

Large Wood Addition and Gulf Hagas eDNA Sampling Report

Methodology and Results for eDNA Samples Collected in Partnership Between Maine-eDNA and the Appalachian Mountain Club

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Data Access, Accreditation, and Use

Raw fastq files and metadata are available through the Maine-eDNA data portal, and have also been sent directly to Sarah Nelson. If issues arise in accessing data, scripts, or other associated materials, contact Beth Y. Davis at bethy.davis4@gmail.com. Use and re-analysis of the data and metadata is up to the discretion of AMC, though it is strongly recommended that the following people are credited in any future outputs due to their contributions:

Karina Ricker - Sampling, field logistics

Carolyn Ziegra - Sampling, field logistics

Miriam Ritchie - Sampling, field logistics, GIS support

Cathy Poppenwimer - GIS support

Sarah Nelson - Project coordination, logistics, editing

Steve Tatko - Project coordination, logistics

Kylie Holt - Sample processing, laboratory management

Andrew Rominger - Project coordination, field logistics, data analysis support

Beth Y. Davis - Project coordination, sampling, field logistics, sample processing, data analysis

If re-analysis of the raw data is performed, use of a versioning tool such as GitHub is strongly recommended.

Sample Procedures

eDNA samples were collected with two primary goals in mind:

- 1) Understand the community impacts of large wood addition restoration (LWA) on stream reaches in the eastern regions of the 100 Mile Wilderness
- 2) Conduct baseline fish and macroinvertebrate community surveys at streams and lakes in the Gulf Hagas (GH) and western 100 Mile Wilderness regions

For these goals, eDNA samples were sequenced using two primers - **MiFish-U**, targeting the 12S mitochondrial ribosomal subunit gene region (12S), and the pair **BF2 + BR2**, targeting the cytochrome c oxidase subunit gene region (COI). For the purposes of this report, the target taxa for the MiFish-U primer are bony fish, particularly salmon, trout, and arctic char species, and the target taxa for BF2 + BR2 are benthic macroinvertebrates, particularly damselflies, dragonflies, and other biomonitoring taxa.

Sampling

eDNA samples were collected by AMC field teams from August to September in 2022 and in July of 2023. LWA sites were roughly classified based on temporal and spatial categories of Pre and Post LWA installation, or if a site was Upstream, Downstream, Internal, or Parallel to the LWA installation area. Control sites for both temporal and spatial categories were also chosen along nearby reaches that had not previously undergone restoration work, with some sites repeated in both 2022 and 2023. GH sites were chosen to collect lake and stream survey data across a large area, and were only sampled in 2022.

At each sampling site, eDNA samples consisted of three subsamples of one liter each for a total of three liters of stream water used to sample eDNA at each site. Subsamples were collected midstream just underneath the stream surface, midstream 7-10 cm above the stream substrate, and on the side of the stream near a bank just underneath the surface. This placement of samples was chosen to capture any microhabitat variation in eDNA caused by either species' stream use or differing flow rates.

Each subsample was either filtered in the field using a hand pump or placed on ice and transported to the lab for vacuum filtration within 24 hours of collection, with site subsamples aggregated by filtering onto the same filter paper(s). For each day of sample collection, one liter of DI or other sterile water was filtered first on the filtering equipment to serve as a negative control for that day, and was carried along with the field team until all other samples for the day were collected.

Filtering

Filtration of eDNA samples utilized **single-use 47 mm 0.45 µm cellulose nitrate filter papers**. All lab surfaces and reusable tools were sterilized by either soaking in 10% bleach solution for at least 10 minutes and drying fully before use, or applying bleach wipes and allowing the bleach to stand for 10 minutes before applying a DI water rinse. Before and after each use of the lab for filtration, a germicidal UV light was run for 30 minutes to an hour. In 2022, filter papers were frozen in absolute ethanol at -20°C before extraction. These samples required additional filtration to remove ethanol, which was followed by letting samples evaporate in the cleaned lab in sterilized petri dishes to remove any remaining ethanol. Afterwards samples were frozen at -20°C until extraction. In 2023, samples were simply frozen in DNA LoBind tubes at -20°C before extraction, and did not require ethanol removal. When a site required use of multiple filter papers due to high sedimentation in the subsamples, all filters for the site were kept within the same tube and physically aggregated during extraction. For each round of filtration, at least one negative control sample was filtered alongside site samples for use as a laboratory blank.

DNA Extraction

See the included ExtractionProtocol document for the full extraction protocol, based on the Qiagen PowerSoil Pro protocol developed by Geneva York for Maine-eDNA use, and adapted by Kylie Holt.

All surfaces were wiped down with bleach wipes followed by DI water before use, and pipette tips and other sensitive equipment cleaned with DNA-Off. Other reusable materials such as forceps and tube racks were cleaned by soaking in 10% bleach for 10 minutes, rinsing five times, then allowed to dry fully before use. An hour of germicidal UV exposure was performed in the lab before and after each round of extraction, and extraction times were chosen to minimize overlap with other projects' lab usage. At least one negative control sample was extracted in each round of extraction to continue serving as a laboratory blank as well as field blank. Extracts were eluted to a 100 µL volume, then frozen at -20°C before PCR and sequencing.

PCR & Sequencing

The PCR protocol for both primers used a 25 µL reaction with 9 µL of nuclease-free MB grade H₂O, 1.25 µL of each forward and reverse primer, 12.5 µL of Quantabio HiFi ToughMix 2X, and 1 µL of extracted DNA. At least one field blank was PCRs with eDNA samples for continued use as a negative control.

The 12S primer pair includes:

- A 21 basepair forward primer: GTCGGTAAAACCTCGGCCAGC
- A 27 basepair reverse primer: GTTGACCCTAATCTATGGGGTGATAC

And amplifies a region approximately 170 basepairs in length

The COI primer pair includes:

- A 20 basepair forward primer: GCHCCHGAYATRGCHTTYCC
- A 20 basepair reverse primer: TCDGGRTGNCCRAARAAYCA

And amplifies a region approximately 420 basepairs in length

PCR for the 12S samples underwent a protocol of 98°C for 10 seconds, 61°C for 5 seconds, and 68°C for 1 second, repeated for 38 cycles. Samples were refrigerated at 20°C until sequencing.

PCR for the COI samples underwent a protocol of 95°C for 10 minutes and a 35-cycle repeat of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, with a final 72°C for five minute stage. Samples were refrigerated at 20°C until sequencing.

Nextera adapters were added to each primer set for Illumina MiSeq sequencing, performed at the University of Maine CORE DNA Sequencing Center. The 12S and COI samples were sequenced on separate runs due to the expected differences in region length, and run for 300 cycles each.

The Nextera adapters were removed during cleanup, leaving just the original primers and the sample sequences. Sample sequences were divided into forward and reverse reads for each sample, available as individual FASTQ files.

Sample Processing

All samples were cleaned and prepared for analysis using the **DADA2 workflow**. For a full list of packages used, see the `FullConfig_AMC.R` file.

Once cleaned, sample sequenced underwent decontamination based on removing identified sequences present in the negative control samples from the rest of the dataset. Samples which were reduced to 0 reads at any step of the DADA2 or decontam process were removed from further analysis consideration.

The included .RMD file includes the code and text for this report, as well as instructions on how to run each section of code if re-analysis is desired. For re-analysis, use of a version-control system such as GitHub is highly recommended.

For additional analyses pertaining specifically to LWA diversity and impacts on stream communities, see Chapter 2 of the included thesis manuscript (Applications of Environmental DNA Metabarcoding in Stream Biomonitoring) and the **associated GitHub repo**.

Gamma Diversity - Overall Species Results

In 2022, a total of 19 unique taxonomic families were detected through the 12S primer across all sites. Of those, the following 15 families were detected in Gulf Hagas sites:

```
## [1] "Anguillidae"      "Catostomidae"     "Centrarchidae"    "Clupeidae"
## [5] "Cottidae"        "Fundulidae"       "Gasterosteidae"  "Leuciscidae"
## [9] "Percidae"         "Petromyzontidae" "Pholidae"        "Plethodontidae"
## [13] "Pleuronectidae"   "Salmonidae"       "Suidae"
```

These families included the following 20 species:

```
## [1] "Alosa pseudoharengus"      "Anguilla rostrata"
## [3] "Brevoortia tyrannus"       "Catostomus commersonii"
## [5] "Chrosomus neogaeus"        "Fundulus diaphanus"
## [7] "Gyrinophilus porphyriticus" "Hippoglossoides platessoides"
```

```

## [9] "Lepomis auritus"           "Margariscus margarita"
## [11] "Myoxocephalus scorpius"   "Notemigonus crysoleucas"
## [13] "Perca flavescens"         "Petromyzon marinus"
## [15] "Pholis ornata"            "Pungitius pungitius"
## [17] "Rhinichthys atratulus"    "Salvelinus fontinalis"
## [19] "Semotilus atromaculatus"   "Sus scrofa"

```

Only three species were detected in GH sites using the COI primer, each belonging to a different taxonomic family. Of the three, only *Daphnia catawba* and *Leptodiaptomus minutus* are invertebrates, a water flea and copepod respectively.

```

## [1] "Daphnia catawba"          "Haemorhous purpureus"   "Leptodiaptomus minutus"

```

LWA sites in 2022 also found 15 unique families and 20 unique species using 12S in 2022, with the following families and species:

```

## [1] "Anguillidae"      "Castoridae"       "Catostomidae"     "Centrarchidae"
## [5] "Clupeidae"        "Cottidae"         "Gadidae"         "Leuciscidae"
## [9] "Percidae"          "Plethodontidae"   "Pleuronectidae"  "Salmonidae"
## [13] "Scombridae"       "Sebastidae"       "Suidae"
## [1] "Anguilla rostrata"   "Brevoortia tyrannus"
## [3] "Castor canadensis"   "Catostomus commersonii"
## [5] "Chrosomus neogaeus"   "Clupea harengus"
## [7] "Cottus cognatus"     "Gadus ogac"
## [9] "Gyrinophilus porphyriticus" "Hippoglossoides platessoides"
## [11] "Margariscus margarita"   "Melanogrammus aeglefinus"
## [13] "Micropterus salmoides"   "Perca flavescens"
## [15] "Rhinichthys atratulus"    "Salvelinus fontinalis"
## [17] "Scomber scombrus"       "Sebastes norvegicus"
## [19] "Semotilus atromaculatus"   "Sus scrofa"

```

In 2023, LWA sites found another 15 unique families and 21 unique species (totaling 19 unique families and 26 unique species detected in LWA sites for both years), detecting the following:

```

## [1] "Anguillidae"      "Castoridae"       "Catostomidae"     "Centrarchidae"
## [5] "Cervidae"          "Clupeidae"        "Cottidae"         "Esocidae"
## [9] "Leuciscidae"       "Merlucciidae"    "Percidae"         "Plethodontidae"
## [13] "Pleuronectidae"   "Salmonidae"       "Soricidae"
## [1] "Anguilla rostrata"   "Brevoortia tyrannus"
## [3] "Castor canadensis"   "Catostomus commersonii"
## [5] "Chrosomus neogaeus"   "Clupea harengus"
## [7] "Cottus cognatus"     "Esox niger"
## [9] "Gyrinophilus porphyriticus" "Hippoglossoides platessoides"
## [11] "Margariscus margarita"   "Merluccius bilinearis"
## [13] "Micropterus salmoides"   "Notemigonus crysoleucas"
## [15] "Odocoileus virginianus"   "Perca flavescens"
## [17] "Rhinichthys atratulus"    "Salmo trutta"
## [19] "Salvelinus fontinalis"    "Semotilus atromaculatus"
## [21] "Sorex cinereus"

```

COI data for LWA sites overlaps moderately with 12S results, but exhibits a greater diversity of results. 33 unique species across 22 families were detected for 2022 LWA sites, and 63 species across 35 families were detected in 2023.

LWA 2022 COI Family detections:

```

## [1] "Baetidae"           "Caeciliusidae"     "Chironomidae"     "Chloroperlidae"

```

```

## [5] "Chydoridae"      "Cicadellidae"      "Cottidae"        "Ephemerellidae"
## [9] "Heptageniidae"    "Hydropsychidae"    "Leptophlebiidae"  "Leuciscidae"
## [13] "Leuctridae"       "Lumbriculidae"     "Perlodidae"      "Philopotamidae"
## [17] "Picidae"          "Plethodontidae"   "Salmonidae"      "Saturniidae"
## [21] "Simuliidae"       "Siphlonuridae"

```

LWA 2022 COI Species detections:

```

## [1] "Anisota virginiensis"      "Baetis phoebus"
## [3] "Baetis tricaudatus"       "Chydorus brevilabris"
## [5] "Cottus cognatus"          "Diphetor hageni"
## [7] "Diplectrona modesta"      "Dolophilodes distinctus"
## [9] "Dryobates pubescens"       "Epeorus fragilis"
## [11] "Ephemerella dorothaea"    "Ephemerella invaria"
## [13] "Eurycea bislineata"       "Eurylophella funeralis"
## [15] "Habrophlebiodes americana" "Isoperla bilineata"
## [17] "Leuctra ferruginea"       "Maccaffertium vicarium"
## [19] "Microtendipes pedellus"    "Paraleptophlebia adoptiva"
## [21] "Paraleptophlebia debilis"  "Polypedilum aviceps"
## [23] "Prosimulium mixtum"       "Rhinichthys atratulus"
## [25] "Salvelinus fontinalis"     "Siphlonurus typicus"
## [27] "Stenacron interpunctatum"  "Stylodrilus heringianus"
## [29] "Sweltsa onkos"            "Teloganopsis deficiens"
## [31] "Tvetenia paucunca"        "Typhlocyba gillettei"
## [33] "Valenzuela flavidus"

```

LWA 2023 COI Family detections:

```

## [1] "Baetidae"           "Brachycentridae"    "Caeciliusidae"    "Capniidae"
## [5] "Catostomidae"        "Chironomidae"       "Cottidae"         "Dipseudopsidae"
## [9] "Elateridae"          "Ephemerellidae"    "Ephemeridae"      "Glossosomatidae"
## [13] "Gomphidae"          "Heptageniidae"     "Hydropsychidae"  "Lepidostomatidae"
## [17] "Leptophlebiidae"     "Leuciscidae"        "Libellulidae"     "Limnephilidae"
## [21] "Lumbricidae"         "Lumbriculidae"     "Naididae"         "Nemouridae"
## [25] "Pediidae"           "Peltoperlidae"     "Perlodidae"       "Philopotamidae"
## [29] "Plethodontidae"     "Pteronarcyidae"    "Salmonidae"       "Sciaridae"
## [33] "Simuliidae"          "Siphlonuridae"     "Spongillidae"

```

LWA 2023 COI Species detections:

```

## [1] "Acerpenna macdunnoughi"  "Agapetus pinatus"
## [3] "Amphinemura wui"         "Aporrectodea caliginosa"
## [5] "Baetis intercalaris"     "Baetis phoebus"
## [7] "Baetis pluto"            "Baetis tricaudatus"
## [9] "Catostomus commersonii"  "Chaetogaster diastrophus"
## [11] "Chironomus melanescens"  "Cottus cognatus"
## [13] "Cratyna vagabunda"      "Cricotopus bicinctus"
## [15] "Desmognathus fuscus"     "Diphetor hageni"
## [17] "Diplectrona modesta"     "Dolophilodes distinctus"
## [19] "Drunella cornutella"     "Epeorus fragilis"
## [21] "Epeorus pleuralis"       "Epeorus vitreus"
## [23] "Ephemera varia"          "Ephemerella dorothaea"
## [25] "Ephemerella invaria"     "Eukiefferiella claripennis"
## [27] "Eurylophella funeralis"  "Habrophlebia vibrans"
## [29] "Habrophlebiodes americana" "Isoperla bilineata"
## [31] "Lanthus parvulus"        "Lepidostoma costale"
## [33] "Litobrancha recurvata"   "Maccaffertium vicarium"

```

```

## [35] "Margariscus margarita"
## [37] "Paracapnia angulata"
## [39] "Paraleptophlebia debilis"
## [41] "Phylocentropus placidus"
## [43] "Polypedilum aviceps"
## [45] "Prosimum mixtum"
## [47] "Pteronarcys proteus"
## [49] "Rhinichthys atratulus"
## [51] "Semotilus atromaculatus"
## [53] "Simulium verecundum"
## [55] "Spongilla lacustris"
## [57] "Stylodrilus heringianus"
## [59] "Tallaperla maria"
## [61] "Tvetenia paucunca"
## [63] "Wormaldia moesta"

## [35] "Micrasema sprulesi"
## [37] "Paraleptophlebia adoptiva"
## [39] "Pedicia albivitta"
## [41] "Plauditus dubius"
## [43] "Pristina aequiseta"
## [45] "Pseudanostirus triundulatus"
## [47] "Pycnopsyche scabripennis"
## [49] "Salvelinus fontinalis"
## [51] "Simulium tuberosum"
## [53] "Siphlonurus barbaroides"
## [55] "Stenacron interpunctatum"
## [57] "Sympetrum vicinum"
## [59] "Teloganopsis deficiens"
## [61] "Valenzuela flavidus"

```

Regional Differences

In 2022, GH and LWA sites showed different eDNA results for both primers, with a small number of families and species that were only found in one region.

12S

2022 GH Only (Family)	2022 GH Only (Species)	2022 LWA Only (Family)	2022 LWA Only (Species)
Centrarchidae	<i>Lepomis auritus</i> (Redbreast sunfish)	Castoridae	<i>Castor canadensis</i> (North American beaver)
Clupeidae	<i>Alosa pseudoharengus</i> (Alewife)	Centrarchidae	<i>Micropterus salmoides</i> (Largemouth bass)
Cottidae	<i>Myoxocephalus scorpius</i> (Shorthorn sculpin ^h)	Clupeidae	<i>Clupea harengus</i> (Atlantic herring)
Fundulidae	<i>Fundulus diaphanus</i> (Banded killifish)	Cottidae	<i>Cottus cognatus</i> (Slimy sculpin)
Gasterosteidae	<i>Pungitius pungitius</i> (Ninespine stickleback)	Gadidae	<i>Melanogrammus aeglefinus</i> (Haddock)
Leuciscidae	<i>Notemigonus crysoleucas</i> (Golden shiner) ^a	Gadidae	<i>Gadus ogac</i> (Greenland cod)
Petromyzontidae	<i>Petromyzon marinus</i> (Sea lamprey)	Scombridae	<i>Scomber scombrus</i> (Atlantic mackerel)
Pholidae	<i>Pholis ornata</i> (Saddleback gunnel ^h)	Sebastidae	<i>Sebastes norvegicus</i> (Golden redfish ^h)

^a *Notemigonus crysoleucas* (Golden shiner) was not found in LWA sites in 2022, but was detected in LWA sites in 2023.

COI

2022 GH Only (Family)	2022 GH Only (Species)
Daphniidae	<i>Daphnia catawba</i> (Water flea)
Fringillidae	<i>Haemorhous purpureus</i> (Purple finch)
Diaptomidae	<i>Leptodiaptomus minutus</i> (Copepod)

2022 LWA Only (Family)	2022 LWA Only (Species)
Saturniidae	<i>Anisota virginiensis</i> (Pink-striped oakworm moth)
Heptageniidae	<i>Epeorus fragilis</i> (Flatheaded mayfly)
Perlodidae	<i>Isoperla bilineata</i> (Two-lined stripetail)
Simuliidae	<i>Prosimulium mixtum</i> (Mixed-up black fly)
Chydoridae	<i>Chydorus brevilabris</i> (Chydorid)
Ephemerellidae	<i>Ephemerella dorothaea</i> (Spiny crawler mayfly)
Heptageniidae	<i>Maccaffertium vicarium</i> (Flatheaded mayfly)
Leuciscidae	<i>Rhinichthys atratulus</i> (Eastern blacknose dace)
Cottidae	<i>Cottus cognatus</i> (Slimy sculpin)
Plethodontidae	<i>Eurycea bislineata</i> (Northern two-lined salamander)
Chironomidae	<i>Microtendipes pedellus</i> (Non-biting midge)
Chloroperlidae	<i>Sweltsa onkos</i> (Ontario sallfly)
Picidae	<i>Dryobates pubescens</i> (Downy woodpecker)
Leptophlebiidae	<i>Habrophlebiodes americana</i> (Prong-gilled mayfly)
Chironomidae	<i>Polypedilum aviceps</i> (Midge)
Chironomidae	<i>Tvetenia paucunca</i> (Bean-sprout gill non-biting midge)

Year Differences

The following tables display families and/or species that were only found in one set of LWA data (either 2022 only or 2023 only)

12S

2022 Only (Family)	2022 Only (Species)	2023 Only (Family)	2023 Only (Species)
Suidae	<i>Sus scrofa</i> (Wild boar)	Esocidae	<i>Esox niger</i> (Chain pickerel)
Gadidae	<i>Gadus ogac</i> (Greenland cod)	Soricidae	<i>Sorex cinereus</i> (Cinereus shrew)
Gadidae	<i>Melanogrammus aeglefinus</i> (Haddock)	Leuciscidae	<i>Notemigonus crysoleucas</i> (Golden shiner)
Sebastidae	<i>Sebastes norvegicus</i> (Golden redfish ^h)	Merlucciidae	<i>Merluccius bilinearis</i> (Silver hake)
Scombridae	<i>Scomber scombrus</i> (Atlantic mackerel)	Cervidae	<i>Odocoileus virginianus</i> (White-tailed deer)
-	-	Salmonidae	<i>Salmo trutta</i> (Brown trout)

COI

2022 Only (Family)	2022 Only (Species)
Saturniidae	<i>Anisota virginiensis</i> (Pink-striped oakworm moth)
Chydoridae	<i>Chydorus brevilabris</i> (Chydorid)
Picidae	<i>Dryobates pubescens</i> (Downy woodpecker)
Plethodontidae	<i>Eurycea bislineata</i> (Northern two-lined salamander)
Leuctridae	<i>Leuctra ferruginea</i> (Eastern needlefly)
Chironomidae	<i>Microtendipes pedellus</i> (Non-biting midge)
Siphlonuridae	<i>Siphlonurus typicus</i> (Minnow mayfly)
Chloroperlidae	<i>Sweltsa onkos</i> (Ontario sallfly)

2022 Only (Family)	2022 Only (Species)
Cicadellidae	<i>Typhlocyba gillettei</i> (Leaf hopper)
2023 Only (Family)	2023 Only (Species)
Baetidae	<i>Acerpenna macdunnoughi</i> (Minnow mayfly)
Glossosomatidae	<i>Agapetus pinatus</i> (Saddle case-maker caddisfly)
Nemouridae	<i>Amphinemura wui</i> (Spring stonefly)
Lumbricidae	<i>Aporrectodea caliginosa</i> (Earthworm ^h)
Baetidae	<i>Baetis intercalaris</i> (Minnow mayfly)
Baetidae	<i>Baetis pluto</i> (Minnow mayfly)
Catostomidae	<i>Catostomus commersonii</i> (White sucker)
Naididae	<i>Chaetogaster diastrophus</i> (Annelid worm)
Chironomidae	<i>Chironomus melanescens</i> (Non-biting midge)
Sciaridae	<i>Cratyna vagabunda</i> (Black fungus gnat)
Chironomidae	<i>Cricotopus bicinctus</i> (Non-biting midge)
Plethodontidae	<i>Desmognathus fuscus</i> (Dusky salamander)
Ephemerellidae	<i>Drunella cornutella</i> (Spiny crawler mayfly)
Heptageniidae	<i>Epeorus pleuralis</i> (Quill gordon)
Heptageniidae	<i>Epeorus vitreus</i> (Flatheaded mayfly)
Ephemeridae	<i>Ephemera varia</i> (Yellow drake)
Chironomidae	<i>Eukiefferiella claripennis</i> (Fly)
Leptophlebiidae	<i>Habrophlebia vibrans</i> (Pronggilled mayfly)
Gomphidae	<i>Lanthus parvulus</i> (Northern pygmy clubtail)
Lepidostomatidae	<i>Lepidostoma costale</i> (Caddisfly)
Ephemeridae	<i>Litobrancha recurvata</i> (Burrower mayfly)
Leuciscidae	<i>Margariscus margarita</i> (Allegheny pearl dace)
Brachycentridae	<i>Micrasema sprulesi</i> (Humpless casemaker caddisfly)
Capniidae	<i>Paracapnia angulata</i> (Angulate snowfly)
Pediciidae	<i>Pedicia albivitta</i> (Giant eastern crane fly)
Dipseudopsidae	<i>Phylocentropus placidus</i> (Caddisfly)
Baetidae	<i>Plauditus dubius</i> (Small minnow mayfly)
Naididae	<i>Pristina aequiseta</i> (Aquatic oligochaete)
Elateridae	<i>Pseudanostirus triundulatus</i> (Three-spotted click beetle)
Pteronarcyidae	<i>Pteronarcys proteus</i> (Appalachian salmonfly)
Limnephilidae	<i>Pycnopsyche scabripennis</i> (Giant red sedge)
Leuciscidae	<i>Semotilus atromaculatus</i> (Common creek chub)
Simuliidae	<i>Simulium tuberosum</i> (Twinn's black fly)
Simuliidae	<i>Simulium verecundum</i> (Black fly)
Siphlonuridae	<i>Siphlonurus barbaroides</i> (Mayfly)
Spongillidae	<i>Spongilla lacustris</i> (Freshwater sponge)
Libellulidae	<i>Sympetrum vicinum</i> (Yellow-legged meadowhawk)
Peltoperlidae	<i>Tallaperla maria</i> (Roach-like stonefly)
Philopotamidae	<i>Wormaldia moesta</i> (Fingernet caddisfly)

^hThese species are unexpected detections due to differences in known habitat or region - detection may be due to contamination, tissue presence from bait or fecal matter, or valid presence.

