

**QUALITY ASSURANCE PROJECT PLAN**  
**eDNA MONITORING OF BIGHEAD AND SILVER CARPS**

**Prepared for:**

**U.S. Fish and Wildlife Service**

**USFWS Great Lakes Region 3**

**Bloomington, MN**

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## Section A. Project Management

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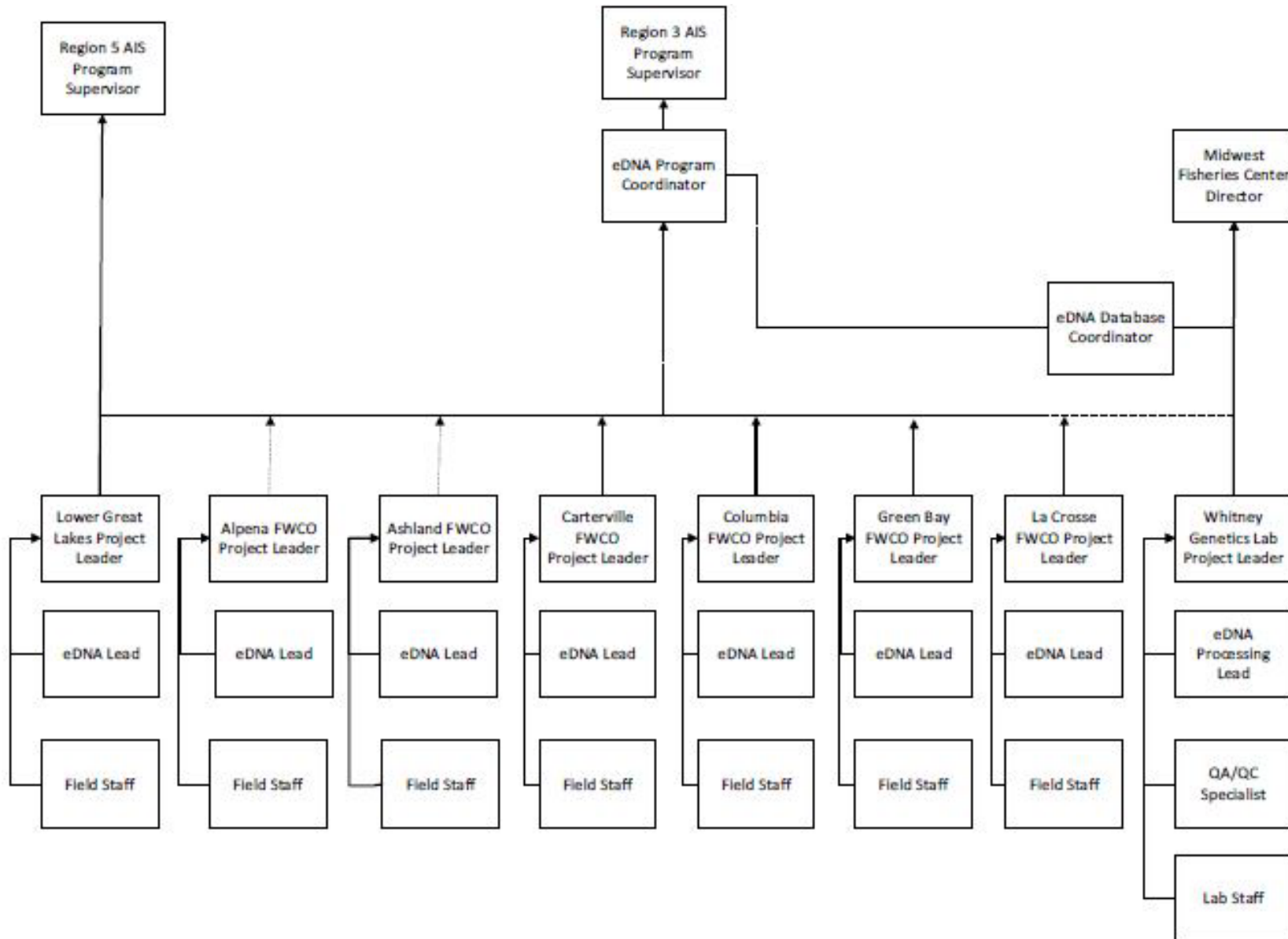
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La Crosse FWCO  
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555 Lester Ave  
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### A3. Invasive Carp eDNA Program Organizational Chart



#### **A4. Project/Task Organization**

Project personnel responsibilities and roles are defined below for each position necessary for completing the US Fish and Wildlife (USFWS) Great Lakes Region 3 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 USFWS 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 Exhibit 3 on file with the USFWS 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 Survey 123 Form (formerly Exhibit 2) 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
- 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 Database Coordinator and Data Steward

#### **Communication Staff**

- eDNA Program Coordinator
- External Affairs

#### **Roles**

##### Regional Office

Fisheries Region 3 Regional Director (RD): Charlie Wooley

Fisheries Assistant Regional Director (ARD): Aaron Woldt

Fisheries Deputy ARD: Brian Elkington

R3 AIS Coordinator: Amy McGovern

R5 AIS Coordinator: Sandra Keppner

R3 eDNA Coordinator: Nick Frohnauer

R3 FWCO Program Supervisor: Kasia Mullet

##### Midwest Fisheries Center

Center Director: Bill Ardren

eDNA Database Coordinator and Data Steward: Jeena Koenig



Whitney Genetics Lab

Project Leader: Pat DeHaan

eDNA Processing Lead: Kyle Von Ruden

eDNA Processing QA/QC Specialist: Maren Tuttle-Lau

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

<b>Office</b>	<b>Project Leaders</b>	<b>Station eDNA Lead</b>
Alpena	Scott Koproski	Anton Gereau
Ashland	Mark Brouder	Jason Ross
Carterville	Mike Thomas	Rebecca Lucas
Columbia	Jason Goeckler	Jessica Howell
Green Bay	Susan Wells	Matt Petasek
La Crosse	Rebecca Neeley	Jenna Bloomfield
Lower Great Lakes	Mike Goehle	Colleen Keefer
Wilmington	Mike Thomas	Nathan Evans

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. Since then, a new threat to the Great Lakes from the Mississippi River basin has become the primary objective of the electric dispersal barrier system. 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. Their potential spread through the dispersal barrier system within the CSSC poses a threat to the Great Lakes ecosystem.

Traditional fishery techniques are used to detect the leading edge of Bighead and Silver Carp population; 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 USFWS offices responsible for field sampling and lab processing of eDNA samples. The FWCO will handle field sampling and the Whitney Genetics Lab (WGL) at the Midwest Fisheries Center (MFC) will process eDNA samples since the USFWS 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, USFWS 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 USFWS Great Lakes Region 3 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 USFWS Great Lakes Region 3 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 USFWS Great Lakes Region 3 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 WGL staff. Any eDNA samples collected and processed that do not follow the

QAPP cannot be directly compared to USFWS 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 USFWS results.

#### **A8. Special Training and Certifications**

In order to successfully complete eDNA monitoring, project personnel must successfully complete certain special and/or non-routine training. Training may include: Field Maps APP, Survey 123, 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 3). The WGL Project Leader is responsible for WGL staff getting the proper training in order to analyze samples. The eDNA Processing QA/QC Specialist is responsible for collecting all training and documentation (Exhibit 4). All training documents will then be provided to the eDNA Program Coordinator.

#### **Personnel Training Requirements**

Minimum training and/or experience requirements for the different components of the eDNA monitoring protocol are detailed in Exhibit 3 and 4. 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 will send documentation of personnel training to the eDNA Program Coordinator. The WGL Project Leader is responsible for documenting and providing training to WGL staff and will send documentation of personnel training to the eDNA Program Coordinator. All forms of documentation may be provided hard copy or in electronic format to the eDNA Program Coordinator or kept on the R3 eDNA SharePoint site

#### **Boat Operator**

Must meet USFWS boat operator requirements. 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 R3 eDNA SharePoint site for the duration of the eDNA monitoring program. Retention and final disposition of some records may be regulated.

#### **QAPP Maintenance and Modifications**

The QAPP will be reviewed annually by the eDNA Program Coordinator, eDNA Program Management Team, eDNA Processing QA/QC Specialist, FWCO Project Leaders, statisticians, station eDNA Leads and other technical 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 USFWS eDNA monitoring personnel will keep up with current research through agency publications and peer reviewed literature.
- The USFWS 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.
- Agencies that would like to contract with other labs for eDNA monitoring may want to use the QAPP and are encouraged to do so. However, it is likely that partnering with other labs will lead to procedures that are not covered in the QAPP. In this case, it is up to each agency to decide on their own requirements needed for eDNA sampling and subsequent sample analysis.
- The USFWS recommends a minimum of following the QAPP to ensure rigorous quality control and sample integrity standards.
- Use of other labs should be carefully reviewed by the contracting agency staff and each agency should ensure that labs are validated and can process samples with at least the same level of quality control and in lab situations that minimize contamination in highly sensitive PCR applications.
- It is likely that other labs will use assays with different PCR markers and even different PCR techniques such as digital PCR. It is up to the management agency contracting the work to ensure that these assays have been rigorously tested and validated for use in sensitive eDNA monitoring programs. At a minimum, the assay methods should be published or validated in multiple labs.

### **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 USFWS 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, USFWS 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 Database Coordinator 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 3 (page 1). 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 Field Summary Plan Survey 123 Form (formerly Exhibit 2) covering quality assurance issues and any changes in personnel roles should be generated by the Station eDNA Lead (or designee) and shared with the eDNA Program Coordinator.

#### **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 Station eDNA 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. 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 (e.g. cool water, negligible flow, known Invasive carp presence), a sampling density of 1 sample per 0.01 km<sup>2</sup> with a minimum of 40 samples was appropriate to detect Bighead and Silver Carp DNA. Most areas in the USFWS 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 0.01km<sup>2</sup> 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 Instructional Manual 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 Database Coordinator, 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.
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 (e.g. 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 Instructional Manual 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 Jeena Koenig by e-mail [jeena\\_koenig@fws.gov](mailto:jeena_koenig@fws.gov).

### **Equipment Preparation**

In order 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 min 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 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). For offices working with the WGL, all field blanks must be filled by WGL. For other agencies, blanks may be filled with any city-provided tap water (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 PFDs 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 previous carp sampling boat to a dedicated eDNA sample collection vessel.
- Dedicated eDNA personal flotation devices (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 Survey 123 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.
- DNAAway.
- 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 C).

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 C for a list of recommended decontaminants. Use personal protective equipment (PPE) and read 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 Field Summary Plan Survey 123 Form (formerly Exhibit 2) 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 (i.e. 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. Survey 123 COC forms will be completed for every sample and every cooler. All samples, including field blanks, will be logged onto Survey 123 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 period (Lance et al. 2017 and Pilliod et al. 2014).

### **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
- Waste water disposal location such as non-specified-use sink
- Clean bench paper
- Clean paper towels
- 2-Gallon Ziploc-type bags
- Dedicated water bottles: one for 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 Clorox 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<sup>o</sup> 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<sup>o</sup>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 min 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 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 COCs. 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 Survey 123 Form (formerly Exhibit 2) and submit via Survey 123 within 10 business days of the last day of sampling. If the trip was executed as planned, use the check boxes provided in the Survey 123 Form, sign as the eDNA Lead and submit the Survey 123 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 Survey 123 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 COCs in Survey 123, then package and ship samples to WGL (See Section B3 for shipping details).
3. QA/QC and/or edit sample collection and processing data in ArcGIS Online within 10 business days of last sampling day. Notify Jeena Koenig (eDNA Database Coordinator) by e-mail (jeena\_koenig@fws.gov) when data have been finalized.

## **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** above Step 13.
3. Preserve samples in the trailer with methods described in section **B2. Sample Processing Procedure**.

### **Shipping Procedure**

1. Centrifuge tubes must be shipped organized in racks, secured in 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 counter-clockwise 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 back into a 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 as 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 Survey 123 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 Survey 123 COC forms are as important as the samples themselves. If Survey 123 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 Survey 123 Shipping COC is submitted. If you do need to contact WGL, contacts are listed below.

Aaron Johnson Phone: 608-518-9800 Email: aaron_e_johnson@fws.gov	Kyle Von Ruden Phone: 608-780-9022 Email: kyle_vonruden@fws.gov	Zeb Woiak Phone: 608-304-4179 Email: zebadiah_woiak@fws.gov
Nick Grueneis Phone: 608-518-0129 Email: nikolas_grueneis@fws.gov	Maren Tuttle-Lau Phone: 608-780-0834 Email: maren_tuttle-lau@fws.gov	Rachel Arnold Phone: 608-304-3368 Email: rachel_arnold@fws.gov
Austin Hannah Email: austin_hannah@fws.gov Phone: 608-769-5344	Pat DeHaan Email: patrick_dehaan@fws.gov Phone: 608-518-6133	Joel Wils Email: joel_wils@fws.gov Phone: 608-284-0197

Upon receipt of the shipment at WGL, once the samples are checked in, staff will sign the Survey 123 COC form.

### **Receipt of Tubes**

Once the samples have been shipped to WGL, designated personnel will receive them. NOTE: alcohol-preserved centrifuged samples are shipped at ambient temperature, there is no need to send refrigerated.

- Upon receipt of samples from the field, the shipped box (es) should be opened. The general condition of the box (es) should also be assessed.
- Centrifuged samples can be stored with preservative at ambient temperatures for up to three weeks, post receipt at the lab. In effort to minimize DNA degradation, after three weeks samples must be decanted, dried, and frozen. Emergency storage of samples in  $-20\text{ }^{\circ}\text{C}$  freezers is allowable until room in the  $-80\text{ }^{\circ}\text{C}$  can be made.
- If samples cannot be spun and dried within 3 weeks, samples still in ethanol can be stored at  $-80\text{ }^{\circ}\text{C}$  until they are processed.
- Review the Survey123 COC forms that correspond to the samples. Note any condition issues (broken tape or seals or damaged containers) with the samples on the COC forms. Note any samples that must be discarded due to condition issues and the reason for discard. Any corrected error on form should be communicated to the person who made the error, and the correction must be noted, dated and initialed in the comments section of the electronic form.
- Enter sample data into internal Case Log including noting any samples that are being discarded and that should not be analyzed, and complete WGL check in form. Note any observations about samples such as condition issues. Log samples into the Bench Top Shelving Log.
- Alert shipping office that samples have been received through Microsoft Teams.

