CASM ENVIRONMENTAL, LLC

Macroinvertebrate Monitoring at Selected Sites in Whychus Creek, Sisters, OR, 2020

Northern Spreadwing on Whychus Creek; C.A. Searles Mazzacano

Prepared for: Lauren Mork, Monitoring Program Manager, Upper Deschutes Watershed Council

Prepared by: Celeste A. Searles Mazzacano, Principal Scientist, CASM Environmental, LLC

Date: March 2, 2021

Consulting Services Agreement 0715-2020

Table of Contents

Summary

The benthic macroinvertebrate community was sampled in multiple reaches of Whychus Creek on 13-14 August 2020: Road 6360 (WC0600), Rimrock Ranch (WC0850, WC0900, WC1025), Whychus Canyon (WC1100, WC1150), Camp Polk (WC1950), Willow Springs (WC2000, WC2050), and Whychus Floodplain (WC2600). Samples represented a mixture of longterm index sites, restored sites, and reaches sampled for the first time prior to restoration. Sampling was done using proportional multihabitat (PM) and single-habitat riffle-targeted (RT) techniques, and the ORDEQ PREDATOR predictive model and Index of Biotic Integrity (IBI) was applied to all RT samples. Sample community taxonomic and biological traits were assessed including: community optima for temperature and fine sediment; tolerances to sediment and organic pollution; functional feeding group; habit (locomotion); annual generations (voltinism); rheophily (flow preference); water temperature association; and maximum body length.

Samples contained 156 unique taxa in 51 families, the most total taxa in any sampling year to date. Order-level diversity was highest among true flies (Diptera; 70 unique taxa in 11 families), especially non-biting midges (Chironomidae). Other families with high diversity included mayflies (Ephemeroptera; 23 unique taxa in six families); caddisflies (Trichoptera; 19 unique taxa in eight families); and stoneflies (Plecoptera; 10 unique taxa in five families). Three taxa not found in any prior sampling year at any Whychus site were collected, mainly in PM samples, and at least one taxon not seen at a given site in a prior sampling year was collected at all sites sampled in multiple years except WC0600.

Sample richness ranged from 35-70 unique taxa (mean 54 taxa; SD 9), and was significantly greater in PM (mean 59; SD 6) vs. RT samples (mean 44 taxa; SD 6). Other metrics such as number of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa and community sediment and temperature optima were not significantly different in RT vs. PM samples. Community composition was strongly influenced by reach location, with greater macroinvertebrate community similarity in samples collected further upstream (RM19.50 through RM2600) compared to those taken in downstream reaches, regardless of the method used (RT or PM). Numerical abundance across the dataset was dominated by *Rhithrogena*, a sediment-sensitive flatheaded mayfly found on rocks in cold, fast flows.

Ongoing restoration along Whychus Creek has resulted in communities that are more diverse and more balanced in recent years, and total and EPT richness increased among all sites since 2015. Taxonomic composition and ecological traits of macroinvertebrate communities changed over the years among all sampling sites due to a combination of restoration, increased habitat heterogeneity with establishment of side channels, better community representation in PM samples, and the broader impacts of changing climate conditions. In-stream restoration activities in Whychus Creek have varied (i.e., re-meandering channels, creating side channels, adding large wood) but the trajectory of macroinvertebrate community response has been similar at most restored sites, with large perturbations for up to two years followed by stabilizing metric values and an altered post-restoration community. Increased habitat heterogeneity corresponds with increased macroinvertebrate diversity; side channel communities are similar to those in the associated primary channel reach but often with greater total and EPT richness and a diversity of both tolerant and sensitive taxa with a wider range of temperature, flow, and sediment associations.

Background

Whychus Creek is a designated priority watershed for conservation and restoration in the upper Deschutes Basin. Projects implemented since 1999 restored perennial flow to the creek and increased in-stream flow volume and channel complexity. The aquatic macroinvertebrate community has been monitored regularly to assess changes and their relationship to altered habitat conditions and, more recently, creation of new heterogenous side channels. The goals of macroinvertebrate monitoring in Whychus Creek include: 1. assessing ongoing changes at the watershed level through continued monitoring at selected long-term index sites; and 2. analyzing communities at the project level prior to and following restoration activities to increase fine-scale resolution at targeted sites.

Methods

Macroinvertebrate sampling techniques

Benthic macroinvertebrate sampling was done on 13-14 August 2020 in multiple regions of Whychus Creek (Table 1): Road 6360 (WC0600), Rimrock Ranch (WC0850, WC0900, WC1025), Whychus Canyon (WC1100, WC1150), Camp Polk (WC1950), Willow Springs (WC2000, WC2050), and Whychus Floodplain (WC2600). Multiple samples were taken at some restoration sites to assess different side channel reaches, and both riffle-targeted (RT) and proportional multihabitat (PM) samples were collected at some index and pre-project sites. RT samples were taken in five reaches (WC0600, WC0900, WC1100-2, WC2000, WC2600), and PM samples were taken in 11 reaches (WC0850, WC1025, WC1100-1B, WC1100-2, WC1100-4, WC1150, WC1950, WC2000, C2050, WC2600), including a duplicate sample taken at WC2050 for quality control.

Table 1. Whychus Creek sampling sites in 2020

Site ID	Description	Coordinates	Year(s) sampled
WC0600	u/s Rd 6360	44.40412, -121.40259	2005, 2009, 2011-2020
WC0850[a]	Rimrock Ranch d/s	44.391278, -121.406182	2011-2017; 2020
WC0900	Rimrock Ranch Meadow	44.384198, -121.407892	2009, 2011-2017; 2020
WC1025[b]	Rimrock Ranch u/s	44.371534, -121.415865	2011-2012, 2014-2017, 2019-2020
WC1100-2[c]	lower Whychus Canyon d/s	44.36587, -121.421706	2014, 2015, 2017-2020
WC1150[d]	upper Whychus Canyon u/s	44.35985 -121.42870	2014-2020
WC1950	d/s Camp Polk bridge; WQ site coordinates	44.318741, -121.514961	2009, 2011-2017, 2019-2020
WC2000	Willow Springs d/s		2020
WC2050	Willow Springs u/s		2020
WC2600	Whychus floodplain, u/s 4606 Rd. footbridge, d/s WQ site coordinates	44.273059, -121.555297	2005, 2009, 2011-2020

[a] designated as WC0875 in 2011-2015 [b] designated as WC1050 in 2016 & 2017, and as WC0950 in 2011-2015; [c] designated WC1025 in 2014; [d] designated WC1075 in 2014

Macroinvertebrate sampling techniques

Sampling was done by CASM Environmental, UDWC staff, and volunteers from natural resource agencies. Because of restrictions on public gatherings due to the COVID-19 pandemic, it was not possible to work with community volunteers as in past years. Social distancing was maintained in the field to the greatest extent possible and participants wore masks. CASM Environmental staff demonstrated sampling techniques, then teams received sampling kits and directions and dispersed into the field. All teams worked in the same area each day, so CASM Environmental and UDWC staff were available to answer questions. Teams returned samples, data sheets, and equipment to CASM Environmental, who inspected each sample to ensure it was properly labeled and preserved.

Riffle-targeted protocol (RT)

Benthic macroinvertebrates were collected from riffle habitats according to Oregon Department of Environmental Quality (ORDEQ) protocols for Oregon's wadeable streams (ORDEQ, 2009). Reach lengths were calculated as 40 times the average wetted stream width at the desired sampling point (min. 500 ft. [150 m]/max 1000 ft. [300 m]) and the upstream and downstream limit was flagged by UDWC. At sites that included side channel reaches, UDWC staff flagged turning points and paths along the channel. A sample consisted of eight individual net sets taken in riffle habitat in the designated reach, each collected from a 1 ft^2 area using a D-frame kick net with 500 µm mesh and a 1-ft (0.3 m) opening. In reaches with eight or more riffles, a single net set was taken in each of eight randomly selected riffles; in reaches with fewer riffles, two net sets were taken in each of four randomly selected riffles.

Large rocks in the sampling area were rubbed and rinsed into the net to collect clinging organisms and set aside, then the remaining substrate was disturbed thoroughly to a depth of 6-10 cm (2-4 in.) for 1-2 minutes. All net sets were pooled in a bucket, large debris was rinsed and removed, and sample material was poured through a sieve lined with a 500 µm Nitex membrane. This concentrated sample was transferred to a 1-liter Nalgene sample jar half-filled with 80% ethanol as a preservative. Jars were filled no more than 2/3 full; sample material was divided among multiple jars if needed. CASM Environmental staff replaced the 80% ethanol in all jars with fresh within 72 hours to ensure preservation.

Proportional multihabitat sampling (PM)

This technique samples a representative portion of all habitat types in a reach, including mineral and organic substrates, which improves sample representativeness and assessment of ecological condition and is also preferred in streams or reaches where riffle habitat is poorly represented (Barbour et al., 2006). Reach lengths were calculated and flagged as described above. At sites where both RT and PM samples were taken, two teams sampled the same reach simultaneously, moving upstream as a unit to avoid disturbing areas not yet sampled. Before sampling, teams walked the reach to determine types of in-stream habitat and their relative proportions. Habitat types were designated as follows:

- bedrock/boulder (continuous rock; large mineral substrate >basketball size)
- cobble (tennis ball- to basketball-size)
- gravel (marble- to tennis ball-size)
- sand/silt (fine sediment)
- filamentous algae (long, flowing strands)
- aquatic vegetation (herbaceous plants rooted or floating in the channel)
- wood (tangles/piles of small wood < 30 cm diameter and large woody debris ≥ 30 cm diameter in wetted channel)
- rootwads (root tangles extruding into flowing channel due to undercut banks)

Each sample was a composite of 10 net sets; the number of net sets taken in each type of habitat was determined by its proportional representation in the reach (Table 2). Flow type in the habitat where each net set was taken was recorded (rapid, riffle, run, glide, pool), but no flow types were targeted. Where there was sufficient current to carry suspended material into the net, cobble and gravel substrates were sampled as described for RT sampling. On bedrock and boulders, the D-net was held perpendicular to the substrate with the mouth facing upstream and the rock surface was rubbed clean in a 1 ft^2 (0.3 m) area in front of the net. In slow/still waters and in sediment, substrate was disturbed manually in a 1 ft^2 (0.3 m) area as the net was swept upwards to capture suspended/disturbed material. Clumps of filamentous algae and plants were shaken into the net mouth to collect clinging organisms, and the net was jabbed firmly into clumps of plants. Woody materials were rubbed or picked into the net, then kicked vigorously while holding the net below. Rootwads were sampled by jabbing the net into the tangle or kicking into the rootwad while holding the net below. All net sets were composited and processed as described for RT samples.

Sample identification

Samples were identified by Cole Ecological, Inc. (www.coleecological.com). Each was first sub-sampled to a target count of 500 individuals; this was done by splitting the entire sample into equal aliquots which were then selected randomly and all individuals in each selected aliquot were picked out. An aliquot in which the target number was reached was picked to completion, which explains differences in organismal abundance between samples (see Table 4 in Results).

Organisms were identified to the lowest practical taxonomic level using the standard taxonomic effort recommended by the Pacific Northwest Aquatic Monitoring Partnership (PNAMP, 2015, level 2; https://tinyurl.com/y6ynt4yo). The dataset reflects changes resulting from recently published updates to taxonomic keys in the seminal reference *Aquatic Insects of North America* (Merritt et al., 2019). For ease of comparison, any changes are noted with the historic name first and the new current name in parentheses. Most of the changes occurred in the former family Tipulidae (crane flies); this group was recently elevated to a superfamily (Tipuloidea) containing four families, and the names of multiple genera have changed.

Table 2. Whychus Creek sample and channel types in 2020. *RT = riffle-targeted, PM = proportional multihabitat; PC = primary channel, SC = side channel; PC/SC = reaches that contained both PC and SC. Average wetted widths were calculated from wetted widths recorded at each point a net set was taken.*

Sample	Site type	sample type	Loc.	# net sets in each habitat type	flow type in net sets	avg wetted width (ft)
WC0600	Index	RT	PC	cobble/gravel/boulder	8 riffle	27.6 ± 2.8
WC0850	Restoration (Baseline)	PM	PC	3 cobble, 4 gravel, sand/silt, 1 wood	4 riffle, 1 run, 3 glide, 2 pool	25.7 ± 4.6
WC0900	Restoration (Baseline)	RT	PC	cobble/gravel	8 riffle	22.3 ± 5.7
WC1025	Restoration (Baseline)	PM	PC	7 cobble, 1 gravel, 1 sand/silt, 1 wood	7 riffle, 2 run, 1 pool	24.7 ± 6.7
WC1100-1B	Restoration	PM	PC/SC	4 sand/silt, 3 gravel, 1 cobble, 1 veg., 1 wood	4 riffle, 1 run, 2 glide, 3 pool	24.6 ± 8.7
WC1100-2TR	Restoration	RT	PC	cobble/gravel	8 riffle	18.3 ± 3.4
WC1100-2PM	Restoration	PM	PC	4 cobble, 4 gravel, 1 sand/silt, 1 wood	5 riffle, 3 run, 1 glide, 1 pool	20 ± 5.7
WC1100-4	Restoration	PM	SC	4 gravel, 3 cobble, 2 sand/silt, 1 wood	5 riffle, 2 run, 3 glide	16.8 ± 6.0
WC1150	Upstream untreated control	PM	PC	5 cobble, 2 bedrock/boulder, 1 gravel, 1 sand/silt, 1 wood	4 riffle, 4 run, 2 glide	26.9 ± 2.9
WC1950	Restoration (Baseline)	PM	PC	6 gravel, 2 cobble, 1 sand/silt, 1 wood	6 riffle, 1 run, 1 glide, 2 pool	22.6 ± 8.7
WC2000PM	Restoration (Baseline)	PM	PC	6 cobble, 3 gravel, 1 sand/silt	9 riffle, 1 glide	28.2 ± 8.8
WC2000TR	Restoration (Baseline)	TR	PC	cobble/gravel/sand	8 riffle	31.9 ± 6.8
WC2050	Restoration (Baseline)	PM	PC	5 cobble, 3 gravel, 1 sand/silt, 1 veg	8 riffle, 1 run, 1 pool	31.2 ± 4.8
WC2050_DUP	site duplicate for QA	PM	PC	5 cobble, 3 gravel, 1 sand/silt, 1 veg	3 riffle, 5 run, 2 glide	30.8 ± 4.3
WC2600-1	Index & Restoration	TR	PC/SC	cobble/gravel/sand	8 riffle	21.8 ± 9.3
WC2600	Index & Restoration	PM	PC/SC	4 sand/silt, 3 cobble, 2 wood, 1 gravel	5 riffle, 4 run, 1 glide	22.4 ± 11.1

Biological/ecological traits of taxa

Assessing functional traits (i.e., biological properties and ecological preferences) of macroinvertebrate taxa helps infer habitat conditions that shape the community, diagnose stressors or environmental filters, and predict restoration-related changes (Poff et al., 2006; Tullos et al., 2009; Culp et al., 2011; Van den Brink et al., 2011; White et al., 2017). Ecological and life history traits of the macroinvertebrate community were assigned to taxa where data were available; values for each trait are not known for every taxon. Trait data were drawn from sources specific to Oregon and/or the west (Vieira et al., 2006; Meyer & McCafferty, 2007; Huff et al., 2008; Richards & Rogers, 2011; Relyea et al., 2012; IDDEQ, 2015; SAFIT, 2016), and general and family-specific references (Pinder, 1986; Wiggins, 1996; Larson et al., 2000; Thorp & Covich, 2001; Stewart & Stark, 2002; Anderson et al., 2013; Merritt et al., 2019). Where multiple modalities existed for a trait, the primary one was used. Community measures calculated included:

- community optima values for temperature and % fine suspended sediment (weighted averages): Temperature and fine suspended sediment can act as environmental filters on macroinvertebrate communities. Some taxa are associated with cool/cold or warm water temperatures, either as stenotherms (narrow tolerance range) or eurytherms (wide tolerance range). Increasing sediment loads can decrease overall richness and abundance of taxa that feed as scrapers or filterers, have a large maximum body size, soft exposed body, external exposed gills, associations with larger mineral substrates, and a crawling or sprawling habit. Taxa with operculate gills, smaller and more sclerotized bodies or cases/tubes, and a swimming or climbing/clinging habit may increase in abundance with increasing fine sediment stress (Beche & Statzner, 2009; Sutherland et al., 2012; Buendia et al., 2013; Bona et al., 2015; Murphy et al., 2017; Doretto et al., 2018; Akamagwuna et al., 2019).

- trophic guild (functional feeding group), i.e., relative abundances of predator (PR), scraper (SC), shredder (SH), and collector (C; includes filterers and gatherers) organisms: Filterers are negatively impacted by sedimentation if their feeding structures become clogged (Rabení et al., 2005; Wilkes et al., 2017); predator abundance can increase as increasing habitat diversity and/or stability creates more abundant and diverse prey (Arce et al., 2014); scrapers can be more abundant on algae- and biofilm-coated mineral substrates; and shredders indicate more plant material and leaf litter input.

- habit (locomotion) i.e., relative abundances of swimmer, clinger, burrower, climber, and sprawler organisms: Swimmers can more rapidly escape disturbances such as sedimentation; burrowers are selected for in sedimented habitat, while sprawlers, clingers, and crawlers can be smothered and/or lose habitat as interstitial spaces are filled (Mathers et al., 2017; Murphy et al., 2017).

- voltinism (# generations per year) i.e., relative abundances of multivoltine (>1 generation/year), univoltine (1 generation/year), and semivoltine <1 generation/yr) organisms. Multivoltinism is associated with more tolerant organisms and/or greater resilience in disturbed habitats, while semivoltine taxa require more stable conditions.

- rheophily (flow preference), i.e, relative abundances of organisms associated with erosional (fast/lotic) , depositional (slow/lentic), and mixed flow (i.e., found in both lotic and lentic habitats);

- temperature associations, i.e., relative abundances of organisms with cool/cold or warm water temperature preferences (taxa with mixed or no particular preference were omitted from this analysis); and

- maximum length, i.e., relative abundances of organisms with small (< 9 mm), medium (9-16 mm), and large (>16 mm) body length: Small body size is associated with greater tolerance and rapid recolonization, which can be an advantage in disturbed sites, while larger-bodied insects are slower to develop and can be more abundant in sites with greater habitat stability (Townsend & Hildrew, 1994; de Castro et al., 2018).

Data analysis

Analyses were done using PAST 4.0 statistical software (Hammer et al., 2001). CLUSTER dendrograms and SIMPER tests were run on a Bray-Curtis similarity matrix of square-root transformed taxa abundances. Principal Component Analysis (PCA) was done using a variance-covariance matrix. When doing t tests to compare means, a cutoff value of $p \leq 0.05$ was used for statistical significance. Means are presented with standard deviation.

Biological conditions in RT sample communities were assessed using the ORDEQ multimetric invertebrate-based index of biotic integrity (IBI) and the probability-based PREDATOR model (Hubler, 2008). In the IBI, raw values of 10 metrics are scored individually (1, 3, 5) then summed to give a number that corresponds to a level of biological impairment (Table 3). This model cannot be applied accurately to PM samples, as they are taken in multiple flow types, but values for individual metrics were calculated for the sake of comparison. PREDATOR calculates the ratio of taxa observed at a site to taxa expected if the site is not impaired (O/E), based on comparison to reference communities; the model uses site elevation, slope, and longitude to select appropriate reference streams. O/E scores correspond to the following biological condition categories: poor (most disturbed; ≤0.78); fair (moderately disturbed; 0.79-0.92); good (least disturbed; 0.93-1.23); and enriched (>1.23). Whychus Creek is an outlier for the PREDATOR model because it has lower annual precipitation than any reference streams the model selects as most appropriate.

Table 3. ORDEQ macroinvertebrate-based IBI metrics and scoring. *[a] relative abundance of the most abundant taxon; [b] modified Hilsenhoff Biotic Index (Hilsenhoff, 1987); reflects tolerance to organic pollution and ranges from 1 (low tolerance) to 10 (high tolerance)*

Metric	**5**	**3**	**1**
Taxa richness	>35	19-35	<19
Mayfly richness	>8	4-8	<4
Stonefly richness	>5	3-5	<3
Caddisfly richness	>8	4-8	<4
# sensitive taxa	>4	2-4	<2
# sediment-sensitive taxa	≥2	1	0
% dominance[a]	<20	20-40	>40
% tolerant taxa	<15	15-45	>45
% sediment-tolerant taxa	<10	10-25	>25
MHBI[b]	<4	4-5	>5
Summed score & condition			
<20 Severely impaired; **20-29** moderately impaired; **30-39** slightly impaired; **>39** Minimally/not impaired			

Results

Macroinvertebrate community in 2020 samples

A total of 156 unique taxa in 51 families (36 insect, 15 non-insect) was collected across all sites. Only three taxa were taken in every sample: *Simulium,* a black fly that clings to stones in flowing water; *Baetis tricaudatus,* a small minnow mayfly found in a variety of flowing waters; and *Glossosoma,* a sediment-intolerant saddlecase-maker caddisfly found in cool rapid streams. Both *B. tricaudatus* and *Glossosoma* are ORDEQ low sediment indicator taxa. Order-level diversity was highest among Diptera, with 70 unique taxa in 11 families, including 50 genera of non-biting midge (Chironomidae). Other well-represented families included mayflies (Ephemeroptera; 23 unique taxa in six families); caddisflies (Trichoptera; 19 unique taxa in eight families); and stoneflies (Plecoptera; 10 unique taxa in five families).

Sampling was done in multiple reaches of Whychus Creek across 12 years but the species accumulation curve is not yet flat, and taxa new to the Whychus dataset were taken in each year since sampling began in 2005 (Figure 1). In addition, the total number of unique taxa and EPT taxa taken in each year among all Whychus sample reaches has increased since 2015. These increases were driven by a combination of recovery from restoration impacts at different sites; creation of new dynamic, heterogeneous side channels; and the consistently greater richness in multihabitat samples, which were taken in a subset of reaches since 2017. Three taxa were taken for the first time in 2020; they occurred in very low abundance, at a small number of sampling sites, and primarily in PM samples:

- *Dicranomyia,* a crane fly that inhabits a variety of flows and habitats including exposed rocks, logs, fine organic sediments and detritus in streams, and muddy or vegetated edges of streams and lakes; one individual in WC0850 PM and three in WC1100-1B PM sample;
- *Protoptila*, a saddlecase-making caddisfly associated with larger, usually somewhat warmer streams and rivers; three individuals in WC0900 RT and one in WC1100-4 PM sample; and
- *Gyraulus*, a tolerant and sediment-tolerant ramshorn snail found in lakes, ponds, marshes, slow portions of streams, and springs on substrates from fine silt to large cobble; one individual in the WC1100-4 PM sample

Invertebrate abundance in 2020 was high; the target sub-sampling number of 500 individuals was attained for all but two samples (WC0900, WC1100-2) with 12-100% of the total sample picked (Table 4). There was no significant difference between the proportion of RT vs. PM samples required for subsampling. Numerical abundance across the dataset was dominated by *Rhithrogena*, a flatheaded mayfly that clings to the tops of stones in cold, fast flows. *Rhithrogena*, which is very sensitive to fine sediment (Relyea et al., 2012) and a DEQ low sediment indicator taxon, was present at all sites except WC2000 and WC2050 at abundances ranging from 1 to 141 individuals. *Cricotopus (Nostocladius)*, a sensitive, sediment-intolerant non-biting midge that burrows into disks of the blue-green alga *Nostoc* in cold fast flows, was taken in all samples except WC1100-1B at abundances ranging from 1 to 148 individuals.

Figure 1. Taxa accumulation in Whychus Creek samples. *Total taxa = # of unique taxa taken among all samples in each year; EPT = number of unique taxa among Ephemeroptera (mayfly), Plecoptera (stonefly), and Trichoptera (caddisfly); new taxa = # of taxa taken for the first time at any Whychus site in each year.*

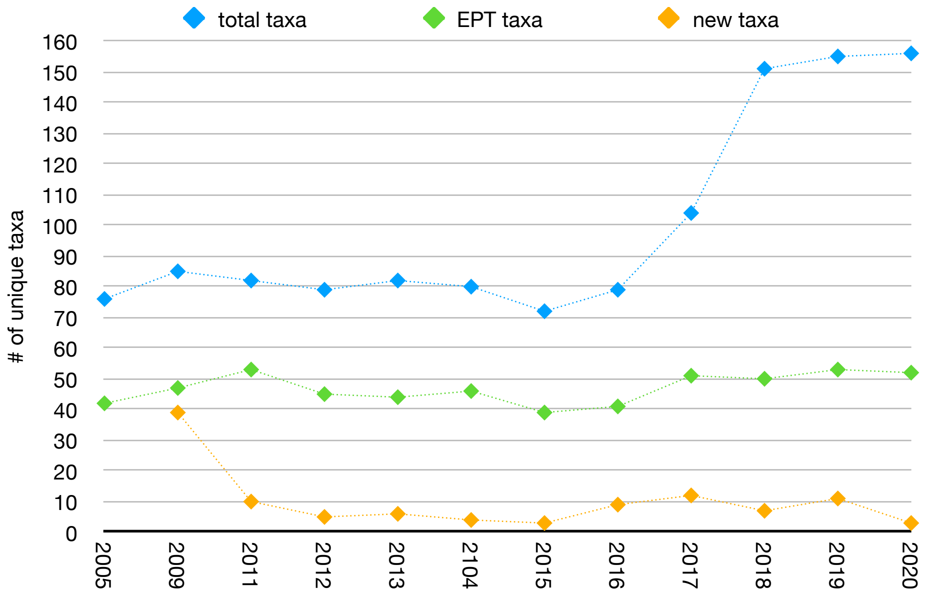


Table 4. Richness, abundance, and evenness in 2020 samples. *Target sub-sampling number is 500. DUP = duplicate sample; RT = riffle-targeted, PM = proportional multihabitat; PC = primary channel, SC = side channel; PC/SC = both channel types in sampling reach. Simpson's Diversity Index (1-D) considers number of species and their abundances; values closer to 1.0 indicate greater diversity.*

Sample	Type	Reach	% of sample picked	Abundance[a] (# individuals)	Richness (# unique taxa)	#EPT taxa	Simpson's Diversity Index (1-D)
WC0600	RT	PC	18.3	546	43	13	0.88
WC0850	PM	PC	33.3	538	55	19	0.93
WC0900	RT	PC	100	261	35	16	0.93
WC1025	PM	PC	31.7	514	57	22	0.94
WC1100-1B	PM	PC/SC	56.7	515	54	19	0.91
WC1100-2	RT	PC	20.0	547	44	21	0.95
WC1100-2	PM	PC	100	282	51	21	0.95
WC1100-4	PM	SC	33.3	550	64	23	0.91
WC1150	PM	PC	73.3	553	56	18	0.94
WC1950	PM	PC	11.7	553	65	24	0.96
WC2000	PM	PC	21.7	535	62	19	0.97
WC2000	TR	PC	15.0	528	48	20	0.93
WC2050	PM	PC	20.0	535	53	21	0.95
WC2050 DUP	PM	PC	26.7	558	62	23	0.96
WC2600-1	RT	PC/SC	83.3	525	50	24	0.87
WC2600	PM	PC/SC	90.0	512	70	23	0.89

The number of unique taxa in each sample ranged from 35 to 70 (mean = 54 ± 9); for reference, in the ORDEQ IBI, >35 taxa receives the highest scaled score. As in past years, richness was significantly greater in PM (59 ± 6 taxa) vs. RT samples (44 ± 6 taxa). Simpson's Diversity Index (1-D), which considers the number of species and their individual abundances, was high for all samples (range = 0.87 to 0.96) and did not differ significantly between RT and PM samples (Table 4), indicating that while PM samples were more diverse, neither sample type was overly dominated by a subset of taxa. This is also reflected by relative abundances of the top (numerically dominant) taxon at each site, which ranged from 7.1% to 28.9% (mean 16.2% ± 7.0) and was not significantly different between PM and RT samples. This is the lowest overall range for this value in any sampling year; for reference, this metric in the ORDEQ IBI receives the highest scaled score at <20% relative abundance.

PM samples might be expected to have fewer sensitive and/or EPT taxa due to the lower representation of riffle habitat, but mean number of EPT (range = 13-24) and sensitive taxa (range = 1-9) was not significantly different between PM and RT samples. Relative abundance of sediment-tolerant organisms (range = 1.7-19.8%) was greater in RT samples and relative abundance of tolerant organisms (range = 9.8-50.3%) was greater in PM samples, but the differences were not significant. Mean community temperature optima (range = 16.3-18.2^{o}C) were the same in RT and PM samples (17.3^{o}C), and the number of both ORDEQ cool (range = 0-8) and warm (range = 1-8) temperature indicator taxa was greater but not significantly in PM samples. Mean community sediment optima (range = 6.5-11.7% FSS) and mean number of DEQ low sediment indicator taxa (range = 3-8) was similar in PM and RT samples, although there were significantly more ORDEQ high sediment indicator taxa (range = 2-9) in PM samples.

As in past years, macroinvertebrate community composition was strongly influenced by reach location (Figure 2), with greater similarity between communities in upstream reaches (RM19.50 through RM2600) compared to those in reaches further downstream, regardless of sample method used (RT or PM). There were also more ORDEQ cool temperature and low sediment indicator taxa and more organisms with a crawler habit in both PM and RT samples in these upstream reaches. The community in some downstream samples varied more, with WC1100-B and WC0900 being least similar to other samples taken from RM6 through RM1100. The paired PM samples taken at WC2050 were most similar to each other (Bray-Curtis Similarity index = 0.75), confirming consistency of sampling technique between different operators.

In a PCA ordination of taxa abundances (Figure 3), axis 1 explained 34% of the total variation among samples; taxa with the highest loading values were *Rhithrogena*, a sediment-sensitive flatheaded mayfly found in cold fast flows that was much less abundant or absent from samples taken at WC1950-WC2050; *Sublettea*, a nonbiting midge found in cold, clear, fast streams that was absent from WC2000-WC2600 samples; and the riffle beetle *Zaitzevia*, an ORDEQ indicator taxon for warm temperatures and high sediment that was absent from WC1950-WC2600 samples. Axis 2 explained an additional 20% of sample variation; taxa with the highest loading values were *Cricotopus (Nostococladius)*, a sensitive, sediment-intolerant nonbiting midge that mines in *Nostoc* algae and was less abundant in samples from WC1100 through WC2050; and the riffle beetles *Ampumixis*, which inhabits sand and gravel in cool, low-sediment, flowing waters and *Optioservus*, which is tolerant and a DEQ indicator taxon for warmer and more sedimented waters. Both of the latter taxa were not present or occurred as a single individual in samples taken at WC2600.

In a PCA ordination of traits measured as relative abundances, axis 1 explained 30% of the total variation between samples (Figure 4); factors with the highest loading values were relative abundances of clingers, which were less abundant in WC2600 samples; and burrowers and multivoltine organisms, both of which were more abundant in WC0600 and WC2600 samples. Axis 2 explained an additional 24% of sample variation; factors with the highest loading were relative abundances of organisms that prefer erosional flows, which were less abundant in WC1950 and WC2000 samples; scrapers, which were less abundant in WC1150-WC2050 samples; and organisms associated with multiple (mixed) flow types, which were more abundant in samples from WC1950-WC2050.

Figure 2. CLUSTER dendrogram of the macroinvertebrate community among all sampling sites in 2020. *Blue = riffle-targeted, aqua = proportional multihabitat. All samples were taken in a primary channel reach except where indicated as SC (side channel) or PC/SC (reach included both primary and secondary channel); DUP = duplicate sample*

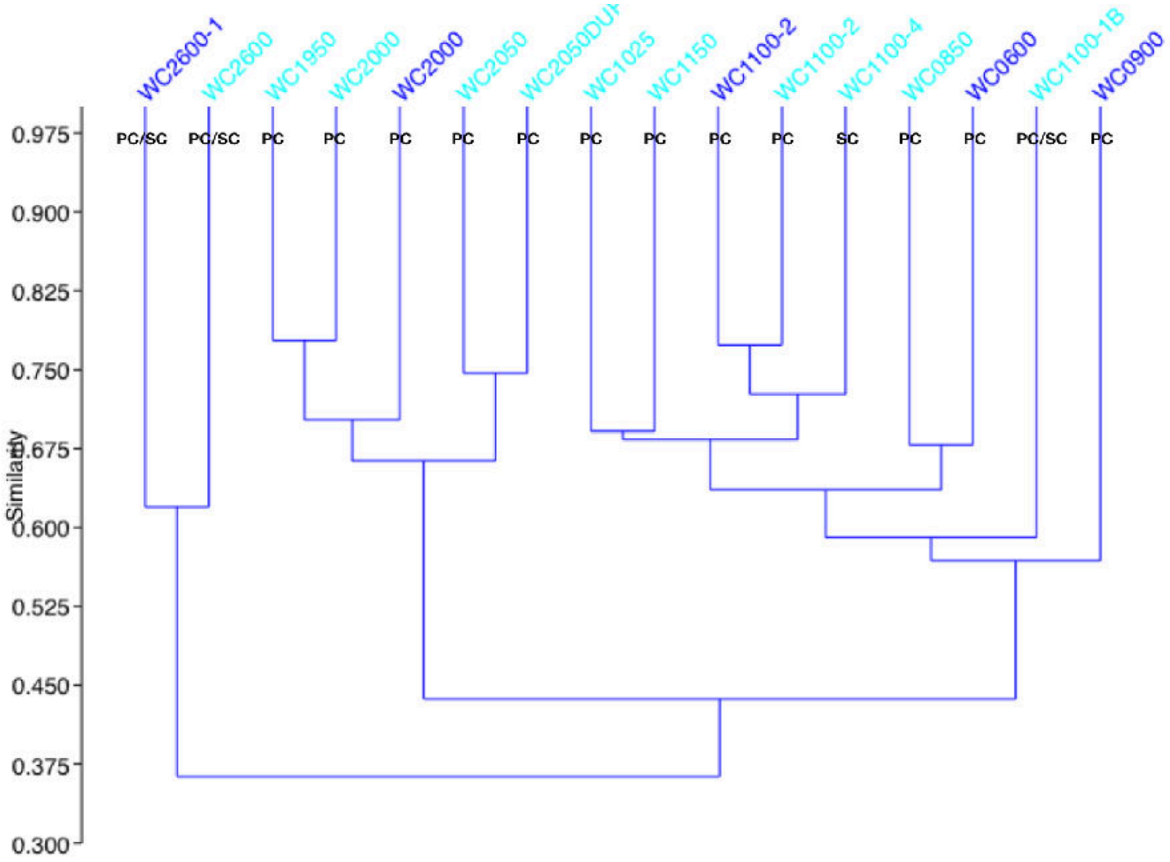


Figure 3. Ordination plot of a Principal Components Analysis (PCA) of taxa abundances among all 2020 samples. *Eigenvectors show dominant taxa contributions, where vector length is related to the strength of the contribution. Blue = riffle-targeted, aqua = proportional multihabitat.*

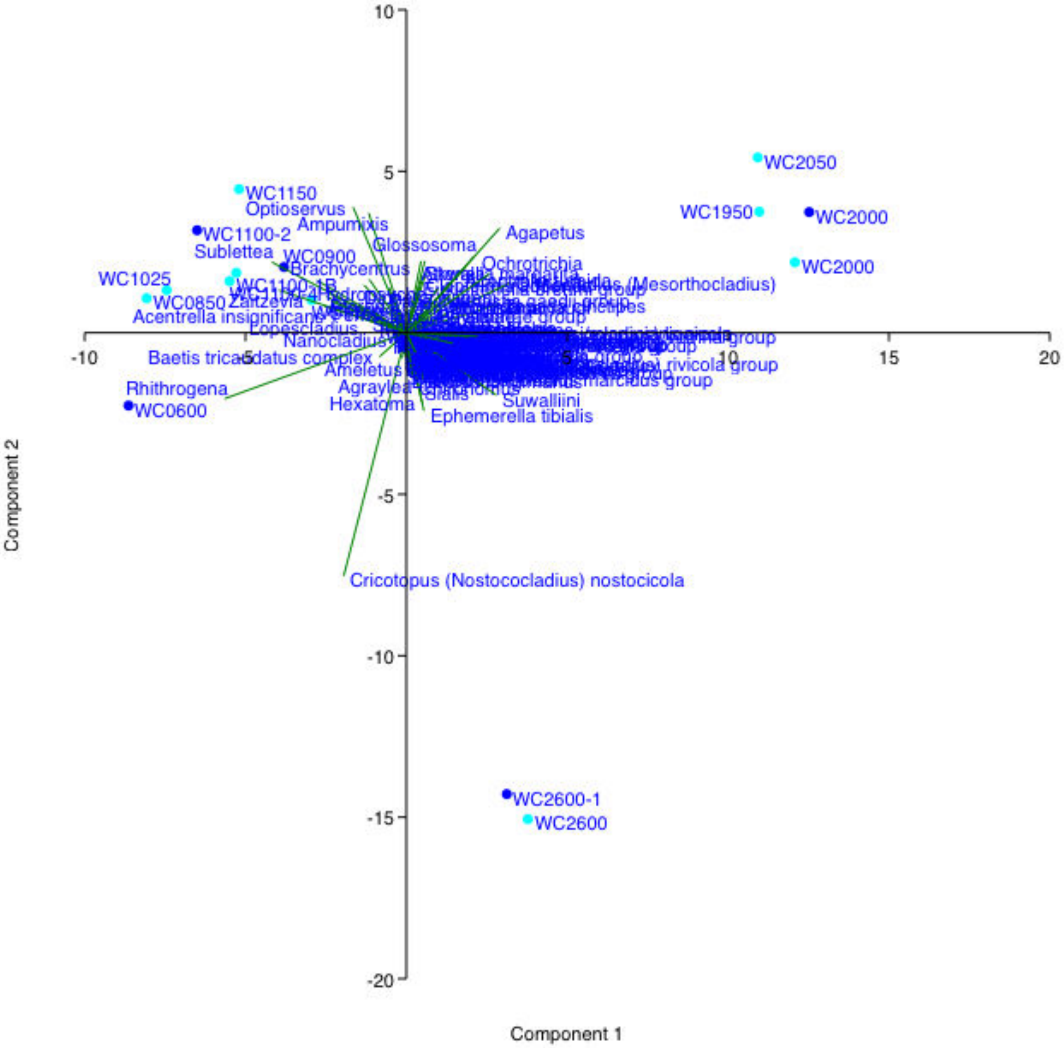
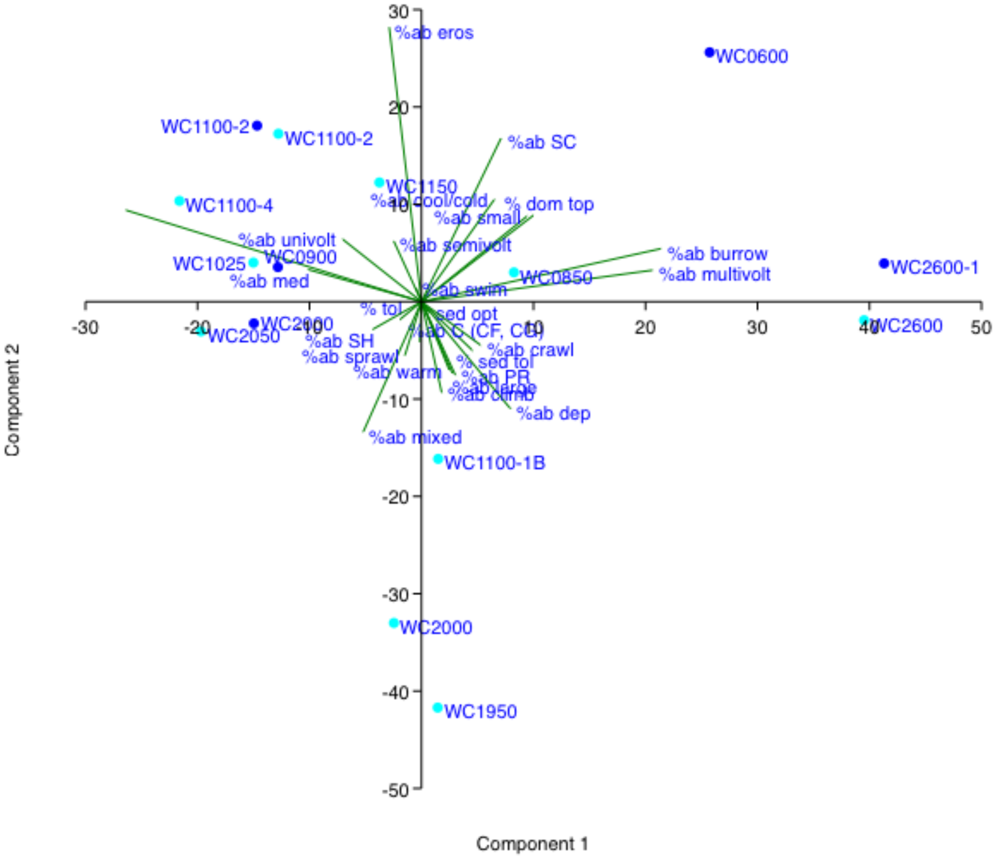


Figure 4. Ordination plot of a Principal Components Analysis (PCA) of traits calculated as relative abundances among all 2020 samples. *Eigenvectors show dominant taxa contributions, where vector length is related to the strength of the contribution. Blue = riffle-targeted, aqua = proportional multihabitat*



Macroinvertebrate community characteristics at sampling sites

<u>WC0600</u>

Riffle-targeted (RT) samples were taken in the primary channel of Whychus Creek at WC0600 in every sampling year since 2005; in 2018 and 2019, a PM sample was also taken in the same reach. Richness in the RT sample (43) was at the lower end of the range for this metric in 2020 (range = 35-70 taxa). The community was dominated by *Cricotopus (Nostococladius),* a sensitive, sediment-intolerant nonbiting midge that mines in *Nostoc* algae disks, at 25.5% of total organismal abundance. The majority of the community consisted of small-bodied, multivoltine taxa that feed as scrapers and collectors and move as clingers and burrowers in cooler, faster flows. All taxa taken in 2020 were found in samples from this site in one or more prior years.

