Final Report

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Bioassessment of four Hill Country streams threatened by proposed municipal wastewater discharges

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Scope of Work

The investigators were charged with completing a comprehensive biological assessment of four (4) Hill Country streams that were subject to permit applications for wastewater treatment plant (WWTP) effluent discharges. The four streams included Onion Creek, Blanco River, Honey Creek, and Barton Creek. Sampling occurred during late spring (high base flows) and late summer (low flows). Each stream was sampled above and below proposed locations of WWTP effluent discharges. Locations were selected with the cooperation of SOSA and landowners. Thus, a total of eight (8) stream reaches (defined stretches of streams with defined upstream and downstream locations) were sampled two (2) times each, or the equivalent of sampling 16 stream reaches during the period of performance.

Each reach was sampled in accordance with current Texas Commission on Environmental Quality (TCEQ) biological assessment protocols. Biological assemblages, or community types, sampled were as follows:

1) Periphyton and macroalgae (high and low flow)
2) Benthic macroinvertebrates (high and low flow)
3) Fish (critical flow period, late summer, per TCEQ guidelines)

Further, discharge, surface-water chemistry (temperature, dissolved oxygen, specific conductance, pH, turbidity, dissolved inorganic phosphorus (PO4-P), total phosphorus (TP), ammonium-nitrogen (NH4-N), nitrate-nitrite-N (NOx-N), and total N (TN) were sampled and analyzed at least two times from each reach during the study period. Finally, YSI EXO1 datasondes were deployed at each stream during high and low flow periods to estimate instantaneous changes in dissolved oxygen, pH, conductivity, and temperature at 15-minute intervals over at least a 24 h period.

The following is the final report summarizing results in accordance with the scope of work and services agreement (#32030263)

Study Sites

Four streams were targeted for this study: Barton Creek, Onion Creek, Blanco River, and Honey Creek (Figure 1). Sites were selected in consultation with the sponsor based on current and potential future wastewater discharge permit applications that threatened the water quality and biological integrity of these four, high-quality Hill Country streams.

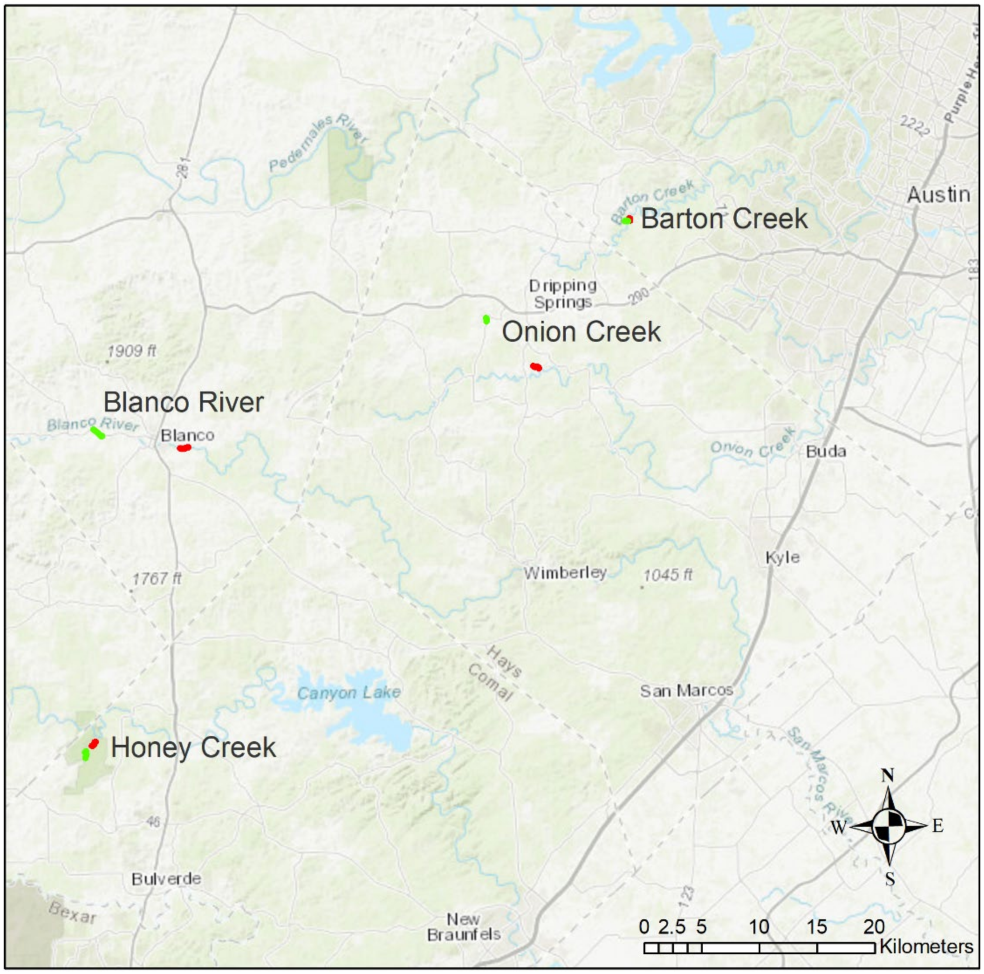

Figure 1. Location of the four streams within the Hill Country region of central Texas. Red and green markers indicate location of the downstream (red) and upstream (green) paired set of reaches per stream.

In March and April 2019, the PIs visited each stream to identify optimal reaches for sampling. The study design, in accordance with the scope of work, was to pair a reach downstream of an existing or pending wastewater discharge with an upstream reach that was as close to the downstream reach as possible while maintaining similar channel form, canopy cover, and other physical characteristics that would necessarily influence the diversity of algae, macroinvertebrates, and fish in each location. In other words, the goal was to have two reaches within each stream that were essentially identical in every way except location relative to an existing or pending wastewater treatment discharge.

Barton Creek

Barton Creek reaches were located on Shield Ranch, Travis County, TX (Figure 2). Reaches were located approximately 16 km upstream (flow distance) from the intersection with SH 71 near Oak Hill, which is the nearest USGS gaging station (USGS 08155200, https://waterdata.usgs.gov/tx/nwis/uv/?site_no=08155200&PARAmeter_cd=00065,00060). Reaches were coded as Barton Creek, Lower (BCL; 30.263458 N, -97.992838 W) and Barton Creek, Upper (BCU; 30.261626 N, -97.994977, W). Each reach was approximately 300 m in length. The upstream marker of BCL was 60 m downstream of the confluence with Long Branch, the tributary that may receive wastewater discharges pending the permit application. The downstream marker of BCU was 130 m upstream of the confluence of Long Branch.

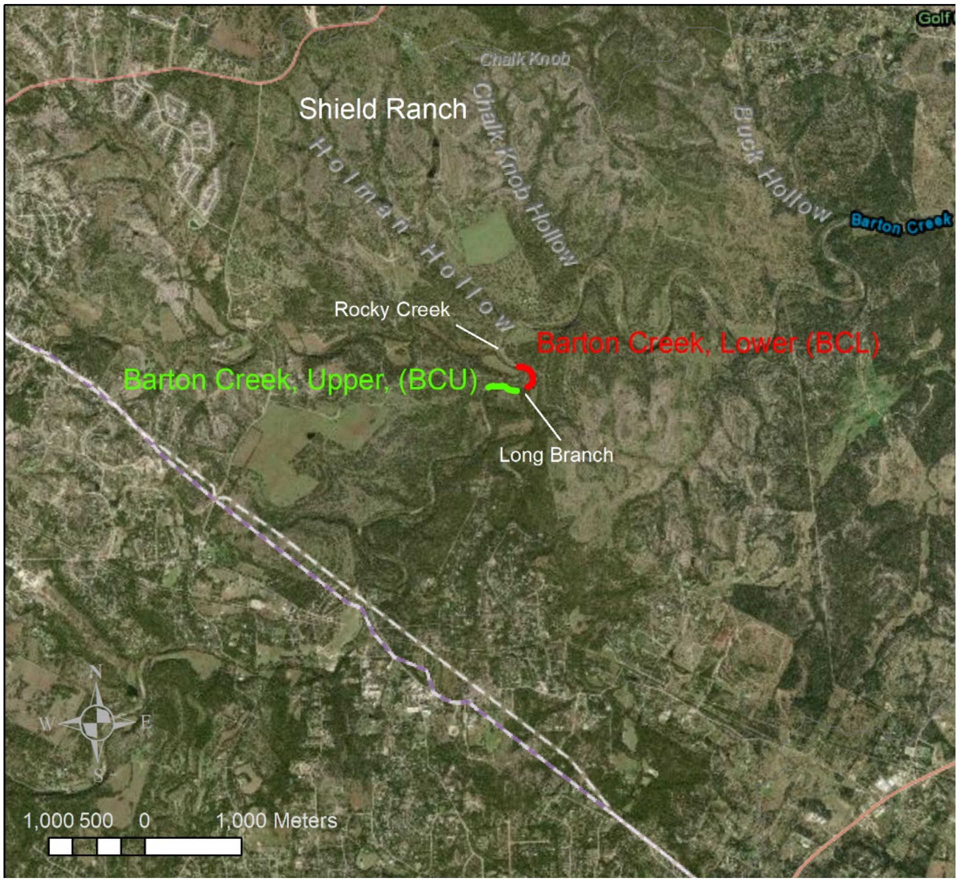

Figure 2. Location of Barton Creek, Lower (BCL) and Barton Creek, Upper (BCU) reaches relative to the proposed wastewater discharge (Long Branch) and a nearby downstream tributary, Rocky Creek, on Shield Ranch, Travis Co., TX.

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Figure 3a. Barton Creek, Shield Ranch, April 2019

Figure 3b. Barton Creek, Shield Ranch, August 2019. View of substrate.

Onion Creek

Onion Creek reaches were located on CharRo Ranch (lower reach) and above the low-water crossing on Creek and Mt Gainor Roads (upper reach), Hays County, near Dripping Springs, TX (Figure 4). The lower reach was located approximately 18 km (flow distance) upstream from the nearest USGS gaging station (USGS 08158700, https://waterdata.usgs.gov/tx/nwis/uv/?site_no=08158700&PARAmeter_cd=00065,00060). Reaches were coded as Onion Creek, Lower (OCL; 30.147500 N, -98.076889 W) and Onion Creek, Upper (OCU; 30.186735 N, -98.123443 W). OCL was approximately 500 m in length, whereas OCU upper was 300 m long. The lower marker of OCL was 50 m downstream of the confluence with South Onion Creek. The downstream marker of OCU was 20 m upstream of the Creek Rd/Mt Gainor low water crossing (Figure 4).

Figure 4. Location of Onion Creek, Lower (OCL) and Onion Creek, Upper (OCU) reaches relative to the proposed wastewater discharge (within Caliterra residential development). OCL was located on CharRo Ranch near the confluence with South Onion Creek whereas OCU was upstream of CharRo Ranch at the nearest upstream location with reasonable access via public easement at junction of Creek and Mt. Gainor Roads, Hays Co., TX.

Figure 5a. Onion Creek, CharRo Ranch, April 2019.

Figure 5b. Onion Creek, Upper Reach (above intersection of Mt. Gainor and Creek Roads), April 2019.

Blanco River

Blanco River reaches were located at Blanco Settlement just downstream of the SH 165 crossing (lower reach) and along Goldwin Smith Road, a private, unpaved road that paralleled the river (upper reach), Blanco County, near Blanco, TX (Figure 6). The upper reach was located approximately 3.5 km (flow distance) downstream from the Crabapple Road USGS gaging station (USGS 08170800, https://waterdata.usgs.gov/tx/nwis/uv/?site_no=08170800&PARAmeter_cd=00065,00060). Reaches were coded as Blanco River, Lower (BRL; 30.090137 N, -98.398604 W) and Blanco River, Upper (BRU; 30.104554 N, -98.483264W). BRU and BRL were approximately 400 m in length.

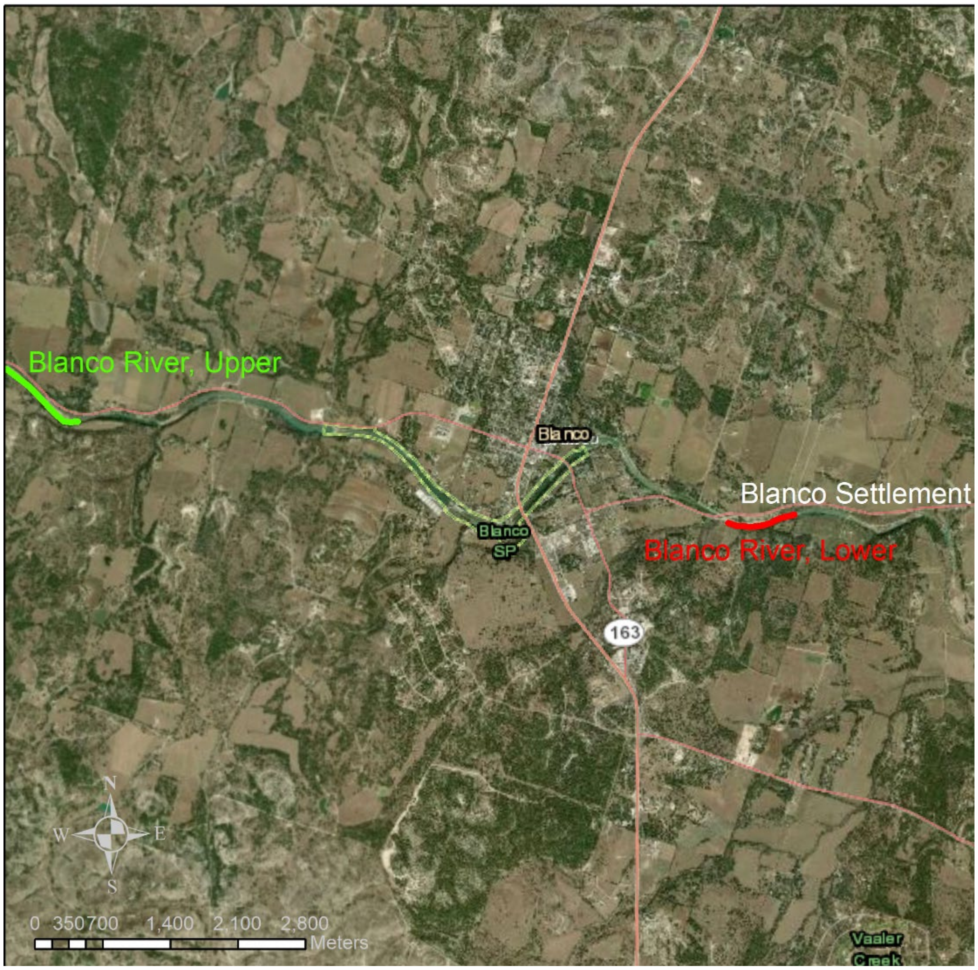


Figure 6. Location of Blanco River, Lower (BRL) and Blanco River, Upper (BRU) reaches. BRL was located immediately adjacent to Blanco Settlement downstream approximately 500 m of the suspected wastewater discharge point from the City of Blanco. BRU was upstream of the City of Blanco immediately adjacent to Goldwin Smith Road, a private, unpaved drive, which was the nearest upstream location with free-flowing habitat that was comparable to BRL, as most of the river between BRU and BRL was impounded. Blanco Co., TX.

Figure 7a. Blanco River, Upper Reach (Goldwin Smith Road), April 2019

Figure. 7b. Blanco River, Blanco Settlement (Lower Reach), April 2019. Note the heavy filamentous algal growth not evident at the upstream location.

Honey Creek

Honey Creek reaches were located in Honey Creek State Natural Area, Guadalupe State Park, Comal County, TX (Figure 8). Both reaches were located below the location of the anticipated wastewater treatment discharge because areas upstream of the discharge are dry for most of the year. Thus, the location of the reaches in Honey Creek differed from the other pairs of reaches in that there was no upstream/downstream comparison. However, the two reaches on Honey Creek captured two different, major spring discharges which could translocate effluent from the proposed discharge in different ways.

The upper reach was located approximately 0.25 km (flow distance) downstream from the first major spring discharge where Honey Creek maintains perennial flow. The downstream reach was located off of a secondary road connected to State Park Rd P31 and just downstream of Beek Spring, which is a significant source of groundwater compared to the upstream spring. Reaches were coded as Honey Creek, Lower (HCL; 29.860162 N, -98.482810 W) and Honey Creek, Upper (HCU; 29.851997 N, -98.489887 W). HCU and HCL reaches were approximately 250 m in length.

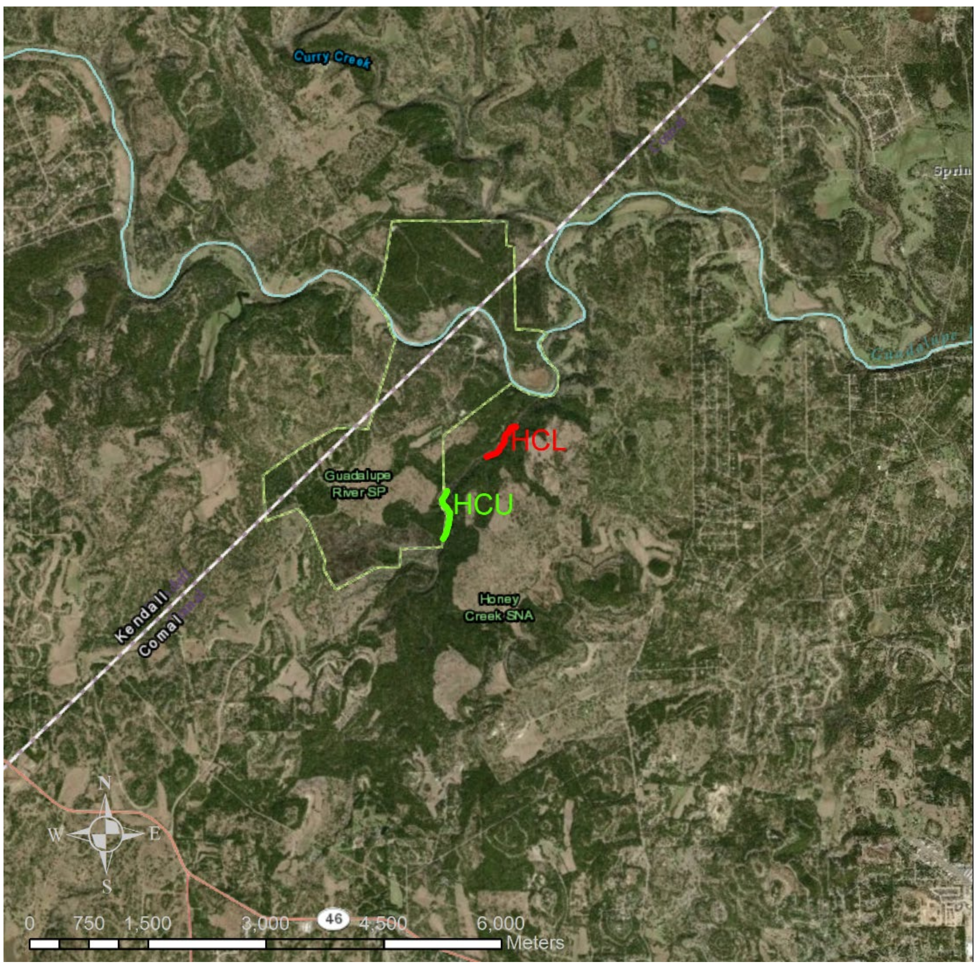


Figure 8. Location of Honey Creek, Lower (HCL) and Honey Creek, Upper (HCU) reaches within Honey Creek State Natural Area and Guadalupe State Park, Comal Co., TX.

Figure 9a. Honey Creek, Upper Reach, May 2019

Figure 9b. Honey Creek, Lower Reach, May 2019

Results

BARTON CREEK

Summary

Barton Creek Upper and Lower Reaches were similar in physical, chemical, and biological characteristics during both high (April-May) and low (August) flow sampling events. Long Branch, the tributary that enters the stream above the lower reach and threatens Barton with potential inputs of nutrients from a pending WWTP permit, had water chemistry that was similar to Barton, with little evidence of nutrient enrichment above background conditions in Barton.

Barton nutrient levels were consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas, with total and orthophosphate (PO_4-P)-phosphorus values < 10 µg/L, total nitrogen (TN) at or below 300 ug/L, nitrite+nitrate-N values at or below 200 µg/L, and ammonium-N < 10 µg/L. All of these values represent high quality, low nutrient conditions.

Dissolved oxygen levels were high and remained at or above levels that are supportive of natural biological communities in Texas streams. EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen and other parameters, revealed similar DO levels between the two reaches during the day and night. The High flow deployment captured an extreme high-water event on May 3, 2019, with stream flow levels jumping from ~100 cfs to ~30,000 cfs in a few hours. The sondes, which were chained to trees, were recovered a few weeks later after flows receded to safe levels for wading. The flood event is very evident in the data, but, surprisingly, DO remained high and even showed daily oscillations that demonstrated modest levels of primary production occurring during the daylight hours even under flood conditions.

Sestonic (sestonic refers to particles in the water column) organic matter (ash-free dry mass particulates), chlorophyll-a (phytoplankton), and total suspended solids were consistent with high-quality, reference stream conditions in both reaches and Long Branch during both high and low flow events. Sestonic chlorophyll-a peaked in high flows at ~ 2 µg/L and was < 1 µg/L during low-flow conditions. For reference, >10 µg/L of sestonic chlorophyll-a is often indicative of eutrophic (nutrient over-enriched) conditions in lakes and rivers.

Periphyton (benthic algae, or algae attached to the stream bottom, particularly on large cobble-sized rocks) biomass was also quite low and consistent with a low-nutrient ecosystem. Total biomass (ash-free dry mass, which the total mass of algae after removing inorganic particles such as carbonates, silt, sand, etc.) and chlorophyll-a were higher in the upper reach during high flow, but values were still quite low in both reaches. Maximum benthic chlorophyll-a (again, benthic refers to algae attached to rocks on the stream bottom) was approximately 45 mg/m^2. For reference, values that exceed 150-200 mg/m^2 are often considered indicative of excessive nutrient pollution, although even lower levels of chlorophyll-a can be associated with a nutrient overenrichment problems, depending upon the reference condition.

Periphyton stable isotope values for carbon (δ13C) and nitrogen (δ15N) were similar between reaches and seasons. The stable nitrogen isotope ratio, δ15N, is often elevated when periphyton obtains its nitrogen from municipal wastewater discharges, was similar above and below Long Branch, suggesting

Long Branch is not contributing a significant source of wastewater to Barton Creek at this time. However, should this change, the lower reach should show an increase in δ15N as compared to the upper reach.

Biomass of *Cladophora glomerata*, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was extremely low in both reaches. It is normal for streams to have some *Cladophora*, so detecting it here was not unexpected. Total algal biovolume, excluding diatoms, was also quite low and consistent with a low-nutrient reference stream.

Diatom species richness was similar between both reaches (30-35 species, depending upon season). Phosphorus (P) sensitive taxa richness and abundance was similar between reaches, as was the richness of P tolerant taxa. It is normal for P tolerant taxa to be found in low-nutrient streams; what matters more is their relative abundance, and, here, they represented well below 50% of the richness and total counts of diatoms.

Macroinvertebrate community composition was quite similar between reaches. Both reaches had about 30 taxa, regardless of season. Using the TCEQ Multimetric Index, both reaches were deemed "Exceptional" in terms of their Aquatic Life Use Designation based on macroinvertebrate communities. The density of macroinvertebrates was low during the high flow event, which was likely due to the huge scouring of the stream channel during the major flood about 1 month before our sampling. The fact that the stream still supported relatively high numbers of species and rated exceptional after this flood is a testament to the high-quality habitat and water found in this stretch of Barton Creek.

Fish assemblages were consistent with high quality Hill Country streams. Species such as Guadalupe Bass, a species endemic to a small region of the Hill Country, were found in both reaches, as were numerous other native species typical of streams in the region.

Barton Creek: Nutrients

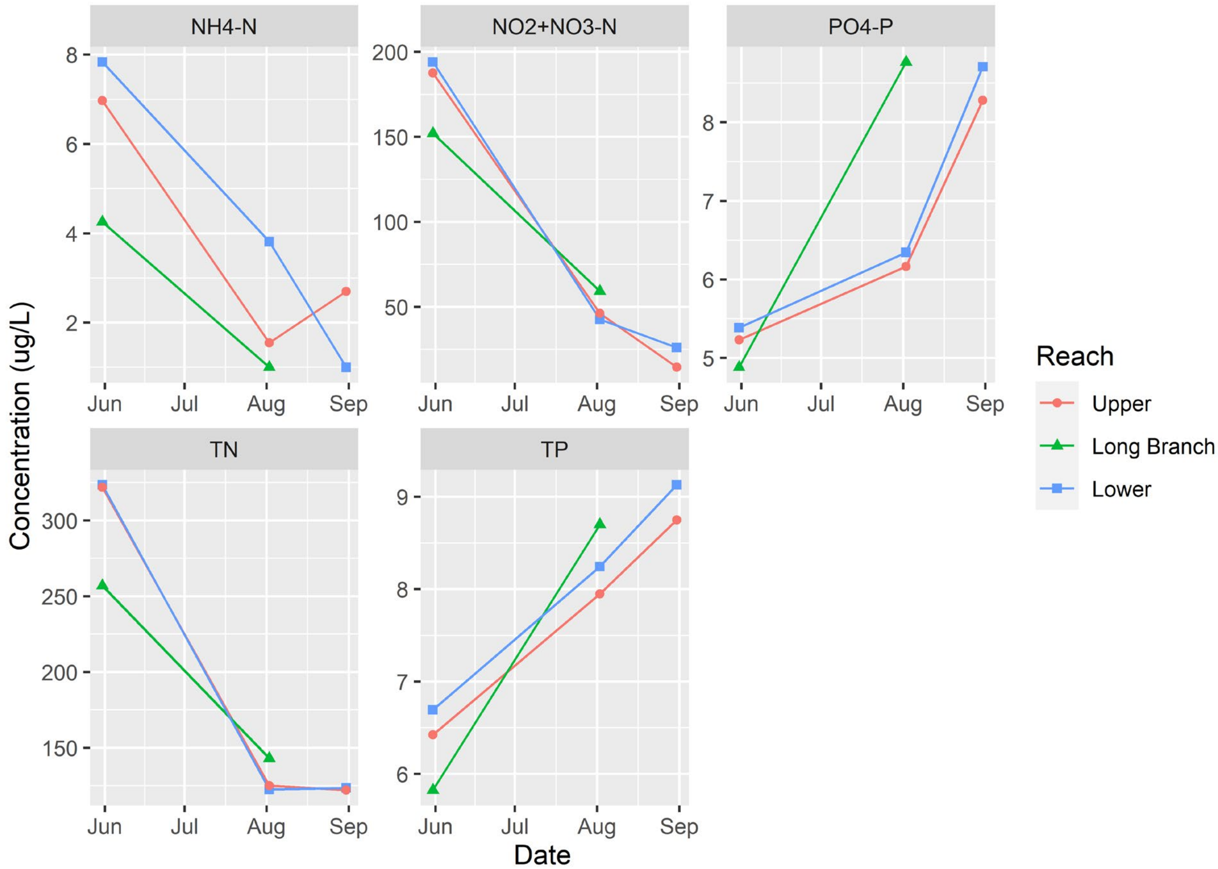


Figure Barton.1: Nutrient levels were consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas, with total phosphorus (TP) and orthophosphate (PO4-P)-phosphorus values < 10 µg/L, total nitrogen (TN) at or below 300 µg/L, nitrite+nitrate-N (NO2+NO3-N) values at or below 200 µg/L, and ammonium-N (NH4-N) < 10 µg/L. All of these values represent high quality, low nutrient conditions. Note that the decline in nitrogen during late summer with simultaneous small increase in phosphorus may indicate that Barton Creek was shifting toward nitrogen limitation during the warmer, dryer months, or that the source of N, which was likely groundwater, was declining.

