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Macroinvertebrate Monitoring in Whychus Creek (Sisters, OR), 2021

Whychus Creek, August 2021; C. A. Searles Mazzacano

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Summary

The benthic macroinvertebrate community was sampled in Whychus Creek on 12-13 August 2021 in the following reaches: Road 6360 (WC0600), Rimrock Ranch (WC0850, WC0900), Whychus Canyon (WC1100, WC1150), Camp Polk (WC1950), Willow Springs (WC2000, WC2050), and Whychus Floodplain (WC2600). Samples represented a mixture of long-term index sites, restored sites, and pre-restoration sites. Sampling was done using multihabitat (M) and single-habitat riffle-targeted (RT) techniques. In past years, multihabitat sampling was done such that the number of net sets taken in different habitat types reflected their representation in the reach (proportional multihabitat). However, the excessively hot summer of 2021 increased snowmelt and levels of glacial till in the creek, such that in August the water was persistently milky and it was impossible to see the substrate and assess different habitat types, so a reach-wide multihabitat protocol (M) was used instead. ORDEQ PREDATOR predictive model and Index of Biotic Integrity (IBI) was applied to RT samples. Macroinvertebrate community taxonomic and biological traits assessed included 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 132 unique taxa in 49 families, which is slightly less than in 2020 (156 taxa in 51 families). Order-level diversity was highest among Diptera (true flies), with 58 unique taxa in eight families, including 43 genera of non-biting midge (Chironomidae), although these numbers were also lower than the prior year. Other well-represented groups included Ephemeroptera (mayfly; 21 genera in six families), Trichoptera (caddisfly; 18 genera in seven families), and Coleoptera (beetles; 10 genera in four families). Six taxa not found in any prior sampling year at any Whychus site were collected; they represented a range of orders and were each present as a single individual in a multihabitat sample, with all but one found around the restored site WC1100.

Richness in individual samples ranged from 38-57 taxa (mean = 45.8 taxa, SD 5.1). For the first time since both riffle-targeted and multihabitat sampling protocols were implemented, there was no significant difference in taxa richness between the two sample types. As in past years, there was also no significant difference between riffle and multihabitat samples for a variety of community metrics that reflect sediment or temperature tolerance. Macroinvertebrate community composition continues to be strongly influenced by reach location, 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 M). Numerical abundance across the dataset was dominated by *Optioservus*, a widespread tolerant riffle beetle that inhabits sediments and detritus in flowing and still habitats in streams and was consistently abundant in downstream and mid-stream reaches (WC0600-WC1150).

Ongoing restoration along Whychus Creek has resulted in more diverse communities, with total and EPT richness increased overall among all sites since 2015. Relative abundance of tolerant organisms is greater than that of sediment-tolerant at the majority of sites, and the impacts of climate-driven pressures such as drought, hotter years, and regular wildfires may be increasing habitat disturbance. Baseline data from several pre-restoration reaches suggest communities currently more tolerant of warmer temperatures and higher fine sediment levels. At long-term

index reaches where no active restoration has been done, a few new taxa continue to be taken in each year, and changes in many metrics indicate improved habitat or water quality. Most restored reaches show a similar trajectory of macroinvertebrate community response, with large perturbations for up to two years post-restoration followed by stabilizing metric values and an altered post-restoration community that generally indicates improved and/or more stable habitat. At restored sites, side channel and associated primary channel communities have a high degree of similarity but side channels often sustain greater total and EPT richness as well as a diversity of both tolerant and sensitive taxa with a wider range of temperature, flow, and sediment associations. The concentration of taxa new to the 2021 Whychus dataset in side channels is a further indicator of the ability of these heterogenous dynamic habitats to sustain a diversity of taxa.

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. Aquatic macroinvertebrates are monitored annually to assess community-level changes and their relationship to altered habitat conditions and, more recently, to 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

Sampling sites

Benthic macroinvertebrate sampling was done 13-14 August 2021 in multiple regions of Whychus Creek (Table 1): Road 6360 (WC0600), Rimrock Ranch (WC0850, WC0900), Whychus Canyon (WC1100, WC1150), Camp Polk (WC1950), Willow Springs (WC2000, WC2050), and Whychus Floodplain (WC2600). Multiple samples were taken at WC1100 to assess different side channel reaches, and both riffle-targeted (RT) and multihabitat (M) samples were collected at some index and pre-project sites. Riffle-targeted samples were taken in four reaches (WC0600, WC0900, WC1100-2, and WC2000), and multihabitat samples were taken in 11 reaches (WC0850, WC1025, WC1100 primary and side channels, WC1150, WC1950, WC2000, C2050, WC2600), including a duplicate sample taken in the WC1100-4 side channel for quality control.

None of the sites experienced restoration between the sampling dates in 2020 and 2021. However, a restoration project was implemented upstream of WC0900 at WC1025 (which was not sampled in 2021) in July-August 2021 prior to sampling. The downstream end of this restoration project is within about 0.5 miles of the WC0900 reach, so WC0900 and WC0850 could have been impacted.

Table 1. Whychus Creek sampling sites in 2021

Site ID	Description	Coordinates	years sampled
WC0600	u/s Rd 6360	44.418750, -121.386350	2005, 2009, 2011-2021
WC0850[a]	Rimrock Ranch d/s	44.391278, -121.406182	2011-2017; 2020-2021
WC0900	Rimrock Ranch Meadow	44.384198, -121.407892	2009, 2011-2017; 2020-2021
WC1100-2[b]	lower Whychus canyon	44.364587, -121.421706	2014-2015, 2017-2021
WC1150[c]	upper Whychus Canyon	44.358320 -121.43105	2014-2021
WC1950	d/s Camp Polk bridge; WQ site coordinates	44.318741, -121.514961	2009, 2011-2017, 2019-2021
WC2000	Willow Springs d/s	44.313269, -121.511074	2020-2021
WC2050	Willow Springs u/s	44.309184, -121.510297	2020-2021
WC2600	Whychus floodplain, 4606 Rd footbridge	44.273059, -121.555297	2005, 2009, 2011-2021

[a] designated as WC0875 in 2011-2015 [b] designated WC1025 in 2014; [c] designated WC1075 in 2014

Macroinvertebrate sampling techniques

Sampling was done by CASM Environmental, UDWC staff, and volunteers from natural resource agencies and the surrounding community. Due to the continuing COVID-19 pandemic, social distancing was maintained in the field and participants wore masks. CASM Environmental staff demonstrated sampling techniques, then teams received sampling kits and maps and dispersed into the field. 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, with a minimum length of 500 ft. (150 m) and maximum of 1000 ft. (300 m). The upstream and downstream limit of each reach as well as turning points and paths along the channel were flagged by UDWC prior to sampling. A sample consisted of eight individual net sets in the designated reach, each collected in a 1 ft^2 area of riffle habitat 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 2-4 in. (6-10 cm) for 1-2 minutes. All net sets were pooled in a bucket, large debris was rinsed and removed, and sample material poured through a sieve lined with a 500 µm Nitex membrane. This concentrated sample was transferred to a 1L 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.

Multihabitat protocol (M)

Restoration objectives in Whychus Creek include increasing the proportion and area of pool habitat while decreasing the proportion and area of riffle habitat to provide refugia for juvenile salmonids. To more accurately assess project impacts, multihabitat sampling, which captures macroinvertebrates from a greater variety of flows and substrates than riffle-targeted, was instituted in 2018 to better assess diversity in the more heterogeneous habitats of created/restored side channels. In past years, multihabitat samples were taken according to the proportions of each habitat type in the reach (proportional multihabitat sampling; Barbour et al., 2006). However, the excessively hot summer of 2021 increased snowmelt and levels of glacial till in the creek, such that in August the water was persistently very milky all day, obscuring the substrate and making assessment of different habitat types impossible. Therefore, to assess the macroinvertebrate community across multiple habitats in these reaches, they were sampled using a reach-wide multihabitat protocol (adapted from USEPA, 2009; Ode et al., 2016), which has been shown to be as robust and provide similar results as targeted single-habitat (riffle) sampling (Gerth & Herlihy, 2006) and was used to sample newly-created side channels at WC1100 in 2017.

Reach lengths were calculated and flagged as described above; at sites where both riffle and multihabitat samples were taken, two teams sampled the same reach simultaneously, moving upstream as a unit to avoid disturbing areas not yet sampled. Ten transects running perpendicular to the direction of flow were set at equal intervals in the sample reach, with the first at the downstream limit of the reach. A D-frame kick net was used to take a single net set in each transect, alternating between the left (i.e., at 25% of the channel wetted width), center (at 50% of the wetted width), and right (at 75% of the wetted width) of the transect as the sampler moved upstream. In each transect, the flow (riffle, run, glide, pool) and habitat type was recorded (Table 2). 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 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

Where there was sufficient current to carry suspended material into the net, cobble and gravel substrates were sampled as described for riffles. 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^2) area in front of the net. In transects with little or no flow, the substrate was continuously disturbed to a depth of several inches using hands or feet while the D-net was swept repeatedly through the suspended material to capture disturbed/dislodged invertebrates. In vegetation, the net was jabbed and swept through the vegetation repeatedly during the one-minute sampling time. Root wads, small wood tangles, and large woody debris were sampled similarly; invertebrates were picked off during a visual examination, then the net was held adjacent to and beneath the wood while the material was kicked vigorously to dislodge invertebrates. Net sets were composited and processed as described for riffle samples.

Table 2. Whychus Creek sampling reach characteristics in 2021. *R= riffle-targeted, M = 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 collected.*

Sample	Site type	Sample type	Loc.	#net sets in each habitat type	flow type in net sets	avg wetted width (ft)
WC0600R	Longterm index	R	PC	cobble/bedrock/gravel	8 riffle	24.5 ± 2.1
WC0850M	Restoration (baseline); WQ	M	PC	7 cobble, 2 gravel, 1 unk.	3 riffle, 1 run, 4 glide, 2 pool	29.3 ± 4.5
WC0900R	Restoration (baseline)	R	PC	cobble/gravel	8 riffle	25.6 ± 4.6
WC1100-1BM	Restoration	M	PC/SC	3 cobble, 5 gravel, 1 sand, 1 wood	3 riffle, 4 run, 3 glide	22.2 ± 6.8
WC1100-2M	Restoration	M	PC	10 cobble/gravel/sand	2 riffle, 8 run	19.4 ± 6.1
WC1100-02R	Restoration	R	PC	cobble/gravel	8 riffle	19.2 ± 4.9
WC1100-3M	Restoration	M	SC	3 sand/silt, 7 cobble/gravel	7 riffle, 1 run , 2 pool	9.6 ± 7.3
WC1100-4M	Restoration	M	SC	4 cobble, 4 gravel, 1 sand, 1 silt	1 riffle, 7 run, 2 glide	11.4 ± 7.3
WC1100-4MDUP	site duplicate for QA	M	SC	4 cobble, 4 gravel, 1 sand, 1 silt	1 riffle, 7 run, 2 glide	11.4 ± 7.3
WC1150M	Adjacent untreated (upstream)	M	PC	7 cobble, 3 boulder	5 riffle, 4 run, 1 glide	27.3 ± 2.4
WC1950M	Restoration (baseline); WQ	M	PC	5 cobble/gravel, 3 gravel, 2 sand/silt	4 riffle, 6 run	not done
WC2000M	Restoration (baseline)	M	PC	4 cobble, 4 gravel, 2 sand/silt	2 riffle, 4 run, 3 glide, 1 pool	23.7 ± 5.9
WC2000R	Restoration (baseline)	R	PC	cobble/gravel	8 riffle	20.2 ± 5.2
WC2050M	Restoration (baseline)	M	PC	1 boulder, 1 cobble, 8 cobble/gravel	1 rapid, 7 run, 2 glide	27.4 ± 4.8
WC2600M	Longterm index	M	PC/SC	2 cobble, 2 cobble/gravel, 3 gravel, 3 sand/silt	2 riffle, 3 run, 4 glide, 1 unk.	15.7 ± 8.6

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 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 (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.

Biological/ecological traits of taxa

Assessing functional traits of macroinvertebrate taxa helps infer habitat conditions that shape the community, diagnose stressors or environmental filters, and relate 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 if available, but 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; Twardochleb et al., 2020). 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 a narrow range of tolerance for either cool/cold or warm waters, while others have a broader temperature tolerance. 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; filterers and gatherers) organisms: Filterers are negatively impacted by sedimentation if their feeding structures become clogged (Rabení et al., 2005); 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 flows (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 broad temperature range associations 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 (Hammer et al., 2001) and PRIMER-e v7 (Clarke et al., 2014) statistical software. CLUSTER dendrograms, non-metric multidimensional scaling (nMDS) ordinations, and SIMPER tests were run on a Bray-Curtis similarity matrix of square-root transformed taxa abundances. Community evenness, which is considered a measure of ecosystem stability (Death, 1996; Wittebolle et al., 2009), was calculated on untransformed taxa abundances. Principal Component Analysis (PCA) was done using a variance-covariance matrix. Differences between mean values of a trait were examined using unpaired t tests, using a cutoff value of $p \leq 0.05$ for statistical significance. Means are presented with standard deviation (SD).

Biological conditions in riffle 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 assigned scaled values then summed to give a number corresponding to a level of biological impairment (Table 3). These models were developed specifically for riffle communities and thus cannot be applied to multihabitat samples, but raw values for individual metrics in multihabitat samples were calculated for reference. 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 established reference communities; the model uses site elevation, slope, and longitude to select appropriate reference streams. O/E scores correspond to biological condition categories of 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) 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 2021 samples

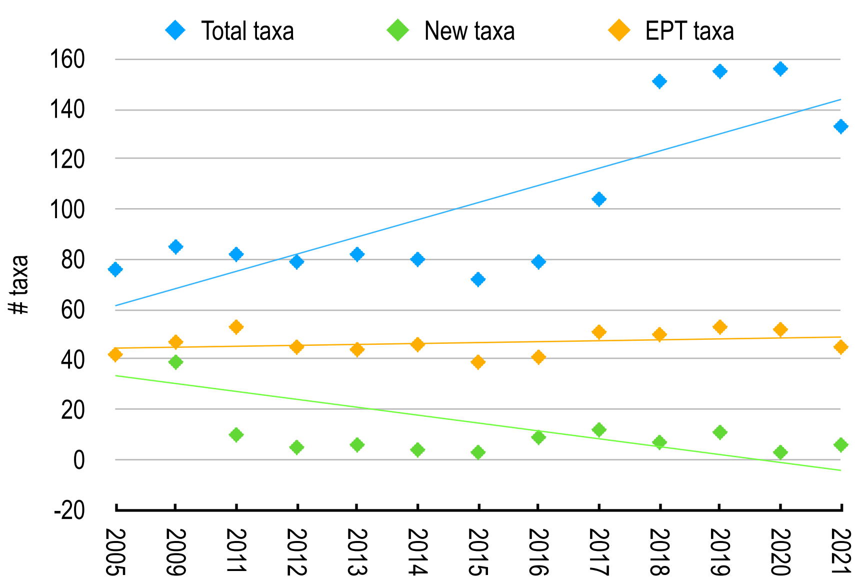
A total of 132 unique taxa in 49 families (35 insect, 14 non-insect) was collected across all sites, which is slightly less than in 2020 (156 taxa in 51 families). Four taxa were present in every sample and were associated with a variety of habitat conditions: Naidinae (widespread tolerant sludge worms associated with slower flows); *Ampumixis dispar* (sediment-sensitive riffle beetle associated with clear, cold flowing water); *Optioservus* (tolerant and sediment-tolerant riffle beetle that inhabits sediments and detritus in flowing and still habitats in streams); and *Cricotopus* (widespread genus of tube-building non-biting midge associated with a variety of waters). As usual, order-level diversity was highest among Diptera, with 58 unique taxa in eight families, including 43 genera of non-biting midge (Chironomidae), although these numbers were also lower than the prior year. Other well-represented groups included Ephemeroptera (mayfly; 21 unique genera in six families), Trichoptera (caddisfly; 18 unique genera in seven families), and Coleoptera (beetles; 10 unique genera in four families).

Multiple reaches of Whychus have been sampled across 13 years but taxa new to the Whychus dataset have been taken in each year (Figure 1). The increase in taxa taken since 2016 has likely been driven by a combination of recovery from restoration impacts at different sites; creation of new dynamic, heterogeneous side channels; and the addition of multihabitat samples, which tend to have greater taxa richness. The number of total and EPT taxa in 2021, while still high, was lower than the previous three sampling years. However, six taxa were taken for the first time in 2021; each occurred as a single individual in a multihabitat sample, and all but one were found around WC1100:

- *Paracymus*, a water scavenger beetle that prefers slower flows and is found in a range of temperatures (WC1100-3 side channel)
- *Yamatotipula*, a subgenus of the crane fly *Tipula* genus (WC1100-3 side channel)
- *Iswaeon*, a small minnow mayfly found in multiple flow types (WC1100-4 side channel duplicate sample)
- *Prostoma*, a ribbon worm associated with debris and plants, especially filamentous algae, in shallow water (WC1100-2 primary channel)
- *Paracricotopus*, a non-biting midge associated with mosses and algae in flowing water (WC2600 primary channel)

Interestingly, two of the three taxa that were new to the Whychus dataset in 2020 were found again in 2021; *Protoptila* (saddlecase-making caddisfly associated with larger, usually somewhat warmer streams and rivers; one individual in WC0600 primary channel riffle sample) and *Dicranomyia* (crane fly found in a variety of flows and habitats; one in WC0900 riffle sample). Numerical abundance across the dataset was dominated by *Optioservus*, a tolerant and sediment-tolerant riffle beetle that inhabits sediments and detritus in flowing and still habitats in streams, which was present in every sample at abundances ranging from 1-184 individuals, with lower abundance in samples from WC1950-WC2600.

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



The target sub-sampling number of 500 individuals was attained for all but two samples, with 27-100% of the total sample picked (Table 4). There was no significant difference between the proportion required for subsampling in riffle vs. multihabitat samples. Samples from the WC1100-3 side channel and WC1950 primary channel contained fewer organisms (364 and 316, respectively). However, inspection after sampling found that volunteers at WC1950 did not follow the protocol properly: wetted widths were not measured; water depths were measured only at the sampling point in each transect instead of at 25%, 50%, and 75% of the way across the transect; and the liquid portion of the composited sample was poured into jars but all mineral material and heavier-bodied invertebrates were left sitting in the bucket. CASM Environmental jarred the material in the bucket when it was found a few hours after sampling, but this departure from protocol means that operator error cannot be ruled out as the source of any changes in WC1950 data compared to prior years.

***Table 4. Richness, abundance, and evenness in 2021 samples.** Target sub-sampling number is 500. DUP = duplicate sample; R = riffle-targeted, M = 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 (# organisms)	Richness (# unique taxa)	# EPT taxa	Simpson's Diversity Index (1-D)
WC0600R	R	PC	63	535	41	15	0.9005
WC0850M	M	PC	70	534	38	15	0.8091
WC0900R	R	PC	40	544	41	19	0.8528
WC1100-1BM	M	PC/SC	43	565	45	19	0.8395
WC1100-2M	M	PC	47	540	44	16	0.8827
WC1100-02R	R	PC	20	527	53	20	0.9275
WC1100-3M	M	SC	100	364	44	18	0.9092
WC1100-4M	M	SC	60	553	43	16	0.8137
WC1100-4MDUP	M	SC	40	536	52	19	0.8525
WC1150M	M	PC	40	538	44	18	0.8491
WC1950M	M	PC	100	316	44	16	0.9322
WC2000M	M	PC	27	544	50	17	0.9233
WC2000R	R	PC	37	556	45	23	0.93
WC2050M	M	PC	67	528	57	16	0.9344
WC2600M	M	PC/SC	70	514	46	17	0.753

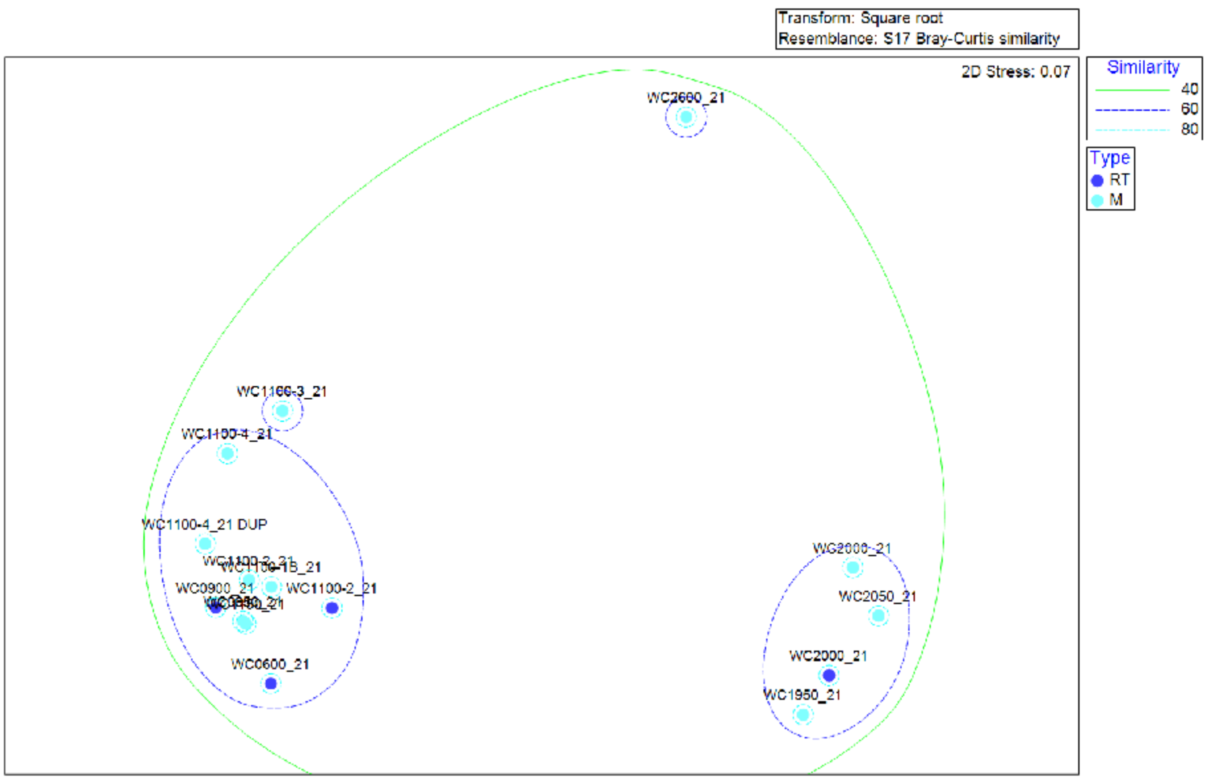
The number of unique taxa in each sample ranged from 38-57 (mean = 45.8; SD 5.1). This range is slightly lower than in 2020 but for reference, this metric in the ORDEQ IBI receives the highest scaled score at >35 unique taxa. For the first time since both sampling protocols were implemented, there was no significant difference in taxa richness between riffle and multihabitat samples (Table 5), and the mean number of EPT taxa in the two sample types continued to show no significant difference. Simpson's Diversity Index (1-D), which considers number of taxa and their individual abundances, was not significantly different between riffle and multihabitat samples but was lower overall than in 2020 (range in 2021 = 0.75 to 0.93), indicating that some samples were more heavily dominated by a single taxon. This is reflected in the relative abundances of the top (numerically dominant) taxon, which were higher overall compared to 2020 (range in 2021 = 12.7-45% abundance; mean 25.1%, SD 9.3). For reference, this metric in the ORDEQ IBI receives the highest scaled score at <20% relative abundance, and six of the 2021 samples were within this range.

Table 5. Comparison of community metrics between riffle and multihabitat samples in 2021. *Means are shown with standard deviation in parentheses. There was no significant difference between any pairs of means, although difference in mean #EPT taxa was close to significant (p=0.0568).*

	Riffle	Multihabitat	2021 range
# total taxa	45 (5.7)	46 (5.1)	38 - 57 taxa
# EPT taxa	19.3 (3.3)	16.8 (1.2)	15 - 23 taxa
# sensitive taxa	3.3 (1.5)	3.7 (0.8)	2 - 5 taxa
# sediment-sensitive taxa	2.3 (0.9)	1.8 (0.8)	1 - 3 taxa
% abundance tolerant taxa	29.2 (17.0)	30.7 (17.9)	4.7 - 65.3%
% abundance sediment-tolerant taxa	4.9 (4.3)	11.1 (12.7)	1.3 - 44.5%
% abundance top taxon	19.4 (4.8)	26.5 (9.9)	12.7 - 45.5%
Community temperature optima (^oC)	17.9 (0.4)	17.5 (1.3)	14.1-18.6^oC
Community fine sediment optima (% FSS)	7.3 (0.7)	7.9 (1.2)	6.2-10.6%
# ORDEQ cool indicator taxa	2.3 (2.6)	2.6 (1.5)	0-6 taxa
# ORDEQ warm indicator taxa	6.5 (1.7)	6.4 (1.8)	3-7 taxa
# ORDEQ low sediment indicator taxa	3.8 (1.5)	3.3 (1.4)	2-6 taxa
# ORDEQ high sediment indicator taxa	3.7 (1.5)	5.8 (2.8)	2-10 taxa

Although multihabitat samples are taken in areas that include slower flows and more sedimented or vegetated substrate, as in past years there was no significant difference between riffle and multihabitat samples for a variety of community metrics that reflect sediment or temperature tolerance (Table 5). The dominant substrate in most net sets regardless of sample type was cobble/gravel, with a smaller number taken in sand/silt or on boulders or wood, and all multihabitat samples included net sets taken in at least one riffle (see Table 2). Macroinvertebrate community composition continues to be 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 (R or M). The paired multihabitat samples taken in the WC1100-4 side channel were most similar to each other (Figure 2), confirming consistency of the sampling technique between different operators. Mean values among all downstream (WC0600-WC1150) and upstream (WC1950-WC2600) sites were significantly different for only a subset of all taxonomic and trait characters examined, and most related to temperature or sediment. Upstream site samples had significantly more DEQ indicator taxa for cool temperatures and low fine sediment levels and significantly greater relative abundance of predators, sprawlers, and multivoltine organisms. Downstream site samples had significantly more DEQ indicator taxa for warm temperatures and higher community temperature optima as well as greater relative abundance of tolerant, small- and medium-bodied, and univoltine and semivoltine organisms, as well as organisms associated with a mixture of flow types.

Figure 2. nMDS ordination of the macroinvertebrate community among all sampling sites in 2021. *Blue = riffle-targeted, aqua = multihabitat; DUP = duplicate sample. Colored circles show community similarity level overlays from a CLUSTER dendrogram of the same data.*



In a PCA ordination of taxa abundances (Figure 3), axis 1 explained 42% of the total variation among samples; taxa with the highest loading values were *Rhithrogena*, a ubiquitous sediment-sensitive flatheaded mayfly found in cold fast flows that was much less abundant in samples from upstream reaches (WC1950-WC2050); the tolerant riffle beetle *Optioservus*, which is a DEQ indicator taxon for warmer and more sedimented waters that had lower abundances in WC1950-WC2600 samples; and the riffle beetle *Zaitzevia*, a DEQ indicator taxon for warm temperatures and high sediment that was not found in samples from WC1950-WC2600. Axis 2 explained an additional 15% of variation; taxa with the highest loading values were *Nostococladius*, a sensitive, sediment-intolerant nonbiting midge that mines in *Nostoc* algae that had high abundances in WC0600 and WC2600 samples;

Optioservus; and *Glossosoma*, a sediment-intolerant saddlecase-maker caddisfly associated with cool rapid streams, which was more abundant in samples from upstream reaches.

In a PCA ordination of traits measured as relative abundances, axis 1 explained 46.3% of the total variation between samples (Figure 4); traits with the highest loading values were relative abundances of multivoltine organisms (higher at upstream sites), clingers (higher at downstream sites), and burrowers (high at WC2600). Axis 2 explained an additional 25.1% of the total variance; factors with the highest loading values were relative abundances of organisms that are tolerant (lower at upstream sites), associated with erosional flows, and sediment-tolerant (both of which were lower in some WC1100 side channels).

Figure 3. Ordination plot of a Principal Components Analysis (PCA) of taxa abundances among all 2021 samples. *Eigenvectors show dominant taxa contributions, where vector length is related to the strength of the contribution. Blue = riffle-targeted, aqua = multihabitat.*

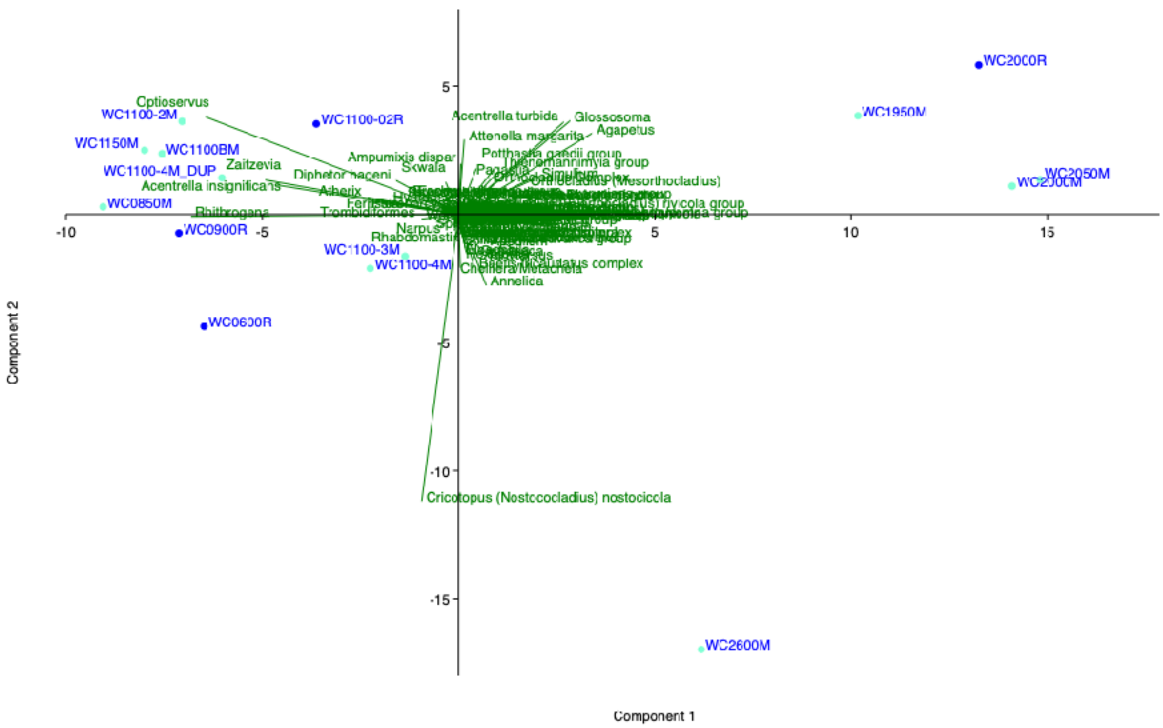
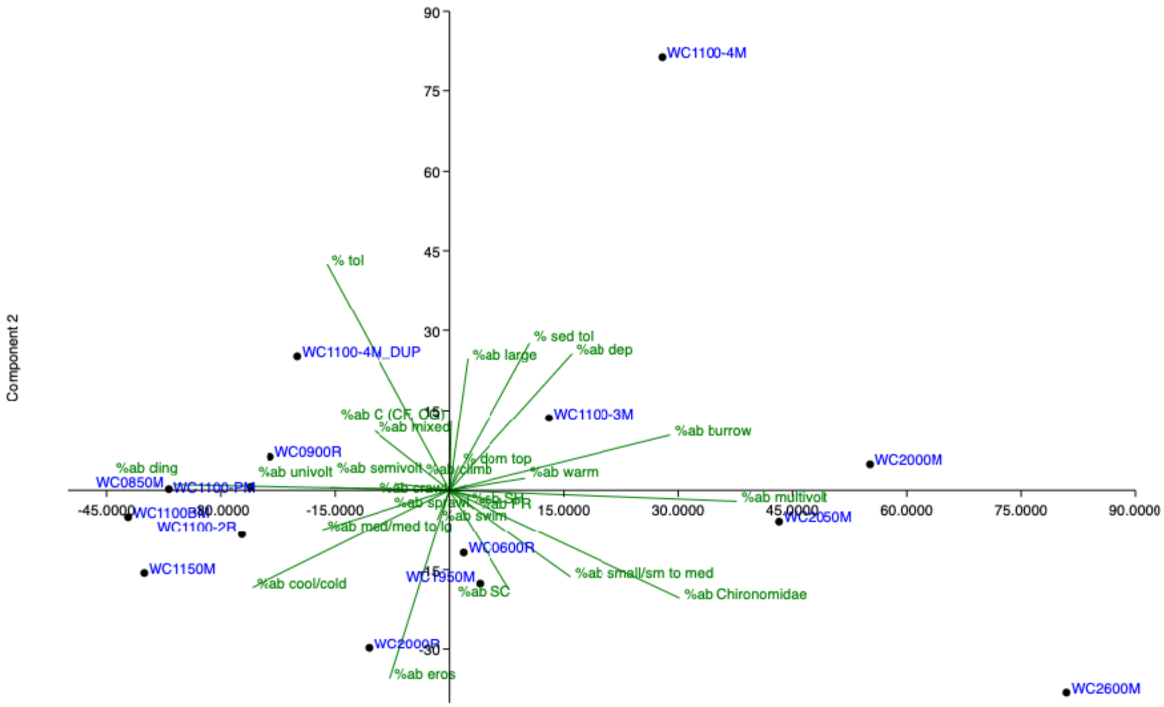


Figure 4. Ordination plot of a Principal Components Analysis (PCA) of traits calculated as relative abundances among all 2021 samples. *Eigenvectors show dominant taxa contributions, where vector length is related to the strength of the contribution. Blue = riffle-targeted, aqua = multihabitat*



Macroinvertebrate community characteristics at sampling sites

<u>WC0600</u>

WC0600 is a longterm index site and riffle-targeted samples were taken in the primary channel at WC0600 in every sampling year from 2005-2021; in 2018 and 2019, a multihabitat sample was also taken in the same reach. The 2021 sample community was dominated by *Optioservus*, a tolerant riffle beetle that is a DEQ indicator taxon for warmer and more sedimented water, at 19.1% of total organismal abundance. The majority of the community consisted of small- to medium-bodied multivoltine taxa that feed as collectors and scrapers and move as clingers in cooler, faster flows. Only one taxon collected in 2021 was not found at this site in at least one prior sampling year: Sphaeriidae (fingernail clam; 2 individuals), a small bivalve commonly found in in shallow muddy substrates in a variety of flows and temperatures that is a DEQ high sediment indicator taxon.

