A1. Title Page

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

2020

Approved by:

Project Manager: Aaron Woldt	AARON WOLDT Digitally signed by AARON WOLDT Date: 2020.11.04 08:27:16 -06'00'
eDNA Program Coordinator: Amy McGovern	AMY MCGOVERN Digitally signed by AMY MCGOVERN Date: 2020.11.03 09:52:28 -06'00'
WGL Project Leader: Teresa Lewis, Acting	Teresa D. Lewis Digitally signed by Teresa D. Lewis Date: 2020.11.03 08:48:05 -06'00'
eDNA Processing QA/QC Specialist: Maren Tuttle-Lau	MAREN TUTTLE-LAU Digitally signed by MAREN TUTTLE-LAU Date: 2020.11.03 07:16:27 -06'00'

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Section A. Project Management

A3. Distribution List

Table 1 Distribution List for Region 3 eDNA Staff that Should Receive the Quality Assurance Plan.

Name	Title	Office	Address
Charlie Wooley	Great Lakes Region 3 Regional Director	Great Lakes Region 3 Regional Office	5600 American Blvd W #990, Bloomington MN 55437
Aaron Woldt	Fisheries Assistant Regional Director	Great Lakes Region 3 Regional Office	5600 American Blvd W #990, Bloomington MN 55437
Amy McGovern	eDNA Program Coordinator/ Region 3 AIS Coordinator	Great Lakes Region 3 Regional Office	5600 American Blvd W #990, Bloomington MN 55437
Sandra Keppner	Region 5 AIS Coordinator	Lower Great Lakes FWCO	1101 Casey Road, Basom NY 14013
Kyle Von Ruden	eDNA Processing Lead	Whitney Genetics Lab	555 Lester Ave, Onalaska WI 54650
Scott Koproski	Project Leader	Alpena FWCO	480 W Fletcher Street, Alpena MI 49707
Mark Brouder	Project Leader	Ashland FWCO	2800 Lake Shore Dr E, Ashland WI 54806
Greg Conover	Project Leader, Acting	Carterville FWCO	292 San Diego Rd, Carbondale IL 62901
Jason Goeckler	Project Leader	Columbia FWCO	101 Park Deville Dr. Ste A, Columbia MO 65203
Susan Wells	Project Leader	Green Bay FWCO	2661 Scott Tower Dr, New Franken WI 54229
Mark Brouder	Project Leader, Acting	Carterville FWCO Wilmington Substation	30239 IL-53, Wilmington IL 60481
Teresa Lewis	Project Leader, Acting	Whitney Genetics Lab	555 Lester Ave, Onalaska WI 54650
Maren Tuttle-Lau	eDNA Processing QA/QC Specialist	Whitney Genetics Lab	555 Lester Ave, Onalaska WI 54650
Rebecca Neeley	Project Leader	La Crosse FWCO	555 Lester Ave, Onalaska WI 54650

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 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

<u>Regional Office</u>

Fisheries Region 3 Regional Director (RD): Charles Wooley
Fisheries Assistant Regional Director (ARD): Aaron Woldt
Fisheries Deputy ARD: vacant
R3 AIS Coordinator: Amy McGovern
R5 AIS Coordinator: Sandra Keppner
R3 Asian Carp/eDNA Coordinator: Amy McGovern, Acting
R3 FWCO Program Supervisor: Brian Elkington

Midwest Fisheries Center
Center Director: Teresa Lewis
eDNA Database Coordinator and Data Steward: Jeena Koenig

Whitney Genetics Lab
Project Leader: Teresa Lewis, Acting
eDNA Processing Lead: Kyle Von Ruden
eDNA Processing QA/QC Specialist: Maren Tuttle-Lau

Table 2 List of Fish and Wildlife Conservation Office (FWCO) Project Leader and Station eDNA Leads.

Office	**Project Leaders**	**Station eDNA Lead**
Alpena:	Scott Koproski	Anjanette (Anjie) Bowen
Ashland:	Mark Brouder	Jason Ross
Carterville:	Greg Conover, Acting	vacant
Columbia:	Jason Goeckler	vacant
Green Bay:	Susan Wells	Matt Petasek
La Crosse:	Rebecca Neeley	Jenna Merry
Lower Great Lakes:	Sandra Keppner	Colleen Keefer
Wilmington:	Mark Brouder, Acting	Nathan Evans

A5. Problem Definition and Background

Invasive aquatic nuisance 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 Asian 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 which 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 Asian 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 Asian 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: Collector 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 in order to conduct eDNA monitoring sample collection. 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. 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. 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, and will send documentation of personnel training to the eDNA Program Coordinator. The WGL Project Leader is responsible for documenting and providing training, 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.

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 projects duration. The QAPP, Collector 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 will be kept on the MFC server 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:

1. The USFWS eDNA monitoring personnel will keep up with current research through agency publications and peer reviewed literature.
2. 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.
3. 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.
    a. These changes are suggested by FWCO staff, and reviewed by the Station eDNA Leads, and upon approval, implemented into the QAPP.
    b. None of the changes presented will reduce the level or rigor or quality control measures.
4. 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.
5. The USFWS recommends a minimum of following the QAPP to ensure rigorous quality control and sample integrity.
6. 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.
7. 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 (00000). Sample ID numbers within cases will be in consecutive numerical order, and will consist of a 3-digit integer (000). Each sample will be identified by the 8-digit number consisting of the case **and** 3-digit sample ID number, without spaces or punctuation (00000000) which results in the regional unique ID (RUID). The 8 digit RUID will be used in the field, the file Geodatabase, and in the final 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. 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 the each basin (e.g. 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 brief post-sampling summary report (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 Asian 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 Asian 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 Asian 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 Asian carp presence), a sampling density of 1 sample per 0.01 km2 with a minimum of 40 samples was appropriate to detect Bighead and Silver Carp DNA. Most of 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 Asian 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 0.01km2 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 and barges. It is not recommended to sample under or downstream of known bird rookeries.

Pre-Trip Planning and Logistics

1. File pre-sampling plan (Exhibit 1).
2. Review ArcGIS Collector 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 aggregate 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 should consist of two people, two 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 taken into account 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 Collector 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.

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. DNA. 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 x g. 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 work day, 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 in Section 2.2.4. 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 x g, 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 Collector 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.
- DNAway.
- Tube rack for dipping samples into target waterbody.
- Bleach bath container for disinfecting tube rack between samples.
- Small container for holding tube caps.
- Copy of QAPP and Collector 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. 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. Use DNAway and paper towels to decontaminate pens, hats, notebook surfaces, electronic equipment surfaces, truck interior and other non-saturable equipment.
7. Contain any equipment that was not treated in bags or totes for later decontamination.
8. Wash hands, launder or change soiled clothing.
9. 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 tub 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-sampling summary (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 tub 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 Collector 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 in a separate cooler.
17. 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 x *g* 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 (Fisher item # listed for: Sorvall Legend XTR centrifuge; rotor GS25F7087G item #75-033-607; 750 ml round bucket GS25F7087G item #75-003-608; 50-ml tube adaptors item #75-003-638)
- 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 check 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 in to centrifuges. **A new set of gloves should be used for each set of tubes comprising one sample any time the tube is opened**. Prior to centrifuging samples, each workstation should have pre-printed labels or one black, waterproof permanent marker for labeling sample tubes, a wastewater container (with lid), and a dedicated alcohol bottle with 95% non-denatured ethanol or 70%-99% isopropanol.
4. Turn on refrigerated centrifuge(s) and ensure they are set to 4^0 C.
5. 50 ml tubes should be removed from the transport cooler, caps checked and tightened, wiped dry with either a clean paper towel or a clean bleach wipe, and placed into the green centrifuge adaptors. The same towel or wipe may be used for all 5 tubes in a sample, unless they become saturated and do not completely dry the tubes. Tubes with heavy soiling on the outside can be rinsed with plain water in the non-dedicated sink and wiped dry.
6. Process tubes in sets of replicates, until a full centrifuge batch is prepared.
7. Place filled adapters into a refrigerated centrifuge set to 4 ^{0}C. Tubes must be evenly distributed within the centrifuge to maintain the rotor balance. NOTE: Always follow the manufacturer's guidelines for centrifuge operation. It is also important to keep replicate tubes for each sample together in one centrifuge. Do not split a sample between centrifuges.
8. Once 50-ml tubes are in position, close and secure centrifuge lid. Set centrifuge to spin the samples for 30 min at max speed (~4500-5000 x *g*) 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 (~4500-5000 x *g*). 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 Collector 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 Collector App and make a note on the COCs. This is not considered a deviation, this is recorded as a note.

