

# Quality Assurance Project Plan

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## Invertebrate Supplementation as Restoration Action in Select B-IBI Basins

Water Quality NEP Stormwater Initiative Interagency  
Agreement No. WQNEP-2017-KCWLRD-00027

August 2018



### Prepared by

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### Prepared for

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Water Quality Program

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This Quality Assurance Project Plan (QAPP) and project report will be available on request from King County and Ecology. The contents of these documents do not necessarily reflect the views and policies of the Environmental Protection Agency or Ecology, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Data for this project will be available on Ecology's Environmental Information Management (EIM) website: [www.ecy.wa.gov/eim/index.htm](http://www.ecy.wa.gov/eim/index.htm). Search on Study ID WHM\_KCY.

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**Cover photo:** Stonefly nymph, taken by King County Stream Team staff

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## 2.0 Abstract

This project will supplement, or seed, macroinvertebrates into four streams where benthic index of biotic integrity (B-IBI) scores are lower than expected based on current habitat conditions and land use. Habitat restoration and stormwater control efforts have been implemented to various degrees in the study basins; however, taxa richness has not increased and B-IBI scores remain low. The presumption is that taxa richness has not increased in basins where habitat is intact or recently restored because the streams are too far from a source of sensitive taxa. To evaluate this idea, a diverse assemblage of macroinvertebrates will be collected from “donor” streams, and seeded in “recipient” streams. Benthic macroinvertebrate samples will be collected from recipient streams prior to, and one year post-seeding to determine if sensitive taxa have become established. If sensitive taxa persist in the recipient streams, B-IBI scores may improve, signaling that no additional restoration actions may be needed. If B-IBI scores do not improve, additional restoration actions (other than seeding) may be needed to improve and sustain these stream communities. The results of this project will help to inform the effectiveness of macroinvertebrate seeding as a tool to assist recovery of stream systems and as well as the need for additional restoration actions.

## 3.0 Background

### 3.1 Introduction and problem statement

Many streams that flow into Puget Sound have been impacted by various stressors for decades, if not longer. The diverse communities of aquatic benthic macroinvertebrates that are native to regional streams respond to these stressors and are therefore good indicators of ecological health. The loss of sensitive taxa, especially some species of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera), indicates degraded conditions; however, it can be challenging to determine the specific stressor. If stressors are reduced and conditions improve, recovery of macroinvertebrate communities may be rapid (within a year) if there is a local source of colonists. However, for streams without a nearby sources of colonists, recovery of the benthic community may be slow and/or limited (Parkyn and Smith 2011).

The ecological processes associated with colonization and recovery are relevant for the region because the presence of sensitive taxa is used to characterize the health and condition of streams. The multi-metric B-IBI is a standardized scoring system that characterizes stream condition and health based on the composition and relative abundance of the benthic macroinvertebrates present. The overall B-IBI score for a site is highly dependent on several taxa richness measures. Although sensitive taxa are ubiquitous in streams that are in excellent condition, many have limited dispersal capabilities. Some taxa can travel up to several kilometers, but most do not disperse more than a few hundred meters from their natal stream (Macneale et al. 2005, Sundermann et al. 2011).

The strong correlation between B-IBI score and the extent of urban development in the contributing basin suggests basin-scale processes are most important in explaining taxonomic richness at a site. However, in some streams B-IBI scores are lower than expected given the land

use in the basin and available habitat (Paul et al. 2009). In some cases these stream reaches are isolated or disconnected from stream networks with more sensitive taxa. Low B-IBI scores in these streams may be due in part to the limited local pool of sensitive taxa. If stream conditions (i.e., habitat, streamflow) are actually better than the B-IBI score indicates, the stream may have the capacity to support a greater number of sensitive taxa.

The work performed for this project includes supplementing (seeding) macroinvertebrate taxa collected from streams with sensitive taxa into four recipient streams that are isolated and have lower than expected B-IBI scores based on the land use in their contributing basins. If seeded taxa are able to establish and persist in the recipient sites, taxa richness and potentially B-IBI scores will increase. If seeded taxa are unable to establish or persist, it will be clear that B-IBI scores accurately reflect current stream conditions and stressor identification analyses can be prioritized.

### 3.2 Study area and surroundings

The study focuses on six streams in the Puget Lowland Ecoregion (Table 1). Macroinvertebrates for seeding will be collected from two streams within the Cedar River watershed (donor sites), including Webster Creek and the main stem of the Cedar River (Figure 1). Macroinvertebrates will be seeded into four streams (recipient sites; Figure 1), including Taylor, Gold and Walker creeks and a tributary of Yarrow Creek (Table 1). All sites are within two adjacent water resource inventory areas (WRIAs 8 & 9). The local climate conditions and native vegetation are similar across the ecoregion, and it is assumed that the study streams historically supported a similar macroinvertebrate assemblage.

Table 1. Study site location information

Creek	Donor or recipient site	Site Code	Location	Latitude	Longitude	WRIA	Basin
Mainstem Cedar River, above Landsburg dam	Donor	Cedar_seed	Unincorporated King County, near Hobart	47.385199	-121.956818	WRIA 8	Cedar River/Lake Sammamish Basin
Webster Creek	Donor	08CED5046	Unincorporated King County, near Hobart	47.4277	-121.91545	WRIA 8	Cedar River/Lake Sammamish Basin
Taylor Creek	Recipient	08WES1340	City of Seattle	47.507869	-122.247582	WRIA 8	Cedar River/Lake Sammamish Basin
Yarrow Creek Tributary	Recipient	YarrowWestTribBeIRM0.2	City of Bellevue	47.641796	-122.204353	WRIA 8	Cedar River/Lake Sammamish Basin
Gold Creek	Recipient	08SAM2865	Unincorporated King County, near Woodinville	47.742702	-122.141764	WRIA 8	Cedar River/Lake Sammamish Basin
Walker Creek	Recipient	WalkerPreserve	City of Normandy Park	47.452032	-122.337736	WRIA 9	Puget Sound (Duwamish-Green) Basin

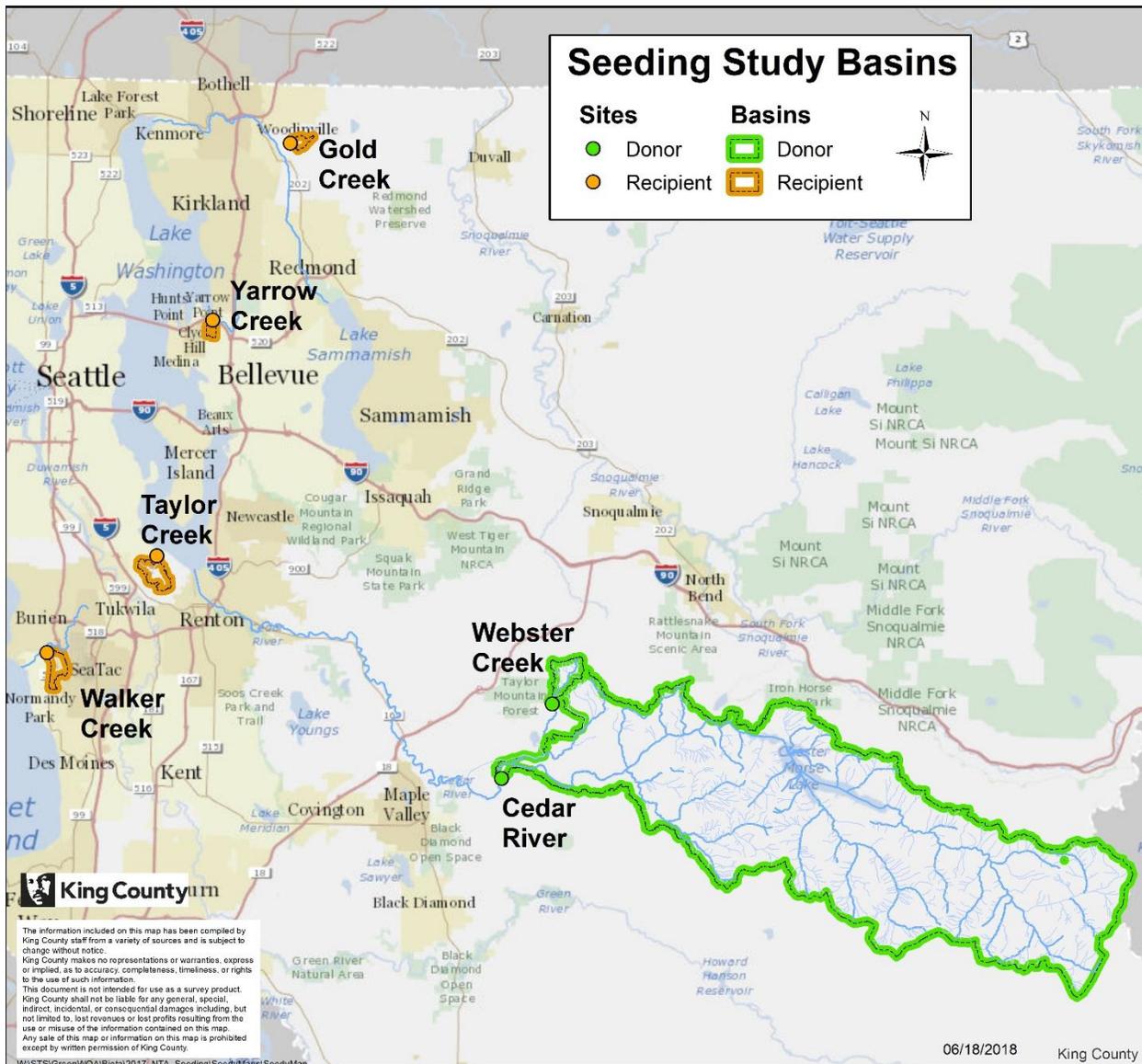


Figure 1. Map of donor and recipient stream sites and their contributing basins.

### 3.2.1 History of study area

Prior to Euro-American settlement, the lowlands of King County were dominated by continuous forests of Western hemlock, Western Red cedar, and Douglas-fir, and interlaced with a network of streams, small rivers and wetlands (King County 2008). The recipient stream basins, like others in the ecoregion, were once almost entirely forested but are now largely dominated by urban and suburban land uses (Table 2; NOAA Coastal Change Analysis Program). Previous efforts to restore habitat and reduce impacts of stormwater runoff in the recipient basins will be described in the final report.

As previously indicated, donor macroinvertebrates will be collected from the upper Cedar River watershed. This watershed serves as the drinking water source for much of Seattle, WA, and

streams in this basin are among the highest quality streams remaining in the Puget Sound region. Extensive logging and several fires occurred in the watershed throughout the 1800s and early 1900s. These events likely impacted water quality and stream conditions, but since the mid 1920s the upper basin has been protected from extensive cutting, mining and urban development. Thus, the macroinvertebrate communities in these streams may be altered from pre-settlement conditions, but they have had nearly a century to recover.

Table 2. Land cover within the donor (green) and recipient (yellow) site basins in 2016.

Land cover (percent of area)	Cedar River	Webster Creek	Gold Creek	Taylor Creek	Walker Creek	Yarrow Creek Tributary
Urban	1.0%	0.0%	21.1%	73.0%	73.8%	66.1%
Developed Open Space	0.0%	0.0%	13.3%	9.6%	8.1%	3.9%
Forest	84.1%	96.0%	64.0%	14.7%	12.2%	28.8%
Grassland	1.6%	0.0%	0.6%	0.0%	0.0%	0.0%
Scrub/Shrub	9.5%	2.0%	0.9%	0.0%	0.1%	1.2%
Bare Land	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%
Wetland	1.2%	1.9%	0.1%	2.5%	5.5%	0.0%
Water	2.5%	0.0%	0.0%	0.0%	0.2%	0.0%
<b>Total Area (acres)</b>	<b>78257.4</b>	<b>843.2</b>	<b>187.3</b>	<b>603.1</b>	<b>458.0</b>	<b>131.8</b>

### 3.2.2 Summary of previous studies and existing data

Similar seeding efforts have been initiated elsewhere, and those projects as well as a review of previous data from King County’s ambient monitoring sites informed this study. In a local project, Sarah Morley of NOAA Fisheries’ Northwest Fisheries Science Center and Katherine Lynch of the City of Seattle added invertebrates from several sites within the Cedar River basin to a newly created hyporheic zone in Thornton Creek, Seattle WA. Preliminary analyses suggest seeding may have been partially successful; four taxa not previously found in Thornton Creek samples were collected post-seeding and were likely from the Cedar River (Morley et al. 2018). In Fairfax County, VA, an ecologist, Jonathan Witt, is leading another seeding study to better understand why macroinvertebrate communities have not recovered as quickly as expected at restored sites (Witt 2017). That study was started in 2017 and no results are available yet.

For this study, the recipient sites were selected because they are more than 2 to 5 km from known sources of diverse and sensitive macroinvertebrate taxa (Figure 2). This range is typically too far for most adult insects to fly, especially if the terrestrial habitat along or between streams is degraded (Sundermann et al. 2011). Despite restoration efforts, these sites also have consistently poor or fair B-IBI scores<sup>1</sup>. In contrast, sites in the upper Cedar River basin, including Webster Creek, regularly score good or excellent and support a diverse community of sensitive taxa. Although macroinvertebrates have been collected from the main stem of the Cedar River for use in previous experiments (Macneale unpublished data; McIntyre et al. 2015), there

<sup>1</sup> B-IBI scores and taxonomic lists were downloaded from the Puget Sound Stream Benthos database (PSSB) ([www.pugetsoundstreambenthos.org](http://www.pugetsoundstreambenthos.org)) for the five study streams.

is not an established B-IBI site and the samples were not used to calculate a B-IBI score. Samples previously collected from Webster Creek were used to generate the expected taxa list for the Cedar River sites. (Note: a subset of the colonization baskets deployed in Webster Creek and the main stem Cedar River will be analyzed in 2018 to establish a list of taxa present at each site to provide baseline information regarding species likely to be transported to the recipient sites.)

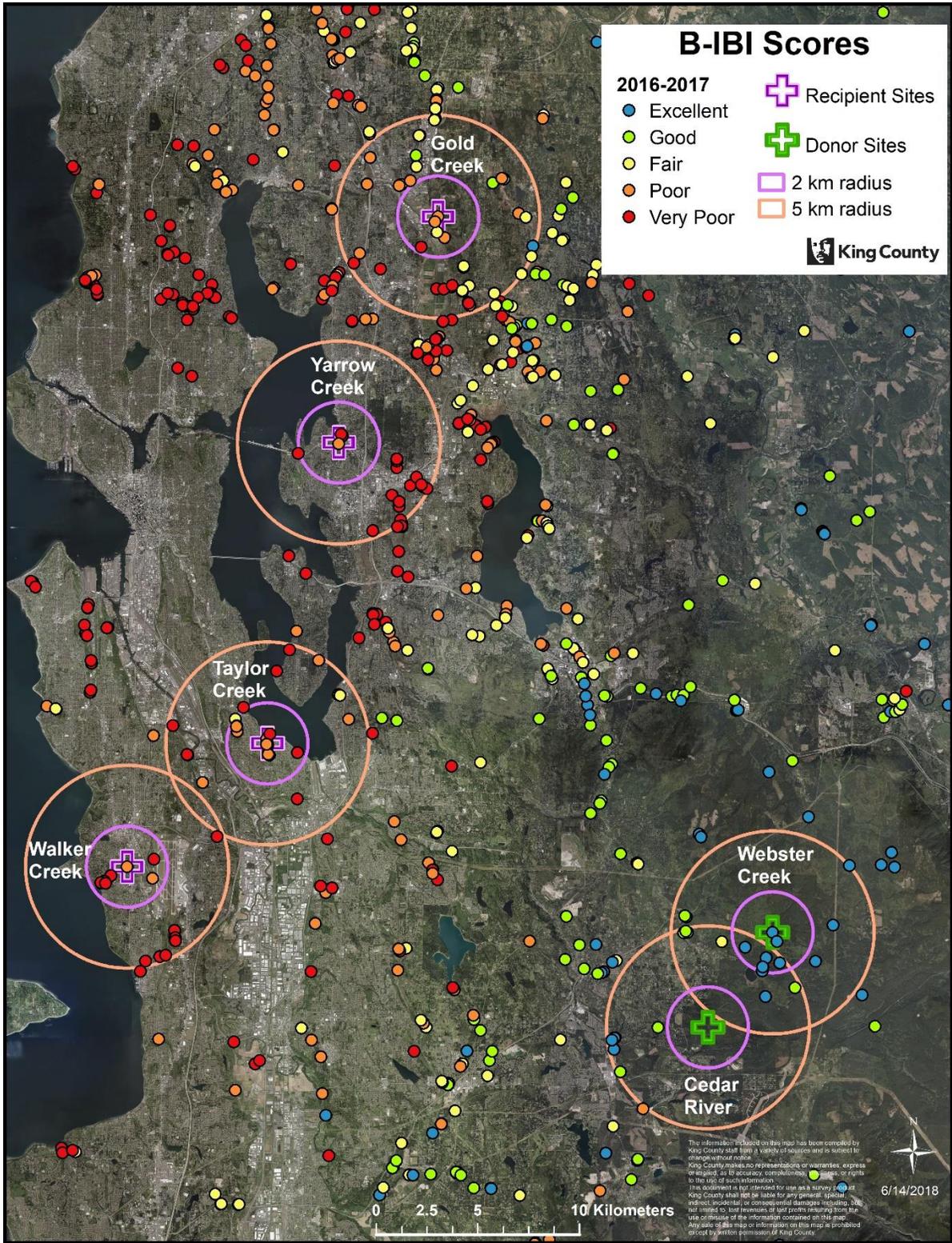


Figure 2. Map of donor and recipient sites and recent B-IBI scores from those and other sites in King County.

The most recent B-IBI scores from each site (Table 3) highlight differences in taxa richness between the donor and recipient sites. The most recent Webster Creek sample included 28 unique EPT taxa (sum of Ephemeroptera [mayfly], Plecoptera [stonefly] and Trichoptera [caddisfly] taxa), while 10 or fewer EPT taxa were present in the recipient stream samples. Although the richness metrics are somewhat redundant (e.g., some clinger taxa are also mayflies, several stoneflies are also long-lived taxa), the differences in these metrics illustrate the large number of sensitive taxa missing from the recipient streams.

Table 3. The most recent B-IBI metric values and scores from the study streams.