The target sub-sampling number of 500 organisms was attained in 10 of 13 sampling years, including every year since 2015 (Figure 5), although more sample needed to be picked in 2021 (63%) compared to the three prior years (18-22% picked). The number of taxa in the 2021 sample (41) was at the lower end of the range for this metric in 2021 and slightly lower than the prior three years at this site. However, total and EPT richness has been fairly stable across the last four years (Figure 6), with significantly greater richness (range = 41-47 taxa) compared to earlier sampling years (range = 24-37 taxa; p = 0.0002). The number of EPT taxa (15) was intermediate compared to earlier years and at the low end of the 2021 samples (range = 15 - 23 EPT taxa). Samples from this site have had consistently lower Trichoptera richness, while abundance of *Acentrella insignificans* (a small minnow mayfly that is slightly sensitive to fine sediment and is associated with erosional flows) 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. *Riffle samples were taken every year; multihabitat samples were taken in the same reach in 2018-2019. Target sub-sampling number is 500.*

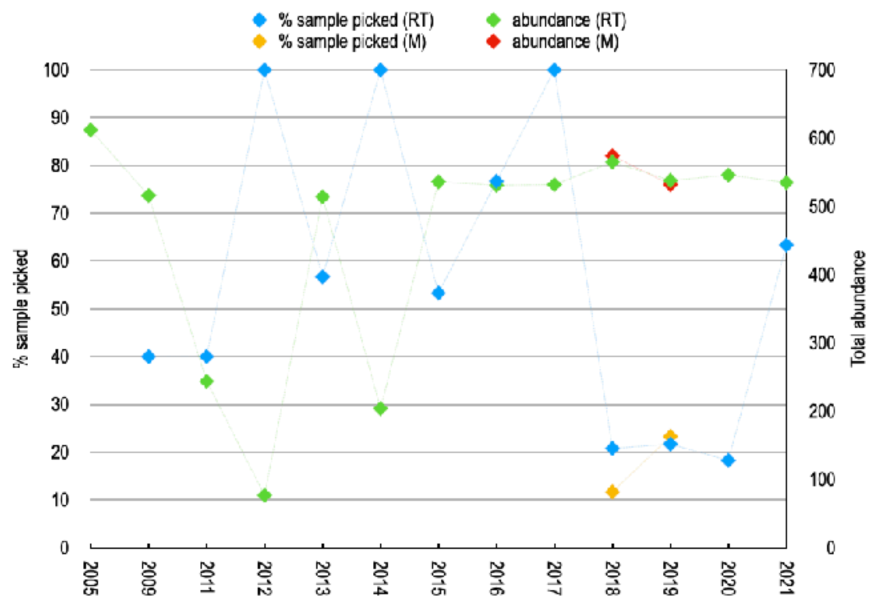
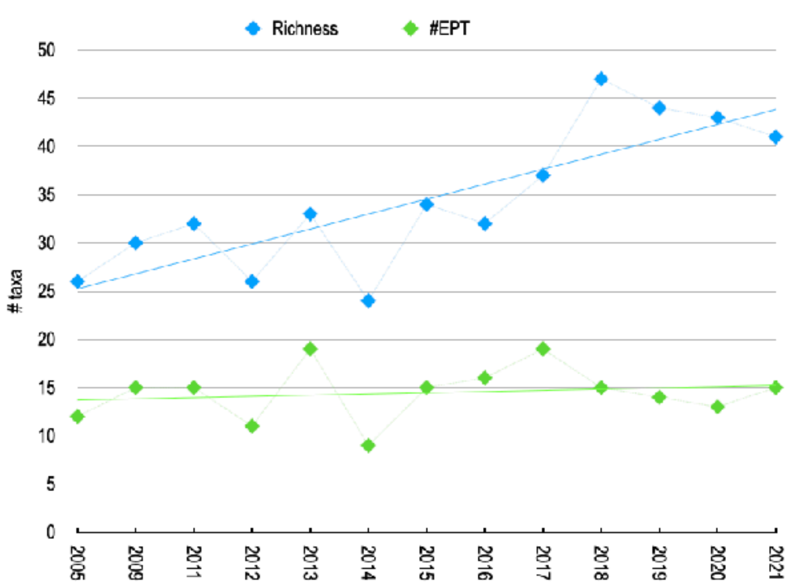


Figure 6. Sample richness and number of EPT taxa at WC0600 in all sampling years. *Riffle samples were taken every year; a multihabitat sample was taken in the same reach in 2018-2019. Values were averaged for the years in which both sample types were taken. Linear trendlines are shown. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



IBI and PREDATOR scores have fluctuated over time (Figure 7). IBI scores were significantly greater from 2018-2021 ($p = 0.0191$) and indicated minimal disturbance in 2018 and 2021. PREDATOR O/E scores decreased slightly but not significantly in the same period, with scores indicating fair conditions in three of the past four years. Few sensitive or sediment-sensitive taxa have been found at this site (range = 1-3), but both were present more consistently in recent years (Figure 8). Relative abundance of sediment-tolerant organisms at this site has always been low enough to receive the highest scaled IBI score (Figure 9), while abundance of tolerant organisms is higher and varies annually.

Figure 7. PREDATOR O/E and ORDEQ IBI scores at WC0600 in all sampling years. *Linear trendlines are shown.*

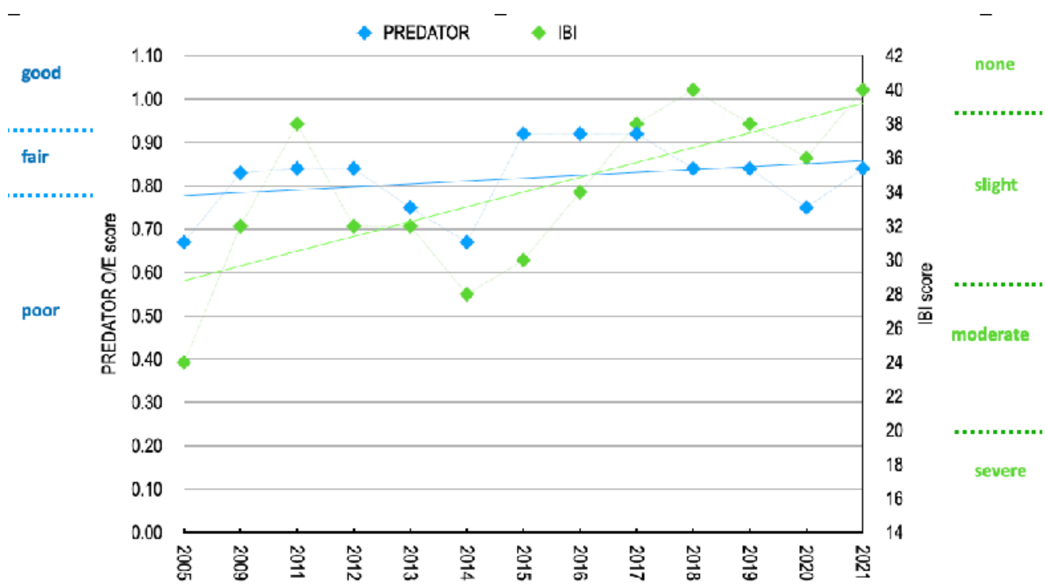


Figure 8. Numbers of sensitive and sediment-sensitive taxa at WC0600 in all sampling years. *Riffle samples were taken every year; multihabitat samples were taken in the same reach in 2018-2019. Values were identical for years in which both sample types were taken. Linear trendlines are shown. For these metrics in the ORDEQ IBI, the highest scaled score correlates with >4 sensitive and ≥2 sediment-sensitive taxa.*

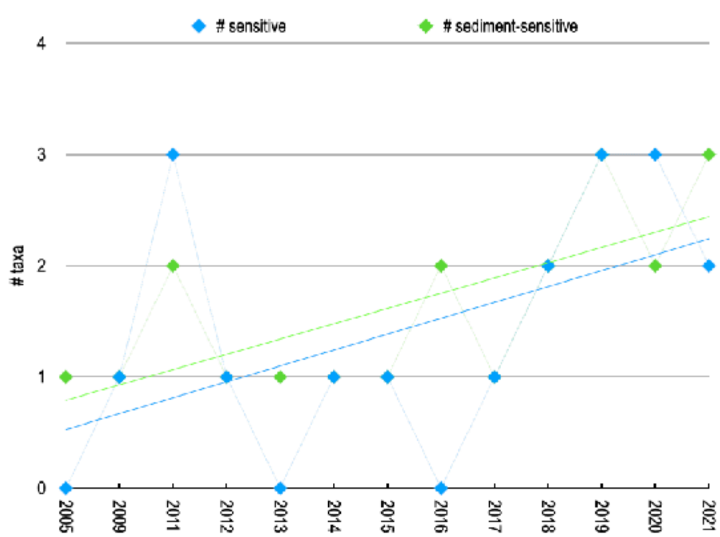
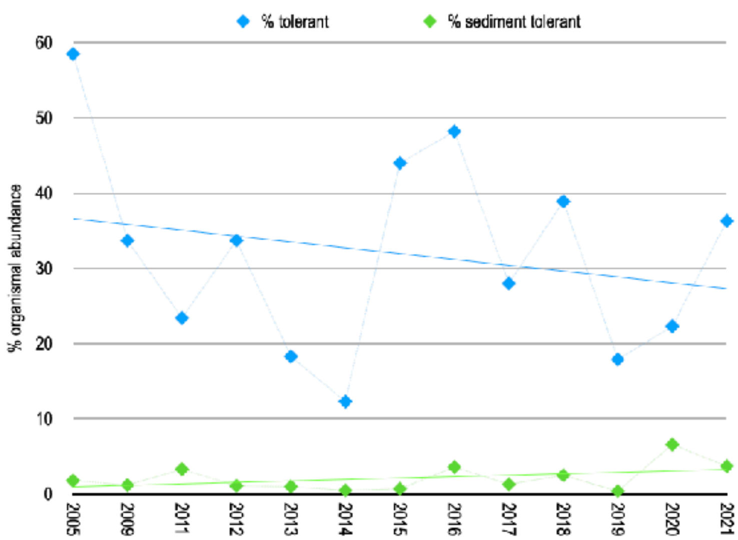


Figure 9. Relative abundance of tolerant and sediment-tolerant organisms at WC0600 in all sampling years. *Riffle samples were taken every year; multihabitat samples were also taken 2018-2019. Values were averaged for the years in which both sample types were taken. Linear trendlines are shown. For these metrics in the ORDEQ IBI, the highest scaled scores correlate with <15% tolerant and <10% sediment-tolerant.*



Macroinvertebrate community composition tends to be moderately balanced and is usually dominated by organisms associated with faster flows (Figure 10). This includes the top taxon in 2021, *Optioservus*, a tolerant riffle beetle that is a DEQ indicator taxon for warmer and more sedimented waters (present at 19% of total abundance). Community temperature optima fluctuate but are lower in the past three years (Figure 11), although there have been no DEQ indicator taxa for colder temperatures recently and warm indicator taxa numbers have risen (Figure 12). Community fine sediment optima increased slightly in recent years, while the number of DEQ low sediment indicator taxa has been stable.

Figure 10. Relative abundance of the numerically dominant taxon at WC0600 in all sampling years. *Riffle samples were taken every year; multihabitat samples were also taken 2018-2019. For this metric in the ORDEQ IBI, the highest scaled score correlates with <20% abundance of the top taxon.*

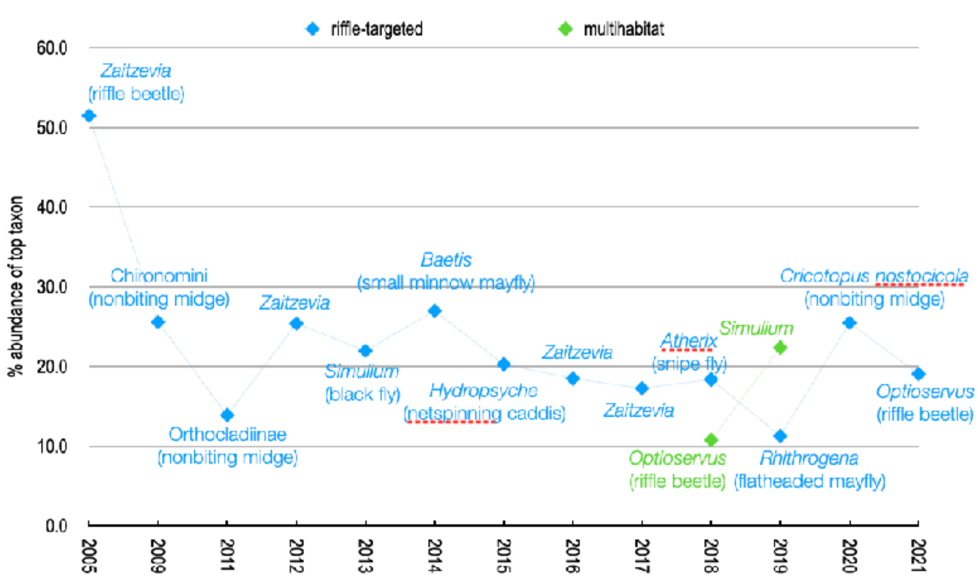


__Figure 11. Temperature and fine sediment optima of the community (weighted means) at WC0600 in all sampling years.__ Riffle samples were taken every year; multihabitat samples were also taken in 2018-2019. Values for years in which both sample types were taken were averaged. Linear trendlines are shown.

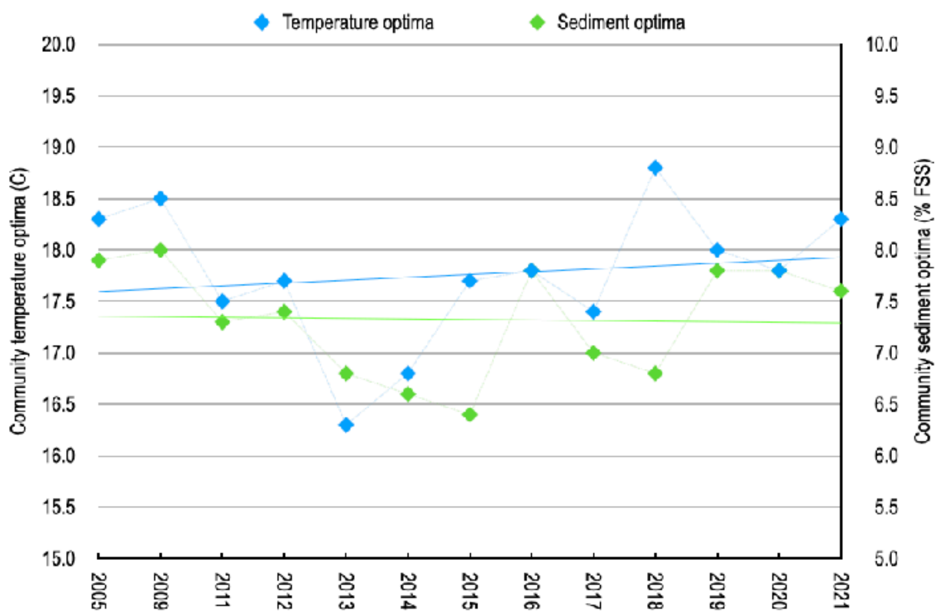
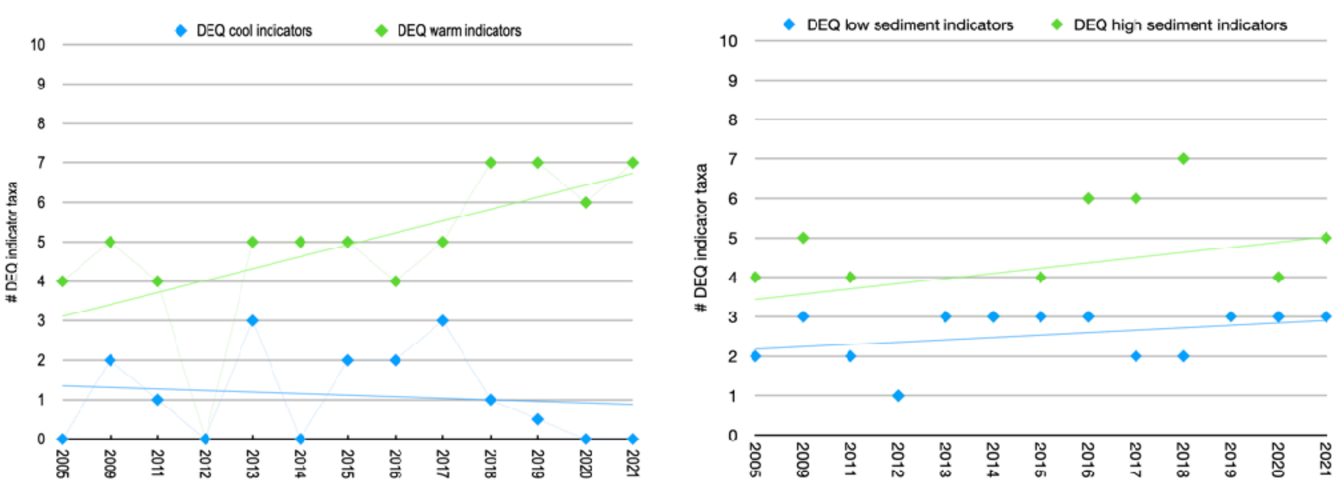


__Figure 12. Number of ORDEQ temperature and fine sediment indicator taxa at WC0600 in all sampling years.__ Riffle samples were taken every year; multihabitat samples were also taken in 2018-2019. Values for the years in which both sample types were taken are averaged here. Linear trendlines are shown. Note that ORDEQ indicator taxa do not account for the temperature associations of all taxa in a sample.



The 2021 sample was most similar to samples taken in 2018-2020 (Bray-Curtis Similarity Index range = 0.657 - 0.731; Figure 13). In a PCA ordination of taxa abundances (Figure 14), axis 1 explained 25.3% of the 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 2016-2021. Axis 2 explained an additional 17.5% 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 (greatest abundance in 2005 and 2009); Chironomini, a tolerant tribe of nonbiting midge in slower waters that is an ORDEQ warm temperature and high sediment indicator (high abundance in 2019 multihabitat sample); and Tanytarsini, a tolerant tribe of non-biting midge associated with warmer waters (greater abundance in 2018-2020).

Figure 13. nMDS ordination of the WC0600 macroinvertebrate community in all sampling years. *Blue = RT, aqua = M. The number at the end of each label indicates the sampling year. Colored circles show community similarity levels from a CLUSTER dendrogram of the same data.*

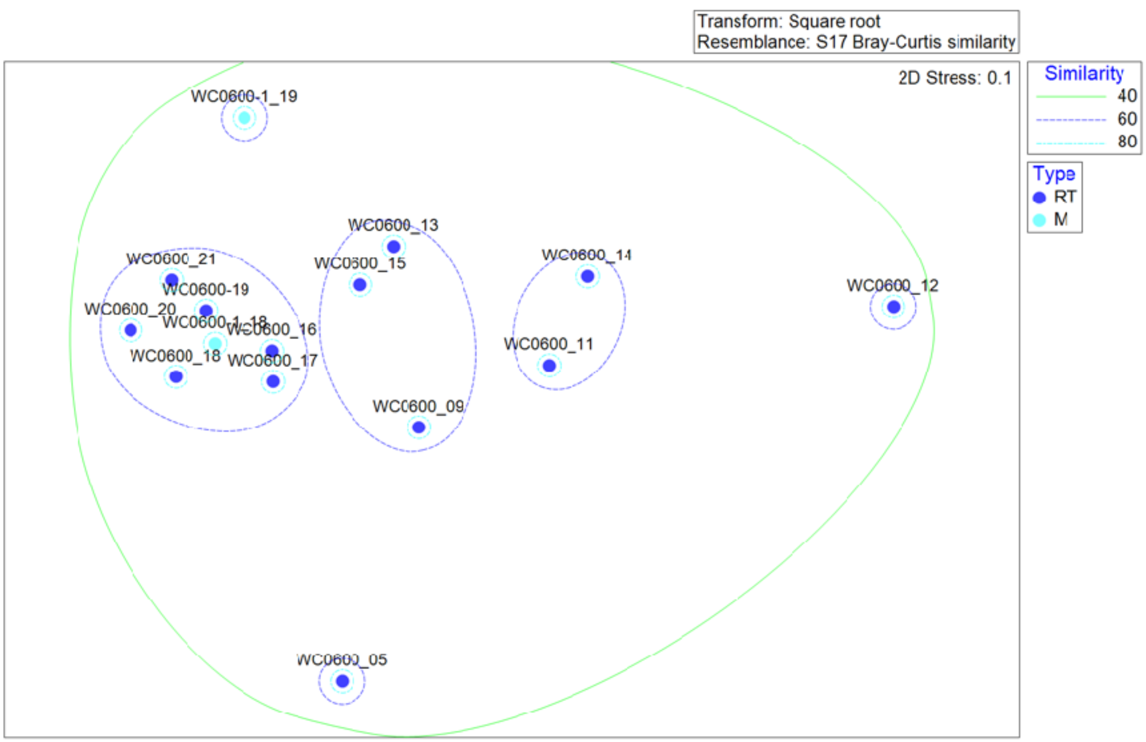
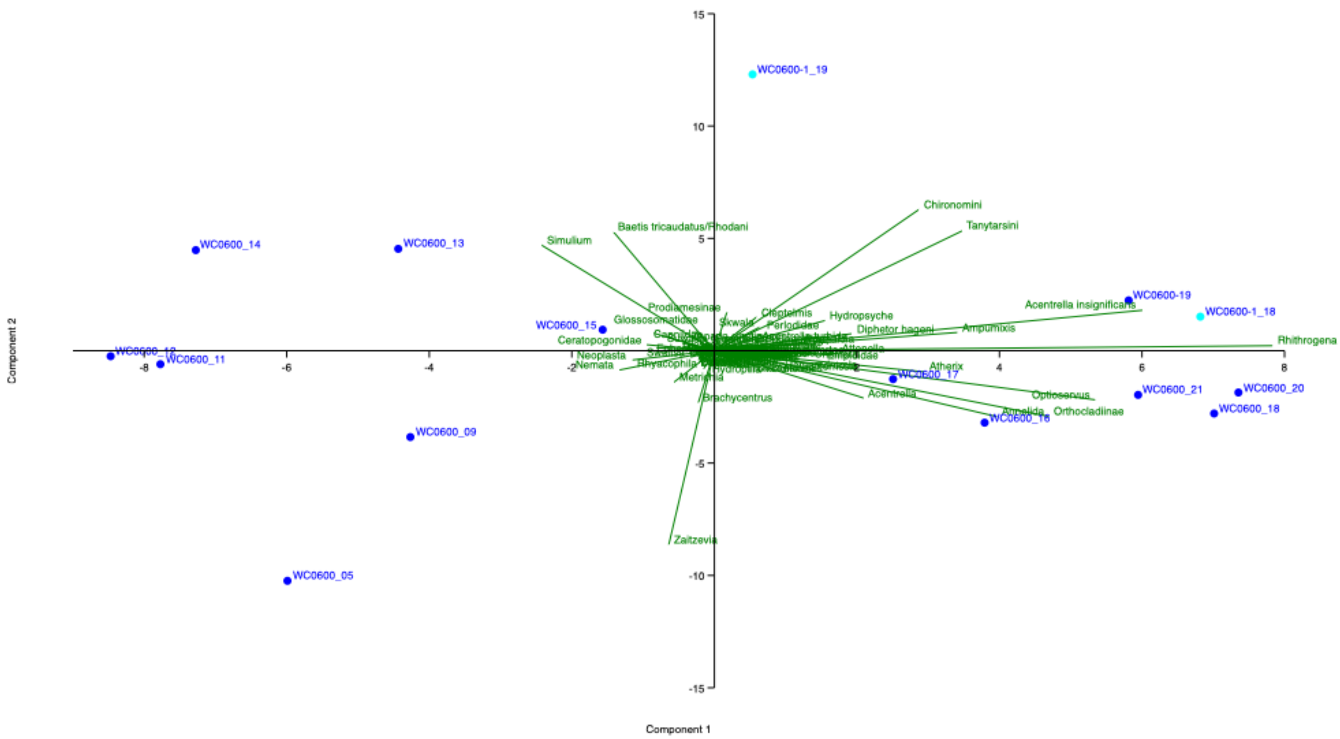
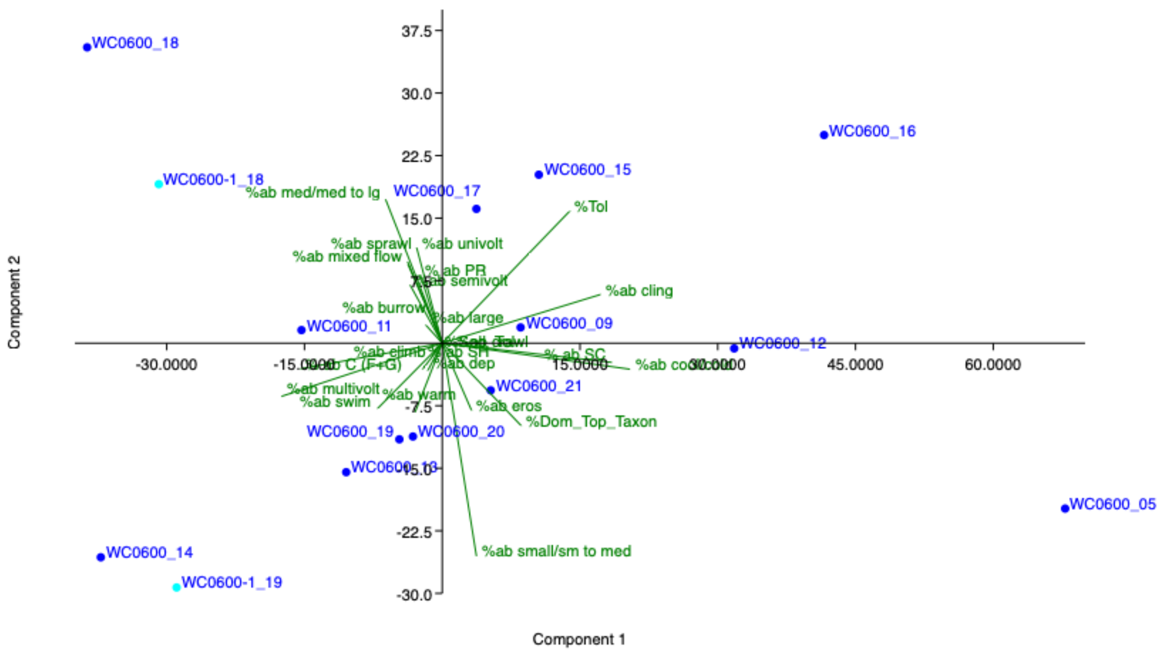


__Figure 14. PCA ordination of the WC0600 macroinvertebrate community in all sampling years.__ Blue = RT, aqua = M. The numeral in each label indicates the sampling year.



In a PCA ordination of traits measured as relative abundances in all years (Figure 15), axis 1 explained 42% of total sample variation; traits with the highest loading values were relative abundance of organisms associated with cooler temperatures, which fluctuates annually but is generally lower in multihabitat samples; scrapers, whose abundance fluctuates but has been similar in the last three years; and multivoltine organisms, which were more abundant in 2018-2021. 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 2015-2018 though abundant overall in each year), medium-bodied (more abundant in 2016-2018 samples), and tolerant (higher abundances in 2005 and 2015-2016).

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



<u>WC0850</u>

Riffle-targeted samples were taken in the primary channel of Whychus Creek around RM 8.5 from 2011-2017; multihabitat samples were taken in 2020-2021 to provide additional baseline data for planned restoration actions. The 2021 multihabitat sample was taken primarily in cobble substrate and a mixture of riffle, run, glide, and pool flows (Table 2). Among all samples taken in 2021, the WC0850 sample community was most similar to WC1150 (Figure 2; Bray Curtis Similarity Index = 0.779) and was dominated by *Optioservus*, a tolerant riffle beetle that is a DEQ indicator taxon for warmer and more sedimented water, at 33.3% of total organismal abundance. The overall community was comprised mainly of small, univoltine clingers that prefer cool flowing waters and feed by scraping and collecting. All taxa in the 2021 sample were found at this site in at least one prior sampling year.

The target sub-sampling number of 500 organisms was attained in six of the eight sampling years after 33-70% of the sample was picked (Figure 16). The 2021 sample had the fewest total taxa (38) among all 2021 samples (range in 2021 = 38 - 57) and represented a decrease from 2020 (Figure 17), although it was still greater than any riffle sample from this site. The number of EPT taxa (15) was also the lowest of any sample taken in 2021 (range = 15 - 23 EPT), and although it was within the range of most other sampling years at the site, it was lower than six of eight earlier years.

Figure 16. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC0850 in all sampling years. *RT samples were taken in 2011-2017; M samples were taken in 2020-2021. Target sub-sampling number is 500 organisms.*

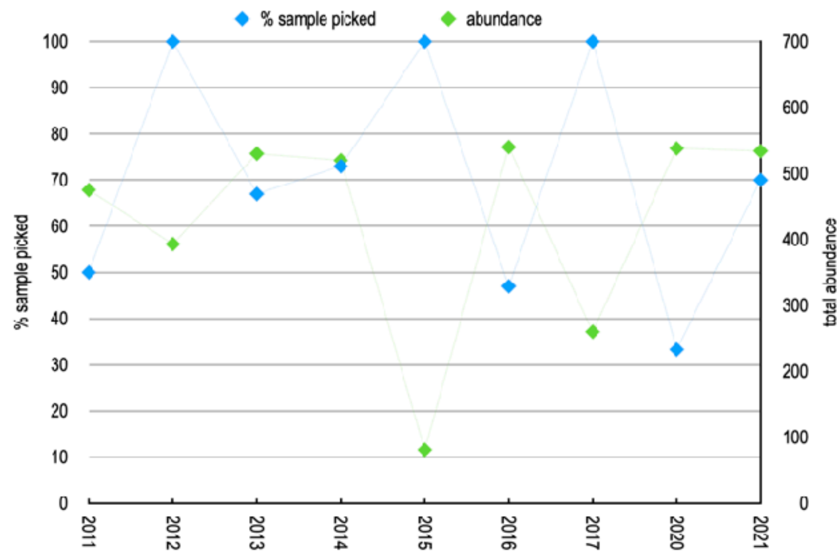
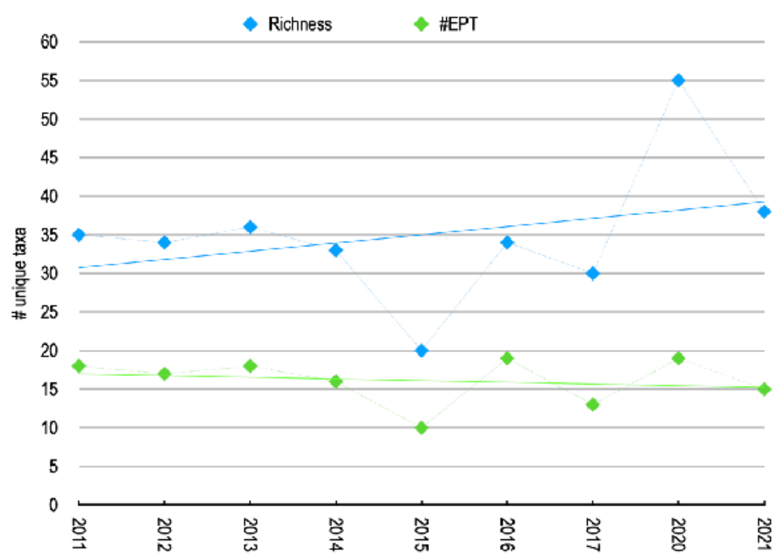


Figure 17. Sample richness and number of EPT taxa at WC0850 in all sampling years. *RT samples were taken in 2011-2017; M samples were taken in 2020-2021. Linear trendlines are shown. For reference, in the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



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 the multihabitat samples taken in the past two years but for reference, values for four of the 10 IBI metrics in the 2021 sample correlated with the highest scaled score: taxa richness, number of sediment-sensitive taxa, relative abundance of sediment-tolerant organisms, and MHBI. The number of sensitive and sediment-sensitive taxa were both greater in 2020-2021 than in prior sampling years (Figure 18). The relative abundance of tolerant organisms at this site in 2021 was greater than in any prior sampling year (Figure 19), while abundance of sediment-tolerant organisms has been low enough to receive the highest IBI score in every sampling year except 2012.

