Invasive Carp eDNA Program
Process for Non-Traditional Sampling

IC eDNA Monitoring Program

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Document Information

IC eDNA Monitoring Program
eDNA Non-Traditional Sampling SOP
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Invasive Carp eDNA Program Non-Traditional Sampling Protocol

Application/Purpose

The Invasive Carp (IC) eDNA Monitoring Program Quality Assurance Project Plan (QAPP) is a controlled document that is reviewed annually by Program Staff (FWCO, PL, WGL, and RO). The document describes standardized field sampling and analysis of those samples. This document will describe the process and necessary steps for using sampling methods that are not outlined in the QAPP to collect Invasive Carp eDNA Monitoring Program samples. Therefore, this protocol and associated SOPs are intended to guide non-traditional sampling requests, collection, and processing with the primary purpose of Invasive carp DNA detection using qPCR processes and marker sets described in the QAPP. (Note: Samples collected for the purpose of Metabarcoding should not be included in this process and should seek guidance and approval through the protocols of that program separately.)

Non-traditional Sampling Description

The methods described herein (See IC eDNA Filter Sample Field Collection SOP and IC eDNA Filter Sample Extraction SOP) are the only approved non-traditional methods for the collection and processing of Invasive Carp eDNA Monitoring Program samples. Any methods used outside of those described herein must receive prior approval from the Invasive Carp eDNA Program Coordinator and proper documentation (publications, research, etc.) should accompany the non-traditional sampling request. Sample size and distribution of non-traditional IC eDNA case samples should follow the recommendation for traditional IC eDNA samples. The process for requesting and carrying out non-traditional sampling should be followed as described below.

Roles/Responsibilities

Although classified as non-traditional, samples collected following this protocol and associated SOPs are still considered part of the Invasive Carp eDNA Monitoring Program and therefore data collection, QA/QC, management, and results reporting will be handled by similar processes and personnel as data are for traditional, QAPP-guided Program samples.

Case Management

Approved cases will be assigned a unique case number to be used for tracking samples through the various phases of collection and processing. These numbers will be assigned by the Invasive Carp eDNA Program Coordinator and posted alongside traditional cases in the Master Tracker, and a comment will be made in the Notes column identifying the case as non-traditional.

FWCO Staff

Similar to traditional Program samples, FWCOs are responsible for entering (automatic with ArcGIS Field Maps app), proofing, and retaining field data. The eDNA Lead for each field station are responsible for ensuring proper data entry and facilitating QA/QC of data. If possible, QA/QC and edits of missing or incorrect data should be completed and synced with the database within **10 business days** after the last day of collection for a specific event or before the next sampling event to be conducted at the same location if that is to occur in less than 10 days. In extenuating circumstances, a designee may be identified to complete data

edits and QA/QC. FWCO staff should retain an electronic copy of the completed dataset for each of their eDNA events.

Survey123 electronic Chain of Custody (COC) forms will be submitted to WGL when samples are shipped. WGL will receive an email via a web hook when the Survey123 COC form is submitted for shipping. WGL will use the eDNA Shipping Alerts channel in Teams to let FWCO offices know when samples are received in the lab. The shipping FWCO may request a hard copy of their Survey123 COC data.

Survey123 electronic Trip Plan Summary Survey 123 forms with post trip information (same form as traditional sample collection) will be submitted to WGL when samples are shipped. WGL will receive an email via a web hook when the Post-Trip & QA/QC Summary Survey 123 form information is submitted.

If FWCO staff are interested in having a reference layer (ex: sample point reference) added to the ArcGIS Field Maps sampling map, please reach to eDNA data steward at least 2 months before sampling event.

WGL Staff

WGL staff are responsible for tracking and documenting a record of sample receipt and handing similar to traditional Program samples (See eDNA Program Data Management SOP No 5). Samples will be processed by WGL following the extraction SOP accompanying this protocol and qPCR protocol referenced in the QAPP.

The eDNA Processing Lead will be responsible for reporting results with the eDNA Program Coordinator and eDNA Data Steward within 48 hours of QC review. The Survey123 COC and Trip Summary Plan data and all laboratory generated data files will be maintained on the WGL and MFC Azure servers.