B2. Post-Sampling Documentation Procedure

1. Complete post-sampling summary (Exhibit 2) and share with the eDNA Program Coordinator within <u>10 business days</u> of the last day of sampling. If the trip was executed as planned, use the check box provided on Exhibit 2, sign and continue to page 2. If any deviations from Exhibit 1 or deviation from standard QAPP procedure occurred during water sample collection in the field or during processing of samples, clearly describe the deviations on Exhibit 2. If deviations will affect lab processing, the eDNA Program Coordinator will contact the eDNA Processing Lead. Even if there were no deviations from the QAPP, Exhibit 2 forms should be shared with the eDNA Program Coordinator following a sampling event. Some of this information may seem duplicated from the Collector App, but it is necessary to provide quality assurance information to the eDNA Program Coordinator and eDNA Database Coordinator at the time lab data is joined with field data to ensure accuracy. 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. Either preserve samples in the trailer with methods described in section **B2.Sample processing procedure** or make arrangements in advance with WGL staff to get the water samples frozen in cryogenic storage within the 24-hour requirement.
4. Put seal tape on the cooler.
5. Hand deliver to WGL staff so that inspection of samples can be completed and WGL check-in form can be sent out within 1 business day.

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, tape it closed, signing across the tape.
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 (https://www.uline.com/BL_7009/Universal-Sorbents) 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. You can tuck most of it into the space created where two racks meet.
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.
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 pick up, you will not be allowed to drop boxes off at FedEx. NOTE: alcohol-preserved centrifuged samples are shipped at ambient temperatures.
13. Items will be shipped to:

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

WGL Contacts

Once items have been shipped, WGL will receive an email via a web hook once the Survey123 Shipping COC is submitted. A follow-up notice through Teams site with approximate delivery time, date and tracking numbers is preferred. If you do need to contact WGL, these are contacts below.

Table 3 Whitney Genetics Laboratory Staff Contact Email and Phone Number Listings for Sample Delivery.

Staff Name	Phone	Email
Aaron Johnson	608-518-9800	aaron_e_johnson@fws.gov
Kyle Von Ruden	608-780-9022	kyle_vonruden@fws.gov
Zeb Woiak	608-304-4179	zebadiah_woiak@fws.gov
Nick Grueneis	608-518-0129	nikolas_grueneis@fws.gov
Maren Tuttle-Lau	608-780-0834	maren_tuttle-lau@fws.gov
Austin Hannah	608-769-5344	austin_hannah@fws.gov
Katie Bockrath	608-304-3368	katherine_bockrath@fws.gov
Teresa Lewis, Acting	608-304-7180	teresa_lewis@fws.gov

Upon receipt of the shipment at WGL, once the samples are checked in, staff will sign the Survey 123 COC form and alert the Station eDNA Lead via Teams site.

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 recorded.
- 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 °C freezers is allowable until room in the -80 °C can be made.
- Review the Survey123 COC forms that correspond to the samples. Note any condition issues (broken tape or seals, damaged containers or containers, etc.) with the samples on the COC forms. Note any samples that must be discarded due to condition issues and the reason for discard. Any corrections that need to be made should be brought up 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. This reporting must be done within 1 business day of completing sample check-in.

B3. Preservative Decanting and Alcohol Evaporation Procedure

1. Centrifuged samples may 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. This entire procedure can be done in advance, and samples stored at -80 °C until ready for processing.
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. 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 for each case, and there can be multiple hood controls for a single case if samples from the same case were placed in multiple hoods. 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 that makes up the control. The lid should be labeled as well, since the tube and lid are separated during the evaporation process.

DNA Assay Considerations

- 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 once a day on week days, and recorded in the Survey123 application. 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 critical components 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 9.

- 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 95 µl of sterile lab DI water and 5 µL of Bluegill 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 ul 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.
- For every extraction batch of samples processed, one extraction negative control and one extraction positive control should accompany the batch.

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.
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 µl. 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., 20034001). Also during this time place the Elution Buffer into the 60°C bead bath.
8. Remove lyse and spin tube from incubator and centrifuge at max speed for at least 1 minute, up to 3 minutes depending on level of sediment in tubes. IF samples are particularly dirty, spin at least 3 minutes. If the basket is clogged, use a pipette to remove the supernatant from the sample, recovering as much as you can. Make notes in the Case Log.
9. Add 500 µL **ethanol** (100%, molecular grade) to the extract. Mix thoroughly by vortexing. If liquid collects around the cap, spin briefly before opening to reduce contamination risk.
10. Transfer up to 780 µL of the mixture by pipette into a GD spin column placed in a 2 mL collection
11. tube. Centrifuge at ≥ 6000 x *g* 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 ≥ 6000 x *g* for 1 minute. Discard flow-through and collection tube.
13. Place GD spin column in a new 2ml collection tube. Add 400 µL Buffer **W1**. Centrifuge at 6000 x*g*

14. for 30 seconds. Discard flow-through and collection tube.
15. Place spin column in a new 2 mL collection tube. Add 600 µL **Wash Buffer**. Centrifuge at 18,000 x *g* for 3 minutes. Discard flow-through and collection tube.
16. Transfer the spin column to a new 1.5 mL MCT.
17. Add 200 µL of the pre-heated **Elution buffer** to the center of the spin column membrane. Incubate for 1 minute at room temperature (15 - 25°C). Centrifuge at ≥ 18000 x *g* for 30 seconds.
18. Discard the spin column and store the eluted DNA samples at −20°C. If DNA is to be immediately
19. used for PCR, keep in 4°refridgerator.

PCR Amplification

In order to determine if the DNA of a specific 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 assayed with an assay that consists of two general Asian 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 work day.
- DNA elutes from samples should either be located in designated −20°C freezer or carried from the DNA extraction room to PCR room. Ensure thermalcyclers 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 deckand 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 prior to use 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 uL of autoclaved sterile lab water into a clean 1.5-ml Eppendorft MCT.
    - A positive PCR control is prepared by previously extracting Asian carp cell lines or tissue slurry (both Bighead and Silver Carp) and diluting to an appropriate volume so as 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 thermos cycler. Log information should include the assay name, sample case, plate number, and the thermal cycler head ID (Exhibit 8).
- Note for Exhibit 10-11: Plate maps have been included for scenarios most processed at WGL. A 384-well plate map is included for initial screening of each case, but 96-well plate sheets are also included for processing any equipment controls for confirmed positive samples.