### **B3. Preservative Decanting and Alcohol Evaporation Procedure**

1. Centrifuged samples need to be re-spun to re-concentrate sample at the bottom of the tube after shipping. Swirl liquid to re-capture any observable debris from the side of the tube wall.
2. Centrifuge at max speed for 5 minutes. Effort should be taken to minimize the time between centrifuging and decanting ethanol.
3. Centrifuged samples preserved with alcohol must have the alcohol evaporated away before extracting the samples.
4. Remove cap from tube and gently pour the preservative from the tube into an appropriate collection vessel with funnel. The tube can be placed back into the original rack or into a new rack depending upon personal preference. Caps should be placed on a clean tray in numerical order of removal. Tubes will be open in the hood at this point, the drying process in the hood aids in the evaporation of the alcohol preservative from the tubes.

5. Move samples in tube racks to the laminar flow hood. Turn on the air flow and leave the samples to dry until all traces of ethanol or isopropanol smell are gone, because these are both PCR inhibitors. Do not let samples sit at room temperature longer than it takes to evaporate the preservative, and never over a weekend. If some samples in a case are taking longer than the rest to dry, put dry samples in the freezer, and allow the remaining samples to evaporate until they are dried. If working in advance, evaporated samples should be stored at -80 °C until extraction.
6. There should be at least one hood control before and after 8-10 sample racks in a hood. A maximum of 3 hood controls for a full hood. A minimum of 2 hood controls should be in place for sample that do not fill a full hood. Hood controls are prepared by placing a clean 50 mL centrifuge tube in a clean rack in the hood along with the range of samples that were placed in the hood to evaporate. The hood control should contain the case number and sample range included with the control. The lid should be labeled as well, since the tube and lid are separated during the evaporation process.

### **General DNA Assay Considerations (Extraction and PCR Amplification)**

- Non-sterile, non-PCR ready microcentrifuge tubes (MCT) and glassware used in the lab must be autoclaved at 121°C for 30 min before being used. Any re-used items must be soaked in a freshly made 10% bleach solution for 10 minutes followed by a thorough rinse. (Sterile, certified DNA- free MCT do not need to be autoclaved prior to use).
- Good laboratory practices should be considered at all times, including but not limited to: Reagents that have passed expiration dates should not be used, unless they have been tested in the lab and demonstrate that they are still effective at the same level as non-expired reagents. Reagents that have been stored at insufficient temperatures should not be used. All reagents must be clearly labeled with lot numbers and expiration dates and recorded on the appropriate data sheet.
- The temperatures of cold storage units must be monitored at a minimum of at least once per week and recorded in the Survey123 application. For cold storage units that are not on an alarm system or generator back-up temperature should be monitored more frequently. Temperature files will be downloaded at the end of each season and kept on an internal server.
- Positive and negative control samples should be used to test the performance of all new batches of reagents prior to their application to eDNA samples. Directions for preparing extraction negative and positive controls are listed in section **B.5 Quality Control**.
- Sterile techniques and good laboratory practices should be used in the DNA laboratory to prevent contamination through the transfer of DNA between surfaces, and to prevent cross-contamination between samples. Contamination can adversely affect the outcome of a case; therefore, it is essential to follow procedures listed in this section to limit the chance of contamination.
- Gloves (e.g., powder-free nitrile or latex) must be worn throughout sample processing. At a minimum, gloves should be changed at the completion of each step of the process. If gloves become contaminated or if contamination is suspected, discard them and replace them with new ones. Clean gloves should be used when using or handling keyboards, notebooks, pens, telephones, etc. and must be replaced immediately before recommencing bench work.
- Centrifuge tubes before opening the reagents. Uncap and close tubes carefully to prevent contamination through the spread of aerosolized DNA.
- To prevent damaging equipment, ensure that centrifuges are always balanced, pipettes are regularly calibrated, and freezers are defrosted yearly or as needed.
- All freezers should have the ability to be locked (with non-universal locks or marine brackets attached that can be used with keyed locks), or be housed in a secure facility.
- Ensure that all equipment, including paper, pens, and lab coats, are dedicated for use only in that particular laboratory (e.g., laboratory coat for each stage of procedure rooms). Each room dedicated to eDNA sample processing should have a lab notebook to contain data sheets related to that specific

area. Lab notebooks shall remain in each dedicated space until the end of each season when they are removed to be archived. Each room should have note-taking materials (e.g., loose-leaf paper, networked PCs) that can be used for consolidation in the Case Log. Other solutions for preventing contamination of sensitive areas may be used following approval by the eDNA Processing Lead and eDNA Processing QA/QC Specialist. Any changes should be incorporated into a revised QAPP.

Laboratory notes/notebooks should be written or printed with tamper-proof procedures:

- Lab notebooks should be unique and have unique identifiers, with consecutive page numbering, note/data entry dates, and initials (of the note-taker) on each page. A user identification page should be included with the printed, signed, and initialed name of each person entering notes/data into the lab book.
- Only permanent blue ink should be used. Special pens may be required for certain paper types.
- Any changes to the notebooks must be dated and initialed by the person who made the change. Any incorrect information should have a single line drawn through it and not be completely obscured.
- Lab notebooks should contain all data images (when appropriate, e.g., gel photographs and denaturing curves). Images should be permanently affixed to the notebook and signed across both the edge of the insert and the page.
- Any large data images or images that are not suitable for permanently affixing into a notebook should be kept on permanent record and its location, document, name, and relation to the assay referenced in the lab notebook.
- Notebooks must be kept in a locked drawer or cabinet with restricted access when not in use.

#### **Extraction Room Considerations:**

- Extraction of DNA must be performed where PCR products and stocks of cloned material are not handled.
- A PCR hood with a built-in ultraviolet (UV) light and HEPA filter may be used to further isolate DNA extraction kit solutions and elutes from ambient DNA when a dedicated DNA extraction space is not available.
- A separate set of necessary laboratory equipment, consumables, and laboratory coats should be dedicated for use in DNA extraction. The extraction data sheet is listed in Exhibit 8.
- Positive and negative extraction controls should be added to each eDNA extraction procedure batch.
- Before proceeding with extraction, a positive control swab is prepared by pipetting mixture which contains a 95:5 ratio of sterile lab DI water to Bluegill (BLG) or Brook Trout (BKT) cell lines or tissue slurry directly onto a sterile cotton swab in 1.5-ml MCT. A batch of extraction positives can be prepared ahead of time and frozen at -20 °C.
- Additionally, an extraction negative control sample should be prepared by pipetting 100 µl of sterile lab DI water onto a sterile cotton swab in a 1.5-ml MCT. A batch of extraction negative controls can be prepared in advance and kept frozen at -20 °C.

#### **B4. Analytical Methods: IBI Scientific gMAX Kit Extraction Procedure**

1. Obtain one Qiagen lyse and spin column per sample to be extracted, open and organize tubes in rack. Label both caps of each sample. You will have time to label the rest (1 lab-supplied 1.5-mL MCT, 1 GD spin column, and 3 IBI collection tubes) during the 30 minute incubation. Be sure to add one positive and negative extraction control to each eDNA extraction procedure batch.
2. Add 350 µL **GSB** to each lyse and spin basket tube. If precipitate has formed in GSB buffer, dissolve by incubating at 60 °C for 10 minutes, before dispensing into tubes.
3. Add 35 µL **proteinase K** to each lyse and spin basket tube.
4. Move dried samples in 50 ml centrifuge tubes to the extraction room. Make sure each tube is dry.

5. Remove a sterile swab from pack, and dip it into a numbered tube of GSB/proteinase K mix. Use the moistened swab to swab the bottom of each tube included in the field sample (e.g. there is 5 tubes per sample, swab the bottom of all 5 tubes with the moistened swab). If the swab becomes covered with debris, rinse swab in tube of GSB/proteinase K solution for that sample, and proceed swabbing the rest of the replicates. Be careful to avoid cross-contamination at this step.

**NOTE:** If a situation arises where a swab falls during the swabbing process (lab bench, floor, garbage, or MCT rack) the swab should be discarded. A note should be made in the logbook and in the appropriate case notes on the eDNA CaseLog spreadsheet. Also indicate what tube number the swab was discarded at (ex: swab fell on floor during tube 2 of 5, new swab for tubes 3 through 5). If a sample has a lot of organic material, there may be a need to make an additional tube of the GSB/Proteinase K mix. The tube would get the same label as the original tube (ex: 22001001a) and both tubes will be combined when the sample is loaded onto the GS spin column in steps 10-12. If multiple tubes of GSB/ProK are used, that should also be noted in the logbook and in the appropriate case notes on the eDNA CaseLog spreadsheet.

6. Place the swab back into the GSB mixture, break the wooden stick as close to the swab as possible, and close the tube. Change gloves between samples. For particularly dirty samples, add mixed GSB and ProK to any samples that appear dry or low so that the level appears to be about 350  $\mu$ L. A sufficient combination of GSB and ProK should be added to maintain enough liquid for incubation. Ensure maximum extraction of the sample.
7. Incubate at 60°C for 30 minutes. Label the rest of the tubes, place GD spin columns in tube rack and print final archive labels for extracts. Archive labels are the case number and three digit sample number (e.g., 22001001). Also during this time place the Elution Buffer into the 60°C bead bath.
8. Remove lyse and spin tube from incubator and centrifuge at max speed for at least 1 minute, up to 3 minutes depending on level of sediment in tubes. IF samples are particularly dirty, spin at least 3 minutes. If the basket is clogged, use a pipette to remove the supernatant from the sample, recovering as much as you can. Make notes in the Case Log.
9. Add 500  $\mu$ L **ethanol** (100%, molecular grade) to the extract. Mix thoroughly by vortexing. If liquid collects around the cap, spin briefly before opening to reduce contamination risk.
10. Transfer up to 780  $\mu$ L of the mixture by pipette into a GD spin column placed in a 2 mL collection
11. tube. Centrifuge at  $\geq$  6000 rpms for 1 minute. Discard flow-through and collection tube.
12. Transfer any remaining mixture by pipette onto the same spin column and place in a new 2 mL collection tube. Centrifuge again at  $\geq$  6000 rpms for 1 minute. Discard flow-through and collection tube.
13. Place GD spin column in a new 2ml collection tube. Add 400  $\mu$ L Buffer **W1**. Centrifuge at 6000 rpms for 30 seconds. Discard flow-through and collection tube.
14. Place spin column in a new 2 mL collection tube. Add 600  $\mu$ L **Wash Buffer**. Centrifuge at 18,000 rpms for 3 minutes. Discard flow-through and collection tube.
15. Transfer the spin column to a new 1.5 mL MCT.
16. Add 200  $\mu$ L of the pre-heated **Elution buffer** to the center of the spin column membrane. Incubate for 1 minute at room temperature (15 - 25°C). Centrifuge at  $\geq$  18000 rpms for 30 seconds.
17. Discard the spin column and store the eluted DNA samples at -20°C. If DNA is to be immediately used for PCR, keep in 4<sup>0</sup> C refrigerator.

### PCR Amplification

In order to determine if the DNA of a species is present in the concentrated water samples taken in the field, the total DNA extracted from the centrifuged samples must be amplified using species- specific primers. All samples will be screened with an assay that consists of two general Invasive carp markers that

detect two loci within the cytochrome oxidase I gene of both species of carp mitochondria (ACTM 1/3). The assay also consists of two markers specific to silver carp that detects two loci within the ND6 and ND2 regions of the mitochondria (SCTM 4/5), and finally two markers specific to bighead carp that detects two loci on the ND4 and ND6 regions of the mitochondria (BHTM 1/2). Any sample that has at least one detection for any loci of eight replicates will be considered a positive detection.

### **Reagent Room**

To prevent carry-over of amplified DNA sequences, when available, PCR reactions should be set up in a separate room from that used for post-PCR manipulations. If a separate and designated space is unavailable, PCR reactions should be set up in a dedicated clean fume hood with UV lights and a HEPA filter. A set of necessary laboratory equipment, consumables, and laboratory coats should be dedicated for pre-PCR manipulations. Reagents and supplies should be taken directly from clean storage into the PCR area and should never be taken to or shared between areas where post-PCR analyses are being performed. Equipment such as pipettes should never be taken to a pre-PCR area after use with amplified material.

### **PCR Amplification Considerations**

This stage of DNA processing is particularly susceptible to contamination and, subsequently, inaccurate results. Carefully follow the bullets listed below:

- This room is a contaminated area; therefore, no reagents, equipment, laboratory coats, etc. from this room should be used in any of the other lab areas.
- A biological or PCR-type hood may be used for setting up cloning or sequencing reactions.
- A set of necessary laboratory equipment, consumables, and laboratory coats should be dedicated for PCR assays.
- Every effort should be made to ensure that equipment, work areas, and solutions are free from DNA contamination. All surfaces should be wiped clean with 10% bleach solution (or commercial DNA eliminating solution such as DNAway) before and after use. If equipped with UV lamps, clean lab rooms or PCR hoods should be irradiated with UV light 30 minutes at the beginning and end of the workday.
- DNA elutes from samples should either be located in designated  $-20^{\circ}\text{C}$  freezer or carried from the DNA extraction room to PCR room. Ensure thermal cyclers are available before preparing the master mix.
- If using lab bench, it should be wiped before and after use with 10% bleach. Validated commercially available sterilization reagent such as DNAway may be used. PCR room should be sterilized using built-in UV lights if available.
- If using automated system for plating it should be UV'd after each use and the sample deck and racks wiped down with DNAway or 10% bleach if necessary.
- After an item or surface is cleaned with bleach, it must be rinsed with purified water or alcohol to prevent the build-up of sodium hypochlorite crystals. Instruments or equipment cleaned with bleach should be rinsed to avoid corrosion.
- Centrifuges, tube racks, pipettes, and any other equipment used for PCR amplification should be cleaned before and after each use.
- Use autoclaved, filtered, or commercially sterile molecular grade water for setting up PCR reactions.
- Aerosol-resistant pipette tips should be used. Place the sterile tip on the pipette immediately prior to use. If the pipette is set down with the tip on, discard the tip. A new pipette tip must be used for the addition of each reagent to a sample tube.
- Use PCR clean or autoclaved sample tubes for PCR master mix.