Stream and Site Code		Webster Creek (08CED5046)	Taylor Creek (08WES1340)	Gold Creek (08SAM2865)	Walker Creek (WalkerPreserve)	Tributary of Yarrow Creek (YarrowWestTribB eIRM0.2)
Last Year Sampled		2017	2017	2017	2017	2013
Quantities	Taxa Richness	56	30	28	29	40
	Ephemeroptera Richness	8	3	1	3	3
	Plecoptera Richness	11	4	2	3	4
	Trichoptera Richness	9	3	4	3	2
	EPT Richness	28	10	7	9	9
	Clinger Richness	26	8	9	12	7
	Long-lived Richness	17	3	4	5	5
	Intolerant Richness	15	0	1	0	0
	Percent Dominant	60.40%	64.40%	46.10%	61.00%	45.20%
	Predator Percent	15.60%	7.80%	14.30%	17.80%	15.80%
	Tolerant Percent	2.20%	46.40%	1.70%	16.40%	20.80%
	Number of Organisms	500	500	293	500	500
Scores (0 - 10)	Taxa Richness Score	10	1	0.3	0.7	4.5
	Ephemeroptera Richness Score	10	2.9	0	2.9	2.9
	Plecoptera Richness Score	10	4.3	1.4	2.9	4.3
	Trichoptera Richness Score	10	2.5	3.8	2.5	1.2
	Clinger Richness Score	10	0.6	1.2	2.9	0
	Long-lived Richness Score	10	1.2	2.5	3.8	3.8
	Intolerant Richness Score	10	0	1.4	0	0
	Percent Dominant Score	2.3	1.2	6.2	2.2	6.4
	Predator Percent Score	7.3	3.4	6.7	8.4	7.4
	Tolerant Percent Score	9.5	0	9.6	6.2	5.2
Overall Score (0 - 100)		89.1	17.2	33.1	32.3	35.6

Taxonomic data previously collected (between 2002 and 2017) at each of the study sites were compiled to generate lists of taxa that may be expected at the donor and recipient sites (Tables 4-7). Each list may represent more taxa than would be present in any one sample, but is intended to be a comprehensive assessment of taxa that could be present in 2018. Most of the study sites have been sampled annually, or every other year; however, the tributary to Yarrow Creek has only been sampled once, as a result the taxa list for this site may underrepresent the true site diversity.

The “new” taxa identified in Tables 4 – 7 (Taxon column), are those taxa for which there is certain taxonomic identity. For example, if an individual present in the recipient site was identified at the “parent” level (often family) and not lower (genus or species), no lower taxa classification within that parent level is included on these lists. For instance, if a stonefly at a recipient site was identified to family level of Perlidae, no Perlidae genera or species would be included in the lists because it is uncertain if they represent a “new” taxa at a site.

These taxa lists use historic data to help identify taxa that may be seeded in recipient streams, and whether or not they would represent a “new” taxa. Additional samples, collected from a subset of the colonization baskets deployed at the Cedar River sites, will be used to confirm which taxa are present in the baskets and will likely be added to the recipient streams. When taxa lists for all site samples are compared (historic donor and recipient samples, basket samples, pre-seeding recipient and post-seeding recipient samples) at the end of the study, conclusions about some taxa may be inconclusive due to taxonomic ambiguities like the Perlidae example above. Taxonomic experts at Rhithron Associates Inc. (hereafter called Rhithron) will be consulted, and when in doubt, conclusions about seeding success will be presented as inconclusive. Additional information describing data analysis is presented in Section 14.

Table 4. New taxa that may be introduced to Taylor Creek from Cedar River sites

Order	Family	Genus	Taxon		
Coleoptera	Dytiscidae		Dytiscidae		
Decapoda	Astacidae	Pacifastacus	Pacifastacus		
Diptera	Pelecorhynchidae	Glutops	Glutops		
Ephemeroptera	Ameletidae	Ameletus	Ameletus		
	Baetidae	Dipheter	Dipheter hageni		
	Ephemerellidae	Attenella	Attenella	Attenella delantala	
		Caudatella	Caudatella	Caudatella	
		Drunella			Drunella coloradensis
					Drunella doddsii
					Drunella flavilinea
					Drunella grandis
					Drunella spinifera
		Ephemerella	Ephemerella	Ephemerella	
Serratella	Serratella	Serratella tibialis			
Haplotaxida	Enchytraeidae	Mesenchytraeus	Mesenchytraeus		
Plecoptera	Capniidae		Capniidae		
	Chloroperlidae	Kathroperla	Kathroperla		
		Paraperla	Paraperla		
		Suwallia	Suwallia		
	Nemouridae	Visoka	Visoka cataractae		
	Peltoperlidae	Yoraperla	Yoraperla brevis		
	Perlidae	Calineuria	Calineuria	Calineuria californica	
		Doroneuria	Doroneuria	Doroneuria	
		Hesperoperla	Hesperoperla	Hesperoperla pacifica	
	Perlodidae	Megarcys	Megarcys	Megarcys	
		Skwala	Skwala	Skwala	
	Pteronarcyidae	Pteronarcella	Pteronarcella	Pteronarcella badia	
		Pteronarcys	Pteronarcys	Pteronarcys princeps	
Trichoptera	Apataniidae	Apatania	Apatania		
	Brachycentridae	Brachycentrus	Brachycentrus americanus		
	Brachycentridae	Micrasema	Micrasema		
	Limnephilidae	Ecclisomyia	Ecclisomyia		
	Uenoidae	Neophylax	Neophylax	Neophylax splendens	
		Neothremma	Neothremma	Neothremma	
		Oligophlebodes	Oligophlebodes	Oligophlebodes	

Table 5. New taxa that may be introduced to Walker Creek from Cedar River sites

Order	Family	Genus	Taxon		
Coleoptera	Hydrophilidae	Ametor	Ametor		
Diptera	Thaumaleidae		Thaumaleidae		
Ephemeroptera	Ameletidae	Ameletus	Ameletus		
	Baetidae	Diphedor	Diphedor hageni		
	Ephemerellidae	Attenella	Attenella	Attenella delantala	
		Caudatella	Caudatella	Caudatella	
		Drunella		Drunella	Drunella coloradensis
				Drunella	Drunella doddsii
				Drunella	Drunella flavilinea
				Drunella	Drunella grandis
			Drunella	Drunella spinifera	
	Ephemerella	Ephemerella	Ephemerella		
Serratella	Serratella	Serratella tibialis			
Plecoptera	Capniidae		Capniidae		
	Nemouridae	Visoka	Visoka	Visoka cataractae	
		Zapada	Zapada	Zapada	Zapada frigida
			Zapada	Zapada	Zapada Oregonensis Group
	Peltoperlidae	Yoraperla	Yoraperla	Yoraperla brevis	
	Perlidae	Calineuria	Calineuria	Calineuria californica	
		Doroneuria	Doroneuria	Doroneuria	
		Hesperoperla	Hesperoperla	Hesperoperla pacifica	
	Pteronarcyidae	Pteronarcella	Pteronarcella	Pteronarcella badia	
		Pteronarcys	Pteronarcys	Pteronarcys	
Trichoptera	Apataniidae	Apatania	Apatania		
	Brachycentridae	Brachycentrus	Brachycentrus	Brachycentrus americanus	
		Micrasema	Micrasema	Micrasema	
	Polycentropodidae	Polycentropus	Polycentropus	Polycentropus	
	Uenoidae	Neophylax	Neophylax	Neophylax splendens	
		Neothremma	Neothremma	Neothremma	
Oligophlebodes		Oligophlebodes	Oligophlebodes		
Trombidiformes	Hygrobatidae	Hygrobates	Hygrobates		
	Protziidae	Protzia	Protzia		
	Torrenticolidae	Monatractides	Monatractides	Monatractides	
		Testudacarus	Testudacarus	Testudacarus	
		Torrenticola	Torrenticola	Torrenticola	

Table 6. New taxa that may be introduced to Gold Creek from Cedar River sites

Order	Family	Genus	Taxon	
Coleoptera	Dytiscidae		Dytiscidae	
Decapoda	Astacidae	Pacifastacus	Pacifastacus	
Diptera	Psychodidae	Maruina	Maruina	
Ephemeroptera	Ameletidae	Ameletus	Ameletus	
	Ephemerellidae	Attenella	Attenella delantala	
		Caudatella	Caudatella	
		Drunella		Drunella coloradensis
				Drunella doddsii
				Drunella flavilinea
				Drunella grandis
			Drunella spinifera	
	Ephemerella	Ephemerella		
	Serratella	Serratella tibialis		
	Heptageniidae	Cinygmula	Cinygmula	
Epeorus		Epeorus grandis		
		Epeorus longimanus		
Plecoptera	Capniidae		Capniidae	
	Chloroperlidae	Kathroperla	Kathroperla	
		Paraperla	Paraperla	
		Suwallia	Suwallia	
	Leuctridae	Despaxia	Despaxia augusta	
		Leuctra	Leuctra	
	Nemouridae	Visoka	Visoka cataractae	
		Zapada	Zapada frigida	
	Perlidae	Calineuria	Calineuria californica	
		Doroneuria	Doroneuria	
	Pteronarcyidae	Pteronarcella	Pteronarcella badia	
Pteronarcys		Pteronarcys		
Trichoptera	Apataniidae	Apatania	Apatania	
	Brachycentridae	Brachycentrus	Brachycentrus americanus	
	Uenoidae	Neothremma	Neothremma	
		Oligophlebodes	Oligophlebodes	

Table 7. New taxa that may be introduced to Yarrow Creek tributary from Cedar River sites

Order	Family	Genus	Taxon
Coleoptera	Dytiscidae		Dytiscidae
	Elmidae	Cleptelmis	Cleptelmis addenda
		Heterlimnius	Heterlimnius corpulentus
		Narpus	Narpus concolor
		Optioservus	Optioservus
		Zaitzevia	Zaitzevia
Hydrophilidae	Ametor	Ametor	
Decapoda	Astacidae	Pacifastacus	Pacifastacus
Diptera	Empididae	Chelifera	Chelifera
		Hemerodromia	Hemerodromia
		Oreogeton	Oreogeton
	Pelecorhynchidae	Glutops	Glutops
	Psychodidae	Maruina	Maruina
	Tipulidae	Antocha	Antocha monticola
		Hexatoma	Hexatoma
		Limnophila	Limnophila
Ephemeroptera	Ameletidae	Ameletus	Ameletus
	Ephemerellidae	Attenella	Attenella delantala
		Caudatella	Caudatella
		Drunella	Drunella coloradensis
			Drunella doddsii
			Drunella flavilinea
			Drunella grandis
			Drunella spinifera
		Ephemerella	Ephemerella
	Serratella	Serratella tibialis	
	Heptageniidae	Cinygmula	Cinygmula
		Epeorus	Epeorus grandis
			Epeorus longimanus
Ironodes		Ironodes	
Rhithrogena		Rhithrogena	
Plecoptera	Capniidae		Capniidae
	Leuctridae	Despaxia	Despaxia augusta
		Leuctra	Leuctra
		Moselia	Moselia infuscata
	Nemouridae	Visoka	Visoka cataractae
		Zapada	Zapada frigida
	Zapada Oregonensis Group		
Peltoperlidae	Yoraperla	Yoraperla brevis	

Order	Family	Genus	Taxon
	Perlidae	Calineuria	Calineuria californica
		Doroneuria	Doroneuria
		Hesperoperla	Hesperoperla pacifica
	Perlodidae	Megarcys	Megarcys
		Skwala	Skwala
	Pteronarcyidae	Pteronarcella	Pteronarcella badia
Pteronarcys		Pteronarcys princeps	
Trichoptera	Apataniidae	Apatania	Apatania
	Brachycentridae	Brachycentrus	Brachycentrus americanus
		Micrasema	Micrasema
	Glossosomatidae	Anagapetus	Anagapetus
		Glossosoma	Glossosoma
	Limnephilidae	Ecclisomyia	Ecclisomyia
	Philopotamidae	Dolophilodes	Dolophilodes
	Philopotamidae	Wormaldia	Wormaldia
	Polycentropodidae	Polycentropus	Polycentropus
	Uenoidae	Neophylax	Neophylax splendens
Neothremma		Neothremma	
Oligophlebodes		Oligophlebodes	

### 3.2.3 Parameters of interest and potential sources

This project will collect new macroinvertebrate data to answer specific study questions related to macroinvertebrate taxa richness in both donor and recipient streams (Table 8). This project does not directly measure environmental variables related to stream condition or stressors, other than field temperature when macroinvertebrates are transported to recipient sites.

Table 8. Parameters of interest

Type of Data	Source	Process
Macroinvertebrate data – donor streams	Macroinvertebrates samples collected at two donor sites (referred to as “basket samples”)	Data collected in 2018
Macroinvertebrate data – recipient streams	Macroinvertebrates samples collected at four recipient sites (referred to as “B-IBI samples”)	Data collected in 2018 and 2019
Temperature data	Stream temperatures on day macroinvertebrates are seeded into recipient sites	On the day macroinvertebrates are seeded into each stream, a hand held thermometer will be used to measure temperature in the donor and recipient streams to ensure donor macroinvertebrates are warmed or cooled sufficiently before transplant

## 4.0 Project Description

### 4.1 Project goals

The immediate and local goal of this project is to increase taxa richness and improve B-IBI scores in four select basins through macroinvertebrate seeding. A broader goal is to improve our understanding of how macroinvertebrate seeding may be used to accelerate recovery of stream communities and improve B-IBI scores.

### 4.2 Project objectives

The project objectives are to:

- 1) Survey baseline communities, quantify taxa richness and calculate B-IBI scores in four recipient streams pre-seeding.
- 2) Transplant sensitive taxa from two Cedar River basin streams to four recipient streams that have very poor, poor or fair B-IBI scores and lack sensitive taxa.
- 3) Survey recipient streams one year post-seeding to determine if seeded taxa became established.
- 4) Compare taxa richness and B-IBI scores, pre-and post-seeding, to determine if richness and B-IBI scores increased.
- 5) Complete analysis and report detailing where and when macroinvertebrate seeding is appropriate.

### 4.3 Information needed and sources

The recipient sites were selected based on previously available macroinvertebrate data (downloaded from Puget Sound Stream Benthos (PSSB) database June 2018 [[www.pugetsoundstreambenthos.org](http://www.pugetsoundstreambenthos.org)]), as well as land use information about the contributing drainage area upstream of each site. Current land use and land cover information for the contributing basins (Table 2) was calculated using 2016 data from the National Oceanic and Atmospheric Administration's (NOAA) Coastal Change Analysis Program (C-CAP).

Kate Macneale (Project Manager) also spoke with two people who have conducted seeding experiments in the last few years, including Sarah Morley of NOAA's Northwest Fisheries Science Center and Jonathan Witt of Fairfax County, VA. Their insights were helpful in designing the study (e.g. Morley et al. 2018).

### 4.4 Tasks required

The primary tasks associated with this project are listed in Table 9.

Table 9. Project tasks and descriptions.

<b>Task</b>	<b>Title</b>	<b>Description</b>
1	Project Development	Detailed project plan and timeline, effectiveness consultation, QAPP
2	Project Administration & Management	Quarterly progress reports and invoicing, documentation, communication, FEATS reporting, Puget Sound Partnership NTA reporting, STORET data collection and reporting
3	Macroinvertebrate seeding in four recipient streams	Deploy colonization baskets, sample macroinvertebrates in recipient streams pre-seeding, and transplant colonization baskets
4	Effectiveness Monitoring	Post-seeding sampling in four recipient streams
5	Broader impacts and communication	Present project findings and post project documents and data to Puget Sound Stream Benthos website

## 4.5 Systematic planning process used

Preparing the study plan and the QAPP is sufficient as a planning process for this project. The required tasks for this project are logical and fairly simple, and therefore do not require a more complicated systematic planning process.

## 5.0 Organization and Schedule

### 5.1 Key individuals and their responsibilities

Table 10. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
Derek Day WA Department of Ecology, Water Quality Program Phone: 360-407-7612	Stormwater Strategic Initiative Lead	Clarifies project scope. Provides internal review of QAPP and approves final QAPP.
Kate Macneale King County - WLRD Science and Tech. Section Phone: 206-477-4769	Project Manager/Principle Investigator	Writes QAPP. Oversees field sampling and transportation of samples to laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into EIM. Writes draft and final reports.
Liora Llewellyn King County - WLRD Science and Tech. Section Phone: 206-263-0594	Investigator	Conducts field sampling, analyzes and interprets data, creates maps and assists in writing reports.
Beth Sosik King County – WLRD Science and Tech. Section Phone: 206-263-01680594	Investigator	Conducts field sampling, analyzes and interprets data, creates maps and assists in writing reports.
Deborah Lester King County - WLRD Science and Tech. Section Phone: 206-477-4752	Unit Supervisor for the Project Manager	Provides internal review of QAPP, approves budget and final QAPP.
Dave White King County - WLRD Science and Tech. Section Phone: 206-477-4847	Section Manager for the Project Manager	Reviews project scope and budget, tracks progress, reviews draft QAPP, and approves final QAPP.
Josh Baldi King County - WLRD Phone: 206-477-9440	Director of WLRD	Approves final report
Wease Bollman Rhithron Associates, Inc. Phone: 406-721-1977	President, Rhithron Associates, Inc.	Reviews macroinvertebrate sample and QC data
Tom Gries Department of Ecology Phone: 360-407-6327	NEP Quality Coordinator, Acting QA Officer	Reviews draft QAPP and approves final QAPP. Reviews draft project report.

EAP: Environmental Assessment Program

EIM: Environmental Information Management database

QAPP: Quality Assurance Project Plan

### 5.2 Special training and certifications

All King County field staff have extensive experience conducting the sampling required for this project. Kate Macneale has a PhD in Entomology and has over twenty years of experience collecting and analyzing macroinvertebrate data for a wide variety of projects. Liora Llewellyn has collected hundreds of benthic macroinvertebrate samples over three years as an ecologist for

King County. Beth Sosik has extensive experience investigating insects as indicators of habitat quality.

Rhithron employs taxonomists with extensive expertise in identification and enumeration of macroinvertebrates. Staff have multiple taxonomic certifications from the Society for Freshwater Science (<http://rhithron.com/taxonomy-staff-2/>).

### **5.3 Organization chart**

See Table 10 for primary staff and their roles.

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### **5.4 Proposed project schedule**

The proposed project schedule is listed in Table 11.

Table 11. Proposed project schedule. Activities will occur during grey shaded months.

Task	Activity	2018												2019												2020					
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun					
1.1	Complete detailed project plan																														
1.2	Write and finalize QAPP																														
1.3	Complete effectiveness consultation																														
2.1	Create project factsheet																														
2.2	Submit quarterly Progress Reports																														
2.3	Write and submit final report including final FEATS report and updated factsheet																														
3.1	Deploy colonization baskets and upload photographs of activities to EAGL																														
3.2	Upload draft report of sampled macroinvertebrates in four recipient streams, pre-seeding taxa richness assessment and B-IBI scores report to EAGL for ECOLOGY review.																														
3.3	Transplant colonization baskets into four recipient streams and upload photos of transplantation to EAGL																														
4.1	Sample macroinvertebrates in the recipient streams to assess post-seeding taxa richness and B-IBI scores (results will be available in early 2020 and included in final report)																														
5.1	Present findings of study and share data publically in Puget Sound Stream Benthos website; upload presentations to EAGL																														

## 5.5 Budget and funding

Funding for this project is through an EPA Water Quality National Estuary Program Stormwater Initiative Interagency Agreement, No. WQNEP-2017-KCWL RD-00027.