Barton Creek: YSI EXO1 Data Sonde Parameters, Instantaneous

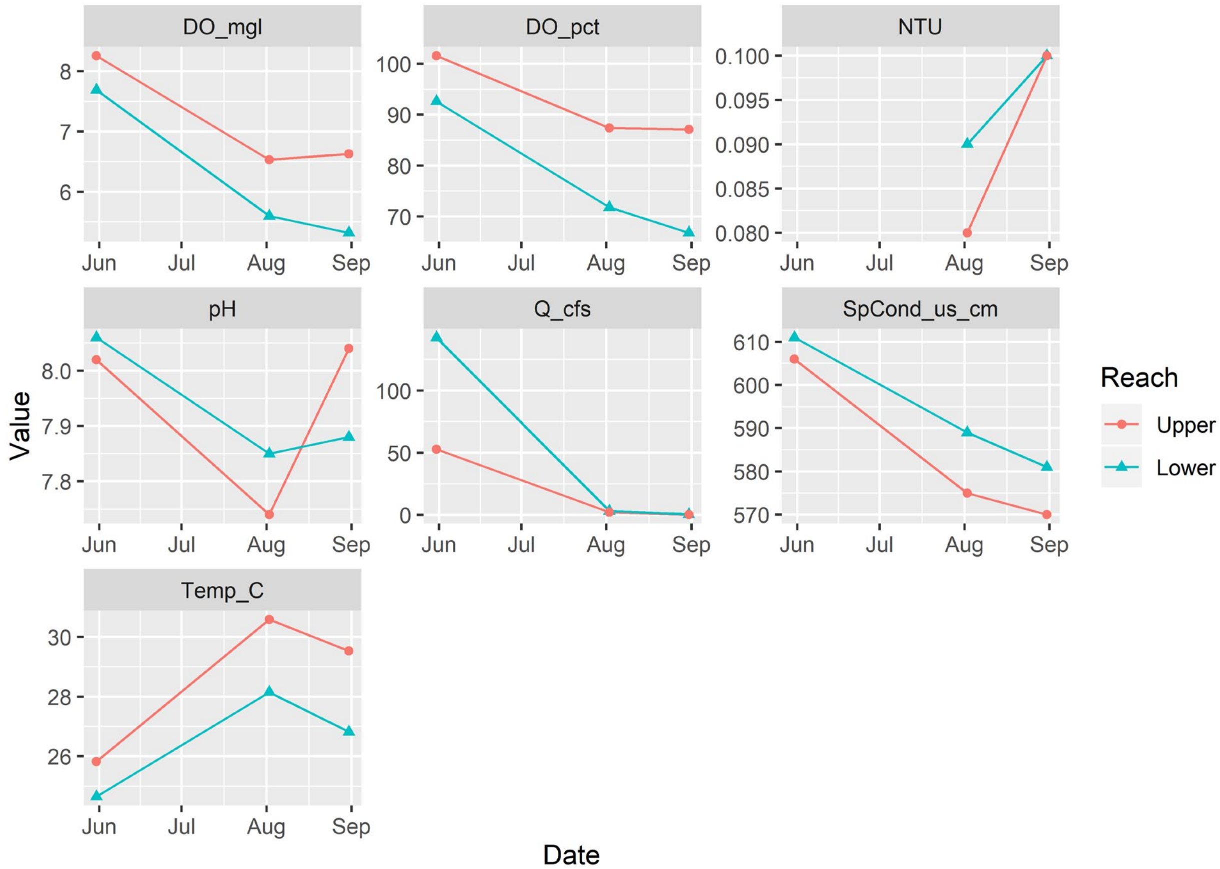


Figure Barton.2: Dissolved oxygen (DO; units are milligrams per liter (mgl) and percent saturation (pct)), turbidity (NTU, a measure of water clarity), pH (acidity), stream flow (Q_cfs, or cubic-feet per second), specific conductance (SpCond_µs_cm; units are microsiemens per centimeter), and water temperature (degrees Celsius) measured in the early morning (Lower) and mid-morning (Upper) reaches of Barton Creek during summer 2019. The tendency for the Upper reach to have higher oxygen and warmer temperatures is related to the time of day when samples were collected (later in the day at the Upper site). NTU levels are extremely low, meaning the water was very clear. NTU was not measured in May. The high value for Q_cfs at the Lower site during late May is not clear, but it may have been related to runoff from Long Branch and springs between the two reaches.

Barton Creek: EXO1 24 h (Diel) Water Quality Parameters

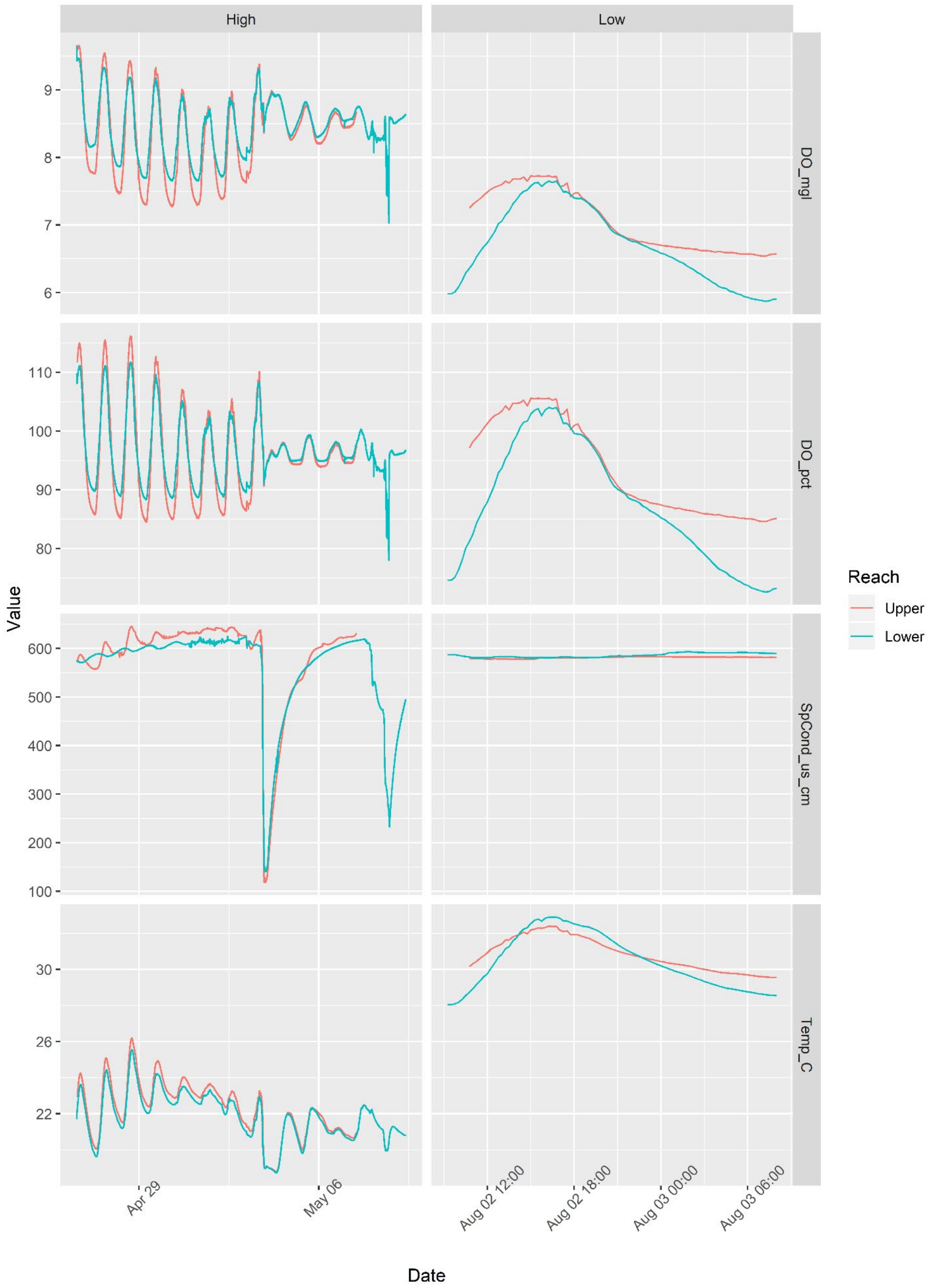


Figure Barton.3: EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen (DO) and other parameters, revealed similar DO levels between the two reaches during the day and night. The High flow deployment captured an extreme high-water event on May 3, 2019, with stream flow levels jumping from ~100 cfs to nearly 30,000 cfs in a few hours. The flood event is very evident in the data, but, surprisingly, DO remained high and even showed daily oscillations that demonstrated modest levels of primary production occurring during the daylight hours even under flood conditions.

Barton Creek: Seston (Organic Matter, Phytoplankton, and Total Particulates)

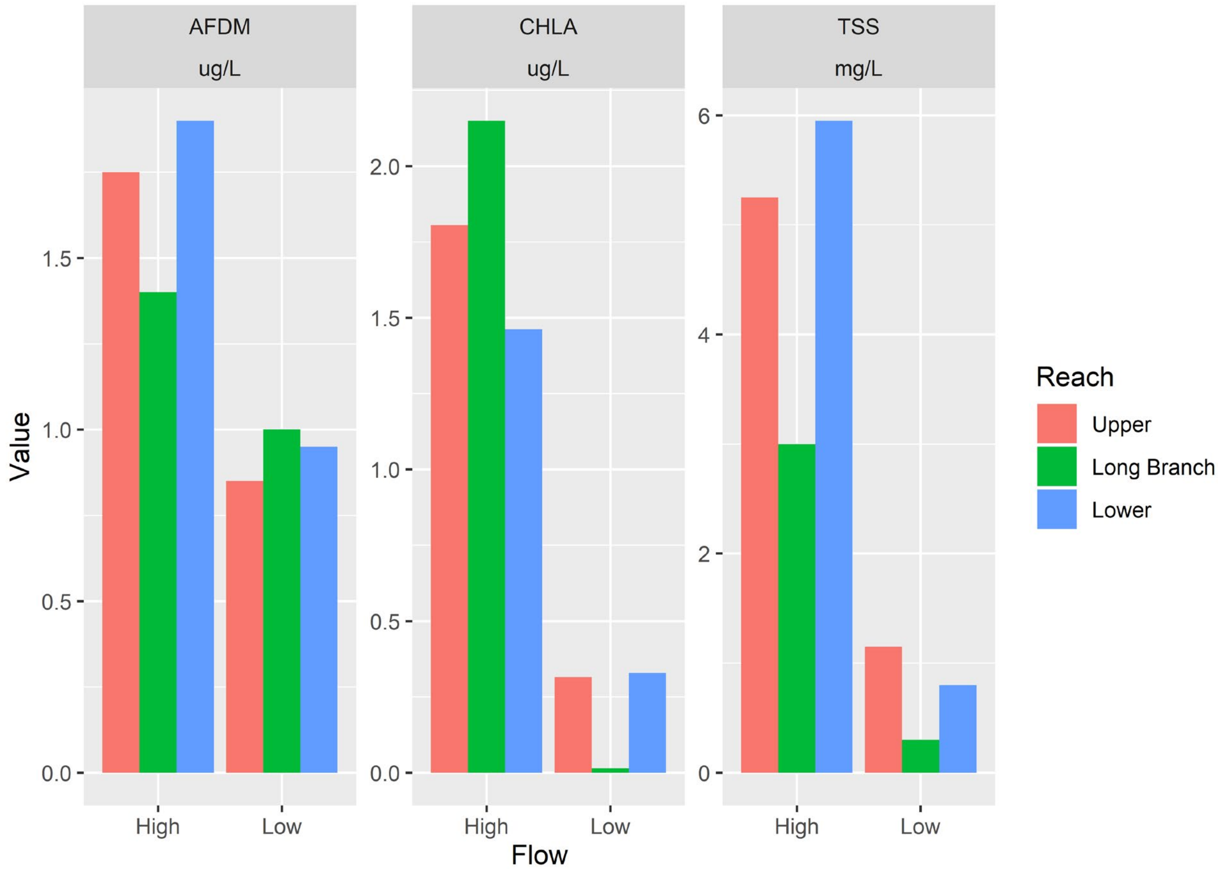


Figure Barton.4: Sestonic (sestonic refers to particles in the water column) organic matter (ash-free dry mass particulates), chlorophyll-a (phytoplankton), and total suspended solids were consistent with high-quality, reference stream conditions in both reaches and Long Branch during both high and low flow events. Sestonic chlorophyll-a peaked in high flows at ~ 2 µg/L and was < 1 µg/L during low-flow conditions. For reference, >10 µg/L of sestonic chlorophyll-a is often indicative of eutrophic (nutrient over-enriched) conditions in lakes and rivers.

Barton Creek: Periphyton (Benthic Algae) Biomass

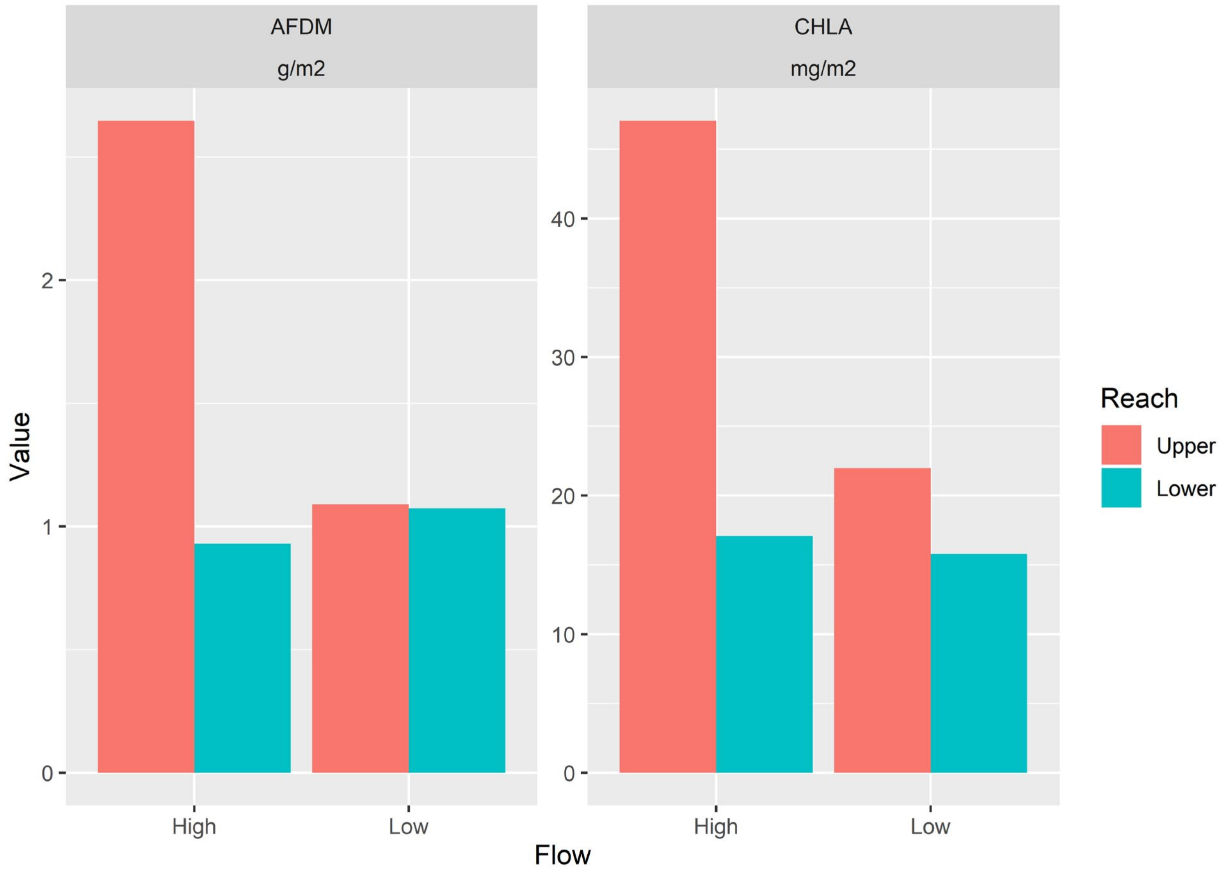


Figure Barton.5: Periphyton biomass (benthic algae, or algae attached to the stream bottom, particularly on large cobble-sized rocks) was relatively low and consistent with a low-nutrient ecosystem. Total biomass (ash-free dry mass, which the total mass of algae after removing inorganic particles such as carbonates, silt, sand, etc.) and chlorophyll-a were higher in the upper reach during high flow, but values were still quite low in both reaches. Maximum benthic chlorophyll-a (again, benthic refers to algae attached to rocks on the stream bottom) was approximately 45 mg/m^2, and around 20 mg/m^2 or less 3 out of 4 measurements..

Barton Creek: Periphyton Stable Isotopic Ratios for Carbon and Nitrogen

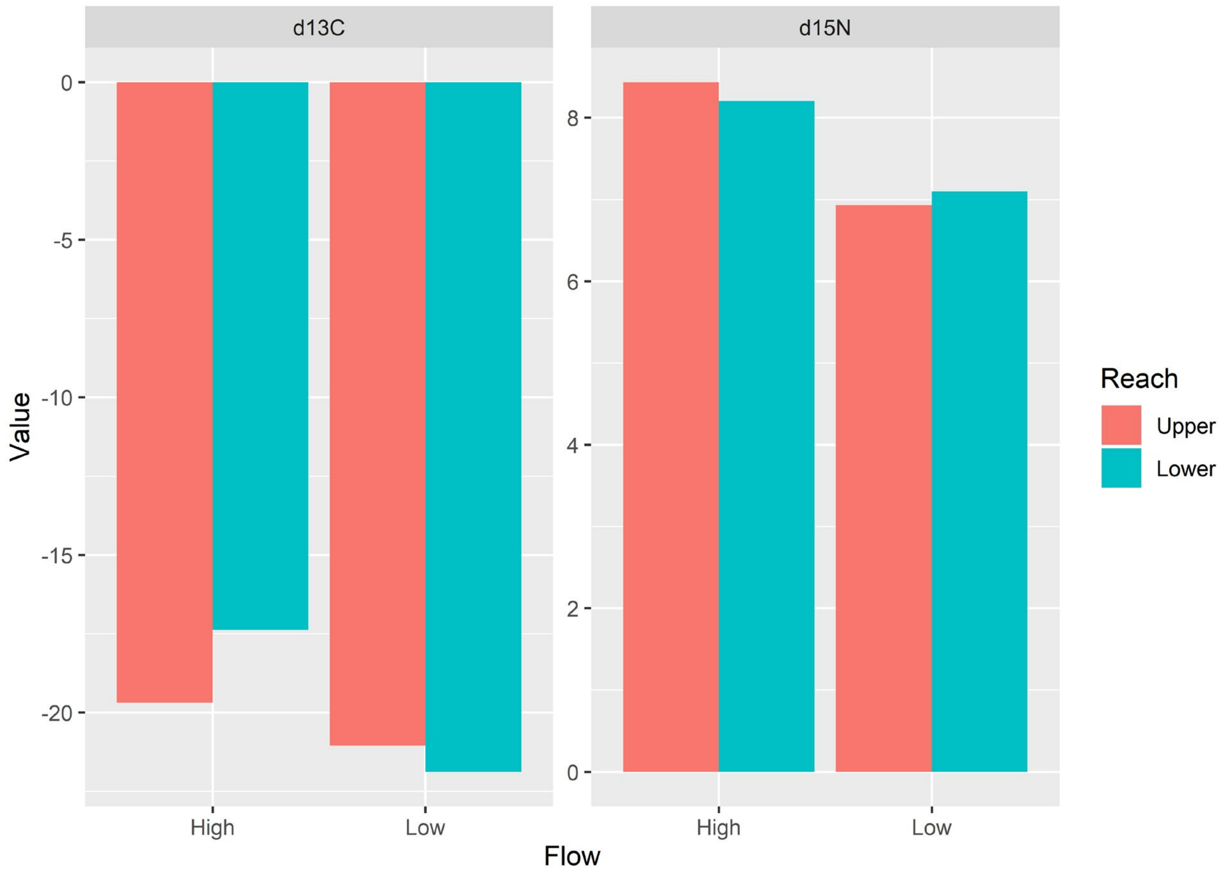


Figure Barton.6: Periphyton stable isotope values for carbon (δ13C) and nitrogen (δ15N) were similar between reaches and seasons. The stable nitrogen isotope ratio, δ15N, is often elevated when periphyton obtains its nitrogen from municipal wastewater discharges, was similar above and below Long Branch, suggesting Long Branch is not contributing a significant source of wastewater to Barton Creek at this time. However, should this change, the lower reach should show an increase in δ15N as compared to the upper reach.

Barton Creek: *Cladophora glomerata* (Nuisance Filamentous Green Alga) and Total Soft Algal Biovolume

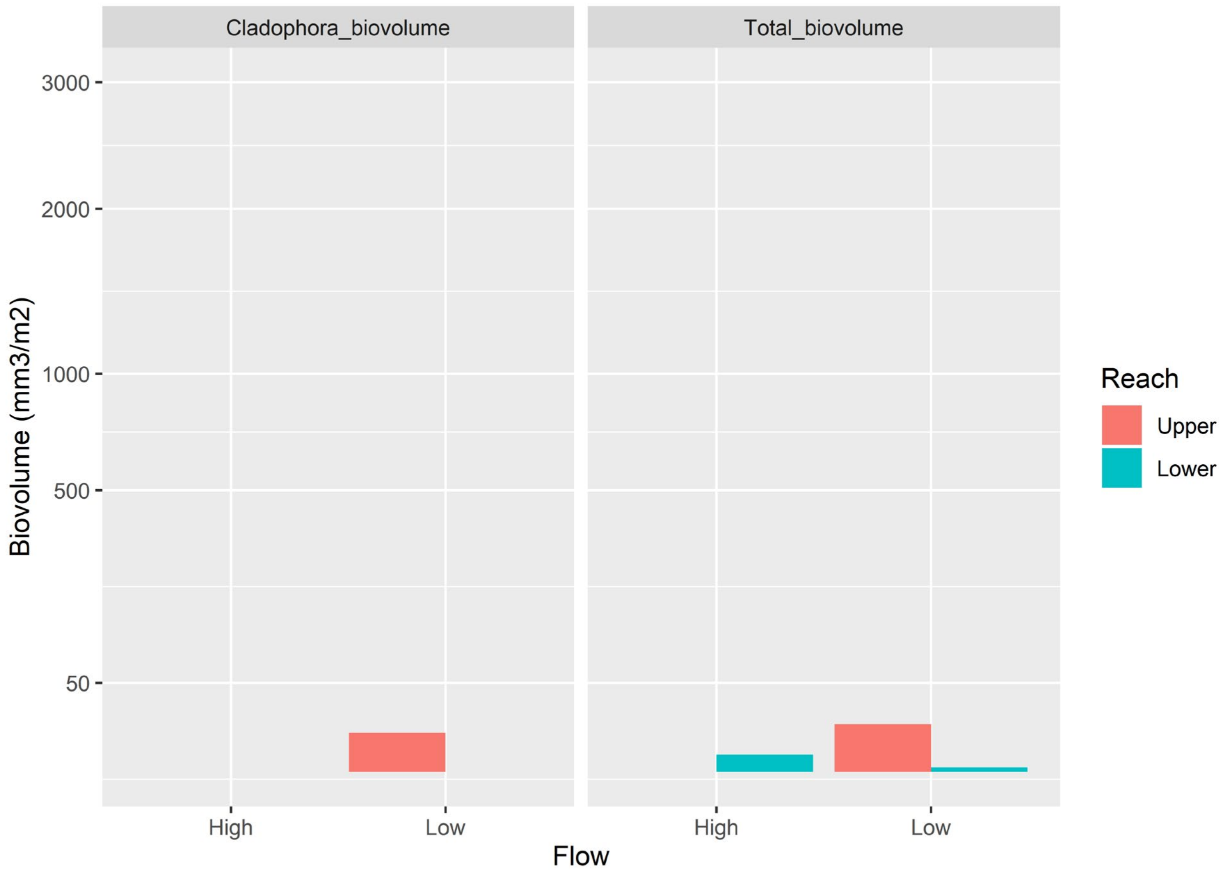


Figure Barton.7: Biomass of Cladophora glomerata, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was extremely low in both reaches. It is normal for streams to have some Cladophora, so detecting it here was not unexpected. Total algal biovolume, excluding diatoms, was also quite low and consistent with a low-nutrient reference stream.

Barton Creek: Diatom Species Community Metrics

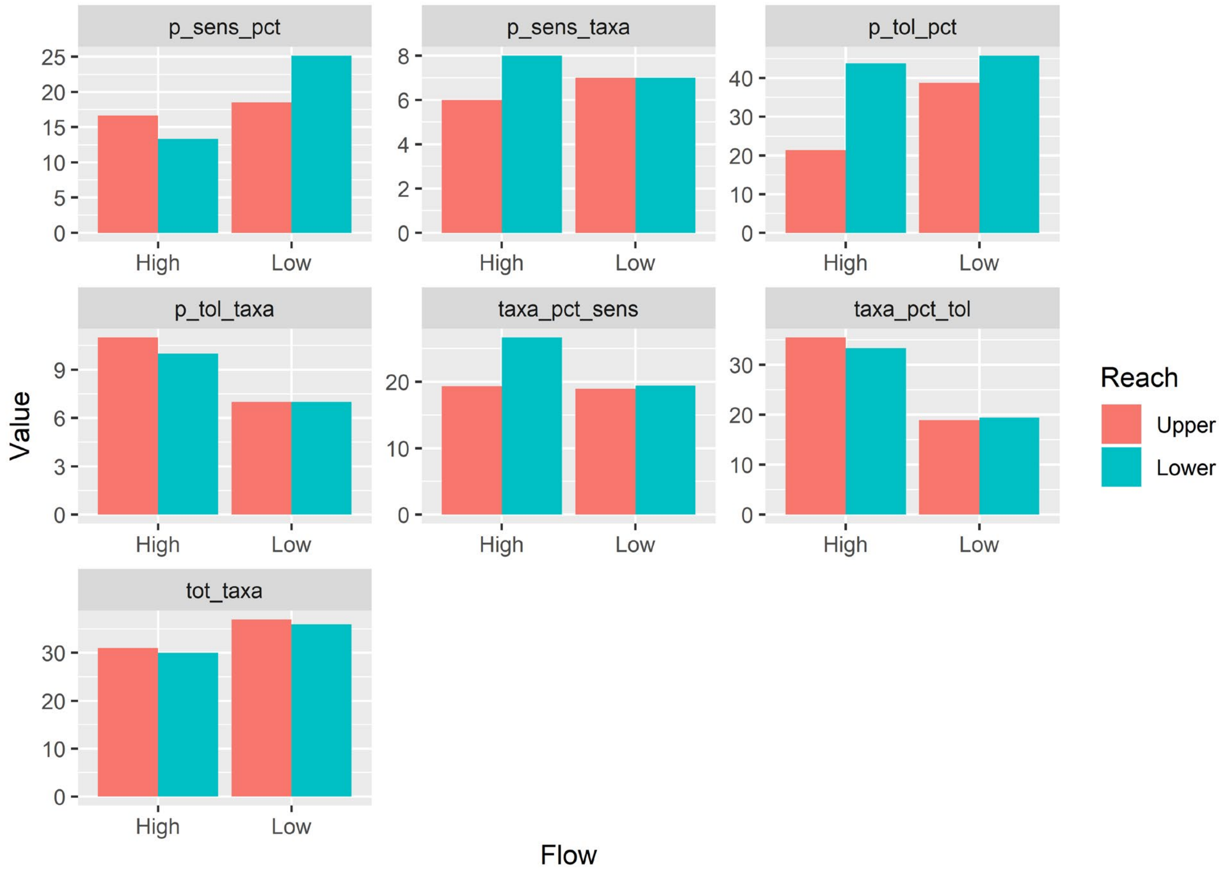


Figure Barton.8: Diatom species richness was similar between both reaches (30-35 species, depending upon season). Phosphorus (P) sensitive taxa richness and abundance was similar between reaches, as was the richness of P tolerant taxa. It is normal for P tolerant taxa to be found in low-nutrient streams; what matters more is their relative abundance, and, here, they represented well below 50% of the richness and total counts of diatoms.

Barton Creek: Macroinvertebrates Community Metrics and ALU Designation

Table Barton.1: Macroinvertebrate community composition was quite similar between reaches. Both reaches had about 30 taxa, regardless of season. Using the TCEQ Multimetric Index, both reaches were deemed "Exceptional" in terms of their Aquatic Life Use Designation based on macroinvertebrate communities.