- Include a positive and negative PCR control in the plate set-up.
- A negative PCR control is prepared by pipetting 200  $\mu$ L of autoclaved sterile lab water into a clean 1.5-ml Eppendorf MCT.
- A positive PCR control is prepared by previously extracting Invasive carp cell lines or tissue slurry (both Bighead and Silver Carp) and diluting to an appropriate volume to not overwhelm the reaction. Positive PCR controls should be tested ahead of using for official eDNA monitoring cases.
- Close each tube immediately after labeling and after the addition of sample or reagents to prevent cross-contamination.
- Be sure to only touch the tip of the MCT cap or use a tube opener, clean Kimwipe®, or other suitable barrier to open MCT.
- Record all solution batch numbers used for reactions in lab notes.
- PCR reagents should be aliquoted (a portion of the original stock) to avoid excessive repetitive freeze-thaw cycles and to protect stock reagents if contamination occurs.
- Lightly vortex (quick touch, because vigorous vortexing can damage *Taq*) to mix sample and quick-spin/centrifuge tubes before opening the reagents to avoid splashes or drips from cap when opening. Uncap and close tubes carefully to prevent aerosol contamination.
- A log should be used to track which PCR reactions happened on each thermal cycler. Log information should include the assay name, sample case, plate number, and the thermal cycler head ID (Exhibit 7).
- Note for Exhibit 9-10: Plate maps have been included for scenarios most processed at WGL.

#### **B4. Analytical Methods: PCR Amplification Procedure**

1. If DNA samples (extraction elutes) are removed from long term storage freezer, note this on sample log (Exhibit 6).
2. Use pre-printed plate map (Exhibits 9 and 10) or build plate map to determine which samples will be pipetted into which wells. Clearly mark plate identification on bottom edge skirt of plate. Write plate identification information (ddMONyy\_initials\_ASSAY\_case(sample#-sample#)\_Plate#) in lab notes.
3. Make sure sample map for each plate is entered into the pipetting robot or attached to lab notes and a signature is written across the map and lab book page.
4. Follow DNA amplification protocol detailed below.
5. Primers specific for the targeting loci described above (ACTM1/3, SCTM4/5, BHTM1/2). The PCR protocol has been optimized to utilize TaqMan Environmental Master Mix 2.0 (Life Technologies, Carlsbad, CA) for eDNA screening. If other brands of real-time PCR master mixes are used, optimization of the recipe and thermal profiles must be executed. Eight reactions are set up for each sample, in addition to negative and positive controls for each master mix.
6. Wipe lab bench area with 10% bleach or commercial DNA sterilization wipes. Also wipe down work area in PCR hood. Use built-in UV lamps to radiate clean room for 30 min prior to PCR set-up.
7. Electronic pipettors should be wiped down.
8. In the clean reagent room, obtain all PCR master mix reagents (using only those that have not expired or that have been tested and found viable).
  - TaqMan Environmental Master Mix 2.0.
  - One tube with combined ACTM1/3 forward and reverse primers at 10  $\mu$ M working dilution.
  - One tube with combined SCTM4/5 forward and reverse primers at 10  $\mu$ M working dilution.
  - One tube with combined BHTM1/2 forward and reverse primers at 10  $\mu$ M working dilution.
  - One tube with a combined FAM-labeled probe for ACTM1/3 and HEX-labeled (or another appropriate fluorophore for your instrument) probe for BHTM1/2 and CY5-labeled probe for SCTM4/5, all diluted



to 2.5  $\mu\text{M}$ . Double-quenched probes are preferred that include a ZEN quencher located about 9 base pairs from the fluorophore as well as an Iowa Blackhole Quencher on the 3' end of the probe, but regular TAMRA-quenched probes may also be used.

- Sterile molecular grade water (commercially sterile or Millipore filtered, autoclaved).
9. Allow reagents to thaw. Do not vortex primers or Taq too violently. Briefly spin down tubes to minimize aerosolization.
  10. Record in lab notebook the lot number and expiration date of all reagents used.
  11. Prepare PCR master mixes in clean reagent room. The master mix volume can be adjusted according to the number of samples to be processed. In order to make sure that master mix does not run out prior to supplying all the desired reactions (this may occur as a result of minor errors or variations in pipetting volumes), it is generally helpful to make more than enough master mix than is needed for the desired number of reactions. For example, if using the 8 channel pipette for the robot, calculate 14 extra reactions for a 384 well plate. If using a 96 well plate, calculate 4 extra reactions. NOTE: If positive extraction controls consist of a different species of DNA, be sure to make a small separate master mix for those samples and use primers specific to the content of the control sample. Negative extraction controls should be amplified with the Bighead and Silver Carp master mix and a second time with the extraction positive control assay.
  12. Each IC Combo Assay PCR 1X reaction should contain (see Exhibit 9):

Table 1. 1X PCR reaction for IC Combo Assay, total volume of 20 $\mu\text{L}$  per reaction

Volume $\mu\text{L}$	Master Mix Reagent Component
10.0	TaqMan Environmental Master Mix 2.0
1.0	each primer (10 $\mu\text{M}$ each, working dilution)
1.0	probe mix (2.5 $\mu\text{M}$ each, working dilution)
3.0	sterile water (*7.0 $\mu\text{L}$ in standard curve mix)
3.0	sample extract as template (*1.0 $\mu\text{L}$ gBlock template in standards)

13. TaqMan Environmental Master Mix is very viscous, and requires careful pipetting to ensure accuracy, and a new tip for each aliquot removed. The tip must be left in the mix for a few seconds after aspiration to ensure complete aspiration of the desired volume, and upon dispensing, a pause is required to allow the mix to pool in the tip before the final plunge to dispense the last drop of mix. If this mix is pipetted too quickly, there will be too little volume to fill the required wells. The electronic pipetting epMotion 5075s were specifically calibrated for this viscous mix. If other mixes are validated for use, new programs for the epMotion 5075s must also be developed.
14. Mix the master mix well by slowly inverting the tube several times. Avoid shaking or inverting too quickly to prevent creating bubbles. Move prepared mix from reagent room into PCR room.
15. Remove DNA extracts from freezer or fridge (fill out sample logs as needed), vortex (quick touch) or finger flick and quick-spin down the extract tubes. Take them into the PCR room. Place the 96- well PCR plate onto a clean surface, positioned from left to right.
16. WGL has epMotion 5075s (automated liquid handling robots) that will fill plates for cycling. If these are unavailable, detailed directions for loading plates follows. Fill all plate wells with 17.0  $\mu\text{L}$  PCR mix. FOR STANDARD WELLS only, add 2.0  $\mu\text{L}$  water. To avoid creating bubbles, which interferes with data collection, place the pipette tip gently in the very bottom of the wells. Fill wells from the bottom up. Do not dispense air into the mix.
17. Carefully pipette 3.0  $\mu\text{L}$  of each extract (or 1.0  $\mu\text{L}$  gblock + 2.0  $\mu\text{L}$  water in standards) to be delivered onto the side of each well of a column, changing the pipette tip between each sample. Again,

placement of the tip is important to avoid creating bubbles, so set the tip against the side of the well, above the level of master mix, and then deposit the aliquot of template.

18. Each column of eight wells should be filled with the same sample (i.e., eight replicates per sample to be tested). Twenty-three columns of the PCR plate can test 46 different samples, the last remaining column is for PCR negatives and positive controls and standard curve dilutions. Pipette 3.0  $\mu\text{L}$  of water into wells G, H, O, and P to serve as PCR negative samples. Five-point, five-fold standard curves with 10, 50, 250, 1250, and 6250 copies/ $\mu\text{L}$  will be used. The highest standard should be placed in well B followed by the next lowest standards through well F, and again in wells J-N. Place a mixed sample of both Silver and Bighead carp cell extract into wells A and I to serve as PCR positive samples. Note that standard wells need extra water (2.0  $\mu\text{l}$ ) added to the mix to account for only 1.0  $\mu\text{l}$  of standard template added to each well compared to 3.0  $\mu\text{l}$  DNA template added to each well for samples.
19. Extraction negative controls and hood controls will be run as a sample. Extraction positive controls will be run separately on a Bluegill assay (Takahara et al. 2013) or Brook Trout (Wilcox et al. 2013) placed consecutively as they were extracted in the case.
20. Each Bluegill PCR 1X reaction should contain (see Exhibit 10):

Table 2. 1X PCR reaction for Bluegill Assay, total volume of 20 $\mu\text{L}$  per reaction

Volume $\mu\text{L}$	Master Mix Reagent Component
13.4	TaqMan Environmental Master Mix 2.0
1.8	forward primer (10 $\mu\text{M}$ each, working dilution)
1.8	reverse primer (10 $\mu\text{M}$ each, working dilution)
1.0	probe (2.5 $\mu\text{M}$ , working dilution)
2.0	template

21. A new tip should be used for each template or standard delivered in each well. Carefully observe the volume of liquid in the pipette tip is accurate. All templates or standard material is delivered on the side of the well, as described in (17).
22. If filling plates by hand in 96-well formats, before the PCR plate is sealed, check every well for bubbles. Bubbles may disrupt the accuracy of the camera detecting fluorophores and should be removed before the plate is sealed and put into the machine.
23. If filling plates on the epMotion robots, place PCR film over the PCR plate and use an automatic plate sealer to ensure the edges of all wells are sealed. Lightly vortex the sealed plate to mix contents, spin down the plate in the plate spinner to ensure mixture is in the bottom of wells and there are no bubbles.
24. WGL has a BioRad brand plate sealer. Use the seal guard to keep the seal from shifting. Turn on the sealer and ensure it is up to temperature. Seal 96 well plates at 180 $^{\circ}\text{C}$  for 3 seconds and 384 well plates at 167 $^{\circ}\text{C}$  for 3 seconds. Inspect the seal to ensure it is not loose, and all wells are sealed. To obtain reliable seals, temperature and time may be adjusted.
25. If you do not have a plate sealer, use adhesive seals and a plate roller or paddle to ensure a good seal. It is imperative to have a good seal to prevent evaporation which will prevent data collection, and potentially contaminate the lab with amplified DNA. Place the PCR plate in the thermal cycler, close and secure lid, and select the appropriate PCR thermal program. The thermal programs for the combo assay reaction is:
  - Initial denaturation at 95 $^{\circ}\text{C}$  for 10 min
  - 95 $^{\circ}\text{C}$  for 15 sec
  - 60 $^{\circ}\text{C}$  1 min with picture
  - Repeat 40 times

26. Record the plate ID, thermal cycler unit or head, and time of run start for the PCR plate in the PCR log (Exhibit 7).
27. Place cycled PCR plates and product in trash without opening the seal. Remove plates promptly. Under no circumstances should a PCR plate be opened or uncovered in the PCR room.

#### **B4. Analytical Methods: Standard Curve Material**

The use of standard curves in quantitative PCR applications allows for calculation of the initial starting copy number of target DNA in the aliquot of template used in each reaction. Standard curve materials will be synthetic strands of DNA purchased from a reliable production company. Standard curves are only used to assess assay performance and address Acceptance Criteria and are not used to for quantification purposes. WGL will purchase gBlock product from Integrated DNA Technologies, but other suitable vendors are allowed. To get reproducible and high-quality standard curve data, it is important to store materials properly, and make serial dilutions fresh each week. Storing for longer periods than one year can produce inaccurate results. The gBlock standard is purchased so that it contains all 6 qPCR marker targets (ACTM1/3, SCTM4/5, BHTM1/2).

1. gBlock is hydrated with TE buffer to make an initial dilution that is 1.00 E+10 copies per microliter. (an excel stock dilution calculator spreadsheet is on the shared drive at WGL).
2. Volume TE to add = fmoles\*0.0000000000000001\*6.022E+23/1.00E+10. Mix well.
3. Immediately use 1.0 µl of the hydrated gBlock into 999.0 µl of TE buffer to make storage stock with 1.0 E+7 copies/µl.
4. Make several aliquots of this stock and store in screw-cap tubes with o-rings to prevent evaporation in the freezer. Freeze remainder of stock from step 1 above.
5. Add 1.0 µl of 1.0 E+7 copies/µl stock to 31.0 µl 100 ng/µl yeast tRNA in water. This working stock has 312,500 copies/µl.
6. Serial dilutions for use in assays should be made fresh weekly with the stock and 100 ng/µl tRNA as a diluent. They may be stored frozen and thawed as needed throughout the week.
7. A **new** pipette tip **is required for each** dilution for accurate results.
8. After each addition, use the pipette to mix a **minimum** of 17 times.
9. Currently, a 5X curve is used at WGL.
  - 30 µl working stock into 270 µl tRNA. Mix 17 times = 31,250 copies
  - 60 µl of 31,250 mix into 240 µl tRNA, mix 17 times = 6250 copies.
  - 60 µl of 6250 mix into 240 µl tRNA, mix 17 times = 1250 copies
  - 60 µl of 1250 mix into 240 µl tRNA, mix 17 times = 250 copies
  - 60µl of 250 mix into 240 µl tRNA, mix 17 times = 50 copies
  - 60 µl of 50 mix into 240 µl tRNA, mix 17 times = 10 copies
10. WGL prepares 5 uL aliquots of the 1.0 E+7 copies/µl stock and uses that 3 times before discarding and using a fresh 1.0 E+7 copies/µl stock tube.