Data Management

All field data and summarized lab detection data from the IC eDNA Monitoring Program can be accessed through ServCat, the U.S. Fish and Wildlife Service's data catalog. The catalog record (URL) contains program information, points of contact, metadata, and how to access the database of field data and summarized lab detection data.

eDNA Data Steward

eDNA Data Steward Reports will be generated as each non-traditional case is completed. Each report will be sent to the eDNA Program Coordinator and saved electronically in the WGL and MFC Azure servers. The eDNA Data Steward will backup and maintain the non-traditional eDNA sample data in at least three backup locations. The eDNA Data Steward will also routinely back up eDNA sample data from the ArcGIS Online eDNA Fisheries Group once a week. Field offices should still download and maintain the data collected by their office as recommended above. The eDNA Data Steward will update metadata annually through ArcGIS and ServCat records.

The Data Custodian will maintain an electronic folder under the Service's Enterprise Data Warehouse that contains the field geodatabase complete with field and lab data, backup of working geospatial files, backup of eDNA result maps, and backup of data summary tables.

Quality Assurance/Quality Control Specialist

Survey123 COC data will be stored in an ArcGIS database and will be maintained by the eDNA Processing QA/QC Specialist. All Trip Summary data will be stored in an ArcGIS database and will be maintained by the eDNA Processing QA/QC Specialist. They will review, validate and certify eDNA detection data before the eDNA Processing Lead reports results.

Overall Process

1. A non-traditional sampling request (See Template E. Non-Traditional Invasive Carp eDNA Sampling Request Microsoft Form) is submitted to the IC eDNA Program Coordinator for review when traditional sampling requests are submitted. The IC eDNA Program Coordinator will determine approval based on IC eDNA Monitoring Program priorities.
2. The IC eDNA Program Coordinator will work with WGL Project Leader to determine lab capacity to accommodate samples.
3. IC eDNA Program Coordinator and sampling POC will work with the Program QA/QC Specialist and Data Branch to review and decide on appropriate data management workflow.
4. eDNA Lead for collecting office will submit a Trip Plan Summary Survey123 form with Pre-Trip information filled out prior to the start of sampling.
5. During collection, the IC eDNA Field Maps form will be used to collect field data with the sampling protocol set to eDNA Non-Traditional Monitoring of Invasive Carps.
6. Sample transfer between the field and lab will be tracked using the IC eDNA COC in Survey123.
7. Samples will be processed by WGL following the IC eDNA Filter Sample Extraction SOP accompanying this protocol and qPCR protocols referenced in the QAPP.
8. Sample results will follow QAPP data QA/QC and internal reporting procedures including the creation of official results maps.
9. Results maps will be shared with the appropriate state and tribal partners at the discretion of the IC eDNA Program Coordinator.
10. Non-traditional sampling results will **not** be posted to the Bighead and Silver Carp Environmental DNA Monitoring Dashboard.
11. Notify the eDNA custodian at the end of the case so data can be added into a ServCat record for non-traditional sampling cases.

Template E

Non-Traditional Invasive Carp eDNA Sampling Request

The content described below is included in a Microsoft Form Template E. Non-Traditional Invasive Carp eDNA Sampling Request Microsoft Form that is required to be completed to request collection of non-traditional Invasive carp eDNA samples. Content is included here as a reference for planning. Please fill out the actual Microsoft Form.

Sampling Location
Where will the non-traditional sampling occur? Please indicate the basin and waterbodies of interest.

Partners/Collaboration
Was this work requested by a state or tribal partner?

Justification for Non-Traditional Sampling
Please explain why QAPP sampling methods cannot be used for this case.

Non-Traditional Sampling Description
Describe the methods (provide citations if available) and sampling design you intend to use to complete this sampling.

Number of samples requested.

Sampling type requested.

Sampling Duration
Will this be a single occurrence event or is it a reoccurring event?

Specify project end date and frequency of events.

IC eDNA Filter Sample Field Collection SOP

Required Equipment

- Smith-Root eDNA Sampler
- Smith-Root self-preserving filter packs with 5 µm PES filters. One filter pack per intended sample and field blank to be collected
- Extra filter packs in case of mistakes or equipment failure
- 500 mL Nalgene bottles, prefilled with municipal tap water, for each field blank sample to be collected
- Nitrile gloves. One pair per sample and field blank to be collected, plus extras
- Collection pole, if needed
- Opaque bag or storage container to store completed samples
- Permanent marker and/or pre-printed sample labels
- iPad for data collection with Field Maps and Survey 123 Apps installed
- Bad Elf GPS