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 7).
2. Use pre-printed plate map (Exhibits 10-11) 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. The PCR reactions are prepared as follows:
6. Wipe lab bench area with 10% bleach, 75% Ethanol, 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 with one of the solutions or wipes listed in Step 1. Liquid
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 µM working dilution.
    - One tube with combined SCTM4/5 forward and reverse primers at 10 µM working dilution.
    - One tube with combined BHTM1/2 forward and reverse primers at 10 µM 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-labeld probe for SCTM4/5, all diluted to 2.5 µM. Double-quenched probes are preferred that include a ZEN quencher located about 9 basepairs 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, make enough master mix for 100 reactions when actually preparing for 96 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 Initial PCR 1X reaction should contain:

Table 4 Master Mix Reagent Components for Combined AC qPCR Assay.

Volume µL	Master Mix Reagent Component
10.0	TaqMan Environmental Master Mix 2.0
1.0	each primer mix (10µM each, working dilution)
1.0	probe mix (2.5 µM each, working dilution)
3.0	sterile water (*7.0 µL in standard curve mix)
3.0	sample extract as template (*1.0 µL gBlock template in standards)

13. For a total volume of 20µL per reaction
14. 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.
15. 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.
16. 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.
17. WGL now 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 µl PCR mix. FOR STANDARD WELLS only, add 2.0 µl 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.
18. Carefully pipette 3.0 µL of each extract (or 1.0 µL gblock + 2.0 µl water in standards) to be screened 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.
19. 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 µL 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/µL will be used. The highest standard should be placed in well B followed by the next lowest standards through wells 7, 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 µl) added to the mix to account for only
20. µl of standard template added to each well compared to 3.0 µl DNA template added to each well for samples.
21. Extraction negative controls and hood controls will be run as a sample, in the order extracted in the case. Extraction positive controls will be run separately on a bluegill assay, placed consecutively as they were extracted in the case (see next step).
22. Extraction positive and negative controls, and hood controls will be removed and run on separate plates with a bluegill marker assay (Takahara et al. 2013).
23. Each Bluegill PCR 1X <u>reaction should contain:</u>

Table 5 Master Mix Reagent Components for Bluegill qPCR Assay.

Volume µL	Master Mix Reagent Component
13.4	TaqMan Environmental Master Mix 2.0
1.8	forward primer (10µM each, working dilution)
1.8	reverse primer (10µM each, working dilution)
1.0	probe (2.5 µM, working dilution)
2.0	template

24. For a total volume of 20µL per reaction
25. 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).
26. 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.
27. 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.
28. 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 180^0C for 3 seconds and 384 well plates at 167^0C for 3 seconds. Inspect the seal to ensure it is not loose, and all wells are sealed. If necessary to obtain reliable seals, temperature and time may be adjusted.
29. 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 program for the AC combo assay reaction is an initial denaturation at 95 °C for 10 minutes. Then 95 °C for 15 seconds, followed by 60 °C for 1 minute with a picture. Repeat 40 times.
30. Record the plate ID, thermal cycler unit or head, and time of run start for the PCR plate in the PCR log (Exhibit 8).
31. Place cycled PCR plates and product in trash without opening the seal. Remove plates promptly. Under no circumstances should you open or uncover PCR plates that have been cycled 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. 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 a.
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. Immediately upon opening a BioRad result file, use the default baseline correction function (Apply Fluorescence Drift Collection found under SETTINGS menu in CFX software). Assay quality must be assessed before data is accepted. Data must be exported as a *.txt file and saved into a folder named with the case number. File names must be carefully entered without any mistakes in spacing or coding. File names will follow this format:
2. 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 an analysis day for a single case.
3. Therefore, if there are 3 rounds of PCR for a case in one day, plate numbers should go from 1 to 24. Dates must be as depicted with a two-digit day, three-letter CAP month, and two digit year.
4. Initials are not case sensitive, but assay MUST BE CAPS.
5. Requirements for a successful analysis have been met (above step X):
6. Once text files are in the folder, the program R should be used analyze the data. Output will include the following:
7. Summary statistics (StandardCurves.csv) on assay quality including the efficiency (E) and R^2 of the duplicate standard curves on each plate.
8. Summary data for the total number of negative controls that were clean and the total number of positive controls that were positive, sorted into extraction vs PCR controls (AnalysisLog.txt). If there are any issues, the samples and controls must be re-processed at the step of failure.
9. 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.
10. A list of samples that had cycle thresholds less than or equal to 15 cycles that were changed to negative.
11. A list of positive detection samples will be provided (DataSummary2.csv).
12. A table of data including the number of octets positive per sample and the average and standard deviation copy number for positive samples (DataSummary1.csv).
13. Enter results and QC summary data into the database in an Excel file.
14. Notify the eDNA Processing Lead immediately of any cases with positive detection results.
15. All results will 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 will be prepared at WGL as described below. 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. WGL will serve as the single preparation facility for creating field blanks used in the Bighead and Silver Carp eDNA monitoring program. At the start of each year WGL will fill new, sterile centrifuge tubes, package them in racks to be secured in a clean plastic sleeve, and then either deliver or ship them to field offices as needed.

Field Blanks:

- Plastic bags that fit to racks of filled tubes will be purchased by WGL. Filled tubes will be securely closed, and placed in the bag. Bags will be twisted closed with wire closures or tape.
- Filled tubes will be distributed at the annual eDNA After Action meeting so that each field station will start out the season with the total estimated number of field blanks needed for the sampling season.
- Field stations can store these prepared field blanks as long as needed, ideally in the field trailers. Bags should be re-sealed after removal of blanks to keep contamination risk low.

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 plate of samples run.
- Successful PCR assays are as follows:
    - Minimum requirements for accepting the data 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.
    - If a field blank, hood control, or extraction or PCR negative controls show product (e.g., DNA copies greater than zero), the associated positive data are negated and, when possible, samples are reprocessed. Contamination of DNA extraction negative controls will require that any positive samples be removed from consideration. If all other samples are negative, contamination was only an issue in the controls, and negative results may be reported. If a PCR negative control show product (DNA copies greater than zero) on either bluegill or Asian carp the plate will be rerun. Unless a specific note is made from the analyst that a procedure error may have occurred. Contamination in an extraction negative will be dealt with on a case by case basis.
    - A positive PCR negative control requires a plate re-run unless the entire plate is negative.
    - Extraction positive controls require a minimum of 1 replicate out of 8 to be considered positive.
    - PCR positive controls must have at least one of two reactions positives.
- Other QC procedures:
    - 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 ±3 ft. Temperature readings of the sonar may be checked against a thermometer. Acceptable temperature readings are ± 3°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 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 Collector App), proofing, and retaining field data. The ArcGIS Collector 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 <u>10 business days</u> 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 Collector 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 Collector 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/QCSpecialist.
4. WGL staff are responsible for keeping an internal Case Log for all samples.

5. WGL staff will keep a record of location of samples stored on benchtops before processing or within freezers after preservative evaporation 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 7).
6. WGL will maintain hard copies of laboratory notebooks and the electronic Case Log 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.
7. 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.
8. The Case Log serves as a way to track cases and samples through the lab workflow.
9. 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.
10. The Survey123 Shipping COC data and all laboratory generated data files will be maintained on the WGL and MFC servers.
11. 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.
12. The eDNA Processing Lead will be responsible for filling final case reports of results with the eDNA Program Coordinator and eDNA Database Coordinator within 24 hours of results completion.
13. USFWS Denver Enterprise Team (FWS Geospatial Warehouse) will maintain an electronic folder that contains the file geodatabase complete with field and lab data.

Table 1. Field descriptions for each of the data fields captured in the ArcGIS Collector application for the eDNA sample point feature class stored in ArcGIS Online.

Field Name	Alias	Type	Length	Description
RUID	Regional 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, #######)
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 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
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.
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
FILTER_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 2. Related table to the Main Data Sheet (Table 4) field descriptions for the individual FWCO office eDNA Chain of Custody Form captured in the ArcGIS Survey123 Application and stored as a feature class in ArcGIS Online.