Figure 18. Numbers of sensitive and sediment-sensitive taxa at WC0850 in all sampling years. *RT samples were taken in 2011-2017; M samples were taken in 2020-2021. Linear trendlines are shown. For reference, these metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

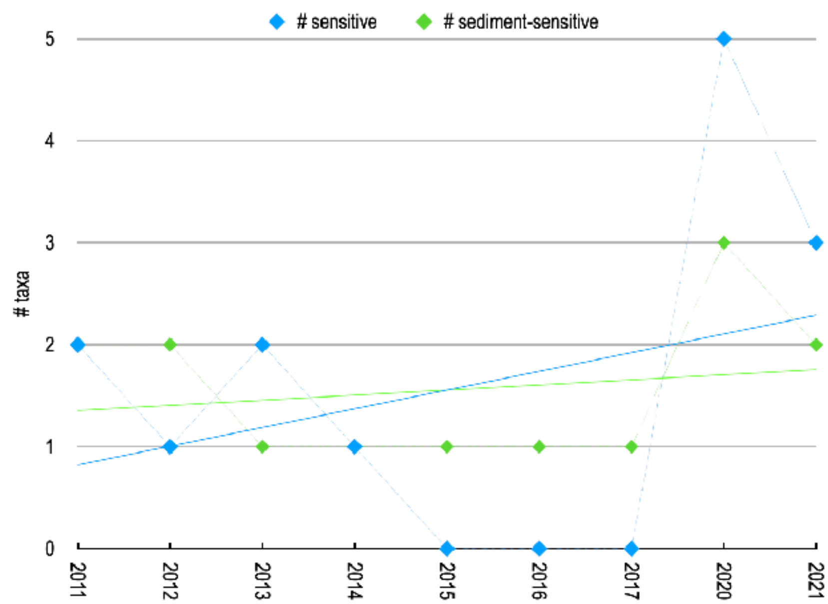
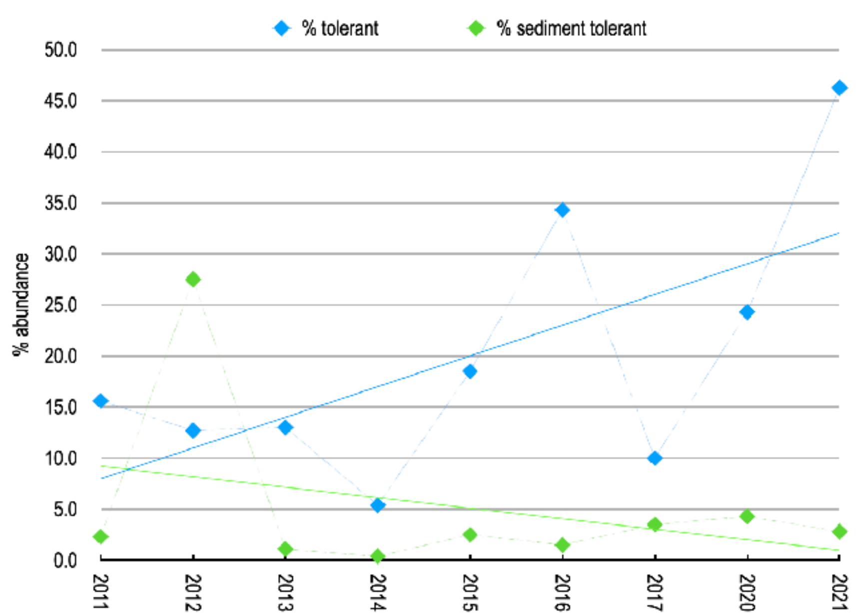


Figure 19. Relative abundance of tolerant and sediment-tolerant organisms at WC0850 in all sampling years. *RT samples were taken in 2011-2017; M samples were taken in 2020-2021. Linear trendlines are shown. For reference, these metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*



Macroinvertebrate community composition has been well to moderately balanced, although relative abundance of the top taxon was greater in 2021 compared to the four prior sampling years (Figure 20). WC0850 samples have been dominated consistently by taxa that prefer faster flows including in 2021, with the the tolerant riffle beetle *Optioservus* (a DEQ indicator taxon for warmer and more sedimented water) dominating organismal abundance. Community temperature and sediment optima have been higher in the last two years (Figure 21), and the number of DEQ indicator taxa for warm temperatures also increased (Figure 22).

Figure 20. Relative abundance of the numerically dominant taxon at WC0850 in all sampling years. *RT samples were taken in 2011-2017; M samples were taken in 2020-2021. For reference, this metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*

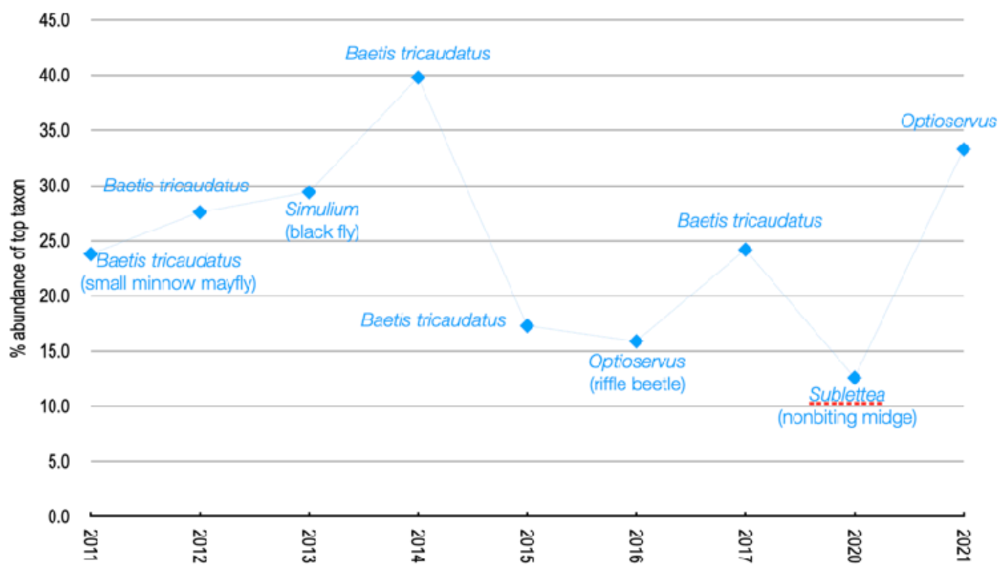


Figure 21. Temperature and fine sediment optima of the community (weighted means) at WC0850 in all sampling years. *RT samples were taken in 2011-2017; M samples were taken in 2020-2021. Linear trendlines are shown. Note that optima are not determined for every taxon in a sample.*

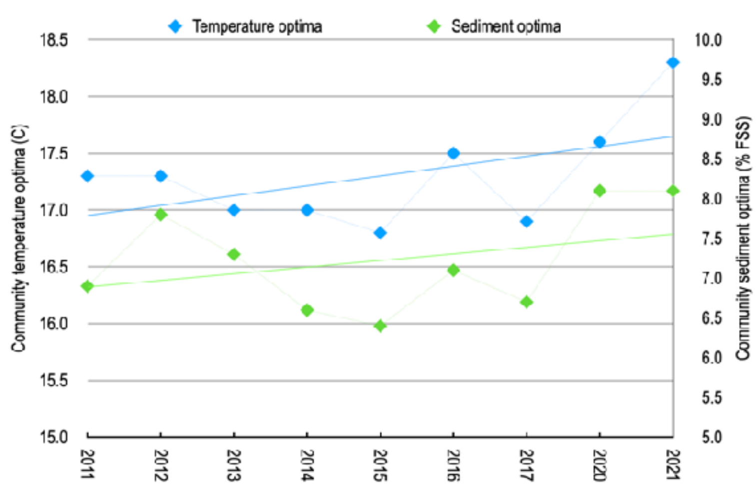
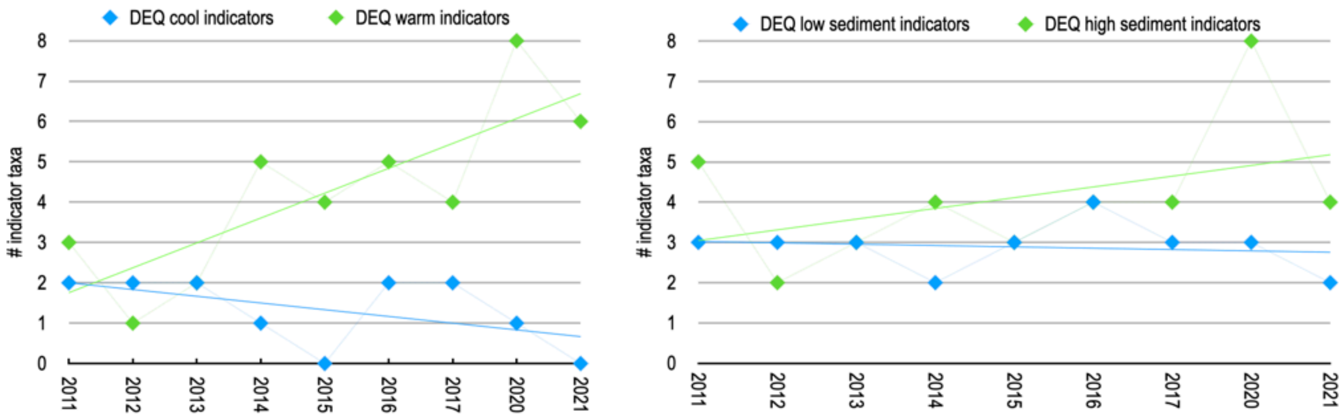


Figure 22. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC0850 in all sampling years. *RT samples were taken in 2011-2017; M samples were taken in 2020-2021. Linear trendlines are shown. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*



The 2021 sample was most similar to samples taken in 2020 and 2016 (Bray-Curtis Similarity Index = 0.674 and 0.608, respectively; Figure 23). In a PCA ordination of taxa abundances (Figure 24), axis 1 explained 43.6% of total sample variation; taxa with the highest loading values were *Optioservus,* a tolerant riffle beetle associated with faster flows (dominant taxon in two recent sampling years); *Simulium,* a black fly associated with erosional habitats that can be abundant following disturbance (much less abundant in 2020 and 2021); and *Baetis tricaudatus*, a sediment-sensitive small minnow mayfly that prefers clear fast water (dominant taxon in five earlier sampling years). Axis 2 explained an additional 16.6% of total variation; taxa with the highest loading values were *Simulium;* Tanytarsini, a tolerant tribe of non-biting midge associated with warmer waters (abundant in 2020 but absent from samples in four other years, including 2021); and *Brachycentrus*, a humpless case-maker caddisfly found in cold flowing water (greatest abundance in 2011).

Figure 23. nMDS ordination of the WC0850 macroinvertebrate community in all sampling years. *Blue = RT, aqua = M. The numeral in each label indicates the sampling year. Colored circles show community similarity levels from a CLUSTER dendrogram of the same data.*

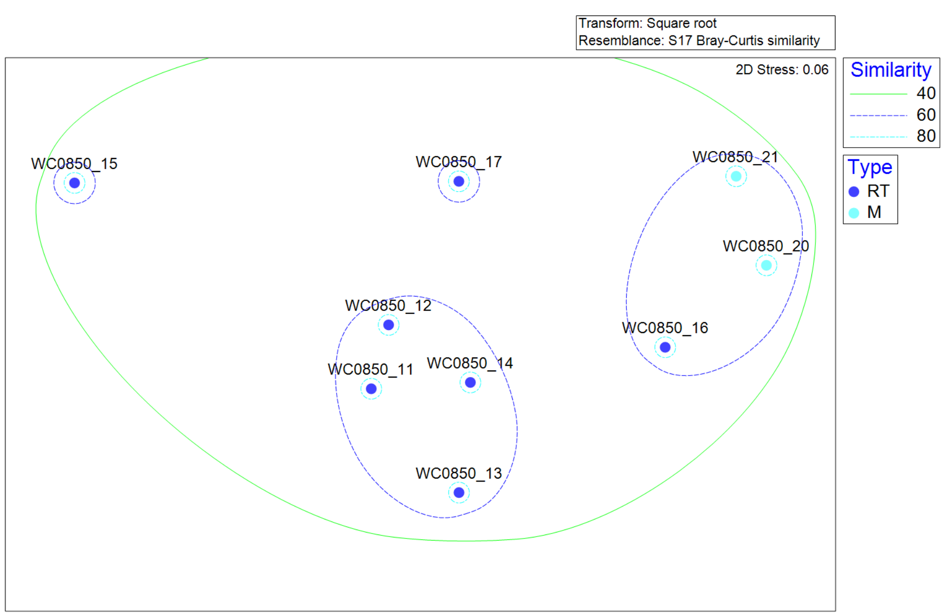
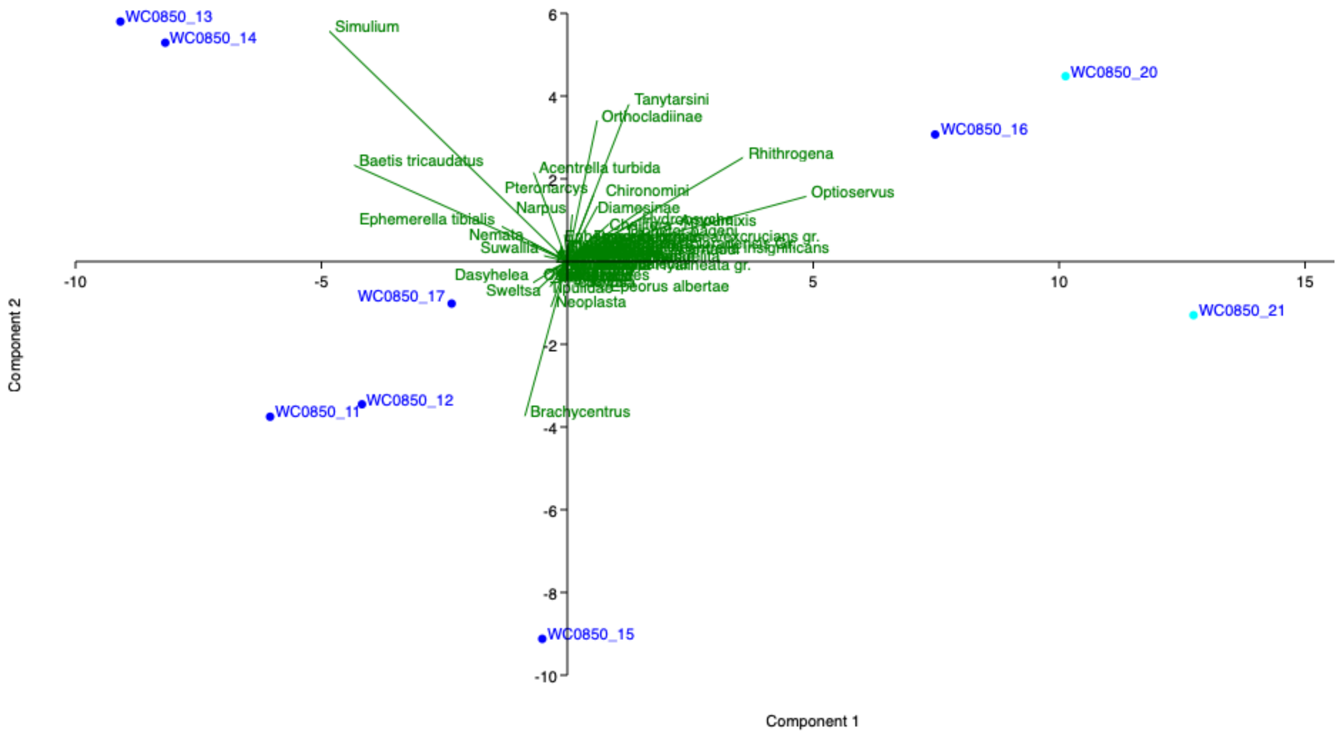
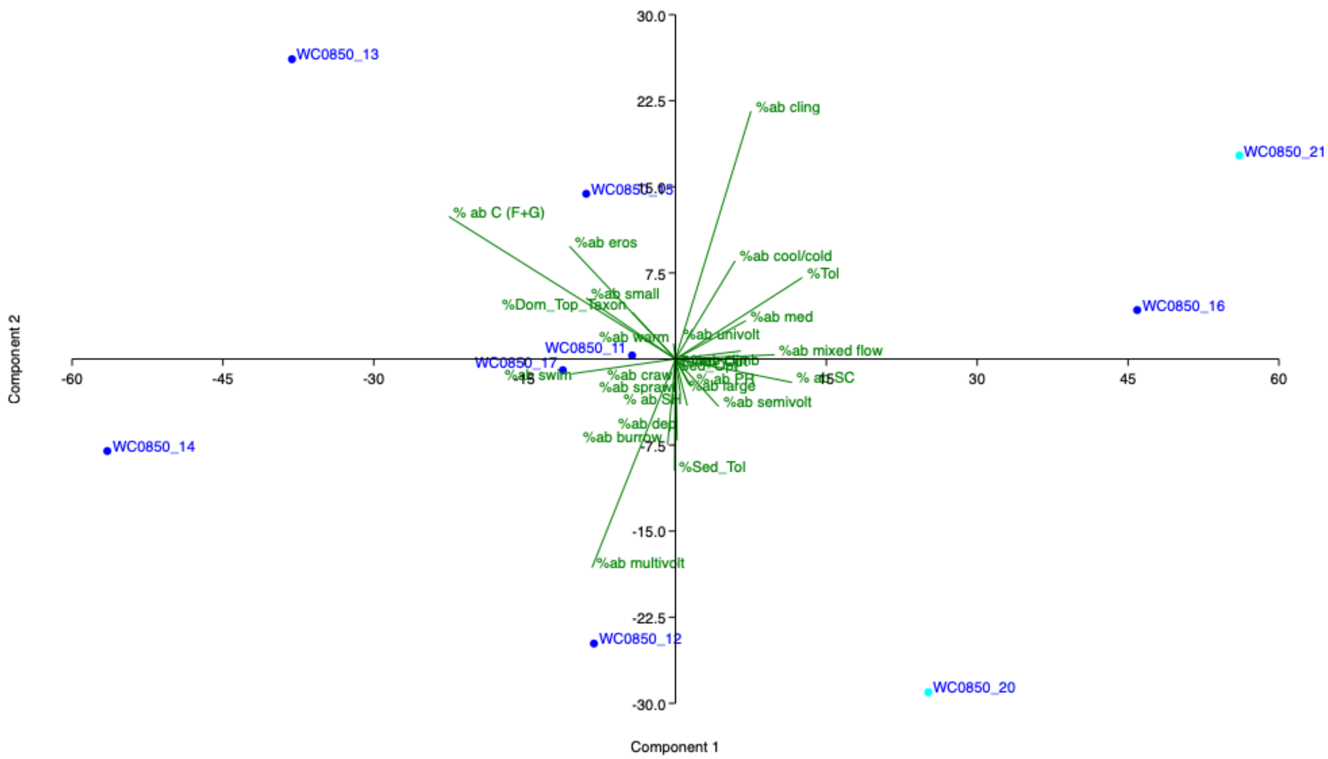


Figure 24. PCA ordination of the WC0850 macroinvertebrate community in all sampling years. *Blue = RT, aqua = M. The numeral in each label indicates the sampling year.*



In a PCA ordination of traits measured as relative abundances in all years (Figure 25), axis 1 explained 53% of total variation between samples; factors with the highest loading were relative abundance of collectors (lowest in 2020 and 2021), tolerant organisms (lower in earlier sampling years), and scrapers (higher in 2015-2021 compared to prior years). Axis 2 explained an additional 14% of total variation, and factors with the highest loading were relative abundances of clingers (lower in early sampling years), multivoltine organisms (low in 2016 and 2021), and collectors (less abundant in 2020-2021).

Figure 25. PCA ordination of the WC0850 macroinvertebrate community traits measured as relative abundances in all sampling years. *Blue = RT, aqua = M. The numeral in each label indicates the sampling year.*



WC0900

Riffle-targeted samples were taken in the primary channel of Whychus Creek at WC0900 in 2005-2017 and again in 2020-2021 to provide additional baseline data for planned restoration work. Among all samples taken in 2021, the WC0900 community was most similar to WC0850 (Figure 2; Bray Curtis Similarity Index = 0.745) and was dominated by *Rhithrogena*, a flatheaded mayfly associated with cold flowing water that is very sensitive to low levels of fine sediment, at 26.1% of total organismal abundance. The majority of the community consisted of small bodied, univoltine taxa that feed as scrapers or collectors and move as clingers in cooler, faster flows. Two taxa collected in 2021 had not been found at this site in at least one prior sampling year: *Anafroptilum*, a tolerant small minnow mayfly associated with sandy substrates in colder, slower flows (one individual); and *Rhyacophila angelita*, a sediment-sensitive free-living caddisfly associated with clear flowing water that is a DEQ indicator taxon for low fine sediment conditions (one individual).

The target sub-sampling number of 500 organisms was attained in eight of 11 sampling years, with 17-100% of the total sample picked (Figure 26). The number of taxa was at the low end of the range among all 2021 samples (41 taxa; range in 2021 = 38 - 57) but greater than any prior year at this site (Figure 27). The number of EPT taxa (19) was intermediate among 2021 samples (range = 15 - 23 EPT), and at the upper end of the range seen at this site (14-20 EPT from 2005-2020).

Figure 26. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC0900 in all sampling years. *RT samples were taken in all years. Target sub-sampling number is 500 organisms.*

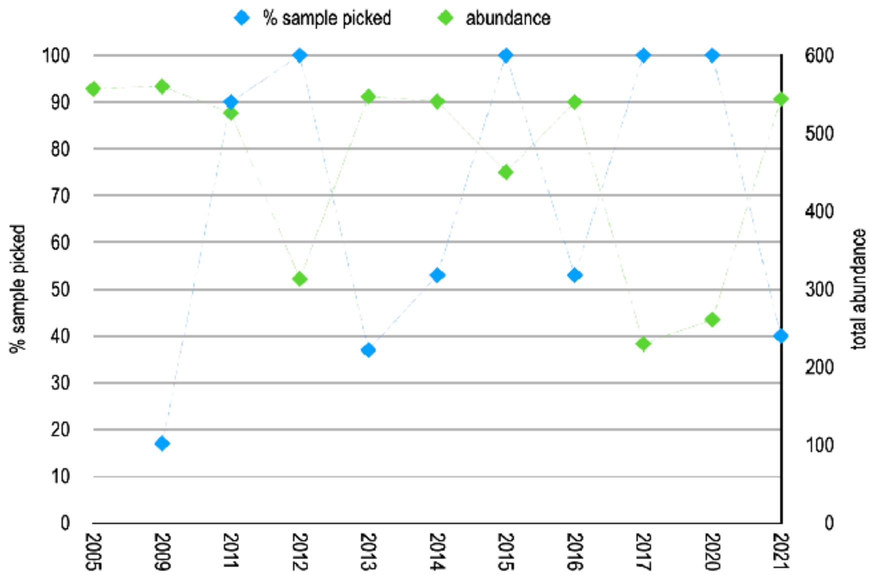
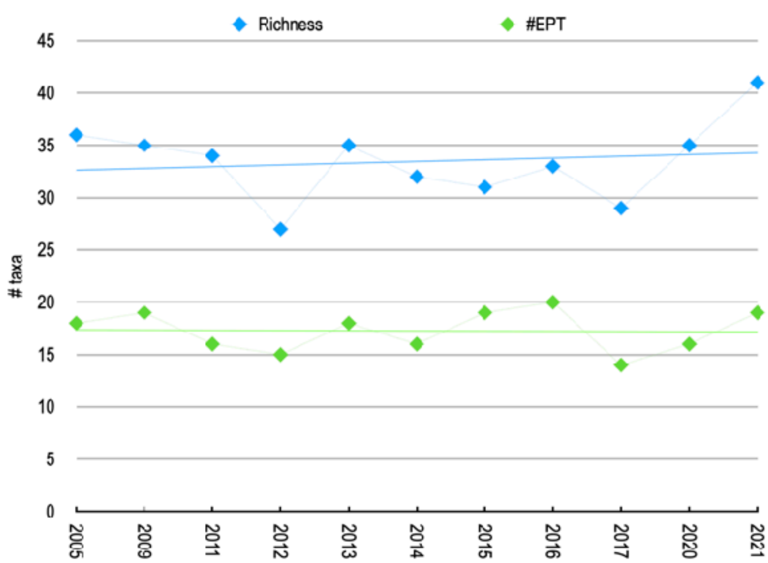


Figure 27. Sample richness and number of EPT taxa at WC0900 in all sampling years. *RT samples were taken in all years. Linear trendlines are shown. For reference, in the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



PREDATOR scores fluctuate but indicated fair biological conditions in all but four sampling years (Figure 28); the O/E score in 2021 (0.92) was the highest since 2012 and at the threshold of fair/good condition. IBI scores similarly fluctuated but always in a range corresponding to slight impairment; in 2021, values for four of the 10 IBI metrics correlated with the highest scaled score (taxa richness, mayfly richness, number of sediment-sensitive taxa, and MHBI). The number of both sensitive or sediment-sensitive taxa was higher in 2020-2021 compared to prior years (Figure 29), although relative abundances of tolerant and sediment-tolerant organisms were also greater (Figure 30).

*Figure 28. **PREDATOR O/E and ORDEQ IBI scores at WC0900.** Linear trendlines are shown.*

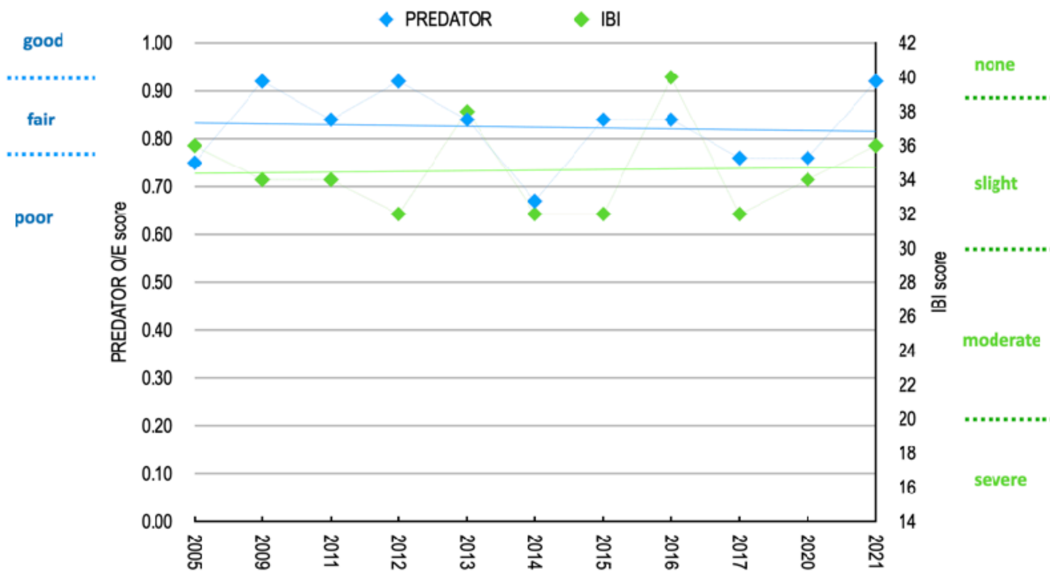


*Figure 29. **Numbers of sensitive and sediment-sensitive taxa at WC0900 in all sampling years.** RT samples were taken in all years. Linear trendlines are shown. For reference, these metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

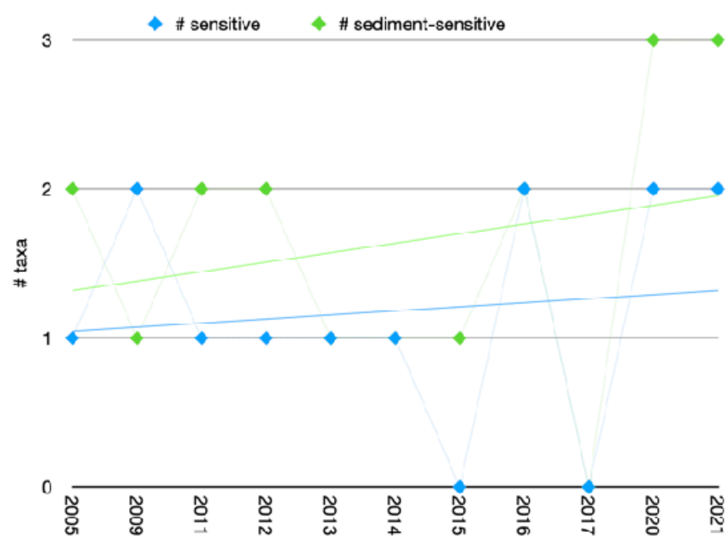
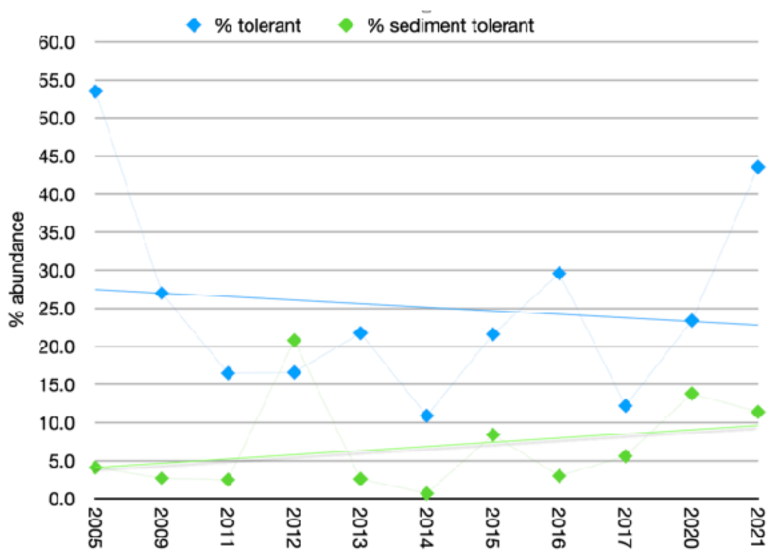


Figure 30. ***Relative abundance of tolerant and sediment-tolerant organisms at WC0900 in all sampling years.*** *RT samples were taken in all years. Linear trendlines are shown. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*



Macroinvertebrate community composition tends to be moderately balanced and dominant taxa are usually associated with faster or mixed flows, including in 2021, though in some years dominant taxa were more tolerant (i.e., *Zaitzevia* and *Optioservus* riffle beetles, worms, nonbiting midge subfamily, snails; Figure 31). Community temperature and sediment optima fluctuate increased overall since 2013, but both were lower in 2021 compared to 2020 (Figure 32). The numbers of DEQ indicator taxa for warm temperatures and high sediment levels were greater in 2021 than any prior sampling year (Figure 33), and at the upper end of the range among all 2021 samples.