The target sub-sampling number of 500 organisms was attained in nine of 12 sampling years, including every year since 2015 (Figure 5), and in the last three years a much smaller proportion of the sample (18-22%) was picked. Total and EPT richness stabilized across the last three years (Figure 6), with more total taxa compared to earlier sampling years. The number of EPT taxa was intermediate compared to earlier years, and samples from this site have had consistently lower Trichoptera richness, while abundance of *Acentrella* (a small minnow mayfly associated with lotic-erosional habitat) and *Rhithrogena* (a flatheaded mayfly found in cold flowing waters that is an ORDEQ low sediment indicator) increased in more recent years.

Figure 5. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC0600 in all sampling years. *RT samples were taken every year; a PM sample was taken in the same reach in 2018-2019.*

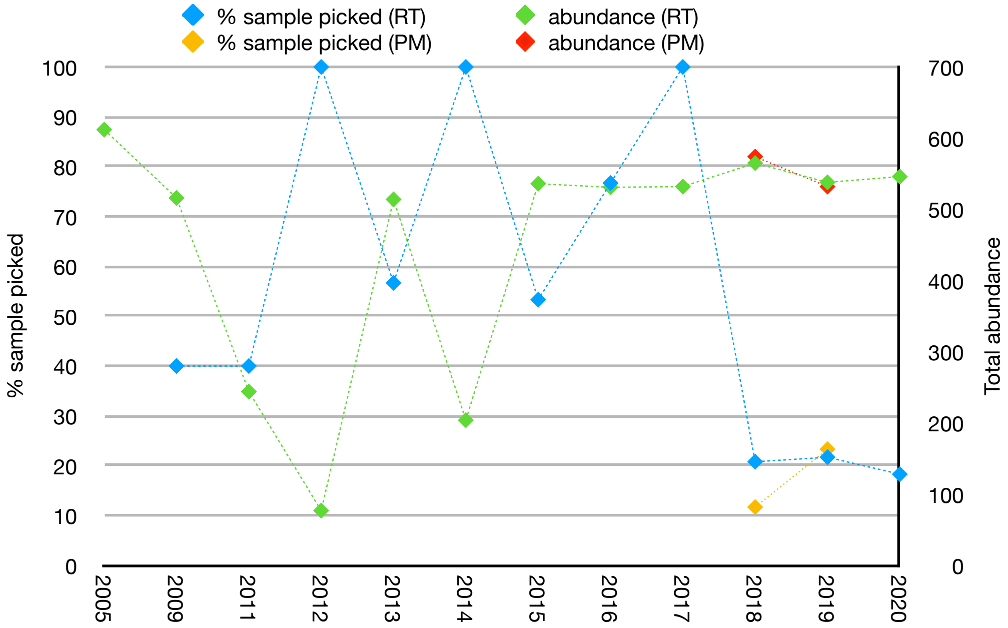
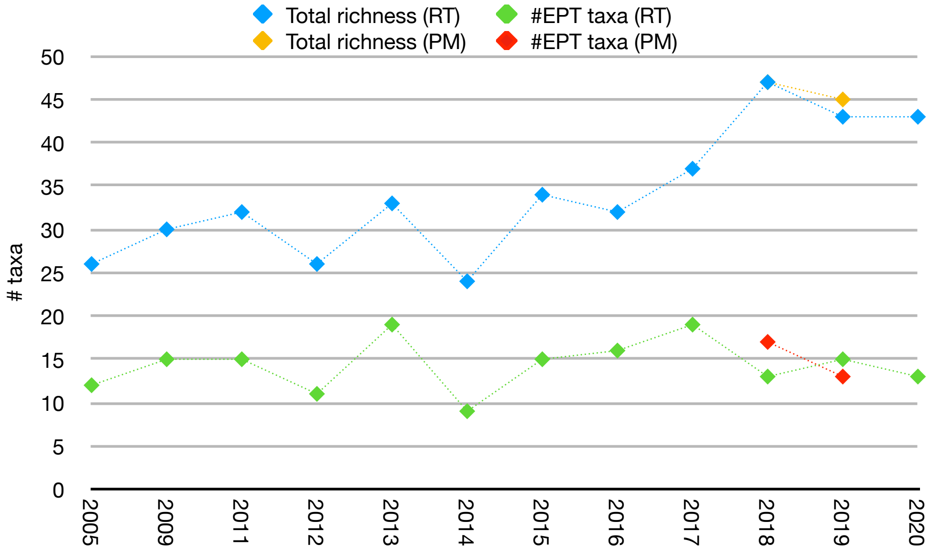


Figure 6. Total richness and number of EPT taxa at WC0600 in all sampling years. *RT samples were taken every year; a PM sample was taken in the same reach in 2018-2019. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



IBI and PREDATOR scores fluctuated but within a more narrow range in recent sampling years (Figure 7). IBI scores rose after 2014 and although they decreased slightly in 2019 and 2020, indicated unimpaired or slightly impaired biological conditions for the last six years. PREDATOR O/E scores indicated fair biological conditions in five of the last six years; O/E scores also decreased in the last two years, with the 2020 sample scoring at the upper end of the poor range. The number of ORDEQ-designated sensitive taxa remained at three in 2020 (Figure 8), which is the most ever taken at this site, although not sufficient to receive the highest scaled score in the IBI. The number of sediment-sensitive taxa has been in the range for the highest IBI score for the last three years, and there were more overall in 2016-2020 (1-3 taxa) than in earlier years (1-2 taxa). Relative abundance of tolerant organisms (Figure 9) was the lowest of any RT sample at this site since 2014. This site has always had low relative abundances of sediment-tolerant organisms and while that metric value was higher in 2020 (6.6%), due primarily to numbers of ramshorn snails and *Antocha* crane flies, it was still within the range of the highest scaled score in the ORDEQ IBI (Figure 9).

Figure 7. PREDATOR O/E and ORDEQ IBI scores at WC0600 in all sampling years.

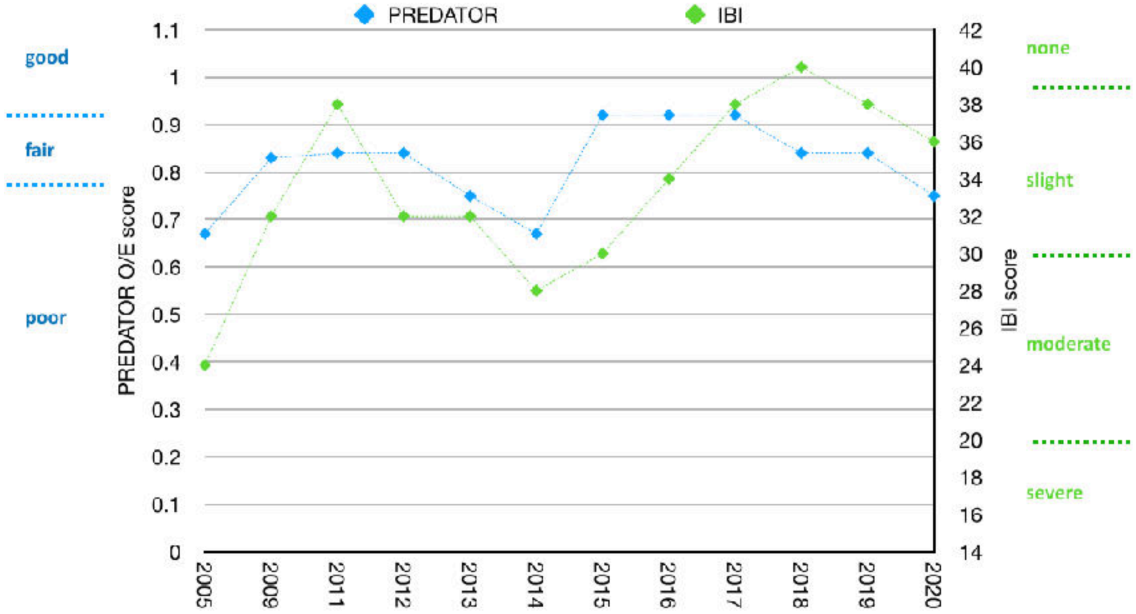


Figure 8. Numbers of sensitive and sediment-sensitive taxa at WC0600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in the same reach as the RT in 2018-2019. In the ORDEQ IBI, the highest scaled score correlates with >4 sensitive and ≥2 sediment-sensitive taxa.*

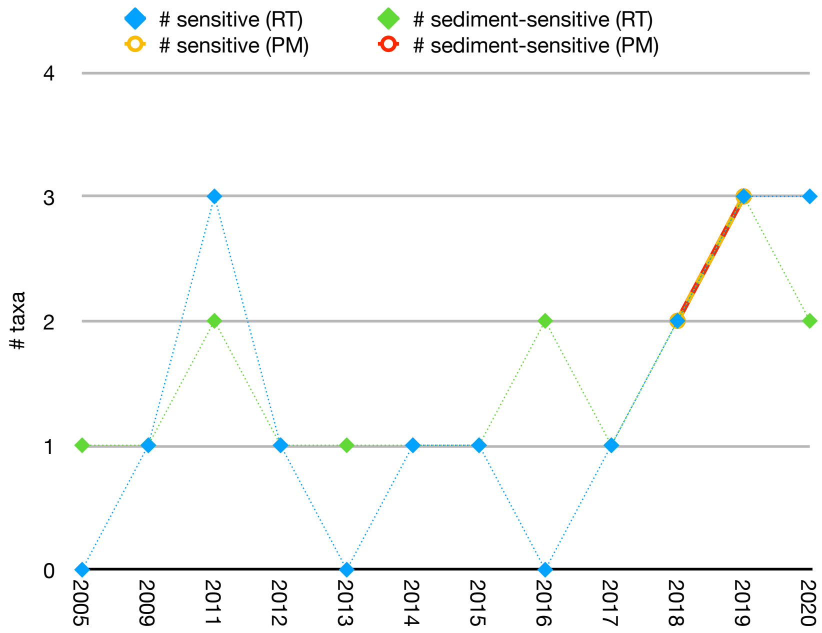
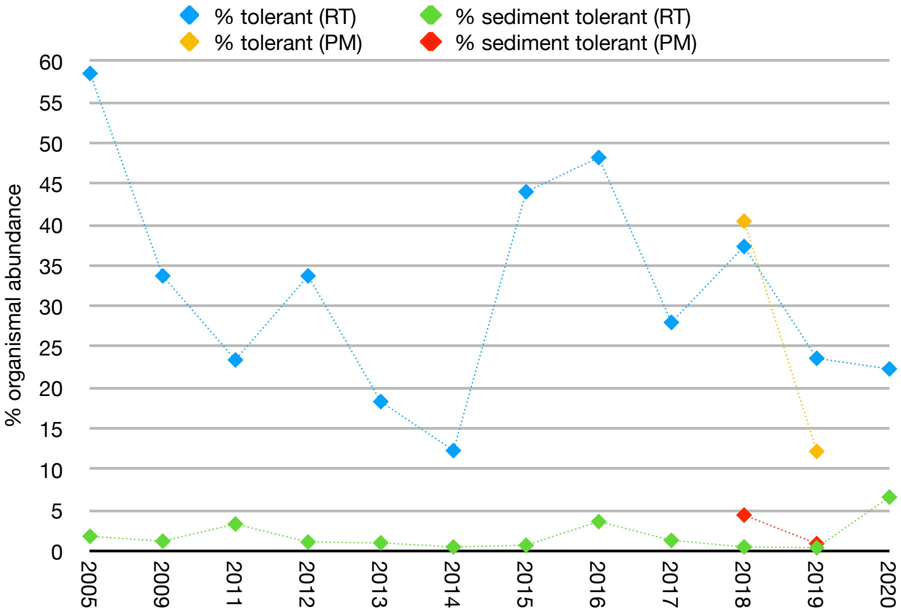


Figure 9. Relative abundance of tolerant and sediment-tolerant organisms at WC0600 in all sampling years. *RT samples were taken every year; a PM sample was taken in the same reach as the RT in 2018-2019. For these metrics in the ORDEQ IBI, the highest scaled scores correlate with <15% tolerant and <10% sediment-tolerant.*



The macroinvertebrate community was less balanced in 2020 and relative abundance of the dominant taxon (25.5%) was above the threshold to receive the highest scaled score in the IBI for the first time in five years (Figure 10). The dominant taxon varies but since 2012 was generally associated with faster flows, including the top taxon in 2020, *Cricotopus (Nostococladius)*, a sensitive, sediment-intolerant nonbiting midge that mines *Nostoc* algae on rocks in cold flowing water. Community temperature optima decreased in recent years (Figure 11), although DEQ warm-water indicator taxa continue to outnumber cool indicators (Figure 12). Community fine sediment optima increased in recent years (Figure 11), although the number of DEQ low sediment indicator taxa has been relatively stable and the number of high sediment indicators decreased in RT samples (Figure 12).

Figure 10. Relative abundance of the numerically dominant taxon at WC0600 in all sampling years. *RT samples (blue) were taken every year; a PM sample (green) was also taken in the same reach as the RT in 2018-2019. For this metric in the ORDEQ IBI, the highest scaled score correlates with <20% abundance of the top taxon.*

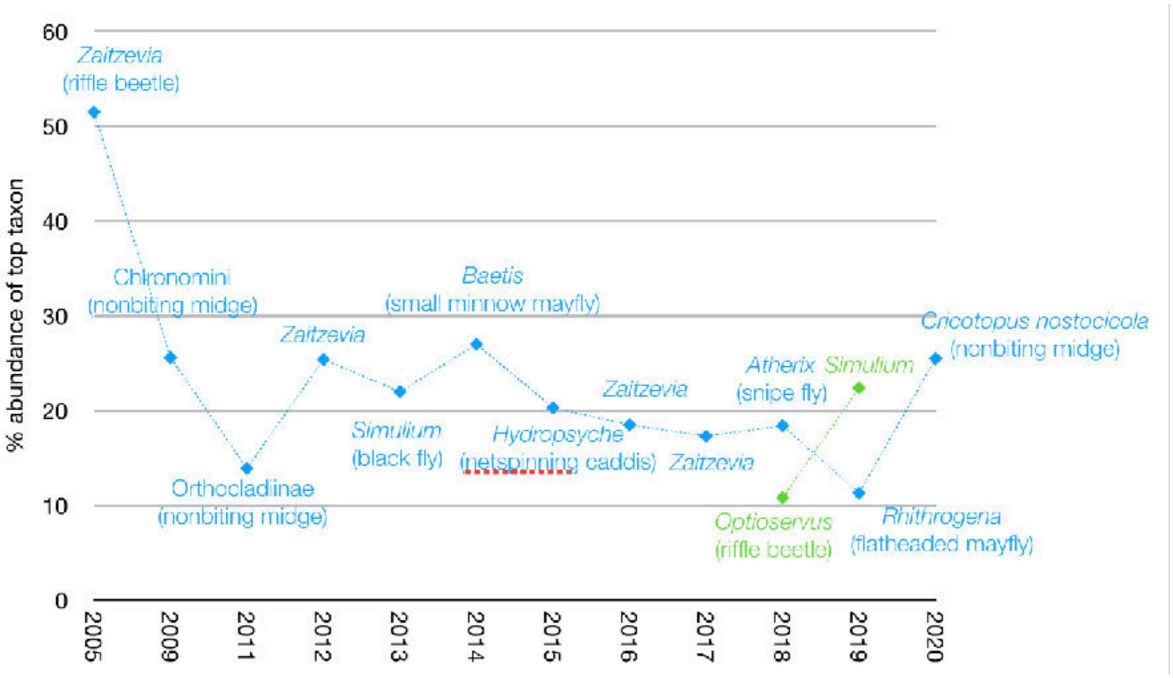


Figure 11. Community temperature and fine sediment optima (weighted means) at WC0600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in the same reach as the RT in 2018-2019.*

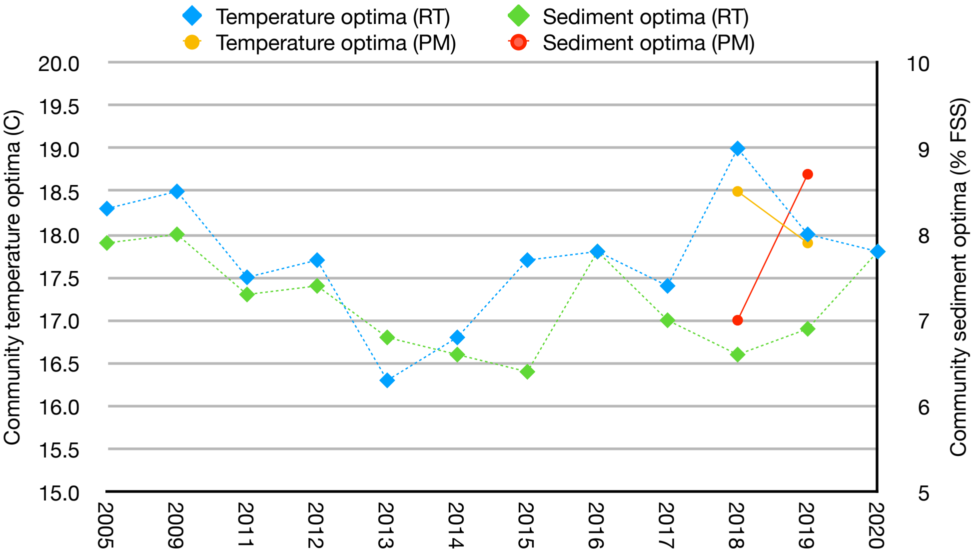
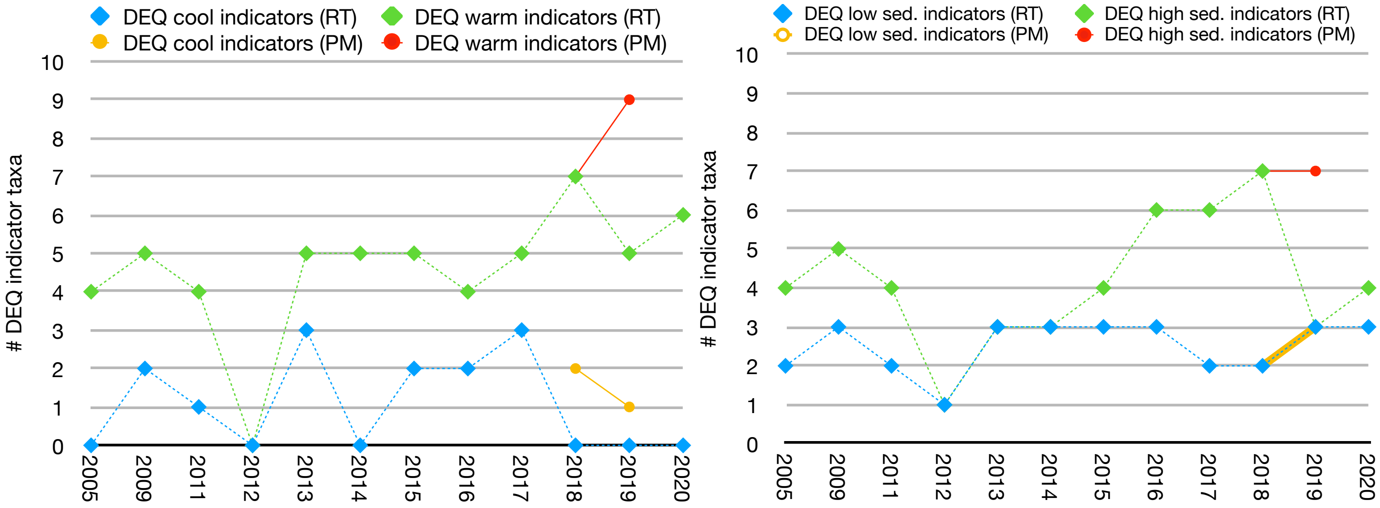


Figure 12. Number of ORDEQ temperature and fine sediment indicator taxa at WC0600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in the same reach as the RT in 2018-2019. Note that ORDEQ taxa do not account for the temperature associations of all taxa in a sample.*



The 2020 sample community was most similar to those in more recent years (2018-2019), and that grouping was more closely related to the 2016-2017 sample communities (Figure 13). In a PCA ordination of taxa abundances (Figure 14), axis 1 explained 25% of total sample variation; taxa with the highest loading values were *Rhithrogena* (a sediment-sensitive flatheaded mayfly found in colder faster water), *Acentrella insignificans* (small minnow mayfly that is slightly sensitive to fine sediment and inhabits faster water), and *Optioservus* (a tolerant riffle beetle that is an ORDEQ warm temperature indicator but found in a variety of flows and temperatures), which were all more abundant in samples from 2016-2020. Axis 2 explained an additional 18% of total variation; taxa with the highest loading values were *Zaitzevia,* a tolerant riffle beetle in faster flows that is an ORDEQ warm water and high sediment indicator (much more abundant in 2005 and 2009 samples); Chironomini, a tolerant tribe of nonbiting midge in slower waters that is an ORDEQ warm temperature and high sediment indicator (very abundant in 2019 PM sample); and *Baetis tricaudatus*, a small minnow mayfly that prefers faster flows (present consistently but greatest abundances in 2013-2015).

In a PCA ordination of traits measured as relative abundances in all years (Figure 15), axis 1 explained 43% of total sample variation; traits with the highest loading values were relative abundance of organisms associated with cooler water, which have fluctuated annually and are also generally lower in PM samples; scrapers, which were more abundant in 2005, 2016, and 2020; and multivoltine organisms, which were more abundant in 2018-2020. Axis 2 explained an additional 18% of variation; traits with the highest loading values were relative abundances of organisms that are small-bodied (less abundant in 2016-2018 sample), medium-bodied (more abundant in 2016-2020 samples), and tolerant (higher abundances in 2005 and 2015-2016).

Figure 13. CLUSTER dendrogram of the WC0600 macroinvertebrate community in all sampling years. *Blue = RT, aqua = PM. The number at the end of each label indicates sampling year.*

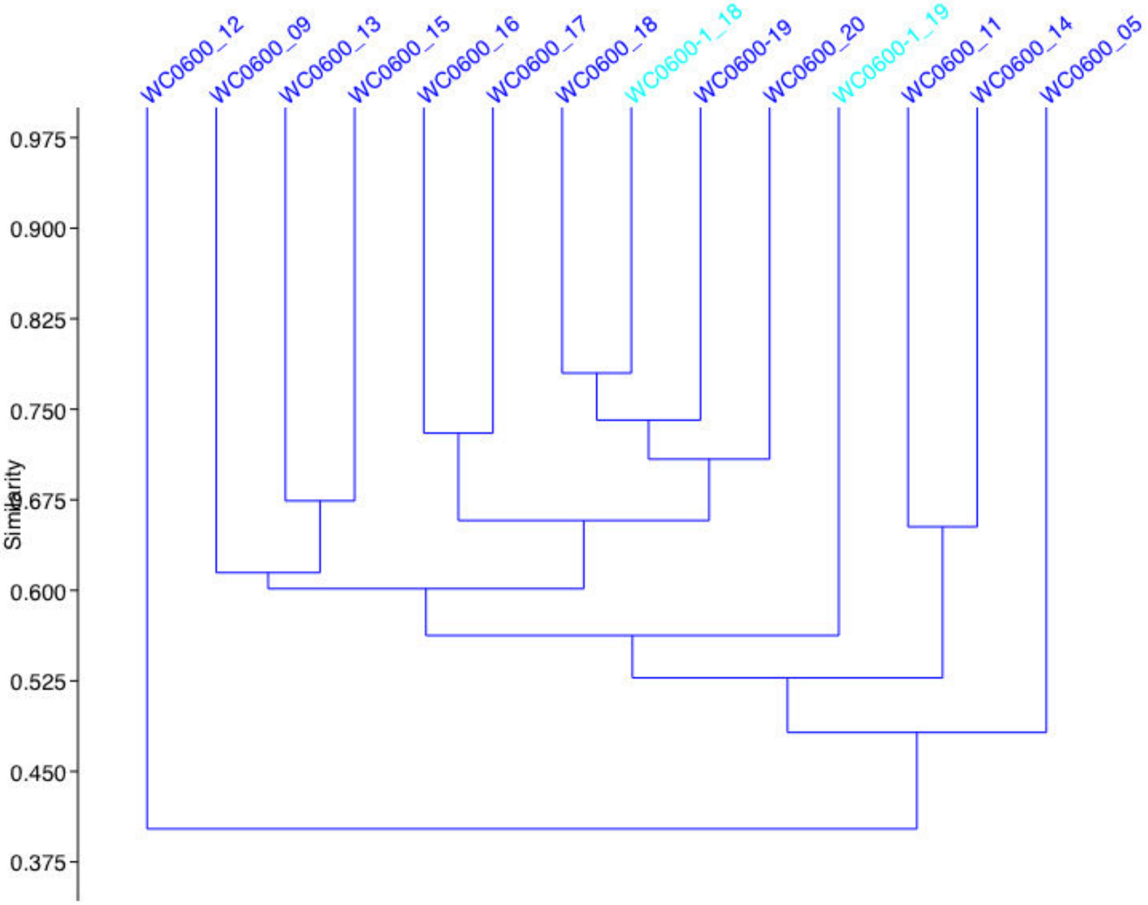


Figure 14. PCA ordination of the WC0600 macroinvertebrate community in all sampling years. *Blue = RT, aqua = PM. The number at the end of each label indicates sampling year.*

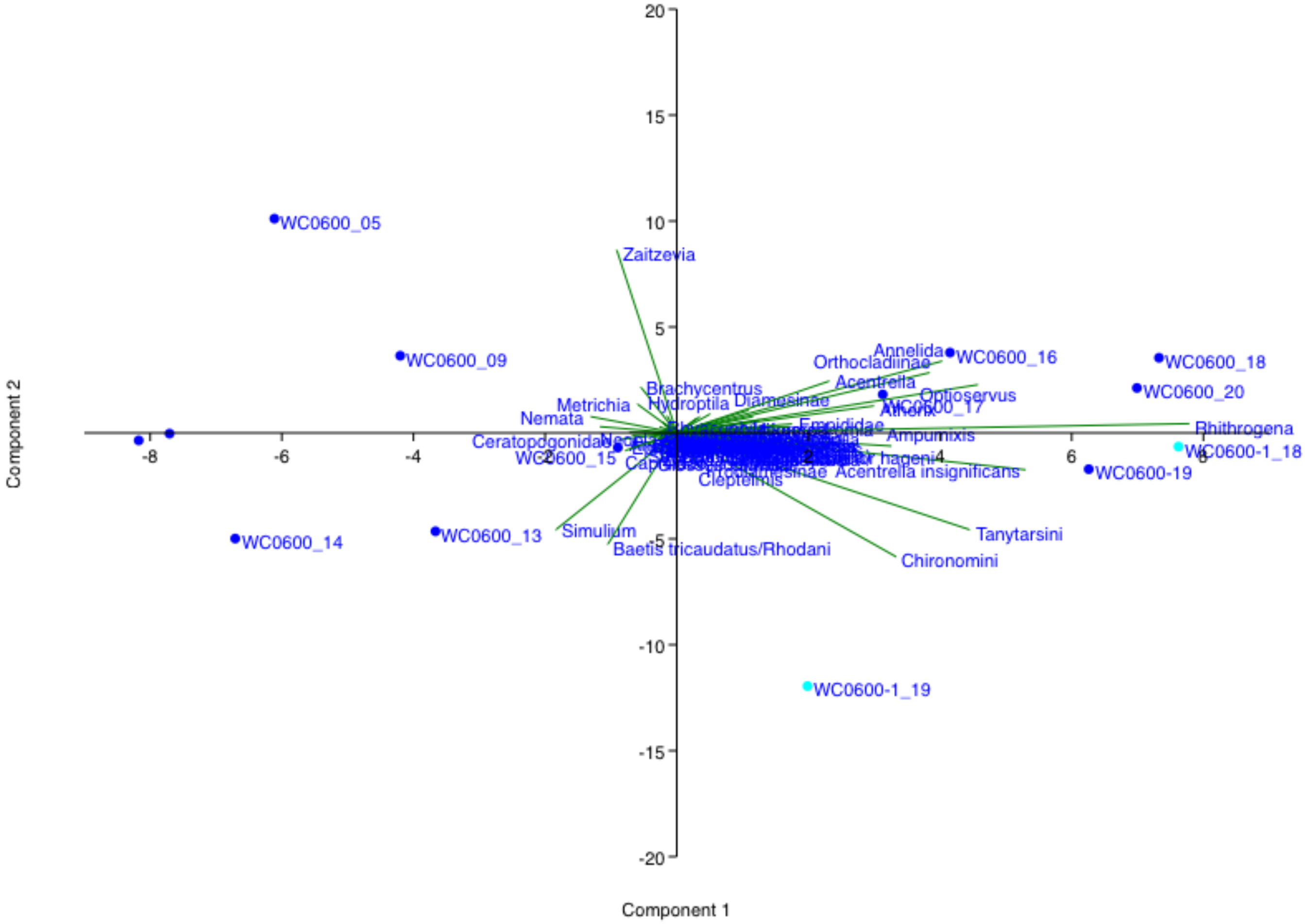
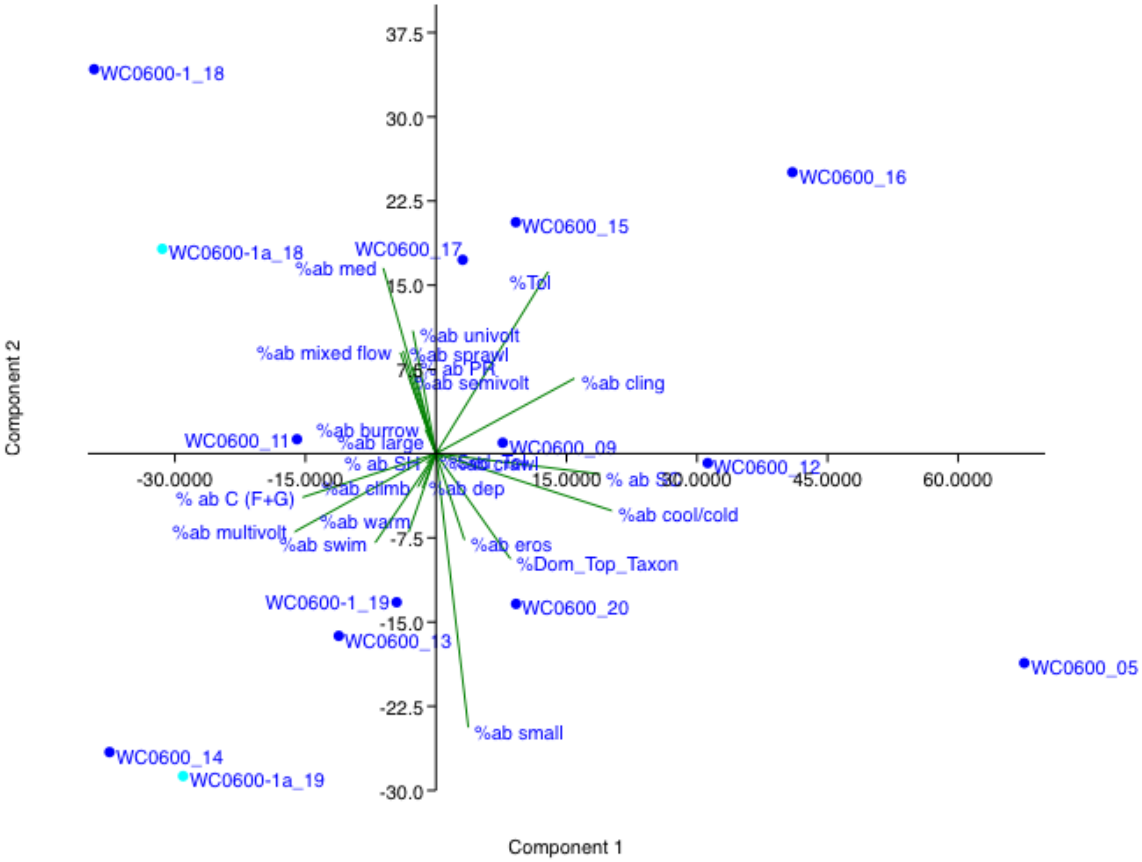


Figure 15. PCA ordination of WC0600 macroinvertebrate community traits measured as relative abundances in all sampling years. *Blue = RT, aqua = PM. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*

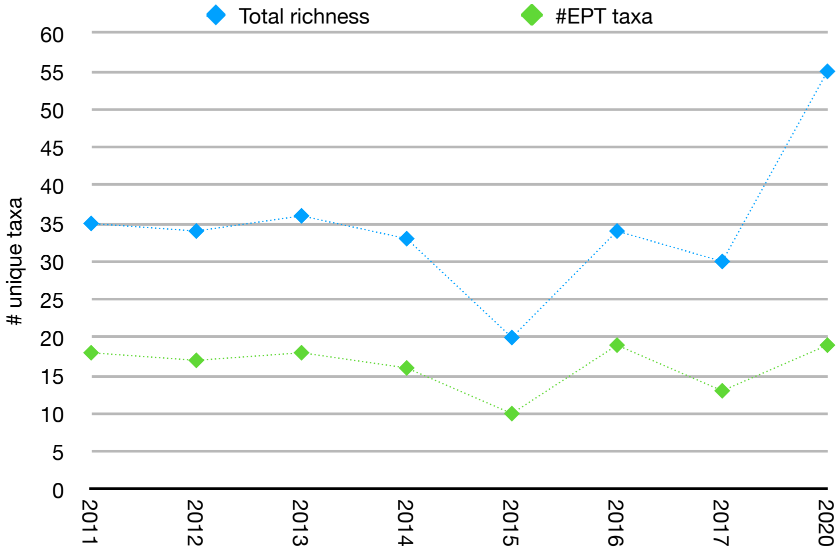


<u>WC0850</u>

RT samples were taken in the primary channel of Whychus Creek around RM 8.5 from 2011-2017; a PM sample was taken in 2020 to provide additional baseline data for upcoming restoration. The 2020 sample community was most similar to the WC0600 sample (Bray-Curtis similarity index = 0.68) and was composed mainly of small, multivoltine clingers that prefer faster-flowing, cool/cold waters and feed by scraping and collecting. This is similar to community characteristics in past years. Sample richness was intermediate for the 2020 dataset (55 unique taxa) but the highest of any sampling year at this site (Figure 16), due at least in part to the fact that PM samples consistently have more taxa than RT samples taken in the same reach. The total number of EPT taxa (19) was also the highest seen at this site, although the 2016 sample had 19 EPT as well; abundance of non-biting midges, especially in the Chironominae and Orthocladiinae subfamilies, also greatly increased over prior years.