HIGH FLOW, Upper Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**28**	4	>21	15-21	8-14	<8
# EPT	**9**	3	>9	7-9	4-6	<4
HBI	**3.58**	4	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**5.08**	3	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Chimarra*)	**39.53**	2	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (FC)	**61.99**	1	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**9.84**	4	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**3.61**	3	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**18.26**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**5**	3	>5	4-5	2-3	<2
% Collector-Gatherers	**15.10**	4	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**2.93**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	39				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

High Flow, Lower Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**26**	4	>21	15-21	8-14	<8
# EPT	**8**	3	>9	7-9	4-6	<4
HBI	**3.34**	4	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**2.49**	4	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Chimarra*)	**47.96**	1	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (FC)	**62.14**	1	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**9.15**	4	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**3.84**	3	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**18.02**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**4**	3	>5	4-5	2-3	<2
% Collector-Gatherers	**14.70**	4	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**3.45**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	39				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

LOW FLOW, Upper Reach

Metric	VALUE	Score	4	3	2	1
Taxa Richness	**29**	4	>21	15-21	8-14	<8
# EPT	**10**	4	>9	7-9	4-6	<4
HBI	**2.64**	4	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**2.28**	4	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Chimarra*)	**48.11**	1	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (FC)	**54.08**	2	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**7.92**	4	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**6.90**	4	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**7.00**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**2**	2	>5	4-5	2-3	<2
% Collector-Gatherers	**19.93**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**7.28**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXPECTIONAL**	40				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Low Flow, Lower Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**31**	4	>21	15-21	8-14	<8
# EPT	**10**	4	>9	7-9	4-6	<4
HBI	**3.07**	4	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**2.57**	4	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Chimarra*)	**32.67**	2	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (FC)	**40.31**	3	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**15.44**	3	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**3.51**	3	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**11.93**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**4**	3	>5	4-5	2-3	<2
% Collector-Gatherers	**22.95**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**9.75**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	41				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Barton Creek: Macroinvertebrate Densities

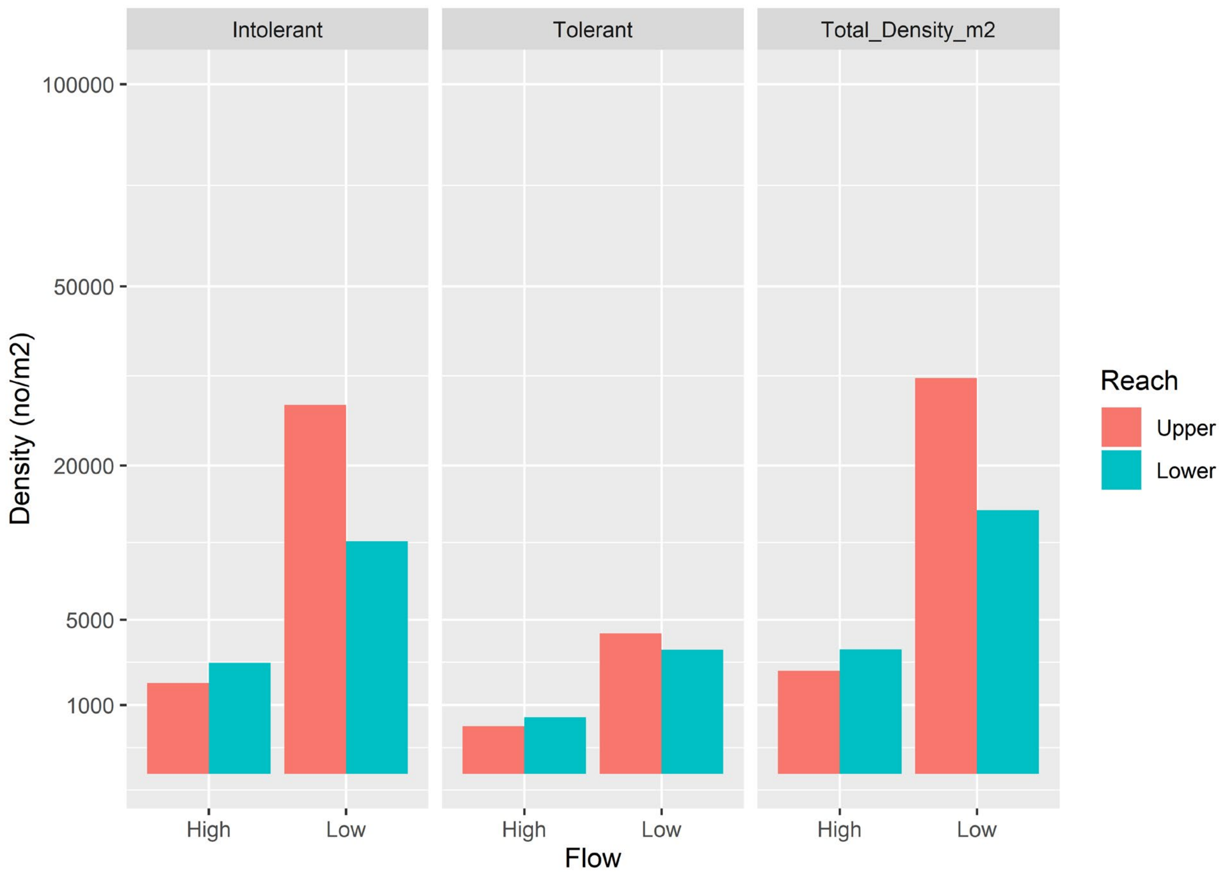


Figure Barton.9: The density of macroinvertebrates was low during the high flow event, which was likely due to the huge scouring of the stream channel during the record flood about 1 month before our sampling. The fact that the stream still supported relatively high numbers of species and rated exceptional after this flood is a testament to the high-quality habitat and water found in this stretch of Barton Creek.

Barton Creek: Macroinvertebrate Taxonomic Composition

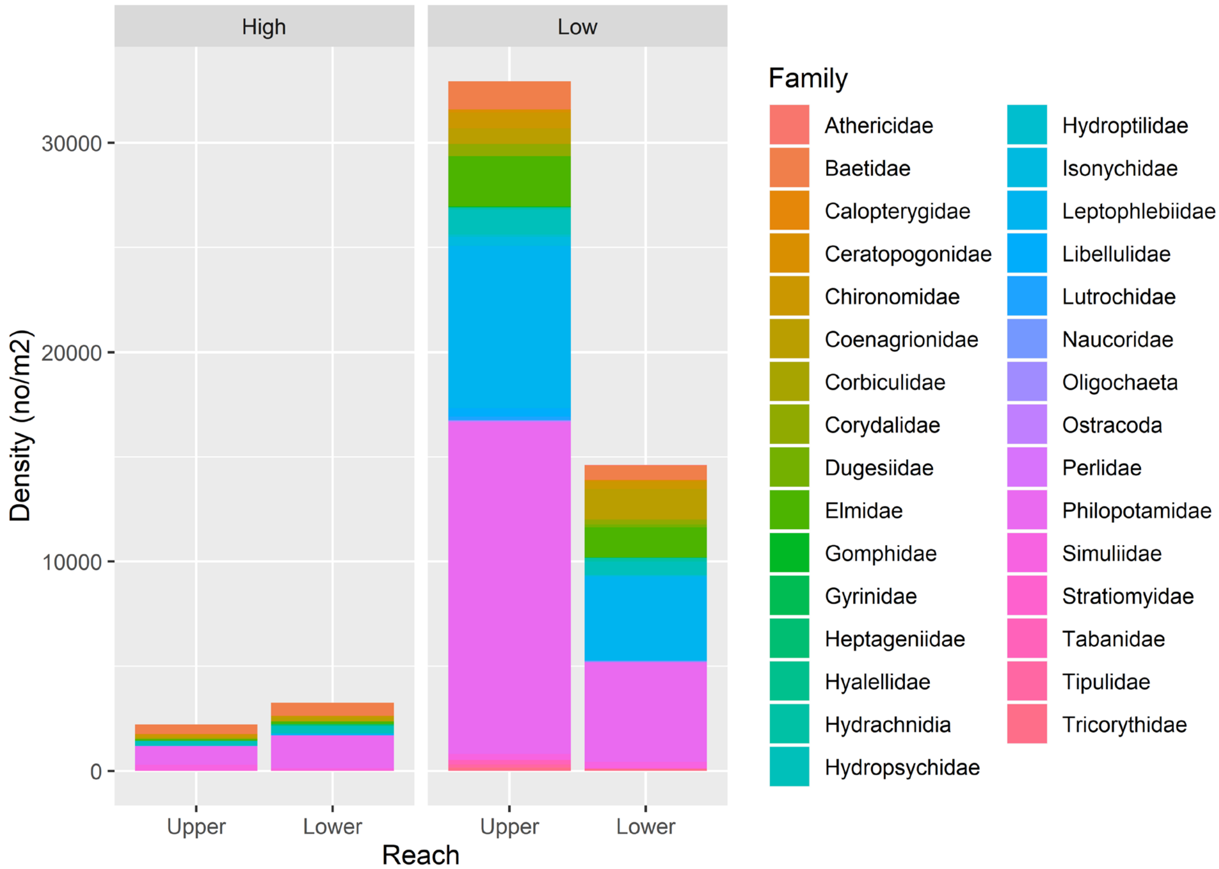


Figure Barton.10: Stacked-bar chart showing the total densities of macroinvertebrates by taxonomic family. Densities differed markedly between high and low flow events because the former occurred within 1 month of a large flood, thus macroinvertebrates had yet to recover completely. However, the proportion of the different families between Upper and Lower reaches was nearly identical within the High and Low flow events, respectively.

Barton Creek: Fish Assemblage Composition

Table Barton.2: Fish assemblages were consistent with high quality Hill Country streams. Species such as Guadalupe Bass, a species endemic to a small region of the Hill Country, were found in both reaches, as were numerous other native species typical of streams in the region.

Barton Creek, Lower		
Species	Count, Total	Count, Juveniles
Blacktail Shiner	135	10
Bluegill	58	19
Central Stoneroller	87	0
Channel Catfish	32	32
Green Sunfish	1	0
Guadalupe Bass	2	2
Largemouth Bass	12	6
Lepomis spp.	8	8
Longear Sunfish	13	2
Redbreast Sunfish	19	1
Rio Grande Cichlid	12	11
Western Mosquitofish	11	0
Yellow Bullhead	9	7
Total	**399**	**98**
Barton Creek, Upper		
Blacktail Shiner	148	5
Bluegill	21	10
Central Stoneroller	160	0
Channel Catfish	54	54
Green Sunfish	1	0
Guadalupe Bass	3	3
Largemouth Bass	5	2
Lepomis spp.	9	8
Longear Sunfish	39	12
Redbreast Sunfish	6	0
Rio Grande Cichlid	7	5
Western Mosquitofish	15	0
Total	**468**	**99**

Image Barton.1: Rio Grande Cichlid from Barton Creek, Upper Reach, September 2019.

Results

BLANCO RIVER

Summary

Blanco River Upper and Lower Reaches were relatively similar in physical habitat and stream flow but differed substantially in some of their chemical (e.g., nutrient) and biological (e.g. algal biomass) characteristics during both high (April-May) and low (August-September) flow sampling events. These differences appeared to be related to nutrient enrichment from wastewater or other source immediately upstream of the lower reach at Blanco Settlement.

Upper Blanco phosphorus levels were consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas, with total and orthophosphate (PO_4-P)-phosphorus values < 10 µg/L. Nitrite+nitrate-N and total N (TN) values were also quite low during April and August, but ticked up in September to levels that were higher than typical of a reference stream in the region. Water flow was extremely low at this time, and fish excretion in pools may have contributed to the elevated nitrogen levels observed in the upper reach, as this is not unusual during very low flow periods in streams in the region.

In contrast, lower Blanco nutrient levels, particularly phosphorus, were elevated above levels typical of reference streams in the region. Total phosphorus (TP) ranged from 17 to over 40 µg/L. TP concentrations above 15-20 are within the threshold zone for rapid, nonlinear changes in algal assemblages in streams in the region. The lowest value (17 µg/L) was observed in April during a large algal bloom, so it is likely that phosphorus was being pulled from the water column by the algae, bringing the level down. The highest value was during the low flow period in September when algal biomass was much lower due to summer scouring events that washed most of the filamentous algae away. This suggests that the load of phosphorus coming from upstream is triggering blooms and being sequestered by algae. Once algal filaments are washed away by high flows, phosphorus levels increase because less algae are present to remove it from the water column.

Dissolved oxygen levels were generally high and remained at or above levels that are supportive of natural biological communities in Texas streams in both reaches; however, nighttime DO dropped below 5 mg/L at the lower reach during the April sampling event that coincided with the bloom. EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen and other parameters, revealed much larger swings in DO levels at the lower reach, consistent with higher levels of primary production (i.e., algal growth).

Sestonic organic matter (ash-free dry mass particulates), chlorophyll-a (phytoplankton), and total suspended solids were consistently higher in the lower reach. The levels of sestonic chlorophyll-a at the lower reach exceeded 5 µg/L during the high flow event (algal bloom); levels above 5 µg/L are unusual for Hill Country streams and are visible to the naked eye (that is, the water looks colored by algae and loses its clarity). The upper reach had much lower sestonic chlorophyll-a in the spring, also suggesting that there was a source of nutrients causing the bloom at the lower reach that was not present at the upper reach.

Periphyton (benthic algae) biomass was much higher in the lower (Blanco Settlement) reach during both events. Chlorophyll-a exceeded 200 mg/m^2 at the lower reach during the early season sampling (during the bloom), whereas the upper supported < 50 mg/m^2 during both seasons.

Periphyton stable isotope values for carbon (δ13C) and nitrogen (δ15N) were also different between reaches and seasons. Stable nitrogen isotopic ratios, which are typically elevated in periphyton where it obtains its nitrogen from municipal wastewater discharges, was approximately 3 units higher at the lower than upper reach, exceeding values of 10 and 11 δ15N during the high and low flow events, respectively, at the lower reach. These levels are **highly indicative** of nitrogen sources from wastewater. Such large differences between reaches also suggest that the sources of nitrogen are much different between reaches.

Biomass of *Cladophora glomerata*, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was quite high during the early, high flow event at the lower reach, again consistent with nutrient enrichment from wastewater. *Cladophora* proliferates near wastewater discharges, and this result implies that wastewater was likely causing the blooms observed at the lower reach during April 2019. During the latter, low-flow event in August, moderate levels of *Cladophora* biovolume were identified at the lower reach whereas **none** was found at the upper reach.

Diatom species composition also revealed substantial differences between the two reaches. The percentage of phosphorus (P) sensitive taxa richness and abundance was much higher at the upper reach, whereas the lower reach had large numbers of P-tolerant taxa. Diatoms are very sensitive to P enrichment, so this finding strongly implies that the lower reach was receiving excessive P enrichment from a source not found at the upper reach.

Macroinvertebrate community composition also differed dramatically between reaches. Using the TCEQ Multimetric Index, the lower reach was deemed "High" in terms of the Aquatic Life Use Designation based on macroinvertebrate communities during April 2019. However, this result seems dubious given the fact that the density of macroinvertebrates at the lower reach during the April algal bloom was abnormally high, approaching 100,000 individuals/m^2, and this was driven almost entirely by taxa that are typically associated with organic pollution and wastewater discharges. Flatworms (Dugesiidae), air-breathing snails (Physidae), segmented worms (Oligochaeta), and a very tolerant mayfly genus (*Baetis*) dominated the densities and biomass at the lower reach during April 2019. Note that TCEQ considers *Baetis* to be "Intolerant" despite the fact that it is arguably the most ubiquitous, tolerant mayfly found in streams throughout the USA (e.g., they even thrive in Appalachian streams that are highly impacted by runoff from coal mines).

Fish assemblages were relatively similar between reaches in terms of species composition. However, numerical abundance of fish, particularly fish that graze heavily on algae (e.g., central stonerollers) and shiner species that eat drifting *Baetis* nymphs were particularly abundant at the lower reach. The upper reach had low numbers of fish but several large individuals of largemouth bass, longear sunfish, redear sunfish, and good numbers of juvenile Guadalupe bass.

Blanco River: Nutrients

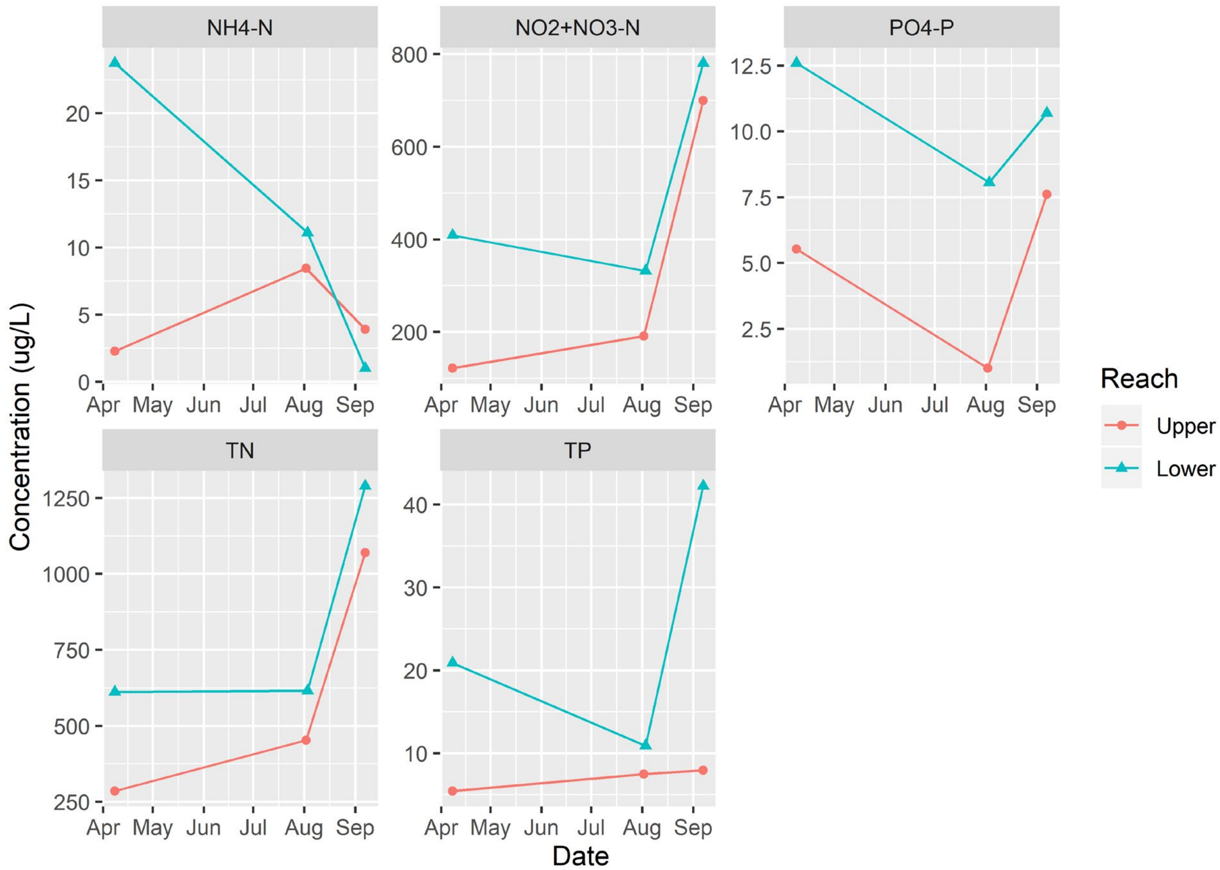


Figure Blanco.1: Upper Blanco phosphorus levels were consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas, with total and orthophosphate (PO_4-P)-phosphorus values < 10 µg/L. Nitrite+nitrate-N and total N (TN) values were also quite low during April and August, but ticked up in September to levels that were higher than typical of a reference stream in the region. In contrast, lower Blanco nutrient levels, particularly phosphorus, were elevated above levels typical of reference streams in the region. Total phosphorus (TP) ranged from 17 to over 40 µg/L. TP concentrations above 15-20 are within the threshold zone for rapid, nonlinear changes in algal assemblages in streams in the region. The lowest value (17 µg/L) was observed in April during a large algal bloom, so it is likely that phosphorus was being pulled from the water column by the algae, bringing the level down. The highest value was during the low flow period in September when algal biomass was much lower due to summer scouring events that washed most of the filamentous algae away.

Blanco River: YSI EXO1 Data Sonde Parameters, Instantaneous

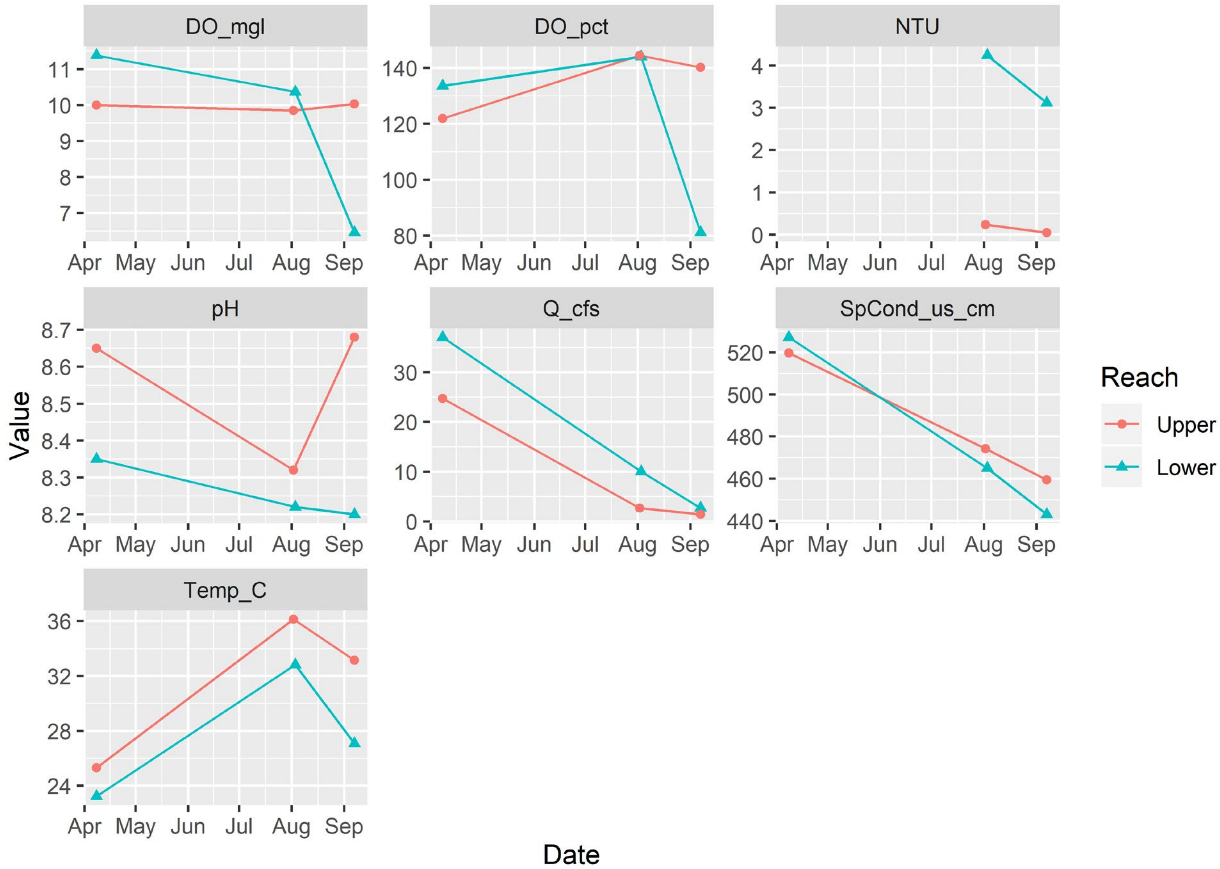


Figure Blanco.2: Dissolved oxygen (DO; units are milligrams per liter (mgl) and percent saturation (pct)), turbidity (NTU, a measure of water clarity), pH (acidity), stream flow (Q_cfs, or cubic-feet per second), specific conductance (SpCond_us_cm; units are microsiemens per centimeter), and water temperature (degrees Celsius) measured in the early morning (Lower) and mid-morning (Upper) reaches of Blanco River during summer 2019. The tendency for the Upper reach to have warmer temperatures is related to the time of day when samples were collected (later in the day at the Upper site). NTU levels at Upper reach (reference) were extremely low, meaning the water was very clear; however, NTU levels were much higher at the Lower reach, indicating cloudy water. NTU was not measured in May. The two reaches were otherwise quite similar in stream flow, specific conductance and pH (although slightly higher pH at Upper reach).

Blanco River: EXO1 24 h (Diel) Water Quality Parameters

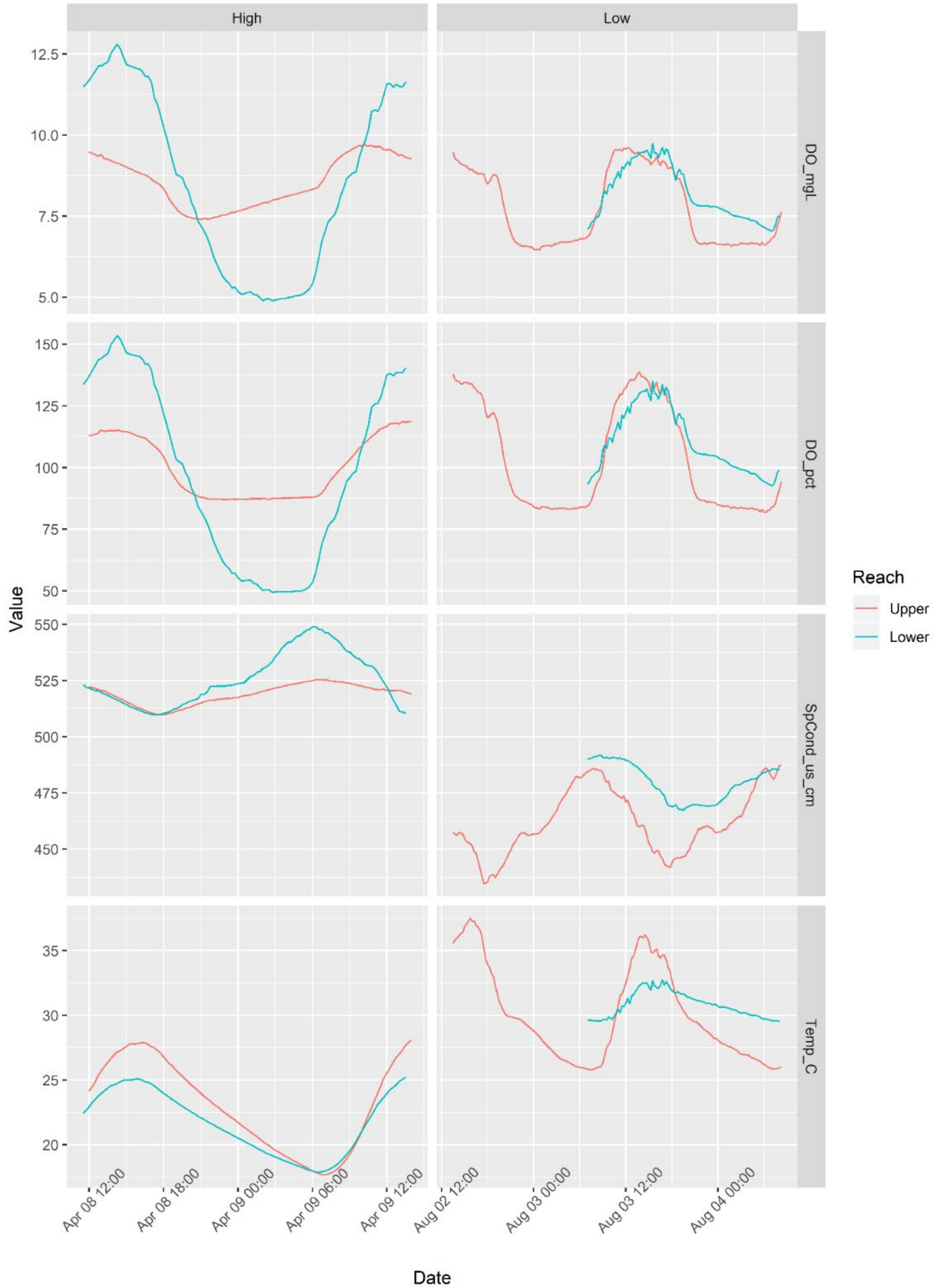


Figure Blanco.3: EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen and other parameters, revealed much larger swings in dissolved oxygen (DO) levels at the lower reach, consistent with higher levels of primary production (i.e., algal growth). Dissolved oxygen levels were generally high and remained at or above levels that are supportive of natural biological communities in Texas streams in both reaches; however, nighttime DO dropped below 5 mg/L at the lower reach during the April sampling event that coincided with the bloom.

Blanco River: Seston (Organic Matter, Phytoplankton, and Total Particulates in Water Column)

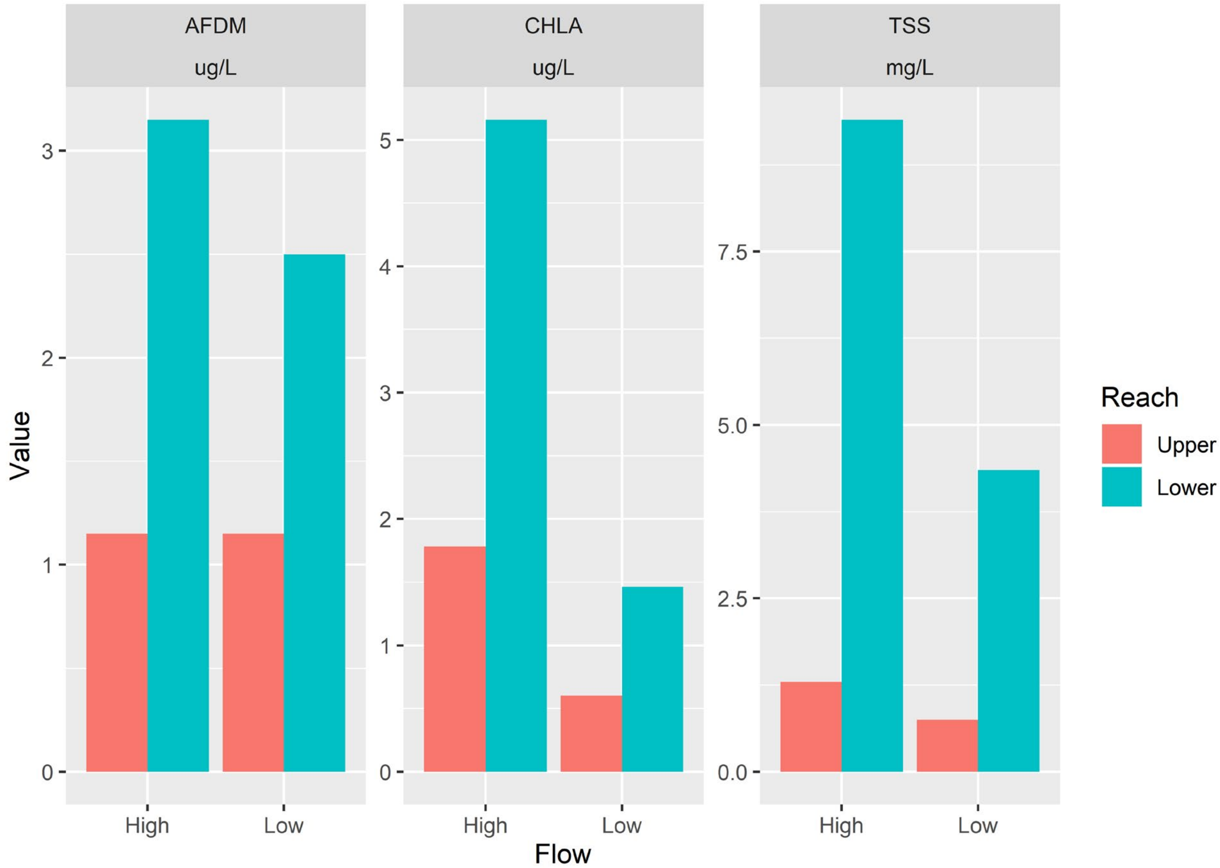


Figure Blanco.4: Sestonic organic matter (ash-free dry mass particulates floating in the water column), chlorophyll-a (phytoplankton or other algal cells in water column), and total suspended solids (TSS, all particulates in water column) were consistently higher in the lower reach. The levels of sestonic chlorophyll-a at the lower reach exceeded 5 µg/L during the high flow event (algal bloom); levels above 5 µg/L are unusual for Hill Country streams and are visible to the naked eye (that is, the water looks colored by algae and loses its clarity). The upper reach had much lower sestonic chlorophyll-a in the spring, also suggesting that there was a source of nutrients causing the bloom at the lower reach that was not present at the upper reach.

