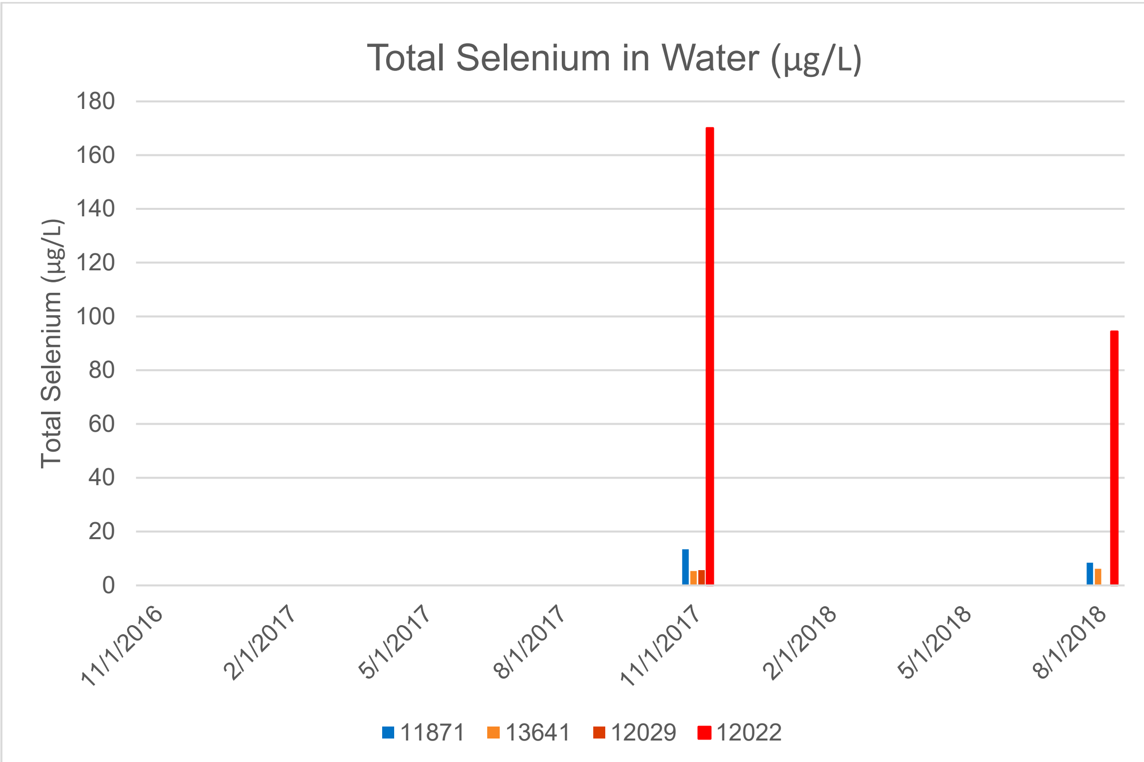

Figure 29. Total selenium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

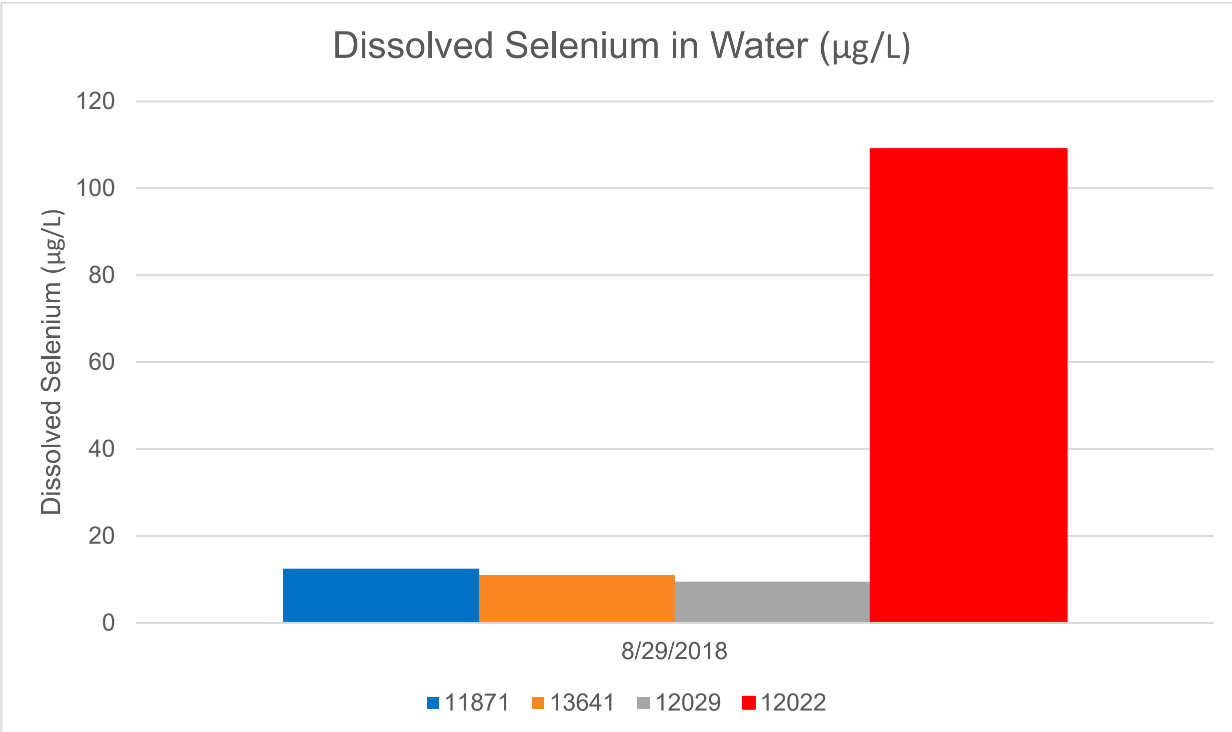

Figure 30. Dissolved selenium in water for four sites in the upper Brazos River drainage collected on August 29, 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Silver

Silver is a naturally existing metal that can occur in deposits of mineral ores and can enter groundwater and surface water through natural processes or through anthropogenic sources such as smelting and mining operations (Howe 2002). Silver in its ionic form can be toxic to aquatic organisms causing osmoregulatory issues (Ford 2019). The USEPA acute criterion for dissolved silver is 3.2 µg/L (USEPA 2004), and the TCEQ established an acute criterion of 0.8w µg/L (w = water effects ratio) for free ionic silver based on Water Effects Ratio (WER) methodology which is based on site specific water quality parameters that affect the toxicity of the constituent in question (TCEQ 2018). The TCEQ standard assumes the WER is equal to 1 except where a site specific WER has been established. WERs are typically based on site specific total suspended solids, hardness, and pH. No WERs for silver have been established in the upper Brazos River or its tributaries.

Total and dissolved silver were collected at each site both years of the study. Only one total silver in water sample of the twenty-eight collected had results that were over the LCRA-ELS minimum detection limit. The Salt Fork Brazos River at US 183 had a total silver concentration of 25 µg/L on May 15, 2018. All dissolved silver in water results for all sites were below the LCRA-ELS minimum detection limits. Total silver in fish tissue was not analyzed.

Sodium

Sodium is an alkaline metal that is common in the environment and forms an important part of water's natural ability to buffer pH. Sodium is a very reactive metal and often forms salts with other constituents. There are no federal or state water quality standards or criteria for sodium by itself.

Total and dissolved sodium in water were collected at each site. Again, not surprisingly, both total and dissolved sodium were several orders of magnitude higher in the Salt Fork Brazos River compared to other sites (Figures 31 and 32). Total sodium in fish tissue was not analyzed.