Eight taxa in the 2020 sample were not seen at this site in any earlier year. They occurred at low abundances (1-6 individuals) and included Blephariceridae, a sensitive net-winged midge family that clings to smooth stones in cold fast flows; Tanypodinae, a subfamily of non-biting midges associated with mosses in cold springs, brooks, and streams; *Dicranomyia*, a crane fly that inhabits rocks, logs, and sediments at the margins of flowing and still waters; *Acentrella insignificans*, a small minnow mayfly that is slightly sensitive to fine sediment and prefers flowing water; *Ochrotrichia*, a tolerant micro-caddisfly that feeds on algae and fine organic detritus in a variety of flow types in cold springs, lakes, rivers, and temporary streams; *Rhyacophila arnaudi*, a free-living caddisfly associated with open, cold, fast streams; Pisidiidae, a sediment-tolerant pea clam family found in a wide range of flows and habitats; and *Ferrissia*, a tolerant and sediment-tolerant limpet found in warmer waters in a variety of flows. Except for *Dicranomyia,* which appeared in the Whychus dataset for the first time in 2020, the remaining taxa were taken in other sites/years.

Figure 16. Sample richness and number of EPT taxa at WC0850 in all sampling years. *A PM sample was taken in 2020; RT samples were taken in all other years. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



The target sub-sampling number of 500 organisms was attained in five of the eight sampling years (Figure 17), with 33-50% of the sample picked. The 2020 sample required the least total sample to be picked and was dominated by *Sublettea*, a nonbiting midge in the Tanytarsini tribe that inhabits cold, clear, fast streams, at only 12.6% of total organismal abundance, the lowest of any sampling year. WC0850 samples have been dominated consistently by taxa that prefer faster flows (Figure 18), especially *Baetis tricaudatus* (dominant taxon in five sampling years), and in recent years at relative abundances within or close to the value that receives the highest ORDEQ IBI score.

IBI scores indicated slight or no impairment in six of the seven years when RT samples were taken. PREDATOR scores were consistently lower, reflecting poor biological conditions in all but two years of RT sampling. These models cannot be applied to PM samples but for reference, values for six of the IBI metrics in the 2020 sample correlated with the highest scaled score: taxa richness (Figure 16); relative abundance of the dominant taxon (Figure 18); number of sensitive and sediment-intolerant taxa (Figure 19); and relative abundance of tolerant and sediment-tolerant organisms (Figure 20). The number of DEQ warm temperature indicator taxa exceeded the number of cool indicators in most sampling years including 2020, which had the most warm indicator taxa of any year at this site.

Figure 17. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC0850 in all sampling years. *A PM sample was taken in 2020; RT samples were taken in all other years.*

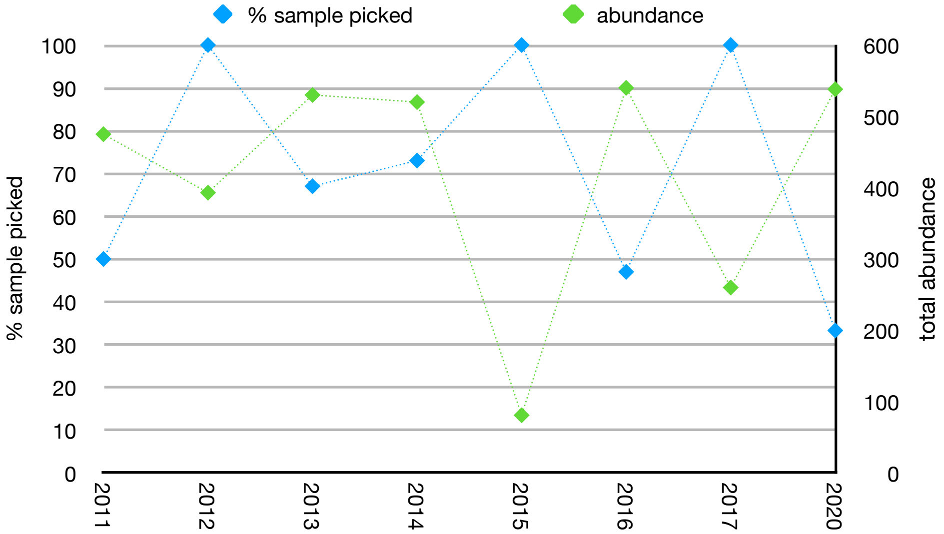


Figure 18. Relative abundance of the numerically dominant taxon at WC0850 in all sampling years. *A PM sample was taken in 2020; RT samples were taken in all other years. For reference, this metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*

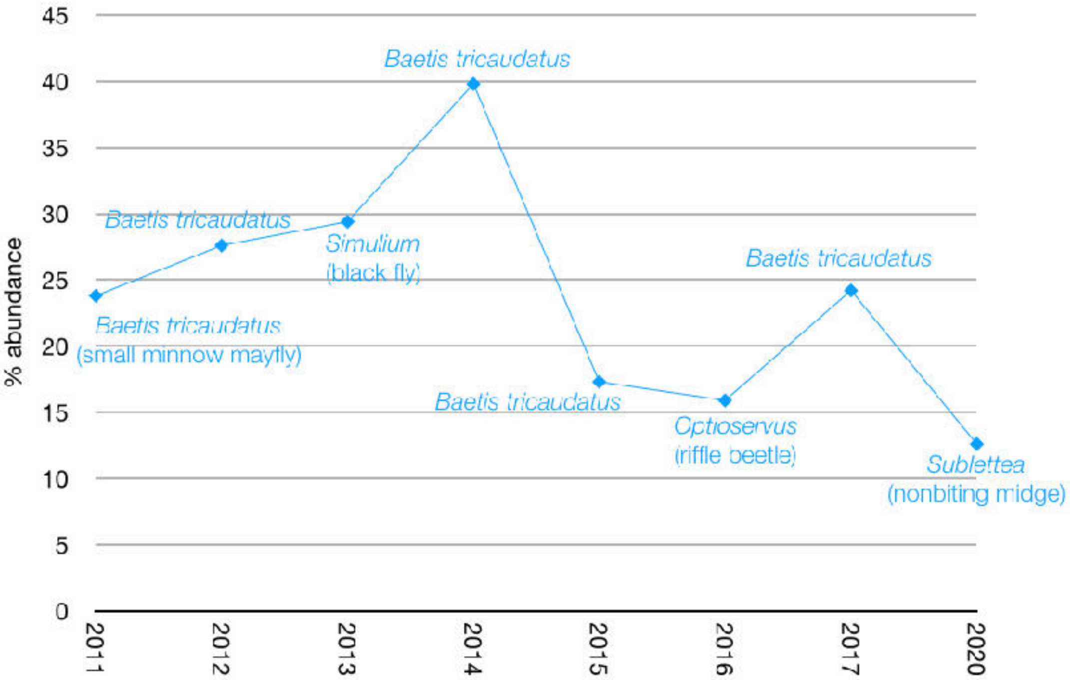


Figure 19. Numbers of sensitive and sediment-sensitive taxa at WC0850 in all sampling years. *A PM sample was taken in 2020; RT samples were taken in all other years. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

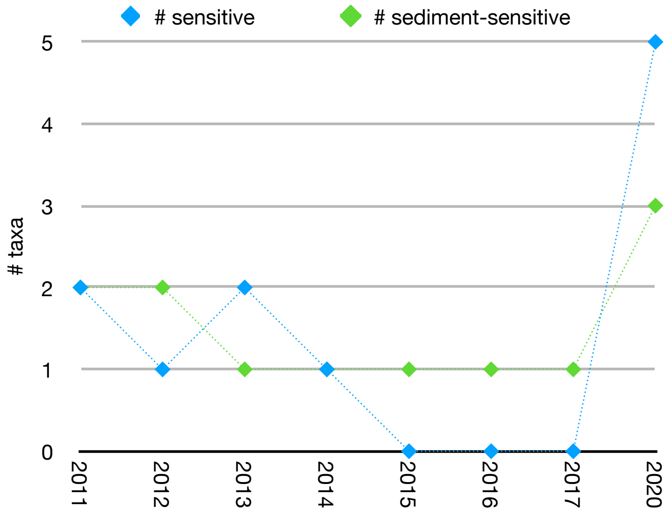
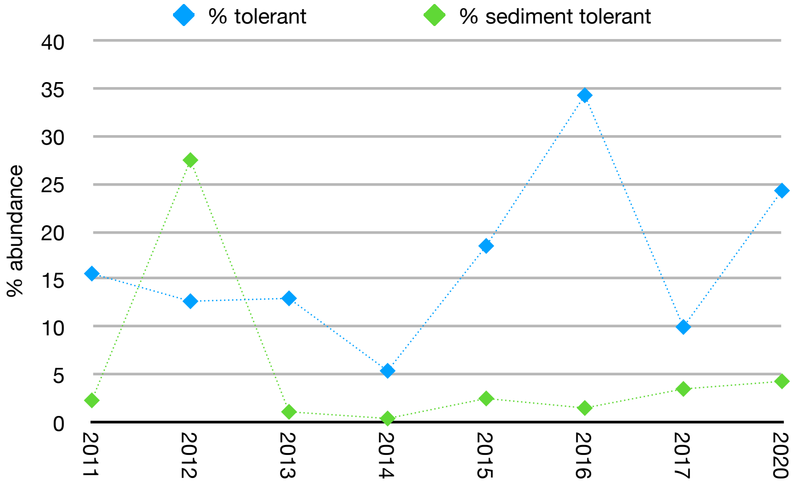


Figure 20. Relative abundance of tolerant and sediment-tolerant organisms at WC0850 in all sampling years. *A PM sample was taken in 2020; RT samples were taken in all other years. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*



The number of ORDEQ low sediment indicator taxa was moderate and fairly consistent over time (range = 2-4), but there were more high sediment indicator taxa in 2020 than any prior year (Figure 21). The number of both warm and high sediment indicator taxa (8 of each) was also at the upper end of the range seen among all 2020 samples (range = 2-9 high sediment and 1-8 warm temperature indicator taxa in 2020). The community temperature and sediment optima, which both decreased at this site from 2013-2015, were also highest in the 2020 sample (Figure 22).

Figure 21. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC0850 in all sampling years. *A PM sample was taken in 2020; RT samples were taken in all other years. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

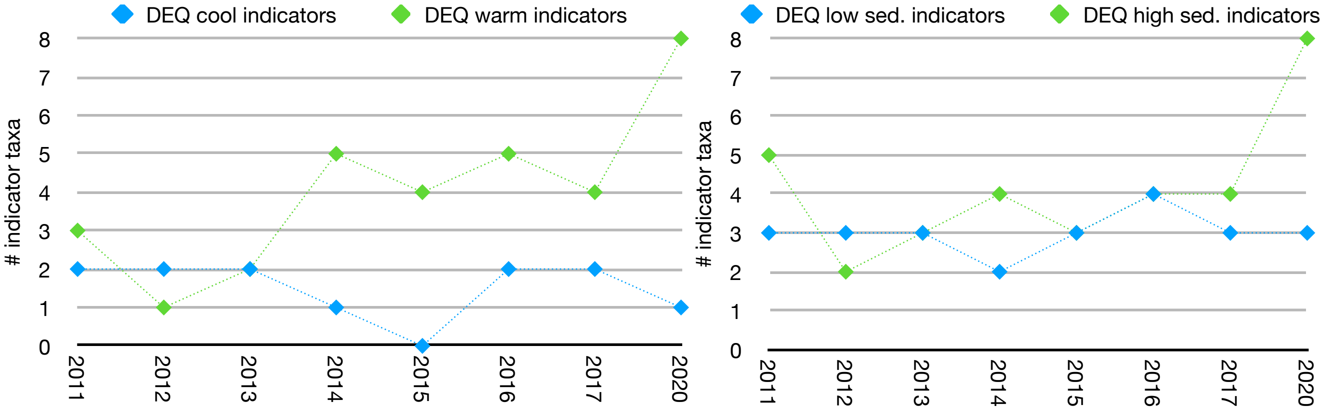
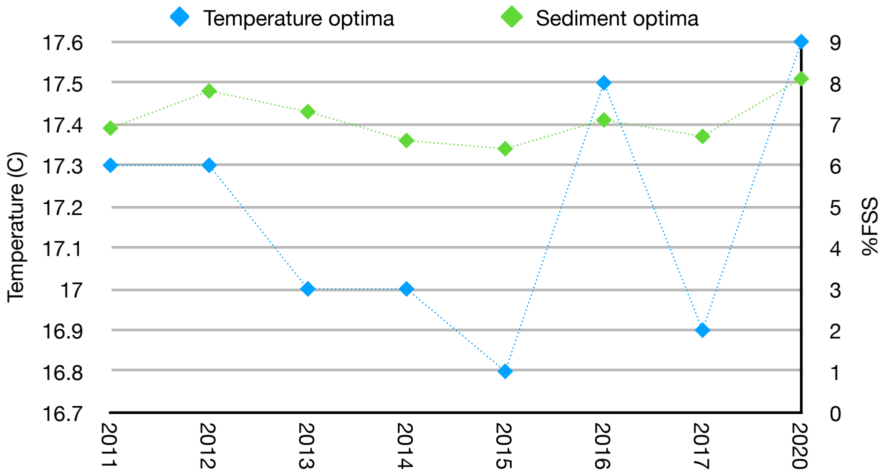


Figure 22. Community temperature and fine sediment optima (weighted means) at WC0850 in all sampling years. *A PM sample was taken in 2020; RT samples were taken in all other years. Note that optima are not known for every taxon in a sample.*



The 2020 sample community was most similar to the 2016 community (Bray-Curtis similarity index = 0.65), and these clustered separately from all other years (Figure 23). In a PCA ordination of taxa abundances, axis 1 explained 40% of total variation between years; taxa with the highest loading values were *Simulium*, *Baetis tricaudatus* (both less abundant in 2016 and 2020) and *Optioservus* (more abundant in 2016 and 2020). Axis 2 explained an additional 21% of total variation; taxa with the highest loading values were *Simulium*, *Brachycentrus* (higher abundance in earlier years), and the Tanytarsini tribe of nonbiting midges (most abundant in 2020). In a PCA ordination of traits measured as relative abundances in all years (Figure 24), axis 1 explained 52% of total variation between samples; factors with the highest loading were relative abundance of collectors (lowest in 2016 and 2020 samples), scrapers (highest in 2016 and 2020), and small-bodied organisms (greatest in 2013 and 2014).

Figure 23. CLUSTER dendrogram of the WC0850 macroinvertebrate community in all sampling years. *Blue = RT, aqua = PM. The number at the end of each label indicates sampling year.*

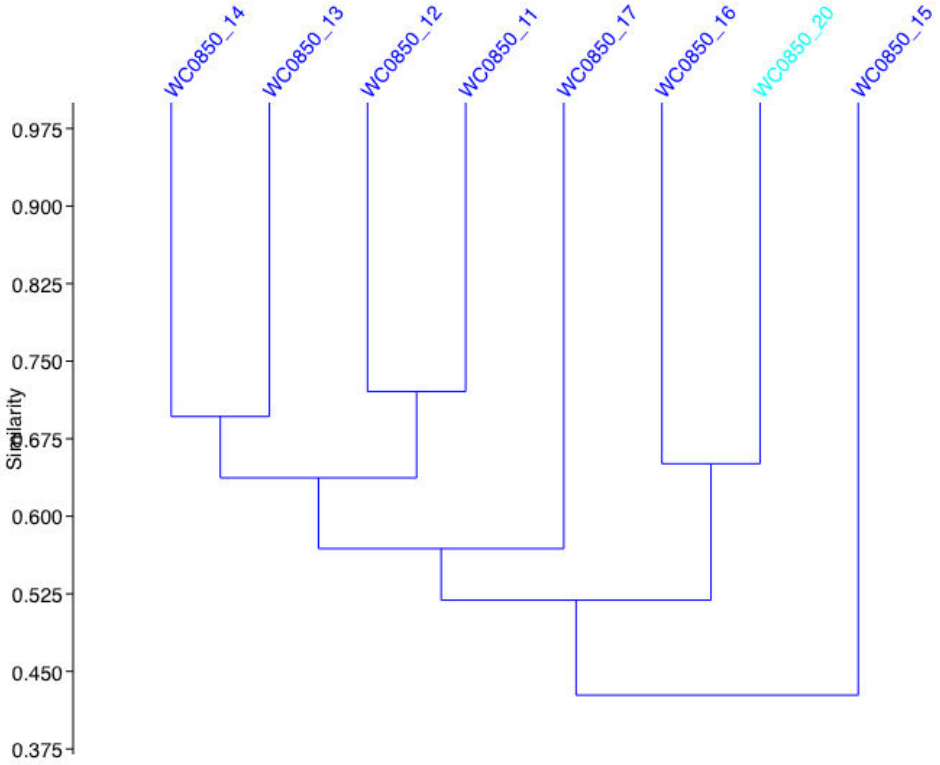


Figure 24. PCA ordination of the WC0850 macroinvertebrate community in all sampling years. *Blue = RT, aqua = PM. The number at the end of each label indicates the sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*

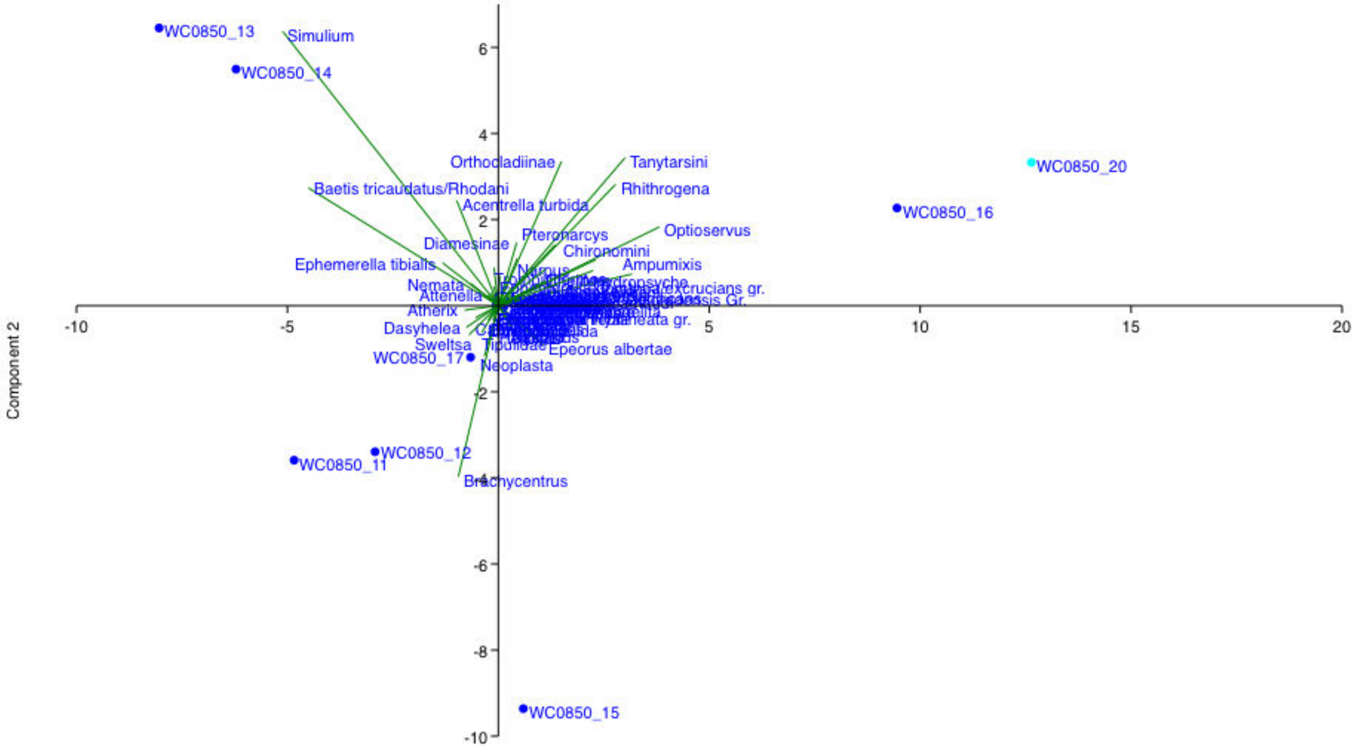
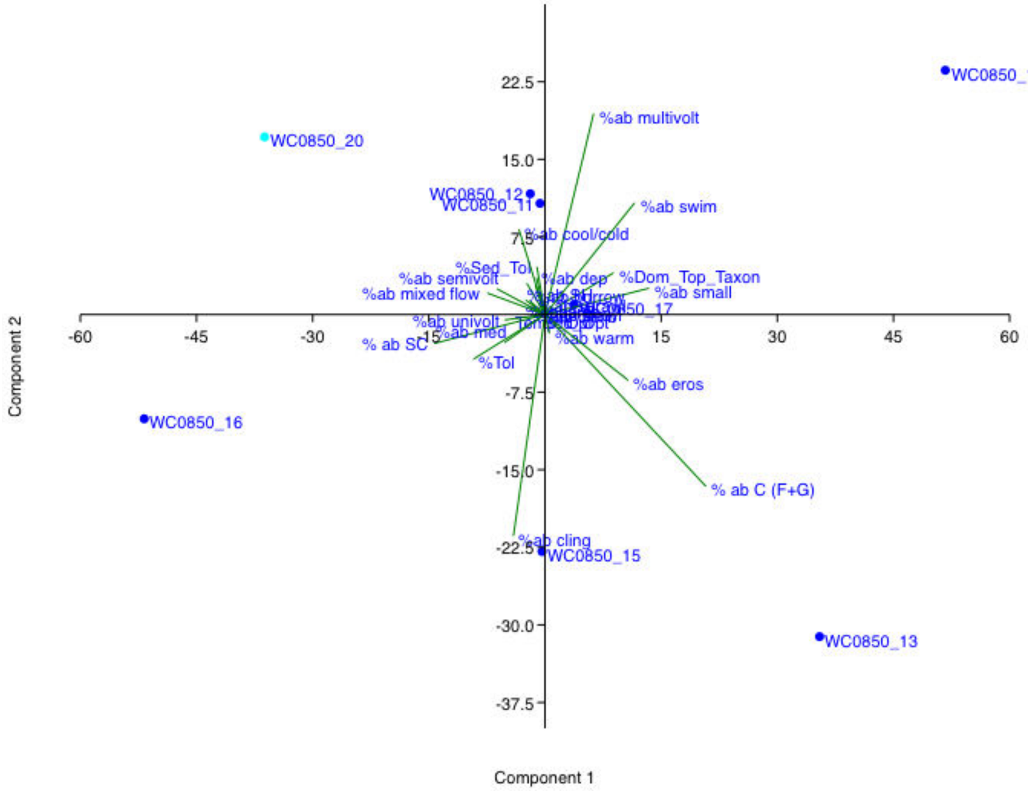


Figure 25. PCA ordination of the WC0850 macroinvertebrate community traits measured as relative abundances in all sampling years. *Blue = RT, aqua = PM. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



WC0900

RT samples were taken in the primary channel of Whychus Creek at WC0900 in 2005, 2009, 2011-2017, and again in 2020 to provide additional baseline data for upcoming restoration work. The 2020 sample had the lowest richness (35 unique taxa) of all 2020 samples, but was at the upper end of the range for this site (Figure 26). The number of EPT taxa (16) was also at the lower compared to all 2020 samples; this metric fluctuates but decreased slightly at this site overall (Figure 26). The 2020 community was dominated by the tolerant, sediment-tolerant ramshorn snail *Ferrissia,* and relative abundance of the top taxon (13%) was the lowest of any year here. The community was composed mainly of small-bodied, multivoltine and univoltine clingers associated with a variety of flow types and temperatures that feed by scraping. Community traits in earlier sampling years were similar but with even more small-bodied organisms and collectors, fewer organisms associated with warm temperatures, and fewer climbers. Only two taxa in the 2020 sample were not seen here in any prior sampling year: *Protoptila*, a somewhat sediment-sensitive saddlecase-maker caddisfly usually found in larger, warmer streams (3 individuals) and *Ferrissia*, a tolerant and sediment-tolerant limpet found in warmer waters in a variety of flows (34 individuals). *Ferrissia* was taken at other sites/years but this is the first year in which *Protoptila* appeared in the Whychus dataset.

The target sub-sampling number of 500 organisms was attained in six of 10 sampling years (Figure 27), and the 2020 sample had fewer organisms (261) than any other sample in 2020. This site has been dominated by different taxa (Figure 28); most are associated with faster or mixed flows, but they have also tended to be either more tolerant and associated with warmer, more sedimented conditions (i.e., *Zaitzevia* and *Optioservus* riffle beetles, Oligochaeta worms, nonbiting midge subfamily Chironominae, ramshorn snail *Ferrissia*) or known as re-colonizers following disturbance (*Baetis tricaudatus* mayfly, *Simulium* black fly). However, since 2015, relative abundance of the dominant taxon has been low enough (<20%) to receive the highest score in the IBI, and was lower in 2020 than in any prior year.

Figure 26. Sample richness and number of EPT taxa at WC0900 in all sampling years. *RT samples were taken in all years. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*

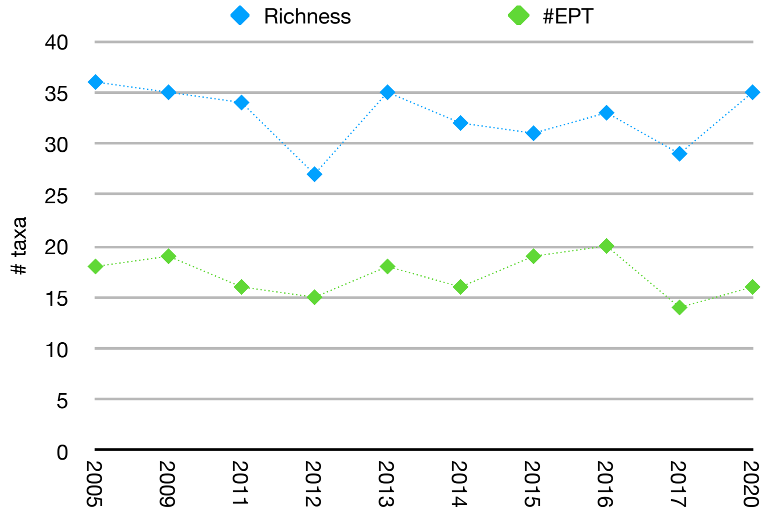


Figure 27. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC0900 in all sampling years. *RT samples were taken in all years.*

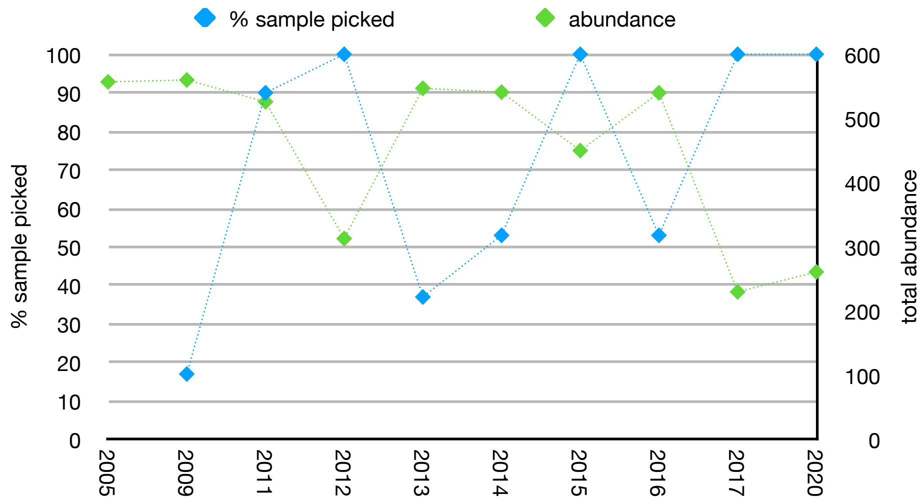
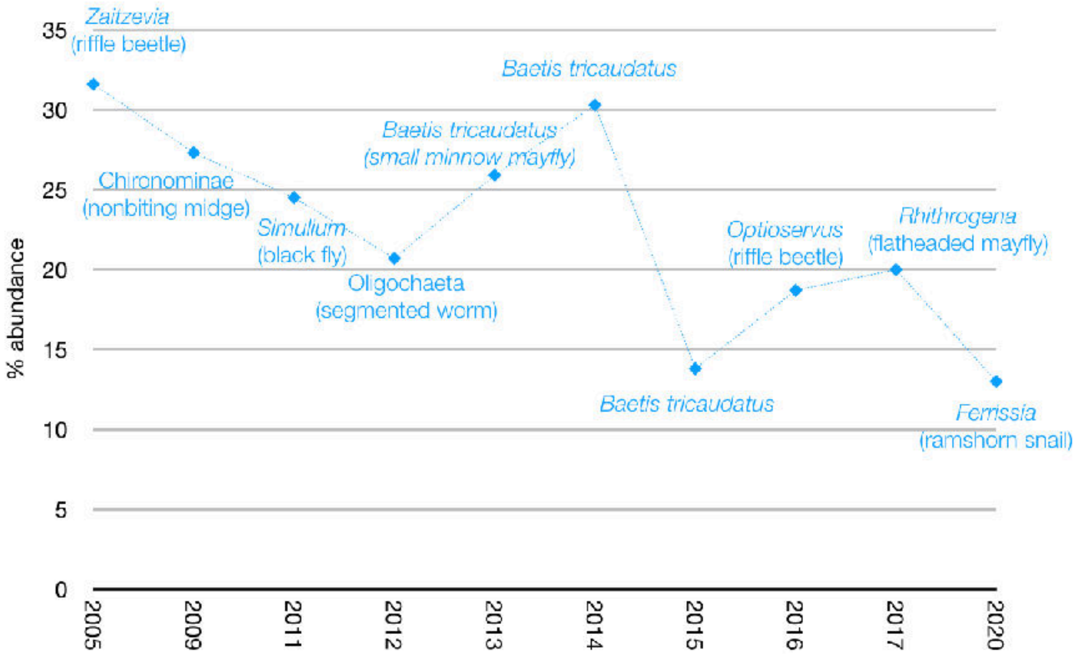


Figure 28. Relative abundance of the numerically dominant taxon at WC0900 in all sampling years. *RT samples were taken in all years. This metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*



IBI scores indicated slight or no impairment in every sampling year, while PREDATOR O/E scores corresponded to poor or fair conditions (Figure 29). Sensitive and sediment-sensitive taxa numbers are consistently low (Figure 30), although samples contained enough sediment-sensitive taxa to receive the highest scaled IBI score in several years, including 2020. Relative abundance of tolerant organisms decreased through the first two sampling years (Figure 31), but was only low enough to receive the highest scaled IBI score in two sampling years, while the proportion of sediment-tolerant organisms was low enough to receive the highest IBI score in all years except 2012 and 2020. There were more ORDEQ warm temperature indicator taxa than cool indicators in every sampling year; the number of ORDEQ high sediment indicator taxa exceeded low sediment indicators in four of the seven earliest sampling years (Figure 32), but their numbers have been equal (3 taxa) in the last three sampling years. Community temperature optima varied more across time than sediment optima (Figure 33), but both were higher in 2020 than in any other sampling year except 2009.

Figure 29. PREDATOR O/E and ORDEQ IBI scores at WC0900.

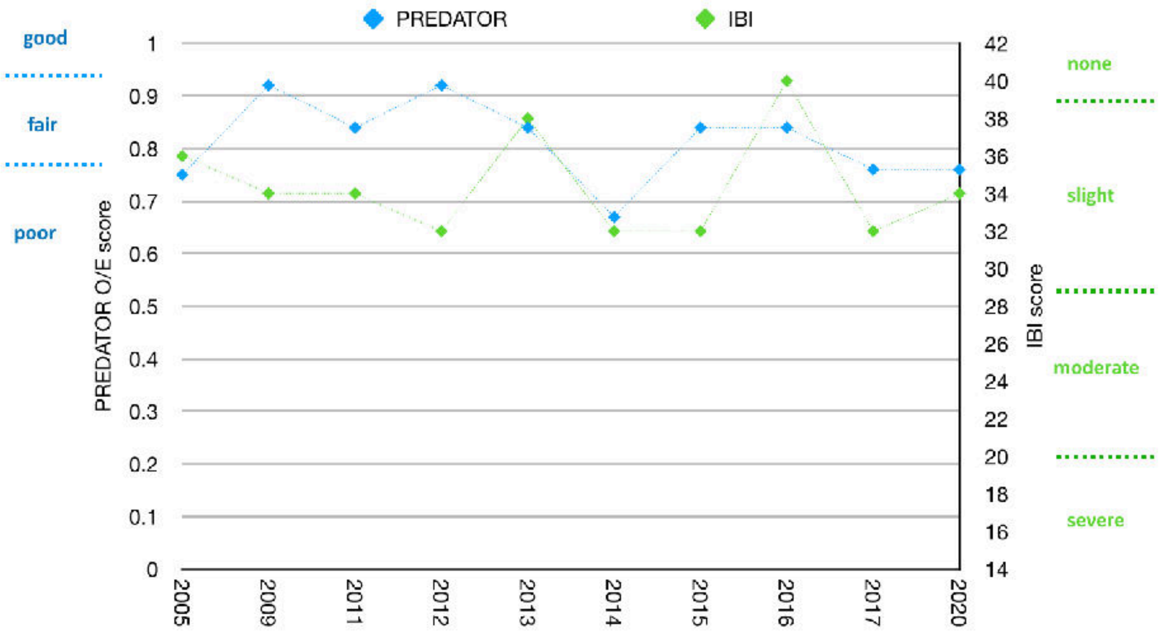


Figure 30. Numbers of sensitive and sediment-sensitive taxa at WC0900 in all sampling years. *RT samples were taken in all years. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

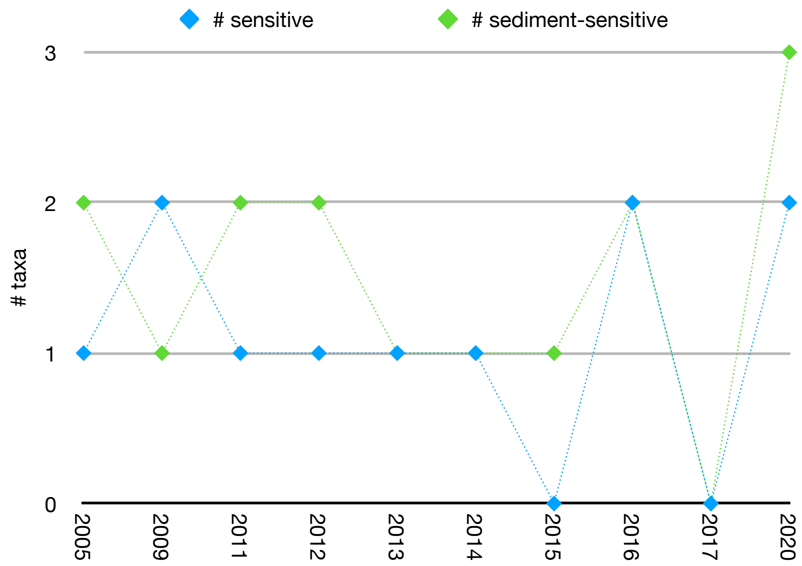


Figure 31. Relative abundance of tolerant and sediment-tolerant organisms at WC0900 in all sampling years. *RT samples were taken in all years. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant organisms.*

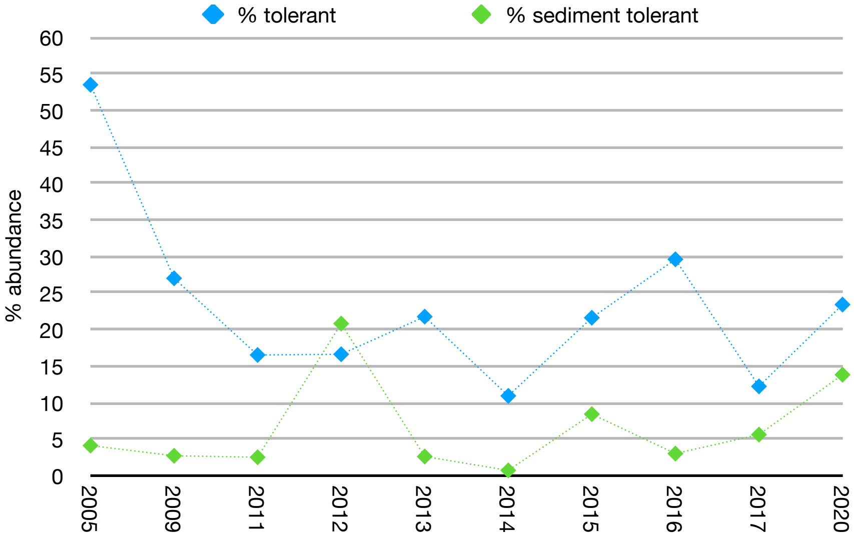


Figure 32. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC0900 in all sampling years. *RT samples were taken in all years. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

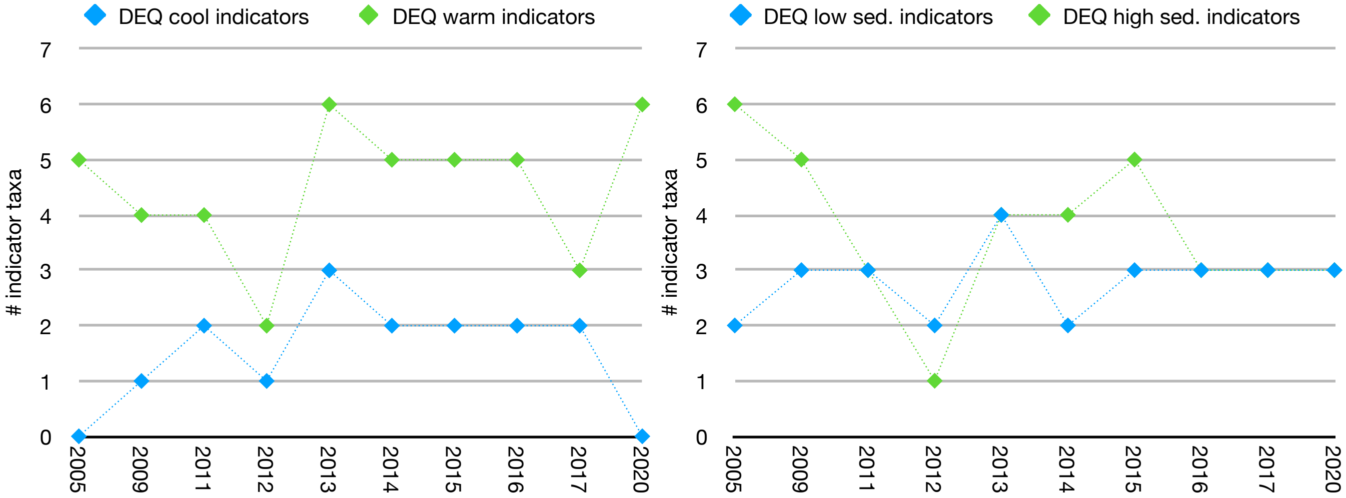
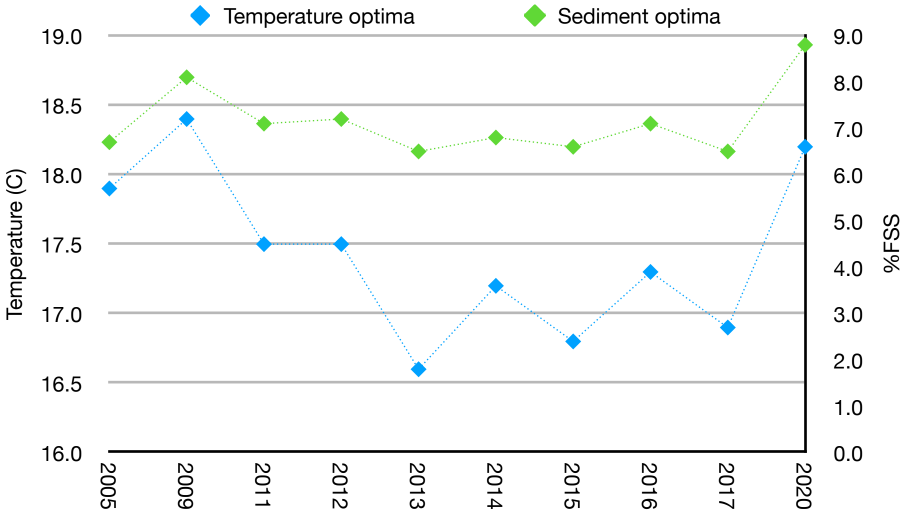


Figure 33. Community temperature and fine sediment optima (weighted means) at WC0900 in all sampling years. *RT samples were taken in all years. Note that individual optima have not been determined for every taxon in a sample.*



The community in 2020 differed greatly from all other sampling years (Figure 34) and was most similar to that in 2015 (Bray-Curtis similarity index = 0.58). Overall, there were more differences in community composition among early (2005, 2009) and later (2017, 2020) sampling years compared to the communities in 2013-2016. PCA ordination of taxa abundances reflects between-sample differences driven largely by the dominant taxon in each year (Figure 35); axis 1 explained 31% of total sample variation, and taxa with the highest loading values were *Baetis tricaudatus* (dominant taxon in 2013-2015; a DEQ low sediment indicator taxon, associated with clear, flowing water and often an early re-colonizer following disturbance), *Simulium* (dominant taxon in 2011), and Chironominae (dominant taxon in 2009). Axis 2 explained an additional 17% of sample variation, and taxa with the highest loading values were Chironominae, *Atherix* (tolerant water snipe fly that is slightly sensitive to fine sediment and inhabits the bottoms of rocks in mixed flows; much lower abundance in three most recent sampling years), and *Rhithrogena* (flatheaded mayfly found in cold flowing water that is very sensitive to low levels of fine sediment; greatest abundance in 2005).

