**NEW JERSEY DEPARTMENT OF ENVIRONMENTAL PROTECTION**
**WATER MONITORING AND STANDARDS ELEMENT**
**BUREAU OF FRESHWATER AND BIOLOGICAL MONITORING**
**P.O. Box 420; Mail Code 35-01**
**TRENTON, NEW JERSEY**

# Quality Assurance/Quality Control Project Plan
## Development of a multi-metric index to assess biological condition in wadeable streams of the New Jersey Pinelands
## 2023 Pilot Study

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Date

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**1.0 Project Name:** Development of a multi-metric index to assess biological condition in wadeable streams of the New Jersey Pinelands: Phase 1 - 2023 Pilot Study

**2.0 Requesting Agency:** NJDEP Water Monitoring and Standards

**3.0 Date of Project:** 4/2023 - 10/2023

**4.0 Project Fiscal Information:** Job Number 35950000, Activity Code V4DT. State funded.

**5.0 Project Manager:** Brian Henning, Research Scientist I, BFBM

**6.0 Quality Assurance:**

- Project QA Officer: Andrew Jensen
- QAPP Reviewer: Jenna Majchrzak, Assistant Quality Assurance Officer, Office of Quality Assurance

**7.0 Special Training Needs/Certification**

Assistants to the project will be trained in the operation and use of all sampling equipment including the proper safety and handling procedures for electroshocking equipment. The training will entail calibration methods, deployment techniques, and data retrieval from the equipment.

Safety training and safety requirements will comply with Bureau of Freshwater and Biological Monitoring Field Work Health and Safety Plan (HASP) and the Bureau of Freshwater and Biological Monitoring Standard Operating Procedures Fish Monitoring, 2019 and any amendments due to Covid safety.

The Bureau of Freshwater and Biological Monitoring (BFBM) is certified by the Office of Quality Assurance (certified lab ID # 11896) for the analyze-immediately parameters to be measured.

NJDOH is certified by the OQA (certified lab ID # 11036) for the nutrient parameters to be measured.

With exception of the water quality field analyses (temperature, pH, DO, conductivity, and turbidity) and the nutrient analyses (nitrate-nitrite, total phosphorus and TKN), all analyses (e.g., visual encounter surveys, eDNA metabarcoding, etc.) are performed in laboratories that are not NJ-certified. Any analyses performed in labs that are not NJ-certified cannot be used for regulatory purposes.

**8.0 Project Description/ Objective:**

The objective of this project is to research methods and sampling techniques to support the development of a multi-metric index using vertebrates (fish and amphibians) to assess biological condition in wadeable streams of the New Jersey Pinelands. While many regions of the State can be currently assessed using two biological assemblages (macroinvertebrates and fish/amphibians) to determine Aquatic Life Use (northern NJ and inner coastal plain), there is a large portion of the outer coastal plain that have no current fish/vertebrate based biological index in which to evaluate stream conditions (Figure 1). A large portion of this area is in the New Jersey Pinelands, a nearly 1-million-acre area, which accounts for 19% of the total land area of the state of New Jersey (New Jersey Pinelands Commission, 2021). All the waters of the Pinelands are designated by the New Jersey Department of Environmental Protection (NJDEP) as Outstanding National Resource Waters (ONRW) and are protected through antidegradation policies (N.J.A.C. 7:9B). Currently, the only NJDEP BFBM biological index to assess Pinelands streams is the Pinelands Macroinvertebrate Index (PMI) which is based on benthic macroinvertebrate assemblages (Jessup et al. 2005). Although these waters are protected through this designation, a second biological indicator is warranted to accurately assess biological conditions in wadeable Pinelands streams to assess Aquatic Life Use and to determine if measurable changes occur in the future. The objective of this study is to determine the best biological indicators of Pineland fish/amphibian impairment, evaluate the best and most efficient methods to collect data of the indicator species, and conduct the monitoring in a cost effective and time efficient manner consistent with Rapid Bioassessment Protocols (Barbour et al. 1999).

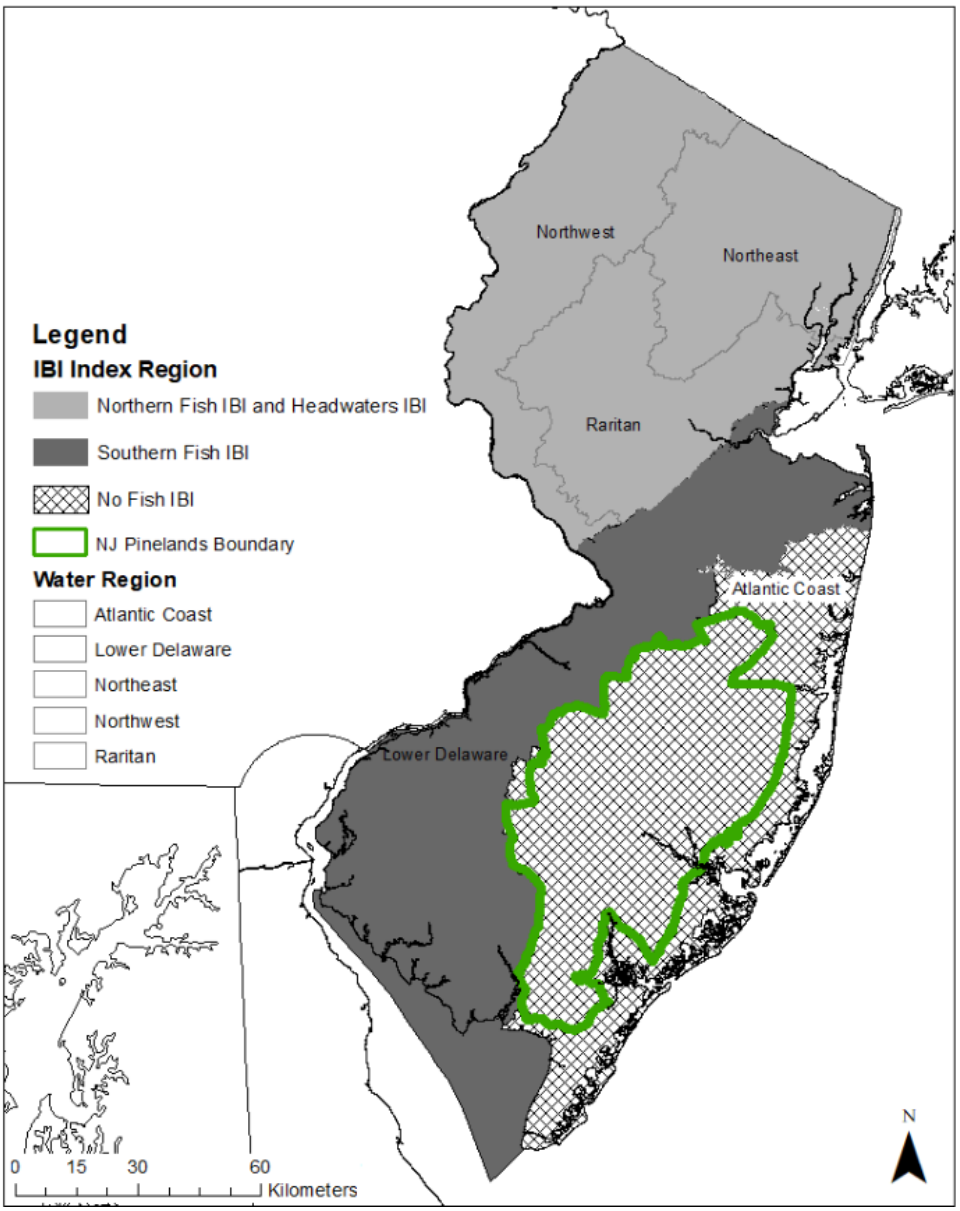


Figure 1. Map of current Fish Indices of Biotic Integrity regions. Note: Large area outlined by NJ Pinelands boundary has no current fish IBI.

## 9.0 Network Design/ Site Selection

A total of 20 sites will be sampled in 2023 for this pilot study. Site selection will be evenly distributed between two disturbance categories: 10 least disturbed and 10 most disturbed sites (Table 1, Figure 3). The disturbance gradient will follow the Aquatic Integrity Classes (AIC) by Zampella et al. (2008). Aquatic integrity is defined as the measure of undeveloped or unaltered land ( non-developed and non-agricultural land use) within a watershed as measured using ArcGIS. Least disturbed sites will be defined by the high Aquatic Integrity Class (AIC 80-100) and

most disturbed sites by the low Aquatic Integrity Class (AIC 0-50). All sites are wadeable streams located within the New Jersey Pinelands jurisdictional boundary within the U.S. level IV pine barrens ecoregion and spread across the four major Pinelands watersheds (Figure 4). Some sites will be co-located at current Ambient Macroinvertebrate Network (AMNET) sites to allow for comparison of biological condition between vertebrate bioindicators and macroinvertebrates as assessed by the Ambient Macroinvertebrate Network's (AMNET) Pinelands Macroinvertebrate Index (PMI); see Quality Assurance Project plans for Ambient Macroinvertebrate Network (AMNET), Atlantic Water Region, 2020-2021 and Ambient Macroinvertebrate Network (AMNET), Lower Delaware Water Region, 2021-22.

Some sites will also be co-located at former Pinelands Commission fish sampling locations for comparison of fish species presence (Zampella and Bunnell, 1998; Zampella et al. 2001,2003,2005,2006a). Selected sites for this pilot study will be small (< 10 sq miles drainage area) to limit the number of staff needed for sampling and to reduce the variability in signal from sampling streams with a wide range of drainage sizes.