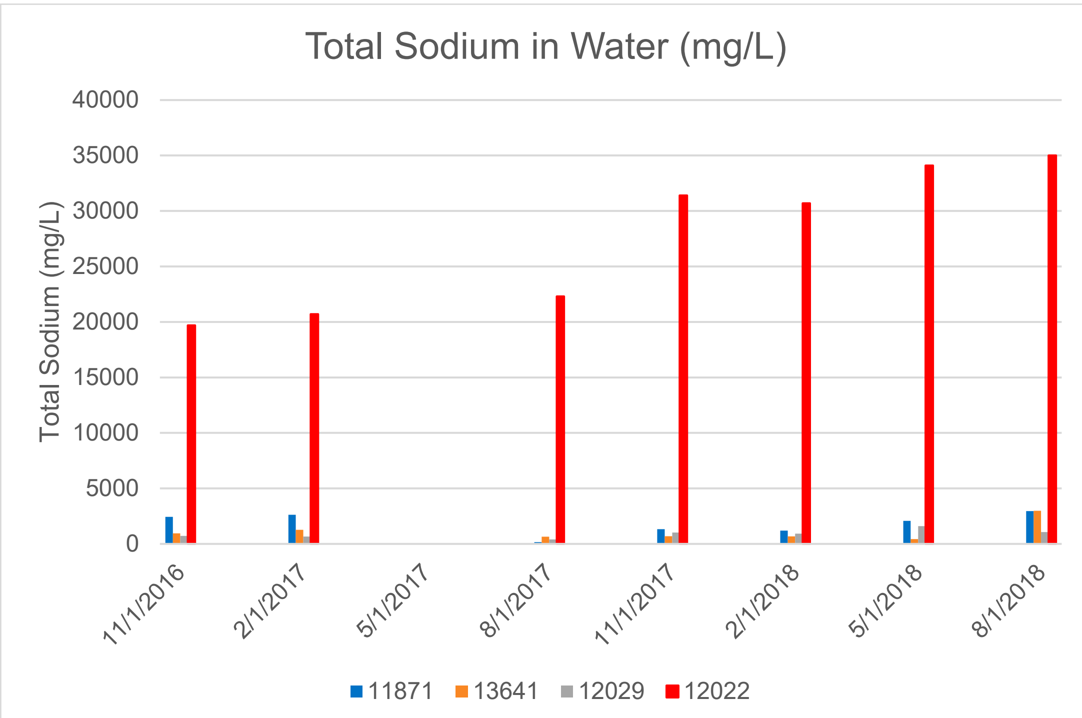

Figure 31. Total sodium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

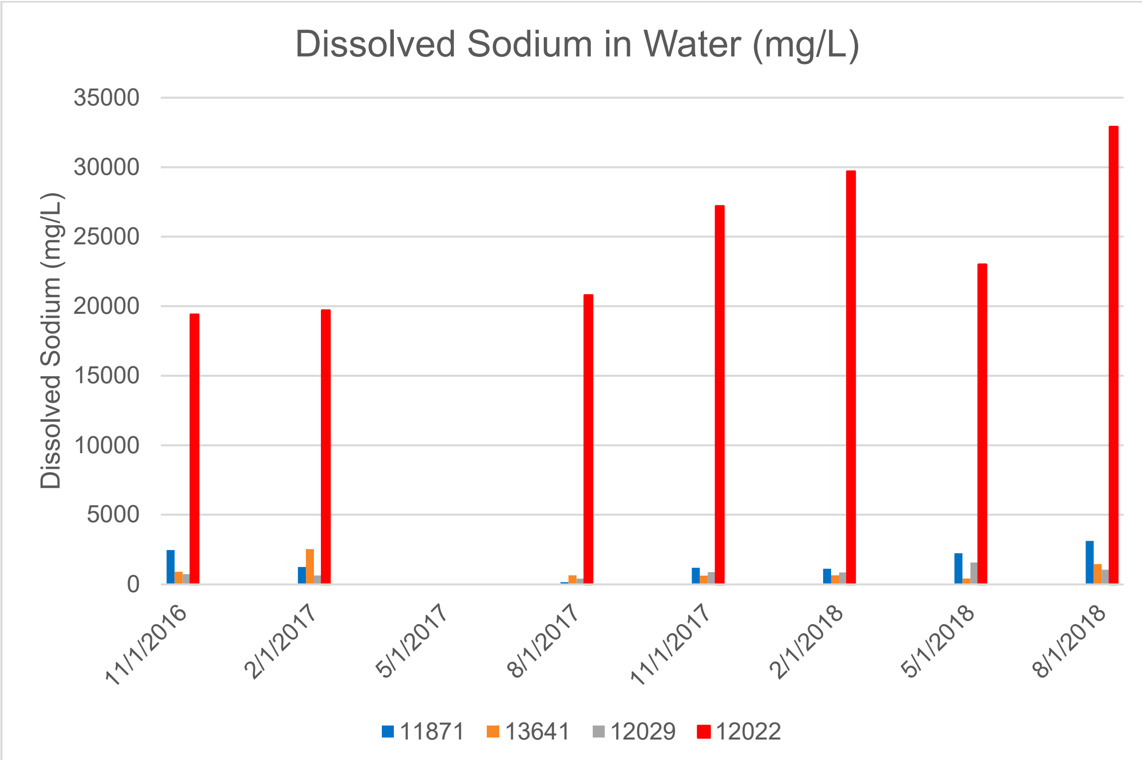

Figure 32. Dissolved sodium in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Zinc

Zinc is a semi-common metal that is found in the environment and can enter groundwater and surface water through natural processes, as well as anthropogenic sources through industrial processes. Zinc is an essential nutrient but can become toxic in larger amounts by disrupting osmoregulation. The USEPA's National Recommended Water Quality Criteria (USEPA 2004) lists a level of 120 µg/L for dissolved zinc in water for both acute and chronic effects. The TCEQ has established water quality standards for dissolved zinc based on ambient water hardness. The TCEQ has acute and chronic criteria for dissolved zinc in water in the portion of the Brazos River that contains sites 11871 and 13641. The acute and chronic criteria for the Brazos River at US 183 are 712.95 µg/L and 440.76 µg/L, respectively, and the acute and chronic criteria for the Brazos River at SH 67 are 615.3 µg/l and 440.76 µg/L (TCEQ 2018). Acute and chronic dissolved zinc in water criteria were calculated using the TCEQ's formula based on ambient water hardness and are presented in Table 14.