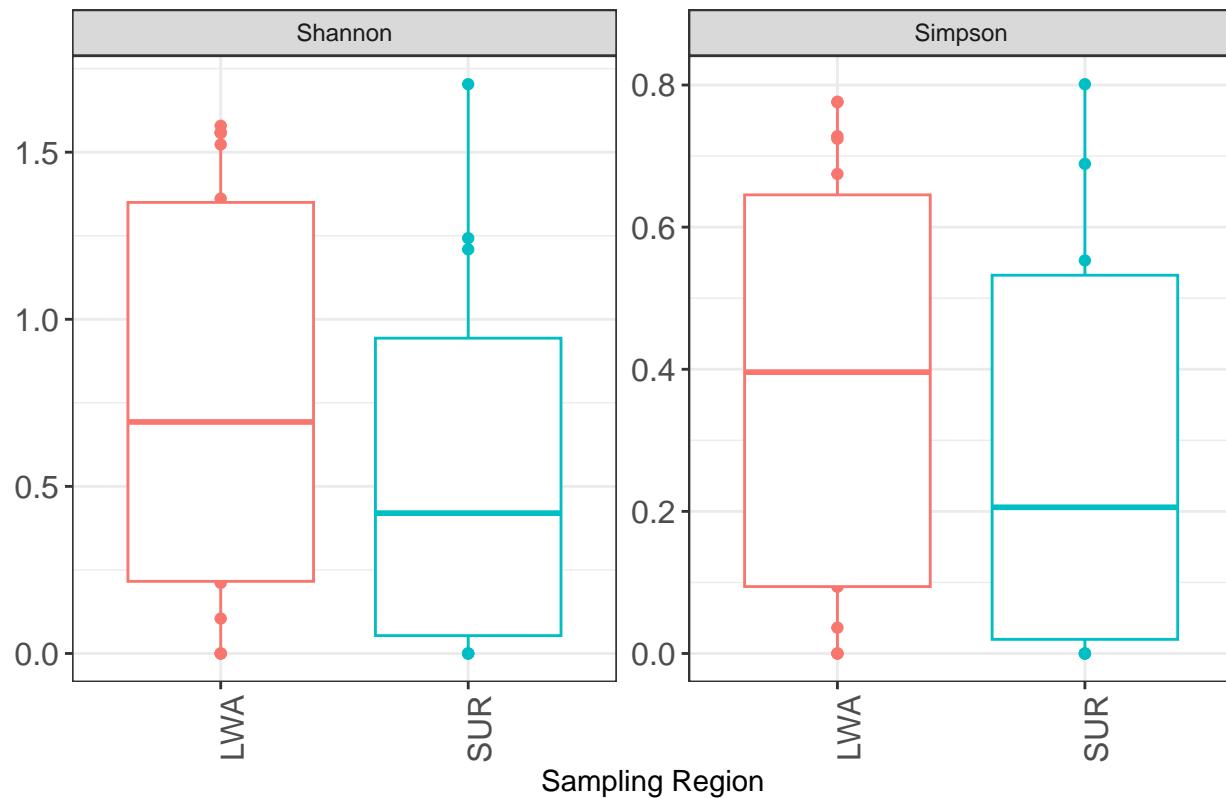
Alpha Diversity

The following plots visualize the differences between detected species richness (number of species) and evenness for each primer (how much a single or few species dominates population counts), comparing sampling region and treatment type. Two measures are currently used:

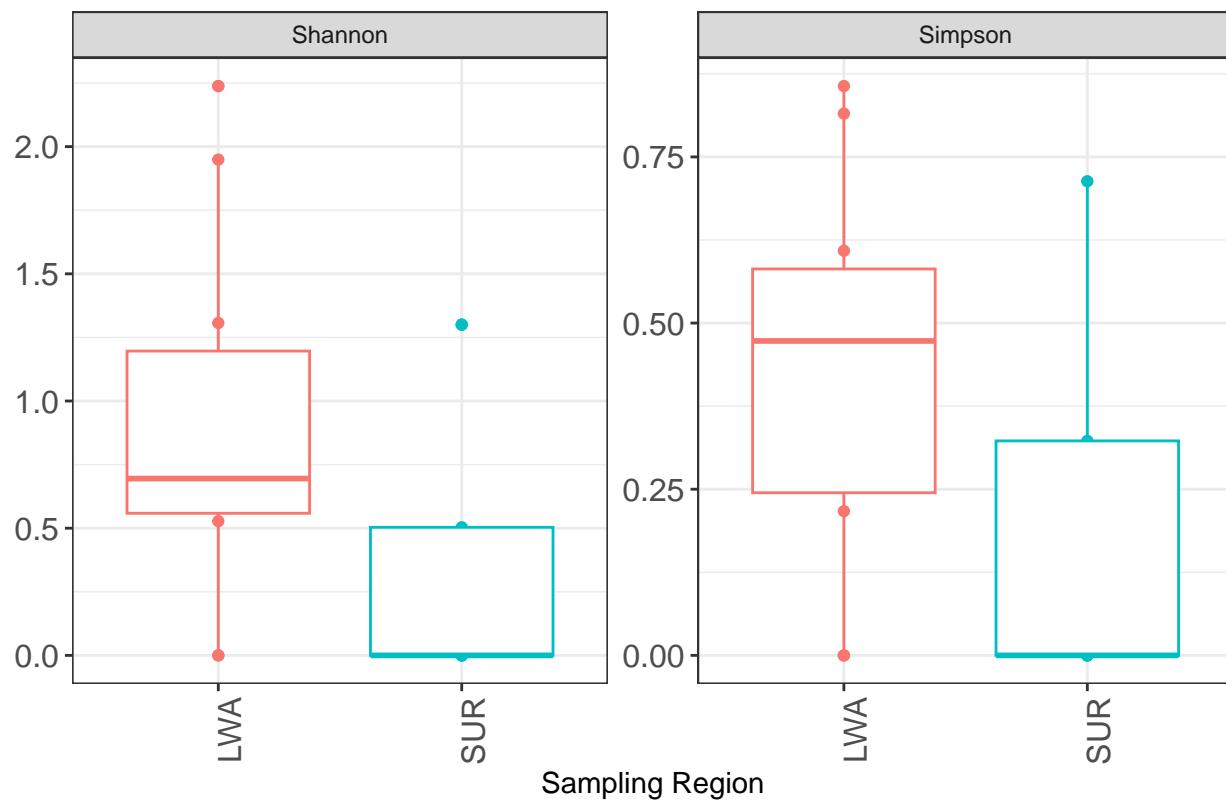
- Shannon estimates species richness, with higher values indicating higher diversity and richness
- Simpson estimates both richness and evenness, ranging from 0 to 1, with higher values indicating greater richness/evenness

(Reminder that SUR = Gulf Hagas sites)

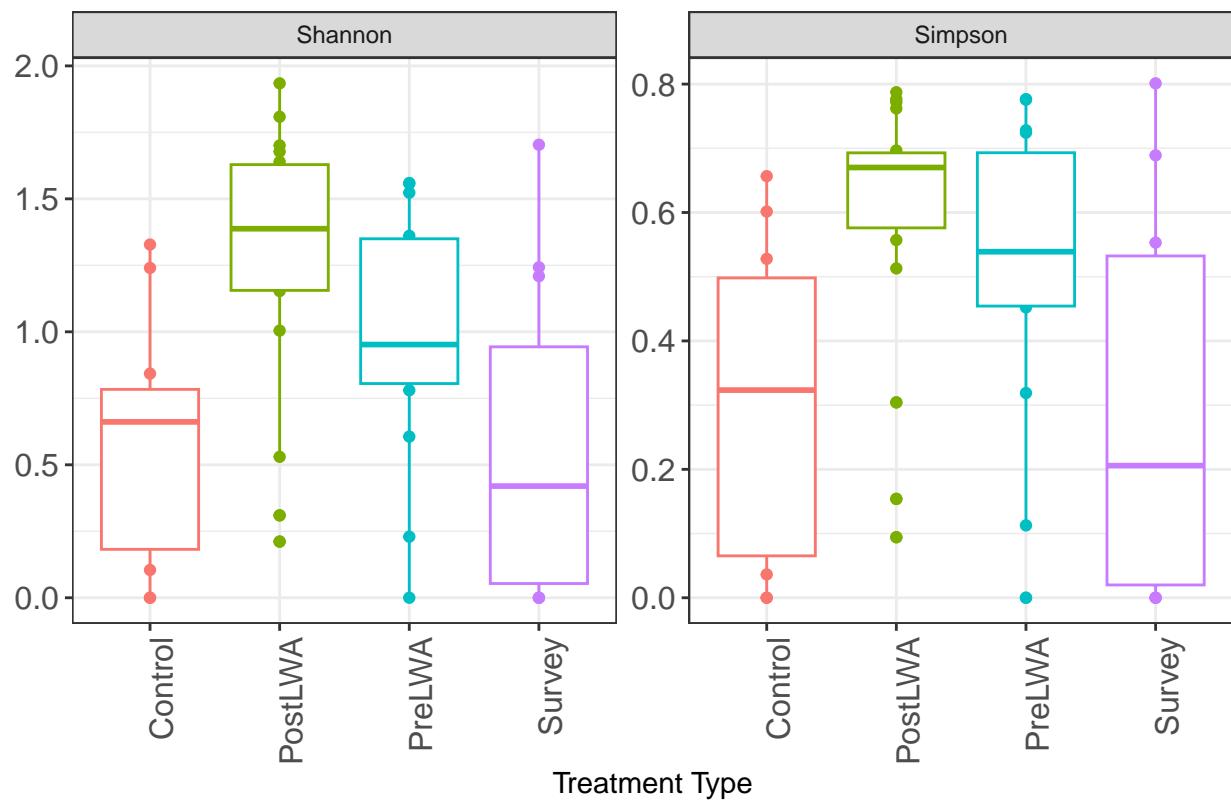
12S Alpha Diversity Measures – 2022 Only



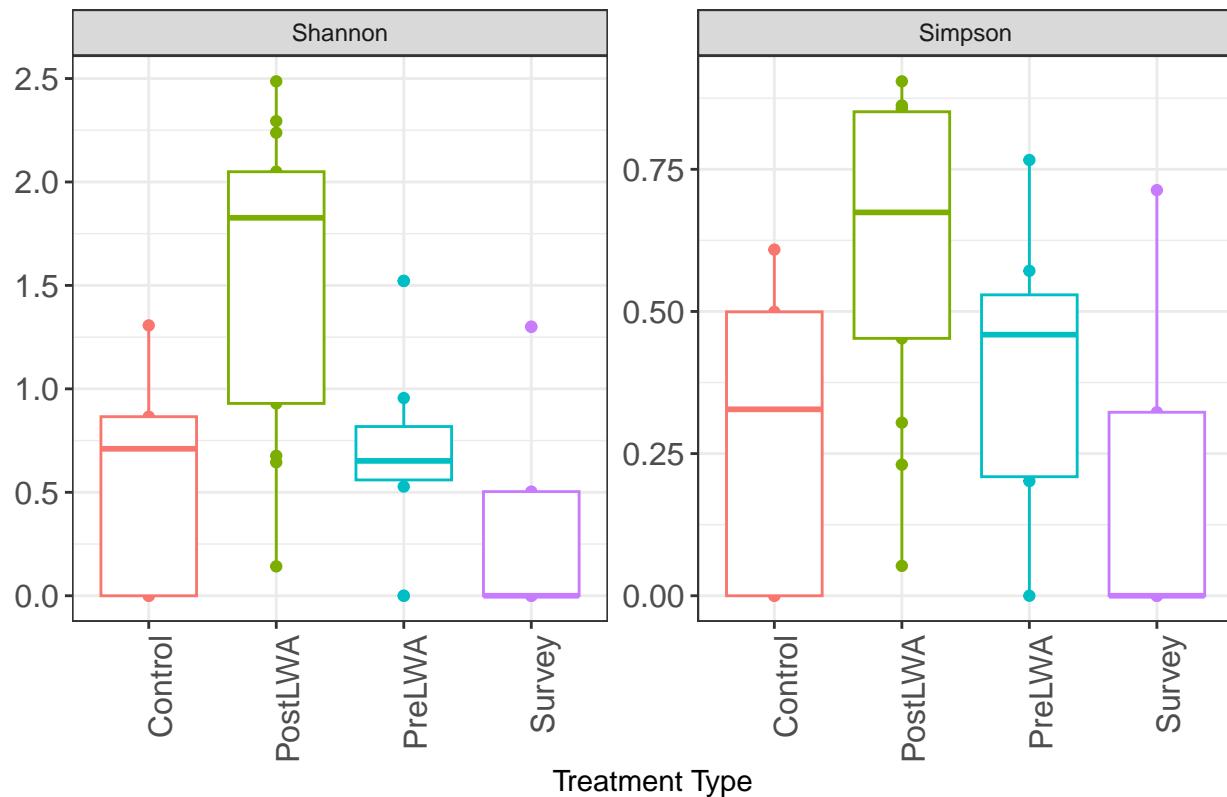
COI Alpha Diversity Measures – 2022 Only



12S Alpha Diversity Measures – All AMC Sites



COI Alpha Diversity Measures – All AMC Sites



Kruskal-Wallis test results on the significance of differences between all LWA and SUR sites, including LWA treatment differences, (TreatType) and all 2022 and 2023 sites (Year), divided by primer and diversity measure. Values less than 0.05 indicate significant difference based on the comparison parameter and diversity measure:

```
##          12S TreatType, All   COI TreatType, All 12S Year, All COI Year, All
## Shannon      0.001265996      0.01300662  0.002081814  0.06692431
## Simpson     0.003808189      0.03600615  0.005834436  0.13385859
```

Kruskal-Wallis test results on the significance of differences between LWA treatments, position of the sampling site in relation to the nearest LWA installment (Position), and sampling year (Year), divided by primer and diversity measure. Values less than 0.05 indicate significant difference based on the comparison parameter and diversity measure:

```
##          12S TreatType, LWA   COI TreatType, LWA 12S Position COI Position
## Shannon      0.002415295      0.02848646  0.001527511  0.2138209
## Simpson     0.005608533      0.05300615  0.002349226  0.2732103
##          12S Year, LWA COI Year, LWA
## Shannon     0.01933069      0.2915433
## Simpson     0.04099037      0.4050190
```

Kruskal-Wallis test results on the significance of differences between sampling region (LWA or GH, Region) and if the site was a stream or lake (Stream/Lake), divided by primer and diversity measure. Values less than 0.05 indicate significant difference based on the comparison parameter and diversity measure:

```
##          12S Region  COI Region 12S Stream/Lake COI Stream/Lake
## Shannon    0.3389555    0.1049351    0.5547137    0.2874954
## Simpson    0.3836271    0.1700818    0.5881729    0.4250307
```

The next section gives site-specific detection results. Each site-specific report includes positional information, a figure of the total (12S and COI) identified reads in each site sample (including non-target taxa such as fungi, bacteria, or algae), the number of unique species in each detected taxonomic class for the sample(s), and the species names of target taxa that were identified in each site sample.

Regional Site Results - Large Wood Addition Sites

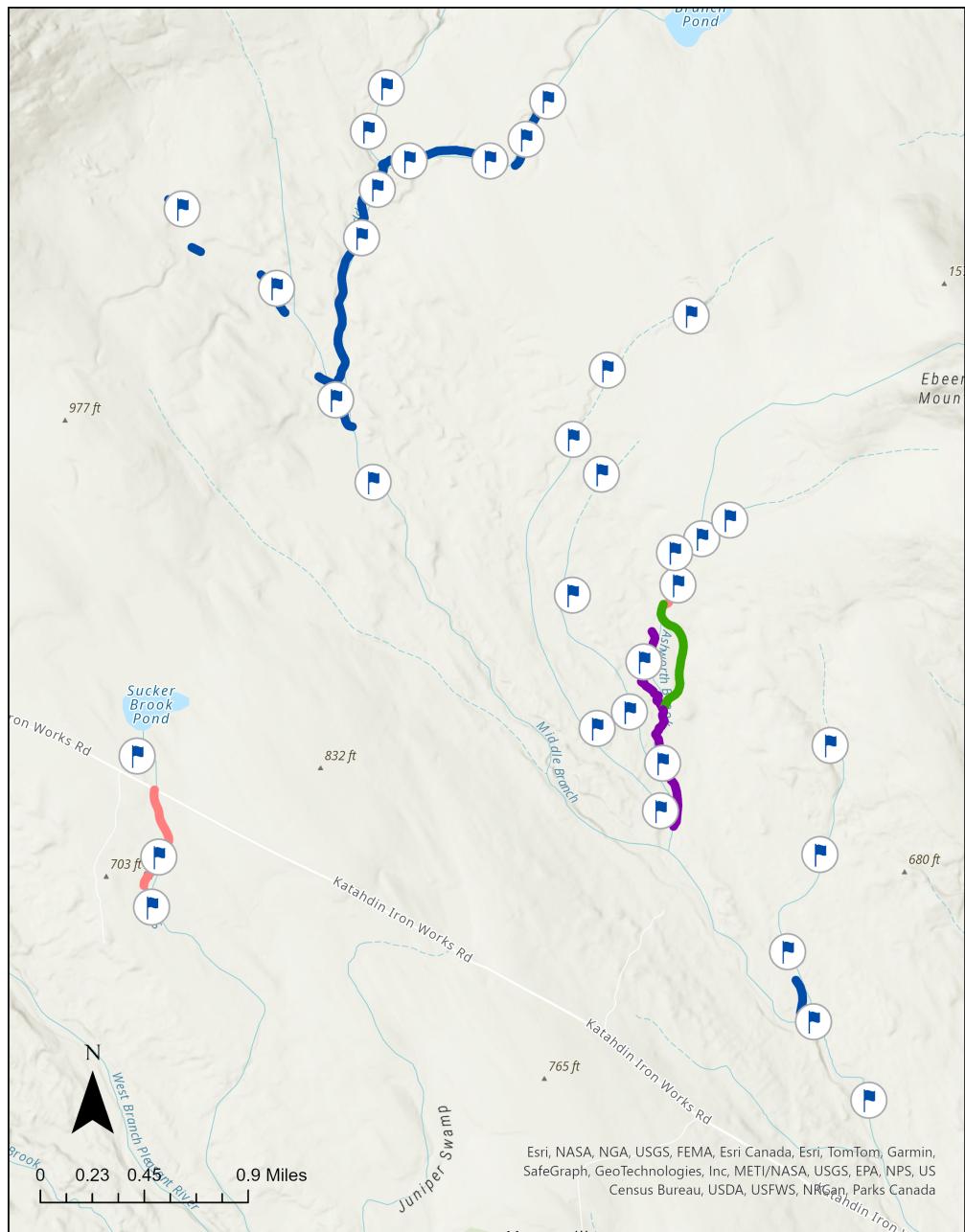


Figure 1: LWA eDNA sampling sites - lines correspond to the years of LWA installment (purple = 2020, green = 2021, blue = 2022 after eDNA sampling, pink = 2023 after eDNA sampling)