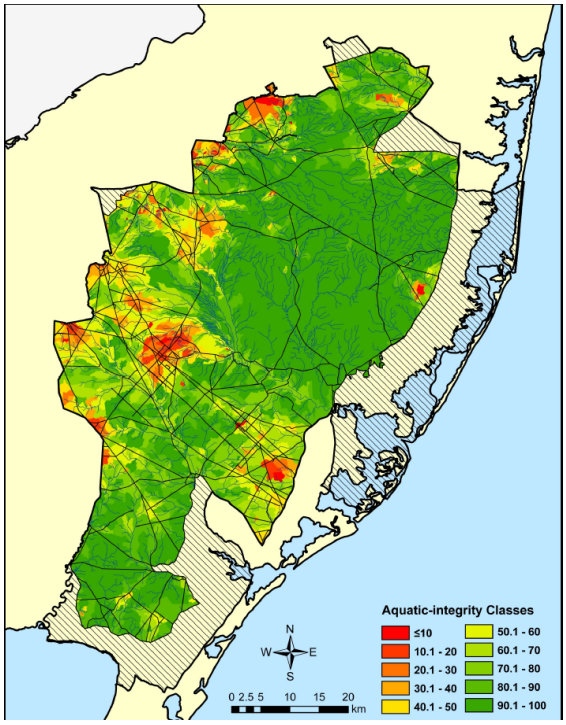


Figure 2. Aquatic integrity based on 2002 land-use/land-cover data. The 90.1-100% class represents the highest level of aquatic integrity. The hatched area represents the portion of the Pinelands National Reserve outside the Pinelands Area. (Source: Zampella, R. A., N. A. Procopio, M. U. Du Brul, J. F. Bunnell. 2008. An ecological-integrity assessment of the New Jersey Pinelands: A comprehensive assessment of the landscape and aquatic and wetlands systems of the region. Pinelands Commission, New Lisbon, NJ, USA.)

Exact site locations will be initially determined via the Global Positioning System (GPS) using a Trimble or hand-held GPS unit, either Garmin model "Oregon 450", Garmin nuvi 2797, or Trimble "Geo XT. All positions are logged into the Geographic Information System (GIS). Hand-

held GPS units, either Garmin model “Oregon 450”, Garmin nuvi 2797, or Trimble “Geo XT”, will be used to confirm correct locations for this sampling based on the availability of the equipment.

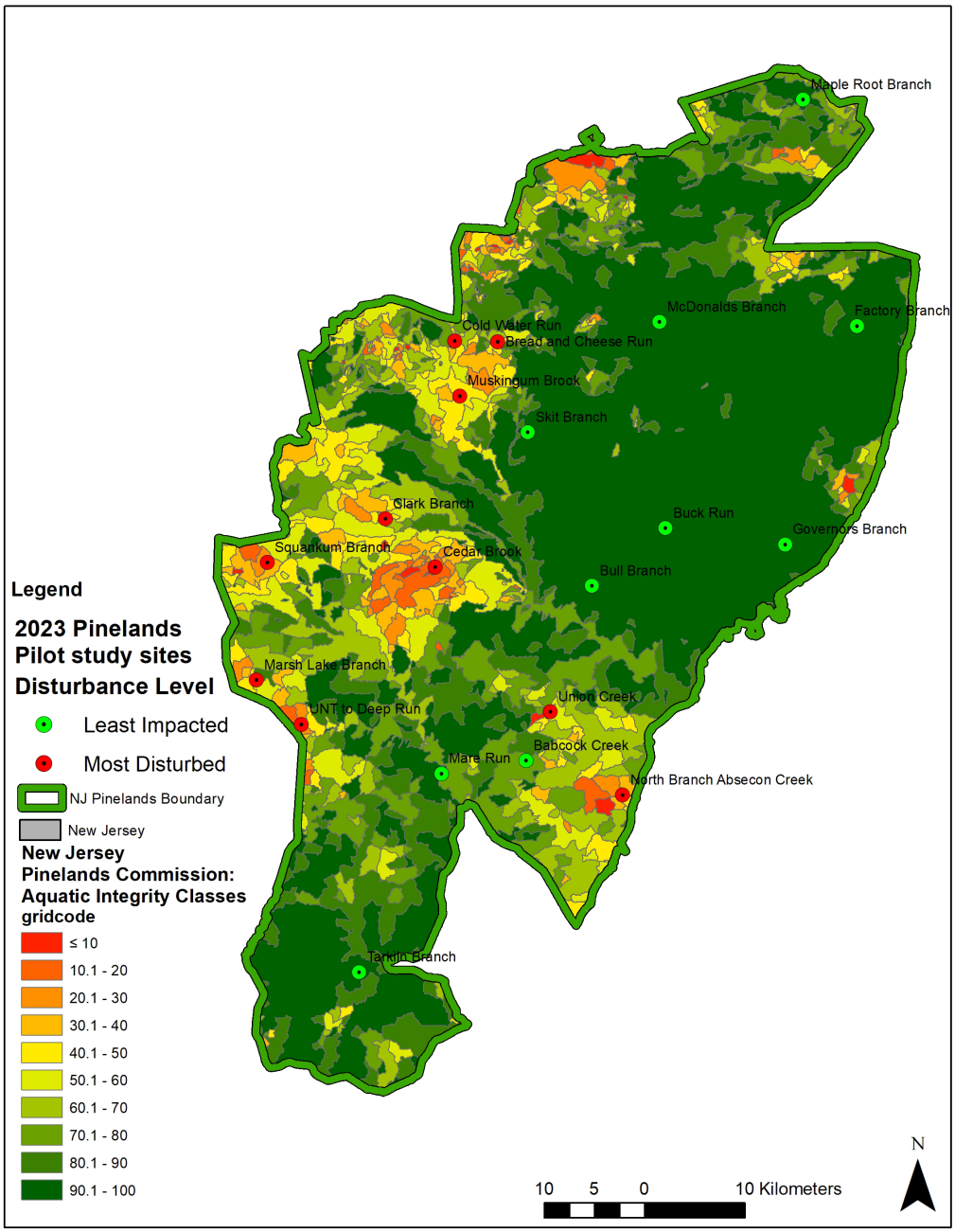


Figure 3. 2023 Bureau of Freshwater and Biological Monitoring pilot study site locations with Aquatic Integrity Classes based on 2002 land-use/land-cover data.

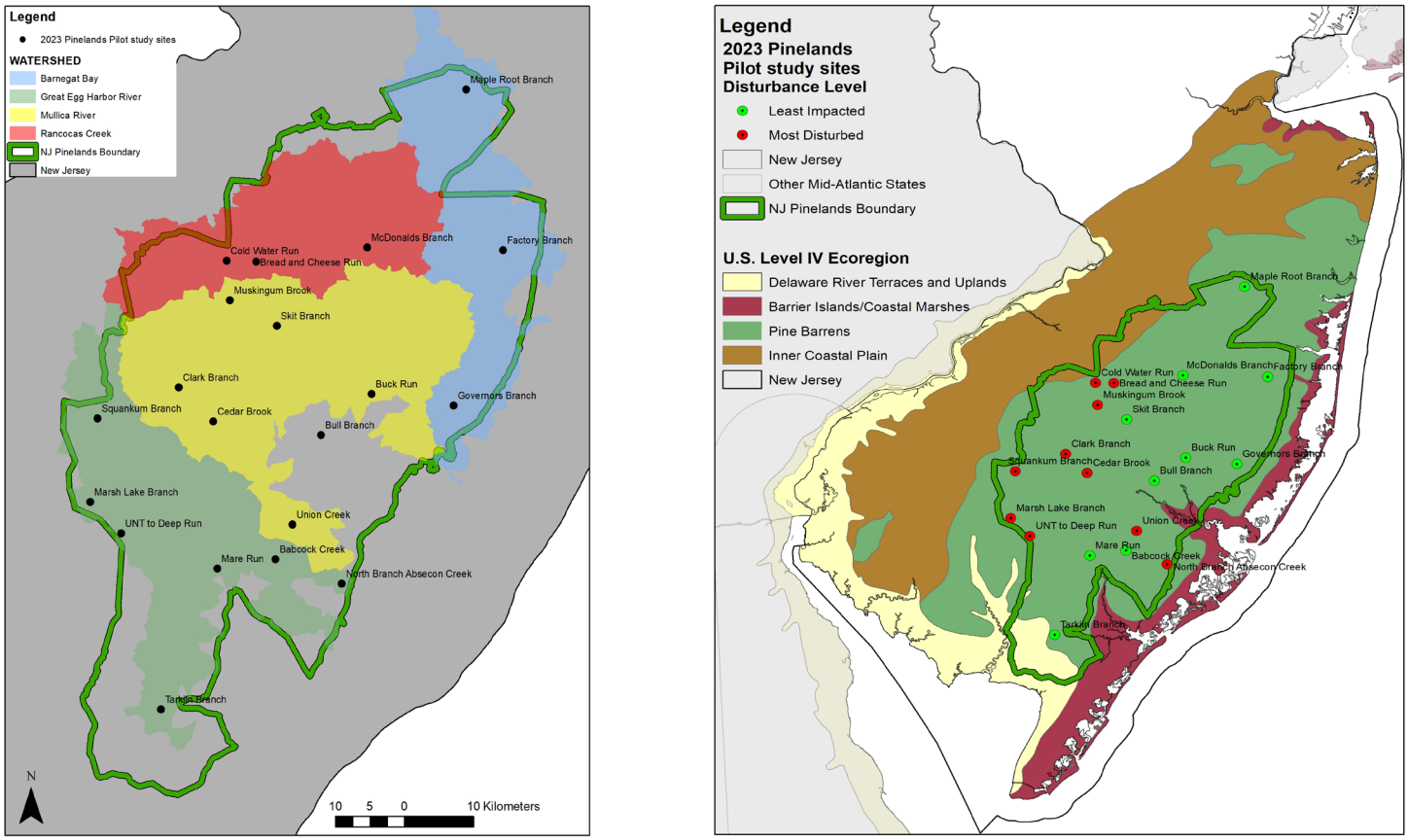


Figure 4. 2023 Bureau of Freshwater and Biological Monitoring pilot study site locations within the four major Pinelands watersheds (left), and within the US level IV ecoregion (right)