Field Name	Alias	Type	Length	Description
Case_Number_Sender	Case Number	String	255	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_Numbers	Sample Numbers	String	255	Yes/No (Yes Bighead Carp eDNA detected, No Bighead Carp eDNA detected)
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).
Received_by	Received by	String	255	Name and Agency of who is receiving the samples.
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).

Table 3. Field descriptions for the Shipping eDNA Chain of Custody Form captured in the ArcGIS Survey123 Application and stored as a feature class in ArcGIS Online.

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.
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 4. Field descriptions for the individual FWCO office eDNA Chain of Custody Form captured with the ArcGIS Survey123 application and stored as a feature class in ArcGIS Online.

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 5. Field descriptions for the eDNA results table generated by the Whitney Genetics Lab. This table is joined with the field data by the Unique ID for each sample (Table 1).

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 Asian Carp), 0= no detection of any assay
AC_Assay	AC_Assay	Integer	1	1= detection of at least 1 Asian Carp qPCR replicate, 0= no detection, corresponds to one of three assays running in multiplex
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.

Section C. Assessment and Oversight

C1. Assessments and Response Actions

Assessment (audits) will be conducted on both the field collection and laboratory analysis portions of eDNA monitoring. 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, lab and field crew performance, technical systems audits of the field, and data management.

C1. Audits

Field Audits

Internal audits of field crew performance and quality controls for sampling will be made annually by the FWCO field staff to make sure that all procedures in the sample collection portions of the QAPP are being followed. On sampling trips where more than two FWCO offices are on the team, the visiting FWCO eDNA leader will serve as the sampling auditor. A brief report will be made to the eDNA Program Coordinator and eDNA Processing QA/QC Specialist of audit findings, including a checklist of audited procedures (Exhibit 4, page 1). If there are no trips with coordinating offices, then a Station eDNA Lead, Project Leader or eDNA Processing QA/QC Specialist may serve as the auditor. At the end of each year a summary will be put together and reported to eDNA Program Coordinator.

Laboratory Audits

Internal audits of WGL laboratory performance and quality controls will be made annually by the eDNA Processing QA/QC Specialist to make sure that all procedures in the DNA processing portions of the QAPP are being followed. A brief report will be made to the eDNA Program Coordinator of audit findings, including a checklist of audited procedures (Exhibit 4, page 2). Participation in validation studies by the lab may suffice as internal audits, and reports of such studies may be substituted and filed with the eDNA Program Coordinator.

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 12).

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 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:

- If a field blank, hood control, extraction or PCR negative controls show product (e.g., DNA copies greater than zero), the associated data (all samples associated with that control) will be negated and, when possible, samples are reprocessed. Contamination of DNA extraction negative controls will require that any positive samples be removed from consideration. If all other samples are negative, contamination was only an issue in the controls, and negative results may be reported. If a PCR negative control show product (DNA copies greater than zero) on either Bluegill or Asian carp the plate will be rerun.
- Positive controls are currently employed for extraction and PCR. If the positive controls fail to behave as expected, any sample showing an apparent lack of results will be rerun. In order for a positive control to be considered passing, current WGL protocol states that at least one replicate of

8 for the positive extraction control and 1 of 2 replicates for the PCR positive control are needed.

- 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.
- Extraction negative and positive controls, PCR plate positive and negative controls along with sample range are all tracked in the internal Case Log. An example of those indicators are listed in Exhibit 6.

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-sampling summary report (Exhibit 2) must be filled out and filed with the eDNA Program Coordinator. The eDNA Processing Lead will track eDNA sampling events and processing in a master case file that will be shared with USFWS personnel and partner agencies.

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 and dried down in order to preserve DNA integrity. A record of all sample location will be kept (Exhibit 7). 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.

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, a weekly communication will be sent to the eDNA Program Coordinator which will contain the number of cases and samples received to date. The communication will also contain the number of cases and samples received in that particular week. Cases in extraction and PCR will also be identified, as well as any cases closed that week. A separate communication to the eDNA Database Coordinator will contain final eDNA results for each case for incorporation into the eDNA database.

Each field sampling office keeps a record of field data. These records are summarized by the eDNA 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 WGL 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. The sample weekly communication outline is listed below.

Section E. Relevant eDNA Literature

Eichmiller, J. J., S. E. Best, and P. W. Sorensen. 2016. Effects of Temperature and Trophic State on Degradation of Environmental DNA in Lake Water. *Environmental Science and Technology* **50**:1859-1867.

Erickson, R. A., Merkes, C. M., & Mize, E. L. (2019). Sampling designs for landscape-level eDNA monitoring programs. *Integrated environmental assessment and management* **15**(5):760-771.

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

Goldberg, C. S., K. M. Strickler, and A. K. Fremier. 2018. Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. *Science of the Total Environment* **633**:695-703.

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.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters* **4**(2):150-157.

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. *Management of Biological Invasions* **8**(3):343-359.

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

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 1 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	
TOTAL	0	0	0	

PERSONNEL:

Field Operations Manager: Phone #:

Sampling Crew (3 person minimum): Boat Operator:

Sample Collector:

Sample Assistant:

Data Recorder:

QAQC Specialist:

Processing Crew (2 person minimum): Sample Processor:

Sample Processor/QAQC Specialist:

Exhibit 1: NOTE: fillable forms for use are stored on the Teams site. Do not use this sample form.

SAMPLING SCHEDULE:

Day 1
Date:

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

Number of

Number of blanks:

Samples: Notes:

Day 2
Date:

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

Number of

Number of blanks:

Samples: Notes:

Day 3
Date:

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

Number of

Number of blanks:

Samples: Notes:

Day 4
Date:

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

Number of

Number of blanks:

Samples: Notes:

Day 5
Date:

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

Number of

Number of blanks:

Samples: Notes:

Post-Trip Field Plan Summary Exhibit 2
NOTE: fillable forms for use are stored on the Teams Site. Do not use this sample form.

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

Exhibit 2 must be completed and sent to the eDNA Program Coordinator and the Database Manager within 10 business days of event completion.

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

1. POST TRIP SUMMARY

☐ Sampling plan was completed as described in Exhibit 1 (Check box if applicable)

☐ Sampling plan was NOT completed as described in Exhibit 1 (Check box if applicable, describe below)

Description of deviations from Exhibit 1 (List and describe deviations, include reasoning)

Field Operations Manager: Print _____ Sign/Date_____

Exhibit 2: NOTE: fillable forms for use are stored on the Teams site. Do not use this sample form.

2. QUALITY CONTROL AUDIT

Sampling Quality

I observed the collection of eDNA samples for case # I 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):

☐ Yes ☐ No Boat decontamination in the field

☐ Yes ☐ No Sample collection procedure

☐ Yes ☐ No Navigation to sample sites

Explanation of deviations (if applicable):

Sampling QAQC Specialist: Print Sign/Date

Processing Quality

I observed the processing of eDNA samples for case # I compared the actions of the processing crew to the QAPP and witnessed adherence to the QAPP in the following (circle the appropriate response for each item; if No, explain below):

☐ Yes ☐ No Decontamination of processing equipment

☐ Yes ☐ No Sample processing procedure

Explanation of deviations (if applicable):

Processing QAQC Specialist: Print Sign/Date

Training Checklist-Field Exhibit 3

Training Checklist for Field Collection and Preservation Staff:

Check Box	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 3

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:

Check Box	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:

Audit Checklist-Field Exhibit 4

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

Field Audit Checklist for Sample Collection and Preservation:

