

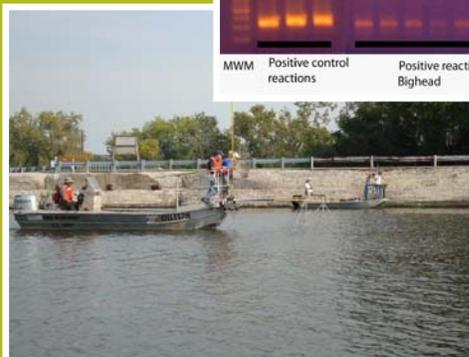
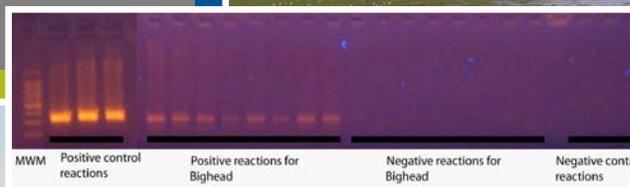
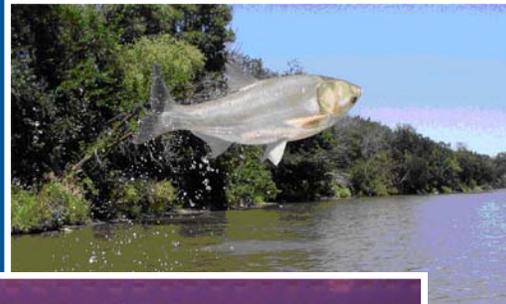
# Revised Final Independent External Peer Review Report Environmental DNA (eDNA) Science and Methodology

Prepared by  
Battelle Memorial Institute

Prepared for  
Department of the Army  
U.S. Army Corps of Engineers  
Ecosystem Restoration Planning Center of Expertise  
Rock Island District

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Task Control Number: 10100  
Delivery Order: 0904

December 7, 2010





**SHORT-TERM ANALYSIS SERVICE (STAS)**

**on**

**Revised Final Independent External Peer Review Report  
Environmental DNA (eDNA) Science and Methodology**

**by**

**Battelle  
505 King Avenue  
Columbus, OH 43201**

**for**

**Department of the Army  
U.S. Army Corps of Engineers  
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**Scientific Services Program**

The views, opinions, and/or findings contained in this report are those of the author and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

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**REVISED FINAL  
INDEPENDENT EXTERNAL PEER REVIEW REPORT  
for the**

**Environmental DNA (eDNA) Science and Methodology**

**EXECUTIVE SUMMARY**

State and federal agencies and stakeholders have been working together to stop the spread of nuisance fish between the Mississippi River Basin and the Great Lakes since the late 1990s. To do this, they have constructed a series of electrical barriers near Romeoville, Illinois. These barriers are a non-lethal deterrent to fish that do not interfere with water flow and minimize impact to navigation in the Chicago Sanitary and Ship Canal (a man-made waterway that provides a direct hydraulic connection between Lake Michigan and the Mississippi River Basin). Without intervention, invasive species such as the Asian carp can transfer between the basins, competing with native species for food, living space, and spawning areas with potential major negative impact to the economy and the environment.

An interagency group of fisheries scientists developed an Asian carp monitoring program to determine the level of threat to the Great Lakes by attempting to identify the leading edge of the invasion of Asian carp. As part of this plan, a team from the University of Notre Dame (UND) led by Dr. David Lodge began testing water samples for the presence of DNA of bighead and silver carp (collectively referred to as Asian carp) in the Chicago Area Waterway System (CAWS) using a technique called the environmental DNA (eDNA) method in the spring of 2009. In the summer of 2009 they found DNA fragments closer to the barrier than had been detected earlier, indicating that Asian carp may be closer to the Great Lakes than previously thought. In November 2009, Asian carp eDNA was detected above the fish barrier, and on June 22, 2010, a live bighead carp was captured in Lake Calumet. Even if additional Asian carp are above the fish barrier, it is not known how many fish may be present. Therefore, it is critical that the barrier continue to be operated effectively to minimize the potential that a sustaining population of Asian carp becomes established above the barrier.

All fish, including Asian carp, release DNA into the environment naturally as mucoidal secretions (slime), feces, and urine. These substances and the DNA within them slowly degrade in the environment, but can be collected in water samples if caught soon enough. Water samples are filtered and the DNA is collected and analyzed for the presence or absence of Asian carp using an eDNA method developed by researchers at UND. The process of testing eDNA is not instantaneous, but DNA can be held in suspension and transported. The presence of eDNA is detected by extracting and amplifying short fragments of the shed DNA from the water sample. In contrast to other surveillance methods, the eDNA method does not rely on direct observation of Asian carp to evaluate presence.

The objective of using the eDNA method, the only available analytical tool, is to use the method as a screening and early detection indicator. Laboratory and field studies using eDNA methods

indicate that Asian carps can be detected in two liter water samples from sites where Asian carp have been captured through electrofishing.

