A1. Title Page

Quality Assurance Project Plan

Invasive Carp eDNA Monitoring Program

Prepared for:

U.S. Fish and Wildlife Service

Midwest and Northeast Regions

2025

Approved by:

Midwest Region AIS Program Supervisor:

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Northeast Region AIS Program Supervisor:

Sandra Keppner SANDRA KEPPNER Digitally signed by SANDRA KEPPNER Date: 2025.03.20 11:33:02 -04'00'

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Nick Frohnauer NICHOLAS FROHNAUER Digitally signed by NICHOLAS FROHNAUER Date: 2025.03.19 15:16:36 -05'00'

Midwest Region eDNA Processing QA/QC Specialist:

Maren Tuttle-Lau MAREN TUTTLE-LAU Digitally signed by MAREN TUTTLE-LAU Date: 2025.03.19 14:59:54 -05'00'

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Section A. Project Management

A3. Distribution List

Name	Title	Office	Address
Will Meeks	Midwest Region Regional Director	Midwest Region Regional Office	5600 American Blvd W #990, Bloomington MN 55437
Aaron Woldt	Fisheries and Aquatic Conservation (FAC) Assistant Regional Director	Midwest Region Regional Office	5600 American Blvd W #990, Bloomington MN 55437
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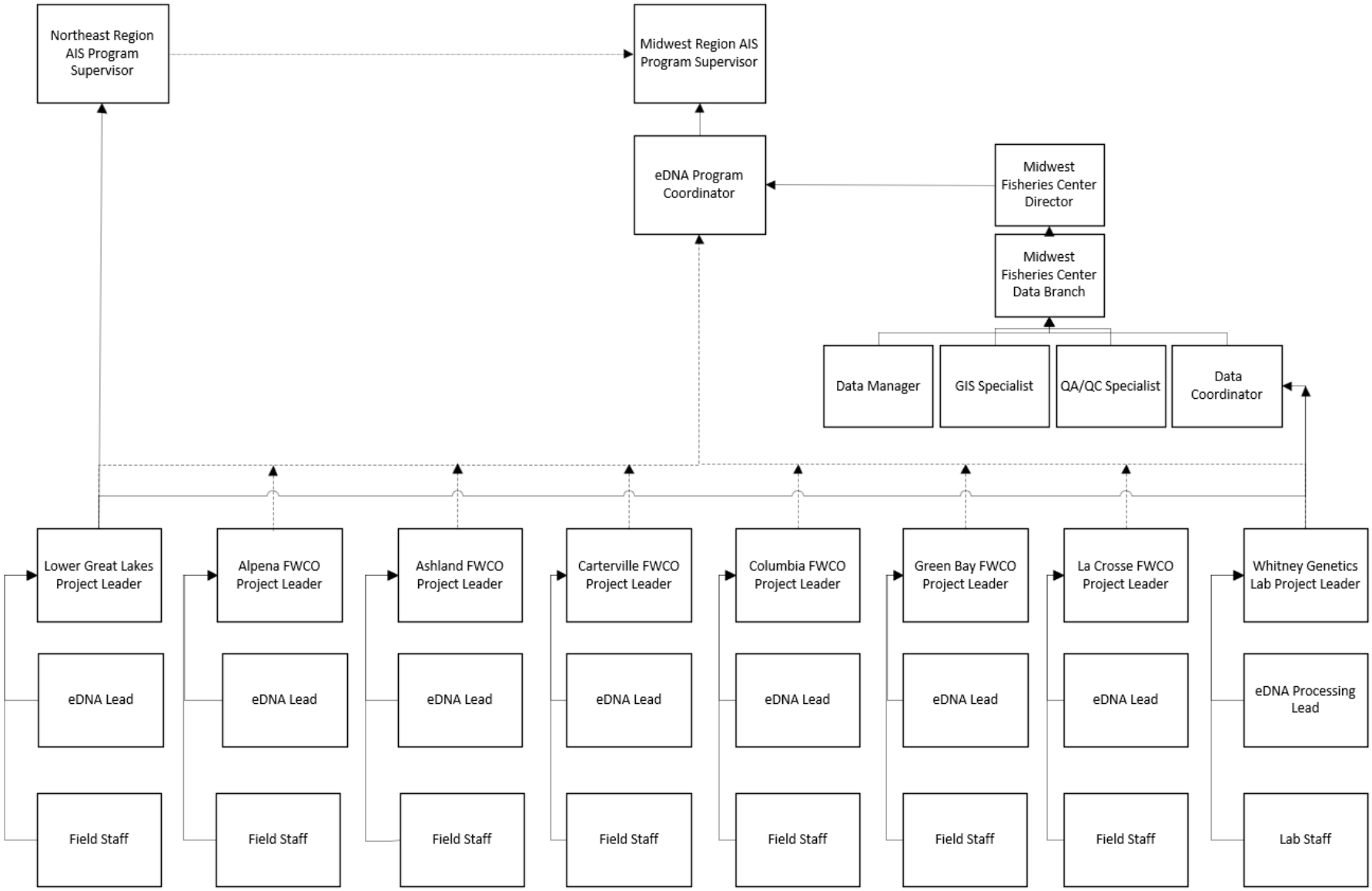
Amy McGovern	Midwest Region AIS Program Supervisor	Midwest Region Regional Office	5600 American Blvd W #990, Bloomington MN 55437
Sandra Keppner	Northeast Region AIS Program Supervisor	Lower Great Lakes FWCO	1101 Casey Road, Basom NY 14013
Kasia Mullet	FWCO Program Supervisor	Midwest Region Regional Office	5600 American Blvd W #990, Bloomington MN 55437
Nick Frohnauer	eDNA and Early Detection Coordinator	Midwest Region Regional Office	5600 American Blvd W #990, Bloomington MN 55437
Scott Koproski	Project Leader	Alpena FWCO	480 W Fletcher Street, Alpena MI 49707
Vacant	Project Leader	Ashland FWCO	2800 Lake Shore Dr E, Ashland WI 54806
Mike Thomas	Project Leader	Carterville FWCO	292 San Diego Rd, Carbondale IL 62901
Jason Goeckler	Project Leader	Columbia FWCO	101 Park Deville Dr. Ste A, Columbia MO 65203
Susan Wells	Project Leader	Green Bay FWCO	2661 Scott Tower Dr, New Franken WI 54229
Rebecca Neeley	Project Leader	La Crosse FWCO	555 Lester Ave, Onalaska WI 54650
Mike Goehle	Project Leader	Lower Great Lakes FWCO	1101 Casey Rd, Basom NY 14013
Mike Thomas	Project Leader	Wilmington Substation	30239 IL-53, Wilmington IL 60481
Patrick DeHaan	Project Leader	Whitney Genetics Lab	555 Lester Ave, Onalaska WI 54650
Maren Tuttle-Lau	QA/QC Specialist	Midwest Fisheries Center	555 Lester Ave, Onalaska WI 54650
Kyle Von Ruden	eDNA Processing Lead	Whitney Genetics Lab	555 Lester Ave, Onalaska WI 54650

A3. Invasive Carp eDNA Program Coordination



A4. Project/Task Organization

Project personnel responsibilities and roles are defined below for each position necessary for completing the US Fish and Wildlife (FWS) Midwest and Northeast Regions environmental DNA (eDNA) monitoring program. To run smoothly and maximize efficiency, it is recommended that staff be assigned to each position.

The roles listed below assume all staff are FWS employees who have been trained according to documented Quality Assurance Project Plan (QAPP) procedures. If other agency personnel are assisting in the field or lab, they must have read and understood the QAPP and have appropriate training on file with the FWS Fish and Wildlife Conservation Office (FWCO) leading the sampling event. When personnel from other agencies or volunteers do not meet the minimum requirements or have equivalent requirements (e.g. state boat operator training), this must be documented on the Post-Trip Field Summary Plan Survey123 Form as a deviation from the QAPP and a brief explanation provided.

Two people can occupy the same position in some of the positions listed below (field sampling collector assistant and field sample collector Quality Assurance/Quality Control (QA/QC) Specialist). However, use judgement as the logistical nature of some of the positions cannot be filled by the same person (the boat operator cannot be the same person as the field sampling collector).

Field Collection Staff

- Field Operations Manager
- eDNA Station Lead
- Field Sampling Collector
- Field Sampling Collector Assistant
- Field Data Recorder
- Field Sampling Collector QA/QC Specialist
- Field Sample Processor, Sample Processor QA/QC Specialist
- Boat Operator

Laboratory Processing Staff

- Lab eDNA Processor
- Lab eDNA Processing Lead

Data Management Staff

- eDNA Processing QA/QC Specialist
- eDNA Data Steward

Communication Staff

- eDNA Program Coordinator
- Office of Communications

Roles

<u>Midwest Regional Office</u>

Midwest Region Regional Director (RD): Will Meeks

Fish and Aquatic Conservation Assistant Regional Director (ARD): Aaron Woldt

Fish and Aquatic Conservation Deputy ARD: Brian Elkington

Midwest Region AIS Coordinator: Amy McGovern

Midwest Region eDNA Coordinator: Nick Frohnauer

Midwest Region FWCO Program Supervisor: Kasia Mullet

Northeast Regional Office

Northeast Region AIS Coordinator: Sandra Keppner

Midwest Fisheries Center

Center Director: Mark Brouder

Data Steward: Ross Ruehmann

eDNA Processing QA/QC Specialist: Maren Tuttle-Lau

Northeast Fisheries Center

Center Director: Meredith Bartron

Data Management

Data Custodians: Ross Ruehmann and Jeena Koenig

Data Trustee: Amy McGovern

Whitney Genetics Lab

Project Leader: Pat DeHaan

eDNA Processing Lead: Kyle Von Ruden

Table 1. List of Fish and Wildlife Conservation Office Project Leader and Station eDNA Lead

Office	**Project Leaders**	**Station eDNA Lead**
Alpena	Scott Koproski	Anton Gereau
Ashland	Vacant	Mike Seider
Carterville	Mike Thomas	Rebecca Lucas
Columbia	Jason Goeckler	Jessica Howell
Green Bay	Susan Wells	Cari-Ann Hayer
La Crosse	Rebecca Neeley	Jenna Bloomfield
Lower Great Lakes	Mike Goehle	Colleen Keefer
Wilmington	Mike Thomas	Jen-Luc Abeln

A5. Background

Aquatic invasive species pose a major threat to aquatic ecosystems worldwide. Within Illinois, the man-

made Chicago Sanitary & Ship Canal (CSSC), constructed in the early 1900s, provided an unnatural portal for invasive species dispersal between the geologically separated Mississippi River and Great Lakes drainage basins. In 2002, in an effort to curtail the spread of invasive species between the two basins, the U.S. Army Corps of Engineers (USACE), constructed an electric dispersal barrier system within the CSSC. The primary objective of the barrier system was to stop the dispersal of the invasive Round Goby (*Neogobius melanostomus*) into the Mississippi River basin; however, the Round Goby had surpassed the barrier before its completion. Invasive carps, including Bighead Carp (*Hypophthalmichthys nobilis*) and Silver Carp (*H. molitrix*) have been steadily dispersing upstream through the Mississippi, Illinois, and Des Plaines rivers.

Traditional fishery techniques are used to detect the leading edge of Bighead and Silver Carp populations; however, these methods are ineffective at targeting these species at low densities. The University of Notre Dame, with funding from the USACE, developed a method that detected eDNA left behind in the aquatic system by the targeted species. Environmental DNA enters the system through a variety of mechanisms, some of which include sloughing of external epidermal cells into the water, sloughing of internal epidermal cells into feces and into the water, and secretion of tissue residues following injury or predation and during spawning activity. The detection of eDNA in water samples is based on whole DNA extraction from particulate organic and inorganic matter found in the water using polymerase chain reaction (PCR) assays for species-specific mitochondrial DNA markers.

Use of eDNA methodologies aides in the detection of Bighead and Silver Carp at low densities and may be used as early detection of the spread of Bighead and Silver Carp into previously uninhabited areas. The original QAPP detailing the eDNA monitoring process, including methodologies and quality controls, was requested from the U.S. Army Engineer Research and Development Center (ERDC) as the USACE assumed eDNA monitoring responsibility from the University of Notre Dame. This modified QAPP will be followed by the FWS offices responsible for field sampling and lab processing of eDNA samples. The FWCO is responsible for field sampling and the Whitney Genetics Lab (WGL) at the Midwest Fisheries Center (MFC) is responsible for processing eDNA samples since the FWS assumed responsibility for monitoring Bighead and Silver Carp in the Chicago Area Waterway System (CAWS) and other water bodies in 2013. This version of the QAPP has been modified to be a guide to field and laboratory methods that should be employed during eDNA monitoring programs. Specific sampling plans and schedules for particular water bodies will be developed by regional sampling agencies and their partners, as well as overseeing governing bodies (such as the Invasive Carp Regional Coordinating Committee and Great Lakes Fishery Commission).

A6. Project History

The University of Notre Dame, Department of Biological Sciences, Center for Aquatic Conservation, prepared a Standard Operating Procedure (SOP) in 2010. The SOP provided details regarding eDNA monitoring protocols and was given to USACE in May 2010 in compliance with Cooperative Ecosystem Study Unit agreement #W912HZ-08-2-0014, modification P00007. In December 2009, a technical and quality systems audit of the Center for Aquatic Conservation Lodge Laboratory at the University of Notre Dame was conducted by the US Environmental Protection Agency (EPA). The laboratory audit report dated 5 February 2010 was provided to USACE in addition to the eDNA monitoring protocol. These documents served as the basis for the QAPP followed by ERDC. Additionally, USACE submitted the eDNA methodology for an Independent External Peer Review (IEPR), which is a requirement to examine decision documents and supporting work products where there are public safety concerns, significant controversy, a high level of complexity, or significant economic, environmental, and social effects to the nation. Released fall of 2011, the eDNA IEPR report, conducted by objective panelists with technical expertise in genetics and population

ecology, confirmed eDNA sampling and testing methodology is sound for detecting Silver and Bighead Carp eDNA but cannot indicate the source of Bighead and Silver Carp eDNA. In addition, eDNA detection results cannot provide information on the size, sex, age, or number of individuals present and cannot distinguish between pure Silver or Bighead carp and their hybrids. In 2013, FWS WGL took over eDNA monitoring activities from the USACE and made changes to the QAPP to adapt to a high throughput processing required of a large-scale monitoring program.