Table 14. Acute and chronic dissolved zinc in water screening State criteria based on the ambient water hardness collected during the study period (Nov 2016 through August 2018) for the Brazos River at US 183 (11871), Brazos River at SH 67 (13641), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Site	Zinc Acute Criteria (µg/L)	Zinc Chronic Criteria (µg/L)
11871	1,042.5	1,051.0
13641	677.1	682.6
12029	994.5	1,002.7
12022	3,834.7	3,866.1

Total and dissolved zinc were collected at each site during the study. Sixteen of the twenty-eight total zinc in water results were below the LCRA-ELS minimum detection limits. The highest and most frequent results for total zinc were from the Brazos River at US 183 (Figure 33). Dissolved zinc in water results for all but the samples collected at all four sites on August 29, 2018, were below detection limits (Figure 34). The results from the Salt Fork Brazos River at US 183 were 223 µg/L which is above the USEPA recommended criteria of 120 µg/L but less than the calculated acute and chronic State criteria in Table 13. Total zinc in fish tissue was not analyzed.

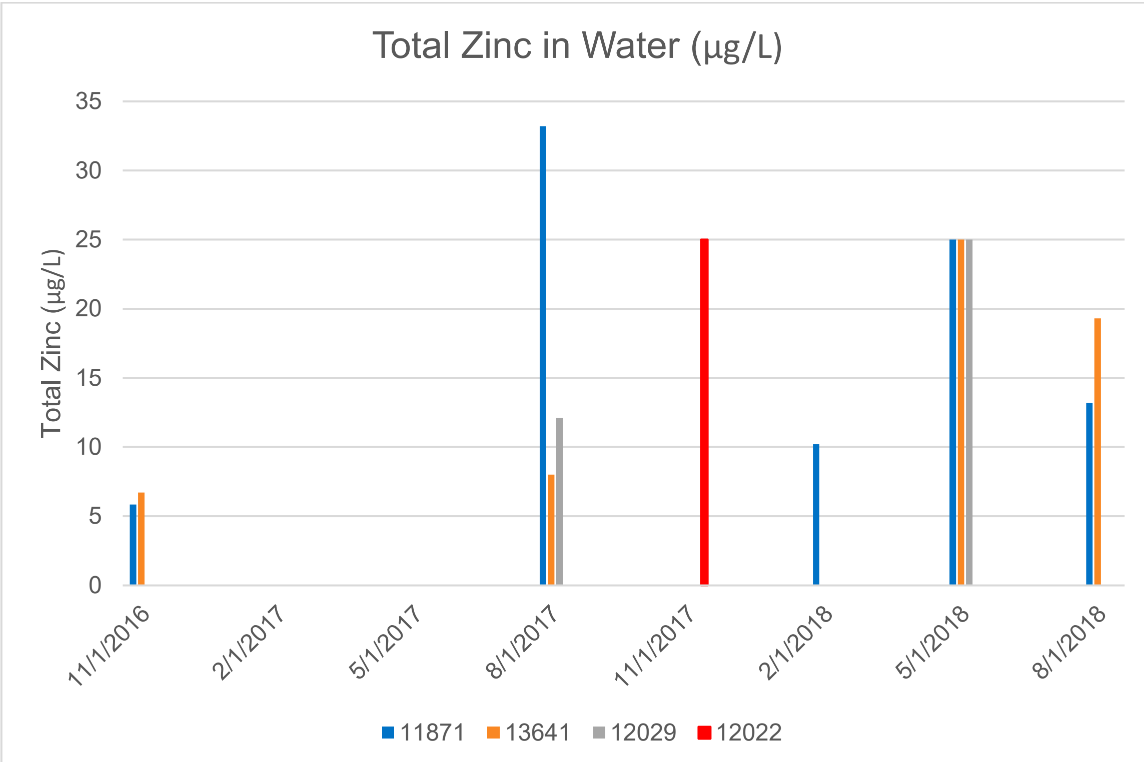

Figure 33. Total zinc in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

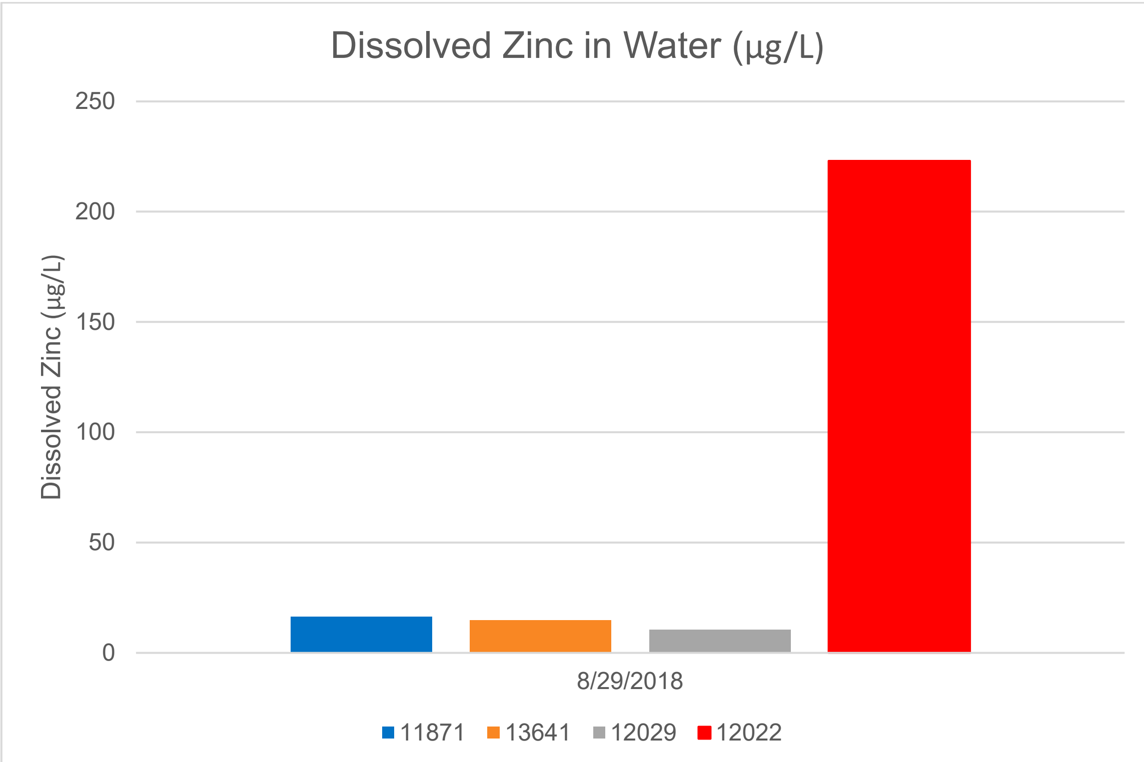

Figure 34. Dissolved zinc in water for four sites in the upper Brazos River drainage collected between November 2016 and August 2018 at the Brazos River at SH 67 (13641), the Brazos River at US 183 (11871), the Double Mountain Fork Brazos River at US 183 (12029), and the Salt Fork Brazos River at US 183 (12022).

Water Body Comparisons

The Salt Fork Brazos River generally had higher concentrations of measured constituents compared to the Brazos River and Double Mountain Fork Brazos River. We used Analysis of Similarity (ANOSIM) to compare water quality between sites, which were significantly different but not greatly so ($R = 0.228$, $p < 0.01$). Non-metric multidimensional scaling was used to graphically depict the differences between sites (Figure 35).