Section B. Sample Collection Procedures

Are correct sample collection methods being followed? ☐

Are controls/blanks being used as necessary? ☐

Are the sample preservation procedures being followed? ☐

Section B5. Quality Control

Are QC activities clearly established and being followed? ☐

Section B7. Data Management

Do electronic/hard copy data sheets contain essential information, including date of analysis, analyst, etc.? ☐

Office Being Audited:

Name of Auditor:

Signature:

Audit Checklist-Lab Exhibit 4

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

Audit Checklist for Sample Analysis in the Lab:

Section D1. Data Review, Verification and Validation

Are data correction procedures appropriate? Are they in practice? ☐

Is there a sample disposal policy? Where is it located? ☐

Are logbooks in use? Do they contain sufficient information? ☐

Are pages sequentially numbered and entries made in permanent ink? ☐

Are controls/blanks being used as necessary? ☐

Section B. Sample Analysis Procedures

Are there detailed analysis procedures for staff to follow? ☐

Are QC activities clearly established and being followed? ☐

Do data sheets contain essential information, including date of analysis, analyst, case information, notes, etc. (Exhibits 5-11)? ☐

Section B7. Data Management

Is there a log for tracking samples? Is it being used? ☐

Office Being Audited:

Name of Auditor:

Auditor Signature:

Laboratory 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 (above); Case Log front page, screen capture (below).

Total Number of Samples Received		Received Cases	Received Samples
Total Number of Received Cases		Drying Cases	Drying Samples
Total Number of Open Cases		Extraction Cases	Extraction Samples
Total Number of Completed Cases		PCR Cases	PCR Samples
Total Number of Samples Completed		Reporting Cases	Reporting Samples

Priority	Case Number	Case Type	Number of Samples	Check-in Date	Shelf Storage Expiration Date	Days You Have to Move Samples to Freezer	Date Samples Were Moved to Freezer
1							
2							
3							
4							
5							
6							

			eDNA Positive Detections (number of samples)				Quality Control Success (Y/N)			
Sample Progress	Status (Open/Closed)	Total Samples Analyzed	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 6

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 (above), Case Log Sample Log open, screen capture (below).

Sample ID				Shelf	Days You								Case eDNA Detection Summary						
Case #:	Sample	Date of Receipt	Initials	Storage Expiration Date	Have to Move Samples to Freezer	Set to Dry Date ,	Initials	Dried Date	Initials	Extraction Date	Initials	Sample	PCR Date	PCR Initials	ACTM Assay	BHTM Assay	SCTM Assay	Code Summary	Status
0	001			1/21/1900	FREEZE							001							
0	002			1/21/1900	FREEZE							002							
0	003			1/21/1900	FREEZE							003							
0	004			1/21/1900	FREEZE							004							
0	005			1/21/1900	FREEZE							005							
0	006			1/21/1900	FREEZE							006							

	EXT + control	EXT - control	EXT - control	Sample Range	Hood	Hood -	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	Control Range	#+'s of 2 reps	#+'s of 4 reps	for PCR controls	Sample
001	.	.	.	.		.	.	.	.	001
002	.	.	.	.		.	.	.	.	002
003	.	.	.	.		.	.	.	.	003
004	.	.	.	.		.	.	.	.	004
005	.	.	.	.		.	.	.	.	005
006	.	.	.	.		.	.	.	.	006
007	.	.	.	.		.	.	.	.	007
008	.	.	.	.		.	.	.	.	008
009	.	.	.	.		.	.	.	.	009
010	.	.	.	.		.	.	.	.	010

Sample Storage Logs Exhibit 7

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.			

-80 °C Sample Log

Freezer ID and Location: _____

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

Thermocycler Log **Exhibit 8**

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 9

"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, 384-Well Exhibit 10

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) BH TM1/2 Probe(2.5 µM) SC TM4/5 Probes(2.5 µM)	1.0 µl	µl		
H20	3.0 µl	µl	NA	NA

Samples: 3.0ul template + 17.0ul master mix

Standards: 1µl standard + 2µl H_2O + 17.0 µl master mix

Plate Name_____

ddMONyy_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 384-Well, Cont'd Exhibit 10

Plate Name_____ _____

ddMONyy_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 _____ Did cycle complete Y N

Plate Name_____

	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 _____ Did cycle complete Y N

qPCR Sheet Bluegill Assay, 384-Well Exhibit 11

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 µl	µl		
Forward Primer 10µM	1.8 µl	µl		
Reverse Primer 10µM	1.8 µl	µl		
Probe (2.5µM)	1.0 µl	µl		
	2 µl template			

Plate Name_____

ddMONyy_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 _____

qPCR Sheet Bluegill Assay, 96-Well Exhibit 11

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 µl	µl		
Forward Primer 10µM	1.8 µl	µl		
Reverse Primer 10µM	1.8 µl	µl		
Probe (2.5µM)	1.0 µl	µl		

2 µl template + 18.0ul master mix

Plate Name_____

ddMONyy_Initials_ASSAY_case(sample#-sample#)_P#

	1	2	3	4	5	6	7	8	9	10	11	12
A											**POS**	**POS**
B												
C												
D												
E												
F												
G											**NTC**	**NTC**
H											**NEG**	**NEG**

Notes for any lab blunders or deviations from protocol:

_____

_____

_____

_____

_____

Cycler ID _____

COPY

Corrective Action Form Exhibit 12

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:

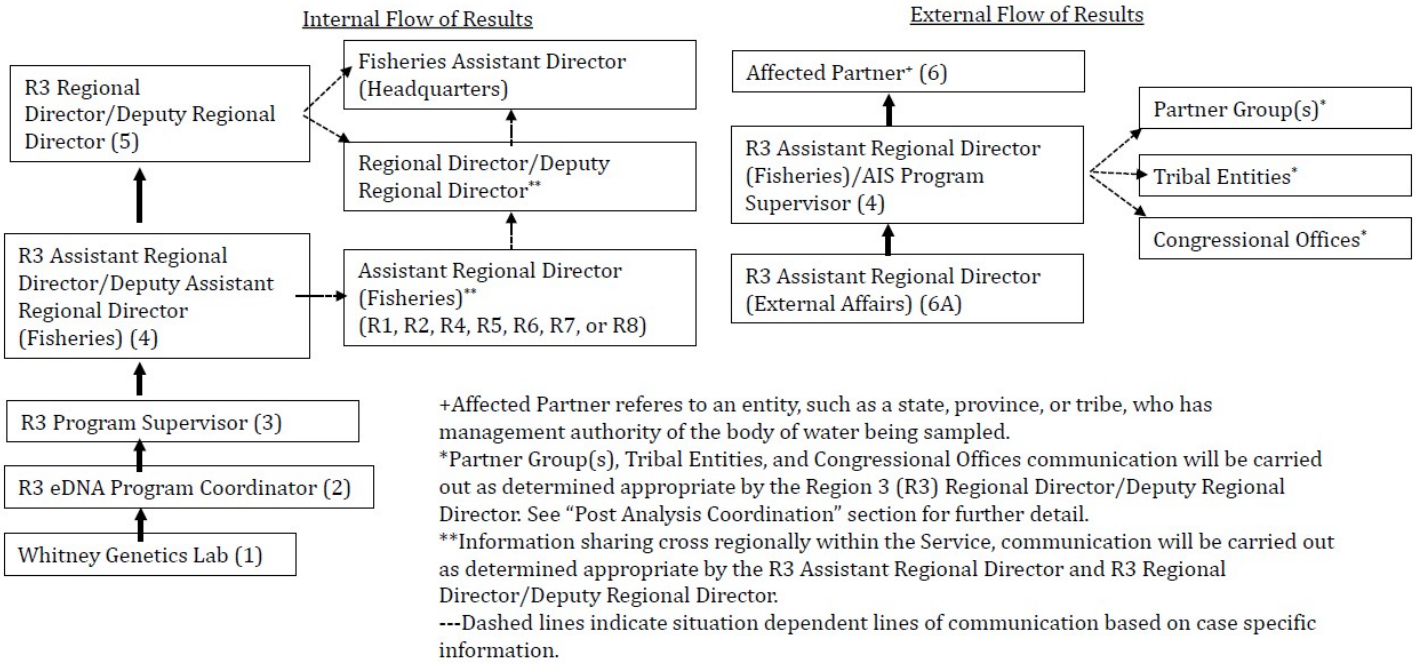
Appendices

Appendix A: Internal Communication of Results SOP

Communication Standard Operating Procedure

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

This Standard Operating Procedure (SOP) is intended to guide U.S. Fish and Wildlife Service (Service) 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 Midwest Region, 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 Asian Carp Regional Coordinating Committees Monitoring and Response Plan and other regionally specific plans. All eDNA sampling should be completed in accordance with the *Quality Assurance Project Plan; eDNA Monitoring of Bighead and Silver Carp* (QAPP; updated annually).