Homes Brook - HB01, HB02, HB03, HB05, & MB01

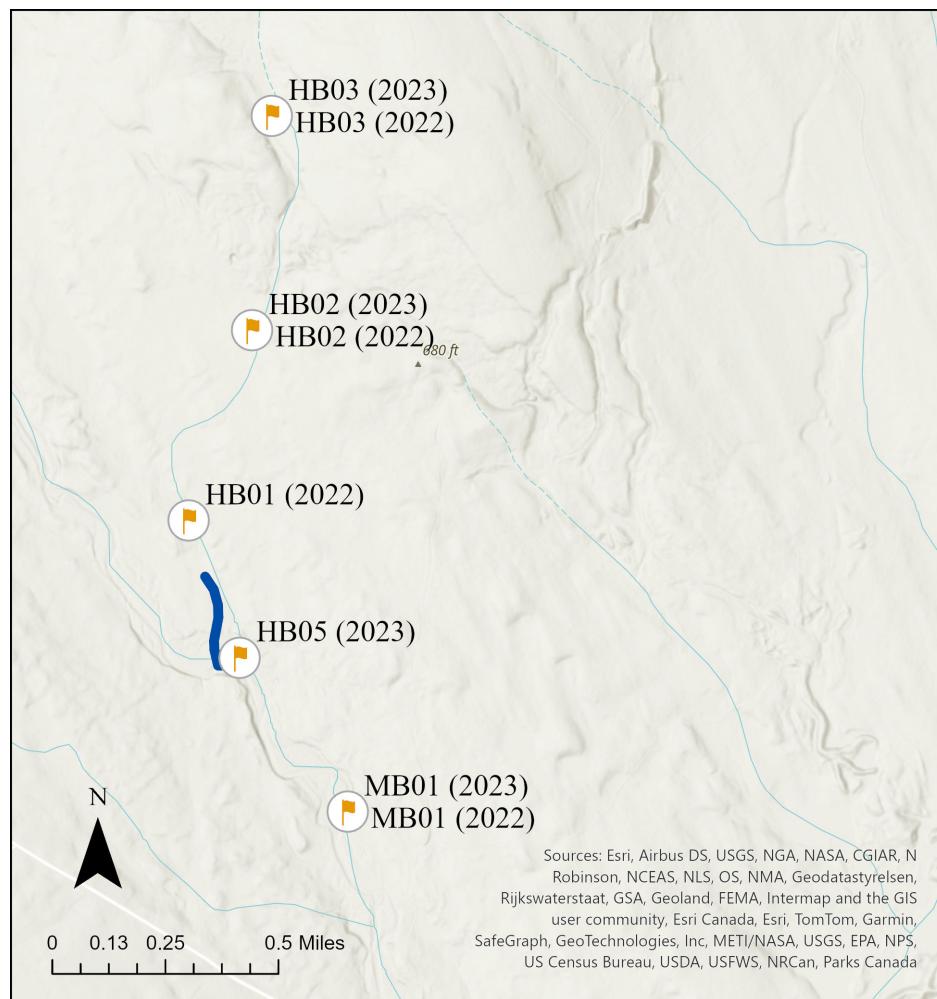
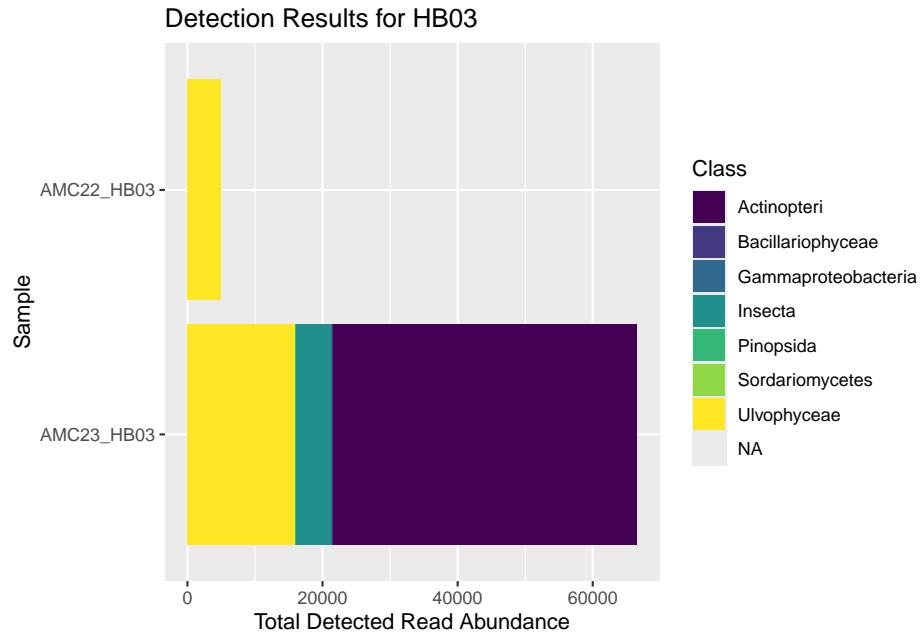


Figure 2: Homes Brook Site Locations

HB03 HB03 was the furthest upstream site on Homes Brook, and sampled at the same coordinates in 2022 and 2023. It is considered Upstream and Pre-LWA of the LWA reach installed in 2022, and Post-LWA for 2023.



```

##           Class     Sample Species
## 1   Ulvophyceae AMC22_HB03      1
## 2   Actinopteri  AMC23_HB03      4
## 3   Bacillariophyceae  AMC23_HB03      1
## 4   Gammaproteobacteria  AMC23_HB03      1
## 5   Insecta  AMC23_HB03     10
## 6   Pinopsida  AMC23_HB03      1
## 7   Sordariomycetes  AMC23_HB03      1
## 8   Ulvophyceae  AMC23_HB03      1

## [1] "Target species detected in 2022:"
```

```

## character(0)
```

```

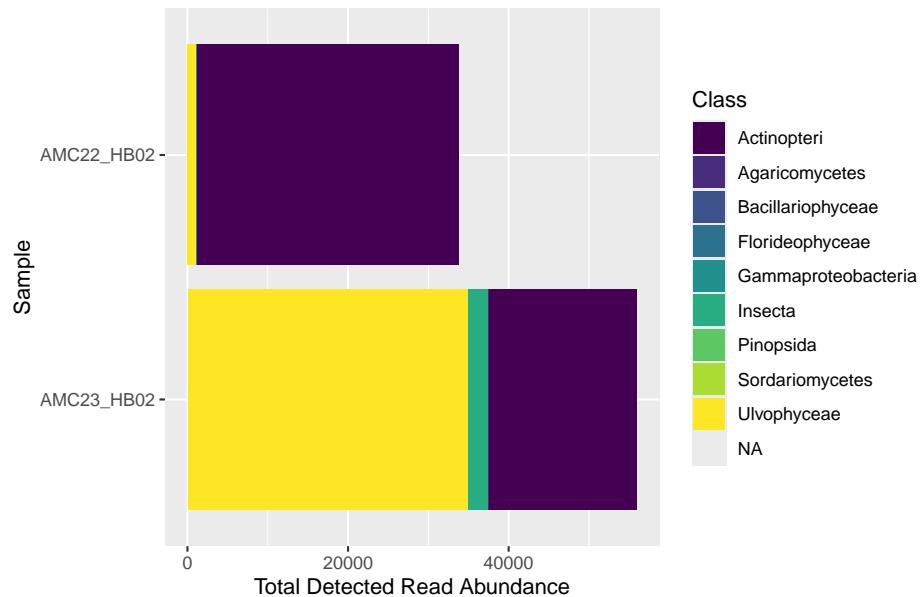
## [1] "Target species detected in 2023:"
```

```

## [1] "Acerpenna macdunnoughi"      "Amphinemura wui"
## [3] "Brevoortia tyrannus"          "Clupea harengus"
## [5] "Cottus cognatus"             "Diphetor hageni"
## [7] "Dolophilodes distinctus"      "Ephemerella invaria"
## [9] "Habrophlebiodes americana"    "Micrasema sprulesi"
## [11] "Paraleptophlebia adoptiva"    "Polypedilum aviceps"
## [13] "Salvelinus fontinalis"        "Stenacron interpunctatum"
```

HB02 HB02 is located just downstream of HB03 and was sampled at the same coordinates in 2022 and 2023. It is considered Upstream and Pre-LWA for 2022, and Post-LWA for 2023.

Detection Results for HB02



```

##           Class     Sample Species
## 1      Actinopteri AMC22_HB02    4
## 2  Gammaproteobacteria AMC22_HB02   1
## 3      Ulvophyceae  AMC22_HB02   1
## 4      Actinopteri  AMC23_HB02   1
## 5    Agaricomycetes  AMC23_HB02   2
## 6  Bacillariophyceae  AMC23_HB02   1
## 7  Florideophyceae  AMC23_HB02   1
## 8      Insecta     AMC23_HB02  20
## 9      Pinopsida   AMC23_HB02   1
## 10     Sordariomycetes  AMC23_HB02   1
## 11     Ulvophyceae  AMC23_HB02   1

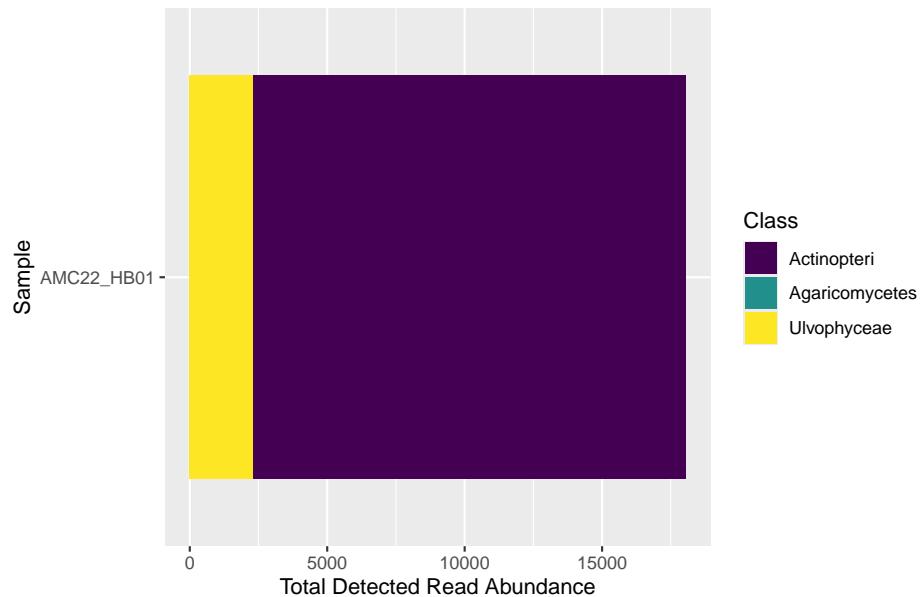
## [1] "Target species detected in 2022:"
## [1] "Micropterus salmoides"    "Perca flavescens"
## [3] "Salvelinus fontinalis"    "Semotilus atromaculatus"

## [1] "Target species detected in 2023:"
## [1] "Acerpenna macdunnoughi"    "Amphinemura wui"
## [3] "Baetis tricaudatus"        "Chironomus melanescens"
## [5] "Diphetor hageni"          "Diplectrona modesta"
## [7] "Dolophilodes distinctus"    "Ephemerella dorothea"
## [9] "Ephemeralia invaria"        "Eukiefferiella claripennis"
## [11] "Isoperla bilineata"       "Lanthus parvulus"
## [13] "Paracapnia angulata"       "Paraleptophlebia adoptiva"
## [15] "Polypedilum aviceps"       "Salvelinus fontinalis"
## [17] "Siphlonurus barbaroides"    "Stenacron interpunctatum"
## [19] "Tallaperla maria"          "Teloganopsis deficiens"
## [21] "Tvetenia paucunca"

```

HB01 HB01 was only sampled in 2022, and is considered Upstream and Pre-LWA.

Detection Results for HB01



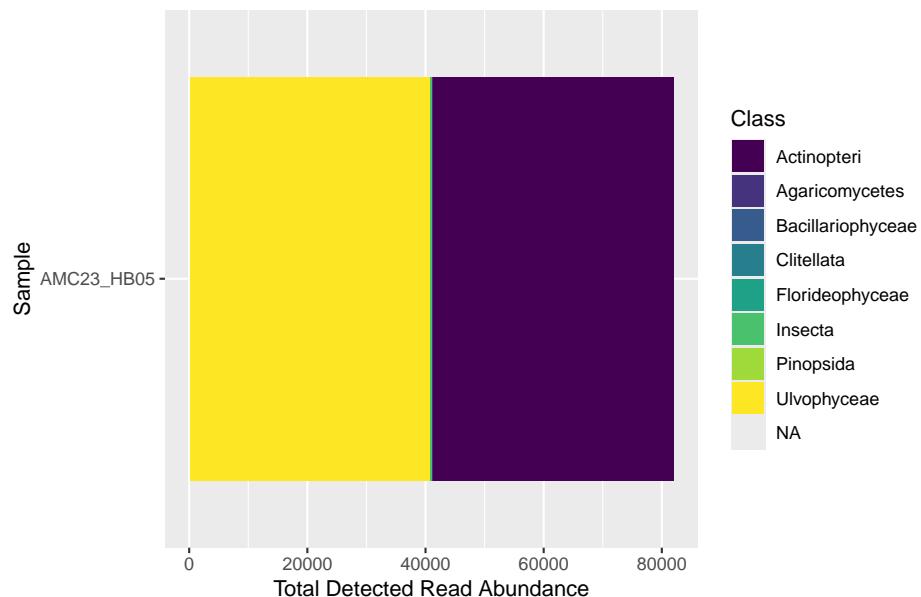
```

##           Class     Sample Species
## 1   Actinopteri AMC22_HB01      3
## 2 Agaricomycetes AMC22_HB01      2
## 3   Ulvophyceae AMC22_HB01      1
## [1] "Target species detected in 2022:"
## [1] "Chrosomus neogaeus"      "Rhinichthys atratulus"
## [3] "Semotilus atromaculatus"

```

HB05 HB05 was only sampled in 2023, and is considered Internal and Post-LWA to the LWA reach installed on Homes Brook.

Detection Results for HB05



```

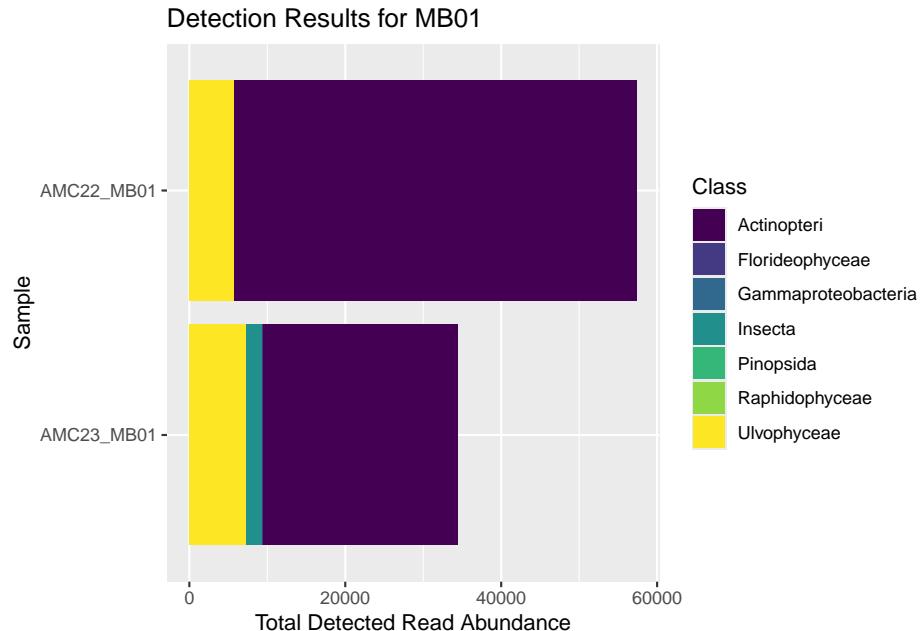
##          Class      Sample Species
## 1      Actinopteri  AMC23_HB05     8
## 2      Agaricomycetes  AMC23_HB05     2
## 3  Bacillariophyceae  AMC23_HB05     2
## 4      Clitellata  AMC23_HB05     2
## 5  Florideophyceae  AMC23_HB05     1
## 6      Insecta  AMC23_HB05    15
## 7      Pinopsida  AMC23_HB05     1
## 8      Ulvophyceae  AMC23_HB05     1

## [1] "Target species detected in 2023:"

## [1] "Acerpenna macdunnoughi"      "Baetis tricaudatus"
## [3] "Catostomus commersonii"      "Chaetogaster diastrophus"
## [5] "Chrosomus neogaeus"          "Cottus cognatus"
## [7] "Dolophilodes distinctus"      "Epeorus fragilis"
## [9] "Epeorus vitreus"             "Ephemerella dorothea"
## [11] "Eurylophella funeralis"      "Lepidostoma costale"
## [13] "Litobrancha recurvata"       "Micropterus salmoides"
## [15] "Paraleptophlebia adoptiva"   "Perca flavescens"
## [17] "Polypedilum aviceps"        "Pristina aequiseta"
## [19] "Rhinichthys atratulus"       "Salvelinus fontinalis"
## [21] "Semotilus atromaculatus"     "Simulium tuberosum"
## [23] "Stenacron interpunctatum"    "Tvetenia paucunca"
## [25] "Wormaldia moesta"

```

MB01 MB01 was sampled at the same coordinates in 2022 and 2023, and is considered Downstream and Pre-LWA for 2022, and Post-LWA for 2023.



```

##          Class      Sample Species
## 1      Actinopteri  AMC22_MB01     9
## 2      Insecta  AMC22_MB01     1
## 3      Ulvophyceae  AMC22_MB01     1
## 4      Actinopteri  AMC23_MB01     5

```

```

## 5      Florideophyceae AMC23_MB01      1
## 6  Gammaproteobacteria AMC23_MB01      1
## 7          Insecta AMC23_MB01      3
## 8          Pinopsida AMC23_MB01      1
## 9      Raphidophyceae AMC23_MB01      1
## 10     Ulvophyceae AMC23_MB01      1

## [1] "Target species detected in 2022:"
```

```

## [1] "Anguilla rostrata"      "Brevoortia tyrannus"
## [3] "Catostomus commersonii" "Clupea harengus"
## [5] "Cottus cognatus"        "Rhinichthys atratulus"
## [7] "Salvelinus fontinalis"   "Scomber scombrus"
## [9] "Semotilus atromaculatus" "Stenacron interpunctatum"
```

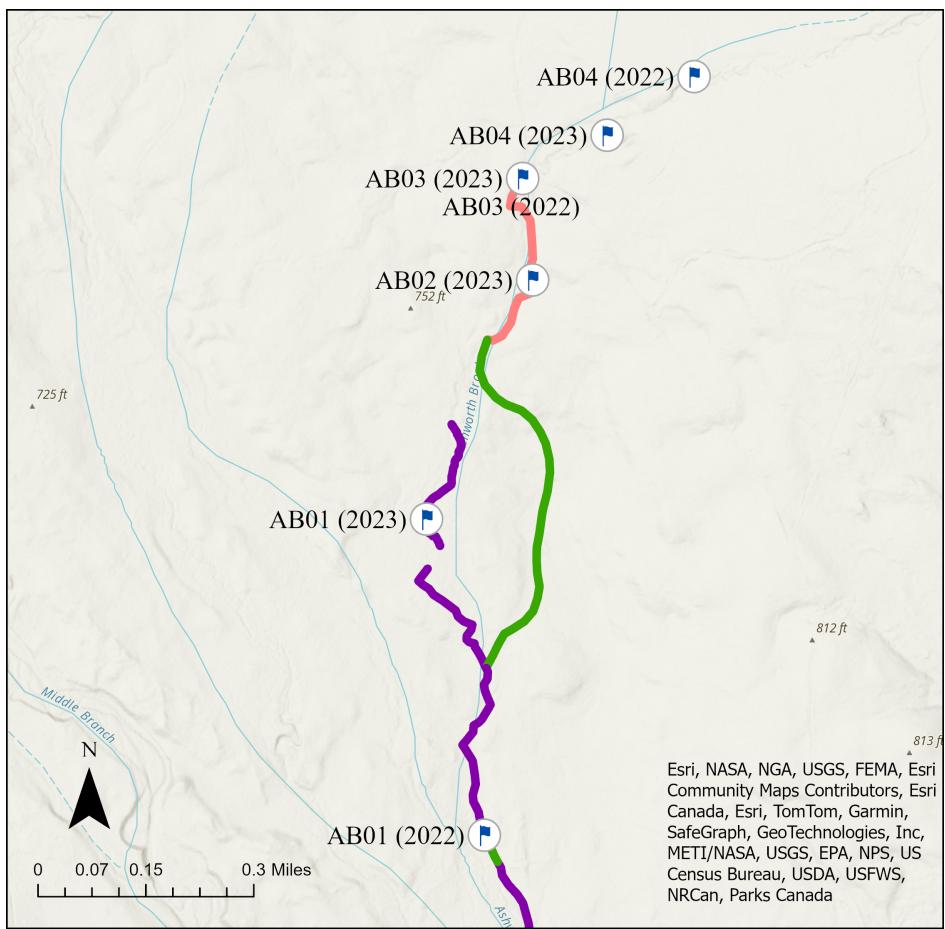
```

## [1] "Target species detected in 2023:"
```

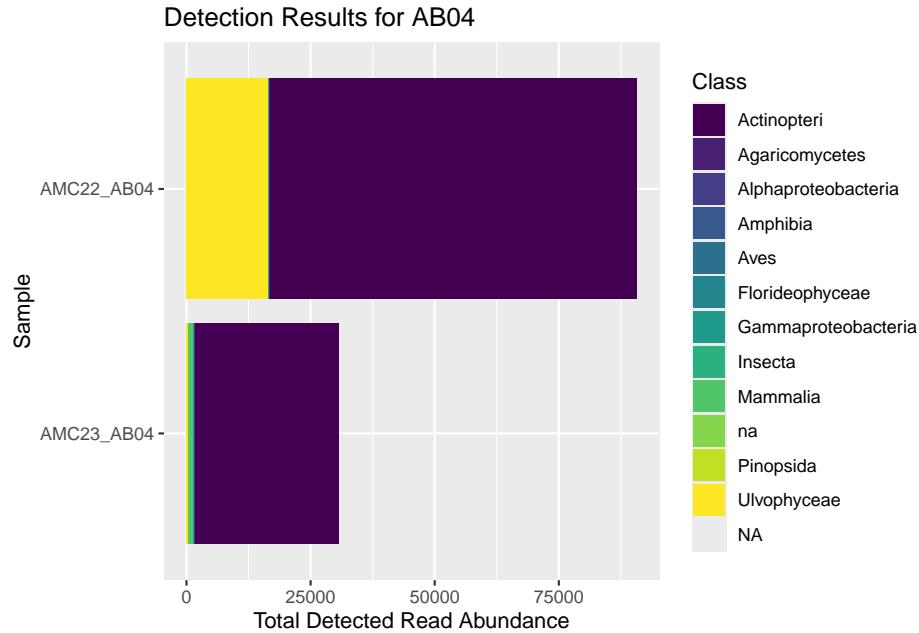
```

## [1] "Baetis tricaudatus"      "Catostomus commersonii"
## [3] "Cottus cognatus"         "Plauditus dubius"
## [5] "Polypedilum aviceps"     "Rhinichthys atratulus"
## [7] "Salvelinus fontinalis"    "Semotilus atromaculatus"
```