Table 1. 2023 Pinelands sampling locations.

| SITE ID | SITE NAME | WATERSHED | Drainage Area($mi^2$) | Latitude | Longitude | Aquatic Integrity Class | Disturbance Category |
|---|---|---|---|---|---|---|---|
| PL001 | Babcock Creek | Great Egg Harbor River | 4.85 | 39.49071 | -74.65974 | 80 | Least Impacted |
| PL002 | Buck Run | Mullica River | 2.98 | 39.69969 | -74.49811 | 100 | Least Impacted |
| PL003 | Bull Branch | Mullica River | 6.64 | 39.6477 | -74.5833 | 100 | Least Impacted |
| PL004 | Factory Branch | Barnegat Bay | 7.31 | 39.88117 | -74.27518 | 100 | Least Impacted |
| PL005 | Governors Branch | Barnegat Bay | 2.05 | 39.685 | -74.3592 | 100 | Least Impacted |
| PL006 | Maple Root Branch | Barnegat Bay | 5.31 | 40.07997 | -74.32308 | 100 | Least Impacted |
| PL007 | Mare Run | Great Egg Harbor River | 5.42 | 39.47872 | -74.75772 | 100 | Least Impacted |
| PL008 | McDonalds Branch | Rancocas Creek | 2.19 | 39.885 | -74.50538 | 100 | Least Impacted |
| PL009 | Skit Branch | Mullica River | 5.04 | 39.7857 | -74.65834 | 100 | Least Impacted |
| PL010 | Tarkiln Branch | Great Egg Harbor River | 5.76 | 39.30021 | -74.852 | 100 | Least Impacted |
| PL011 | Bread and Cheese Run | Rancocas Creek | 2.28 | 39.8669 | -74.6937 | 40 | Most Disturbed |
| PL012 | Cedar Brook | Mullica River | 3.78 | 39.66456 | -74.76559 | 20 | Most Disturbed |
| PL013 | Clark Branch | Mullica River | 2.44 | 39.7077 | -74.8237 | 40 | Most Disturbed |
| PL014 | Cold Water Run | Rancocas Creek | 1.22 | 39.86775 | -74.74366 | 50 | Most Disturbed |
| PL015 | Marsh Lake Branch | Great Egg Harbor River | 3.08 | 39.56231 | -74.97239 | 50 | Most Disturbed |
| PL016 | Muskingum Brook | Mullica River | 4.21 | 39.81813 | -74.73767 | 50 | Most Disturbed |
| PL017 | North Branch Absecon Creek | Great Egg Harbor River | 3.52 | 39.4599 | -74.548 | 40 | Most Disturbed |
| PL018 | Squankum Branch | Great Egg Harbor River | 3.10 | 39.66788 | -74.96061 | 30 | Most Disturbed |
| PL019 | Union Creek | Mullica River | 2.54 | 39.5345 | -74.6315 | 50 | Most Disturbed |
| PL020 | UNT to Deep Run | Great Egg Harbor River | 1.72 | 39.5225 | -74.9201 | 30 | Most Disturbed |

Site list may change due to site conditions and access (Final site list will be amended in the Addendum after the field season and all signees will be emailed an amended document if changes occur).

**10.0 Sampling Methods**

*Visual Encounter Survey (VES)*

Visual Encounter Surveys (VES) are a low cost, low effort survey technique to evaluate the species richness and relative abundance of amphibians within a search area (Crump and Scott, 1994). Visual encounter surveys will be performed during daylight, between the hours of 08:00-14:00. Visual encounter surveys will follow environmental DNA and water quality sampling and performed before coverboard surveys. Caution will be used to not disturb the VES search area by not walking within a 10 meter buffer of the 5m VES search area while traveling between the start and end of the reach to access eDNA and water quality sampling location. Observer(s) will search all available cover by searching the ground and hand turning cover (logs, debris) within a single (straight line) 150m stream transect on each side of the stream (Figure 6). The two 150 m transects of the VES are conducted parallel to bank and between 0-5 m from the wetted edge. Observer(s) will record the time searched for each VES using a stopwatch. The VES surveys will be conducted on the same day as cover board surveys and eDNA sampling and will follow the same sampling time periods; 1) Spring: April /May 2) Summer: June/July, and 3) late summer: August/September. All salamanders and anurans will be captured with the aid of dip nets and the species and habitat type found will be recorded. All objects turned in the survey are returned to their original position to reduce habitat disturbance.

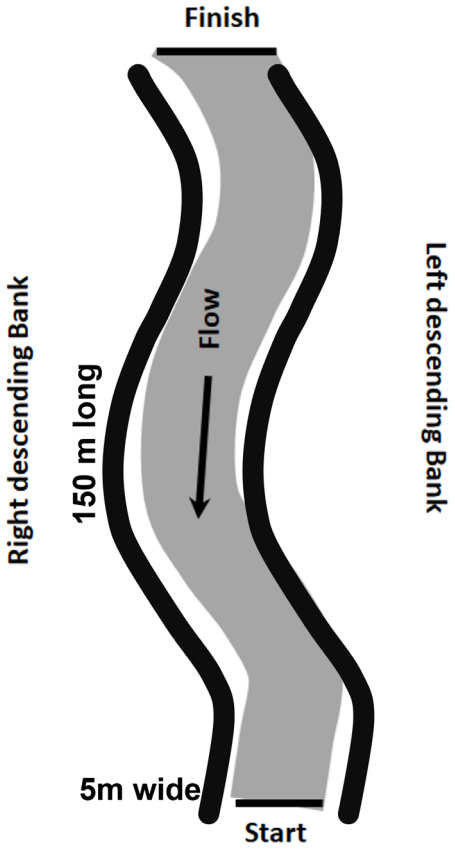


Figure 6. Layout of Visual Encounter Survey (VES) sampling design. The thick black line represents a 5m search area along the entire 150m stream reach.

Amphibian taxa observed by cover board survey or VES that escaped catchment are recorded and identified to the lowest taxonomic level based on observed characters. All biota sampled are identified to species and enumerated for each sampling method. The life stage (larval or adult) of each amphibian sampled is recorded on the field datasheet. Larval specimens not readily identified in the field will be preserved in 10% formalin in a reusable 1 L high-density polyethylene bottle for later identification in the laboratory using taxonomic keys (Altig, 1970; Petranka, 1998).

*Cover board Survey*

Coverboard surveys will be conducted to evaluate the relative abundance and biodiversity of amphibians at each site. Cover board surveys are a traditional sampling technique used in monitoring amphibians (Grant et al.1992; Hampton, 2007). A total of 12 cover boards, each measuring 1.8cm x 61cm x 61cm made of pine plywood will be placed parallel to the stream bank with 6 cover boards on each side of the stream, approximately every 25 meters along the length of the 150 m stream reach (Figure 5). Cover boards will be placed within approximately 5 meters of the stream bank, caution will be taken to avoid placing on sensitive vegetation. The area under each cover board will be raked to bare soil using a hand rake (except when placed on sphagnum moss) upon placing the cover board (Hyde and Simons, 2001). Cover boards will be set out (established) at each site in March. The cover board array at each site will be sampled once in each of the three time periods; 1) Spring: April /May 2) Summer: June/July, and 3) late summer: August/September. Cover boards will be removed from the site after the third sampling event. Cover boards will be lifted by hand and all individuals under the coverboard will be collected with the aid of dip nets. All individuals collected will be placed into buckets, identified, enumerated, and the habitat surrounding the coverboard will be recorded on field data sheets. Following identification individuals will be released at the edge of the coverboard.

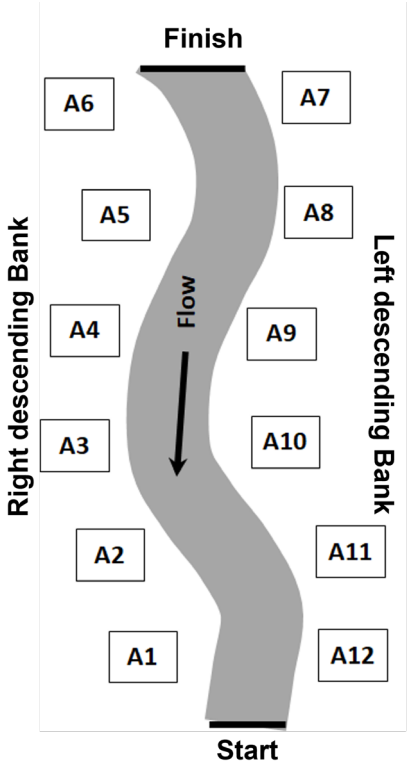


Figure 5. Layout of cover board sampling design

*Electrofishing*

The Pinelands electrofishing sampling will take place between June 1 and July 31, 2023. All sites will be sampled via electrofishing once during this time period. All sampling is conducted during daylight hours between 0800 and 1600. Electrofishing will be the last site activity performed so as to not disturb the water and sediment for eDNA and water quality sampling and to not disturb the stream bank areas where the visual encounter survey and cover board sampling is performed.

A stream reach of 150 m will be sampled using one or two backpack electrofishing units with a crew of two to five individuals during the summer period of June/July. The Midwest Lake Electrofishing Systems (MLES) Infinity Xstream backpack electrofisher will be exclusively used for Pinelands sampling as test results have shown it to be successful in water conductivities as low as 14 µS/cm (Dean et al. 2019). Electrofishing will be conducted in an upstream manner in which the operator systematically samples all available habitats. A block net will be placed at the end of the reach to prevent fish from escaping upstream of the sampled area. All stunned fish, crayfish, frogs and salamanders will be collected by the crew using dip nets and placed into live wells for later identification. All biota sampled by electrofishing are identified to species and enumerated. All fish are examined for anomalies or DELTs (DELT-Deformities, eroded fins, lesions or tumors), and the total length (TL; mm) of sport fish are measured and recorded on field data sheet. Any fish, crayfish or amphibian not readily identified in the field (except for NJ listed threatened or endangered species) will be preserved in 10% formalin in a reusable 1 L high-density polyethylene bottle for later identification in the laboratory. Reusable 1 L high-density polyethylene bottles will be washed with Liquinox and rinsed with tap water between each use. Additional details are outlined in the Bureau of Freshwater and Biological Monitoring Standard Operating Procedures Fish Monitoring, 2019.

