# Macroinvertebrate Monitoring in Whychus Creek, Sisters, Oregon, 2018



*Whychus Creek side channel; C.A. Searles Mazzacano*

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## Summary

Benthic macroinvertebrates were sampled on 24-25 August 2018 in three regions of Whychus Creek: Road 6360 (WC0600), Whychus Canyon (WC1100, WC1150), and Whychus Floodplain (WC2600). A riffle-targeted (RT) and proportional multihabitat (PM) sample was taken simultaneously in the same mainstem reach at each site, and PM samples were taken in side channels created at WC1100 (in 2016) and WC2600 (in 2014). The ORDEQ PREDATOR predictive model and Index of Biotic Integrity (IBI) was applied to all RT samples, and taxonomic and biological traits were assessed for all samples, including: optima values for temperature and fine sediment; tolerances to sediment and organic pollution; functional feeding group; habit (swimmer, clinger, sprawler, etc.); flow preference; temperature association; and maximum body length. Samples contained a total of 151 unique taxa in 50 families. Order-level diversity was highest among the true flies (Diptera), especially non-biting midges (Chironomidae). Other families with high diversity included mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera). Seven taxa not found in any prior sampling year were collected (five EPT, 1 dragonfly, and one beetle), including one new family; all of these new taxa were seen only in PM samples.

The macroinvertebrate communities in each mainstem RT and PM sample were most similar to each other. PM samples had significantly more taxa and greater organismal abundance than RT samples. The mean number of EPT was higher in PM samples in mainstem reaches as well as across the complete sample set, even though PM samples did not target riffles. Dominant taxa in PM samples were more reflective of diverse substrates, flows, and water temperatures, while taxa that dominated RT samples were more strongly associated with colder lotic habitats. No significant difference was found between mainstem RT and PM samples for any biological traits assessed, although mean relative abundances of sediment-tolerant, warm water-, depositional-, and mixed flow-associated taxa were higher in PM samples. These results indicate that multihabitat sampling gives a more accurate representation of site diversity without sacrificing the ability to detect sensitive riffle-associated taxa.

Macroinvertebrate communities in side channel habitat created in at WC2600 and WC1100 had a high degree of similarity to the mainstem community at each site, which is a nearby source of colonists. However, side channels at each site had greater richness than the mainstem, which is likely a function of greater habitat heterogeneity. There was no significant difference in the mean numbers of EPT taxa in side channel vs. mainstem samples at either site, indicating that these more sensitive taxa can also colonize and persist in these new habitats. Characteristics of side channel communities reflect the filtering effects of more disturbed and changing habitat, including lower evenness, dominance of more tolerant and/or pioneer taxa, and more multivoltine taxa than the mainstem reaches.

Longterm monitoring sites show increases in several biotic assessment metrics across time, including greater taxa richness, improved evenness, and more EPT, sensitive, and sediment-intolerant taxa. PREDATOR and IBI scores reflect improved overall conditions as well, though the level of change is greater in IBI scores. Macroinvertebrate communities at WC1100 and WC2600, which were severely negatively impacted by restoration-related disturbances, have recovered substantially in recent years. Community temperature optima at some longterm sites have been increasing in recent years, but changes vary among sites and it is not known if landscape level or climate-related factors are a driving force.

## Background

The Whychus Creek watershed is a designated priority watershed for conservation and restoration in the upper Deschutes Basin. Projects implemented since 1999 have restored perennial flow to the creek and increased in-stream flow volume. The aquatic macroinvertebrate community has been monitored regularly in Whychus Creek to assess changes following these restoration projects. Recently, creation of side channel habitat for salmonids has led to additional focus on macroinvertebrate communities in these more heterogenous habitats, and their implications for habitat use by fish. The goals of macroinvertebrate monitoring in Whychus Creek are now thus twofold: 1. to continue monitoring at selected long-term indicator sites using the standard riffle-targeted protocol to assess ongoing changes at the watershed level; and 2. to monitor communities at the project level using a proportional multihabitat technique to increase fine-scale resolution at targeted sites.

## Methods

*Sampling Sites*

Benthic macroinvertebrate sampling was done on 24-25 August 2018 along three regions of Whychus Creek (Table 1): Road 6360 (WC0600), Whychus Canyon (WC1100, WC1150), and Whychus Floodplain (WC2600). A single habitat riffle-targeted sample (RT) and a proportional multihabitat sample (PM) was taken in the mainstem channel at each site, and proportional multihabitat sampling was done in side channel habitat at WC1100 (created in 2016) and WC2600 (created in 2014). A duplicate RT sample was taken at WC2600 for quality control.

*Table 1. Whychus Creek sampling sites in 2018*

| Site ID | Description | Coordinates | Year(s) sampled |
|---------|-------------|-------------|-----------------|
| **WC0600** | RM 6, u/s Rd 6360 | 44.40412, -121.40259 | 2005, 2009, 2011-2018 |
| **WC1100[a]** | Whychus Canyon d/s | 44.36493, -121.422232 | 2015, 2017-2018[c] |
| **WC1150[b]** | Whychus Canyon u/s | 44.35862, -121.43023 | 2014-2018 |
| **WC2600** | RM 26, 4606 Rd. footbridge | 44.27322, -121.5552 | 2005, 2009, 2011-2018 |

[a] *designated WC1025 in 2014;* [b] *designated WC1075 in 2014;* [c] *not sampled in 2016, as project had just been implemented*

*Macroinvertebrate sampling techniques*

Sampling was done by CASM Environmental and UDWC staff in conjunction with volunteers solicited from natural resource agencies (24 August) and the local community (25 August). On each day, CASM Environmental staff demonstrated the sampling techniques and explained each item on the data sheet. Teams received sampling kits and instructions for finding their sites and dispersed into the field. In 2018, all teams sampled in the same general area each day, so CASM Environmental and UDWC staff were close by to answer questions. After teams returned their samples, data sheets, and equipment to CASM Environmental, each sample was inspected to ensure it was properly labeled and preserved.

Single habitat riffle-targeted protocol (RT)

Benthic macroinvertebrates were collected from mainstem riffle habitats according to ORDEQ protocols for Oregon's wadeable streams (OWEB, 2003). Reach lengths were calculated as 40 times the average wetted stream width at the desired sampling point (min. = 500 ft., max = 1000 ft) and the upstream and downstream extent was flagged by UDWC staff prior to sampling. A site sample consists of eight individual net sets taken in riffle habitat in the designated reach, each collected from a 1 ft$^2$ area using a D-frame kick net with 500 μm mesh and a 1-ft opening. In reaches with eight or more riffles, a single net set is taken in each of eight randomly selected riffles; in reaches with fewer riffles, two net sets are taken in each of four randomly selected riffles.

Large rocks and debris in the sampling area are rubbed and rinsed into the net to dislodge and collect any clinging organisms and set aside. The substrate is then disturbed thoroughly using a boot heel to a depth of 6-10 cm (2-4 in.) for 1-2 minutes. All eight net sets are pooled in a bucket, large debris is rinsed and removed, and sample material is concentrated by pouring through a 500 μm sieve lined with a flexible square of 500 μm Nitex membrane. The membrane is lifted out and the concentrated sample is carefully transferred into a 1-liter Nalgene sample jar half-filled with 80% ethanol as a preservative. Jars were filled such that sample material occupied no more than 2/3 of the total jar volume, and were split into multiple jars as needed. CASM Environmental staff replaced the 80% ethanol in all jars with fresh within 48 hours to ensure good preservation.

Proportional multihabitat sampling (PM)

Reach lengths for sampling were calculated and flagged as described above; mainstem reaches were the same as for the RT protocol and were sampled at the same time. For braided side channels, UDWC staff also flagged turning points and paths along the target channel. Prior to sampling, the team walked the length of the reach and used a worksheet to determine the types of in-stream habitat present and their relative proportions. Habitat types were designated as follows:

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

Each sample was a composite of 10 net sets, and the number of net sets taken in each type of habitat was determined by its proportional representation within the reach (Table 2). For example, in a reach with 20% boulder, 50% cobble, 20% gravel, and 10% sand/silt habitat, the composite sample would consist of two, five, two, and

one net set taken from randomly chosen areas of each respective type of habitat. Flow type in the habitat where each net set was taken was recorded (rapid, riffle, run, glide, pool), but no particular flow types were targeted.

In areas where there was sufficient current to carry suspended material into the net, cobble and gravel substrates were sampled as described above for RT sampling. For 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$^2$ area in front of the net. In slow/still waters and in sediment, the substrate was disturbed manually in a 1 ft$^2$ area and the net was swept upwards to capture suspended/disturbed material. Clumps of filamentous algae or plants were shaken into the mouth of the net to collect any clinging organisms, and the net was jabbed into clumps of plants. Wood tangles and woody debris were rubbed or picked off into the net, and then the material was kicked vigorously while holding the net beneath it. Rootwads were sampled by jabbing the net into the tangle or kicking into the rootwad while holding the net below it. All net sets were composited and processed as described for RT samples.

*Table 2. Whychus Creek sampling sites in 2018.*

*RT = riffle-targeted, PM = proportional multihabitat; MS = mainstem, SC = side channel*

| Sample | Type | Loc | # net sets in each habitat type | flow type in net sets | avg wetted width (ft) |
|---|---|---|---|---|---|
| WC0600-1 | RT | MS | 8 riffle | 8 riffles | 25.0 ± 2.6 |
| WC0600-1 | PM | MS | 1 bedrock, 3 cobble, 3 gravel, 2 sand/silt, 1 rootwad | 2 riffle, 4 run, 2 glide, 2 pool | 25.7 ± 2.7 |
| WC1100-1 | PM | SC | 2 cobble, 2 gravel, 3 sand/silt, 1 veg, 2 wood | 1 riffle, 6 run, 2 glide, 1 pool | 26.2 ± 13 |
| WC1100-2 | RT | MS | 5 riffle | 5 riffle | 18.9 ± 4.8 |
| WC1100-2 | PM | MS | 5 cobble, 1 gravel, 3 sand/silt, 1 fil. algae | 3 riffle, 4 run, 1 glide, 2 pool | 19.1 ± 4.7 |
| WC1100-3 | PM | SC | 1 cobble, 2 gravel, 5 sand/silt, 1 veg, 1 wood | 2 riffle, 3 glide, 5 pool | 17.7 ± 6.9 |
| WC1100-4 | PM | SC | 5 cobble, 4 sand/silt, 1 wood | 4 riffle, 1 run, 4 glide | 18.7 ± 6.6 |
| WC1100-5 | PM | SC | 4 cobble, 2 gravel, 4 wood | 5 riffle, 3 run, 2 glide | 18.1 ± 4.4 |
| WC1150-1 | RT | MS | 8 riffle | 8 riffle | 25.3 ± 1.8 |
| WC1150-1 | PM | MS | 3 boulder, 3 cobble, 2 gravel, 1 sand/silt1 rootwad | 3 riffle, 6 run, 1 pool | 26.5 ± 1.7 |
| WC2600-1 | RT | MS | 8 riffle | 8 riffle | 24.2 ± 7.4 |
| WC2600-1 | PM | MS | 5 cobble, 2 gravel, 2 sand/silt, 1 wood | 5 riffle, 2 glide, 3 pool | 23.8 ± 7.7 |
| WC2600-2 | PM | SC | 1 cobble, 2 gravel, 5 sand/silt, 2 wood | 4 riffle, 2 run, 1 glide, 3 pool | 12.9 ± 5.1 |
| WC2600-3 | PM | SC | 4 cobble, 4 gravel, 1 sand/silt, 1 wood | 4 riffle, 5 run, 1 pool | 19.1 ± 6.9 |
| WC2600-4 | PM | SC | 1 cobble, 5 gravel, 3 sand/silt, 1 wood | 4 riffle, 3 run, 3 pool | 11.4 ± 2.8 |
| WC2600-5 | PM | SC | 2 cobble, 6 gravel, 1 sand/silt, 1 wood | 7 riffle, 1 run, 2 glide | 8.7 ± 3.0 |

*Sample identification*

Samples were identified by Cole Ecological, Inc. (www.coleecological.com). They were first sub-sampled to a target count of 500 individuals (Barbour & Gerritsen, 1996; Vinson & Hawkins, 1996) by spreading the entire sample on a gridded tray and using a random number generator to select squares to pick. A square was picked to completion after the target number was reached if material remained, which accounts for differences in abundance between samples (see Table 3 in Results). Note that for the WC1150 RT sample, only 1/8 of the total sample was spread and picked, as it was discovered later that the sampling team had collected 64 net sets instead of eight.

Individuals were identified to the lowest practical taxonomic level using standard taxonomic effort recommended by the Southwest Association of Freshwater Invertebrate Taxonomists (Richards & Rogers, 2011; SAFIT, 2016). For some taxa, identification is taken only to genus because they contain species that are poorly described and/or are known to contain a number of undescribed species; immature stages of certain taxa are not yet associated with their adult forms; some taxa can only be identified to species from adult specimens (i.e., details of genitalia are diagnostic); and the statewide distribution for many species has not been delineated. In some cases identification is only done to family (i.e., Ephydridae [shore flies], Capniidae [small winter stoneflies], due to these same issues.

*Biological/ecological traits of taxa*

Assessing taxa traits can help infer habitat conditions and diagnose stressors or environmental filters influencing macroinvertebrate community taxonomic composition within a site (Poff et al., 2006; Tullos et al., 2009; Culp et al., 2011). The following traits were assigned to species, genera, or families where data were available: optima values for temperature and fine sediment; tolerance to sediment and organic pollution; trophic guild (functional feeding group); habit (swimmer, clinger, sprawler, etc.); flow preference (erosional, depositional, mixed); temperature preference (cool/cold, warm); and maximum body length. See attached database for a complete list of taxa and associated characteristics. Trait data were drawn from sources specific to Oregon and/or the west (ORDEQ, 2003; Vieira et al., 2006; Meyer & McCafferty, 2007; Huff et al., 2008; Richards & Rogers, 2011; IDDEQ, 2015; SAFIT, 2016), as well as general and family-specific references (Pinder, 1986; Wiggins, 1996; Larson et al., 2000; Thorp & Covich, 2001; Stewart & Stark, 2002; Merritt et al., 2008; Anderson et al., 2013). Where multiple modalities existed for a given trait, the primary one given for the genus or family was used in analyses.

*Data analysis*

Analyses and calculations were done using the PAST statistical software package (Hammer et al., 2001). CLUSTER, ANOSIM, and SIMPER tests were run on a Bray-Curtis similarity matrix of square-root transformed taxa abundances. Principal Component Analysis (PCA) was run on square root-transformed data using a variance-covariance matrix.