#### **B4. Analytical Methods: PCR Data Analysis**

1. Export data file from Bio-Rad and open using Bio-Rad CFX Manager software.
2. File names must be carefully entered without any mistakes in spacing or coding. File names will follow this format: ddMONyy\_initials\_ASSAY\_case#(sample#-sample#)\_Plate#\_cyclerSN. There should not be any spaces. Sample range should be integers separated with a dash. Plate numbers are designated as P1, P2, P3.....and no plate numbers should be repeated within any single case.
3. Dates must be as depicted with a two-digit day, three-letter CAP month, and two-digit year. Initials are not case sensitive, but assay **MUST BE CAPS**.
4. Immediately upon opening a BioRad result file, click on PLATE SETUP, then REPLACE PLATE FILE and replace with the appropriate template. Under EDITING\_TOOLS, click SETUP WIZARD and enter the sample names.

5. Then use the baseline correction function (Apply Fluorescence Drift Collection found under SETTINGS menu in CFX software). This will adjust for wells that have abnormal drifting RFU values during the initial few cycles of a run and derives an estimated baseline.
6. Assay quality must be assessed before data is accepted. With a 3 assay multiplex it may still be necessary to manually adjust the baseline threshold. To do this click and hold on the assay's baseline and adjust the threshold to where a maximum R<sup>2</sup> coefficient is reached or adjust the baseline to the linear region of exponential growth on the amplification plot. The threshold line should not be placed in the plateau phase nor in the initial linear phase of amplification, which may be too low and in the background fluorescence.
7. Next, be sure that all assays have efficiencies between 80-120% and R<sup>2</sup> equal to or greater than 0.95. Then manually check results of PCR positives, PCR negatives and any Extraction Negatives that may be within the samples so that all are acceptable.
8. Once quality measures are deemed acceptable, export the data as a \*.txt file and save into a folder named with the case number.
9. Once text files are in the folder, the program R should be used to analyze the data. Output will include the following:
  - a. Summary statistics on assay quality including the efficiency (E) and R<sup>2</sup> of the duplicate standard curves on each plate.
  - b. Summary data for the total number of negative controls that were clean and the total number of positive controls that were positive.
  - c. A list of samples that had cycle thresholds less than or equal to 15 cycles that were changed to negative.
  - d. A list of positive detection samples will be provided.
10. Rerun data should be exported as a \*.txt file with the same naming convention, but saved within a folder nested within the folder with case results. The folder with re-run data must be named "reruns", all lowercase, one word.
11. Enter results and QC summary data into the CaseLog file (Exhibit 5). Biologists who are entering results should review entries for accuracy and completion. The eDNA Processing Lead will then review all associated detection data (Case Log entries and BioRad files).
12. Notify the eDNA Processing Lead immediately of any cases with positive detection results.
13. All results will be reviewed by the eDNA Processing QA/QC Specialist then reported by the eDNA Processing Lead.

## **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 very 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 Case Log (Exhibit 6).

#### **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 12).

#### **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. The acceptable range for hand-held or console measurements are  $\pm 3$  ft. Temperature readings of the sonar may be checked against a thermometer. Acceptable temperature readings are  $\pm 3^{\circ}\text{F}$ . These records should be kept in ink in a bound notebook, where the originals are kept on site in a secure location and copies are sent to the eDNA Coordinator at the end of each sampling season.
- 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 be also be calibrated annually.
- Daily temperature recordings will be taken 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

In order 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 below of which personnel are responsible for the data management practice, how often it will be performed and where/how the data will be stored.

### Data Management Responsibilities:

1. FWCOs are responsible for entering (automatic with ArcGIS Field Maps APP), proofing, and retaining field data. The ArcGIS Field Maps APP Instructional manual is a provided as a separate document. The ArcMap eDNA data editing manual can be used to properly enter data and facilitate QA/QC of the data. If possible, QA/QC and edits of missing or incorrect data should be completed and synced with the database within 10 business days after the last day of collection for a specific event or before the next sampling event to be conducted at the same location if that is to occur in less than 10 days. This removes the possibility of confusing old and new data points and declutters the base maps used for data collection. Once data have been proofed and edited, any changes must be synced back to the database. After this is complete, the station eDNA lead will inform the eDNA Database Coordinator so that completed data can be cleared from the ArcGIS Field Maps APP map. If other field work prevents proofing and QA/QC of data to take place within the required 10 business days, FWCOs must notify the eDNA Database Coordinator and eDNA Program Coordinator. FWCOs should retain an electronic copy of the completed dataset for each of their eDNA events. A list of data collected and an explanation of data fields are provided in Table 1 for ArcGIS Field Maps APP.
2. Hard copy and electronic data (final QA/QC of field data) should be backed up regularly.
3. Survey 123 electronic Shipping COC forms will be submitted to WGL when samples are shipped. WGL will receive an email via a web hook when the Survey123 Shipping COC form is submitted. Survey 123 COC instructions are provided in Appendix B. A list of field descriptions for Survey123 Shipping COC forms are included in Tables 2 through 4. WGL will use the eDNA Shipping Alerts channel in Teams to let FWCO offices know when samples are received in the lab. The shipping

- FWCO may request a hard copy of their Survey123 Shipping COC data, however all COC data will be stored in an ArcGIS database and will be maintained by the eDNA Processing QA/QC Specialist.
4. Survey 123 electronic Post-Trip Field Summary Survey 123 forms will be submitted to WGL when samples are shipped. WGL will receive an email via a web hook when the Survey123 Post-Trip Field Summary form is submitted. Post-Trip Field Summary form instructions are provided in Appendix E. All Post-Trip Field Summary Survey 123 data will be stored in an ArcGIS database and will be maintained by the eDNA Processing QA/QC Specialist.
  5. WGL staff are responsible for keeping an internal CaseLog for all samples.
  6. WGL staff will keep a record of location of samples stored on benchtops before processing or within freezers after samples are dried prior to extraction should be maintained (such as a log-in/log-out record book). WGL staff will track all sample movement to or from a specified location, and movements will be recorded via signature on the freezer or benchtop shelving log (see Exhibit 8).
  7. WGL will maintain hard copies of laboratory notebooks and the electronic CaseLog in an excel file. Raw data files from the real-time instruments will be processed with standardized R-code, and all result files will be kept in a folder labeled with the case number. These files will be backed up on the WGL and MFC servers.
  8. WGL will scan data notebooks into PDFs by case and these will be saved on the lab server and backed up on the MFC server.
  9. The CaseLog serves as a way to track cases and samples through the lab workflow.
  10. Final reports will be generated as each case is completed. Each report will be sent to the eDNA Program Coordinator and saved electronically in the WGL and MFC servers.
  11. An Enterprise Data Management Plan has been completed for the IC eDNA Monitoring Program.
  12. The Survey123 Shipping COC and Post-Trip Field Summary data and all laboratory generated data files will be maintained on the WGL and MFC servers.
  13. The eDNA Database Coordinator will backup and maintain the eDNA sample data in at least three backup locations. The eDNA Database Coordinator will also routinely back up eDNA sample data from the ArcGIS Online eDNA Fisheries Group once a week. Field offices should still download and maintain the data collected by their office (per 2. Above). A list of field descriptions that contain data descriptions for reported results is listed in Table 5.
  14. The eDNA Processing Lead will be responsible for reporting results with the eDNA Program Coordinator and eDNA Database Coordinator within 48 hours of results completion.
  15. USFWS Denver Enterprise Team (FWS Geospatial Warehouse) will maintain an electronic folder that contains the file geodatabase complete with field and lab data.

Table 3. Data dictionary for the invasive carp eDNA field sample point feature class.

Field Name	Alias	Type	Length	Description
RUID	Regional Unique ID	Long Integer	8	Composite number that has 2-digits representing the year (YY), a 3-digit case (CCC) number; a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event, followed by a 3 digit sample ID number (SSS) assigned by the FWCO numerically as they take samples in the field (no punctuation, YYCCSSS). Used as the primary key to join to the eDNA Data Steward Report table.
FWCO_ID	Sampling Station ID Code	Text	3	Alpena FWCO – ALP; Ashland FWCO – ASH; Carterville FWCO – CAR; Columbia FWCO – COL; Green Bay FWCO – GRB; La Crosse FWCO - LAX; Lower Great Lakes FWCO – LGL The office who lead the collection of samples and collected the eDNA sample field data.
STATE	State	Text	2	Two letter U.S. state code in which samples were collected.
BASIN	Basin	Text	4	LH = Lake Huron; LM = Lake Michigan; LE = Lake Erie; LS = Lake Superior; LO = Lake Ontario; CAWS = Chicago Area Waterway System; UMR=Upper Mississippi River; OHR=Ohio River; MOR=Missouri River; OTHR=Other (applicable to older eDNA field data) -Other designations to be added as needed by Fisheries Data Steward; River basin where samples were collected.
WATERBODY	Waterbody	Text	100	Waterbody name of where sample was collected. A set domain list is used to standardize waterbody name for each sampling site.
DATE_COLL	Date Collected	Date	n/a	Date of collection (MM/DD/YYYY)
WIND_DIR	Wind Direction	Text	20	Abbreviated wind direction at time of sampling, “Calm”, “Variable”, or shorthand (NE, NW, SE, SW, N, S, E, W)
LATITUDE	LATITUDE	Double	n/a	WGS 84 Decimal Degrees, 5 decimals
LONGITUDE	LONGITUDE	Double	n/a	WGS 84 Decimal Degrees, 5 decimals
TEMP_F	TEMP_F	Double	n/a	Temperature of the water in degrees Fahrenheit (1 decimal). Temperature could be in Fahrenheit or Celsius. Fahrenheit became standard measure in 2016. A -999999 means no data.



Field Name	Alias	Type	Length	Description
DEPTH	DEPTH	Double	n/a	Depth where sample was taken in Feet (1 decimal). Depth for all cases for all years could be reported either in meters or feet. Feet became standard measurement in 2016. A -999999 means no data.
BLANK	BLANK	Text	5	Yes/No (Field Control: Tube filled with distilled water)
HABITAT	HABITAT	Text	30	Habitat type where sample was collected, i.e. LDB (Left Descending Bank), RDB (Right Descending Bank), MC (Main Channel), SC (Side Channel), RAB (Right Ascending Bank), LAB (Left Ascending Bank), LAB-SC (Left Ascending Bank-Side Channel), RAB-SC (Right Ascending Bank-Side Channel), MC-SC (Main Channel-Side Channel), OW-B/BW/M (Open Water-Bay/Backwater/Marina), SH-B/BW/M (Shoreline-Bay/Backwater/Marina), DAM-TW (Dam Tailwaters), OTHER (Other), IMP (Impoundment)
COLLECT_TIME	Collection Time	Long Integer	4	Time stamp in military time, no punctuation when sample was collected
PROCESSING_TIME	Processing Time	Long Integer	4	Time stamp in military time, no punctuation when sample was processed in the eDNA trailer either by filtering or centrifuge. Please see the Quality Assurance Plan for more information (QAPP).
PROCESSOR	PROCESSOR	Text	3	Initials of person who filtered/processed the sample
COMMENTS	COMMENTS	Text	255	General notes, issues, or observations while sampling or processing

Table 4. Data dictionary for the eDNA result data steward report table generated by the Whitney Genetics Lab.

Field Name	Alias	Type	Length	Description
Case_Number	Case Number	Long Integer	5	Composite number that has 2-digits representing a year (YY) and a 3-digit case number (CCC); a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event.
Sample_Number	Sample Number	Integer	3	A 3 digit sample ID number assigned by the FWCO numerically as they take samples in the field (no punctuation, #####)
Unique_ID	Unique ID	Long Integer	8	Composite number that has 2-digits representing the year (YY), a 3-digit case (CCC) number; a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event, followed by a 3 digit sample ID number (SSS) assigned by the FWCO numerically as they take samples in the field (no punctuation, YYCCSSS). Used as the foreign key to join the field data to eDNA sample field data (Primary key: RUID).

Field Name	Alias	Type	Length	Description
Any_Detection	Any Detection	Integer	1	1= detection of at least 1 of the 3 assays (Bighead, Silver, or Invasive Carp), 0= no detection of any assay, null= no detection data
ACTM_Assay	ACTM Assay	Integer	1	1= detection of at least 1 Invasive Carp qPCR replicate, 0= no detection, corresponds to one of three assays running in multiplex, null= no detection data
BHTM_Assay	BHTM Assay	Integer	1	1= detection of at least 1 Bighead carp qPCR replicate, 0= no detection, corresponds to one of three assays running in multiplex, null= no detection data
SCTM_Assay	SCTM Assay	Integer	1	1= detection of at least 1 Silver carp qPCR replicate, 0= no detection, corresponds to one of three assays running in multiplex, null=no detection data
eDNA_Detection_Summary	eDNA Detection Summary	Integer	1	Summary results from the ACTM_Assay, BHTM_Assay, and SCTM_Assay. 0= no detections, 1= detection ACTM only, 2= Bighead or BHTM and ACTM only, 3= Silver or SCTM and ACTM only, 4= Both Bighead (BHTM) and Silver (SCTM) with or without ACTM detection. null= no detection data

Field Name	Alias	Type	Length	Description
eDNA_Detection_Status	eDNA Detection Status	Text	255	Summary results from the ACTM Assay, BHTM Assay, and SCTM Assay in text for symbolizing on detection maps. No eDNA detected= no detections, IC carp eDNA only detected= detection of ACTM only, Bighead carp eDNA only detected= Bighead (BHTM) and ACTM only, Silver carp eDNA only detected= Silver (SCTM) and ACTM only, No eDNA detected= no detections, Bighead carp and Silver carp eDNA detected= Both Bighead (BHTM) and Silver (SCTM) with or without ACTM detection. No detection data= detection data not available
Reportable_Data	Reportable Data	Text	255	Yes/No, Yes = data is reported to the public, No = data is not reported to the public.
Notes	Notes	Text	255	Any relevant lab notes pertaining to the eDNA detection data.