A7. Objective

The specific goals and objectives of eDNA sampling plans for specific water bodies are currently created and directed by their governing bodies. For example, the CAWS sampling plan is created by FWS Midwest and Northeast Regions in cooperation with the Invasive Carp Regional Coordinating Committee's Monitoring and Response Workgroup and can be found in the Monitoring and Response Plan. Similarly, the Great Lakes, Ohio River, and Upper Mississippi River eDNA sampling plans are created by the FWS Midwest and Northeast Regions in cooperation with state and other partners. The objective of this QAPP is to provide detailed procedures for Bighead and Silver Carp eDNA sample collection, sample processing, data analysis and interpretation, result reporting, and QA/QC procedures to ensure that data are as technically defensible, consistent, and usable as possible. The FWS Midwest and Northeast Regions will continue to maintain and update the QAPP for use in eDNA monitoring programs that allows for inclusion of any beneficial technical or strategic modifications that become apparent from past monitoring events, research, and/or relevant published literature vetted by FWS Midwest and Northeast Regions Fisheries and Aquatic Conservation (FAC) staff. Any eDNA samples collected and processed that do not follow the QAPP cannot be directly compared to FWS monitoring results. Therefore, the procedures outlined in the QAPP should be followed for eDNA monitoring and/or early detection of Silver and Bighead Carp by agencies and institutions that would like to compare their eDNA detection results to FWS results.

A8. Special Training and Certifications

To successfully complete eDNA monitoring, project personnel must complete certain special and/or non-routine training. Training may include Field Maps App, Survey123, field sample collection/processing, trailer towing, boat operation, DNA extraction and PCR analysis competency. Station eDNA Leads are responsible for field staff getting the proper training to conduct eDNA monitoring sample collection (Exhibit 2). The WGL Project Leader is responsible for WGL staff getting the proper training to analyze samples. The eDNA Processing QA/QC Specialist is responsible for collecting all training and documentation (Exhibit 3). All training documents will be held on the eDNA SharePoint site.

Personnel Training Requirements

Minimum training and/or experience requirements for the different components of the eDNA monitoring protocol are detailed in Exhibit 2 and 3. All agencies need to submit documentation of proposed staff member training to the eDNA Program Coordinator. Station eDNA Leads will be responsible for documenting and providing training to FWCO staff and training documentation will be housed on the eDNA SharePoint site. FWCO training will include:

- R3 eDNA Program Sample Collection
- R3 eDNA Program Sample Shipment
- R3 eDNA Program Decontamination
- R3 eDNA Program Sample Preservation
- ArcGIS Field Maps APP Manual No 1
- ArcGIS Pro eDNA Editing Manual No 2
- Survey 123 Chain of Custody and Post-Trip & QA/QC Summary Creation/Submission SOP No 3
- IC eDNA Program Data Management SOP No 5

The WGL Project Leader is responsible for documenting and providing training to WGL staff and training documentation will be housed on the eDNA SharePoint site. WGL training will include:

- R3 eDNA Program Preservative Decanting and Evaporation
- Survey 123 Chain of Custody and Post-Trip & QA/QC Summary Creation/Submission SOP No 3
- WGL IC eDNA Program Sample Processing SOP No 4
- IC eDNA Program Data Management SOP No 5

All forms of documentation may be provided hard copy or in electronic format to the eDNA Program Coordinator or kept on the eDNA SharePoint site.

Boat Operator

Must meet FWS boat operator requirements. All FWS field staff that are serving as boat operators in the IC eDNA Monitoring Program are required to take Motorboat Operator Certification Course (MOCC) and those records will be kept on station.

A9. Documents and Records

The eDNA Program Coordinator and the eDNA Processing QA/QC Specialist are responsible for making sure that all project personnel receive the most recently approved QAPP, training certification, protocols and other supporting documents that are used throughout the project duration. The QAPP, Field Maps App Manual and Survey123 COC documents will be updated annually, and this information will be communicated before the start of each sampling season. A record of the updates to each document will be kept on both the MFC Server and the IC eDNA Monitoring Program SharePoint site for the duration of the IC eDNA Monitoring Program. Retention and disposition of some records may be regulated.

QAPP Maintenance and Modifications

The QAPP will be reviewed annually by all IC eDNA Monitoring Program staff. Technical staff are especially important due to the continual advancement of eDNA techniques and methodologies being developed. New approaches should be evaluated and applied to monitoring efforts to improve the use of the tool in management applications and to increase efficiency. The following technical areas will be reviewed annually:

- The FWS eDNA monitoring personnel will keep up with current research through agency publications and peer reviewed literature.
- The FWS will work closely across Fish Technology Centers and with collaborating agencies such as the US Geological Survey and the USACE to drive scientific advancements in eDNA technology.
- The QAPP will be updated annually to reflect advancements in eDNA research as well as to improve efficiency in field and lab efforts based on lessons learned from the previous year of implementation.
    - These changes are suggested by Program staff, and reviewed by the eDNA Leads, and upon approval, implemented into the QAPP.
    - None of the changes presented will reduce the level of rigor or quality control measures.

Records Retention

All copies of case files, reports, electronic copies of data, analysis files are considered permanent Project Records (NCI-22-78-1/37) of FWS Combined Disposition Manual. Records are maintained at the project management level and present a complete picture of each project from initiation to termination. Once completed, these records should be separated in 1 year file sets by the eDNA Processing QA/QC Specialist and transferred to Federal Records Center when 5 years old. At 10 years, records should be offered to

National Archives and Records Administration (NARA). Considering eDNA monitoring is an ongoing project, record sets will be kept on station at MFC in a secure location, then transferred to NARA when each sampling year reaches 10 years.

Section B. Data Generation, Acquisition and Analysis

B1. Sampling Design and Planning

Sample design will encompass all considerations needed for site selection, equipment and specific procedures for collecting and shipping samples.

Case Number Assignment and Management

Sampling priority will be finalized by the eDNA Program Coordinator, FWS Regional Office, and partner personnel. The WGL eDNA Processing Lead will create a master case list each season and assign case numbers as needed to each FWCO. Each sampling event is centered around a specific water body and will be assigned a case number consisting of a 5-digit integer with the year samples are collected as the first two digits (YY000). Sample ID numbers within cases will be in consecutive numerical order and will consist of a 3-digit integer (001 to 999). Each sample will be identified by the 8-digit number consisting of the case **and** 3-digit sample ID number, without spaces or punctuation (ex 22001001) which results in the regional unique ID (RUID). The 8-digit RUID will be used in the field, the file Geodatabase, and in the results report. However, the laboratory will use a simplified version of the RUID consisting of the 5-digit case ID number and a one-to-three-digit sample ID number (ex 1 instead of 001). Lab processing priority (which may differ from sampling priority) will be finalized by the eDNA Program Coordinator and appropriate partner personnel. All eDNA field and lab generated data will be collated by the eDNA Data Steward and housed in a file Geodatabase maintained in Denver, CO. The database is organized around the RUID for each individual sample; thus a unique identifier is required for each eDNA sample.

Sampling Documents

Prior to any field sampling work, all field employees must review the QAPP and acknowledge procedures and processes to be followed for every sample and every event. Field employees will acknowledge their understanding and intent to comply by signing training certification form given as Exhibit 2. A general and holistic annual sampling plan for each basin (ex Ohio River, Lake Erie, etc) will be developed prior to the start of each field season with consultation from state partners, and oversight from the WGL eDNA Processing Lead and the eDNA Program Coordinator. An event-specific, pre-sampling plan (Exhibit 1) will be drafted for each sampling event by the Station eDNA Lead (or designee) of the station leading the event. This pre-sampling Exhibit will contain all logistic and personnel information pertaining to that individual collection event. All field staff participating in a sample collection event must read and become familiar with the Exhibit 1 pertaining to that event. After each trip, a Post-Trip & QA/QC Summary Survey123 Form covering quality assurance issues and any changes in personnel roles should be generated by the Station eDNA Lead (or designee). A designee can be assigned under extenuating circumstances, one temporary designee can be assigned by the eDNA Station Lead when the eDNA Station Lead is unavailable to perform their designated role and responsibility. The designee is responsible for taking all required training to perform the eDNA Station Leads role.

Sample Design and Site Selection

When planning eDNA sampling events, accurate and thoughtful planning should go into determining where, when, and how many samples to collect in a waterbody of interest. Collaboration with a statistician is encouraged. Effective planning allows the crew to collect and process samples efficiently and ensure that the data collected are sufficient to address the objectives of the eDNA program or project. All available data regarding biology, life history and habitat use of Bighead and Silver Carp in a particular system should be considered during the planning process. Additionally, site reconnaissance and/or aerial imagery should be utilized when selecting sites in unfamiliar areas to determine suitability for sampling.

eDNA Sampling for Early Detection vs Monitoring Programs

The goals and objectives for any eDNA project should be clearly defined prior to the sample design process. It is important to state if eDNA collection is for early detection of Bighead and Silver Carp or monitoring of Bighead and Silver Carp populations. The objectives of a project and subsequent sampling design will differ based on this distinction. Early detection refers to the detection of relatively few individuals newly inhabiting an area, potentially leading to establishment. Early detection sampling will likely occur well ahead of any established, self-sustaining Invasive carp population front. Monitoring refers to the surveillance of an established population front and sampling for this purpose will likely occur much closer to the established population in a transition area where Invasive carp are dense below and scarce above. Both types of programs involve repeated, long-term data collection in order to observe developing trends and changes in the occurrence of Invasive carp. This determination should be made through a collaborative discussion between eDNA Station Leads, FWCO Project Leaders, the WGL eDNA Processing Lead, the eDNA Program Coordinator, and the appropriate state partner representatives.

eDNA Sampling in Lentic vs Lotic Systems

Consideration should be made during the sample design process based on the nature of the waterbody that is being targeted for eDNA sampling. Lentic systems are typically referred to as lake-like, non- flowing bodies of water that are commonly isolated or have limited connectivity to flowing water. Lotic systems are those, such as rivers, that maintain some level of flow at all times. The intensity of the flow can fluctuate dramatically within lotic systems, and rates of flow between systems can vary. Depending on the spatial scale being considered, a system can contain areas with lentic qualities and areas with lotic qualities (e.g. a backwater of the Mississippi River is a more lentic-like area, but is part of a larger lotic system). The difference in the hydrodynamic forces between lentic and lotic systems may affect the ability to detect eDNA. The following are general considerations, and any recommendation made may not exactly apply to all systems.

Due to the flow-through nature of lotic systems, the residence time of a particle of eDNA in any one location may be reduced, as the movement of the water transports eDNA downstream, compared to a non-flowing lentic system (Stoeckle et al. 2017). Additionally, the volume of water contained in an isolated lentic system is less likely to drastically change compared to a lotic system that is heavily influenced by rain events and annual flooding. Increased water volume at certain times of the year may dilute eDNA signal and decrease detection probability.

When sampling in lentic systems, like lakes or bays, sampling should take place in a uniform fashion. Ideally, the entire lake would be sampled with individual sampling points arranged in a uniformly spaced grid. However, on exceptionally large lakes where sampling the entirety is not feasible, sampling should target areas where eDNA may be likely to accumulate, such as along shorelines or marinas. Because areas will be revisited and re-sampled over time to maintain a long-term dataset, it is important that the design of sampling be repeatable in subsequent months or years. While it is recommended to work with a quantitative scientist to optimize eDNA sampling for each specific system, general minimum sampling density recommendations when eDNA sampling for early detection are based upon the 2018 field optimization study conducted on the Upper Mississippi River for the detection of Bighead and Silver Carp (Mize et al. 2019). The study recommended that, for the UMR, under ideal sampling conditions (ex cool water, negligible flow, known Invasive carp presence), a sampling density of 1 sample per 0.01 km^2 with a minimum of 40 samples was appropriate to detect Bighead and Silver Carp DNA. Most areas in the FWS eDNA monitoring program are sampled for early detection purposes and present conditions that are unideal for Bighead and Silver Carp eDNA detections. The lack of information and data about Bighead and Silver Carp presence or behavior in the system, and the presence of Invasive carp in these systems is assumed to be extremely rare or nonexistent. Therefore, in early detection focused areas, or areas where little to no information is available about the

presence or behavior of Bighead or Silver Carp, the minimal sampling recommendation is 1 sample per 0.01 km^2 with a minimum of 80 samples collected at each study site. This is based on the assumption that positive detections will be representative of a population of Bighead and Silver Carp that is at least 1 order of magnitude smaller in size than the population estimated in the site studied in Mize et al. (2019). If the goal is to detect a smaller population, sampling density will need to be either increased or managers may elect to conduct consistent sampling over time and should assume that it will take a longer period of time before the population of Bighead or Silver Carp are large enough to detect with this sampling regiment. The best practice is to use continual feedback, from optimization efforts and tight collaboration with quantitative scientists, in order to adaptively adjust sampling sizes to increase the probability of detecting Bighead and Silver Carp eDNA.

When sampling lotic systems, like streams and rivers, sampling in the thalweg or other high flow areas, especially in large rivers should be avoided, when possible, except areas downstream of bridge pilings. Materials become trapped in the turbulence of these areas and DNA has been detected in these areas. In general, DNA in these areas is much more likely to be flushed downstream rapidly, lowering detection probability. Instead, sampling should target off-channel areas where eDNA is most probable to accumulate and persist, such as (but not limited to): backwaters, eddies, bays, and marinas. Minimum sampling recommendations for off-channel areas like these is similar to the sampling of lentic areas as long as they present similar characteristics including negligible flow, little connectivity to flowing water, etc. Other areas to target may include smaller eddies around structures like piers and docks, or impounded areas above dams. Juvenile and age-0 Bighead and Silver Carp prefer shallow, productive areas such as those adjacent to, and within wetlands. Areas to be considered for sample collection also include those with locally high productivity, such as water treatment effluents or areas that may be seasonally attractive to Bighead or Silver Carp such as warm water effluents in cold water. If there are no off-channel areas (with lentic-like characteristics and low- flow) to target, samples should be collected from shorelines, particularly those in side channels or along depositional banks. If shorelines are used, total sample number should be increased to compensate for lower detection probabilities and care should be taken to avoid sampling during high flow conditions. At this time there are no specific minimum sampling recommendations for flowing areas, but as a particular site is repeatedly sampled over time minimum sampling efforts can be determined using statistical models (Erickson et al. 2019). These recommendations can then be adaptively managed and changed over time to optimize sampling for a specific site or system (Erickson et al. 2019).