+Affected Partner referes to an entity, such as a state, province, or tribe, who has management authority of the body of water being sampled.
*Partner Group(s), Tribal Entities, and Congressional Offices communication will be carried out as determined appropriate by the Region 3 (R3) Regional Director/Deputy Regional Director. See "Post Analysis Coordination" section for further detail.
**Information sharing cross regionally within the Service, communication will be carried out as determined appropriate by the R3 Assistant Regional Director and R3 Regional Director/Deputy Regional Director.
---Dashed lines indicate situation dependent lines of communication based on case specific information.

Data sharing from FWCO's and Whitney Genetics Laboratory (WGL)

1. Once sample processing is complete and positives identified, results will be communicated through the flowchart (Figure 1) within Region 3 (R3) Fisheries to the Assistant Regional Director (ARD) level. Reporting internally to the eDNA Coordinator level as the results are determined by the Whitney Genetics Lab (WGL) will help streamline the process and allow time for the eDNA Coordinator to prepare for disseminating the results up and out to the affected partner.
2. eDNA results information is to be kept confidential, being transmitted as shown in the flow chart (Figure 1). Any potential communication with partner groups or other entities outside the Service will not occur prior to informing the affected partner. For further information on the dissemination of results to others, see the "Post Analysis Coordination" Section below.
3. eDNA results information will be formatted as described in the Results Procedure Section (below) by the eDNA Coordinator and prepared as a briefing for the Fisheries Deputy Assistant Regional Director (DARD), and ARD.
4. Once briefed, the R3 Fisheries ARD will brief the R3 Regional Director (RD), or Deputy Regional Director (DRD), which will allow the RD or DRD to provide input. The R3 Fisheries ARD or delegated.eDNA Program Coordinator will disseminate the results as appropriate to affected partner and partner group(s) as outlined in the Post Analysis Coordination Section (below).
5. The communication and briefing process from eDNA Program Coordinator to RD and/or DRD should occur quickly. This will allow R3 to disseminate results promptly and maintain our responsiveness to affected partner and partner group(s).
6. When communications are made which transmit results or data from one step to the next in the flowchart (Figure 1), the person sending it must confirm receipt of the information via email response to ensure the message was received and maintain a record of the exchange.
7. Communication of results beyond the eDNA Program Coordinator will only be made once all samples from the sampling event are fully analyzed.

Results Procedure

1. The sampling event data will be packaged together by the eDNA Program Coordinator in a uniform template to be used to inform the affected partner. Sampling events are defined by the geographic boundaries 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 by the WGL once processing at the lab is completed.
4. The eDNA Database Coordinator, as part of gathering this information, will compare the datasets provided by the WGL and the FWCO to ensure the data matches each other, i.e. labeling and unique identifiers match, and combine them to create the final data package to be sent the eDNA Program Coordinator.
5. The data package to be sent to the affected partner will include:
    a. Geo-referenced map indicating all sites eDNA samples were taken, highlighting those sites that were determined to have positive detections for eDNA (Figure 2).
    b. Digital Excel file with each sample uniquely identified. Data columns will include: Sample ID, Latitude and Longitude in decimal degrees, Date of Collection, Water Body Name, Silver Carp Results, and/or Bighead Carp Results.
    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 attached as Appendix B). This memo will be sent via email.

d. A press release template with eDNA specific language will be provided upon request.