Table 5. Field descriptions for the Shipping eDNA Chain of Custody Form

Field Name	Alias	Type	Length	Description
Unique_Username	Username	String	255	The ArcGIS username of the individual logged in to the Survey123 application.
Case_Number	Case Number	Integer	5	Composite number that has a 5-digit “case” number, a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event.
Sample_Range	Sample Numbers	String	50	Sample number range being shipped.
Date_of_Collection_Start	Date of Collection Start	Date	255	Start date of collection of samples (M/DD/YYYY)
Date_of_Collection_End	Date of Collection End	Date	255	End date of collection of samples (M/DD/YYYY)
Shipped_by	Shipped by (first and last names)	String	255	Individuals who shipped the samples.
Collection_Office	Collection Office	Select1	255	FWCO office who shipped the samples.
Notes	Notes	String	255	General notes, issues, or observations about samples.
Shipping_Notes	Shipping Notes	String	255	Note of how many boxes are being shipped and which boxes contain which samples.
Sample_Numbers	Sample Numbers	String	255	Sample number range being shipped.
Released_by	Released by: (Name, Agency)	String	255	Name and Agency of who is releasing samples
Release_Signature	Release Signature	Text	255	Digital signature of who is releasing samples.
Release_Signature_Certified	I certify the digital release signature above represents my signature for Chain of Custody purposes.	Select1	255	A yes check box to certify the digital signature represents the individual signature for the chain of custody purposes.
Release_Date	Release Date	Date	255	The release date of the samples (M/DD/YYYY).
Delivered_by	Delivered via	Select1	255	How were samples transferred: options include FedEx, In Person, and Other
Delivered_by_Other	Delivered via Other	String	255	How were samples transferred by other.
Received_by	Received by	Select1	255	Name of WGL staff who is receiving the samples.

Field Name	Alias	Type	Length	Description
Receipt_Signature	Receipt Signature	String	255	Digital signature of who is receiving the samples.
Receipt_Signature_Certified	Select1	String	255	A yes check box to certify the digital signature represents the individual signature for the chain of custody purposes.
Receipt_Date	Receipt Date	Date	255	The receipt date of the samples (M/DD/YYYY).
Editing_Notes	Editing Notes	String	255	Any changes to the form denoted with the initials of the individual making the change and the date when the change was made

Table 6. Field descriptions for the individual FWCO eDNA Chain of Custody Form

Field Name	Alias	Type	Length	Description
Unique_Username	Username	String	255	The ArcGIS username of the individual logged in to the Survey123 application.
Case_Number	Case Number	Integer	5	Composite number that has a 5-digit “case” number, a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event.
Sample_Range	Sample Numbers	String	50	Sample number range being transferred.
Date_of_Collection	Date of Collection	Date	255	Date of collection of samples (M/DD/YYYY)
Collected_by	Collected by (first and last names)	String	255	Individuals who assisted in collecting the samples.
Collection_Office	Collection Office	String	255	FWCO office who lead the collection of samples.
Notes	Notes	String	255	General notes, issues, or observations about samples being transferred.
Editing_Notes	Editing Notes	String	255	Any changes to the form denoted with the initials of the individual making the change and the date when the change was made

Table 7. Field descriptions for eDNA results table.

Field Name	Alias	Type	Length	Description
Case_Number	Case_Number	Long Integer	5	Composite number that has a 5- digit “case” number, a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event.
Sample_Number	Sample_Number	Integer	3	A 3 digit sample ID number assigned by the FWCO numerically as they take samples in the field (no punctuation, #####)
Unique_ID	Unique_ID	Long Integer	8	Composite number that has a 5- digit “case” number, a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event, followed by a 3 digit sample ID number assigned by the FWCO numerically as they take samples in the field (no punctuation, #####). Used as the join field to link the field data to eDNA sample result data.
Any_Detection	Any_Detection	Integer	1	1= detection of at least 1 of the 3 assays (Bighead, Silver, or Invasive Carp), 0= no detection of any assay
AC_Assay	AC_Assay	Integer	1	1= detection of at least 1 Invasive Carp qPCR replicate, 0= no detection, corresponds to one of three assays running in multiplex



Field Name	Alias	Type	Length	Description
BH_Assay	BH_Assay	Integer	1	1= detection of at least 1 Bighead carp qPCR replicate, 0= no detection, corresponds to one of three assays running in multiplex
SC_Assay	SC_Assay	Integer	1	1= detection of at least 1 Silver carp qPCR replicate, 0= no detection, corresponds to one of three assays running in multiplex
eDNA_Detection_Summary	eDNA_Detection_Summary	Integer	1	Summary results from the AC_Assay, BH_Assay, and SC_Assay. 0= no detections, 1= detection AC only, 2= Bighead or Bighead and AC only, 3= Silver or Silver and AC only, 4= Both Bighead and Silver with or without AC detection.
Notes	Notes	Text	255	Any relevant lab notes pertaining to the eDNA detection data.

Table 8. Field descriptions for Exhibit 2 Survey123 Form.

Field Name	Alias	Type	Length	Description
Case	Case Number	Integer	5	Composite number that has a 5-digit "case" number, a series of unique numbers assigned by the Whitney Genetics Lab (WGL) to each Fish and Wildlife Conservation Office (FWCO) at the start of the sampling year and provided for each individual planned sampling event.
Basin	Sample Basin	String	255	The watershed the sample was collected from.
Begin_Date	Date of Collection Start	Date	255	Start date of collection of samples (M/DD/YYYY)
End_Date	Date of Collection End	Date	255	End date of collection of samples (M/DD/YYYY)
Post_Trip_Summary	Post Trip Summary	Select1	255	Sampling plan was completed as described in Exhibit 1 OR Sampling plan was NOT completed as described in Exhibit 1 (Describe below)
Description_of_deviation_s	Description of deviations from Exhibit 1	String	1000	List and description of any deviations from Exhibit 1, only available if "Sampling plan was NOT completed as described in Exhibit 1 (Describe below)" was selected in Post Trip Summary
Field_Operations_Manager	Field Operations Manager (first and last name)	Text	255	Individual who was the Field Operations Manager
Sampling_Quality	Sampling Quality	Select1	255	The Sampling QA/QC Specialist observed and witnessed adherence to the QAPP in the following criteria. A "Yes" or "No" check box is selected for each criteria "Boat decontaminated in the field", "Sample collection procedure", "Navigation to sample sites"
Explanation_of_Deviation	Explanation of Deviation	String	1000	List and describe any deviations from the QAPP, include reasoning. Only available if any "No" answer is selected in Sampling Quality
Sampling_QAQC_Specialist	Sampling QA/QC Specialist (first and last name)	Text	255	Individual who was the Sampling QA/QC Specialist

Field Name	Alias	Type	Length	Description
Processing_Quality	Processing Quality	Select1	255	The Processing QA/QC Specialist observed and witnessed adherence to the QAPP in the following criteria. A "Yes" or "No" check box is selected for each criteria "Decontamination of processing equipment", "Sample processing procedure"
Explanation_of_Deviation	Explanation of Deviation	String	1000	List and describe any deviations from the QAPP, include reasoning. Only available if any "No" answer is selected in Processing Quality
Processing_QAQC_Specialist	Processing QA/QC Specialist (first and last name)	Text	255	Individual who was the Processing QA/QC Specialist
Data_Quality_for_case_number	Data Quality Specialist certifies case number	Integer	255	The Data QA/QC Specialist completed the QA/QC of sample date for case number:
Data_quality_confirm	Data Quality Confirmation	Select1	255	The Data QA/QC Specialist confirms that data are accurate and represent the locations and conditions in which samples were collected. A "Yes" or "No" check box is selected
Explanation_of_Deviation	Explanation of Deviation	String	1000	List and describe any deviations, include reasoning. Only available if "No" is selected in Data Quality Confirmation
Data_QAQC_Specialist	Data QA/QC Specialist (first and last name)	Text	255	Individual who was the Data QA/QC Specialist
Notes	Notes	String	1000	General notes, issues, or observations about sample collection and processing
eDNA_Lead_Signature	eDNA Lead Signature (first and last name)	Text	255	Individual who was the eDNA Lead, certifying that the information input is correct and accurate and that they communicated with the field staff for the information that is entered.
eDNA_Lead_Signature_Certified	Select1	String	255	A yes check box to certify the digital signature represents the individual signature for Exhibit 2 purposes.
Date	Date of eDNA Lead Signing	Date	255	Date the eDNA Lead submits Exhibit 2 (M/DD/YYYY).

## Section C. Assessment and Oversight

### C1. Assessments and Response Actions

Assessment (audits) will be conducted every year on 3 of the 6 main identified IC eDNA 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.

#### C1. 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.

#### C1. 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 (Exhibit 12).

Laboratory problems and issues should be documented first in the paper laboratory notebooks, 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 any problem renders a result non-reportable, this will be highlighted in the weekly report made and the eDNA Processing Lead will provide notation in the final case report so that samples with no results are easily seen by the eDNA Program Coordinator and the eDNA Database Coordinator (Exhibit 11).

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 Database Coordinator.

#### D1. 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 Database Coordinator. 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 Database Coordinator 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 Database Coordinator 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 logbooks 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 Database Coordinator will ultimately serve as a validated data set.

#### D1. 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 Database Coordinator, other USFWS personnel as needed, and partner agencies as appropriate. To provide proof of Quality Assurance, upon completion of all sampling events, the Post-Trip Field Summary Plan Survey 123 Form (formerly Exhibit 2) be filled out and filed with the eDNA Program Coordinator. eDNA sampling events and processing will be tracked in the Master Tracker file hosted on the eDNA Program SharePoint site.

## **D2. 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 in order to save space. A record of all sample location will be kept (Exhibit 6). 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 12) 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 final 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 Database Coordinator will contain final eDNA results for each case for incorporation into the eDNA Database. In the event that final eDNA results are not able to be reported to the public (research or non-IC eDNA Monitoring work), those results will be indicated on the eDNA dashboard.

Each field sampling office keeps a record of field data. These records are summarized by the eDNA Database Coordinator and reported to the eDNA Program Coordinator. The eDNA Database Coordinator 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 USFWS Regional Office management staff. A specific results communication SOP is listed in Appendix A.



## Section E. Relevant eDNA Literature

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- Farrington, H.L., C.E. Edwards, X. Guan, M.R. Carr, and R.F. Lance. 2015. Mitochondrial Genome Sequencing and Development of Genetic Markers for the Detection of DNA of Invasive Bighead and Silver Carp (*Hypophthalmichthys nobilis* and *H. molitrix*) in Environmental Water Samples from the United States. *PLoS One* **10**:e0117803
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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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- Klymus, K. E., C. A. Richter, D. C. Chapman, and C. Paukert. 2015. Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *Biological Conservation* **183**:77-84.
- Lance, R. F., Klymus, K. E., Richter, C. A., Guan, X., Farrington, H. L., Carr, M. R., & Baerwaldt, K. L. (2017). Experimental observations on the decay of environmental DNA from bighead and silver carps.

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Refinement of eDNA as an early monitoring tool at the landscape-level: Study design considerations.

*Ecological Applications* **29**(6):1374-1388.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2014). Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources* **14**(1):109-116.

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.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley, A. R., & Schwartz, M. K. (2013). Robust detection of rare species using environment DNA: The importance of primer specificity. *PLoS ONE*, **8**(3), e59520.

**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	
<b>TOTAL</b>	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:   
QA/QC Specialist:

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 3

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

#### Training Checklist for Field Collection and Preservation Staff:

	Training type	Date Completed
	Collector App/ArcGIS Editing Manual	
	Survey123 COC procedure	
	Disinfection procedures (boat/coolers)	
	Trailer towing procedures	
	Boat operator	
	Water sample collection procedure	
	Centrifugation/preservation procedure	
	Sample shipment procedure	
	QAPP review	
	Midwest Fisheries Center building SOP for minimizing contamination	

Name of Trainee:

Signature of Trainee:

**Notes:**

Not all trainings are required for each eDNA sample collection and processing staff member. Consult the eDNA station lead for specific training requirements required by each position.

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 4

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

#### Training Checklist for Sample Analysis Staff in the Lab:

	Training type	Date Completed
	Sample receipt procedure	
	Survey 123 COC procedure	
	Sample drying procedure	
	Sample logbooks (check in/check out)	
	GLP refresher	
	DNA extraction competency	
	PCR analysis competency	
	QAPP review	
	Midwest Fisheries Center building SOP for minimizing contamination	

Name of Trainee:

Signature of Trainee:

### WGL eDNA Monitoring Sample Case Log Exhibit 5

Screen captures of Excel log file. First worksheet in workbook lists all sampling events or cases which correspond to samples from a particular system. Subsequent worksheets are created for each case and allow for sample tracking through the eDNA lab. This is our Laboratory Information Management Tracking system.

Case Log front page, screen capture 1

Priority	Case Number	Case Type	Number of Samples	Check-in Date	Shelf Storage Expiration Date
1					1/21/1900
2					1/21/1900

Case Log front page, screen capture 2

Days You Have to Move Samples to Freezer	Date Samples Were Moved to Freezer	Sample Progress	Status (Open/Closed)	Total Samples Analyzed
FREEZE				
FREEZE				
FREEZE				

Case Log front page, screen capture 3

eDNA Positive Detections (number of samples)					Quality Control Success (Y/N)		
No eDNA Detected	ACTM eDNA ONLY	Bighead Carp eDNA Only	Silver Carp eDNA Only	Both Bighead & Silver Carp eDNA	Extraction Control	PCR Control	Hood Control



### Laboratory Case Log: Individual Case File Exhibit 5

Screen capture of Excel log file with the first case sample log open. Samples are tracked by date and process step through the lab. Corresponding quality control results by extraction batch and PCR batch are reported with sample results. Quality control columns included in screen capture 2.