In a PCA ordination of traits measured as relative abundances in all years (Figure 36), axis 1 explained 42% of the total variation between samples; traits with the highest loading values were relative abundances of scrapers (high in 2009, 2017, and 2020), clingers (highest in 2005 and 2016), and multivoltine organisms (highest in 2012-2015). Axis 2 explained an additional 30% of the variation; traits with the highest loading were relative abundances of organisms that are small (lower overall in more recent sampling years), associated with erosional flows, and feed as collectors (both of which were greatest in samples taken from 2011-2017).

Figure 34. CLUSTER ordination of the WC0900 macroinvertebrate community in all sampling years. *RT samples were taken in all years. The number at the end of each label indicates the sampling year.*

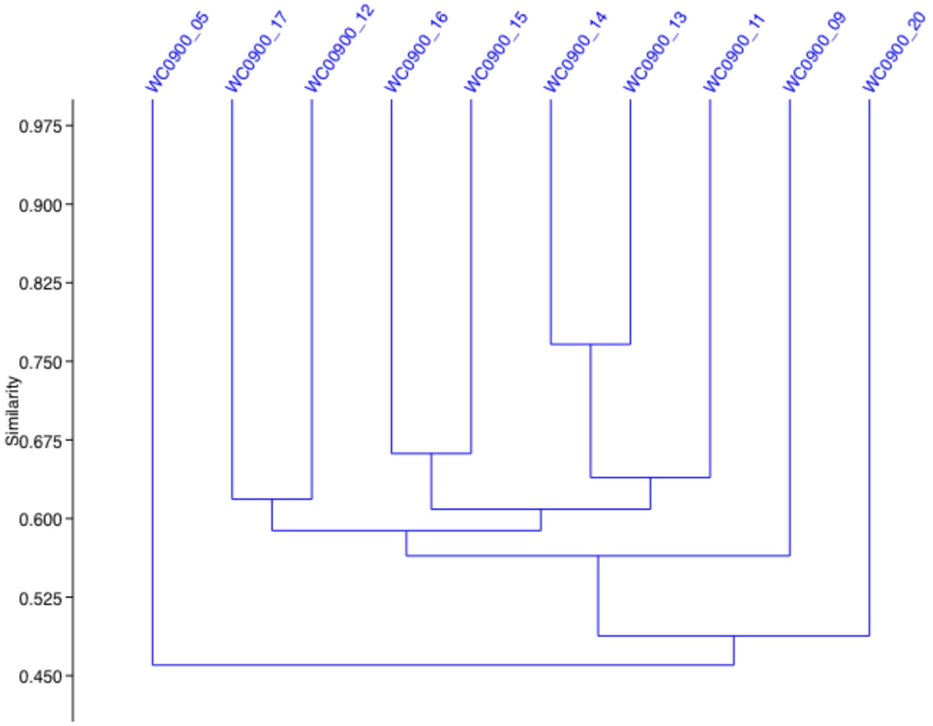


Figure 35. PCA ordination of the WC0900 macroinvertebrate community in all sampling years. *RT samples were taken in all years. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*

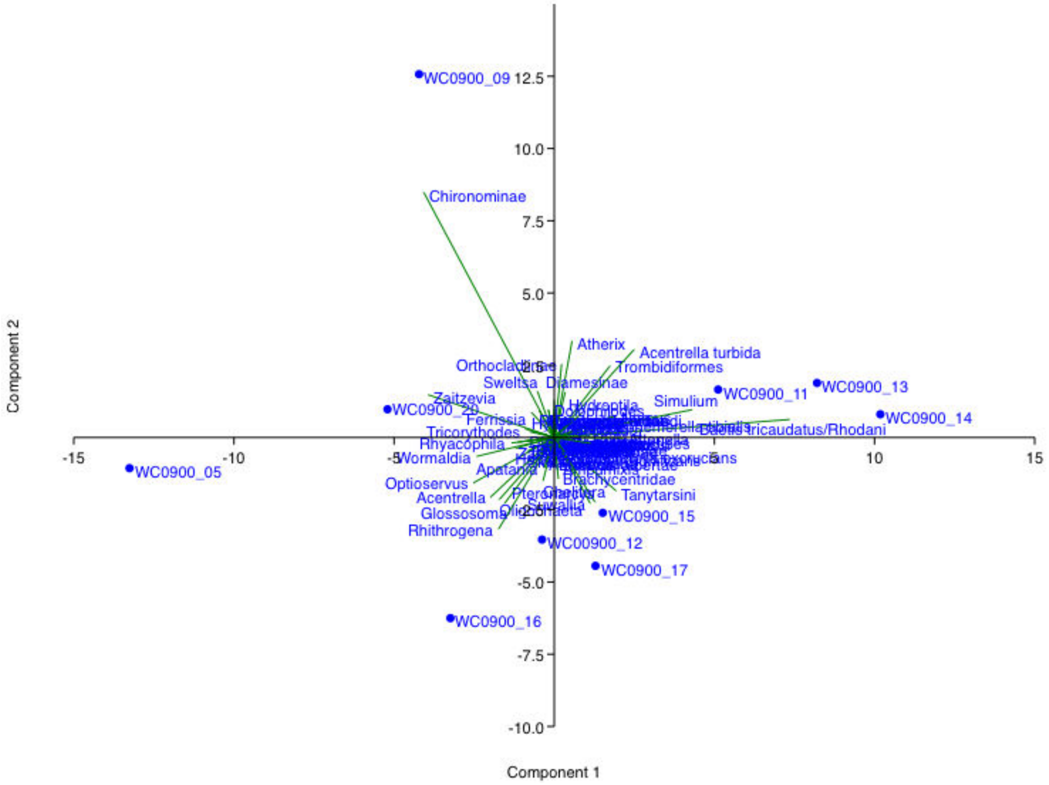
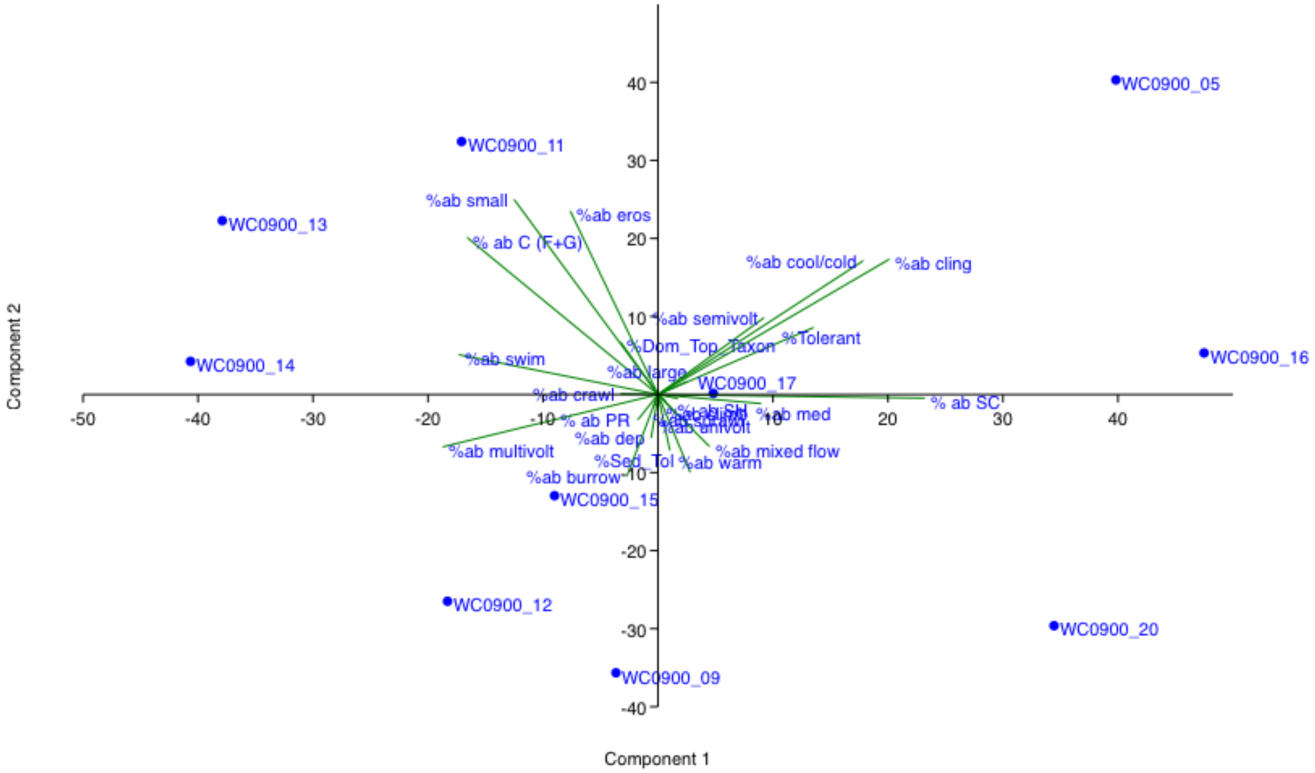


Figure 36. PCA ordination of the WC0900 macroinvertebrate community traits measured as relative abundances in all sampling years. *RT samples were taken in all years. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



<u>WC1025</u>

RT samples were taken in the primary channel of Whychus Creek around RM 10.25 in 2011, 2012, and 2014-2017; PM samples were taken in the same reach in 2019 and 2020 to provide additional baseline data for upcoming restoration work. Sample richness was intermediate for the 2020 dataset (57 taxa) but one of the highest at this site (Figure 37), though higher richness in 2019-2020 is due in part to the fact that PM samples often have more taxa than RT samples taken in the same reach. The number of EPT taxa (22) was at the upper end of the range among all 2020 samples and the highest for this site (Figure 37). The 2020 community was dominated by *Rhithrogena,* a sediment-sensitive flatheaded mayfly associated with fast cold flows, at 15% of total organismal abundance. The community consisted mainly of small-bodied, multivoltine and univoltine clingers that prefer fast cool flows and feed as collectors. This is similar to community characteristics in earlier sampling years.

The community in 2020 was most similar to that at WC1150 (Bray-Curtis similarity index = 0.69). Five taxa taken in 2020 were not found at this site in any prior sampling year: *Centroptilum (Anafroptilum)*, a tolerant small minnow mayfly associated with sandy substrates in colder, slower flows; *Pedomoecus*, a sensitive mountain casemaker caddisfly in cool rapid streams; *Wormaldia*, a sediment-sensitive finger-net caddisfly found in faster flows; *Rhyacophila atrata*, a freeliving caddisfly found in cold clear flowing water; and *Pacifasticus*, a native crayfish that hides in rocky crevices or woody debris in a wide range of habitats. All occurred at low abundance (1-3 individuals) and were taken at other sites/years.

The target sub-sampling number of organisms was attained in all years except 2017, with 21-100% of the sample picked (Figure 38). PM samples, which generally included many net sets taken in riffles (seven of 10 net sets in 2020 sample), consistently had more organisms than RT samples. Relative abundance of the dominant taxon at this site was in the range of the highest scaled score in the ORDEQ IBI in six of eight sampling years (Figure 39). In earlier years, the community was dominated by caddisfly or mayfly taxa associated with colder temperatures and lower fine sediment (*Brachycentrus*, *Baetis tricaudatus*), but dominant taxa in 2016-2019 were characteristic of warmer, more sedimented waters (tolerant riffle beetle, worms). In contrast, the dominant taxon in the 2020 sample, *Rhithrogena*, is again a more sensitive organism.

Figure 37. Sample richness and number of EPT taxa at WC1025 in all sampling years. *A PM sample was taken in 2019 and 2020; RT samples were taken in all other years. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*

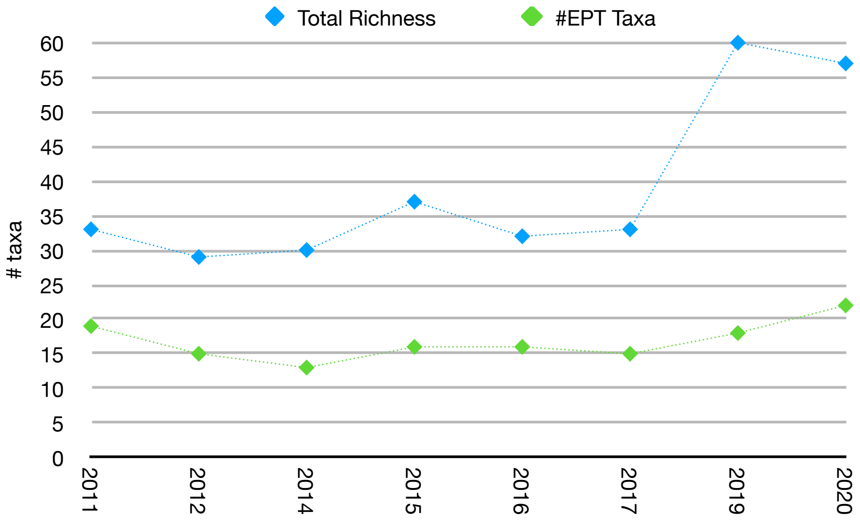


Figure 38. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC1025 in all sampling years. *PM samples were taken in 2019 and 2020, RT samples were taken in all other years.*

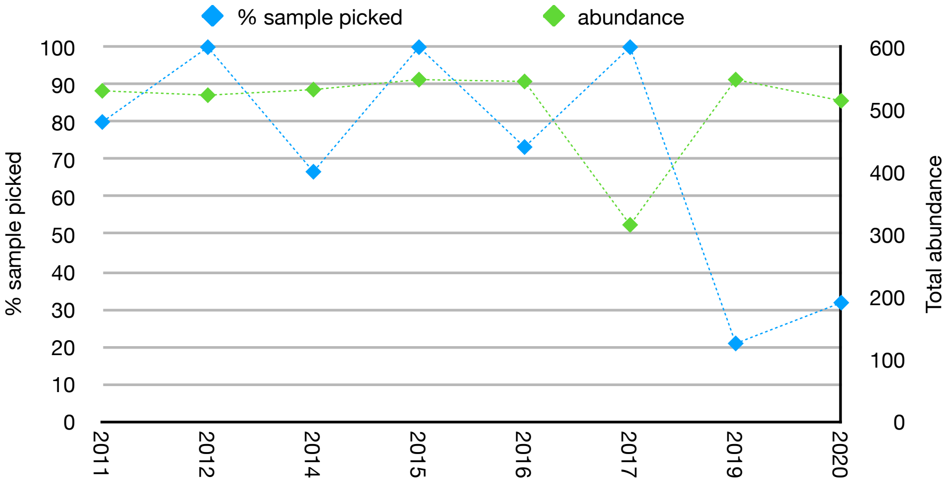
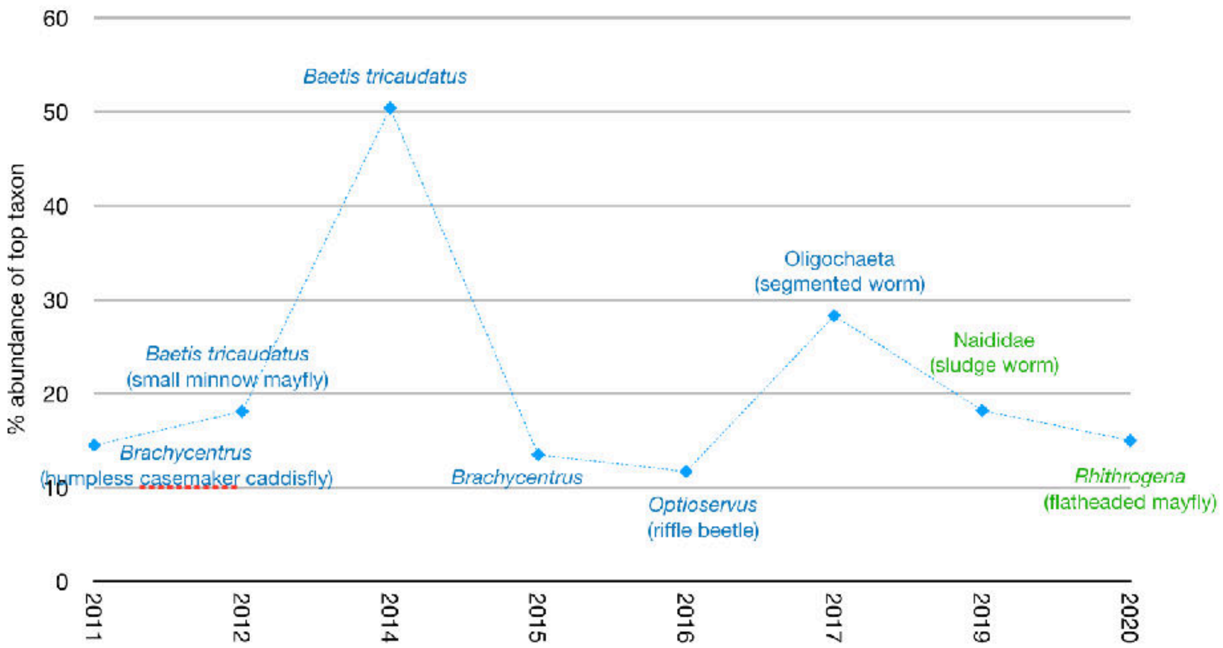


Figure 39. Relative abundance of the numerically dominant taxon at WC1025 in all sampling years. *PM samples (green) were taken in 2019 and 2020, RT samples (blue) were taken in all other years. This metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*



In years when RT samples were taken, IBI scores indicated slight impairment except in 2014 and 2017, which scored as moderately disturbed; PREDATOR scores varied more, indicating good, fair, or poor conditions over time. There were 2-4 times more sensitive and sediment-sensitive taxa in 2019-2020 compared to prior years (Figure 40), and the 2020 sample was the first with enough sensitive taxa to score in the highest range of the ORDEQ IBI. The proportions of tolerant and sediment-tolerant organisms increased overall from 2014-2017 (Figure 41); relative abundance of tolerant organisms remained higher in recent compared to early sampling years, but both decreased in 2020 and the proportion of sediment-tolerant organisms fell into the range corresponding to the highest scaled score in the ORDEQ IBI for the first time since 2016 (Figure 41). Numbers of both ORDEQ cool and warm temperature indicator taxa increased over time (Figure 42), with more warm than cool indicators in most years. The number of low sediment indicator taxa has been steady, and though numbers of high sediment indicator taxa increased overall, there were about half as many in the 2020 sample compared to 2019 (Figure 42). Community temperature and sediment optima rose overall throughout the sampling period, but both were lower in 2020 compared to 2019 (Figure 43).

Figure 40. Numbers of sensitive and sediment-sensitive taxa at WC1025 in all sampling years. *PM samples were taken in 2019 and 2020, RT samples were taken in all other years. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

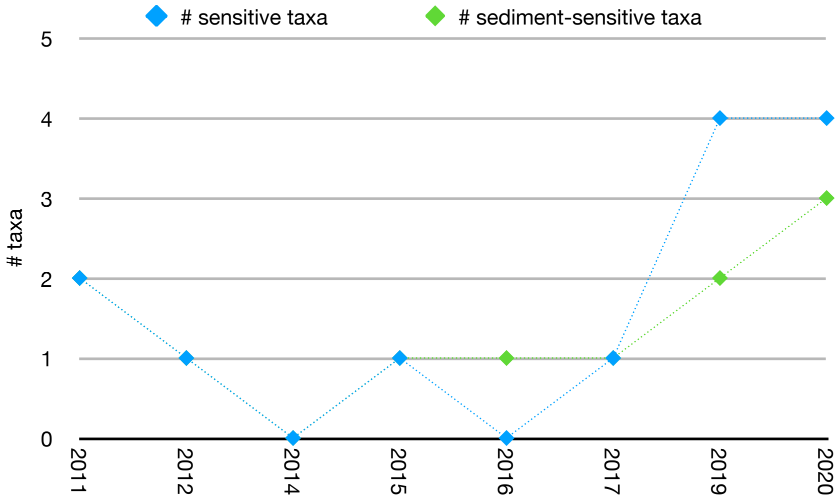


Figure 41. Relative abundance of tolerant and sediment-tolerant organisms at W1025 in all sampling years. *PM samples were taken in 2019 and 2020, RT samples were taken in all other years. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*

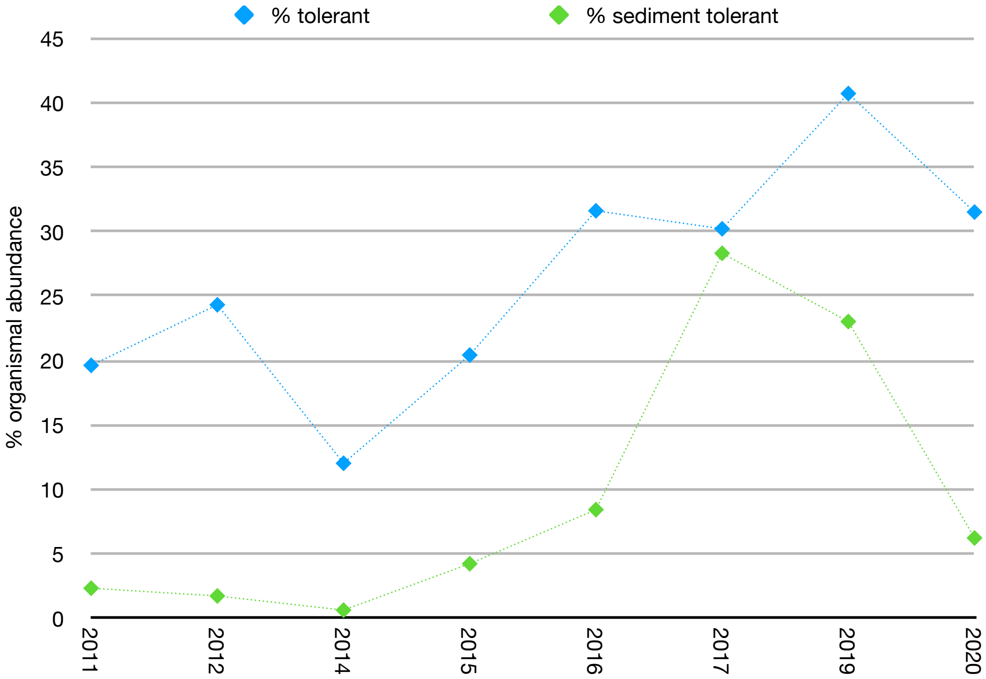


Figure 42. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC1025 in all sampling years. *PM samples were taken in 2019 and 2020, RT samples were taken in all other years. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

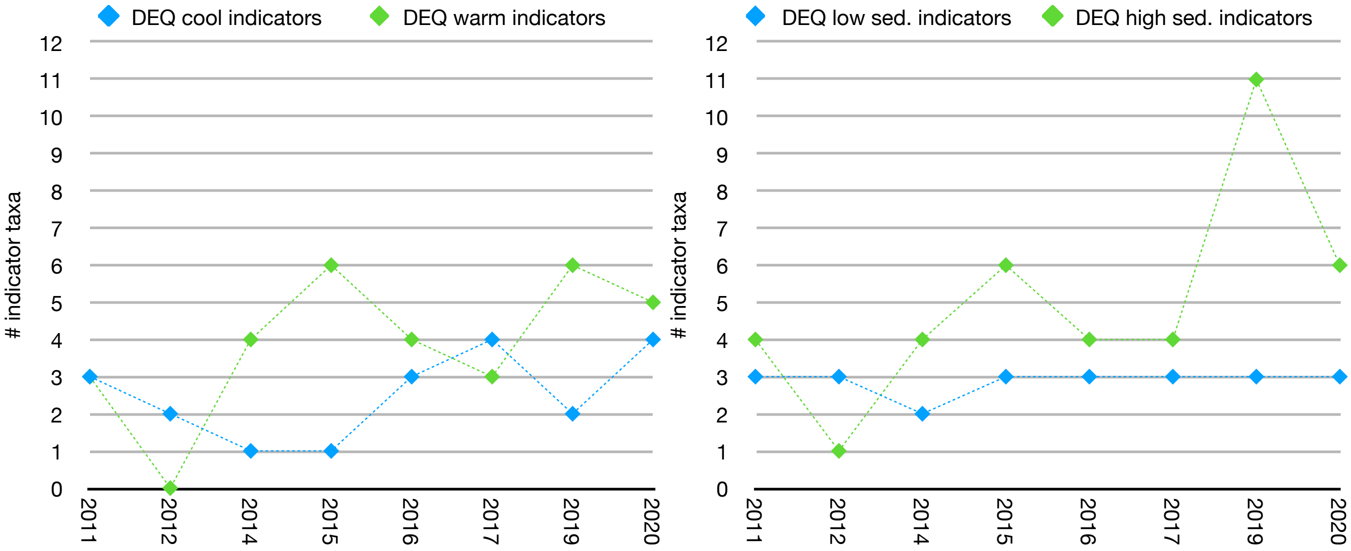
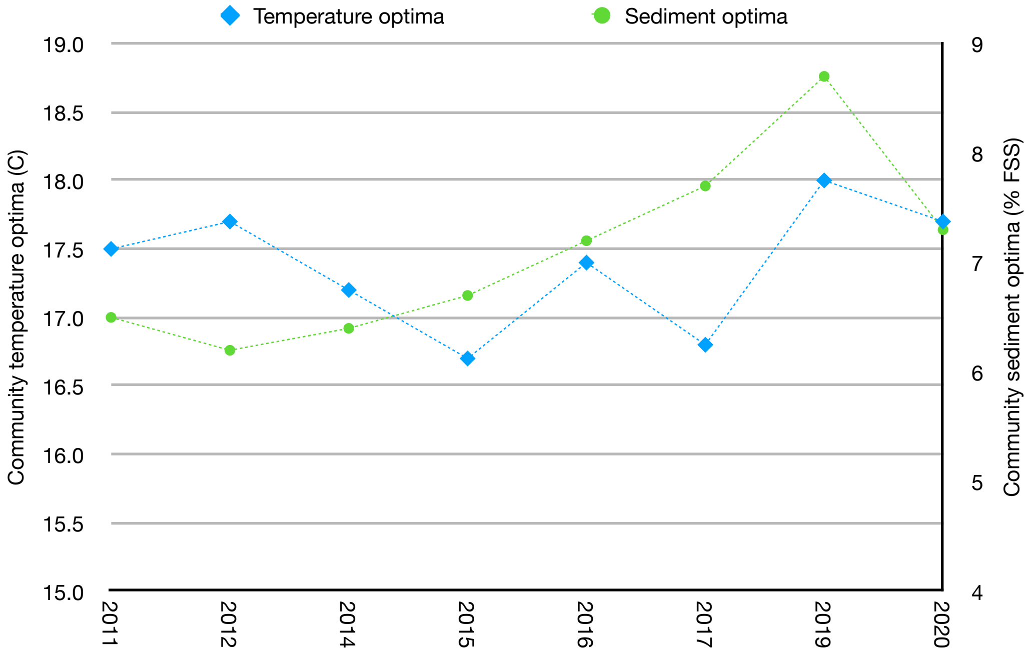


Figure 43. Community temperature and fine sediment optima (weighted means) at W1025 in all sampling years. *PM samples were taken in 2019 and 2020, RT samples were taken in all other years. Note that individual optima have not been determined for every taxon in a sample.*



The 2019 and 2020 PM sample communities were most similar (Bray-Curtis similarity index = 0.71; Figure 44) and differ most from all other years (overall average dissimilarity = 46%). This could be influenced by sample method, as RT samples were taken in all prior years, but the 2017 RT sample is also an outlier from all earlier years. In a PCA ordination of taxa abundances (Figure 45), axis 1 explained 39% of total sample variation and was strongly influenced by the dominant taxa in different years; taxa with the highest loading values were *Baetis tricaudatus* (sediment-sensitive small minnow mayfly that prefers clear fast water and can be an early colonizer after disturbance; more abundant in 2011-2015), Annelida (tolerant, sediment-tolerant aquatic earthworms; more abundant in 2016-2019), and *Brachycentrus* (humpless case-maker caddisfly found in cold flowing water; abundant in 2011-2012 and 2015). Axis 2 explained an additional 19% of sample variation; taxa with the highest loading were *Brachycentrus*, *Baetis tricaudatus*, and *Zaitzevia* (tolerant riffle beetle found in faster flows that is a DEQ warm temperature and high sediment indicator; lower abundance in 2014 and 2017-2020).

In a PCA ordination of traits measured as relative abundances (Figure 46), axis 1 explained 48% of total sample variation; factors with the highest loading were relative abundances of swimmers (low in 2016 and 2019), multivoltine organisms, and the dominant taxon (both highest in 2014 and 2017). Axis 2 explained an additional 33% of sample variation; factors with the highest loading were relative abundances of organisms associated with faster flows (low in 2017 and 2019), slower water (high in 2017 and 2019), and of burrowers (very low in 2011-2014 and in 2020).