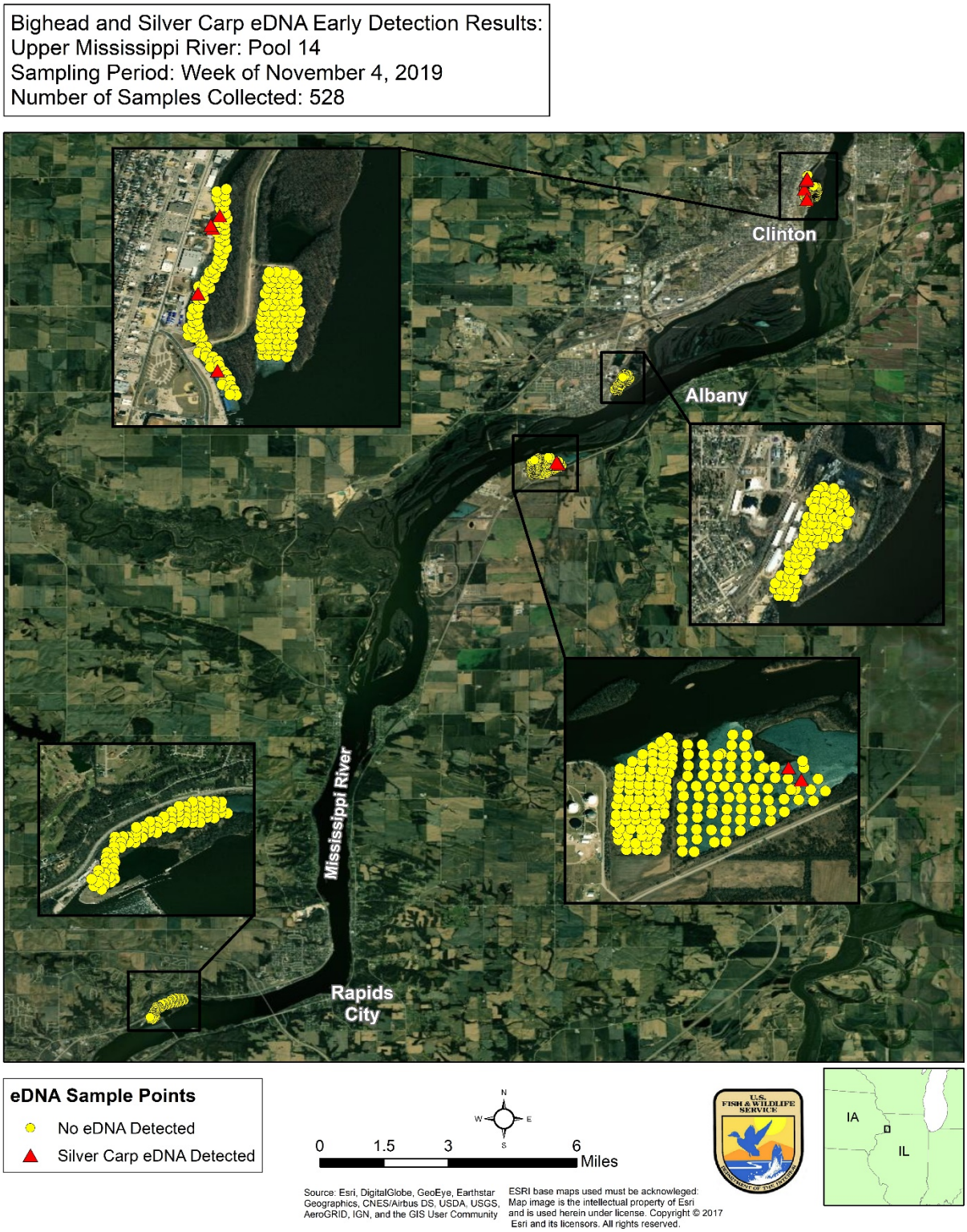
Post Analysis Coordination

1. The data package of results will be emailed from the AIS Program Supervisor directly to the affected partner point of contact (as determined by the affected partner) with a carbon copy to the Assistant Regional Director of Fisheries. Prior to this transmission to the affected partner, the AIS Program Supervisor will send a notification of the results information to the eDNA Station Lead and the Project Leader of the FWCO that collected the samples. This communication is confidential and allows Service leadership to prepare for partner inquiries following notification.
2. When notifying the affected partner, the data package will also be transmitted via email to our website manager and External Affairs Office. The Service External Affairs team will be prepared to assist the affected partner with outreach or press release materials upon request. This transmission will start a 5 business day waiting period at the end of which the results will be posted to a Service website. However, upon written request from an affected partner, the Service 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.
3. Following transmission of the data package to the affected partner, the eDNA Processing Lead 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. We will also assist as requested in a partner led press release sharing the results with the general public.
4. The website manager (External Affairs) will review the materials and format them as needed to prepare them for posting on the USFWS public website: www.fws.gov/midwest/fisheries/eDNA.html
5. 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.
6. Immediately upon posting the results online, the website manager will inform the ARD/DARD of the posting.
7. In addition, partner groups, tribal entities and congressional member offices will be notified on a case dependent basis.
    a. Partner Groups – Groups, such as the Asian Carp Regional Coordinating Committee, the Council of Great Lakes Fisheries Agencies, the Great Lakes Fishery Commission Lake Committees, the Mississippi River Interstate Cooperative Resource Association, etc. will be notified via email and/or phone call from the DRD (delegated to ARD as appropriate) to their chairperson upon issuance of a press release by the affected partner.
    b. 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 (See Results Procedure section). 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 website.
    c. 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).

Communication SOP Appendix A

Figure 2. Example of the standard map template used to report Bighead and Silver Carp eDNA Early Detection results for a case. This example map displays the eDNA detection results for Bighead and Silver Carp in the Upper Mississippi River Pool 14 for the week of November 4, 2019.



Communication SOP Appendix A

Dear (Insert name of Partner),

Enclosed with this letter 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 (Enter Name of Sampling Location or Water Body). These results are presented geographically to provide an overview of the locations where each individual sample was collected, as well as indicating those that were found to have positive eDNA detections for Bighead and/or Silver Carp eDNA (include if positives detections are found). Additionally, there is a table provided which identifies each individual sample collected by unique identifier and its corresponding data.

<u>If one or more samples were positive for Bighead and Silver Carp eDNA, use this paragraph:</u>

Of the X samples taken, positive eDNA detections were found in X samples. Of those, X were positive for silver carp only and/or X were positive for bighead carp only and/or X were positive for ACTM marker only. (If ACTM marker positive) The ACTM marker is a general Bigheaded Carp marker which tells us that eDNA has been detected for either Bighead Carp, Silver Carp or both but is not specific enough to say which species of the two. Working with you as our partner, we would recommend that…

<u>If no samples were positive for Bighead and Silver Carp eDNA, use this paragraph:</u>

After reviewing the data, you will see that none of the water samples collected in this effort were found to be positive for Bighead and/or Silver Carp eDNA. We will keep you appraised as further information is collected through future sampling efforts.

Please be aware that these results will be posted on our public website no later than (Enter approximate time (noon) and date of posting if no press release if issued), 3 business days after the transmission of this message. Upon your request, we will work with you to interpret these results and issue a press release.

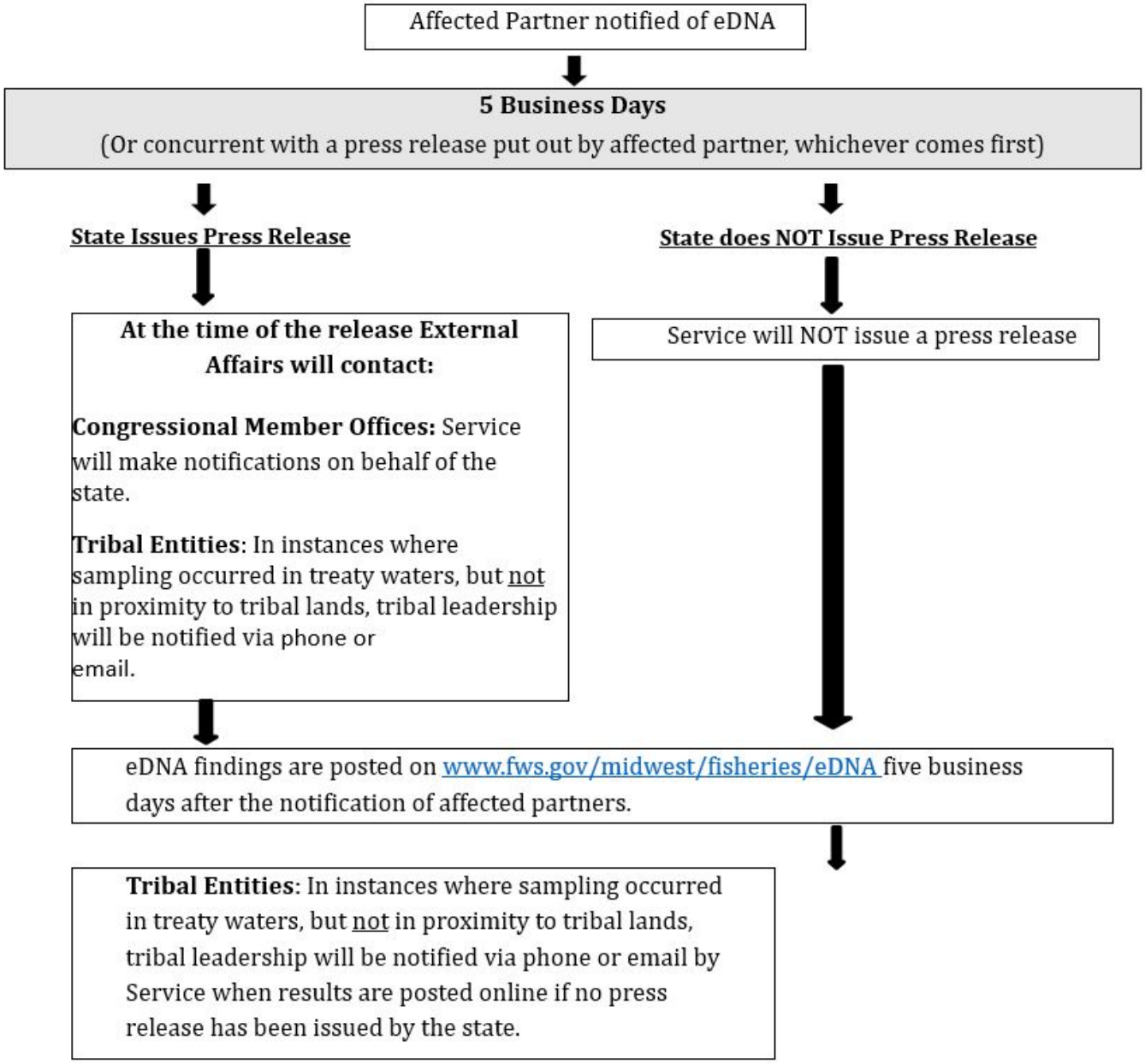
<u>Include if more than one affected partner:</u>

This information has also been sent to (Enter names of other affected partner) as affected partners. Please work together with the Service and the other affected partners to coordinate next steps in responding to these results and providing information to the public to ensure a clear and consistent message.