Biological condition of long-term RT sampling sites was assessed using the ORDEQ multimetric invertebrate-based index of biotic integrity (IBI), and the probability-based PREDATOR model (Predictive Assessment Tool for Oregon; Hubler, 2008) developed for the Western Cordillera and Columbia Plateau (Klamath Mountain,

Cascades, East Cascades, Blue Mountains, and Columbia Plateau ecoregions; WCCP). The IBI consists of 10 traits whose raw values are scored individually (1, 3 or 5) based on similarity to reference streams, then summed to give a single number that reflects the level of site impairment (Table 3). PREDATOR calculates the ratio of taxa observed at a sampling 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 the most appropriate reference streams. O/E scores associated with a probability of capture ($P_c$) > 0.5 were used to avoid rare taxa bias (i.e. the model considers only invertebrates with over 50% likelihood of being collected at reference sites). O/E scores reflect the following biological conditions: $\leq$0.78 = poor (most disturbed); 0.79-0.92 = fair (moderately disturbed); 0.93-1.23 = good (least disturbed); and >1.23 = enriched. Note that Whychus Creek is an outlier for the PREDATOR model because it has lower annual precipitation than any of the reference streams the model selects as most appropriate (Shannon Hubler, pers. comm. 2017).

*Table 3. ORDEQ genus-level macroinvertebrate-based IBI metrics and scoring.*

| I-IBI | | | |
|---|---|---|---|
| **Scoring Criteria** | | | |
| 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** | | | |
| Severely impaired | | <20 | |
| Moderately impaired | | 20-29 | |
| Slightly impaired | | 30-39 | |
| Minimally/not impaired | | >39 | |

[a] *relative abundance of the most abundant taxon;* [b] *modified Hilsenhoff Biotic Index (Hilsenhoff, 1987), which reflects tolerant to organic pollution and ranges from 1 (lot tolerance) to 10 (high tolerance)*

## Results

*Overall macroinvertebrate community diversity*

Samples collected in 2018 contained a total of 151 unique taxa in 50 families (36 insect, 14 non-insect). Order-level diversity was highest among the true flies (Diptera), with 71 unique taxa in nine families, and was driven by the large number of non-biting midge genera (Chironomidae; 48 unique genera). Other families with high diversity included mayflies (Ephemeroptera; 22 unique taxa in five families), stoneflies (Plecoptera; 15 unique taxa in seven families), and caddisflies (Trichoptera; 14 unique taxa in eight families). Seven taxa not found in any prior sampling year were collected, all of which were seen only in PM but not RT samples. New taxa include:

- *Brychius*, a crawling water beetle (Haliplidae; also a new family for the project list) found in fast areas of rivers and streams and moving water in lakes and ponds (one individual in a WC1100 side channel);

- *Centroptilum*, a small minnow mayfly (Baetidae) that prefers colder, slower waters in lotic and lentic habitats (low abundance in WC1150 and WC2600 mainstem and WC1100 side channels);

- *Procloeon*, a small minnow mayfly found in slow waters of lotic and lentic habitats (two individuals in a WC1100 side channel);

- *Ephemerella aurivillii*, a spiny crawler mayfly (Ephemerellidae) that prefers cold waters in rivers, streams, and headwaters (low to moderate abundance in all WC2600 side channels);

- Gomphidae, a family of clubtail dragonflies that generally prefer clear flowing waters (three individuals in WC1150 mainstem);

- *Moselia infuscata*, a sensitive rolled-winged stonefly (Leuctridae) that prefers cold water in small to mid-sized streams (one individual in a WC2600 side channel); and

- *Malenka,* a winter stonefly (Nemouridae) found in cold flowing waters (two individuals in WC2600 mainstem)

Invertebrate abundances were high, and the target sub-sampling number of 500 individuals was attained for all but one site (WC2600-1, mainstem RT), with anywhere from 11.7-100% of the total sample picked (Table 4). The proportion of sample picked was significantly greater for RT samples (mean = 58.8% $\pm$ 29.9) compared to PM (mean = 31.8% $\pm$ 20.9; unpaired t-test, p = 0.0490). However, it should be noted that the proportion of total sample picked to attain the target number for the RT sample at both WC0600 and WC1100 was lower in 2018 compared to all other sampling years for those sites (see *Longterm Sampling Sites* below).

Numerical abundance in the complete data set was dominated by several genera of non-biting midge, especially *Micropsectra* and *Tanytarsus*, which are common, widespread genera in the tolerant Chironominae subfamily; the black fly *Simulium*, a lotic species that is often a pioneer after restoration; *Rhithrogena*, a flat-headed mayfly that prefers fast waters with low sediment; and *Baetis tricaudatus*, a small minnow mayfly common in the drift in lotic waters that tends to be an early re-colonizer following disturbance and is a DEQ low sediment and cool temperature indicator taxon. All were found in both RT and PM samples, and have been abundant and ubiquitous throughout the years of Whychus sampling.

**Table 4. Richness, abundance, and diversity among sampling sites in 2018.**
*DUP = duplicate sample; RT = riffle-targeted, PM = proportional multihabitat; MS = mainstem, SC = side channel*

| Sample | Type | Reach | Abundance (# individuals) | % of sample picked | Richness (# unique taxa) | #EPT taxa | Simpson's Diversity Index  (1-D) |
|---|---|---|---|---|---|---|---|
| WC0600 | PM | MS | 574 | 11.7 | 47 | 17 | 0.93 |
| WC0600-1 | RT | MS | 565 | 20.8 | 47 | 13 | 0.91 |
| WC1100-1 | PM | SC | 544 | 30 | 61 | 18 | 0.91 |
| WC1100-2 | RT | MS | 560 | 56.7 | 48 | 19 | 0.93 |
| WC1100-2 | PM | MS | 570 | 36.7 | 57 | 18 | 0.95 |
| WC1100-3 | PM | SC | 528 | 23.3 | 64 | 18 | 0.96 |
| WC1100-4 | PM | SC | 556 | 18.3 | 57 | 14 | 0.89 |
| WC1100-5 | PM | SC | 543 | 43.3 | 51 | 19 | 0.76 |
| WC1150-1 | PM | MS | 583 | 11.7 | 65 | 23 | 0.96 |
| WC1150-1 | RT | MS | 545 | 73.3 | 44 | 17 | 0.95 |
| WC2600-1 | RT | MS | 218 | 100 | 39 | 17 | 0.93 |
| WC2600-1DUP | RT | MS | 538 | 43.3 | 43 | 20 | 0.91 |
| WC2600-1 | PM | MS | 540 | 76.7 | 54 | 24 | 0.94 |
| WC2600-2 | PM | SC | 552 | 15 | 73 | 20 | 0.90 |
| WC2600-3 | PM | SC | 599 | 15 | 62 | 19 | 0.93 |
| WC2600-4 | PM | SC | 570 | 36.7 | 59 | 22 | 0.88 |
| WC2600-5 | PM | SC | 532 | 63.3 | 59 | 24 | 0.94 |

*Proportional multihabitat (PM) vs. riffle-targeted (RT) sampling communities*

The ORDEQ benthic macroinvertebrate sampling protocol for wadeable streams (ORDEQ, 2003) targets riffle habitat. Riffles are faster, colder, and better-oxygenated, so they are more likely to contain sensitive EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) that are considered hallmarks of a healthy stream. Riffle-targeted data are the basis of the PREDATOR and IBI state-wide bioassessment models (Hubler, 2008); since 2005, this methodology has been used in Whychus Creek to assess macroinvertebrate community changes following restoration. However, it is not necessarily the most appropriate technique at this point. Early projects involved restoring in-stream flow, which is expected to have a positive impact on riffle communities; indeed, the greatest overall change in community composition occurred after Whychus returned to perennial flow. However, more recent activities such as channel braiding, floodplain reconnection, adding large wood, and creating side channel habitat are being done to increase habitat complexity and diversity, which does not necessarily translate to more or "better" riffles. While assessment of the Whychus Creek macroinvertebrate community included biological as well as taxonomic traits in recent years, and different metrics in the IBI have been assessed individually, it was decided in 2018 to alter the sampling protocol to include all available habitat types in reaches of interest.

At the same time, RT sampling done since 2005 generated an extensive long-term data set rarely seen in monitoring projects. Therefore, in order to both more accurately determine impacts of salmonid-focused habitat creation projects on their macroinvertebrate food base and to continue long-term monitoring, side channel habitat at WC1100 and WC2600 was sampled using the PM technique to capture all available habitats, while the mainstem at both sites as well as at WC0600 and WC1150 were sampled using the customary RT protocol. It was anticipated that RT and PM samples from the same site will reflect different communities, but the extent of difference was not known. For this reason, each mainstem PM and RT sample was taken in the same reach at the same time, with team moving as a unit to avoid disturbing unsampled sections of the reach.

Richness among all samples ranged from 39 to 73 unique taxa (Table 4), and was significantly higher (unpaired t-test; $p = 0.0004$) in PM samples (means = $59 \pm 6.8$ taxa in PM; $44 \pm 3.6$ taxa in RT). Mean richness was also significantly greater in PM samples when only mainstem reaches are compared ($55.8 \pm 7.5$ taxa in PM, $44.2 \pm 3.6$ taxa in RT, $p = 0.0176$) However, the Simpson diversity index (1-D), which considers both richness and evenness, did not differ significantly between RT and PM samples in the mainstem or across the complete sample set (Table 4), indicating that even though PM samples were more diverse, the community was not overly dominated by a subset of taxa at any site. The relative abundance of the most numerically dominant taxon at each site ranged from 7.2-46.2%, and did not exceed 30% at any site except in one WC1100 side channel sample.

Interestingly, although RT samples may be expected to contain more sensitive EPT taxa due to the greater representation of riffle habitat, the mean number of EPT was higher in PM samples for mainstem reaches only and across the complete sample set, although the difference was not significant in either case ($p > 0.10$). Mainstem RT and PM samples had similar means for numbers of sensitive and sediment-intolerant taxa, community temperature optima, and numbers of DEQ low sediment and low temperature indicator taxa. The mean community sediment optima and number of DEQ high temperature and high sediment indicator taxa were greater in mainstem PM samples, but in no case was the difference significant.

CLUSTER analysis of the macroinvertebrate community at each site showed that, as in past years, community composition is strongly influenced by reach location (Figure 1), with the WC2600 mainstem and side channel samples clustering apart from all other site samples. The mainstem RT and PM sample at each site were most similar to each other; a partial exception is WC2600, where the duplicate RT sample taken for quality control was most similar to the mainstem PM sample. The side channel communities at each site differed more in their degree of similarity to mainstem samples and to each other,

SIMPER analysis showed an overall average dissimilarity of 50.6% between all mainstem RT and PM samples. Of the four taxa that contributed the most to this difference, three had higher mean abundance in PM samples (non-biting midge genera *Tanytarsus* and *Cricotopus Nostococladius,* and the riffle beetle *Optioservus*) and one had a higher mean abundance in RT samples (the water-snipe fly *Atherix*). All of these are found in a variety of flow conditions, though *Cricotopus Nostococladius* has a specialized association with the blue-green algae *Nostoc*, as larvae are miners in the algal disks (Anderson et al., 2013). However, their cumulative contribution to the overall dissimilarity was only 11.6%, suggesting there is not a large difference in taxonomic composition in samples taken in the same reach using the two methods, and a one-way ANOSIM indicated no significant difference between

mainstem RT and PM sample composition (p=0.8006). Mainstem PM samples contained anywhere from two to five net sets taken if n riffles (see Table 2), which helps explain the overlap in the macroinvertebrate community.

*Figure 1. CLUSTER analysis of macroinvertebrate community composition in 2018 samples.*
*Dark blue = riffle-targeted, aqua = proportional multihabitat*
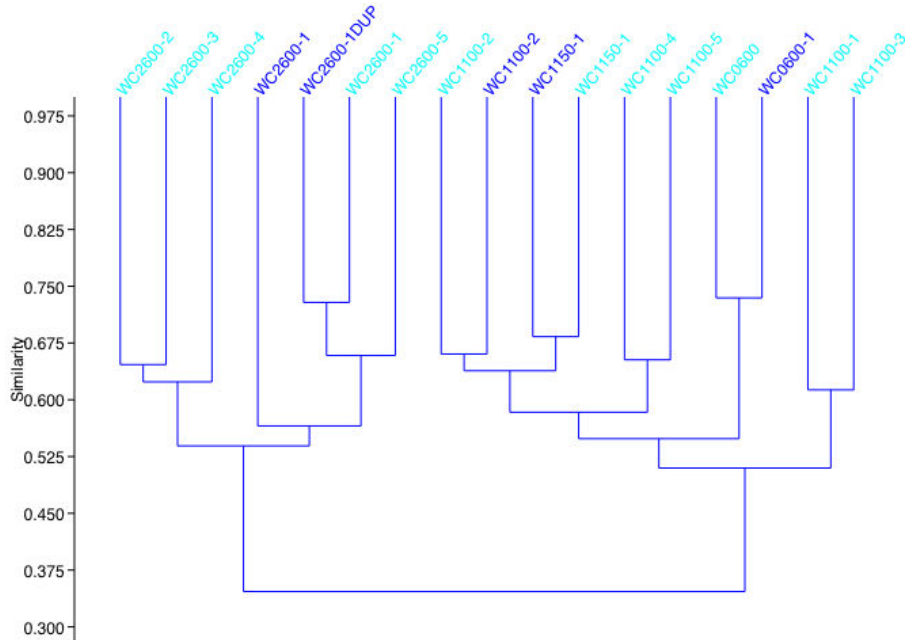


Trait differences between RT and PM samples were assessed (unpaired t-test), including: relative abundance of the dominant taxon (%Dom_top) and of tolerant (%Tol) and sediment-tolerant (%Sed_tol) taxa; relative abundances of different functional feeding groups (PR [predator], SC [scraper], SH [shredder], C [collector-filter & collector-gatherer]), habits (burrower, sprawler, clinger, climber, swimmer, swimmer), maximum body sizes (small [Sm], medium [Med], large [Lg]), voltinism types (univoltine [univol], multivoltine [multivol], semivoltine [semivol]), flow association (erosional [eros], depositional [dep]), and temperature preference (cool/cold, warm). No significant difference was found between mainstem RT and PM samples for any traits, although relative abundance of the dominant taxon was close to significant (RT = 14.5% $\pm$ 3.1, PM = 10.2% $\pm$ 2.3, p=0.0543), and the mean relative abundance of sediment-tolerant, warm water-, depositional-, and mixed flow-associated taxa were 1.5-2 times higher in PM samples. However, when comparing all RT and PM samples in the dataset, mean relative abundance of multivoltine, warm water-, and depositional-associated taxa were significantly greater in PM samples compared to RT. Differences between the sampling techniques thus appear to be driven by the greater variety of flow and substrate types represented in PM samples, as they include net sets taken in slow and still areas where waters are warmer and substrates and flow rates are more heterogenous.