Ashworth Brook - AB04, AB03, AB02, AB01

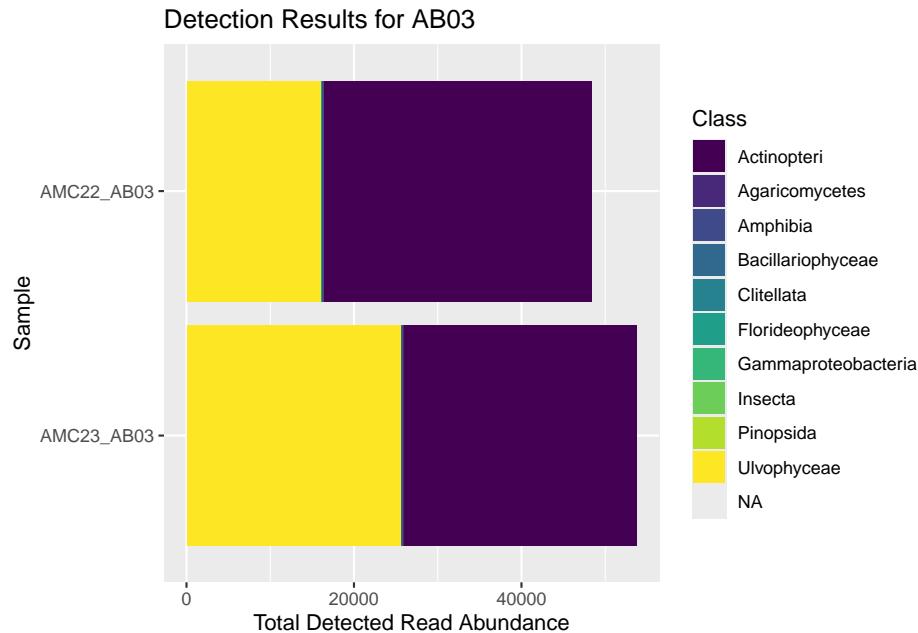


AB04 AB04 samples were collected in both 2022 and 2023, though the site for 2023 shifted slightly downstream compared to that of 2022 due to field conditions. In both years, these samples are considered Upstream of all prior or planned LWA work, though due to the relative time difference between collection and installation plans, the 2022 sample was considered Post-LWA and the 2023 sample considered Pre-LWA.



```
##          Class   Sample Species
## 1      Actinopteri AMC22_AB04     1
## 2    Agaricomycetes AMC22_AB04     2
## 3      Amphibia  AMC22_AB04     1
## 4       Aves    AMC22_AB04     1
## 5  Florideophyceae AMC22_AB04     1
## 6  Gammaproteobacteria AMC22_AB04     1
## 7      Insecta  AMC22_AB04     8
## 8        na    AMC22_AB04     1
## 9    Ulvophyceae  AMC22_AB04     1
## 10     Actinopteri  AMC23_AB04     1
## 11  Alphaproteobacteria  AMC23_AB04     1
## 12  Gammaproteobacteria  AMC23_AB04     1
## 13      Pinopsida  AMC23_AB04     1
## 14    Ulvophyceae  AMC23_AB04     1
## [1] "Target species detected in 2022:"
## [1] "Dolophilodes distinctus"      "Dryobates pubescens"
## [3] "Epeorus fragilis"           "Eurylophella funeralis"
## [5] "Gyrinophilus porphyriticus"  "Habrophlebiodes americana"
## [7] "Paraleptophlebia debilis"    "Prosimulium mixtum"
## [9] "Salvelinus fontinalis"       "Typhlocyba gillettei"
## [11] "Valenzuela flavidus"
## [1] "Target species detected in 2023:"
## [1] "Salvelinus fontinalis"
```

AB03 AB03 was sampled in 2022 and 2023, with no significant sampling location changes. The sampling site is upstream of the Ashworth LWA reach installed in 2021, and is considered Upstream for both 2022 and 2023 as the 2023 LWA installation occurred after sampling.



```

##          Class     Sample Species
## 1      Actinopteri AMC22_AB03    3
## 2      Amphibia   AMC22_AB03    2
## 3      Clitellata AMC22_AB03    1
## 4  Florideophyceae AMC22_AB03    1
## 5  Gammaproteobacteria AMC22_AB03    1
## 6      Insecta   AMC22_AB03    9
## 7      Pinopsida AMC22_AB03    1
## 8      Ulvophyceae AMC22_AB03    1
## 9      Actinopteri AMC23_AB03    2
## 10     Agaricomycetes AMC23_AB03    1
## 11     Amphibia   AMC23_AB03    1
## 12  Bacillariophyceae AMC23_AB03    1
## 13  Florideophyceae AMC23_AB03    1
## 14  Gammaproteobacteria AMC23_AB03    1
## 15      Insecta   AMC23_AB03    4
## 16      Pinopsida AMC23_AB03    1
## 17      Ulvophyceae AMC23_AB03    1

## [1] "Target species detected in 2022:"

## [1] "Baetis tricaudatus"      "Cottus cognatus"
## [3] "Diphetor hageni"         "Diplectrona modesta"
## [5] "Dolophilodes distinctus"  "Eurycea bislineata"
## [7] "Eurylophella funeralis"   "Gyrinophilus porphyriticus"
## [9] "Leuctra ferruginea"       "Rhinichthys atratulus"
## [11] "Salvelinus fontinalis"    "Siphlonurus typicus"
## [13] "Stylodrilus heringianus"  "Sweltsa onkos"
## [15] "Tvetenia paucunca"

## [1] "Target species detected in 2023:"

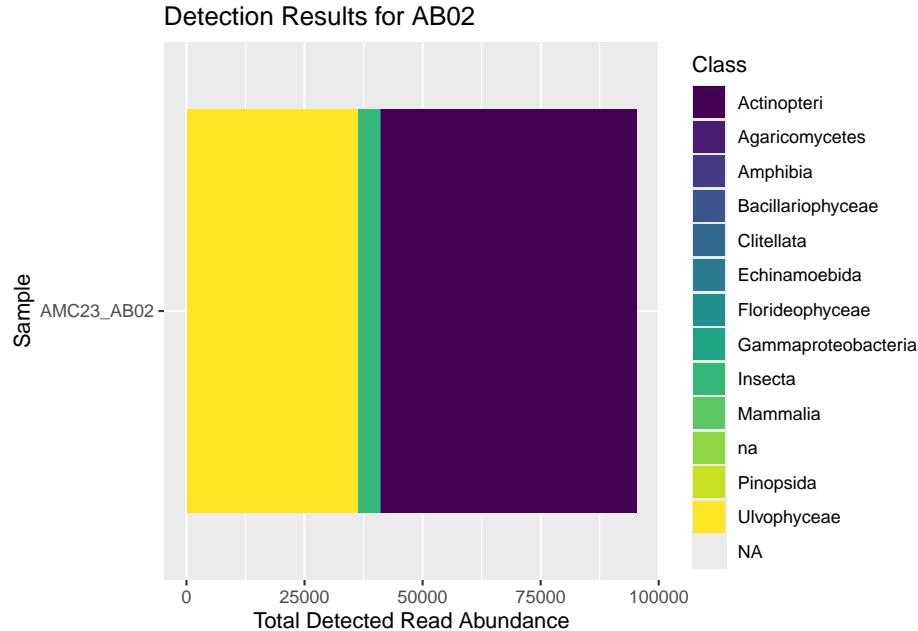
```

```

## [1] "Baetis tricaudatus"      "Cottus cognatus"
## [3] "Dolophilodes distinctus" "Epeorus fragilis"
## [5] "Eurylophella funeralis"  "Gyrinophilus porphyriticus"
## [7] "Salvelinus fontinalis"

```

AB02 AB02 was sampled only in 2023, and is placed upstream of both the 2020 and 2021 LWA installment reaches. It is internal to the planned 2023 LWA installation, but can be considered Pre or Post-LWA. In current analyses, it is considered Upstream and Pre-LWA



```

##          Class     Sample Species
## 1      Actinopteri AMC23_AB02    3
## 2      Agaricomycetes AMC23_AB02   2
## 3      Amphibia    AMC23_AB02    1
## 4  Bacillariophyceae AMC23_AB02   3
## 5      Clitellata  AMC23_AB02    4
## 6      Echinamoebida AMC23_AB02   1
## 7  Florideophyceae AMC23_AB02   1
## 8  Gammaproteobacteria AMC23_AB02   1
## 9      Insecta    AMC23_AB02   18
## 10     Mammalia   AMC23_AB02    1
## 11      na        AMC23_AB02    1
## 12     Pinopsida  AMC23_AB02    1
## 13     Ulvophyceae AMC23_AB02    1
## [1] "Target species detected in 2023:"
## [1] "Agapetus pinatus"           "Aporrectodea caliginosa"
## [3] "Baetis tricaudatus"         "Chaetogaster diastrophus"
## [5] "Cottus cognatus"            "Cratyna vagabunda"
## [7] "Desmognathus fuscus"         "Diphetor hageni"
## [9] "Diplectrona modesta"        "Dolophilodes distinctus"
## [11] "Epeorus pleuralis"          "Eurylophella funeralis"
## [13] "Habrophlebiodes americana"  "Lanthus parvulus"
## [15] "Lepidostoma costale"         "Maccaffertium vicarium"

```

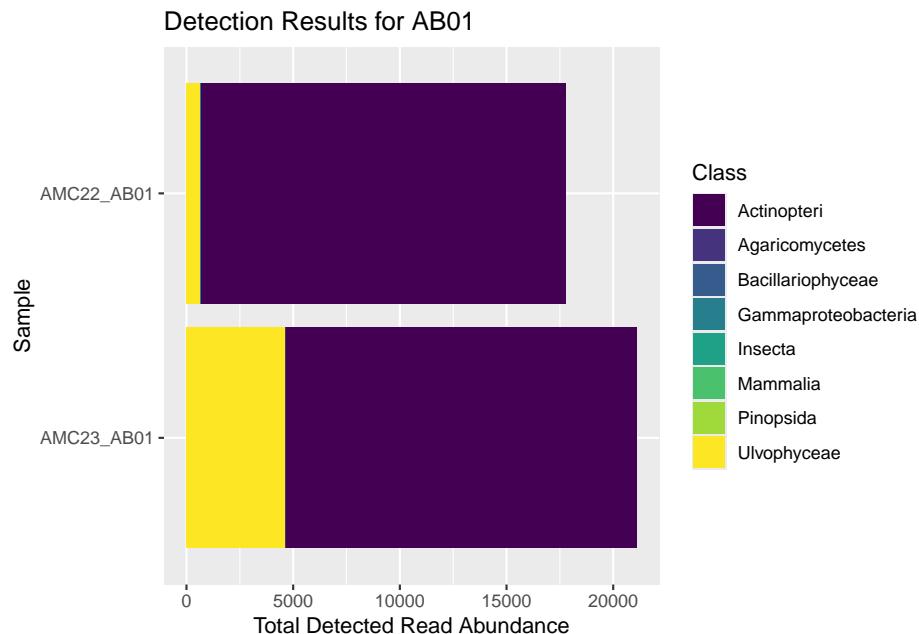
```

## [17] "Pedicia albivitta"           "Polypedilum aviceps"
## [19] "Pristina aequiseta"          "Pseudanostirus triundulatus"
## [21] "Pteronarcys proteus"         "Rhinichthys atratulus"
## [23] "Salvelinus fontinalis"        "Stylodrilus heringianus"
## [25] "Tvetenia paucunca"          "Valenzuela flavidus"

```

AB01 Samples labeled AB01 were collected in both 2022 and 2023 as the furthest downstream sample on Ashworth Brook, but field conditions required moving the 2022 collection site upstream for 2023 sampling. In both years, these samples were collected within the 2020 LWA installment area, and are thus considered Internal and Post-LWA for both years despite the change in location.

The BLK01 sample was located downstream of AB01, after the confluence of Ashworth and Nelson Brook.



```

##             Class     Sample Species
## 1      Actinopteri AMC22_AB01      9
## 2  Gammaproteobacteria AMC22_AB01     1
## 3      Mammalia   AMC22_AB01      1
## 4    Ulvophyceae   AMC22_AB01      1
## 5      Actinopteri   AMC23_AB01     3
## 6  Agaricomycetes   AMC23_AB01     1
## 7 Bacillariophyceae   AMC23_AB01     1
## 8      Insecta   AMC23_AB01     4
## 9      Pinopsida   AMC23_AB01     1
## 10     Ulvophyceae   AMC23_AB01     1

## [1] "Target species detected in 2022:"

## [1] "Castor canadensis"           "Catostomus commersonii"
## [3] "Chrosomus neogaeus"          "Clupea harengus"
## [5] "Cottus cognatus"            "Hippoglossoides platessoides"
## [7] "Margariscus margarita"       "Rhinichthys atratulus"
## [9] "Salvelinus fontinalis"        "Semotilus atromaculatus"

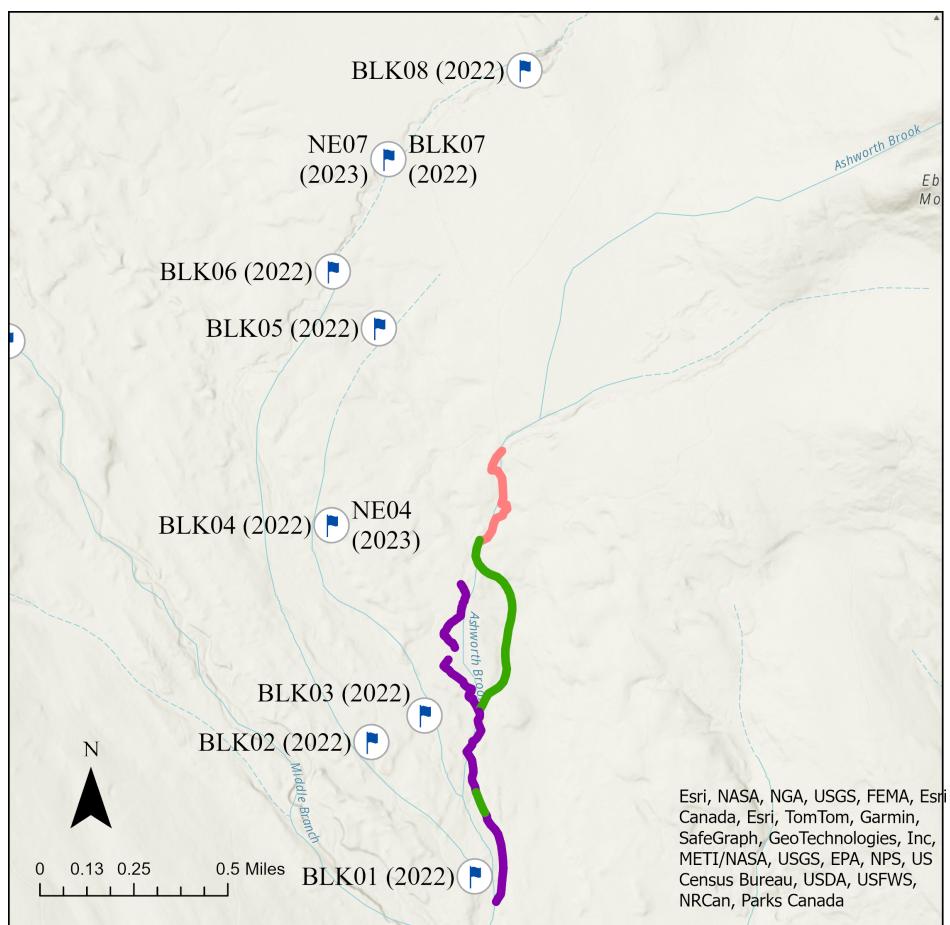
## [1] "Target species detected in 2023:"

## [1] "Cottus cognatus"            "Dolophilodes distinctus"

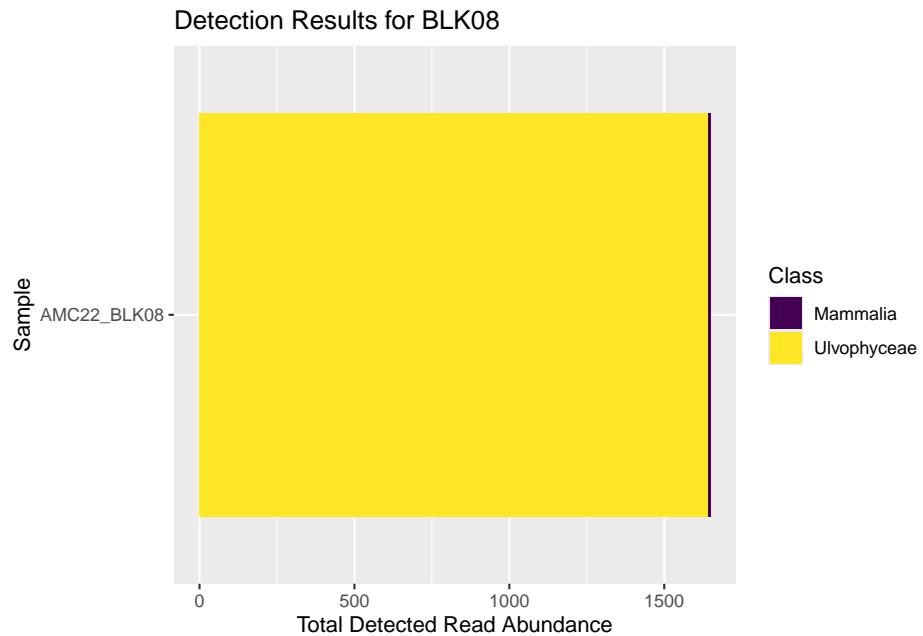
```

```
## [3] "Habrophlebia vibrans"      "Habrophlebiodes americana"  
## [5] "Lepidostoma costale"        "Rhinichthys atratulus"  
## [7] "Salvelinus fontinalis"
```

Nelson Brook - BLK08, BLK07/NE07, BLK06, BLK05, BLK04/NE04, BLK01

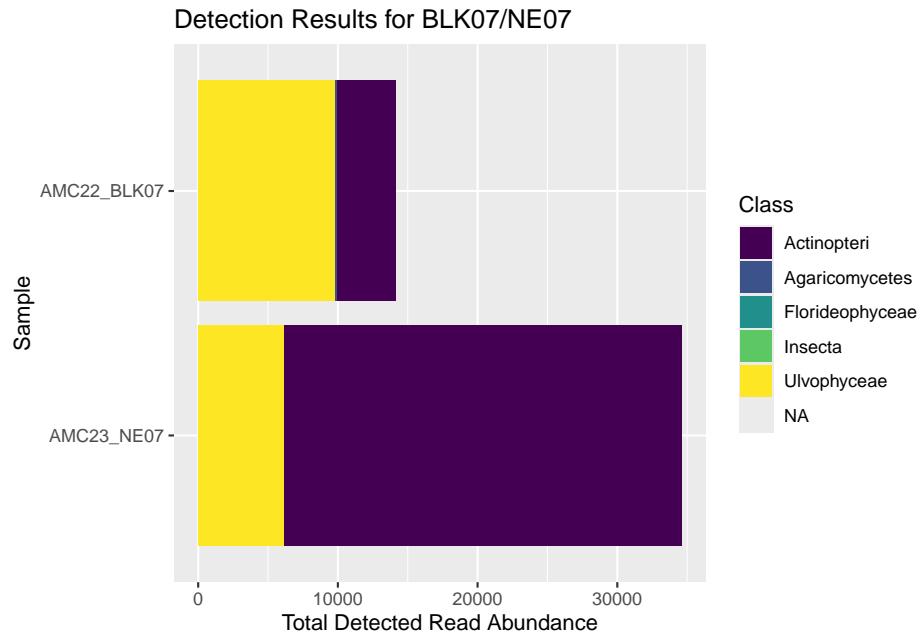


BLK08 BLK08 was sampled only in 2022, and is considered a Parallel Control to Ashworth Brook sites due to relative proximity and confluence of the streams. It is the farthest upstream site on Nelson Brook (previously misidentified as Black Brook)



```
##           Class      Sample Species
## 1   Mammalia  AMC22_BLK08      1
## 2 Ulvophyceae  AMC22_BLK08      1
## [1] "Target species detected in 2022:"
## [1] "Sus scrofa"
```

BLK07/NE07 BLK07/NE07 was sampled in both 2022 and 2023 at the same coordinates for each year, and is considered a Parallel Control site. It is placed just downstream of BLK08.



```

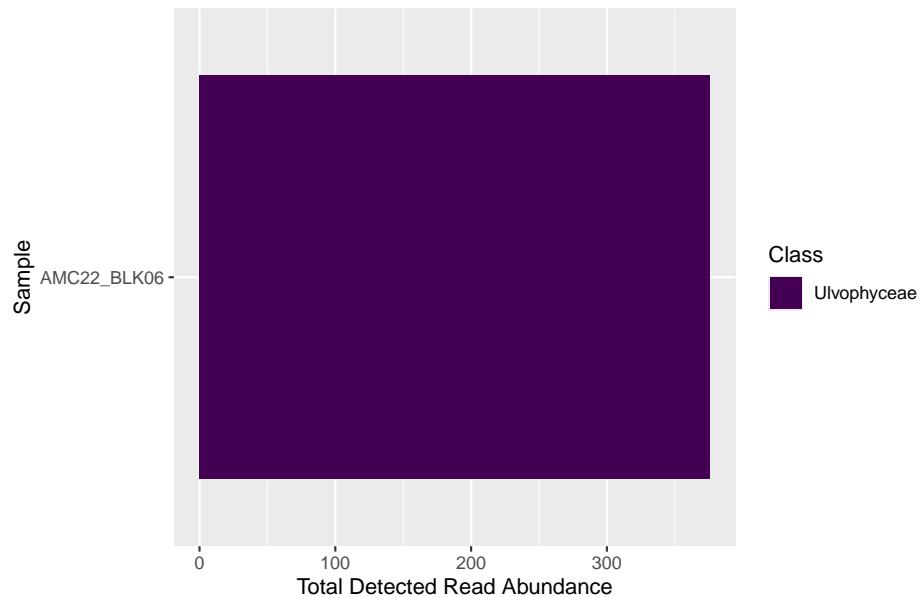
##          Class      Sample Species
## 1    Actinopteri  AMC22_BLK07     1
## 2  Agaricomycetes  AMC22_BLK07     3
## 3  Florideophyceae  AMC22_BLK07     1
## 4      Insecta  AMC22_BLK07     1
## 5   Ulvophyceae  AMC22_BLK07     1
## 6    Actinopteri  AMC23_NE07     2
## 7   Ulvophyceae  AMC23_NE07     1

## [1] "Target species detected in 2022:"
## [1] "Cottus cognatus"      "Valenzuela flavidus"
## [1] "Target species detected in 2023:"
## [1] "Cottus cognatus"      "Salvelinus fontinalis"

```

BLK06 BLK06 was only sampled in 2022, and is downstream of BLK08 and BLK07/NE07. It is also considered a Parallel Control to Ashworth Brook sites.