The U.S. Army Corps of Engineers (USACE) is conducting an Independent External Peer Review (IEPR) of the eDNA science and methodology. Battelle, as a 501(c)(3) non-profit science and technology organization with experience in establishing and administering peer review panels for USACE, was engaged to coordinate the IEPR of the eDNA science and methodology. Independent, objective peer review is regarded as a critical element in ensuring the reliability of scientific analyses. The IEPR was external to the agency and conducted following USACE and Office of Management and Budget (OMB) guidance described in USACE (2010), USACE (2007), and OMB (2004). This final report describes the IEPR process, describes the panel members and their selection, and summarizes the Final Panel Comments of the IEPR Panel (the Panel). The IEPR of the eDNA science and methodology reviewed the scientific ideas, methods, and evaluations and did not include an assessment of management decisions or actions.

Four panel members were selected for the IEPR from 27 identified candidates. Based on the technical content of the eDNA science and methodology and the overall scope of the project, the final panel members were selected for their technical expertise in genetics and population ecology. Although the panel was disclosed to USACE, Battelle made the final decision on selecting the panel.

The Panel received electronic versions of the eDNA science and methodology documents, along with a charge that solicited comments on specific sections of the documents to be reviewed. The charge was prepared by Battelle to assist the USACE in the development of the charge questions that was to guide the peer review, according to guidance provided in USACE (2010) and OMB (2004). USACE was given the opportunity to provide comments and make revisions, and subsequently approved the final charge questions.

The USACE Project Delivery Team briefed the Panel and Battelle during a kick-off meeting held via teleconference prior to the start of the review. In addition, an in-person meeting to discuss the eDNA science and methodology was held at UND on July 22 and 23, 2010. Other than the teleconference and the in-person meeting, there was no direct communication between the Panel and USACE during the peer review process. The Panel produced approximately 24 individual comments in response to 6 charge questions.

IEPR panel members reviewed the eDNA science and methodology documents individually. The panel members then met via teleconference with Battelle to review key technical comments, discuss charge questions for which there were conflicting responses, and reach agreement on the Final Panel Comments to be provided to USACE. Each Final Panel Comment was documented using a four-part format consisting of: (1) a comment statement; (2) the basis for the comment; (3) the significance of the comment (high, medium, or low); and (4) recommendations on how to resolve the comment. Overall, eight Final Panel Comments were identified and documented. Of these, two were identified as having high significance and six had medium significance.

When USACE learned of the eDNA research, USACE evaluated the use of eDNA testing to help determine the possible location of Asian carp. USACE viewed it as an emerging technology still in the research stage. It had never been applied in the field before in a water body like the CAWS. Nor had it undergone independent scientific studies or peer reviews of the type that the Corps would normally require before applying a technology which would inform management decisions. USACE decided to use eDNA in the CAWS despite the uncertainties associated with research that had not been fully tested and despite results that would leave many questions unanswered. Subsequently, USACE decided to commission this peer review to enhance understanding of the meaning of eDNA results. This report documents the methods and results associated with conducting the peer review of the eDNA technology.

Table ES-1 summarizes the Final Panel Comments by level of significance. Detailed information on each comment is contained in Appendix A of this report.

**Table ES-1. Overview of 8 Final Panel Comments Identified by the eDNA Science and Methodology IEPR Panel**

<b>Significance – High</b>	
1	The current mitochondrial DNA (mtDNA) methodology cannot distinguish pure silver or bighead carp from hybrids of the two species.
2	The eDNA methodology does not unequivocally indicate the physical presence of live bighead or silver carp.
<b>Significance – Medium</b>	
3	The assumption that the eDNA methodology is of limited use in the winter months should be evaluated.
4	The sampling design used is not statistically based.
5	The eDNA methodology should be used to screen ichthyoplankton and egg samples to provide a means to identify sites with successful reproduction.
6	The current eDNA methodology should be modified to estimate the number of individual fish contributing eDNA to a positive sample.
7	The current PCR methodology should be changed to a quantitative PCR (qPCR) approach to estimate the quantity of silver and bighead DNA in a sample and to speed detection of eDNA in water samples.
8	The production, movement, and degradation of eDNA in the system should be evaluated.

USACE guidance (2010) states the final report will contain the Panel's "assessment of the adequacy and acceptability of the economic, engineering, and environmental methods, models, and analyses used." However, for the eDNA IEPR, the Panel focused on the environmental aspects of the eDNA science and methodology; no economic or engineering assessment was conducted. The Panel generally agreed on its assessment of the adequacy and acceptability of the environmental methods, models, and analyses used in the eDNA documents. Disagreements among panel members existed regarding the levels of significance for Final Panel Comments 1 and 2. For each of these Final Panel Comments, three panel members thought it warranted a "high" level of significance, while one panel member thought a "medium" level of significance was appropriate.