Blanco River: Periphyton (Benthic Algae) Biomass

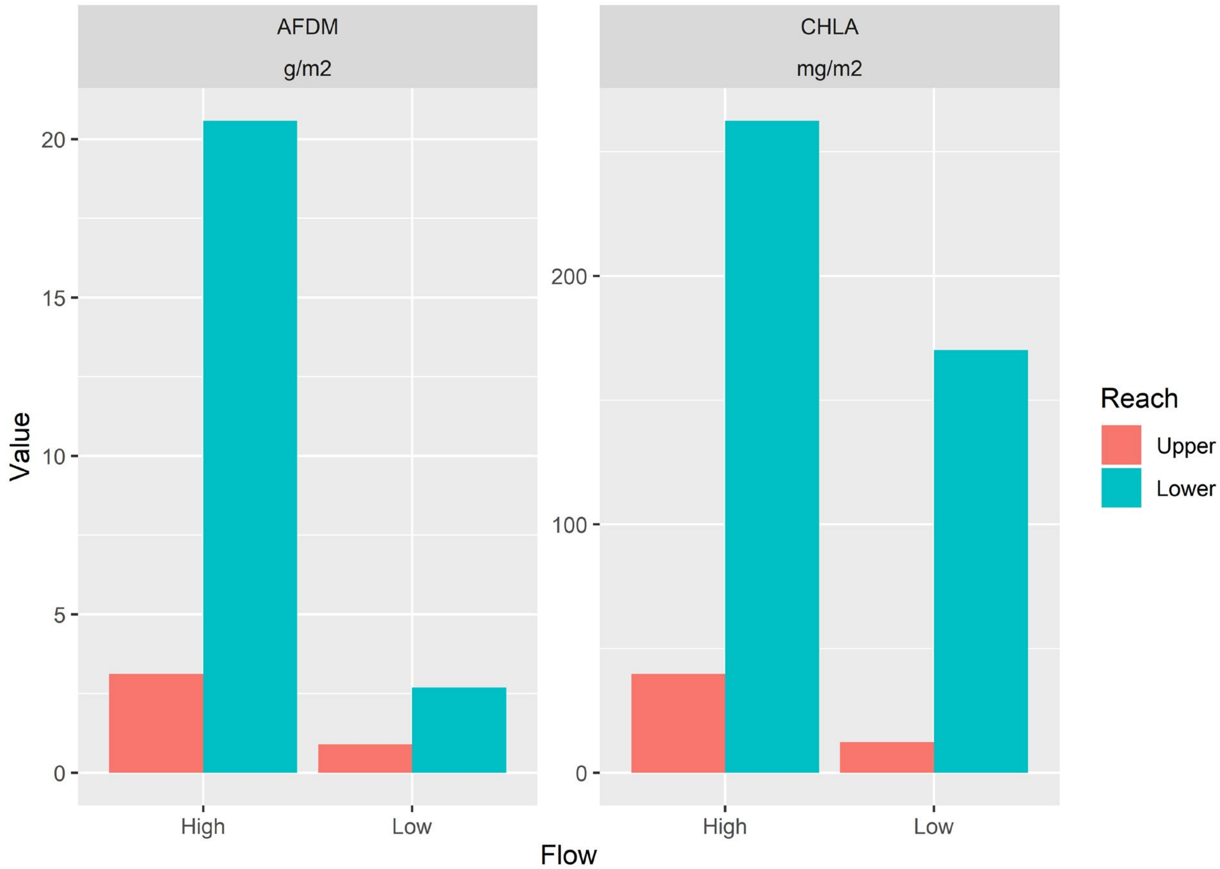


Figure Blanco.5: Periphyton (benthic algae, or algae attached to rocks on stream bottom) biomass was much higher in the lower (Blanco Settlement) reach during both events. Chlorophyll-a exceeded 200 mg/m^2 at the lower reach during the early season sampling (during the bloom), whereas the upper supported < 50 mg/m^2 during both seasons. .

Blanco River: Periphyton Stable Isotopic Ratios for Carbon and Nitrogen

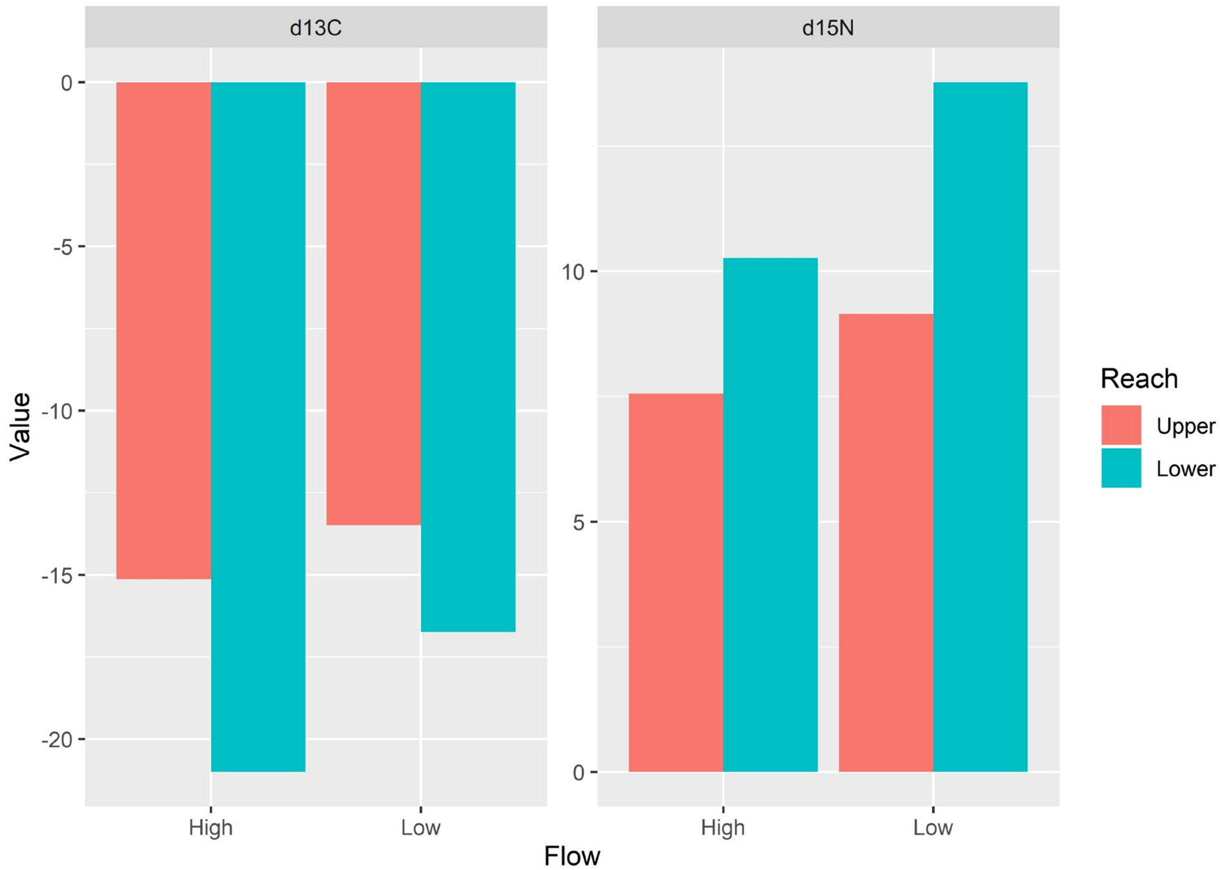


Figure Blanco.6: Periphyton stable isotope values for carbon (δ13C) and nitrogen (δ15N) were different between reaches and seasons. Stable nitrogen isotopic ratios, which are typically elevated in periphyton where it obtains its nitrogen from municipal wastewater discharges, was approximately 3 units higher at the lower than upper reach, exceeding values of 10 and 11 δ15N during the high and low flow events, respectively, at the lower reach. These levels are highly indicative of nitrogen sources from wastewater. Such large differences between reaches also suggest that the sources of nitrogen are much different between reaches.

Blanco River: *Cladophora glomerata* (Nuisance Filamentous Green Alga) and Total Soft Algal Biovolume

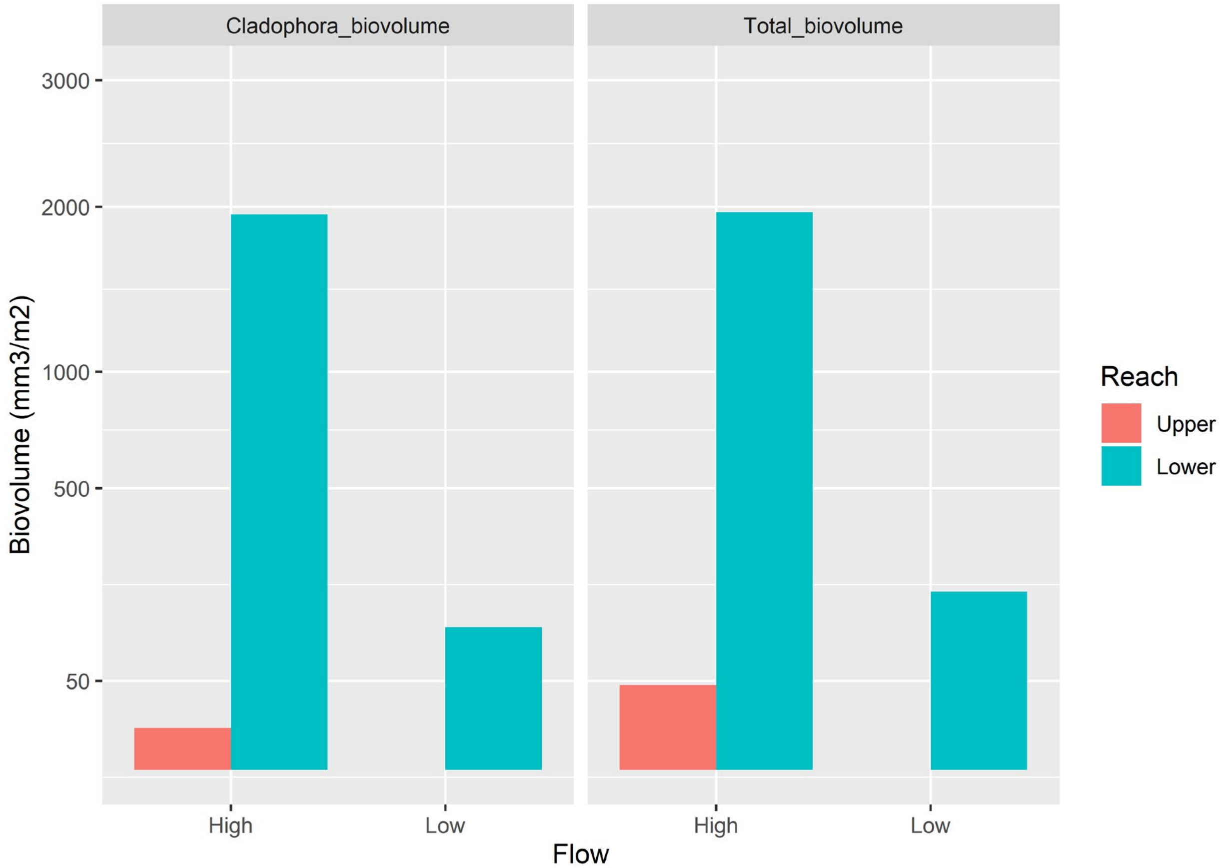


Figure Blanco.7: Biomass of Cladophora glomerata, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was quite high during the early, high flow event at the lower reach, again consistent with nutrient enrichment from wastewater. Cladophora proliferates near wastewater discharges, and this result implies that wastewater was likely causing the blooms observed at the lower reach during April 2019. During the latter, low-flow event in August, moderate levels of Cladophora biovolume were identified at the lower reach whereas ***none*** *was found at the upper reach.*

Blanco River: Diatom Species Community Metrics

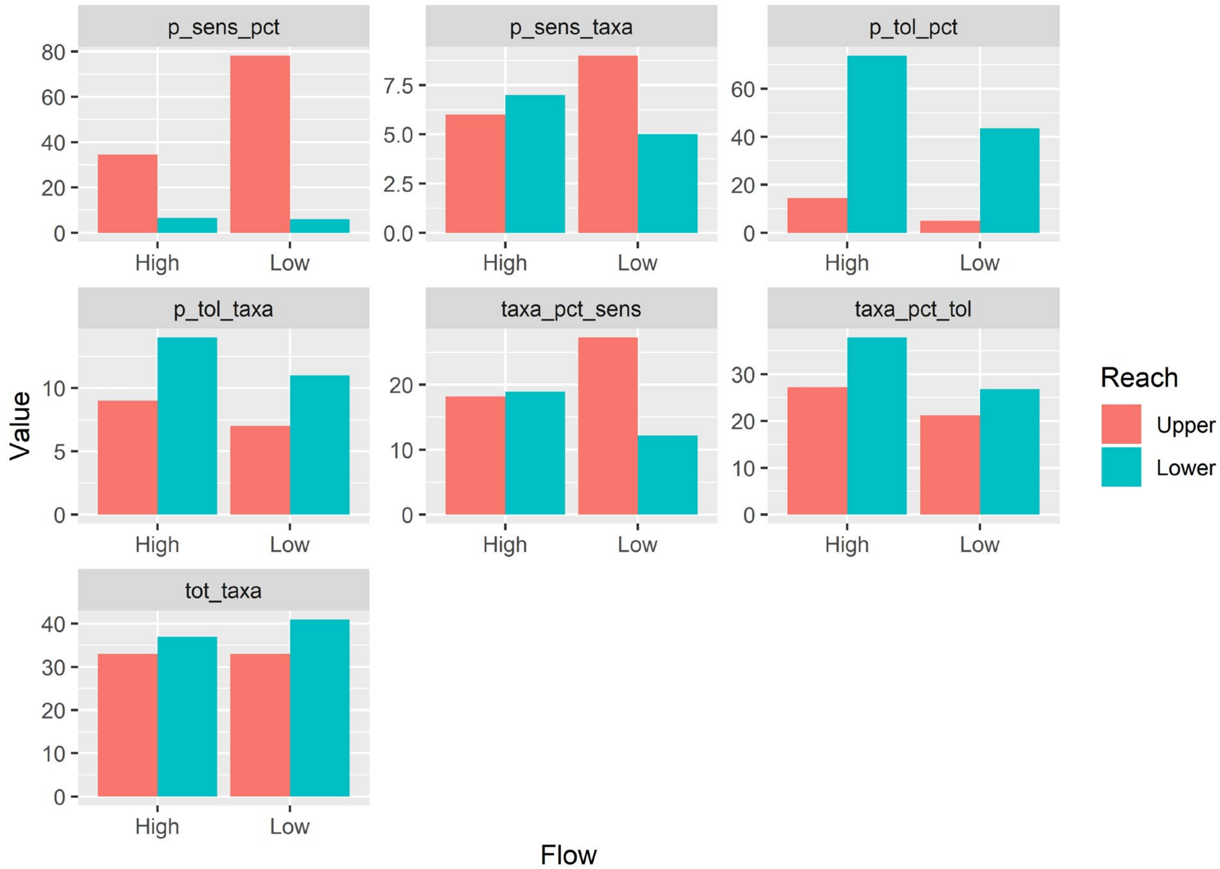


Figure Blanco.8: Diatom species composition also revealed substantial differences between the two reaches. The percentage of phosphorus (P) sensitive taxa richness and abundance was much higher at the upper reach, whereas the lower reach had large numbers of P-tolerant taxa. Diatoms are very sensitive to P enrichment, so this finding strongly implies that the lower reach was receiving excessive P enrichment from a source not found at the upper reach.

Blanco River: Macroinvertebrates Community Metrics and ALU Designation

Table Blanco.1: Macroinvertebrate community composition also differed dramatically between reaches. Using the TCEQ Multimetric Index, the lower reach was deemed "High" in terms of the Aquatic Life Use (ALU) Designation based on macroinvertebrate communities during April 2019. However, this result seems dubious because the density of macroinvertebrates at the lower reach during the April algal bloom was abnormally high, approaching 100,000 individuals/m^2, and this was driven almost entirely by taxa that are typically associated with organic pollution and wastewater discharges (See next figure).

High flow, Upper reach.

Metric	Value	Score	4	3	2	1
Taxa Richness	**30**	4	>21	15-21	8-14	<8
# EPT	**13**	4	>9	7-9	4-6	<4
HBI	**4.78**	3	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**6.27**	3	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Baetis*)	**31.75**	2	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (SCR)	**31.44**	4	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**15.44**	3	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**1.76**	2	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**56.96**	2	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**6**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**28.41**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**3.33**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	**38**				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

High flow, Lower Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**28**	4	>21	15-21	8-14	<8
# EPT	**7**	3	>9	7-9	4-6	<4
HBI	**6.51**	1	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**5.23**	3	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Physella*)	**40.42**	1	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (SCR)	**63.30**	1	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**5.81**	4	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**0.74**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**6.98**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**11**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**25.91**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**0.16**	1	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**HIGH**	**30**				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

LOW FLOW, Upper Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**39**	4	>21	15-21	8-14	<8
# EPT	**9**	3	>9	7-9	4-6	<4
HBI	**4.51**	3	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**21.26**	1	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Microcylleopus*)	**20.28**	4	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (Predator)	**30.37**	4	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**30.37**	2	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**0.81**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**28.74**	3	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**7**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**29.20**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**23.43**	2	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**HIGH**	34				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Low Flow, Lower Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**35**	4	>21	15-21	8-14	<8
# EPT	**8**	3	>9	7-9	4-6	<4
HBI	**4.98**	2	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**42.10**	1	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (Chironominae)	**27.56**	3	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (Predator)	**29.43**	4	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**29.43**	2	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**0.55**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**13.86**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**7**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**28.71**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**5.74**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**HIGH**	35				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Blanco River: Macroinvertebrate Densities

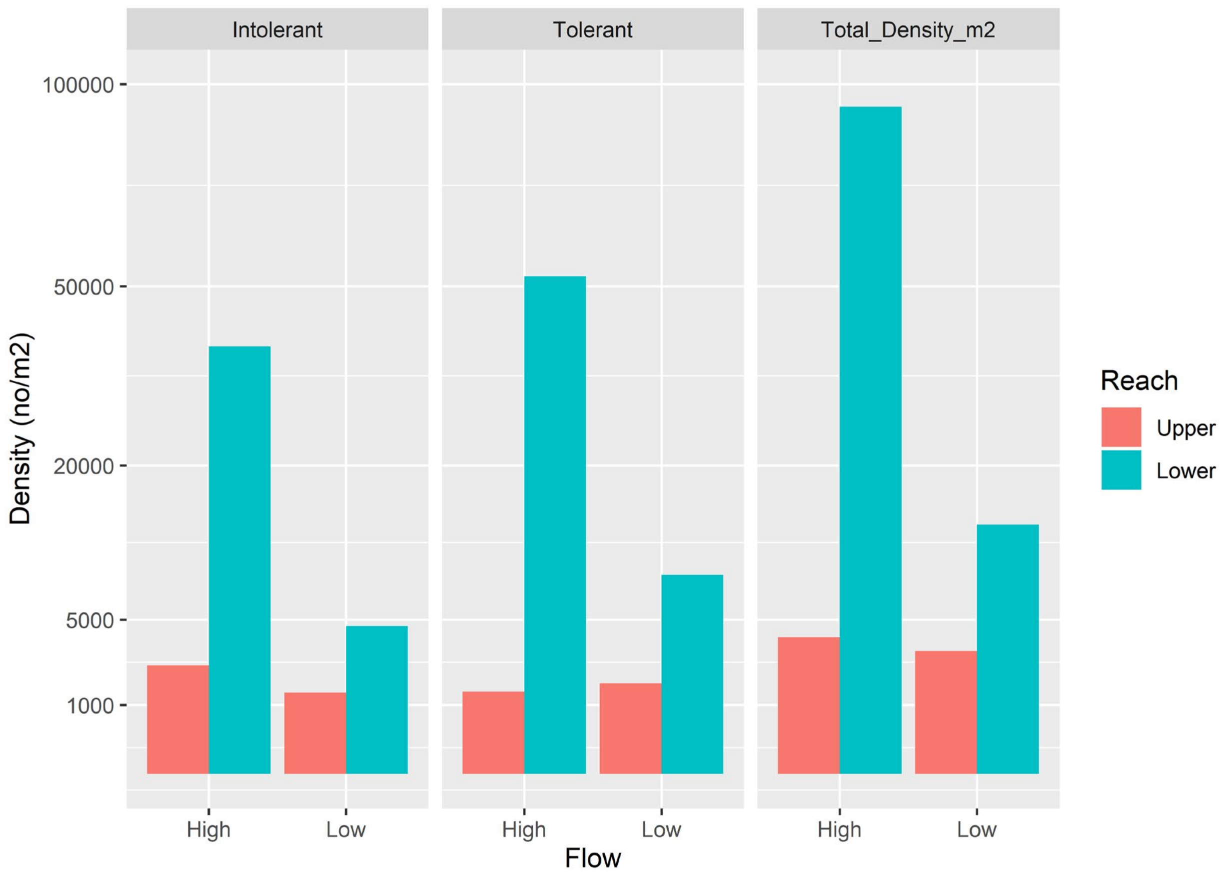


Figure Blanco.9: Densities of Tolerant, Intolerant, and Total macroinvertebrates during April (high flow) and August (low flow) at the Upper and Lower Reaches of the Blanco River. Densities of all macroinvertebrates were extraordinarily high at the lower reach, especially during April (high flow), which coincided with the algal bloom.

Blanco River: Macroinvertebrate Taxonomic Composition

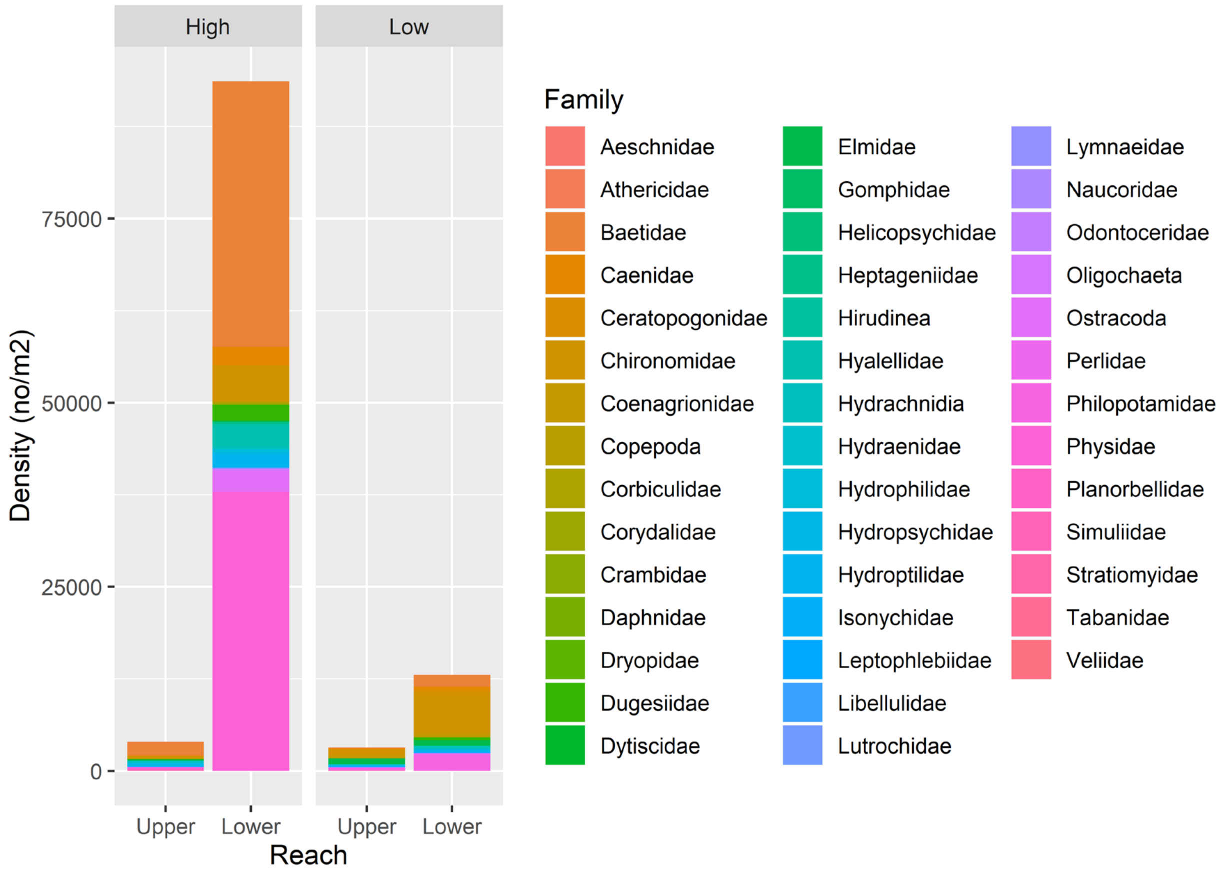


Figure Blanco.10. Stacked bar plot of macroinvertebrate taxonomic composition by families. See next plot for illustration of difference between upper and lower reaches during April (the bloom event) for a few key taxa that are typical of wastewater discharges.

Blanco River: Densities of Macroinvertebrate Taxa Commonly Found Below Wastewater Treatment Plants

(Data from early season, higher flow period following the significant algal bloom at Blanco Settlement)

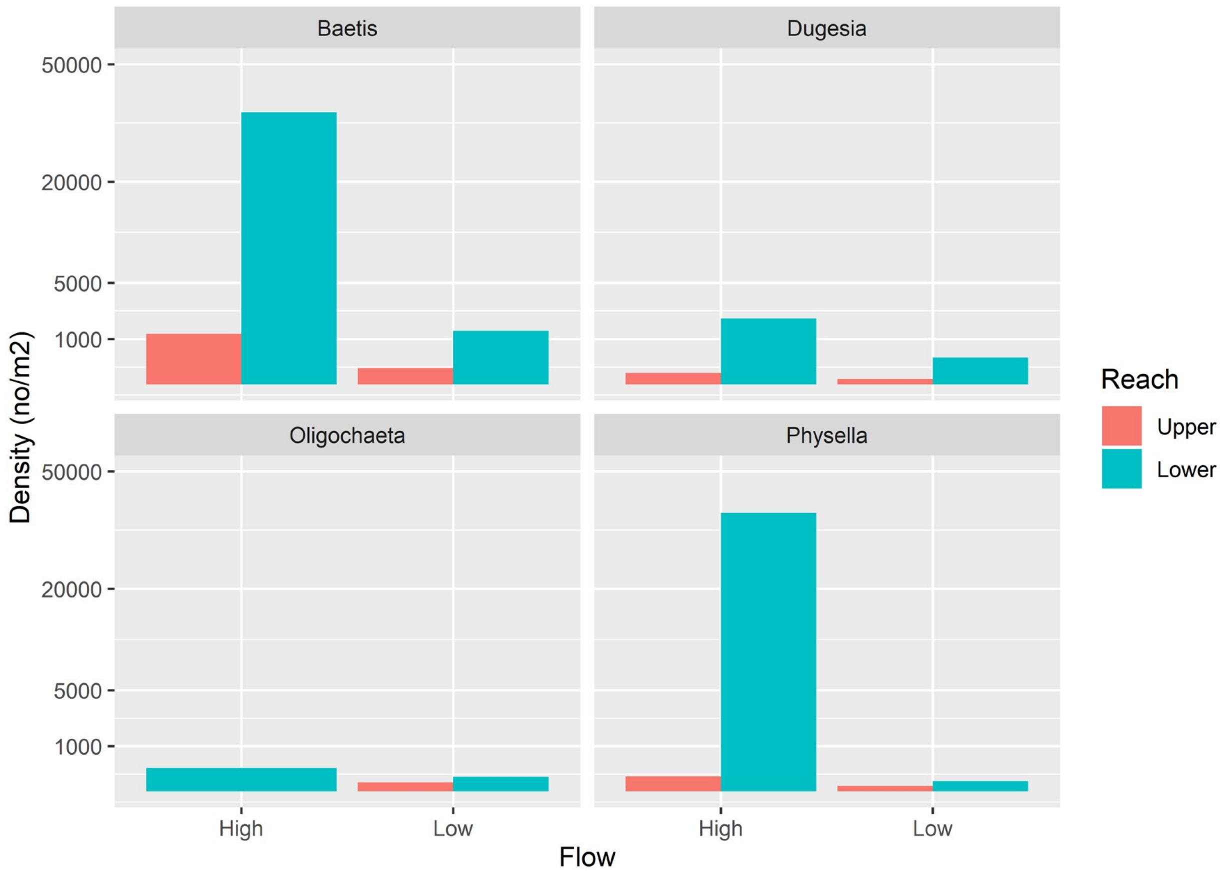


Figure Blanco.11. Densities of flatworms (Dugesiidae), air-breathing snails (Physidae), segmented worms (Oligochaeta), and a very tolerant mayfly genus (Baetis) dominated the taxonomic composition at the lower reach during April 2019, which coincided with a large bloom of nuisance algae.