Figure 44. CLUSTER ordination of the WC1025 macroinvertebrate community in all sampling years. *PM samples (aqua) were taken in 2019 and 2020, RT samples (blue) were taken in all other years. The number at the end of each label indicates the sampling year.*

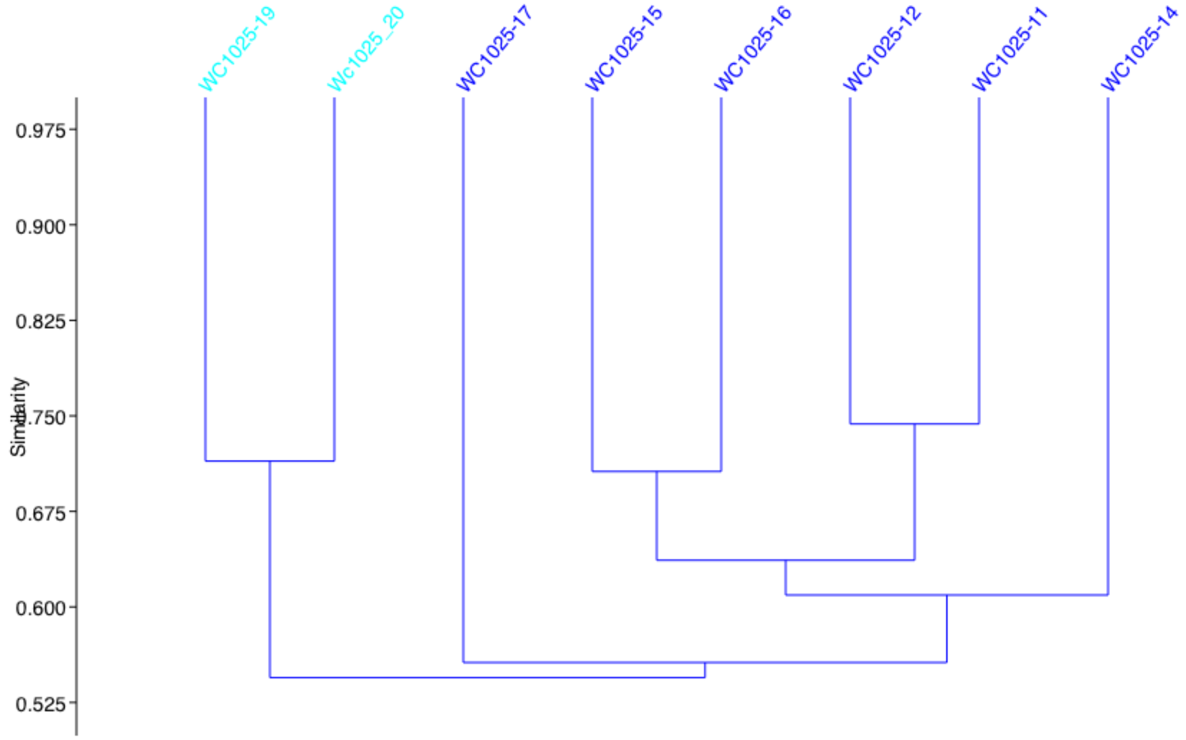


***Figure 45. PCA ordination of the WC1025 macroinvertebrate community in all sampling years.** PM samples (aqua) were taken in 2019 and 2020, RT samples (blue) were taken in all other years. The number at the end of each label indicates the sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*

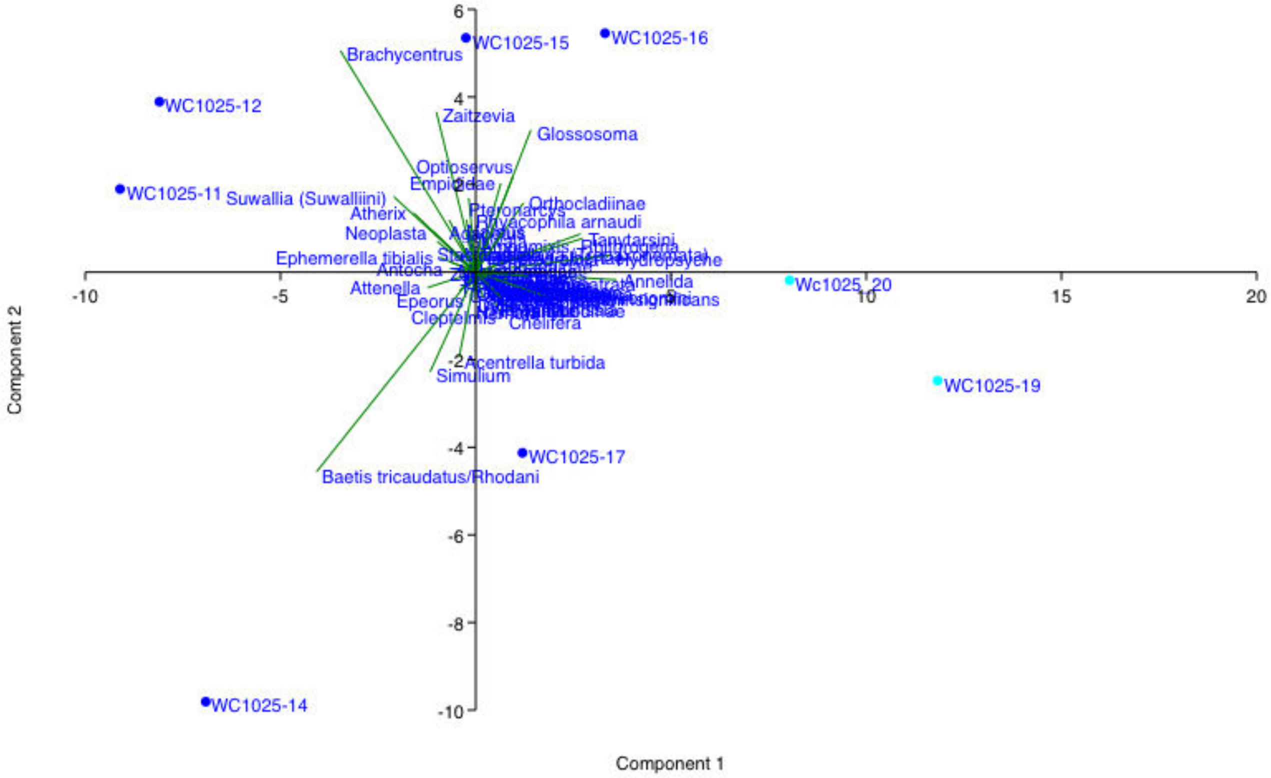
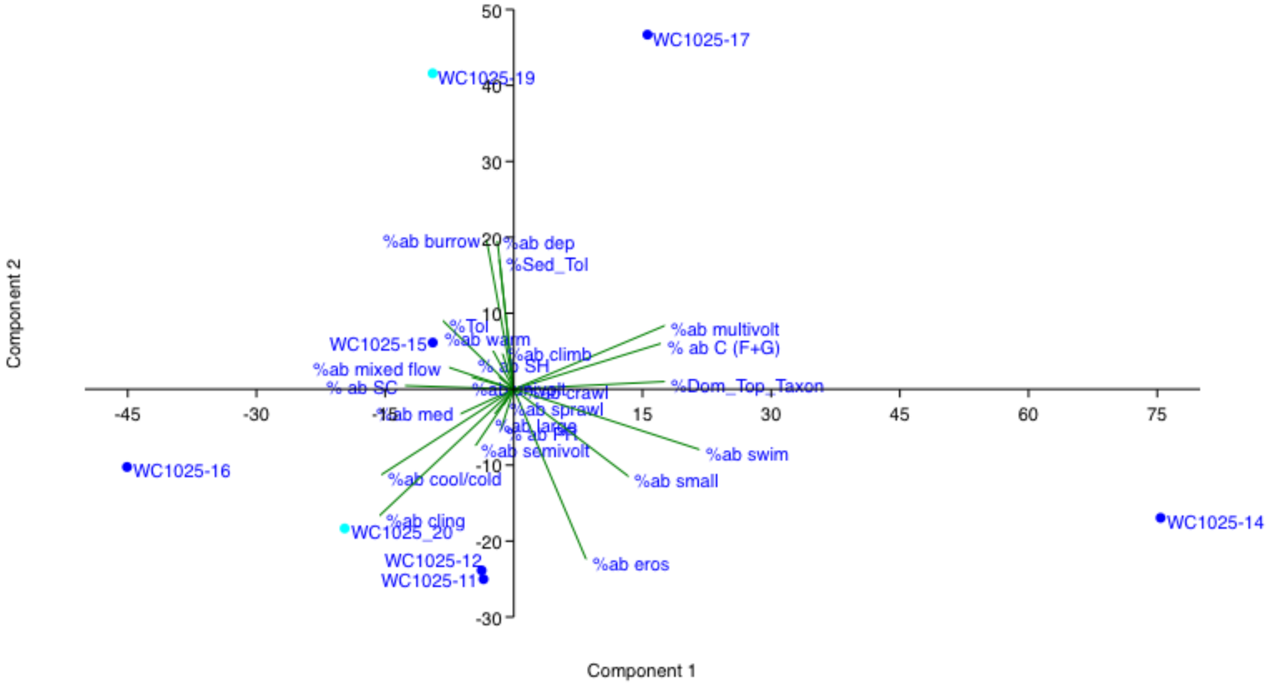


***Figure 46. PCA ordination of the W1025 macroinvertebrate community traits measured as relative abundances in all sampling years.** PM samples (aqua) were taken in 2019 and 2020, RT samples (blue) were taken in all other years. The number at the end of each label indicates the sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



WC1100

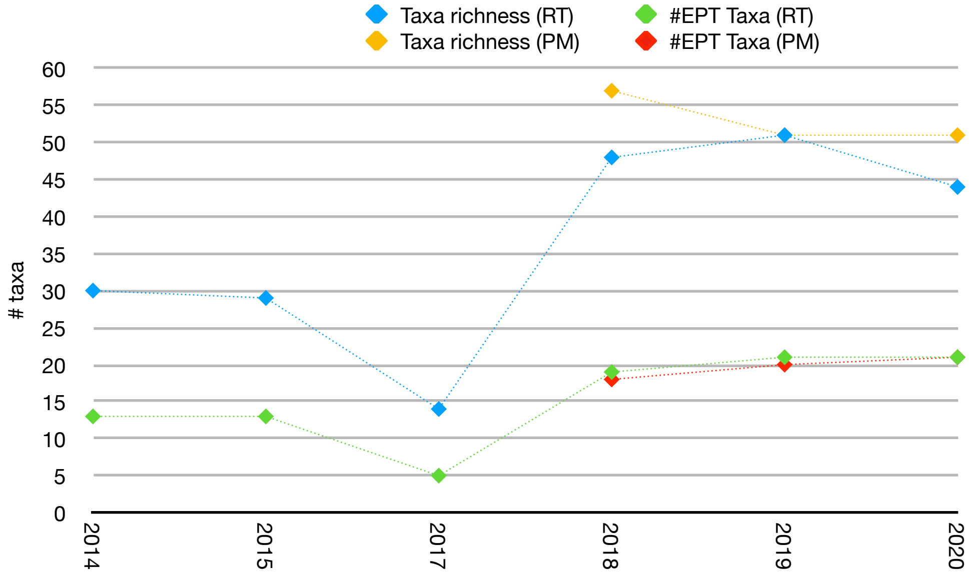
RT samples were taken in the primary channel of Whychus Creek around RM 11.0 in 2014-2015 to collect baseline data prior to the Whychus Canyon restoration project that created new side channel habitat in 2016. Following restoration, RT and PM samples were taken in the same reach of the primary channel (2019-2020) and PM samples were taken in side channels (2017-2020). Stream channels have been braided and dynamic here and samples taken in 2020 included a reach with both primary and secondary channel habitat (PC/SC; WC1100-1B), a side channel reach (SC; WC1100-4), and the primary channel (WC1100-2RT, WC1100-2PM).

In 2020, the community in the primary channel RT sample was most similar to the PM sample in the same reach (Bray-Curtis similarity index = 0.76) and to the side channel (Bray-Curtis similarity index = 0.79). Although the RT sample had the fewest taxa of all samples at this site (44 taxa vs. 51-64 in PM samples), the WC1100-1B community was least similar to all other WC1100 samples (Bray-Curtis similarity index range = 0.64-0.65). The side channel had the most taxa at this site, and community was more similar to that in the primary channel samples (Bray-Curtis similarity index = 0.71 with PM sample and 0.74 with RT sample). The top taxon was not overly dominant in any samples (range = 12.6-25.6%), but WC1100-1B was the only one not dominated by the flatheaded mayfly *Rhithrogena;* instead, the most abundant organism in this sample was tolerant Naididae sludge worms. WC1100-B also had the highest community sediment optima (11.7%) of any sample in 2020. There were similar numbers of EPT taxa in all the samples (range = 19-23); the WC1100-1B sample had the least, and the side channel had the most.

Eight taxa taken in 2020 were not seen here in prior years; all occurred at low abundance (1-3 individuals) and the majority were in samples that included side channel habitat. New taxa included Sciomyzidae, a predatory marsh fly associated with warmer slower flows; *Dicranomyia*, a crane fly that inhabits rocks, logs, and sediments at the margins of streams and lakes; *Cinygma*, a sensitive flatheaded mayfly associated with woody substrates in colder faster flows; *Neoleptophlebia*, a prong-gilled mayfly that inhabits sediment and detritus in faster flowing water; *Siphlonurus*, a tolerant primitive minnow mayfly associated with sediments and plants in slower flows; Crambidae, a grass moth family whose tolerant aquatic caterpillars are found on vascular hydrophytes in slow flows; *Protoptila*, a somewhat sediment-sensitive saddlecase-maker caddisfly usually in larger, warmer streams; and *Gyraulus*, a tolerant and sediment-tolerant ramshorn snail found in a range of temperatures and habitats in slower flows. *Protoptila* and *Gyraulus* appeared in the Whychus dataset for the first time in 2020, but the other taxa were taken in other sites/years.

Total and EPT richness in primary channel samples were greater in 2018-2020 compared to earlier sampling years (Figure 47), and PM and RT samples had almost identical numbers of EPT taxa in each year. Samples from side channel and PC/SC habitat at this site had high total and EPT richness; this included in 2017, when recovery from restoration was ongoing and the primary channel RT sample had only 14 taxa while three side channel PM samples contained 37-42 total taxa. The 2020 community in all WC1100 samples was composed mainly of small-bodied, multivoltine and univoltine clingers that prefer colder, faster flows and feed as scrapers or collectors. The communities in 2014-2015 prior to restoration had greater relative abundances of collectors and swimmers, and no taxa with a climbing habit were seen in any sample from 2014-2017, though they were present at low to moderate abundance in every sample and year from 2018 to 2020. The mean relative abundances of taxa associated with cooler water was also significantly greater among all samples from 2019-2020 (40%±14) compared to those from 2014-2018 (19% ±6.6).

Figure 47. Sample richness and number of EPT taxa at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. PM and RT samples were taken in the same reach at the same time in 2018-2020. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



The target sub-sampling number of 500 organisms was attained in all primary channel RT samples except in 2017, with 20-80% of the total sample picked; this target was attained in primary channel PM samples only in 2018 (Figure 48). Relative abundance of the dominant taxon in primary channel RT and PM samples decreased over time and was low enough to receive the highest scaled score in the ORDEQ IBI in 2018-2020 (Figure 49). This site has primarily been dominated by sensitive taxa, though more tolerant taxa were abundant in the years immediately following restoration. Recent PM and RT samples were dominated by *Baetis tricaudatus* (sediment-sensitive small minnow mayfly that prefers clear fast water; 2019) and *Rhithrogena* (a sediment-sensitive flatheaded mayfly found in cold flowing water; 2020). Although newly-formed side channels might be expected to have low evenness and be dominated by more tolerant taxa, this metric varies widely among side channel and PC/SC samples taken in 2017-2020 (range = 10.6-46.2% abundance of top taxon), and dominant taxa are a mixture of tolerant (i.e., non-biting midges, sludge worms, *Optioservus* riffle beetles), early colonizers (i.e., *Simulium* black flies, *Baetis tricaudatus* mayflies), and sensitive types (*Attenella margarita* spiny crawler mayfly, *Zapada cinctipes* stonefly).

Figure 48. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. PM and RT samples were taken in the same reach at the same time in 2018-2020.*

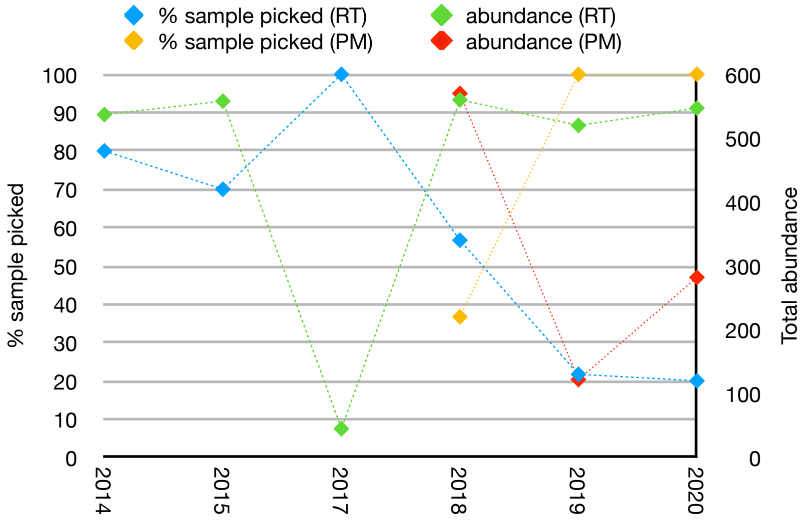
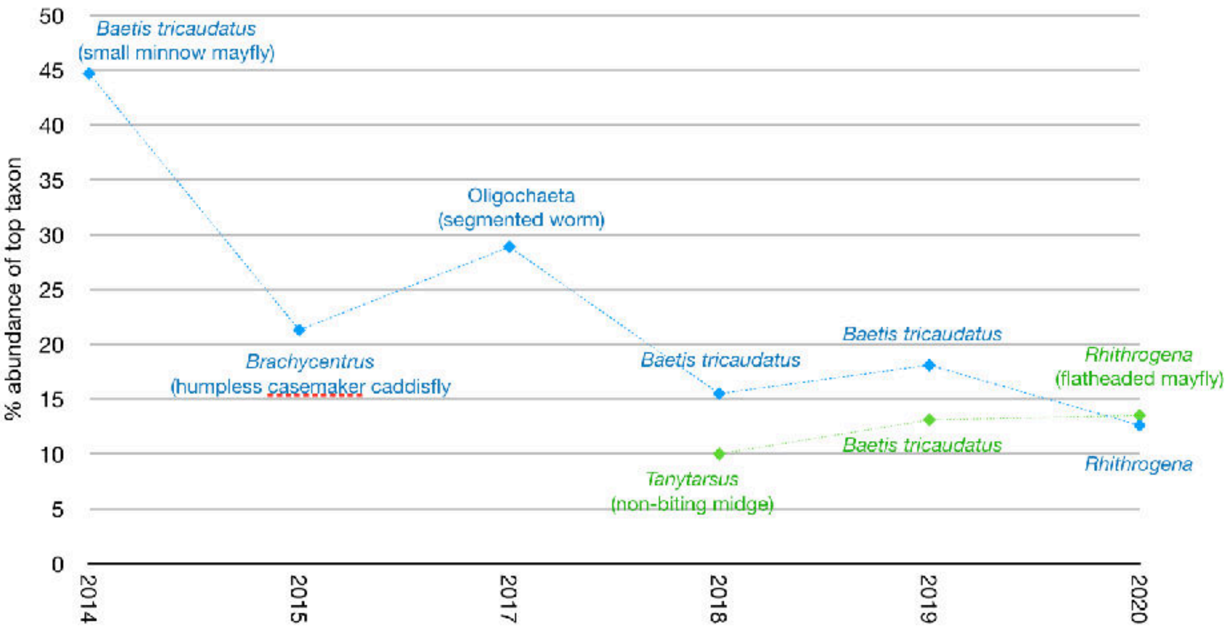


Figure 49. Relative abundance of the dominant taxon at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. RT samples (blue) and PM samples (green) were taken in the same reach at the same time in 2018-2020. This metric in the ORDEQ IBI receives the highest scaled score at <20% relative abundance.*



An increase in IBI and PREDATOR scores was punctuated by a sharp drop in both in 2017 immediately after restoration (Figure 50). Both means are greater in samples from 2018-2020 (mean IBI = 41±1; mean O/E = 0.93 ± 0.17) compared to 2014-2015 samples (mean IBI = 28 ± 5.7; mean O/E = 0.85 ± 0.12), but the difference is significant only for the IBI. Numbers of sensitive and sediment-sensitive taxa increased sharply in RT samples following restoration (Figure 51) but were lower in PM samples taken in the same years. There are consistently more sensitive taxa (2-7) than sediment-intolerant taxa (0-2) in side channel and PC/SC samples. Relative abundance of sediment-tolerant organisms is consistently lower than that of tolerant organisms in RT and PM primary channel samples, and both returned to levels similar to pre-restoration years following a spike in 2017 (Figure 52). Somewhat surprisingly, side channels samples have significantly lower relative abundance of tolerant organisms (mean = 12.5% ± 4.0) compared to samples in primary+side channel reaches (mean = 31.8% ± 17.8) and lower abundance of sediment-tolerant organisms (means = 3.3 ± 4.0 and 8.3 ± 10.0, respectively), though this difference is not significant.

Figure 50. PREDATOR O/E and ORDEQ IBI scores at WC1100 in all sampling years.

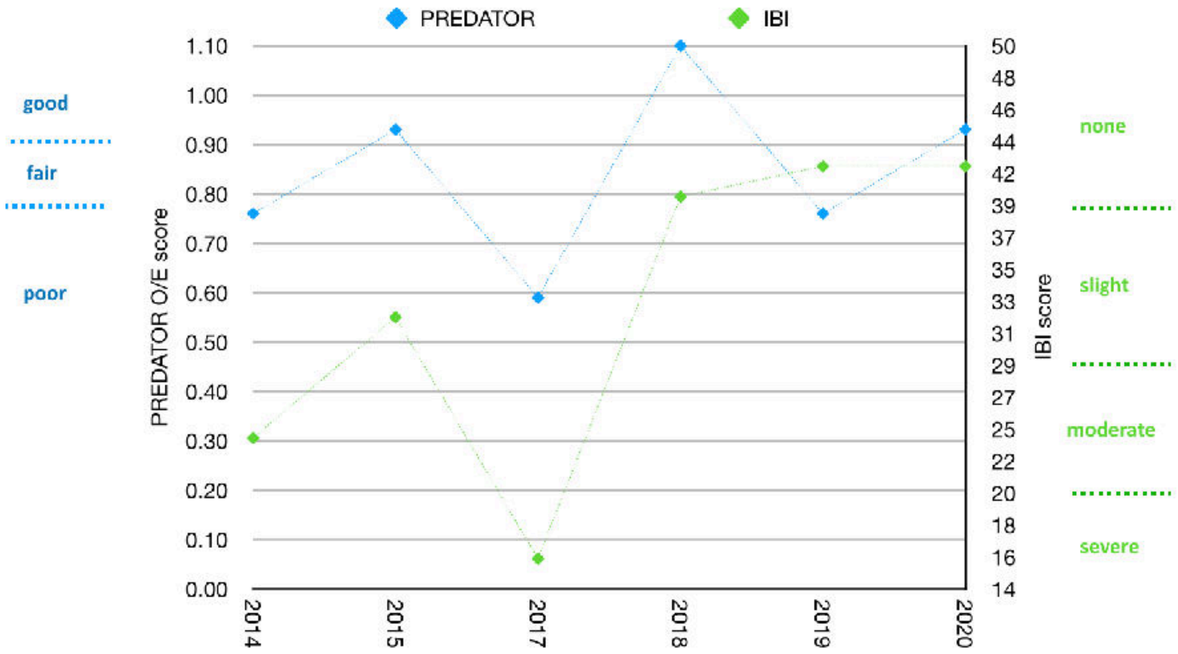


Figure 51. Numbers of sensitive and sediment-sensitive taxa at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. PM and RT samples were taken in the same reach at the same time in 2018-2020. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

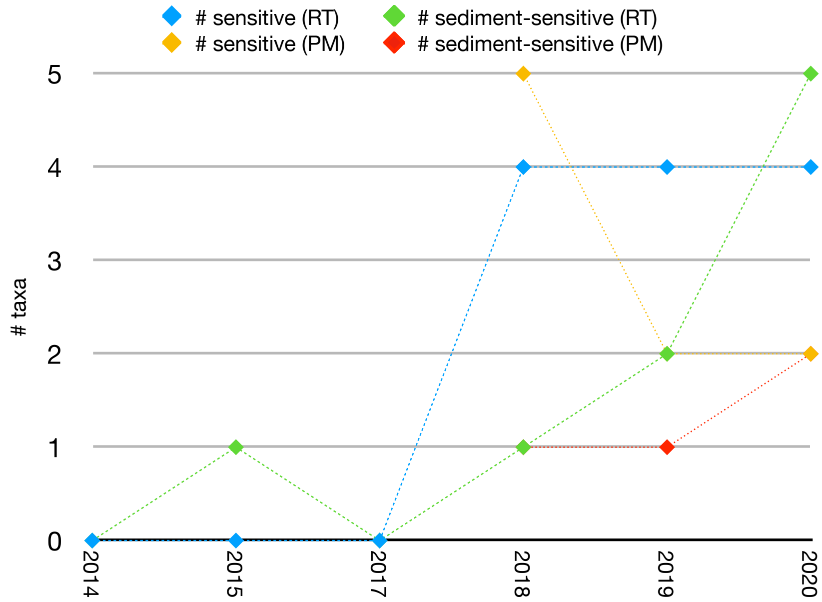
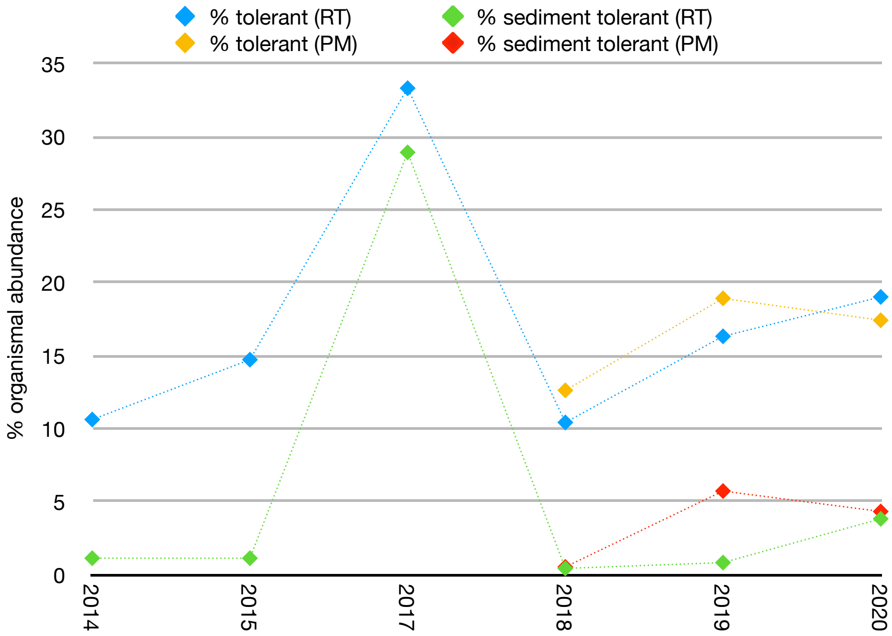


Figure 52. Relative abundance of tolerant and sediment-tolerant organisms at W1100 in all sampling years. *Only samples taken in the primary channel are shown. PM and RT samples were taken in the same reach at the same time in 2018-2020. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*



Numbers of DEQ warm temperature and high sediment indicator taxa numbers consistently exceeded those of cool temperature and low sediment indicators in RT and PM samples in the primary channel (Figure 53), as well as in samples that included side channel reaches. Community temperature optima increased slightly in recent years but did not seem to be affected by the disturbance associated with restoration (Figure 54); in contrast, the community fine sediment optima increased sharply in 2017 (Figure 54) but decreased since.

Figure 53. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. PM and RT samples were taken in the same reach at the same time in 2018-2020. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

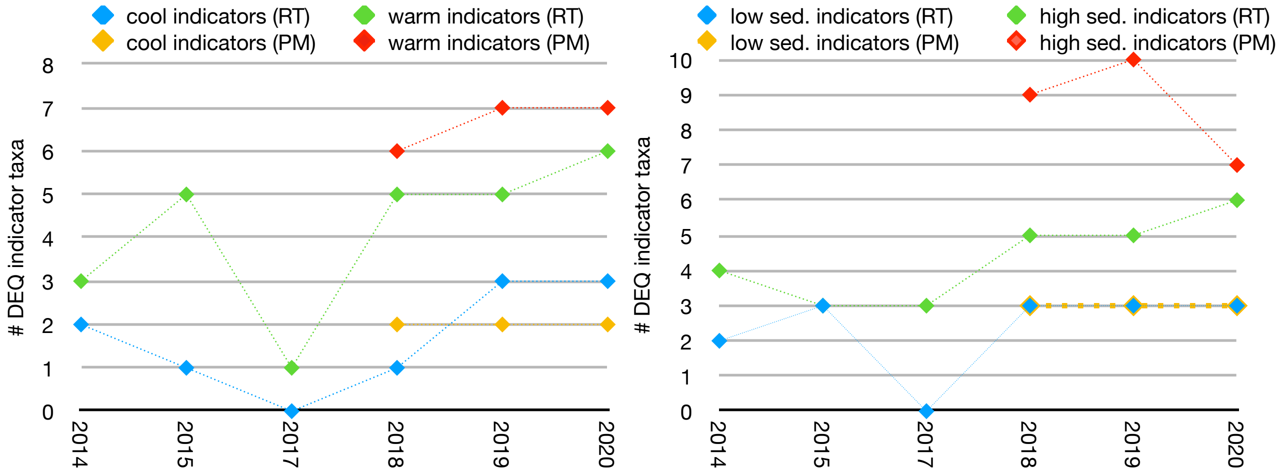
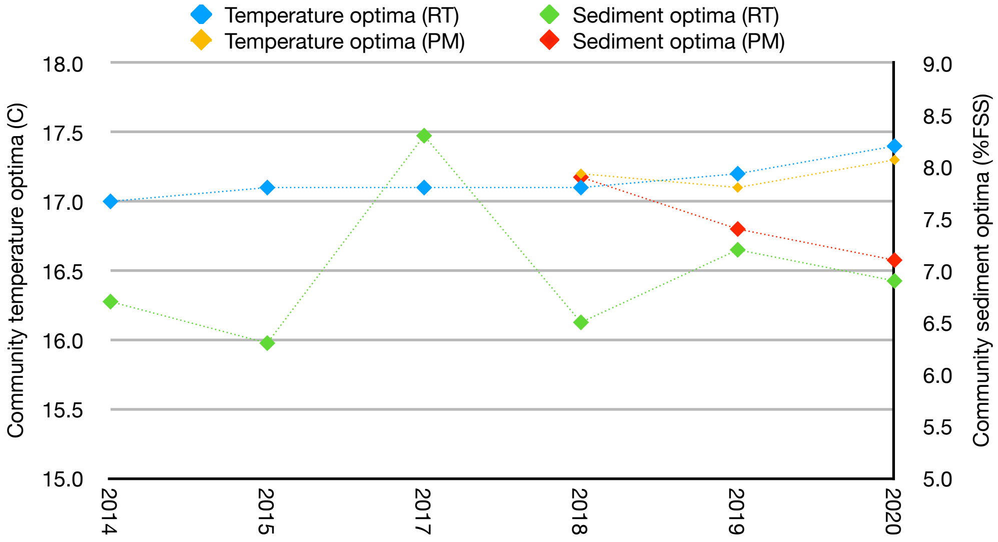


Figure 54. Temperature and fine sediment optima of the community (weighted means) at W1100 in all sampling years. *PM and RT samples were taken in the same reach at the same time in 2018-2020. Note that individual optima have not been determined for every taxon in a sample.*



The community in the 2017 primary channel RT sample was an outlier to all other sampling years at this site, reflecting restoration-associated disturbances, and pre-restoration primary channel communities differ from all those in 2018-2020 (Figure 55). The communities in new side channels in 2017 also differed more from those in side channel or primary channel reaches in 2018-2020. Most primary channel samples in a given year were more similar to each other than to side channel samples, regardless of whether the PM or RT method was used. In a PCA ordination of taxa abundances (Figure 56), axis 1 explained 23% of total variation, and taxa with the highest loading were the tolerant non-biting midge tribes Tanytarsini and Chironomini (both more abundant post-restoration, especially in side channels), and *Baetis tricaudatus* (more abundant pre-restoration). Axis 2 explained an additional 17% of variation; taxa with the highest loading included the sensitive flatheaded mayfly *Rhithrogena* (much more abundant post-restoration), *Hydropsyche* (tolerant netspinner caddisfly, DEQ warm temperature indicator taxon; increased abundance in samples that include primary channel post-restoration), and *Optioservus* (tolerant riffle, beetle, DEQ warm temperature and high sediment indicator taxon; increased abundance post-restoration).

Figure 55. CLUSTER dendrogram of the WC1100 macroinvertebrate community in all sampling years. *PM (aqua) and RT (blue) samples were taken in primary and side channel reaches in 2017-2020; RT samples were taken all other years. The last number in each label indicates the sampling year. PC = primary channel, SC = side channel, PC/SC = sample reach that included both primary and side channel.*

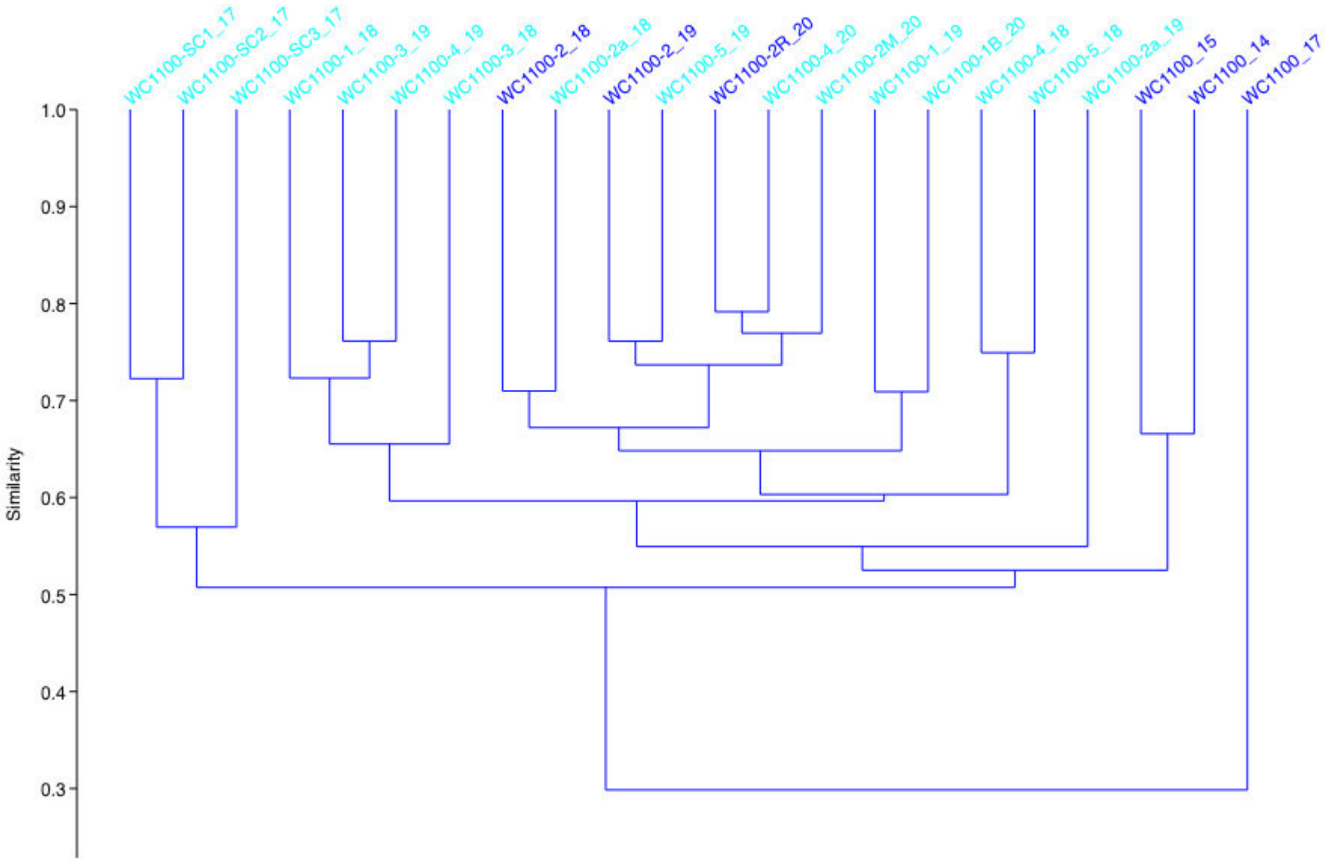
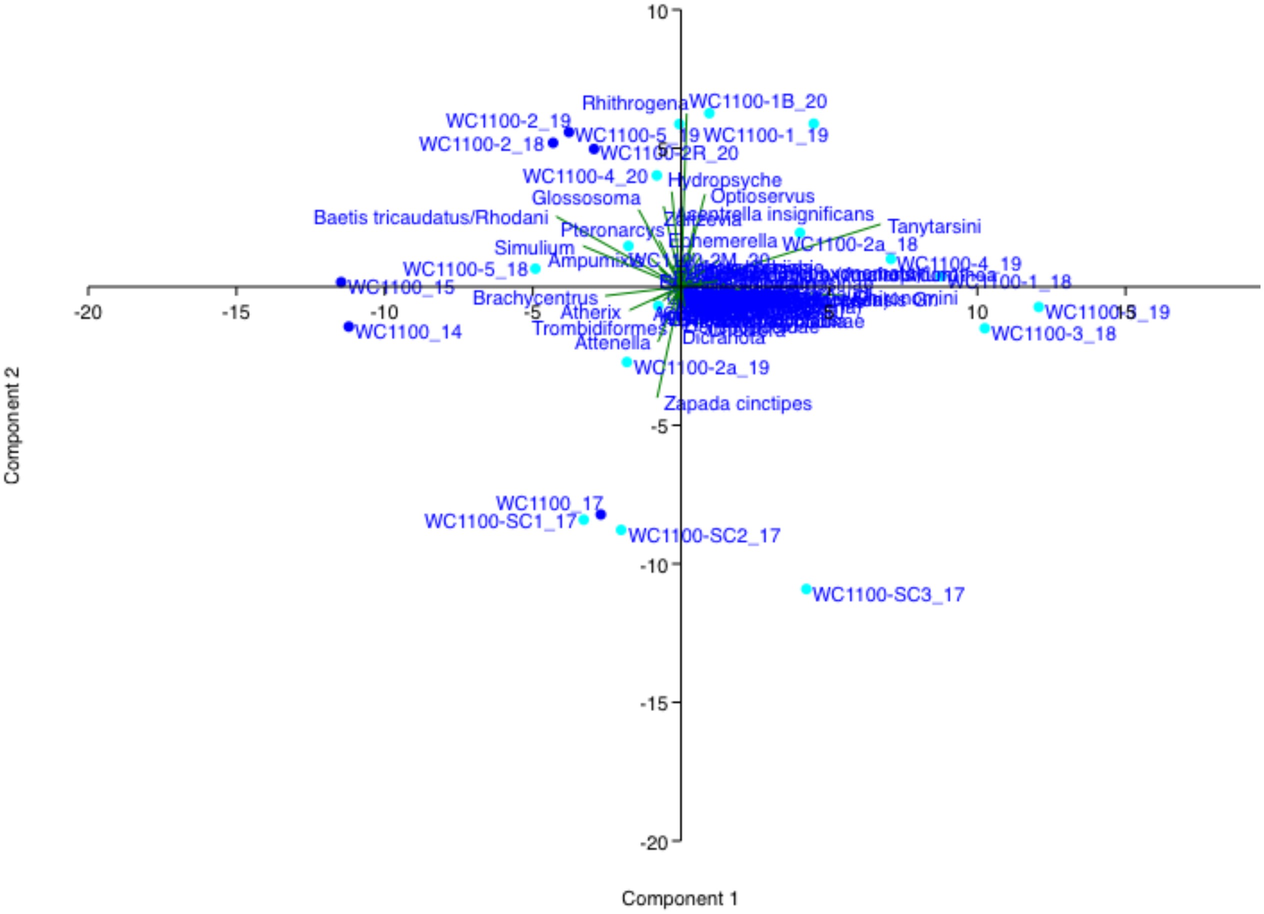
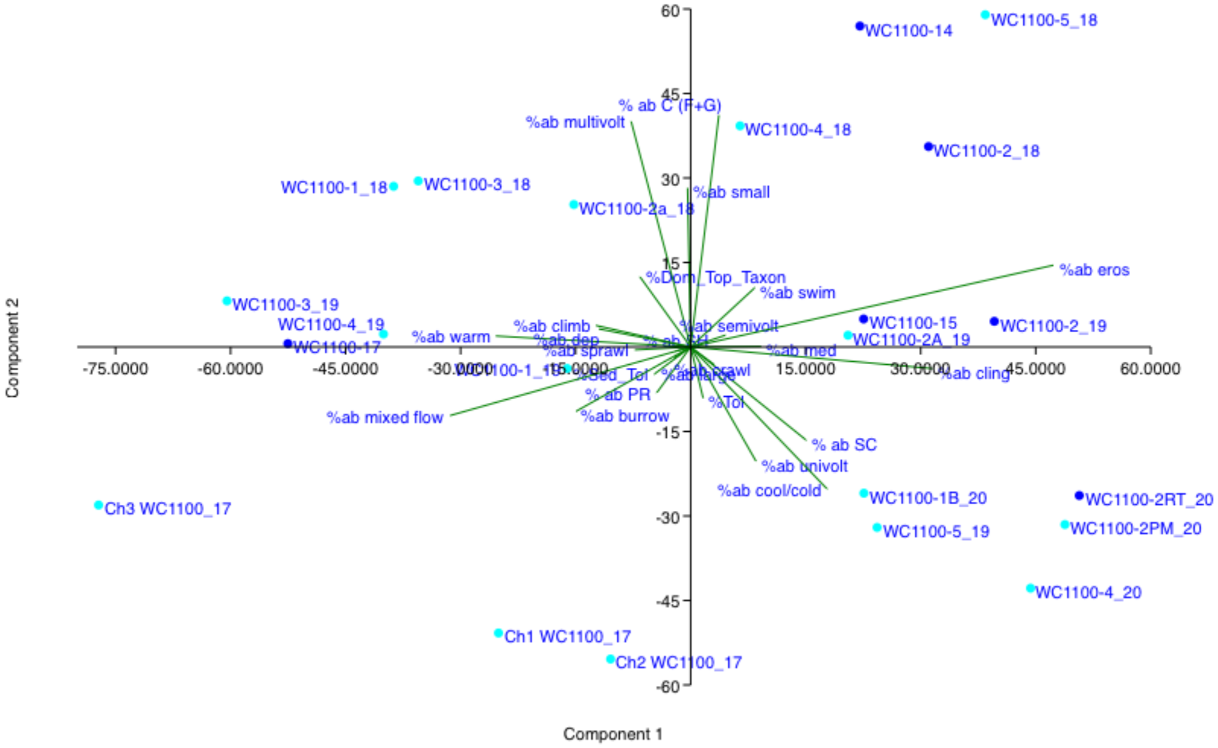


Figure 56. PCA ordination of the WC1100 macroinvertebrate community in all sampling years. *PM (aqua) and RT (blue) samples were taken in primary and side channel reaches in 2017-2020. Rt samples were taken in the primary channel in all earlier years. The number at the end of each label indicates the sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



In a PCA ordination of all traits measured as relative abundances (Figure 57), axis 1 explained 37% of the total variation; traits with the highest loading were relative abundances of organisms associated with erosional flows (more in primary channel samples from 2017-2020), mixed flows (more in side channel samples from 2017-2020), and clingers (more in primary channel samples from 2017-2020). Axis 2 explained an additional 28% of variation; traits with the highest loading were relative abundances of collectors (low in all 2017 side channel samples), multivoltine organisms (significantly lower in 2019-2020 samples compared to all earlier years), and small-bodied organisms (lower in 2017 samples).