PCA ordination of mainstem sample traits also showed separation based more on overall reach location than on sampling methodology (Figure 2). The upstream-most samples (WC2600) separated from the other mainstem samples along the PC1 axis, which explained 42.5% of the total variance; factors with the highest loading values were relative abundance of tolerant and semivoltine organisms. PC2 explained an additional 27.7% of the

variance, and factors with the highest loading values on this axis were relative abundance of burrowers (likely driven by dominance of tubificid worms in the WC2600 RT sample) and of predators.

**Figure 2. PCA ordination of traits in mainstem riffle-targeted and proportional multihabitat samples.**
*Blue dot = riffle-targeted, aqua = proportional multihabitat.*



*Individual site characteristics*

<u>WC0600</u>

<u>*Mainstem riffle-targeted vs. multihabitat sample*</u>

Community composition of the PM and RT sample taken in the same mainstem reach was very similar (Bray-Curtis similarity index = 0.73), even though only two of the 10 net sets in the PM sample were from riffles (see Table 2). Sample richness was the same (47 unique taxa) and Simpson's Diversity Index was similar in both (0.91 in RT, 0.93 in PM). The higher PM sample index reflects better evenness, as the dominant taxon (*Optioservus*) was present at only 10.8% of total abundance, while the relative abundance of the dominant taxon in the RT sample (*Atherix*) was almost twice as high (18.4%). These taxa were present in both samples, and prefer erosional and depositional habitats in running waters. The difference in dominant taxa is likely a function of the greater substrate heterogeneity in PM samples. *Optioservus* is more common in sediment and detritus, which was better represented in the PM sample; *Atherix* prefers bottoms of rocks, and the riffles sampled in this reach were reported on the data sheet as having mostly cobble/gravel substrate. Four of the 17 EPT taxa in the PM sample

were not found in the RT sample; all are clingers in cool to cold flowing waters (*Ephemerella tibialis*, *Sweltsa*, *Micrasema*, *Rhyacophila arnaudi*), and each occurred as just a single individual in the PM sample.

Total abundance was also similar in each sample, although a smaller proportion of the total PM sample was picked to attain the 500-individual target (total sample sorted = 11.7% of PM, 20.8% of RT). There was little to no difference between the samples for most measured traits, including numbers and relative abundances of tolerant and sensitive taxa, DEQ indicator taxa, sediment and temperature optima, and relative abundances of small, medium, and large taxa. The PM sample had two-fold higher abundance of swimmers, while the RT sample had twice as many sprawlers. Warm water-associated taxa were more abundant in the PM sample, but this sample also contained two DEQ indicator taxa for low temperatures, while the RT sample had none.

Most taxa common to both samples had similar abundances; those substantially more abundant (3-5X) in the RT sample were characteristic of flowing waters, including several non-biting midge genera (*Nanocladius*, *Parametriocnemus*, *Potthastia Gaedii Gr.*, *Sublettea*), the flatheaded mayfly *Diphetor hageni*, humpless case-maker caddisfly *Brachycentrus*, and purse case-maker caddisfly *Hydroptila* (although this genus is also known from lakes and ponds). Taxa that were 3-12 times more abundant in the PM sample included non-biting midges (*Cricotopus Nostococladius*, *Tanytarsus)*, *Simulium* black flies, the small minnow mayfly *Acentrella turbida*, the free-living caddisfly *Hydropsyche,* and the finger-net caddisfly *Wormaldia.* These taxa are mainly associated with faster-flowing, colder waters, with the exception of *Hydropsyche* (found in both warm and cool lotic waters) and *Tanytarsus* (found in all types of freshwater habitats).

Only four taxa were unique to the RT sample (*Chelifera/Metachela*, *Cryptolabis*, *Dicranota*, *Ferrissia*) and all occurred in very low abundance (1-2 individuals). All of these taxa were seen in this reach in at least one previous sampling year with the exception of *Ferrissia*, which was taken for the first time at WC0600 in 2018. Seven taxa were unique to the PM sample (*Cricotopus bicinctus*, *Limnophyes*, *Ephemerella tibialis*, *Sweltsa*, *Micrasema*, *Rhyacophila arnaudi*, Nemata), but these also occurred at very low abundances (1-6 individuals) and have been found in multiple previous sampling years at this site.

*Longterm sampling*

Riffle-targeted samples were taken at WC0600 in every project sampling year since 2005. The target number of organisms was attained in seven of the 10 years, including every year since 2015 (Figure 3), and the proportion of sample picked was lower in 2018 (20.8%) than any other sampling year (data not available for 2005). Sample richness fluctuates (Figure 4) but has increased steadily since 2014 (24 taxa) and was two-fold higher in 2018 (47 taxa). Annual variations in EPT taxa generally resemble those for total richness; numbers increased steadily from 2014 through 2017, but decreased in 2018 (Figure 4). Increases in EPT have been driven mainly by Ephemeroptera, with more lotic-erosional associated taxa such as *Rhithrogena*, *Acentrella* species, *Attenella*, and *Diphetor hageni* seen in later sampling years. IBI scores show a trend towards improved biological condition, and in 2018 indicated minimal impairment (Figure 5). PREDATOR score has also increased over time; all O/E scores in earlier sampling years (2005-2014) indicated poor to fair condition, but scores rose in 2015 and three of the last four years the community scored on the border between fair and good (O/E = 0.92).

*Figure 3. Proportion of sample sorted and sub-sampled organismal abundance for WC0600, 2005-2018.*
*Sample sorting data were not available for 2005.*
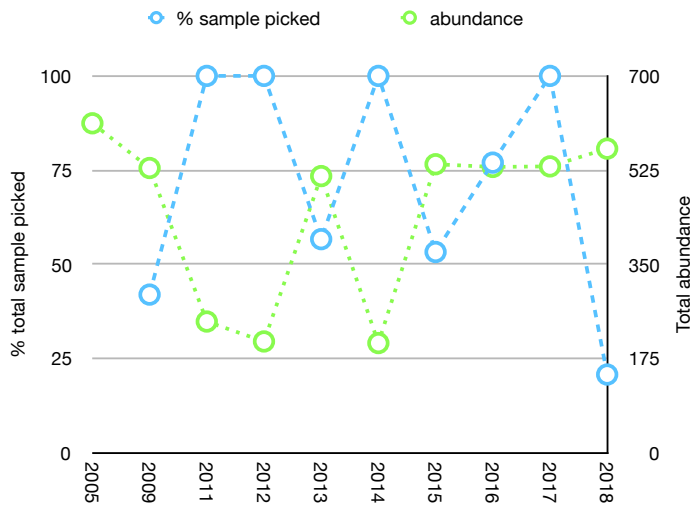


*Figure 4. Richness and number of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa in WC0600 samples, 2005-2018.*
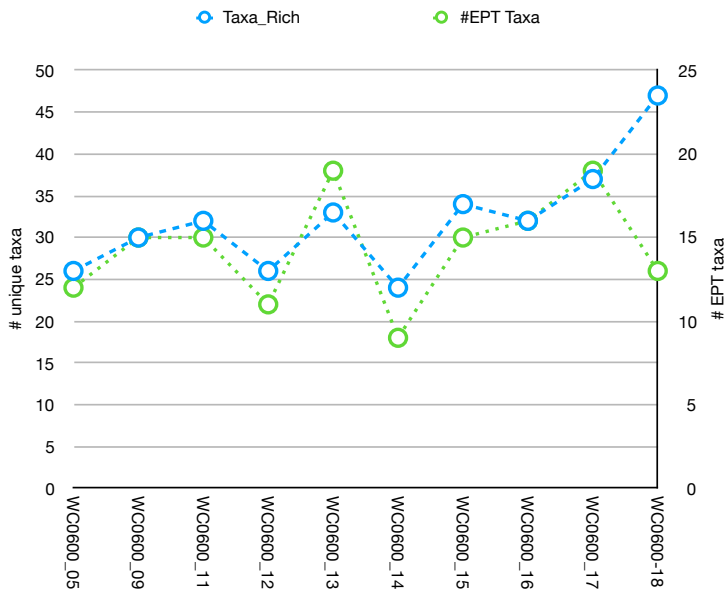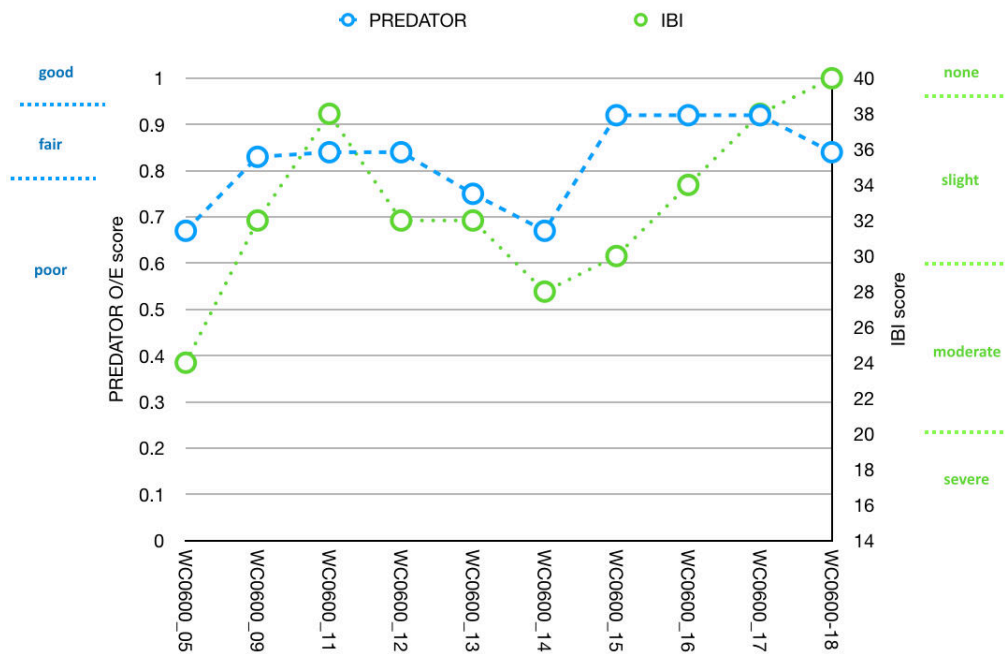
*Figure 5. PREDATOR O/E and IBI scores for WC0600 samples, 2005-2018.*



CLUSTER analysis of macroinvertebrate taxa abundances shows an overall separation between the communities in recent (2013-2018) and early (2005-2012) sampling years (Figure 6A). However, in a PCA ordination, the first two axes explain only a combined 44.6% of total variance (Figure 6B). Factors with the highest loading value on PC1 were Chironominae and *Zaitzevia*, which were the dominant taxa in 2009 and 2005, respectively. *Zaitzevia* also dominated the community in several later years (2012, 2016, and 2017), but at lower relative abundance in each subsequent year (i.e., from 51% of total abundance in 2005 to 17.3% in 2017). Factors with the highest loading on PC2 were the flatheaded mayfly *Rhithrogena* and the riffle beetle *Ampumixis*, both of which prefer cold, fast, flowing waters and have been seen in higher abundances in more recent sampling years (i.e., 2013-2018).

In a PCA ordination of traits measured as relative abundances (dominant taxon, tolerant organisms, and functional feeding groups), the PC1 axis explained 61.5% of variance, with relative abundances of scrapers and tolerant organisms having the highest loading values. However, abundances of all feeding guilds including scrapers have varied greatly annually with no consistent pattern. Relative abundance of tolerant taxa decreased from 2005 (58.5% of total abundance) through 2014 (12.3%) but increased again since then (28-48.2% from 2015-2018). Temperature optima of the community shows a similar pattern, with a sustained decrease from 2005 (18.3$^{\circ}$C) through 2013 (16.3$^{\circ}$C) reversing in recent years to a high of 19$^{\circ}$C in 2018. Community sediment optima were also higher in earlier sampling years and have decreased overall, but the difference is not significant (mean = 7.5 $\pm$ 0.5 %FSS for 2005-2013 and 6.9 $\pm$ 0.6 %FSS for 2014-2018; p = 0.1078)
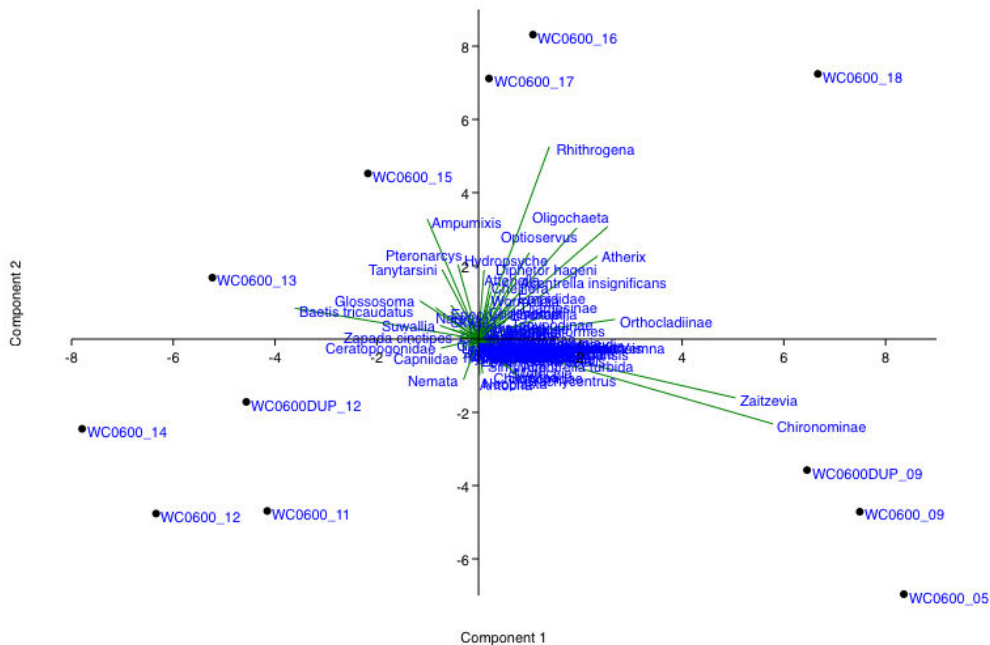
*Figure 6. Multivariate analysis of WC0600 macroinvertebrate community composition, 2005-2018.*