Image Blanco.1. Flatworms, leeches, aquatic worms, and lunged snails from the bottom of the Blanco River at Blanco Settlement, April 2019. These are not typical taxa from Hill Country streams.

Blanco River: Fish Assemblage Composition

Table Blanco.2: Fish assemblages were relatively similar between reaches in terms of species composition. However, numerical abundance of fish, particularly fish that graze heavily on algae (e.g., central stonerollers) and shiner species that eat drifting Baetis nymphs were particularly abundant at the lower reach. The upper reach had low numbers of fish but several large individuals of largemouth bass, longear sunfish, redear sunfish, and good numbers of juvenile Guadalupe bass.

Blanco River, Lower		
Blacktail Shiner	1052	495
Bluegill	127	11
Central Stoneroller	46	0
Channel Catfish	6	1
Flathead Catfish	1	0
Green Sunfish	15	0
Guadalupe Bass	8	8
Largemouth Bass	9	2
Longear Sunfish	149	12
Redbreast Sunfish	154	0
Redear Sunfish	3	0
Rio Grande Cichlid	18	2
Western Mosquitofish	52	17
Total	**1640**	**548**
Blanco River, Upper		
Blacktail Shiner	210	45
Bluegill	1	0
Central Stoneroller	10	0
Channel Catfish	1	1
Green Sunfish	4	0
Guadalupe Bass	4	4
Largemouth Bass	12	3
Lepomis spp.	17	1
Longear Sunfish	17	6
Redbreast Sunfish	3	1
Redear Sunfish	9	0
Western Mosquitofish	27	0
Total	**315**	**61**

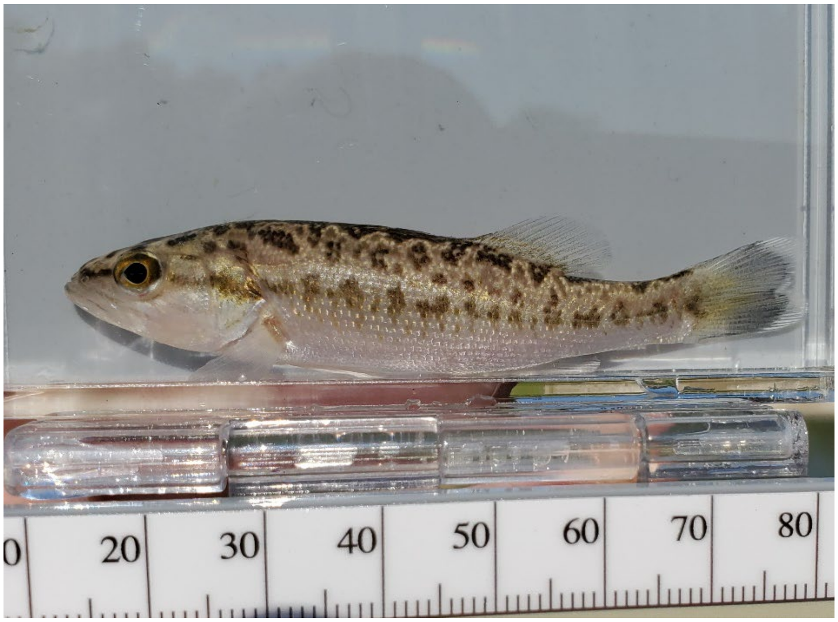


Image Blanco.2: Juvenile Guadalupe Bass, Blanco River, October 2019

Image Blanco.3. Blacktail Shiner adult male in spawning colors, Blanco R., October 2019.

Image Blanco.4: Rio Grande Cichlid adult, Blanco River, October 2019.

Results

HONEY CREEK

Summary

Honey Creek Upper and Lower Reaches were relatively dissimilar in their physical habitat and flow regime. The channel of the upper reach tended to be broken up in braids that spanned a wide, peat-based floodplain with substantial canopy cover from *Taxodium distichum* (bald cypress). The lower reach was also well-canopied by bald cypress, but had a more defined channel, much higher stream flow from a large spring that discharges just upstream of the reach, faster stream velocity, and larger, hard substrate (cobble and small boulders). Substrate in the upper reach tended to be softer, more depositional, and organic than the lower reach. These differences played a role in some differences in chemical and biological condition of the two reaches, but also make them uniquely sensitive to potential nutrient enrichment from wastewater.

Honey Creek phosphorus levels were consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas, with total and orthophosphate (PO_4-P)-phosphorus values < 10 µg/L. However, total nitrogen (TN) and nitrite+nitrate-N values were relatively high and approached 1 mg/L at the lower reach during low flow conditions. This implies that the groundwater is already contaminated with nitrate-N, which is probably associated with application of industrial fertilizers on residential lawns or croplands rather than wastewater or septic systems. This hypothesis is based on the fact that periphyton δ15N values in both reaches during both events were similar and below levels usually associated with human or animal waste sources of nitrogen (e.g., see Results from lower Blanco River and lower Onion Creek). Nevertheless, Honey Creek may already have a nitrogen enrichment problem that would only be exacerbated by any additional nutrient inputs, particularly phosphorus.

Dissolved oxygen levels were high and remained at or above levels that are supportive of natural biological communities in Texas streams. EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen and other parameters, revealed similar DO levels between the two reaches during the day and night. Temperature fluctuations were greater in the upper reach, which had lower flow than the lower reach, especially during the heat of August. Thus, the lower reach's larger discharge from groundwater helps buffer its temperature better than the upper part of the stream.

Sestonic organic matter (ash-free dry mass particulates), chlorophyll-a (phytoplankton), and total suspended solids were consistent with high-quality, reference stream conditions in both reaches during both high and low flow events.

Periphyton (benthic algae) biomass was moderately high in both reaches during the spring, high flow period. Both reaches supported similar levels of algal biomass. Maximum benthic chlorophyll-a was approximately 150 mg/m^2. The algal biomass observed during May 2019 was approaching levels that one might observe in streams impacted by excessive nutrient enrichment. This implies that small inputs of nutrients, particularly phosphorus, could cause nuisance levels of algae to proliferate.

Periphyton stable isotopic ratios for nitrogen (δ15N) were similar between reaches and seasons. Both reaches had δ15N values around 8, which is similar to levels found in Barton Creek, but much lower than levels found in the lower reach of Blanco River.

Biomass of *Cladophora glomerata*, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was moderately high in both reaches during May 2019. However, levels of *Cladophora* biovolume were still far lower than Blanco River at Blanco Settlement during the April 2019 bloom. Moreover, our estimates of Cladophora biovolume at the Blanco River came after a runoff event that scoured much of the stream bottom and washed filaments of Cladophora downstream, so our Blanco River estimates are low relative to the peak of the bloom in the water body. Regardless, increases in nutrient levels in Honey Creek could facilitate proliferation of *Cladophora* and harm biological integrity of the ecosystem.

Diatom species richness was very high in both reaches, with the lower reach supporting 71 species during one of the events. Richness and abundance of phosphorus (P) sensitive taxa were slightly lower than that of tolerant taxa. However, the extremely high species richness (diversity) of diatoms and the unique environmental conditions found here due to spring-fed conditions and low levels of light (high canopy cover) may also be responsible for the types of species found here. Clearly, no stream in the current study supported nearly as many species of diatoms as Honey Creek, regardless of how they are classified in terms of P sensitivity or tolerance. Thus, Honey Creek supported exceptional diversity of diatoms.

Macroinvertebrate community composition was similar between reaches. Both reaches had about 30 taxa, regardless of season. Using the TCEQ Multimetric Index, both reaches were deemed "Exceptional" in terms of their Aquatic Life Use Designation based on macroinvertebrate communities with the exception of the upper reach during May 2019, when it was classified as "High". Note that the Upper reach has naturally high levels of organic matter, almost resembling a soft-bottomed stream of the coastal plain, and thus some of the taxa present may be unduly classified as indicative of organic pollution when, in fact, the organic matter is natural. Both reaches supported relatively high densities of a unique, spring-dwelling caddisfly (*Leucotrichia sarita*) that grazes on biofilms attached to rocks in fast-flowing water. This taxon may represent a species of concern and certainly is one that could be affected by wastewater inputs. Several other caddisfly genera were also only found at Honey Creek (compared to Barton, Blanco, and Onion) and were thus unique to the study. These genera, which were not identified to species because they cannot be identified as larvae, should be viewed as potentially vulnerable to any wastewater inputs into the stream.

Fish assemblages supported several species that are either endemic only to the Hill Country or have limited distribution in Texas and northern Mexico. These species include Guadalupe Bass, greenthroat darter, Texas shiner, and Guadalupe roundnose minnow. We also collected several longear sunfish with very unique color patterns that may be an unknown subspecies yet to be described.

Honey Creek: Nutrients

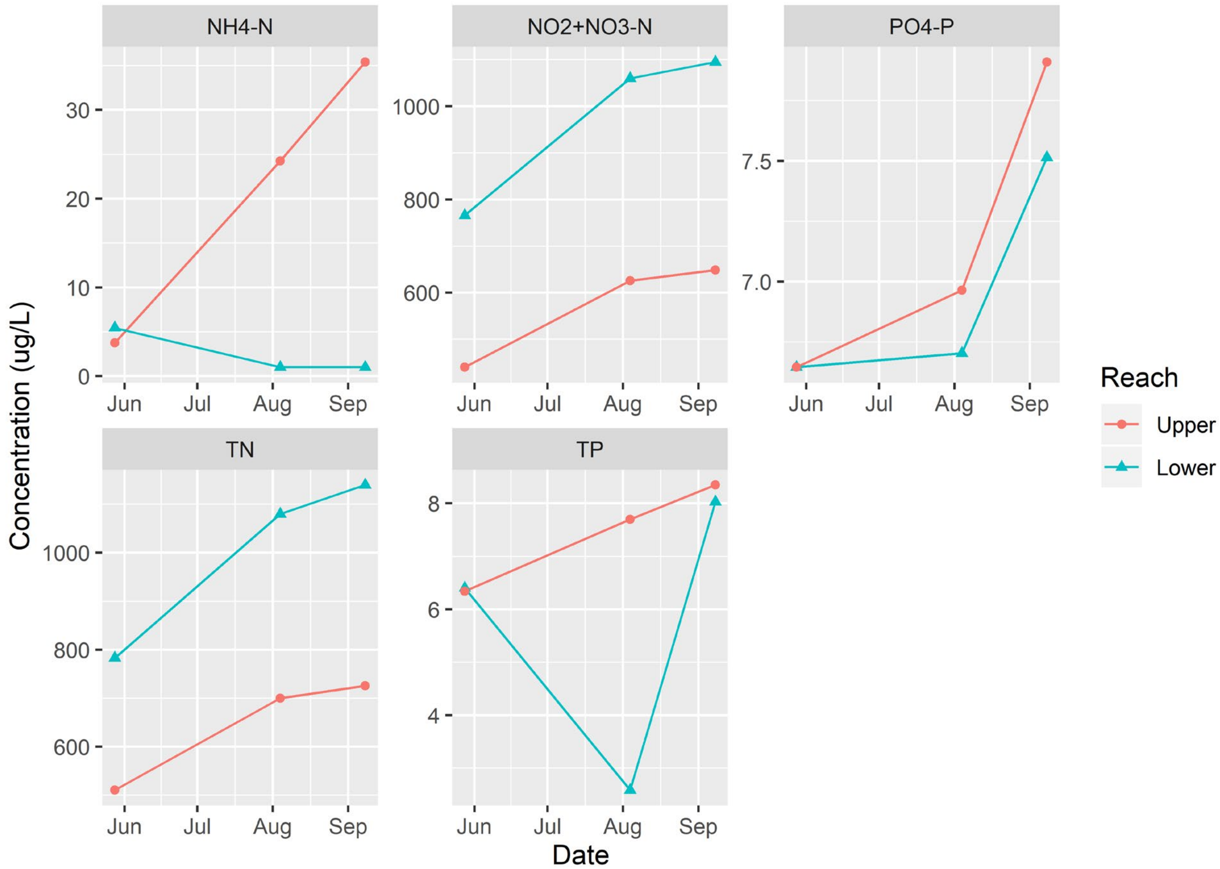


Figure Honey.1: Phosphorus levels were consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas, with total and orthophosphate (PO_4-P)-phosphorus values < 10 µg/L. However, total nitrogen (TN) and nitrite+nitrate-N values were relatively high and approached 1 mg/L at the lower reach during low flow conditions. This implies that the groundwater is already contaminated with nitrate-N, which is probably associated with application of industrial fertilizers on residential lawns or croplands rather than wastewater or septic systems. This hypothesis is based on the fact that periphyton δ15N values in both reaches during both events were similar and below levels usually associated with human or animal waste sources of nitrogen (e.g., see Results from lower Blanco River and lower Onion Creek). Nevertheless, Honey Creek may already have a nitrogen enrichment problem that would only be exacerbated by any additional nutrient inputs, particularly phosphorus.

Honey Creek: YSI EXO1 Data Sonde Parameters, Instantaneous

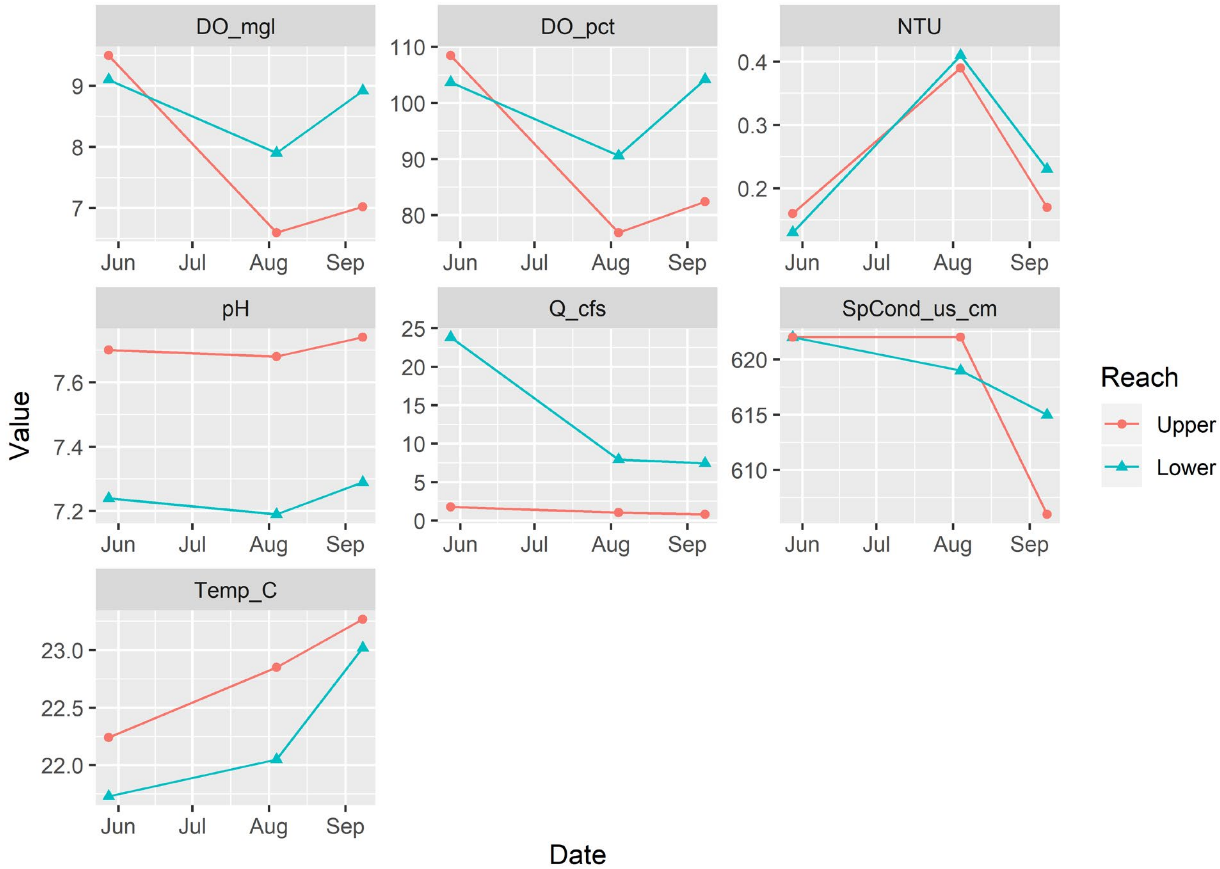


Figure Honey.2: Dissolved oxygen (DO; units are milligrams per liter (mgl) and percent saturation (pct)), turbidity (NTU, a measure of water clarity), pH (acidity), stream flow (Q_cfs, or cubic-feet per second), specific conductance (SpCond_us_cm; units are microsiemens per centimeter), and water temperature (degrees Celsius) measured in the early morning (Upper) and mid-morning (Lower) reaches of Honey Creek during summer 2019. The tendency for the Upper reach to have warmer temperatures is related to lower groundwater inputs relative to the Lower reach, which has a much larger spring that contributes substantially higher stream flow (Q_cfs) and thus buffers the water temperature more than the upstream reach. NTU levels at both reaches were extremely low. NTU was not measured in May. The two reaches were otherwise quite similar in specific conductance, but the upper reach tended to have slightly higher pH.

Honey Creek: EXO1 24 h (Diel) Water Quality Parameters

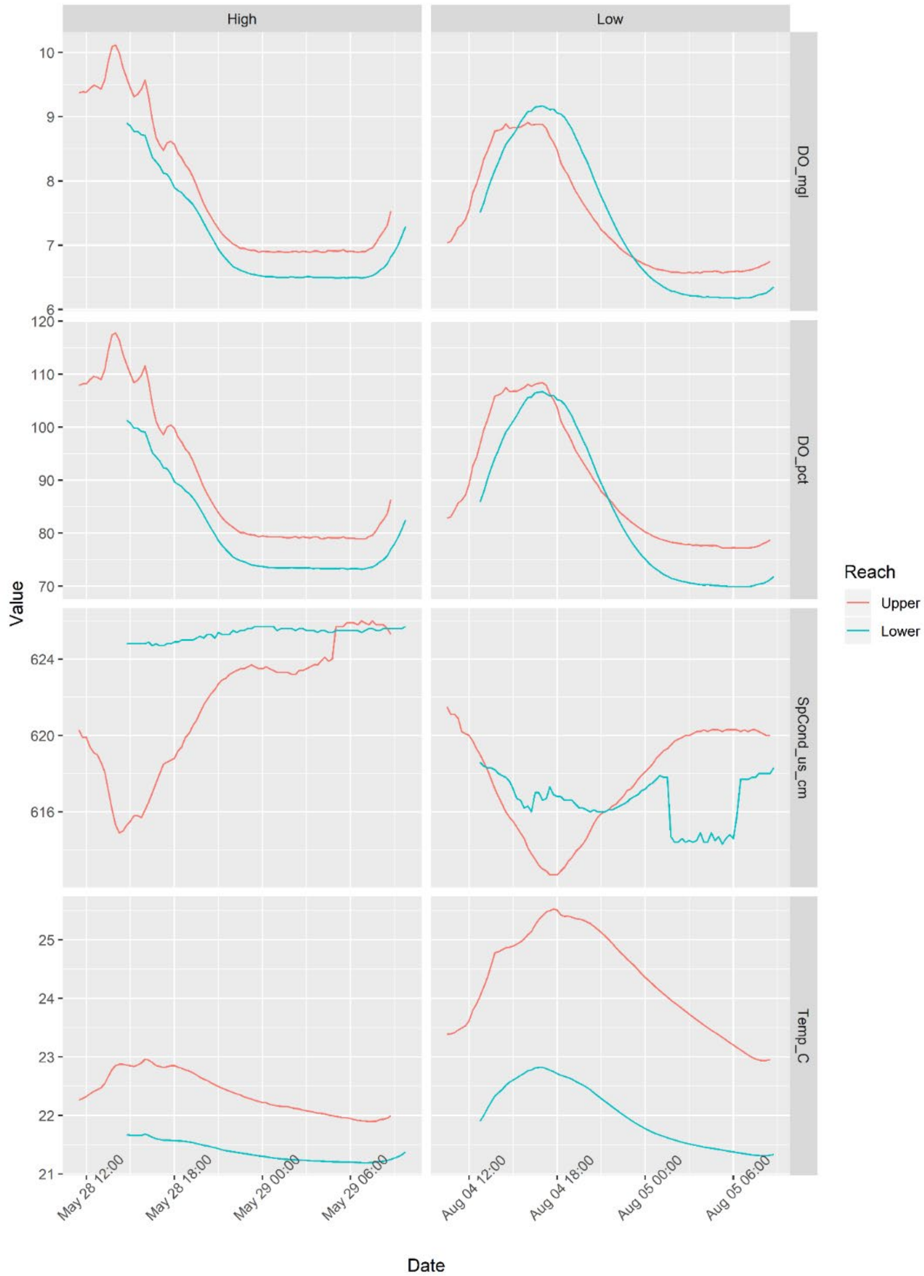


Figure Honey.3: Dissolved oxygen levels were high and remained at or above levels that are supportive of natural biological communities in Texas streams. EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen and other parameters, revealed similar DO levels between the two reaches during the day and night. Temperature fluctuations were greater in the upper reach, which had lower flow than the lower reach, especially during the heat of August. Thus, the lower reach's larger discharge from groundwater helps buffer its temperature better than the upper part of the stream.

Honey Creek: Seston (Organic Matter, Phytoplankton, and Total Particulates in Water Column)

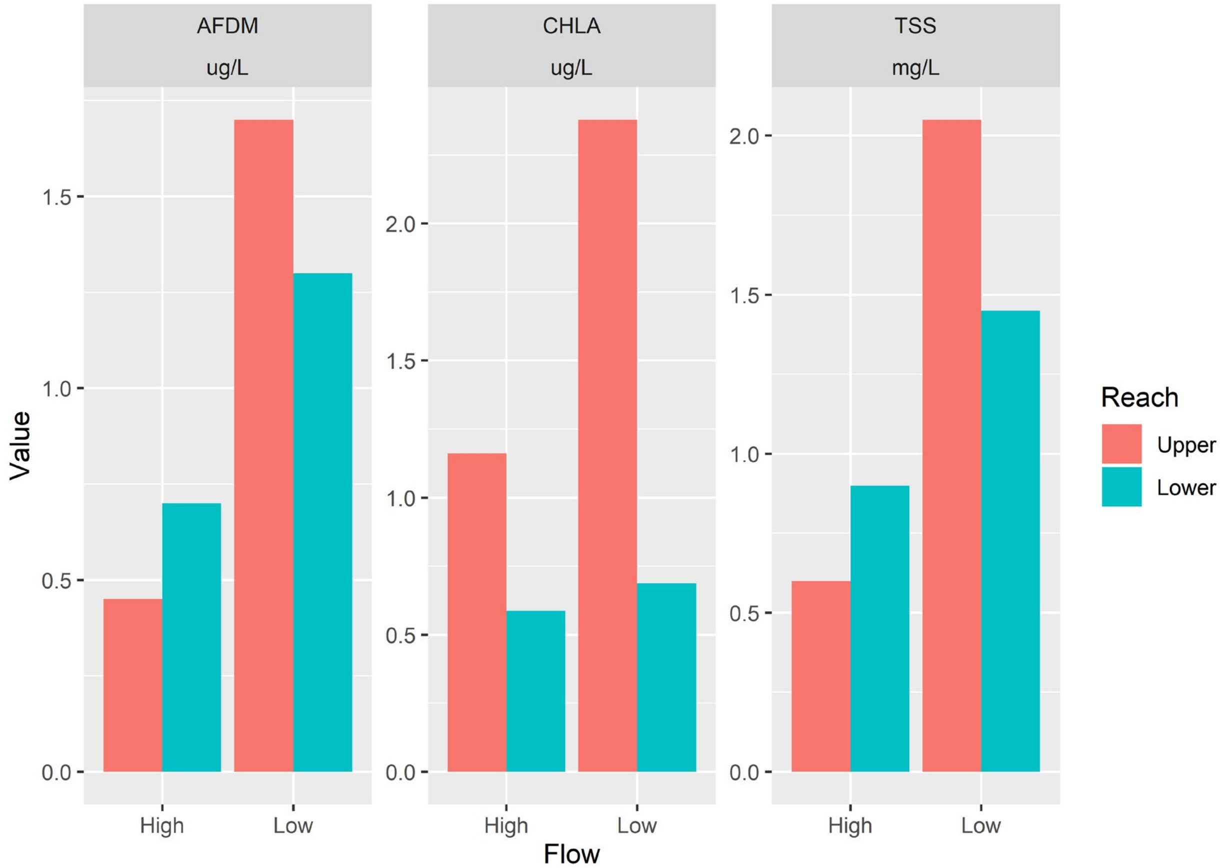


Figure Honey.4: Sestonic organic matter (ash-free dry mass particulates floating in the water column), chlorophyll-a (phytoplankton and other algae floating in water column), and total suspended solids (TSS; all particles, including silt, clay, etc.) were consistent with high-quality, reference stream conditions in both reaches during both high and low flow events. Chlorophyll-a trended slightly higher in the upper reach, which had lower flow and longer residence time, but even there, it was quite low.

Honey Creek: Periphyton (Benthic Algae) Biomass

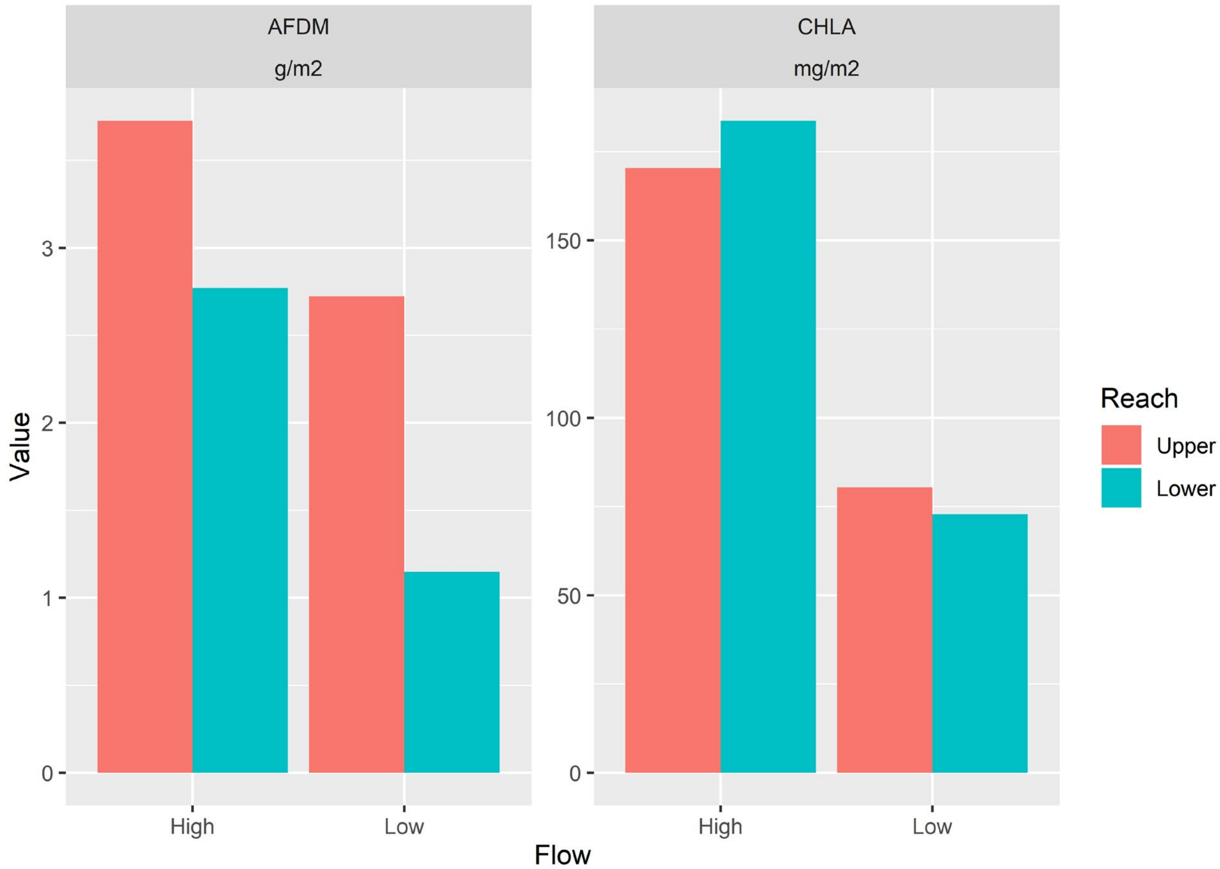


Figure Honey.6: Periphyton (benthic algae) biomass was moderately high in both reaches during the spring, high flow period. Both reaches supported similar levels of algal biomass. Maximum benthic chlorophyll-a was approximately 150 mg/m^2.The algal biomass observed during May 2019 was approaching levels that one might observe in streams impacted by excessive nutrient enrichment. This implies that small inputs of nutrients, particularly phosphorus, could cause nuisance levels of algae to proliferate.