Budget estimates were based on anticipated costs for field time needed to place and transplant colonization baskets, and macroinvertebrate sampling and processing. The budget also includes costs for preparation of the QAPP, data analysis, report writing and project management. Cost estimates assume the project will be completed within two years. Costs for future monitoring of the recipient sites (in 2020 and later) will be incurred by the ambient monitoring programs supported by jurisdictions that routinely monitor the sites.

Table 12. Project budget by task and sub-task.

<b>Task</b>	<b>Activity and Deliverable</b>	<b>Cost per Sub-task</b>	<b>Total cost</b>
1	Project Development		\$23,228.12
1.1	Project plan	\$2,167.88	
1.2	QAPP	\$19,877.76	
1.3	Effectiveness consultation	\$1,182.48	
2	Project Administration & Management		\$30,416.60
2.1	Project factsheet	\$985.40	
2.2	Quarterly Progress Reports	\$11,481.80	
2.3	Final report, final FEATS report and updated factsheet	\$17,949.40	
3	Macroinvertebrate seeding in four recipient streams		\$38,943.98
3.1	Colonization baskets deployed	\$4,283.44	
3.2	Report of initial pre-seeding findings	\$5,439.64	
3.3	Invertebrates seeded into recipient streams	\$29,220.90	
4	Effectiveness Monitoring		\$4,947.50
4.1	Recipient streams sampled to assess project success	\$4,947.50	
5	Broader impacts and communication		\$2,463.80
5.1	Study findings presented and data shared	\$2,463.80	
Total projected project costs			\$100,000.00

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1.2	QAPP	\$19,877.76	
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2.1	Project factsheet	\$985.40	
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2.3	Final report, final FEATS report and updated factsheet	\$17,949.40	
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3.3	Invertebrates seeded into recipient streams	\$29,220.90	
4	Effectiveness Monitoring		\$4,947.50
4.1	Recipient streams sampled to assess project success	\$4,947.50	
5	Broader impacts and communication		\$2,463.80
5.1	Study findings presented and data shared	\$2,463.80	
Total projected project costs			\$100,000.00

## 6.0 Quality Objectives

### 6.1 Data quality objectives

The Data Quality Objectives (DQO) for this project are for new and existing data that will be collected, reviewed and analyzed as part of the project. The DQOs for this project are that data are of high quality and representative of sample sites. Another important objective is that the taxonomic data will be based on sufficient taxonomic resolution and quality to allow conclusive statements to be made about seeding success. While some level of uncertainty is expected, a key DQO is the need for sufficient, high quality information from both the donor sites and pre- and post-seeding samples collected from the recipient sites. These data are necessary to make conclusive statements about the majority of taxa identified in the samples and determine the degree to which the seeding efforts was successful. To achieve these objectives, data will be evaluated according to standard indicators of quality assurance, including:

- **Precision** -measure of the variability in the results of replicate measurements due to random error.

- **Bias** -the difference between the population mean and the true value.
- **Sensitivity** -measure of the capability of a method to detect what is being measured.
- **Comparability** -the ability to compare data from the current study to data from other similar studies and historical data.
- **Representativeness** -the degree to which environmental samples and other data are representative of existing conditions.
- **Completeness** -the amount of data required for your study to be a success.

Measurement quality objectives (MQOs) are criteria used to evaluate performance or acceptance of data and are based on the indicators above.

## 6.2 Measurement quality objectives for Macroinvertebrate Data

Measurement quality objectives for benthic macroinvertebrate data for this study vary somewhat depending on the sample type. Two types of samples will be collected and analyzed for this project; “basket samples” and “B-IBI samples”. Basket samples include the macroinvertebrates collected from a subset of the colonization baskets deployed in the donor sites. At each of the two donor sites, macroinvertebrates from five of the deployed baskets (for a total of 10) will be retained and preserved. Taxa from these samples will be enumerated and identified to the lowest taxonomic level possible (typically genus or species). These data will be used to estimate the taxa and number of individuals to be seeded in the recipient streams.

The “B-IBI samples” will be collected at each recipient site. Taxonomic data from these samples will be used to generate a B-IBI score and to characterize the taxa present pre-seeding in 2018, as well as post-seeding in 2019. B-IBI samples will be collected using standard protocols previously used at these sites. Taxa in all samples will be identified to the lowest possible taxonomic level based on a 500+ count subsample. However, additional processing of samples collected in 2019 (post-seeding) will include enumeration and taxonomic identification of the entire sample. This two-step processing will ensure that representative B-IBI scores are comparable to historic data and also provides a comprehensive taxa list for each site based on whole samples. As a result, any taxa unaccounted for due to subsampling will be included in the whole-sample taxa list.

### 6.2.1 Precision

**Basket samples:** The variability in counts and taxa richness for the basket samples collected from the two donor sites will be used to estimate the precision of taxa lists used to predict the likelihood of a taxa being seeded in the recipient streams. Taxonomic data from all basket samples will be combined to generate a list of taxa likely to be seeded in the recipient streams. The mean and standard deviation of taxa counts from each donor sites will be used to estimate the density of organisms that may be seeded in a recipient stream.

**B-IBI samples:** Replicate B-IBI samples will not be collected. The recipient streams are relatively small and it can be challenging to collect an 8 ft<sup>2</sup> sample at these sites. During the 2019 sampling event it will be critical to minimize sampling disturbance and lethal collections that might unnecessarily reduce seeded taxa density. Although replicate samples will not be

collected, precision of the taxa lists reported for each recipient site post-seeding will be improved through identification of all individuals in the 2019 B-IBI samples.

MQOs for precision of invertebrate counts and taxonomic identification are based on the re-identification and re-enumeration of 10% of a sample (randomly-selected) in a blind procedure. Based on raw counts, samples must have >90% similarity (Bray-Curtis), >90% Percent Taxonomic Disagreement (PTD), >95% Percent Difference in Enumeration (PDE) (Appendix C, Rhithron 2018).

## 6.2.2 Bias

Basket samples: To minimize bias in basket sample estimates, the five samples collected from each donor site will be randomly selected.

B-IBI samples: Sampling bias will be minimized by following standard protocols for benthic macroinvertebrate collection, preservation, transportation, storage, and sample analysis, as well as the use of trained staff.

Analytical bias will be minimized by laboratory quality control procedures. Initial laboratory sample processing and subsampling will include checking sorting efficiency. QC checks will be conducted on 100% of the samples by independent observers who microscopically re-examine at least 20% of sorted substrate from each sample.

## 6.2.3 Sensitivity

Basket samples: All macroinvertebrates collected in the five baskets at each donor site will be identified and counted to maximize the capability of detecting all taxa present in the baskets, as well as seeded in the recipient streams. The stream area represented by the five baskets is approximately the same area sampled in an 8 ft<sup>2</sup> sample. It is assumed that the combined data from the five baskets will be more sensitive in detecting the presence of taxa than a typical B-IBI sample because all individuals will be counted and identified while a minimum of 500 organisms are targeted for typical B-IBI samples.

B-IBI samples: The standard methods used here, in the field and laboratory, are capable of collecting, counting and identifying macroinvertebrates to calculate B-IBI scores.

## 6.2.4 Comparability

Basket samples: The macroinvertebrate taxa and relative abundances found in the random subset of basket samples are intended to be comparable to the communities present in the remaining baskets to be placed in the recipient sites.

B-IBI samples: The B-IBI samples collected for this study will be comparable to other B-IBI samples and historic samples collected with standard methods. Comparability will be maintained through use of standard sampling equipment and established protocols, along with standardized data validation and reporting procedures. The King County protocols (2018) were designed to produce consistent and repeatable results in each stream reach and ensuring data comparability by targeting riffle or non-depositional habitat, limiting the collection window to the summer low

flow period, disturbing the substrate for a standard time period (60 seconds), and using the same net mesh size (500 µm). Training in field data collection protocols for all field staff will occur prior to sample collection to ensure consistency across sampling locations. Sample collection at all sites will be led by the same field staff, further enhancing site-to-site consistency by limiting variation that can arise from use of multiple personnel. All samples will be sent to the same taxonomic laboratory (Rhithron) to ensure taxonomic identification consistency and comparability.

### 6.2.5 Representativeness

Basket samples: As mentioned above, the basket samples are intended to represent the population of taxa in the baskets that will be placed in the recipient streams. Basket samples will be selected randomly from each donor site and are essentially the same as the other baskets except that they will not be transported and deposited in the recipient streams.

B-IBI samples: The B-IBI samples are collected according to established protocols to ensure they are representative of the macroinvertebrate community present in the stream reach during low-flow periods (summer and early fall). Historic samples collected at the recipient sites have also been collected during the low-flow period. Collection of composite samples across the reach is intended to sample a sufficient area and number of invertebrates to be representative of the benthic macroinvertebrate community present at the site.

Standard sorting protocols (Appendix D, Rhithron 2017) are applied to achieve representative subsamples; samples will be subsampled to at least 500 organisms. In addition, to ensure all taxa in the 2019 samples are identified, any unprocessed portion of the sample will be processed and used to generate taxa lists, but these data will not be used to calculate B-IBI scores.

### 6.2.6 Completeness

Basket samples: The macroinvertebrate data from the basket samples will be considered complete if all ten samples are collected and processed.

B-IBI samples: The macroinvertebrate data from the B-IBI samples will be considered complete if the pre- and post-seeding samples are collected and processed from each of the recipient sites. Sampling in favorable weather when flow conditions are appropriate (summer base flow), along with adherence to standardized protocols will aid in providing a complete set of data for this project.

The loss of macroinvertebrates from a sample will be minimized by making sure the sampling cup is firmly attached to the net, washing and inspecting the net between sampling sites, carefully transferring net contents to the sample bottle(s), and preserving the sample with an adequate amount of ethanol. Sample bottle and labeling information are described below in Section 8. If validity of the sample information is in question, the sample will be excluded from analysis. The goal for macroinvertebrate data completeness is 100% of the total samples collected and analyzed. Completeness is defined as the total number of samples appropriate for use in further data analysis following field collection.

## **6.7 Acceptance criteria for quality of existing data**

King County will assess the quality of historic macroinvertebrate data, and consult with Rhithron if there are any questions about historic samples they processed. Historic data should be consistent, obtained using comparable standard techniques and technology, and be subject to similar QA/QC standards using methods that are reliable and transparent.

## **6.8 Model quality objectives**

Not applicable.

## 7.0 Study Design

### 7.1 Study boundaries and site selection

The study sites are shown and described in Figure 1 and Table 1. The donor sites were selected because they support an abundance of sensitive taxa. The recipient sites were selected because they meet four criteria:

- 1) They lack many sensitive taxa and consistently have very poor, poor or fair B-IBI scores,
- 2) they are disconnected or far from sources of sensitive taxa,
- 3) there have been some instream and/or stormwater management actions in the basin intended to improve stream conditions, and
- 4) the cities or agencies involved in managing the creek are willing to have Cedar River macroinvertebrates seeded at the site.

### 7.2 Field data collection

#### 7.2.1 Sampling locations and frequency

Macroinvertebrates from the donor sites (Table 1), will be collected and transported using colonization baskets (approximately 15" x 12" x 4"). Approximately 145 baskets will be placed in the two donor sites in late July or early August 2018. Baskets will be filled with rocks from the donor site, nestled in the benthos (stream substrate), and left in place for at least six weeks to allow a diverse community to colonize.

A "pre-seeding" B-IBI sample will be collected at each recipient site before macroinvertebrates are seeded to assess the pre-seeding community. B-IBI samples will be collected within a week (in late August or early September 2018) of seeding. This timing is consistent with previous macroinvertebrate sampling at each site.

In September 2018, colonization baskets will be removed from the benthos and transported carefully to the recipient sites. Baskets will be placed in large coolers, kept cool and aerated, temperature adjusted if necessary, and placed in the recipient streams within a few hours. The contents of approximately 33 baskets (but not the baskets themselves) will be placed gently in each of the four recipient streams. It is estimated that macroinvertebrates will be seeded in one recipient stream per day, and all recipient streams will be seeded within two weeks of each other. During this time period, a random sub-set of baskets (5 from each donor site) will be collected and the contents preserved for analysis.

In 2019, B-IBI samples will be collected from each of the same recipient sites in late August or September to assess post-seeding success.

#### 7.2.2 Field parameters to be measured

Donor macroinvertebrates will be collected using colonization baskets. When each basket is removed from the donor stream, a kick net (500 µm mesh) will be placed downstream so that any escaping macroinvertebrates will be captured. The basket and any macroinvertebrates in the

kick net will be placed in a cooler or plastic container to contain all individuals. Any fish or amphibians collected will be returned to the donor streams. The donor stream temperature will be measured when baskets are collected, and also at the recipient site when the macroinvertebrates are seeded. If needed, the coolers will be warmed slowly with recipient stream water to ease acclimatization.

Table 13. Field parameters and methods.

Parameters	Method	Number of samples/measurements per site
Macroinvertebrates, pre- and post-seeding	Targeted riffle: King County 2018	8, 1ft <sup>2</sup> samples collected from up to 4 riffles, composited from each site in 2018 and 2019
Macroinvertebrates, in colonization baskets	Collect and preserve all macroinvertebrates present in basket	5 baskets from each of the two donor sites in 2018
Stream temperature	Hand held thermometer	Measured at donor and recipient sites on seeding day

### 7.3 Modeling and analysis design

No modeling will be done as part of this project.

### 7.4 GIS analysis and design

No geospatial analysis will be done as part of this project, other than measuring the distances between the donor and recipient sites.

### 7.5 Assumptions in relation to objectives and study area

It is assumed that macroinvertebrate taxa lists, compiled from multiple years of sampling at each site, are accurate and reflect the taxa present and their relative abundances. It is also assumed that multiple taxa present in the Cedar River streams are not present within the recipient (or nearby) streams.

It is also assumed that for at least some sensitive taxa, the number of taxa seeded will be sufficient to allow some to survive, mate, and produce offspring. Some taxa may be added at such low densities (less than 20) that there will be little chance for them to become established and persist. It is assumed that taxa seeded at higher densities (at least 100 individuals) will have a greater likelihood to become established and persist.

Finally, it is assumed that the five baskets sampled from each donor site will adequately represent the invertebrate composition and abundance in the baskets transported and added to the recipient sites.

### 7.6 Possible challenges and contingencies

### 7.6.1 Logistical problems

This project may encounter logistical problems associated with deploying, retrieving, and transporting colonization baskets. Planning and ensuring sufficient staff are available to support these activities will be critical to avoid and/or address unplanned challenges. Significant rain events may delay sampling, resuming a few days later when flows are stable.

### 7.6.2 Practical constraints

The project duration is limited to two years (June 2018-June 2020). Within this time period King County will plan, sample, analyze, and report on findings. This project is subject to review by different agencies, as well as outreach efforts to other jurisdictions and organizations interested in the project findings (Cities of Seattle, Bellevue and Normandy Park and the Port of Seattle).

Rhithron will conduct taxonomic identification of all samples and will also upload B-IBI scores to the PSSB database. Macroinvertebrate samples will be collected between August and September each year, and will be transferred to the taxonomy lab as soon as possible. Processing can take several months and is dependent on the laboratory's schedule.

### 7.6.3 Schedule limitations

This project is subject to review internally as well as by Ecology. Colonization baskets cannot be deployed until the QAPP has been reviewed and approved. The colonization, pre-seeding sampling and seeding window is confined to the summer months between July and September. If development, review, and approval of this QAPP extends beyond the sampling window, it will not be possible to initiate this study in 2018.

## 8.0 Field Procedures

### 8.1 Invasive species evaluation

King County appreciates the risks involved whenever species are transported from one location to another, and has, wherever possible, built in safeguards to minimize the risk of transporting non-native species. The largest risk reduction comes from the unique condition of the donor sites. The donor streams are in pristine basins that supply the region with drinking water, and consequently the basins are free of agricultural, industrial, residential and recreational use. Access to the watershed has been strictly controlled by Seattle Public Utilities for decades. Thus there have been far fewer opportunities for invasive species to be introduced in these reaches. The distribution of known invasive species and historical taxa lists were used to validate our selection.

King County will adhere to Ecology's methods to address the spread of invasive species as outlined in the SOP EAP070 (Ecology 2016). At the end of every sampling day or upon moving from one waterbody to another, staff will follow a suite of decontamination procedures, including thorough cleaning, inspection, and freezing (<-10°C for > 8 hours) of all equipment possibly exposed to aquatic or terrestrial invasive species.

In addition, when the five sample baskets are collected from each donor site, the contents will be carefully checked in the field before preservation. King County staff, including Kate Macneale, will inspect the samples for invasive or nuisance species, such as New Zealand mudsnails and *Didymosphenia*. No aquatic invasive or nuisance species have been found previously at the two donor sites, but careful checks will help confirm that is still the case and ensure no unwanted taxa are transported to the recipient sites.

### 8.2 Measurement and sampling procedures

Macroinvertebrate sample collection in the recipient streams will follow the standard protocol used by King County (2018). Samples will be collected in 2018 at each of the four recipient sites within one week prior to seeding (pre-seeding), and at a similar time of year in 2019 (post-seeding). Eight, 1ft<sup>2</sup> samples will be collected from four riffles at each the site and composited.

Macroinvertebrates will be collected from five baskets from each of the donor sites during the seeding period. All of the rocks within a basket will be scrubbed and rinsed into a net, and all material will be retained, preserved with ethanol, and transported to Rhithron for analysis. The contents of each basket will be processed separately.

### 8.3 Containers, preservation methods, holding times

Benthic macroinvertebrate samples will be deposited in 1- and 2-liter plastic sampling jars. The samples will be preserved with 95% ethanol. The containers will be stored in coolers and transferred to King County's locked storage facility until they can be delivered to the taxonomic laboratory (Rhithron) for processing. Samples will be held for no more than three months before shipping, and will be analyzed within four months of delivery to Rhithron. A chain-of-custody

(COC) form will be filled out prior to transfer sample transfer to the taxonomic laboratory as detailed in section 8.6.

## 8.4 Equipment decontamination

NA. Sampling equipment will not be cleaned to prevent cross-contamination. However, King County will adhere to Ecology’s methods to address equipment decontamination as outlined in the SOP EAP090 (Ecology 2017) and summarized in Section 8.1 above.

## 8.5 Sample ID

A new project will be created in the PSSB database for this study. Samples will be identified using the established site code (if available) in addition to a modifier to distinguish that the sample was collected as part of this study.