Figure 57. PCA ordination of the WC1100 macroinvertebrate community traits measured as relative abundances in all sampling years. *PM (aqua) and RT (blue) samples were taken in primary and side channel reaches in 2017-2020; RT samples were taken in the primary channel all other years. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



WC1150

Sampling began at WC1150 in 2014 to provide an upstream reference for the WC1100 project site. An RT sample was taken in the primary channel from 2014-2017; in 2018, PM and RT samples were taken in the same reach, and in 2019-2020 only a PM sample was taken. Sample richness was intermediate for the 2020 dataset (56 taxa) but the second highest recorded at this site (Figure 58); increased richness in 2018-2020 is likely due in part to the fact that PM samples consistently have more taxa than RT samples in the same reach. The total number of EPT taxa (18) was at the lower end of the range among all 2020 samples, and continues a trend seen in 2019 of a slight decrease in this metric (Figure 58). The 2020 community was dominated by *Ampumixis dispar*, a sediment-sensitive riffle beetle associated with clear, cold flowing water, at 17.5% of total organismal abundance. The community consisted mainly of small, multivoltine clingers associated with cold flowing water that feed as collectors. This is similar to the overall community characteristics in prior years, although samples from more recent years had greater abundance of semivoltine organisms, with the most in 2020 (22.2%), and the appearance low numbers of climbers (1.3-6.2% relative abundance), which were not seen in any sample prior to 2018. The only taxon taken in the 2020 sample that was not seen at this site in other years was a single *Rhyacophila nevadensis*, a free-living caddisfly that inhabits rock surfaces in fast-flowing waters; this species was seen only once before in a Whychus sample (one individual at WC0600 in 2011).

The target sub-sampling number of organisms was attained in every year except 2017, with 12-100% of the total sample picked (Figure 59). Relative abundance of the dominant taxon at this site was low enough to receive the highest scaled score in the ORDEQ IBI in four of the seven sampling years (Figure 60). With the exception of 2017, when the sample had large numbers of tolerant segmented worms (Oligochaeta), dominant taxa have been more sensitive, sediment-intolerant, and associated with colder flowing water (*Baetis tricaudatus*, *Ampumixis dispar*, *Glossosoma*, *Cricotopus* (*Nostococladius*)).

Figure 58. Sample richness and number of EPT taxa at WC1150 in all sampling years. *RT samples were taken in 2014-2018; PM samples were taken in the same reach in 2018-2020. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*

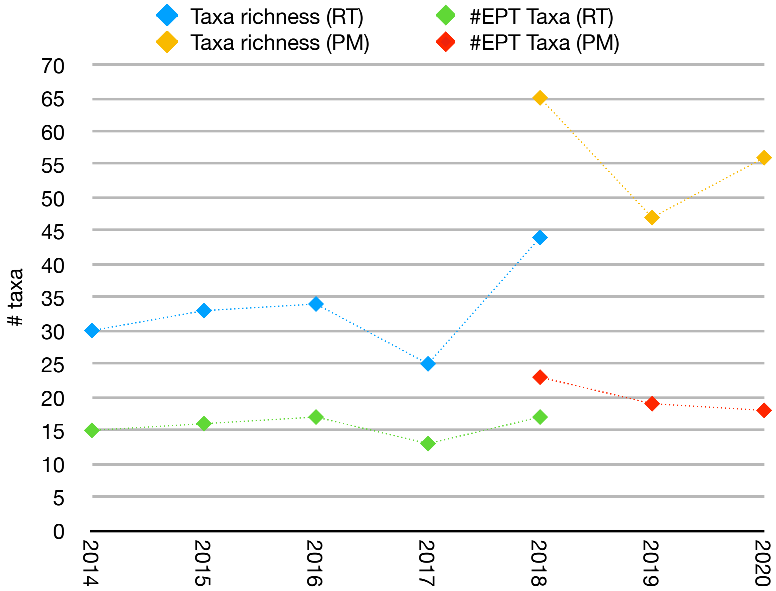


Figure 59. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC1150 in all sampling years. *RT samples were taken in 2014-2018; PM samples were taken in the same reach in 2018-2020.*

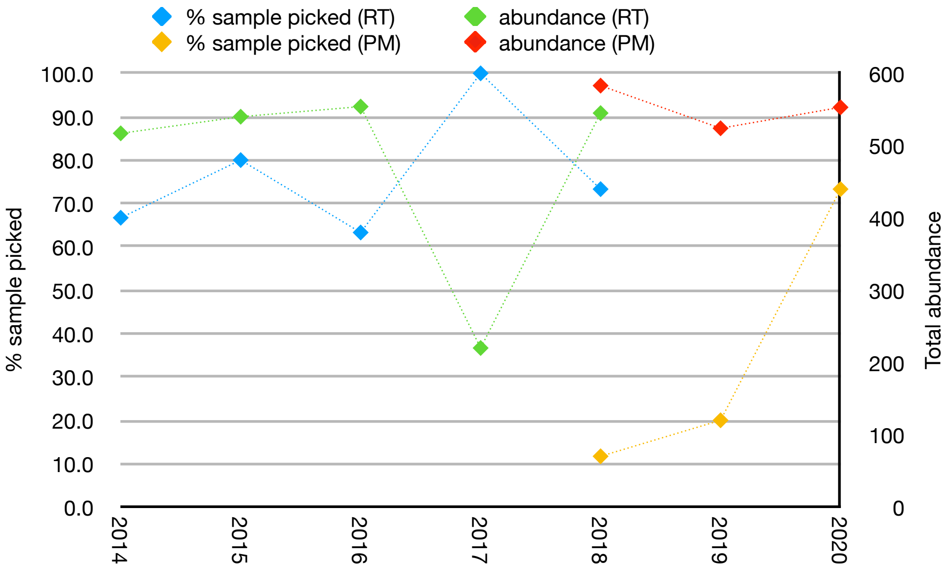
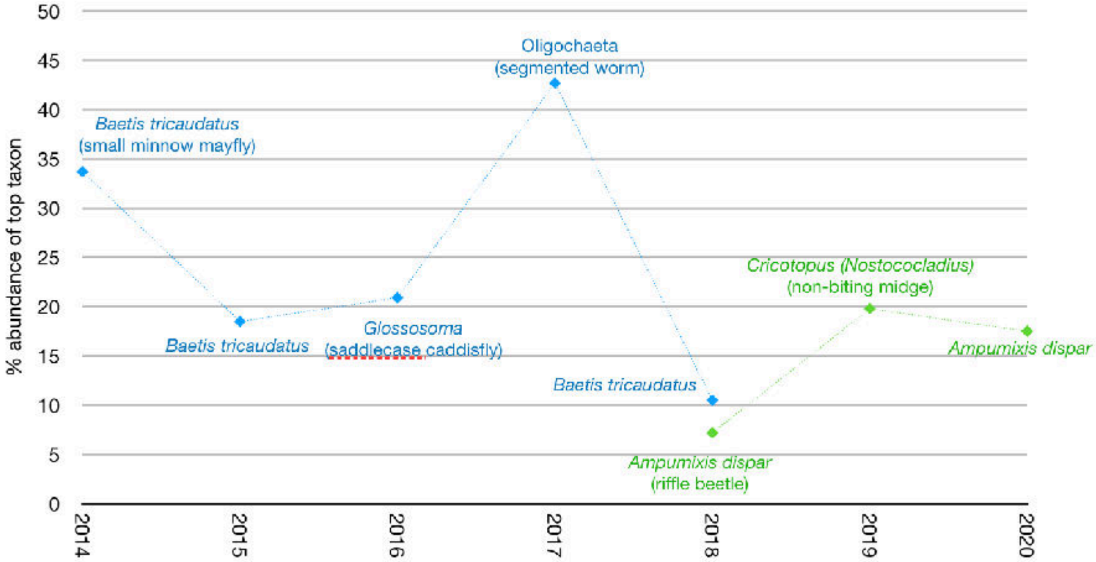


Figure 60. Relative abundance of the dominant taxon at WC1150 in all sampling years. *RT samples (blue) were taken in 2014-2018; PM samples (green) were taken in the same reach in 2018-2020. This metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*



In years when RT samples were collected, IBI scores indicated slight to no impairment except in 2017, which scored as moderately impaired. PREDATOR scores reflected fair to good biological conditions except in 2014 (poor). Sensitive and sediment-sensitive taxa increased two- to five-fold since 2017 (Figure 61), and PM samples from 2018-2020 had the most sensitive taxa at this site. Overall, relative abundance of tolerant organisms increased slightly while abundance of sediment-tolerant organisms decreased and was particularly low in samples from 2018-2020, but these trends were interrupted by a large anomalous increase in both values in 2017 (Figure 62). The number of DEQ cool temperature indicator taxa increased over time, but warm indicator taxa numbers varied more (Figure 63). The number of low sediment indicator taxa was consistent in PM and RT samples (Figure 63), and high sediment indicator numbers also changed little but fluctuated more in recent years. Community temperature optima decreased slightly in 2018-2020 PM samples (Figure 64); community sediment optima fluctuated and was unusually high in 2017 (8.3% FSS) but stabilized at a lower value in subsequent years (range = 6.0-7.0% FSS).

Figure 61. Numbers of sensitive and sediment-sensitive taxa at WC1150 in all sampling years. *RT samples were taken in 2014-2018; PM samples were taken in the same reach in 2018-2020. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and >2 sediment-sensitive taxa.*

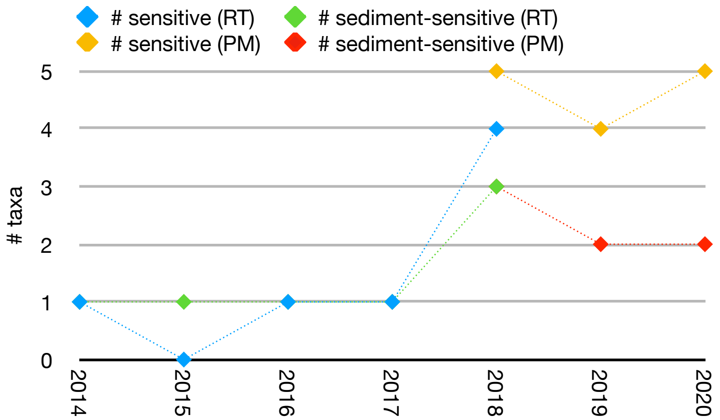


Figure 62. Relative abundance of tolerant and sediment-tolerant organisms at W1150 in all sampling years. *RT samples were taken in 2014-2018; PM samples were taken in the same reach in 2018-2020. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*

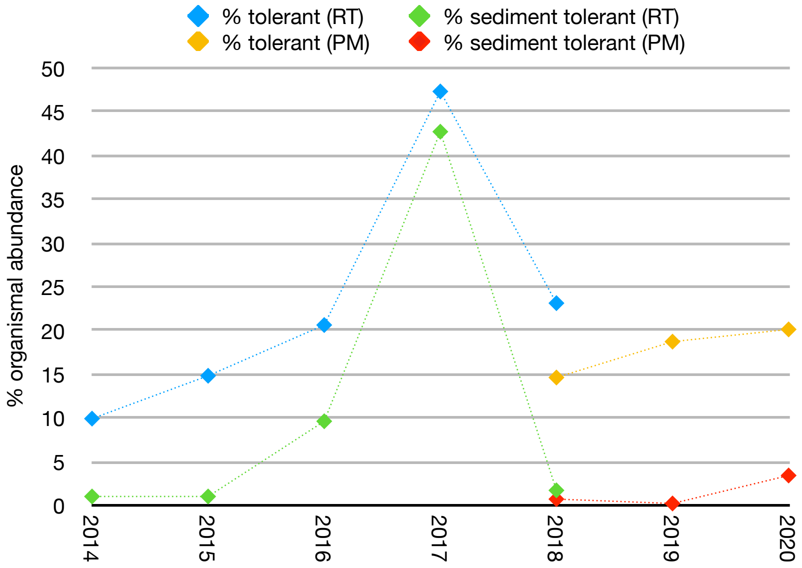


Figure 63. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC1150 in all sampling years. *RT samples were taken in 2014-2018; PM samples were taken in the same reach in 2018-2020. Note that ORDEQ indicators do not reflect the temperature or sediment associations of all taxa in a sample.*

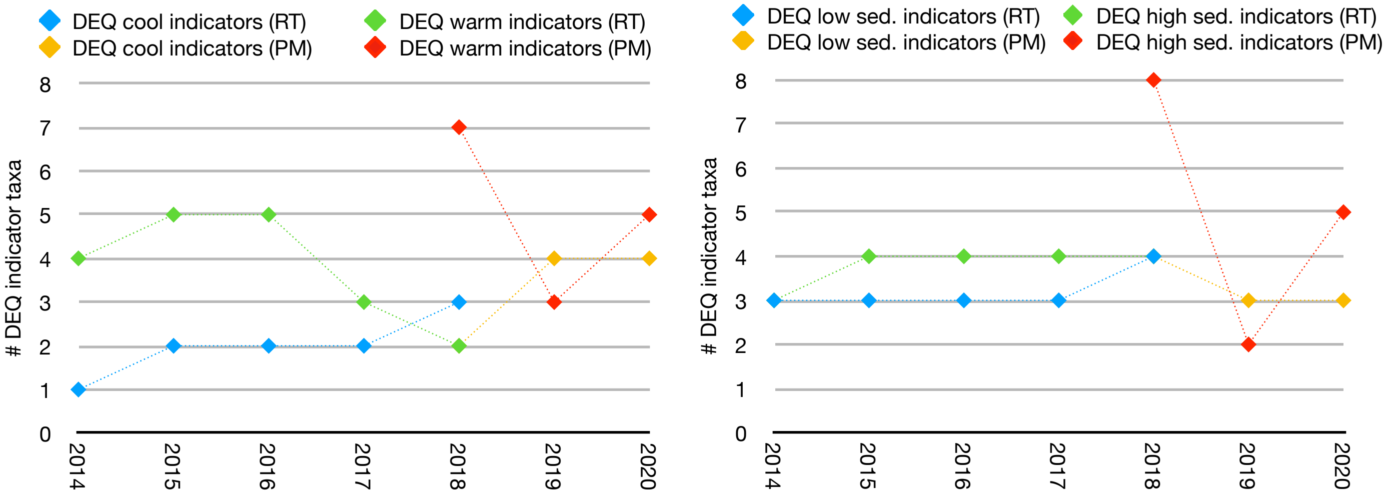
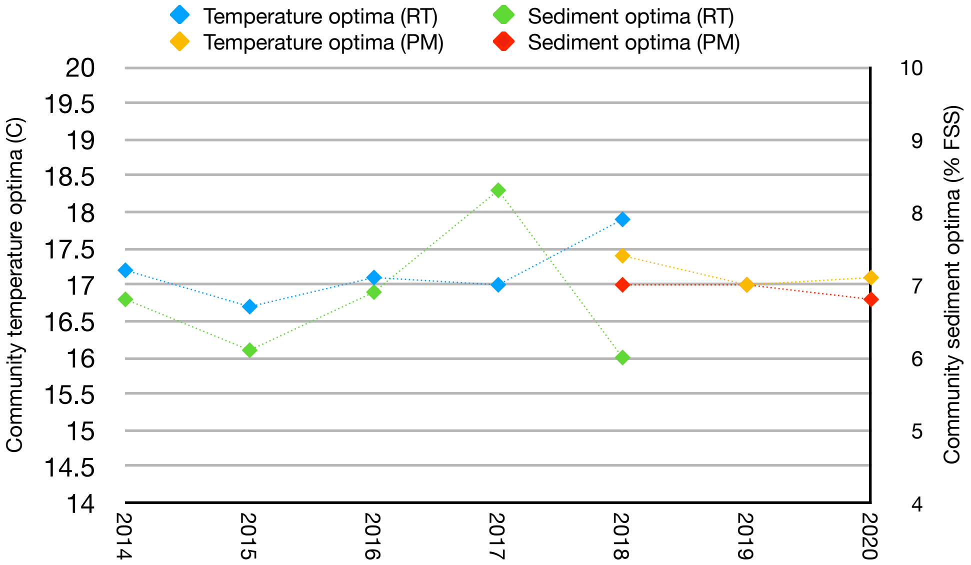


Figure 64. Community temperature and fine sediment optima (weighted means) at W1150 in all sampling years. *RT samples were taken in 2014-2018; PM samples were taken in the same reach in 2018-2020. Note that individual optima are not known for every taxon in a sample.*



Sample communities in 2018-2020 are more similar to each other than to communities in earlier years (Figure 65), and the 2014 and 2017 communities differ most from all others. In a PCA ordination of taxa abundances (Figure 66), axis 1 explained 27% of total sample variation; taxa with the highest loading values were *Ampumixis* (sediment-sensitive riffle beetle associated with clear, cold, flowing water; most abundant 2019-2020), Tanytarsini (tribe of non-biting midges that build tubes on soft sediments and are associated with mixed flows and warmer waters; abundant in 2018 and 2020), and *Baetis tricaudatus* (sediment-sensitive small minnow mayfly that prefers clear fast water and can be an early colonizer following disturbance; more abundant in 2014-2015). Axis 2 explained an additional 21% of total variation; taxa with the highest loading values were Annelida (tolerant segmented worms; highest abundance in 2016 and 2017), *Baetis tricaudatus*, and *Glossosoma* (sediment-sensitive saddlecase-maker caddisfly in cool fast water; most abundant in 2016).

In a PCA ordination of all traits measured as relative abundances (Figure 67), axis 1 explained 61% of total sample variation; traits with the highest loading values were relative abundances of taxa associated with erosional flows (very low in 2017), burrowers (very abundant in 2017), and clingers (abundant in all sampling years except 2017). Axis 2 explained an additional 26% of total variation; traits with the highest loading were relative abundance of scrapers (lower in 2014, 2017, and 2018), organisms that prefer cooler temperatures (lower abundances in 2014 and 2017), and burrowers.

Figure 65. CLUSTER dendrogram of the WC1150 macroinvertebrate community in all sampling years. *RT samples (blue) were taken in 2014-2018; PM samples (aqua) were taken in the same reach in 2018-2020. The number at the end of each label indicates sampling year.*

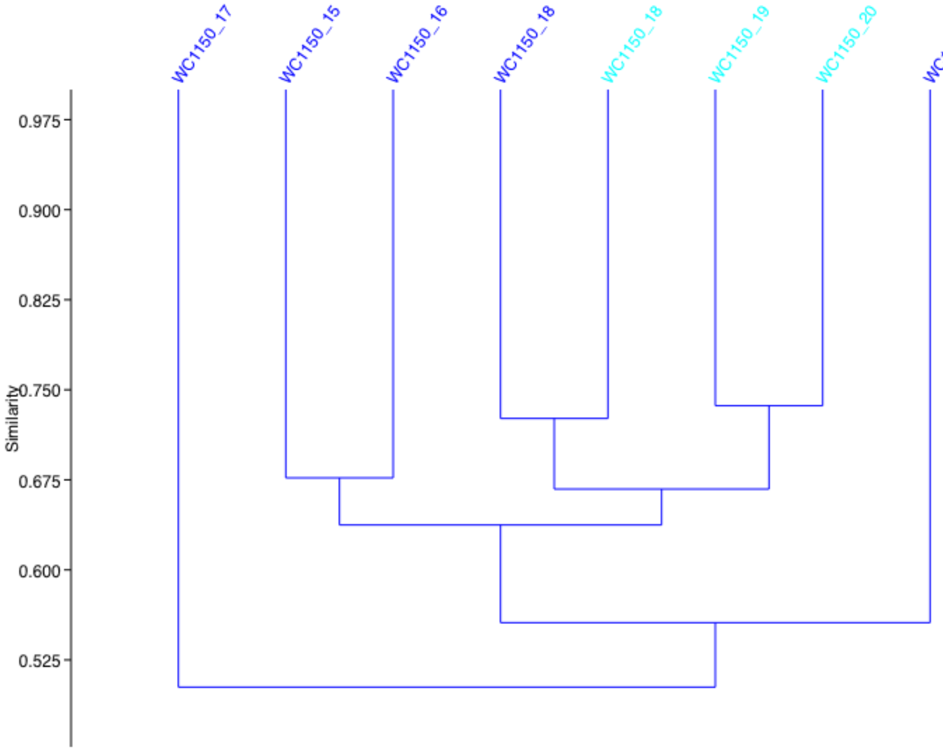


Figure 66. PCA ordination of the WC1150 macroinvertebrate community in all sampling years. *RT samples (blue) were taken in 2014-2018; PM samples (aqua) were taken in the same reach in 2018-2020. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*

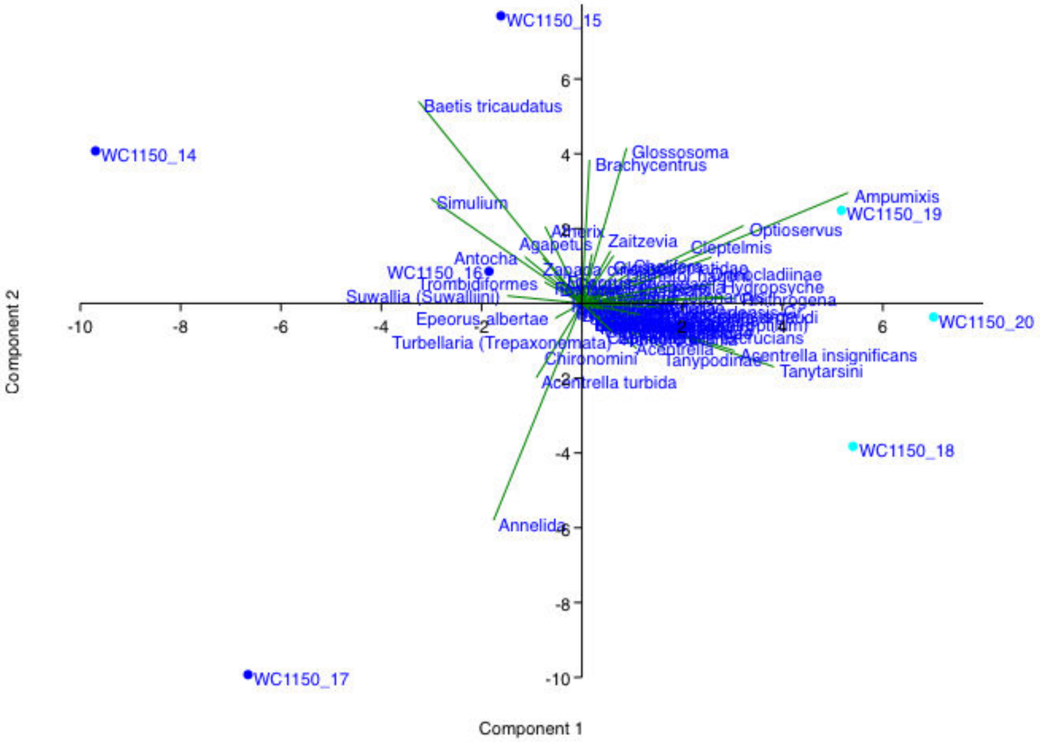
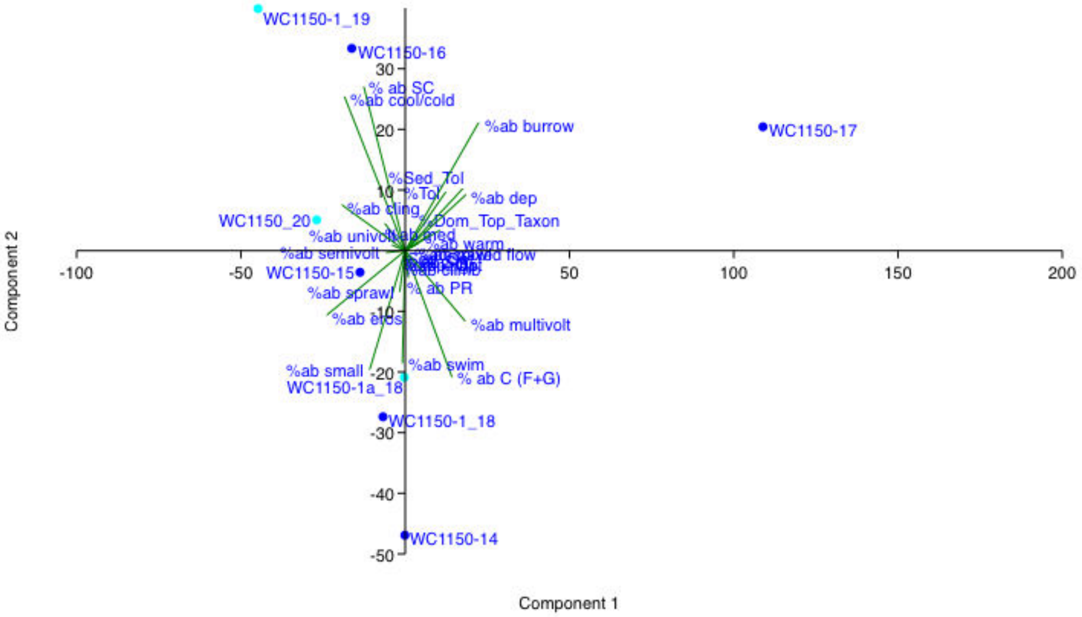


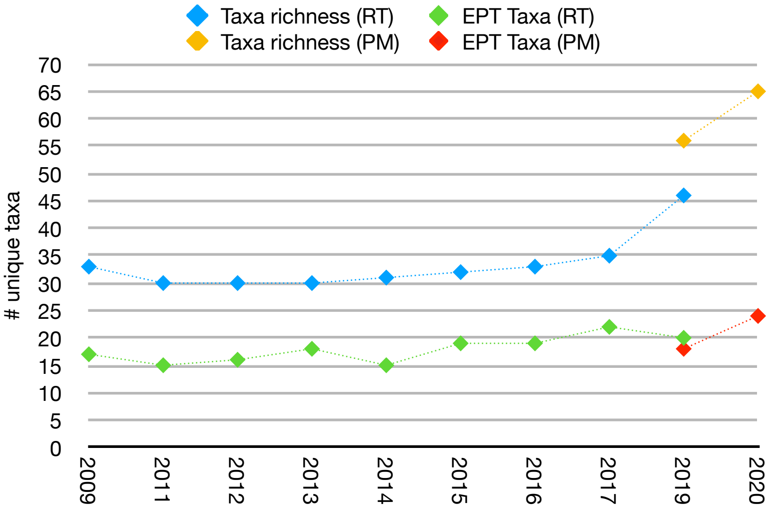
Figure 67. PCA ordination of the WC1150 macroinvertebrate community traits measured as relative abundances in all sampling years. *RT samples were taken in 2014-2018; PM samples were taken in the same reach in 2018-2020. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



<u>WC1950</u>

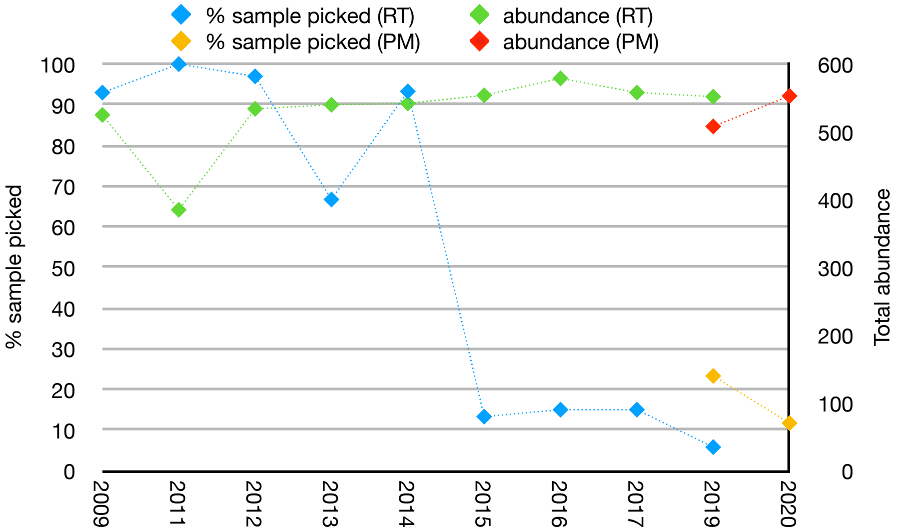
An RT sample was taken in a primary channel reach at WC1950 from 2009-2019 except in 2018, when other restored reaches were prioritized for sampling. A PM sample was taken in the same primary channel reach in 2019 and 2020. Total richness (65 unique taxa) and number of EPT taxa (24) were both at the upper end of the range in the 2020 dataset and the most ever taken at this site (Figure 68). Increased richness is influenced by the greater diversity in PM vs. RT samples but this is likely not the only factor, as RT sample richness increased overall from 30 to 35 in 2009-2017, and was even greater in the 2019 RT sample (46 taxa). The 2020 community was dominated by *Optioservus*, a tolerant and sediment-tolerant riffle beetle that inhabits sediments and detritus in flowing and still habitats in streams; relative abundance of this taxon (7.1%) was the lowest among all 2020 samples and of any sampling year at this site. The 2020 community was composed mainly of small, multivoltine clingers that prefer colder water and multiple flow types. No single feeding guild dominated, and there were similar relative abundances of collectors, predators, and scrapers. The 2020 PM sample also had the most large-bodied organisms, shredders, and organisms associated with colder temperatures compared to all earlier sampling years, and PM samples in both 2019 and 2020 had more climber and sprawlers than earlier years. Two taxa were taken for the first time at this site, both of which occurred at low abundance (1-2 individuals): *Anafroptilum (Centroptilum)*, a tolerant small minnow mayfly associated with cooler waters and sandy substrate in slower flows; and Physidae, a snail family tolerant of warmer temperatures and fine sediment and found in a variety of habitats on soft silty substrates. Both were taken in other sites/years.

Figure 68. Sample richness and number of EPT taxa at WC1950 in all sampling years. *RT samples were taken in 2009-2019; PM samples were taken in the same reach in 2019-2020. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



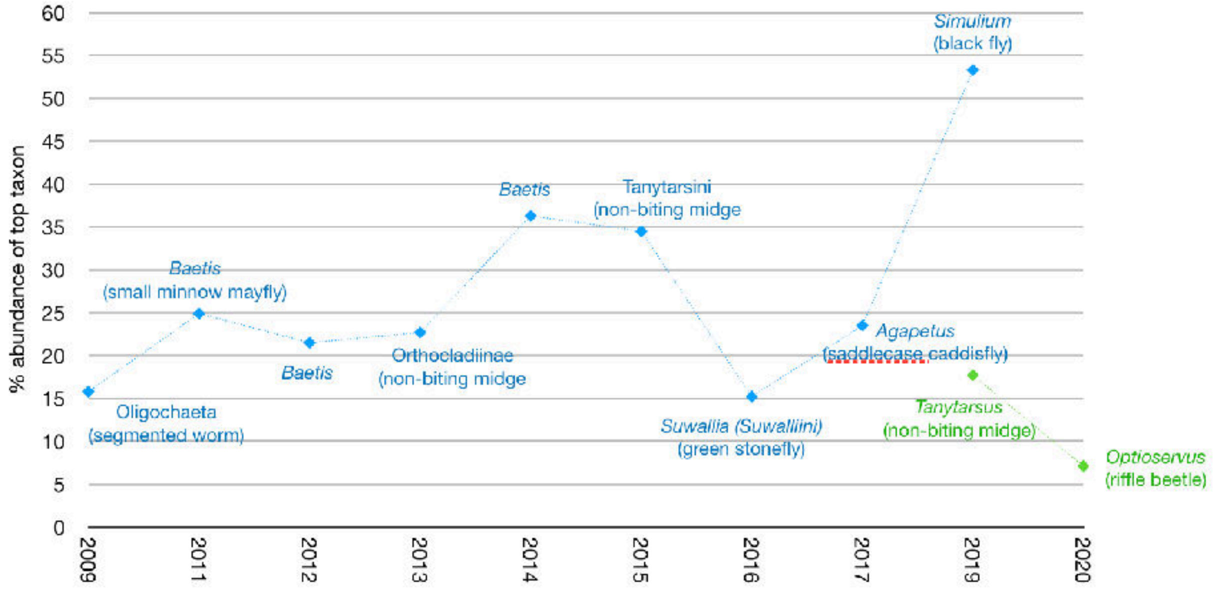
The target sub-sampling number of organisms was attained every year except 2011, with 6-100% of the total sample picked (Figure 69), with much less sample needed in 2015-2020. Relative abundance of the dominant taxon in RT samples was low enough to receive the highest scaled score in the ORDEQ IBI (<20%) in only two sampling years (Figure 70), one prior to restoration (2009) and the other four years post-restoration (2016), and this value more than doubled in RT samples from 2017 to 2019. The 2017 sample was dominated by *Agapetus* caddisflies (23.5% of total abundance), which feed as scrapers in cold fast flows, while the 2019 RT sample was heavily dominated by *Simulium* black flies (53.3% of total abundance), which feed as collectors in flowing water. The different feeding modes combined with the fact that an abundance of *Simulium* can indicate recent recovery from disturbance suggests changes in habitat occurred between these sampling years. In contrast, relative abundance of the dominant taxon in PM samples taken in 2019 and 2020 was below 20%. In both years the dominant taxon in PM samples was a more

Figure 69. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC1950 in all sampling years. *RT samples were taken in 2009-2019; PM samples were taken in the same reach in 2019-2020.*



tolerant type, and in 2019 shared the same feeding guild as the *Simulium* that dominated the RT sample the same year. Dominant taxa at this site are generally associated with mixed or erosional flows and their characteristics range from tolerant and/or burrowing taxa (i.e., Oligochaeta, Tanytarsini, Tanytarsus, Orthocladiinae, *Optioservus*) to more sensitive and associated with cold water (*Agapetus*, *Baetis*, *Suwallia*).

Figure 70. Relative abundance of the dominant taxon at WC1950 in all sampling years. *RT samples were taken in 2009-2019; PM samples were taken in the same reach in 2019-2020. This metric in the ORDEQ IBI receives the highest scaled score at <20% relative abundance.*



In years when RT samples were taken, IBI scores indicated slight or no impairment, and samples taken in 2012 as well as in the two years that followed restoration had the same IBI score (36; slight impairment). PREDATOR scores mostly indicated poor or fair conditions, including in 2012 (O/E = 0.82; fair). O/E scores were lower in the two years following restoration, but returned to the almost the same value (0.81) in each sample taken in 2015-2019. An increase in the number of sensitive taxa from 2011-2013 was followed by a decrease for several years, but the numbers have been rising since 2017, and the 2020 RT sample had the most sensitive taxa ever taken at this site (Figure 71). Numbers of sediment-sensitive taxa are lower but have increased overall since 2013 (Figure 71).

Despite relative abundance of tolerant and sediment-tolerant organisms exhibiting an increasing trend post-restoration, especially since 2015, abundance of tolerant organisms has remained with the range of the highest scaled score in the DEQ IBI in every RT sample taken after 2005 except in 2017 (Figure 72), although both PM samples in 2019-2020 exceed this threshold. Abundance of sediment-tolerant organisms was in or very near the range for the top scaled score in the IBI for this metric in both PM and RT samples from 2011-2020 (Figure 72). There were more DEQ cool temperature than warm indicator taxa and more low sediment than high sediment indicator taxa in almost every RT sample (Figure 73), while PM samples had more warm temperature and high sediment indicators. Community temperature optima increased over the last three sampling years but were slightly lower in 2020, while community sediment optima were highest in samples from the past two years (Figure 74).