Honey Creek: Periphyton Stable Isotopic Ratios for Carbon and Nitrogen

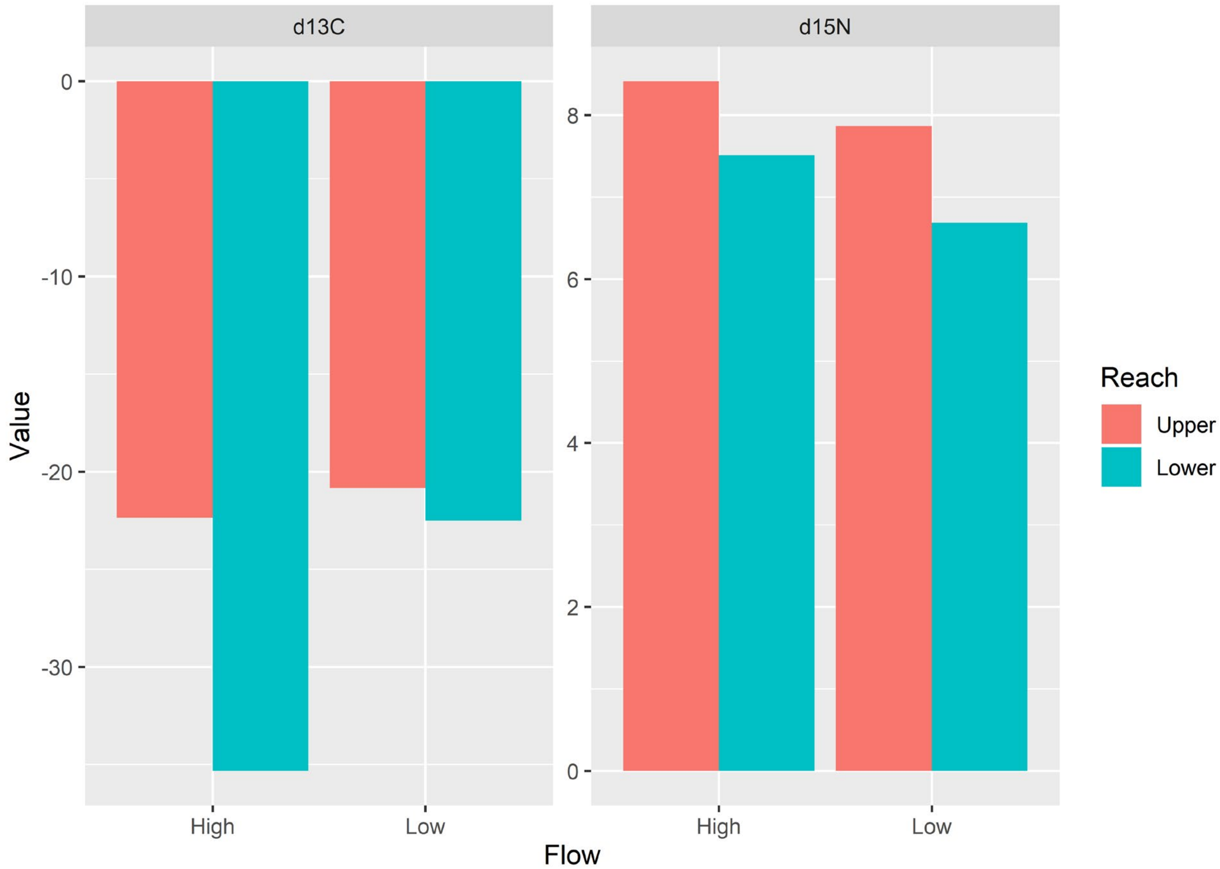


Figure Honey.6: Periphyton stable isotopic ratios for nitrogen (δ15N) were similar between reaches and seasons. Both reaches had δ15N values around 8, which is similar to levels found in Barton Creek, but much lower than levels found in the lower reach of Blanco River. The δ15N values may be indicating some early signs of septic or other animal source of nitrogen in the system.

Honey Creek: *Cladophora glomerata* (Nuisance Filamentous Green Alga) and Total Soft Algal Biovolume

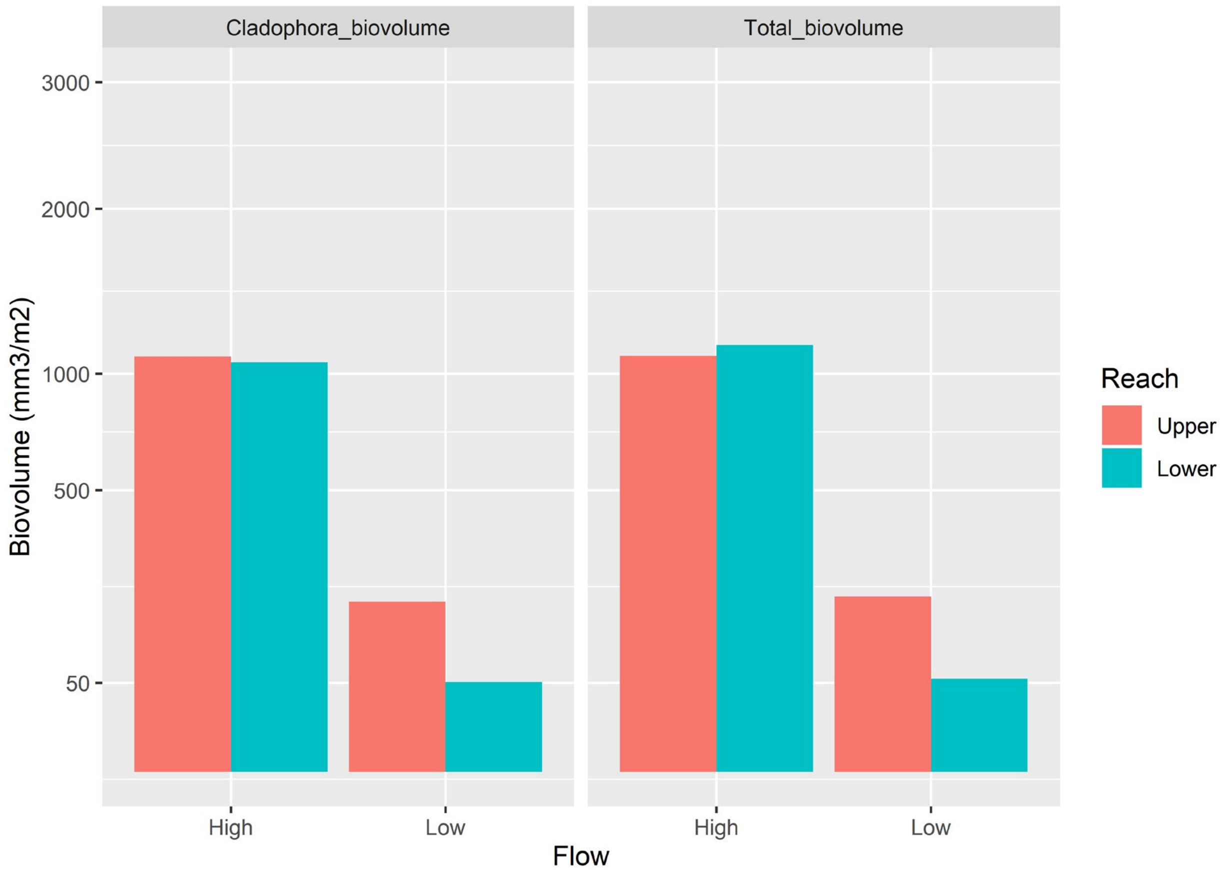


Figure Honey.7: Biomass of Cladophora glomerata, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was moderately high in both reaches during May 2019. However, levels of Cladophora biovolume were still far lower than Blanco River at Blanco Settlement during the April 2019 bloom. Moreover, our estimates of Cladophora biovolume at the Blanco River came after a runoff event that scoured much of the stream bottom and washed filaments of Cladophora downstream, so our Blanco River estimates are low relative to the peak of the bloom in the water body. Regardless, increases in nutrient levels in Honey Creek could facilitate proliferation of Cladophora and harm biological integrity of the ecosystem.

Honey Creek: Diatom Species Community Metrics

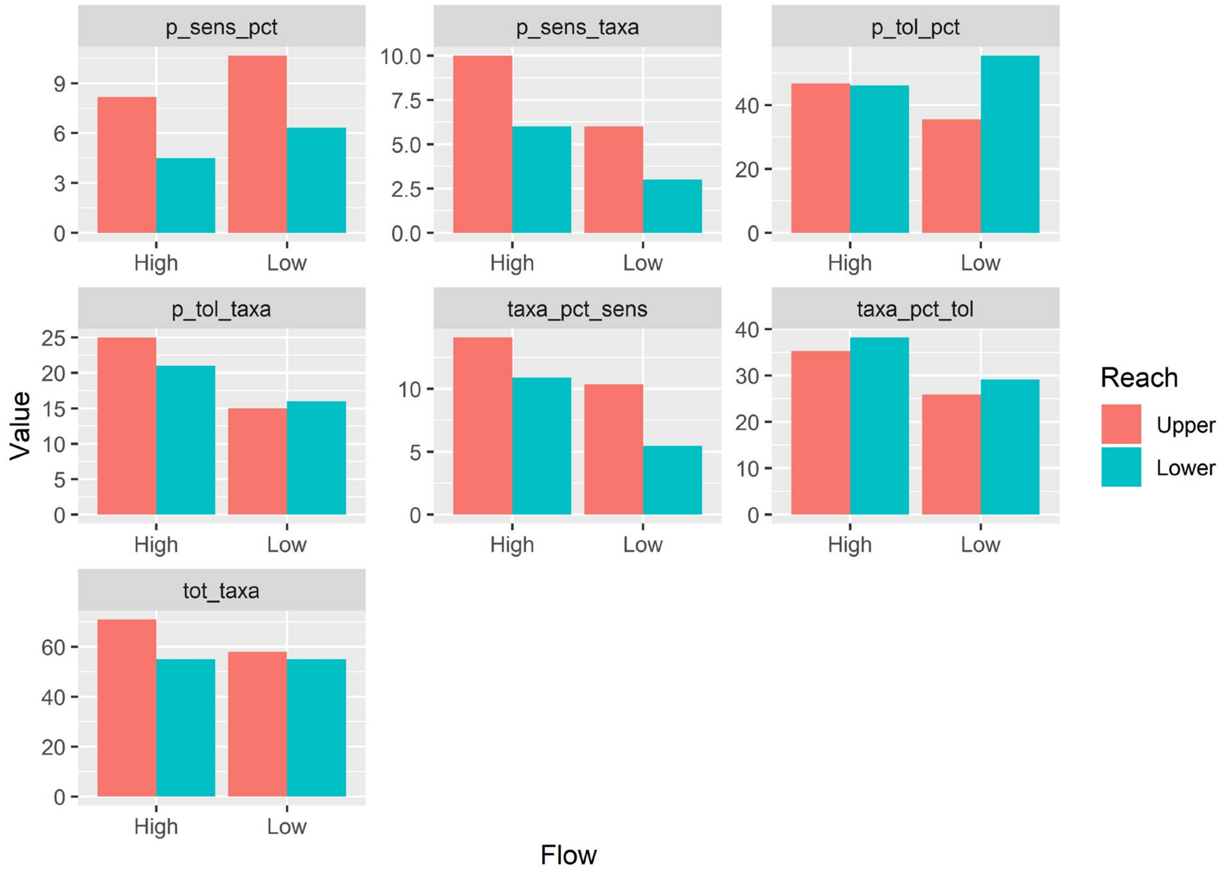


Figure Honey.8: Diatom species richness was very high in both reaches, with the lower reach supporting 71 species during one of the events. Richness and abundance of phosphorus (P) sensitive taxa were slightly lower than that of tolerant taxa. However, the extremely high species richness (diversity) of diatoms and the unique environmental conditions found here due to spring-fed conditions and low levels of light (high canopy cover) may also be responsible for the types of species found here. Clearly, no stream in the current study supported nearly as many species of diatoms as Honey Creek, regardless of how they are classified in terms of P sensitivity or tolerance. Thus, Honey Creek supported exceptional diversity of diatoms.

Honey Creek: Macroinvertebrates Community Metrics and ALU Designation

Table Honey.1

HIGH FLOW, Upper Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**32**	4	>21	15-21	8-14	<8
# EPT	**11**	4	>9	7-9	4-6	<4
HBI	**5.26**	2	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**39.07**	1	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (Orthocladinae)	**27.21**	3	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (CG)	**44.02**	3	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**15.15**	4	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**0.43**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**3.51**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**7**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**44.02**	1	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**1.55**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**HIGH**	**35**				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

High flow, Lower Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**33**	4	>21	15-21	8-14	<8
# EPT	**14**	4	>9	7-9	4-6	<4
HBI	**4.60**	2	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**4.37**	3	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Tricorythodes*)	**19.15**	4	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (CG)	**34.91**	4	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**16.04**	3	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**1.99**	2	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**42.19**	3	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**9**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**34.91**	2	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**1.34**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	**39**				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

LOW FLOW, Upper Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**38**	4	>21	15-21	8-14	<8
# EPT	**9**	3	>9	7-9	4-6	<4
HBI	**4.42**	3	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**9.28**	3	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Heliocpsyche*)	**26.22**	3	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (SCR)	**34.17**	4	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**21.52**	3	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**1.19**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**0.53**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**9**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**23.92**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**4.10**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	39				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Low Flow, Lower Reach

Metric	Value	Score	4	3	2	1
Taxa Richness	**34**	4	>21	15-21	8-14	<8
# EPT	**13**	4	>9	7-9	4-6	<4
HBI	**3.30**	4	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**5.82**	3	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Helicopsyche*)	**37.48**	2	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (SCR)	**53.84**	2	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**9.70**	4	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**4.89**	4	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**1.42**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**8**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**24.39**	2	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**0.68**	1	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	38				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Honey Creek: Macroinvertebrate Densities

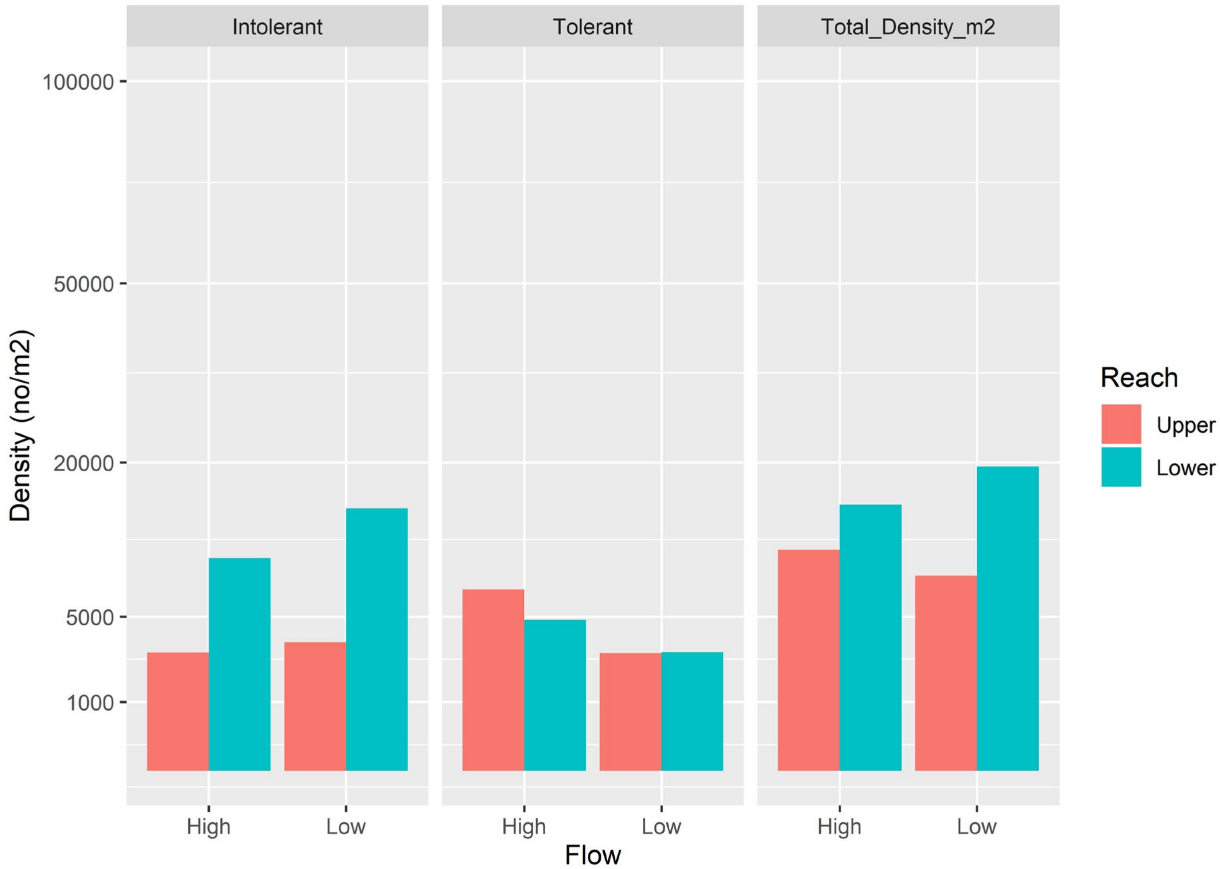


Figure Honey.9: Macroinvertebrate community composition was similar between reaches. Both reaches (see Table Honey.1, previous) had about 30 taxa, regardless of season. Using the TCEQ Multimetric Index, both reaches were deemed "Exceptional" in terms of their Aquatic Life Use (ALU) Designation based on macroinvertebrate communities with the exception of the upper reach during May 2019, when it was classified as "High". Note that the Upper reach has naturally high levels of organic matter, almost resembling a soft-bottomed stream of the coastal plain, and thus some of the taxa present may be unduly classified as indicative of organic pollution when, in fact, the organic matter is natural.

Honey Creek: Macroinvertebrate Taxonomic Composition

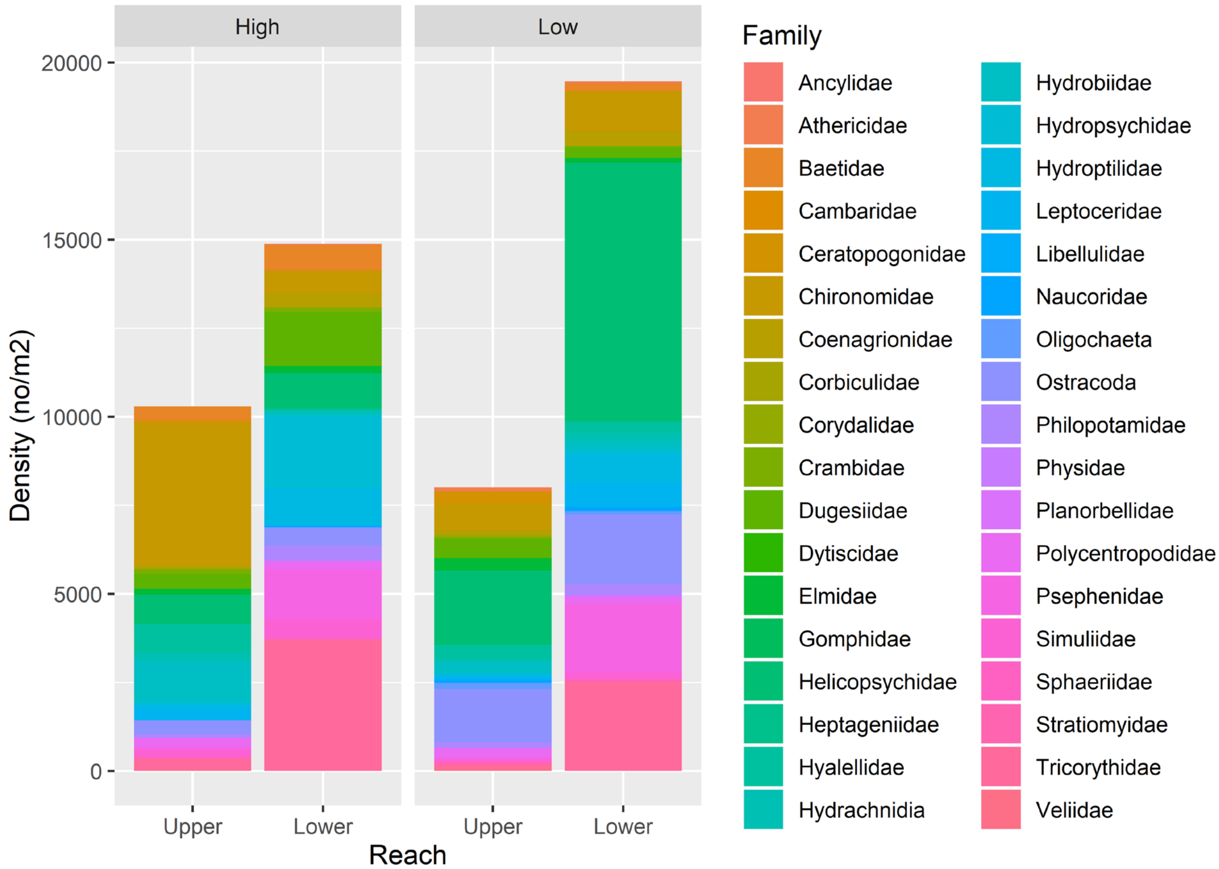


Figure Honey.10: Stacked bar plot of macroinvertebrate taxonomic composition by family densities. Both reaches were similar in composition. The lower reach, in particular, supported densities of a unique, spring-dwelling caddisfly (Leucotrichia sarita) that grazes on biofilms attached to rocks in fast-flowing water (see Image Honey.1, next). This taxon may represent a species of concern and certainly is one that could be affected by wastewater inputs. Several other caddisfly genera were also only found at Honey Creek (compared to Barton, Blanco, and Onion) and were thus unique to the study. These genera, which were not identified to species because they cannot be identified as larvae, should be viewed as potentially vulnerable to any wastewater inputs into the stream.

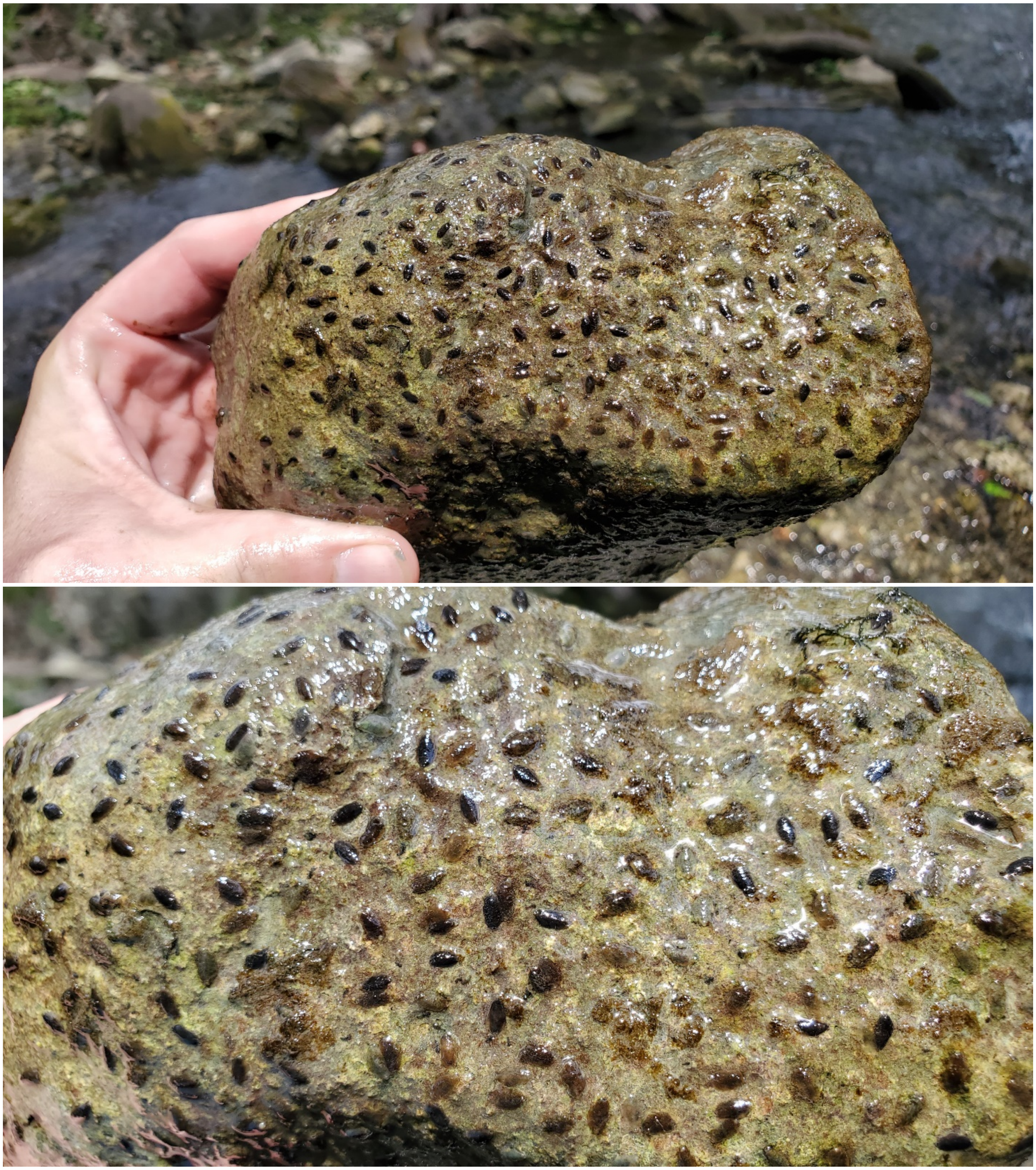


Image Honey.1: Leucotrichia sarita *larval fixed retreats (cases) attached to rocks in the lower reach at Honey Creek during August 2019. This species is a spring-dwelling specialist that requires high levels of dissolved oxygen and is likely vulnerable to nutrient enrichment.*

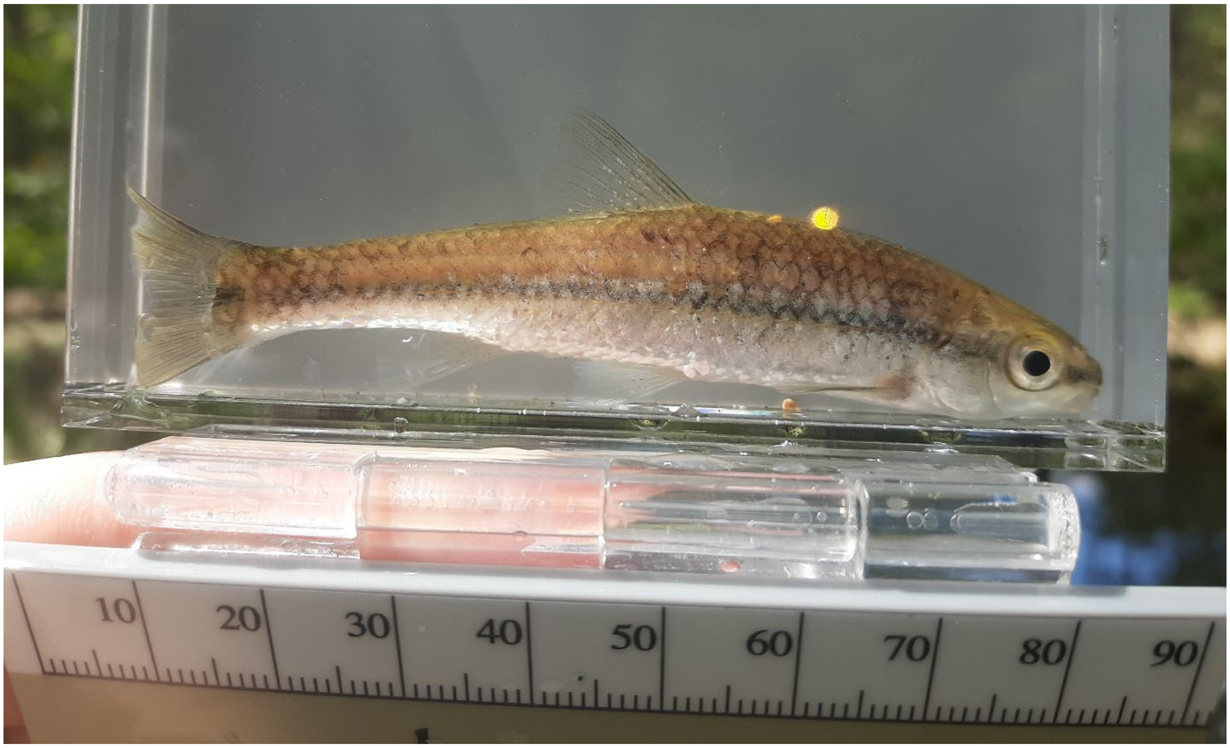
Honey Creek: Fish Assemblage Composition

Table Honey.2: Fish assemblages supported several species that are either endemic only to the Hill Country or have limited distribution in Texas and northern Mexico. These species include Guadalupe Bass, greenthroat darter, Texas shiner, and Guadalupe roundnose minnow. We also collected several longear sunfish with very unique color patterns that may be an unknown subspecies yet to be described. Note that the first column represents the total number of individuals collected, whereas the second column is the number of juveniles (as part of the total number).