Figure 31. Relative abundance of the numerically dominant taxon at WC0900 in all sampling years. *RT samples were taken in all years. For reference, this metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*

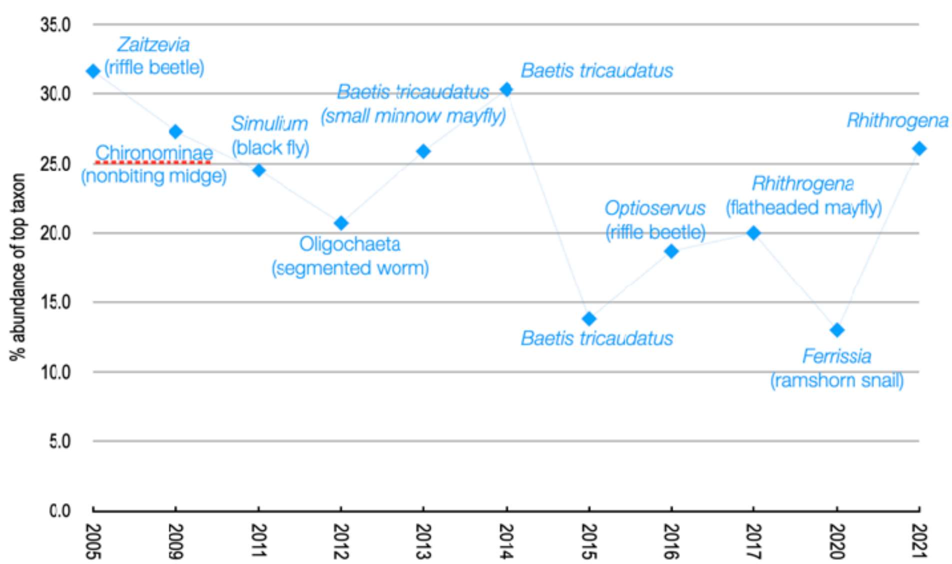


Figure 32. Temperature and fine sediment optima of the community (weighted means) at WC0900 in all sampling years. *RT samples were taken in all years. Linear trendlines are shown. Note that individual optima have not been determined for every taxon in a sample.*

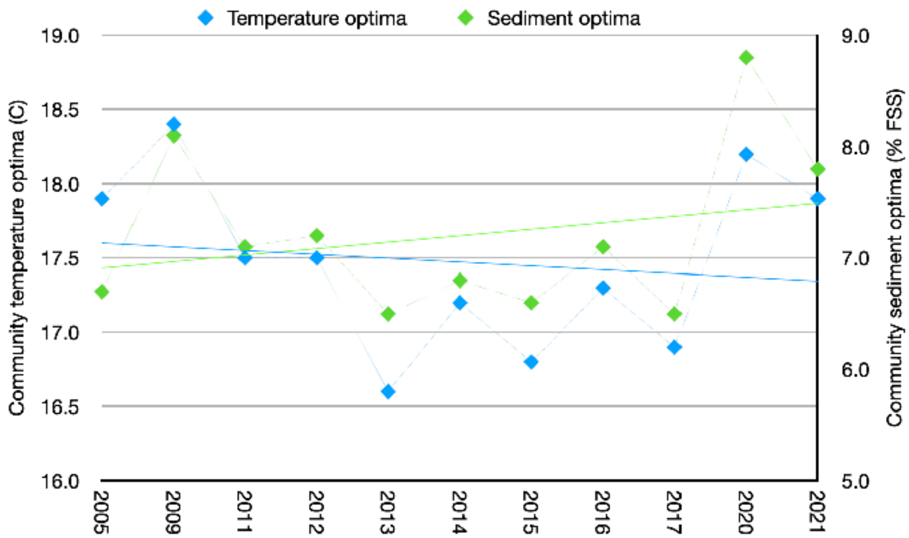
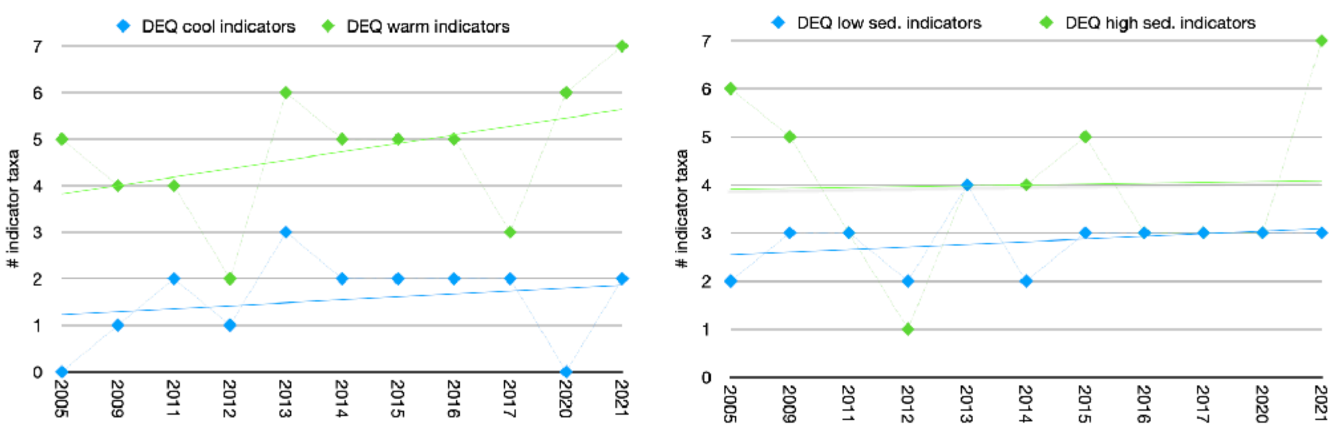


Figure 33. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC0900 in all sampling years. *RT samples were taken in all years. Linear trendlines are shown. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*



The 2021 sample was most similar to the sample taken in 2020 (Bray-Curtis Similarity Index = 0.564) and these two differed more from all prior years (Figure 34). In a PCA ordination of taxa abundances (Figure 35), axis 1 explained 35.5% of total sample variation; taxa with the highest loading values were *Baetis tricaudatus*, a small minnow mayfly associated with clear, flowing water and a DEQ low sediment indicator (dominant taxon 2013-2015); *Simulium,* a black fly associated with erosional habitats (dominant taxon in 2011); and *Rhithrogena,* a sediment-sensitive flatheaded mayfly found in colder faster water (dominant taxon in 2017 and 2021). Axis 2 explained an additional 14.7% of total variation; taxa with the highest loading values were *Zaitzevia*, a tolerant riffle beetle found in faster flows that is a DEQ warm temperature and high sediment indicator (most abundant in 2005); Tanytarsini, a tribe of chironomid midges associated with mixed flows and warmer waters that build tubes on soft sediments (absent from

2005-2011); and *Brachycentrus*, a humpless case-maker caddisfly associated with cold flowing water (lower abundance 2020-2021).

In a PCA ordination of traits measured as relative abundances in all years (Figure 36), axis 1 explained 46% of total sample variation; traits with the highest loading values were relative abundance of organisms associated with cooler water (higher in 2021 than any prior year), clingers (high in 2005 and 2021), and scrapers (higher in more recent sampling years). Axis 2 explained an additional 26% of variation; traits with the highest loading values were relative abundances of organisms that are small-bodied (less abundant in 2015-2020), associated with erosional flows (lower in 2009 and 2020), and feed as collectors (lower in 2016-2021).

Figure 34. nMDS ordination of the WC0900 macroinvertebrate community in all sampling years. *RT samples were taken in all years. The numeral in each label indicates the sampling year. Colored circles show community similarity levels from a CLUSTER dendrogram of the same data.*

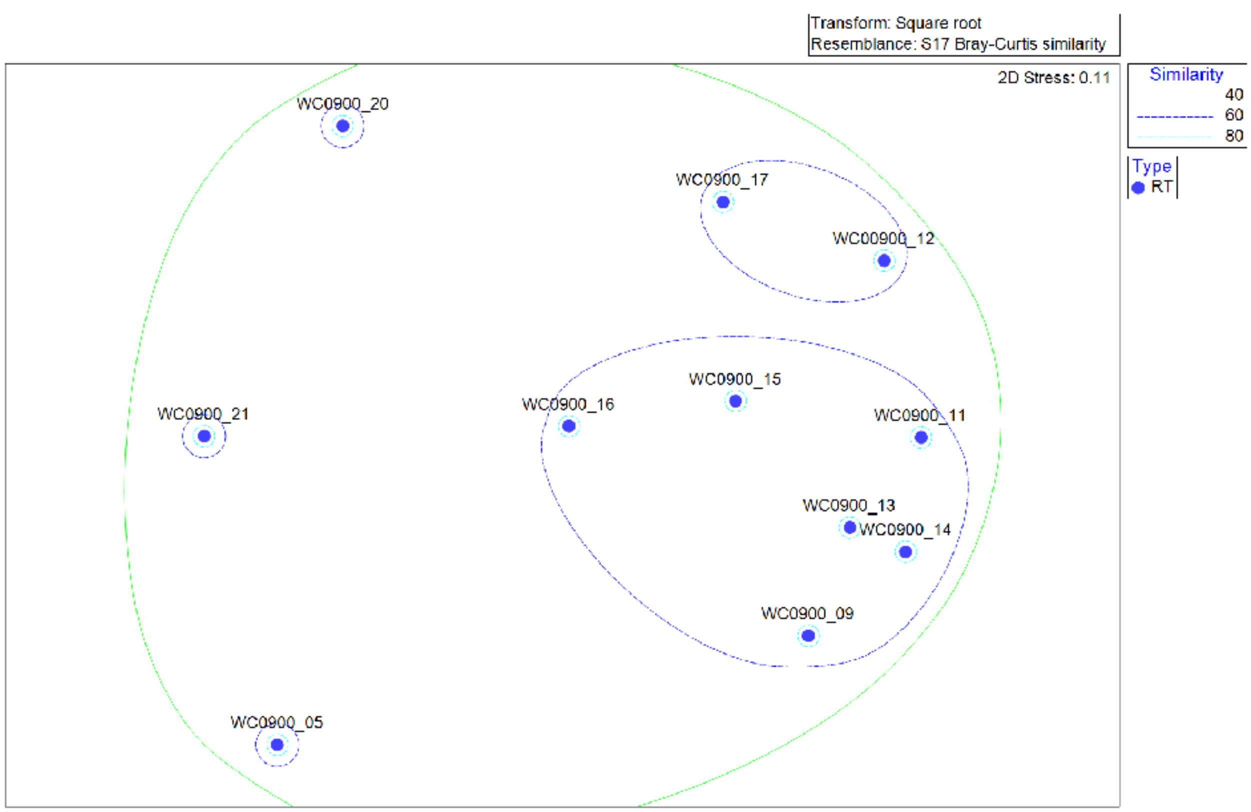


__Figure 35. PCA ordination of the WC0900 macroinvertebrate community in all sampling years.__ RT samples were taken in all years. The numeral at the end of each label indicates the sampling year.

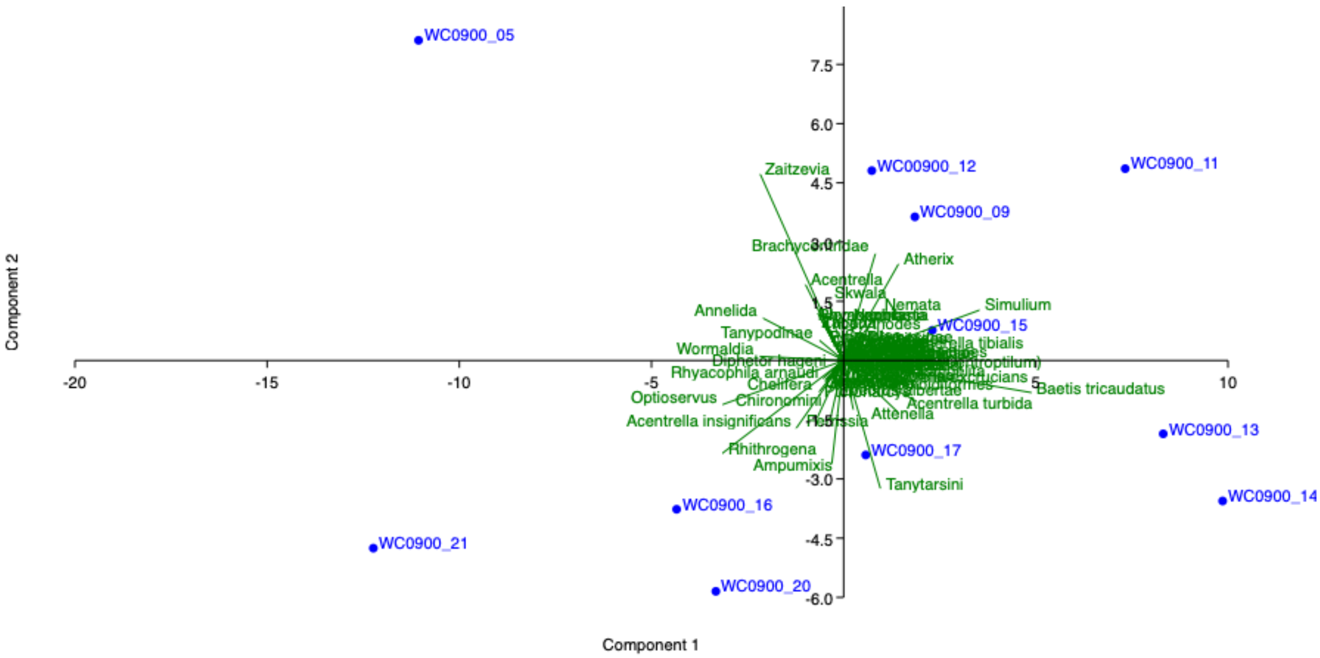
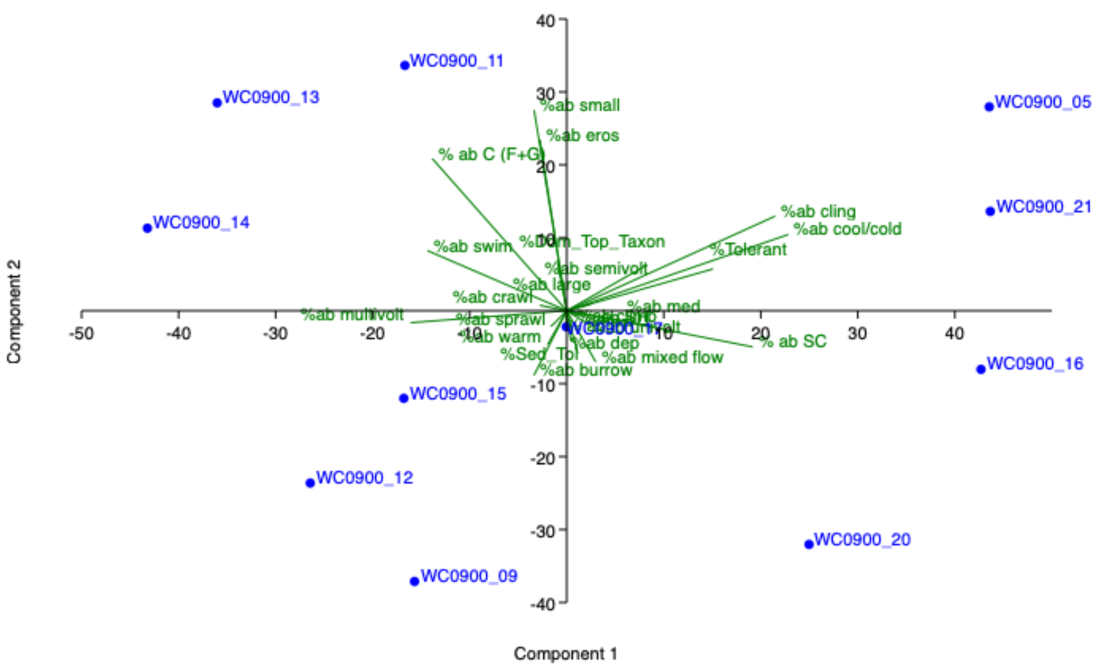


__Figure 36. PCA ordination of the WC0900 macroinvertebrate community traits measured as relative abundances in all sampling years.__ RT samples were taken in all years. The numeral at the end of each label indicates the sampling year.



WC1100

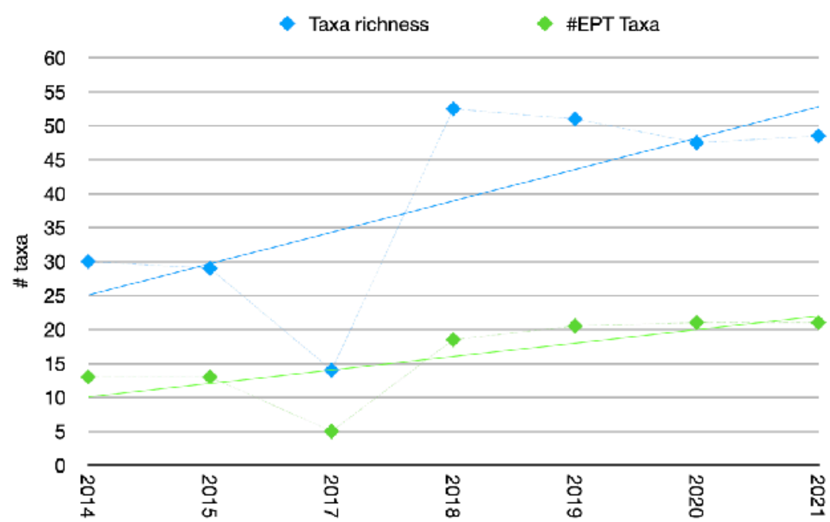
Riffle-targeted samples were taken in the primary channel of Whychus Creek around RM 11.0 in 2014-2015 to collect baseline data prior to the Whychus Canyon restoration project that created new side channel habitat in 2016. After restoration, riffle and multihabitat samples were taken in the same reach of the primary channel (PC; 2017-2021) and multihabitat samples were taken in side channels (SC; 2017-2021). Stream channels are braided and dynamic; samples taken in 2021 included a reach with both primary and secondary channel (PC/SC) habitat (WC1100-1B; multihabitat), reaches in two side channels (SC; WC1100-3, WC1100-4; multihabitat), and the primary channel (WC1100-2 riffle and multihabitat). The PC multihabitat sample was taken in cobble/gravel/sand substrate and a mixture of riffle and run flows (Table 2). Side channel samples were taken in similar habitats and flows, although WC1100-3 contained more net sets taken in riffles while the WC1100-4 sample had more net sets in runs (Table 2).

Among all 2021 samples, the community in the primary channel RT sample was most similar to the WC1100-1B PC/SC sample (Bray-Curtis Similarity index = 0.772; Figure 2) and also had a high degree of similarity to the multihabitat sample taken in the same reach and at WC1150 (Bray-Curtis Similarity index = 0.726 and 0.713, respectively). The PC multihabitat sample was most similar to multihabitat samples taken at WC0850 and WC1150 (Bray-Curtis Similarity index = 0.730 and 0.762, respectively). Samples in the two side channels differed more but were overall more similar to communities in downstream reaches (WC0600-WC1150) than to reaches further upstream (WC1950-WC2600; Figure 1). The duplicate samples taken at WC1100-4 for quality assurance were most similar to each other (Bray-Curtis Similarity index = 0.681).

The number of total taxa (range = 44-43) and EPT taxa (range = 16-20) among all WC1100 samples was similar in PC vs. SC samples, although the riffle-targeted sample had the most of each (Table 5). These values were in the middle of the range for all 2021 samples (Table 4), and both were higher in PC samples taken at WC1100 in 2018-2021 compared to earlier sampling years (Figure 37). Relative abundance of the dominant taxon was very low among all WC1100 samples (range = 2.1-13.5%) except in the WC1100-4 side channel, where the top taxon accounted for 44.5% of total organismal abundance. However, relative abundance of the top taxon in the duplicate WC1100-4 sample was only 8.8%. The dominant taxon in samples that included any primary channel reaches was *Optioservus*, a tolerant and sediment-tolerant riffle beetle that inhabits sediments and detritus in lotic and lentic stream habitats. The two side channels differed; WC1100-3 was dominated by *Rhithrogena* (a sediment-sensitive flatheaded mayfly associated with cold flowing water) while WC1100-4 was dominated by the tolerant sludge worm family Naididae, although the duplicate sample in the same reach was also dominated by *Optioservus*.

The community in all WC1100 samples in 2021 was composed mainly of small-bodied, multivoltine and univoltine organisms that move as clingers, prefer colder flows, and feed as collectors. Side channel samples, while still containing a majority of clinger organisms, had more burrowers and fewer organisms associated with erosional flows than any samples that contained primary channel habitat. Seven taxa taken in 2021 were not seen here in prior years, four of which were also new to the complete Whychus data set. All new site taxa occurred at very low abundances (1-2 individuals) and primarily in side channels: *Paracymus*, a water scavenger beetle associated with slower flows in a range of temperatures (WC1100-3); *Yamatotipula*, a subgenus of tipulid crane fly associated with detritus in a range of flows (WC1100-3 side channel); *Iswaeon*, a small minnow mayfly associated with a variety of flows (1100-4 side channel duplicate); Aeshnidae, an overall tolerant family of darner dragonfly whose nymphs are associated with vascular hydrophytes in a variety of fresh waters (WC1100-4 SC duplicate); *Metrichia*, a pursecase-making caddisfly often associated with mats of filamentous algae in flowing waters (WC1100-4 SC); *Prostoma*, a ribbon worm associated with filamentous algae and organic debris in slow flows (WC1100-2 PC multihabitat); and *Onocosmoecus*, a common Northern caddisfly associated with slower-flowing sections of cool rivers and streams (WC1100-2 PC multihabitat).

Figure 37. Sample richness and number of EPT taxa at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. M and RT samples were taken in the same reach at the same time in 2018-2021; values for those years are averaged here. Linear trendlines are shown. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



The target subsampling number of 500 organisms was attained in all PC riffle-targeted samples (20-80% of total sample picked) except in 2017, and in two of the four years of PC multihabitat samples (36-47% of total sample picked; Figure 38). Organismal abundance in side channels is consistently high, with all multihabitat samples taken in each year except WC1100-3 in 2021 attaining the target subsampling number (18-60% of total sample picked). Relative abundance of the dominant taxon in PC riffle and multihabitat samples decreased over time and was low enough to receive the highest scaled IBI score (<20%) in all samples since 2018, with the exception of the 2021 multihabitat sample, which was just outside the top-scoring cutoff (24.6%; Figure 39). This metric tends to vary among side channels, where the top taxon has been present at anywhere from 14-46.2% relative abundance from 2018-2021.

Figure 38. ***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. M and RT samples were taken in the same reach at the same time in 2018-2021. Target sub-sampling number is 500 organisms.*

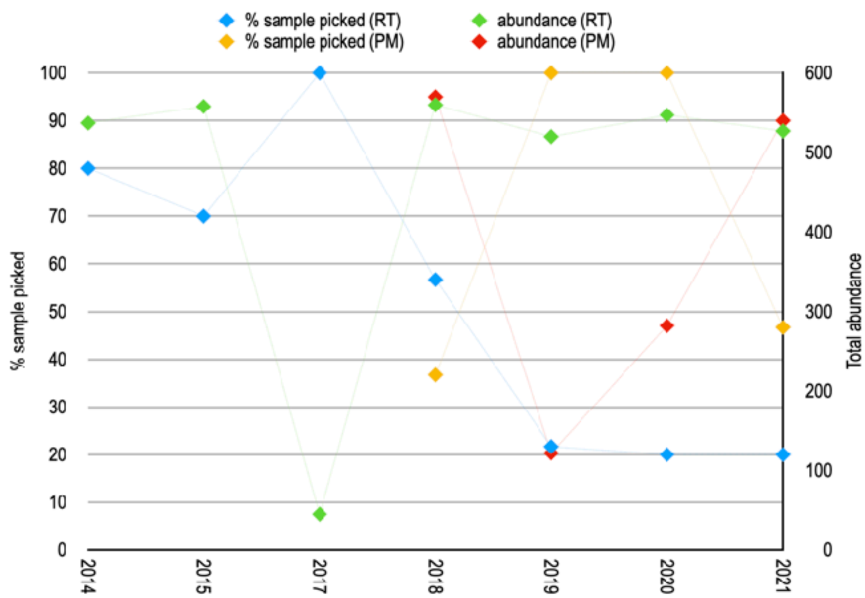
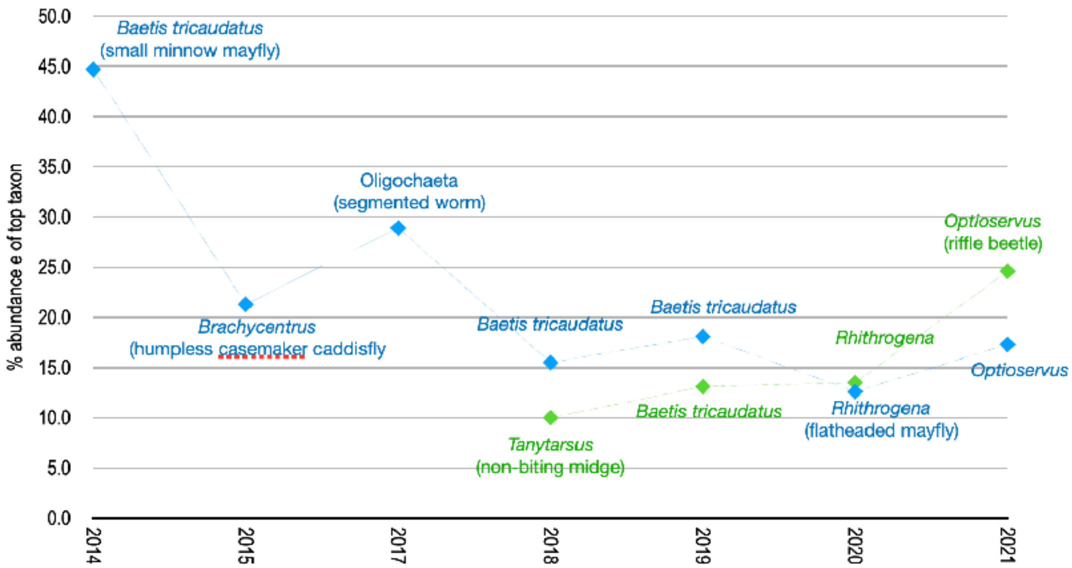


Figure 39. Relative abundance of the numerically dominant taxon at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. RT samples (blue) and M samples (green) were taken in the same reach at the same time in 2018-2021. This metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*



PREDATOR scores for riffle-targeted PC samples indicated good biological conditions for the past two years, and IBI scores correlated with minimal disturbance for the last four years (Figure 40). The means of both are greater in 2018-2021 compared to 2014-2015, but the difference is significant only for the IBI (p = 0.007). Numbers of sensitive and sediment-sensitive taxa increased sharply in RT samples following restoration (Figure 41) but were lower in M samples taken in the same years. The mean number of sensitive (3) and sediment-sensitive taxa (1) was the same in

PC/PCSC samples and SC samples, and samples from SC reaches also had more sensitive taxa (2-7) than sediment-intolerant taxa (0-2). Relative abundance of sediment-tolerant organisms is lower than that of tolerant organisms in PC samples from all years (Figure 42) as well as in all samples from all years (Figure 43); in 2021, there was a higher proportion of tolerant organisms and greater variation in abundance of sediment-tolerant organisms among all WC1100 reaches sampled compared to prior years. The mean relative abundance of tolerant and of sediment-tolerant organisms did not differ significantly between SC and PC/PCSC samples.

Figure 40. PREDATOR O/E and ORDEQ IBI scores at WC1100 in all sampling years. *Linear trendlines are shown.*

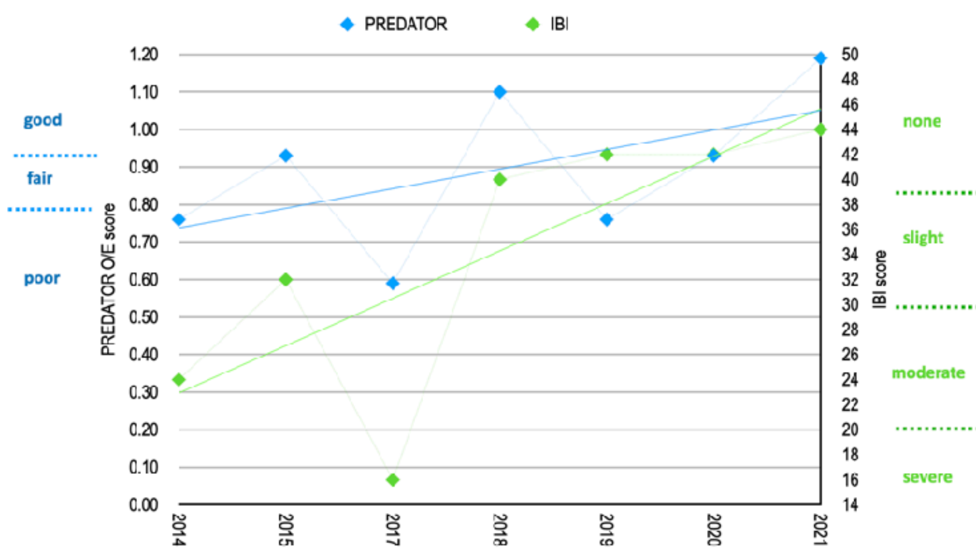


Figure 41. Numbers of sensitive and sediment-sensitive taxa at WC1100 in all sampling years. *Only samples taken in the primary channel are shown. M and RT samples were taken in the same reach at the same time in 2018-2021. Metric values in the two sample types were similar and are averaged here. For reference, these metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

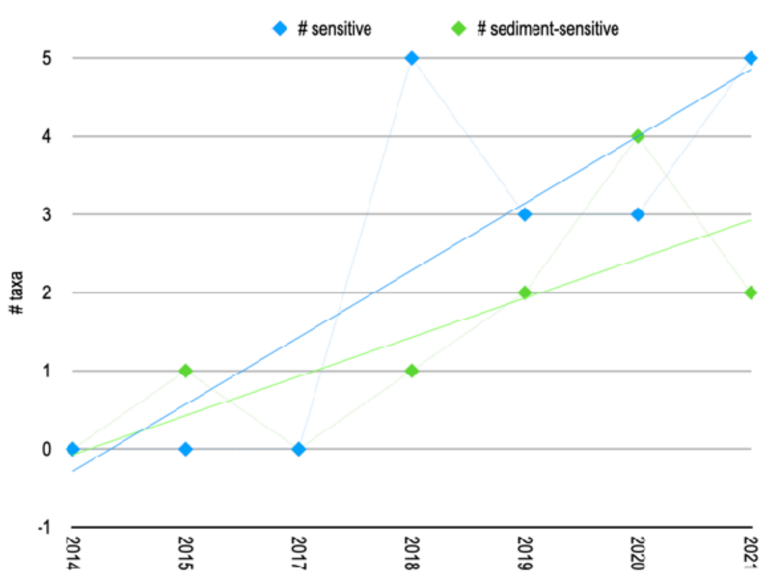


*Figure 42. **Relative abundance of tolerant and sediment-tolerant organisms at W1100 in all sampling years.** Only samples taken in the primary channel are shown. M and RT samples were taken in the same reach at the same time in 2018-2021. Metric values in the two sample types are averaged here. Linear trendlines are shown. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*

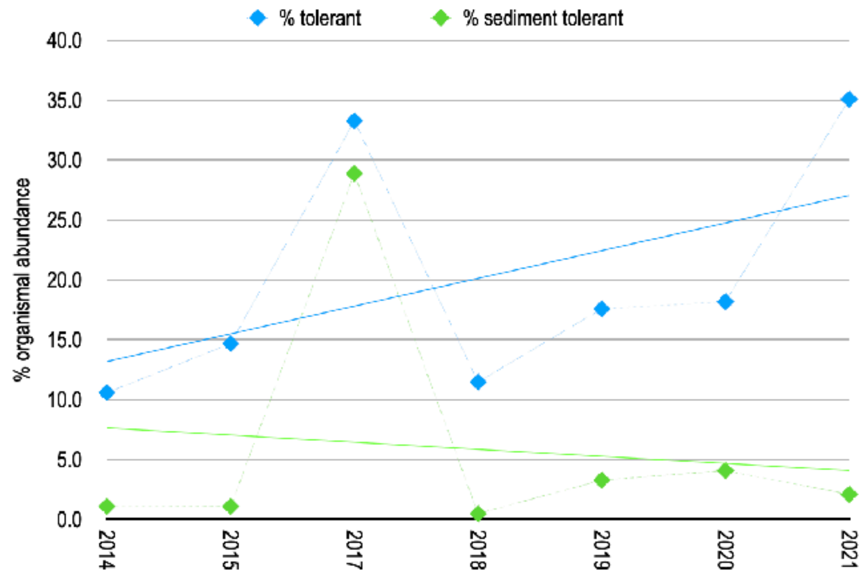
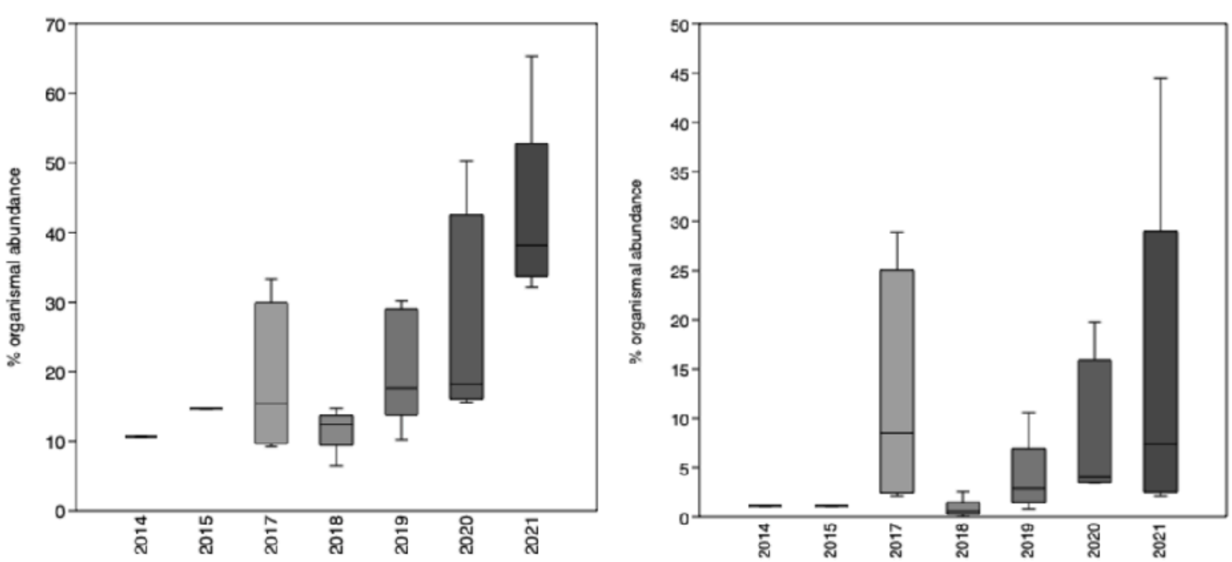


*Figure 43. **Relative abundance of tolerant (left) and sediment-tolerant (right) organisms at W1100 in all sampling years.** All reaches (PC, SC, PC/SC) and sample types (M, RT) taken in each year were included. Horizontal line in each box indicates median value; filled box shows interquartile range; whiskers depict data range.*



The numbers of DEQ indicator taxa for cool temperature and low fine suspended sediment in PC samples were exceeded in every year by the number of warm temperature and high sediment indicators (Figure 44), and community temperature and sediment optima have both trended upwards (Figure 45). However, when temperature associations are considered across the entire WC1100 dataset (of which DEQ indicator taxa are only a small subset), median relative abundance of cool/cold-associated organisms exceeded that of warm-associated since 2019, and the proportion of cool/cold-associated organisms has increased steadily since then while proportions of warm-associated organisms decreased in the same span (Figure 46). The mean relative abundance of cool/cold associated organisms was greater in PC/PCSC while warm-associated organisms were more abundant in SC samples, but the differences were not significant.