Table 14. Sample IDs for samples collected in 2018 and 2019.

Creek Name	Site Code	Type of sample	Sample IDs in 2018	Sample ID in 2019
Cedar River main stem	Cedar_seed	baskets samples	Cedar_seed_basket_01 Cedar_seed_basket_02 Cedar_seed_basket_03 Cedar_seed_basket_04 Cedar_seed_basket_05	NA
Webster Creek	08CED5046	baskets samples	08CED5046_seed_01 08CED5046_seed_02 08CED5046_seed_03 08CED5046_seed_04 08CED5046_seed_05	NA
Taylor Creek	08WES1340	B-IBI sample	08WES1340Preseed	08WES1340Postseed
Walker Creek	WalkerPreserve	B-IBI sample	WalkerPreservePreseed	WalkerPreservePostseed
Gold Creek	08SAM2865	B-IBI sample	08SAM2865Preseed	08SAM2865Postseed
Yarrow Creek Tributary	YarrowWestTribBelRM0.2	B-IBI sample	YarrowWestTribBelRM0.2Preseed	YarrowWestTribBelRM0.2Postseed

## 8.6 Chain-of-custody

To maintain the legal integrity of collected macroinvertebrate samples, a COC procedure is followed by all project staff. Before sampling begins, a blank COC form (Appendix A) is printed and placed in the sample storage area. During the sampling period the form is filled out daily as samples are collected and stored. The form includes sample ID, collection date, number of containers used (large samples can require more than one container), field staff that collected the sample, and box number associated with sample transport. The COC form also includes contact information for the project managers. If a correction is required, a single line is drawn across the correction so it remains legible, and the correction is written adjacent to the error, with the author's initials and date. This practice ensures the project's data are legally defensible.

When samples are ready for transport, the completed COC form is scanned and copied. A digital copy is retained for the project file, and a printed copy is provided to the recipient when samples are transferred. The COC form must accompany the samples at all times. The sample list entered in the PSSB database will be crosschecked against the COC form before samples are transferred to the taxonomic laboratory. Upon receipt at the taxonomic laboratory the COC record will be crosschecked with each sample.

## 8.7 Field log requirements

King County will utilize waterproof field sheets to record relevant field notes, including date of activity or collection, water temperature, any observations about the donor or recipient sites.

## 8.8 Other activities

During field activities, staff will use GPS to confirm sampling locations are consistent before and after seeding. Staff will also take photos and make field notes regarding anything that may inform the study or help evaluate the results.

## 9.0 Laboratory Procedures

### 9.1 Macroinvertebrate laboratory

Rhithron Associates, Inc. of Missoula, Montana are currently contracted by King County to process macroinvertebrate samples. Rhithron provides sample sorting, taxonomic identification, sample QA/QC, and data upload into the PSSB database.

B-IBI samples collected at recipient sites in 2018 will be processed in the same way that King County's ambient monitoring program samples are typically processed. Samples are subsampled to at least 500-count, and organisms are identified according to the "fine" taxonomic effort. Laboratory procedures are based on the Rhithron protocol (Appendix D, Rhithron 2017). The 2019 B-IBI samples will be processed similarly. However, if any unprocessed sample remains (following subsample analysis), the remainder of the sample will be processed. This additional effort will insure that any taxa missed through subsampling will be included in the whole-sample taxa list.

The entire content of each basket samples will be processed and all individuals will be identified and counted to the “fine” taxonomic resolution.

Preserved samples are picked up by Rhithron staff for transport to the taxonomic laboratory. Prior to transport, samples are boxed and inventoried to verify that all samples on the COC form are accounted for.

## 9.2 Sample preparation method

Standard sorting protocols (Appendix D, Rhithron 2017) will be applied to B-IBI samples to achieve representative subsamples. Rhithron uses Caton subsampling devices, divided into 30 grids, each approximately 5 cm by 6 cm, for all sample handling. To obtain subsamples with a minimum of 500 organisms, samples are poured into the device, grids are randomly chosen, and substrate materials lifted out into petri dishes. Technicians use dissecting microscopes (10x-30x magnification) to remove all organisms from the contents of each grid until 500 organisms are collected. If less than 500 organisms are counted, the entire sample is sorted. Unsorted sample fractions are currently retained and stored at the Rhithron laboratory.

Once sorted, individual organisms are examined by certified technicians and identified to their appropriate taxonomic level using 10x-80x dissecting scopes. Representative specimens are slide mounted and examined at 200x-1000x magnification using a compound microscope. Once samples are sorted, identified, and recorded, organisms are preserved in 95% ethanol in labeled vials and archived at Rhithron. QA/QC procedures are carried out for each sample to assess sorting efficiency, identification, and data entry (Section 6). All samples are identified to Ecology's “fine” standard taxonomic effort (STE) requirement (chironomids, Acari, and oligochaetes are identified to lowest practical level) (Table 15, Ecology 2010, Appendix D, Rhithron 2017).

Table 15. Standard taxonomic effort (STE) level for benthic sample identification (Ecology 2010).

Taxa Group	Fine STE
Oligochaeta (segmented worms)	Subfamily/genus
Acari (mites)	Genus
Gastropoda (snails)	Genus
Dytiscidae (predaceous diving beetles)	Genus (adults and larvae)
Simuliidae (blackflies)	Genus (adults and larvae)
Chironomidae (midges; larvae and pupae)	Genus/species/species group
Trichoptera (caddisflies)	Genus/species/species group
All other taxonomic groups	Lowest practical level: typically genus or species

Organisms that cannot be identified to the taxonomic targets because of immaturity, poor condition, or lack of complete current regionally applicable published keys are left at appropriate taxonomic levels that are coarser than those specified. Identified organisms are preserved in 95% ethanol in labeled vials and archived at the Rhithron laboratory.

Taxonomic data are uploaded directly into the PSSB database by Rhithron. Once uploaded, data are immediately accessible by King County staff for use. Data are also stored in Rhithron's own electronic database.

### **9.3 Special method requirements**

No special method requirements are needed for processing the macroinvertebrate samples.

### **9.4 Laboratories accredited for methods**

Ecology does not accredit laboratories for identification and enumeration of freshwater invertebrates. Rhithron taxonomists hold multiple Society for Freshwater Science taxonomic certifications for the groups of invertebrates encountered in Puget Sound samples.

## **10.0 Quality Control Procedures**

### **10.1 Field and laboratory quality control**

Quality Control associated with macroinvertebrate collection will include a series of measures:

- All field staff will be trained in established sampling protocols.
- A core project team member will accompany and assist in sample collection to ensure consistent and common application of protocols.
- To reduce the chance of organisms being lost during sampling, nets will be visually inspected for holes and all rocks and nets will be thoroughly examined before additional samples are collected or before being discarded or stored.
- A taxonomic laboratory with certified taxonomists and established QC procedures will be used for identification and census of collected taxa

Quality control procedures for initial lab sample processing and subsampling involve checking sorting efficiency. These checks will be conducted on 100% of the samples by independent observers who microscopically re-examine at least 20% of sorted substrate from each sample. Quality control procedures for each sample will proceed as follows: the quality control technician will pour the sorted substrate from a processed sample out into a Caton tray, redistributing the substrate so that 20% of it can be accurately lifted out by removing entire grids in a random fashion. Grids will be selected, and re-examined until 20% of the substrate is re-sorted. All organisms that were missed will be counted and this number will be added to the total number obtained in the original sort. If 95% sorting efficiency is not achieved for a given sample, a failure will be recorded on the bench sheet and in the database.

### **10.2 Corrective action processes**

If questions regarding field protocols are not easily resolved, staff will meet to discuss and consult with Ecology if needed. Corrective actions will be noted and documented to track any interruptions in data collection.

## **11.0 Management Procedures**

### **11.1 Data recording and reporting requirements**

King County will create and maintain a project page on the PSSB database web site to provide access to data and reports produced for the project.

### **11.2 Laboratory data package requirements**

Rhithron will upload data to the PSSB database as they become available. Rhithron will also prepare a QA/QC report for each data set submitted. The project manager (Kate Macneale) will review all data before they are released to the public on the PSSB.

### **11.3 Electronic transfer requirements**

Rhithron will load macroinvertebrate data to the PSSB web site. Data available through the PSSB are downloadable in comma delimited, tabular format, sorted by location, agency, project, site code and date. The files can be easily imported into Microsoft Excel, Access or other database software.

### **11.4 EIM/STORET data upload procedures**

Data generated as part of this project will be uploaded to EIM and to STORET, unless Ecology determines storing data in PSSB is sufficient.

### **11.5 Model information management**

Not Applicable.

## **12.0 Audits and Reports**

### **12.1 Field, laboratory, and other audits**

Constructive reviews from internal staff as well as staff from Ecology and EPA are appreciated, but it is not anticipated that data collection and analyses or reports will be controversial. Therefore, an audit is unlikely to be necessary.

### **12.2 Responsible personnel**

Not Applicable – there will be no audit, and therefore no personnel will be responsible for the audit.

### **12.3 Frequency and distribution of reports**

Quarterly progress reports will describe progress in sample collection, seeding activities, and reporting throughout the project period. A draft and final report will be prepared to describe the project and results. The draft report will be submitted to Ecology for review. Comments will be discussed with Ecology and a final report will be prepared and submitted.

### **12.4 Responsibility for reports**

Kate Macneale and Beth Sosik will author the final reports.

## 13.0 Data Verification

### 13.1 Field data verification, requirements, and responsibilities

All data will be subject to verification before data analysis, distribution to an outside party (i.e., not part of the King County project team) or posting to a publicly accessible database. Prior to such use, the Project Manager will contact the appropriate project staff and field technicians responsible for collecting data to verify procedures were followed and data were checked for errors.

The project manager (Kate Macneale) will verify field data to ensure that:

- Data are consistent, correct, and complete, with no errors or omissions.
- Established criteria for QC results were met.
- Data specified in the Study Design were obtained.
- Methods and protocols specified in this QAPP were followed.

If MQOs are not met, data will be flagged and the metrics calculated with the data will be qualified (Table 16).

Table 16. Acceptance criteria and corrective actions for data.

Parameter	Frequency	Acceptance Criteria	Corrective Action
Macroinvertebrate data – basket samples	Each sample	Individuals identified to fine STE resolution	Individuals not identified to fine STE resolution will be flagged
Macroinvertebrate data - B-IBI samples	Each sample	At least 500 count per sample	Flag and qualify metrics calculated from data

### 13.2 Laboratory data verification

The taxonomic laboratory project manager will verify all taxonomic results, prior to reporting. If performance objectives for sorting, counting or identification were not met, samples will be reprocessed according to the laboratory QA/QC plan (Appendix C, Rhithron 2018). Once the taxonomic data are entered into the PSSB database, the project manager (Kate Macneale) will review the uploaded data to assure that there are no errors in the data entry.

### 13.3 Validation requirements, if necessary

Not Applicable.

### 13.4 Model quality assessment

Not Applicable.

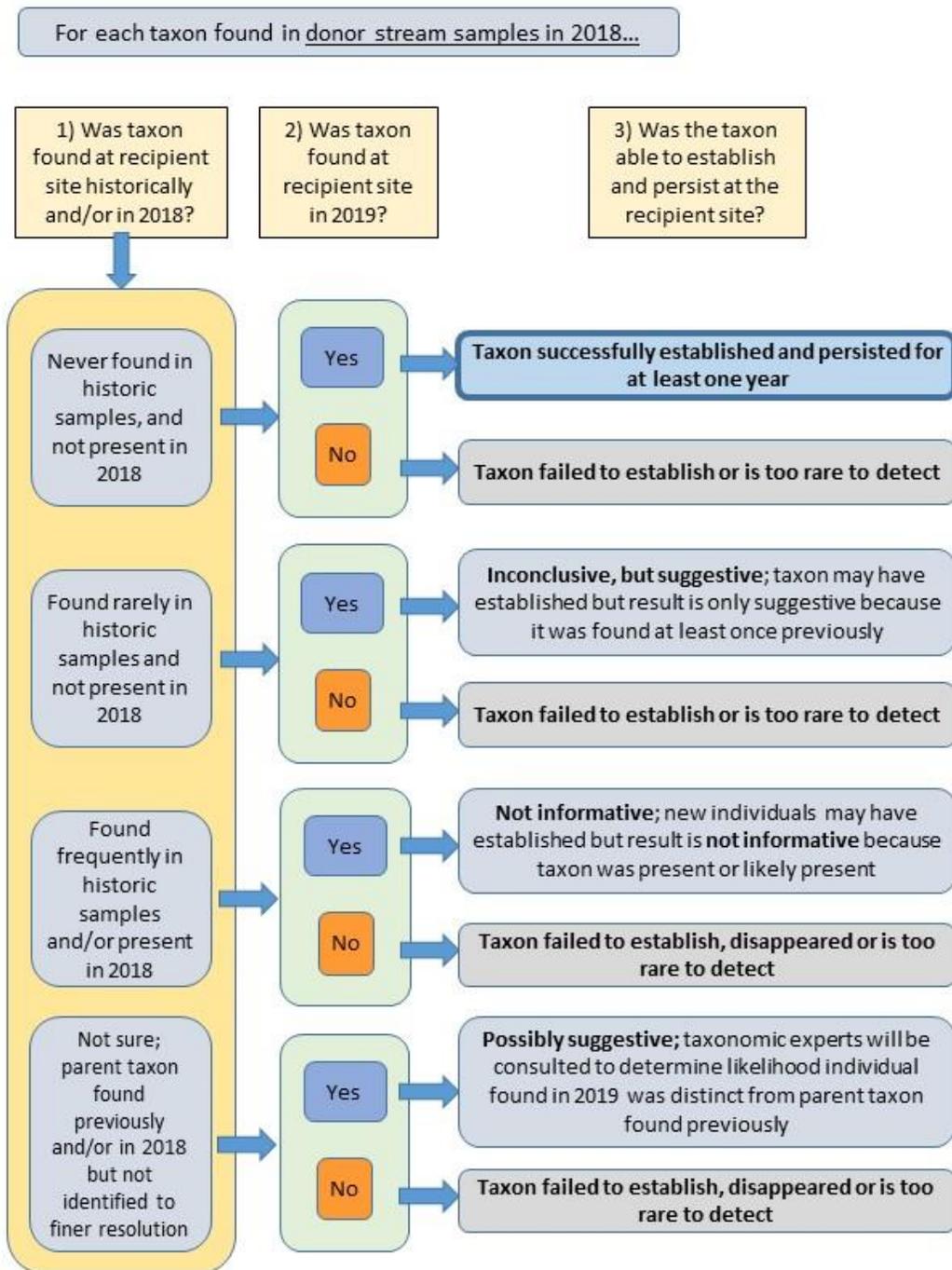
## **14.0 Data Quality (Usability) Assessment**

### **14.1 Process for determining project objectives were met**

The evaluation process to determine whether project outcomes have met the original objectives will include several steps. New macroinvertebrate data will ultimately be deemed useful if they illustrate which taxa were present pre-seeding at the recipient sites, which taxa were added, and whether the added taxa persisted. To be usable and useful, data will have been collected according to the QAPP, consistent with the study design, and will have met QA/QC criteria.

Macroinvertebrate data will be considered usable if the MQOs for collection and processing were met and at least 500 organisms were identified and counted in the pre- and post-seeding B-IBI samples in the recipient sites. Macroinvertebrate data will be useful to identify which taxa are present and their relative abundances at each site. Potential exploratory analyses are described in section 14.3.

To evaluate if seeded taxa were indeed able to establish and persist, King County will carefully review the taxa lists from each site. The following chart will aid in determining whether seeded taxa present in the basket samples were able to establish and persist in recipient streams (Figure 3).



**Figure 3. Decision tree for 2019 to determine if seeded taxa were able to establish and persist in recipient streams.**

## 14.2 Treatment of non-detects

The project's primary objective is to determine if macroinvertebrate taxa seeded in recipient streams in 2018 are detected in 2019. Because most taxa spend a year in their aquatic life stage, the presence of taxa in 2019 previously not observed prior to seeding may indicate seeded individuals were able to survive, mate and produce offspring. Not detecting a new taxon may indicate the taxon was either unable to become established or is too rare to detect in the B-IBI samples. Figure 3 illustrates conclusions that may be drawn depending on the availability and quality of data.

If B-IBI samples from the recipient sites contain fewer than 500 identifiable organisms, the data will be insufficient to calculate the B-IBI. If this occurs, the data will be reported, but not used for analysis.

## 14.3 Data analysis and presentation methods

Macroinvertebrate data will be uploaded and stored in the PSSB database and will all be available for public use and distribution. Data analysis will focus on generating taxa lists from pre- and post-seeding samples from the donor and recipient sites to determine if any seeded taxa were able to establish in the recipient sites. In addition, multivariate analyses (using PRIMER/PERMANOVA, or the vegan package in R) may be used to test if there were significant differences in macroinvertebrate communities among the donor and recipient sites, and among pre-seeding and post-seeding communities. B-IBI scores will be used to characterize ecological integrity at each of the recipient sites pre- and post-seeding.

## 14.4 Sampling design evaluation

The project's design will be evaluated based on the study outcome (e.g., did seeding work?), and lessons learned in the process. The evaluation will include a description of logistical considerations and limitations, uncertainties, long-term considerations and a discussion of when seeding may not be appropriate.

The final report will also include a discussion of how many colonists may be needed for successful establishment. This will include the estimated number of individuals of each taxon added to each recipient site (based on the basket samples and scaled to the number of baskets added). Depending on establishment success (Figure 3), the discussion will include projected sample sizes needed in future projects.

## 14.5 Documentation of assessment

The data usability assessment will be documented in the final report.

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## 16.0 Appendices





## Appendix B. Glossaries, Acronyms, and Abbreviations

### Glossary of General Terms

**Ambient:** Background or away from point sources of contamination. Surrounding environmental condition.

**Benthic Index of Biotic Integrity (B-IBI):** A standardized scoring system which can be used to compare and rank the health of different streams using the relative diversity and abundance of species of benthic macroinvertebrates in freshwater streams and rivers.