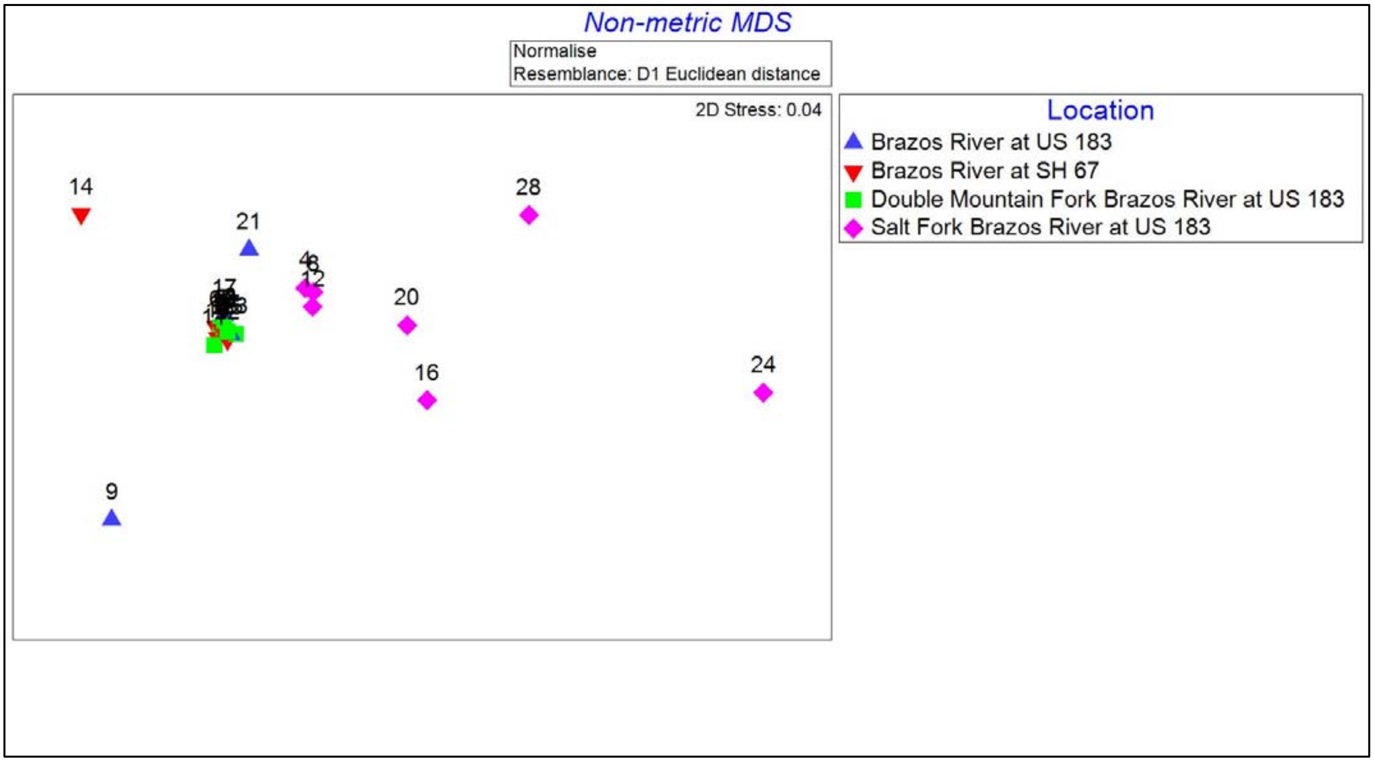

Figure 35. Non-metric Multidimensional Scaling plot of all water quality data from each site.

Total and dissolved forms of calcium, magnesium, manganese, potassium, and sodium were much higher in the Salt Fork Brazos River at US 183 than in the Brazos River sites and the Double Mountain Fork Brazos River. ANOSIM results showed significant differences between concentrations of these metals in the Salt Fork Brazos River compared with other water bodies sampled for both the global test ($R = 0.378$, $p < 0.01$) and pair-wise tests (Table 15).

Table 15. ANOSIM (Analysis of Similarity) results showing pair-wise comparisons of concentrations of alkaline metals in each water body.

Water Body Comparisons	R Statistic	p-value
Brazos River at US 183 and the Salt Fork Brazos River at US 183	0.767	0.002
Brazos River at SH 67 and Salt Fork Brazos River at US 183	0.834	0.001
Double Mountain Fork Brazos River at US 183 and Salt Fork Brazos River at US 183	0.794	0.001

Total and dissolved metal concentration data from each site were grouped in PRIMER as either Salt Fork Brazos River or Non-Salt Fork Brazos River and compared to each other. ANOSIM results for global tests between total metal and dissolved metal concentrations for Salt Fork and Non-Salt Fork groups were significantly different ($R = 0.764$, $p < 0.01$).

Discussion

Water quality and fish tissue samples were collected from the upper Brazos River and its tributaries in order to determine whether altered water quality is affecting populations of aquatic organisms in these water bodies, particularly the federally endangered Smalleye Shiner and Sharpnose Shiner. Results from this study have shown exceedances of the acute and chronic standards for dissolved aluminum and total selenium in water (TCEQ 2018). High concentrations of these metals in the water could be a result of natural introduction via the weathering of rocks and soil in the watershed, from anthropogenic sources, or a combination of these two sources. A cursory look at groundwater data from the Texas Water Development Board (TWDB) in the upper Brazos River drainage shows elevated amounts of dissolved aluminum as high as 1,095 ug/L and total selenium in the groundwater with a range of 12.5 µg/l to 26.2 µg/L (TWDB Website Accessed November 6, 2019). Additionally, a spike in the concentration is seen in aluminum and iron in May of 2018 and there appears to be no corollary evidence, such as precipitation or point source discharges, that could potentially lead to these increased concentrations.

Total arsenic, chromium, copper, lead, and mercury were all detected in fish tissue samples from the upper Brazos River, Double Mountain Fork Brazos River, and Salt Fork Brazos River, and were above the 85th percentiles reported by Hinck et al (2009) which sampled larger bodied fish from across the United States. These elevated levels of metals in fish tissue may be a result of naturally high metals in the groundwater from the geology surrounding the upper Brazos River. Metals in water data from the TWDB Groundwater Database shows high results for dissolved forms of copper and lead in groundwater in the upper Brazos River drainage area with values that were above established surface water quality criteria and screening values (TWDB 2019).The dissolved metals in water and total metals in fish tissue data from the Salt Fork Brazos River in particular were much higher than the other water bodies. Total copper appears to be accumulating in fish tissue with concentrations in fish above reported toxicity thresholds limits that affected growth and reproduction in Rainbow Trout and Fathead Minnows and above the 85th percentile of total copper in fish tissue for a nationwide study (Beckvar et al. 2005, Lapointe et al. 2011, and Hinck et al 2009). Total lead was detected in fish tissue above the 85th percentile from a nationwide study (Hinck et al 2009). All total mercury in fish tissue results were below the 85th percentile of 0.27 mg/kg (Hinck et al 2009).