*A. CLUSTER analysis (Bray-Curtis similarity index of square root-transformed taxa abundance data).*
*Number after site name indicates year.*
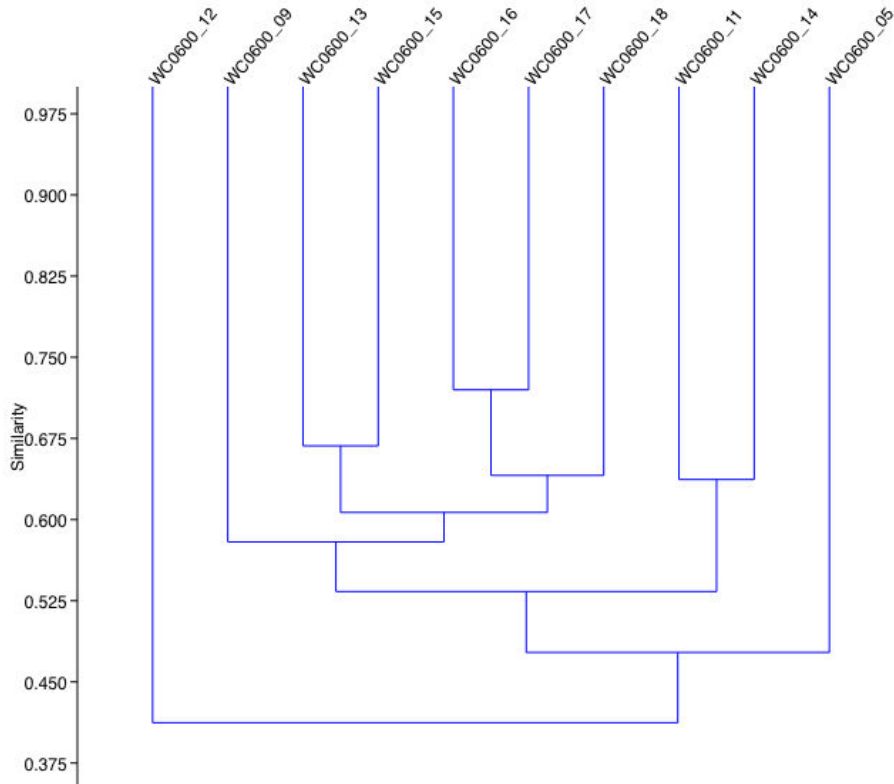


*B. PCA ordination (square root-transformed taxa abundance data).*

## WC1100

The Whychus Canyon restoration project at WC1100, which created new side channel habitat, was implemented in 2016. Pre-project data were collected in 2014-2015, but no sampling was done in 2016, as the work was just completed. In 2017, mainstem sampling resumed and side channel sampling was implemented. Multihabitat samples were taken in side channels in 2017 and 2018 but the methodology differed. In 2017, a reach-wide multihabitat protocol was used (adapted from USEPA, 2009 and Ode, 2016; see Searles Mazzacano, 2018 for details), with 10 net sets taken at evenly-spaced points along the reach from alternating spots within the channel. Because the reach-wide method also captures multiple flow and substrate types and because it was the first assessment of the macroinvertebrate community in this newly-created habitat, the data are included here for comparison.

*Mainstem riffle-targeted vs. multihabitat sample*

Community composition of samples taken in the same mainstem reach using the PM or RT protocol was similar (Bray-Curtis Similarity Index = 0.66), even though only three of the 10 net sets in the PM sample were from riffles. The PM sample had more taxa (57 vs. 48 in RT), but the number of EPT taxa and Simpson's Diversity Index (1-D) were almost identical (see Table 3). The high Simpson's diversity index indicates that neither community is dominated by a small number of taxa, and this is reflected by the low relative abundance of the most dominant taxon in each sample. The RT sample was dominated by the small minnow mayfly *Baetis tricaudatus*, a swimmer and clinger in faster-flowing waters, at 15.5% of total abundance. The PM sample was dominated by the non-biting midge *Tanytarsus* at 10% of total abundance; these larvae spin nets and build tubes on the substrate in all types of freshwater habitats.

Total abundances after subsampling were also similar, although a smaller proportion of the PM sample was required to reach the target number (30% vs. 57% of total RT sample sorted), The number of sensitive and sediment-intolerant taxa and community temperature optima were similar in both, as were the number of DEQ indicator taxa for high and low temperature and for low sediment. However, the PM sample had almost twice as many DEQ high sediment indicator taxa as the RT sample (9 vs. 5 taxa), and the community sediment optima was also higher (7.9 %FSS vs. 6.3 %FSS).

Most taxa common to both samples had similar abundances; those substantially more abundant (3-14X) in the RT sample were characteristic of faster flows (i.e., *Simulium*, *Baetis tricaudatus*, *Rhithrogena*, *Pteronarcys californica*, *Hydropsyche*), while taxa that were more abundant (9-50X) in the PM sample had broader flow tolerances (i.e., *Tanytarsus*, Capniidae). Only three taxa in the RT sample were absent from the PM (*Antocha*, *Diphetor hageni*, and *Epeorus albertae*), and all occurred at very low abundance (1-3 individuals). More taxa were unique to the PM sample, at abundances ranging from 1-9; these included groups found in a greater variety of flow types and in slower waters, such as lumbriculid worms, several dipteran genera (non-biting midge, dance fly [*Hemerodromia*], crane fly [*Hesperoconopa, Tipula*]), the green rockworm caddisfly *Rhyacophila arnaudi*, nematodes, and turbellarian flatworms. All taxa unique to the PM sample had either not been seen at WC1100 before or else in only a single year at low abundance, and all had low occurrence and abundance in the complete dataset (i.e., all Whychus sampling sites 2005-2018).

The two sample types had similar relative abundances of the dominant taxon and of tolerant taxa, as well as for maximum body size and voltinism types. However, trait-based differences were seen in feeding guild, temperature preference, flow association, and habit. The RT sample had more shredder, erosional-associated, and clinger and swimmer taxa, while the PM sample had more scrapers, warm water-, mixed flow-, and depositional-associated taxa, as well as more burrowers and climbers. These differences reflect the presence of additional habitat and flow types in the multihabitat sample, as the runs, glides, and pools that were sampled have slower flows where sediments can settle out, more algae and macrophyte growth can occur, and organisms do not need to cling to substrates to avoid being swept away by the current.

*Side channel vs. mainstem communities*

The mainstem RT and PM samples were most similar to each other, and the WC1100-4 and WC1100-5 side channel communities were more similar to the mainstem than to the other two side channels (Figure 7A). Side channel samples had greater richness, but SIMPER analysis found only a 37.2%% overall average dissimilarity between the mainstem vs. side channel macroinvertebrate communities. Taxa that contributed most to the difference were *Simulium* and Chironomini, which both had a greater mean abundance in side channel samples, and *Baetis tricaudatus* and *Rhithrogena*, which both had greater mean abundance in mainstem samples. The number of EPT taxa was not significantly different in mainstem and side channel samples, and the EPT community had an overall average dissimilarity of only 38.5%, with greater mean abundance of *Leptophlebiidae*, *Baetis tricaudatus*, *Rhithrogena*, and *Acentrella insignificans* in the mainstem accounting for 35.3% of the observed difference. In a PCA ordination of mainstem and side channel sample taxa (Figure 7B), PC1 and PC2 explained 75% of the variance, and the same factors has the highest loading value on each axis: *Simulium* and Chironomini. *Simulium* is often seen in high abundance in newly-created habitats as a pioneer species (Wotton et al., 1998; Hammock & Bogan, 2014), while Chironomini is a sediment-inhabiting, highly tolerant tribe of non-biting midges, able to withstand warmer temperatures, low dissolved oxygen levels, and wide pH ranges (Pinder, 1986).

There was no significant difference in the mean values of most traits between the mainstem and side channel samples. However, side channel samples had significantly greater relative abundance of multivoltine taxa (means = 61.7% $\pm$ 0.6 mainstem, 75.5% $\pm$ 4.6 side channel; p = 0.0161) and lower relative abundance of univoltine taxa (means = 19.9% $\pm$ 3.0 mainstem, 9.4% $\pm$ 3.4 side channel; p = 0.0213), and the relative abundance of swimmers was significantly greater in the mainstem (means = 21.1% $\pm$ 8.8 mainstem, 6.8% $\pm$ 1.5 side channel; p = 0.0226). Mean relative abundance of the top taxon in side channels (27.5% $\pm$ 14.6) was over two times greater than mainstem samples (12.8% $\pm$ 3.9), but the difference was not significant. In a PCA ordination of all trait values measured as relative abundances (Figure 8), the first axis explained 65.9% of the total variance; factors with the highest loading values were relative abundances of erosional-associated and mixed flow-associated taxa. The mainstem samples differentiated more on PC2, which explained an additional 19% of the variance, with relative abundance of swimmers and of the top taxon having the highest loading values.

*Figure 7. Multivariate analysis of WC1100 macroinvertebrate community in mainstem and side channels, 2018.*
*A. CLUSTER analysis (Bray-Curtis similarity index of square root-transformed abundance data).*

*Blue = riffle-targeted sample, aqua = proportional multihabitat. WC1100-2 samples were taken in the mainstem.*
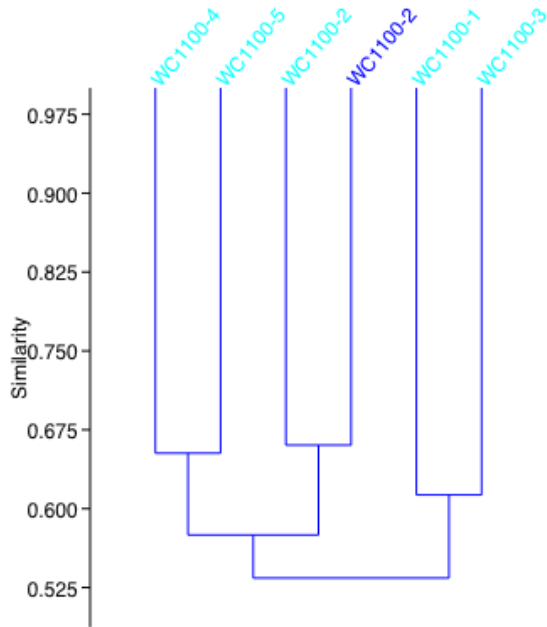


*B. PCA ordination of mainstem and side channel macroinvertebrate taxa.*

*Blue = riffle-targeted sample; aqua = proportional multihabitat. WC1100-2 samples were taken in the mainstem.*
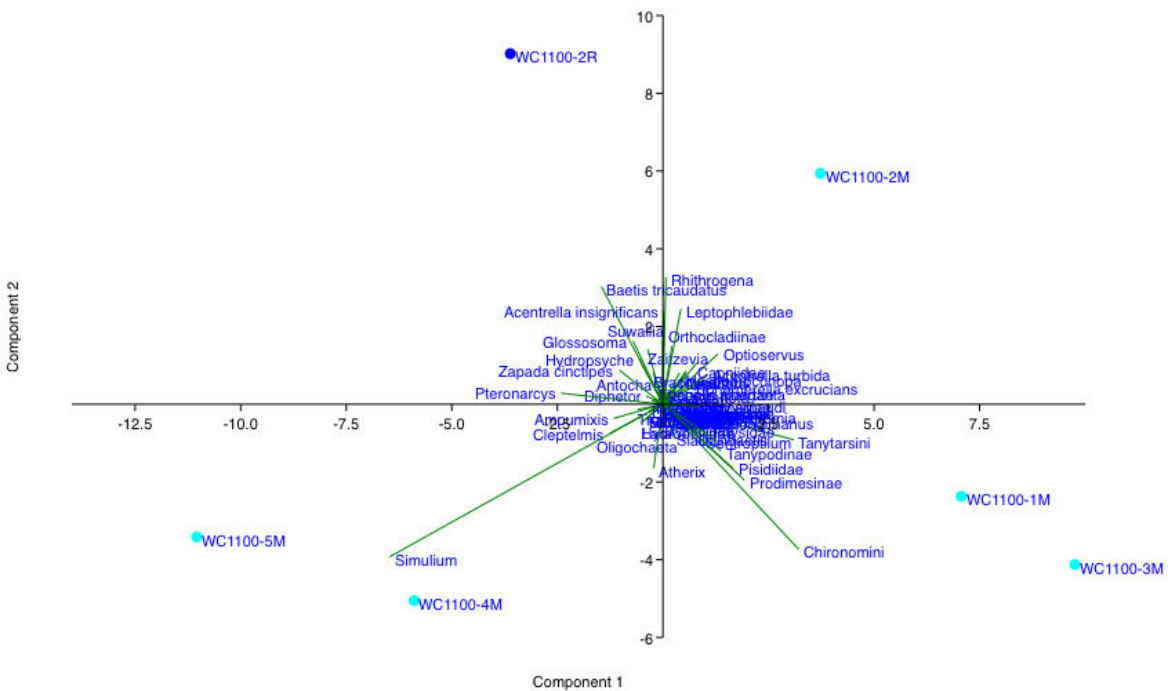
**Figure 8. PCA ordination of WC1100 macroinvertebrate community traits in mainstem and side channel communities, 2018.** *Blue = riffle-targeted sample, aqua = proportional multihabitat. WC1100-2 samples were taken in the mainstem.*
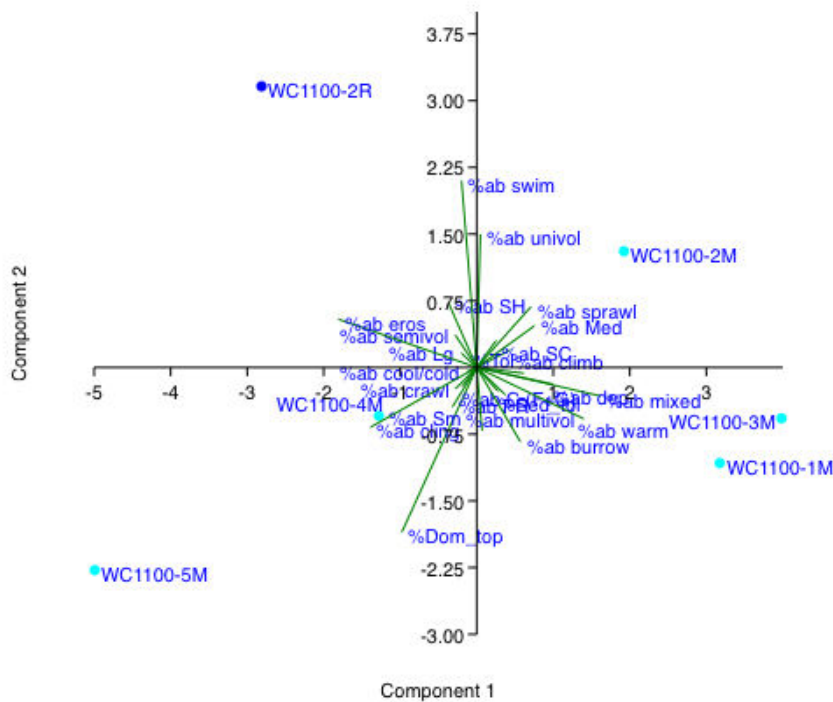


*Changes in side channel communities*

The WC1100 side channels are dynamic habitat and this is reflected by the macroinvertebrate community, which shows a clear separation between years (Figure 9). The communities in 2017 and 2018 were significantly different (ANOSIM; p = 0.0273), with an overall average dissimilarity of 48.5%. Taxa that contributed most to the difference were *Simulium* (higher mean relative abundance in 2018); *Zapada cinctipes*, a winter stonefly found in cold flowing waters; and *Attenella margarita,* a spiny crawler mayfly found in lotic habitats (both of the latter with higher mean relative abundance in 2017). The following trait means differed significantly between years:

- richness: 2017 = 39.0 ± 2.7 taxa; 2018 = 58.3 ± 5.6 taxa; p = 0.0029
- number of DEQ high temperature indicator taxa: 2017 = 2.7 ± 1.2 taxa; 2018 = 7.8 ± 2.1 taxa; p = 0.0128
- relative abundance of predators: 2017 = 27.7% ± 10.3; 2018 = 8.3% ± 2.5; p = 0.0132
- relative abundance of climbers: 2017 = 0; 2018 = 9.2% ± 4.0; p = 0.0132

There were also more burrowers in 2017 (30.9 ± 22) than in 2018 (4.9 ± 3.1), but the difference was not quite significant (p=0.06). The shift from more burrowers to more climbers in 2018 may be due to the establishment of additional non-mineral substrate, such as macrophytes and wood tangles.