With any sampling, it is important to consider secondary vectors of eDNA in the water, such as birds, sewer outflows, urban influences, and barges. It is not recommended to sample under or downstream of any of the previous mentioned situations.

Pre-Trip Planning and Logistics

1. File pre-sampling plan (Exhibit 1).
2. Review ArcGIS Field Maps App Manual No 1 for eDNA Monitoring of Bighead and Silver Carp to ensure ArcGIS online accounts are set-up and offline maps are downloaded.

Spatial planning

1. Use of aerial maps detailed enough to show unique features (e.g., barge slips, factory, bridge pilings etc) that can be identified in the field and used as markers for location when sampling.
2. Aerial maps should be marked with sample locations and should ensure spatial coverage and overall representativeness of the sample area.
3. Print map(s) with detailed sample plan.
4. Locate access points for boat launch and acquire permission to use if necessary. If sampling around locks, or if sampling will require lockage, notify the Lockmaster at least 1 day before

sampling.
5. Coordinate sample plan with sampling crew, eDNA Program Coordinator, eDNA Data Steward, or any partner agency.

Temporal Planning

1. Biology and ecology of Bighead and Silver Carp should be considered when timing sampling events throughout the year.
2. Time sampling events to target low water, cooler temperatures and/or times and places where fish congregate to increase detection probability. While spawning may release gametes, high water events that trigger spawning also dilute eDNA and transport it rapidly downstream. Water temperatures above 25 °C cause faster degradation of DNA, however fish metabolism is also higher in warm vs cold water. Consideration should be given to all these factors when determining when to sample (Curtis et al. 2020; Van Driessche et al. 2022; Xiong et al. 2022).
3. If timing of sampling cannot be optimized around fish biology and environmental conditions, then consideration should be given to determine the effectiveness of sampling. If sampling is to proceed, then larger sample sizes will be necessary to overcome lower detection probability in high flow, warm water conditions, or low fish activity (ex during cold water conditions).

General Planning

1. Sampling crews should be a minimum of three people: a field sampling collector, field sampling collector assistant, one boat operator and one of which will also serve as the field sample collector QA/QC Specialist.
2. Processing crews are recommended to consist of two people; the two people being sample processors, one of which will also serve as the field processing QA/QC Specialist.
3. Names of personnel assigned to each of these field positions will be documented in Exhibit 1. All training and documentation listed in previous sections should be taken into account during this stage.
4. River stage and weather forecast should be checked and considered prior to and throughout sampling.
5. In the event of significant rainfall, the Station eDNA Lead (or designee) must assess the situation and determine if sampling should be pursued. The first consideration is crew safety. If it is determined that sampling can occur safely, sampling may be pursued. The sampling crew, FWCO Project Leader and eDNA Program Coordinator need to discuss the effects of high water on the ability to detect eDNA, because increased runoff will dilute the signal or transport it too rapidly downstream, and thus decrease the chances of detecting target DNA. This call must be made on a case-by-case basis. If sampling must occur, sample size should be increased. In general, and especially in the CAWS, sampling should be avoided within 7 days of a combined sewer overflow (CSO) event, due to adverse health effects from raw sewage. Weather data and river stage for the area to be sampled can be checked at: http://waterdata.usgs.gov/nwis.
6. Ensure that the designated data recorders for the field crew and processing trailer have read and understood the ArcGIS Field Maps App Manual No 1 for eDNA Monitoring of Bighead and Silver Carp. All participating offices have been provided with a digital and hard copy of the manual. A copy of the manual can be requested from Ross Ruehmann by email ross_ruehmann@fws.gov.

Equipment Preparation

To perform laboratory molecular analyses to detect eDNA, vessels and equipment must be decontaminated in accordance with the following protocols to eliminate introduction of outside DNA sources in the sampling regime. Bleach will only decontaminate clean surfaces, so ensure that all surfaces are well cleaned first to completely remove any film or biological build up so the bleach can destroy any potential contamination. Water used for bleach solutions may be any source of clean tap

water. Water used to rinse equipment, such as the centrifuge buckets and other processing equipment should also be from a clean source to avoid introducing any inhibitors to the samples.

Cautions: Precautions should be made to avoid direct skin contact with bleach; bleach solution may also stain clothing or other materials. Be aware of pollutants in the aquatic environment and related health hazards.

Cooler Decontamination and Sample Labelling

- Remove mud and other biological residues from surfaces by rinsing and scrubbing. Equipment surfaces must be free of debris and other material before decontaminating with bleach.
- Mix a bleach solution (10 or 20%) with water in a 3-gallon low-pressure sprayer that is dedicated to the project. The bleach solution must be prepared immediately prior to use, and each time decontamination activities will be occurring. Use caution if spraying 20% bleach to avoid creating an inhalation hazard.
- Sample transport coolers will be decontaminated with freshly made bleach solution in the low pressure sprayer. Use the low-pressure sprayer to thoroughly cover the inside and outside surfaces. For 10% bleach, allow 10 minutes of contact time before rinsing with water. A 20% bleach solution only needs to sit for 10 seconds before rinsing. Coolers may be left to air dry, or dried using clean paper towels while wearing clean gloves.
- Purchase sterile, chemical-free disposable 50-ml polypropylene tubes with maximum relative centrifugal force (RCF) of at least 6,000 rpms. Even sterile tubes can still have traces of the chemical used to free the plastic tubes from the metal forms during production. Some manufacturers sell sterile tubes that do not have this residual chemical in them, and these should be used to avoid introducing PCR-inhibitors from the sample tube.
- Sample tube labels can be printed by the sampling agency. Sample tubes will be labeled with the RUID (Section B1 for case and sample numbering) for each individual sample. Labels will be printed on Rite-N-Rain® or some type of waterproof labels and affixed to the outside of the sample tubes. It is also helpful in most cases to write the 1-3 digit sample number on the cap with a permanent marker for easier identification.
- Once tubes have been labeled, they will be placed in an appropriate transport vessel (e.g. clean cooler or box). Decontaminated coolers, described above, should be used to transport samples to and from the boat, however cardboard boxes or bags may be used to transport samples in a vehicle if there are more samples than the available clean coolers can handle. Samples must be contained in clean coolers when being taken on the water for sample collection.
- A minimum of 10% of the number of samples collected should be field blanks (control sample). Blanks may be filled with any city-provided tap water, see section B5. Quality Control Field for instructions on filling blanks. If water source is a well or you are unsure of the source, use distilled or deionized water. Note, if the sampling design for your particular body of water requires a specific sample size in order to meet a precise detection probability, then containers for control samples to meet the 10% will be added to the total number of sample containers dictated by your sampling plan. Blank samples should be distributed evenly among regular samples so that a blank sample is encountered about every 10 regular samples.

B2. Motorized Sample Collection

In order to perform laboratory molecular analyses to detect eDNA, samples must first be collected from the appropriate aquatic environment in accordance with the following protocols. Gear (such as personal flotational devices (PFD) and hats) should be decontaminated prior to and between sampling events according to section B2. However, if any gear is compromised during sampling (accidentally falling in, or a spill, etc.) and staff feel that it may contribute to contamination of the samples, the gear should be

decontaminated as soon as possible at the end of the workday or replaced with new gear as soon as possible.

Field Equipment Needed

- Waterbody-appropriate vessel. If possible, vessel and associated equipment should be dedicated to eDNA collections to minimize risk of DNA contamination of samples from other sources. If boat dedication is not possible, or eDNA collection occurred in known carp positive waters, procedures for decontaminating boats and equipment are included below. These procedures will also work for converting a vessel previously used for non-eDNA work (including work in carp waters) to a dedicated or temporary eDNA sample collection vessel. It should be common practice to decontaminate vessels, regardless of whether or not they are dedicated equipment or where sampling occurs, prior to each sampling event.
- Dedicated eDNA PFD and rain gear for each crew member should be available for each vessel. A minimum of one Type II PFD is required for each person on board the vessel and one Type IV throwable device per vessel, unless otherwise noted by Coast Guard or individual station policy (e.g. Type I is required within the Safety Zone near the existing electric barrier on the CSSC).
- It may be necessary to sample eDNA in carp-infested waters. In this case, crews should carefully consider whether or not to use clean dedicated eDNA gear and personal protective equipment. It may be prudent to avoid exposing clean eDNA gear to carp- infested waters and instead other "relatively" clean gear could be used instead. All gear used, regardless of dedicated or not, must be thoroughly disinfected following procedures described in Section B (Decontamination of Boat and Field Equipment Procedure) before and after each use for eDNA sampling. If there is uncertainty or questions, call the eDNA Program Coordinator.
- Sufficient 100-qt coolers capable of holding 5 50-ml centrifuge tubes per planned field sample with one additional empty cooler to store the first set of collected samples.
- Sterile, chemical-free polypropylene 50-ml centrifuge tubes that are rated to withstand 6000 rpms, labeled by sampling agency. Tubes will not be reused, but WGL will return 50 mL tube racks to be reused by sampling agencies if requested.
- Additional tubes in case tubes are dropped in the field and the sample tube needs to be replaced.
- Pre-printed, waterproof labels for centrifuge tubes (Rite-In-The-Rain or Avery laser white weatherproof address labels #5520).
- 3-gallon sprayer (low pressure hand sprayer for spraying down boats in the field). Car washing brushes or mops may be used in lieu of low pressure hand sprayer for chemical decontaminant application.
- Habitat measurement equipment (Global Positioning System [GPS], Digital Depth Sounder).
- Pre-Sampling Plan (Exhibit 1).
- Charged I-Pad inside a LifeProof case or similar waterproof cover equipped with ArcGIS Field Maps APP and Survey123 App.
- Charger or battery backup for iPad.
- Charged GNSS Receiver inside a clear waterproof bag or container.
- Sharpie® permanent marker in black.
- Powderless nitrile or latex gloves.
- Ice for coolers with samples.
- Bleach (concentrated) or Virkon Aquatic
- Bleach may be mixed in two strengths: 10% solutions require 10-minute contact time or 20% solutions require 10-second contact time (and require extra caution when spraying).
- The oxidative feature of bleach deteriorates with exposure to organic material and over time, so bleach solutions must be made fresh daily in order to decontaminate DNA.

- Virkon must be applied in a 2% solution for DNA decontamination or reduction methods. Equipment must be fully submersed in a 2% Virkon solution for 30 minutes for complete decontamination, however metal surfaces subject to corrosion should not be soaked longer than 10 minutes. In the field, where submersion may not be possible, a 2% Virkon spray or swab application for a 10-minute contact will reduce DNA to negligible levels. Virkon mixed in solution is good for one week.
- Water sources: water used to mix bleach or Virkon solutions can be any source of well or city water, it does not have to be DI water. Well or city water may be used to wipe down surfaces or to rinse equipment after bleach or other disinfectant application.
- Garbage bags.
- Paper towels.
- DNAway.
- Tube rack for dipping samples into target waterbody.
- Bleach bath container for disinfecting tube rack between samples.
- Small container for holding tube caps.
- Copy of QAPP and Field Maps App manual.
- Proper clean personal gear for the weather.
- Cell phone or other communications device.

Decontamination of Boat and Field Equipment Procedure

This section applies to all motorized or hand-powered boats, paddles, and any associated field equipment used to collect eDNA.

A set of field equipment used by staff collecting eDNA such as personal flotation devices, rain gear, hats, sunglasses, etc., should be dedicated for eDNA field work only, to avoid contamination risks. This is especially important if the same staff are involved in field work in rivers with well-established populations of Bighead and Silver Carp, or if staff conduct field work where they come into direct contact with Bighead and Silver Carp. This dedicated gear should be stored and transported in designated containers (such as totes) so that contamination from trucks or boats contaminated with Bighead and Silver Carp DNA is avoided. After the trip, gear and transport containers should be decontaminated according to the section Cooler Decontamination and Sample Labeling above.

It is preferable to have designated vessels, trailers, and trucks set aside for eDNA work if possible. Even if there is designated equipment, because eDNA can be moved among sites on vessels, boats and equipment must be decontaminated prior to sampling, and between sampling sites. If complete decontamination cannot be performed between cases while in the field, choose the most preferred method of DNA reduction available in between cases (Appendix B).

Upon return to the field station office, a complete DNA decontamination for all associated equipment must be performed before returning to the field. Follow steps 1-8 for decontamination or reduction of DNA on equipment surfaces before and between sampling events. Refer to Appendix B for a list of recommended decontaminants. Use personal protective equipment (PPE) and read Safety Data Sheet (SDS) before use of any product. Follow equipment safety instructions and read equipment manual before using an industrial hot water pressure washer:

1. Put on appropriate PPE. Decontamination PPE should be designated, stored separately and decontaminated after each use to prevent reintroduction of DNA to equipment, and transfer of splashed DNA around your facility.
2. Remove equipment from boats, trucks, etc. and lay them out separately so that all surfaces of

equipment will be exposed to treatment. It may be necessary to treat one side and then flip.