**Reach:** A specific portion or segment of a stream.

**Riparian:** Relating to the banks along a natural course of water.

**Sediment:** Soil and organic matter that is covered with water (for example, river or lake bottom).

**Stormwater:** The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

**Streamflow:** Discharge of water in a surface stream (river or creek).

**Watershed:** A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

## Acronyms and Abbreviations

B-IBI	(See Glossary above)
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
GIS	Geographic Information System software
i.e.	In other words
PSSB	Puget Sound Stream Benthos website database
QA	Quality assurance
QC	Quality control
SOP	Standard operating procedures
USGS	United States Geological Survey
WDFW	Washington Department of Fish and Wildlife
WRIA	Water Resource Inventory Area

### *Units of Measurement*

km	kilometer, a unit of length equal to 1,000 meters
m	meter
ft	feet

## Quality Assurance Glossary

**Accuracy:** The degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms precision and bias be used to convey the information associated with the term accuracy. (USGS, 1998)

**Bias:** The difference between the population mean and the true value. Bias usually describes a systematic difference reproducible over time, and is characteristic of both the measurement system, and the analyte(s) being measured. Bias is a commonly used data quality indicator (DQI). (Kammin, 2010; Ecology, 2004)

**Comparability:** The degree to which different methods, data sets and/or decisions agree or can be represented as similar; a data quality indicator. (USEPA, 1997)

**Completeness:** The amount of valid data obtained from a project compared to the planned amount. Usually expressed as a percentage. A data quality indicator. (USEPA, 1997)

**Data Quality Indicators (DQI):** Commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity. (USEPA, 2006)

**Data Quality Objectives (DQO):** Qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions. (USEPA, 2006)

**Data set:** A grouping of samples organized by date, time, analyte, etc. (Kammin, 2010)

**Data validation:** An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment, and objective criteria, to determine whether the MQOs for precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability and integrity, as these criteria relate to the usability of the data set. Ecology considers four key criteria to determine if data validation has actually occurred. These are:

- Use of raw or instrument data for evaluation.
- Use of third-party assessors.
- Data set is complex.
- Use of EPA Functional Guidelines or equivalent for review.

Examples of data types commonly validated would be:

- Gas Chromatography (GC).
- Gas Chromatography-Mass Spectrometry (GC-MS).
- Inductively Coupled Plasma (ICP).

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier, data is usable for intended purposes.
- J (or a J variant), data is estimated, may be usable, may be biased high or low.
- REJ, data is rejected, cannot be used for intended purposes (Kammin, 2010; Ecology, 2004).

**Data verification:** Examination of a data set for errors or omissions, and assessment of the Data Quality Indicators related to that data set for compliance with acceptance criteria (MQOs).

Verification is a detailed quality review of a data set. (Ecology, 2004)

**Measurement Quality Objectives (MQOs):** Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness. (USEPA, 2006)

**Measurement result:** A value obtained by performing the procedure described in a method. (Ecology, 2004)

**Method:** A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed. (EPA, 1997)

**Parameter:** A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene and nitrate + nitrite are all “parameters.” (Kammin, 2010; Ecology, 2004)

**Population:** The hypothetical set of all possible observations of the type being investigated. (Ecology, 2004)

**Precision:** The extent of random variability among replicate measurements of the same property; a data quality indicator. (USGS, 1998)

**Quality assurance (QA):** A set of activities designed to establish and document the reliability and usability of measurement data. (Kammin, 2010)

**Quality Assurance Project Plan (QAPP):** A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives. (Kammin, 2010; Ecology, 2004)

**Quality control (QC):** The routine application of measurement and statistical procedures to assess the accuracy of measurement data. (Ecology, 2004)

**Replicate samples:** Two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled. (USGS, 1998)

**Representativeness:** The degree to which a sample reflects the population from which it is taken; a data quality indicator. (USGS, 1998)

**Sample (field):** A portion of a population (environmental entity) that is measured and assumed to represent the entire population. (USGS, 1998)

**Sample (statistical):** A finite part or subset of a statistical population. (USEPA, 1997)

**Sensitivity:** In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit. (Ecology, 2004)

**Standard Operating Procedure (SOP):** A document which describes in detail a reproducible and repeatable organized activity. (Kammin, 2010)

**Systematic planning:** A step-wise process which develops a clear description of the goals and objectives of a project, and produces decisions on the type, quantity, and quality of data that will be needed to meet those goals and objectives. The DQO process is a specialized type of systematic planning. (USEPA, 2006)

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# Appendix C. Laboratory Quality Assurance Plan for Macroinvertebrate Sample Processing

Rhithron Associates, Inc.

## *Laboratory Quality Assurance Plan*

*Quality Assurance/Quality Control  
Policies and Procedures*

*Version 18.1*

*Revised January, 2018*

*Corporate Approval*



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Document Name: Laboratory Quality Assurance Plan	Document revised: January 2018 Effective date: Date of last signature
Document number: Revision 18.1 Replaces Revision 16.1.a	Issued by: Rhithron Associates, Inc. Chief Biologist, Vice President

## Macroinvertebrate Project Management

### *Scope and quality objectives*

Rhithron processes and identifies macroinvertebrate samples from clients throughout North America. The data generated from these samples need to be consistently and reliably generated to support the uses to which the data are put, typically, to assess water quality and habitat integrity in surface water systems. The methods and protocols applied to samples vary and depend on client-specifications and project goals. Thus, samples must be handled with the utmost attention and care and the client-specified protocol, including required taxonomic resolution, must be faithfully followed. All client-required deliverables must be quality-assured and delivered within specific timeframes. Quality control/quality assurance (QA/QC) systems must be implemented, and all procedures and protocols, including QA/QC procedures and results, must be documented and delivered to clients along with other project deliverables.

The potential for introducing uncertainty into macroinvertebrate sample analysis arises at multiple places in the process. Implementation of all provisions of the Standard Operating Procedures for Macroinvertebrate Sample Analysis (SOP, current draft 17.2.b) will allow the qualified, trained staff to meet data quality objectives (DQO) for all projects. Rhithron's internal DQOs for macroinvertebrate sample analysis are summarized as follows:

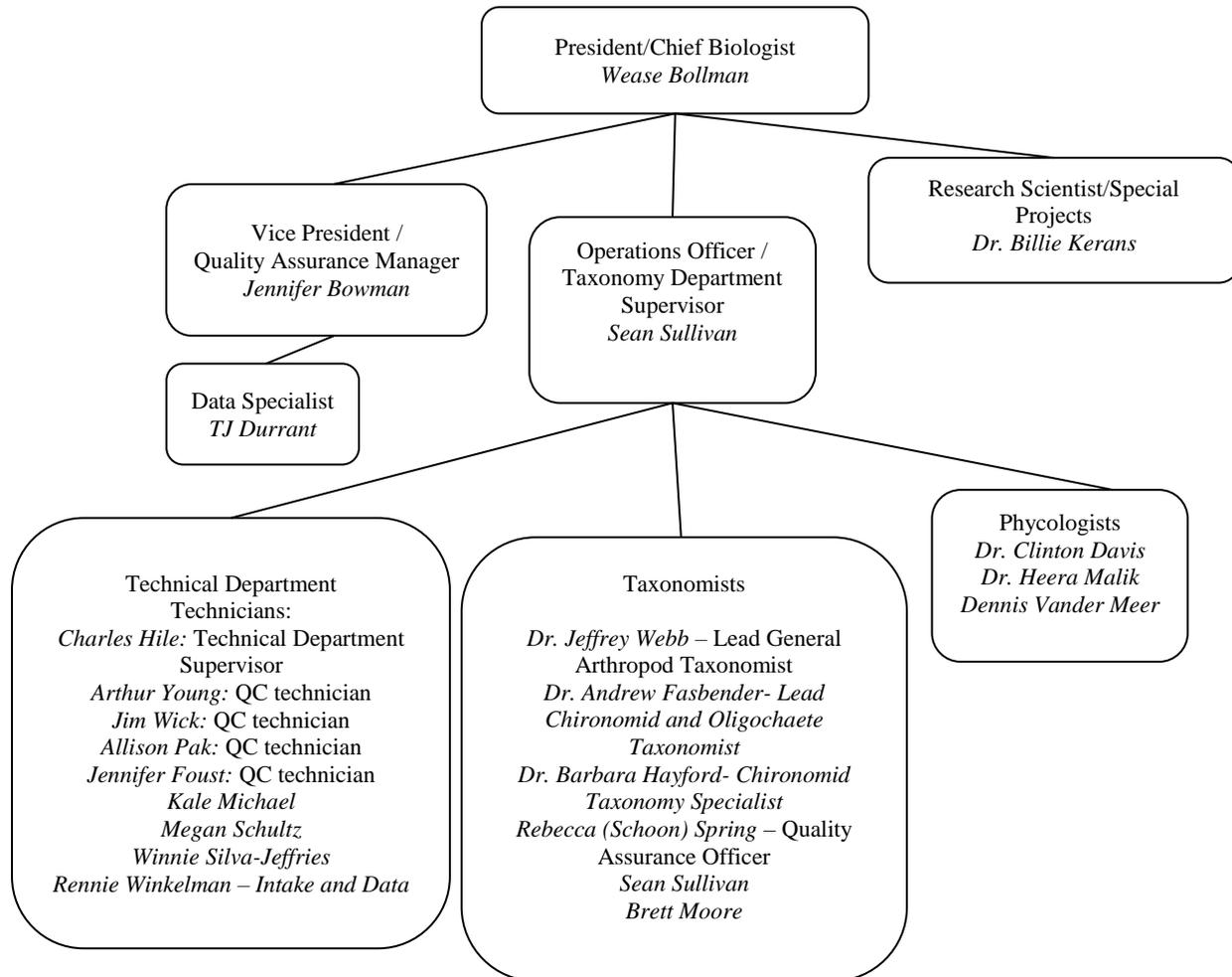
- All chain-of-custody documentation is maintained.
- Sample sorting efficiency is maintained at greater than 90% for each sample.
- Taxonomic accuracy and precision are maintained at  $\geq 95\%$  similarity (Bray-Curtis similarity X 100),  $\leq 5$  Percent Difference in Enumeration (PDE) and  $\leq 10$  Percent Taxonomic Disagreement (PTD).
- Bias is minimized, and representativeness, comparability, and completeness of data are maximized.
- All client project requirements and specifications are met or exceeded.
- Quality-assured, completed projects are delivered by the client's specified due date.

### Laboratory organization

The organizational chart in Figure 1 shows the Rhithron personnel responsible for the various tasks associated with macroinvertebrate and periphyton sample analysis, and illustrates the pathways of communication that are used to assure the quality of Rhithron's work.

#### **Responsibilities related to the analytical protocols**

The Lead Technician communicates the variations for individual projects to the Technical staff at weekly meetings, where projects scheduled for the upcoming week or on-going projects are discussed and reviewed. Specific project guidelines are printed on the inventory/sign-out sheet, which is available at all times to the technicians. QA/QC procedures are implemented in the Technical Department by trained QC technicians; a minimum of 10% of samples processed by technicians at Rhithron are randomly selected by the Operations Officer and subjected to QA/QC procedures that evaluate sorting efficiency. QC Technicians record sorting efficiency for each sample on sample benchsheets. QA/QC failures are addressed immediately by technicians. Periodic comparisons of subsample similarity are performed on randomly selected samples at least once per week. Random selection of samples for this QA/QC check is provided by the Lead Technician. Oversight of these functions is provided by the Lead Technician and the Operations Officer. The Lead Technician enters sorting efficiency statistics for every sample into the Rhithron database.



**Figure 1. Rhithron Associates, Inc. organizational chart: July 2016**

The Taxonomy Project Manager assigns taxonomists to projects and communicates the specific protocols and procedures related to sample analysis and QA/QC for individual projects to the taxonomy staff. A Protocol and Procedure (P&P) document specific for each project records project specifics, including QC protocols. P&P documents are kept in a manual in the taxonomy laboratory and are also available to taxonomists on the Rhithron network server. The Vice President randomly selects a minimum of 10% of completed samples, and re-identification of these samples is assigned to taxonomists by the Taxonomy Department Quality Assurance Officer. The Quality Assurance Officer calculates sample similarity statistics and provides these to the Taxonomy Department Supervisor, who institutes corrective action where needed. Corrective action may involve review of taxonomic determinations, additional QA/QC for the project, or sending specimens to systematic authorities for verification.

### **Responsibilities related to the QC functions for sample analysis**

The Lead Technician is responsible for the implementation of sample processing QA/QC procedures involving sorting efficiency. Standard operating procedure requires at least 10% of samples to be evaluated for sorting efficiency; these checks are performed immediately after a sample is processed. The QC Technician is selected by random rotation; thus the QA/QC process

is shared by all trained QC Technicians. Sorting efficiency results are compiled by the Lead Technician, who institutes additional training for technicians with poor sorting efficiency statistics. Failure of the subsample similarity procedure results in review of sample handling procedures by all technical staff.

QA/QC procedures for taxonomy fall under the authority of the Taxonomy Department Supervisor and Taxonomy Department Quality Assurance Officer, who review all sample similarity statistics other QC parameters and identify areas in taxonomic determinations or enumeration that require corrective action. The Quality Assurance Officer generates similarity statistics and other QC parameters for comparison of identifications and enumerations, and implements corrective actions when needed. The Taxonomy Department Supervisor assures that corrective action is taken by taxonomy staff members. Corrective action may include additional QA/QC for a project, or submittal of specimens to systematists for verification of identification. The Taxonomy Department Supervisor indicates to the Quality Assurance Officer when additional QA/QC is needed for a project.

## **Training/Certification**

Quality analysis of macroinvertebrate samples requires a laboratory staff with extensive training and experience. Laboratory technicians perform macroinvertebrate sample sorting: each technician completes an extensive step-by-step in-house training program. Each laboratory technician is required to maintain an average sorting efficiency of  $\geq 90\%$ . Quality Control Technicians perform sample sorting, and are additionally responsible for the quality checks on at least 10% of samples in each project. QC technicians have at least one year of experience in the technical laboratory, and have passed written and practical examinations that document their understanding and proficiency at providing sorting QC. They are also required to maintain sorting efficiency for their own samples at  $\geq 90\%$ . Staff taxonomists hold SFS (Society for Freshwater Science, formerly the North American Benthological Society or NABS) Level 2 certifications in taxonomic groups in which they work. The Taxonomy Department Quality Assurance Officer holds multiple SFS Level 2 certifications, and has at least 3 years of experience in the Taxonomy Laboratory.

## **Documentation and records**

Samples are received and sample metadata are logged into the Rhithron database (RAILISv.1.2.1) by the Data Technician. Logging in samples involves comparing the information on a chain-of-custody document to the information on sample container labels. An internal inventory is produced, and each sample is assigned a unique Rhithron identifier (RAI number). The internal inventory is printed out and serves as an inventory/sign-out sheet when the project is in the custody of the technical department. A chain-of-custody (COC) document for each sample is created by the Taxonomy Department Project Manager. The Data Technician signs the COC after sample log-in and confers with the client about discrepancies, damage, or other problems with the samples as they have arrived. A copy of the COC is made, and the original is returned to the client. Internal COC records for transfer of samples between departments are kept on the COC copy made at this time.

Transfer of sample custody within the Technical Department is recorded and tracked on the inventory/sign-out sheet. Sample processing information is recorded by sorting technicians on paper benchsheets and these data are transferred to the Rhithron database by the Lead Technician. Transfer of sample custody within the Taxonomy Department is recorded on the sample COC sheet.. Taxonomic and count data, and all associated data generated by taxonomists are entered into the EPIC (v.1.7) data entry program and subsequently uploaded to the Rhithron database. Data output in the form of taxa and lists of metrics are generated for each sample. Both paper and electronic formats can be generated. Processed and unprocessed sample remnants are either retained and stored in a secure facility or

returned to clients. The Rhithron database resides on the Rhithron network server, which is backed up daily both externally and to 3 internal drives.

## **Data generation and acquisition for macroinvertebrate analysis**

Processing, analytical and archival methods for macroinvertebrates follows specified standard operating procedures and relies on standard resources and references. Detailed procedures are found in the Rhithron Standard Operating Procedures (SOP: current draft 17.2.b).

### **Performance objectives: Technical laboratory (sample sorting)**

The goal of sample processing is to sort invertebrates from substrate in such a manner that results in an unbiased, representative subsample containing the appropriate number of organisms. The number of organisms is typically determined by the project specifications.

#### Objectives

There are several aspects of sample processing by Rhithron's technical staff that are important to subsequent data quality and thus, provide the objectives for each project. First, the target count of organisms is achieved within the specified tolerance limits. Second, the client-specified protocol is faithfully followed. Third, sorting efficiency is maintained at an average level no lower than 90% for each project, thus assuring sorting accuracy and precision. Fourth, the appropriate paperwork is associated with the correct sample. Finally, all data pertinent to the sub-sampling procedure, including fraction of sample used to obtain the target number of organisms, condition of the sample, any problems associated with sorting, and quality assurance procedure outcomes and statistics, etc. are recorded on sample benchsheets.

#### QA/QC plan

Accomplishment of the performance objectives is evaluated by a QA/QC plan that examines the adherence to Rhithron-specific and client-specific protocols.

**Target count:** Under-processed samples are detected at the time of taxonomic identifications by the taxonomists or at the time of data entry by the Lead Technician. If the sample was not fully picked in the processing stage, under-processed samples are revisited by the sorting technician, who distributes the unpicked sample portion into the appropriate number of Caton tray grids, and sorts the sample until the target count is reached.

**Adherence to specified procedure:** Daily oversight by the Lead Technician assures that client-specific protocols are followed in the technical department. Documentation for each project in progress is reviewed periodically.

**Sorting efficiency:** Quality control procedures for initial sample processing and subsampling involves checking sorting efficiency. These checks are conducted on at least 10% of the samples by independent observers who microscopically re-examine 100% of sorted substrate from each QC sample. All organisms that were missed are counted. Sorting efficiency is evaluated by applying the following calculation:

$$SE = \frac{n_1}{n_2} \times 100$$

where: SE is the sorting efficiency, expressed as a percentage,  $n_1$  is the total number of specimens in the first sort, and  $n_2$  is the total number of specimens in the second sort plus the first sort. Sorting efficiency is recorded on the benchsheet, and these data are entered into the Rhithron database.

Correspondence of sample and paperwork: Two technicians check the correspondence of sample and paperwork before each sample is processed. Technicians check the RAI number, the client's sample identifiers, and the number of jars associated with that sample. Both technicians sign the benchsheet, which is generated by Rhithron's database for each specific sample, when this step is completed. Using a "buddy" system insures that there are no mismatches between labels, spreadsheets, other data materials and the corresponding sample. Correct labeling of the sample fractions resulting from the processing procedures is assured by the provision of database-generated labels, which are attached to the benchsheet for each sample.

Complete recording of appropriate sub-sampling data: Benchsheets for samples that have been processed are collected daily by the Lead Technician, who checks for completeness of sub-sampling data, checks for missing data, and enters these data into the Rhithron database. Since these checks are performed daily, obtaining the data for each sample is assured.

Corrective actions: If 90% sorting efficiency is not achieved for a given sample, a failure is recorded on the benchsheet and in the database. A failure of any sample triggers assessment of an additional 10% of samples. For large projects, additional QC samples may be stratified by the technician whose sample failed the QA/QC check. Sorting efficiency statistics for each technician and for the entire laboratory are reviewed monthly. Sorting efficiency for each project is reported to the client in the technical summary document. Technicians who do not maintain the target sorting efficiency are given remedial training, and larger portions of the samples they process are examined for the sorting efficiency test until they are able to maintain the target sorting efficiency.