Filter Sample Collection Procedure

1. Ensure that the outlet hosing, which drains the filtered water from the machine, is draining away from the water in which the sample is being collected
2. Turn on the Smith-Root eDNA Sampler and check that settings are as follows:
    a. Target volume set to 1.0 Liter
    b. Target flow set to 1.0 Liter per minute
    c. Collection mode set to Auto
    d. Maximum pressure set to 10.0 PSI
    e. Volume Offset set to 250 ml (this is the default for using the long inlet hosing that came with the unit. This volume will differ if you have altered the length of the original long inlet hosing piece. If alteration has occurred, the appropriate offset volume should be determined prior to the sampling event)
3. Prior to the collection, a sample label should be placed on the sample packaging or a sample RUID written on the packaging with permanent marker.
4. A new pair of nitrile gloves should be donned and then the sample package may be opened.
5. Carefully remove the filter housing and one-time use snorkel tube from the packaging and put them together, taking care not to touch the collection ends of either item.
6. Attach the filter housing to the collection pole, if using, then attach the sampler inlet hosing.
7. Lower the filter pack and attached snorkel tube to the water surface so the end of the snorkel tube enters the first few inches of the water column, taking care to avoid debris entering the tube. Do not submerge the filter housing.
8. When ready to begin collecting water, press Start on the sampler interface.
9. The sample timer on the machine interface will begin to count up, water will begin to pump up through the inlet hosing, through the machine and out the outlet hosing.

10. Continue collecting water until one of the following three scenarios occurs, at which time the snorkel tube should be moved from the water, allowed to drain, and then inverted until the remaining water in the inlet hosing passes through the machine:
    a. **The machine beeps twice indicating the target volume will be reached.** This alert takes into consideration the offset volume of 250 mL so it will likely occur when the machine interface reads 0.75 L of water have been collected. After inverting the snorkel tube and allowing the water in the rest of the inlet hose to pass through the meter, the total volume will be approximately 1.0 L.
    b. **The filter clogs.** This can be indicated by the filter clog alert (indicated by 5 beeps from the machine), which is dependent on what the Filter Clog Point is set to on the machine. For more consistency between samples, watch the total volume collected on the interface and if the value stops increasing while the pump is one and remains stalled for 10 seconds, consider the filter clogged.
    c. **Three minutes of collection time has passed.** The timer on the sampler interface, which begins counting up when the pump was started in Step 8, reaches 3:00 minutes of collection time.
11. After the inlet hose has drained and snorkel inverted, allow the suction to remain on for an additional 15 seconds to allow the filter membrane additional drying time in case any excess moisture remains in the housing.
12. Press Stop on the sampler interface to stop the machine from pumping, detach the filter housing from the collection pole and inlet hosing.
13. Remove and discard the snorkel tube.
14. Place the completed sample back into the original labelled filter packaging, squeeze out excess air, then re-seal.
15. Store samples in an opaque container or bag.
16. Remove nitrile gloves and discard.
17. Record sample data, including final achieved volume, in Field Maps.
18. Repeat Steps 3-17 for all filter pack eDNA samples.

Filter Field Blank Collection Procedure

1. Follow Steps 3-6 above, however do not attach to collection pole (if using for regular samples).
2. Open a 500 mL pre-filled Nalgene and expose the water to the air for 10 seconds.
3. Carefully lower the end of the filter pack snorkel tube into the bottle of water. Do not touch the inside of the Nalgene with your fingers.
4. Press Start on the sampler interface.
5. Continue collecting water until the bottle is empty. In order to filter the entire volume of the blank sample, you may need to remove the collection tube from the bottle, invert it, and pour the remaining blank water from the bottle into the tubing.
6. Follow Steps 11-17 above to complete the sample.

Filter Sample Storage Prior to Shipping to WGL

Completed and sealed filter pack samples may be stored at room temperature while awaiting shipment to WGL. Samples should be kept in an opaque bag or container to block exposure

to UV light. Care should be taken to avoid samples being exposed to high ambient temperatures, such as those that may occur in a hot vehicle or when in direct sunlight.

Filter Sample Shipment to WGL

Please organize samples prior to shipping. Place 10-12 filter pack samples, grouped in sequential order (e.g. 1-12, 13-24, etc.), into 2-gallon Ziploc-style bags and label the bags with the case number and appropriate sample range. Grouped and organized samples can then be shipped in any size cardboard box(es) that is appropriate for the case size. No additional special packaging or messaging needs to accompany the samples. Prior to shipping, the IC eDNA Sampling COC must be completed for the case and submitted through Survey123.