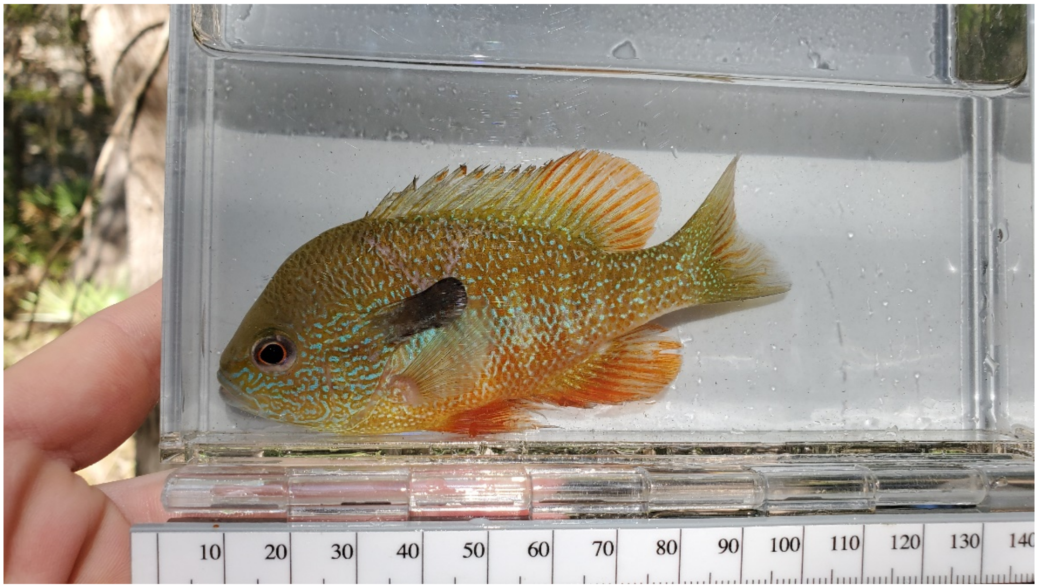
Honey Creek, Lower		
Blacktail Shiner	9	0
Central Stoneroller	81	0
Greenthroat Darter	117	0
Guadalupe Bass	6	5
Guadalupe Roundnose Minnow	56	3
Largemouth Bass	1	1
Longear Sunfish	29	0
Mexican Tetra	23	0
Redspotted Sunfish	18	0
Texas Shiner	11	3
Warmouth	3	1
Yellow Bullhead	1	1
Total	**355**	**14**
Honey Creek, Upper		
Blacktail Shiner	3	1
Central Stoneroller	243	18
Greenthroat Darter	34	6
Guadalupe Bass	11	11
Guadalupe Roundnose Minnow	2	0
Largemouth Bass	4	4
Longear Sunfish	89	2
Mexican Tetra	15	0
Redspotted Sunfish	19	3
Warmouth	3	0
Western Mosquitofish	26	0
Yellow Bullhead	14	8
Total	**463**	**53**

Mexican tetra (*Astyanax mexicanus*) from Honey Creek, September 2019. The only species of the order Characiformes (includes Piranhas) native to the United States, although this represents a range expansion (formerly limited to Rio Grande basin, south Texas).

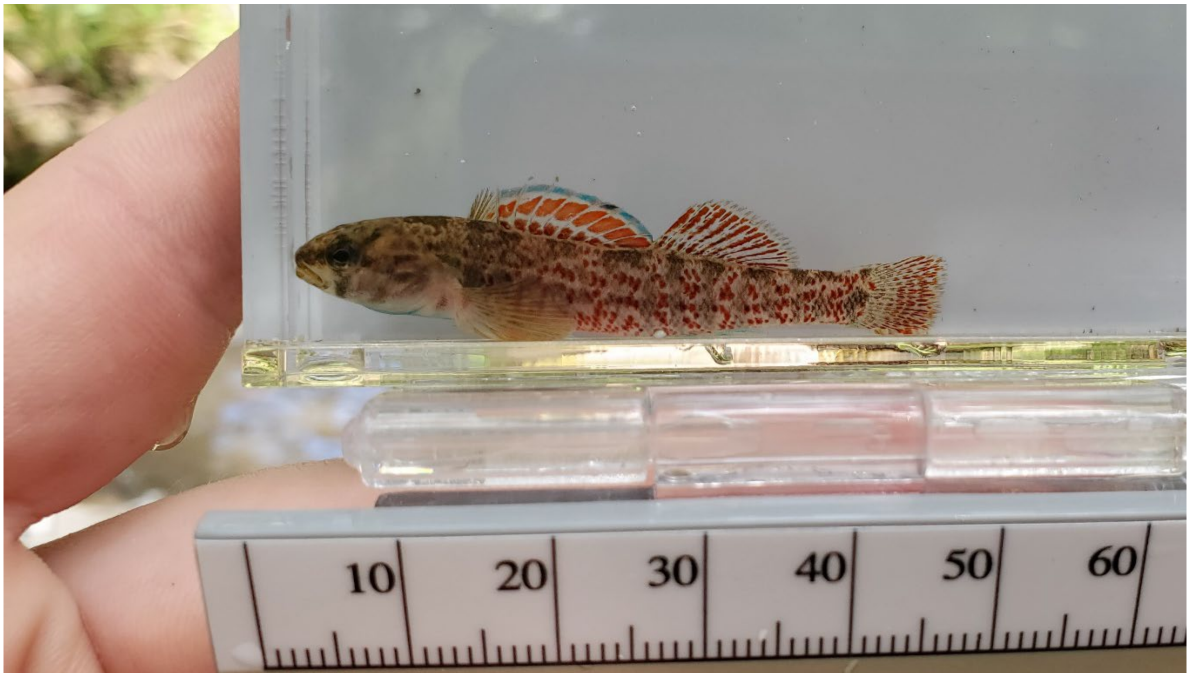
Texas shiner (*Notropis amabilis*) from lower Honey Creek, September 2019. Endemic to south-central Texas.



Guadalupe roundnose minnow (*Dionda nigrotaeniata*), a Texas endemic native to the Guadalupe River basin in central Texas.



Longear sunfish (*Lepomis megalotis*), sporting spectacular deep-orange colors on pelvic, soft-dorsal, and anal fins, from Honey Creek in September 2019. Colors are dulled in this photo compared to individuals immediately following capture. The color patterns and markings on specimens from Honey Creek are sufficiently distinct that they may be a subspecies.



Greenthroat darter (*Etheostoma lepidum*) from Honey Creek in September 2019, a species only found in spring-fed streams in the Colorado, Guadalupe, and Nueces drainages of Texas.

Results

ONION CREEK

Summary

Onion Creek Upper and Lower Reaches were generally similar in physical, chemical, and biological characteristics during both high (April-May) flow but were more dissimilar during low (August) flow sampling events. The lower reach, on CharRo Ranch, spans a groundwater recharge zone and loses most of its flow during low flow periods. Thus, the lower reach became fragmented into a series of disconnected pools during low flow events, whereas the upper reach maintained at least some flow during early and late August 2019.

Nutrient levels in both reaches were generally consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas. However, total P was slightly above 10 µg/L and TN was slightly above 300 µg/L in April 2019 in the lower reach. However, overall, nutrient levels never exceeded levels associated with biological thresholds (e.g., 20 µg/L TP, 500 µg/LTN) for biological condition in central Texas streams.

Dissolved oxygen levels were high and remained at or above levels that are supportive of natural biological communities in Texas streams. EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen and other parameters, revealed similar DO levels between the two reaches during the day and night. Even during low flow, when the lower reach had been reduced to a series of disconnected pools, it maintained DO levels similar to that of the upper, flowing reach.

Sestonic organic matter (ash-free dry mass particulates), chlorophyll-a (phytoplankton), and total suspended solids were consistent with high-quality, reference stream conditions in both reaches during both high and low flow events. Sestonic chlorophyll-a peaked in high flows at ~ 3 µg/L in the lower reach and was < 1 µg/L during low-flow conditions.

Periphyton (benthic algae) biomass was also quite low and consistent with a low-nutrient ecosystem. Total biomass (ash-free dry mass) and chlorophyll-a were higher in the upper reach during high flow, but values were still quite low in both reaches. Maximum benthic chlorophyll-a was approximately 40 mg/m2.

Periphyton stable isotopic ratio values for nitrogen (δ15N) were one of the only variables that definitely suggested a human source of nutrients in the lower reach (CharRo Ranch) when compared to the upper reach. The lower reach had δ15N values near 11 per mil during high flow (April), whereas the upper reach had values closer to 8. The difference between reaches was smaller during the low flow period, which implies that the lack of runoff into the lower reach may have contributed to the increased similarity between the two reaches. That is, if wastewater application to fields or other land was reaching Onion Creek, we might expect this to be more evident during higher flow events when rain would facilitate runoff and increase seepage from uplands near the river.

Biomass of *Cladophora glomerata*, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was relatively low in both reaches.

Diatom species richness was similar between both reaches (20-25 species in April, 30-35 species in August). Phosphorus (P) sensitive taxa richness and abundance was similar between reaches, as was the richness of P tolerant taxa. There was no compelling difference in the abundance of any species indicative of high P levels between the two reaches.

Macroinvertebrate community composition differed slightly between reaches. The Upper reach had higher densities of macroinvertebrates in both seasons, but the species richness was not consistently higher or lower. In April, the upper reach had more taxa than the lower, but August low flow, the lower reach had more taxa. Despite the apparent increase in richness in the Lower reach during low flow, the TCEQ Multimetric Index scored only a "High" Aquatic Life Use Designation based on macroinvertebrate communities compared to an "Exceptional" in the Upper reach. Both reaches were classified as "Exceptional" during the high flow period in April.

Fish assemblages were consistent with high quality Hill Country streams. Both reaches supported Guadalupe Bass, an endemic to central Texas, as well as surprisingly high numbers of good-sized largemouth bass and most of the sunfish species known from the region. One difference was that the lower reach had several very large, adult flathead catfish as well as numerous juveniles of both flathead and channel catfish.

Onion Creek: Nutrients

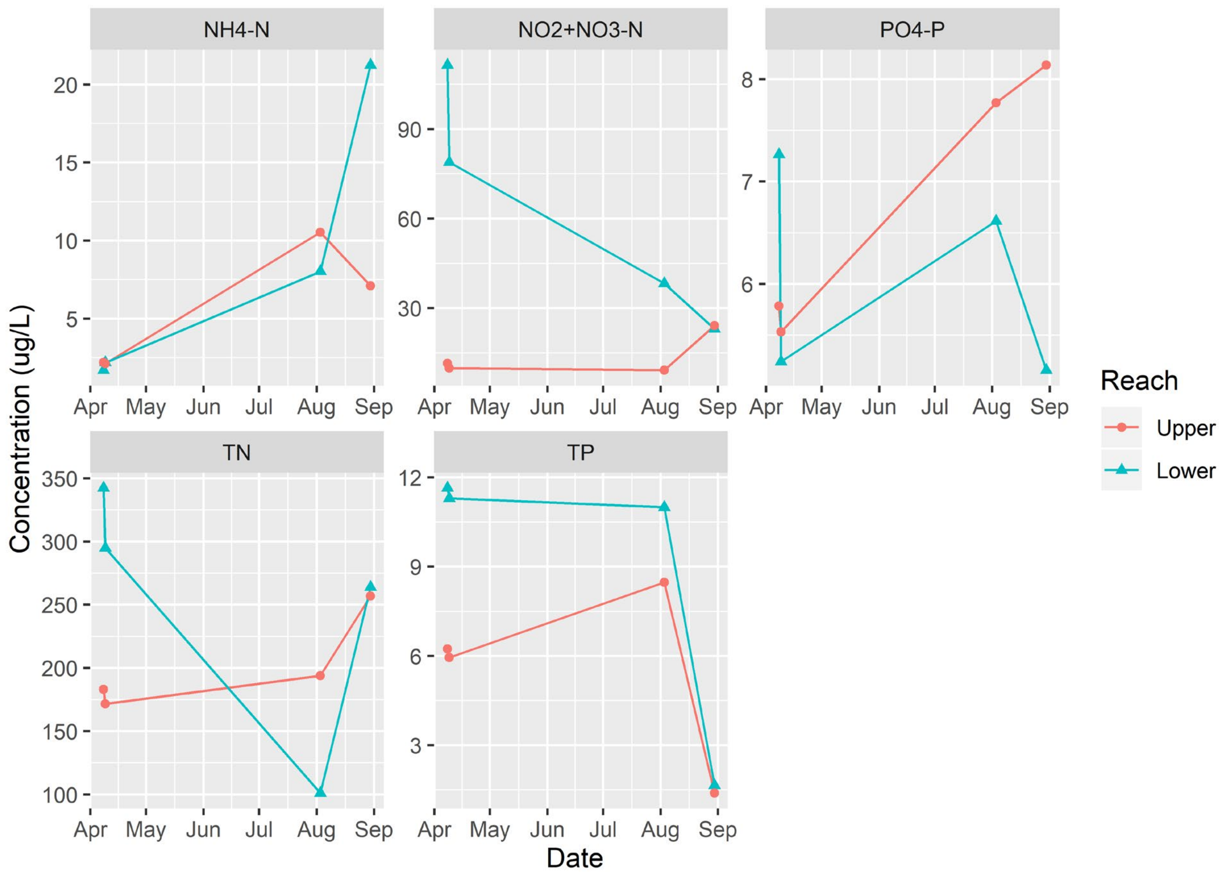


Figure Onion.1: Nutrient levels in both reaches were generally consistent with a high-quality, reference stream in the Edwards Plateau or Cross Timbers Ecoregions of central Texas. However, total P was slightly above 10 µg/L and TN was slightly above 300 µg/L in April 2019 in the lower reach. However, overall, nutrient levels never exceeded levels associated with biological thresholds (e.g., 20 µg/L TP, 500 µg/LTN) for biological condition in central Texas streams.

Onion Creek: YSI EXO1 Data Sonde Parameters, Instantaneous

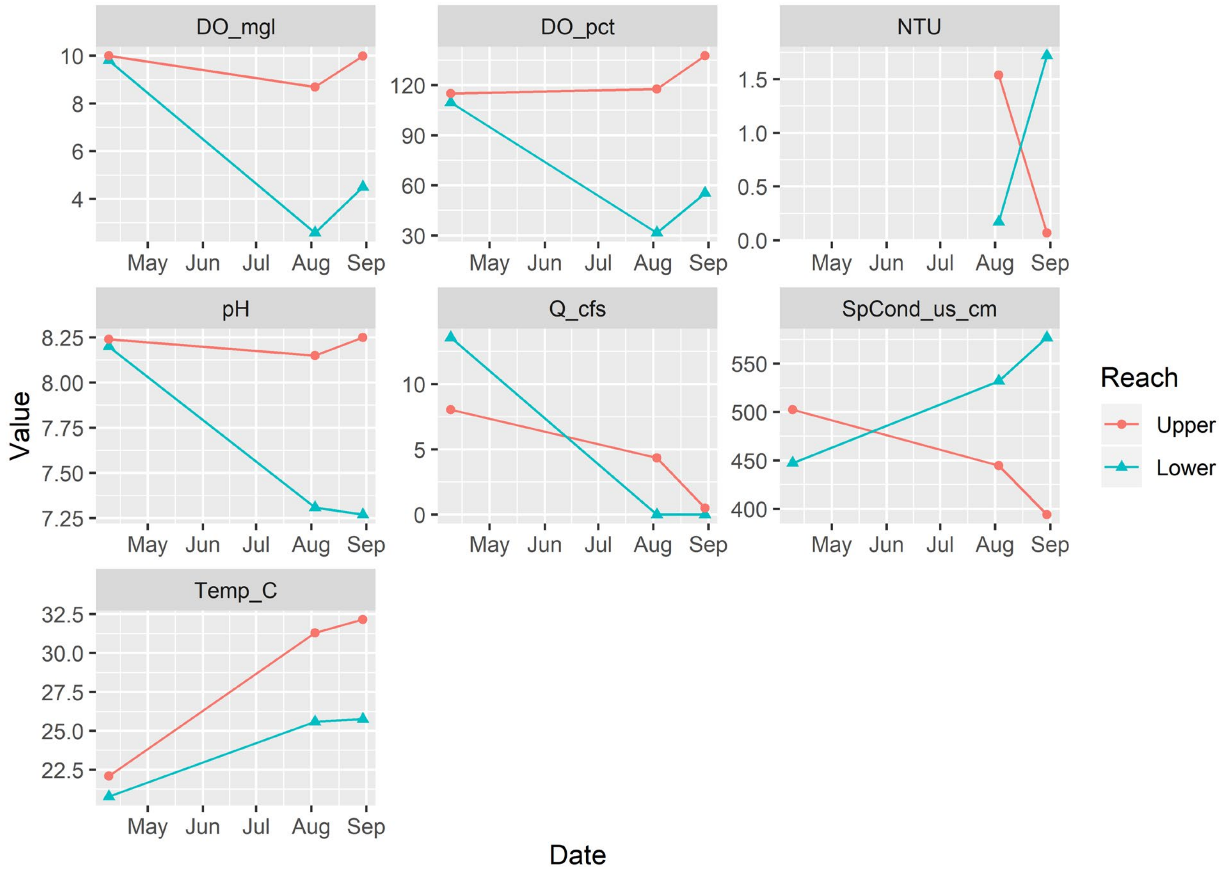


Figure Onion.2: Dissolved oxygen (DO; units are milligrams per liter (mgl) and percent saturation (pct)), turbidity (NTU, a measure of water clarity), pH (acidity), stream flow (Q_cfs, or cubic-feet per second), specific conductance (SpCond_us_cm; units are microsiemens per centimeter), and water temperature (degrees Celsius) measured in the early morning (Lower) and mid-morning (Upper) reaches of Honey Creek during summer 2019. The tendency for the Upper reach to have warmer temperatures and DO is related to daytime (see next for 24-h estimates which account for time of day). NTU levels at both reaches were extremely low. NTU was not measured in May. The two reaches were overall quite similar, although the trend in differences in specific conductance over time may suggest greater influence of groundwater in one of the reaches (lower, probably).

Onion Creek: EXO1 24 h (Diel) Water Quality Parameters

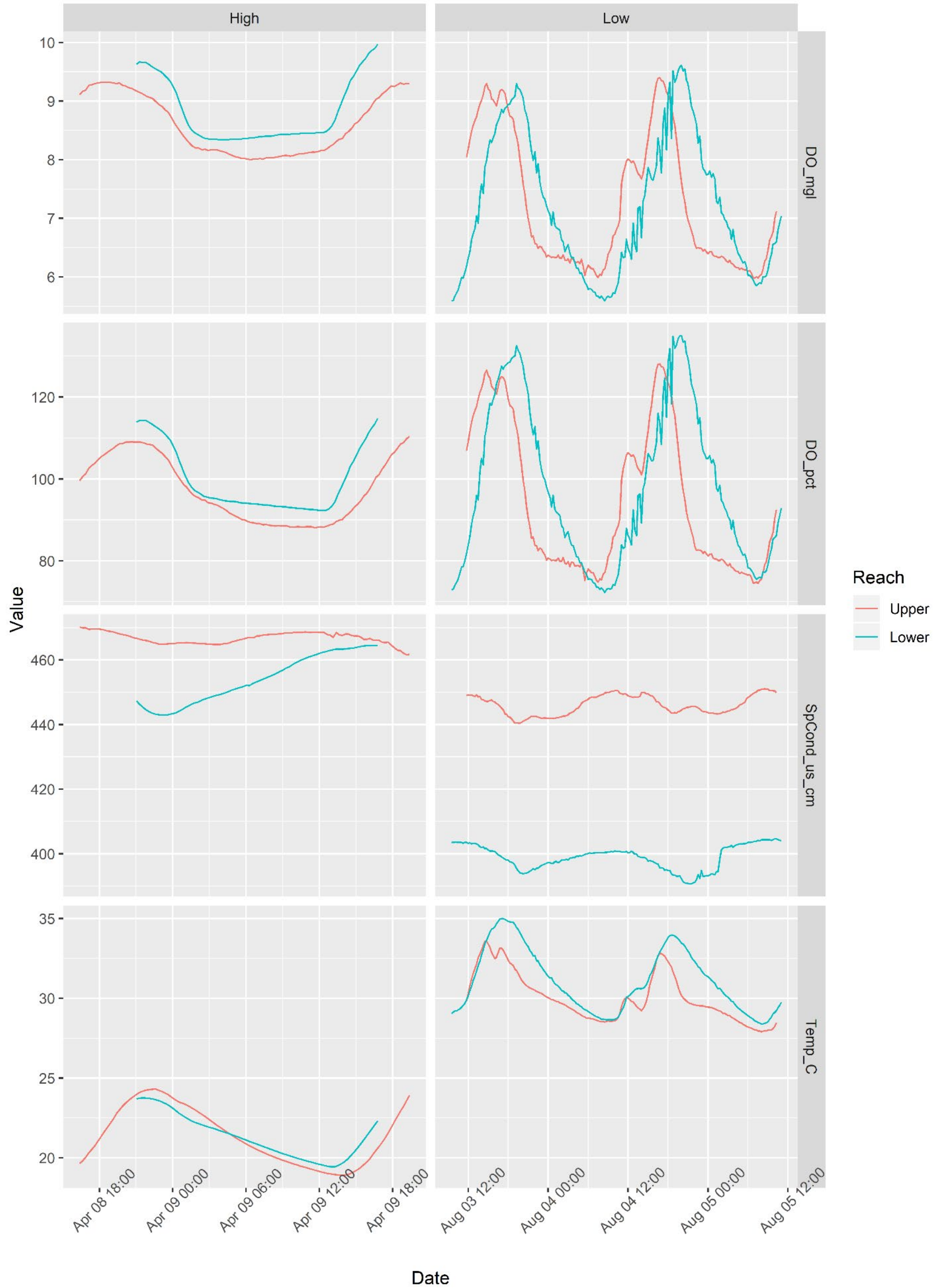


Figure Onion.3: Dissolved oxygen levels were high and remained at or above levels that are supportive of natural biological communities in Texas streams. EXO1 sondes, which were deployed to capture 15-minute intervals of dissolved oxygen and other parameters, revealed similar DO levels between the two reaches during the day and night. Even during low flow, when the lower reach had been reduced to a series of disconnected pools, it maintained DO levels similar to that of the upper, flowing reach.

Onion Creek: Seston (Organic Matter, Phytoplankton, and Total Particulates in Water Column)

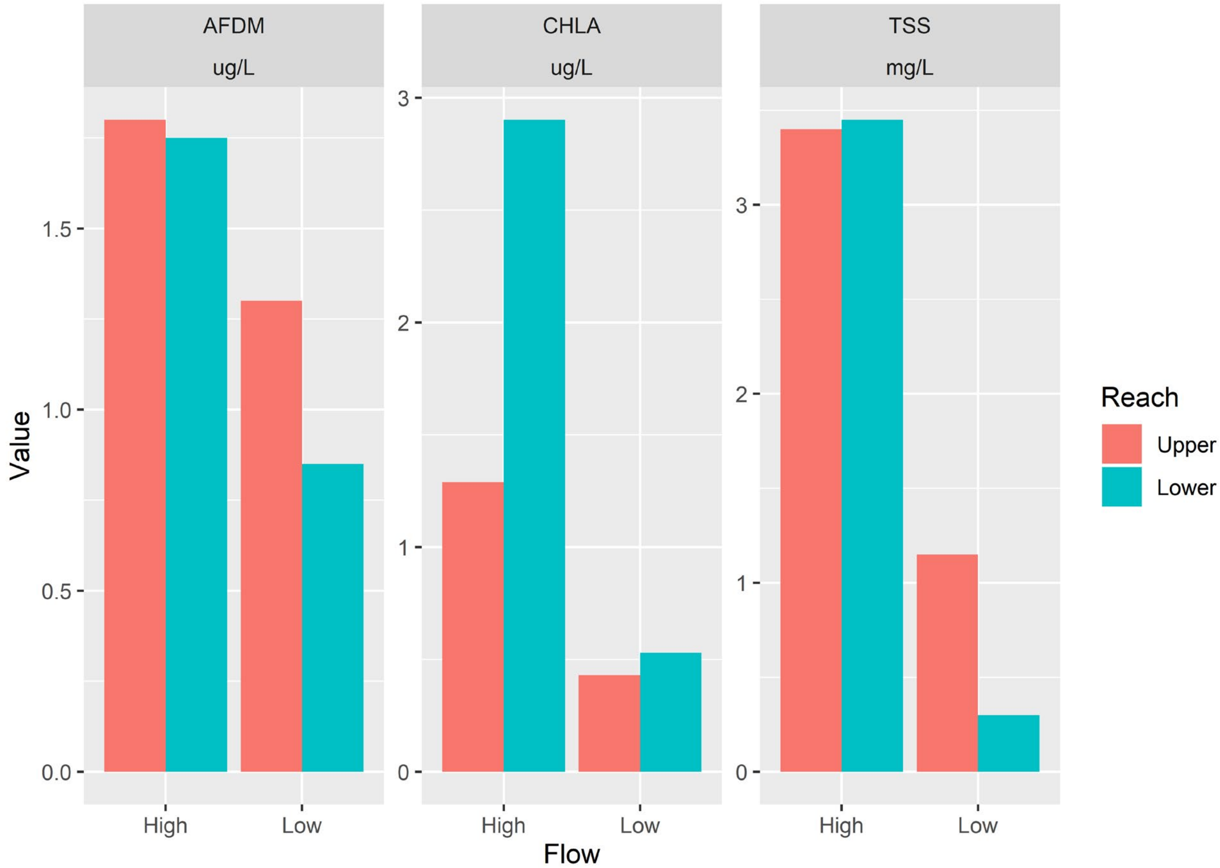


Figure Onion.4. Sestonic organic matter (ash-free dry mass particulates floating in water column), chlorophyll-a (phytoplankton or other algae floating in water column), and total suspended solids (TSS, all particulates in water column) were consistent with high-quality, reference stream conditions in both reaches during both high and low flow events. Sestonic chlorophyll-a peaked in high flows at ~ 3 µg/L in the lower reach and was < 1 µg/L during low-flow conditions.