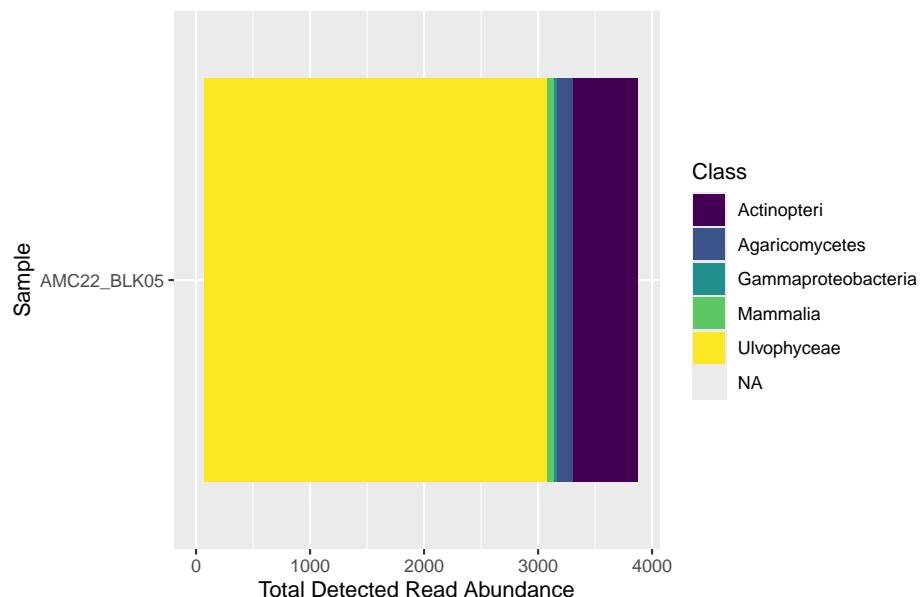
Detection Results for BLK06



```
##           Class      Sample Species
## 1 Ulvophyceae AMC22_BLK06      1
## [1] "Target species detected in 2022:"
## character(0)
```

BLK05 BLK05 was sampled in 2022 and is roughly parallel (and slightly downstream of) BLK06. It is considered a Parallel Control to the Ashworth sites.

Detection Results for BLK05



```
##           Class      Sample Species
## 1 Actinopteri AMC22_BLK05      2
## 2 Agaricomycetes AMC22_BLK05      2
```

```

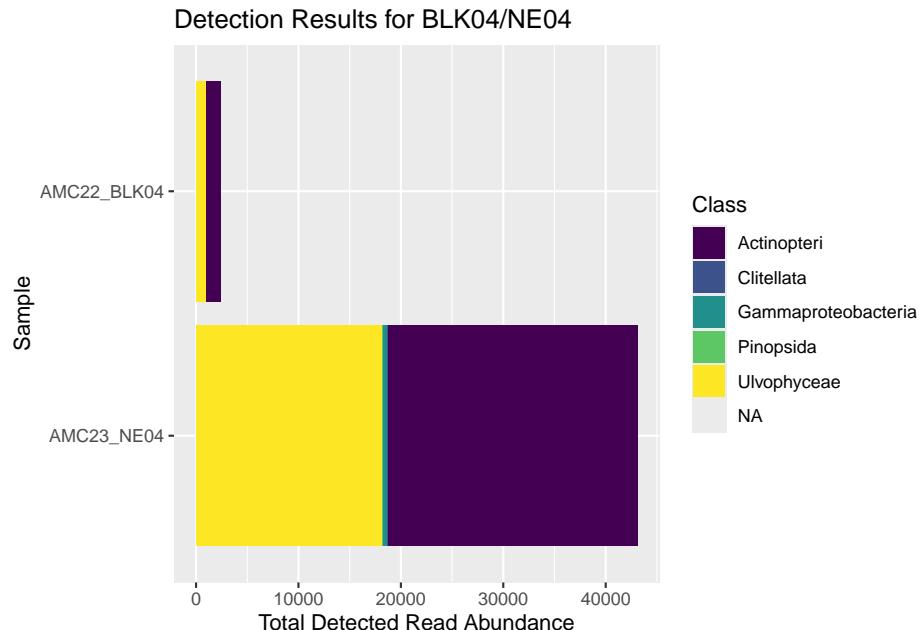
## 3 Gammaproteobacteria AMC22_BLK05      1
## 4          Mammalia AMC22_BLK05        1
## 5       Ulvophyceae AMC22_BLK05        1

## [1] "Target species detected in 2022:"

## [1] "Hippoglossoides platessoides" "Melanogrammus aeglefinus"
## [3] "Sus scrofa"

```

BLK04/NE04 BLK04/NE04 was sampled in both 2022 and 2023 at the same coordinates each year, and is considered a Parallel Control. It is located downstream of BLK05 and roughly latitudinally parallel with site AB02



```

##           Class     Sample Species
## 1   Actinopteri AMC22_BLK04      3
## 2   Ulvophyceae AMC22_BLK04      1
## 3   Actinopteri AMC23_NE04      2
## 4   Clitellata  AMC23_NE04      1
## 5 Gammaproteobacteria  AMC23_NE04      1
## 6   Pinopsida   AMC23_NE04      1
## 7   Ulvophyceae  AMC23_NE04      1

## [1] "Target species detected in 2022:"

## [1] "Clupea harengus"          "Gadus ogac"
## [3] "Hippoglossoides platessoides"

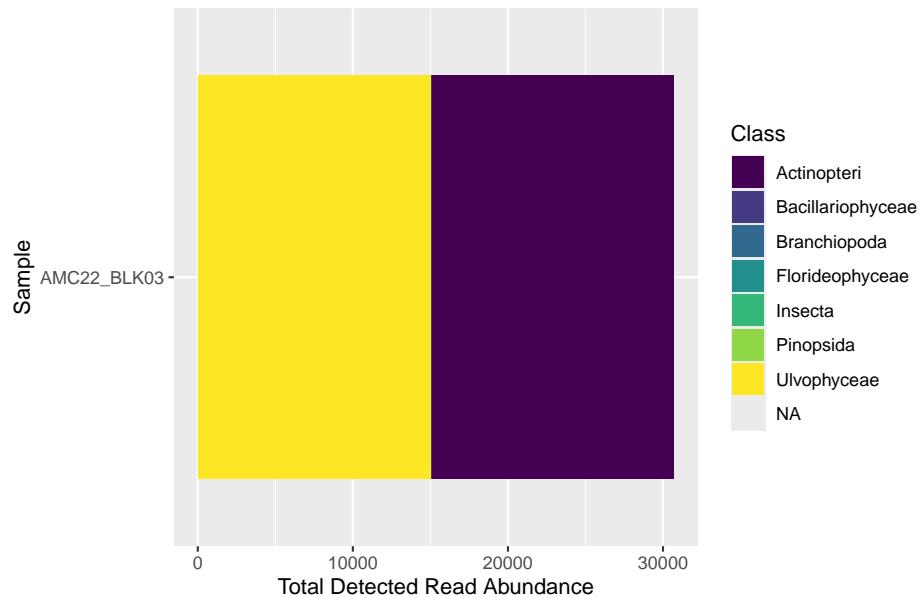
## [1] "Target species detected in 2023:"

## [1] "Rhinichthys atratulus"    "Semotilus atromaculatus"
## [3] "Styelodrilus heringianus"

```

BLK03 BLK03 was sampled in 2022, closely Parallel to the lower Ashworth Brook sites. It is downstream of BLK04/NE04, and parallel to BLK02.

Detection Results for BLK03



```

##           Class      Sample Species
## 1   Actinopteri  AMC22_BLK03     3
## 2 Bacillariophyceae  AMC22_BLK03     1
## 3   Branchiopoda  AMC22_BLK03     1
## 4   Florideophyceae  AMC22_BLK03     1
## 5       Insecta    AMC22_BLK03     1
## 6      Pinopsida  AMC22_BLK03     1
## 7   Ulvophyceae  AMC22_BLK03     1
## [1] "Target species detected in 2022:"
## [1] "Anisota virginiensis"    "Chydorus brevilabris"
## [3] "Rhinichthys atratulus"   "Salvelinus fontinalis"
## [5] "Semotilus atromaculatus"

```

BLK02 BLK02 was sampled only in 2022, downstream of BLK06 and upstream of BLK01. It is classed as a Parallel Control.



```

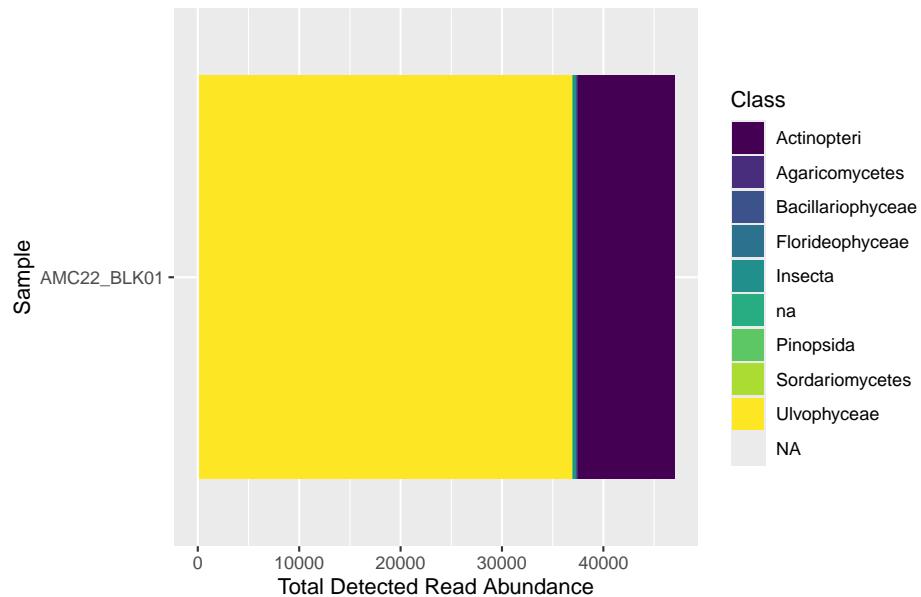
##           Class      Sample Species
## 1   Actinopteri  AMC22_BLK02     2
## 2   Agaricomycetes  AMC22_BLK02     1
## 3   Bacillariophyceae  AMC22_BLK02     2
## 4   Florideophyceae  AMC22_BLK02     1
## 5   Insecta  AMC22_BLK02     5
## 6   Pinopsida  AMC22_BLK02     1
## 7   Sordariomycetes  AMC22_BLK02     1
## 8   Ulvophyceae  AMC22_BLK02     1

## [1] "Target species detected in 2022:"
## [1] "Dolophilodes distinctus"    "Ephemerella invaria"
## [3] "Isoperla bilineata"        "Paraleptophlebia adoptiva"
## [5] "Polypedilum aviceps"       "Rhinichthys atratulus"
## [7] "Semotilus atromaculatus"

```

BLK01 BLK01 was sampled only in 2022, downstream of BLK02 as well as all AB sites. Placement of this site aimed to capture fish movement up into the Nelson and Ashworth streams, and it is classed as a Control sample.

Detection Results for BLK01



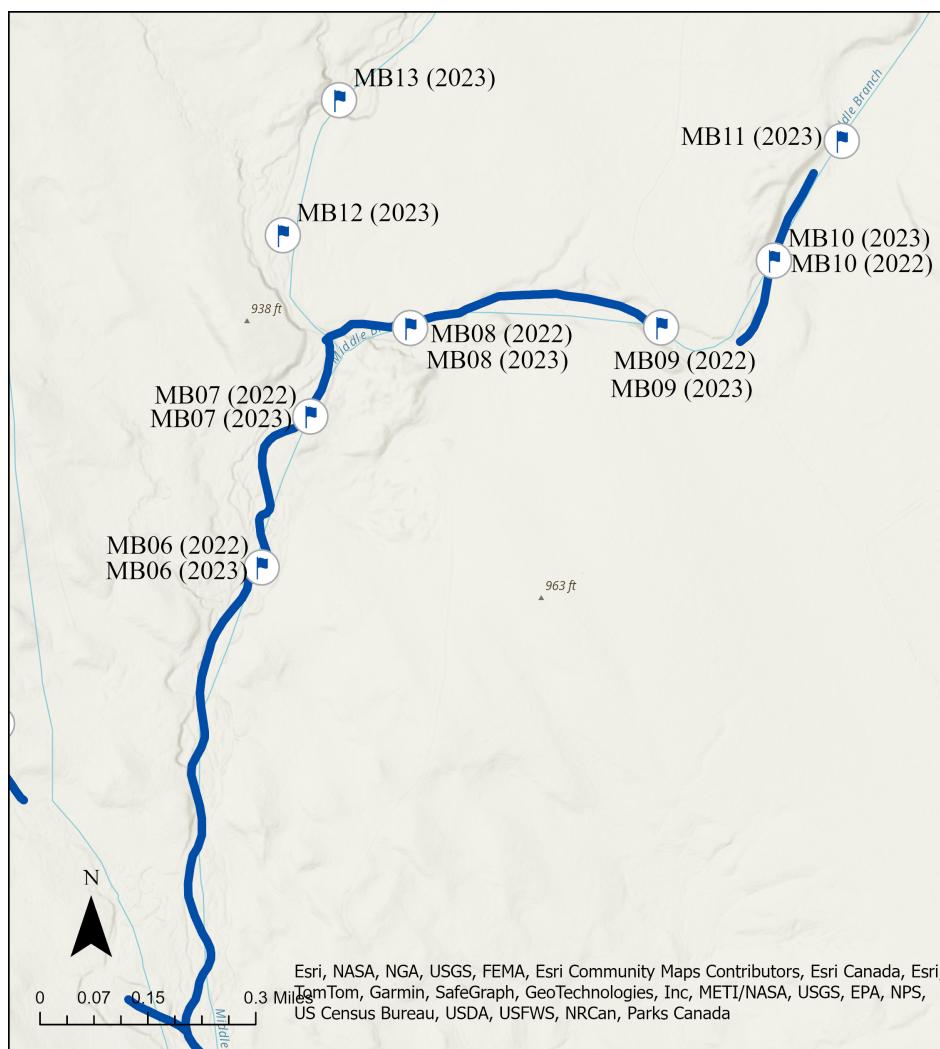
```

##           Class      Sample Species
## 1   Actinopteri  AMC22_BLK01     2
## 2   Agaricomycetes  AMC22_BLK01     4
## 3   Bacillariophyceae  AMC22_BLK01     2
## 4   Florideophyceae  AMC22_BLK01     1
## 5       Insecta  AMC22_BLK01     3
## 6          na  AMC22_BLK01     1
## 7   Pinopsida  AMC22_BLK01     1
## 8   Sordariomycetes  AMC22_BLK01     2
## 9   Ulvophyceae  AMC22_BLK01     1

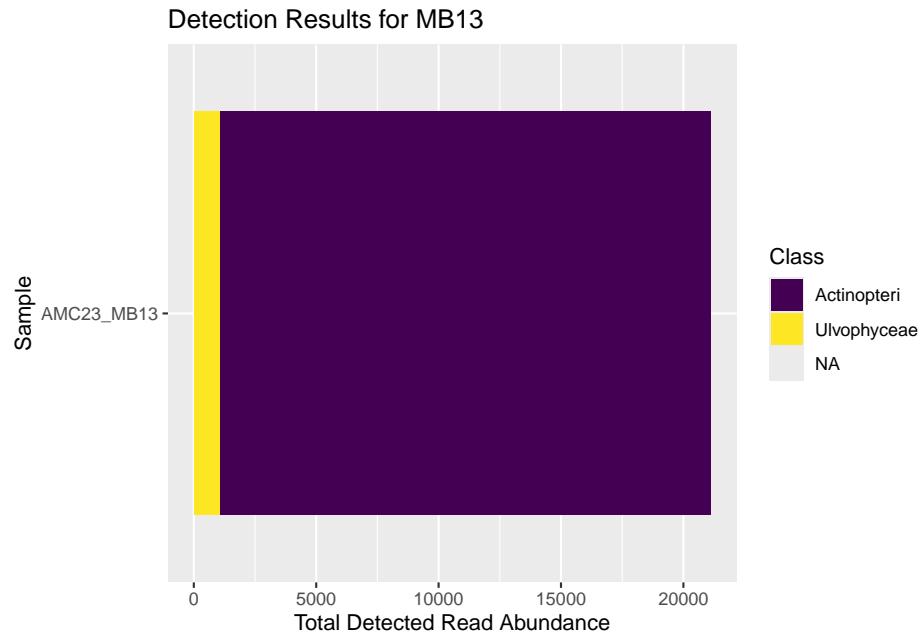
## [1] "Target species detected in 2022:"
## [1] "Ephemerella dorothea"          "Hippoglossoides platessoides"
## [3] "Leuctra ferruginea"           "Paraleptophlebia adoptiva"
## [5] "Rhinichthys atratulus"

```

Middle Branch - MB13, MB12, MB11, MB10, MB09, MB08, MB07, MB06



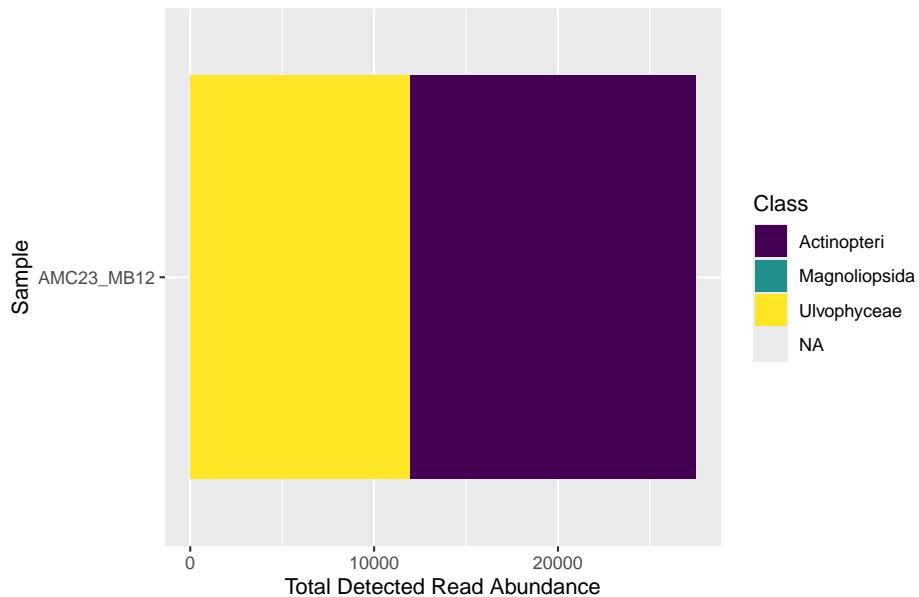
MB13 Placed above the confluence, MB13 is a Parallel Control to the LWA installation on the Middle Branch, and was only sampled in 2023.



```
##           Class     Sample Species
## 1 Actinopteri AMC23_MB13      5
## 2 Ulvophyceae AMC23_MB13      1
## [1] "Target species detected in 2023:"
## [1] "Cottus cognatus"          "Hippoglossoides platessoides"
## [3] "Rhinichthys atratulus"    "Salvelinus fontinalis"
## [5] "Semotilus atromaculatus"
```

MB12 MB12 is downstream of MB13 and another Parallel Control, and was only sampled in 2023.

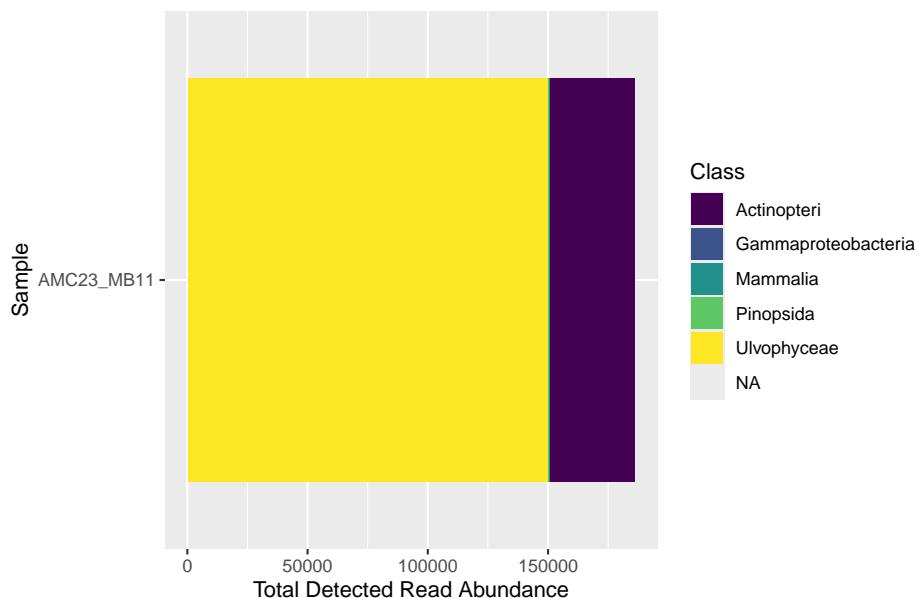
Detection Results for MB12



```
##           Class      Sample Species
## 1 Actinopteri  AMC23_MB12      5
## 2 Ulvophyceae  AMC23_MB12      1
## [1] "Target species detected in 2023:"
## [1] "Anguilla rostrata"    "Clupea harengus"    "Cottus cognatus"
## [4] "Rhinichthys atratulus" "Salvelinus fontinalis"
```

MB11 MB11 is upstream of the 2022 LWA installation on the Middle Branch, and was only sampled in 2023. It is considered Upstream and Post-LWA.