***Figure 9. CLUSTER analysis of macroinvertebrate communities in WC1100 side channels, 2017 and 2018.***
*Aqua = 2018 samples, purple = 2017 samples.*
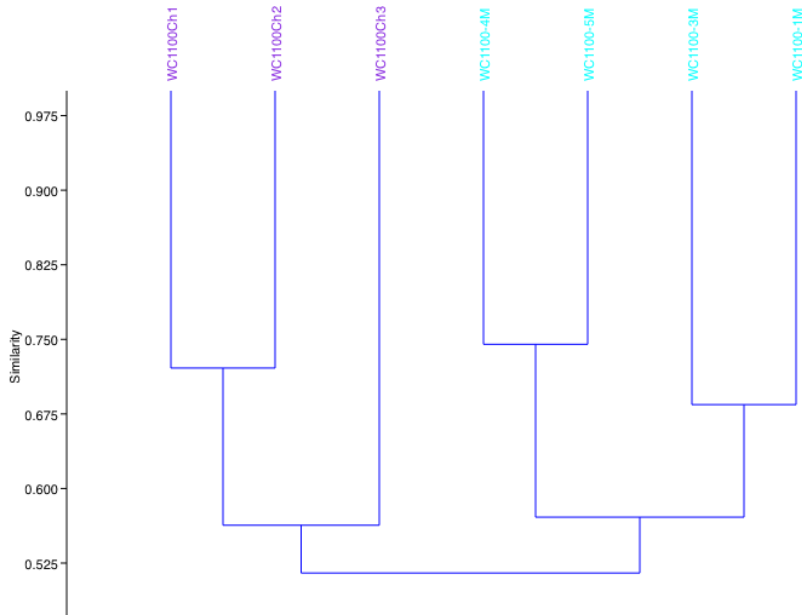


<u>*Longterm sampling*</u>

Riffle-targeted samples were taken in the WC1100 mainstem since 2014 with the exception of 2016, the year in which the floodplain reconnection project was done. The pre-project sampling community in both years was similar (Bray-Curtis similarity index = 0.65), and the target sub-sampling number was attained with about 80% of the total sample picked (Figure 10). In 2017 the community had not recovered from project activities and was severely impoverished; the entire sample contained only 45 individuals in 14 taxa and was dominated by oligochaete worms. The number of EPT taxa was three-fold lower in 2017 (5 taxa) compared to prior years, and the relative abundance of sediment-tolerant taxa was an order of magnitude greater than in any other year (Figure 11). IBI and PREDATOR scores were much lower, and both indicated severe disturbance (Figure 12).

In contrast, the community in 2018 had more total taxa and EPT than any other year, and the highest IBI and PREDATOR scores, which indicated undisturbed/good biological quality. The first sensitive and sediment-intolerant taxa at this site were also seen in 2018. The relative abundance of the dominant taxon (*Baetis tricaudatus*; 15.5% of total abundance) was the lowest in any sampling year; *B. tricaudatus* was also the top taxon in 2014, but it dominated the community to a much greater extent (44.7% of total abundance). The relative abundance of of the dominant taxon was similar in 2015 and 2017 (21.3% and 28.9% of total abundance, respectively); however, in 2017 the community was dominated by *Brachycentrus*, a sensitive caddisfly found on woody debris or aquatic plants in fast-flowing waters, while in 2017, it was dominated by Oligochaeta, a tolerant burrower in sediments of slower, warmer waters.

*Figure 10. Proportion of sample sorted and sub-sampled organismal abundance for WC1100, 2014-2018.*

*No sampling was done in 2016 due to recent restoration activity.*
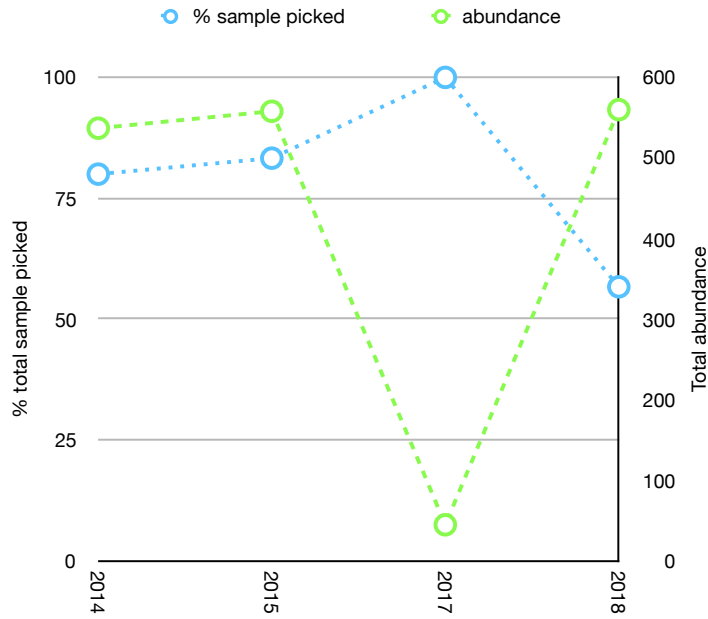


*Figure 11. Richness and number of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa in WC1100 samples, 2014-2018.*
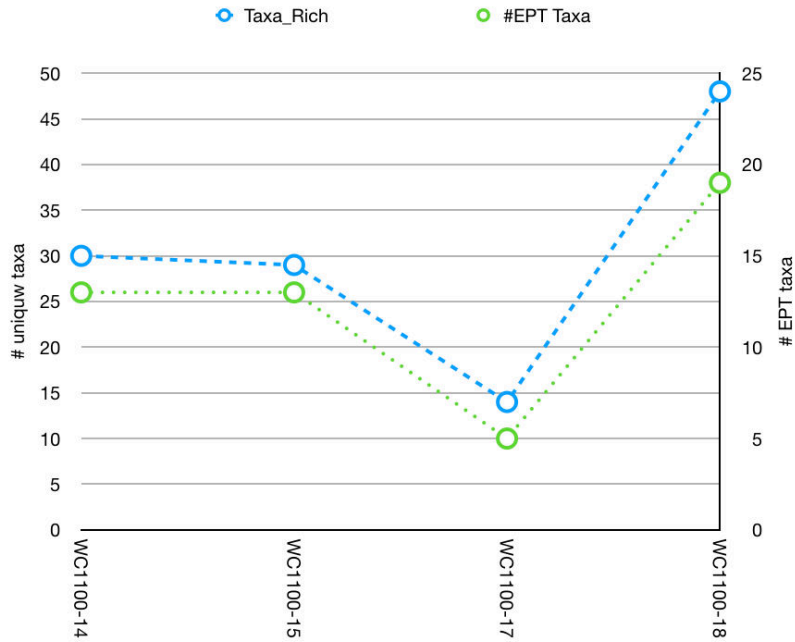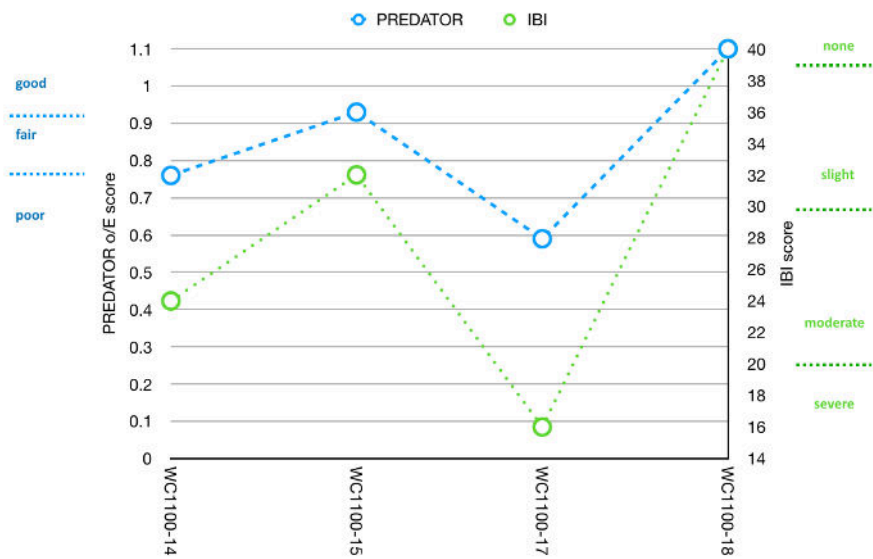
*Figure 12. PREDATOR O/E and IBI scores for WC1100 samples, 2014-2018.*



The macroinvertebrate community in 2018 overlapped substantially with 2014 and 2015 (Bray-Curtis similarity index = 0.58 and 0.61, respectively); in contrast, Bray-Curtis indices between 2017 and any other year were no greater than 0.37. PCA ordination (Figure 13) separates 2017 from all other years along the first axis, which explains 48% of the total variance; this is due to the richness and dominance of tolerant taxa in 2017. The 2018 community separates from pre-project sampling years along the PC2 axis, which explains an additional 32% of total variance; factors with the highest loading are Chironominae, *Rhithrogena*, and *Baetis tricaudatus*. Biological traits of the 2018 community did not differ greatly from 2014 or 2015 (17.8% and 15% overall average dissimilarity, respectively). Relative abundance of shredders accounted for 37-42% of the overall dissimilarity between 2018 and pre-project year samples; this greater abundance (8-49 times more compared to 2014-2015) post-project suggests increased allochthonous and/or woody inputs. Community temperature optima were the same in every year (17.0-17.1°C) and sediment optima were similar (6.3-6.7% FSS), with the exception of 2017 (8.3% FSS).

*Figure 13. PCA ordination of WC1100 macroinvertebrate community, 2014-2018.*
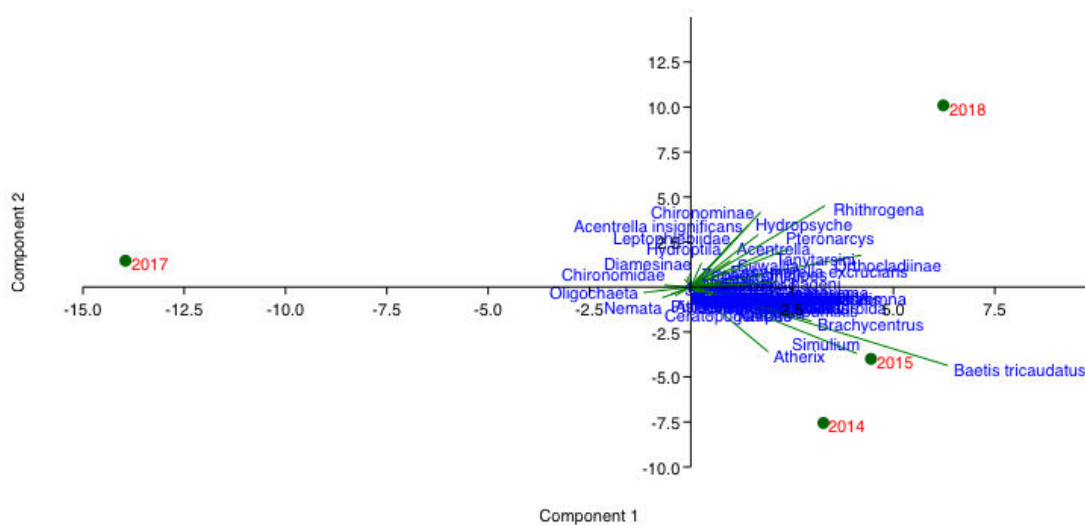
Sampling at WC1150 began in 2014 to provide an upstream reference for the WC1100 project site, and has been done in every year from 2014-2018.

*Mainstem riffle-targeted vs. multihabitat sample*

Community composition was similar between mainstem PM and RT samples (Bray-Curtis index = 0.68). Most of the PM net sets were taken in running water habitat (i.e., riffles, glides, runs; 9 of 10 total), even though only three were from riffles, which helps account for the high community similarity. Total sample abundances were similar, but less of the PM was picked to attain the target subsampling number (see Table 3). The PM sample had 21 more taxa (65 vs. 44 taxa in RT sample), including six more EPT (23 vs. 17 EPT taxa). However, Simpson's Diversity Index (1-D) was almost the same for both (0.96 and 0.95, respectively) indicating a high degree of evenness, and the dominant taxon in each was present at very low abundance. The RT sample was dominated by *Baetis tricaudatus (*10.5% of total abundance), a small minnow mayfly that prefers cool, clear, fast-flowing waters and is a DEQ indicator for both low temperature and low sediment. The PM sample was dominated by the riffle beetle *Ampumixis dispar* (7.2% of total abundance); this species is found in cold, clear, fast-flowing water where it is a clinger and burrower in sand and gravel.