Figure 71. Sensitive and sediment-sensitive taxa in WC1950 samples. *RT samples were taken in 2009-2019; PM samples were taken in the same reach in 2019-2020. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≤2 sediment-sensitive taxa.*

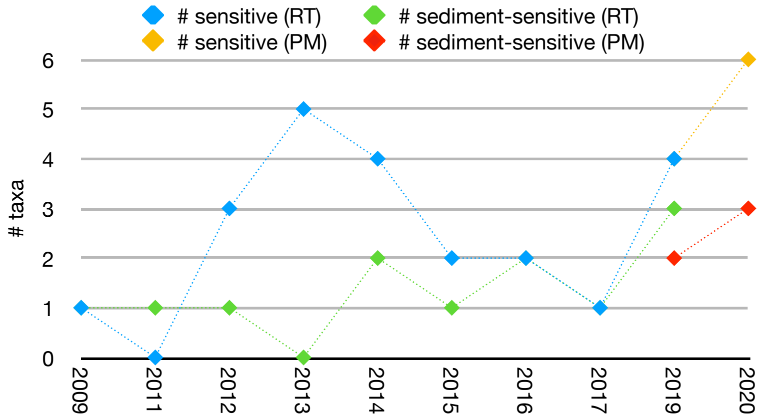


Figure 72. Relative abundance of tolerant and sediment-tolerant organisms at W1950 in all sampling years. *RT samples were taken in 2009-2019; PM samples were taken in the same reach in 2019-2020. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*

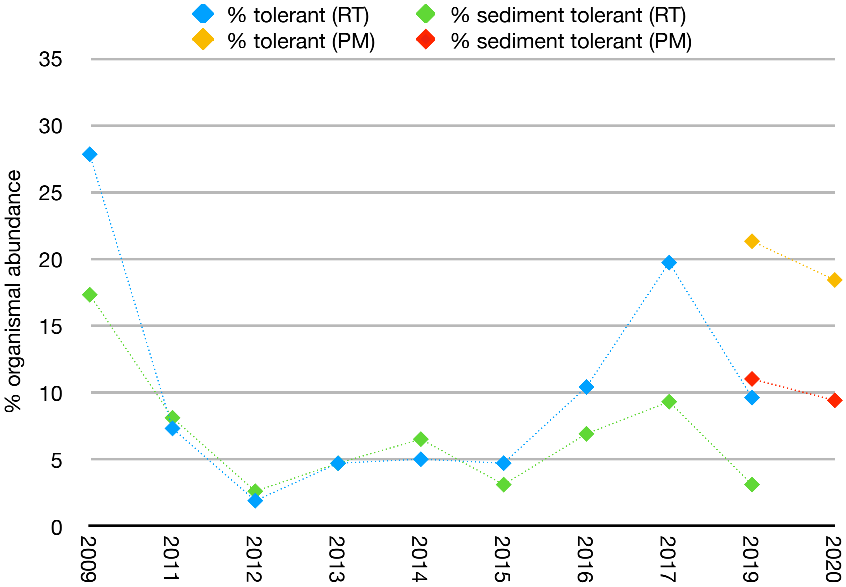


Figure 73. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC1950 in all sampling years. *RT samples were taken in 2009-2019; PM samples were taken in the same reach in 2019-2020. Note that ORDEQ indicators do not account for temperature or sediment associations of all taxa in a sample.*

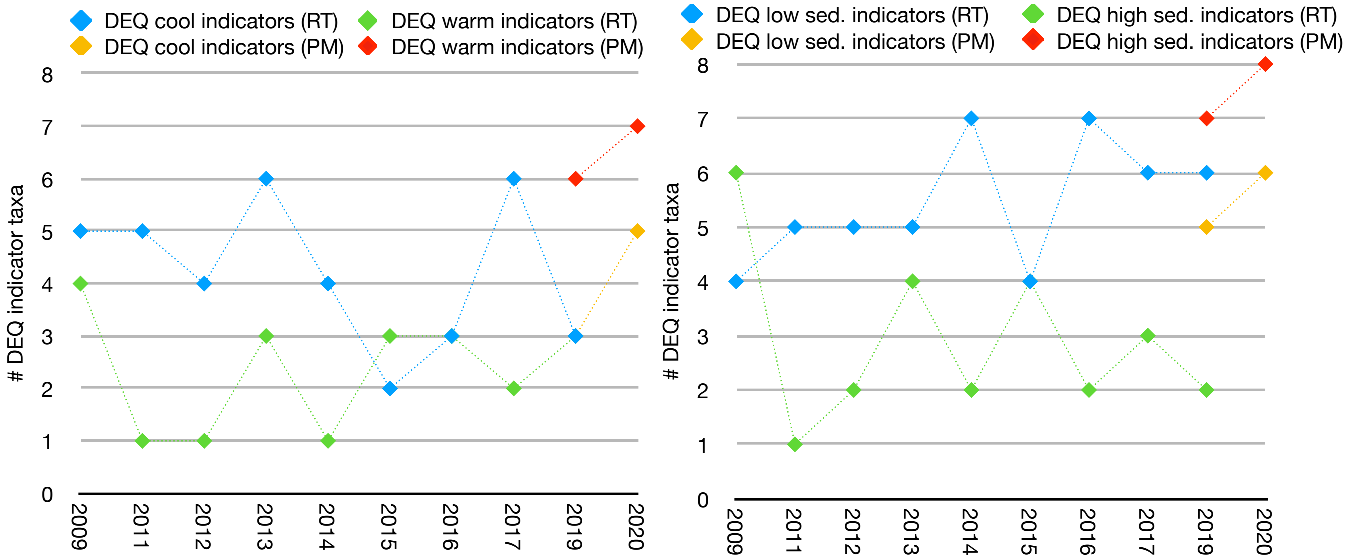
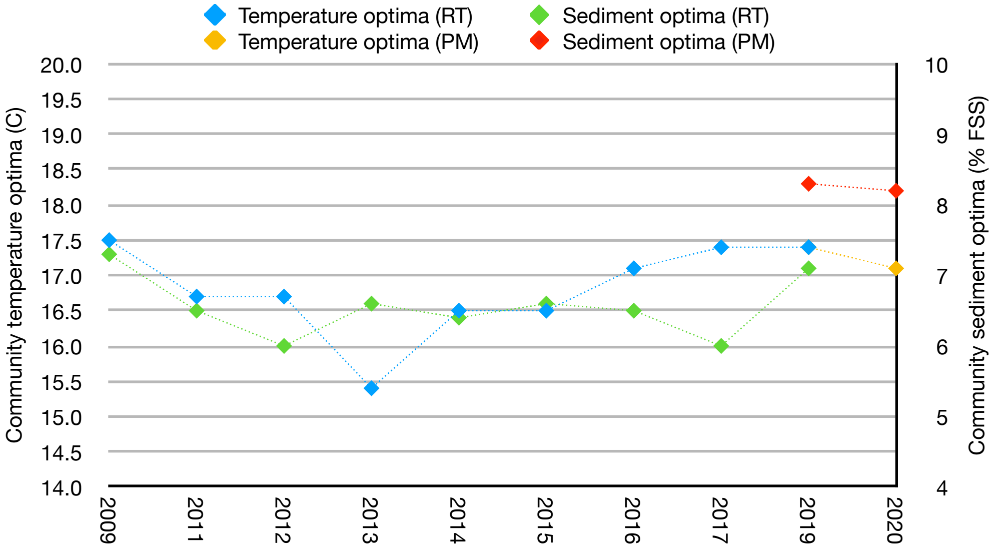
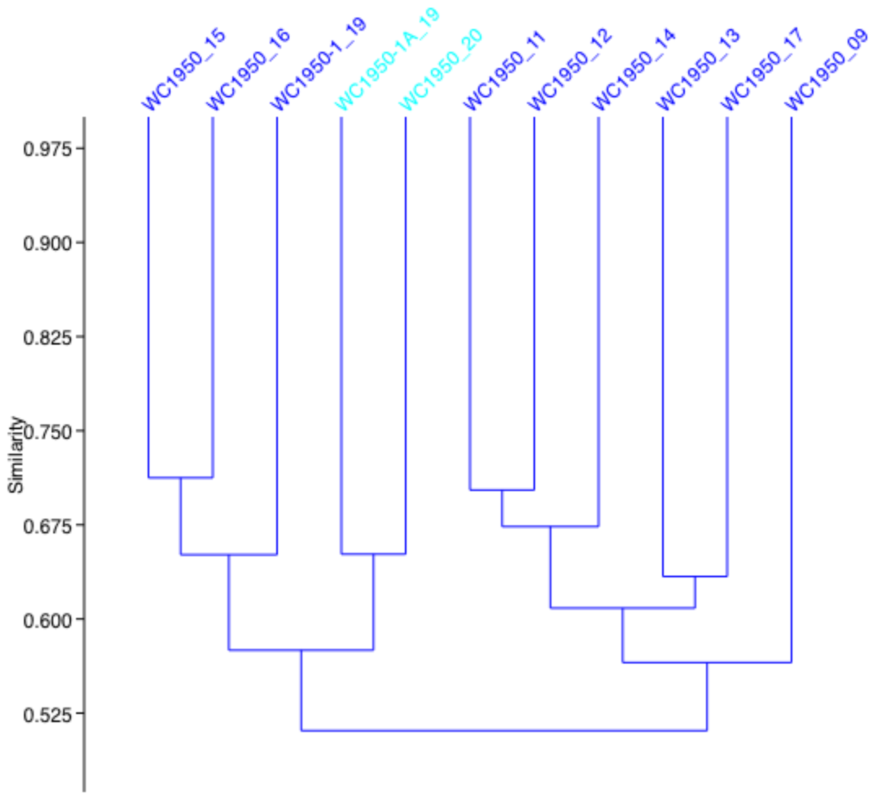


Figure 74. Temperature and fine sediment optima of the community (weighted means) at W1950 in all sampling years. *RT samples were taken in 2009-2019; PM samples were taken in the same reach in 2019-2020. Note that individual optima are not known for every taxon in a sample.*



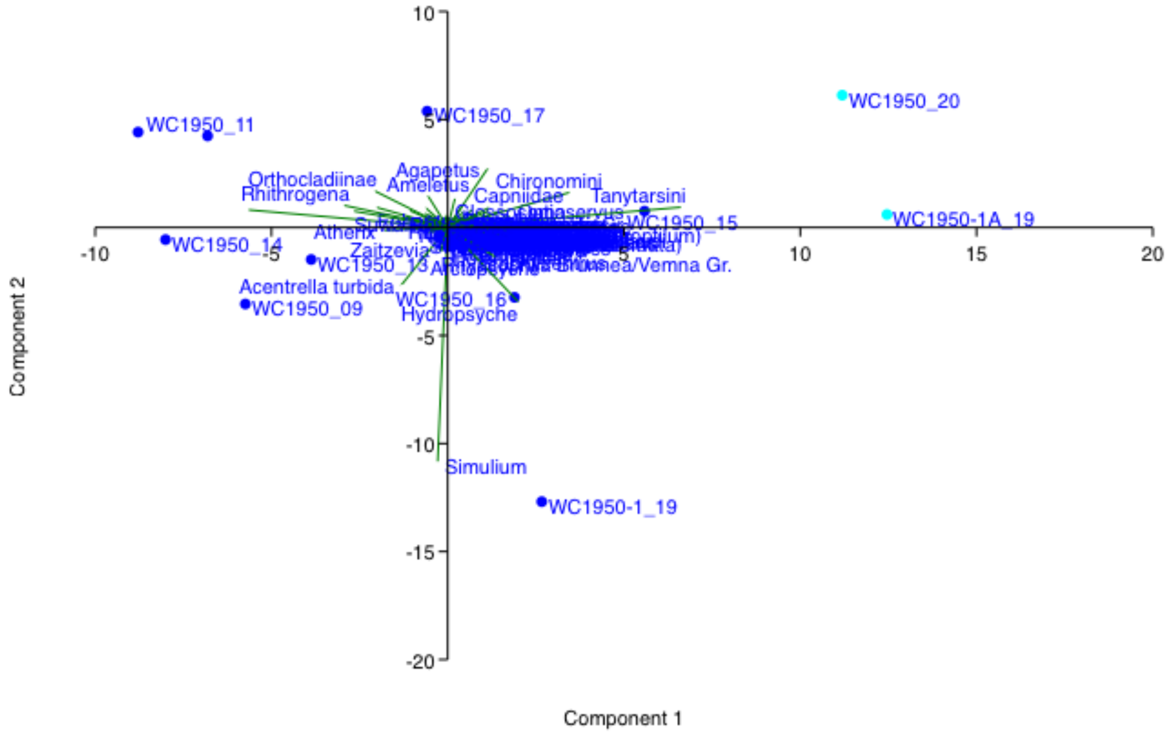
The community in recent years (2019-2020) is most similar to those in 2016 and 2015 (Figure 75), and unlike many other restored sites at Whychus, the communities in years immediately prior to and following restoration did not differ greatly. The most notable differences among all post-restoration sample communities (PM and RT) compared to prior years include greater abundances of several riffle beetle genera (*Cleptelmis*, *Ampumixis*, *Optioservus*), *Chelifera* dance flies (a predator found in multiple flow and habitat types), some mayflies (spiny crawler mayfly *Attenella* and Leptophlebiidae prong-gilled mayflies), and the non-biting midge subfamilies Tanypodinae (often associated with mosses in cold springs, brooks, and streams) and Diamesinae (associated with cold water in multiple flow types) as well as the Tanytarsini tribe (more tolerant, associated with warmer waters). In addition, there are fewer *Atherix* snipe flies (tolerant predators found in multiple flow types), *Rhithrogena* flat-headed mayflies, and a steady decrease of two orders of magnitude in *Baetis tricaudatus* abundance. Changes in abundances of many of the above taxa were not seen until 2016, however. The 2019 and 2020 PM samples were the first in which the Prodiamesinae subfamily of non-biting midges was seen (associated with springs, streams, ponds, and the littoral zone of lakes), and relative abundance of the tolerant non-biting midge tribe Chironomini was 30-50 times higher than in previous years.

Figure 75. CLUSTER dendrogram of the WC1950 macroinvertebrate community in all sampling years. *RT samples (blue) were taken in 2009-2019; PM samples (aqua) were taken in the same reach in 2019-2020. The number at the end of each label indicates sampling year.*



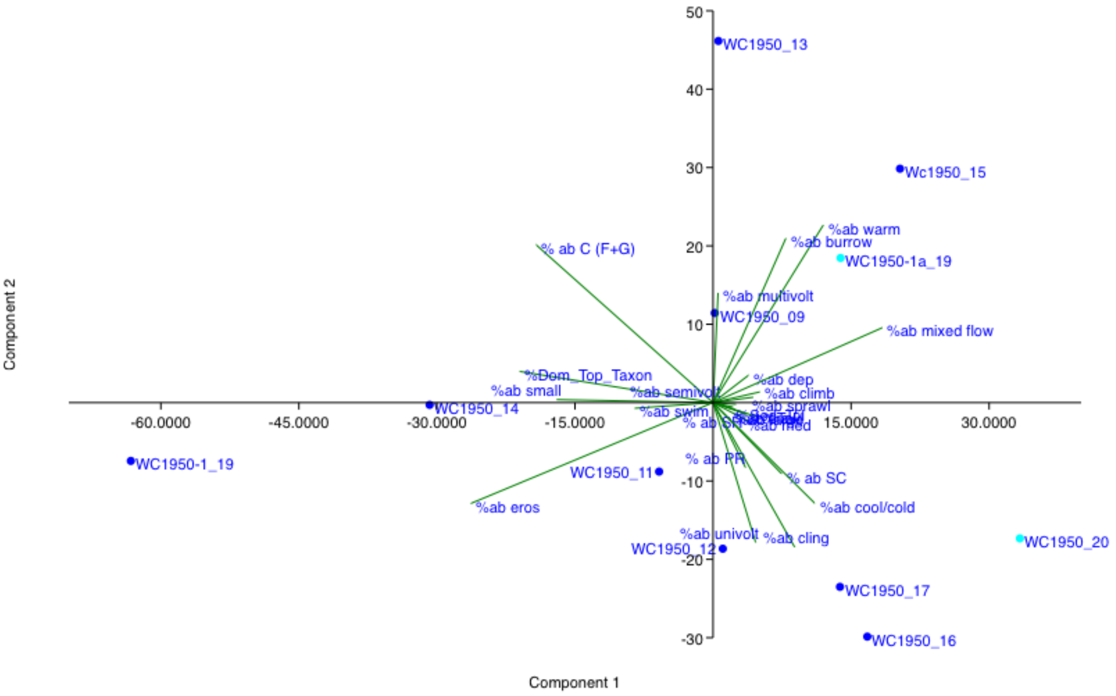
In a PCA ordination of taxa abundances (Figure 76), axis 1 explained 34% of total sample variation, and taxa with the highest loading values were Tanytarsini, a tolerant non-biting midge tribe associated with warmer waters (more abundant 2015-2020), *Baetis tricaudatus* (deceasing abundances after 2014), and Chironomini (more abundant in PM samples). Axis 2 explained an additional 18% of variation, and taxa with the highest loading were *Simulium* black flies (present in all sampling years but much more abundant in 2019 RT sample); *Agapetus*, a somewhat sediment-sensitive saddlecase-maker caddisfly associated with cold fast water (higher abundances in 2016 and 2017), and *Hydropsyche*, a tolerant netspinner caddisfly and DEQ warm temperature indicator taxon (absent from five of the eight samples taken from 2009-2017 and from the 2020 sample).

Figure 76. PCA ordination of the WC1950 macroinvertebrate community in all sampling years. *RT samples (blue) were taken in 2009-2019; PM samples (aqua) were taken in the same reach in 2019-2020. The numeral in each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



In a PCA ordination of all traits measured as relative abundances (Figure 77), axis 1 explained 31% of total sample variation; traits with the highest loading were relative abundances of organisms associated with faster flows and of the top taxon (both lower in PM samples), and organisms that feed by collecting (highest in the 2019 RT sample, due to dominance by *Simulium*). Axis 2 explained an additional 24% of total variation, and traits with the highest loading were relative abundances of organisms associated with warmer temperatures and a burrowing habit (both highest in 2013 and 2015) as well as collectors (especially high in the 2019 RT sample).

Figure 77. PCA ordination of the WC1950 macroinvertebrate community traits measured as relative abundances in all sampling years. *RT samples (blue) were taken in 2009-2019; PM samples (aqua) were taken in the same reach in 2019-2020. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



WC2000

Sampling was done for the first time to provide baseline data prior to restoration; a PM and RT sample were taken simultaneously in the same primary channel reach. The two sample communities were very similar (Bray Curtis similarity index = 0.72), although the PM sample was even more similar to the WC1950 PM sample (Bray-Curtis similarity index = 0.78). Both had high evenness (see Table 4) and low relative abundance of the dominant taxon (7.7% of total abundance in PM sample, 14.6% in RT). The IBI score of the RT sample (42) indicated unimpaired biological condition, and the PREDATOR score (0.90) corresponded to fair biological condition. An IBI score cannot be calculated for the PM sample, but raw values for six of the 10 IBI metrics were in the range that receives the highest scaled score (#Trichoptera taxa, #sediment-sensitive taxa, % dominance top taxon, %tolerant and %sediment-tolerant organisms. and MHBI).

The PM sample was composed mainly of small-bodied, multivoltine, clinging organisms that prefer cooler, fast and mixed flows and feed as collectors. It was dominated by *Tanytarsus*, a common non-biting midge that tolerates a wide range of habitats and ecological conditions and builds tubes on soft sediments. The RT sample was composed mainly of small- and medium-bodied, multivoltine and univoltine, clinging organisms that prefer cooler, faster flows and feed as scrapers and collectors. It was dominated at by *Glossosoma*, a sediment-sensitive saddlecase-making caddisfly associated with cold, clear, rapid streams. Relative abundance of tolerant organisms in both samples were among the lowest of all 2020 samples (14.2% and 9.8% in PM and RT, respectively; range in 2020 = 9.8-50.3%).

Total richness was higher in the PM sample (62 unique taxa) than the RT (48 unique taxa), but the number of EPT taxa was similar (19 in PM, 20 in RT) and at the upper end of the range in the 2020 sample set (range = 13-23). The number of DEQ cool temperature indicator taxa was high (5 taxa in PM, 6 in RT) and was exceeded only at WC2600 (8 taxa), and the number of warm indicator taxa in the RT sample (3 taxa) was among the lowest of all 2020 samples (range = 1-8 taxa). Community temperature optima (17.1^{o}C in PM, 16.9^{o}C in RT) were closer to the lower end of the range for all 2020 samples (range = 16.3-18.2^{o}C). Both samples had five DEQ low sediment indicator taxa, which was intermediate for the range seen in 2020 (range = 3-8 taxa). The RT sample had fewer high sediment indicator taxa (3 taxa) and the lowest community sediment optima (6.5% FSS) of all 2020 samples (range = 6.5-11.7% FSS). Although the PM sample had more high sediment indicator taxa (8 taxa), the community sediment optima (7.6%) was still closer the lower end of the range for this value among 2020 samples.

WC2050

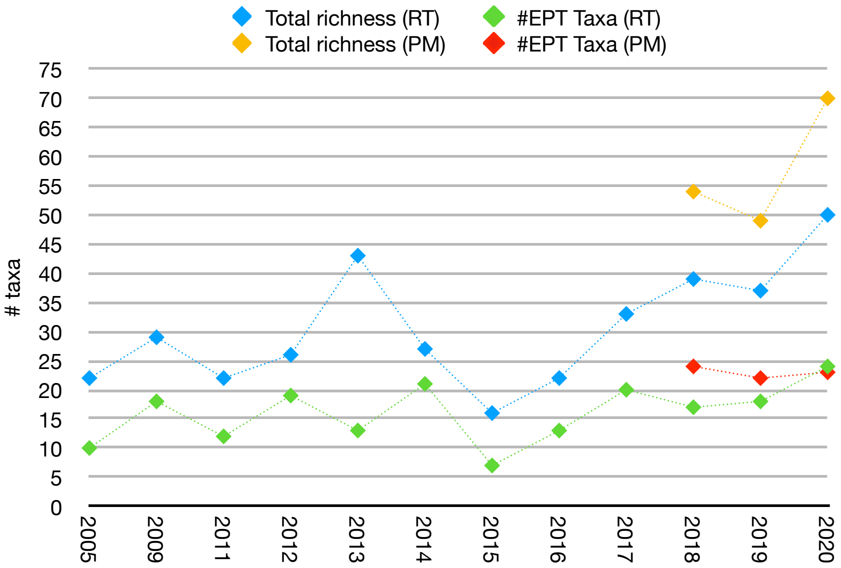
Sampling was done for the first time to acquire baseline data prior to restoration. Two PM samples were taken simultaneously in a primary channel reach to provide a site duplicate for quality control, though different areas of habitat were sampled for each (see Table 2). Duplicate sample communities paired in a CLUSTER dendrogram of all 2020 samples (Bray Curtis similarity index = 0.75; see Figure 1), and both were more similar to the communities in the WC2000 and WC1950 samples. Although it is not appropriate to calculate an IBI score for a PM sample, raw values for seven of the 10 metrics corresponded to the highest scaled IBI score (numbers of total, Trichoptera, sensitive, and sediment-sensitive taxa; relative abundance of top taxon and sediment-tolerant organisms, and MHBI).

Sample richness was intermediate among the 2020 sample set (53 unique taxa) and EPT richness was at the upper end of the 2020 range (21 EPT taxa). The sample community was composed mainly of small-bodied, multivoltine and univoltine clingers that prefer cooler fast and mixed flows and feed as collectors and scrapers. It was dominated at 10.3% relative abundance by *Ochrotrichia*, a tolerant micro-caddisfly that feeds on algae and fine organic detritus in a variety of flow types in cold springs, lakes, and rivers, including temporary streams. The numbers of DEQ cool temperature (6 taxa) and low sediment (5 taxa) indicator taxa were in the upper portion of the 2020 sample ranges, and the numbers of warm temperature and high sediment indicator taxa were intermediate, as was the community temperature optima (17.3^{o}C). However, this sample had lower relative abundance of organisms associated with warmer waters (2.6%) and of sediment-tolerant taxa (1.7%) than any other 2020 sample, and the community sediment optima was at the lower end of the 2020 range (6.6% FSS). This site also had the fewest scrapers and the most shredders of all 2020 samples.

WC2600

Riffle-targeted sampling was done in a primary channel reach at WC2600 every sampling year from 2005 through 2020 including in 2014, when the stream was directed into a new meandering channel. In 2018, additional PM samples were taken in side channels; in 2019 and 2020, as the restored stream structure developed, the reach in which RT and PM samples were taken included primary and side channel habitat. The 2020 sample communities were most similar to each other (Bray-Curtis similarity index = 0.62) and least similar to any other 2020 sample (see Figure 1). The PM sample had the most unique taxa (70) of any 2020 sample, and while there were fewer total taxa in the RT sample (50), both had more EPT (24 taxa in PM, 23 in RT) than any other 2020 sample. Total and EPT richness increased steadily since 2015 and were higher in 2020 than in any prior year (Figure 78). Seven taxa taken in 2020 were not found here in any earlier sampling year, including the riffle beetles *Ampumixis* and *Cleptelmis* (both associated with colder, faster flows); Hydrophilidae (water scavenger beetle family associated with vascular hydrophytes in warmer slower flows); *Epeorus albertae* (a flatheaded mayfly that is moderately sensitive to sediment and tolerant of warmer waters, found on bottoms of rocks in faster flows); *Siphlonurus* (a tolerant primitive minnow mayfly associated with vascular hydrophytes in slower flows); Aeshnidae (a tolerant family of darner dragonfly associated with vascular hydrophytes in a variety of flow types); and *Rhyacophila atrata* (a free-living caddisfly associated with cold, clear, fast water). All new taxa were present at very low abundance (1-3 individuals) and with the exception of *R. atrata*, which was seen in both the PM and RT sample, were found only in the PM sample. All of these taxa were found in other sites/years.

Figure 78. Sample richness and number of EPT taxa at WC2600 in all sampling years. *Only samples taken in the primary channel are shown. RT samples were taken every year; a PM sample was also taken in 2018-2020. This metric in the ORDEQ IBI receives the highest scaled score at >35 total taxa.*



The dominant taxon in each sample was the same and occurred at similar relative abundance (27.0% abundance in RT, 28.9% in PM): *Cricotopus (Nostococladius)*, a sediment-intolerant non-biting midge that burrows into *Nostoc* blue-green algae in cold clear waters. Communities in both samples consisted primarily of small, multivoltine clingers and burrowers that feed as collectors and scrapers and are associated with cooler, faster flows. Both had more DEQ cool temperature indicator taxa (8 taxa in each) and low sediment indicator taxa (7 taxa in RT, 8 in PM) than any other 2020 sample, although the PM sample also had the most high sediment indicator taxa. The WC2600 samples also had the lowest community temperature optima among all 2020 samples (16.3^{o}C in RT, 16.7^{o}C in PM).

Organismal abundances varied, with the sub-sampling target of 500 individuals met in seven of the 11 years in which RT samples were taken, after picking 21.7-100% of the total sample (Figure 79). This target was attained in all PM samples, with 30-90% of the total sample picked. Prior to restoration, the community was dominated by Orthocladiinae, a common family of non-biting midges that are primarily burrowers and tube-builders in warmer waters and mixed flows (Figure 80). In the years following restoration, the community was less balanced, and the high abundance of *Simulium* black flies in 2016 suggests ongoing recovery from disturbance. In recent years, the dominant taxon occurred at lower relative abundances and in all but one sample (2019 PM) was associated with faster and/or colder waters and greater sensitivity to fine sediments.

Figure 79. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC2600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in 2018-2020.*

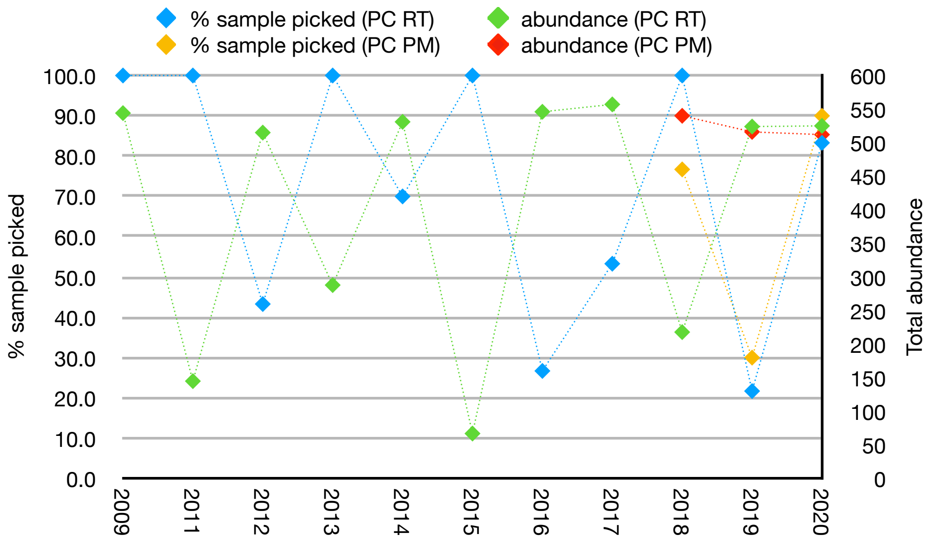
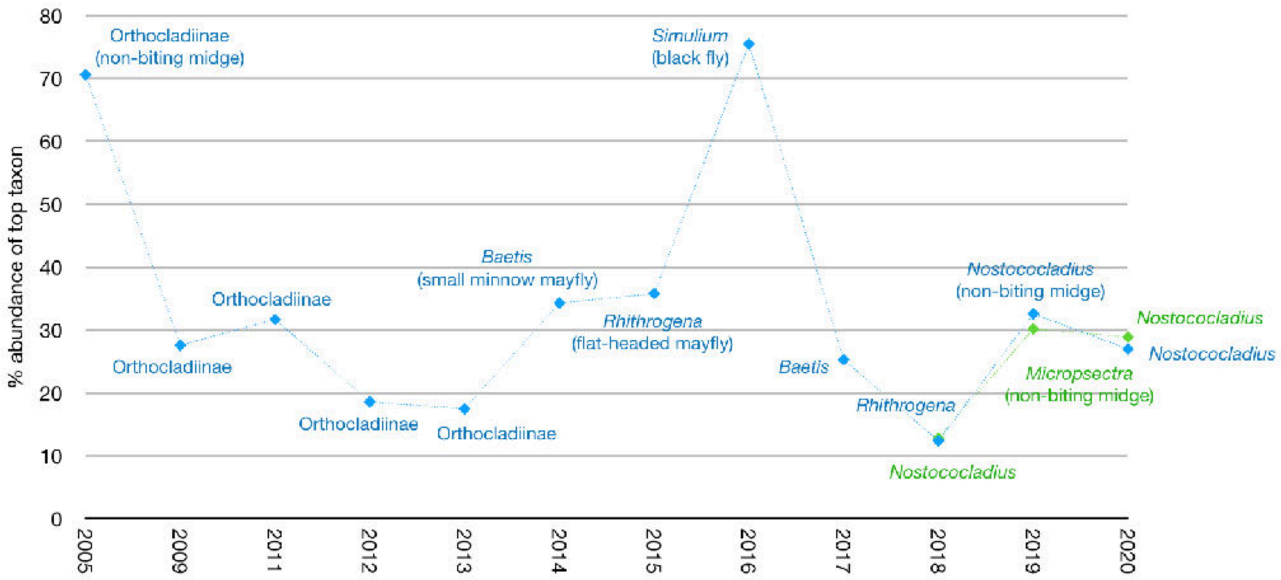
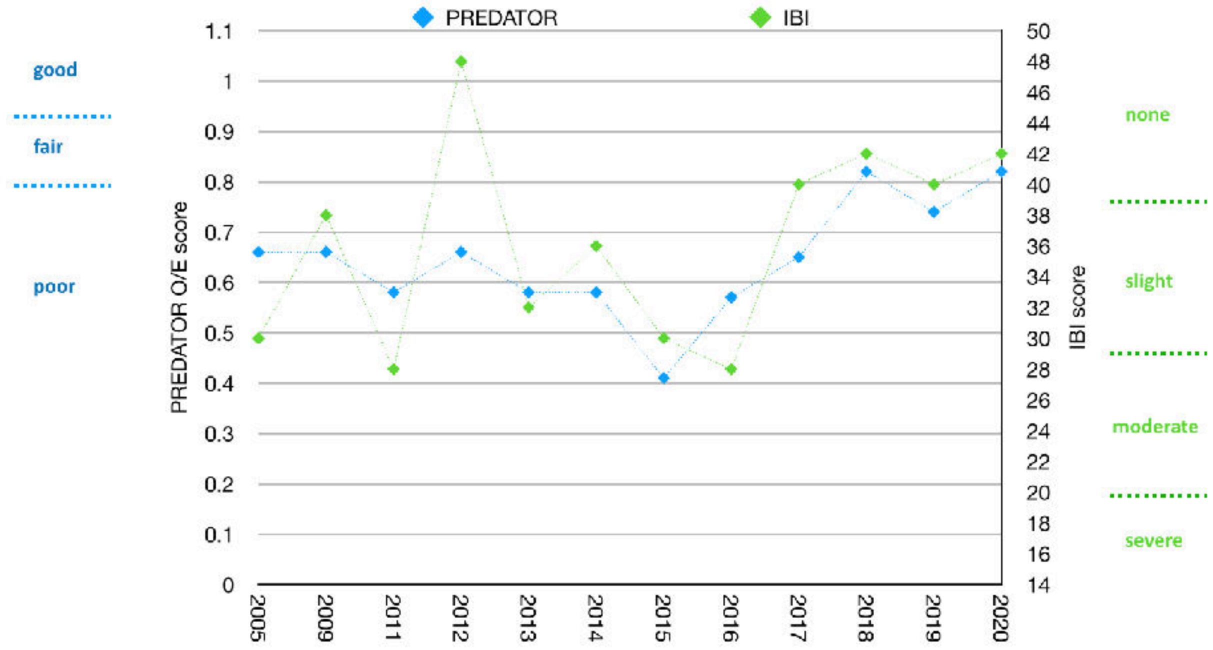


Figure 80. Relative abundance of the dominant taxon at WC2600 in all sampling years. *RT samples (blue) were taken every year; a PM sample (green) was also taken in 2018-2020. This metric receives the highest scaled score in the ORDEQ IBI at <20% abundance.*



IBI and PREDATOR scores in RT samples increased since 2016 (Figure 81), and were higher in 2020 than in any other year. PREDATOR scores indicated poor conditions from 2005 through 2017 but were in the fair range for two of the last three years, including 2020. IBI scores fluctuated in earlier years, but indicated unimpaired conditions in samples since 2017. Similarly, numbers of sensitive taxa, which fluctuated greatly between 2005 and 2015, increased steadily since 2015 and are consistently high in PM samples; the number of sediment-sensitive taxa increased as well but especially in RT samples (Figure 82). Relative abundance of tolerant organisms fluctuated greatly from year to year but was in the range to receive the highest scaled score for this metric in the ORDEQ IBI in 10 of the 12 years in which RT samples were taken and in all PM samples (Figure 83), although there were more in both samples taken in 2020 compared to 2019. Relative abundance of sediment-tolerant organisms also fluctuated and was in the range to receive the highest scaled IBI score in eight of the years in which RT samples were taken and in all PM samples.

Figure 81. PREDATOR O/E and ORDEQ IBI scores at WC2600 in all sampling years.



There were more DEQ cool temperature indicator taxa than warm indicators in every sampling year except 2005 in both RT and PM samples, although the 2020 PM sample had more warm indicator taxa than any prior year (Figure 84). The number of low sediment indicator taxa also greatly exceeded the number of high sediment indicators in RT samples in every sampling year (Figure 84). However, the number of high sediment indicator taxa in PM samples tripled in 2019 and again in 2020, and there were more high sediment than low sediment indicator taxa in the 2020 PM sample. Community temperature optima at WC2600 are generally among the lowest of all the Whychus sites and are similar or identical in PM and RT samples taken in the same year, but have increased slightly overall since sampling began (Figure 85). Community sediment optima also increased over time, and were higher in PM compared to RT samples in 2019 and 2020.