### **Performance objectives: Taxonomy Department (macroinvertebrate identification and enumeration)**

The goal of the taxonomic portion of sample processing is to accurately and precisely identify and enumerate organisms to the taxonomic resolution required by the project. Bias is minimized and data are reported completely. Materials related to the project, including labeled microscope slides, labeled vials with identified organisms, and laboratory benchsheets are handled carefully and are archived on the completion of identification and enumeration, and after all QA/QC procedures and data reviews have been completed. Deliverables such as voucher collections are assembled accurately and completely. Higher levels of taxonomy applied to organisms that cannot be identified to taxonomic targets are explained and qualified in all cases. Life stages are accurately recorded in the data.

#### *Objectives*

There are several aspects of invertebrate identification and enumeration by Rhithron's taxonomy staff that are important to subsequent data quality. First, the accuracy and precision of identifications and enumerations are maintained such that Bray-Curtis similarity (X100) between quality checked samples is 95% or greater, the Percent Difference in Enumeration (PDE) is 5% or less, and the Percent Taxonomic Disagreement (PTD) is 10% or less. Second, bias is minimized, and data completeness is assured. Third, the client-specified protocol, including specified target number of organisms and the required taxonomic resolution, is faithfully followed. Fourth, all client-requested deliverables are provided, including reference collections. Finally, summaries of QA/QC procedures and results, and sample processing procedures are documented and delivered along with client-requested deliverables.

#### *QA/QC plan*

Accomplishment of the performance objectives is evaluated by the following QA/QC plan that examines the adherence to Rhithron-specific and client-specific protocols.

Accuracy of taxonomy is evaluated by adherence to target taxonomic resolution requirements, and by the use of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). Bias is minimized by the use of taxonomic literature and resources that are accepted by the industry and reflects the most current accepted nomenclature. A bibliography of Rhithron's taxonomic library is maintained in a literature database. Consultation with experts and systematists occurs frequently. High quality optical equipment is used and regularly maintained. Geographic distributions of identified animals are checked and experts consulted when uncertainties arise, to assure credible identifications. Taxonomic discrepancies are examined and discussed by the original taxonomist and the QC taxonomist. Discussions may include the Taxonomy Department Supervisor, Project Manager, Quality Assurance Officer as well as other staff taxonomists. Discrepancies and disagreements that cannot be resolved internally are submitted via vouchered specimens or digital photographs to experts or systematists for resolution. Taxa lists may be changed when disagreements are resolved.

Taxonomic precision is assessed by the re-identification of a randomly-selected 10% of samples in a blind procedure. The results of the QC process are evaluated by the calculation of the Bray-Curtis similarity, the PDE and the PTD. The percent taxonomic disagreement (PTD) is calculated by the following equation:  $PTD = (1 - \frac{comp_{pos}}{N}) \times 100$  where  $comp_{pos}$  is the number of agreements and  $N$  is the total number of organisms in the larger of the 2 counts. The lower the PTD, the more similar are taxonomic results and the overall taxonomic precision is better. Rhithron's quality objective for PTD is 10% or less. The percent difference in enumeration (PDE) is calculated by the following equation:  $PDE = \frac{|n1-n2|}{n1+n2} \times 100$  where  $n1$  is the number organisms counted by the original taxonomist, and  $n2$  is the number of organisms counted by the QC taxonomist. The lower the PDE, the more precise the enumeration. Rhithron's quality objective for PDE is 5% or less. The Bray-Curtis similarity index is calculated by the following equation:  $Similarity_{ij} = \frac{2C_{ij}}{S_i+S_j}$  where  $C_{ij}$  is the sum of the lesser counts for only those taxa in common between both samples and  $S_i$  and  $S_j$  are the total number of organisms counted in each sample. This index produces a similarity value that varies between 0 and 1, where a value of 0 means the two samples are completely different and a value of 1 means the samples are completely similar.

When QC parameters exceed Rhithron's quality objectives, additional samples are randomly selected and re-identified.

Data completeness is addressed by indicating reasons why taxonomic targets are occasionally not met. These are essential data components that are required by the EPIC (v.1.7) data entry program. Reasons include: damage to specimens, poor preservation, early instar or immaturity, and life stage. When metric calculation is required by a project scope, these specimens are included in the calculation of compositional metrics or tolerance indices, but are not included in calculations of richness metrics unless their uniqueness from other specimens is confidently ascertained.

## Data management

### Scope

The goal of data management is to consistently, reliably, and accurately generate valid data products in conformance with client-specified requirements. Data management includes tracking the status of data as they are collected, transmitted, and processed.

## **Objectives**

There are several aspects of data management that are important to subsequent data quality. First, data files are accurate and data entry is error free. Second, data are delivered to the client in the format specified by the scope of work. Third, QA/QC protocols and results, and any corrective actions taken, are reported to the client, along with a detailed description of sample processing procedures. Fourth, client approval is obtained for any changes to the project protocols. Clients are informed of any problems that could affect the quality of the data. Data storage is appropriately protected.

## **QA/QC Plan**

Accomplishment of the performance objectives is evaluated by the following QA/QC plan that examines the adherence to Rhithron-specific and client-specific protocols.

### *Sample intake and chain of custody documentation*

Sample intake procedures insure that each project is complete and in appropriate condition for further processing, and that internal documentation is created that adequately tracks sample location at all times while a project is in Rhithron's custody. Sample intake is managed by the Data Technician, who checks the condition of each sample and compares sample container labeling against the client-provided chain-of-custody (COC) document. Any discrepancies, damage, or missing containers are reported by the Data Technician to the client immediately. After difficulties are rectified, the Data Technician signs the COC, makes a copy of the COC, and returns the original signed COC to the client.

The Data Technician transfers sample shipment metadata to the Rhithron database; at this time, each sample is assigned an internal laboratory identifier (RAI number), which is used to track project and individual sample progress through the laboratory to project completion. Sample metadata may include site name, client sample identifiers, replicate numbers, sample collection dates, number of jars in each sample, and other distinguishing notations, or other information that the client may require in a subsequent data deliverable. The Data Technician is responsible for generation of internal laboratory COC documents, and for assuring that COC documents are filed at project completion with other project paperwork.

Final decisions about alterations to sample processing or identification protocols are made by the client. Any circumstances or problems that may compromise the validity or usefulness of the data are reported to the client by the Chief Biologist and/or the Operations Officer.

Before sending the project, the project specifications received from the client are reviewed to make sure that all deliverables are completed to the specifications of the client's scope of work. A technical summary of QA/QC statistics for each sample and the protocols employed in sample processing and identification is prepared by the Chief Biologist and is sent to the client along with data deliverables.

Data are stored on a Dell PowerEdge 6000SC Server supported by Windows 2003 Small Business Server Operating System. The server is configured with RAID 5 hard drive and a remote server backup. A hard drive configuration setup with RAID 5 allows for fault tolerance in case of server failure and uses at least three hard drives with striping of data across two

drives and parity on the third drive, thus ensuring data recovery. Rhithron employs automated off-site data backups.

#### Technical department data

Technicians use an internal COC document to sign-out samples and to sign samples back in on completion of the sorting and sub-sampling procedures. Technicians record sample sorting and sub-sampling information on the technical department benchsheet. This information includes: the number of grids sorted, preliminary counts of organisms sorted, technician identification, time expended for sample sorting and sub-sampling, and notes related to the condition of the sample. QC technicians record the outcome of QC procedures, which are carried out on at least 10% of sorted samples. The QC parameter is reported as sorting efficiency.

The Lead Technician is responsible for transfer of technicians' data from benchsheets directly to RAILIS. The Lead Technician performs evaluations to ensure that QC is maintained throughout the sorting/sub-sampling procedure, and that client-specified protocols are followed. Technical data entry is reviewed and verified by the Data Technician, who compares benchsheet information to entered data.

#### Taxonomic data

Taxonomic data are entered by taxonomists, using a proprietary data-entry software application (EPIC v.1.7). The EPIC software uses drop-down taxa lists and incorporates several required fields for each taxonomic data entry. Required fields include correctly-spelled taxonomic name, count, uniqueness code, life stage, qualifier, and comments. Direct data entry by taxonomists minimizes errors due to misspellings, data loss or corruption at transfer, and maximizes completeness and thoroughness of the data.

Data errors associated with misidentification of specimens are corrected after QC procedures are complete. Verification of specimens by outside authorities may also result in changes to entered data. QC sample parameters are reviewed by the Taxonomy Department Quality Assurance Officer, who determines whether quality criteria for samples and projects are met.

Final data review is a line-by-line review and verification of all deliverable data: the Taxonomy Department Quality Assurance Officer performs this review. Each line of data is scanned for completeness, and each taxonomic entry is reviewed for reasonableness, which includes considerations of geographic distributions as well as ecological information implied by other taxa reported in the sample (e.g. indicators of lotic vs. lentic environs).

#### Post-analysis archiving

Sorted and unsorted sample fractions, all vials and slides are securely contained, clearly labeled with the RAI number, organized by project, and archived at the Rhithron laboratory for a period of time specified by the client or for one year, whichever period is longer. Archived sample materials are examined for integrity biannually.

#### Assessment and oversight provisions

Oversight of each project, at every stage of its progress, is provided by the project management group, which consists of the Taxonomy Department Supervisor, Vice President, and Chief Biologist. A weekly meeting of this team is held at which progress is reviewed and deficiencies, protocols, QA/QC statistics, and other pertinent topics are reported and reviewed. A project progress log, in which daily issues pertinent to each project are recorded, is kept by the Vice President and updated daily. Corrective actions are determined, and surveillance for these actions provided for by this team.

When laboratory procedures for a project are completed, the oversight group performs a complete project audit, in which the client-provided scope of work and the project progress log are reviewed. Decisions made regarding the project as it progressed through the laboratory are reviewed, uncorrected mistakes, if any, are identified, and data deficiencies, subsequently reported to the client by means of the technical summary, are discussed.

# Appendix D. Laboratory Standard Operating Procedures



Rhithron Associates, Inc.

## ***Standard Operating Procedures:***

***Working Draft***

***Revised February, 2017***

### ***Corporate Approval***

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Document Name: Standard Operating Procedures and Laboratory Quality Assurance Plan: Macroinvertebrates	Document revised: February 20, 2017 Effective date: Date of last signature
Document number: Revision 17.2.b	Issued by: Rhithron Associates, Inc. Chief Biologist

## **Macroinvertebrate samples**

### ***Health and safety warnings***

In addition to the laboratory's usual requirements, the following health and safety procedures must be followed. All proper personal protection clothing and equipment (e.g., lab coat, protective eyewear/goggles) must be worn or applied. When working with potential hazardous chemicals (e.g., 95% ethanol) or biological agents (benthic organisms or sediment) avoid inhalation, skin contact, eye contact, or ingestion. If skin contact occurs remove clothing immediately and wash/rinse thoroughly. Wash the affected skin areas thoroughly with large amounts of soap and water.

### ***Project set-up***

#### **Goals**

The goals of invertebrate project set-up procedures are to prepare samples for processing by the Technical Department and the Taxonomists while maintaining the integrity of the samples and to generate the required paperwork and computer files. The General Assistant (Figure 1) receives the samples when they arrive and is responsible for making sure that required procedures are followed.

#### **Sample intake, inventory and chain of custody**

Samples that Rhithron receives for processing are collected by clients, and delivered by commercial or postal carriers. Samples generally arrive at the laboratory's front door and the General Assistant signs delivery documents.

#### **Scope**

Using the following procedures, the General Assistant assesses the condition of the samples and preservative needs, makes sure that all samples correspond with a chain of custody or inventory (COC) provided by the client, and that all expected parts of the delivery have arrived safely. The procedures in this section pertain to macroinvertebrate sample deliveries, with alcohol (ethanol or isopropyl) preservation.

#### **Personnel**

The General Assistant is responsible for the completion of sample intake procedures, including generation of the project inventory report (which serves as the sample sign-out document for the Technical Department) upon completion of the intake and inventory procedures.

#### **Procedures**

When packages arrive, all shipping containers should be opened by the General Assistant and the COC should be located. The COC should be referred to during all following steps. Each sample jar should be removed and the level and integrity of preservative must be checked, recharging ethanol preservative when required. The label of each sample

container must be checked against the COC, and marked off as they are identified. For samples with multiple jars, all jars need to be organized together. Any leakage or damage, and any discrepancies between sample labeling and the COC document must be noted. This information must be reported to the Quality Assurance Manager (Figure 1) immediately who reports to the client by telephone or email immediately. Any discrepancies must be rectified before custody documents are signed, copied, and returned to the client.

Once all samples have been checked off against the COC, and all discrepancies have been rectified by the client, the client-provided COC must be signed and copied. The original COC is returned to the client via fax, email or USPS by the Quality Assurance Manager.

The General Assistant creates the Rhithron Associates Incorporated (RAI) Inventory file (an Excel file saved to the client folder). Entries in this file include the site name, client sample identifiers, replicate numbers, sample collection dates, the number of jars in each sample (e.g., 1 of 2, 2 of 2, etc.), and any other distinguishing data. The RAI Inventory file contains a number of worksheets: 1) "Client" Client COC information, or information gathered by the General Assistant from jar labels, 2) "Pre-check-in" Client information that is copied into Rhithron-Laboratory Information System (LIMS) format for subsequent upload and to create sample jar labels and RAI numbers (internal identifiers) that are assigned to each sample, 3) "Check-in" Client information with an additional column correct client info and consistent with the jar labels, it is a record of the jar/COC checking activity and also for recording discrepancies (communicates to Quality Assurance Manager the discreps he/she has to rectify w client) and also the Data Technician (Figure 1) adds discrepancies to the macroinvertebrate project LIMS (RAILIS) for documentation and 4) "Sample Upload" This sheet is used by the Data Technician. The General Assistant also places the physical copy of the COC into the General Assistant's filing cabinet

The General Assistant uploads the internal inventory into RAILIS and makes the sample jar labels (RAILIS output) which consist of the identification RAI numbers and labels for the all the sample jars. The General Assistant attaches these labels to the sample jars during check-in.

The General Assistant takes samples to storage site. He/she records the location of the samples in the storage site, the type of preservative, whether the samples were decanted by the client in the project table in RAILIS. The General Assistant then notifies the Data Technician (and copies the Lead Technician (Figure 1)) that the sample data are ready for upload.

The Data Technician then gets custody of the samples and data. He/she uploads the internal inventory created by the General Manager into RAILIS and sample metadata into EPIC. RAILIS also generates the benchsheets, Project Inventory Report and vial labels for use during sorting that are printed by Lead Technician when the samples are about to be processed.

### **Sample storage and transfer to the Technical Department**

Samples and projects awaiting processing are stored in the storage building behind the Rhithron laboratory. The storage building is locked and alarmed for unauthorized entry, and is equipped with a fire suppression system and fire alarms.

### **Procedures**

The General Assistant or designee transfers samples to the secure storage area. Each project is assigned to an individual storage shelf or area designated only for that project and the location is noted in RAILIS. The samples and project remain in the custody of the Technical Department until samples have been processed, at which time custody of the samples and project is transferred to the Taxonomy Department.

Upon completion of taxonomy procedures, custody transfers back to the Technical Department, which transfers all completed sample materials to the secure storage area. Stored samples and sample fractions are checked monthly for sample integrity, and preservative is added as needed. Sample and project custody are tracked and recorded in the LIMS project mapping function.

### ***Sorting for a subsample***

#### **Goal**

The goal of invertebrate sample processing procedures is to produce a random sub-sampling of a raw benthic, drift, jab, artificial substrate, or tow sample as delivered to Rhithron by our clients. Sub-samples must be produced in a standardized, repeatable manner, and sorting is quality-assured by the application of QC procedures to at least 10% of sorted samples.

Sample sorting procedures must be applied so as to achieve the following outcomes:

- The target count of organisms is achieved within the specified tolerance limits.
- The client-specified protocol is faithfully followed.
- Sorting efficiency is maintained at an average level no lower than 95% for each project, thus assuring sorting accuracy and precision.
- The appropriate paperwork is associated with the correct sample.
- All data pertinent to the sub-sampling procedure, including fraction of sample used to obtain the target number of organisms, condition of the sample, any problems associated with sorting, and quality assurance procedure outcomes and statistics, etc. are recorded on sample benchsheets. See Figure X. for an example of the sub-sampling portion of a sample benchsheet.
- Cross-contamination between samples does not occur.

#### **Considerations**

##### ***The Protocol and Procedures***

Rhithron's standard operating procedure for sorting/subsampling aquatic invertebrate samples is described in the following sections. These procedures are applicable to ethanol-preserved samples which are to be sorted to a 500-organism sub-sample. It is important to note that client specifications usually require alteration of some or all of these procedures. For each project, client specifications are reviewed by the Lead Technician with the staff before processing of samples begins. Project-specific protocols may include changes in the target number of organisms to be sorted, changes in the acceptable maximum/minimum number of organisms to be sorted, differences in the taxa which are to be included or excluded from the sorted sub-sample, whether or not an additional search for large or rare organisms is to be conducted, and other information. The project-specific protocols are found on the inventory/sign-off sheet. Every technician assigned to a project must review the project-specific protocols before beginning to process any samples. If there are any questions or uncertainty about any procedure or protocol detail, the Lead Technician should be consulted for clarification before proceeding.

### **The sample inventory and sign-out sheet**

Each project has an associated internal sample inventory, which provides spaces for sample sign-out, located in the unsorted sample staging area. See an example of a project inventory/sign-out sheet in Figure Y. The General Assistant prints the inventory sheet during the process of sample intake, and delivers it to the technical laboratory with the benchsheets and labels for the project. The inventory sheet serves as an internal chain-of-custody document for the Technical Department and includes the protocol for that project. When a project is ready to be sorted, the inventory sheet is posted. The project Technician signs out a sample when processing commences and signs the sample back in when the sorting and sub-sampling are complete. When all sorting for a project has been completed, the inventory is placed into the project folder, which is placed in the holder on the sample refrigerator for the taxonomists.

### **Technical Department sample metadata**

Technicians record sorting information by entering the information into an electronic data entry interface. Each sample is identified uniquely within the interface, in order to prevent data association with the wrong sample. Metadata for each sample is recorded by the Data Technician immediately after all steps of sample check-in. All interface fields pertaining to sample preparation and sorting, and QC procedures must be filled out completely. These procedures were revised in February 2013, and replace all preceding protocol documents.

### **Scope and personnel qualifications**

These procedures may be used by any person who has successfully completed the technical department training program. A laboratory staff member qualified to perform quality control (QC) checks (see below) must be present when samples are processed by an inexperienced staff member, or when QC checks are needed for an experienced sorter's samples.