Case Log Sample Log open, screen capture 1

Sample ID		Date of Receipt	Initials	Shelf Storage Expiration Date	Days You Have to Move Samples to Freezer	Set to Dry Date	Initials	Dried Date	Initials	Extraction Date	Initials	Sample
Case #:	Sample											
	001			21	FREEZE							001
	002			21	FREEZE							002

Case Log Sample Log open, screen capture 2

Case eDNA Detection Summary							Sample
PCR Date	PCR Initials	ACTM Assay	BHTM Assay	SCTM Assay	Code Summary	Status	
							001
							002

Case Log Sample Log open, screen capture 3

EX POS - 1 OF 8 CONSIDERED ACCEPTABLE						PCR NEG - ANY HIT IS A RERUN						
EX NEG - ANY HIT IS CONSIDERED POS						PCR POS - NO HITS IS A RERUN, 1 OF 2 IS ACCEPTABLE						
Enter data for QC in the line corresponding to the last sample in the processing batch.						HC - any hits on any HC within the hood is considered positive for that sample range						
Quality Control Checks												
EXT + control	EXT - control	EXT - control	Sample Range	HC	HC	BLG PCR + control	BLG PCR - control	Sample Range	PCR + control	PCR - control	Sample Range	Sample
BLG #+'s of 8 reps	BLG #+'s of 8 reps	#+'s of 8 reps	for EXT controls	Positive 1/0	Hood Control Range	#+'s of 2 reps	#+'s of 4 reps	for BLG PCR controls	#+'s of 2 reps	#+'s of 4 reps	for PCR controls	
												001
												002

### Sample Storage Logs Exhibit 6

Sample Chain-of-Custody logs used in the lab. Samples are CHECKED IN to storage area once, and then each successive removal or return are logged in the CHECKED OUT spaces across the line.

#### -80 °C Sample Log

Freezer ID and Location: \_\_\_\_\_

Line Number	Case # and sample numbers CHECKED IN	Sample number range, state action taken (ex: extraction). Date and Initial CHECKED OUT.			

Example of ambient temperature (alcohol-preserved centrifuge samples only) sample storage log. Once samples are checked out of ambient storage into cold storage, the entry is closed in the ambient log.

Bench-top Shelving Log Room \_\_\_\_\_

Line Number	Case # and sample numbers CHECKED IN	Sample number range, state action taken (ex: extraction). Date and Initial CHECKED OUT.			

# Thermocycler Log Exhibit 7

## PCR Log

Month: \_\_\_\_\_ Year: \_\_\_\_\_ Machine ID: \_\_\_\_\_ Machine Location: \_\_\_\_\_

Plate ID	PCR Machine ID	Plate Orientation	PCR Program	Run Start Time	Run Finish Time	Date Initials

# Extraction Data Sheet Exhibit 8

"COPY" watermark is grey on original.

Extraction data sheet: to be filled out and taped into Extraction room data book

Analyst \_\_\_\_\_ Room# \_\_\_\_\_ Date \_\_\_\_\_ Sample batch \_\_\_\_\_

Reagents used: note lot ID and expiration date for kit components

Reagent/tubes	Lot #	Expiration date
GSB Buffer		
Proteinase K		
Ethanol		
W1 Buffer		
Wash Buffer		
Elution Buffer		

Tube ID	Sample ID	Tube ID	Sample ID
1		25	
2		26	
3		27	
4		28	
5		29	
6		30	
7		31	
8		32	
9		33	
10		34	
11		35	
12		36	
13		37	
14		38	
15		39	
16		40	
17		41	
18		42	
19		43	
20		44	
21		45	
22		46	
23		47	
24		48	

Write notes for any deviations from QAPP or lab blunders on facing page of notebook. Be sure to add the same notes to the case log.

|

# qPCR Sheet IC Combo Assay, 384-Well Exhibit 9

Combined ERDC ACTM1/3, BHTM1/2, SCTM 4/5 qPCR data sheet: to be filled out and taped into PCR room data book

Analyst \_\_\_\_\_ Date \_\_\_\_\_ Sample batch \_\_\_\_\_

Reagents and recipe: note batch (for diluted working primers), or lot ID and expiration date

SAMPLE PLATE MM

Reagent name	Volume per rxn	Volume for _____ rxns	Lot or ID #	Expiration date
TM Env. Master Mix	10.0 µl	_____ µl		
AC TM1/3 Primer Mix(10 µM)	1.0 µl	_____ µl		
BH TM1/2 Primers(10 µM)	1.0 µl	_____ µl		
SC TM4/5 Primers(10 µM)	1.0 µl	_____ µl		
AC TM1/3 Probe(2.5 µM)	1.0 µl	_____ µl		
BH TM1/2 Probe(2.5 µM)				
SC TM4/5 Probes(2.5 µM)				
H2O	3.0 µl	_____ µl	NA	NA

Samples: 3.0ul template + 17.0ul master mix

Standards: 1µl standard + 2µl H<sub>2</sub>O + 17.0 µl master mix

Plate Name \_\_\_\_\_

ddMONvy Initials ASSAY case(sample#-sample#) P#

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A																								POS	
B																									6250
C																									1250
D																									250
E																									50
F																									10
G																									NTC
H																									NEG
I																									POS
J																									6250
K																									1250
L																									250
M																									50
N																									10
O																									NTC
P																									NEG

Cycler ID \_\_\_\_\_

# qPCR Sheet Bluegill Assay, 96-Well Exhibit 10

Bluegill qPCR data sheet: to be filled out and taped into PCR room data book

Analyst \_\_\_\_\_ Date \_\_\_\_\_ Samples \_\_\_\_\_

Reagents and recipe: note batch (for diluted working primers), or lot ID and expiration date

Reagent name	Volume per rxn	Volume for _____ rxns	Lot or ID #	Expiration date
TaqMan Env. Master Mix	13.4 $\mu$ l	$\mu$ l		
Forward Primer 10 $\mu$ M	1.8 $\mu$ l	$\mu$ l		
Reverse Primer 10 $\mu$ M	1.8 $\mu$ l	$\mu$ l		
Probe (2.5 $\mu$ M)	1.0 $\mu$ l	$\mu$ l		

2  $\mu$ l template

Plate Name \_\_\_\_\_

ddMONvy Initials ASSAY case(sample#-sample#) P#

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A																								POS	
B																									
C																									
D																									
E																									
F																									
G																									NTC
H																									NEG
I																									POS
J																									
K																									
L																									
M																									
N																									
O																									NTC
P																									NEG

Notes for any lab blunders or deviations from protocol:

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Cycler ID \_\_\_\_\_

# Corrective Action Form Exhibit 11

## 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 12

## 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 SOP**

## **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 (USFWS) 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 Great Lakes Region 3, 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 Database Coordinator by WGL once processing in the lab is completed.
4. The eDNA Database Coordinator will compare the datasets provided by WGL and the FWCO to ensure accuracy and completeness, then combine to create the final results package to be sent 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 R3 FAC Leadership consisting of the FAC Deputy Assistant Regional Director (Deputy ARD) and ARD, Regional AIS Supervisor, National IC Coordinator, FWCO Program Supervisor, USFWS Invasive Carp Regional Coordinating Committee (ICRCC) Co-Chair, impacted FWCO (Project Leader and eDNA Lead) and External Affairs.
6. eDNA results information is to be kept confidential. Any potential communication with partner groups or other entities outside of USFWS will not occur prior to informing the affected partner.
7. If necessary, R3 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 External Affairs Congressional staff member.
10. The communication and briefing process from eDNA Program Coordinator to the R3 Regional Director and/or Deputy Regional Director should occur quickly. This will allow R3 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 USFWS IC eDNA Monitoring Program Dashboard. However, upon written request from an affected partner, the USFWS 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. USFWS does not issues press releases for eDNA results but USFWS External Affairs will assist. The eDNA Program Coordinator will coordinate the public release of results with the partner led press release.
  15. USFWS 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 USFWS 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 USFWS 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 USFWS 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 External Affairs 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 not in proximity to tribal lands, tribal leadership will be notified via phone or email by USFWS when results are posted online if no press release has been issued by the state.
3. Results are then posted on the USFWS IC eDNA Monitoring Program Dashboard in conjunction with the media release.

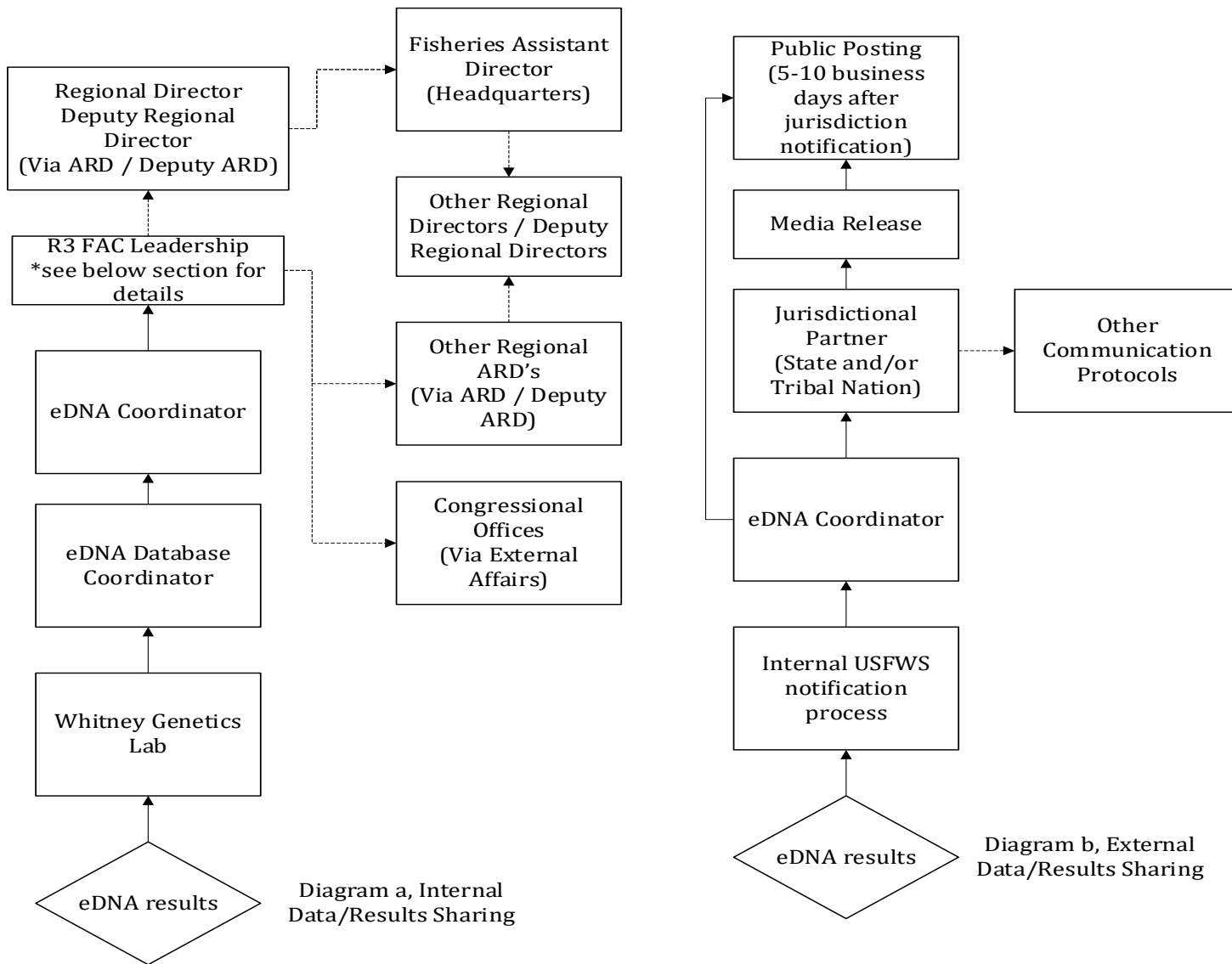


Figure 1. Internal and External Flow of IC eDNA Monitoring Program Data/Results.

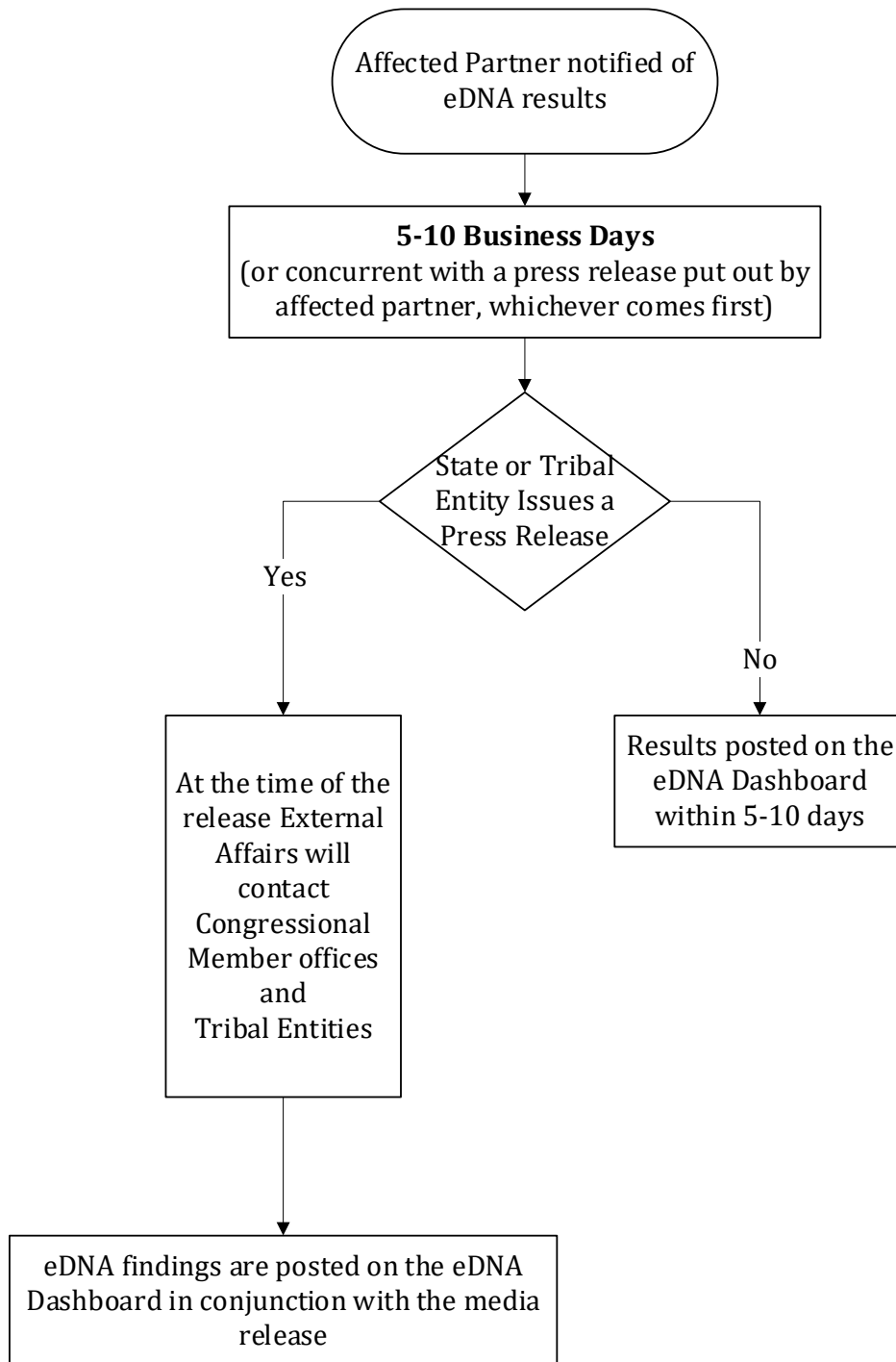


Figure 2. Media Release Flow of IC eDNA Monitoring Program Data/Results.