*Environmental DNA (eDNA)*

General field sampling guidelines for environmental DNA (eDNA) consists of procedures to prevent the contamination of the sample with DNA from other equipment and samples. Samplers will wear clean nitrile gloves and take precautions to not touch any surfaces that may contaminate the DNA sample. Waders, boots, and all sampling equipment will be rinsed in deionized water in between sample locations. Equipment will be decontaminated before each use with 3% bleach solution and rinsed with distilled water.

Each site will be sampled for amphibian environmental DNA (eDNA) during three time periods, 1) Spring: April /May 2) Summer: June/July, and 3) late summer: August/September (Table 2). The spring and summer time sampling periods are consistent with the breeding phenology of many of the anurans in the Pinelands (Zampella et al. 2001). Sampling of amphibian eDNA will occur on the same day as cover board and VES surveys (Table 2). Each site will be sampled for both fish and amphibian eDNA during June/July sampling period. Sampling of eDNA for fish metabarcoding will occur on the day of electrofishing sampling (prior to electrofishing) or within one week prior of electrofishing. A total of 3 filters will be sampled at each site for metabarcoding analysis of the amphibian/fish assemblage. One sample will be taken at the start (0 m), middle (75 m) and end of the reach (150 m) with an effort made to sample different types of habitats within the stream (pool, riffle, run; Figure 7). One “blank” or negative control will be taken at each site by sampling a 500 ml bottle of distilled water. Sampling will be performed facing upstream to reduce possible contamination from the sampler. Sampling kits consisting of a 60 mL syringe, Whatman 1.0 µm filter, gloves and preservative were packaged and purchased from Jonah Ventures Laboratory. A 60 mL syringe will be used to draw water in and then pushed out the syringe with attached 1.0 µm filter until the filter is clogged. When the filter is clogged, the syringe is removed and 1 mL of

preservative is injected through the filter. The final volume of water filtered through the syringe will be recorded on a datasheet and in the JonahDNA App (Appendix G). Sampling water for eDNA will follow the instructions provided from the laboratory in Appendix F.

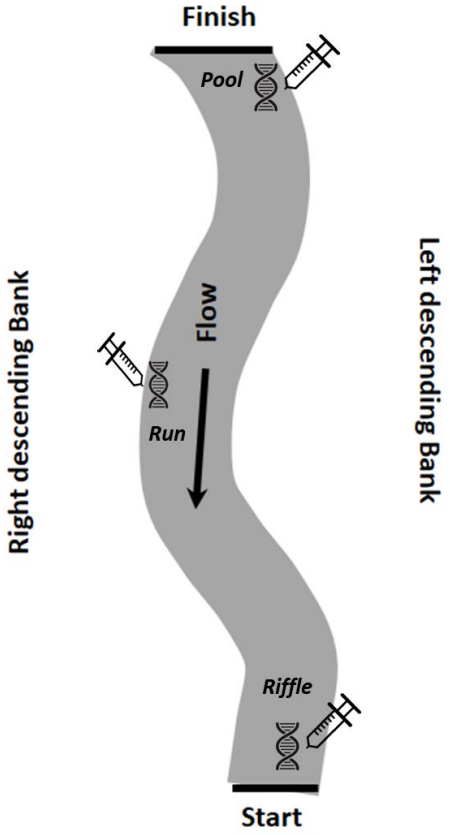


Figure 7. Sampling locations for eDNA throughout the stream reach.

*eDNA sampling method comparison*

To test and compare the difference in eDNA sampling techniques a subset of 10 sites will be sampled using the 60 mL syringe with attached 1.0 µm and a handheld eDNA sampler with tripod mounted pole extension (Smith-Root, Inc) with 5 µm self-desiccating filters concurrently at the exact same locations during the summer sampling period (June/July). The methods used for sampling with the 60 mL syringe with attached 1.0 µm filter are described above and in Appendix F.

The handheld eDNA sampler with tripod mounted pole extension (Smith-Root, Inc) will be used to filter 3 individual samples taken at the start (0 m), middle (75 m) and end of the reach (150 m) with an effort made to sample different types of habitats within the stream (pool, riffle, run; Figure 7). The goal will be to filter at least 10-liters of water total from each site, with each individual sample filtering approximately 3.33 L of water. If the filter becomes clogged before 3.33 L is achieved, pumping will be stopped and the volume filtered will be recorded. Every effort will be made to filter water from all available habitat types including riffles, runs, pools and snags. Care will be taken to ensure bottom sediments are not disturbed while collecting samples. Once the filter has clogged the filter will be removed from the water, inverted and the pump will run to completely suck any residual water out of the filter housing. While wearing clean nitrile gloves, the filter housing will be cracked

open while the pump is running to remove any excess water and to dry the filter. The housing cap will then be re-attached and the filter placed in the labeled foil zip-loc package. Each foil packet is then labeled using the following naming convention: site ID-month/day/year-sample number (PL001-60123-01, PL002-60123-02, etc.) Once back at the laboratory, samples will be stored in the freezer until transport to the Jonah Ventures laboratory (5485 Conestoga Ct STE 210, Boulder, CO 80301) for DNA extraction and metabarcoding analysis. All samples will be shipped with a spreadsheet of the samples collected, with date, sample ID, volume of water filtered, habitat sampled and analysis requested ( ie. metabarcoding for fish and amphibians).

Table 2. Sampling timeline by method.

| | Month/Season | | | | | |
|---|---|---|---|---|---|---|
| **Sampling Method** | Spring | | Summer | | Late Summer | |
| | April | May | June | July | August | September |
| **Electrofishing** | | | **x** | | | |
| **Visual Encounter Survey (VES)** | **x** | | **x** | | **x** | |
| **Coverboard** | **x** | | **x** | | **x** | |
| **eDNA - Fish** | | | **x** | | | |
| **eDNA - Amphibian** | **x** | | **x** | | **x** | |

Table 3. List of Pinelands taxa. Biogeographic classification of fish follows designations by Hastings (1979, 1984) and anurans by Conant (1979).

**Fish**

| Biogeography | Common name | Scientific name |
|---|---|---|
| Restricted native | | |
| | Mud sunfish | *Acantharchus pomotis* |
| | Yellow bullhead | *Ameiurus natalis* |
| | Pirate perch | *Aphredoderus sayanus* |
| | Blackbanded sunfish | *Enneacanthus chaetodon* |
| | Banded sunfish | *Enneacanthus obesus* |
| | Swamp darter | *Etheostoma fusiforme* |
| Widespread native | | |
| | American eel | *Anguilla rostrata* |
| | Chain pickerel | *Esox niger* |
| | Bluespotted sunfish | *Enneacanthus gloriosus* |
| | Creek chubsucker | *Erimyzon oblongus* |
| | Redfin pickerel | *Esox americanus* |
| | Tadpole madtom | *Noturus gyrinus* |
| | Eastern mudminnow | *Umbra pygmaea* |
| Peripheral nonnative | | |
| | Brown bullhead | *Ameiurus nebulosus* |
| | Tessellated darter | *Etheostoma olmstedi* |
| | Pumpkinseed | *Lepomis gibbosus* |
| | Golden shiner | *Notemigonus crysoleucas* |
| Introduced nonnative | | |
| | Bluegill | *Lepomis macrochirus* |
| | Largemouth bass | *Micropterus salmoides* |
| | Black crappie | *Pomoxis nigromaculatus* |

**Anurans**

| Biogeography | Common Name | Scientific Name |
|---|---|---|
| Pine Barrens species | | |
| | Pine Barrens treefrog | *Hyla andersonii* |
| | Carpenter frog | *Lithobates virgatipes* |
| Wide-ranging species | | |
| | Green frog | *Lithobates clamitans* |
| | Fowler's toad | *Bufo woodhousii fowleri* |
| | Southern leopard frog | *Lithobates sphenocephalus* |
| | Eastern spadefoot | *Scaphiopus holbrookii* |

| | | |
|---|---|---|
| | Northern spring peeper | *Pseudacris crucifer* |
| Border-entrant species | | |
| | Bullfrog | *Lithobates catesbeiana* |
| | Pickerel frog | *Lithobates palustris* |
| | Northern cricket frog | *Acris crepitans crepitans* |
| | Wood frog | *Lithobates sylvaticus* |
| | New Jersey chorus frog | *Pseudacris kalmi* |
| | Gray treefrog | *Hyla versicolor and H. chrysoscelis* |

**Salamanders**

| **Biogeography** | **Common Name** | **Scientific Name** |
|---|---|---|
| Pine Barrens species | | |
| | Eastern Mud Salamander | *Pseudotrition montanus montanus* |
| Wide-ranging species | | |
| | Four-toed Salamander | *Hemidactylium scutatum* |
| | Northern Dusky Salamander | *Desmognathus fuscus* |
| | Northern Red Salamander | *Pseudotriton ruber ruber* |
| | Northern Two-lined Salamander | *Eurycea bislineata* |
| | Red-spotted Newt | *Notophthalmus viridescens* |

**Crayfish**

| **Biogeography** | **Common name** | **Scientific name** |
|---|---|---|
| Widespread native | | |
| | Common Crayfish | *Cambarus bartonii bartonii* |
| | Spinycheek Crayfish | *Faxonius limosus* |
| | White River Crayfish | *Procambarus acutus* |
| Introduced nonnative | | |
| | Allegheny Crayfish | *Faxonius obscurus* |
| | Rusty Crayfish | *Faxonius rusticus* |
| | Virile Crayfish | *Faxonius virilis* |
| | Red Swamp Crayfish | *Procambarus clarkii* |

The NJDEP will collect tissue samples from fish and amphibian species throughout the Pinelands for genomic sequencing to develop a catalog of eDNA primers for aquatic vertebrates. Pinelands fish, salamanders and anurans will be collected during site visits via electrofishing, VES, coverboard surveys or hand dip netting. Designated fish species captured for tissue sampling will be rinsed thoroughly with distilled water and using sterile scissors and forceps (sterilized by flame), a small clip of the caudal fin will be removed and placed in a non-buffered ethanol filled vial. The vial will be labeled with the species, date, and location of collection. Designated amphibian species captured for tissue sampling will be collected by a buccal swab, following the procedures of Ambu and Dufresnes (2023). Buccal swabs will be collected with a sterile 6" Puritan Medical Fab-Swab with Dry Transport Tube. Each swab will be placed inside the transport tube, labeled with the species, date, and location of collection and placed in a cooler for transport back to the laboratory. Swabs will be held frozen in the laboratory chest freezer until delivered to the genomic sequencing laboratory.