QC procedures are performed by QC Technicians (Figure 1), who have received additional training and have at least 1 year of experience in the Technical Department and have achieved a mean sorting efficiency of at least 90% over the previous 6 months. QC systems in the Technical Department include examining the sorted substrate from at least 10% of samples in order to determine sorting efficiency for those samples. The fewer pickable organisms missed by the sorting technician, the better the sorting efficiency. QC technicians check all sorted substrate for technicians in training, and check 10% of sorted substrate for experienced technicians. The QC technician calculates the sorting efficiency for each QC sample, and records it on the benchsheet. The QC technician also participates in QC procedures for sample check-out, by double-checking sample identifiers, number of jars, and other parameters for each sample that is checked out prior to sorting and sub-sampling.

The Lead Technician serves as the department quality assurance officer. The Lead Technician provides oversight of daily operations related to sample processing, monitors QC activities to ensure conformance, periodically conducts performance and system audits, verifies the entry of data on benchsheets for completeness and appropriateness, determines sorting efficiency for each technician, performs evaluations to ensure that QC is maintained throughout the sorting and sub-sampling procedures and that the appropriate protocols are applied to all aspects of sample processing.

### **Materials and equipment**

Caton tray  
Plastic holding tray(s) for Caton screen(s)  
1000 ml Nalgene jars

ethanol, in wash bottle  
scissors  
scoops, spoons or spatula  
nitrile examination gloves  
stereoscopic microscope (Leica EZ4 or Leica S6)  
Fiberoptic or LED illuminator  
Rhithron Associates, Inc. Revision 17.2.b (replaces 14.6.b)  
Laboratory Standard Operating Procedures February 2017  
10  
3x lighted magnifier  
500 micron soil sieve  
specimen cup  
specimen handling tools (forceps, needles, pipette)  
petri dishes  
LIMS-generated sample labels (see Figure Z)  
benschsheet (pre-printed and specific to a particular sample)  
sample splitting pan (for samples with large volumes)

## **Methods**

### ***Selecting a sample and sample sign-out***

Before a Technician (Figure 1) begins sample selection, he/she should be sure that his/her workstation has been cleared of any and all materials related to another sample, including jars, vials, benschsheets, and labels. The Technician must determine the next sample to be sorted and check the inventory/sign-out sheet for protocols. The Technician initials the inventory/sign-out sheet indicating that he/she has reviewed the protocols specific to the project. The Technician then removes the correct sample from the sample staging area project shelf.

The Technician must check the inventory/sign-out sheet to make sure that the correct sample and all jars associated with that sample have been obtained and must compare the outside and inside sample labels with the inventory/sign-out sheet information for that sample. If the Technician discovers any discrepancies, he/she must notify the Lead Technician immediately. Samples that have discrepancies cannot be processed further until the problems are rectified. The Technician then selects the bench sheet associated with the sample, matching RAI numbers and other sample identifiers.

Finally, the Technician must obtain a second check of his/her work to this point from a QC Technician. The QC Technician checks to see that all jars are collected, that all jar identifiers match one another and the benschsheet. The QC Technician checks all information against the inventory and checks off each item on the inventory/sign-out sheet. The QC Technician checks that the proper bench sheet and labels for the sample have been selected. When all of the information has been checked, the QC Technician initials next to the RAI number on the top left hand corner of front page of benschsheet.

### **Sample preparation**

Before beginning the preparation procedure, the Technician should be sure that all sorting equipment is thoroughly cleaned and free of organisms. He/she should carefully examine sieves, Caton tray components, and all other sorting equipment using a 3x lighted magnifier before proceeding.

The Technician should wear nitrile gloves while preparing samples. The sample should be gently mixed in its jar(s). The Technician decants the alcohol preservative while pouring the sample out of each jar, using the 500-micron soil sieve (US #35) and the plastic

Caton holding tray in the rinsing sink. If the sample is contained in several jars, empty and wash each jar one at a time. If the alcohol is not excessively stained or diluted, retain it for reuse as preservative for the unsorted portion of sample, otherwise, discard the alcohol down the rinsing sink drain. Pour the sample out into the 500-micron sieve. Retrieve all internal sample labels and rinse them of all debris and organisms into the sieve. Check once again to make sure that the internal labels correspond with the bench sheet and the inventory. Save all labels and staple them to the back lower left of bench sheet once they are dried. Gently rinse the sample jar, retaining all contents on the sieve. All material from the jar(s) is now contained on the 500-micron sieve.

Using the 500-micron sieve, gently wash the sample, running cold tap water over it to remove any fine material. Transfer the sieve contents onto the Caton screen. If there are several sample jars, empty each onto the Caton screen as rinsing proceeds. Rinse the sieve onto the Caton screen to collect any organisms or debris that may have been retained in the sieve. Inspect the sieve with the 3x lighted magnifier. Be sure the sieve is clean to prevent cross contamination between samples. Place all organisms retrieved from the sieve onto the Caton screen.

Place the Caton screen into the plastic holding tray. Add enough water to spread the sample evenly over the Caton screen. Move the sample into the corners of the pan using gloved hands, forceps or other equipment. Agitate the tray and screen to help spread the sample. If the sample is composed of different types of material, be sure that there is thorough mixing of all types. Lift the Caton screen out of the plastic tray to drain. Pour off the water from the plastic tray and set the screen back into the tray. Add just enough water to the tray so that it barely covers the screen while it is in the tray. Be careful not to add so much water that the sample material floats around.

### **Precautions**

Never allow a sample to dry out during any stage of preparation or sorting. To ensure that there is no cross-contamination between samples, before beginning sample preparation, and after completion of preparation, be sure to examine sieves, Caton screens, spatulas, spoons, scoops, and all other materials to make sure that no organisms or sample residues are adhering to surfaces. Sample preparation and sorting is often complicated by the materials present in the samples. In every case, your goal is to mix materials as thoroughly as possible and randomly distribute mixed materials over the Caton screen. Do not separate different kinds of materials in the Caton tray.

### **Obtaining the sub-sample by sorting**

Use a random number generator, such as a pair of dice, to select 3 grids (i.e., 10% of the contents of the Caton tray) for sorting. Three grids are sorted to ensure that the subsample material is representative of the overall sample. Use the Caton cookie-cutter device to delineate the selected grids, moving the sample material very slightly to push the material in the selected grid together, in order to make it easier to get it out of the tray. Using a scoop, scraper, spoon, or other appropriate equipment, lift the contents of the selected grids into petri dishes, one grid in each dish, and add water from a wash bottle to the sample material to avoid desiccation and to disperse the material in the petri dishes. Depending on the consistency of the sample material, it may be necessary to use scissors during these steps.

Examine the Caton screen for any organisms remaining within each sampled grid. Use the following rules when dealing with organisms that lie on the line between two grids. First, an organism belongs to the grid where its head is. Second, if you can't determine where the head is, the organism belongs to the grid containing most of its body. Third, if part of an organism's head is on either side of the line, pick the organism if the line is on

the "top" of the grid or the right side of the grid. Be sure not to let any sample fractions dry out or get spilled. Cover the Caton tray while sorting the selected grids, and do not allow the material in the Caton tray to move around.

Examine the contents of each of the selected grids under the microscope, using at least 6x magnification, and determine whether the total number of organisms in these grids appears to exceed the target count (500 organisms). If the number of organisms appears to exceed the target number (500 organisms) in the collective three grids, then each grid is quartered, and a quarter is randomly selected for initial sorting. Sort the quarter volume of the first grid, and sort a quarter volume of each of the next 2 grids. If the number of organisms that have been picked in one quarter volume of each of the 3 grids is below the target, then another fraction of each grid would be processed until the target number of 500 and a maximum of 600 (500+20%) is reached. All organisms from the selected fractions, or grids, must be sorted to minimize bias.

If the target is not reached when the three grids are completely picked and fully processed (including organisms recovered during QC checks), subsequent grids would be randomly selected and each picked to completion until 500+20% organisms is reached. If the target number of organisms is reached within the fraction of the first or second grids, sorting is stopped for that sample, on completion of the sorting of the corresponding fraction (i.e., the third grid quarter would not be processed).

All samples are sorted using 6-10x magnification (Leica EZ-4 or Leica S6 stereomicroscopes). Remove the invertebrates from the sample material in each grid, using forceps. Place organisms for identification in the taxonomy vial(s). Sort through the substrate material thoroughly. Using mechanical counters, keep a running count of the total number of organisms picked as well as a separate count of the number of chironomids and the number of worms.

Do not remove or count empty snail or bivalve shells, specimens of surface-dwelling or strict water column arthropod taxa (e.g., Collembola, Veliidae, Gerridae, Notonectidae, Corixidae, Cladocera, or Copepoda), or incidentally-collected terrestrial taxa. Also, do not count fragments such as legs, antennae, gills, or wings. For Oligochaeta, attempt to remove and count only whole organisms and fragments that include the head; also, do not count fragments that do not include the head. If a sorter is unsure as to whether a specimen should be counted or not, he or she should place the organism in the sort vial without counting it (the final count is made by the taxonomist).

If the last grid (or quarter grid) processed results in a count greater than 20% above target number (i.e., more than 600 organisms), use the overcount adjustment procedure described here. First, obtain a petri dish with a "sliced-pie" template. Second, pour out all the sorted organisms, and distribute the organisms as evenly as possible in the petri-dish. Add ethanol as needed. Third, using the dice, randomly select a pie slice and remove all of the organisms from the associated pie slice, counting the removed organisms as you go. If an organism is lying on the dividing line between 2 slices, use the criteria described above to determine whether or not to remove it. Fourth, continue random selection of pie slices and removal of organisms until the number of organisms in the final subsample will be within the protocol tolerance for the project. Similar to picking grids and quarters, the entire slice must be picked. Fifth, place all removed organisms back into the unsorted substrate. Place all organisms left in the petri-dish in the 20 mL scintillation vial for the taxonomists. Finally, document this procedure in the "Sample Notes" section of your bench sheet (Figure x).

During the sorting/sub-sampling procedure, empty sorted substrate into a labeled jar. Use ethanol (preferably recycled from the initial sample rinsing) to preserve the sorted substrate. Place the sorted substrate jar on the shelves reserved for sorted substrate. Randomly-chosen samples for QC are selected from these jars. QC Technicians will perform sorting efficiency checks on 10% of samples in the project. See procedures for QC in Rhithron's Laboratory Quality Assurance Plan document. All material that is not sorted (i.e. all material remaining in the Caton tray and all quartered grid portions that were not sorted) must be returned to the original sample jar(s) and preserved with fresh ethanol. Place the original sample jar(s) on the shelves reserved for processed sample fractions. Unsorted and sorted sample fractions are archived for QC and potential return to the client. Place subsampled organisms into the labeled specimen cup, and place the specimen cup in the sorted sample storage area.

The Technician should initial the inventory/sign-out sheet (Figure y) in the appropriate place, indicating that all sample fractions have been returned to the right places.

<p><b>Technician Information:</b></p> <p>Sort Tech: _____</p> <p>Sort Date: _____</p> <p>Sort Hours: _____</p> <p># of Vials: _____</p> <p>Large / Rare Vial: Y N or NA</p> <p>Minutes Searched: _____</p> <p>Preservative Level: _____</p> <p>Volume Protocol: S or L ____/120</p> <p>Substrate Amount: _____</p> <p>No. Grids: _____</p> <p>Total Count: _____</p> <p style="padding-left: 20px;"># Bugs: _____</p> <p style="padding-left: 20px;"># Midges: _____</p> <p style="padding-left: 20px;"># Worms: _____</p> <div style="border: 1px dashed black; padding: 5px; margin-top: 10px;"> <p>QC Tech: _____</p> <p>QC Date: _____</p> <p>QC Pct.: 25 % or ____ %</p> <p>QC Result: # _____ P / F</p> <p>QC Rectified: Y / N</p> <p>Sort Efficiency: _____ %</p> </div> <p>Technician Notes: _____</p>	<p><b>Multiple Technicians:</b></p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Tech:</th> <th style="width: 10%;">Total:</th> <th style="width: 10%;">BB:</th> <th style="width: 10%;">M:</th> <th style="width: 10%;">W:</th> <th style="width: 10%;">Time:</th> <th style="width: 10%;">Grids:</th> </tr> </thead> <tbody> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> </tbody> </table> <p><b>Grid Locations and Counts:</b></p> <table border="1" style="width: 100%; border-collapse: collapse; text-align: center;"> <tbody> <tr><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr> <tr><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr> <tr><td>13</td><td>14</td><td>15</td><td>16</td><td>17</td><td>18</td></tr> <tr><td>19</td><td>20</td><td>21</td><td>22</td><td>23</td><td>24</td></tr> <tr><td>25</td><td>26</td><td>27</td><td>28</td><td>29</td><td>30</td></tr> </tbody> </table>	Tech:	Total:	BB:	M:	W:	Time:	Grids:																						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
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**Figure X.** Sample benchsheet detail: Technician information and sorting/sub-sampling data fields.

**Figure Y.** Project inventory/sign-out sheet  
Figure to be added.



**Figure Z.** LIMS-generated labels, including RAI number label for specimen cup lid, sample label for inside the specimen cup, and RAI number labels for sample fraction vials.

### ***Identification and enumeration***

#### **Goal**

The goal of the taxonomic portion of sample processing is to identify and enumerate organisms accurately and precisely, to the taxonomic resolution required by the project. Bias is minimized, data are reported completely, and the quality and integrity of data are consistent. Materials related to the project, including labeled microscope slides, labeled vials with identified organisms, and laboratory benchsheets are handled carefully and are archived on the completion of identification and enumeration, and after all QA/QC procedures and data reviews have been completed. Deliverables such as reference collections and voucher collections are assembled accurately and completely. Higher levels of taxonomy applied to organisms that cannot be identified to taxonomic targets are explained and qualified in all cases. Life stages are accurately recorded in the data. A citation list of primary and secondary taxonomic literature sources is maintained for each project, to maximize comparability of data.

#### **Considerations**

##### ***The Protocol and Procedures document***

The taxonomic resolution for general arthropod organisms may vary from project to project, and is typically specified by the client. Before a Taxonomist (Figure 1) begins any sample, he/she should review the client-specified taxonomic requirements: requirements for each project are located on the P&P (Protocol and Procedures) document. P&P documents are found in the "Protocol and Procedures" binder, which is kept in the sorted sample storage area. For reference purposes, Taxonomists can also access P&P documents on the Rhithron network server at \\RHITHRON1\Data\Taxonomy\Taxonomic resolution templates. Look for the Rhithron project name to retrieve the appropriate document. Every Taxonomist assigned to a project must sign off on the appropriate P&P document before beginning any sample.

### ***The sample sign-out sheet***

Each project has an associated sample sign-out sheet, which is located in the sorted sample staging area. When a Taxonomist obtains a sample to begin identification and enumeration, he/she should consult this sheet for an available sample. Locate the sample, and put your initials and the date in the appropriate column next to your sample's RAI number. This form serves as an internal chain-of-custody document for the Taxonomy Department. Make sure that you check the RAI number on the specimen cup against the RAI number on the sign-out sheet. When identification of the sample is completed, initial the sample sign-out sheet, indicating that the sample has been returned to the sorted sample storage area.

The Taxonomist must ensure that his/her workstation is clear of any material related to any other sample before beginning. Projects requiring reference collections will have related procedures described in a later section. Taxonomists will record their data in the EPIC data entry software (EPIC v. 1.7). Procedures for the use of the EPIC data entry software (EPIC v. 1.7) are included later in this document. The EPIC data entry software application uploads data automatically to the Rhithron LIMS. ITIS (<http://www.itis.usda.gov>) taxonomic serial numbers (TSN) are associated with each taxon in the Rhithron LIMS. Taxon entries in the Rhithron LIMS are periodically matched to the ITIS TSNs. Each new taxon entry into the Rhithron LIMS includes the ITIS TSN when it is available. Some taxonomic entries in the Rhithron LIMS do not have associated ITIS TSNs. Such entries include valid names which occur in the literature but are not included in ITIS "Slashed" taxonomies, such as *Pericoma/Telmatoscopus*, *Chelifera/Metachela*, etc., taxonomic groups, such as the *Rhyacophila vemna* Group, *Cricotopus tremulus* Group, etc. and taxa that are apparently undescribed, such as *Nanocladius* sp. D (Epler). For these, no ITIS TSN is reported with the taxon entry.

### ***Technical Department benchsheets***

Technicians record sorting information on paper benchsheets (Figure x), which are delivered to the sorted sample storage area along with the sorted samples. Records from these benchsheets that are pertinent to the Taxonomists are: the number of vials used for the sorted sample, the count of general arthropods, the count of chironomids, and the count of oligochaetes. Technical Department benchsheets are located in file folders in the sorted sample storage area.

## **Transfer of sorted samples to the Taxonomy Department**

Sorted samples are delivered from the Technical Department to the Taxonomy Department by the Project Manager or his designee. Sorted samples are stored in green-lidded medical specimen cups. The RAI sample identification number (RAI number) is located on a label on the specimen cup lid. The specimen cup contains vials with sorted specimens; each vial contains a small label with the RAI number. Typically, there is a vial with chironomids, a vial with oligochaetes, one or more vials with general arthropods, and potentially other vials as

well. There is also a large sample label inside the specimen cup (Figure Z). All RAI numbers on and in the specimen cup should be identical. Immediately report any discrepancies to the Taxonomy Department Supervisor (Figure 1), who will resolve these problems. Samples with label discrepancies cannot be processed further until problems are resolved.

## ***General arthropod identifications***

### **Scope, related procedures and personnel qualifications**

Rhithron's standard operating procedure for identification and enumeration of the general arthropod portions of aquatic invertebrate samples is described in the following sections. It is important to note that client specifications usually require alteration of some or all of these procedures. For each project, client specifications are reviewed with the taxonomy staff before identification of samples begins. The review includes the taxa lists from previous projects from the same area or client when available, the protocols specified for the project, and the quality control results from previous projects from the same area or client when available. The Taxonomy Department Supervisor oversees this review; in addition, taxonomic protocols for each project are documented in a project log, which is available in the taxonomy laboratory throughout the progress of the project. Project-specific protocols are also available on the Rhithron network server. These precautions ensure that the appropriate, client-specified protocols are followed for each project.

These procedures are applicable to pre-sorted non-chironomid, non-oligochaete portions of benthic, drift, and tow samples. However, chironomids and/or oligochaetes may be included in the general arthropod identification procedures, if taxonomic resolution requirements specify family level or coarser level identifications for these groups. Information about taxonomic resolution specifications for a project may be found in the Protocol and Procedure manual or on the Rhithron network server.