Detection Results for MB11



```
##           Class      Sample Species
```

```

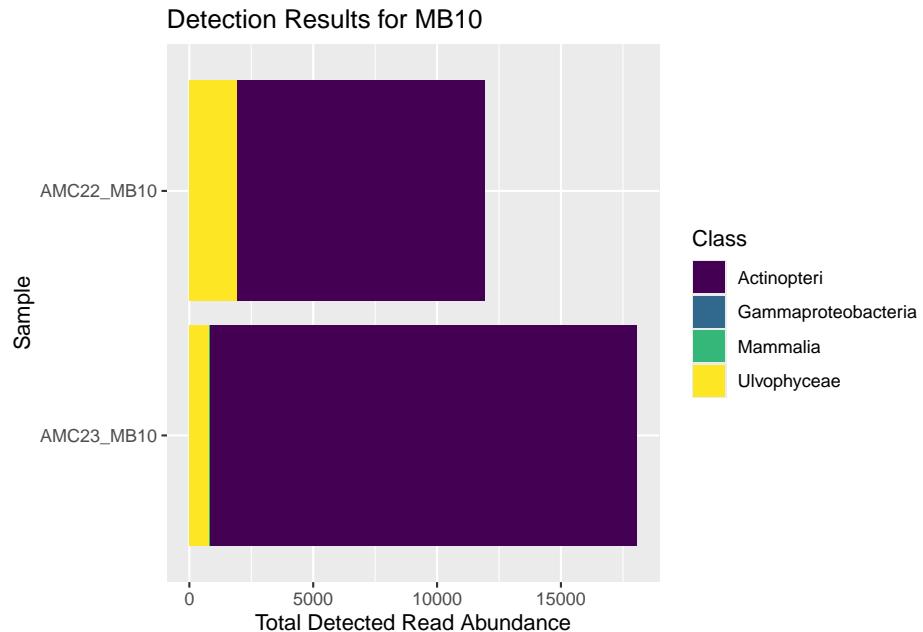
## 1      Actinopteri AMC23_MB11      6
## 2  Gammaproteobacteria AMC23_MB11    1
## 3      Mammalia AMC23_MB11       2
## 4      Pinopsida AMC23_MB11      1
## 5     Ulvophyceae AMC23_MB11      1

## [1] "Target species detected in 2023:"

## [1] "Brevoortia tyrannus"      "Castor canadensis"
## [3] "Chrosomus neogaeus"       "Notemigonus crysoleucas"
## [5] "Rhinichthys atratulus"    "Salvelinus fontinalis"
## [7] "Semotilus atromaculatus"

```

MB10 MB10 was sampled in both 2022 and 2023 at the same coordinates, and is the farthest upstream site within the 2022 LWA installation reach on the Middle Branch. It is considered Internal and Pre-LWA for 2022, and Internal and Post-LWA for 2023.



```

##          Class      Sample Species
## 1      Actinopteri AMC22_MB10      5
## 2  Gammaproteobacteria AMC22_MB10    1
## 3     Ulvophyceae AMC22_MB10      1
## 4      Actinopteri AMC23_MB10      6
## 5      Mammalia AMC23_MB10      1
## 6     Ulvophyceae AMC23_MB10      1

## [1] "Target species detected in 2022:"

## [1] "Hippoglossoides platessoides" "Perca flavescens"
## [3] "Rhinichthys atratulus"        "Salvelinus fontinalis"
## [5] "Semotilus atromaculatus"

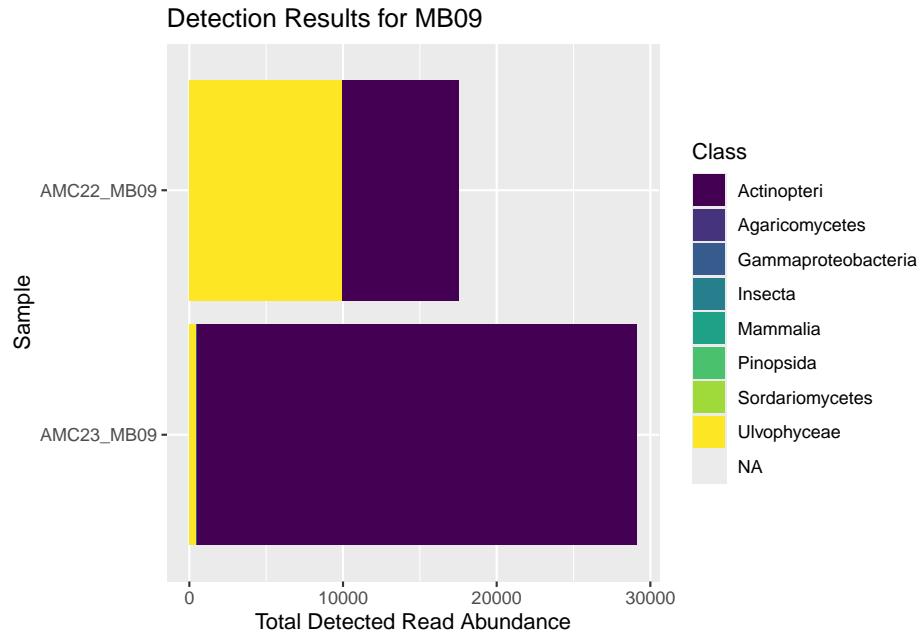
## [1] "Target species detected in 2023:"

## [1] "Anguilla rostrata"           "Castor canadensis"
## [3] "Chrosomus neogaeus"          "Notemigonus crysoleucas"
## [5] "Rhinichthys atratulus"       "Salvelinus fontinalis"

```

```
## [7] "Semotilus atromaculatus"
```

MB09 MB09 was sampled in both 2022 and 2023 at the same coordinates, and is considered Internal and Pre-LWA for 2022, and Internal and Post-LWA for 2023.

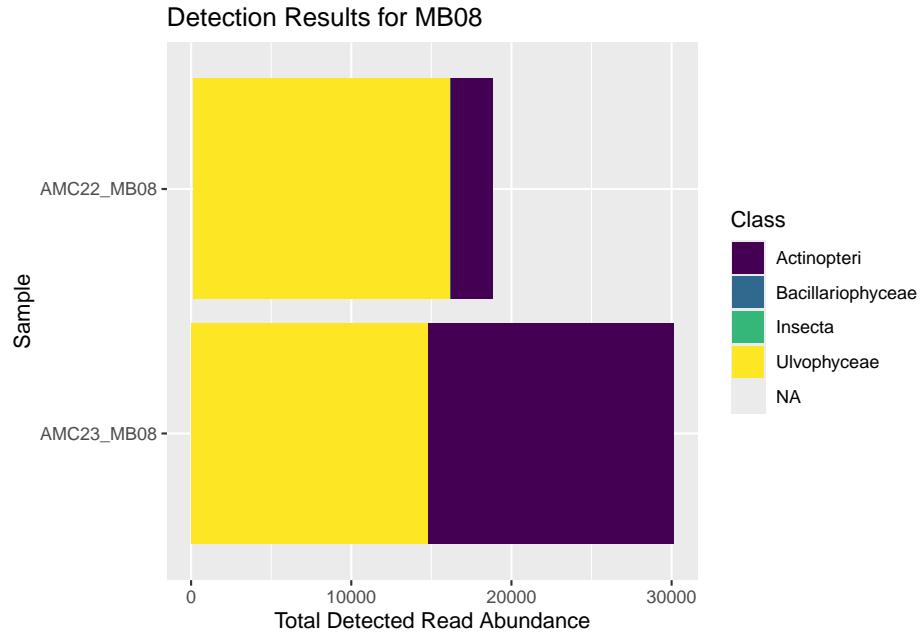


```
##          Class     Sample Species
## 1      Actinopteri AMC22_MB09      3
## 2      Agaricomycetes AMC22_MB09      1
## 3      Insecta    AMC22_MB09      1
## 4      Pinopsida   AMC22_MB09      1
## 5  Sordariomycetes AMC22_MB09      1
## 6      Ulvophyceae AMC22_MB09      1
## 7      Actinopteri AMC23_MB09      5
## 8  Gammaproteobacteria AMC23_MB09      1
## 9      Mammalia   AMC23_MB09      1
## 10     Ulvophyceae AMC23_MB09      1

## [1] "Target species detected in 2022:"
## [1] "Eurylophella funeralis"      "Hippoglossoides platessoides"
## [3] "Rhinichthys atratulus"      "Salvelinus fontinalis"

## [1] "Target species detected in 2023:"
## [1] "Hippoglossoides platessoides" "Notemigonus crysoleucas"
## [3] "Odocoileus virginianus"      "Rhinichthys atratulus"
## [5] "Salvelinus fontinalis"        "Semotilus atromaculatus"
```

MB08 MB08 was sampled in 2022 and 2023 at the same coordinates, and is just upstream of the confluence. It is considered Internal and Pre-LWA for 2022, and Internal and Post-LWA for 2023.



```

##           Class     Sample Species
## 1   Actinopteri AMC22_MB08      1
## 2 Bacillariophyceae AMC22_MB08      2
## 3    Insecta    AMC22_MB08      2
## 4   Ulvophyceae AMC22_MB08      1
## 5   Actinopteri AMC23_MB08      6
## 6   Ulvophyceae AMC23_MB08      1

## [1] "Target species detected in 2022:"
## [1] "Habrophlebiodes americana" "Paraleptophlebia adoptiva"
## [3] "Rhinichthys atratulus"
## [1] "Target species detected in 2023:"
## [1] "Brevoortia tyrannus"      "Cottus cognatus"
## [3] "Merluccius bilinearis"    "Rhinichthys atratulus"
## [5] "Salvelinus fontinalis"    "Semotilus atromaculatus"

```

MB07 MB07 was sampled in 2022 and 2023 at the same coordinates. It is considered Internal and Pre-LWA for 2022, and Internal and Post-LWA for 2023, and is the second-most downstream site on the Middle Branch reach.

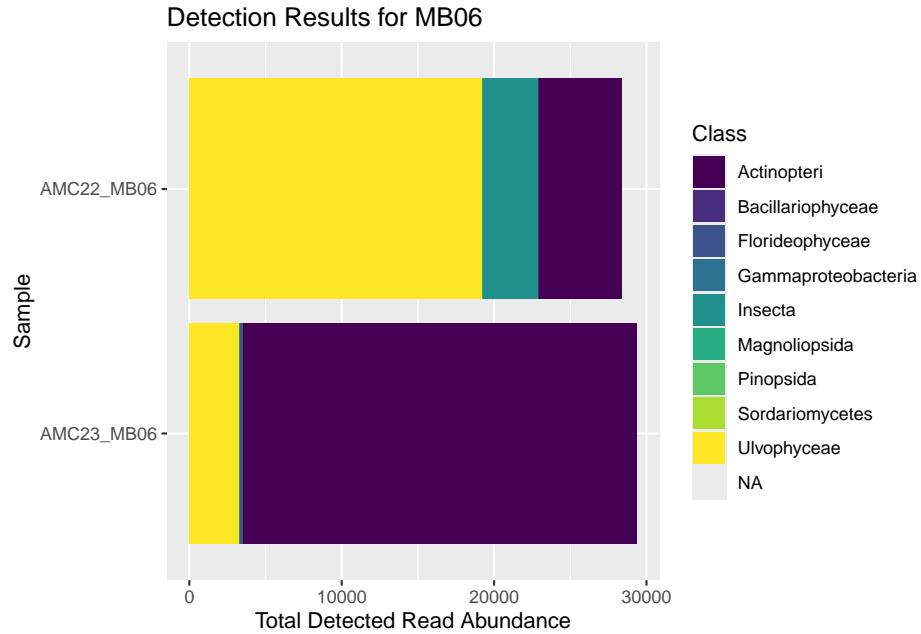


```

##           Class     Sample Species
## 1      Actinopteri AMC22_MB07    4
## 2      Ulvophyceae AMC22_MB07    1
## 3      Actinopteri AMC23_MB07    5
## 4      Agaricomycetes AMC23_MB07   2
## 5      Florideophyceae AMC23_MB07   1
## 6  Gammaproteobacteria AMC23_MB07   1
## 7      Insecta    AMC23_MB07    7
## 8      Magnoliopsida AMC23_MB07   1
## 9          na    AMC23_MB07    2
## 10     Pinopsida  AMC23_MB07   1
## 11      Ulvophyceae AMC23_MB07   1
## [1] "Target species detected in 2022:"
## [1] "Cottus cognatus"          "Rhinichthys atratulus"
## [3] "Salvelinus fontinalis"    "Semotilus atromaculatus"
## [1] "Target species detected in 2023:"
## [1] "Cottus cognatus"          "Dolophilodes distinctus"
## [3] "Eurylophella funeralis"    "Habrophlebiodes americana"
## [5] "Hippoglossoides platessoides" "Lanthus parvulus"
## [7] "Paraleptophlebia debilis"    "Prosimilium mixtum"
## [9] "Rhinichthys atratulus"      "Salvelinus fontinalis"
## [11] "Semotilus atromaculatus"    "Valenzuela flavidus"

```

MB06 MB06 is the farthest downstream sample for the Middle Branch reach, and was sampled in both 2022 and 2023 at the same coordinates. It is considered Internal and Pre-LWA for 2022, and Internal and Post-LWA for 2023.



```

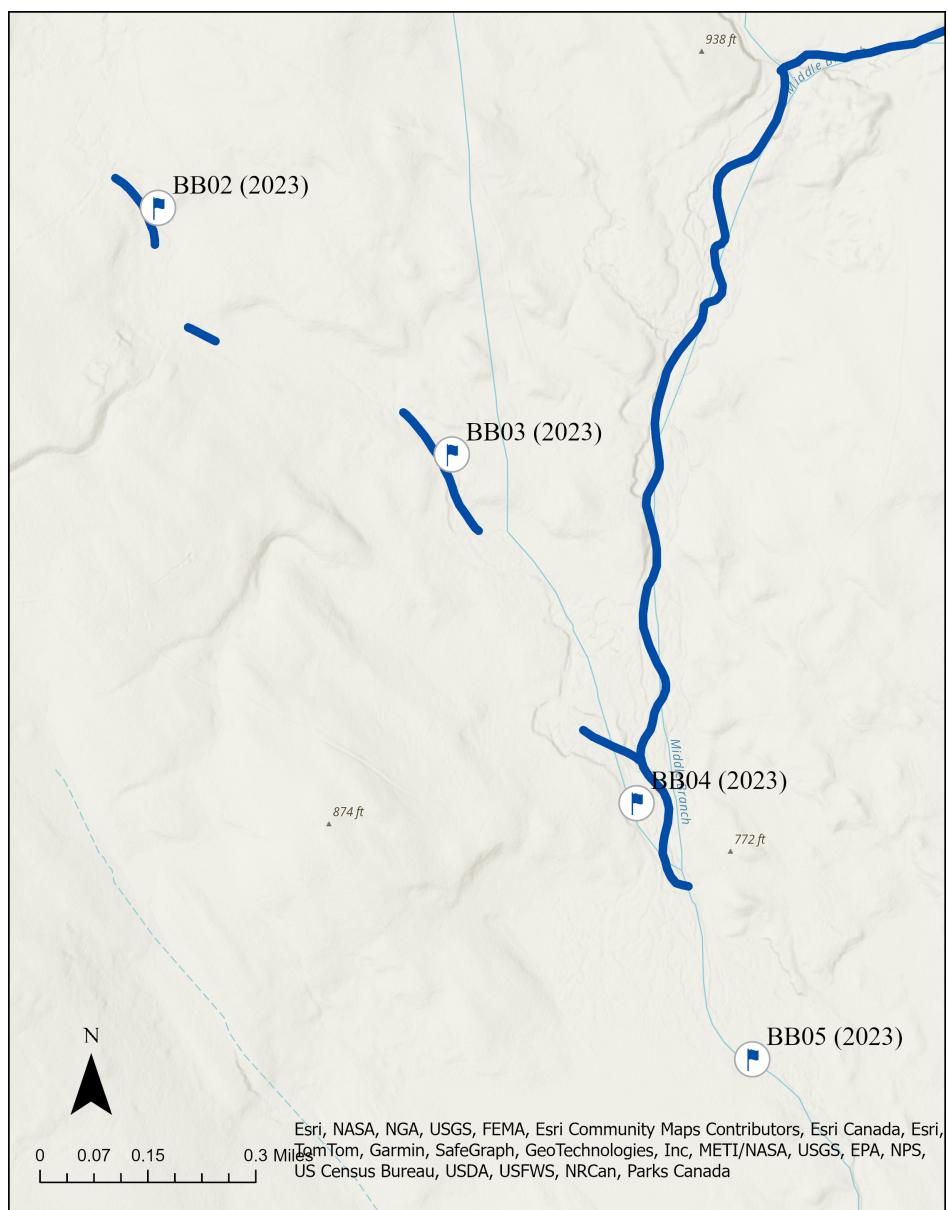
##          Class     Sample Species
## 1      Actinopteri AMC22_MB06    5
## 2  Bacillariophyceae AMC22_MB06   2
## 3  Florideophyceae AMC22_MB06   1
## 4      Insecta    AMC22_MB06   6
## 5      Pinopsida  AMC22_MB06   1
## 6  Sordariomycetes AMC22_MB06   1
## 7      Ulvophyceae AMC22_MB06   1
## 8      Actinopteri AMC23_MB06   6
## 9  Florideophyceae AMC23_MB06   1
## 10 Gammaproteobacteria AMC23_MB06   1
## 11      Insecta    AMC23_MB06   2
## 12      Pinopsida  AMC23_MB06   1
## 13      Ulvophyceae AMC23_MB06   1

## [1] "Target species detected in 2022:"
## [1] "Baetis phoebus"           "Baetis tricaudatus"
## [3] "Clupea harengus"         "Cottus cognatus"
## [5] "Ephemерella invaria"      "Maccaffertium vicarium"
## [7] "Microtendipes pedellus"    "Rhinichthys atratulus"
## [9] "Sebastes norvegicus"      "Semotilus atromaculatus"
## [11] "Teloganopsis deficiens"

## [1] "Target species detected in 2023:"
## [1] "Baetis tricaudatus"       "Cottus cognatus"
## [3] "Drunella cornutella"       "Perca flavescens"
## [5] "Rhinichthys atratulus"     "Salmo trutta"
## [7] "Salvelinus fontinalis"      "Semotilus atromaculatus"

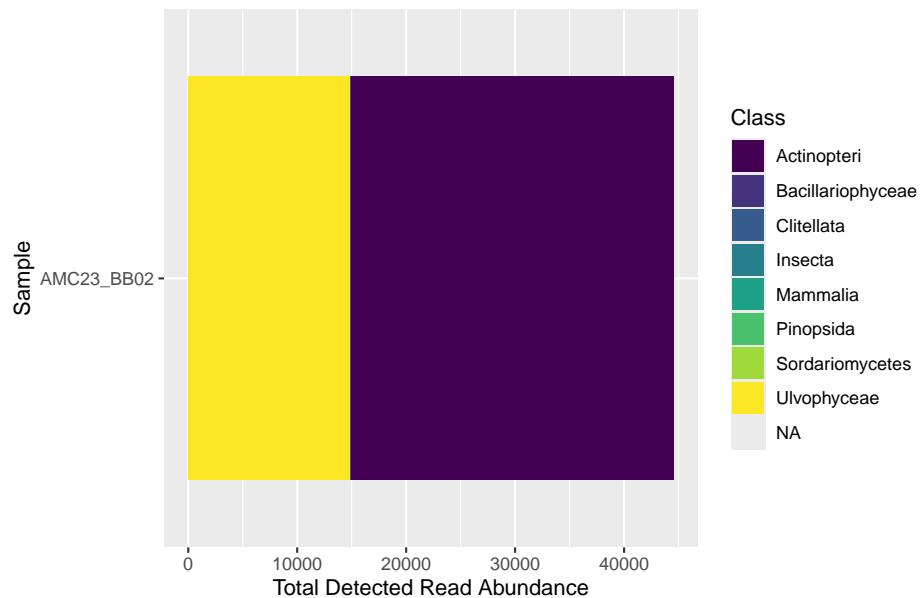
```

Black Brook - BB02, BB03, BB04



BB02

Detection Results for BB02



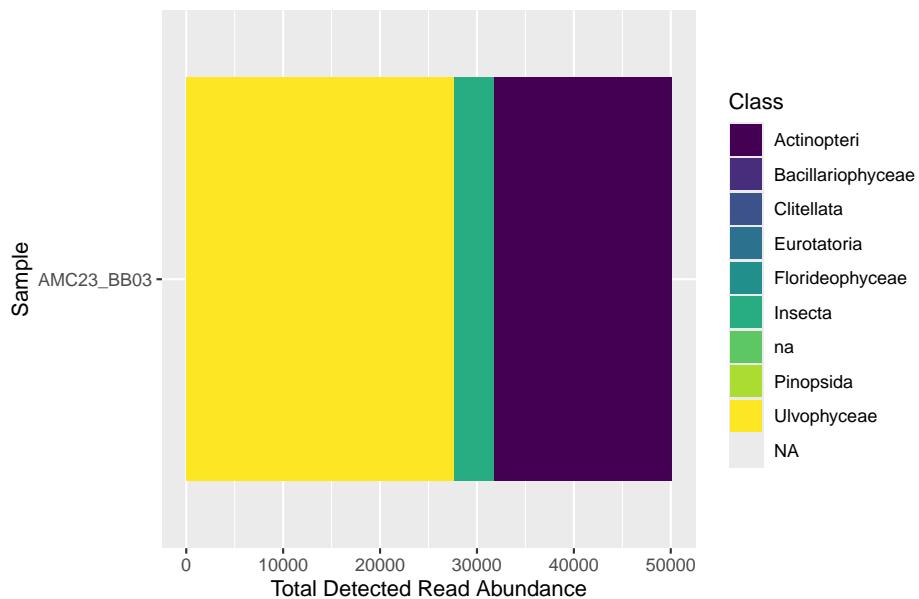
```
##           Class     Sample Species
## 1      Actinopteri AMC23_BB02      5
## 2  Bacillariophyceae AMC23_BB02      1
## 3      Clitellata AMC23_BB02      2
## 4      Insecta   AMC23_BB02      4
## 5      Mammalia  AMC23_BB02      1
## 6      Pinopsida  AMC23_BB02      1
## 7  Sordariomycetes AMC23_BB02      1
## 8  Ulvophyceae  AMC23_BB02      1

## [1] "Target species detected in 2023:"
```

Species	Count
"Baetis tricaudatus"	1
"Castor canadensis"	1
"Catostomus commersonii"	1
"Chaetogaster diastrophus"	1
"Eukiefferiella claripennis"	1
"Habrophlebiodes americana"	1
"Margariscus margarita"	1
"Phyllocentropus placidus"	1
"Rhinichthys atratulus"	1
"Salvelinus fontinalis"	1
"Semotilus atromaculatus"	1
"Stylodrilus herringianus"	1