Bighead and Silver Carp eDNA Early Detection Results:  
 Upper Mississippi River: Pool 14  
 Sampling Period: Week of November 4, 2019  
 Number of Samples Collected: 528

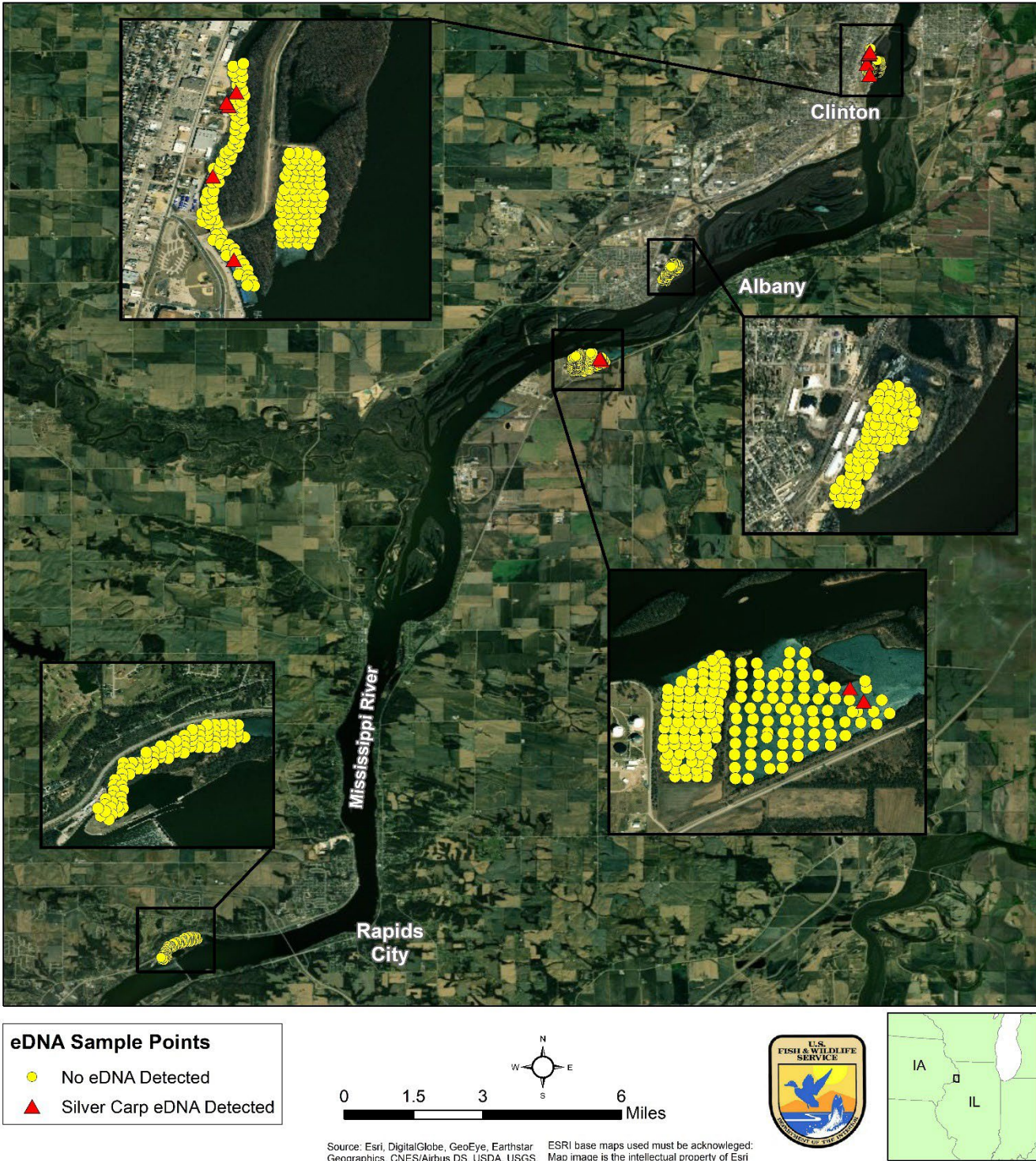


Figure 3. Example Geo Referenced Map of IC eDNA Monitoring Program Data/Results.



## 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 USFWS 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 apprised of your release date.

Please be aware that the USFWS 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 USFWS 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

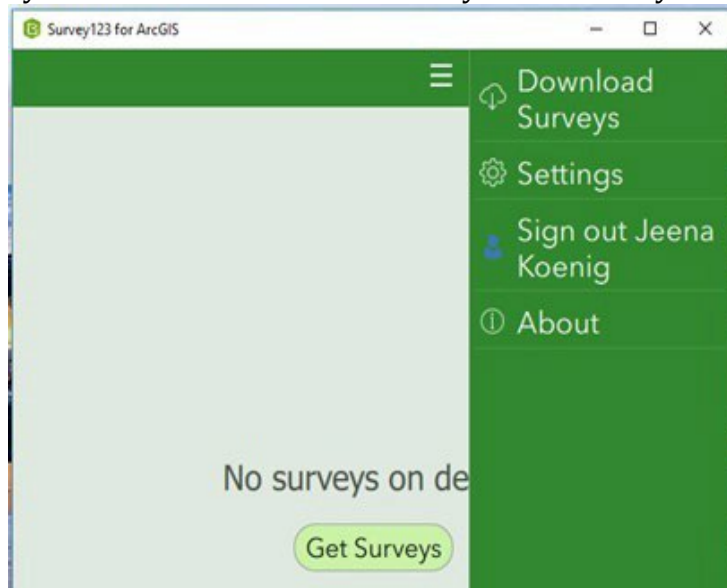
Nick Frohnauer  
eDNA and Early Detection Coordinator  
U.S. Fish & Wildlife Service Region 3  
Norman Point II  
5600 American Blvd, West; Suite 990  
Bloomington, MN 55437-1458

## **Appendix B: Survey 123 COC Creation and Submission**

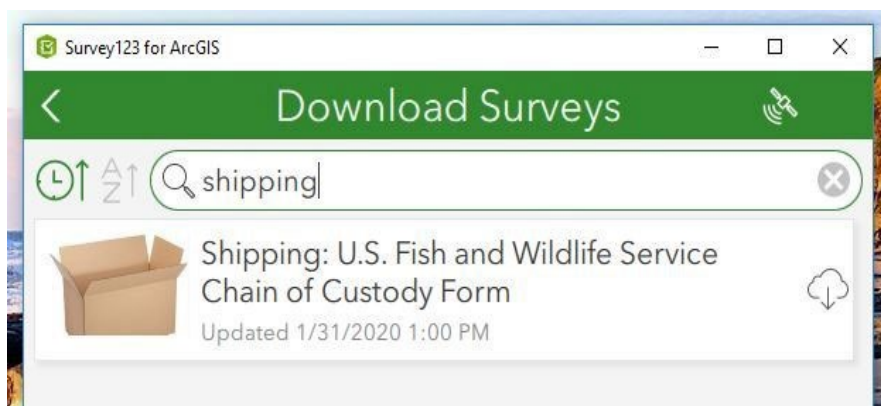
## Survey 123 Instructions for COC Creation/Submission

To create and send COC's in Survey 123: (instructions work for I-Phone and I-Pad)

1. Download Survey 123 Form from ArcGIS Survey123 Application
2. On the login page select Enterprise Login
3. Type FWS into text box.
4. Select U.S. Fish and Wildlife Service
5. Sign in using FWS Account and Password if needed
6. Select "Download Surveys" from the menu or "Get Surveys" if no surveys are on device



7. Each office will have their own collection form called: Office Name: U.S. Fish and Wildlife Service Chain of Custody Form. Each individual office form will have their own unique picture for the form icon. If you work for a FWCO or are collecting samples you will also need to download the Shipping Chain of Custody form (Select the cloud download icon next to the form) (has a cardboard box form icon). If you work for WGL you'll want to download the shipping form mentioned above.



8. Once you have the appropriate form downloaded, go to the upper left hand corner of the screen and select the back arrow. You should see the form you downloaded in step 7.
9. To create a new COC form when collecting eDNA samples, open the Survey123 app on your I-Pad at the beginning of your week and click on the COC form and select "collect" you will then see the electronic version of what used to be our paper COC.
10. Enter the information required in the various boxes i.e. case number, sample numbers (1-xyz), date

- of collection, enter the crew names in the collected by box, select your office (should be a drop down menu with all the FWCO's) and fill in any notes that pertain to the collection of your samples.
11. Once the collection team (boat, or otherwise) is ready to hand off to trailer personnel, select a delivery method (FedEx, in person, or other), one of the collection team members should type their name in the release signature box and select the check box certifying their digital signature, enter in Release agency (default set to USFWS), and select the release date. The person receiving the samples, either a person working in the processing trailer or a "runner" that will transfer the samples from the boat personnel to processing trailer personnel, will then type their name in the receipt signature box and select the check box certifying their digital signature, enter in Receipt agency (default set to USFWS), select the receipt date, and fill out the Delivery method. The Person receiving the samples from the boat will then select the submit button in the bottom right corner of the form (looks like a "check mark") and select send now if there is a Cellular signal, otherwise select Send Later and use the Outbox to send your forms to ArcGIS Online when within Cellular range. If a "runner" is to be used to bring samples from the waterbody to the trailer, that person will either need to create a new COC form or use the repeat option under the Sample Numbers group for the existing COC form by selecting the "+" icon at the bottom of the group right before Editing Notes.
  12. If a runner was used, the trailer staff should now fill out the receipt information in either the new form or the repeat sample field once the runner delivers the samples identified in the form. The trailer crew can then submit the form and select send now if there is a WiFi or Cellular signal. From here, the boat crew can create a new form for a new set of samples following steps 8-10. **Important: Never leave Survey123 forms in your Outbox if cellular signal is available, make sure to send forms to ArcGIS Online as soon as possible. Go to Outbox, select the Send icon in the bottom right hand corner to send forms to ArcGIS Online.**
  13. Once the samples have been processed in the trailer and packaged for shipment to WGL, the processing lead (or designee) will need to create a shipping COC form (shipping box icon) and enter all the pertinent information. Any pertinent notes created during sample processing in the trailer should be transferred to the shipping COC notes i.e. a tube broke during centrifugation or sample xyz has 3 tubes instead of 5. Before the case is shipped, the shipping notes must be filled out to indicate how many containers and samples are included in the shipment. For example, if a case is being shipped that has 200 samples in 2 boxes, the shipping notes should read: "Shipping 2 boxes: Box 1:samples 1-100. Box 2: samples 101- 200." Then WGL staff will know to expect 2 boxes for case xyz with the matching samples and COC's in the Survey123 shipping COC form inbox. Survey 123 will not let a form be submitted unless all required fields are filled out (Notes will pop up for your guidance).
  14. When the Survey123 Shipping COC form is submitted, an e-mail notification will automatically be sent to the WGL staff informing them of the shipment details entered into the form. The processing lead should use the R3 eDNA Program, eDNA shipping alerts Teams channel to input the tracking numbers for the case shipped.
  15. When the case arrives at WGL, the person checking in the samples should open the shipping COC form on Survey 123 using the inbox and select the case from the FWCO that shipped it. **Note: Always select the Refresh icon in the bottom right hand corner of the Inbox to see the latest forms, Inbox does not show newly submitted forms automatically.**
  16. From here WGL check-in staff need to make sure that the samples collected/shipped match the samples received from FedEx. Any discrepancies should be addressed with a follow up call or email to the person whose name appears in the Released box on the Shipping COC and/or the Station eDNA Lead to make corrections. Corrective actions should be recorded in the editing notes section of the Shipping COC form. The FWCO staff member making the corrections should initial and date all editing notes. Ex: "Added agency to release agency-JK 3/5/2020"
  17. Once the accuracy is verified for the shipping COC form then the WGL check-in staff can sign and date under the WGL Only receipt signature and select the submit button ( Select Send Now after

- submission) finalizing the case's documentation of receipt and by the WGL.
18. All final sample and shipping COC forms will be QA/QC by the eDNA Processing QA/QC Specialist and backed up and removed from ArcGIS Online by 5 business days.

### Survey123 Form Examples:

#### FWCO Office COC Form:

14:00 Mon Jun 8 62%

La Crosse FWCO: U.S. Fish and Wildlife Service Chain of Custody Record

U.S. Fish and Wildlife Service Chain of Custody Record

Case Number: \*  
Case Number should be 5 characters long.  
50000

Sample Numbers: \*  
1-100

Date of Collection: \*  
Monday, June 8, 2020

Collected by (first and last names): \*  
Jane Doe, John Smith, Martin Brody

Collection Office: \*  
La Crosse FWCO

Notes:  
Test

The chain of signatures should be continuous.