High concentrations of total selenium in water were measured in the Salt Fork Brazos River at levels sufficient to produce deformities in fish and birds. However, total selenium in fish tissue results for all sites were below the laboratory's minimum detection limits. Selenium toxicity in fish is based mainly on diet in that the organism must consume food contaminated with selenium (USEPA 2016b). It is possible that the fish in these water bodies are not consuming selenium contaminated food and thus are not contaminated themselves despite very high levels in water, but our analysis is hampered by laboratory minimum detection limits for fish samples that are above known selenium toxicity thresholds, therefore additional research will be needed to draw any conclusions about potential impacts to shiners. Similarly, further study of this topic is required to determine if the food sources of the smalleye and sharpnose shiners are contaminated with selenium. It is safe to say that regardless of the sources or routes of exposure, the relatively short life-cycle of sampled fish (1-2 years; USFWS 2014c, Marks 1999) likely serves to reduce exposure time, and hence, lowers the potential for selenium accumulation in the tissues of small, short-lived fish species common to the upper Brazos River.

We were unable to detect changes to water quality or tissue residues in fish that could be directly tied to constituents in wastewater related to reverse osmosis, however, we cannot conclude that reverse osmosis is having no effect on water quality due to limitations of the study, including low sample number, low resolution due to limited sample sites, sampling of short-lived species (i.e., lower bioaccumulation potential), and other variables that limited our ability to draw definitive conclusions on causality.

References

Aslam, S., and A. M. Yousafzai. 2017. Chromium toxicity in fish: a review article. Journal of Entomology and Zoology Studies 5(3):1483–1488.

Beckvar, N., T. M. Dillon, and L. B. Read. 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds: Environmental Toxicology and Chemistry 24(8): 2094–2105.

Durham, B. W. 2007. Reproductive ecology, habitat associations, and population dynamics of two imperiled cyprinids in a Great Plains river. PhD dissertation. Texas Tech University, Lubbock, Texas.

Durham, B. W., and G. R. Wilde. 2009. Population dynamics of Smalleye Shiner, an imperiled cyprinid fish endemic to the Brazos River, Texas. Transactions of the American Fisheries Society 138:666–674.

Ford, L. 2001. Development of chronic aquatic water quality criteria and standards for Silver. Water Environment Research 73(2):248–253.

Grubh, A.R., Robertson, C.R., Aziz, K., Kleinsasser, L.J., Mauk, R.J., and Howell, M.H. 2014. Response of water quality and the aquatic community to a reverse osmosis unit discharge into the Wichita River, Texas. Texas Parks and Wildlife Department. River Studies Report No. 21. Austin, Texas.

Hinck, J. E., C. J. Schmitt, K. A. Chojnacki, and D. E. Tillitt. 2009. Environmental contaminants in freshwater fish and their risk to piscivorous wildlife based on a national monitoring program. Environmental Monitoring and Assessment 152:469–494.

Howe, P. D., and S. Dobson. 2002. Silver and silver compounds: environmental aspects. World Health Organization, Geneva, Switzerland.

Kumari B., V. Kumar, A. K. Sinha, J. Ahsan, A. K. Gosh, H. Wang, and G. DeBoeck. 2017. Toxicology of arsenic in fish and aquatic systems. Environmental Chemistry Letters 15(1):43–46.

Lapointe, D., F. Pierron, and P. Couture. 2011. Individual and combined effects of heat stress and aqueous or dietary copper exposure in fathead minnows (*Pimephales promelas*). Aquatic Toxicology. 104: 80-85.

Lemly, D. A. 1997. Environmental implication of excessive selenium: a review. Biomedical and Environmental Sciences 10:415–435.

Marks, D. E. 1999. Life history characteristics of the Sharpnose Shiner (*Notropis oxyrhynchus*) and the Smalleye Shiner (*Notropis buccula*) in the Brazos River, Texas. PhD dissertation. Texas Tech University, Lubbock, Texas.

Moss, R. E., and K. B. Mayes.1993. Current status of *Notropis buccula* and *Notropis oxyrhynchus* in Texas. Job No. 7.0: Endangered and threatened species conservation project No. E-1-4. Texas Parks and Wildlife Department. Austin, Texas.

Ostrand, K. G., and G. R. Wilde. 2001. Tolerances of five prairie stream fishes to abiotic conditions. Transactions of the American Fisheries Society 130:742–749.

TCEQ (Texas Commission on Environmental Quality). 2010. Procedures to implement the Texas Surface Water Quality Standards. RG - 194. TCEQ, Austin, Texas.

TCEQ (Texas Commission on Environmental Quality). 2018. Texas Surface Water Quality Standards. 2016-002-307-OW. TCEQ, Austin, Texas.