*Decontamination of field equipment*

Field precautions for preventing the spread of aquatic invasive species will follow the Northeast Partners in Amphibian and Reptile Conservation (NEPARC) recommendations to minimize the spread of chytridiomycosis and ranavirus (NEPARC, 2014). All waders, boots, buckets, nets and other gear used in sampling will be decontaminated in the field after each site by spraying a 3% bleach solution on all equipment surfaces, allowing the bleach to soak into equipment for 1-5 minutes, then thoroughly rinsing with distilled water (Appendix B).

## 11.0 Field Measurements

Dissolved oxygen (DO), pH, water temperature, turbidity and specific conductivity will be measured in-field at each site by biomonitoring staff, concurrent with faunal sampling, in accordance with N.J.A.C. 7:18 *Regulations Governing the Certification of Laboratories and Environmental Measures* (NJDEP, 2018), Subchapter 8, Analyze-Immediately Environmental Measurements, and NJDEP's *Field Sampling Procedures Manual* (NJDEP, 2005 and 2022, as available). These physical/chemical parameters will be taken from the start of the reach, *in situ*, mid-depth, in a free-flowing area of the stream. Water quality measurements will be taken first prior to any other sampling activities where the stream is disturbed. BFBM is certified by the Office of Quality Assurance for each parameter sampled (Certified Lab ID # 11896). Water temperature, pH, specific conductance, and dissolved oxygen are measured using a Hydrolab MS5 or YSI ProDSS Multiparameter Digital Water Quality Meter. The Hydrolab MS5 and the YSI ProDSS Multiparameter Digital Water Quality Meter are multi-parameter water quality systems that combine temperature, pH, conductance, and luminescent dissolved oxygen (LDO) probes into one meter.

At the time of sampling, visual based habitat assessments will be performed at each site using the format given in the Rapid Bioassessment Protocols (Barbour et al, 1999) for low gradient streams. The form that will be used is included in Appendix E. Several qualitative measurements will be made based on visual observation including substrate composition, weather conditions, water clarity, and presence of aquatic vegetation.

**Dissolved oxygen (Hach 10360 – 10/2011 Rev 1.2)** – Dissolved Oxygen is measured at each sampling location at mid depth in the thalweg of the channel in flowing water using the Hydrolab MS5 Water Quality Monitoring System or YSI ProDSS Multiparameter Digital Water Quality Meter. The meter and probe will be maintained and calibrated in accordance with the Operating Manual (February 2006 Edition 3, HACH Environmental, Loveland, CO). The meter will be calibrated with 100% saturated water and the mg/L reading will be checked against dissolved oxygen solubility tables (https://water.usgs.gov/water-resources/software/DOTABLES/ )

The meter is barometrically compensated and checked at each sampling site using fully oxygenated water. Weekly Winkler titrations will be performed to check the dissolved oxygen probe. All titrations and field DO measurements are recorded in the Field Logbook and are initialed and dated by the field staff that performed the calibration, titration, and analysis.

**pH (SM 4500-H B-11)** - The pH is measured at each sampling location at mid depth in the thalweg of the channel in flowing water using the Hydrolab MS5 Water Quality Monitoring System or YSI ProDSS Multiparameter Digital Water Quality Meter. The meter and probe will be maintained and calibrated in accordance with the Operating Manual (February 2006 Edition 3, HACH Environmental, Loveland, CO). The pH probe will be calibrated prior to each sampling event with certified pH buffers for the expected range of pH at the sampling location (e.g. pH buffers 4.0 and 7.0 for acidic streams and 7.0 and 10.0 for alkaline streams) and then checked with a mid-range buffer. The acceptance criteria for this check is a reading within ± 0.1 pH units from the true value. After three hours of continuous use, the pH of the mid-range buffer will be checked again. The acceptance criterion for this re-check is a reading within ± 0.2 pH units from the true value. All pH calibration readings and field pH measurements are recorded in the Field Logbook and are initialed and dated by the field staff that performed the calibration and analysis.

**Specific Conductance (SM 2510 B-11)** - The specific conductance is measured at each sampling location at mid depth in the thalweg of the channel in flowing water using the Hydrolab MS5 Water Quality Monitoring System or YSI ProDSS Multiparameter Digital Water Quality Meter. The meter and probe will be maintained and calibrated in accordance with the Operating Manual (February 2006 Edition 3, HACH Environmental, Loveland, CO). The specific conductance probe will be calibrated prior to each sampling event with certified conductivity standards (1800 µmhos/cm). To ensure accuracy, the probe will be checked each day of use with a certified standard (1800 µmhos/cm). The check standard is required to read within ± 1% of the true value of the standard prior to using the meter. All specific conductance calibration readings and field specific conductance measurements are recorded in the Field Logbook and are initialed and dated by the field staff that performed the calibration.

**Water Temperature (SM 2550 B-10)**: The water temperature is measured at each sampling location at mid depth in the thalweg of the channel in flowing water using the Hydrolab MS5 Water Quality Monitoring System or YSI ProDSS Multiparameter Digital Water Quality Meter. The meter and probe will be maintained and calibrated in accordance with the Operating Manual (February 2006 Edition 3, HACH Environmental, Loveland, CO). The temperature readings of the Hydrolab MS5 Water Quality Monitoring System and the YSI ProDSS Multiparameter Digital Water Quality Meter will be tested against a NIST certified thermometer quarterly to ensure accuracy. All temperature checks and field water temperature

measurements are recorded in the Field Logbook and are initialed and dated by the field staff that performed the calibration.

**Turbidity**- HACH Model 2100Q turbidimeter is calibrated on a quarterly basis per manufacturer recommendations. The meter is then checked with certified standards for accuracy within the calibration range during each day of use following, "Standard Operating Procedure for Field Turbidity Measurement EPA Method 2130B-11." Records of all calibrations and calibration checks will be maintained and dated in individually assigned field log books.

**Canopy Cover**- The canopy cover of the stream is measured at 50 m intervals from the starting location to the end point of sampling (0 m, 50 m, 100 m and 150 m) during every sampling event. The canopy cover will be estimated using a convex spherical crown densitometer at breast height from the middle of the stream channel.

**Habitat Assessment** – Low gradient Pineland stream habitat will be assessed at every site visited. Rapid habitat assessments were performed using EPA's Rapid Habitat Assessment Form (Appendix Barbour et al, 1999), including the following measures: epifaunal substrate, pool substrate characterization, pool variability, sediment deposition, channel flow status, channel alteration, channel sinuosity, bank stability, bank vegetative protection, and riparian vegetative zone width.

**Nutrients -** Total Phosphorus (TP) and Total Nitrogen (TN) will be collected at Pinelands sites. Total Nitrogen will be considered the sum of Nitrite- Nitrate (NO2-NO3) and Total Kjeldahl Nitrogen (TKN) concentrations. Sampling will consist of a single grab sample, during a biological sampling visit, or a reconnaissance visit no more than three weeks prior to biological sampling. If collected at time of biological sampling, nutrients will be collected prior to sampling to avoid disturbance of the substrate. Samples will be preserved in the field with 14 drops of sulfuric acid to a pH less than 2 immediately upon returning to the vehicle and stored on ice for transport to BFBM laboratory. Sample bottles will be single-use plastic bottles. Sample volume and container type will be as described in the respective laboratory's "Quality Manual" and/ or SOP, approved by the Office of Quality Assurance (OQA). A table of the laboratory, methods and reporting limits are in Appendix D. All persons collecting, handling, or transporting the nutrient samples to the NJDOH laboratory will complete the appropriate sections of the NJDOH chain-of-custody form, prior to relinquishing the samples. A copy of the chain-of-custody to be used is included as Appendix E.

## 12.0 Identification of Stream Fauna\ QAQC

References used for the identification of species collected in sampling.


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## 13.0 Laboratory Analysis

Environmental DNA sample filters will be shipped to Jonah Ventures Laboratory within 6 months of the sample date for metabarcoding analysis of fish and amphibian assemblages.

## 14.0 Data Analysis

Fish and amphibian sampling results (e.g., counts, lengths, DELTs) from all sample collection methods( electrofishing, VES, coverboards and eDNA), habitat measures and water quality measures will be entered in a Microsoft Access database. Data entered will be independently reviewed for consistency and accuracy by the project officer and another designated BFBM staff member. An additional third reviewer will be used should any discrepancies occur in the data entered.

## 15.0 Project Timeline

The Pinelands sampling takes place between April and September. The spring and summer time sampling periods are consistent with the breeding phenology of many of the anurans in the Pinelands (Zampella et al. 2001). In addition, the sampling timeline provides stable flows that permit safe wading conditions and increases electrofishing sampling efficiency.

## 16.0 Resource Needs

BFBM will need two hourly staff in addition to 2 full-time staff to complete this project.