3. Rinse surfaces with the highest water pressure available for the location. Surfaces must be clean for decontaminant/DNA reducers to work. Remove any environmental debris such as plant material, mud, or fish slime with brushes or gloves. This may need to be done with an initial high-pressure spray if one is available, or if possible, perform this step at the sampling site to leave as much DNA material behind as possible. Buckets and brushes or a water pump can be used at the boat ramp to help rinse boat surfaces of blood and slime before leaving the water.
4. Choose a decontamination method that is appropriate for the equipment, location and services available (Proceed to steps 5, 6, 7 or 8). If equipment has been exposed to Bighead and Silver Carp DNA use one of the high-pressure sprayer methods ***in conjunction*** with one of the chemical methods (steps A or B ***and*** C or D or E) for decontamination. If equipment has been used in a non-carp area, it is highly recommended to use a minimum of one method (high pressure or chemical) to clean. Be sure to thoroughly rinse after chemical application:
    a. Use an industrial hot water pressure washer set at 212 °F to decontaminate appropriate surfaces. Minimum exposure time for decontamination is 10 seconds.
    b. Apply detergent at low pressure to saturate surfaces with an industrial cold water pressure sprayer with detergent injector. Wait 3 to 5 minutes, then rinse at high pressure for 10 seconds.
    c. Mix a 10-20% solution of concentrated household bleach (6-8% sodium hypochlorite minimum before mixing) in tap water in a hand pressure sprayer (low pressure saturation). Spray or swab to saturate at low pressure or immerse all appropriate surfaces. Exposure time for complete decontamination is 10 minutes for 10% and 10 seconds for 20%. Rinse with fresh water and allow surfaces to dry. The mixed solution is good for one day.
    d. As an alternative to step C for smaller items, prepare a 20% bleach solution in a small tub and completely immerse items for 10 seconds. Rinse and allow surfaces to dry.
    e. Mix a 2% Virkon and water solution and immerse smaller equipment in a bath for 30 minutes. Metals should be immersed no longer than 10 minutes. For larger equipment, prepare a 2% Virkon solution in a low-pressure sprayer or swab and saturate surfaces. Minimum exposure time is 10 minutes. Rinse with fresh water and allow surfaces to dry. Caution: Do not aerosolize this product. Use at the largest droplet setting to avoid respiratory exposure. The mixed solution good for one week.
5. NOTE: In absence of availability of methods for decontamination previously mentioned in this document, rinse equipment with copious amounts of water at the highest pressure available and allow to dry. Exposure to the sun or UV radiation and heat will help reduce residual DNA. Follow complete decontamination procedures beginning at step 1 upon return to field station office or at first availability.
6. It is preferable to have a designated vessel, processing trailer, truck, and associated field equipment set aside for eDNA work if possible. This also includes PFDs, boots, and rain gear. Even if there is designated equipment, it must be decontaminated prior to sampling and between cases.
7. It is also important to fully decontaminate all gear and equipment between sampling of different cases to mitigate risk for cross contamination.
8. Use DNAway and paper towels to decontaminate pens, hats, notebook surfaces, electronic equipment surfaces, truck interior and other non-saturable equipment.
9. Contain any equipment that was not treated in bags or totes for later decontamination.
10. Wash hands, launder or change soiled clothing.
11. Remove PPE and decontaminate before storing separately for next use.

B2. Sample Collection Procedure

Note: Centrifuge samples for early detection and monitoring are comprised of 5 replicate 50-mL tubes, which may be collected at once by using a tube rack to hold and dip all five tubes at once. Field blanks will be comprised of one, pre-filled 50-mL tube. The following procedure assumes this design.

1. Prior to launch, crew members will have reviewed this QAPP, will have been trained and will understand their assigned roles in the sample collection procedure. All sample identification information and other field logistics will be recorded on the pre-sampling summary (Exhibit 1).
2. Prior to launch each day, the hull of the boat or vessel will be sprayed with a prepared bleach (10% or 20%) or 2% Virkon solution. 10% bleach or 2% Virkon will need 10 minutes of contact time and 20% will need 10 seconds of contact time. It will be allowed the appropriate contact time for the solution strength then launched into the water which will provide the necessary rinsing.
3. Vessel will be launched from an appropriate area that allows access to the reaches to be sampled, preferably downstream of the target sampling reach in lotic systems.
4. Sampling will commence at the first site located at the downstream end of the reach to be sampled and will proceed in an upstream direction. This is done in order to minimize water disturbance caused by the vessel's wake within the sample reach. The only exception to this protocol is when the boat must be launched upstream of the sampling reach. If the vessel can travel to the downstream end of the sampling site without driving through it, then do so and begin sampling at the most downstream point, working upstream. If driving through the site cannot be avoided or conducted with minimal disturbance, then sampling will commence at the first site located at the upstream end of the reach to be sampled and will proceed in a downstream direction. The direction traveled for sampling should be recorded on the pre-sampling plan (Exhibit 1) and any deviations that occur while in the field should be noted and justified on the Post-Trip & QA/QC Summary Survey123 Form upon event completion.
5. It is imperative to avoid disturbing sediments and avoid collecting samples where the sediments have been stirred up. Sediments add to the inhibition load of the sample and could increase the rate of false negative results for either filtered or centrifuged samples. If sediments are accidentally disturbed by the boat motor, it is required that the driver, re-position the boat in a new area without disturbing sediments. The data recorder must make a note on the data sheet and record actual GPS coordinates where the sample was taken. When collecting samples near the bank, be observant of wake-disturbed surfaces. To compensate for the wake created by a passing boat, samples may need to be collected 2–3 ft off the bank to obtain displaced surface film and avoid turbid water.
6. Mix a small 20% bleach bath in a container with enough depth to fully submerge the tube rack used for dipping samples. Tube rack must be decontaminated between each sample collected by submerging it in the bleach bath for 10 seconds then rinsing in the target waterbody at each sample point.
7. When first arriving at a sample location, the lead sampler and sampling assistant will put on new gloves (powderless latex or nitrile). **Gloves must be changed by the lead sampler before each new sample is taken to prevent cross contamination. The same gloves may be worn when collecting blank samples in tandem with a regular sample in a site.**
8. Going in consecutive numerical order based on the tube labels, the sampling assistant will remove a set of labeled sample tubes (5 50-mL tubes per actual sample) from the clean sample cooler.
9. Sampling assistant will remove caps from the sample tubes and either hold the caps in a clean gloved hand or place them in a clean container. Do not under any circumstances touch the interior of a sample container, even with a clean glove.
10. At the same time the lead sampler will remove the tube rack from the bleach bath and rinse in the

waterbody

11. Sampling assistant will then place the un-capped tubes in the rack, which is held by the lead sampler, taking care not to touch the rack or the gloves of the lead sampler. **If the sampling assistant touches a contaminated surface, they must immediately change gloves.**
12. Lead sampler will then reach over the upstream side or the bow of the transport vessel with the rack of uncapped sample tubes and fill the tubes by skimming the surface of the water. The sample container must not be submerged or dipped beyond the upper 2 inches of the surface water for sample collection, since the intent of the sampling is to collect floating organic matter that is on the water surface. Samplers should avoid collecting large organic debris such as twigs, leaves, seeds, etc., because they cause problems in extraction, however a small amount of duckweed is fine. Tubes should be filled all the way, there is no reason to measure water, and as long as all tubes are filled the same, they will be safe to use in the centrifuge. To avoid contamination, the individual collecting the sample should avoid touching any other surfaces with the clean gloves (ex the gunwale) and should only handle the sample tubes and caps.
13. Field blanks (single, 50-mL tubes filled with tap water prior to trip) will be encountered throughout the course of sampling. They will be numbered similar to actual samples but only be comprised of a single 50-mL tube each. When a blank is encountered it should be opened to expose the container contents to the atmosphere for 10 seconds, and resealed. Then it should be placed in the rack with the 5 uncapped sample tubes and fully submerged while the actual sample is being collected. Blanks are taken in tandem with the next actual sample and do not replace a sample in that location. In other words, the boat will remain at the same location for the blank and the corresponding actual sample.
14. While the water sample is being collected by the lead sampler, the sampling assistant will record habitat measurements and sample data in the Field Maps App.
15. Once the sample tubes are filled, the sampling assistant will place caps into the lead sampler's gloved hand or hand them caps one by one without touching their gloves.
16. Lead sampler will screw the tube caps back on, making sure they are tight and not cross-threaded. The capped tubes will then be placed in numerical order in a rack. When the rack is full, place in a cooler. NOTE: Lead sample is considered to be contaminated while sampling and should be the only one placing samples into the cooler.
17. The Lead sampler will submerge the dirty tube rack in the 20% bleach bath for 10 seconds.
18. Steps 1 through 17 will be repeated at each sampling location until sampling has been completed for the targeted reach.
19. Once a cooler is full, add ice to completely cover all containers. Replace ice as it melts, removing excess water only as needed, since ice water will provide better cooling than ice alone.
20. Survey123 COC forms will be completed for every sample and every cooler. All samples, including field blanks, will be logged onto Survey123 COC forms. The forms will be collected and signed whenever the coolers are transferred between parties.

B2. Sample Processing/Centrifugation

In order to isolate eDNA from water samples collected in the field, particulate matter must be concentrated. Centrifugation of water samples collects matter at the bottom of the tubes, which can be collected on sterile cotton swabs and eDNA can be extracted from the swab. Validation of this method has shown that a maximum of five tubes can be swabbed with a single swab.

Water samples collected in the field need to be centrifuged and preserved with 95% non-denatured ethanol or 70-99% isopropyl alcohol within 24 hours after field collection ends. Samples MUST be stored in the dark and on ice while being stored during the 24-hour period (Lance et al. 2017 and Pilliod et al. 2014). If samples are to exceed the 24-hour period, field staff MUST notify the eDNA Program Coordinator.

Samples will likely not be further processed if the 24-hour period is exceeded.

Processing Equipment:

- Sterile, chemical-free centrifuge tubes (Midwest Scientific #TP91050) made of polypropylene that can withstand 6000 rpms with caps and labels (recommended Rite-In-The-Rain or Avery laser white weatherproof address labels #5520)
- Paper towels
- 95% ethanol (non-denatured) or 70-99% isopropyl alcohol (mix prior to trip with purchased molecular grade water). It is **IMPERATIVE** only pure ethyl or isopropyl alcohols are used and diluted with molecular grade water. There can be **NO** methanol in the mix, as methanol damages DNA so that it cannot be amplified in PCR or sequenced.
- 2 reusable bottle-top re-pipettor for dispensing alcohol
- Black permanent markers (e.g., Sharpie®)
- Powderless latex or nitrile gloves
- Refrigerated centrifuge(s) with rotors and adaptors for 50-ml tubes (centrifuges that have refrigeration and hold 50 mL tubes and have a rotor with max speed of ~4200-5000 rpm)
- Bleach
- DNAway
- Dedicated lab equipment cleaning sink
- Wastewater disposal location such as non-specified-use sink
- Clean bench paper
- Clean paper towels
- 2-Gallon Ziploc-type bags
- Dedicated water bottles: one for Deionized (DI) water; one for bleach solution
- Tape

Decontamination and Preparation of eDNA Processing Trailer

- Prior to departure the trailer should be cleaned, decontaminated and stocked with all supplies needed to process eDNA samples for that particular event.
- Decontaminate surfaces and floors with bleach. For 20% bleach solutions, allow it to sit for 10 seconds before wiping dry and then rinsing with water. For a 10% bleach solution, allow it to soak for 10 minutes, then dry and rinse with water to prevent build-up of bleach salts.
- Green centrifuge inserts should be disinfected in 10% or 20% bleach bath for 10 minutes or 10 seconds, respectively, rinsed with water, and allowed to dry thoroughly.
- Gloves, paper towels, ethanol/isopropyl, garbage bags, Sharpies, clean rube racks and packaging material should be restocked for each event
- Upon arrival at the sample processing site, select an area to park the trailer that has limited risk for contamination from other activities on-going in the area. For example, avoid parking next to boat washing stations where commercial carp fishing boats will be sprayed clean.
- Processing trailer should be checked for horizontal alignment and be as level as possible.
- Rotors and buckets for centrifuges should be wiped with DNAway and installed into the centrifuges.
- Bottle top dispensers and ethanol/isopropyl dispensing containers should be prepared on bench tops
- Centrifuge tube racks should be available for organizing and working with sample tubes

B2. Sample Processing Procedure

1. Hands must be washed thoroughly prior to starting.

2. A dedicated plastic wash bottle with 10% (or 20%) bleach solution, or commercially available product such as DNAway, should be prepared for wiping down lab tables and other surfaces prior to processing samples. All equipment must be cleaned with bleach, DNAway, or bleach wipes between sampling events.
3. Each workstation must be rinsed with bleach solution and the surface covered with one or more clean paper towels or bench paper prior to beginning the centrifuging process. One set of gloves may be used to move tubes from the cooler to the centrifuge adaptors and to load adaptors into centrifuges. **A new set of gloves should be used for each set of tubes comprising one sample any time the tube is opened**. Prior to centrifuging samples, each workstation should have pre-printed labels or one black, waterproof permanent marker for labeling sample tubes, a wastewater container (with lid), and a dedicated alcohol bottle with 95% non-denatured ethanol or 70%-99% isopropanol.
4. Turn on refrigerated centrifuge(s) and ensure they are set to 4 ^{0}C.
5. 50 ml tubes should be removed from the transport cooler, caps checked and tightened, wiped dry with either a clean paper towel or a clean bleach wipe, and placed into the green centrifuge adaptors. Towels or wipes used for drying may be used for multiple tubes and samples but should be changed once they become saturated and do not completely dry the tubes.
6. Process tubes in sets of replicates, until a full centrifuge batch is prepared.
7. Place filled adapters into a refrigerated centrifuge set to 4 ^{0}C. Tubes must be evenly distributed within the centrifuge to maintain the rotor balance. NOTE: Always follow the manufacturer's guidelines for centrifuge operation. It is also important to keep replicate tubes for each sample together in one centrifuge. Do not split a sample between centrifuges.
8. Once 50-ml tubes are in position, close and secure centrifuge lid. Set centrifuge to spin the samples for 30 minutes at max speed (~4200-5000 rpm) and begin centrifuging the samples. During this period the next set of tubes may be dried and placed in a clean rack or additional green centrifuge inserts if available. Change gloves after handling multiple samples. Field blanks should be handled in the same manner as samples.
9. Once samples have been centrifuged for 30 minutes, the eDNA will be on the bottom of the tube. Use a new pair of gloves for each set of tubes comprising one sample, carefully remove cap and GENTLY pour off water into a wastewater container. It is imperative to decant as much water as possible while ensuring pellet retention. A carboy is useful to prevent splashing, or a plain bucket with bleach in the bottom will also work. Change gloves after each sample set.
10. Add 20 ml of 95% non-denatured ethanol or 70%-99% isopropanol to the tube. The alcohol is required to stabilize the eDNA, and if too little is used, the DNA will degrade before it is detected. Replace cap and swirl alcohol around tube covering the entire internal wall. Centrifuge the samples with alcohol for 10 minutes (or more as needed if pellet is too loose) at max speed (~4200-5000 rpm). Blanks should be handled in the same manner as samples.
11. Decant excess alcohol until the level of the alcohol is within the conical end of the tube, re-cap and place in a clean rack for shipping. If it was difficult to decant water in step (8) and more than 3 ml water remained, then the alcohol used in step (9) will not have provided a 95% alcohol preservation level, so a second addition of alcohol will be required at this time. Add another 5-10 ml of alcohol, swirl gently, and leave excess alcohol in the tube. Place tubes back into a sterilized rack, keeping samples organized by sample number and ensure control samples are labeled. Change gloves between samples.
12. Record the time centrifuging was completed and the initials of the designated processing QA/QC data recorder in the Field Maps App as samples are completed.
13. When a rack is full, secure lids to tubes with tape (per shipping requirements), place racks in an individual 2- gallon size Ziplock type plastic bags so that the tubes are secured in the rack. Refer to sample handling and custody for further shipping instructions.
14. Prior to centrifuging the next batch of 50 ml samples, remove the 50 ml tube adaptor inserts and

examine for any water or debris. If anything is found, clean with 10% bleach for 10 minutes, 20% bleach for 10 seconds, or wipe down with DNAway. Rinse well to remove all residual bleach or DNAway, dry with clean paper towels, and replace. If adaptor inserts are clean and dry only perform this step during daily lab cleanup. (It may be helpful to have two to three full sets of inserts so that processing can proceed while any dirty inserts are being cleaned). Steps 4-13 should be repeated until all samples in that cooler have been processed.