These procedures apply to the Taxonomy staff who report to the Taxonomy Department Supervisor. Taxonomy staff members who identify general arthropod portions of invertebrate samples hold SFS Level II-certifications in Eastern and Western EPT and General Arthropod taxa groups. Under the guidance of the Taxonomy Department Supervisor, Taxonomists are responsible for working as a team to ensure currency with changes in taxonomic nomenclature, geographic distributions, and other issues relevant to performing these procedures. Taxonomists interact with other professionals in the field via listservs, meetings and workshops offered by professional societies (e.g. SFS, NBAW, SAFIT, etc.), and informal communications.

Quality control (QC) systems include "blind" re-identification of at least 10% of samples (see procedures for QC in the Rhithron Laboratory Quality Assurance Plan document). The Taxonomy Department Supervisor is responsible for the oversight for these procedures.

### **Materials and equipment**

- 20mL scintillation vial with cap
- Specimen handling tools (forceps, pipette, needles, etc.)
- Ethanol wash bottle with ethanol
- Watch glass
- Sorting palette
- Vial or tube rack
- Label supplies
- Stereoscopic microscope (Leica S8A)
- Fiberoptic illuminator (Dolan-Jenner MI 150 or MI 151)

Networked computer, located at the microscope, for access to EPIC data entry software

## **Methods**

To prevent any sample cross-contamination, a Taxonomist should ensure that hi/her workstation is clear of all material related to any other sample before beginning. The Taxonomist should obtain a sample from the sorted sample storage area. The Taxonomist puts his/her initials and the date on the sample sign-out sheet, to indicate that he/she has accepted custody of, and responsibility for, the sample. Obtain the Technical Department benchsheet for that sample.

At the workstation, carefully open the specimen container and remove the large sample label and the vials of sorted specimens. Make sure that all RAI numbers on labels, in vials, and on benchsheets correspond with the sample that was signed out. Make sure that the number of vials entered by the Technician on the Technician benchsheet corresponds with the number of vials found in the specimen cup. Immediately notify the Taxonomy Department Supervisor if any discrepancies are encountered. Samples with discrepancies cannot be processed further until problems are resolved.

The Taxonomist initials the front of the large label and places it in the 20mL scintillation vial so that it can be read from the outside of the vial. Fill the vial about halfway with fresh ethanol. He/she should locate the vials with general arthropods. These are usually labeled "BB" with marker pen on the cap of the vial. Carefully spill the contents into a watch glass, checking for organisms that may stick to the cap or to the vial. Add ethanol as needed to keep the sample organisms covered.

Using 10x – 80x magnification, the Taxonomist sorts the organisms, using the sorting palette as needed. He/she should examine each organism and identify each to the required taxonomic resolution, referring as needed to the P&P document on the server or in the Protocol and Procedures binder. Identifications are made with reference to resources in Rhithron's taxonomic resource library, which is a collection of books and documents in hard copy (in the Taxonomy Department Library) and/or in electronic form on Rhithron's network server at: \\SERVER1\Data\Taxonomy\Taxonomic Resources. As you work, replenish the ethanol level in the sorting palette as needed. Cover the palette and watch glass if your work is interrupted for more than a few minutes. Do not allow any sample portion to dry out at any time.

When identifying organisms, the Taxonomist should use the following conventions, unless the client-specified protocol calls for something different. Damaged organisms are identified and counted only if 1) an arthropod fragment includes the head and thorax, 2) a mollusk shell actually contains an animal, and 3) if it is the only representative of that taxon in the sample, it should be identified if possible. Immature and early instar organisms are identified and counted only if 1) they can confidently be associated with other mature identifiable specimens or 2) if they are the only representative of a taxon in the sample.

The Taxonomist counts each taxon. Record taxa names, counts, life stage, uniqueness, qualifiers, reference collection information, and taxonomic certainty ratings in the EPIC data entry program, using the procedures found in a later section of this document. Terms such as "uniqueness" and "qualifiers" are defined there. Place identified organisms into the 20mL scintillation vial. Add ethanol to the vial as needed. If specimens have been removed from the sample for inclusion in the reference collection, place a label in the 20mL scintillation vial with the taxon name and number of specimens removed for each taxon.

When the Taxonomist has completed the general arthropod identification for the sample, cap the 20mL scintillation vial and place it into the green-lidded specimen cup. Make sure that all other vials (chironomids, oligochaetes, etc.) are replaced in the specimen cup. Return the cup and the technical department benchsheet to the sorted sample storage area.

The Taxonomist should clean-up after the general arthropods of a sample have been identified includes thorough washing and drying of watch glasses, sorting palettes, forceps and needles, and any other equipment that was used in the identification process.

### ***Chironomid identifications***

#### **Scope, related procedures and personnel qualifications**

Rhithron's standard operating procedure for identification and enumeration of the chironomid portions of aquatic invertebrate samples is described in the following sections. It is important to note that client specifications usually require alteration of some or all of these procedures. For each project, client specifications are reviewed with the taxonomy staff before identification of samples begins. The review includes the taxa lists from previous projects from the same area or client when available, the protocols specified for the project, and the quality control results from previous projects from the same area or client when available. The Taxonomy Department Supervisor oversees this review; in addition, taxonomic protocols for each project are documented in a project log, which is available in the taxonomy laboratory throughout the progress of the project. Project-specific protocols are also available on the Rhithron network server. These precautions ensure that the appropriate, client-specified protocols are followed for each project.

These procedures are applicable to pre-sorted chironomid-only portions of benthic, drift, and tow samples. These procedures are applicable when the client-specified taxonomic resolution for chironomids is genus resolution or finer. If chironomids are to be identified to family or sub-family/tribe, follow the procedures for "General arthropod identifications" above. Information about taxonomic resolution specifications for a project may be found in the Protocol and Procedure manual or on the Rhithron network server.

These procedures apply to the Taxonomy staff, which reports to the Taxonomy Department Supervisor. Taxonomy staff members who analyze chironomid sample portions hold SFS Level II-certifications in Eastern and Western Chironomidae taxa groups. Under the guidance of the Taxonomy Department Supervisor, Taxonomists are responsible for working as a team to ensure currency with changes in taxonomic nomenclature, geographic distributions, and other issues relevant to performing these procedures. Taxonomists interact with other professionals in the field via listservs, meetings and workshops offered by professional societies (e.g. SFS, NBAW, SAFIT, etc.), and informal communications.

Quality control (QC) systems include "blind" reidentification of at least 10% of samples (SEE PROCEDURES FOR QC IN RHITHRON'S LABORATORY QUALITY ASSURANCE PLAN DOCUMENT). Internal tracking and chain-of-custody documentation is required in the Taxonomy Department. The taxonomy department supervisor is responsible for the oversight for these procedures.

#### **Materials and equipment**

Polycone-capped vial  
Specimen handling tools (forceps, pipette, needles, etc.)  
Ethanol wash bottle with ethanol  
Watch glass

Sorting palette  
Vial or tube rack  
Stereoscopic microscope (Leica S8A)  
Fiberoptic illuminator (Dolan-Jenner MI150 or MI151)  
Standard microscope slides  
Standard microscope slide cover slips  
CMC-10 mounting medium (Masters Chemical Company)  
Mounting medium applicator  
Glass marking pen  
Label supplies  
Slide map benchsheet  
Compound microscope (Leica DM1000, Olympus BX51) with 40x, 60x and 100x magnification, and other objectives as needed  
Networked computer, located at the microscope, for access to EPIC data entry software

### **Methods**

The Taxonomist should ensure that his/her workstation is clear of all material related to any other sample. This step is intended to prevent any potential sample cross-contamination. The Taxonomy staff member should obtain a sample from the sorted sample storage area. The Taxonomist puts his/hers initials and the date on the sample sign-out sheet and obtains the Technical Department benchsheet for that sample.

At his/her workstation, carefully open the specimen container and remove the vial of sorted chironomid specimens. This vial is usually labeled "M" with marker pen on the vial cap. The Taxonomist should make sure that all RAI numbers on labels, in vials, and on benchsheets correspond with the sample that was signed out and that the number of vials entered by the technician on the Technician benchsheet corresponds with the number of vials found in the specimen cup. The Taxonomist should immediately notify the Taxonomy Department Supervisor if any discrepancies are encountered. Samples with discrepancies cannot be processed further until problems are resolved.

The Taxonomist should carefully spill the contents into a watch glass, checking for organisms that may stick to the cap or to the vial. If the "morphotyping" protocol is used, replace the small vial label and fill the vial about halfway with fresh ethanol. If the "complete slide mounting" protocol is used, the label may be discarded.

Using 10x – 80x magnification, the Taxonomist should sort the chironomids, using the sorting palette as needed. He/she should make slide mounts as needed, using CMC-10 mounting medium, applying a coverslip. A label, indicating the sample identifier (RAI number) and the number of the slides in the sequence for that sample, should be placed on each slide. Allowing CMC mounted material to cure overnight enables the medium to digest soft tissues that may interfere with identification. If permanent slide mounts are required, clear enamel is used to ring the coverslip.

The Taxonomist should examine slide-mounted organisms under the compound microscope. Locate all essential diagnostic characteristics, using the appropriate key or taxonomic resource literature. Identify each to the required taxonomic resolution, referring as needed to the P&P document on the server or in the Protocol and Procedures binder. Identifications are made with reference to resources in Rhithron's taxonomic resource library, which is a collection of books and documents in hard copy (in the Taxonomy Department Library) and/or in electronic form on Rhithron's network server at:  
\\SERVER1\Data\Taxonomy\Taxonomic Resources.

The Taxonomist should use the slide map benchsheet to record identifications for slide-mounted chironomids. The slide map serves as a record of the number of midges on slides, and their locations, which facilitates QC procedures. Recording the location of each slide-mounted midge also allows taxonomists to easily locate problematic organisms for discussions and rectifications.

When the Taxonomist is identifying chironomids he/she should use the following conventions, unless the client-specified protocol calls for something different. First, damaged organisms are identified and counted only if a chironomid fragment includes the head and thorax or the damaged chironomid is the only representative of that taxon in the sample. Second, early instar chironomids are identified and counted only if they can confidently be associated with other mature identifiable specimens or they are the only representative of a taxon in the sample.

The Taxonomist counts each taxon and records taxa names, counts, life stage, uniqueness, qualifiers, reference collection information, and taxonomic certainty ratings for slide-mounted and non-slide-mounted chironomids in the EPIC data entry program, using the procedures found in a later section of this document. Terms such as "uniqueness" and "qualifiers" are defined there. Midges not mounted on slides are placed in a polycarbonate vial with the sample identifier label. The Taxonomist should initial this label on the back.

If a reference collection is required, the Taxonomist should circle slide-mounted organisms that are to be included in the collection, using the glass marker pen. The Taxonomist should place non-mounted specimens in reference collection vials with the appropriate taxon labels. If specimens from a sample have been included in a reference collection, the Taxonomist should place a label in the vial of sorted chironomid specimens indicating the taxa that have been included, and the number of each taxon included in the collection. After identification and when the CMC medium is no longer fluid, the Taxonomist should organize slides by sample number in a slide box exclusive for the project.

When the Taxonomist has completed the chironomid identification for the sample, he/she caps the polycarbonate vial and places it into the green-lidded specimen cup. He/she should make sure that all other vials (general arthropods, oligochaetes, etc.) are replaced in the specimen cup. The specimen cup and the technical department benchsheet are returned to the sorted sample storage area.

Clean-up after a sample's chironomids are identified includes thorough washing and drying of watch glasses, sorting palettes, forceps and needles, and any other equipment that was used in the identification process.

### ***Oligochaete identifications***

#### **Scope, related procedures and personnel qualifications**

Rhithron's standard operating procedure for identification and enumeration of oligochaete portions of aquatic invertebrate samples is described in the following paragraphs. It is important to note that client specifications usually require alteration of some or all of these procedures. For each project, client specifications are reviewed with the taxonomy staff before identification of samples begins. The review includes the taxa lists from previous projects from the same area or client when available, the protocols specified for the project, and the quality control results from previous projects from the same area or client when available. The Taxonomy Department Supervisor oversees this review; in addition, taxonomic protocols for each project are documented in a project log, which is available in the taxonomy laboratory throughout the progress of the project. Project-specific protocols are

also available on the Rhithron network server. These precautions ensure that the appropriate, client-specified protocols are followed for each project.

These procedures are applicable to pre-sorted oligochaete-only portions of benthic, drift, and tow samples. These procedures are applicable when the client-specified taxonomic resolution for oligochaetes is genus resolution or finer. If oligochaetes are to be identified to sub-class (i.e. "Oligochaeta"), follow the procedures for "General arthropod identifications" in Procedure 1. above. Information about taxonomic resolution specifications for a project may be found in the Protocol and Procedure manual or on the Rhithron network server. See part b.1 below to locate this information.

Two procedures for oligochaete identification are described here: one protocol uses morphotyping as a time-and-effort saving procedure; the other protocol calls for slide mounting of each and every specimen. Client specifications may require complete slide mounting, otherwise, Rhithron's standard procedure is to carefully morphotype specimens, slide mounting representatives of each taxon or identifying unmounted specimens when possible.

These procedures apply to the Taxonomy staff, which reports to the Taxonomy Department Supervisor. Taxonomy staff members who analyze oligochaete sample portions hold SFS Level II-certifications in Oligochaeta. Under the guidance of the Taxonomy Department Supervisor, Taxonomists are responsible for working as a team to ensure currency with changes in taxonomic nomenclature, geographic distributions, and other issues relevant to performing these procedures. Taxonomists interact with other professionals in the field via listservs, meetings and workshops offered by professional societies (e.g. SFS, NBAW, SAFIT, etc.), and informal communications.

Quality control (QC) systems include "blind" reidentification of at least 10% of samples. See procedures for QC in Rhithron's Laboratory Quality Assurance Plan document. Internal tracking and chain-of-custody documentation is required in the Taxonomy Department. The Taxonomy Department Supervisor is responsible for the oversight for these procedures. These procedures were revised in February 2013, and replace all preceding protocol documents.

### **Materials and equipment**

Polycone-capped vial  
Specimen handling tools (forceps, pipette, needles, etc.)  
Ethanol wash bottle with ethanol  
Watch glass  
Sorting palette  
Vial or tube rack  
Stereoscopic microscope (Leica S8A)  
Fiberoptic illuminator (Dolan-Jenner 150 or 151)  
Standard microscope slides (part number)  
Standard microscope slide cover slips (part number)  
CMC-10 mounting medium (Masters Chemical Company)  
Mounting medium applicator  
Glass marking pen  
Label supplies  
Slide map benchsheet  
Compound microscope (Leica DM1000, Olympus BX51) with 40x, 60x and 100x magnification, and other objectives as needed  
Networked computer, located at the microscope, for access to EPIC data entry software

## **Methods**

The Taxonomist should ensure that his/her workstation is clear of all material related to any other sample. This step is intended to prevent any potential sample cross-contamination. The Taxonomy staff member should obtain a sample from the sorted sample storage area. The Taxonomist puts his/hers initials and the date on the sample sign-out sheet and obtains the Technical Department benchsheet for that sample.

At his/her workstation, carefully open the specimen container and remove the vial of sorted oligochaete specimens. This vial is usually labeled "W" with marker pen on the vial cap. The Taxonomist should make sure that all RAI numbers on labels, in vials, and on benchsheets correspond with the sample that was signed out and that the number of vials entered by the technician on the Technician benchsheet corresponds with the number of vials found in the specimen cup. The Taxonomist should immediately notify the Taxonomy Department Supervisor if any discrepancies are encountered. Samples with discrepancies cannot be processed further until problems are resolved.

The Taxonomist should carefully spill the contents into a watch glass, checking for organisms that may stick to the cap or to the vial. If the "morphotyping" protocol is used, replace the small vial label and fill the vial about halfway with fresh ethanol. If the "complete slide mounting" protocol is used, the label may be discarded.

Using 10x – 80x magnification, the Taxonomist should sort the oligochaetes, using the sorting palette as needed. Slide mounts should be made by the Taxonomist as needed, using CMC-10 mounting medium and a coverslip. A label should be placed on each slide, indicating the sample identifier (RAI number) and the number of the slide in the sequence for that sample. Allowing CMC mounted material to cure overnight enables the medium to digest soft tissues that may interfere with identification. If permanent slide mounts are required, clear enamel is used to ring the coverslip.

The Taxonomist should examine slide-mounted organisms under the compound microscope. And locate all essential diagnostic characteristics, using the appropriate key or taxonomic resource literature. The Taxonomist should identify each to the required taxonomic resolution, referring, as needed, to the P&P document on the server or in the Protocol and Procedures binder. Identifications are made with reference to resources in Rhithron's taxonomic resource library, which is a collection of books and documents in hard copy (in the Taxonomy Department Library) and/or in electronic form on Rhithron's network server at: \\SERVER1\Data\Taxonomy\Taxonomic Resources.

The Taxonomist should use the slide map benchsheet to record identifications for slide-mounted oligochaetes. The slide map serves as a record of the number of worms on slides, and their locations, which facilitates QC procedures. Recording the location of each slide-mounted worm also allows taxonomists to easily locate problematic organisms for discussions and rectifications.

The Taxonomist should use the following conventions, unless the client-specified protocol calls for something different. First, damaged organisms are only identified and counted if the head and enough additional segments for identification are present or the damaged organism is the only representative of that taxon in the sample. Second, immature organisms are identified and counted only if they can confidently be associated with other mature identifiable specimens or if they are the only representative of a taxon in the sample.

The Taxonomist should count each taxon and record taxa names, counts, life stage, uniqueness, qualifiers, reference collection information, and taxonomic certainty ratings for

slide-mounted and non-slide-mounted oligochaetes in the EPIC data entry program, using the procedures found in a later section of this document. Terms such as “uniqueness” and “qualifiers” are defined there. Worms not mounted on slides are placed in a polycone vial with the sample identifier label. The Taxonomist should initial this label on the back.

If a reference collection is required, the Taxonomist should circle slide-mounted organisms that are to be included in the collection, using the glass marker pen. The Taxonomist should place non-mounted specimens in reference collection vials with the appropriate taxon labels. If specimens from a sample have been included in a reference collection, the Taxonomist should place a label in the vial of sorted oligochaete specimens indicating the taxa that have been included, and the number of each taxon included in the collection. After identification and when the CMC medium is no longer fluid, the Taxonomist should place slides, organized by sample number, in a slide box exclusive for the project.

When the Taxonomist has completed the oligochaete identification for the sample, he/she should cap the polycone vial and place it into the green-lidded specimen cup. In addition, he/she should make sure that all other vials (general arthropods, chironomids, etc.) are replaced in the specimen cup. The cup and the Technical Department benchsheet should be returned to the sorted sample storage area.

Clean-up after a sample’s oligochaetes are identified includes thorough washing and drying of watch glasses, sorting palettes, forceps and needles, and any other equipment that was used in the identification process.