TCEQ (Texas Commission on Environmental Quality). 2020. Draft 2020 Texas Integrated Report – Assessment Results for Basin 12 – Brazos River. TCEQ, Austin, Texas.

TWDB (Texas Water Development Board). 2018. Texas Water Development Board Groundwater Database. (http://www.twdb.texas.gov/groundwater/data/gwdbrpt.asp). Accessed November 6, 2019.

USEPA (United States Environmental Protection Agency). 1986. Quality criteria for water. EPA 44/5-86-001.USEPA, Office of Water, Washington, D.C.

USEPA (United States Environmental Protection Agency). 2004. National recommended water quality criteria. 4304T. USEPA, Office of Water, Washington, D.C.

USEPA (United States Environmental Protection Agency). 2007. Aquatic life ambient water quality criteria - Copper. EPA 822-R-07-001. Office of Water. Washington, D.C.

USEPA (United States Environmental Protection Agency). 2016a. Aquatic Life Ambient Water Quality Criteria - Cadmium. EPA 820-R-16-002. USEPA, Office of Water, Washington, D.C.

USEPA (United States Environmental Protection Agency). 2016b. Aquatic life ambient water quality criterion for Selenium – freshwater. EPA 822-R-16-006. USEPA, Office of Water, Washington, D.C.

USEPA (United States Environmental Protection Agency). 2018. Final aquatic life ambient water quality criteria for Aluminum. EPA 822-R-18-001. USEPA, Office of Water, Washington, D.C.

USFWS (United States Fish and Wildlife Service). 2014a. Endangered and Threatened Wildlife and Plants; Determination of Endangered status for the Sharpnose Shiner and Smalleye Shiner. Final Rule, 50 CFR Part 17. Vol. 79, No. 149, Monday, August 4, 2014. pp. 45274–45286.

USFWS (U.S. Fish and Wildlife Service). 2014b. Endangered and Threatened Wildlife and Plants; Designation of critical habitat for the Sharpnose Shiner and Smalleye Shiner; Final Rule, 50 CFR Part 17. Vol. 79, No. 149, Monday, August 4, 2014. pp. 45242–45271.

USFWS (U.S. Fish and Wildlife Service). 2014c. Species status assessment report for the Sharpnose Shiner (*Notropis oxyrhynchus*) and Smalleye Shiner (*N. buccula*). Ecological Services Field Office Report. U.S. Fish and Wildlife Service, Arlington, Texas.

USFWS (U.S. Fish and Wildlife Service). 2015. Recovery outline for Sharpnose Shiner and Smalleye Shiner. Ecological Services Field Office Report. U.S. Fish and Wildlife Service, Arlington, Texas.

Appendix

Formulae used by the Texas Commission on Environmental Quality (TCEQ) to calculate site specific acute and chronic criteria for certain dissolved metals in water. Hardness indicates ambient water hardness (TCEQ 2018).

Dissolved Metal in Water	Acute Criteria	Chronic Criteria
Cadmium	$(1.136672-(\ln(hardness)(0.041838)))\ (we^{(1.0166(\ln(hardness))-2.4743)})$	$(1.101672-(\ln(hardness)(0.041838)))\ (we^{(0.7409(\ln(hardness))-4.719)})$
Chromium	$0.316we^{(0.8190(\ln(hardness))+3.7256)}$	$0.860we^{(0.8190(\ln(hardness))+0.6848)}$
Copper	$0.960me^{(0.9422(\ln(hardness))-1.6448)}$	$0.960me^{(0.8545(\ln(hardness))-1.6463)}$
Nickel	$0.998we^{(0.8460(\ln(hardness))+2.255)}$	$0.997we^{(0.8460(\ln(hardness))+0.0584)}$
Zinc	$0.978we^{(0.8473(\ln(hardness))+0.884)}$	$0.986we^{(0.8473(\ln(hardness))+0.884)}$

e The mathematical constant that is the basis of the natural logarithm. When rounded to four decimal points, e is equal to 2.7183.

m Indicates that a criterion may be multiplied by a water-effect ratio (WER) or based on a biotic ligand model result in order to incorporate the effects of local water chemistry on toxicity. The WER multiplier is equal to 1 except where sufficient data is available to establish a site-specific multiplier. WER multipliers and criteria based on biotic ligand models for individual water bodies are listed in Appendix E of §307.10 of the Texas Surface Water Quality Standards. The number preceding the m in the freshwater equation is an EPA conversion factor. The biotic ligand model is based on the dissolved portion of copper, and the equation is not used in this case.

w Indicates that a criterion is multiplied by a WER in order to incorporate the effects of local water chemistry on toxicity. The WER is equal to 1 except where sufficient data is available to establish a site-specific WER. WERs for individual water bodies are listed in Appendix E of §307.10 of the Texas Surface Water Quality Standards. The number preceding the w in the freshwater criterion equation is an EPA conversion factor.

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