15. Steps 1-14 should be repeated for each cooler of samples collected and processed.
16. Decontamination and clean up should occur daily during collection events (either in the evening after processing is complete for the day, or in the morning before sampling beings). This includes, at minimum, wiping down all floors and surfaces with 10% bleach for 10 minutes or 20% bleach for 10 seconds, followed by rinsing. Additionally, all plastic centrifuge inserts should be submerged in a 20% bleach bath for 10 seconds, rinsed thoroughly, and allowed to dry. Centrifuge buckets should be wiped down with DNAway.
17. If a sample is accidentally spoiled during the centrifuging process (e.g., the pellet was lost during decanting, bleach was squirted into a tube, tube breaks during centrifugation, or cross-contamination is suspected), it should immediately be thrown away. Record the appropriate sample ID as well as the reason for the ruined sample in the Field Maps APP and make a note on the Chain of Custody (COC) forms. This is not considered a deviation; this is recorded as a note.
18. Clean as needed at the end of each sampling day or at the end of the sampling trip.

B2. Post-Sampling Documentation Procedure

1. Complete the Post-Trip Field Summary Plan Survey123 Form and submit via Survey123 within 10 business days of the last day of sampling. If the trip was executed as planned, use the check boxes provided in the Survey123 Form, sign as the eDNA Lead and submit the Survey123 Form. If any deviations from Exhibit 1 or QAPP procedures occurred during water sample collection in the field or during processing of samples, clearly describe the deviations on Post-Trip Field Summary Plan Survey123 Form. If deviations will affect lab processing, the QA/QC Specialist will alert the eDNA Program Coordinator and the Lab eDNA Processing Lead. Some of this information may seem duplicated from the Field Maps App and Chain of Custody, but it is necessary to provide quality assurance information to ensure accuracy and completeness. These documents standardize information for a long-term monitoring program implemented by several different offices with frequent staff turnover across a large geographic area.
2. Complete and submit appropriate Shipping COC forms in Survey123, then package and ship samples to WGL (See Section B3 for shipping details).
3. eDNA Station Lead, or designee, will QA/QC and/or edit sample collection and processing data in ArcGIS Online within 10 business days of last sampling day. See ArcGIS Pro eDNA Editing Manual Number 2 for reference.

B3. Sample Handling and Custody

Samples must be maintained accordingly depending on sample type. All samples should be shipped as soon as logistics in the field allow. Please try to ship as often as samples are collected to prevent samples from sitting in preservative longer than needed. Ensure that samples are properly packed and shipped according to the procedure below.

Hand Delivered Procedure

1. Notify WGL staff of hand delivery with as much fore warning as possible.
2. Samples will be in cooler from section **B2. Sample Processing Procedure**.
3. Preserve samples in the trailer with methods described in section **B2. Sample Processing Procedure**.

4. Fill out the appropriate COC forms and state Hand Delivered.

Shipping Procedure

1. Centrifuge tubes must be shipped organized in racks, secured in individual plastic bags that will keep tubes in the racks, and shipped in containers that will hold the tubes and prevent damage to the tubes, as well as meeting regulations for shipping "small quantities" of flammable liquids according to 49 CFR 173.4. These samples may only be shipped ground, and if using FedEx, you must call for pick-up, they will NOT accept drop-offs at any office.
2. Ensure tubes are securely closed to prevent leaking. If necessary, twist caps counterclockwise to properly seat the threads indicated with a "click". Tighten. Use a single piece of tape to secure the lid to the tube. Place tubes back into the rack in numerical order. Place the rack of tubes into an individual plastic bag, seal or wrap it tight to keep tubes from falling out and tape the bag closed.
3. Construct the shipping box, double taping all seams. Box must meet federal guidelines; two options can be purchased from Uline: model #S-4798 (holds 120 samples or 600 tubes) or model #S16458 (holds 90 samples or 450 tubes).
4. Use two sections of absorbent material (model #S-14749, https://www.uline.com) and make a cross over the box. Push the center of the cross into the bottom of the box, making sure to press the matting into the bottom corners.
5. On top of the matting, place a 24x24x48 gusseted bag.
6. Place racks in a 3x2 configuration (3 layers high) for a 22x18x16 box. They only fit like this one way, 18 racks in total. A 22x22x22 box will be able to add another layer, for 4 layers of 3x2 racks. Add more matting or use spare zip bags to fill in the side space left by the larger box.
7. Twist tie shut the 24x24x48 gusseted bag to fully enclose all the tube racks. Use tamper tape to lay the twist knot as flat as possible. Tuck as much of the knot as possible into the space created where two racks meet. Sign across the outermost bag and across the tamper tape.
8. Fold tag ends of matting onto each other to fully enclose the top of the box with matting.
9. Place a 20x20 divider on top of everything to make a flat surface, if necessary.
10. Fill out a Survey123 COC form and provide an exact list of the samples shipped in that particular container (Refer to Appendix B for additional directions). The individual employee packing and sealing the containers should list their name in the "Released by"" line, be sure to also include agency. The container should be packed and released on the same date.The Survey123 COC forms are as important as the samples themselves. If Survey123 COC forms are not filled out properly, then sample integrity is lost, and the samples should not be processed because their custody cannot be accounted for. If you have questions, do not hesitate to call the eDNA Processing Lead or the eDNA Processing QA/QC Specialist.
11. Finally, double tape the top seams to close the box. Clearly write "This package conforms to 49 CFR 173.4 for domestic highway or rail transport only" on one side of the box. Be sure to draw arrows on all sides of the box, indicating which side is the top of the box.
12. Fill out a FedEx GROUND shipping label with appropriate information and request a pick-up. FedEx will only ship flammables if you request a pickup, you will not be allowed to drop boxes off at FedEx. NOTE: alcohol-preserved centrifuged samples are shipped at ambient temperatures.
13. Items will be shipped to:

Midwest Fisheries Center
Whitney Genetics Lab
555 Lester Avenue
Onalaska, WI 54650
608-783-8444

WGL Contacts

Once items have been shipped, WGL will receive an email via a web hook once the Survey123 Shipping COC is submitted. Upon receipt of the shipment at WGL, once the samples are checked in, staff will sign the Survey123 COC form.

B4. Analytical Methods

See Invasive Carp eDNA Sample Processing SOP No 4.

B5. Quality Control

This section identifies the QC activities required for field sampling and laboratory analysis along with the frequency these activities should occur.

Quality Control: Field

Field blanks will account for 10% of the samples per case. Blanks should be uniformly incorporated into sampling so that one blank occurs every 10 samples. Blanks are assigned to tubes when labeling ahead of time and consist of a single, pre-filled 50mL tube. Blanks will be prepared on station at each FWCO office. Before the sampling event, FWCO staff will fill new, sterile centrifuge tubes, package them in racks and secure in a clean plastic sleeve as needed.

Field Blanks

1. Wear clean gloves and find a clean unopened (if possible) bag of TPP tubes.
2. Use either commercially purchased distilled/deionized water or a source of clean tap water.
3. Uncap tube with gloved hand and place the cap on clean bench paper or clean wipe/napkin.
4. Fill the TPP tube to just below the threaded part of the tube opening.
5. Replace cap with gloved hand. Make sure these tubes are labeled as field blanks or are in an identifiable container labeled as field blanks.

Quality Control: WGL

Controls Used

- Hood controls are added when samples are opened and set in a hood to evaporate the preservative before extraction.
- Positive and negative extraction controls should be added to each eDNA extraction procedure batch.
- A positive and negative PCR plate control should be added to each PCR plate of samples run.
- Two standard curves should be added to each PCR plate (384 or 96 well).
- Data QC procedures are listed in Section D (Data Quality Indicators).
- Additional QC measures for collected data are recorded in the internal WGL Case Log.

eDNA Security Plan(s)

- A detailed eDNA security plan for the MFC has been developed due to the co-location of the La Crosse FWCO and the WGL. Staff in the FWCO often work in Bighead and Silver Carp contaminated waters and conduct field work where Bighead and Silver Carp are directly handled. Thus, this plan includes detailed procedures for decontaminating field equipment as well as boats, trailers, and trucks used for all field work including eDNA sample collection. All staff members of the MFC are required to read this plan and adhere to the recommendations therein.
- An eDNA security plan for each field or lab station involved in eDNA sampling or processing should be developed using the MFC eDNA security plan as a model but adapted for each station's unique situation. This plan should be kept on file at the regional office. The documents should be signed by

all personnel at the field or lab station and approved by the eDNA Program Coordinator.

B6. Instrument/Equipment Calibration and Frequency

Field and laboratory equipment used in eDNA monitoring should be inspected or tested before use and maintenance should be conducted on a routine basis. Descriptions are provided below on how equipment will be tested, inspected, and maintained so project personnel will know that equipment is working properly. If equipment is not working properly, equipment will be re-inspected for effectiveness and a corrective action provided (Exhibit 4).

Field Instruments

- Hand-held or console installed sonar: Batteries will be changed at least once a month in hand-held units (if not required sooner) to ensure accurate readings of the instrument. In addition, reading accuracy should be checked prior to sampling season for all units. Depth readings may be checked by traveling to a known depth area, anchoring with a rope to measure the depth of water and checking the rope measurement against the instrumentation.
- Acceptable range for hand-held or console measurements are ±3 ft. Temperature readings of the sonar may be checked against a thermometer.
- iPads and GNSS Receiver: Charged daily and ensure data points are uploaded to the cloud as soon as a signal is available.
- GPS equipment: Batteries will be changed at least once a month (if not required sooner) to ensure accurate readings of the instrument. In addition, coordinate accuracy will be checked against known benchmarks.
- Centrifuges: Annual service is a good idea if they are heavily used.

Laboratory Instruments

- Separate freezers are designated for storage of field samples, DNA extracts, PCR product, and reagents/components. Temperature recordings can potentially indicate any issues with the units. All refrigerator and freezer units will be cleaned and defrosted as needed.
- Pipettes: Annually all pipettes in use will be inspected, calibrated, and certified. Any pipette failing inspection and certification will be disposed of and replaced.
- Centrifuges: Annual service is a good idea if they are heavily used.
- iPads should be charged daily and updated on a regular basis to ensure COC forms and sampling data are uploaded as soon as possible.
- Any thermal-cycler head that fails the manufacturers self-test upon instrument startup will be removed and replaced with the manufacturer's certified replacement part.
- epMotion 5075s (automated liquid handling robots) maintenance will occur annually. Pipettes contained in the robot will also be calibrated annually.
- Temperature recordings will be taken weekly on all WGL refrigerator and freezer units (4, -20, -80 °C).
- All other laboratory equipment will be inspected monthly and undergo proper maintenance for ideal equipment working condition. Any equipment not performing accurately or to established standards will be disposed of and/or replaced or repaired.

B7. Data Management

To keep accurate records of eDNA sample collection, personnel associated with sampling, processing, data generated from field collection, and data generated with laboratory analysis must be kept for quick reference and to prevent loss. Responsibilities are outlined in the IC eDNA Program Data Management SOP No 5 of which personnel are responsible for the data management practice, how often it will be performed and where/how the data will be stored.

Section C. Assessment and Oversight

C1. Assessments and Response Actions

Assessment (audits) will be conducted every year on 3 of the 6 main identified Invasive Carp (IC) eDNA Monitoring Program areas. For each audit, 20 percent of each case numbers for that year will be reviewed. For example, in the 2021-2022 sample season there were 36 total cases. Of those 36 cases, 8 cases will be audited for 3 of the below areas. These assessments will ensure the procedures listed in the QAPP for sample collection and analysis are being followed. Some assessments may include observations of procedure compliance, technical systems audits, and data management.

C2. Audits

See Appendix D for the full audit schedule, evaluation criteria, indicators, and audit checklist items. The 6 identified areas for audits are as follows:

- Sample Design, Site Selection and Pre-trip Planning
- Sample Collection
- Sample Processing
- Sample Handling and Custody
- Alcohol Evaporation
- Extraction and Amplification

Sample Collection and Sample Processing audits will encompass 2 FWCO's per year and be completed either by the eDNA Program Coordinator or designee.

C3. Corrective Actions

Corrective actions are measures taken to correct conditions that are adverse to data quality and when possible, prevent the occurrence of issues in the future. Analytical, personnel, and equipment-related problems may develop during sampling, sample handling, sample preparation, laboratory instrumental analysis, and data review. Some types of corrective actions would be to collect additional data, investigate other data sources or loosen acceptance criteria.