Figure 44. 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. M and RT samples were taken in the same reach at the same time in 2018-2021. Metric values in the two sample types are averaged here. Linear trendlines are shown. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

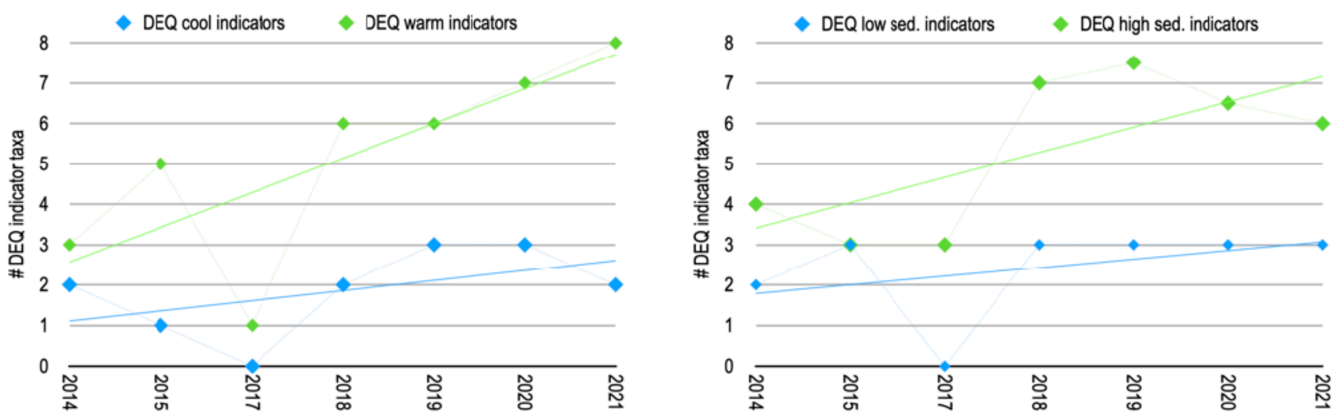


Figure 45. Temperature and fine sediment optima of the community (weighted means) at WC1100 in all sampling years. *M and RT samples were taken in the same reach at the same time in 2018-2021. Metric values in the two sample types are averaged here. Linear trendlines are shown. Note that individual optima have not been determined for every taxon in a sample.*

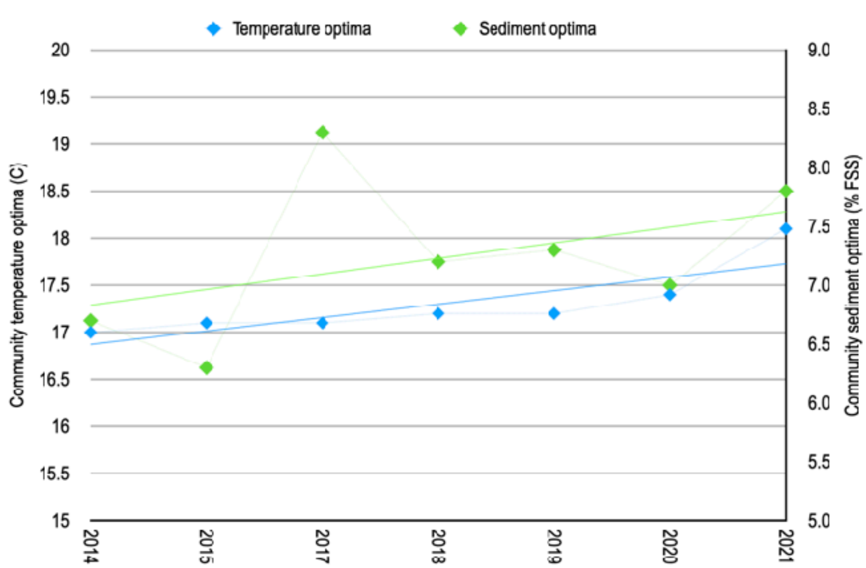
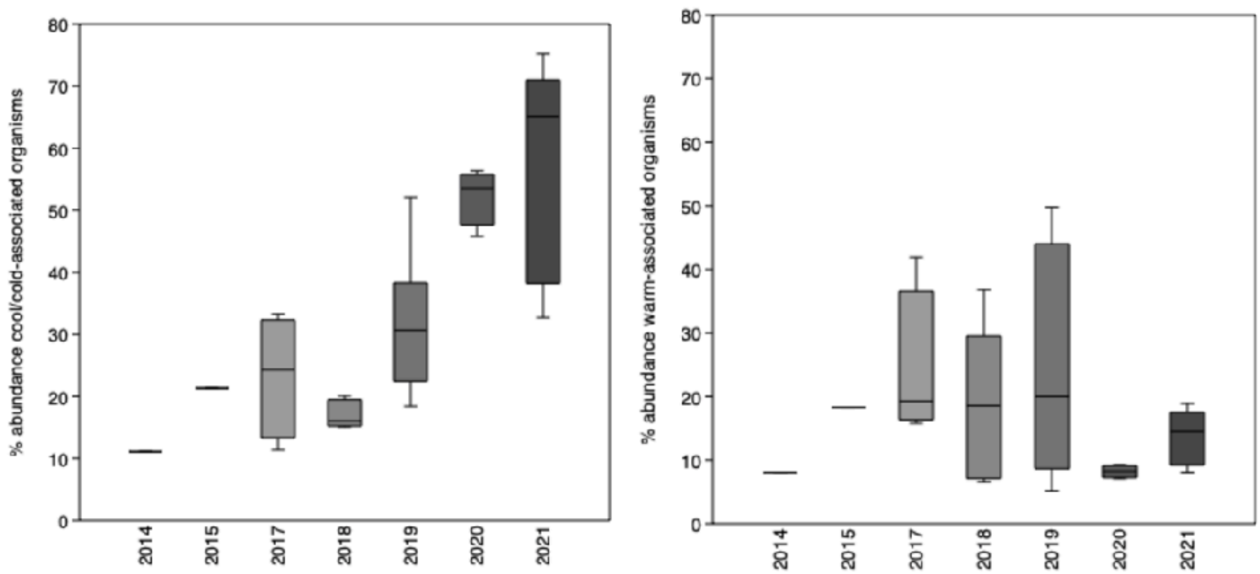


Figure 46. Relative abundances of organisms associated with cool (left) and warm (right) temperatures at WC1100 in all sampling years. *All reaches (PC, SC, PC/SC) and sample types (M, RT) taken in each year were included. Horizontal line in each box indicates median value; filled box shows interquartile range; whiskers depict data range.*



Macroinvertebrate community composition was significantly different between PC and SC reaches (one-way ANOSIM; $p = 0.0014$; R = 0.132) although the R value was low. SIMPER analysis showed greater dissimilarity between PC and SC communities (47.7% dissimilarity) compared to PC vs. PC/SC (40.2% dissimilarity) or PC/SC vs. SC (42.3% dissimilarity). Taxa that contributed most to PC vs. SC differences were Tanytarsini, Chironomini, and *Simulium*, which were more abundant in side channel samples. This likely reflects the impacts of sampling in newly-formed dynamic side channels with less mineral substrate, as Tanytarsini and Chironomini midges are tube-builders and burrowers, and *Simulium* is an early colonizer.

Macroinvertebrate community composition in primary channel samples differed pre- and post-restoration, with the 2017 community an outlier due to restoration-associated disturbances. Side channel communities tend to be more closely related to each other than to post-restoration PC or PC/SC communities (Figure 47). In a PCA ordination of taxa abundances (Figure 48), axis 1 explained 22.4% of total variation, and taxa with highest loading were the tolerant non-biting midge tribe Tanytarsini (more abundant post-restoration, especially in side channels); the sensitive flatheaded mayfly *Rhithrogena* (more abundant post-restoration, especially 2020-2021); and *Optioservus,* a tolerant widespread riffle beetle found in a variety of stream habitats whose increasing abundance post-restoration through 2021 may reflect greater habitat stability that supports their semivoltine life cycle. Axis 2 explained an additional 17.9% of total variation; taxa with highest loading included *Baetis tricaudatus,* a sediment-sensitive small minnow mayfly that prefers clear fast water (more abundant pre-restoration); *Simulium* black flies, early colonizers of flowing stream habitat (very abundant in 2018 side channels); and Chironomini, a tolerant, burrowing nonbiting midge tribe (not found prior to restoration but taken consistently since 2017, with greater overall abundance in side channels).

In a PCA ordination of all traits measured as relative abundances (Figure 49), axis 1 explained 38% of the total variation; traits with the highest loading were relative abundances of organisms that move as clingers (more abundant post-restoration, especially in 2020-2021); are associated with faster flows (more abundant post-restoration, in primary and some side channels); and are associated with cool/cold water temperatures (increased abundance post-restoration, especially 2020-2021). Axis 2 explained an additional 25% of variation; traits with the highest loading were relative abundances of organisms that feed as collectors (low in all 2017 side channel samples), are associated with faster flows, and have multivoltine life histories (nearly two-fold lower in 2019-2021 samples compared to all earlier years).

Figure 47. nMDS ordination of the WC1100 macroinvertebrate community in all sampling years. *M and RT samples were taken in primary and side channel reaches in 2017-2021; RT samples were taken all other years. The last number in each label indicates the sampling year. Colored circles show community similarity levels from a CLUSTER dendrogram of the same data.*

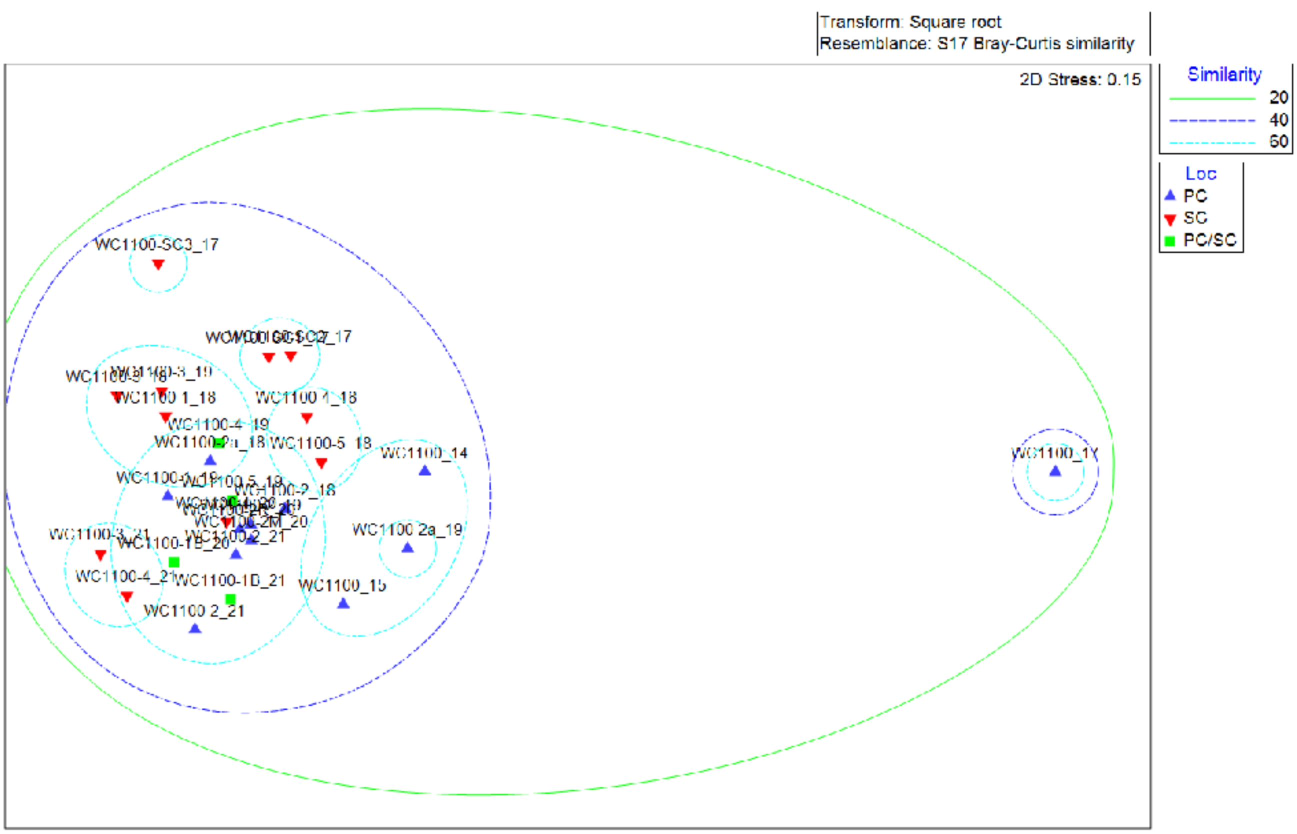


Figure 48. PCA ordination of the WC1100 macroinvertebrate community in all sampling years. *M (aqua) and RT (blue) samples were taken in primary and side channel reaches in 2017-2021. RT samples were taken in the primary channel in all earlier years. The number at the end of each label indicates sampling year.*

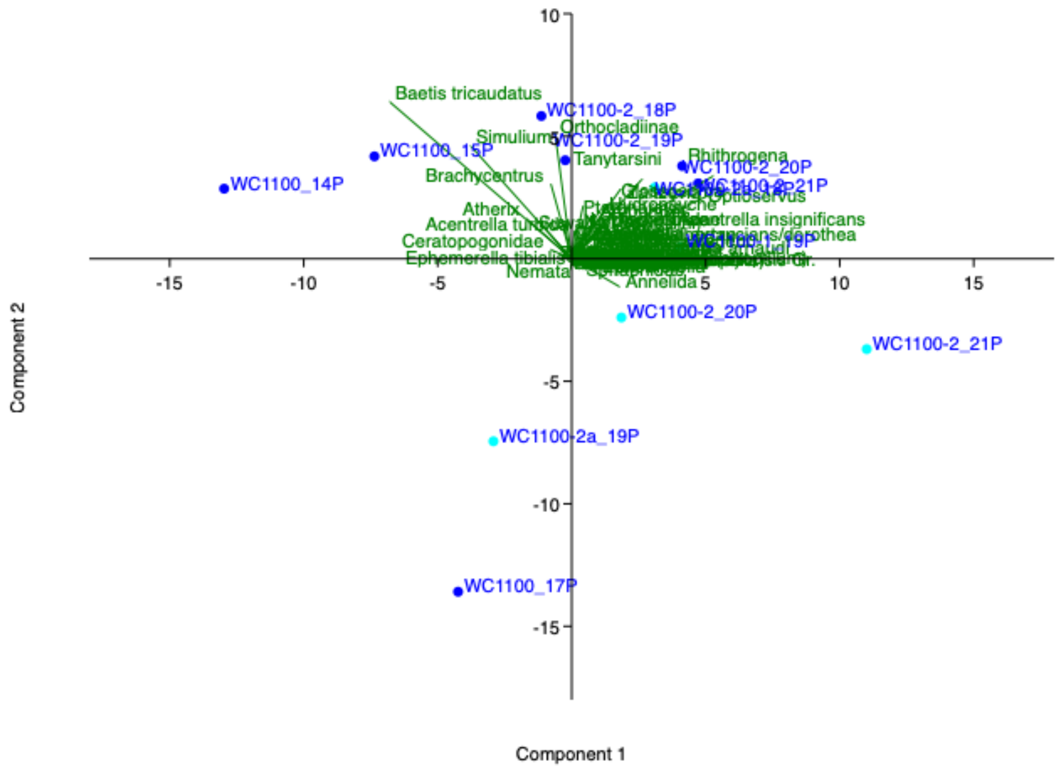
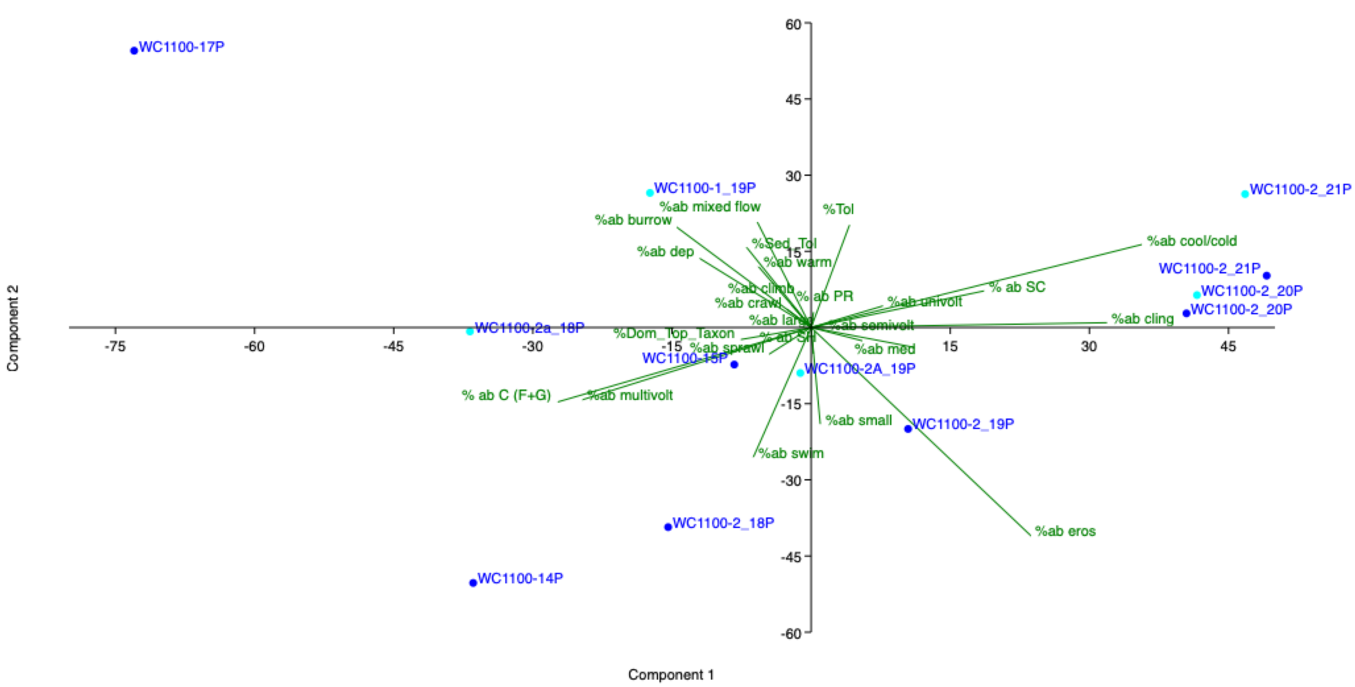


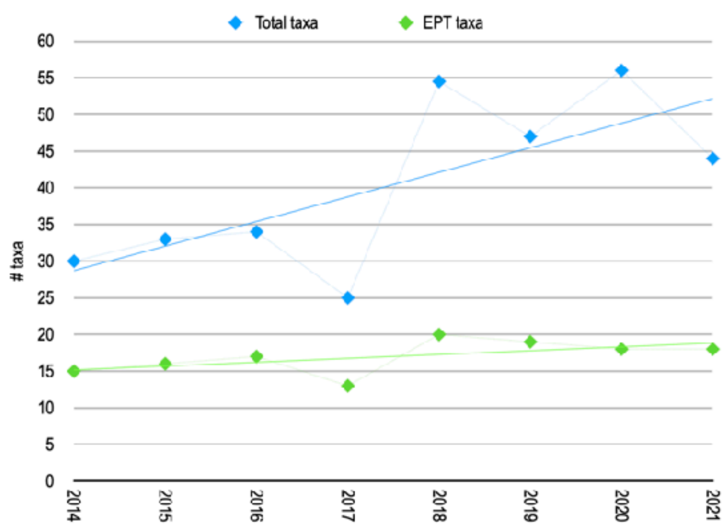
Figure 49. PCA ordination of the WC1100 macroinvertebrate community traits measured as relative abundances in all sampling years. *M (aqua) and RT (blue) samples were taken in primary and side channel reaches in 2017-2021; RT samples were taken in the primary channel all other years. The number at the end of each label indicates sampling year.*



WC1150

Sampling began at WC1150 in 2014 to provide an upstream reference for the WC1100 project site. Riffles were sampled from 2014-2017; in 2018, multihabitat and riffle-targeted samples were taken in the same reach; and from 2019-2021, only multihabitat samples were collected. The 2021 sample was taken primarily in flowing water (riffle and run) on cobble and boulder substrates (Table 2). Sample richness was intermediate for the 2021 dataset (44 taxa) and lower than the past two years (Figure 50), but has been in the range for the highest scaled BI score since 2018. The number of EPT taxa (18) was at the lower end of the 2021 range but consistent with EPT richness at this site since 2019. The 2021 community was dominated by *Rhithrogena*, a sediment-sensitive flatheaded mayfly associated with stones in fast cool flows. The community was composed primarily of small, univoltine clingers associated with cool erosional flows that feed as collectors and scrapers. Two taxa taken in 2021 were not found at this site in any prior year: *Wormaldia*, a sediment-sensitive fingernet caddisfly associated with cooler, faster flows (2 individuals); and one *Neoleptophlebia*, a prong-gilled mayfly often found in sediment and detritus in faster flows.

Figure 50. Sample richness and number of EPT taxa at WC1150 in all sampling years. *Riffle samples were taken in 2014-2018; multihabitat samples were taken in the same reach in 2018-2021. Values for the year in which both sample types were taken (2018) are averaged here. Linear trendlines are shown. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*



The target sub-sampling number of organisms was attained in every year except 2017, with 12-80% of the total sample picked (Figure 51). Relative abundance of the dominant taxon at this site was low enough to receive the highest scaled score in the ORDEQ IBI in five of the eight sampling years (Figure 52), but has increased overall since 2018. With the exception of 2017, when almost half the sample consisted 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), Rhithrogena*).

Figure 51. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC1150 in all sampling years. *RT samples were taken in 2014-2018; M samples were taken in the same reach in 2018-2021. Target sub-sampling number is 500 organisms.*

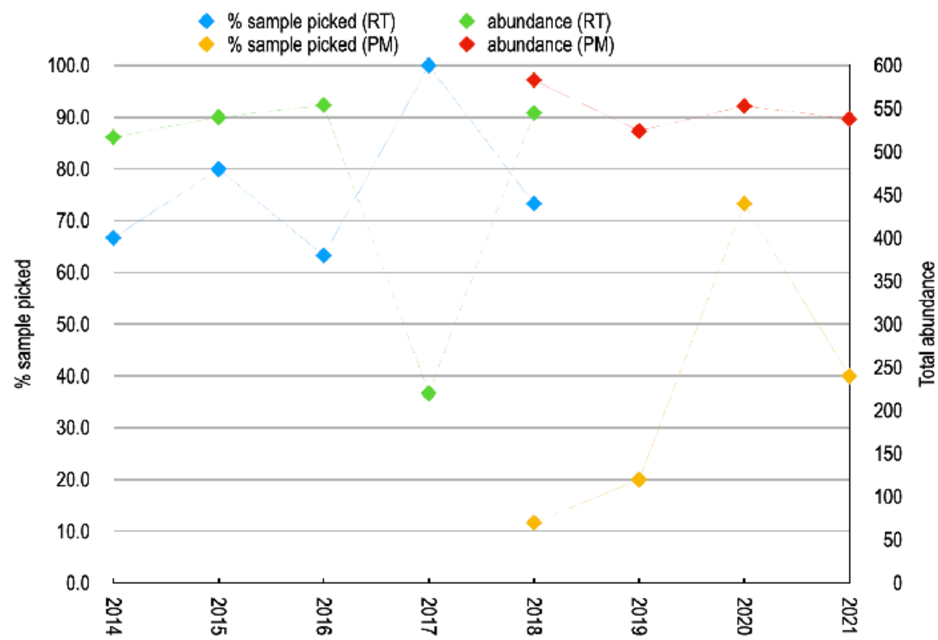
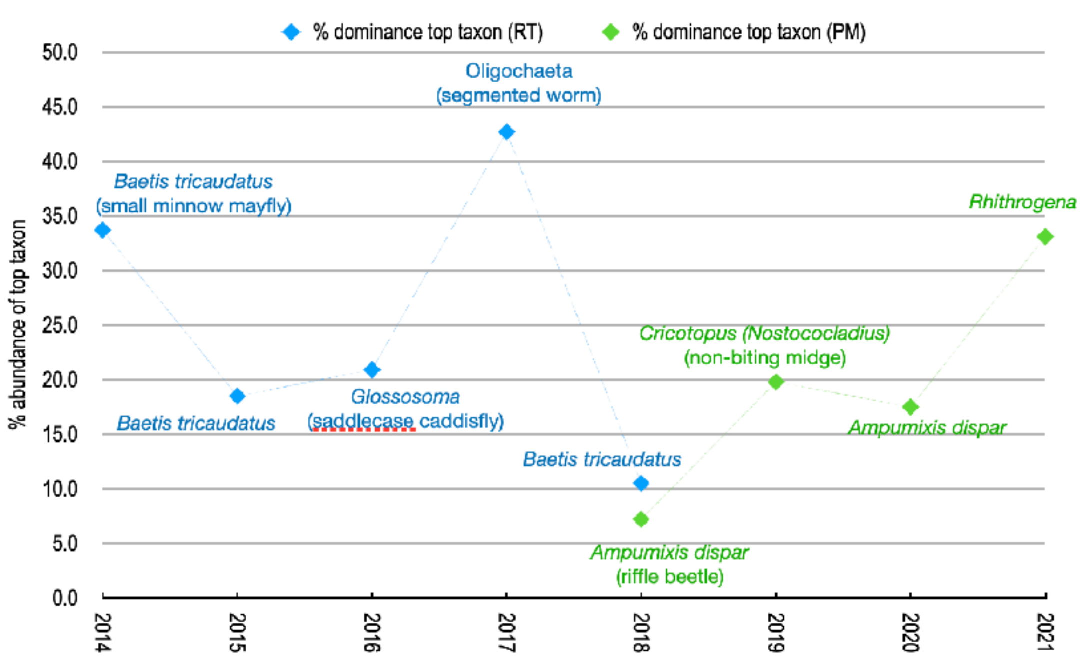


Figure 52. Relative abundance of the numerically dominant taxon at WC1150 in all sampling years. *RT samples (blue) were taken in 2014-2018; M samples (green) were taken in the same reach in 2018-2021. This metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*



In years when RT samples were collected, IBI scores indicated slight to no impairment except in 2017, which scored as moderately impaired. In 2021, values for four of the 10 IBI metrics correlated with the highest scaled score: taxa richness, # sediment-sensitive taxa, % sediment-tolerant organisms, and MHBI. PREDATOR scores reflected fair to good biological conditions except in 2014 (poor). Numbers of both sensitive and sediment-sensitive taxa were higher from 2018-2021 compared to earlier sampling years (Figure 53). Relative abundance of sediment-tolerant organisms has been very low since 2018, while abundance of tolerant organisms has risen (Figure 54). The number of DEQ cool temperature indicator taxa increased over time, but is usually exceeded by the number of warm indicator taxa in each year (Figure 55). High sediment indicator taxa have also generally outnumbered low sediment indicator taxa, but the difference between the two in each year is small. Community temperature optima increased slightly overall since sampling began and was higher in 2021 than in any prior sampling year. Community sediment optima increased through early sampling years and while the mean in 2018-2021 is lower than in 2014-2017, the difference is not significant (Figure 56).

Figure 53. Numbers of sensitive and sediment-sensitive taxa at WC1150 in all sampling years. *Riffle samples were taken in 2014-2018; multihabitat samples were taken in the same reach in 2018-2021. Values for the single year (2018) in which both types of samples were taken were averaged here. Linear trendlines are shown. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

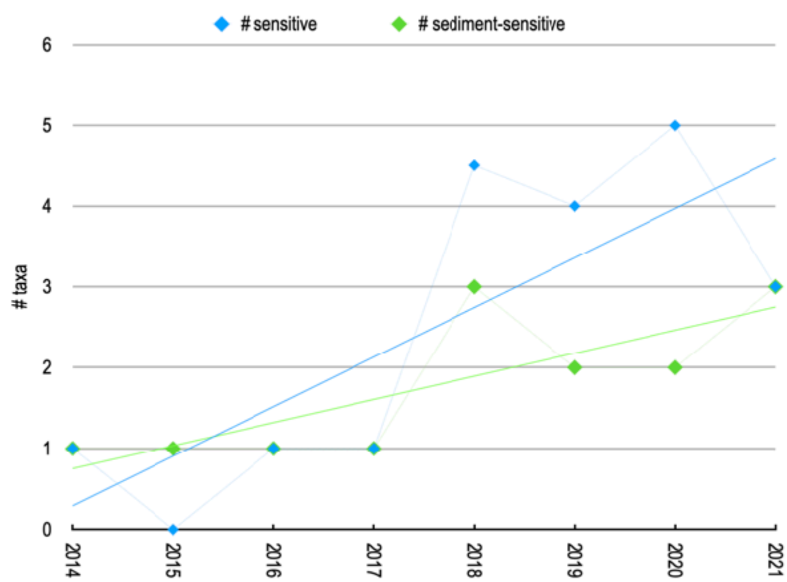


Figure 54. Relative abundance of tolerant and sediment-tolerant organisms at WC1150 in all sampling years. *Riffle samples were taken in 2014-2018; multihabitat samples were taken in the same reach in 2018-2021. Values for the single year (2018) in which both types of samples were averaged here. Linear trendlines are shown. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*

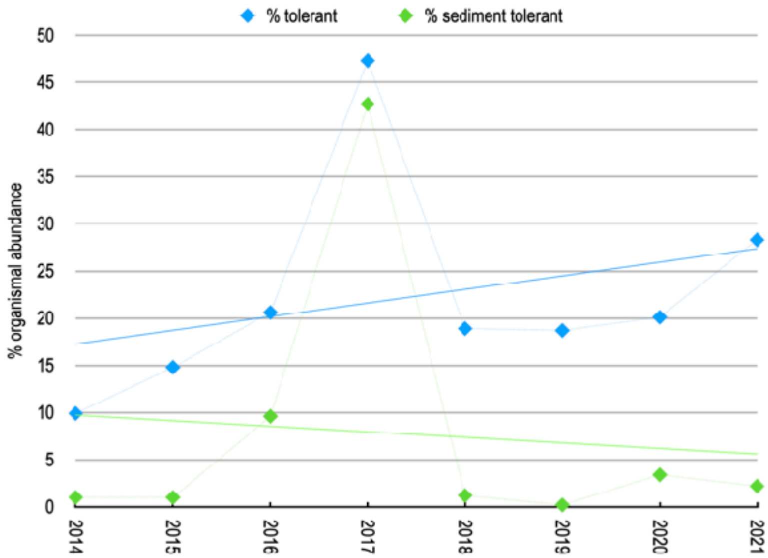


Figure 55. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC1150 in all sampling years. *Riffle samples were taken in 2014-2018; multihabitat samples were taken in the same reach in 2018-2021. Values for the year (2018) in which both types of samples were taken were averaged here. Linear trendlines are shown. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

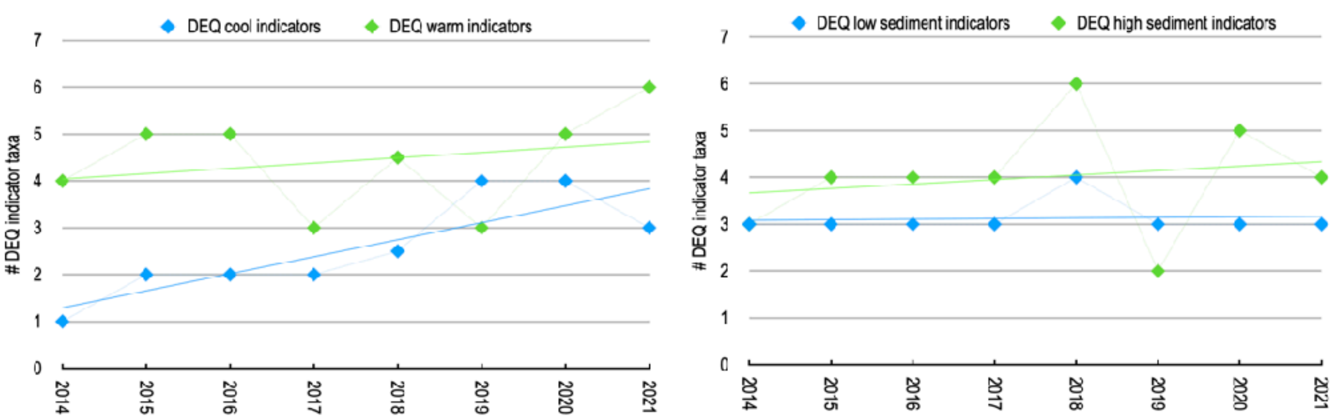
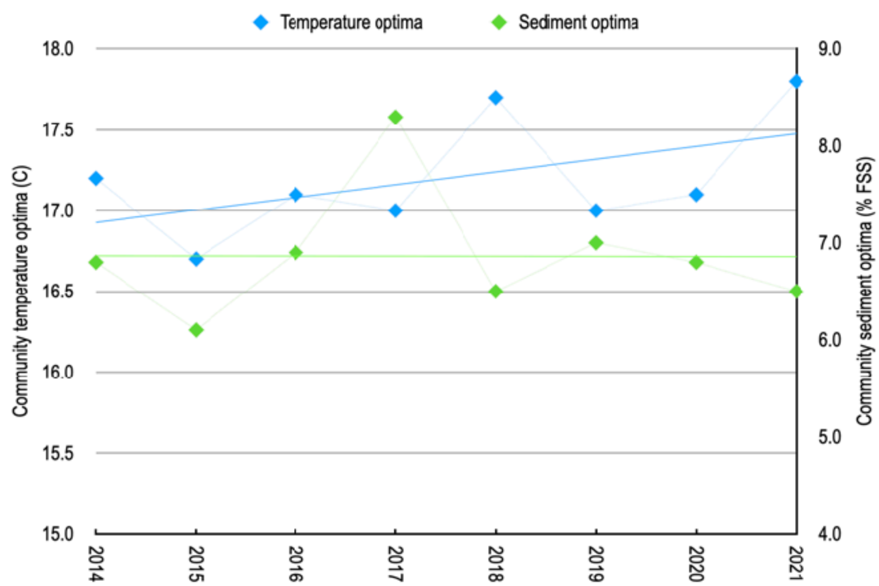


Figure 56. Temperature and fine sediment optima of the community (weighted means) at WC1150 in all sampling years. *Riffle samples were taken in 2014-2018; multihabitat samples were taken in the same reach in 2018-2021. Values for the year (2018) in which both types of samples were taken were averaged here. Linear trendlines are shown. Note that individual optima are not known for every taxon in a sample.*



The sample community in 2021 was more similar to the 2018-2020 communities (Figure 57; Bray Curtis Similarity Index range = 0.55 - 0.65) than to those in 2014-2017 (Bray Curtis Similarity Index range = 0.45 - 0.61). In a PCA ordination of taxa abundances (Figure 58), axis 1 explained 27.8% of total sample variation; taxa with the highest loading values were *Rhithrogena,* a sediment-sensitive flatheaded mayfly associated with the tops of stones in fast flows of cool streams (higher abundances 2018-2021, dominant taxon in 2021); *Baetis tricaudatus,* a sediment-sensitive small minnow mayfly that prefers clear fast water (most abundant 2014-2015)*, and Simulium,* a black fly found in flowing water that can tolerate warmer temperatures (higher abundance 2014-2016). Axis 2 explained an additional 18.3% of total variation; taxa with the highest loading values were Annelida, common, widespread tolerant segmented worms generally associated with soft sediments (very low abundance except in 2016 and 2017); *Ampumixis*, aa sediment-sensitive riffle beetle associated with sand and gravels in cool flowing water (more abundant overall 2018-2021); and *Cleptelmis*, a riffle beetle associated with cobble and submerged roots in in cool flowing water (higher abundance 2018-2020, though absent from the 2021 sample).