Community temperature and sediment optima were similar in both samples, as were the number of DEQ low temperature and low sediment indicator taxa. Little difference was also seen in the relative abundances of different functional feeding groups and maximum length categories, voltinism types, habits, and flow preferences between the two samples. However, the relative abundance of warm water-associated taxa, though low in both samples, was nearly three times greater in the PM sample (11.5% vs. 4.2%), and there were 3.5 times more DEQ high temperature indictor taxa and twice as many high sediment indicator taxa in the PM sample.

The three taxa that were substantially more abundant (4-19X) in the RT sample are all associated with lotic habitats; two inhabit both slow and fast stream regions (*Atherix*, a tolerant water snipe fly known from rock bottoms and interstices; and *Lopescladius*, a non-biting midge), and one (saddlecase-maker caddisfly *Glossosoma*) is more restricted to riffles in cold fast streams. Of the four taxa that were more abundant (4-22X) in the PM sample, three prefer cold fast water (*Cleptelmis addenda*, a riffle beetle that clings to plants material on rocks and gravel in cold streams; and the non-biting midges *Cricotopus Nostococladius* and *Pagastia*), while one, the non-biting midge *Tanytarsus*, prefers warmer waters and is known from lentic and lotic habitats.

Only four taxa in the RT sample were not collected in the PM sample, and all occurred at very low abundance (1-2 individuals). Three were collected from this site in past years, including two genera of non-biting midge, and the cold water-associated mayfly *Ephemerella tibialis.* However, this was the first year that one, the stonefly *Sweltsa*, was collected at WC1150, although it has been found in multiple years at low abundance at WC0600 and WC2600. Taxa unique to the PM sample occurred at abundances ranging from 1-24 individuals, and included several genera of non-biting midge, crane fly, caddisfly, mayfly, and stonefly, as well as the first *Micrasema* and *Lepidostoma* caddisflies taken at this site and the first shore fly (Ephydridae) and clubtail dragonfly (Gomphidae) found at any of the longterm riffle sites discussed here.

Riffle-targeted samples were taken at WC1150 from 2014-2018. Macroinvertebrate community composition was most similar among the 2015, 2016, and 2018 communities (Figure 14; Bray-Curtis similarity index 0.62-0.67 between years), with the 2017 community more of an outlier. In a PCA ordination (Figure 15), the first two axes explained 59.7% of the total variance. Factors with highest loading on PC1, which separated 2014 and 2017 from other sample years, were Oligochaeta, which dominated the community in 2017 at 42.7% relative abundance; and *Baetis tricaudatus*, which dominated the community in 2014, 2015, and 2018 (at 33.7%, 18.5%, and 10.5% of total abundance, respectively). Factors with the highest loading value on PC2 were *Glossosoma*, which dominated the community in 2016, and *Simulium*, which was more abundant in 2014 by an order of magnitude compared to any other year.

**Figure 14. CLUSTER analysis of WC1150 macroinvertebrate community, 2014-2018.**
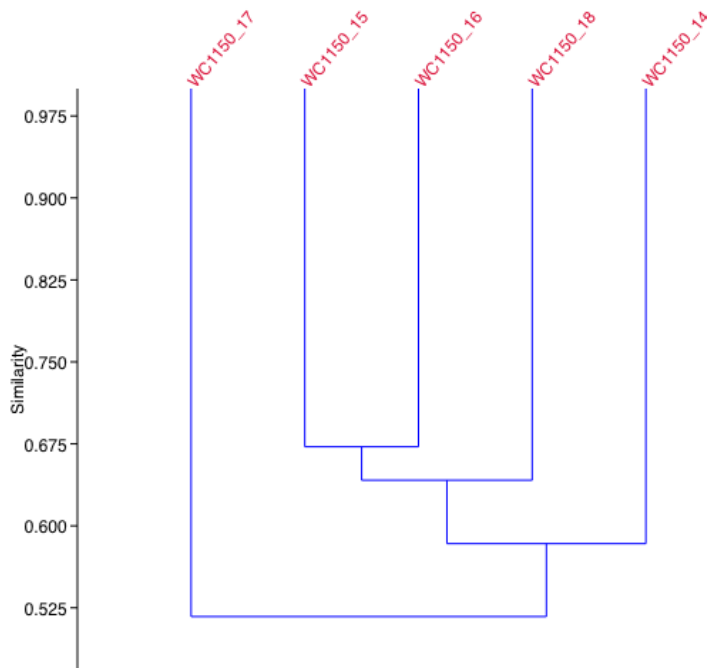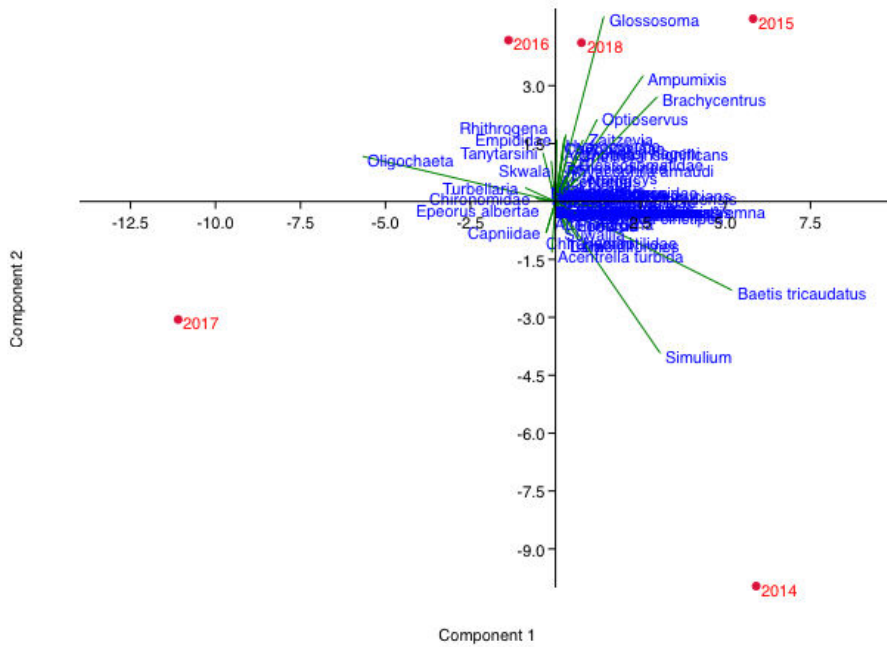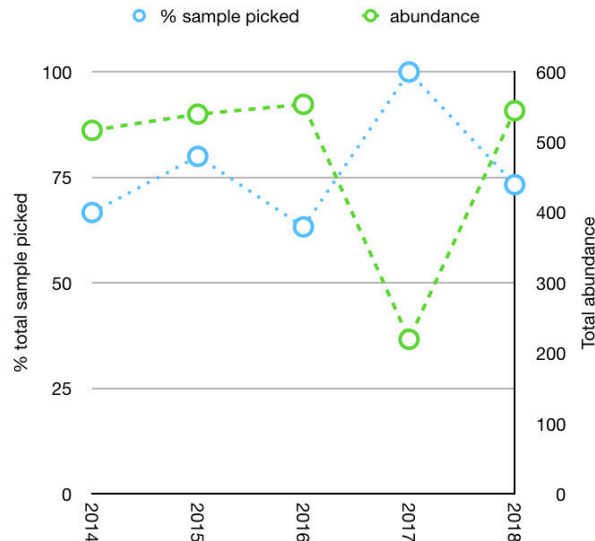*Number at the end of the sample name indicates year.*

*Figure 15. PCA ordination of WC1150 macroinvertebrate community, 2014-2018.*



The target number of organisms was attained in every year with 63-80% of the total sample picked, except 2017, when the sample yielded only 220 organisms (Figure 16). The 2017 community also had the lowest richness and fewest EPT (Figure 17), and was dominated by tolerant aquatic earthworms (Oligochaeta). However, richness in 2018 was higher than any other year (44 taxa), EPT diversity recovered, and community evenness improved, as the dominant *Baetis tricaudatus* mayfly occurred at just 10.5% of total abundance. PREDATOR scores indicated poor condition in 2014 but have since increased and leveled off, and indicate fair or good condition (Figure 18). IBI scores indicated slight disturbance until 2017, which had an anomalously low score indicating moderate disturbance, but the IBI score in 2018 was the highest in any year (40) and indicated minimal disturbance.

*Figure 16. Proportion of sample sorted and sub-sampled organismal abundance for WC1150, 2014-2018.*

*Figure 17. Richness and #EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa in WC1150 samples, 2014-2018.*
*Number at the end of the sample name indicates year*
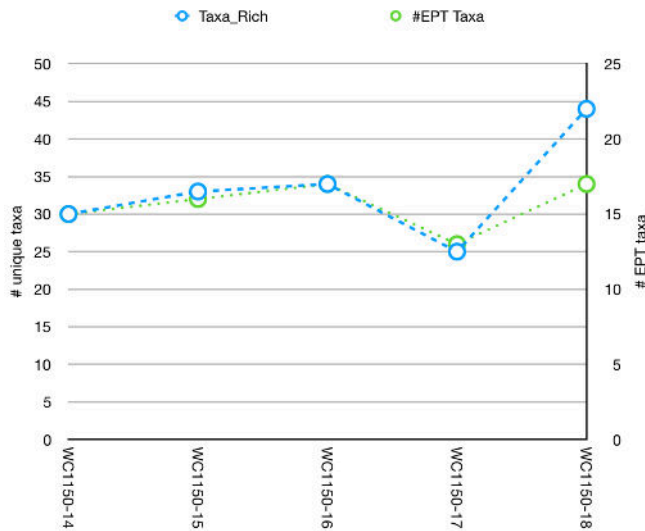


*Figure 18. PREDATOR O/E and IBI scores for WC1150 samples, 2014-2018.*
*Number at the end of the sample name indicates year.*
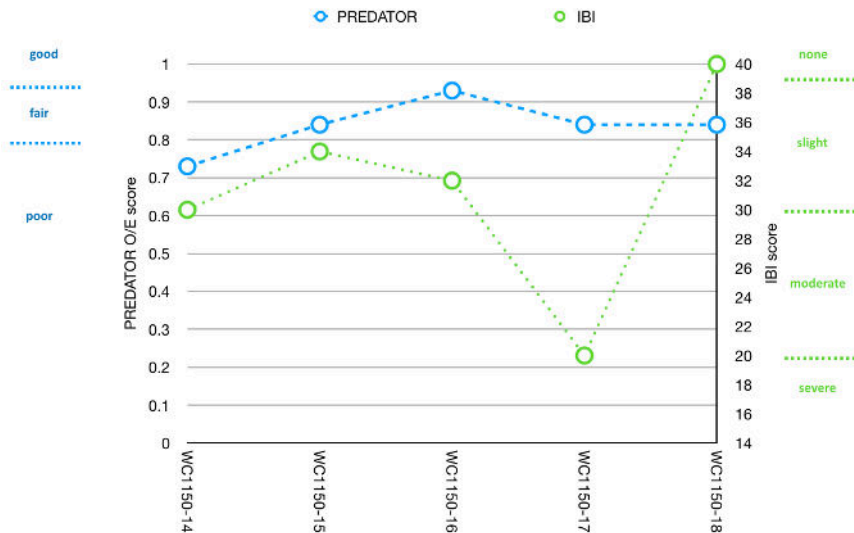
Since 2014, the number of DEQ low temperature indicator taxa has tripled (from one to three) and the number of high temperature indicator taxa was two-fold lower in 2018 (two taxa) compared to prior years (4-5 taxa in 2014-2016). The number of sediment-intolerant and sensitive taxa was 3-4 times higher in 2018 compared to any prior year, and no sediment-tolerant taxa were present (but note that the relative abundance of sediment-tolerant taxa was also very low [1%] in both 2014 and 2015). The community sediment optima in 2018 was the lowest of any sampling year (6% FSS); in contrast, the community temperature optima was higher in 2018 (17.9°C) than in any other sampling year (16.7-17.2°C in 2014-2017).

In a PCA ordination of traits of measured as relative abundances (dominant taxon, tolerant organisms, and functional feeding groups), the first axis explained 62.5% of the total variance, with relative abundance of sediment-tolerant organisms and tolerant organisms having the highest loading values. Values for both of these factors were relatively low in every sampling year except 2017, when relative abundance of tolerant organisms was 2-3 times greater than in any other year, and relative abundance of sediment-tolerant organisms was an order of magnitude greater.

<u>WC2600</u>

Riffle-targeted samples were taken at WC2600 in every project year since 2005. The creek was also directed into a new meandering channel at this site in 2014.

*Mainstem riffle-targeted vs. multihabitat sample*

Community composition of samples from the same mainstem reach was most similar between the PM and duplicate RT sample taken for quality control (Bray-Curtis similarity index = 0.73); the original RT sample had the same lower level of similarity to both (Bray-Curtis index = 0.59). The PM sample contained five net sets taken in riffle habitats, with the remainder in slow water (glides and pools; see Table 2). The target sub-sampling number of individuals was attained in the PM and duplicate RT sample with roughly equal abundance in each (Table 4), though less of the duplicate RT sample required sorting (43.3% vs. 76.7% of the PM sample). In contrast, the entire original RT sample contained only 218 individuals.

The duplicate RT sample had more total taxa (43) and EPT (20 taxa) than the original RT sample (39 taxa, 17 EPT), but the PM sample had greater richness than either (54 taxa, 24 EPT). Interestingly, although the number of EPT taxa in all three samples was similar to that in the other mainstem site samples in 2018, WC2600 samples had almost twice as many stonefly taxa (7-10) as the other mainstem sampling sites (3-5 taxa). The dominant taxon in each sample was present at low relative abundance (12.4-15.8% of total) and was highest in the duplicate RT sample, which is reflected by its lower Simpson's Diversity Index (Table 4). The duplicate RT sample was dominated by *Rhithrogena* (15.8% of total abundance), a flat-headed mayfly that prefers fast waters with low sediment; this taxon also dominated the WC2600 community in 2015. The PM sample was dominated by *Cricotopus Nostococladius*, a non-biting midge that mines in discs of *Nostoc* blue-green algae in clear cold waters. In contrast, the original RT sample was dominated by tolerant and sediment-tolerant tubificid sludge worms (12.4% of total abundance); these worms were seen in the other mainstem samples but were 3-9 times less abundant.

Despite the differences described above, all three mainstem samples had the same community temperature optima and similar values for relative abundances of different feeding guilds, voltinism types, flow preferences, habit types, and maximum body sizes, as well as number of DEQ temperature and low sediment indicator taxa. The original RT sample had more DEQ high sediment indicator taxa, however, as well as greater relative abundance of tolerant and sediment-tolerant organisms and a higher community sediment optima, due to the characteristics of the dominant tubificid worms in this sample.