## 17.0 Data Storage and Distribution

Sampling results will be stored locally on a Microsoft Access database. No biological data will be entered into USEPA's Water Quality Data Exchange (WQX) as this data is for research purposes only. Analytical data from grab samples submitted to the laboratory and data from analyze immediately

parameters will be entered into USEPA's Water Quality Data Exchange (WQX) and will be accessible through the USEPA and the National Water Quality Monitoring Council's Water Quality Portal by June of the following year it is received from the analytical laboratory.

## 18.0 Data Reporting

All habitat assessment data, physical/chemical analysis, and site observations will be recorded on the BFBM's Biological Field Observations and Data Sheet, and also recorded electronically on a Microsoft Access database.

All fauna identifications will be recorded on the BFBM's Data Sheet and entered electronically into a Microsoft Access database. A report will be written within 1 year of receiving the results returned from the Jonah Ventures Laboratory to summarize the results of this pilot study and to compare the results of several sampling techniques to capture aquatic vertebrates in Pinelands wadable streams, provide analysis of the best available bioindicators of Pinelands streams and their relationship with developed land use in the Pinelands and provide recommendations for future studies, enhancements and continued monitoring.

## 19.0 Audits

The Office of Quality Assurance may reach out during the project timeline in order to conduct an audit.

## 20.0 Corrective Actions

The Project Officer will be responsible for the oversight of all activities relating to this project. The Project Officer will assess field collection functions and make corrections when necessary to maintain the data accuracy as defined in this plan. If any changes or modifications are made to this plan regarding data collection, as it relates to the objectives(s) and data accuracy required in this project, all original signees of the QAPP will be notified.

## 21.0 Literature Cited


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Zampella, R.A., Bunnell, J.F., Laidig, K.J., Procopio, N.A., 2005. The Great Egg Harbor Watershed Management Area: a report to the Pinelands Commission on the status of selected aquatic and wetland resources. Pinelands Commission, New Lisbon, New Jersey, USA.

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**ADDENDUM**

## 22.0 2023 Final Site Selection (as requested by OQA):

| Station ID | Waterbody/Location | Latitude-dd | Longitude-dd | County | Municipality | Network |
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# APPENDIX A

# Water Quality Meter Calibration Procedures

## ***HYDROLAB MS5 Datasonde***

## ***Things to Remember and Maintenance***

**Things to Remember**

1. **Maintenance of all probes must be performed at least monthly.**
2. As specified by NJDEP OQA, Hydrolab MS5 must be calibrated weekly and checked daily. Results of lab/field calibrations and checks must be recorded in field notebook with proper units.
3. **All water and standards used for rinse or LDO checks must be room temp.**
4. Always rinse 3X with room temp DI after each standard.
5. ALWAYS pour fresh standards into corresponding "rinse" container after use in calibration/check.
6. **Calibrate specific conductivity before any other parameters. All probes and calibration cup must be dried with Kimwipes so there is NO residual water as indicated in the *"Weekly Calibration Guide."***
7. If there is significant difference from the standard when checking, record extra readings to ensure stabilization.
8. When storing sonde, fill calibration cup with appx 15mL of pH 4 buffer or tap water.
9. **LDO must be calibrated with site-specific barometric pressure and checked using the air-saturated water method.**

**Monthly Maintenance**

- **Record Date and Type of Maintenance in the Field Notebook**
- Invert the sonde vertically and secure in a stable lab stand.
- Gently clean probes with Simple Green and toothbrush then rinse thoroughly.
- For integrated pH probe, cover pH bulb with thumb and use flathead screwdriver to carefully unscrew the Teflon junction cap.
- For standard pH probe, hold the base of reference probe and unscrew Teflon junction cap.
- Empty contents into sink, shaking or use q-tip end, and flush with fresh pH reference solution (AgCl).
- Flush pH tube (use syringe for integrated probe) with AgCl. Add two KCl crystals and refill with AgCl to prevent introduction of air bubbles.
- Teflon junction cap should be replaced annually.
- When replacing integrated Teflon junction, plastic will cross thread easily so begin by hand then tighten with screwdriver to top of probe.
- LDO probe maintenance consists of unscrewing the cap by hand and blotting any moisture inside with Kimwipes.
- LDO cap should be replaced annually.

# ***HYDROLAB MS5 Datasonde***
# ***Daily Calibration Guide***

**Calibration Prep**

1. Connect sonde to computer by lining up dimple on probe and dot on plug then "burp" the plug to ensure a proper fit. Sonde will audibly "beep" when connected.
2. Open HYDRAS 3 LT on desktop and sonde will beep again.
3. Once sonde appears (COM1), double click to initialize the sonde. If the sonde does not appear in the list, click "Re-Scan for Sondes." Full initialization takes appx 30 seconds and during this process you should see a blue progress bar at the bottom of the screen.
4. **THIS STEP DESCRIBES INITIAL SETTINGS AND SHOULD BE SET FROM PREVIOUS USE.** In "System" tab, click on "Level 3", then enter the password "Hydrolab." In "Parameter Setup", click the "pH" tab and "Cal Points" field should contain a "2." If not, type "2" and click "Save Settings".

**Specific Conductivity**

1. Navigate to the "Calibration" tab and then the "SpC µS/cm" tab.
2. Rinse 3X with DI then dry probes and calibration cup with Kimwipes.
3. Click the "Reset" button and asterisk will appear next to reading.
4. Calibrate to zero first by typing "0" in the standard field and click "Calibrate."
5. Rinse with the 1800 µS/cm "rinse" then fill with fresh standard.
6. Let readings stabilize, type standard value (1800), then click "Calibrate."
7. Once proper calibration is complete, the asterisk will disappear.
8. Perform CHECK as described on the *"Daily Checks in Lab"* instruction sheet.

**pH**

1. Navigate to the "Calibration" tab and then to the "pH" tab
2. Click the "Reset" button and an asterisk will appear next to reading.
3. Get the pH standards of 7 and 4 or 7 and 10 ready, depending on expected field conditions.
4. Rinse probes 3X with DI, then rinse with the bottle labeled "pH 7 Rinse."
5. Fill the probe with fresh pH 7 standard and record the temperature of the standard.
6. In front of the large boxed pH container, there will be a chart comparing the pH value to temperature. Use the pH value listed next to the nearest temperature.
7. Let the readings stabilize, type in the temperature corrected value, and click "Calibrate."
8. Repeat steps 4 - 7 with the next pH standard.
9. Once proper calibration is complete, the asterisk will disappear.
10. Perform CHECK with pH standard 5 or 8 depending on expected field conditions as described on the *"Daily Checks in Lab"* instruction sheet.

**Luminescent Dissolved Oxygen**

The LDO must be calibrated daily by compensating for barometric pressure. LDO should be checked in the lab bubbler water bath. After traveling to your sampling location, LDO should be calibrated with site-specific barometric pressure and checked using the air-saturated water method. See the *"Daily Checks in Lab"* instruction sheet for both methods.

# ***HYDROLAB MS5 Datasonde***

# ***Daily Checks in Lab***

**Specific Conductivity**

1. After calibrating, navigate to "Online Monitoring" and check SpCond in µS/cm in the Parameters list.
2. Set "Monitoring Interval" to 30 seconds and click "Start" at top right-hand side.
3. "Online monitoring in progress" will begin to blink red. Once the "# Samples" reaches "1," the buttons below will become clickable.
4. Click "New Table" and a new window will appear which will display readings according to the set monitoring interval. Record for 2 minutes or until reading stabilizes.
5. Write both time and reading in note book and be sure to include proper units.
6. If the check gives results outside of the acceptable range (99% - 101%), recalibrate and repeat the check.

**pH**

1. After calibrating, rinse probes 3x with DI, no need to dry.
2. Depending on calibration or expected field conditions, check with pH with 5 or 8 standard.
3. Rinse with standard from "rinse" container then fill with fresh standard and allow 2 minutes for stabilization.
4. Navigate to "Online Monitoring" and check pH in the Parameters list.
5. Repeat steps 5 through 9 from *Specific Conductivity* section above.

**LDO**

*Lab Bubbler Water Bath*

1. This procedure should be performed before leaving the office (AM) and upon return (PM).
2. Remove the calibration cup and install the probe guard then attach the Surveyor handheld.
3. Immerse the sonde and immerse in the water bath and allow it to stabilize.
4. Enter the barometric pressure in the "LDO%" calibration screen on the Surveyor handheld and select "Calibrate."
5. Compare the sonde temp and DO readings to the USGS Oxygen Solubility Table and record barometric pressure, sonde temp and DO along with USGS Table DO.

*Field Air-saturated Water*

1. **Uncap then recap** 1L bottle of water (keep bottle in truck cab to maintain room temp) and shake bottle appx 1 minute to saturate with oxygen.
2. Fill calibration cup so water covers the LDO probe by appx 0.5 inches and allow it to stabilize.
3. Set the cap on top and enter the barometric pressure in the "LDO%" calibration screen on the Surveyor handheld and select "DONE."
4. Perform CHECK.

# APPENDIX B

## Decontamination Procedures

# Disinfection of Field Equipment to Minimize Risk of Spread of Chytridiomycosis and Ranavirus[1]

## Importance of Disinfection

The spread of pathogens is a major threat to amphibians and reptiles worldwide.[2-5] This is particularly true for Ranavirus (RV) and *Batrachochytrium dendrobatidis* (Bd) responsible for chytridiomycosis. Humans can transmit diseases from one place to another and from one organism to another in a short amount of time and over distances the organisms cannot traverse. With the increasing spread of pathogens and reports of die-offs among amphibians and select reptiles worldwide, it is imperative that field biologists, researchers, hobbyists, and anyone interested in recreational herpetology-related field activities employ basic disinfecting procedures to prevent the spread of pathogens.