Sample Numbers

Sample Numbers: \*  
1-100

Release Signature

Release Signature: \*  
Please enter in first and last name.  
Jane Doe

I certify the digital release signature above represents my signature for Chain of Custody purposes. \*

Yes

14:03 Mon Jun 8 62%

La Crosse FWCO: U.S. Fish and Wildlife Service Chain of Custody Record

Release Agency: \*  
If not USFWS, enter in Agency.  
USFWS

Release Date: \*  
Monday, June 8, 2020

Receipt Signature

Receipt Signature: \*  
Please enter in first and last name.  
Tim Miller

I certify the digital receipt signature above represents my signature for Chain of Custody purposes. \*  
 Yes

Receipt Agency: \*  
If not USFWS, enter in Agency.  
USFWS

Receipt Date: \*  
Monday, June 8, 2020

Delivered via: \*  
 FedEx  
 In Person  
 Other

1 of 1

Please initial and date each Editing Note.

Editing Notes:  
Added agency to release agency JD 6/8/2020

✓

14:10 Mon Jun 8 60%

Shipping: U.S. Fish and Wildlife Service Chain of Custody Record

**Shipping: U.S. Fish and Wildlife Service Chain of Custody Record**

Case Number: \*  
Case Number should be 5 characters long.

Sample Numbers: \*

Date of Collection Start: \*

Date of Collection End: \*

Shipped by (first and last names): \*

Collection Office: \*

Notes:

Shipping Notes: \*

Shipping Notes Example: Shipping 2 boxes: Box 1: 1-100, Box 2: 101-200

**The chain of signatures should be continuous.**

**Sample Numbers**

Sample Numbers: \*

**Release Signature**

Release Signature: \*  
Please enter in first and last name.

I certify the digital release signature above represents my signature for Chain of Custody purposes.  
 \*

✓



# Shipping COC Form:

14:10 Mon Jun 8 59%

Shipping: U.S. Fish and Wildlife Service Chain of Custody Record

1-100

**Release Signature**

Release Signature: \*  
Please enter in first and last name.

Jane Doe

I certify the digital release signature above represents my signature for Chain of Custody purposes.  
\*

Yes

Release Agency: \*  
If not USFWS, enter in Agency.

USFWS

Release Date: \*  
Friday, June 5, 2020

Delivered via: \*

FedEx  
 In Person  
 Other

Are you WGL?

Yes  
 No

**Please initial and date each Editing Note.**

**Editing Notes:**

✓

**WGL Only:**

14:11 Mon Jun 8 59%

Shipping: U.S. Fish and Wildlife Service Chain of Custody Record

Release Agency: \*  
If not USFWS, enter in Agency.  
USFWS

Release Date: \*  
Friday, June 5, 2020

Delivered via: \*  
 FedEx  
 In Person  
 Other

Are you WGL?  
 Yes  
 No

Receipt Signature (WGL Only)

Receipt Signature: \*  
Please enter in first and last name.

I certify the digital receipt signature above represents my signature for Chain of Custody purposes.  
\*  
 Yes

Receipt Date: \*  
Date

Please initial and date each Editing Note.

Editing Notes:

✓

## **Appendix C: 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 sec	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 min Detergent contact/ 10 sec 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 min	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 sec	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 min	Lasts 1 week in solution Environmental safety	Corrosive to metals when exposed longer than 10 min	Use PPE – wear a dust mask when mixing powder
2% Virkon Low Pressure Saturation	Potassium Peroxymonosulfate and Sodium Chloride	10 min	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
<b>Heat + Air</b> (Drying in hot sun/air)	<b>30C (86F) 24 hours minimum</b> (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
<b>Heat + Water</b> Spray and/or Immerse	<b>50C (122F) contact time 10 minutes</b> (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
<b>Steam Spray</b> *(recommended for eDNA decontamination)	<b>100C (212F) contact time 10 seconds</b> (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
<b>Virkon Aquatic</b> *(recommended for eDNA decontamination in a 2% solution for 10-30 min)	<b>Follow product direction for proper mixture and minimum contact time.</b> (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
<b>Quaternary Ammonium + Water</b> (family of products)	<b>Follow product direction for proper mixture and minimum contact time</b> (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
<b>Sodium Hypochlorite + Water</b> *(recommended for eDNA decontamination)	<b>200 ppm for pathogens; 5000 ppm for eDNA – contact time: 10 min</b> (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 Woiak, Whitney Genetics Lab: Zebadiah\_woiak@fws.gov

Last updated: June 2020

## **Appendix D. 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 Database Coordinator 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)

### 2a. 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

### 3. Sample Processing

**Evaluation Criteria:** Section B2. Sample Processing/Centrifugation of the QAPP details field sample processing.

**Indicator:** Sampling times recorded on Survey123 forms.

**Audit Checklist Items:**

- Sample times will not exceed 24 hours from time of collection to sample processing

**Responsible Party:** 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:**

- Survey 123 data will be reviewed within 5 business days of sample receipt
- Survey 123 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:** Logbooks, 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 logbooks 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 final results accurately

**Responsible Party:** QA/QC Specialist

#### **6a. 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:**

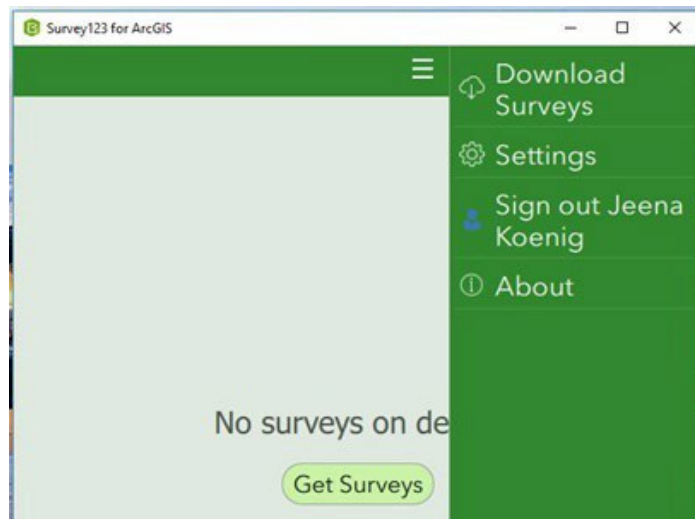
- Review extraction procedures are being followed against QAPP
- Review amplification procedures are being followed against QAPP
- Review logbooks for accuracy and completeness for following procedures. Data sheets contain essential information (date of analysis, analyst, case information, notes)

**Responsible Party:** QA/QC Specialist

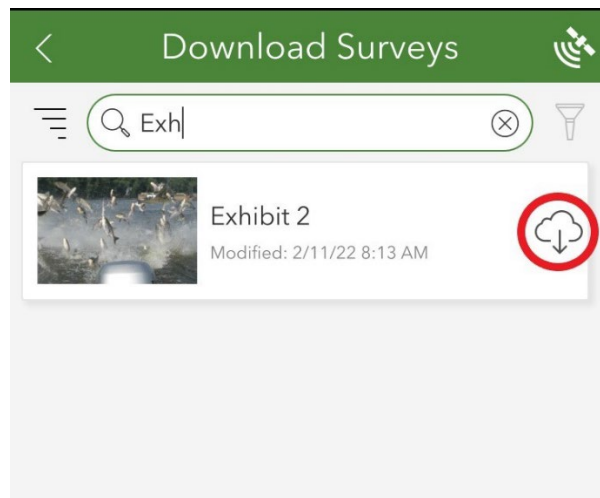
**Appendix E. Survey 123 Form Post-Trip Field Summary Plan**

## Survey 123 Instructions for Post-Trip Field Summary Plan (formerly Exhibit 2)

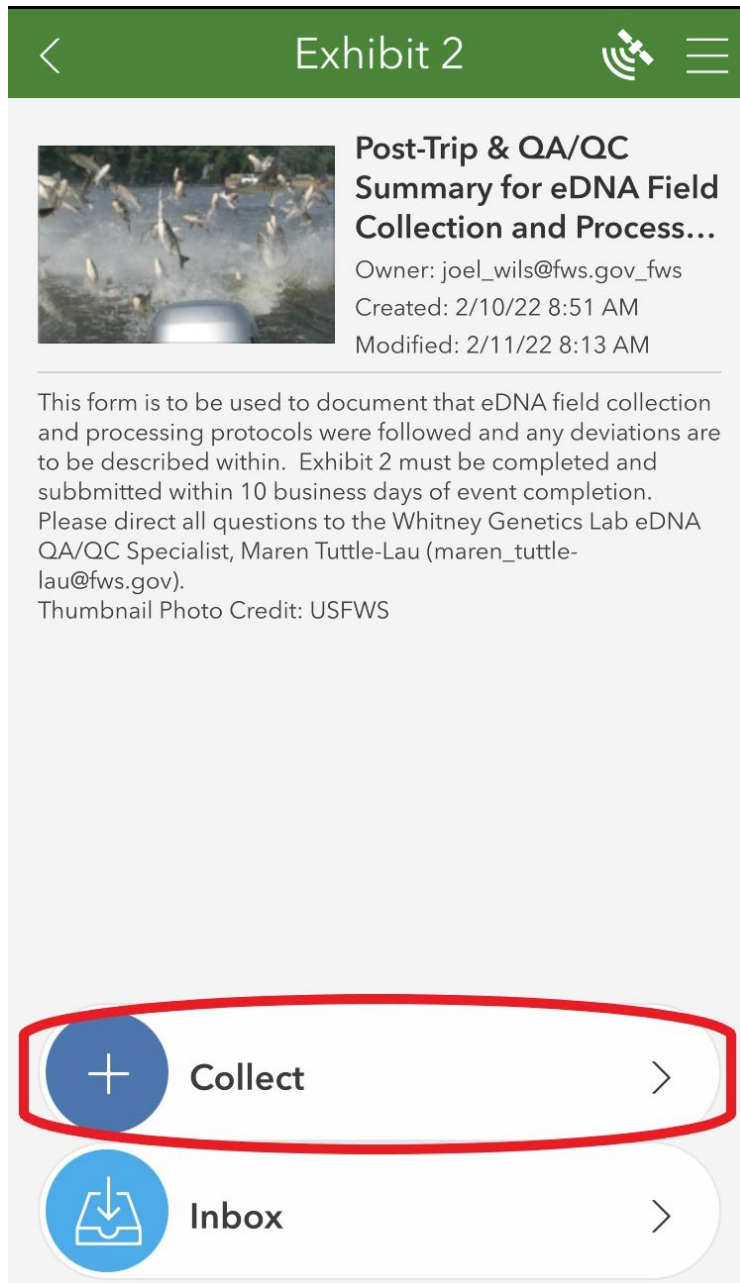
1. To create and send Post-Trip Field Summary Plan forms in Survey 123: (instructions work for I-Phone and I-Pad)
2. Download Survey 123 Form from ArcGIS Survey123 Application
3. On the login page select Enterprise Login
4. Type FWS into text box.
5. Select U.S. Fish and Wildlife Service
6. Sign in using FWS Account and Password if needed
7. Select “Download Surveys” from the menu or “Get Surveys” if no surveys are on device



8. Type: Exhibit 2 in the search bar and select the download icon on the right.



9. Once you have the appropriate form downloaded, go to the upper left hand corner of the screen and select the back arrow. You should see the form you downloaded in step 7.
10. To create a new Exhibit 2 form after collecting eDNA samples, open the Survey123 app on your I-Pad or I-Phone within 10 business days of sample collection and click on the Exhibit 2 form and select “collect”. You will then see the electronic version of what used to be our paper Exhibit 2.



11. Enter the information required in the various boxes (i.e. case number, the basin samples were collected from, dates of collection, etc.) and proceed to answer the questions

Exhibit 2

**Post Trip & QA/QC Summary for eDNA Field Collection and Processing**

Case: \*

Basin: \*

Begin Date \*

End Date \*

**1. Post Trip Summary \***

Sampling plan was completed as described in Exhibit 1

Sampling plan was NOT completed as described in Exhibit 1 (Describe below)

Field Operations Manager \*

Exhibit 2

Field Operations Manager \*

Please enter in their first and last name.

**2. Quality Control Audit**

Sampling Quality

The Sampling QA/QC Specialist observed the collection of eDNA for the above case number. They compared the actions of the sampling crew to the QAPP and witnessed adherence to the QAPP in the following (check the appropriate response for each item; if No, explain below):

Boat decontamination in the field \*

Yes  No

✓

Exhibit 2

Boat decontamination in the field \*

Yes  No

Sample collection procedure \*

Yes  No

Navigation to sample sites \*

Yes  No

Sampling QA/QC Specialist \*

Please enter in their first and last name.

Processing Quality

The Processing QA/QC Specialist observed the processing of eDNA samples for the above case number. They compared the actions of the processing crew to the QAPP and witnessed

✓

Exhibit 2

adherence to the QAPP in the following (check the appropriate response for each item; if No, explain below):

Decontamination of processing equipment \*

Yes  No

Sample processing procedure \*

Yes  No

Explanation of deviations (if applicable)

Processing QA/QC Specialist \*

Please enter in their first and last name.

Data Quality

✓

Exhibit 2

Data Quality

The Data QA/QC Specialist completed the QA/QC of sample data for eDNA case number: \*

The Data QA/QC Specialist confirms that data are accurate and represent the locations and conditions in which samples were collected. Any corrections or edits to the data are explained in the Notes column for the affected sample(s). \*

Check the appropriate response; if No, explain below

Yes  No

Explanation of deviations (if applicable)

Exhibit 2

Data QA/QC Specialist \*

Please enter in their first and last name.

I certify that the information input is correct and accurate and that I communicated with the field staff for the information that is entered.

**▼ eDNA Lead Signature**

Signature \*

Please enter in first and last name.

I certify the digital release signature above represents my signature \*

Yes

Date \*

12. Once all fields have been filled out, select the ✓ to submit the form. All questions are required, so if you accidentally miss a question, the form will automatically direct you to what you missed

**End of Document**