BB03

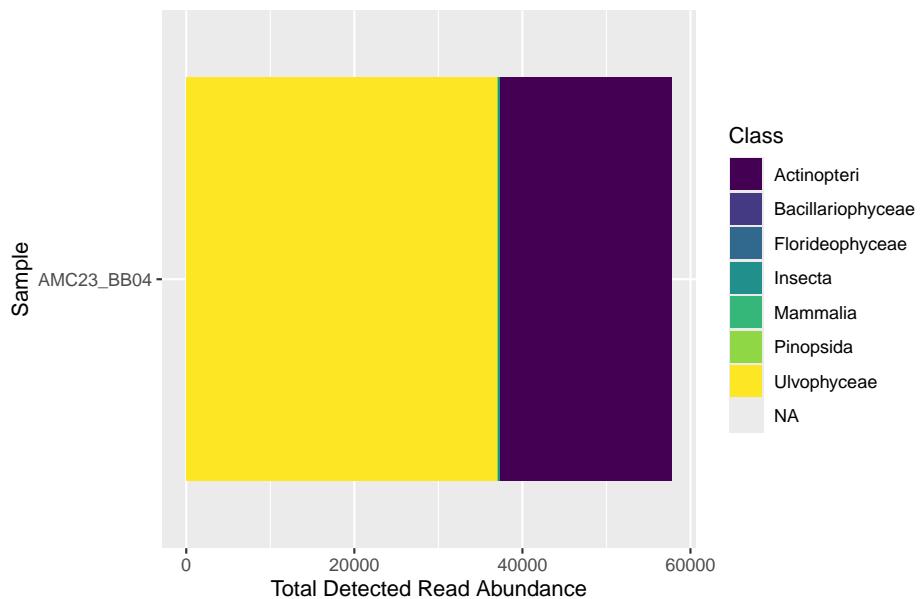
Detection Results for BB03



```
##           Class     Sample Species
## 1      Actinopteri AMC23_BB03     4
## 2  Bacillariophyceae AMC23_BB03     1
## 3      Clitellata AMC23_BB03     2
## 4     Eurotatoria AMC23_BB03     1
## 5  Florideophyceae AMC23_BB03     3
## 6      Insecta   AMC23_BB03    14
## 7        na     AMC23_BB03     1
## 8      Pinopsida AMC23_BB03     1
## 9     Ulvophyceae AMC23_BB03     1
## [1] "Target species detected in 2023:"
## [1] "Acerpenna macdunnoughi"      "Aporrectodea caliginosa"
## [3] "Baetis intercalaris"         "Baetis phoebus"
## [5] "Baetis pluto"               "Baetis tricaudatus"
## [7] "Catostomus commersonii"     "Dolophilodes distinctus"
## [9] "Drunella cornutella"         "Ephemera varia"
## [11] "Ephemerella invaria"        "Eurylophella funeralis"
## [13] "Paraleptophlebia adoptiva"   "Polypedilum aviceps"
## [15] "Pristina aequiseta"          "Pycnopsyche scabripennis"
## [17] "Rhinichthys atratulus"       "Salvelinus fontinalis"
## [19] "Semotilus atromaculatus"     "Teloganopsis deficiens"
```

BB04

Detection Results for BB04



```

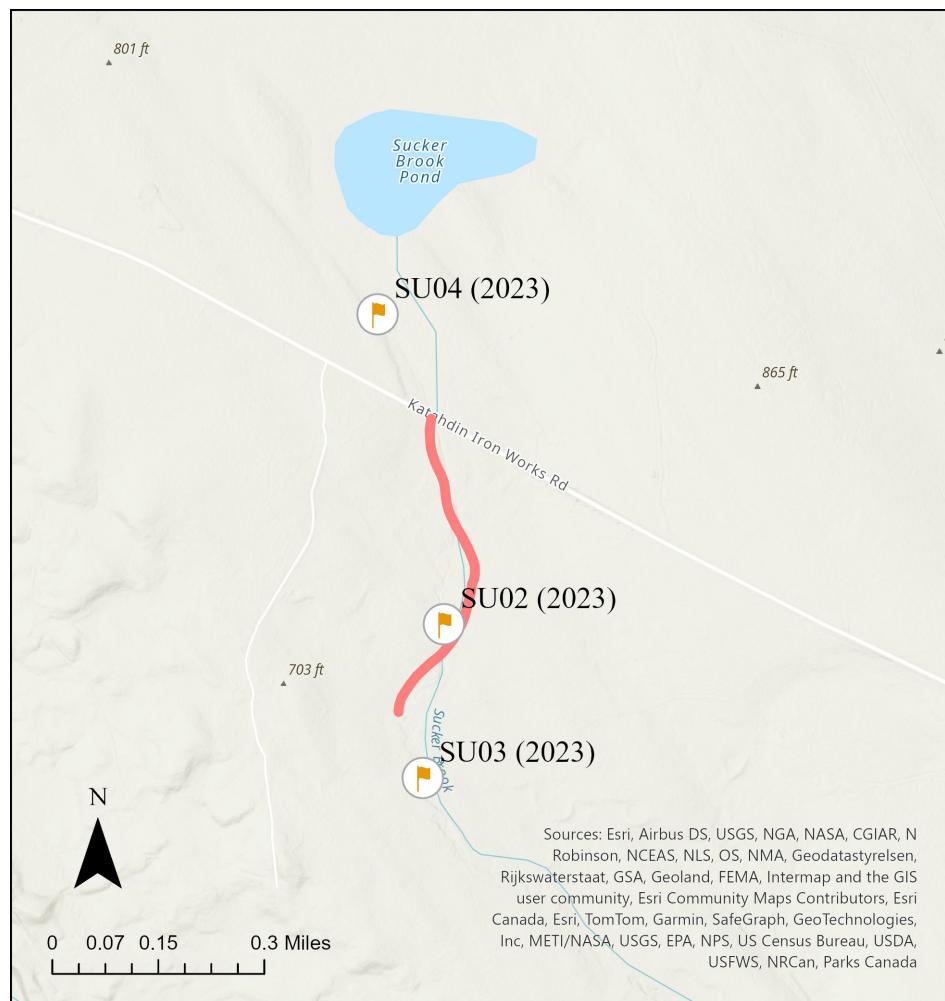
##           Class      Sample Species
## 1   Actinopteri  AMC23_BB04      5
## 2 Bacillariophyceae  AMC23_BB04      1
## 3   Florideophyceae  AMC23_BB04      1
## 4     Insecta  AMC23_BB04     12
## 5    Pinopsida  AMC23_BB04      1
## 6   Ulvophyceae  AMC23_BB04      1

## [1] "Target species detected in 2023:"

## [1] "Acerpenna macdunnoughi"  "Baetis intercalaris"
## [3] "Baetis phoebus"          "Baetis tricaudatus"
## [5] "Catostomus commersonii"  "Cottus cognatus"
## [7] "Cricotopus bicinctus"    "Drunella cornutella"
## [9] "Ephemerella invaria"     "Plauditus dubius"
## [11] "Polypedilum aviceps"    "Rhinichthys atratulus"
## [13] "Salvelinus fontinalis"   "Semotilus atromaculatus"
## [15] "Simulium tuberosum"      "Simulium verecundum"
## [17] "Stenacron interpunctatum"

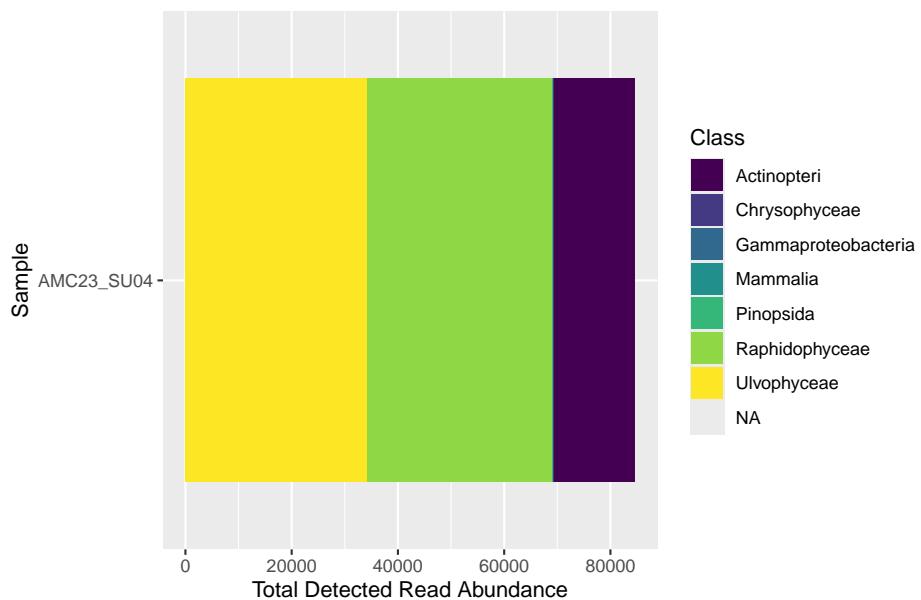
```

Sucker Brook - SU03, SU02, SU04



SU04

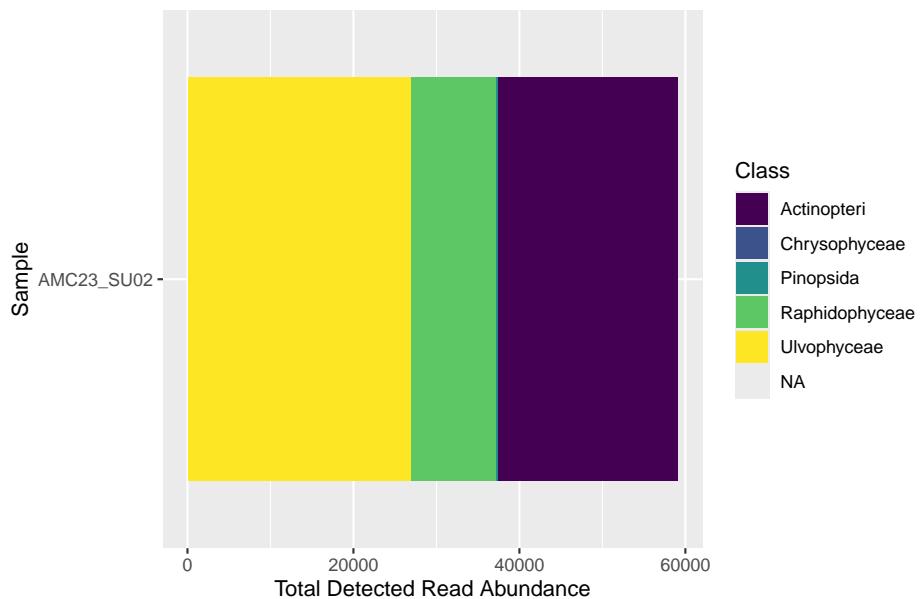
Detection Results for SU04



```
##           Class     Sample Species
## 1      Actinopteri AMC23_SU04      3
## 2   Chrysophyceae AMC23_SU04      1
## 3 Gammaproteobacteria AMC23_SU04      1
## 4      Mammalia  AMC23_SU04      1
## 5      Pinopsida  AMC23_SU04      1
## 6  Raphidophyceae  AMC23_SU04      1
## 7   Ulvophyceae  AMC23_SU04      1
## [1] "Target species detected in 2023:"
## [1] "Catostomus commersonii"  "Esox niger"
## [3] "Semotilus atromaculatus" "Sorex cinereus"
```

SU02

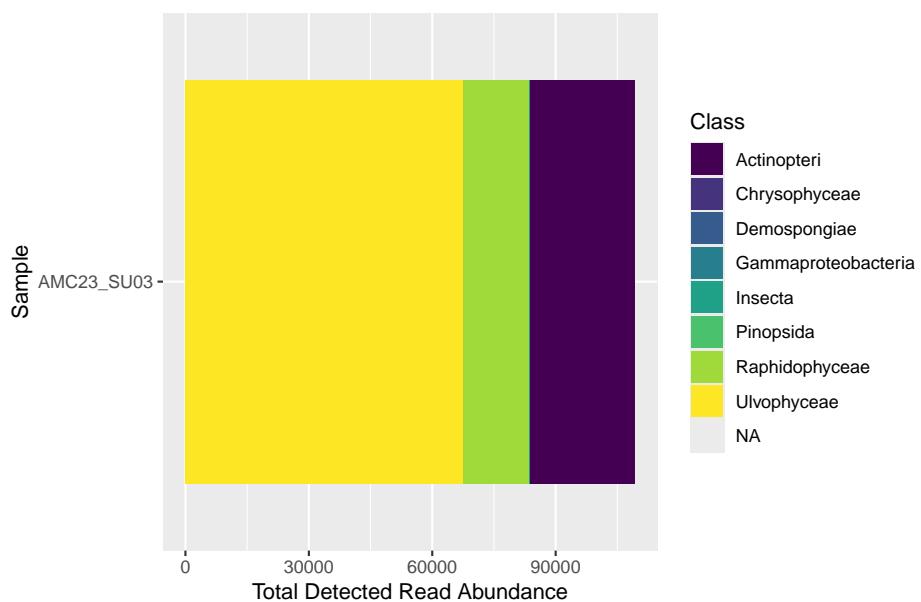
Detection Results for SU02



```
##           Class     Sample Species
## 1   Actinopteri AMC23_SU02      4
## 2 Chrysophyceae AMC23_SU02      1
## 3   Pinopsida  AMC23_SU02      1
## 4 Raphidophyceae AMC23_SU02      1
## 5   Ulvophyceae AMC23_SU02      1
## [1] "Target species detected in 2023:"
## [1] "Catostomus commersonii"  "Chrosomus neogaeus"
## [3] "Margariscus margarita"   "Semotilus atromaculatus"
```

SU03

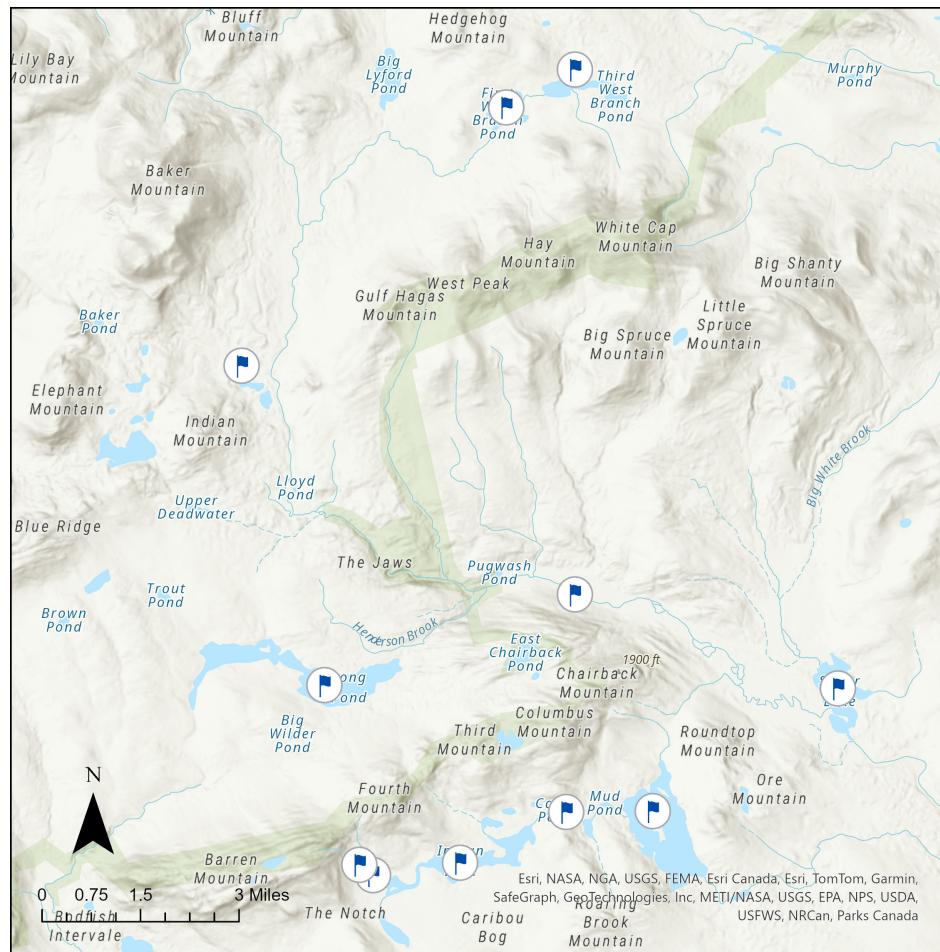
Detection Results for SU03



```
##          Class      Sample Species
## 1      Actinopteri AMC23_SU03      3
## 2      Chrysophyceae AMC23_SU03     1
## 3      Demospongiae AMC23_SU03     1
## 4      Gammaproteobacteria AMC23_SU03 1
## 5          Insecta  AMC23_SU03      2
## 6      Pinopsida   AMC23_SU03      1
## 7      Raphidophyceae AMC23_SU03     1
## 8      Ulvophyceae AMC23_SU03      1

## [1] "Target species detected in 2023:"
## [1] "Chrosomus neogaeus"      "Notemigonus crysoleucas"
## [3] "Semotilus atromaculatus" "Spongilla lacustris"
## [5] "Sympetrum vicinum"       "Tvetenia paucunca"
```

Regional Site Results - Gulf Hagas Sites

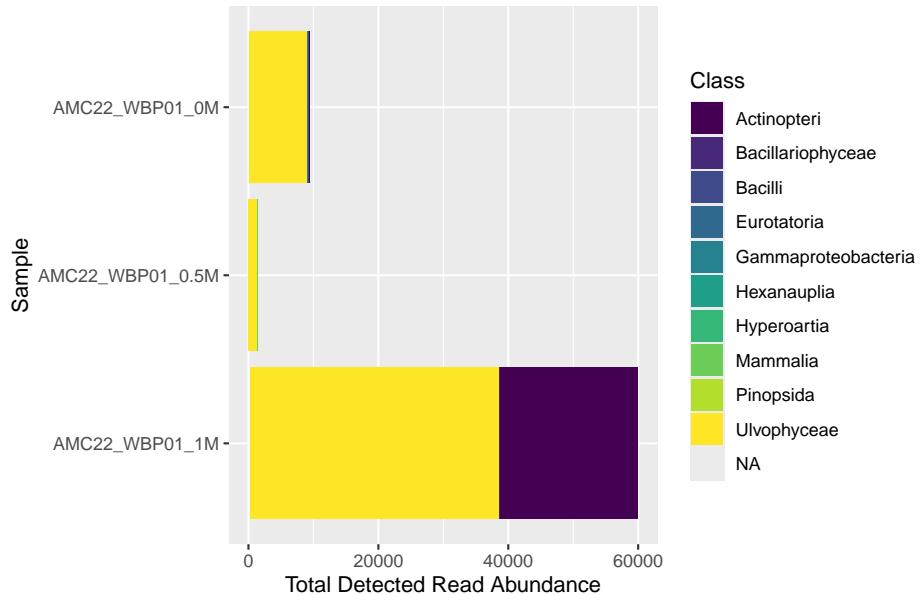


WBP01 & WBP02



WBP01 WBP01 is the northernmost site in both the Gulf Hagas and LWA sampling regions, and is located in the Second West Branch Pond, connected to the First West Branch Pond (WBP02). WBP01 samples were kept separate to examine differences in detection at 0, 0.5, and 1 meter depths.

Detection Results for WBP01



##	Class	Sample	Species
## 1	Mammalia	AMC22_WBP01_0.5M	1
## 2	Ulvophyceae	AMC22_WBP01_0.5M	1
## 3	Actinopteri	AMC22_WBP01_0M	1
## 4	Bacilli	AMC22_WBP01_0M	1
## 5	Gammaproteobacteria	AMC22_WBP01_0M	1

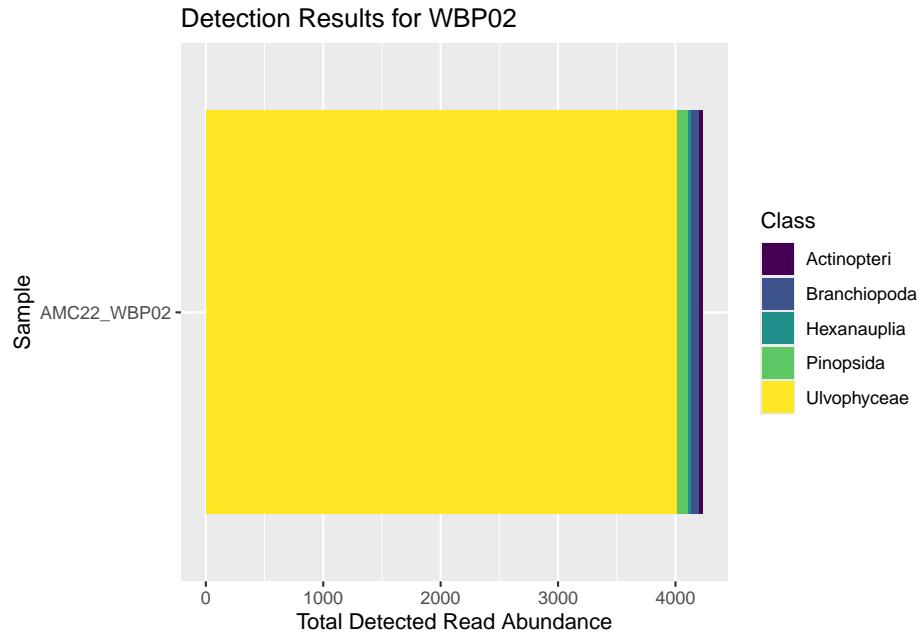
```

## 6 Pinopsida AMC22_WBP01_OM 1
## 7 Ulvophyceae AMC22_WBP01_OM 1
## 8 Actinopteri AMC22_WBP01_1M 4
## 9 Bacillariophyceae AMC22_WBP01_1M 1
## 10 Eurotatoria AMC22_WBP01_1M 1
## 11 Hexanauplia AMC22_WBP01_1M 1
## 12 Hyperoartia AMC22_WBP01_1M 1
## 13 Ulvophyceae AMC22_WBP01_1M 1

## [1] "Target species detected at 0M:"
## [1] "Hippoglossoides plateaoides"
## [1] "Target species detected at 0.5M:"
## [1] "Sus scrofa"
## [1] "Target species detected at 1M:"
## [1] "Chrosomus neogaeus"      "Leptodiaptomus minutus"
## [3] "Petromyzon marinus"      "Rhinichthys atratulus"
## [5] "Salvelinus fontinalis"    "Semotilus atromaculatus"

```

WBP02 WBP02 is located in the First West Branch pond, and is the second northern-most site in the Gulf Hagas region.