In a PCA ordination of all traits measured as relative abundances (Figure 59), axis 1 explained 59% of total sample variation; traits with the highest loading values were relative abundances of organisms associated with cooler flows (significantly greater abundance 2019-2021; p = 0.0076), clingers (more abundant 2019-2021), and organisms that prefer faster erosional flows (unusually low abundance in 2017). Axis 2 explained an additional 26% of total variation; traits with the highest loading were relative abundance of small (more abundant 2014 and 2018), burrower (most abundant in 2017, reflecting dominance of Annelida), and scraper organisms (more than twice as abundant in samples from 2019-2021, but not quite significantly different [p = 0.0531]).

__Figure 57. nMDS ordination of the WC1150 macroinvertebrate community in all sampling years.__ RT samples (blue) were taken in 2014-2018; M samples (aqua) were taken in the same reach in 2018-2021. The number at the end of each label indicates the sampling year. Colored circles show community similarity levels from a CLUSTER dendrogram of the same data.

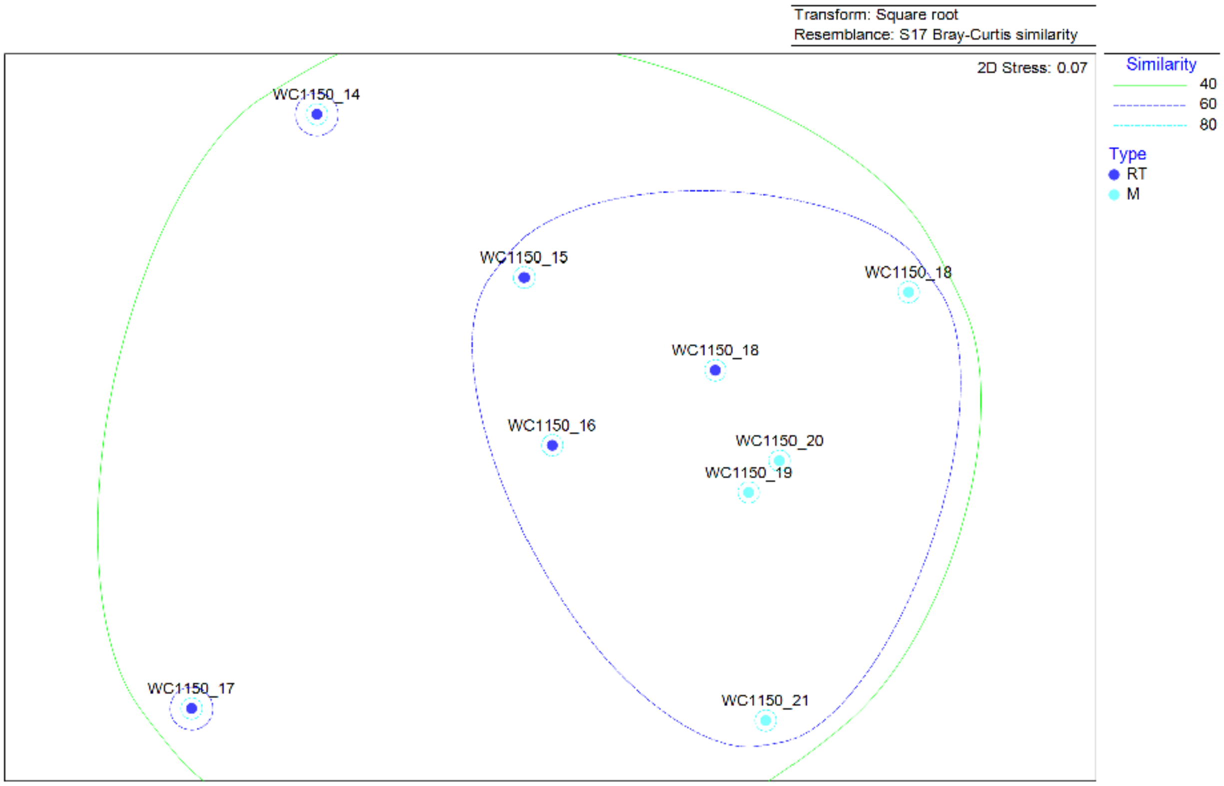


__Figure 58. PCA ordination of the WC1150 macroinvertebrate community in all sampling years.__ Riffle samples (blue) were taken in 2014-2018; multihabitat samples (aqua) were taken in the same reach in 2018-2021. The number at the end of each label indicates sampling year.

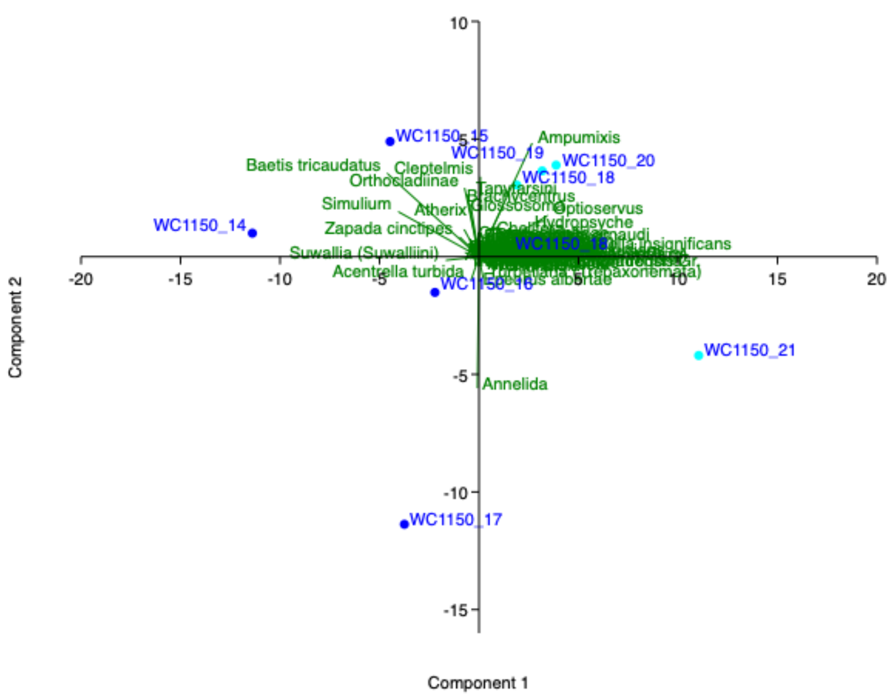
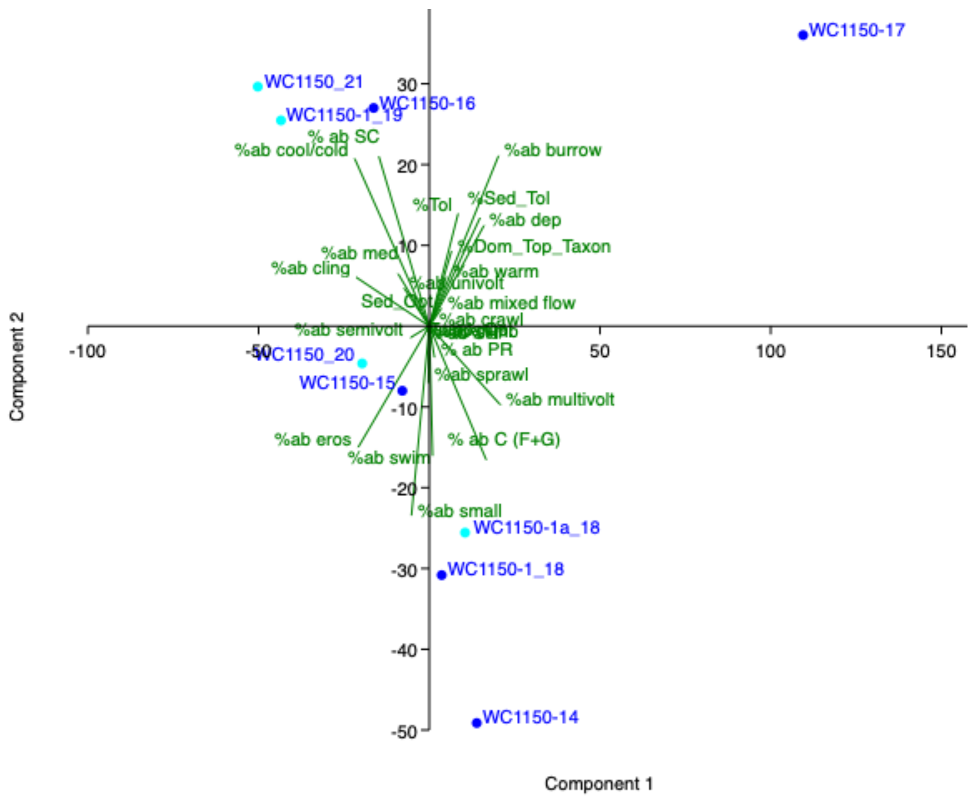


Figure 59. ***PCA ordination of the WC1150 macroinvertebrate community traits measured as relative abundances in all sampling years.*** *Riffle samples were taken in 2014-2018; multihabitat samples were taken in the same reach in 2018-2021. The number at the end of each label indicates sampling year.*



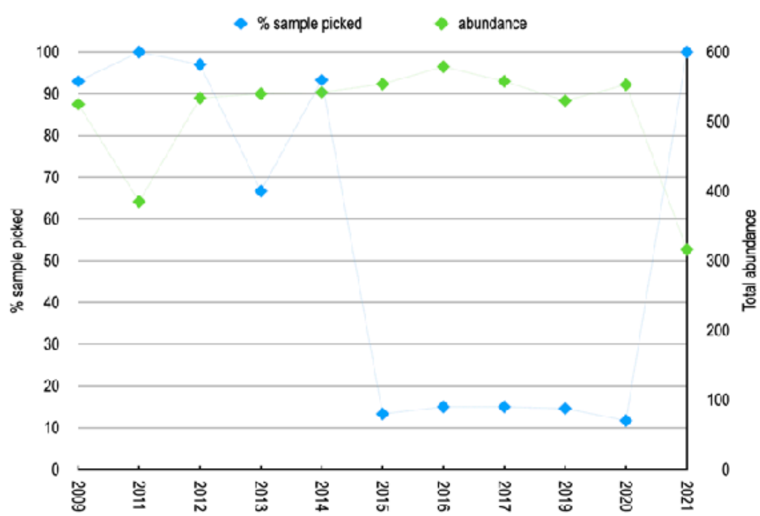
<u>WC1950</u>

This site was restored in 2012, and extensive backwatering and deposition has occurred since. An RT sample was taken in a primary channel reach from 2009-2019 except in 2018, when other restored reaches were prioritized for sampling. A multihabitat sample was taken in the same reach in 2019-2021. Data from 2021 must be treated with caution, as the volunteer team that sampled here did not adhere to the protocol, omitting several measurements and processing the final sample improperly. All net sets were taken in moving water (riffle and run) in a mixture of cobble/gravel/sand substrates (Table 2).

Total richness (44 taxa) was the lowest at this site since 2017 (Figure 60) and at the lower end of the 2021 sample dataset. The number of EPT taxa (16) was the lowest at this site since 2014, and at the low end of the 2021 sample set as well. The community was dominated by *Acentrella turbida*, a small minnow mayfly that is slightly sensitive to fine sediment and is associated with warmer waters and erosional flows, at a relative abundance greater than the last two sampling years but still within the range of the highest scaled IBI score and at the lower end of the range for 2021 samples. Values for five of the 10 IBI metrics correlated with the highest scaled score in the 2021 sample: richness, % dominance top taxon, % tolerant organisms, % sediment-tolerant organisms, MHBI.

Figure 60. Sample richness and number of EPT taxa at WC1950 in all sampling years. *Riffle samples were taken in 2009-2019; multihabitat samples were taken in the same reach in 2019-2021. Values for the year (2019) in which both types of samples were taken were averaged. Linear trendlines are shown. In the ORDEQ IBI, >35 total taxa receives the highest scaled score.*

Figure 61. Proportion of sample needed for sub-sampling and resulting organismal abundance at WC1950 in all sampling years. *Riffle samples were taken in 2009-2019; multihabitat samples were taken in the same reach in 2019-2021. Values for the year (2019) in which both types of samples were taken were similar and were averaged here. Target sub-sampling number is 500 organisms.*

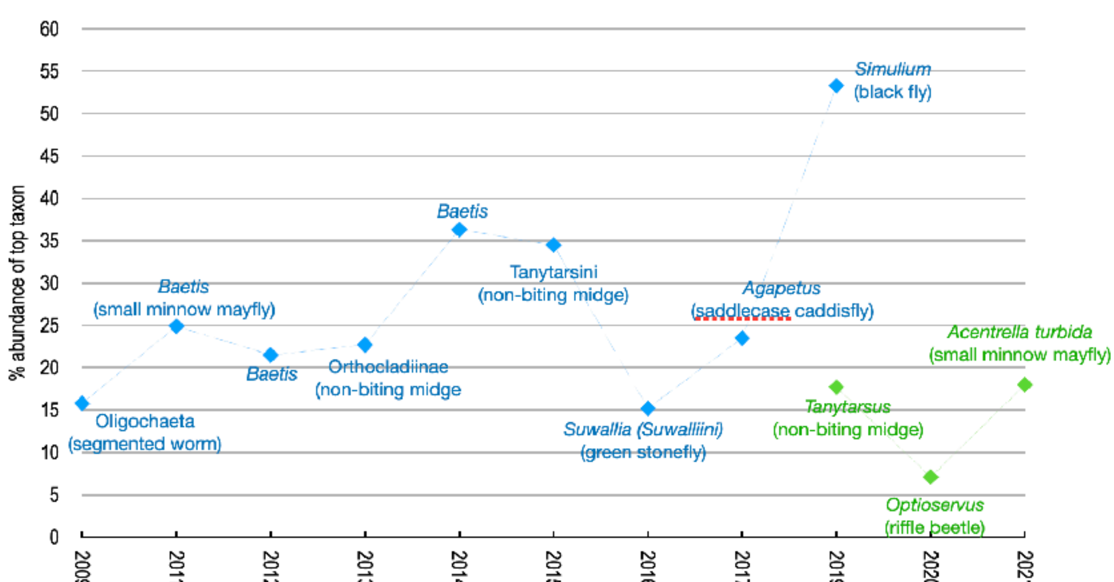


The 2021 community was composed mainly of small, multivoltine clingers that feed as collectors and are associated with cooler, faster flows. All taxa were taken at this site in at least one prior year. The target sub-sampling number of organisms was attained every year except 2011 and 2021, with 6-100% of the sample picked (Figure 61), and less sample was needed to attain the target in 2015-2020. Relative abundance of the top taxon (Figure 62) was low enough to receive the highest scaled score in the ORDEQ IBI in six sampling years, including all multihabitat samples taken in 2019-2021, although the 2019 riffle-targeted sample was much less balanced (53.3% *Simulium*) compared to the multihabitat sample taken in the same year and reach (17.7% *Tanytarsus*). *Simulium is* often a colonizer of recently disturbed habitat and *Tanytarsus* are broadly tolerant, suggesting changes in habitat between 2017 and 2019 possibly associated with a winter high-flow event exceeding 400 cfs. The dominant taxa since 2019 have been somewhat tolerant but associated with faster flows.

There are more sensitive than sediment-sensitive taxa in most years (Figure 63); despite annual fluctuations, both increased overall throughout the years, although the 2021 sample had fewer sediment-sensitive taxa than the prior two years. Tolerant organisms outnumbered sediment-tolerant in the last six sampling years (Figure 64); relative abundance of sediment-tolerant organisms was in the range to receive the highest scaled IBI score in every sampling year except 2009, and decreased overall since sampling began. DEQ cool temperature indicator taxa outnumbered warm indicators in almost every year that riffle-targeted samples were taken (Figure 65), but overall the number of warm indicator taxa is trending upwards while the number of cool indicator taxa is decreasing. In contrast, there are more low sediment indicator taxa than high sediment indicators in most sampling years, including 2021 (Figure 65). Community temperature and sediment optima have both increased overall since 2013 (Figure 66).

The 2021 sample community was something of an outlier, but still overall more similar to communities in recent sampling years (Figure 67). Unlike many other restored sites at Whychus, macroinvertebrate communities in years immediately prior to and following restoration did not differ greatly, although changes in community composition have continued. The relative similarity of macroinvertebrate communities at this site in years immediately prior to and following restoration might reflect the relatively smaller footprint of disturbance during restoration implementation within this sampling reach compared to the other Camp polk reaches and other restoration project reaches. The most consistent differences between the community in the most recent sampling years (2019-2021) compared to earlier years includes greater abundance of riffle beetles *Ampumixis* and *Optioservus* (associated with erosional

Figure 62. Relative abundance of the numerically dominant taxon at WC1950 in all sampling years. *Riffle samples (blue) were taken in 2009-2019; multihabitat samples (green) were taken in the same reach in 2019-2021. This metric in the ORDEQ IBI receives the highest scaled score at <20% abundance of the top taxon.*



habitats, and requiring sufficient habitat stability to support their semivoltine life cycles), *Acentrella turbida* (small minnow mayfly that is slightly sensitive to fine sediment and associated with warmer waters and erosional flows), *Attenella* (spiny crawler mayfly tolerant of a range of flow types and temperatures), and *Skwala* (spring stonefly associated with a broader range of flows and temperatures); and fewer *Baetis tricaudatus* (small minnow mayfly associated with faster flows and low sediment that can be an early colonizer), *Rhithrogena* (flatheaded mayfly

Figure 63. Numbers of sensitive and sediment-sensitive taxa at WC1950 in all sampling years. *Riffle samples were taken in 2009-2019; multihabitat samples were taken in the same reach in 2019-2021. Values for the year in which both types of samples were taken (2019) were averaged. Linear trendlines are shown. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa.*

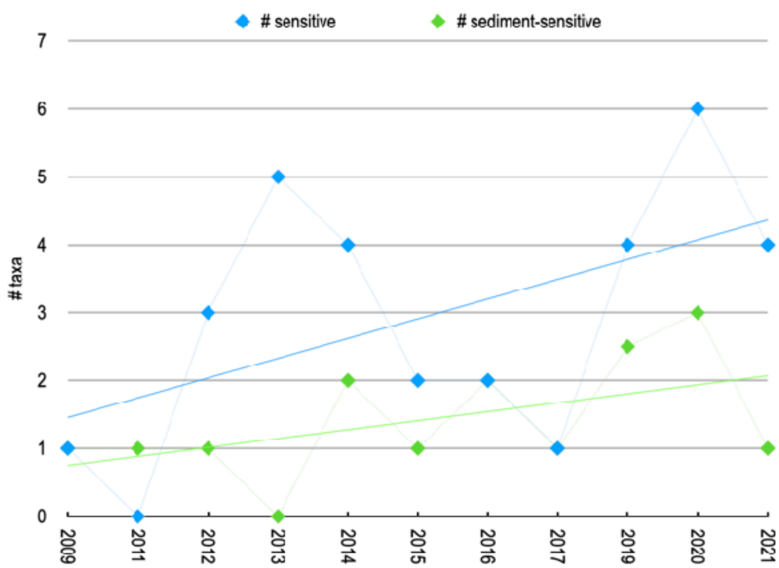
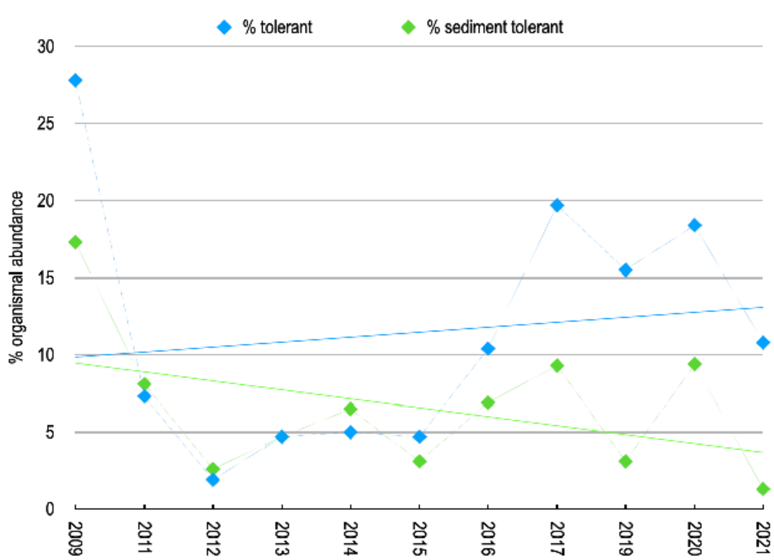


Figure 64. Relative abundance of tolerant and sediment-tolerant organisms at WC1950 in all sampling years. *Riffle samples were taken in 2009-2019; multihabitat samples were taken in the same reach in 2019-2021. Values for the year in which both types of samples were taken (2019) were averaged here. Linear trendlines are shown. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*



associated with cool clear flows), and the stoneflies *Suwallia* and *Zapada cinctipes* (both found in a range of habitats and flows).

Figure 65. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC1950 in all sampling years. *Riffle samples were taken in 2009-2019; multihabitat samples were taken in the same reach in 2019-2021. Values for the single year in which both types of samples were taken (2019) were averaged here. Linear trendlines are shown. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

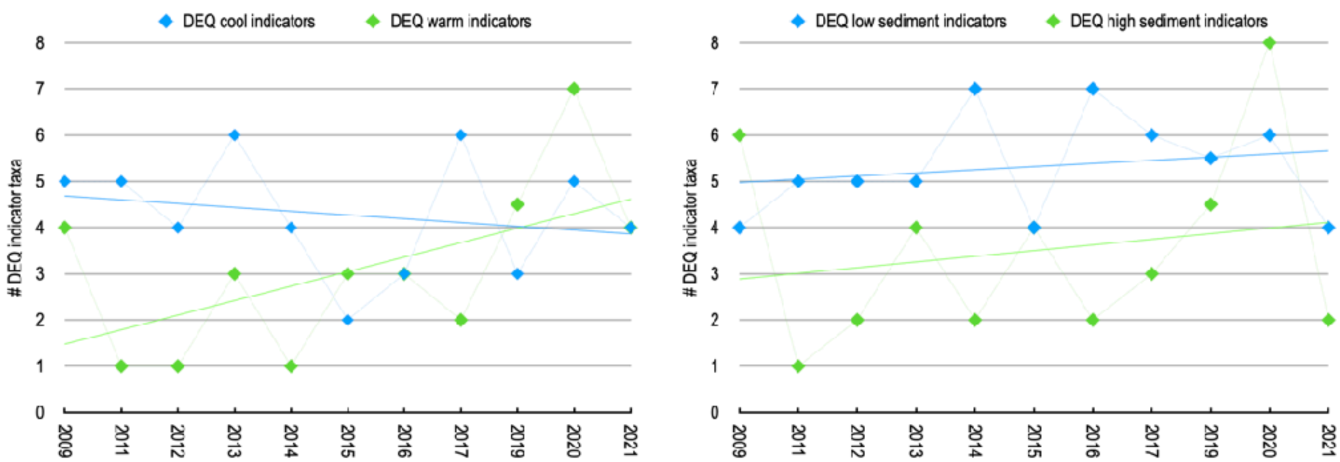


Figure 66. Temperature and fine sediment optima of the community (weighted means) at WC1950 in all sampling years. *Riffle samples were taken in 2009-2019; multihabitat samples were taken in the same reach in 2019-2021. Values for the year in which both types of samples were taken (2019) were averaged here. Note that individual optima are not known for every taxon in a sample.*

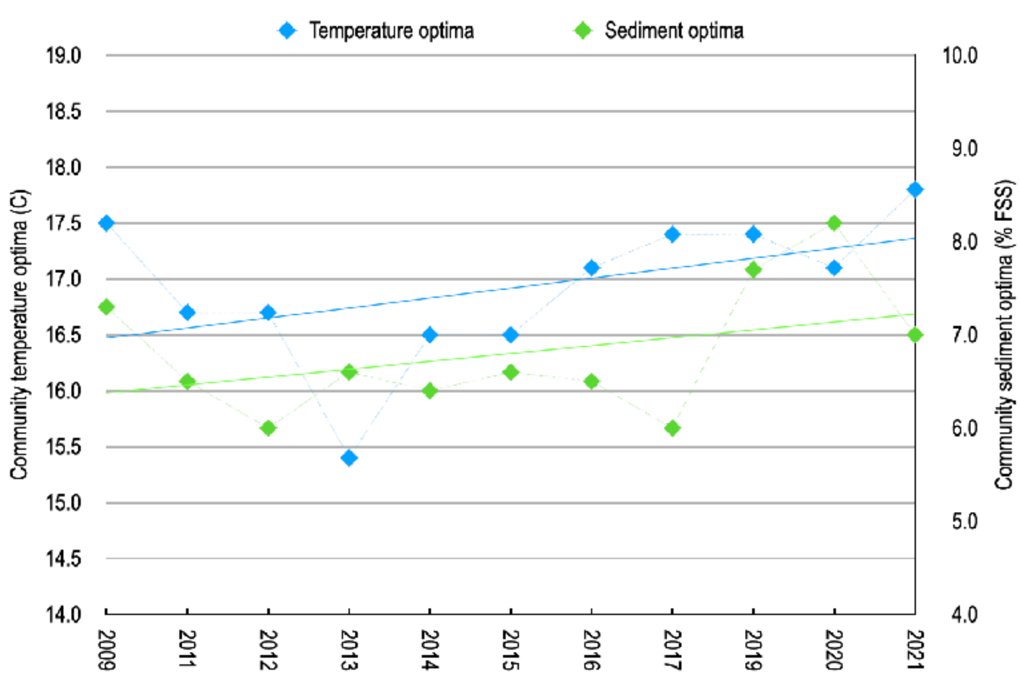
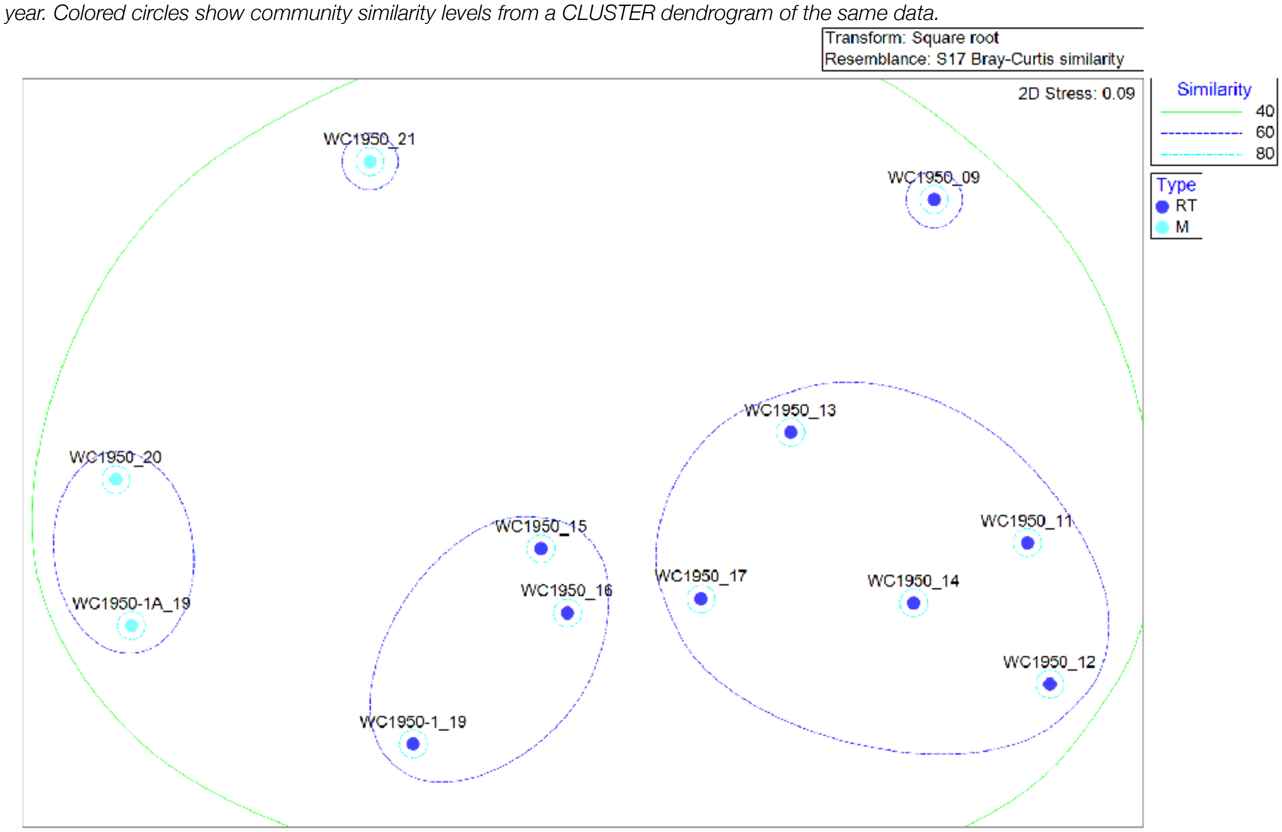


*Figure 67. **nMDS ordination of the WC1950 macroinvertebrate community in all sampling years.** Riffle samples (blue) were taken in 2009-2019; multihabitat samples (aqua) were taken in the same reach in 2019-2021. The number at the end of each label indicates sampling year. Colored circles show community similarity levels from a CLUSTER dendrogram of the same data.*



In a PCA ordination of taxa abundances (Figure 68), axis 1 explained 31% 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 post-restoration), *Baetis tricaudatus* (lower abundance post-restoration), and Chironomini (tolerant non-biting midge tribe found only in 2019-2020). Axis 2 explained an additional 16% of total sample variation, and taxa with the highest loading values were *Simulium* (more abundant post-restoration), *Acentrella turbida*, and *Agapetus* (saddlecase-maker caddisfly associated with cold fast water that was more abundant post-restoration).

In a PCA ordination of all traits measured as relative abundances (Figure 69), axis 1 explained 29.1% of total sample variation; traits with the highest loading were relative abundances of organisms associated with fast flows (greater in most riffle-targeted samples), found in a range of flow types (greater post-restoration), and of the top taxon (lower overall in multihabitat samples). Axis 2 explained an additional 25.2% of total variation, and traits with the highest loading were relative abundances of organisms that feed as collectors (more abundant in riffle-targeted samples), and that move as clingers and burrowers (lower abundance 2019-2021).