Apart from the dominant taxon in each RT sample, no taxa occurred at substantially higher abundance compared to the PM sample. Taxa that were 3-8 fold more abundant in the PM sample included *Orthocladius* and *Tanytarsus*, non-biting midges found in a variety of warmer lotic and lentic waters; *Ameletus*, a comb-mouth mayfly known from cool lotic and lentic habitats; and *Isoperla*, a stripetail stonefly found in cold lotic and lentic waters.

Thirteen taxa were unique to one or both of the RT samples; all occurred at very low abundances (1-2 individuals), including two cold water-associated caddisflies (*Hydroptila*, *Glossosoma*), a stripetail stonefly (*Rickera sorpta*), three mayflies known from cold lotic waters (*Epeorus grandis*, *Epeorus deceptivus*, *Drunella spinifera*), three non-biting midges (*Paratanytarsus*, *Eukiefferiella Devonica Gr*.), three riffle beetles (*Lara*, *Narpus*, *Optioservius*), and Ostracoda (seed clam crustaceans*. All of these, with the exception of ostracods (a DEQ high sediment indicator known from a variety of lotic and lentic waters) and the chironomid midges, are associated with colder lotic habitats. The 10 taxa unique to the PM sample all prefer cold lotic habitats and also occurred at low abundance (1-4 individuals): the Northern caddisfly subfamily Dicosmoecinae; the winter stonefly *Malenka*; two flatheaded mayflies (*Epeorus longimanus*, *Cinygma*); the small minnow mayfly *Centroptilum*; the crane fly *Rhabdomastix*; and four genera of non-biting midge (*Sublettea, Rheocricotopus, Parametriocnemus, Krenosmittia*). Many taxa unique to either sample type are also unique to site WC2600 itself, as they were only found here in any prior sampling year, including *Rickera*, all three *Epeorus* species, *Drunella spinifera*, and *Rhabdomastix.* Three unique to the PM sample (*Malenka, Cinygma, Centroptilum*) are new additions to the project list, as they were not seen in any other sampling year or site.

*Side channel vs. mainstem communities*

The WC2600-5 side channel sample was most similar to mainstem samples; the other three side channels clustered separately (Figure 19). Seven of the 10 net sets in WC2600-5 were taken in riffles, while the other side channel samples had net sets in only four riffles, which may contribute to the observed difference. SIMPER analysis found an overall average dissimilarity of 44.5% between all mainstem and side channel communities. The taxa that accounted most for the difference contributed only a cumulative 13.1% to the dissimilarity, with higher mean abundance of the stonefly *Suwallia* and mayfly *Rhithrogena* in the mainstem sample, and a higher mean abundance of the non-biting midges *Micropsectra and Thienemannimyia* in side channel samples. The number of EPT taxa in mainstem and side channel samples was not significantly different, and the EPT community had an overall average dissimilarity of only 39.9%; taxa that accounted for 28% of the total EPT dissimilarity included *Rhithrogena*, *Suwallia*, and *Baetis tricaudatus* (all with greater mean abundances in the mainstem) and *Cinygma* (flatheaded mayfly associated with wood in cold fast water; greater mean abundance in side channels).

PCA ordination of taxa abundance showed the mainstem and closely related WC2600-5 samples and the remaining side channels separating mostly along PC1, though this axis explained only 38.4% of the total variance (Figure 20); factors with the highest loading were *Micropsectra,* which was the dominant taxon in two of the four side channels*, and *Rhithrogena,* which dominated the duplicate RT sample. PC2 explained an additional 21% of total variance; factors with the highest loading values were *Cricotopus Nostococladius,* which dominated the mainstem PM sample, and Ostracoda. Several trait means differed significantly between mainstem and side

channel samples, including:

- richness: 45.3 ± 7.8 taxa in mainstem; 63.3 ± 6.7 taxa in side channels; p = 0.0216
- # of sediment-tolerant taxa: 3.0 ± 0 in mainstem; 1.8 ± 0.5 taxa in side channels; p = 0.0083
- relative abundance of multivoltine taxa: means = 52.6% ± 1.8 in mainstem; 65.3% ± 7.9 in side channels; p = 0.0451

*Figure 19. CLUSTER analysis of the macroinvertebrate community in WC2600 mainstem and side channel habitat, 2018. Blue = riffle-targeted sample; aqua = proportional multihabitat.*
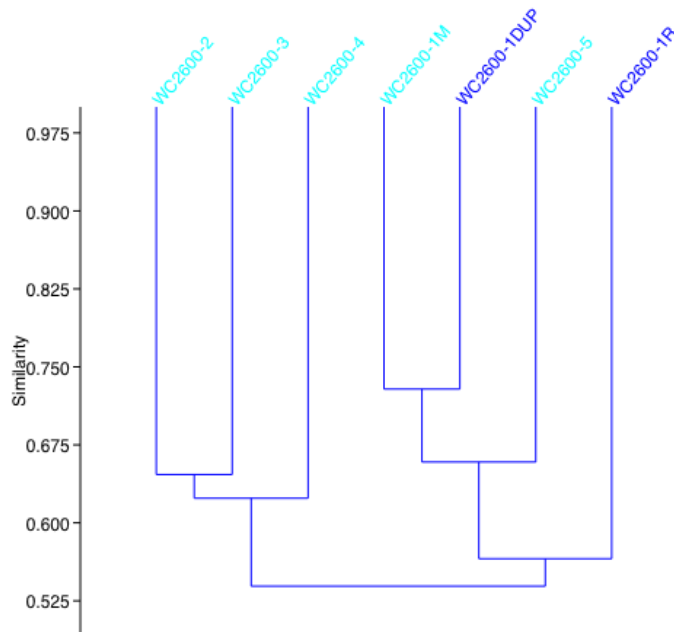


Mainstem samples also had greater mean relative abundance of univoltine taxa and fewer DEQ high sediment indicator taxa, but differences were not quite significant (p >0.08). It is notable that while there was no difference in the mean number of DEQ low temperature indicator taxa in mainstem vs. side channel samples, these values were four times higher at this site compared to the other sites sampled in 2018 (mean at WC2600 = 8.4 ± 0.5 taxa; mean at all other sites combined = 2.1 ± 1.6; p = 0.0001).

PCA ordination of all traits measured as relative abundances showed that axis one, which differentiated between the mainstem + WC2600-5 side channel samples and the remaining side channels, explained 58% of the total variance (Figure 21). Factors with the highest loading value on PC1 were relative abundance of depositional-associated taxa and of warm water-associated taxa, were both greater in three of the four side channels.
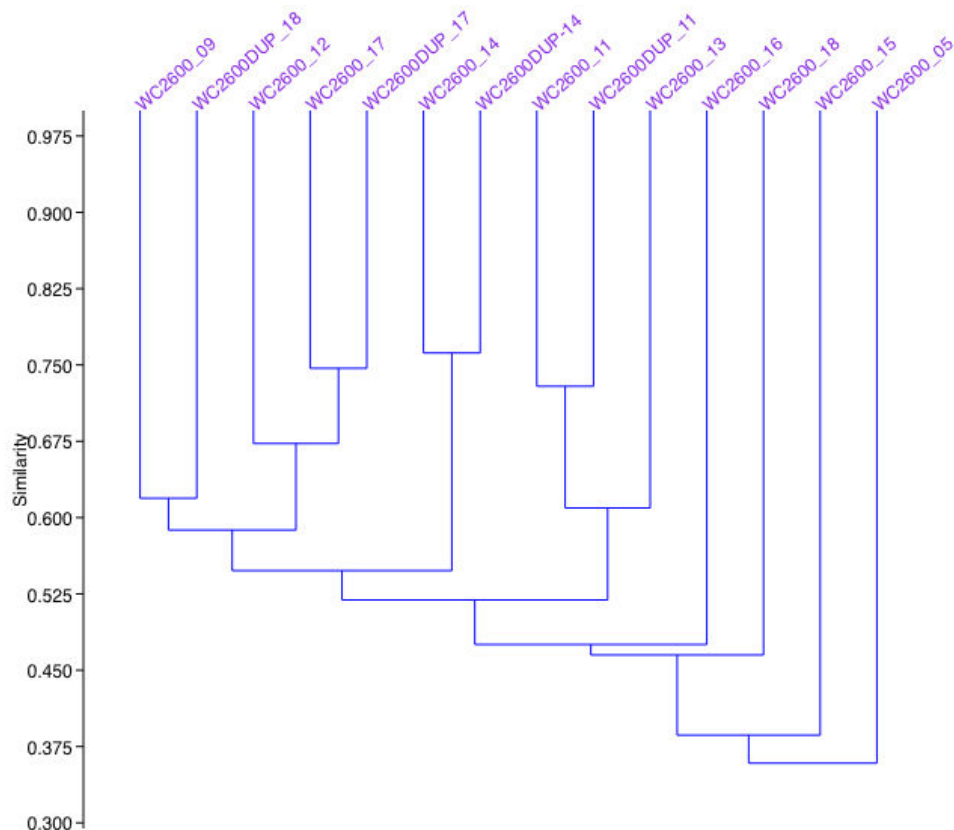
**Figure 20. PCA ordination of the macroinvertebrate community in WC2600 mainstem and side channel habitat, 2018.** *Blue dots= riffle-targeted sample; aqua = proportional multihabitat.*



**Figure 21. PCA ordination of macroinvertebrate community traits in WC2600 mainstem and side channel habitat, 2018.** *Blue dots= riffle-targeted sample; aqua = proportional multihabitat.*

The macroinvertebrate community at WC2600 has changed over time, but with no strong pattern between years (Figure 22). The communities in 2005 and 2015 are outliers, with the least similarity overall to all other years (Bray-Curtis similarity index from 0.28-0.0.48). The macroinvertebrate community in 2005 is in general less similar to that in any other sampling year among all Whychus Creek sampling sites due to changes resulting from restoration of perennial flow. The community in 2015 had not yet recovered from the channel restoration project done in 2014, which explains its outlier status. In a PCA ordination of all years (Figure 23), PC1 and PC2 explain just 53% of the total variance, and the two factors with the highest loading values are the same on both axes: _Simulium_, which dominated the community in 2016 as a high-abundance pioneer species following restoration; and _Baetis tricaudatus_, which was the dominant taxon in 2014 and 2017.

It should also be noted that except for 2018, the duplicate sample taken at this site is most similar to the paired sample taken in the same year (i.e., 2011, 2014, and 2017). The greater difference in paired samples in 2018 could be due to operator error or difference in technique, as the duplicate is taken by CASM Environmental staff while the volunteer teams differ every year, This is always a risk when working with changing pools of volunteers, but overall the protocol has shown itself to be quite resistant to this element.

**Figure 22. CLUSTER analysis of WC1150 macroinvertebrate community, 2014-2018.**
_Number at the end of the sample name indicates year._

*Figure 23. PCA ordination of WC2600 macroinvertebrate community, 2005-2018.*

*Number at the end of the sample name indicates year.*
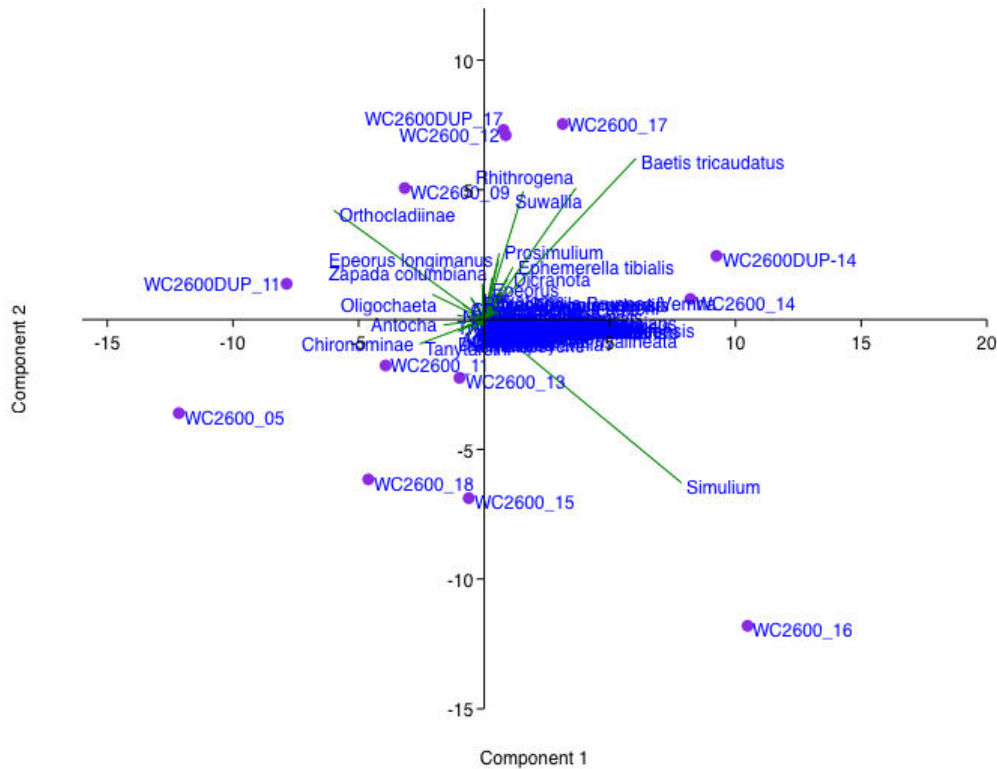


The target number of organisms was attained in six of the nine years prior to 2018, with 26.7-100% of the total sample sorted (Figure 24). In 2018, 43.3% of the total duplicate RT sample was sorted, but the entire original sample had only 218 organisms. Richness increased overall from 2005 to 2013 (22 and 43 taxa, respectively; Figure 25), and although the impacts of restoration activity in 2014 led to a sharp decline (16 taxa in 2015), richness has increased steadily since then (paired RT samples in 2018 had 39 and 43 taxa). EPT richness fluctuated more in earlier sampling years but generally followed the same pattern as total richness, rising from 10 to 21 taxa from 2005-2014 and dropping to just seven in 2015. EPT richness has also recovered, with 20 and 17 EPT taxa in the paired 2018 samples. Taxa contributing to increased EPT richness over time include *Visoka* and several *Epeorus*, *Zapada*, and *Rhyacophila* species, all of which are characteristic of faster-flowing and often cold, clear waters. The number of DEQ low temperature and low sediment indicator taxa followed a similar pattern, increasing overall from 2005 through 2014, with a post-restoration decrease recovering in recent years. Community temperature and sediment optima fluctuate from year, but both have increased overall since 2005.