Onion Creek: Periphyton (Benthic Algae) Biomass

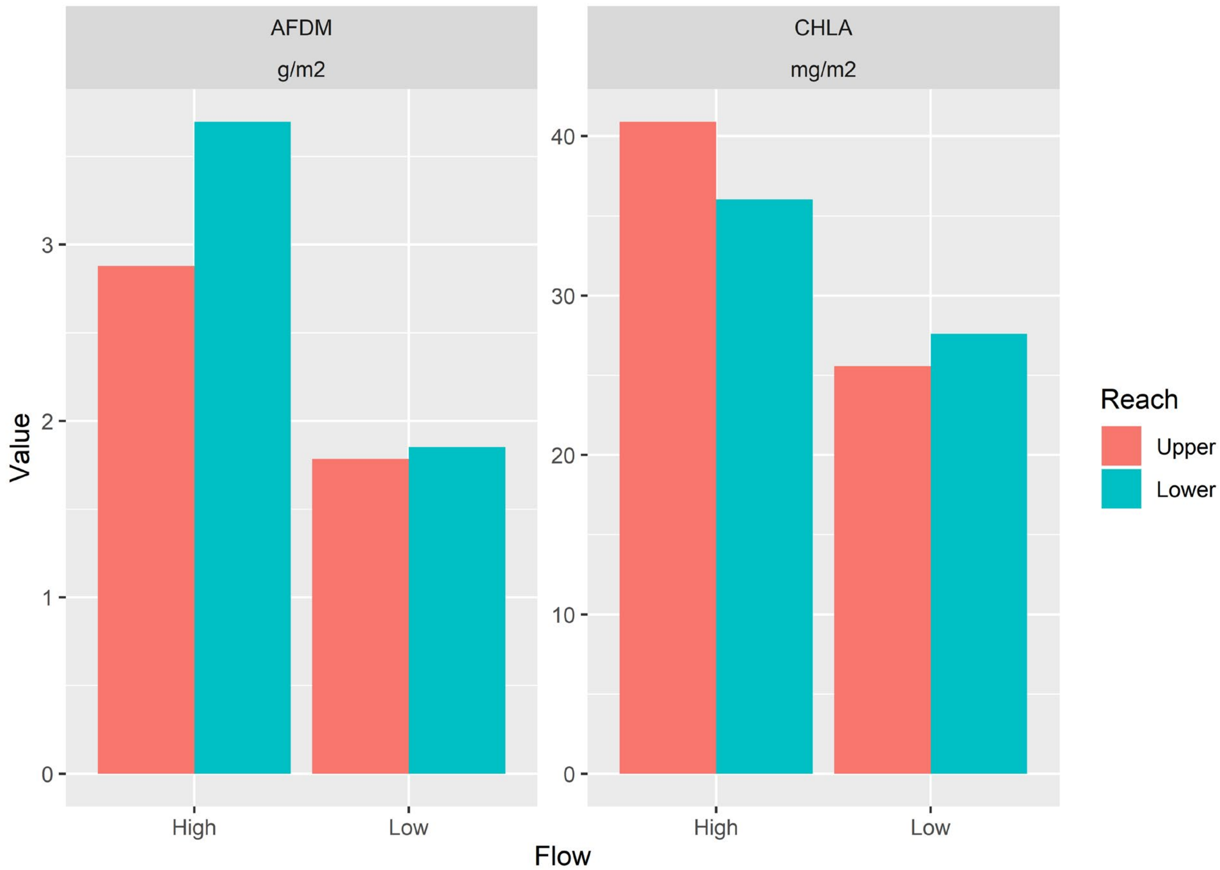


Figure Onion.5: Periphyton (benthic algae) biomass was low and consistent with a low-nutrient ecosystem. Total biomass (ash-free dry mass) and chlorophyll-a were higher in the upper reach during high flow, but values were still quite low in both reaches. Maximum benthic chlorophyll-a was approximately 40 mg/m^2.

Onion Creek: Periphyton Stable Isotopic Ratios for Carbon and Nitrogen

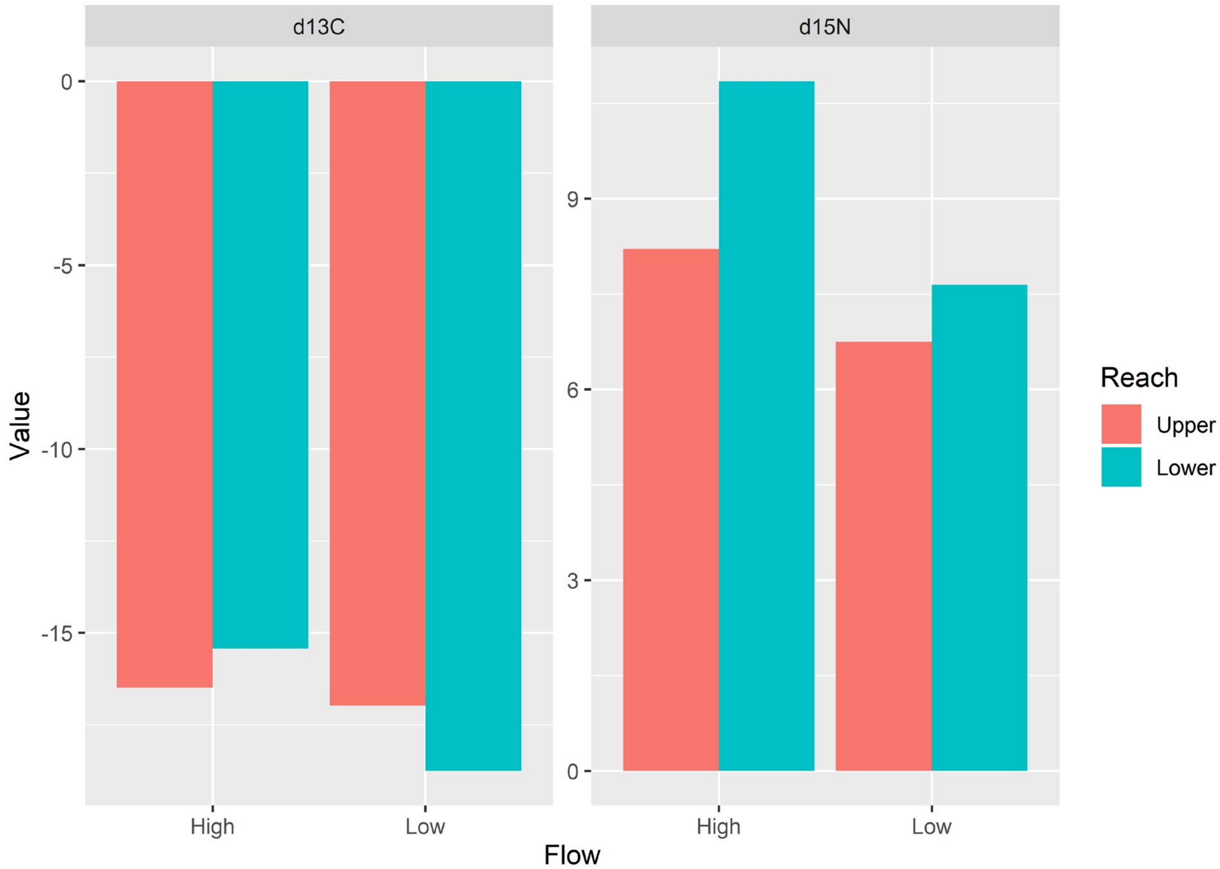


Figure Onion.6: Periphyton stable isotopic ratio values for nitrogen (δ15N) were one of the only variables that definitely suggested a human source of nutrients in the lower reach (CharRo Ranch) when compared to the upper reach. The lower reach had δ15N values near 11 per mil during high flow (April), whereas the upper reach had values closer to 8. The difference between reaches was smaller during the low flow period, which implies that the lack of runoff into the lower reach may have contributed to the increased similarity between the two reaches. That is, if wastewater application to fields or other land was reaching Onion Creek, we might expect this to be more evident during higher flow events when rain would facilitate runoff and increase seepage from uplands near the river.

Onion Creek: *Cladophora glomerata* (Nuisance Filamentous Green Alga) and Total Soft Algal Biovolume

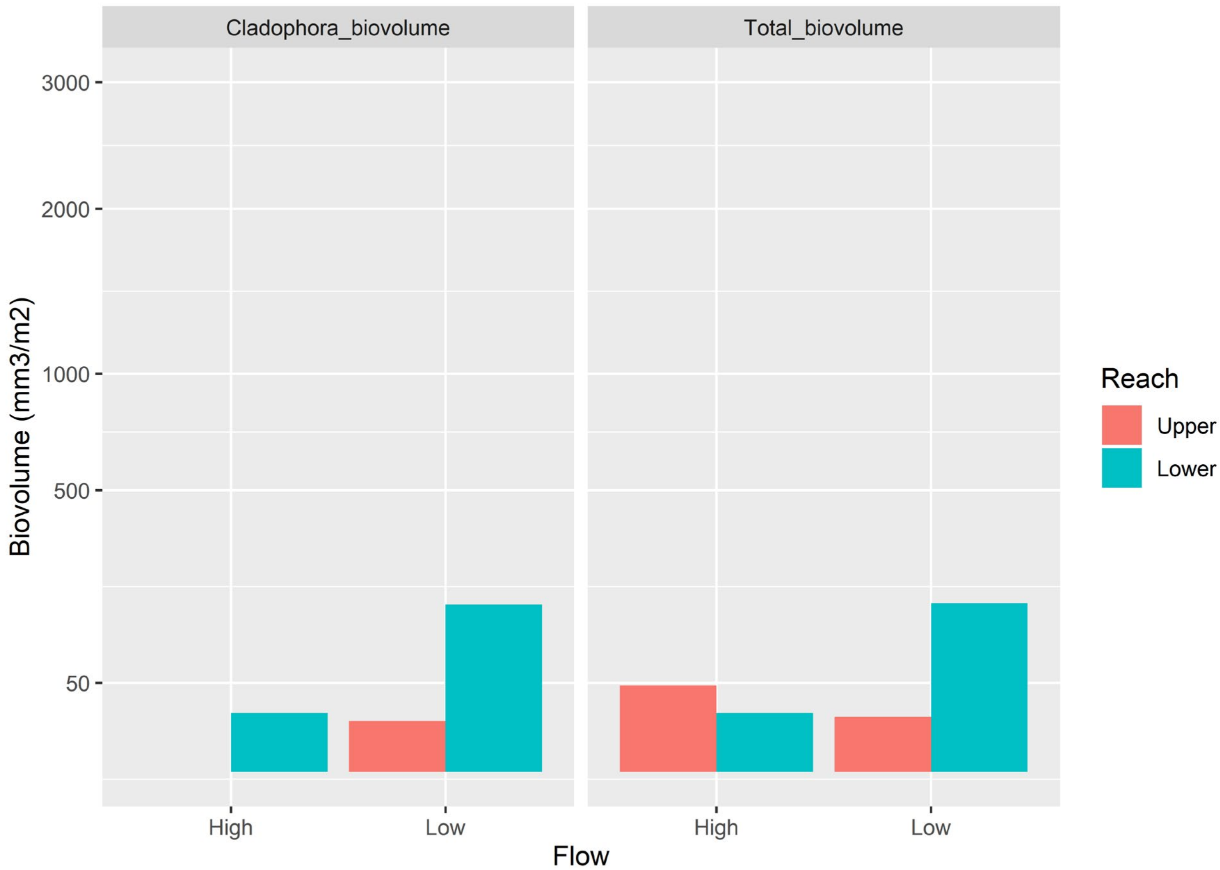


Figure Onion.7: Biomass of Cladophora glomerata, the most common nuisance filamentous green algal species associated with excessive nutrient enrichment, was relatively low in both reaches.

Onion Creek: Diatom Species Community Metrics

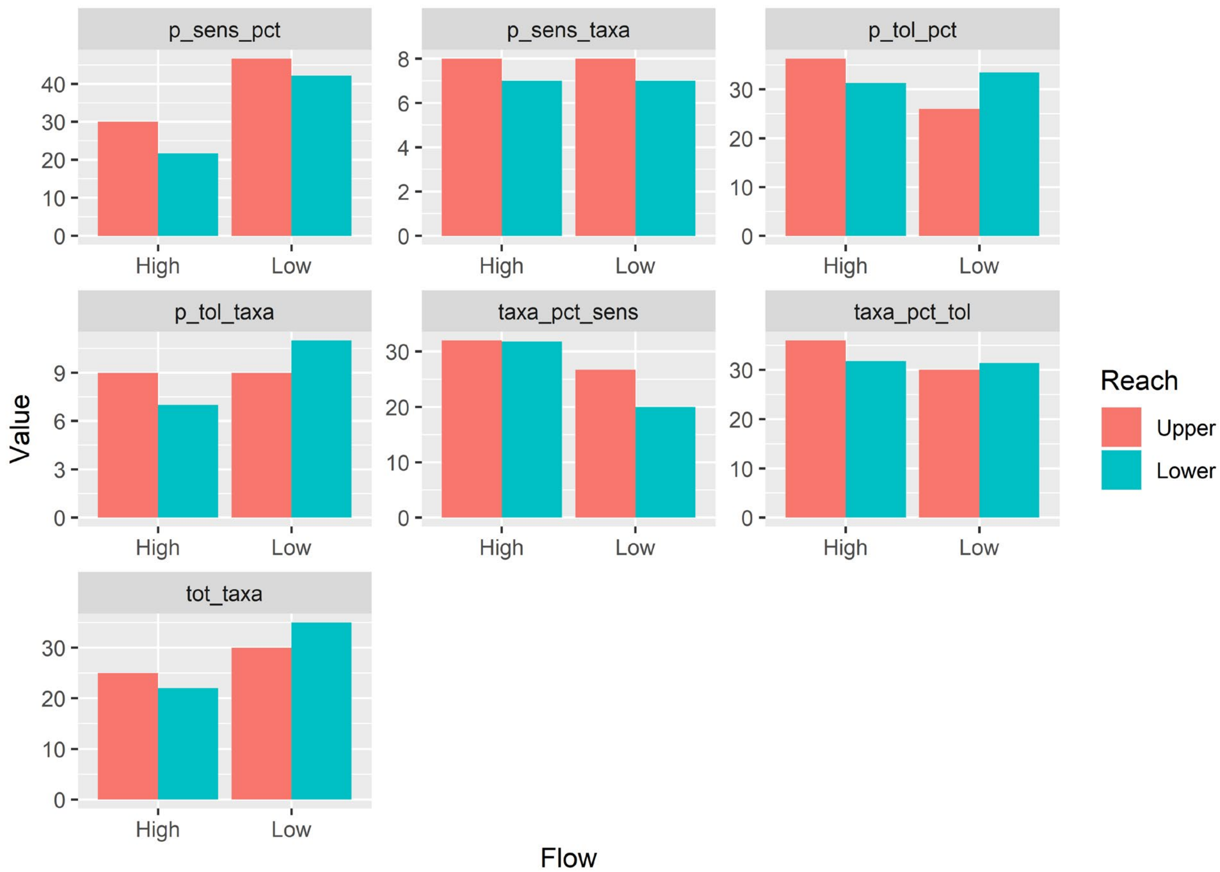


Figure Onion.8: Diatom species richness was similar between both reaches (20-25 species in April, 30-35 species in August). Phosphorus (P) sensitive taxa richness and abundance was similar between reaches, as was the richness of P tolerant taxa. There was no compelling difference in the abundance of any species indicative of high P levels between the two reaches.

Onion Creek: Macroinvertebrates Community Metrics and ALU Designation

Table Onion.1: The TCEQ Multimetric Index scored only a “High” Aquatic Life Use Designation in the Lower reach during low flow compared to an “Exceptional” in the Upper reach. Both reaches were classified as “Exceptional” during the high flow period in April.

HIGH FLOW, Upper (Mt. Gainor)

Metric	Value	Score	4	3	2	1
Taxa Richness	**31**	4	>21	15-21	8-14	<8
# EPT	**10**	4	>9	7-9	4-6	<4
HBI	**4.44**	3	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**21.53**	1	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Baetis*)	**25.19**	3	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (CG)	**23.48**	4	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**18.70**	3	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**1.30**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**28.97**	3	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**9**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**23.48**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**1.97**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	**37**				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

High Flow, Lower (CharRo)

Metric	Value	Score	4	3	2	1
Taxa Richness	**23**	4	>21	15-21	8-14	<8
# EPT	**8**	3	>9	7-9	4-6	<4
HBI	**4.09**	3	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**13.46**	2	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Baetis*)	**25.95**	3	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (FC)	**49.03**	2	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**14.77**	4	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**3.14**	2	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**20.00**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**4**	3	>5	4-5	2-3	<2
% Collector-Gatherers	**19.31**	3	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**0.98**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**EXCEPTIONAL**	**37**				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

LOW FLOW, Upper (Mt. Gainor)

Metric	Value	Score	4	3	2	1
Taxa Richness	**32**	4	>21	15-21	8-14	<8
# EPT	**11**	4	>9	7-9	4-6	<4
HBI	**4.05**	3	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**28.81**	1	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (*Chimarra*)	**43.68**	1	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG (FC)	**54.82**	1	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**23.39**	3	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**1.17**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**6.43**	4	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**6**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**16.43**	4	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**0.92**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**HIGH**	34				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Low Flow, Lower (CharRo)

Metric	Value	Score	4	3	2	1
Taxa Richness	**41**	4	>21	15-21	8-14	<8
# EPT	**11**	4	>9	7-9	4-6	<4
HBI	**5.06**	2	<3.77	3.77-4.52	4.53-5.27	>5.27
% Chironomidae	**43.91**	1	0.79-4.10	4.11-9.48	9.49-16.19	<0.79 or >16.19
% Most Dominant Taxa (Chironominae)	**37.58**	2	<22.15	22.15-31.01	31.02-39.88	>39.88
% Most Dominant FFG ()	**32.67**	4	<36.50	36.50-45.30	45.31-54.12	>54.12
% Predators	**19.90**	3	4.72-15.20	15.21-25.67	25.68-36.14	<4.73 or >36.14
Ratio Intolerant (<6) /Tolerant (≥6)	**0.27**	1	>4.79	3.21-4.79	1.63-3.20	<1.63
% Trichoptera as Hydropsychidae	**81.43**	1	<25.50	25.51-50.50	50.51-75.50	>75.50 or none
# Non-insect Taxa	**8**	4	>5	4-5	2-3	<2
% Collector-Gatherers	**32.67**	2	8.00-19.23	19.24-30.46	30.47-41.68	<8.00 or >41.68
% Elmidae	**9.43**	4	0.88-10.04	10.05-20.08	20.09-30.12	<0.88 or >30.12
Aquatic Life Use Designation	**HIGH**	32				
Exceptional	>36					
High	29-36					
Intermediate	22-28					
Low	<22					

Onion Creek: Macroinvertebrate Densities

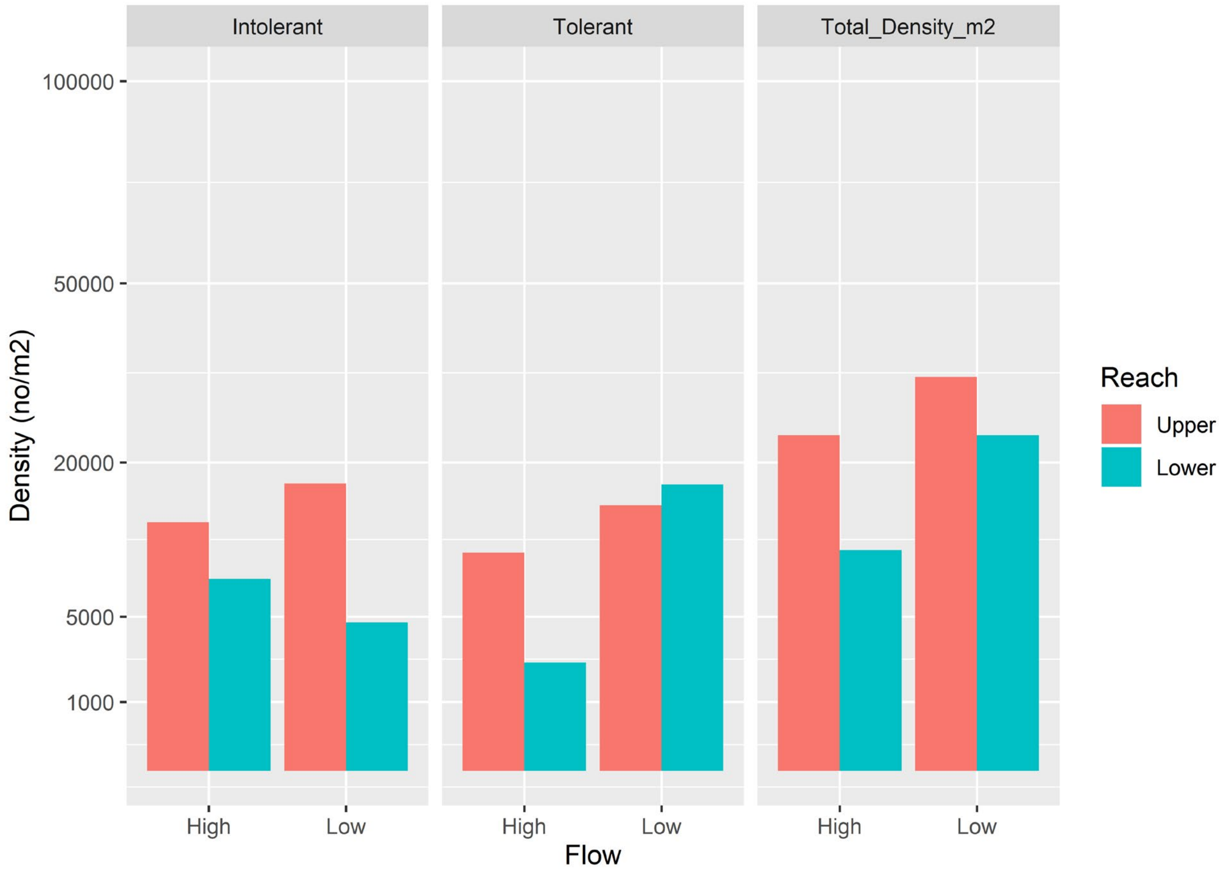


Figure Onion.9: Macroinvertebrate community composition differed slightly between reaches. The Upper reach had higher densities of macroinvertebrates in both seasons, but the species richness was not consistently higher or lower. In April, the upper reach had more taxa than the lower, but August low flow, the lower reach had more taxa.

Macroinvertebrate Taxonomic Composition

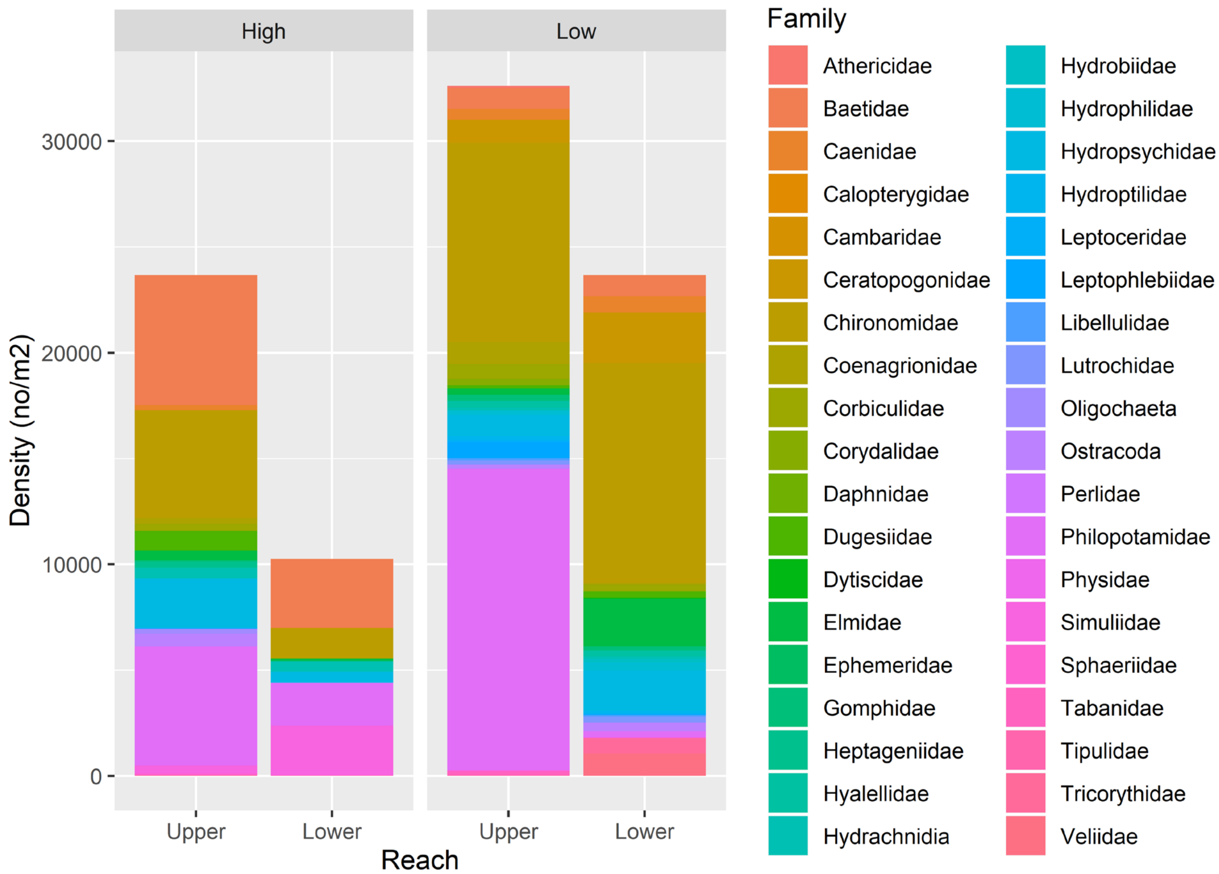


Figure Onion.10. Stacked bar plot of macroinvertebrate densities by family. The upper reach tended to have slightly higher densities, but composition was quite similar between both reaches during early season, high flows and late season, low flows.

Onion Creek: Fish Assemblage Composition

Table Onion.2: Fish assemblages were consistent with high quality Hill Country streams. Both reaches supported Guadalupe Bass, an endemic to central Texas, as well as surprisingly high numbers of good-sized largemouth bass and most of the sunfish species known from the region. One difference was that the lower reach had several very large, adult flathead catfish as well as numerous juveniles of both flathead and channel catfish. Note that column 1 refers to the total number of individuals collected whereas column 2 represents the number of juveniles (as part of the total number).

Onion Creek, Lower		
Blacktail Shiner	65	5
Bluegill	406	57
Bullnose minnnow	1	1
Channel Catfish	20	15
Flathead Catfish	8	1
Green Sunfish	63	20
Largemouth Bass	13	8
Lepomis spp.	386	385
Longear Sunfish	90	5
Redbreast Sunfish	5	0
Redear Sunfish	44	2
Redspotted Sunfish	5	0
Warmouth	4	0
Western Mosquitofish	25	24
Total	**1135**	**523**
Onion Creek, Upper		
Blacktail Shiner	59	0
Bluegill	99	38
Green Sunfish	8	0
Guadalupe Bass	1	1
Largemouth Bass	11	1
Lepomis spp.	3	3
Longear Sunfish	100	3
Redbreast Sunfish	33	0
Redear Sunfish	32	0
Total	**346**	**46**

Image Onion.1: Largemouth bass from Onion Creek, Lower reach, September 2019.

Image Onion.2. Electrofishing Lower reach, September 2019.

Image Onion.3: Flathead Catfish from Lower reach, upper pool, September 2019.

Conclusions

All four of these Hill Country streams are vulnerable to nutrient enrichment. In the cases of the three streams where the reach upstream was above the input of existing and potential wastewater inputs (all but Honey Creek), these reaches had ambient nutrient levels indicative of a low-nutrient, pristine to nearly pristine Hill Country ecosystem. However, in two of these three cases, the lower reach (Blanco River and Onion Creek) already had signs of wastewater pollution.

The Blanco River at Blanco Settlement, in particular, was already impacted by sources of nutrients that were not detected above the city of Blanco. Multiple indicators (dissolved and total phosphorus concentrations, sestonic chlorophyll-a and total suspended solids, 24-hour changes in dissolved oxygen, algal biomass, stable isotopes of nitrogen in algae, nuisance algal biovolume, diatom species composition, macroinvertebrate densities, macroinvertebrate species composition, and fish species densities) all suggested that the Blanco River at Blanco Settlement was impacted already by wastewater.

Huge mats of Cladophora glomerata, such as shown here, were found throughout the lower reach (Blanco Settlement) of the Blanco River during April 2019.

Onion Creek at CharRo Ranch had fewer indicators of wastewater impacts than Blanco Settlement, but it appears to be in the early stages of eutrophication from excessive nutrient inputs. Nutrients trended higher during certain flow regimes, especially low flow, as did stable nitrogen isotopes, which are one of the most sensitive early-warning signals of external wastewater nutrient sources. Of further concern is the tendency for the lower reach to dry up into a series of pools during low flow, which leaves it even more vulnerable to nutrient enrichment.

Barton Creek may have some influence of wastewater from the upstream catchment, as evidenced by slightly elevated nitrogen isotopes in the periphyton, but there were no clear differences between the upstream and downstream reaches as they relate to Long Branch, the tributary proposed to be the conduit for wastewater into Barton Creek on Shield Ranch. Currently, Barton Creek at Shield Ranch has relatively high-water quality and algal, macroinvertebrate, and fish assemblages typical of a reference-caliber Hill-Country stream.

Finally, Honey Creek, arguably the most unique and special of these four streams, had differences between the upstream and downstream reach that were likely mostly related to the amount of groundwater feeding into the stream, with the upper reach being just downstream of the first major spring, and the downstream reach being downstream of one or several more larger springs. The lower reach had much higher flows, but also higher levels of nitrogen, potentially indicative of groundwater contamination. Both reaches had higher-than-expected nitrogen concentrations and are highly vulnerable to any additional nutrient enrichment, especially phosphorus, given the already elevated levels of nitrogen.

Honey Creek, Lower Reach, May 2019. Green coloration on the stream bottom was predominantly aquatic mosses (bryophytes) and vascular plants (macrophytes), which are indicative of high-water quality.