Figure 68. PCA ordination of the WC1950 macroinvertebrate community in all sampling years. *Riffle samples (blue) were taken in 2009-2019; multihabitat samples (aqua) were taken in the same reach in 2019-2021. The numeral at the end of each label indicates sampling year.*

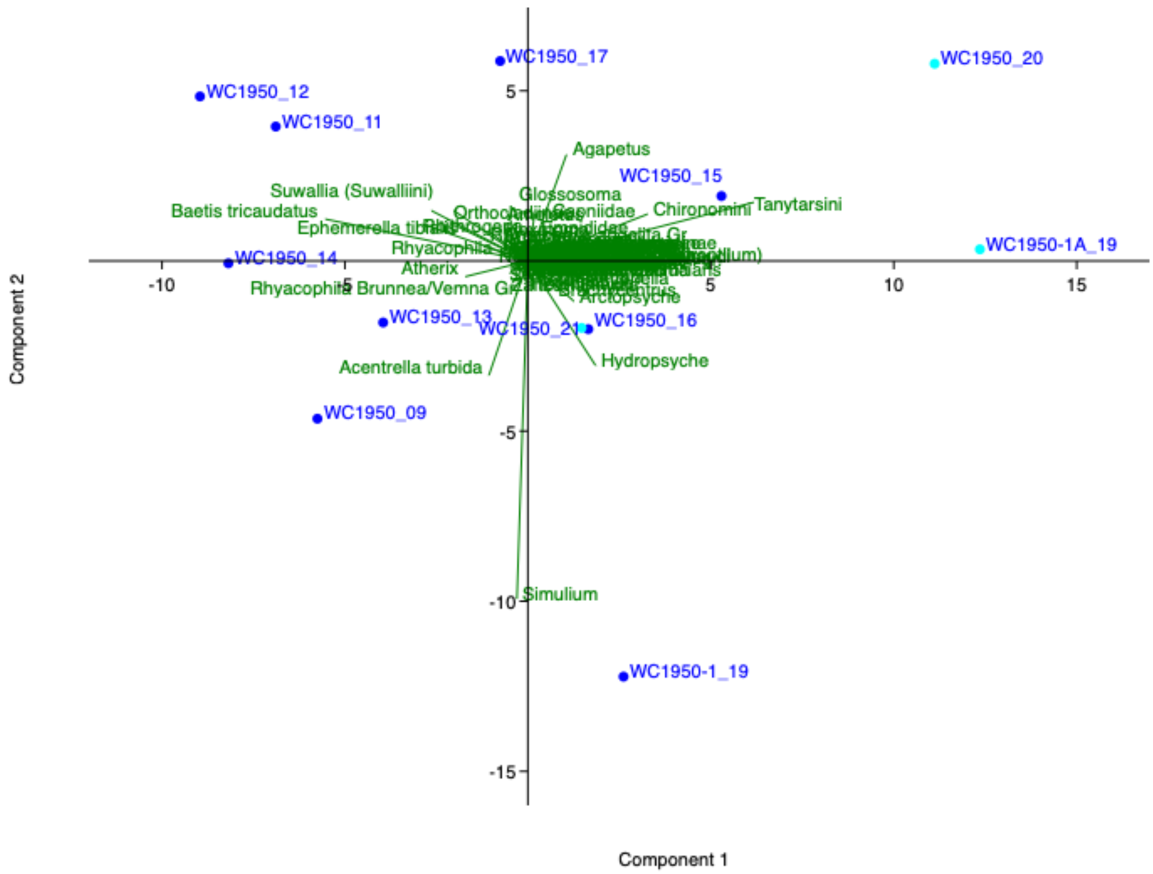
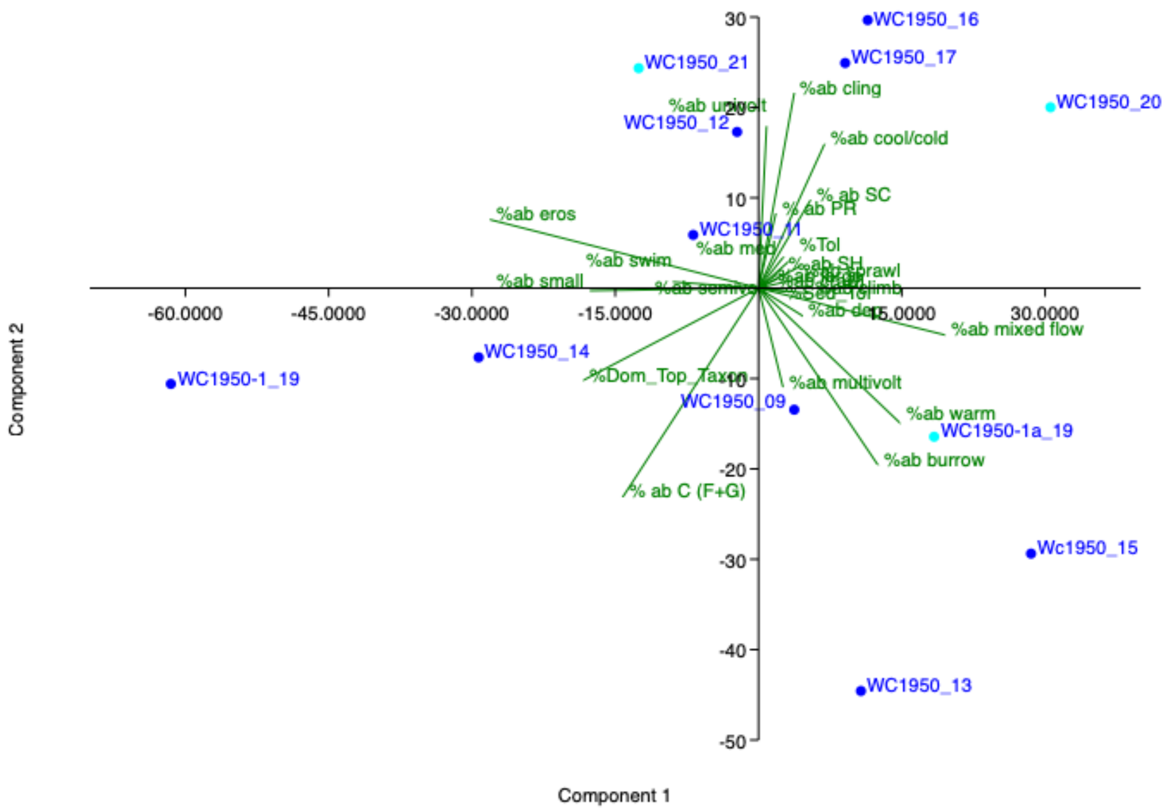


Figure 69. PCA ordination of the WC1950 macroinvertebrate community traits measured as relative abundances in all sampling years. *Riffle samples (blue) were taken in 2009-2019; multihabitat samples (aqua) were taken in the same reach in 2019-2021. The number at the end of each label indicates sampling year.*



<u>WC2000</u>

This is the second year in which sampling was done to provide baseline data prior to restoration; a PM and RT sample were taken simultaneously in the same reach each year. The 2021 multihabitat sample was taken in a mixture of moving and still water (riffle, run, glide, and pool) and a variety of mineral substrates (cobble, gravel, and sand/silt; Table 2). The two sample communities in 2021 were similar (Bray Curtis similarity index = 0.59), although the riffle-targeted sample was more similar to the WC1950 multihabitat sample (Bray Curtis similarity index = 0.69), while the WC2000 multihabitat was more similar to the WC2050 multihabitat sample (Bray Curtis similarity index = 0.64; see Figure 2). The PREDATOR O/E score for the riffle-targeted sample indicated fair biological condition while the IBI score correlated with no impairment (Table 6); similar results were seen in 2020. These scores can't be calculated for multihabitat samples, but raw values for four of the 10 IBI metrics were in the range that receives the highest scaled score (taxa richness, # sediment-sensitive taxa, % dominance of the top taxon, MHBI).

Table 6. Proportion of sample needed for sub-sampling, organismal abundance, sample richness, and number of EPT taxa at WC2000 in all sampling years. *Riffle and multihabitat samples were taken in the same reach in 2020-2021. For reference, the target sub-sampling number is 500 organisms; in the ORDEQ IBI, >35 total taxa and <20% abundance of the top taxon receive the highest scaled score.*

	Riffle-targeted		**Multihabitat**	
	2020	**2021**	**2020**	**2021**
% of sample picked	15	36.7	21.7	26.7
Total abundance	528	556	535	544
PREDATOR O/E	0.90 (fair condition)	0.81 (fair condition)	—	—
DEQ IBI	42 (no impairment)	42 (no impairment)	—	—
# unique taxa	48	45	62	50
# EPT taxa	20	23	19	17
% dominance top taxon	14.6	14.9	7.7	17.6
top taxon	*Glossosoma* (saddlecase-maker caddisfly)	*Agapetus* (saddle-case-maker caddisfly)	*Tanytarsus* (nonbiting midge)	*Phaenopsectra* (nonbiting midge)

The 2021 riffle and multihabitat samples were comprised mainly of small-bodied, rapidly developing organisms that move as clingers and feed as scrapers and collectors. More organisms in the riffle-targeted samples were associated with cooler, faster flows, while the multihabitat sample had more organisms tolerant of a broader range of temperatures and flows. Eight taxa were taken at this site for the first time in 2021, at abundances raging from 1-19 individuals, and most were associated with riffle samples: *Lara*, a wood-feeding riffle beetle associated with clear cool fast flows (1, riffle); *Callibaetis* a small minnow mayfly associated with filamentous algae in slower warmer flows (1, riffle); *Anafroptilum*, a small minnow mayfly associated with depositional habitats in cooler waters (9, riffle); *Caudatella*, a spiny crawler mayfly associated with cool fast flows (1, riffle); *Rhithrogena*, a sediment-sensitive flatheaded mayfly associated with cool fast flows (7, riffle); *Metrichia*, a pursecase-making caddisfly associated with mats of filamentous algae in faster flows and large open streams (16 in multihabitat, 18 in riffle); *Psychoglypha*, a northern caddisfly found in a range of cool water habitats (1, multihabitat); and *Rhyacophila angelita* Gr., a sediment-sensitive freeliving caddisfly associated with cold clear flows (1, riffle). All of these taxa were taken in other sites and years.

The target subsampling number of 500 organisms was attained in every sample from this site, with 15-36.7% of the total sample picked (Table 6). Multihabitat samples had greater total richness than riffle-targeted in each year and there were more EPT taxa in riffle-targeted samples (Table 6), but differences were not significant. Richness in both sample types was intermediate in the 2021 sample set, but the riffle-targeted sample had more EPT taxa than any other sample taken in 2021. No sample was overly dominated by a single taxon (relative abundance of top taxon = 7.7-17.6% among all samples), and the value for this metric in 2021 samples was at the low end of the range across the 2021 dataset, but characteristics of the dominant taxa differ with sample type (Table 6). Riffle-targeted samples in 2020-2021 were dominated by two genera of saddlecase-maker caddisfly, *Glossosoma* and *Agapetus*, which are slightly to moderately sensitive to fine sediment and are associated with cool rapid flows. Multihabitat samples in the same years were dominated by two nonbiting midge genera, *Tanytarsus* and *Phaenopsectra*, which are both tube-builders associated with sediments in a broader range of flows and temperatures.

Numbers of sensitive and sediment-sensitive taxa are similar in both sample types. Multihabitat samples have more tolerant and sediment-tolerant organisms than riffle-targeted (Table 7), but the difference is not significant, and the riffle sample was at the low end of the range for both metrics in the 2021 dataset. The numbers of DEQ warm temperature and high sediment indicator taxa are higher in multihabitat samples (Table 8), while the 2021 riffle sample was at the top of the range among all 2021 samples for numbers of DEQ cool temperature and low fine sediment indicator taxa. Community sediment and temperature optima were similar among sample types and years with the exception of the 2021 multihabitat sample, which had a lower community temperature optima that was also the lowest in the 2021 dataset (Table 8).

Table 7. Macroinvertebrate community metrics at WC2000 in all sampling years. *RT and PM samples were taken in the same reach in 2020-2021. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa; and at <15% tolerant and <10% sediment-tolerant organisms.*

	Riffle-targeted		Multihabitat	
	2020	2021	2020	2021
# sensitive taxa	3	4	5	4
# sediment-sensitive taxa	3	1	3	2
% tolerant organisms	6.1	4.7	9.5	16.5
% sediment-tolerant organisms	9.8	2.7	14.2	14.3

Table 8. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC2000 in all sampling years. *Riffle-targeted and multihabitat samples were taken in the same reach in 2020-2021. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample, and individual temperature and fine sediment optima are not known for every taxon.*

	Riffle-targeted		Multihabitat	
	2020	2021	2020	2021
# DEQ cool indicator taxa	6	6	5	3
# DEQ warm indicator taxa	3	4	7	7
# DEQ low-sediment indicator taxa	5	6	5	4
# DEQ high-sediment indicator taxa	3	3	8	8
Community temperature optima (C)	16.9	17.4	17.1	14.1
Community sediment optima (% FSS)	6.5	6.2	7.6	6.5

The two samples taken in 2021 differed more from each other than the pair of samples taken in the prior year (Figure 70), but all four samples from this site are at least 65% similar in taxonomic composition. In a PCA ordination of taxa abundances (Figure 71), axis 1 explained 53.5% of total variation between samples, and the taxa with the highest loading values were Chironomini, a tolerant non-biting midge tribe (highly abundant only in the 2021 multihabitat sample); the sediment-sensitive saddlecase-maker caddisfly *Glossosoma* (more abundant in riffle-targeted samples); and the tolerant segmented worm group Annelida (more abundant in multihabitat samples). Axis 2 explained an additional 37.2% of total variation and taxa with the highest loading values were Tanytarsini, a tolerant non-biting midge tribe associated with warmer water (much less abundant in the 2021 riffle-targeted sample); *Metrichia*, a pursecase-making caddisfly (taken here for the first time in both 2021 samples); and *Ephemerella tibialis,* a spiny crawler mayfly that is slightly sensitive to fine sediment and associated with cooler faster flows (more abundant in both 2021 samples).

In a PCA ordination of traits calculated as relative abundances (Figure 72), axis 1 explained 71% of between-sample variation (71%), and traits with the highest loading values were abundances of organisms with multivoltine life cycles (greatest in 2021 multihabitat sample), and with erosional and cold water habitat associations (both more abundant in riffle-targeted samples). Axis 2 explained an additional 22% of variation, and traits with the highest loading were abundances of small-bodied (greater in 2021), clinging (more in 2021 riffle-targeted sample), and scraper organisms (fewer in 2020 multihabitat sample).

Figure 70. CLUSTER dendrogram of the WC2000 macroinvertebrate community in all sampling years. *Riffle (blue) and multihabitat (aqua) samples were taken in the same reach in 2020-2021. The number at the end of each label indicates the sampling year.*

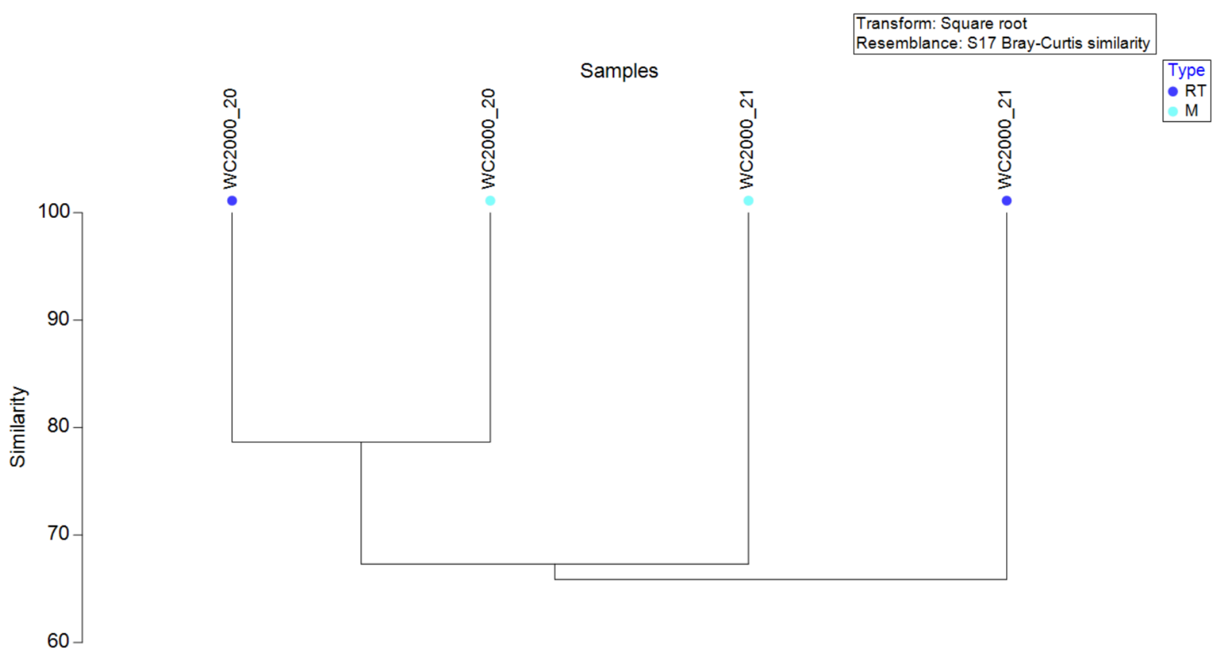


__Figure 71. PCA ordination of the WC2000 macroinvertebrate community in all sampling years.__ Riffle (blue) and multihabitat (aqua) samples were taken in the same reach in 2020-2021. The numeral at the end of each label indicates sampling year.

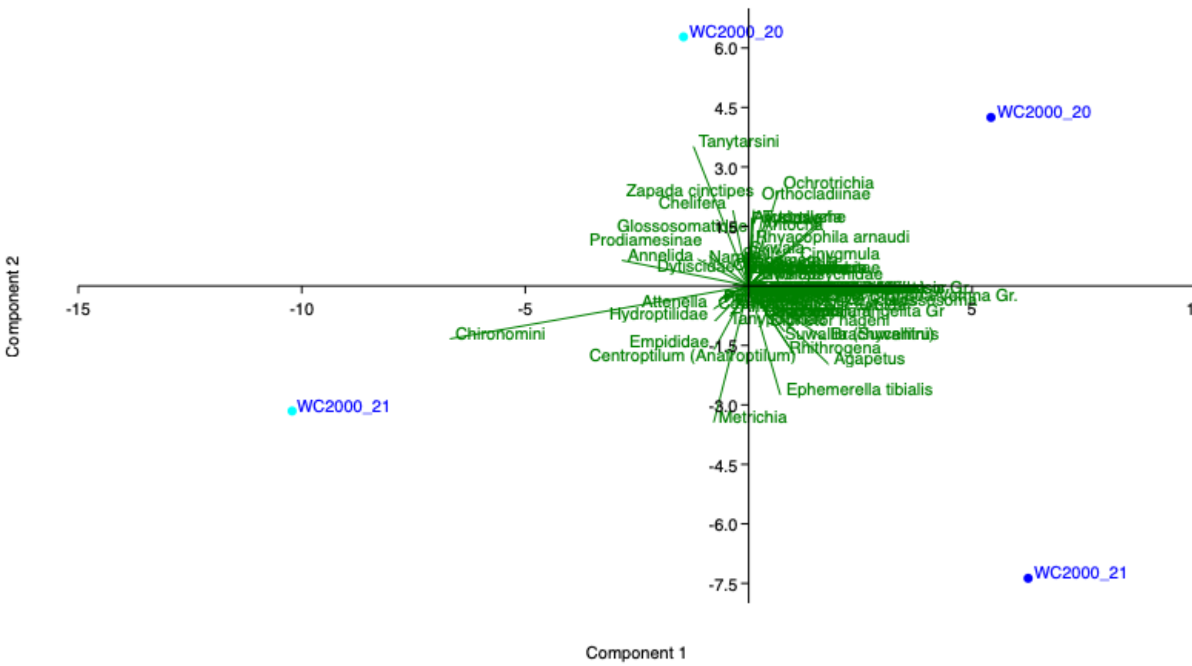
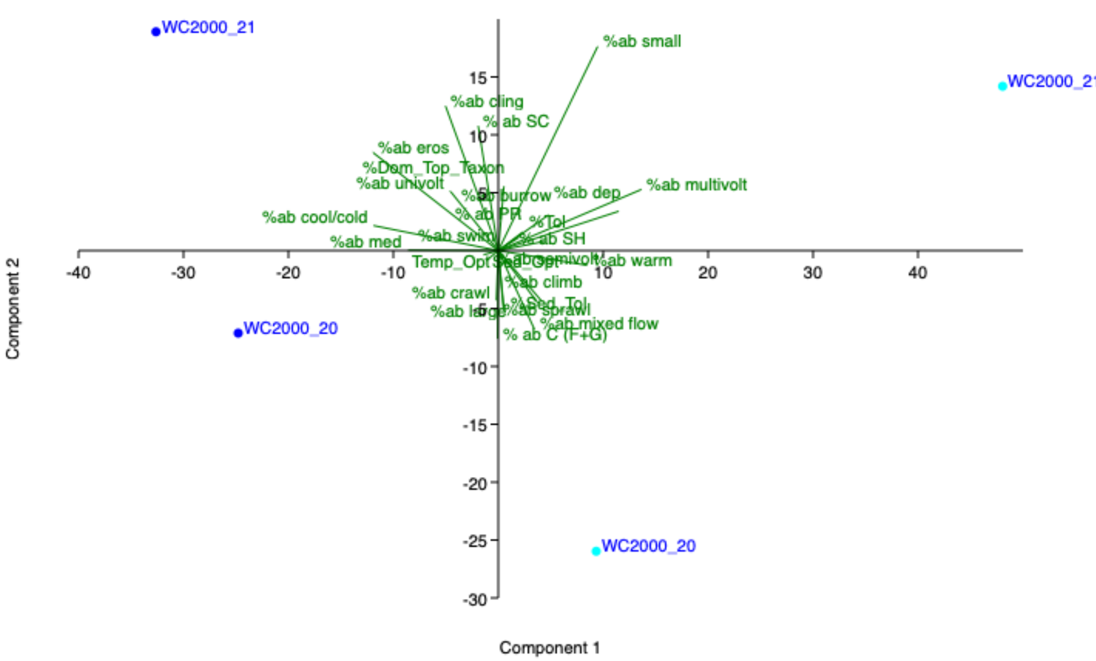


__Figure 72. PCA ordination of the WC2000 macroinvertebrate community traits measured as relative abundances in all sampling years.__ Riffle (blue) and multihabitat (aqua) samples were taken in the same reach in 2020-2021. The numeral at the end of each label indicates the sampling year.



WC2050

Multihabitat samples were taken at this site in 2020 and 2021 to obtain baseline data prior to restoration. The target subsampling number of organisms was attained in both years, with more of the sample picked in 2021 (Table 9). Net sets in 2021 were taken in moving water (run, glide, and rapid) in primarily cobble/gravel substrates (Table 2). Although IBI score can't be calculated for multihabitat samples, raw values for two of the 10 metrics corresponded to the highest scaled IBI score (taxa richness, % dominance of the top taxon); in 2020, the site sample scored in the top range for seven metrics. The 2021 sample was comprised mainly of small-bodied, multivoltine organisms that move as clingers and feed as collectors in faster flows with a range of temperatures. In contrast, the 2020 sample community included more shredders, crawlers, and organisms associated with cooler water and a range of flows.

Table 9. Proportion of sample needed for sub-sampling, organismal abundance, sample richness, and number of EPT taxa at WC2050 in all sampling years. *Multihabitat samples were taken in 2020-2021. Target sub-sampling number is 500 organisms. For reference, in the ORDEQ IBI, >35 total taxa and <20% abundance of the top taxon receive the highest scaled score.*

	2020	2021
% of sample picked	20	67
Total abundance	535	528
# unique taxa	53	57
# EPT taxa	21	16
% dominance top taxon	10.3	12.7
top taxon	*Ochrotrichia* (pursecase-making caddisfly)	*Acentrella turbida* (small minnow mayfly)

Twelve taxa taken in 2021 were not seen in 2020: Ostracoda, a widespread, sediment-tolerant group of seed clams (3 individuals); tolerant native *Pacifastacus* crayfish (1); *Lara*, a wood-feeding riffle beetle associated with clear cool fast flows (1); haliplid crawling water beetles and hydrophilid water scavenger beetles, both associated with plant material in slower flows (1individual in each family); Chironomini, a tolerant, burrowing nonbiting midge tribe (41); *Anafroptilum*, a small minnow mayfly associated with depositional habitats in cooler waters (1); *Caudatella*, a spiny crawler mayfly associated with cool fast flows (4); *Ephemerella excrucians*, a spiny crawler mayfly associated with cooler faster flows (3); *Rhithrogena*, a sediment-sensitive flatheaded mayfly associate with cool fast flows (1), *Metrichia*, a pursecase-making caddisfly associated with mats of filamentous algae in faster flows and large open streams (43); and sediment-tolerant, burrowing Sphaeriidae fingernail clams, which are found in a wide range of habitats (1). All of these were found in other sites and years, but several were also taken for the first time in the nearby WC2000 reach in 2021. A SIMPER analysis revealed a 33.2% dissimilarity between the 2020 and 2021 samples, and taxa that contributed the most to the differences were Chironomini and *Metrichia* (both absent in 2020), as well as Tanytarsini (more abundant in 2020).

The 2021 sample had the most taxa of any Whychus sample in 2021, but the number of EPT taxa was lower than in the prior year and at the low end of the 2021 dataset (Table 9). The community was dominated by *Acentrella turbida*, a small minnow mayfly that is slightly sensitive to fine sediment and associated with warmer waters and erosional flows, at a low relative abundance (12.7%). There were fewer sensitive and sediment-sensitive taxa in the 2021 sample compared to the prior year. Tolerant organisms were more abundant in 2021, but the relative abundance of sediment-tolerant organisms was lower than in 2020 (Table 10). Samples in both years had similar numbers of DEQ cool temperature and low sediment indicator taxa (Table 10), but there were more warm temperature and high sediment indicator taxa in 2021, and the community temperature and sediment optima were higher as well.

Table 10. Numbers of sensitive and sediment-sensitive taxa and relative abundance of tolerant and sediment-tolerant organisms at WC2050 in all sampling years. *Multihabitat samples were taken in 2020-2021. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and ≥2 sediment-sensitive taxa; and at <15% tolerant and <10% sediment-tolerant organisms.*

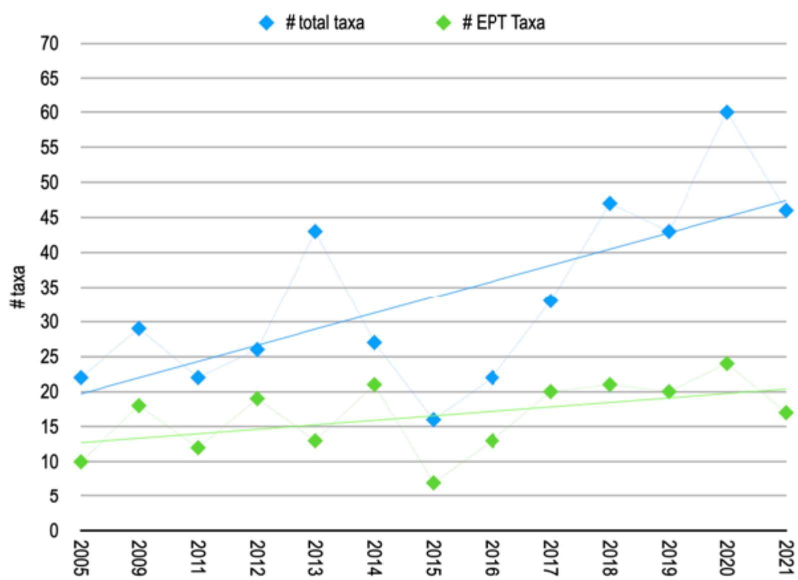
	2020	2021
# sensitive taxa	5	4
# sediment-sensitive taxa	3	1
% tolerant organisms	1.7	17.8
% sediment-tolerant organisms	21.3	11.9
# DEQ cool indicator taxa	6	5
# DEQ warm indicator taxa	4	7
# DEQ low-sediment indicator taxa	5	5
# DEQ high-sediment indicator taxa	5	10
Community temperature optima (C)	17.3	18.1
Community sediment optima (% FSS)	6.6	8.2

<u>WC2600</u>

The primary channel at WC2600 was sampled from 2005-2021. Riffle-targeted samples were taken through 2020, and multihabitat samples were taken in the same reach in 2018-2021. In 2014, the stream was directed into a new meandering channel, and additional multihabitat samples were taken in newly-formed side channels in 2018. Sampling continues to focus on the primary channel but since 2018, as the stream structure developed following restoration, the initial primary channel sampling reach has included elements of side channel habitat as well. The 2021 multihabitat sample was taken in moving water (glide, run, and riffle) in a variety of mineral substrates (cobble, gravel, sand/silt; Table 2).

The 2021 sample was most similar to samples taken in other upstream reaches (i.e., RM19.5 - 20.5; see Figure 2). Total richness was intermediate for the 2021 sample set, and the number of EPT taxa was at the lower end of the range for all samples in 2021. Although there were fewer total and EPT taxa in 2021 compared to the prior year, both have increased overall since 2015 (Figure 73). The only taxon in the 2021 sample that was not found at this site in any earlier sampling year was the pursecase-making caddisfly *Metrichia* (11 individuals), which was a new sample taxon at WC2000 and WC2050 as well. The sample was dominated by *Nostococladius*, a sediment-intolerant nonbiting midge associated with cool clear rivers and streams that burrows into discs of the blue-green algae *Nostoc. Nostococladius* dominated organismal abundance in riffle-targeted and/or multihabitat samples in the last four sampling years, but was more abundant in 2021 (Figure 74). The sample community consisted mainly of small, multivoltine burrowers that feed as scrapers and are associated with cooler, faster flows.

Figure 73. Sample richness and number of EPT taxa at WC2600 in all sampling years. *Only samples taken in the primary channel are shown, although from 2018-2021, the sampling reach contained portions of both primary and side channel habitat. Riffle samples were taken 2005-2020; a multihabitat sample was taken in the same reach in 2018-2021. Values for the years with both types of samples were averaged here. Linear trendlines are shown. This metric in the ORDEQ IBI receives the highest scaled score at >35 total taxa.*



The sub-sampling target of 500 individuals was met in all multihabitat samples and in seven of the 11 years in which RT samples were taken, after picking 21.7-100% of the total sample (Figure 75). Although IBI scores can't be calculated for multihabitat samples, raw values for six of the 10 metrics corresponded to the highest scaled IBI score: taxa richness, # sensitive taxa, # sediment-sensitive taxa, % tolerant organisms, % sediment-tolerant organisms, and MHBI. The number of sensitive taxa is generally greater than the number of sediment-sensitive taxa, and though both were lower at this site in 2021 compared to 2020, they were at the upper end of the range seen across the 2021 sample dataset and have both increased overall following restoration disturbances (Figure 76). The relative abundances of tolerant and sediment tolerant organisms have also increased overall since restoration was completed, but raw values of both were in the range of the highest scaled IBI score in most sampling years (Figure

77). Both were lower in 2021 compared to the prior year, and at the lower end of the range seen across all 2021 samples. DEQ indicator taxa for cool temperatures and low fine sediment consistently outnumber indicator taxa for warm water and high sediment (Figure 78), although community temperature and fine sediment optima have increased overall (Figure 79).