Non-conformance issues arise when eDNA sampling, processing in the field or lab procedure execution deviates from procedures described in the QAPP. Creating and implementing a corrective action in a timely fashion can reduce the impact of the identified non-conformances. With timely identification, the impact of those non-conformances can be resolved. Resolutions could include re-inspection of performance to gauge the effectiveness of corrective actions.

In the case of analytical/equipment problems or non-conformance issues, the responsible lead will determine if the problem or deviation will affect the accuracy of the resulting data. If it is determined that the problem or deviation does affect data accuracy, two courses of action may be followed:

1. The procedure is repeated until it is performed without any problem or deviation.
2. The sample or samples are removed and not processed any further.
3. In either case, a corrective action report must be completed. Careful notes of any corrective actions and what incident led to them, as well as an explanation of the resolution or preventative measure(s) identified should be carefully noted in the corrective action report, which must be provided electronically to all Leaders (Project Leader, eDNA Processing Lead

or eDNA Processing QA/QC Specialist) as an after-action report.

4. In the case that the responsible lead determines that data accuracy is not affected by the analytical/equipment problem or deviation from procedure, the sample or samples may continue to be processed. The responsible lead will make careful note of the incident in project records and include the rationale for continuing processing.

Laboratory problems and issues should be documented first in OneNote, and then transferred to the electronic Case Log at the end of each processing batch for all phases of lab work. The eDNA Processing QA/QC Specialist will verify lab notes and electronic notes, and the electronic record will serve as the report and corrective action note for any future audit.

If a corrective action concerning an analyst is shown to resolve the issue or non-conformance, the analyst will be allowed to continue to collect data. If the corrective action does not resolve the issue, the analyst will be asked to re-train themselves on the methods described in the QAPP and certify that the issue is resolved before the analyst is allowed to collect data. Benchmark competency is defined as the ability to collect data without significant errors that result in loss of samples, loss of data, contamination of samples or contamination of assays. It is understood that human error exists and can occur frequently. However, each event will be examined and if continued errors are found, benchmark competency will be re-evaluated.

Section D. Data Review, Validation and Usability

D1. Data Review, Verification and Validation

Data generated for both field collection of samples and laboratory analysis will be reviewed, verified and validated before reporting out to partners. The procedures for review, verification and validation are listed below. Data review is the responsibility of each person collecting data, the QA/QC reviewer listed for each collection portion, the eDNA Processing QA/QC Specialist, and the eDNA Data Steward.

Data Review, Verification and Validation Procedures

eDNA Field Operations Managers must QA/QC data in the database within 10 business days of returning from the field unless other arrangements have been made with the eDNA Program Coordinator and eDNA Data Steward. If the eDNA Field Operations Manager cannot complete QA/QC, then one other personnel designated by the eDNA Field Operations Manager may also proof the data.

All field data are first QA/QC in ArcGIS for accuracy and completeness by the Station eDNA Lead (or designee) of the collection office specified for the data. The field data are then briefly checked by the eDNA Data Steward while field data are being paired with the eDNA result data. This step involves checking to ensure field blanks are all negative for eDNA and validating that the results pair with field data through a join validation in ArcGIS. The join validation checks for duplicate RUIDs or RUIDs that do not pair with the eDNA result data. The number of samples and dates of samples are also checked by the eDNA Data Steward when result maps are produced.

All laboratory generated data will be reviewed first by the staff performing the task and then the eDNA Processing QA/QC Specialist. The eDNA Processing QA/QC Specialist will review all paper data sheets against the internal Case Log. All notes in the OneNote electronic logbook should be transcribed into the internal Case Log. All dates, lot numbers for reagents, name of analyst will be checked against the internal Case Log entries. All Case Log data will be reviewed and verified before completing and sending out any results reports.

Data generated will be verified by evaluating the completeness, correctness and compliance to the specific acceptance criteria outlined in the Analytical Methods sections. The results report that is sent to the eDNA Data Steward will ultimately serve as a validated data set.

Data Quality Indicators

Details on quality control are found within section B5. The use of field blanks, hood controls, extraction negative controls, and PCR negative controls assess whether contamination is present in the process.

Positive controls for extraction and PCR are effective quality control for assessing the efficacy of protocols.

Data Quality Indicators are measured in several ways:

- Field blanks: Field blanks will only be run on the IC combo assay to look for target species contamination. If a field blank amplifies product, the range of samples on both sides of the contaminated field blank will be negated until the next clean field blank. For example, if field blanks are 11, 22 and 33 and 22 is positive while 11 and 33 are negative, then samples 12-32 will be negated.
- Hood controls: A hood control will only be run on the IC combo assay to look for target species contamination. If any of the three hood controls per range of samples show target species product, the associated data (all samples associated with that hood range) will be negated.

- Negative extraction controls: Negative extraction controls will be run with the extraction batch on IC combo assay plates and on BLG or BKT plates. A successful negative extraction control would have zero amplification on either IC combo assay, BLG or BKT assay plates. If any extraction negative shows product (e.g., DNA copies greater than zero), the associated data (all samples associated with that control) will be negated.
- Positive extraction controls: Positive extraction controls are run only the BLG or the BKT assay. A successful positive extraction control would have at least 1 replicate amplified out of the 8 replicates on the BLG or BKT plate.
- PCR negative control: A successful PCR negative control will have no amplification on either IC combo assay plates, BLG or BKT assay plates.
- PCR positive control: A successful PCR positive control will have one replicate amplified out of 2 replicates on both the IC combo assay, BLG or BKT assay plates.
- Standard curves: Minimum requirements for accepting the standard curves are E=80-120% and $R^2 \geq 0.95$. If necessary, errant standard curve points may be dropped to recover curve statistics, but there must be at least one 3-point curve remaining. This is acceptable, since the standard curves are for assessing assay performance only, and not for quantification of starting copy number. If the standard curves fail, the plate must be re-amplified.

D2. Usability: Reports to Management

Field Reporting

For each sampling event, a pre-trip plan (Exhibit 1) that includes the sampling sites, dates, personnel

roles, and contact information for field staff should be supplied to the eDNA Program Coordinator, eDNA Data Steward, other FWS personnel as needed, and partner agencies as appropriate. To provide proof of Quality Assurance, upon completion of all sampling events, the Post-Trip & QA/QC Summary Survey123 Form be filled out. eDNA sampling events and processing will be tracked in the Master Tracker file hosted on the eDNA Program SharePoint site.

D3. eDNA Results Reporting

eDNA Sample Archiving

All analyzed eDNA samples will be kept in -80 °C storage at WGL in eDNA sample storage designated freezers only. Samples will be moved from 1.7 mL MCT into 96 well plates to save space. A record of all sample location will be kept. Samples will be held at WGL for no more than five years from the date results were reported to the submitting partner. Upon the expiration of five years from the date of report, WGL will either (a) return samples to the submitting partner or (b) certify the destruction of the submitted samples. Unless a written request is made the by the submitting partner to return the samples, the default expectation will be to destroy the samples. Submitted samples may be used for research purposes, unless otherwise stated from the submitting partner.

At the conclusion of the sampling year, the eDNA Program Coordinator will contact state partners to inquire if partners want samples returned or destroyed. The eDNA Program Coordinator will then give states time to declare preference. Depending upon states response, samples are (a) returned to submitting partners/state, (b) destroyed or (c) sent back to third party designee (determined by submitting partner/state). Form 1 (Exhibit 5) will be sent back to the partner that indicates samples were either shipped back or that samples will be destroyed. Sample disposal will include autoclaving the samples to ensure DNA is not usable.

eDNA Results Reporting

The lab will update the eDNA Program Coordinator with information regarding any positive detections during PCR analysis so that timely handling of eDNA detection results can be mapped and communicated internally, with other offices, and partners as needed. Independent of any positive detections, the eDNA Program Coordinator will be notified when cases are closed. A separate communication to the eDNA Data Steward will contain eDNA detection results for each case for incorporation into the eDNA Database.

Each field sampling office keeps a record of field data. These records are summarized by the eDNA Data Steward and reported to the eDNA Program Coordinator. The eDNA Data Steward collates field and lab data for reporting results to partners and the public. The eDNA Processing Lead will provide updates and communications to the eDNA Program Coordinator. Any revisions to the communication procedures must be approved by the eDNA Processing Lead and approved by the assigned FWS Regional Office management staff. A specific results communication SOP is listed in Appendix A.

Section E. Relevant eDNA Literature

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Guan, X., Monroe, E. M., Bockrath, K. D., Mize, E. L., Rees, C. B., Lindsay, D. L., & Lance, R. F. 2019. Environmental DNA (eDNA) Assays for Invasive Populations of Black Carp in North America. *Transactions of the American Fisheries Society* **148**(6):1043-1055.

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Mize, E. L., Erickson, R. A., Merkes, C. M., Berndt, N., Bockrath, K., Credico, J., & Von Ruden, K. 2019. Refinement of eDNA as an early monitoring tool at the landscape-level: Study design considerations. *Ecological Applications* **29**(6):1374-1388.

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Takahara, T., Minamoto, T., & Doi, H. 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PloS One* **8**:e56584.

Van Driessche, C., Everts, T., Neyrinck, S., and Brys, R. 2022. Experimental assessment of downstream environmental DNA patterns under variable fish biomass and river discharge rates. Environmental DNA. 5(1):102-116.

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Exhibits

Pre-Trip Field Plan Summary Exhibit 1

NOTE: fillable forms for use are stored on the Teams site. Do not use this sample form.

Pre-Trip Plan for eDNA Field Collection and Processing

Exhibit 1a must be completed and sent to the eDNA Program Coordinator and the Database Manager at least one business day prior to the start of the event. Please attach maps of the target sampling areas.

Case: **Basin:** **Dates:**

PRE-TRIP PLAN

Target Waterbody	Samples	Blanks	Total	Sample #s
			0	0
			0	
			0	
			0	
			0	
			0	
			0	
			0	
TOTAL	0	0	0	

PERSONNEL:

Field Operations Manager: Phone #:

Sampling Crew:

Boat Operator:

Sampling Lead:

Sampling Assistant:

Data Recorder:

QA/QC Specialist:

Processing Crew:

Processing Lead:

QAQC Specialist:

1

NOTE: fillable forms for use are stored on the Teams site. Do not use this sample form.

SAMPLING SCHEDULE:

Day 1

Date:

Boat ramp(s) Coordinates (dec. deg):

Number of Samples:

Number of blanks:

Notes:

Day 2

Date:

Boat ramp(s) Coordinates (dec. deg):

Number of Samples:

Number of blanks:

Notes:

Day 3

Date:

Boat ramp(s) Coordinates (dec. deg):

Number of Samples:

Number of blanks:

Notes:

Day 4

Date:

Boat ramp(s) Coordinates (dec. deg):

Number of Samples:

Number of blanks:

Notes:

Day 5

Date:

Boat ramp(s) Coordinates (dec. deg):

Number of Samples:

Number of blanks:

Notes:

Training Checklist-Field Exhibit 2

NOTE: fillable forms for use are stored on the Teams site. Do not use this sample form.

Training Checklist for Field Staff:

Check Box		Training type	Date Completed
	1	ArcGIS Field Maps APP Manual No 1	
	2	Survey 123 Chain of Custody and Post-Trip & QA/QC Summary Creation/Submission SOP No 3	
	3	R3 eDNA Program Decontamination (video)	
	4	R3 eDNA Program Sample Collection (video)	
	5	R3 eDNA Program Sample Preservation (video)	
	6	R3 eDNA Program Sample Shipment (video)	
	7	QAPP review	
	8	ArcGIS Pro eDNA Editing Manual No 2	
	9	IC eDNA Program Data Management SOP No 5	

Signature of Trainee:

Notes:
All Field Staff are responsible for: 1 through 7.

eDNA Leads are responsible for: 1 through 9.

All FWS field staff that are serving as boat operators in the IC eDNA Monitoring Program are required to take Motorboat Operator Certification Course (MOCC) and those records will be kept on station.

Any staff members that will be driving a government owned vehicle with or without a trailer must have also complete Defensive Driving training. This training is mandatory for all DOI employees.

Training Checklist-Lab Exhibit 3

NOTE: fillable forms for use are stored on the Teams site. Do not use this sample form.

Training Checklist for Lab Staff:

Check Box	Training type	Date Completed
	WGL IC eDNA Sample Processing SOP No 4	
	WGL IC eDNA Data Management SOP No 5	
	R3 eDNA Program Preservative Decanting and Evaporation (video)	
	Survey 123 Chain of Custody and Post-Trip & QA/QC Summary SOP No 3	
	WGL specific training (sample receipt/GLP refresher/OneNote)	
	DNA extraction competency	
	PCR analysis competency	
	QAPP review	

Signature of Trainee:

Notes:
DNA extraction and PCR analysis competencies are only required once.

Corrective Action Form Exhibit 4

Corrective Action Report

Reporting Employee: _____ Date: _____

Description of the result/incident or suspect equipment:

Description of the Corrective Actions taken:

Reporting Employee Signature: _____

Approving Employee Signature: _____

Sample Destruction Form 1 Exhibit 5

Form 1. Release (Return or Disposition) of eDNA Samples Documentation

Case Information:

Case Number: Year Collected:

Number of Samples Collected: Location:

Submitting Partner:

Release (Return or Disposition) of eDNA Samples:

Will eDNA samples be released for Return or Disposition? Please check the appropriate box.

1) Release for Disposition (destruction): 2) Return to Submitting Partner:

3) Sent to third party designee (determined by submitting partners):

Date eDNA samples were released by the above specified method:

Signature of person releasing eDNA samples:

If returned to the submitting partner (or third party designee), please include shipping information. If released for disposition, please leave this section blank.

To: Tracking Number:

Address: Dry Ice or Ice (please check box)

A note about returned samples: In an effort to conserve space, eDNA samples have been dried down and frozen at -80 °C. Upon receipt or at first use, we suggest samples be hydrated to approximately 100 µL volume.

Complete Documentation: For samples returned to the submitting partner, attach a completed COC to this document. Send completed COC and Form 1 to the submitting partner. Once completed and returned by the requesting partner, place a copy in the appropriate case folder/COC folder. If samples are destroyed, send completed Form 1 to submitting partner.