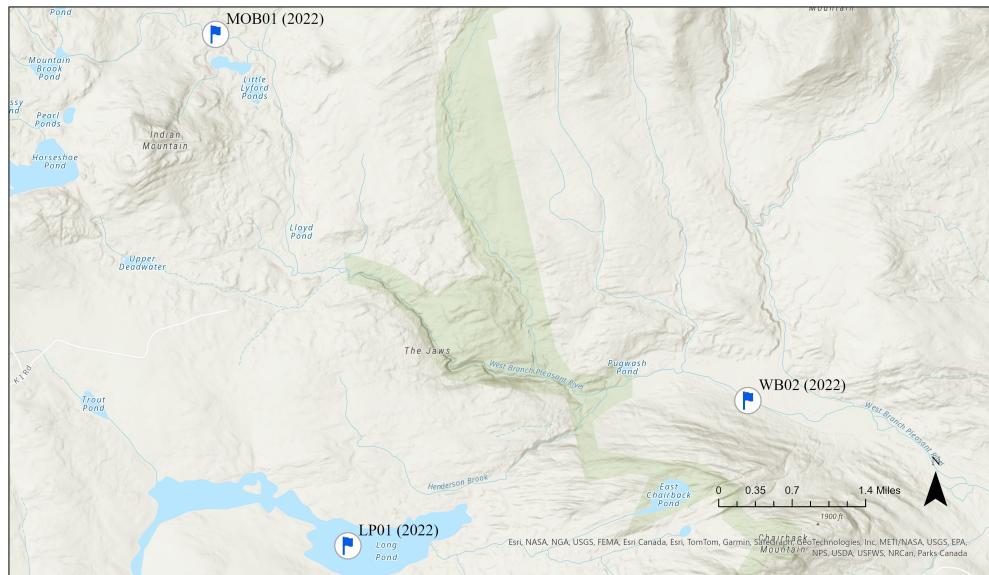
```

##          Class     Sample Species
## 1  Actinopteri AMC22_WBP02      1
## 2 Branchiopoda AMC22_WBP02      1
## 3  Hexanauplia AMC22_WBP02      1
## 4   Pinopsida AMC22_WBP02      1
## 5 Ulvophyceae AMC22_WBP02      1

## [1] "Target species detected:"
## [1] "Brevoortia tyrannus"      "Daphnia catawba"      "Leptodiaptomus minutus"

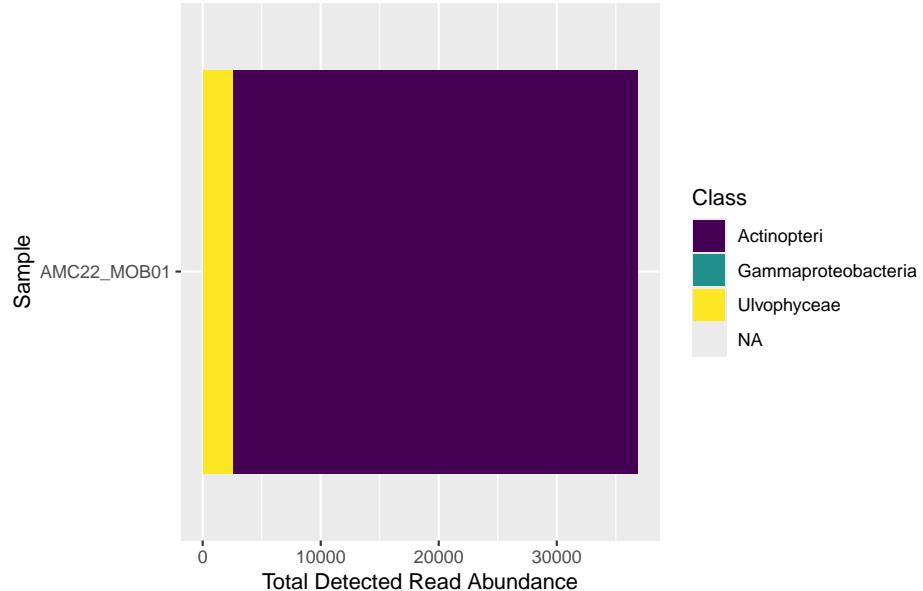
```

MOB01, LP01, WB02



MOB01 MOB01 is placed just above Little Lyford Pond, and is the western-most site in the GH region. It is distantly connected to WB02 in the West Branch Pleasant River.

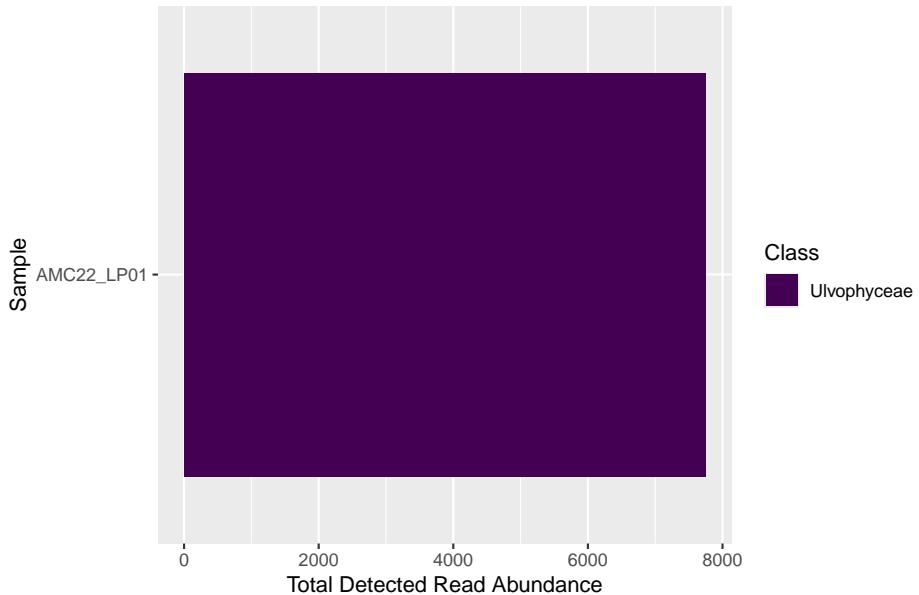
Detection Results for MOB01



```
##          Class     Sample Species
## 1      Actinopteri AMC22_MOB01     1
## 2  Gammaproteobacteria AMC22_MOB01     1
## 3      Ulvophyceae AMC22_MOB01     1
## [1] "Target species detected:"
## [1] "Salvelinus fontinalis"
```

LP01 LP01 is placed in Long Pond, southeast of MOB01.

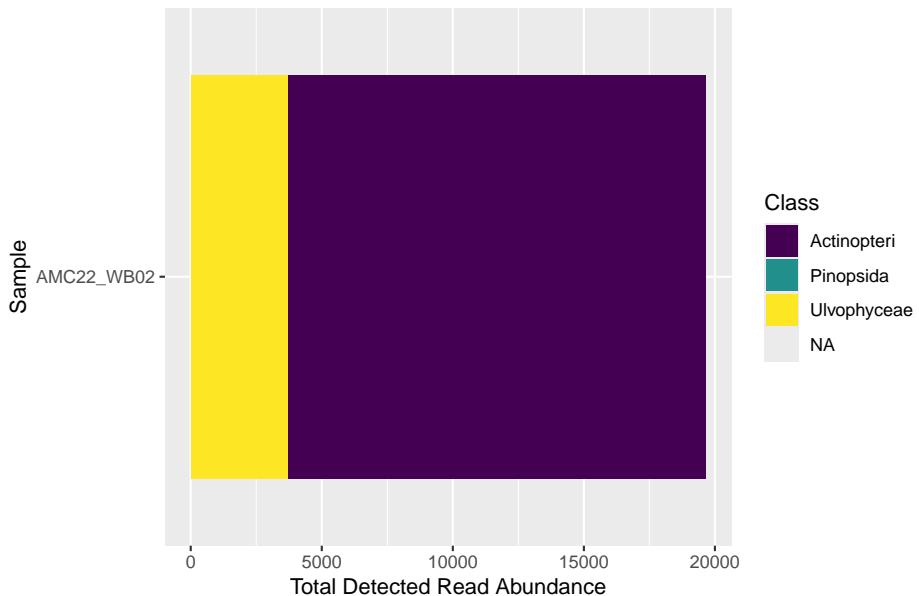
Detection Results for LP01



```
##           Class      Sample Species
## 1 Ulvophyceae AMC22_LP01      1
## [1] "Target species detected:"
## character(0)
```

WB02 WB02 is placed in the West Branch Pleasant River between MOB01 and SL01 in Silver Lake.

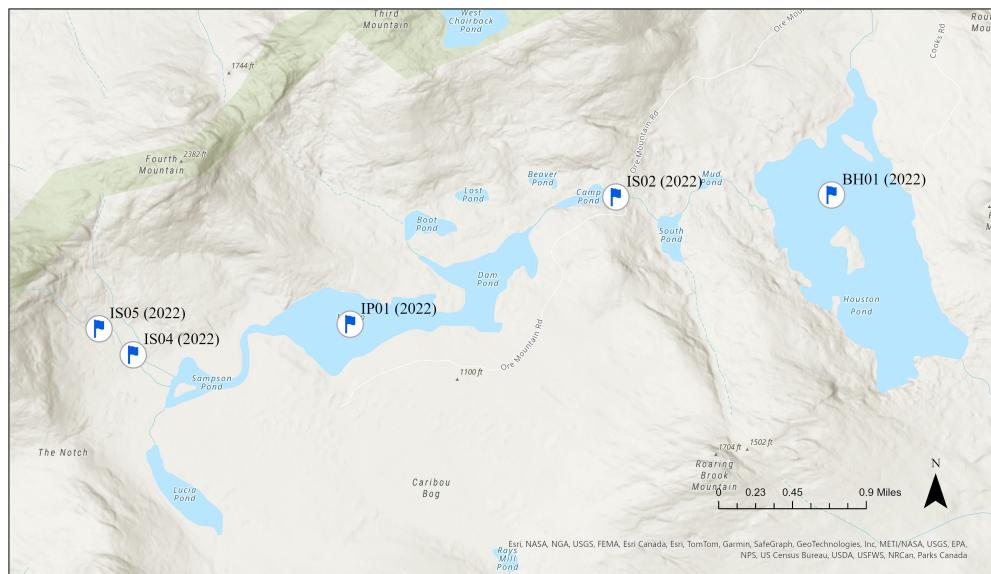
Detection Results for WB02



```
##           Class      Sample Species
## 1 Actinopteri AMC22_MOB01      1
## 2 Gammaproteobacteria AMC22_MOB01      1
```

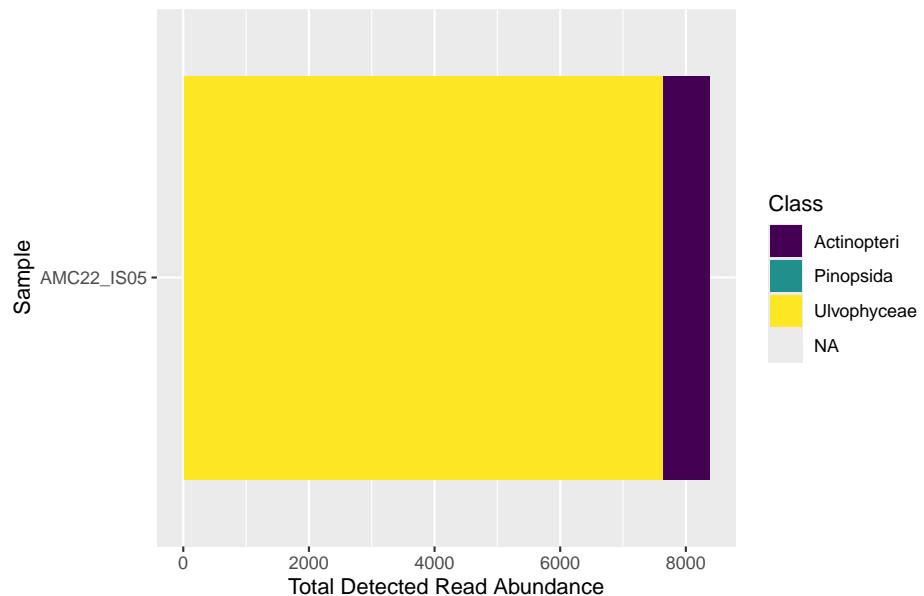
```
## 3           Ulvophyceae AMC22_MOB01      1
## [1] "Target species detected:"
## [1] "Notemigonus crysoleucas" "Rhinichthys atratulus"
## [3] "Semotilus atromaculatus"
```

IS02, IS04, IS05, IP01 & BH01



IS05

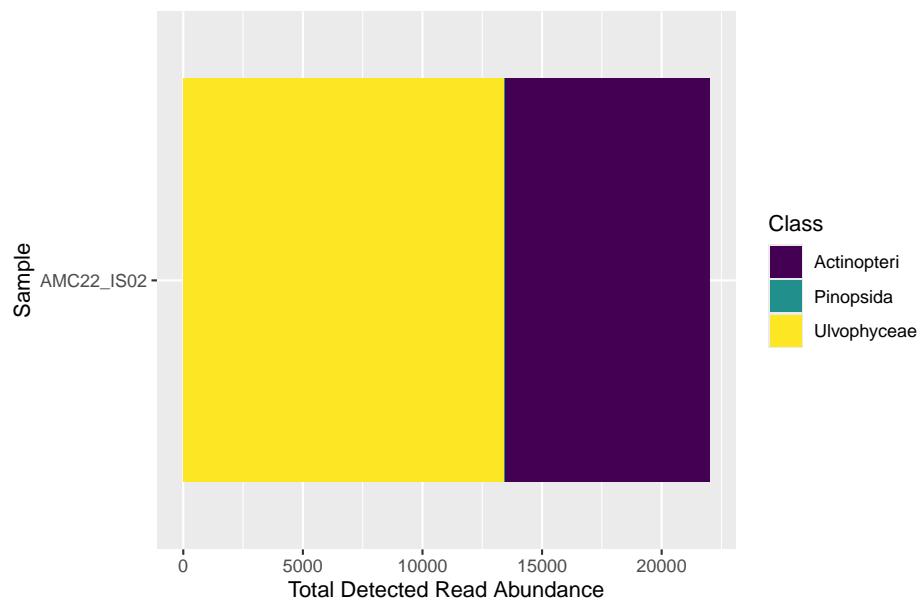
Detection Results for IS05



```
##           Class     Sample Species
## 1  Actinopteri  AMC22_IS05      2
## 2   Pinopsida  AMC22_IS05      1
## 3 Ulvophyceae  AMC22_IS05      1
## [1] "Target species detected:"
## [1] "Brevoortia tyrannus"          "Hippoglossoides platessoides"
```

IS02

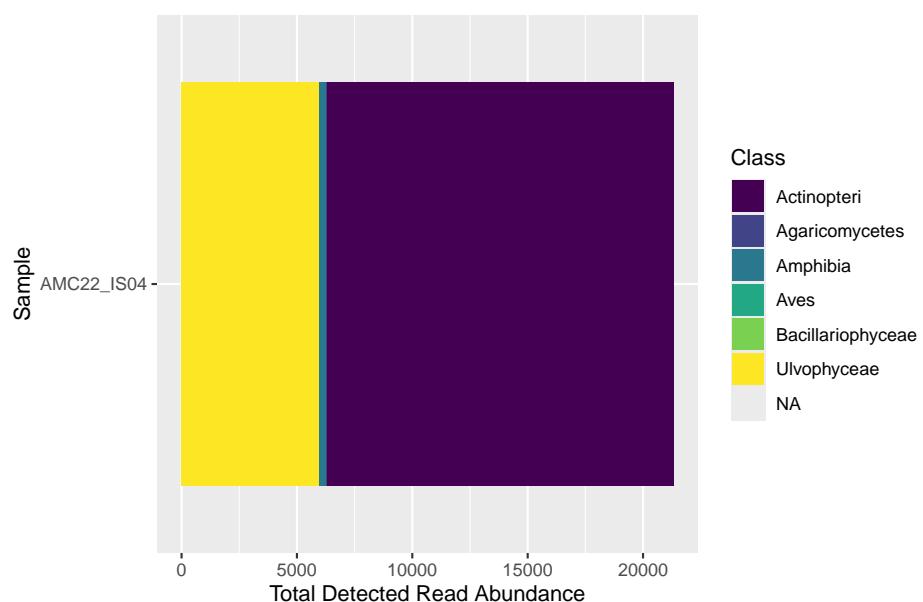
Detection Results for IS02



```
##           Class     Sample Species
## 1 Actinopteri AMC22_IS02      4
## 2   Pinopsida  AMC22_IS02      1
## 3  Ulvophyceae  AMC22_IS02      1
## [1] "Target species detected:"
## [1] "Catostomus commersonii"  "Fundulus diaphanus"
## [3] "Lepomis auritus"          "Notemigonus crysoleucas"
```

IS04

Detection Results for IS04



```

##          Class      Sample Species
## 1    Actinopteri  AMC22_IS04      1
## 2  Agaricomycetes  AMC22_IS04      1
## 3     Amphibia   AMC22_IS04      1
## 4       Aves    AMC22_IS04      1
## 5 Bacillariophyceae  AMC22_IS04      1
## 6    Ulvophyceae  AMC22_IS04      1

## [1] "Target species detected:"
## [1] "Gyrinophilus porphyriticus" "Haemorhous purpureus"
## [3] "Salvelinus fontinalis"

```

IP01



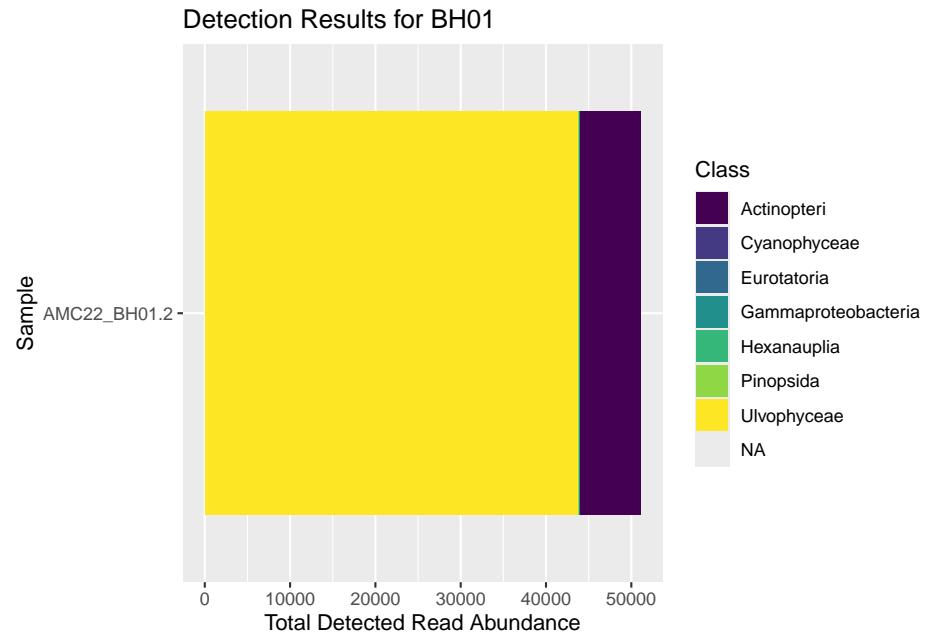
```

##          Class      Sample Species
## 1    Actinopteri  AMC22_IP01      4
## 2  Branchiopoda  AMC22_IP01      1
## 3  Chrysophyceae  AMC22_IP01      1
## 4   Eurotatoria  AMC22_IP01      1
## 5  Hexanauplia  AMC22_IP01      1
## 6    Pinopsida   AMC22_IP01      1
## 7 Raphidophyceae  AMC22_IP01      1
## 8    Ulvophyceae  AMC22_IP01      1

## [1] "Target species detected:"
## [1] "Anguilla rostrata"      "Catostomus commersonii" "Daphnia catawba"
## [4] "Fundulus diaphanus"     "Lepomis auritus"        "Leptodiaptomus minutus"

```

BH01

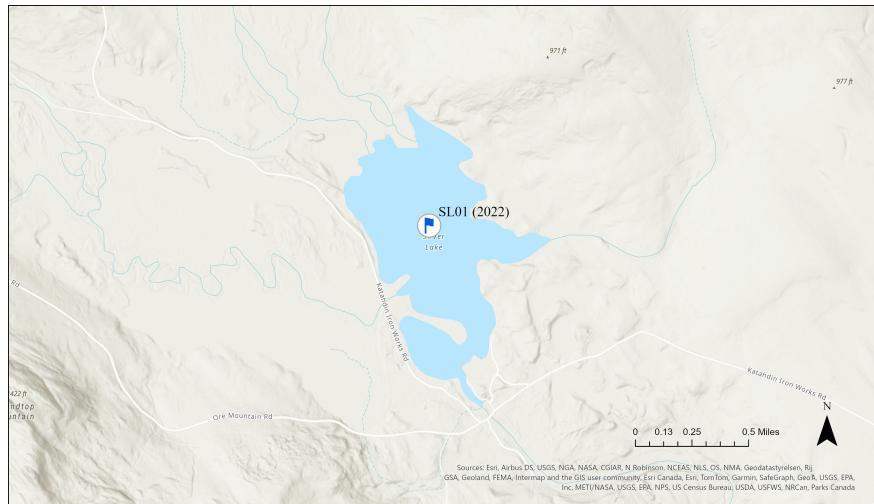


```

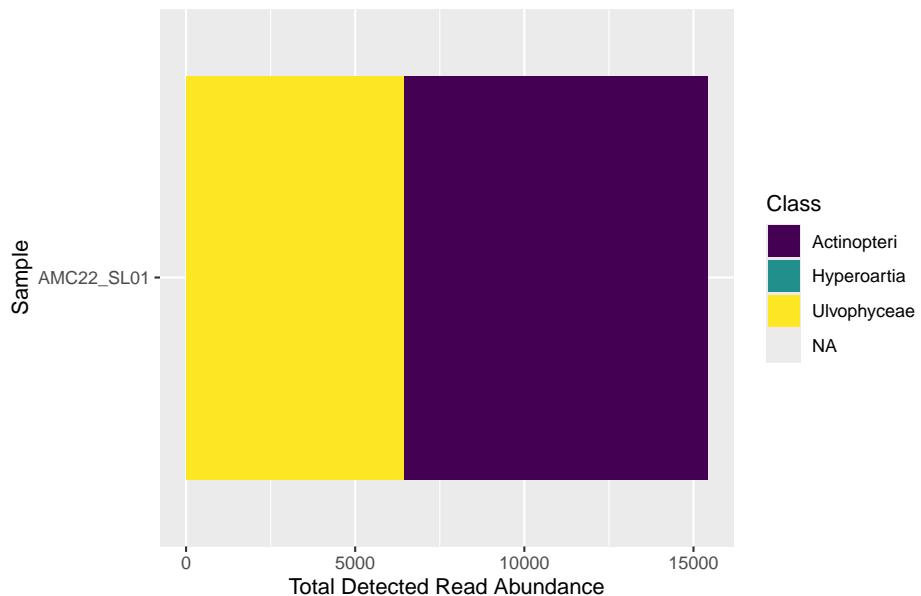
##           Class      Sample Species
## 1   Actinopteri AMC22_BH01.2     2
## 2   Cyanophyceae AMC22_BH01.2     1
## 3   Eurotatoria AMC22_BH01.2     1
## 4 Gammaproteobacteria AMC22_BH01.2     1
## 5   Hexanauplia AMC22_BH01.2     1
## 6   Pinopsida    AMC22_BH01.2     1
## 7   Ulvophyceae AMC22_BH01.2     1
## [1] "Target species detected:"
## [1] "Fundulus diaphanus"      "Lepomis auritus"        "Leptodiaptomus minutus"

```

SL01



Detection Results for SL01



```
##          Class     Sample Species
## 1 Actinopteri AMC22_SL01      9
## 2 Hyperoartia AMC22_SL01      1
## 3 Ulvophyceae AMC22_SL01      1
## [1] "Target species detected:"
## [1] "Alosa pseudoharengus"    "Anguilla rostrata"
## [3] "Catostomus commersonii"  "Margariscus margarita"
## [5] "Myoxocephalus scorpius"   "Perca flavescens"
## [7] "Petromyzon marinus"       "Pholis ornata"
## [9] "Pungitius pungitius"      "Semotilus atromaculatus"
```