## Before Leaving for the Field

Although other chemicals are effective (see table), NEPARC recommends a 3% bleach solution to inactivate Bd and most RV's.[3-7] Concentrated bleach is inexpensive and readily available. However, diluted bleach solutions lose their potency if exposed to air, sunlight, or organic material, and should be discarded after 5 days if exposed.[8] To ensure maximum efficacy, prepare only as much solution as you will need for the sampling event.

*Suggested equipment:*

- Brushes for scrubbing and/or removing mud and vegetation from equipment.
- Hand sanitizers and antiseptic alcohol wipes.
- Handheld bottles and/or pump sprayers for applying bleach and water. Bring clean rinse water.

- Gloves for handling animals. These should be disinfected or discarded between animals.
- Plastic bags of different sizes: examining animals in bag minimizes contact.
- Prepare additional sets of equipment if sampling at multiple locations.
- Trash bags.

## Instructions for Large Equipment

Brush off mud, wash with biodegradable soap, disinfect with bleach and rinse all exterior surfaces of boats, canoes, vehicles or trailers and their tires that may have come in contact with potentially affected water (e.g. stream or wetland).

## After Each Sampling Event and Before Moving to the Next Site

1. Brush off mud and vegetation from field equipment (e.g., nets, buckets, boots). Soil or mud can reduce the effectiveness of the disinfection process.
2. Generously spray or immerse all items in bleach solution.
   - Bleach is highly toxic to aquatic organisms; stand at least 50 m from any natural water source.
   - Lab studies indicate 1 minute contact time to be sufficient to inactivate pathogens but NEPARC recommends 5 minutes in field situations.
3. Rinse bleached items with water to minimize damage to the equipment and to prevent exposing the next wetland to residual bleach.
4. Use alcohol wipes to disinfect calipers, measuring boards, and other sensitive equipment.




## End of the Day

After returning from the field, all equipment should be washed and thoroughly disinfected. If available, set up 2 buckets or large tubs: one with soapy water and one with 3% bleach solution.

Brush or scrub off any soil or vegetation. Immerse into soap, wash then rinse.

- Immerse in bleach and leave for 5 minutes. Rinse thoroughly with water.
- Hang equipment and gear, and allow them to air dry completely.

# DISINFECTION OPTIONS FOR RANAVIRUS (RV) AND *BATRACHOCHYTRIUM DENDROBATIDIS* (*Bd*)

Although these chemicals were not developed specifically for RV or Bd, these recommendations represent the minimum concentration and contact time demonstrated as effective

| | Clorox Bleach® | Nolvasan® | Virkon S® | Ethanol |
|---|---|---|---|---|
| **Active Ingredient (AI)** | Sodium hypochlorite | Chlorhexidine | Potassium peroxymonosulfate | Ethyl alcohol |
| **Concentration of AI** | 6.0% | 2.0% | 20.4% | 70.0% |
| **Relative cost** | $4.99/gal | $65.95/gal | $76.50/10 lb or $1.60/gal | $23.45/L or $88.83/gal |
| **Min. Contact Time RV[9]/Bd[10]** | 1 min / 30 sec | 1 min / not determined | 1 min / 20 sec | 1 min[11] / 20 sec |
| **Min. Concentration RV[9]/Bd[10]** | 3.0% / 1.0% | 0.75% / not determined | 1.0% / 1.0% | 70% / 70% |
| **Effective dilution ratio for both RV and Bd** | 1:32 dilution (bleach:water) for 3% solution using 6% concentration of household bleach. | 1:127 (Nolvasan®: water) for 0.75% solution (RV only) | 1 scoop (1.3 oz) or 1 tablet per gal of water | Effective when applied undiluted (70%) |
| **Toxicity to Humans** | • Vapor may cause severe irritation or damage to eyes and skin<br>• Harmful if swallowed | • May be fatal if inhaled<br>• Avoid breathing spray mist<br>• Causes irreversible eye damage<br>• Harmful if swallowed | • Harmful if swallowed<br>• Irritating to respiratory system and skin<br>• May cause serious eye damage | • May be fatal if swallowed or inhaled<br>• Can damage liver, kidneys and nervous system by repeated or prolonged exposure<br>• May be absorbed through skin. Repeated or prolonged contact can cause eye irritation or dermatitis[12] |
| **Toxicity to Amphibians** | • Fatal at high concentrations | • Safe for short durations [13] | • Non-toxic[14] | • May destroy mucus and wax resulting in dehydration and microbial infection[11] |
| **Effects on Equipment** | • Corrodes metals<br>• Will fade colors and break down cloth fibers | • None reported | • Safe on fabric<br>• May cause pitting on galvanized or soft metal if not rinsed with water | • May damage rubber and plastics<br>• May cause deterioration of glues[12] |

## Special Instructions:

• Remove debris from equipment prior to treatment.[15] • Wear safety glasses and gloves when handling chemicals. • Water pH can affect chemicals; all information in this table assumes the use of tap or municipal water. • Keep out of lakes, streams, or ponds; stand at least 50 m from any natural water source. • Do not clean equipment or dispose of waste solutions at field sites. • For disposal, follow local, state, and federal guidelines.

**Bleach:** Inactivated by organic material. • Inactivated by sunlight. • If in an opaque container, diluted bleach will last 1 month[16]. If exposed to sunlight or air, it will only last 5 days.

**Nolvasan:** Can be inactivated by organic material.[15] • Store at room temperature in sealed container.[17] • Dilute concentrate with water of pH 5-7.[18] • Remains stable for 1 week if dilute with tap water, and for up to 6 weeks if diluted with deionized water.[17] • Use concentrate within 36 months.[17] • Toxic to fish.[18]

**Virkon-S:** Store at room temperature.[19] • Keep solution away from extreme cold or heat. • Shelf life for tablets is 2 years and for powder is 3 years. • Remains stable for 1 week if diluted with tap water.

**Ethanol:** Highly flammable. • Use and store in a well ventilated area. • Evaporation may diminish effective concentration.[12,18]

## CITATIONS FOR DISINFECTION OF FIELD EQUIPMENT TO MINIMIZE RISK OF SPREAD OF CHYTRIDIOMYCOSIS AND RANAVIRUS


1. This information has been compiled in part from Miller, D. L., and M. J. Gray. 2009. Southeastern Partners in Amphibian and Reptile Conservation, Disease, Pathogens and Parasites Task Team, Information Sheet #10.

2. Converse K.A. and D.E. Green. 2005. Diseases of tadpoles. p 72-88. In: Wildlife Diseases: Landscape Epidemiology, Spatial Distribution and Utilization of Remote Sensing Technology. S.K. Majumdar, J.E. Huffman, F.J. Brenner and A.I. Panah (eds.). Pennsylvania Academy of Science, Easton, PA.

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# APPENDIX C

## Nutrient Laboratory Analysis

| Parameter | Laboratory | Lab Number | Method | Media | Lower Reporting Limit | units | Method Detection Limit | units | Holding Time | Preservative |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrite + Nitrate, as N | NJ DEPARTMENT OF HEALTH - 11036 | 11036 | SM 4500-NO3(F)-16 | Water | 0.012 | mg/l | 0.0069 | mg/l | 28 days | pH<2, Ice to 4ºC |
| Total Kjeldahl Nitrogen | NJ DEPARTMENT OF HEALTH - 11036 | 11036 | EPA 351.2 | Water | 0.1 | mg/l | 0.041 | mg/l | 28 days | pH<2, Ice to 4ºC |
| Phosphorus, Total | NJ DEPARTMENT OF HEALTH - 11036 | 11036 | EPA 365.1 | Water | 0.01 | mg/l | 0.007 | mg/l | 28 days | pH<2, Ice to 4ºC |

# APPENDIX D

# NJDOH Laboratory Chain-of-Custody

Field ID Number

**New Jersey Department of Health**
**Environmental and Chemical Laboratory Services**
**PO Box 361, Trenton, NJ 08625-0361**
**Phone: 609-530-2820**
**ORGANIC AND INORGANIC CHEMISTRY SAMPLE SUBMITTAL**
***(See Instructions)***

Lab Sample Number
(For Lab Use Only)

**AGENCY INFORMATION**

| Submitting Agency | Send Results To | Agency No. | Project Name |
|---|---|---|---|
| NJDEP-BFBM | | 207 | Biological Nutrient Correlation Project |
| Street Address: 35 Arctic Parkway | Final Report Option: ☐ Tier 1 ☐ Tier 2<br>Electronic Report Option: ☐ EDD ☐ E-2 | Would you like copies of the internal chain of custody forms sent with your report? ☐ Yes ☒ No | Project Code: BIONTR<br>Memo Number |
| City, State, Zip Code: Trenton, NJ 08625 | Phone: 609-292-0427 | Fax: 609-633-1095 | Email: brian.henning@dep.nj.gov |

**SAMPLE INFORMATION**

| | | |
|---|---|---|
| Sample Point/Station ID Number/Water Facility ID | Collection Date (YY/MM/DD): 23 / __ __ / __ __ | **Sample Type** |
| Sampling Site/Facility/Supply/Location/Sampling Point ID | Coll. Time (24h) Start: __ __ __ __ Coll. Time (24h) End: 1200 | **Non-Potable:** ☒ Stream/Surface ☐ Ground Water ☐ Private Well ☐ Septic ☐ Ocean/Saline ☐ Sediment ☐ Tissue ☐ Sewage: ☐ Raw ☐ Effluent ☐ Industrial: ☐ Raw ☐ Effluent |
| Waterbody Name | Sample Retention: Retain? ☒ No ☐ Yes Duration _____ | |
| Municipality/County | Type of Sampling Event: ☒ Regular ☐ Compliance ☐ Repeat ☐ Non-Regulatory ☐ Other | **Potable:** ☐ Groundwater Rule ☐ Source ☐ Confirmation ☐ Raw ☐ Finished ☐ Private Well ☐ At Source ☐ Flushed ☐ 1st Draw ☐ Lead Source Line ☐ Surface $H_2O$ Intake ☐ Distribution System |
| Sampling Point Street Address: FIBI | If Repeat or GWR, List Original Lab Sample No. | |
| | Sample Collector | **Fraction:** ☒ Total ☐ Dissolved |
| PWSID | Trip # | **Other:** ☐ _____ |
| | | **Priority:** ☒ Routine ☐ Priority ☐ Emergency |