Figure 82. Numbers of sensitive and sediment-sensitive taxa at WC2600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in 2018-2020. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

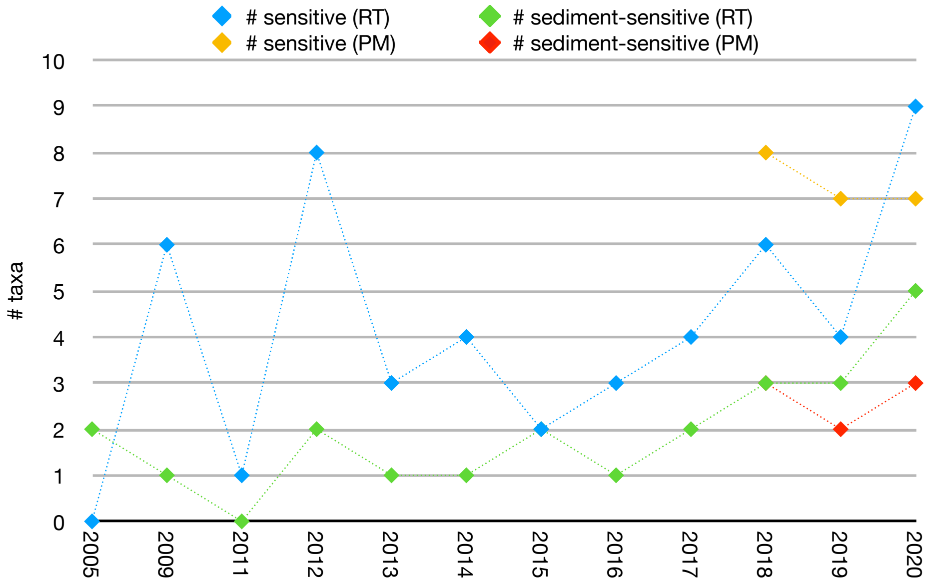


Figure 83. Relative abundance of tolerant and sediment-tolerant organisms at WC2600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in 2018-2020. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*

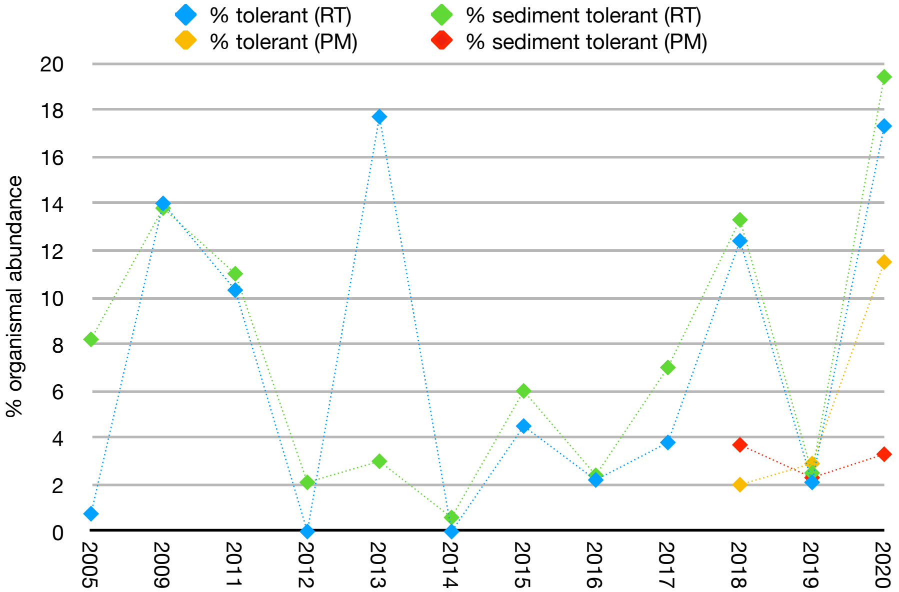


Figure 84. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC2600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in 2018-2020. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

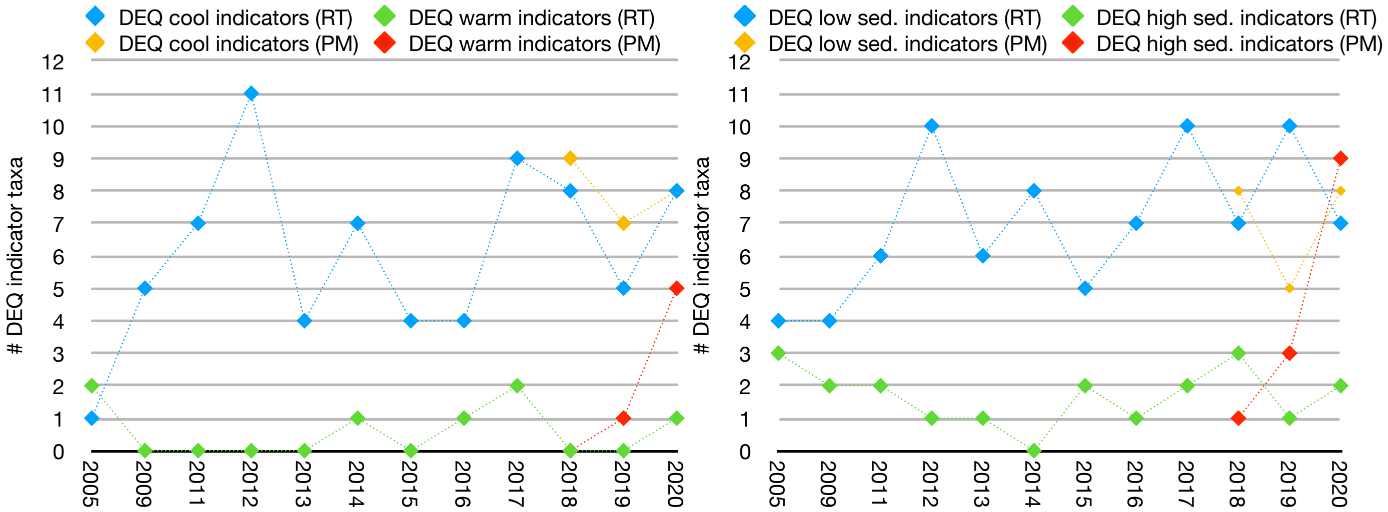
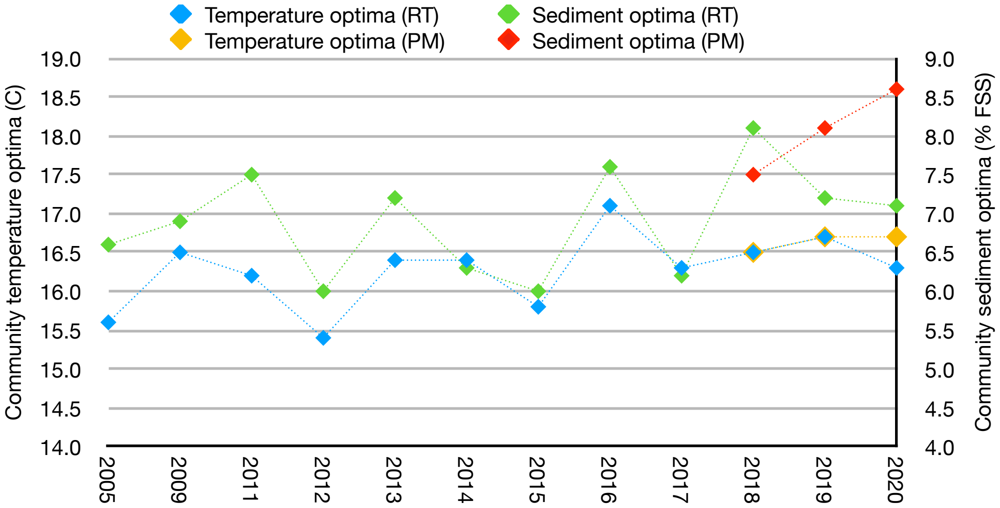


Figure 85. Temperature and fine sediment optima of the community (weighted means) at W2600 in all sampling years. *RT samples were taken every year; a PM sample was also taken in 2018-2020. Note that individual optima are not known for every taxon in a sample.*



Macroinvertebrates communities in all PM samples were more similar to each other than to RT sample communities (Figure 86), despite being taken in a variety of reach types (side channel, primary channel, PC/SC). The 2020 PM sample community (PC/SC) was more similar to 2018 side channels (average Bray-Curtis similarity index = 0.66) than to the 2020 RT sample taken in the same reach (Bray-Curtis similarity index = 0.65). The 2020 and 2019 RT communities, both of which were taken in a PC/SC reach, were more similar to each other (Bray-Curtis similarity index = 0.65) than to any PM sample. In a PCA ordination of taxa abundances (Figure 87), axis 1 explained 33% of total sample variation, and taxa with the highest loading values were Tanytarsini (tolerant non-biting midge tribe associated with warmer waters), *Zapada cinctipes* (forest stonefly inhabiting a variety of substrates in cold fast flows), and *Rhithrogena* (flatheaded mayfly that clings to stones in cold fast flows). Tanytarsini abundance was very low from 2005-2017 (0-1 individuals) and while members were present in every sample from 2018-2020, there were fewer in RT (9-23 individuals) compared to PM samples (40-177 individuals). There were few *Zapada cinctipes* in RT samples from 2005-2020 and in the 2020 PM sample (0-5 individuals) but abundance was an order of magnitude greater in PM samples from 2018-2019. *Rhithrogena* was found in every sampling year but at much lower abundance in 2018 primary and side channel reach samples. Axis 2 explained an additional 16% of variation; taxa with the highest loading values were *Simulium*, a black fly that clings to stones in flowing waters and is an early colonizer following disturbance (increased abundance in 2016 and 2019 PC/SC samples); Tanytarsini; and Orthocladiinae, a common family of non-biting midge that are burrowers and tube-builders in warmer waters and mixed flows (present in all sampling years but most abundant in 2005).

Figure 86. CLUSTER dendrogram of the WC2600 macroinvertebrate community in all sampling years. *PM (aqua) and RT samples (blue) were taken in primary and side channel reaches in 2018-2020. The number at the end of each label indicates sampling year. PC = primary channel, SC = side channel, PC/SC = reach with both primary and side channel.*

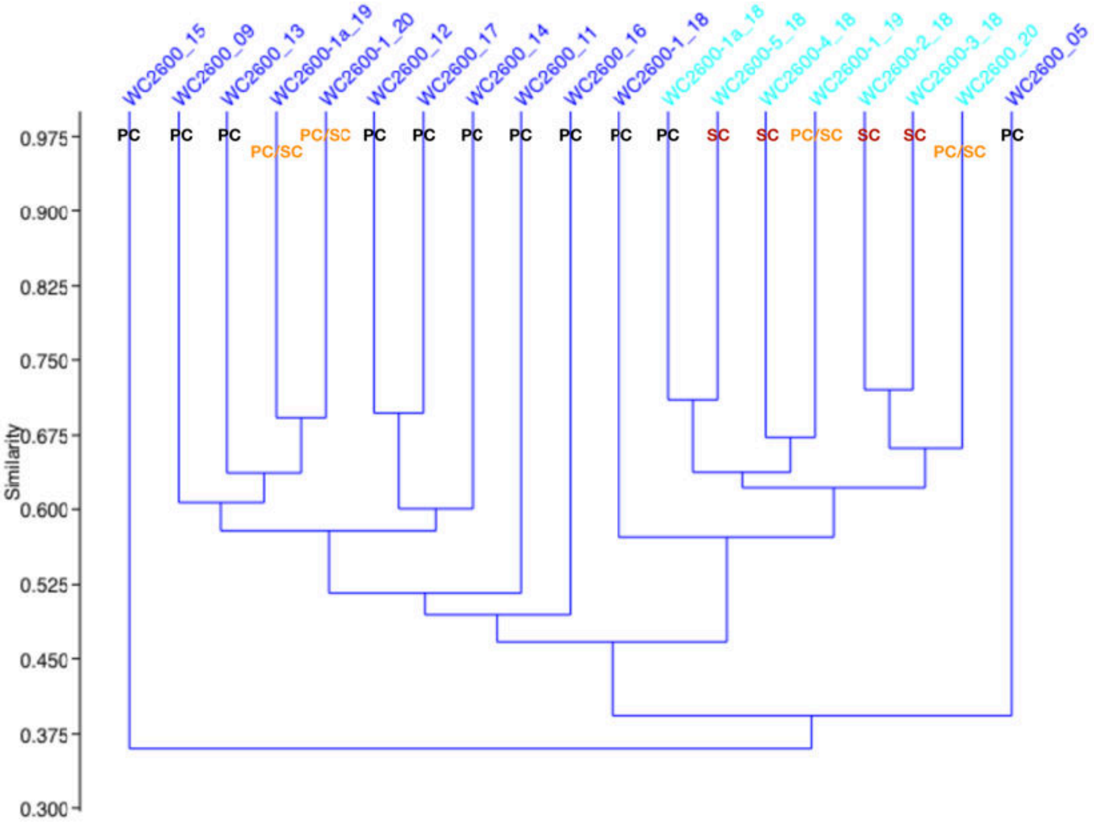
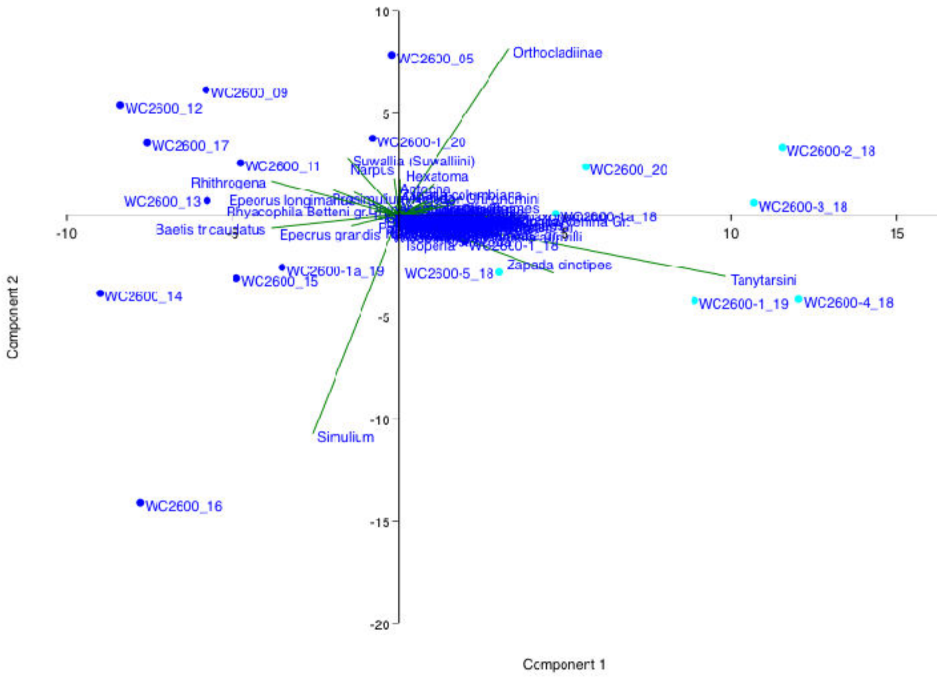
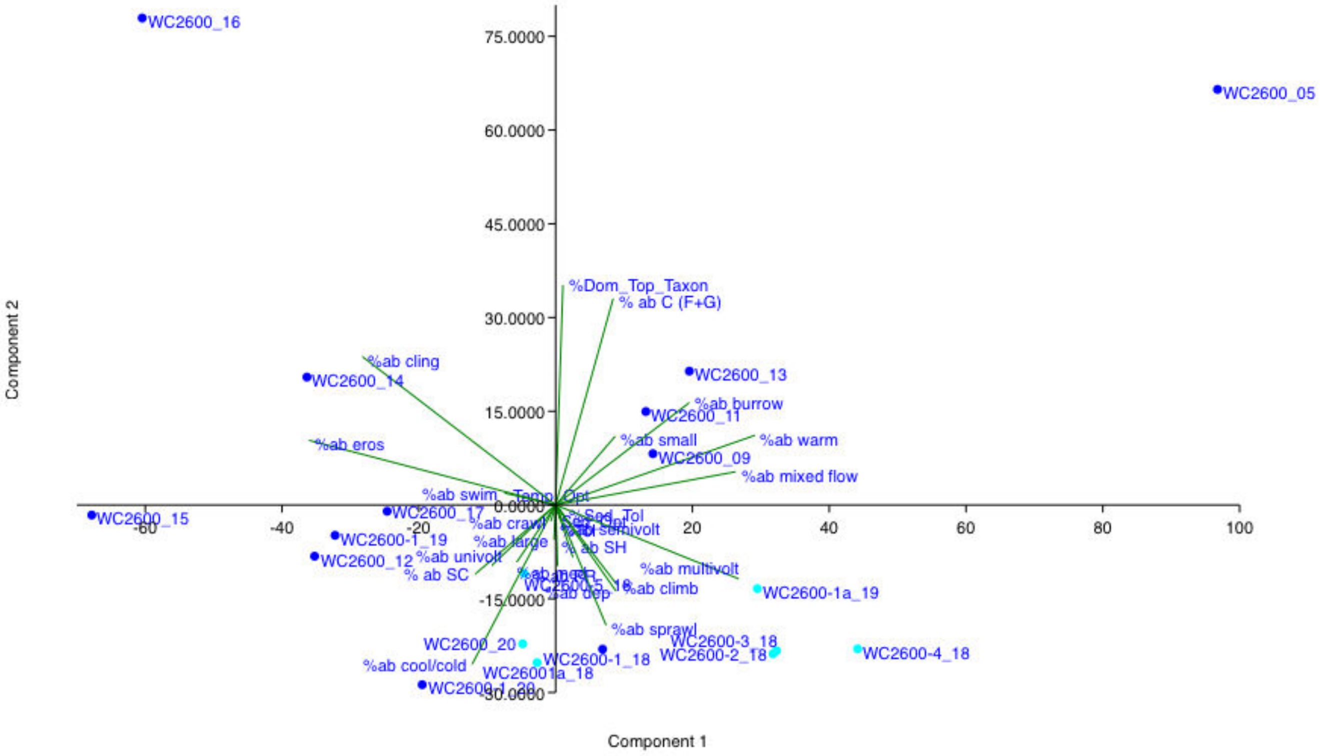


Figure 87. PCA ordination of the WC2600 macroinvertebrate community in all sampling years. *RT samples (blue) were taken in 2005-2020; PM samples (aqua) were taken in 2018-02020. The number at the end of each label indicates sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



In a PCA ordination of all traits measured as relative abundances (Figure 88), axis 1 explained 40% of total sample variation. Traits with the highest loading values were relative abundances of taxa associated with faster flows and warmer water (both were lower in early sampling years and in most of the side channel samples) and of clingers, which were less abundant in 2005 as well as in 2018 side channel samples. Axis 2 explained an additional 23% of variation, and traits with the highest loading included relative abundances of the dominant taxon, which was 2-5 times higher in 2005 and 2016 compared to other years; collectors, which were more abundant from 2005-2016 compared to later years; and organisms associated with cooler temperatures, which were greatest in samples taken in 2019-2020.

Figure 88. PCA ordination of the WC2600 macroinvertebrate community traits measured as relative abundances in all sampling years. *RT samples (blue) were taken in 2005-2020; PM samples (aqua) were taken in 2018-2020. The number at the end of each label indicates the sampling year. Eigenvectors show dominant contributions, where vector length is related to the strength of its contribution.*



Discussion

Changes in macroinvertebrate community characteristics along Whychus Creek

Sampling was done in Whychus Creek in 12 of the past 15 years; during that time, restoration activities at both the basin and reach level were implemented at different sites along much of the stream's length including restoring perennial flow, increasing channel complexity, and creating new side channel habitat. The taxonomic composition and ecological traits of the macroinvertebrate community changed over the years among all sampling sites, reflecting the influence of changing climate conditions (i.e., annual temperature and rainfall), habitat alterations, and use of an additional sampling technique (proportional multihabitat). New taxa (i.e., not taken at any site along the creek in any prior year) continue to be taken in each sampling year; most are found in PM samples, attesting to the ability of this technique to capture a greater variety of taxa from the more heterogenous habitats sampled (Barbour et al., 2006).

Sample richness (number of unique taxa) as well as EPT richness (Ephemeroptera, Plecoptera, Trichoptera) increased among all sites across the last five years, due to a combination of the greater richness in PM samples, recovery from restoration-related disturbance, and increased habitat heterogeneity with establishment of side channels. Increases in richness are not due solely to sampling method. Although PM samples have more total taxa than RT samples taken in the same reach, the number of EPT in the two have been quite similar; in addition, total richness in RT samples increased across time at several sites where RT samples were taken in all sampling years (i.e. WC0600, WC0850, WC1025, WC1100, and WC2600). Community evenness, which is considered a measure of ecosystem stability (Death, 1996; Wittebolle et al., 2009), also increased; relative abundance of the top (most abundant) taxon decreased at most sites across sampling years, and the range for this metric among all 2020 samples was the lowest in any year. Most sites are dominated by small-bodied, rapidly-developing (univoltine and multivoltine), scraper and collector taxa that prefer cool temperatures and fast or mixed flows. Abundance of organisms with a climbing habit increased in recent years; these were absent from many sites prior to 2018, and this change likely reflects greater habitat heterogeneity and more woody and/or plant substrates. The number of taxa taken across all sites in 2020 was the most of any sampling year, and a few taxa new to the Whychus dataset are still appearing despite the long span over which sampling has been conducted. These generally have low abundance in samples, so they may be rarer taxa with less chance of being collected in any sampling year, although many taxa that have been found in multiple years also occur at low abundances (i.e., <10 individuals). It is likely that the changes in habitats across both time (i.e., climate) and space (flow and channel restoration) are supporting some new colonization events.

PM samples are consistently more diverse than RT samples taken simultaneously in the same reach, and are more representative of the total community in the habitat, which is an important factor when considering macroinvertebrates as a food source for native fish. Because PM samples include net sets taken in a variety of substrates and flow types, as opposed to the concentration on fast flows with larger mineral substrates in riffle-targeted sampling, they often contain greater abundances of organisms with a variety of flow and temperature associations. This includes greater relative abundances of organisms with higher sediment and warmer temperature tolerances reflective of slow-water habitat such as glides and pools, but PM samples also capture sensitive taxa and organisms with cold temperature, low sediment, and fast flow habitat associations. In 2020, the number of DEQ cool temperature and low sediment indicator taxa increased from downstream to upstream reaches and the community temperature optima decreased along the same gradient, regardless of sampling method used.

PM samples are designed to be representative of different types of substrate in a reach (as opposed to flow types), but composite samples generally include net sets from riffles (range = 3-9 net sets taken in riffles among all 2020 samples), and PM and RT samples taken in the same reach consistently have similar macroinvertebrate community composition. Overall community similarity among samples continues to be strongly influenced by reach location; with a few exceptions in each year, both PM and RT communities have greater community similarity in samples from RM6-1150 and RM1825-2600, although in 2020 the WC2600 sample was an outlier.

Changes in macroinvertebrate community characteristics at longterm index sites

Among all the sites sampled along Whychus Creek in 2020, only WC0600 and WC1150 have not undergone reach-level restoration. However, they are affected by basin-level activities as well as restoration projects implemented upstream and downstream, and community composition has changed over time. Taxa richness at both sites increased, but these sites had the fewest new taxa among all 2020 samples; only one taxon was taken at WC1150 for the first time this year, while WC0600 was the only site at which no new taxa were seen.

Samples taken at WC0600 have had high organismal abundance since 2015, and PREDATOR and IBI scores also increased in that span and then stabilized in the last three years. Community composition changed enough that samples from recent years (2016-2020) are more similar to each other than to samples from earlier years. Recent years have also seen increased numbers of sensitive and sediment-sensitive taxa, and a dominant taxon associated with faster flows. Signals from some metrics are more mixed; site samples have always had low relative abundances of sediment-tolerant organisms, and relative abundance of tolerant organisms has been decreasing since 2016, but community sediment optima increased in recent years while community temperature optima decreased in the same span. EPT diversity at this site has been consistently at the lower end of the range among recent Whychus samples and the 2020 sample had the fewest EPT of any site this year.

WC1150 was established as an upstream control site for WC1100, where new side channel habitat was created in 2016. However, observed taxa and trait differences in the 2017 sample community compared to all other sampling years suggest that the downstream restoration project also impacted the community at WC1150. With the exception of 2017, this site has generally had higher IBI and PREDATOR scores compared to other Whychus reaches as well as more sensitive, sediment-intolerant, and cold temperature-associated taxa and fewer sediment-tolerant organisms. Community temperature optima, which increased in RT samples to a high in 2018, are lower in the PM samples taken since 2018; the community sediment optima is higher but stable, and this increase is influenced by a switch to PM sampling, which captures more depositional habitat.

Baseline macroinvertebrate community characteristics at pre-restoration sites

Samples were taken at WC0850 and WC0900 in multiple years, but neither site had been sampled since 2017. With a gap in sampling it was anticipated that the 2020 community would differ more from prior years, especially since the PM technique was used at WC0850 for the first time. As expected, the WC0850 sample had almost twice as many taxa as in earlier years, and EPT richness was also at the upper end of the range for this site. Eight taxa in the 2020 ample had not been taken at the site in any prior year; most were associated with colder, faster flows, and one was also new to the complete Whychus dataset. In contrast, richness in the WC0900 sample was similar to that in earlier years and only two taxa new to the site were taken, both of which had warmer temperature associations. This may be due in part to the fact that an RT sample was taken, but WC0900 also had the fewest taxa and lowest organismal abundance of any 2020 sample, and only one site had fewer EPT.

The most abundant taxon in the WC0850 sample occurred at the lowest relative abundance of any year, and in general this site has been dominated by taxa that prefer faster colder flows. There is no clear signal from other metrics; the 2020 sample had more sensitive, sediment-sensitive, and cool temperature indicator taxa compared to prior years but also more sediment-tolerant organisms (though at a comparatively low relative abundance), and community temperature and sediment optima were higher as well. These changes are likely influenced by the fact that there were almost twice as many taxa in the 2020 sample taken from a greater variety of habitats. Six of the net sets in the composite sample were taken in flow types other than riffles, so representation of taxa associated with warmer slower flows and more sedimented substrates would be expected to increase.

While the WC0900 sample was the most balanced of any prior year, the dominant taxon was a tolerant, sediment-tolerant snail and the majority of the community was tolerant of a variety of flow types and temperatures. Sample communities in earlier years had fewer organisms associated with warm temperatures, and in 2020 the community temperature optima was not only higher at this site than in any year except 2009 but also the highest in the 2020 sample set. There were more sediment-sensitive taxa in 2020 but also a greater relative abundance of sediment-tolerant organisms than in any year since 2012, and the highest community sediment optima of any year at this site. The taxonomic composition of the 2020 sample differed more from all other years at this site except for 2005; given that the number of taxa taken in each year has varied little, this suggests that temperature and sediment conditions may be acting as stronger environmental filters.

The recent gap in sampling years at the WC1025 site was shorter, as it was sampled in both 2019 and 2020. Similar to what was seen at WC0850, use of the PM sampling technique was accompanied by increases in total and EPT richness, and four taxa new to the site were taken in 2020. Relative abundance of the top taxon was the lowest since 2017 and this was also the first time in that span that the most abundant taxon was sensitive and associated with cold fast flows. Numbers of sensitive and sediment-sensitive taxa have also increased since 2017, while relative abundance of sediment-tolerant organisms decreased in the same span. Community sediment optima, which increased steadily through 2019, were lower for the first time in 2020, and the community temperature optima of the 2020 sample was lower than the previous year. Despite differences in individual metrics, the communities in the 2019 and 2020 PM samples were most similar to each other and differed from all other years, and the 2017 community was also an outlier. It is possible that this reach was disturbed by the restoration activities conducted upstream at the WC1100 site, and that the community here has been recovering as well as potentially receiving positive impacts from the restored habitat upstream.

The reaches at WC2000 and WC2050 were sampled for the first time in 2020, and all sample communities were most similar to the WC1950 community. Total richness in both samples was intermediate for the 2020 data set and number of EPT taxa was towards upper end of 2020 sample range. Sample communities were balanced, with a low relative abundance of the dominant taxon (<15%), and they scored in top range for 6-7 of the 10 metrics in the DEQ IBI. Both samples had more ORDEQ cool temperature and low sediment indicators than almost all reaches further downstream. The WC2000 sample had a lower community temperature optima and fewer tolerant organisms than most other samples in 2020, as well as fewer scrapers and more shredders, suggesting the presence of more plant material in the channel here. The WC2050 sample had the lowest relative abundances of burrowers, non-biting midges, and organisms associated with warm water among all 2020 samples.

Changes in macroinvertebrate community characteristics at restored sites

Although in-stream restoration activities implemented in reaches of Whychus Creek have varied (i.e., channel re-meandering, creation of side channels, addition of large wood), the overall trajectory of macroinvertebrate community response has been similar among most restored sites, with large perturbations for up to two 2 years followed by greater stabilization of metric values and an altered post-restoration community. Increased habitat heterogeneity has corresponded to increased macroinvertebrate diversity, with side channel communities that are similar to those in the associated primary channel reach but often with greater richness and more EPT taxa. These side channels generally have more organisms that prefer slower or more mixed flow types, and many taxa in these samples are associated with higher sediment and warmer temperatures, which is not unexpected given the variety of flows and substrates in these channels. However, side channels also sustain a diversity of sensitive taxa with cool/cold temperature associations, again pointing to the capacity of more heterogeneous habitats to sustain a wider diversity of taxa. In addition, several metrics in primary channel communities post-restoration indicate improved habitat conditions, with increased richness and lower abundance of the dominant taxon, reflecting more balanced communities in habitats supporting a wider range of taxa.

At WC1100, where restoration in 2016 was followed by a severely disturbed community in 2017, eight taxa were taken for the first time in 2020, most of which were in samples that included side channel habitat. In addition, while side channel samples had the most total and EPT taxa at this site, the total and EPT richness in primary channel samples were 1.5-2 times greater than in pre-restoration samples. Samples taken in 2020 at WC1950, where restoration was implemented immediately downstream in 2012, had the most total taxa as well as the most EPT and sensitive taxa ever taken at this site. The recovery trajectory at this site differed from that seen in several other restored reaches in Whychus. There was little change in total richness from 2009-2014, but the number of taxa has increased steadily since 2015, and while PREDATOR scores dropped in the two years following restoration, IBI scores were unaffected. Community composition in samples taken in 2011, 2012, and 2014 was also similar (see Figure 75). The 2017 sample seemed to be an inflection point, as samples taken after this year showed stronger apparent recoveries for several metrics such as numbers of sensitive and sediment-sensitive taxa, but proportions of tolerant and sediment-tolerant taxa increased in this span as well. The community was less balanced in the three years following restoration (relative abundance of the top taxon in 2012 = 21.5%; range in 2013-2015 = 22.7-36.3%) but not as unbalanced as the 2019 sampling community, which was dominated at by *Simulium* black flies at 53.3% of total organismal abundance. Data from these metrics suggest that while the macroinvertebrate community here may be responding more gradually to restoration-related habitat changes occurring over a longer time (i.e., generation of new habitats due to backwatering and deposition), some additional disturbance or environmental filter affected the community in more recent years.

Recovery at WC2600 followed a trajectory more similar overall to other restored sites, with macroinvertebrate community composition in the sample taken one year post-restoration differing the most from all others at this site. However, this site is unusual in that all PM samples taken in any reach (primary, secondary, or PC/SC) from 2018-2020 are more similar to each other than to the corresponding RT sample taken in the same year. Communities in this reach differ more from those further downstream, and that appears to be compounded by the fact that post-restoration channel development resulted in a reach that now includes both primary and secondary channel. This site is one of only two sampled in every project year, but the channel re-meandering in 2014 complicates assessment of longterm trends. WC2600 samples generally have among the lowest community temperature optima of all Whychus sites; within-site changes since 2005 include more total, EPT, sensitive, and DEQ cool indicator taxa, increased sediment optima, and a greater relative abundance of cool/cold associated organisms and shredders and fewer collectors. WC2600 samples taken in 2020 had the most total taxa, EPT taxa, and DEQ cool temperature and low sediment indicator taxa of any sample taken at this site as well as among all samples taken this year. There were also more sediment-tolerant taxa than any other 2020 sample but the relative abundance of both sediment-tolerant and tolerant organisms was low, again reflecting a greater diversity of taxa occupying heterogeneous habitats.

Literature Cited

Akamagwuna, F.C., P.K. Mensah, C.F. Nnadozie, and O.N. Odume. 2019. Trait-based responses of Ephemeroptera, Plecoptera, and Trichoptera to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. River Research and Applications 35: 999-1012.

Anderson, T., P.S. Cranston, and J.H. Epler (eds.). 2013. The larvae of Chironomidae (Diptera) of the Holarctic region: keys and diagnoses. Insect Systematics & Evolution, Suppl. 66, 573 pp.

Arce, E., V. Archaimbault, C.P. Mondy and P. Usseglio-Polatera. 2014. Recovery dynamics in invertebrate communities following water-quality improvement: taxonomy- vs trait-based assessment. Freshwater Science 33(4): 1060-1073.

Barbour, M.T., J.B. Stribling, and P.F.M. Verdonschot. 2006. The multihabitat approach of USEPA's rapid bioassessment protocols: benthic macroinvertebrates. Limnetica 25(3): 839-850.

Beche, L.A. and B. Statzner. 2009. Richness gradients of stream invertebrates across the USA: taxonomy- and trait-based approaches. Biodiversity Conservation 18: 3909-3930.

Bona F., A. Doretto, E. Falasco, V. La Morgia, E. Piano, R. Ajassa, R., and S. Fenoglio. 2015. Increased sediment loads in alpine streams: An integrated field study. River Research and Applications 32(6): 1316-1326.

Buendia , C., C.N. Gibbons, D. Vericat, R.J. Batalla, and A. Douglas. 2013. Detecting the structural and functional impacts of fine sediment on stream invertebrates. Ecological Indicators, 25, 184–196.

Culp, J.M., D.G. Armanini, M.J. Dunbar, J.M. Orlofske, N.L. Poff, A.I. Pollard, A.G. Yates, and G.C. Hoe. 2011. Incorporating traits into aquatic biomonitoring to enhance causal diagnosis and prediction. Integrated Environmental Assessment and Management 7(2): 187-197.

de Castro D.M.P., S. Dolédec, and M. Callisto. 2018. Land cover disturbance homogenizes aquatic insect functional structure in neotropical savanna streams. Ecological Indicators, 84, 573–582.

Death, R.G. 1996. The effect of habitat stability on benthic macroinvertebrate communities: the utility of species abundance distributions. Hydrobiologia 317: 97-107.

Doretto, A., E. Piano, F. Bona, and S. Fenoglio. 2018. How to assess the impact of fine sediments on the macroinvertebrate communities of alpine streams? A selection of the best metrics. Ecological Indicators, 84: 60–69.

Hammer, Ø., Harper, D.A.T., and P. D. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 9pp.

Hubler, S., D.D. Huff, P. Edwards, and Y. Pan. 2016. The Biological Sediment Tolerance Index: assessing fine sediments conditions in Oregon streams using macroinvertebrates. Ecological Indicators 67: 132-145.

Huff, D.S., S.L. Hubler, Y. Pan, and D.L. Drake. 2008. Detecting shifts in macroinvertebrate assemblage requirements: implicating causes of impairment in streams. ORDEQ Laboratory and Environmental Assessment Division, DEQ06-LAB-0068-TR. 36 pp.

IDDEQ. 2015. Water body assessment guidance. Idaho Department of Environmental Quality, Boise ID, 104 pp.

Larson D.J., Y. Alarie, and R.E. Roughley 2000. Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic region, with emphasis on the fauna of Canada and Alaska. National Research Council of Canada, NRC Press, Ottawa Canada.

Mathers, K.L., S.P. Rice, and P.J. Wood. 2017. Temporal effects of enhanced fine sediment loading on macroinvertebrate community structure and functional traits. Science of the Total Environment, 599: 513–522.

Merritt, R.W., K.W. Cummins, and M.B. Berg. 2019. An introduction to the aquatic insects of North America, 5th ed. Kendall/Hunt Publishing Company, Dubuque, Iowa, 1480 pp.

Meyer, M.D. and W.P. McCafferty. 2007. Mayflies (Ephemeroptera) of far western United States. Part 2: Oregon. Transactions of the American Entomological Society 133(1-2): 65-114.

Murphy, J.F., J.I. Jones, A. Arnold, C.P. Duerdoth, J.L. Pretty, P.S. Naden, and A.L. Collins. 2017. Can macroinvertebrate biological traits indicate fine-grained sediment conditions in streams? River Research and Applications, 33(10): 1606–1617.

OR DEQ (State of Oregon Department of Environmental Quality). 2009. 2009. Water Monitoring and Assessment Mode of Operations Manual (MOMs). Laboratory and Environmental Assessment Division. DEQ03-LAB-0036-SOP. Available at: https://www.oregon.gov/deq/FilterDocs/DEQ03LAB0036SOP.pdf.

Pinder, L.C.V. 1986. Biology of freshwater Chironomidae. Annual Review of Entomology 31: 1-23.

Poff, N.L., J.D. Olden, N.K.M. Vieira, D.S. Finn, M.P. Simmons, and B.C. Kondratieff. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society 25(4): 730-755.

Rabení, C.F., K.E. Doisy, and L.D. Zweig. 2005. Stream invertebrate community functional responses to deposited sediment. Aquatic Sciences, 6705: 395–402.

Relyea, C.D., G.W. Minshall, and R.J. Danehy. 2012. Development and validation of an aquatic fine sediment biotic index. Environmental Management 49: 242-252.

Richards, A.B. and D.C. Rogers. 2011. List of freshwater macroinvertebrate taxa from California and adjacent states including Standard Taxonomic Effort levels. Southwest Association of Freshwater Invertebrate Taxonomists, 266 pp.

Southwestern Association of Freshwater Invertebrate Taxonomists (SAFIT). 2016. Tolerance values and functional feeding groups. Southwestern Association of Freshwater Taxonomists. Available at http://www.safit.org/Docs/Tolerance_Values_and_Functional_Feeding_Groups

Stewart, K.W. and B.P. Stark. 2002. Nymphs of North American stonefly genera (Plecoptera). The Caddis Press, Ohio, 510 pp.

Sutherland, A.B., J.M. Culp, and G.A. Benoy. 2012. Evaluation of deposited sediment and macroinvertebrate metrics used to quantify biological response to excessive sedimentation in agricultural streams. Environmental Management, 50, 50–63.

Thorp, J.H. and A.P. Covich. 2001. Ecology and classification of North American Freshwater Invertebrates, 2nd ed. Academic Press, 1055 pp.

Townsend, C.R. and A.G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems. Freshwater Biology 31: 265-275.

Tullos, D.D., D.L. Penrose, and G.D. Jennings. 2009. Analysis of functional traits in reconfigured channels: implications for the bioassessment and disturbance of river restoration. Journal of the North American Benthological Society 28: 80-92.

Van den Brink, P.J., A.C. Alexander, M. Desrosiers, W. Goedkoop, P.L.M. Goethals, M. Liess, and S.D. Dyer. 2011. Traits-based approaches in bioassessment and ecological risk assessment: strengths, weaknesses, opportunities and threats. Integrated Environmental Assessment and Management 7(2): 1980208.

Vieira N.K.M., N.L. Poff, D.M. Carlisle, S.R. Moulton III, M.L. Koski, and B.C. Kondratieff. 2006. A database of lotic invertebrate traits for North America. US Geological Survey Data Series 187. Available at http://pubs.water.usgs.gov/ds197.

White, J.C., M.J. Hill, M.A. Bickerton, and P.J. Wood. 2017. Macroinvertebrate taxonomic and functional trait compositions within lotic habitats affected by river restoration practices. Environmental management 60: 513-525.

Wiggins, G.B. 1996. Larvae of the North American caddisfly genera (Trichoptera), 2nd ed. University of Toronto Press, 457 pp.

Wilkes, M.A., M. McKenzie, J.F. Murphy, and R.P. Chadd. 2017. Assessing the mechanistic basis for fine sediment biomonitoring: Inconsistencies among the literature, traits and indices. River Research and Applications, 33: 1618–1629.

Wittebolle, L.W., M. Marzoti, L. Clement, A. Balloi, D. Daffonchio, K. Heylen, P. De Vos, W. Verstraete, and N. Boon. 2009. Initial community evenness favors functionality under selective stress. Nature 458(7238): 623-626.