Appendices

Appendix A: Internal Communication of Results Plan

Notification of Reportable U.S. Fish and Wildlife Service eDNA Results

This Standard Operating Procedure (SOP) is intended to guide U.S. Fish and Wildlife Service (FWS) employees involved in the sampling and analysis of environmental DNA (eDNA) as they disseminate the results of early detection and monitoring for Bighead and Silver Carp genetic surveillance. Included is a flowchart (Figure 1) depicting how information will be transmitted through the Program as well as specific guidance relating to the format and types of information that will be crucial to include as eDNA results are communicated. Further guidance regarding Service eDNA sampling can be found in the Invasive Carp Regional Coordinating Committees Monitoring and Response Plan and other regionally specific plans.

eDNA Results Preparation

1. The sampling event data will be packaged together by the eDNA Program Coordinator in a uniform template for internal and external communications. Sampling events are defined by the geographic boundaries and time sampled.
2. Field data related to the sampling event are automatically uploaded to the eDNA database and are proofed by the collecting FWCO.
3. The analysis results associated with each sampling event will be provided to the eDNA Program Coordinator and eDNA Data Steward by WGL once processing in the lab is completed.
4. The eDNA Data Steward will compare the datasets provided by WGL and the FWCO to ensure accuracy and completeness, then combine to create the results package to be sent to the eDNA Program Coordinator:
    a. Geo-referenced map book indicating all sites eDNA samples were taken, highlighting all the sampling sites for the case in separate maps (Figure 3).
    b. Digital Excel file with each sample uniquely identified. Data columns will include Regional Unique ID, Sampling Station ID Code, State, Basin, Waterbody, Date collected, Latitude and Longitude in decimal degrees, Blanks, Case Number, eDNA Detection Status, and Lab notes.

Internal Data/Results Sharing (Figure 1-Diagram a)

5. The eDNA Program Coordinator sends the results package to Midwest Region Fish and Aquatic Conservation (FAC) Leadership consisting of the FAC Deputy Assistant Regional Director (Deputy ARD) and ARD, Regional AIS Supervisor, National IC Coordinator, FWCO Program Supervisor, FWS Invasive Carp Regional Coordinating Committee (ICRCC) Co-Chair, impacted FWCO (Project Leader and eDNA Lead) and Office of Communications.
6. eDNA results information is to be kept confidential. Any potential communication with partner groups or other entities outside of FWS will not occur prior to informing the affected partner.
7. If necessary, Midwest Region FAC Leadership may inform the Regional Director and/or Deputy Regional Director. Additionally, depending upon the results, the Regional Director and/or Deputy Regional Director may inform the FAC Assistant Director at the Headquarters level.
8. Depending on the region that the results originate from, the Regional Director or designee will inform Other Regional Director and/or Deputy Regional Directors.
9. Communication of results may also include Congressional Offices via the Office of Communications staff member.
10. The communication and briefing process from eDNA Program Coordinator to the Midwest Region Regional Director and/or Deputy Regional Director should occur quickly. This will allow the Midwest Region to disseminate results promptly and maintain our responsiveness to affected partner and partner group(s).

External Results/Data Sharing (Figure 1-Diagram b)

11. The eDNA Program Coordinator will disseminate the eDNA results communication package to affected partner(s):
    a. Geo-referenced map book indicating all sites eDNA samples were taken, highlighting all the sampling sites for the case in separate maps (Figure 3).
    b. Digital Excel file with each sample uniquely identified. Data columns will include: Regional Unique ID, Sampling Station ID Code, State, Basin, Waterbody, Date collected, Latitude and Longitude in decimal degrees, Blanks, Case Number, eDNA Detection Status, and Lab notes.
    c. A transmission memo from the eDNA Program Coordinator to the affected partner relating a summary of the information that has been collected and our proposed next steps (Memo template below). This memo will be sent via email.
    d. Consultation will be provided upon request.
12. When notifying the affected partner, the internal communication list will be copied. This transmission will start a 5-business day waiting period at the end of which the results will be posted to the FWS IC eDNA Monitoring Program Dashboard. However, upon written request from an affected partner, the FWS will extend the timeframe for up to an additional 5 business days. The request will specify the reason(s) for the extension (e.g. new geographic area of eDNA detection, high number of positive results, etc.), the name and position of the requesting official, and the number of additional days requested.
13. Following transmission of the data package to the affected partner, the eDNA Program Coordinator will be prepared to work with them, to assist in interpreting the results or supporting further sampling if possible and necessary, understanding that in some cases the affected partner may not decide to carry out a follow up investigation.
14. A media release may be done by the State or Tribal Entity. FWS does not issues press releases for eDNA results, but FWS Office of Communications will assist. The eDNA Program Coordinator will coordinate the public release of results with the partner led press release.
15. FWS will not release results to other partners or communication chains, but partner(s) may choose to share these results with other agencies/groups and communication chains. In the Great Lakes Basin, the FWS will notify the ICRCC co-chairs prior to public release.
16. Tribal entities and congressional member offices will be notified on a case dependent basis.
    a. Tribal Entities – Notifications will be made to each tribe's leadership respectively as an affected partner when sampling has taken place within or in direct proximity to tribal lands. In this case, they will receive the same data package via email as any other affected partner. If the samples were taken in treaty waters, but not in proximity to tribal lands, their leadership will be notified via email and/or phone call from the DRD (delegated to ARD as appropriate) either upon issuance of a press release by the affected partner or when the results are posted on the FWS IC eDNA Monitoring Program Dashboard.
    b. Congressional Member Offices – Congressional office contacts will be notified upon issuance of a press release by the affected partner on their behalf. Notification will be made via email and/or phone call from DRD (delegated to ARD as appropriate).
17. Results will be posted concurrent with any press releases issued by affected partner, **OR** by 3 PM CST on the fifth business day after delivery of the data package to the affected partner, whichever comes first, unless more time is requested by the partner. This is to ensure transparency in the system and to not restrict information sharing. Day 1 will be considered the next business day following delivery of the data package to the affected partner.

Results/Data Media Release (Figure 2)

1. When affected partner is notified of results, results are publicly posted on the FWS IC eDNA Monitoring

Program Dashboard within 5 to 10 business days.

2. If the Jurisdictional Partner, which may include the State partner and/or a Tribal Entity, issues a press release at the time Office of Communications will contact the Congressional Member offices and/or Tribal Entity.
    a. Congressional Offices will make notifications on behalf of the state.
    b. In instances where sampling occurred in treaty waters, but not in proximity to tribal lands, tribal leadership will be notified via phone or email
    c. In instances where sampling occurred in treaty waters, but <u>not</u> in proximity to tribal lands, tribal leadership will be notified via phone or email by FWS when results are posted online if no press release has been issued by the state.

3. Results are then posted on the FWS IC eDNA Monitoring Program Dashboard in conjunction with the media release.

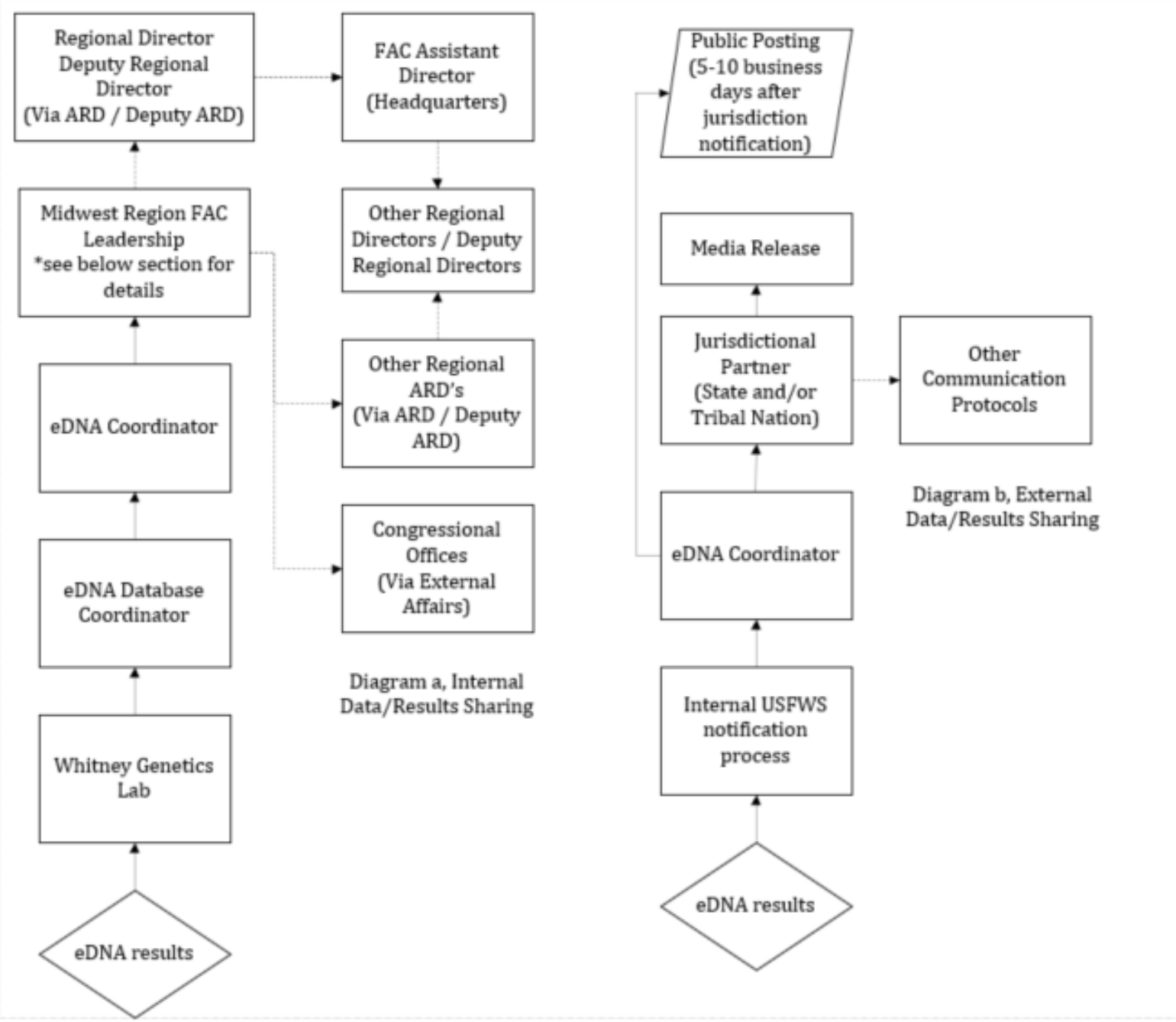

Figure 1. Internal and External Flow of IC eDNA Monitoring Program Data/Results.

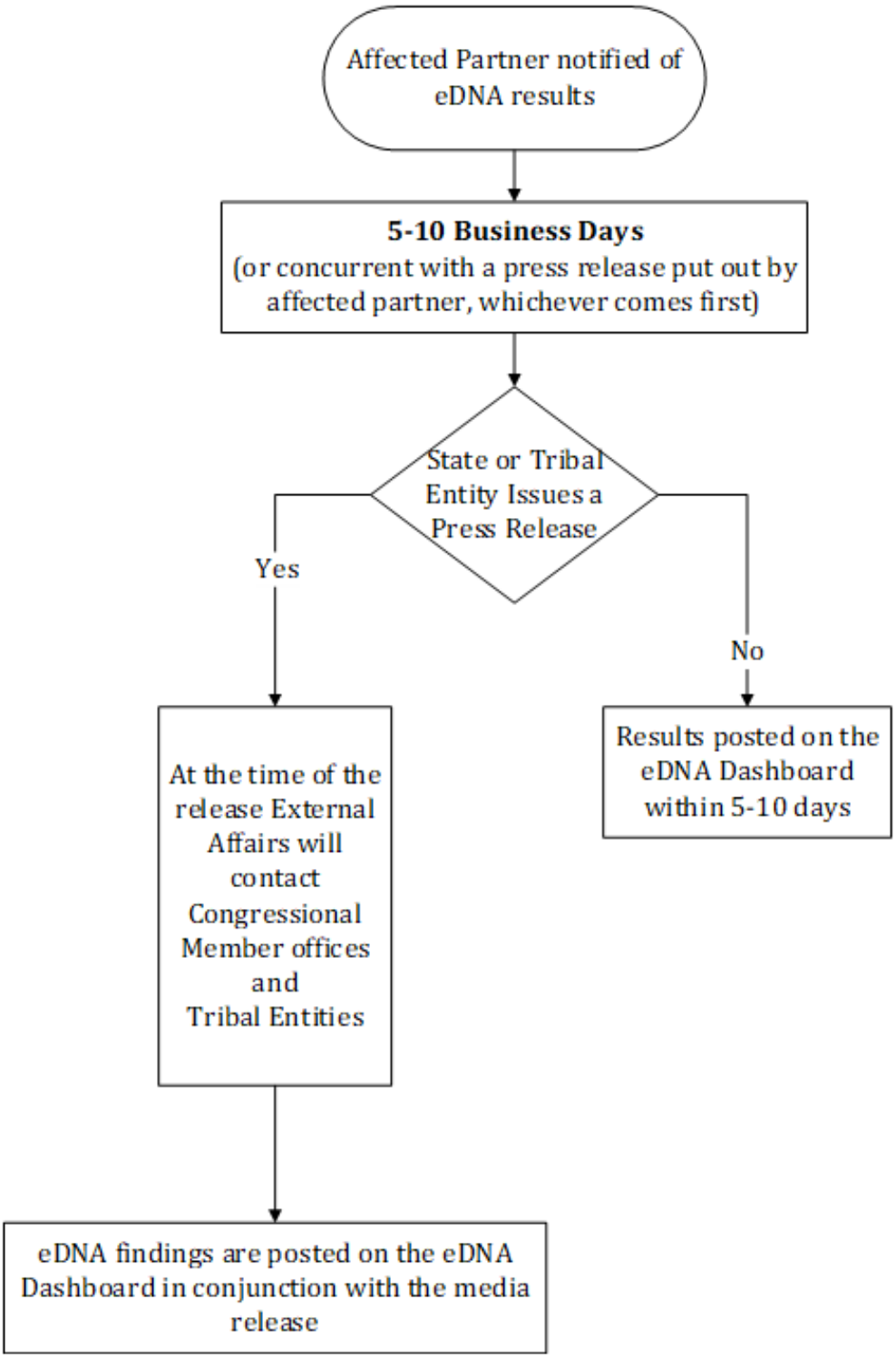

Figure 2. Media Release Flow of IC eDNA Monitoring Program Data/Results.