**FIELD INFORMATION**

| | | |
|---|---|---|
| Air Temp °C | Water Temp °C | Stream Flow-CFS |
| Weather Conditions | Sample pH (Field) | Gage Height-Ft. |
| Preserved in: ☒ Field ☐ Lab<br>Date: 23 __ / __ __ / __ __<br>Time: __ __ __ __ | DO (mg/l) | Spec.Cond. (µS/CM) |
| | DO% Sat | Salinity (ppm) |
| Chlorine Residual | Sample Depth Ft. | Tide Stage |
| Comments/Field Checks | Barometric Pressure (mmHg) | Turbidity (NTU) |

**ANALYSIS REQUESTS**

**Metals**

| | |
|---|---|
| ☐ Ag ..... Silver | ☐ Mg ..... Magnesium |
| ☐ Al ..... Aluminum | ☐ Mn ..... Manganese |
| ☐ As ..... Arsenic | ☐ Mo ... Molybdenum |
| ☐ B ..... Boron | ☐ Na ..... Sodium |
| ☐ Ba ..... Barium | ☐ Ni ..... Nickel |
| ☐ Be ..... Beryllium | ☐ Pb ..... Lead |
| ☐ Ca ..... Calcium | ☐ Sb ..... Antimony |
| ☐ Cd ..... Cadmium | ☐ Se ..... Selenium |
| ☐ Co ..... Cobalt | ☐ Si ..... Silica |
| ☐ CR-T .... Chromium | ☐ Tl ..... Thallium |
| ☐ Cu ..... Copper | ☐ U ..... Uranium |
| ☐ Fe ..... Iron | ☐ V ..... Vanadium |
| ☐ K ..... Potassium | ☐ Zn ..... Zinc |

**Preferred Methodology**
☐ EPA 200.7 / 200.9 ☐ EPA 200.8

**Residues**
☐ Total Suspended Solids (TSS)
☐ Total Solids (TS)
☐ Total Dissolved Solids (TDS)
☐ Settleable Solids (SS)
☐ Total Volatile Solids (TVS)

**General**

| | |
|---|---|
| ☐ Alkalinity | ☐ Fluoride by IC |
| ☐ Bromide by IC | ☐ Hardness |
| ☐ Chloride | ☐ MBAS |
| ☐ Chloride by IC | ☐ Odor |
| ☐ Chromium, Hexavalent | ☐ pH |
| ☐ Chromium, Hexavalent by IC | ☐ Phenols (PW) |
| ☐ Color | ☐ Phenols (NPW) |
| ☐ Conductance | ☐ Sulfate by IC |
| ☐ Cyanide | ☐ Sulfate Lachat |
| ☐ Dissolved Oxygen | ☐ Turbidity |
| ☐ Fluoride | |

**Mercury**
☐ Mercury by EPA 245.1
☐ Low Level Mercury EPA 1631E

**Nutrients**

| | |
|---|---|
| ☐ Nitrite | ☒ Nitrite + Nitrate |
| ☒ Total Phosphorus | ☐ Ortho Phosphorus |
| ☐ Ammonia | ☒ Total Kjeldahl Nitrogen (TKN) |
| ☐ Nitrate (Calculated) | |
| ☐ Nitrogen, Total (Calculated) | |

**Organics (Drinking Water)**
☐ EPA 504.1 - EDB, DBCP,123TCP
☐ EPA 505 - Chlordane
☐ EPA 505 - Toxaphene
☐ EPA 507 - N and P containing Pesticides
☐ EPA 515.3 - Chlorinated Acid Herbicides
☐ EPA 524.2 - Purgeables
☐ EPA 525.2 - Liquid-Solid Extractables
☐ EPA 531.1 - N-Methylcarbamoyloximes and N-Methylcarbamates

**Organics (Non-Potable Water)**
☐ EPA 624 – Purgeables
☐ EPA 625 - Base/Neutral and Acid Extractables

**Demands**
☐ Total Organic Carbon (TOC)
☐ Dissolved Organic Carbon (DOC)
☐ Chemical Oxygen Demand (COD)

**Suggested Dilutions**
☐ BOD5 ☐ BOD20 ____ ____ ____
☐ CBOD5 ☐ CBOD20 ____ ____ ____

**Other**
☐ _____ ☐ _____ ☐ _____

| Relinquished By: | Affiliation: | Received By: | Affiliation: | Date/Time | Reason for Custody Change |
|---|---|---|---|---|---|
| Name (Print): _____ | NJDEP-BFBM | Name (Print): _____ | | | |
| Signature: _____ | | Signature: _____ | | | |
| Name (Print): _____ | | Name (Print): _____ | | | |
| Signature: _____ | | Signature: _____ | | | |

CHEM-44
FEB 16

# APPENDIX E
# EPA's Rapid Habitat Assessment Form (low gradient)

## HABITAT ASSESSMENT FOR *LOW* GRADIENT STREAMS

| | Condition Category | | | |
|---|---|---|---|---|
| | **Optimal** | **Suboptimal** | **Marginal** | **Poor** |
| **1. Epifaunal Substrate /Available Cover** | Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient) | 30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale). | 10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed. | Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **2. Pool Substrate Characterization** | Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common. | Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present. | All mud or clay or sand bottom; little or no root mat; no submerged vegetation. | Hard-pan clay or bedrock; no root mat or vegetation. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **3. Pool Variability** | Even mix of large-shallow, large-deep, small-shallow, small-deep pools present. | Majority of pools large-deep; very few shallow. | Shallow pools much more prevalent than deep pools. | Majority of pools small-shallow or pools absent. |
| SCORE: | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **4. Sediment Deposition** | Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition. | Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools. | Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent. | Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **5. Channel Flow Status** | Water reaches base of both lower banks, and minimal amount of channel substrate is exposed. | Water fills >75% of the available channel; or <25% of channel substrate is exposed. | Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed. | Very little water in channel and mostly present as standing pools. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **6. Channel Alteration** | Channelization or dredging absent or minimal; stream with normal pattern. | Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present. | Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted. | Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. In stream habitat greatly altered or removed entirely. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **7. Channel Sinuosity** | The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas. | The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line. | The bends in the stream increase the stream length 2 to 1 times longer than if it was in a straight line. | Channel straight, waterway has been channelized for a long distance. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **8. Bank Stability** (score each bank) Note: determine left or right side by facing downstream. | Banks stable, evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected. | Moderately stable, infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion. | Moderately unstable; 30-60% of bank in reach has areas of erosion, high erosion potential during floods. | Unstable, many eroded areas, "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars. |
| SCORE (LB) | Left 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE (RB) | Right 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| **9. Bank Vegetative Protection** (score each bank) | More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, under story shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally. | 70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining. | 50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining. | Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height. |
| SCORE (LB) | Left 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE (RB) | Right 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| **10. Riparian Vegetative Zone Width** (score each bank riparian zone) | Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone. | Width of riparian zone 12-18 meters; human activities have impacted zone only minimally. | Width of riparian zone 6-12 meters; human activities have impacted zone a great deal. | Width of riparian zone <6 meters: little or no riparian vegetation due to human activities. |
| SCORE (LB) | Left 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE (RB) | Right 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |

**HABITAT SCORE**

| HABITAT SCORES | VALUE |
|---|---|
| OPTIMAL | 160 – 200 |
| SUB-OPTIMAL | 110 – 159 |
| MARGINAL | 60 – 109 |
| POOR | < 60 |

# APPENDIX F

## Jonah Ventures eDNA sampling instructions

# Sampling Water for Environmental DNA


**ACTIVATE ACCOUNT**

Create an account on JonahDNA.com. Download the JonahDNA app on the Apple App Store or Google Play.


**DRAW WATER**

Put on gloves. Use the syringe (without the filter) to draw 60 mL of water


**ATTACH FILTER**

Attach the filter to the syringe. Do not overtighten.


**PUSH WATER**

Push the water through the filter. Stop when all the water has been pushed through or flow is reduced to less than about a drop per 2 seconds.


**REPEAT UNTIL FILTER CLOGS**

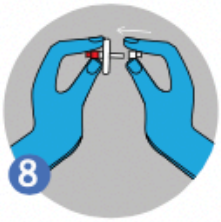
If all 60 mL of water has been pushed through, remove the filter, draw 60 mL more water, and repeat until filter is clogged.


**PUSH AIR**

Push 50 mL of air through the filter to help dry it out.


**INJECT PRESERVATIVE**

When complete, inject all supplied preservative into the filter.




**PUT ON LOCK CAPS**

Fit red lock cap over the top of f the filter. Screw on tight. Push the clear cap over the bottom.


**PUT FILTER IN FOIL BAG**

Place filter in foil bag and seal. Sample can be kept at room temperature for several days before mailing.


**ENTER DATA INTO APP**

Open JonahDNA app. Sign in. Record data. Submit data.


**MAIL FILTER**

Place foil bag with filter into provided envelope. Send via USPS.

JONAH VENTURES
KNOWLEDGE IN SEQUENCE

The detergent Triton X-100™ is used as a preservative in this kit. Safety and handling instructions can be found here: https://tinyurl.com/477as6ye

**WARNING. DO NOT CONSUME.** Keep away from food and drink. This preservative can cause skin and eye irritation. Please wear protective gloves to prevent skin exposure. Wash eyes or skin thoroughly if contact occurs. Remove and wash any contaminated clothing before re-use.

Questions? Contact us at support@jonahventures.com

# APPENDIX G

## JonahDNA App for sample recording