*Figure 74. **Relative abundance of the numerically dominant taxon at WC2600 in all sampling years.** Only samples taken in the primary channel are shown, although from 2018-2021, the sampling reach contained portions of both primary and side channel habitat. Riffle samples (blue) were taken in 2005-2020; a multihabitat sample (green) was taken in the same reach in 2018-2021. This metric receives the highest scaled score in the ORDEQ IBI at <20% abundance of the top taxon.*

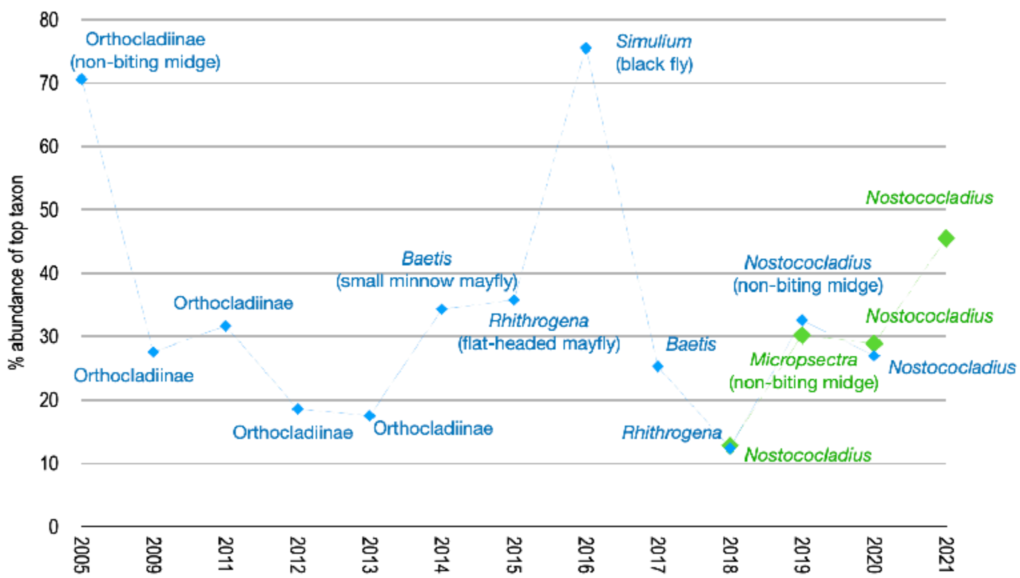


*Figure 75. **Proportion of sample needed for sub-sampling and resulting organismal abundance at WC2600 in all sampling years.** Only samples taken in the primary channel are shown, although from 2018-2021, the sampling reach contained portions of both primary and side channel habitat. Riffle samples (blue) were taken in 2005-2020; a multihabitat sample (green) was taken in the same reach in 2018-2021. Target sub-sampling number is 500 organisms.*

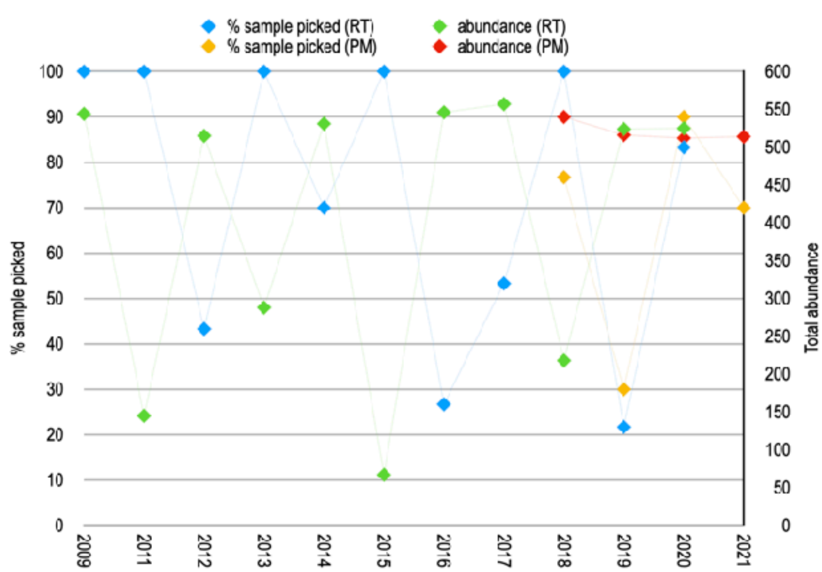


Figure 76. Numbers of sensitive and sediment-sensitive taxa at WC2600 in all sampling years. *Only samples taken in the primary channel are shown, although from 2018-2021, the sampling reach contained portions of both primary and side channel habitat. Riffle samples were taken 2005-2020; a multihabitat sample was also taken in the same reach in 2018-2021. Values for the years with both types of samples were averaged here. These metrics in the ORDEQ IBI receive the highest scaled score at >4 sensitive and >2 sediment-sensitive taxa.*

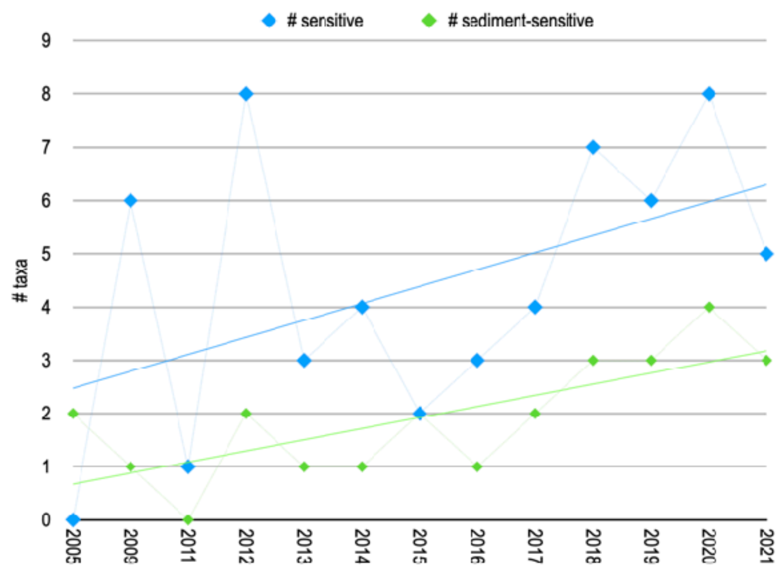


Figure 77. Relative abundance of tolerant and sediment-tolerant organisms at W2600 in all sampling years. *Riffle samples were taken 2005-2020; a multihabitat sample was also taken in the same reach in 2018-2021. Values for the years with both types of samples were averaged here. These metrics in the ORDEQ IBI receive the highest scaled score at <15% tolerant and <10% sediment-tolerant.*

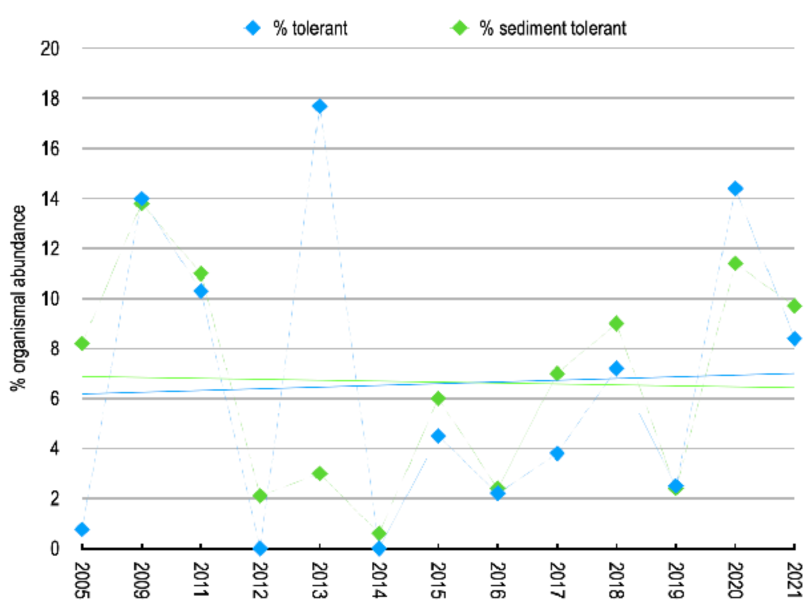


Figure 78. Number of ORDEQ indicator taxa for cool and warm temperatures and low and high sediment at WC2600 in all sampling years. *Riffle samples were taken 2005-2020; a multihabitat sample was also taken in the same reach in 2018-2021. Values for the years with both types of samples were averaged. Note that ORDEQ indicators do not account for the temperature or sediment associations of all taxa in a sample.*

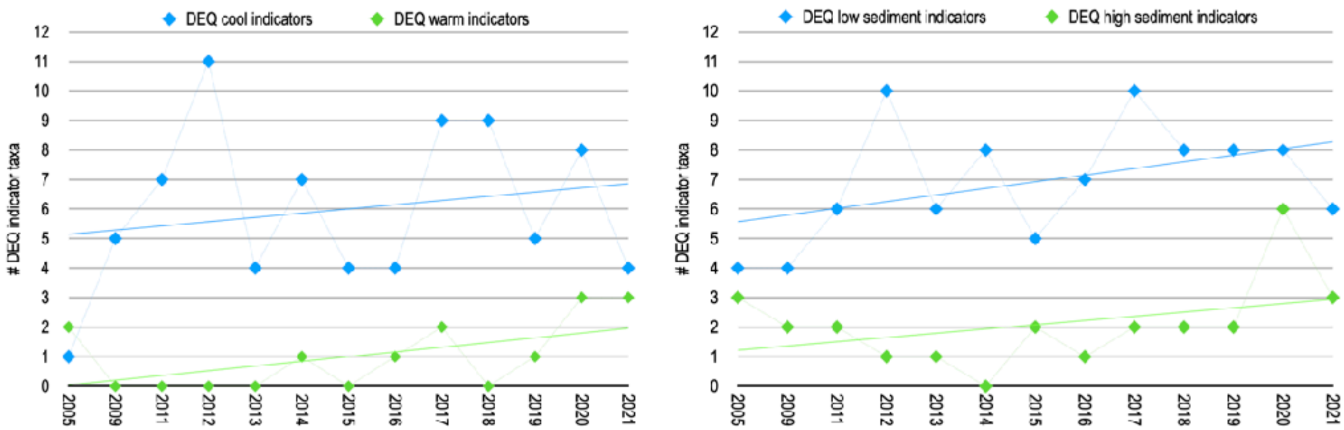
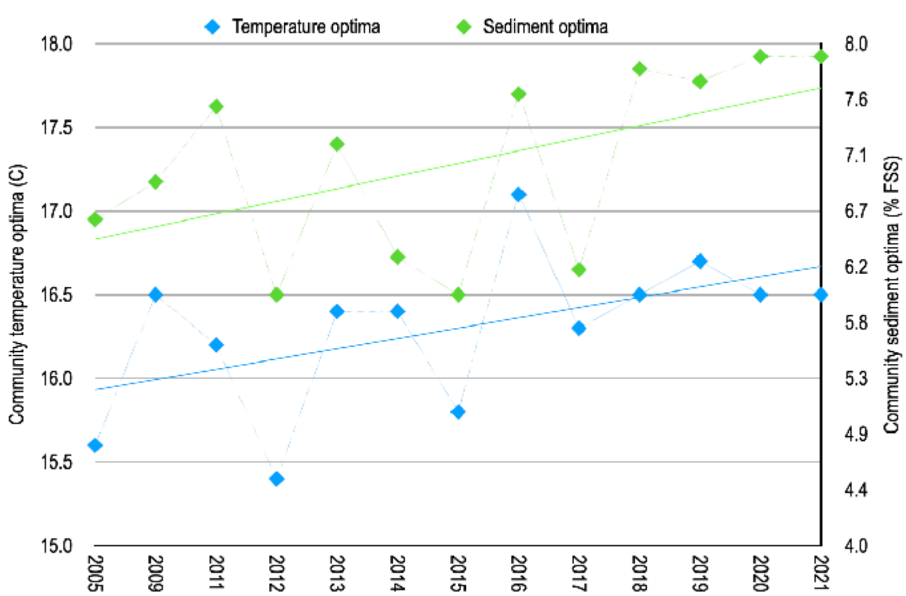


Figure 79. Temperature and fine sediment optima of the community (weighted means) at W2600 in all sampling years. *Riffle samples were taken 2005-2020; a multihabitat sample was also taken in the same reach in 2018-2021. Values for the years with both types of samples were averaged. Note that individual optima are not known for every taxon in a sample.*



Samples taken at this site have an overall community similarity of at least 40% with the exception of 2005 and 2015 (Figure 80). Multihabitat samples taken from 2018-2020 in any type of channel cluster together, but the 2021 multihabitat sample community was most similar to the riffle-targeted samples taken in the primary/side channel reach in 2019 and 2020 (Bray Curtis Similarity Index = 0.63-0.64). In a PCA ordination of taxa abundances (Figure 81), axis 1 explained 31.8% of total sample variation, and taxa with the highest loading values were Tanytarsini, a tolerant non-biting midge tribe associated with warmer waters (most abundant in side channels), *Zapada cinctipes,* a forest stonefly found in a variety of substrates in cold fast flows (more abundant in 2018-2019), and *Baetis* *tricaudatus,* a small minnow associated with faster flows and low sediment that can be an early colonizer after disturbance (more abundant in years spanning restoration). Axis 2 explained an additional 17% of total 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); Orthocladiinae, a nonbiting midge family that includes *Nostococladius*, which has numerically dominated samples for the last four years; and Tanytarsini non-biting midges.

Figure 80. nMDS ordination of the WC2600 macroinvertebrate community in all sampling years. *Riffle samples were taken 2005-2020; a multihabitat sample was taken in the same reach in 2018-2021, although by 2018 the former primary channel reach contained side channel elements as well. Multihabitat samples were taken in side channel reaches in 2018. The number at the end of each label indicates sampling year. Colored circles show community similarity levels from a CLUSTER dendrogram of the same data.*

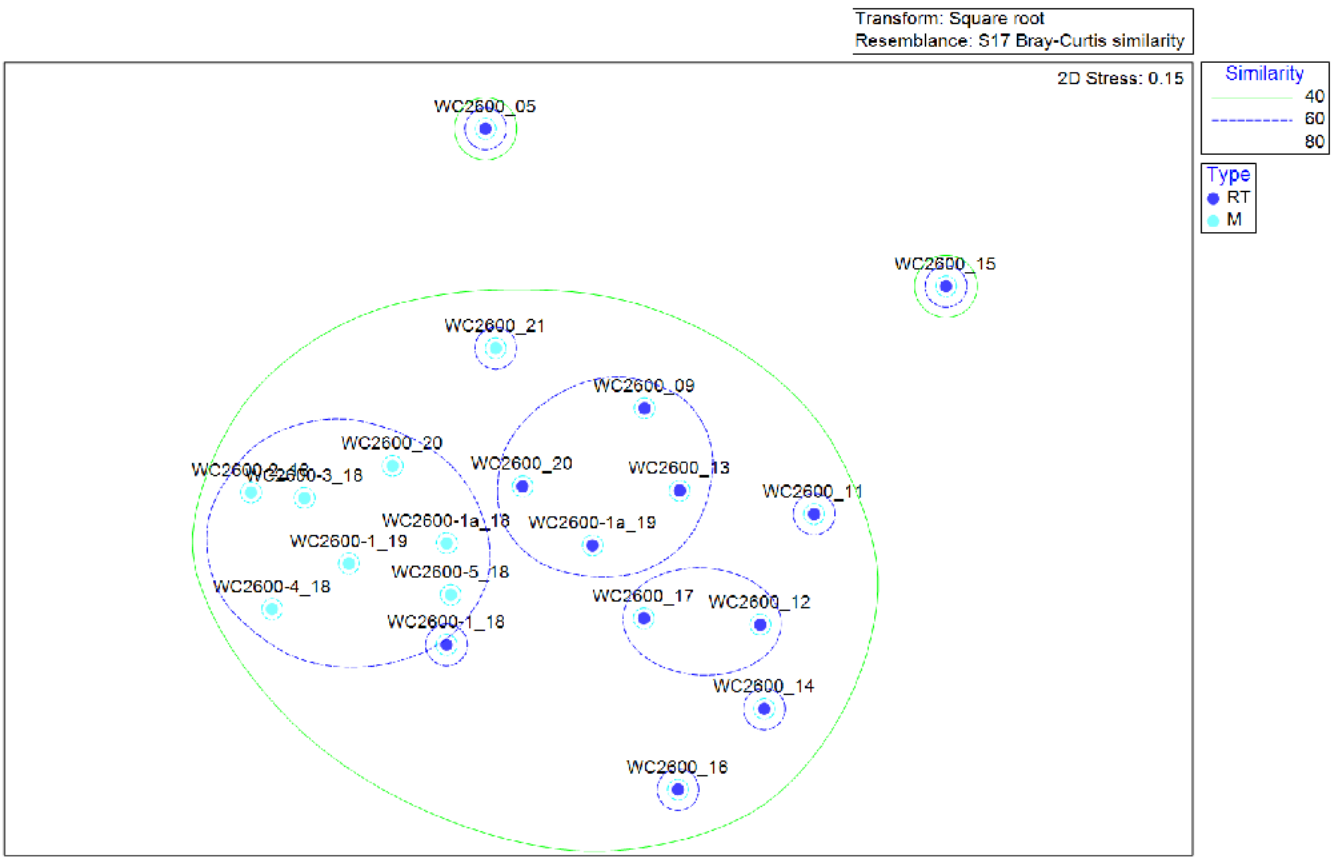
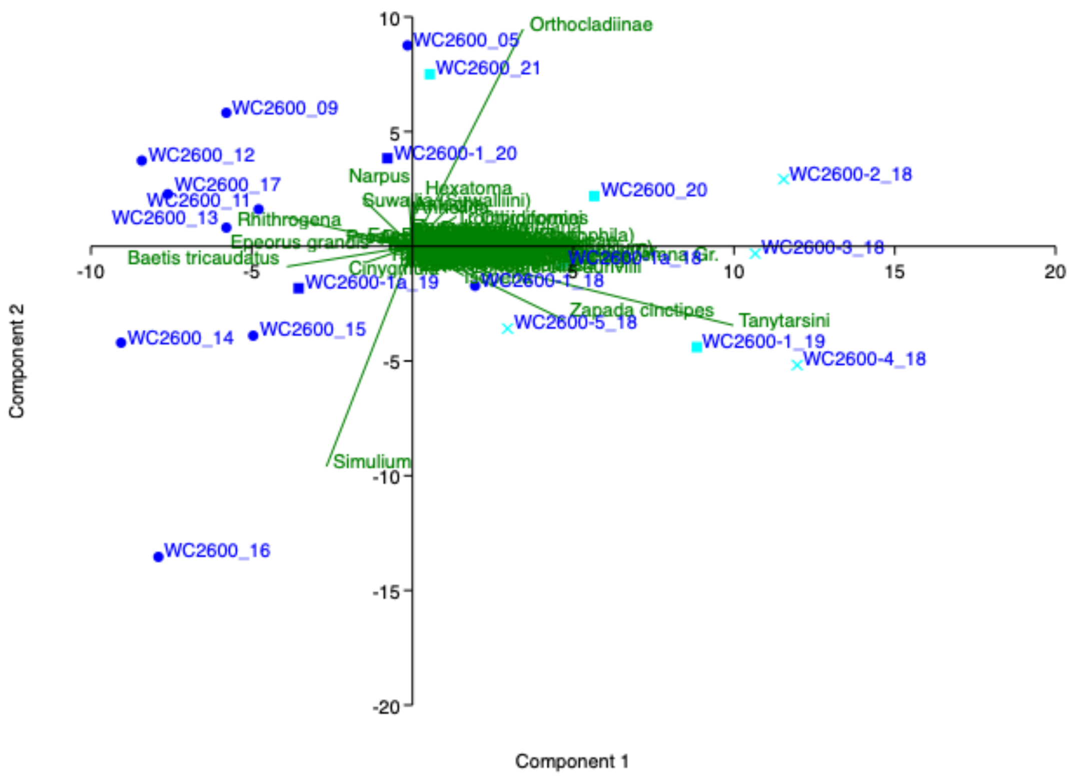
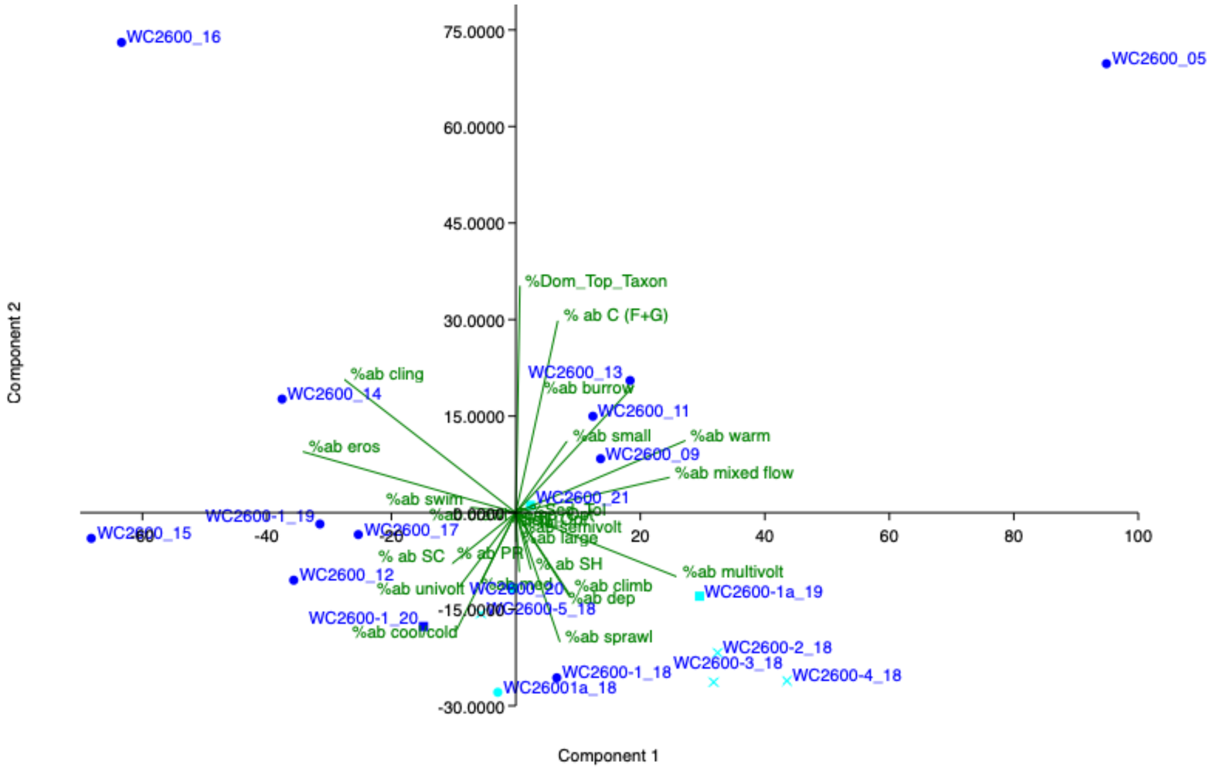


Figure 81. PCA ordination of the WC2600 macroinvertebrate community in all sampling years. Riffle samples were taken 2005-2020; a multihabitat sample was taken in the same reach in 2018-2021, although by 2018 the former primary channel reach contained side channel elements as well. Multihabitat samples were taken in side channel reaches in 2018. The number at the end of each label indicates sampling year.



In a PCA ordination of all traits measured as relative abundances (Figure 82), axis 1 explained 37% of total sample variation. Traits with the highest loading values were relative abundances of organisms associated with faster flows (less abundant in most side channel samples), warmer water (more abundant pre-restoration and in post-restoration side channels), and with the clinger habit (more abundant in riffle-targeted samples). Axis 2 explained an additional 21% of variation, and traits with the highest loading included relative abundances of the dominant taxon (2-5 times higher in 2005 and 2016); collectors (less abundant in 2020-2021 primary/secondary channel samples), and clingers.

Figure 82. PCA ordination of the WC2600 macroinvertebrate community traits measured as relative abundances in all sampling years. *Riffle samples were taken 2005-2020; a multihabitat sample was taken in the same reach in 2018-2021, although by 2018 the former primary channel reach contained side channel elements as well. PM samples were taken in side channel reaches in 2018. The number at the end of each label indicates sampling year.*



Discussion

Macroinvertebrate community characteristics along Whychus Creek

Macroinvertebrate sampling and bioassessment in Whychus Creek has been done since 2005, with annual sampling beginning in 2011. During that time, restoration activities have been implemented at the basin level, such as restoring perennial flow; and at the reach level, such as re-meandering mainstem channels, restoring floodplain connectivity, and creating new side channels. Restoration activities have driven changes in macroinvertebrate community composition, but this extended sampling period has seen substantial climatic changes, with increased drought, heat, and wildfires, which are also likely impacting macroinvertebrates. Even at longterm index sites where no active restoration has been done, the macroinvertebrate community in sampling reaches continues to change.

Overall taxa richness and community evenness was slightly lower in the 2021 sample set compared to the prior year, and it is difficult to know whether this is a result of increased climate-related disturbance, or the fact that stream conditions at the time of sampling meant that multihabitat samples could not be taken to ensure proportional representation of habitat types. However, multihabitat sample net sets were still taken in different habitat and flow types, and all but one site sample contained taxa that were new for that particular reach. All new taxa taken for the first time in the Whychus dataset were found in multihabitat samples, and all but one were taken in the restored WC1100 primary and side channels, attesting to the ability of heterogenous habitat with a mixture of flows and substrates to support a wide range of taxa and the ability of multihabitat sampling to capture a greater variety of taxa than riffle-targeted (single habitat) sampling. Interestingly, several taxa that were new to the 2020 Whychus dataset were found again at sites in 2021, which suggests establishment of new taxa as opposed to an opportunistic take of a very rare taxon, especially as some taxa present at very low abundances throughout sampling are still captured consistently.

Macroinvertebrate community composition continues to be strongly influenced by reach location, with greater similarity among communities from upstream reaches (i.e., WC1950-WC2600) compared to those in reaches further downstream (i.e., WC0600-WC1150), regardless of sampling technique or whether a primary or side channel reach was sampled. Differences in temperature- and sediment-related traits suggest that upstream communities are experiencing lower pressures from fine sediment or elevated temperatures, as 2021 samples from upstream reaches had significantly more DEQ indicator taxa for cool temperatures and low fine sediment and greater relative abundance of sprawler organisms. Despite these overall differences, the community at most sites in 2021 had high relative abundances of organisms associated with faster and/or cooler flows.

Changes in macroinvertebrate community characteristics at longterm index sites

The sample sites at WC0600 and WC1150 have not undergone reach-level restoration, but conditions have been influenced by basin-level activities, restoration projects implemented upstream and/or downstream, and ongoing climate changes. For example, observed taxa and trait differences in the 2017 sample community at WC1150 compared to all other sampling years suggest that the downstream restoration project at WC1100 also affected the community at WC1150. The community at both sites has changed over time, with earlier sampling years differing more from recent years at both sites, and a few new taxa that had not been taken in prior years were seen in 2021 samples from each site as well. Overall, changes in many metrics indicate improved habitat or water quality. Taxa and EPT richness increased at both sites over time, especially since 2017-2018; PREDATOR and IBI scores at WC0600 also rose in that same span, with IBI scores indicating low levels of impairment in recent years. At both longterm sites, sediment-tolerant organisms are much less abundant than tolerant organisms, and more dominant taxa in recent years are associated with faster flows.

Notable taxonomic shifts at WC0600 in more recent (2018-2021) compared to earlier (2005-2017) sampling years include much greater numbers recently of the mayflies of *Rhithrogena* and *Acentrella insignificans*, which are sensitive to fine sediment and associated with faster flows. Some more tolerant groups associated with a range of flows and temperatures have also increased in abundance in recent years, such as the riffle beetle *Optioservus* and Tanytarsini and Orthocladiinae non-biting midges. However, part of the increase in Orthocladiinae is driven by greater *Nostococladius* abundance, and the *Nostoc* algae those midges inhabit is generally found in cool waters with rocky substrate and low levels of fine sediment. Taxonomic shifts at WC1150 in recent vs. earlier sampling years show a similar pattern, with greater abundance in recent years of several types of cool water-associated riffle beetles; *Acentrella* and *Rhithrogena* mayflies; *Pteronarcys*, a long-lived shredder stonefly associated with high levels of coarse particulate matter (CPOM) and low scouring/re-sorting of sediment; and *Hydropsyche*, a more tolerant type of netspinning caddisfly. WC1150 has also seen an overall decrease in collector abundance with increased numbers of scrapers and clingers, which is suggestive of lower sediment levels.

Community temperature optima have increased in recent years at both sites while community sediment optima have been relatively flat, despite the addition of multihabitat sampling at WC1150, suggesting that temperature stress associated with climate change may be occurring. However, while relative abundance of organisms associated with cooler flows is decreasing at WC0600, cool-associated organisms have been increasing in abundance at WC1150.

Baseline macroinvertebrate community characteristics at pre-restoration sites

WC0850 and WC0900 have been sampled across more years than WC2000 and WC2050, although neither was sampled in 2018 or 2019. New taxa were taken at WC0900 in 2021 but not at WC0850. Taxa and EPT richness at both sites are in the low to intermediate range compared to other sampling sites but have also increased at both sites in recent years. Community composition is overall more similar among samples taken in recent vs. earlier sampling years at both sites, although the 2020 and 2021 samples differ more from the 2017 sample than they do from each other. IBI scores at both sites indicated little to no impairment in most years, while PREDATOR scores have been lower in more years at WC0850 (poor) compared to WC0900 (fair condition). Dominant taxa at both sites are generally associated with faster flows but have included taxa more tolerant of warmer temperatures, flow types, and/or sediment, such as snails, worms, and chironomid midges. Abundance of tolerant organisms is increasing at both sites in recent years, while abundance of sediment-tolerant organisms is low, especially at WC0850. Community temperature and sediment optima are also higher in the most recent sampling years.

This is only the second year of baseline sampling at the WC2020 and WC2050 reaches, with riffle-targeted and multihabitat samples taken at WC2000 and riffle-targeted alone taken at WC2050. Samples have been well-balanced and diverse in both years, with overall high taxa richness and low relative abundance of the top taxon. Dominant taxa at WC2050 and in the WC2000 riffle samples are EPT members and are associated with faster flows and a range of temperature and sediment tolerances, although the saddlecase-making caddisfly genera that dominated the WC2000 riffle sample were more strongly associated with cooler water and lower fine sediment pressure. In contrast, the multihabitat sample at WC2050 in both years was dominated by more tolerant, sediment-associated non-biting midge genera, even though the habitats sampled included flowing (riffle, run, glide) as well as standing (pool) waters.

Samples from both sites have high between-year community similarity, although all WC2000 samples have greater mean similarity than the two samples taken at WC2050. Communities at both sites are most similar to those in more recent sampling years at nearby WC1950. Multihabitat samples from both sites had a greater percentage of tolerant organisms in 2021 than in 2020, and unlike most of the other Whychus sites, abundance of sediment-tolerant organisms was similar to that of tolerant. Notable taxa differences between years at WC2000, regardless of sample type, included lower abundance of sediment-associated Tanytarsini midges and greater abundance of a mayfly (*Ephemerella tibialis)* and caddisfly (*Metrichia*) associated with faster flows. *Metrichia* was absent from 2020 samples but present in both riffle and multihabitat samples at similar abundances in 2021; this genus is often associated with mats of filamentous algae, which in turn suggests nutrient-enriched conditions. Relative abundances of organisms with different ecological traits were similar between samples and years. Notable taxa differences between years at WC2050 included fewer tolerant *Cleptelmis* riffle beetles as well as fewer of some sensitive stonefly taxa taken the previous year, but greater numbers of sediment-tolerant segmented worms and non-biting midges as well as more *Simulium*, which can indicate recent disturbances, and the appearance of *Metrichia*. Trait differences also suggest increasing habitat disturbance and potentially increased fine sediment pressure, with more small-bodied, rapidly developing, burrower organisms associated with warmer temps and slower in 2021, and decreased abundance of the shredders and cool/cold-associated organisms present in 2020. It is possible that the sustained high turbidity observed in the creek during 2021 contributed to disturbance.

Changes in macroinvertebrate community characteristics at restored sites

The overall trajectory of macroinvertebrate community response is similar among most restored sites regardless of the type of restoration, with large perturbations for one to two years followed by stabilization of metric values as an altered post-restoration community establishes. Increased habitat heterogeneity has been accompanied by 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. Side channels generally have more organisms that prefer slower or mixed flow types, and many taxa 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 sensitive taxa with cool/cold temperature associations, again pointing to the capacity of more heterogeneous habitats to support a diverse community. 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 with a diversity of taxa.

Conditions in the WC1100 primary channel improved and stabilized following restoration-related disturbances that impacted the community strongly through 2018, with more total and EPT taxa, higher PREDATOR and IBI scores, and more sensitive and sediment-sensitive taxa, although both community sediment and temperature optima have trended upwards. As was seen with several other sites, relative abundance of tolerant organisms is increasing in recent years in both primary and side channel samples, but abundance of sediment-tolerant organisms remains lower, even in side channels. The proportion of organisms associated with cooler flows also increased among all primary and side channel samples since 2018, while the proportion of warm-associated organisms decreased overall in that span. WC1100 side channels support a different community composition than reaches that contain any primary channel habitat, and generally have more organisms associated with slower flows, broader temperature tolerances, and a burrowing habit. However, they support sensitive organisms as well, with EPT richness similar to primary channel communities. The ability of heterogeneous side channel habitat to support a variety of taxa is further shown by the fact that four of the five taxa new to the complete Whychus dataset in 2021 were taken at this site and most occurred in side channels.

Restoration at WC1950 occurred earlier (2012) than at most other restored Whychus sites. Many traits and metrics calculated for the 2021 sample community differed substantially from other recent years, but interpreting these changes is com plicated by the fact that sampling was done improperly by the volunteer team. All taxa taken in 2021 were found at the site in other sampling years, and compared to recent years at this site organismal abundance was lower; there were fewer total, EPT, sensitive and sediment-sensitive taxa; fewer DEQ indicator taxa for temperature and sediment; and lower relative abundance of tolerant and sediment-tolerant organisms. With the exception of 2017, in which community composition and metrics suggested habitat disturbance, the communities in recent sampling years are more similar to each other than to early sample communities (2009-2014), but the 2021 sample, while still clustering with the 2015-2020 samples, differed more overall compared to the 2020 sample. Despite this, trends at the site suggest increased habitat quality and stability, with increased richness; more EPT, sensitive, and sediment-sensitive taxa; greater relative abundance of tolerant compared to sediment-tolerant organisms; fewer collectors and more shredders; and fewer burrowers and more clingers. Other trait metrics fluctuate more annually and show no clear trends.

The recovery trajectory at WC2600 following channel re-meandering in 2014 included a more disturbed community in 2015/2016 as well as in side channels in 2018 that gave way to a more braided main channel with primary and side channel characteristics. Macroinvertebrate community changes include a large increase in total richness as well as in the number of EPT, sensitive, and sediment-sensitive taxa. Proportions of tolerant and sediment-tolerant organisms are similar, unlike most other Whychus sites, and generally low (i.e., <15%). This is also one of the only sites where DEQ indicator taxa for cold temperature and low sediment outnumber warm and high sediment indicators. As was seen with several other sites, both community temperature and sediment optima are trending upwards, but community temperature optima at WC2600 is consistently among the lowest of all Whychus samples. Other trends include more multivoltine and fewer univoltine organisms, which could suggest more disturbed or changing habitat conditions; fewer collectors and more scrapers, which could reflect lower sediment levels on mineral surfaces; and more organisms associated with erosional flows and fewer associated with warm water.

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