Please contact eDNA Program Coordinator XXXX at (Phone and email) if you have any questions.

Communication SOP Appendix A

U.S. Fish and Wildlife Service External Affairs Flow Chart

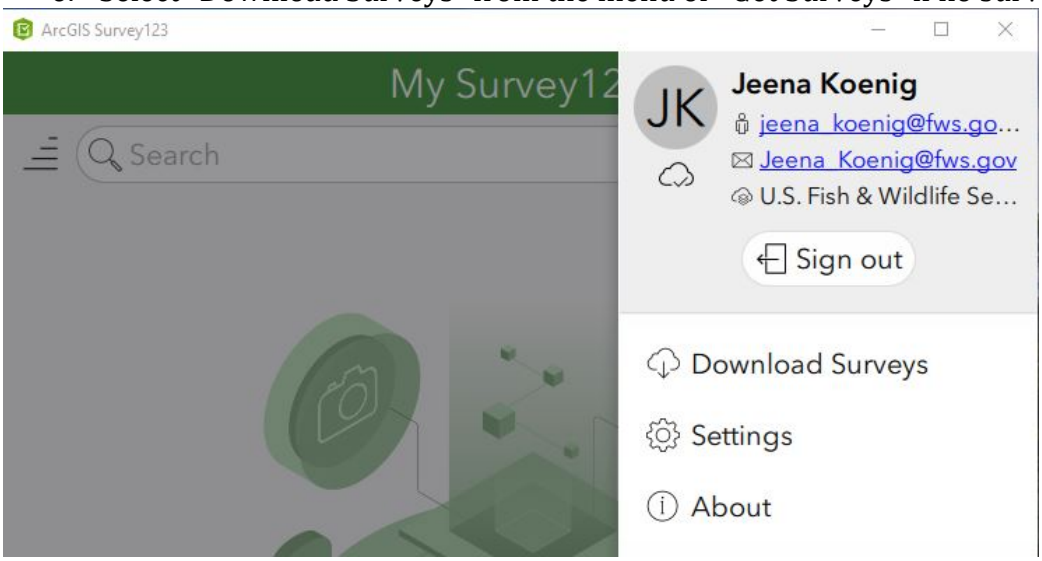


Appendix B: Survey 123 COC Creation and Submission

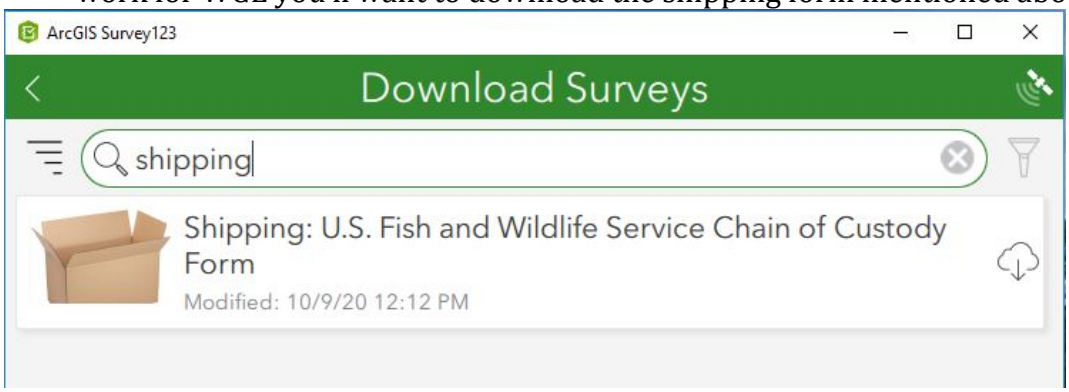
Survey 123 Instructions for COC Creation/Submission

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 Example:

La Crosse FWCO: U.S. Fish and Wildlife Service Chain of Custody Record
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: *
Monday, October 19, 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

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

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, October 19, 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 10/19/2020
211

COC Review (WGL Only):

COC Review (WGL Only):

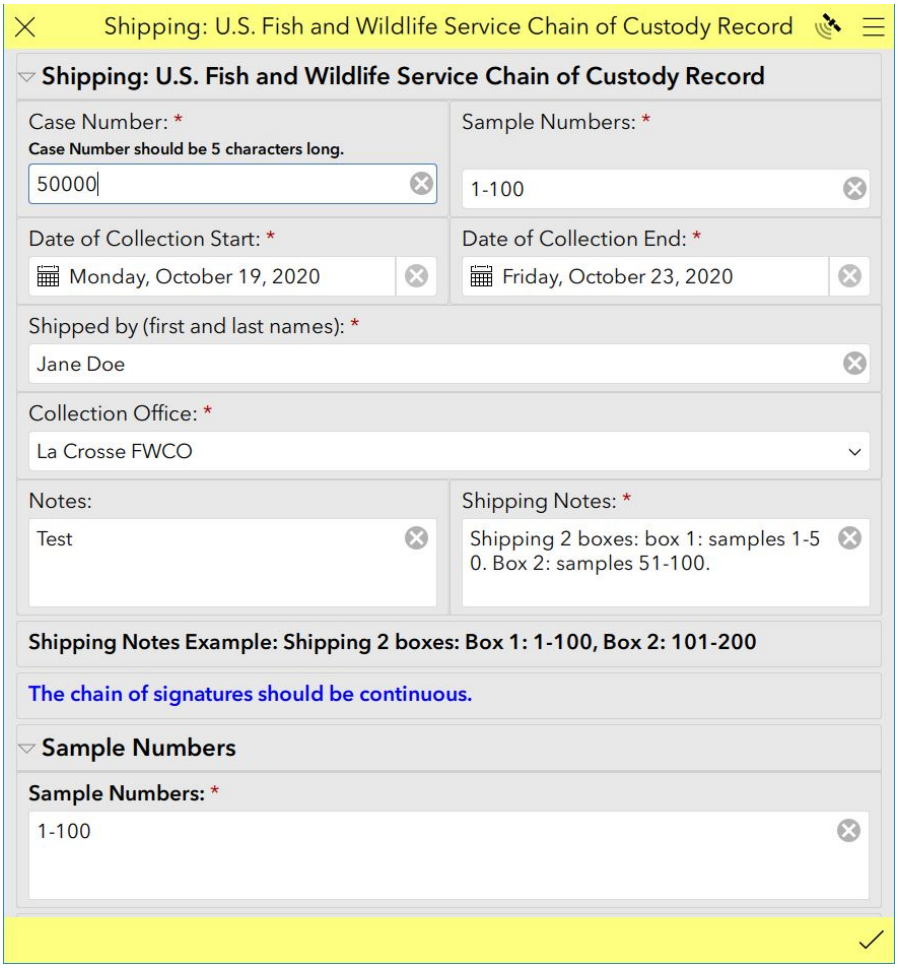
Reviewed by:
Maren Tuttle-lau

Date of Review:
Date

Review Status:
( ) Approved ( ) Needs Correction (●) Pending

Review Notes:

Shipping COC Form:



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

Release Signature

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

Jane Doe

247

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, October 23, 2020

Delivered via: *

- (●) FedEX
- ( ) In Person
- ( ) Other

Are you WGL?

- ( ) Yes
- (●) No

Please initial and date each Editing Note.

Editing Notes:

Test

WGL Only:

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:

Test

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

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

Last updated: June 2020

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