IC eDNA Filter Sample Extraction SOP

Purpose

To guide an individual on how to conduct a DNA extraction from filters using an IBI Scientific gMAX Kit.

Safety

Warnings for consideration:

- Exposure to Ethanol
- Exposure to other potentially harmful chemicals

Recommended Personal Protective Equipment:

- Gloves
- Lab coat
- Safety glasses
- Please view and understand JHA numbers 1- Ethanol, 19- Chemical Use and any SDS's for chemicals you will use before proceeding.

Procedure

Analytical Methods: IBI Scientific gMAX Kit Extraction Procedure for Filtered Samples

1. Obtain one **Qiagen lyse and spin column** per filter to be extracted and organize tubes in rack. You will have time to label the rest (1 lab-supplied 1.5-mL MCT, 1 GD spin column, and multiple IBI collection tubes) during the 60-minute incubation. Be sure to add one positive and negative extraction control to each eDNA extraction procedure batch.
2. Add 350 µL **GSB** to each lyse and spin basket tube. If precipitate has formed in GSB buffer, dissolve by incubating at 60°C for 10 minutes, before dispensing into tubes.
3. Add 35 µL **proteinase K** to each lyse and spin basket tube.
4. Move dried filter samples to the extraction room. Make sure each container is dry.
5. Remove filter housing from package and place in upside down vacuum filter cups, you may setup multiple filter this way at once. Use a bleach wipe to clean the outside of filter housing package or change gloves between each sample when setting them in the cups.
6. Then for each sample, put on a new pair of gloves and remove the filter from the housing. Fold the interior portion of the filter tightly (use tweezers or your new/clean gloves) and carefully fit into the lyse and spin basket with the GSB mixture and stuff the filter down, below the top line of the lyse and spin basket, with clean pipette tip, new wooden swab end, or clean forceps if needed. Make sure the lid securely closes. If filters are too high in the lyse and spin basket, foam over may occur during incubation. Be careful to avoid cross-contamination at this step. Change gloves between samples.
7. Incubate sample racks with lyse and spin columns, in the incushaker at 60°C for 60 minutes(non-shaking). Label the rest of the tubes, place GD spin columns in tube rack

and print final archive labels for extracts. Archive labels are the case number and three-digit sample number (e.g., 20034001). Also, during this time place the Elution Buffer into the 60°C bead bath.

8. Remove lyse and spin tube from incubator and centrifuge at max speed for at least 1 minute, up to 3 minutes depending on level of sediment in tubes, to spin through all of the liquid. If samples are particularly dirty, spin at least 3 minutes. If the basket is clogged, use a pipette to remove the supernatant from the sample, recovering as much as you can. Make notes in the Case Log.
9. Add 500 µL **ethanol** (100%, molecular grade) to the extract. Mix thoroughly by vortexing. If liquid collects around the cap, spin briefly before opening to reduce contamination risk.
10. Transfer up to 780 µL of the mixture by pipette into a GD spin column placed in a 2 mL collection tube. Centrifuge at ≥ 16000 x *g* for 1 minute. Discard flow-through and collection tube. This will have to be done multiple times for samples with more than one filter.
    a. For example: Sample 003 has five filters. Transfer the ethanol/extract mixture from the first filter to the GD spin column, centrifuge, discard flow-through and collection tube, place the GD spin column into new collection tube, transfer the ethanol/extract mix from the second filter into the GD spin column, centrifuge, and continue for each filter until all five ethanol/extract mixtures have been centrifuged through the same GD spin column.
11. Transfer any remaining mixture by pipette onto the same spin column and place in a new 2 mL collection tube. Centrifuge again at ≥ 16000 x *g* for 1 minute. Discard flow-through and collection tube.
12. Place GD spin column in a new 2ml collection tube. Add 400 µL Buffer **W1**. Centrifuge at 16,000 x *g* for 30 seconds. Discard flow-through and collection tube.
13. Place spin column in a new 2 mL collection tube. Add 600 µL **Wash Buffer**. Centrifuge at 16,000 x *g* for 3 minutes. Discard flow-through and collection tube.
14. Transfer the spin column to a new 1.5 mL MCT.
15. Add 150 µL of the pre-heated Elution buffer to the center of the spin column membrane. Incubate for 1 minute at room temperature (15 - 25°C). Centrifuge at 16,000 x g for 30 seconds.
16. Discard the spin column and store the eluted DNA samples at −20°C. If DNA is to be immediately used for PCR, keep in 4°C refrigerator.