PREDATOR and IBI scores fluctuated more at WC2600 than at any of the other longterm RT sites (Figure 26). O/E scores indicated poor biological condition in each year from 2005-2015, and the values decreased over time (O/E in 2005 = 0.66; O/E in 2015 = 0.41). However, scores have increased steadily since 2015, and in 2018 the site score indicated fair biological condition for the first time (O/E = 0.82). IBI scores fluctuated even more; scores indicated slight disturbance in five of the 10 sampling years, and for the last two years the WC2600 has scored as minimally disturbed.

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*Figure 24. Proportion of sample sorted and sub-sampled organismal abundance for WC2600, 2005-2018.*
*Sample sorting data not available for 2005.*
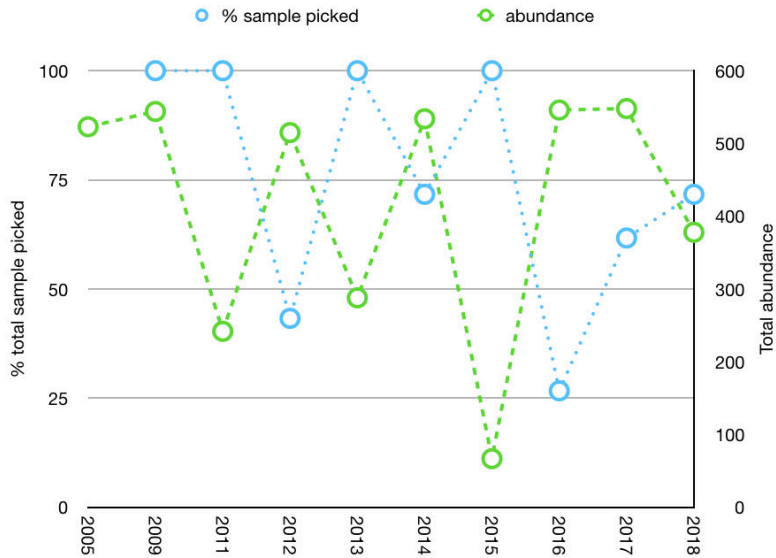


*Figure 25. Richness and #EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa in WC2600 samples, 2005-2018.*
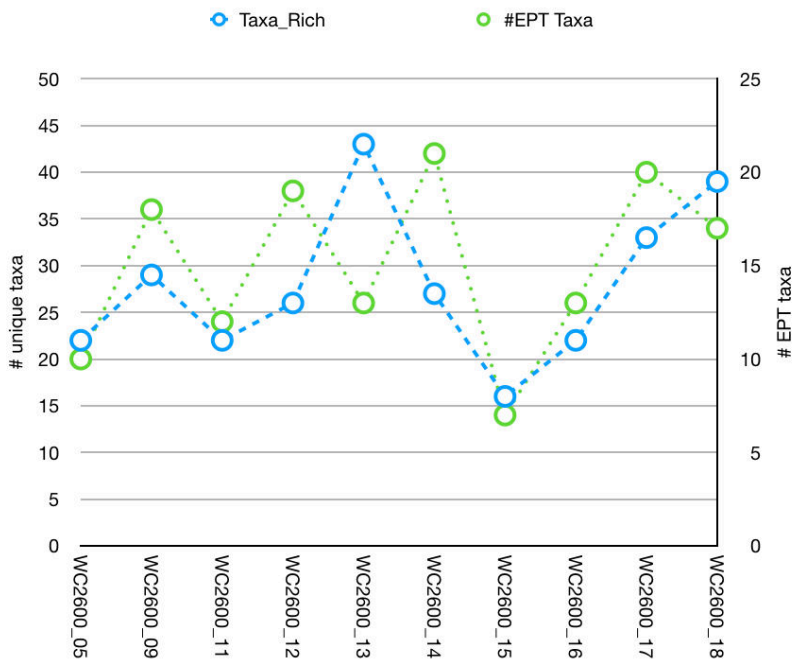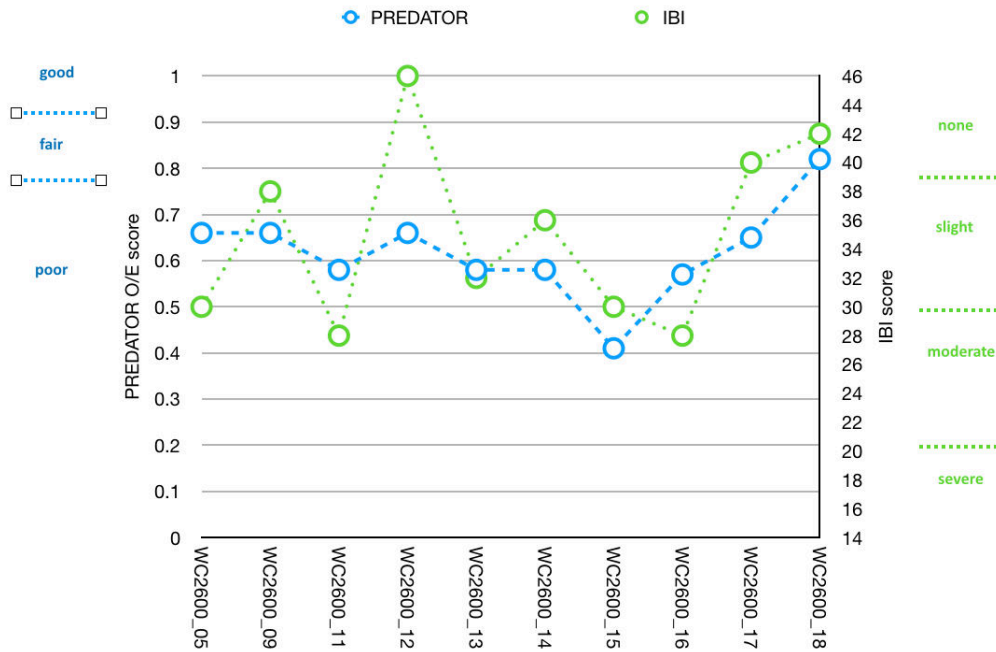
*Figure 26. PREDATOR O/E and IBI scores for WC2600 samples, 2005-2018.*



The dominant taxon in each sampling year has shifted towards more sensitive and/or lotic species. From 2005 through 2013, samples were dominated by non-biting midges in the Orthocladiinae subfamily, a ubiquitous group that tolerates a wide range of habitat conditions. However, during those years the relative abundance of this group decreased from 70.6% in 2015 to just 17.5% in 2013. Dominant taxa since then have been the lotic groups *Baetis tricaudatus*, *Rhithrogena*, and *Simulium*. These taxa dominated the community to a greater extent than in previous years, especially in 2016, when the sample was composed of 75.5% *Simulium*, but community evenness has recovered and in 2018 the dominant taxon in the paired samples was present at a much lower relative abundance (12.4% in original sample, 15.8% in duplicate).

In a PCA ordination of traits measured as relative abundances (dominant taxon, tolerant organisms, and functional feeding groups), axis one axis explained 44.9% of the variance. The factors with highest loading on PC1 were relative abundance of the dominant taxon, which has changed over time as described above; and relative abundance of collectors (filterers + gatherers. This has always been the dominant feeding guild at this site but the proportion decreased overall from 2005 (91.6%) through 2018 (54.1%), likely due to the reduced dominance of Orthocladiinae, which are primarily collector-gatherers and scrapers.

## Discussion

*Mainstem samples: riffle-targeted vs. proportional multihabitat*

Areas of slower water in a stream such as pools and glides allow accumulation of more sediment and organic material, and water is expected to be warmer and thus lower in dissolved oxygen. Faster-moving areas such as riffles and runs have colder, better oxygenated water and more coarse mineral substrate and algae. These differences in hydrology, temperature, and substrate are expected to sustain different taxonomic communities of aquatic macroinvertebrates, with associated differences in traits such as developmental rate (i.e., voltinism), feeding guild, locomotion, thermal preference, rheophily, and environmental tolerances. Faster, colder water can sustain more sensitive organisms, which may develop more slowly and to a greater maximum body size; cling on the surfaces and within interstitial spaces of rocks to avoid being dislodged by current; and feed by scraping algae or collecting suspended particles of organic material. Slower, warmer habitats are more likely to be associated with faster-developing, smaller taxa with a higher tolerance to environmental stressors that burrow into or sprawl on the substrate and climb on macrophytes and feed as collectors and shredders.

In this study, RT and PM samples taken in mainstem reaches had a high degree of similarity, with Bray-Curtis similarity index for the two at each site ranging from 0.66 to 0.73, and between-sample differences were influenced strongly by reach location. PM samples tended to have more sediment-tolerant and/or ORDEQ high sediment indicator taxa, as well as taxa that tolerate warmer temperatures and slower or mixed flows. However, PM samples also consistently had greater invertebrate abundance (based on the smaller proportion of total sample sorted during sub-sampling), and similar or greater taxa richness compared to RT samples. Furthermore, every mainstem PM sample had more taxa in the sensitive EPT group than the RT sample taken simultaneously in the same reach, even though the composited PM samples contained net sets from fewer riffles (3-5 among different mainstem samples). These results indicate that multihabitat sampling provides a more accurate assessment of site biodiversity without sacrificing capture of sensitive riffle-associated taxa. It is possible that if sampling were done at a different time of year, differences between the two sample types would be more pronounced, as periods of highest or lowest flow may make hydrologic conditions in pools and riffles less distinct and drive greater similarity in taxa (Herbst et al., 2018). However, the random selection of available habitat types in PM sampling also included a range of flow types, including riffles and runs, which may help consistently minimize differences between the two.

Dominant taxa in mainstem PM samples tended to be more reflective of diverse substrates and flows; these included *Optioservus*, a riffle beetle found in sediments and detritus in cold lotic erosional and depositional habitats (WC0600); *Tanytarsus*, a chironomid midge that spins nets and builds tubes on the substrate in all kinds of freshwater habitats (WC1100); *Ampumixis*, a tolerant clinger and burrower in sandy substrates of cold fast water (WC1150); and *Cricotopus Nostococladius*, a sensitive and sediment-intolerant chironomid midge that mines in the discs of *Nostoc* blue-green algae in cold clear rivers and streams (WC2600).

Most traits had similar values in the PM and RT sample taken in the same mainstem reach, but any differences between mainstem them again reflected the additional flow and substrate types sampled in the multihabitat protocol, with more multivoltine, depositional, and warm water-associated taxa in PM samples. The community in PM samples also reflected inclusion of slower depositional habitats, with generally more sediment-tolerant and ORDEQ high sediment indicator taxa and a higher community sediment optima in PM.

The PM and RT sample taken in each mainstem site each contained some taxa not seen in the other sample, but PM samples tended to have a greater number unique taxa than the RT and they occurred at slightly higher abundances (i.e., >2 individuals, while taxa unique to each RT sample were mostly seen as 1-2 individuals). In addition, all of the seven taxa that were new additions to the project list in 2018 were found only in PM samples (mainstem and side channel). Unique taxa differed mainly in their rheophily, with taxa unique to RT samples at a site being more associated with more lotic-erosional flows, and those unique to the PM sample having a broader range of flow preferences.

*Side channels at recent project sites*

Macroinvertebrate communities in side channel habitat created in 2014 (at WC2600) and 2016 (at WC1100) had an overall high degree of average similarity compared to the mainstem at each site. This is not unexpected, as the mainstem reach is a source for new colonists, but differences in flow, substrate, and habitat stability in the new channels also act as environmental filters that may result in different overall communities. The side channels at each site had a greater number of total taxa compared to the mainstem, although the difference was significant only for the WC2600 side channels, which may be a function of greater habitat heterogeneity. However, there was no significant difference in the mean numbers of EPT taxa in side channel vs. mainstem samples at either site, and these communities also had a great deal of overlap (~39% average dissimilarity for both).

Simpsons Diversity Index (1-D) was generally high across the entire sampling set, but all values <0.91 were associated with side channel communities, indicating less evenness and greater numerical dominance by a subset of taxa, which may be expected in more disturbed habitats. Dominant taxa in side channels tended to be more tolerant and/or pioneer species (i.e. *Simulium* black flies, chironomid midge genera), although the WC2600-5 side channels community, which was more similar to the mainstem reach community, was dominated by a lotic taxon (the small minnow mayfly *Baetis tricaudatus*).

Trait differences included more univoltine taxa in mainstem sample communities and more multivoltine samples in side channels. These side channels are relatively new and may currently be considered more disturbed and unstable habitat, which favors taxa with greater mobility and/or rapid reproduction and development (Tullos et al., 2009). Thus, faster development (i.e., more multivoltine taxa) would be favored in the side channels, with the more stable environment of the mainstem supporting more taxa with a longer development time (i.e., univoltine taxa). Greater abundance of swimmers and erosional-flow associated taxa in the mainstem also suggests faster flows overall compared to side channel reaches.

The WC1100 side channels have changed substantially over the last two years, and macroinvertebrate community composition has changed as well (overall average dissimilarity of 48.5%). Community differences may be due to

repeated local colonization and extirpation events as changing flow, substrate, nutrient, and inter-species competition conditions favor or inhibit different taxa. Significant increases in 2018 in taxa richness and relative abundance of climber taxa, accompanied by a decrease in abundance of burrower taxa, suggests that substrates may contain less sediment and more structural elements such as macrophytes.

*Longterm monitoring sites*

Several biotic assessment metrics have improved overall among the longterm monitoring sites in this project, including total richness and number of EPT taxa. Numbers of sensitive and sediment-intolerant taxa have also increased at several sites across time, which is likely driven in part by the increase in EPT. PREDATOR O/E scores have always indicated lower biological condition than IBI scores at the same site, but both of these assessments reflect improved community conditions as well. Community evenness has also improved, with lower relative abundances of the top taxon seen at all sites in 2018. Macroinvertebrate communities at WC1100 and WC2600 that were strongly negatively impacted by restoration-related disturbances recovered across a two-year period, with values for the metrics above meeting or exceeding those seen prior to project implementation.

Individual sites do show some anomalies. Community temperature optima at WC0600 was much higher in 2005 (18.3°C) compared to the WC2600 community in the same year (15.6°C), but decreased steadily in each sampling year to a low of 16.3°C in 2013. However, it has increased in every sampling year that followed, reaching a new high of 19.0°C in 2018. Temperature optima for the WC2600 community have been the lowest of all four sites and fluctuated more in every year, but this site trending towards an overall increase as well, as is WC1150, though the magnitude of the change is smaller (<1°C). In contrast, community temperature optima for WC1100 samples is the same every year (17.0-17.1°C). Because these changes have not been consistent between sites, it is possible that they are due to landscape-level factors, but given that the last four years have been the hottest ever recorded (World Meteorological Organization, 2018), the impacts of climate change can't be ruled out.

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