Figure 3. Example Geo Referenced Map of IC eDNA Monitoring Program Data/Results.

Form Letter to Project Leaders

Hi Insert Project Leader,

As a heads up, we will be transmitting results from the week of Insert Date eDNA sampling of the Insert Location(s) to the state of Insert State and/or Tribe on Insert Date. These samples were collected for the Insert Program or Project.

By way of preview, results are listed below. Please do not distribute to anyone without clearance from myself.

Insert Location

Sampled Insert Date
total samples
no eDNA detected
invasive carp eDNA only detected
silver carp eDNA only detected
bighead carp eDNA only detected
bighead and silver carp eDNA detected
See attached maps.

Results will be posted to the FWS eDNA dashboard likely on Insert Date unless we receive a request from Insert State and/or Tribe to delay or post sooner.

Thank you and your team for fulfilling this component of the Insert Program or Project.

As always, if you have any questions, please contact me by email.

Insert eDNA Coordinator Signature and Contact

Form Letter to Partners

Insert Partner Names:

Enclosed with this email you will find the U.S. Fish and Wildlife Service Whitney Genetics Lab results relating to the recent environmental DNA (eDNA) sampling which occurred in the Insert Location(s) during Insert Dates. These results are presented geographically to provide an overview of the locations where each individual sample was collected. Additionally, there is a table provided which identifies each individual sample collected by unique identifier and its corresponding data.

A total of # samples were collected. After reviewing the data, you will see that **zero (0)** samples tested positive for bighead carp, silver carp, or invasive carp eDNA. -OR- After reviewing the data, you will see that **number (#)** water samples tested positive for bighead carp, **number (#)** water samples tested positive for silver carp, and **number (#)** water samples tested positive for invasive carp eDNA.

Please be aware that these results will be posted on our public website, https://fws.maps.arcgis.com/apps/dashboards/index.html#/52b22abe9c4d4575adfe851a946f444d on Insert Date, five business days after the transmission of this message; however, if Insert State and/or Tribe plans to release the results sooner, we will post them sooner. Upon your request, we will delay the posting of these results for an additional 5 business days to Insert Date. If requested, we will work with you to interpret these results and issue a press release. Please keep us appraised of your release date.

Please be aware that the FWS will not release results to other partners or communication chains, but your state may choose to share these results with partners and other communication chains. -OR for GL Partners- The FWS will alert the Invasive Carp Regional Coordinating Committee Co-Chairs the day before the public release. We will not release results to any other partners or communication chains, but your state may choose to share these results with partners and other communication chains.

If you would like to discuss these results, we would be happy to set up a conference call for you and appropriate staff at your earliest convenience.

Thank you for your continued interest and dedication to the monitoring and prevention of invasive species in the Insert Basin.

Nick

Nick Frohnauer
eDNA and Early Detection Coordinator
U.S. Fish & Wildlife Service Midwest Region
Norman Point II
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Appendix B: Elimination of eDNA on Boats and Equipment

Disinfection Recommendations

Recommended methods for reduction of residual or environmental DNA on boats and other equipment associated with environmental conservation work in the field. For boats and equipment that have been previously exposed to carp DNA, choose one high pressure sprayer method ***in conjunction*** with one chemical method to reduce DNA levels to negligible or below LOD. Read MSDS and Use personal protective equipment (PPE). Read job hazard assessment (JHA) for applicable methods.

Method	Active Ingredient	Contact Time	Advantages	Disadvantages	Cautions
Steam + Pressure Washer @ 212 F	Pressure, Heat and Water	10 seconds	Environmental safety	Need electrical and water hookups Can melt or tear materials	Use PPE – need proper safety training, can cause burns, cuts, air embolisms
Cold Water High Pressure Sprayer with Low Pressure Detergent Application	Detergent, Pressure and Water	3-5 minutes Detergent contact/ 10 seconds high pressure rinse	Environmental safety	Need water hookups Can tear materials	Use PPE – need proper safety training, can cause cuts, air embolisms
10 % Household Bleach Low Pressure Saturation	Sodium hypochlorite (5-8% before mixing)	10 minutes	No hookups necessary – can use off station	Not preferred for environmental safety Single day use in solution	Use PPE and avoid breathing fumes
20 % Household Bleach Immersion Bath	Sodium hypochlorite (5-8% before mixing)	10 seconds	No hookups necessary – can use off station	Not preferred for environmental safety Single day use in solution	Use PPE and avoid breathing fumes
2% Virkon Immersion Bath	Potassium Peroxymonosulfate and Sodium Chloride	30 minutes	Lasts 1 week in solution Environmental safety	Corrosive to metals when exposed longer than 10 minutes	Use PPE – wear a dust mask when mixing powder
2% Virkon Low Pressure Saturation	Potassium Peroxymonosulfate and Sodium Chloride	10 minutes	Lasts 1 week in solution Environmental safety	Not quite as effective as bleach in laboratory study; equal or better than bleach in field (boat) equipment study	Use PPE – wear a dust mask when mixing powder. Do Not Aerosolize! Use a low pressure dispenser (hose attachment sprayer at largest droplet setting)

Preventing the Spread of Pathogens, Bacteria and Invasive on/in Boats, Motors, Trailers and Equipment

Developed by the US Fish and Wildlife Service Great Lakes Region 3

Disinfection Techniques and Options:

Boat, motor, trailer, and gear must have all aquatic vegetation, visible organisms/animals, soil, and water drained and removed BEFORE TRANSPORT. Upon leaving a water-body possibly infected with pathogens or invasive species, a proper disinfection must be completed before re-use of boat, motor, trailer, and any exposed gear in another waterway. **Contact time** is crucial for complete disinfection. **Contact time** reflects exposure of air, water, or disinfectant to a specific area, and not the total amount of time spent disinfecting. For example, if you are using 50C water to disinfect your boat, you must apply 50C water to each area for 10 minutes or longer (see options and procedures below). **Read SDS, wear personal protective equipment (PPE), and comply with federal and state regulations.**

Disinfection is MANDATORY for all exposed equipment and gear!

Methods	Procedures	Positives	Negatives
Heat + Air (Drying in hot sun/air)	**30C (86F) 24 hours minimum** (time at temp contact period crucial) (exposure to hot sun/air while dry)	Chemical free Effective, but only if properly done under ideal conditions	Time consuming Weather/Temperature criteria Critical to reliable results
Heat + Water Spray and/or Immerse	**50C (122F) contact time 10 minutes** (time and temp contact crucial) (source of very hot water needed)	Chemical free Same as above	Must maintain high water temp/contact; hotter than normal tap or carwash. Use PPE
Steam Spray *(recommended for eDNA decontamination)	**100C (212F) contact time 10 seconds** (time and temp contact crucial) (steamer washer/sprayer needed)	Chemical free Same as above	Must maintain very high water temp/contact; (i.e. steamer washer/sprayer). Risk of burns, use PPE
Virkon Aquatic *(recommended for eDNA decontamination in a 2% solution for 10-30 min)	**Follow product direction for proper mixture and minimum contact time** (apply directly, maintain saturation and rinse thoroughly)	Environmentally friendly Designed for aquatic use Quick inactivation time Sewer compatible	Follow SDS directions for health risks and use PPE when mixing. Chemical based. Corrosive in concentrate form
Quaternary Ammonium + Water (family of products)	**Follow product direction for proper mixture and minimum contact time** (apply directly, maintain saturation and rinse thoroughly)	Effective, user friendly Low health risks Sewer compatible	Follow SDS directions for health risks and use PPE. Chemical based
Sodium Hypochlorite + Water *(recommended for eDNA decontamination)	**200 ppm for pathogens; 5000 ppm for eDNA – contact time: 10 min** (apply directly, maintain saturation and rinse thoroughly)	Widely available Effective	Follow SDS directions for health risks and use PPE. Highly Corrosive

USFWS Contacts: Corey Puzach, Fish Health Center: corey_puzach@fws.gov, Dave Wedan, Watercraft Safety Coordinator: dave_wedan@fws.gov
Jen Bailey, Fish Health Center: Jennifer_bailey@fws.gov, *for eDNA decontamination contact Zeb Wojak, Whitney Genetics Lab: Zebadiah_woiak@fws.gov

Last updated: June 2020

Appendix C. Audit Schedule and Evaluation Criteria

Audit Schedules and Evaluation Criteria

Evaluation Criteria: Looking for written organizational policies, documented objectives, organizational quality specifications, standards, procedures, data specifications or requirements.
Indicator: The informational piece that would be looked at during an Audit.
Audit Checklist Items: Areas that would be looked at during an Audit.
Responsible Party: Person(s) who would be performing the Audit.
Audit Timeline: Every year, 3 of the 6 main identified IC eDNA Program areas will be Audited. For each Audit, 20 percent of each case numbers for that year will be reviewed.

1. Sample Design, Site Selection and Pre-trip Planning
Evaluation Criteria: Section B1. Sampling Design and Planning details procedures Sample Documents, Sample Design and Site Selection
Indicator: Exhibit 1
Audit Checklist Items:

- Detailed aerial maps for use as markers for sampling location to ensure spatial coverage and overall sample area present
- Coordinate sample plans complete with sampling crew, eDNA Program Coordinator, eDNA Data Steward and/or partner agency identified
- Sampling crews should be a minimum of three people: a field sampling collector, field sampling collector assistant, one boat operator and one of which will also serve as the field sample collector QA/QC Specialist
- Processing crews should consist of two people, two sample processors, one of which will also serve as the field processing QA/QC Specialist. Documentation exists if less than 2 people are used
- Date uploaded to eDNA Program SharePoint site will precede date of field collection

Responsible Party: eDNA Program Coordinator

2. Sample Collection – In-person Field Sample Collection and Processing Review

Evaluation Criteria: Section B2. Sample Collection Procedure and Section B2. Sample Processing/Centrifugation details sample collection and processing procedures
Indicator: Review staff in boat for sample collection and in trailer for sample processing
Audit Checklist Items:

- Review field collection procedures in boat against QAPP
- Review sample processing procedures in trailer against QAPP
- Proper number of staff in boat and trailer, fulfilling roles identified
- Exhibit 1 filled out completely. Documentation of deviations match any field notes
- Disinfection procedures are being followed
- Gloves are being changed at the appropriate times
- Sample times are not exceeding 24 hours from time of collection to time of processing
- eDNA Lead signature is present, indicating review of initials of Sampling QA/QC Specialist, Processing QA/QC Specialist and Data QA/QC Specialist

Responsible Party: eDNA Program Coordinator, or designated party (Project Leader)

3. Sample Collection
Evaluation Criteria: Field Sample Collection data
Indicator: Field GeoDatabase
Audit Checklist Items:

- Check the map and ensure all your sample points are displaying on the targeted waterbody, if any points are missing or in the wrong place
- Snap blank samples to the appropriate field data point if not already snapped
- Check state line layer in map to ensure the correct state has been identified for the sampling point
- Check for null values in each data field
- Check the date collected column for any dates outside of the date range of collection
- Look for repeat Regional Unique ID's
- Make sure calculate geometry has been run on the Latitude and Longitude fields
- Please make note of any environmental conditions that may affect the quality of the samples in the comments field
- If not all the samples could be collected, identify the sample numbers in the comments field

Responsible Party: eDNA Data Steward or QA/QC Specialist

4. Sample Handling and Custody

Evaluation Criteria: Section B3. Sample Handling and Custody details procedures on shipping or hand delivering samples to WGL for analysis

Indicator: COC Survey123 data

Audit Checklist Items:

- Survey123 data will be reviewed within 5 business days of sample receipt
- Survey123 data will be reviewed for accuracy and completeness. eDNA Leads will be notified of any incomplete data
- Data will be downloaded at a minimum of twice per season, mid-season and at the end of each eDNA season

Responsible Party: QA/QC Specialist

5. Alcohol Evaporation

Evaluation Criteria: Section B3. Preservative Decanting and Alcohol Evaporation details procedures on evaporating alcohol off tubes in preparation for extraction and further downstream analysis

Indicator: WGL Case log

Audit Checklist Items:

- WGL Case Log will be checked to make sure date of sample receipt to when samples are dried does not exceed 3 weeks

Responsible Party: QA/QC Specialist

6. Extraction and Amplification

Evaluation Criteria: Section B4. Analytical Methods: IBI Scientific gMax Kit Extraction Procedure, Section B4. PCR Amplification Procedure, Section B4. Analytical Methods: Standard Curve Material, and Section B4. Analytical Methods: PCR Data Analysis all detail procedures on extracting, amplifying, and interpreting results for collected samples.

Indicators: OneNote electronic logbook, WGL Case Log, Hood Controls, Field Controls, Extraction Negative and Positive Controls, PCR Negative and Positive Controls, Standard Curves, BioRad files.

Audit Checklist Items:

- Appropriate logs or OneNote electronic logbook in use
- Controls and blanks being used as necessary
- QC activities are clearly established

- Data sheets contain essential information (date of analysis, analyst, case information, notes)
- Sample tracking system in place
- BioRad data files so that proper files are downloaded and edited to reveal results accurately

Responsible Party: QA/QC Specialist

7. Extraction and Amplification- In-person Review

Evaluation Criteria: Section B4. Analytical Methods: IBI Scientific gMax Kit Extraction Procedure, Section B4. PCR Amplification Procedure, Section B4. Analytical Methods: Standard Curve Material, and Section B4. Analytical Methods: PCR Data Analysis all detail procedures on extracting, amplifying, and interpreting results for collected samples.

Indicators: Review staff in lab for extraction and amplification procedures.

Audit Checklist Items:

- Review extraction procedures are being followed against QAPP
- Review amplification procedures are being followed against QAPP
- Review OneNote electronic logbooks for accuracy and completeness for following procedures. Data sheets contain essential information (date of analysis, analyst, case information, notes)

Responsible Party: QA/QC Specialist