When it was apparent that a consensus would not be reached on the levels of significance for these two Final Panel Comments, Battelle recommended that the opinion of the majority of the Panel be the deciding factor and the Panel agreed. For Final Panel Comment 1, the panel member who thought it should be a “medium” level of significance thought that while distinguishing silver or bighead carp from hybrids of these species may be important in many contexts, it is not essential in all situations. In particular, if passage of fish above the barrier in the CAWS is of concern, distinguishing a hybrid from “pure” silver or bighead carp would not be critical. For Final Panel Comment 2, the panel member who thought it should be a “medium” level of significance thought that the concerns raised in that comment were already addressed as much as possible by the methodology itself and he did not think that the recommendations for resolution would resolve the issue.

The following statements summarize the Panel’s findings, which are described in the Final Panel Comments and are discussed in more detail in Appendix A.

**Genetics:** The overall methodology to detect eDNA in the water column and attribute that eDNA in the form of mtDNA back to silver and bighead maternal lineages is sound. However, opportunities to estimate the number of fish contributing DNA to a positive sample, quantify levels of silver and bighead carp DNA in the water samples, rule out alternate alternate pathways to the occurrence of silver and bighead carp DNA in CAWS, address the awareness of the possible presence of hybrids of these two species, and use more sophisticated DNA detection protocols have been missed or overlooked. It is surprising that the available mtDNA sequences were not examined for intraspecific variation, and that the limitations of the mtDNA data alone to deal with the problem of potential hybrids were not presented.

**Population Ecology:** The eDNA methodology has great potential to complement traditional fishery sampling methods. Strengths of the method include the ability to rapidly collect water samples for analysis from a large geographic area with minimal cost. Also, the method is potentially more sensitive at detecting bighead and silver carp in environments typical of the CAWS than traditional fishery methods. Key limitations of the method, however, are that the location of fish contributing eDNA to a water sample is not known with certainty, and there is the potential for transport of eDNA over substantial distances or across barriers. Also, because sampling is seasonal with no sampling during the colder months (especially preceding spring spawning runs) when the fish’s susceptibility to the electrical barriers is at its lowest physiologically, there may be significant upstream movement that is being discounted inappropriately due to unfounded assumptions. Finally, not using the eDNA technology as a tool to screen ichthyoplankton collections misses a great opportunity to determine if successful spawning and hatch is occurring above the barrier system.

**Alternative Surveillance Methods:** In addition to these overall genetics and population ecology findings, the following paragraphs include the Panel’s evaluation of whether any alternative surveillance methods exist that might more accurately or precisely detect the location of the invasion front of Asian carp in the CAWS.

The question of whether a feasible alternative exists to the eDNA methodology that is more reliable and precise needs to be answered in at least two parts. Firstly, the precision of a

sampling program depends not only on the measure methods (e.g., eDNA vs. electrofishing), but also on the sample size. Thus, a comparison of electrofishing (for example) with the eDNA methodology would require additional information such as the sample size (or total cost) allocated to each method. Further, there is insufficient information on the “catchability” of Asian carp in any gear to allow for an accurate comparison.

The question of whether an alternative exists (emphasis on the word an) implies a view that either one method or another should be employed in a sampling program, when in fact, various methods may in fact complement one another. Some of the advantages of the eDNA methodology include:

- sample collection can occur over a large spatial area very rapidly
- the cost per sample is relatively low compared to traditional fishery methods
- dispersal of eDNA within the water allows for the eDNA sample to integrate fish presence over a larger area than traditional fishery methods where capture of a fish depends on gear being present at the exact location and time as an Asian carp

In the Panel’s opinion, no other single method provides this suite of advantages offered by eDNA samples.

Some of the limitations of the eDNA method include:

- detection of eDNA does not provide conclusive proof of the physical presence of a live fish at a given location in space and time
- eDNA detections do not provide information on the size or age (for example) of individuals present
- as currently implemented using mitochondrial DNA, the method cannot distinguish between pure silver or bighead carp and their hybrids, nor can it specify the gender of individuals caught
- there is a time delay inherent between water sample collection and processing for eDNA, and thus detection is not immediate as it would be with traditional fish sampling gears

Because of these limitations, a sampling program using traditional fish sampling methods is likely required to provide all of the information needed to make critical management decisions. The eDNA method thus provides a useful complement to traditional fish sampling gears, and can greatly improve the efficiency of a sampling program for Asian carp.

## TABLE OF CONTENTS

EXECUTIVE SUMMARY .....	i
1. INTRODUCTION .....	1
2. PURPOSE OF THE IEPR .....	2
3. METHODS.....	2
3.1 Planning and Schedule.....	3
3.2 Identification and Selection of IEPR Panel Members .....	5
3.3 Preparation of the Charge and Conduct of the IEPR .....	7
3.4 Review of Individual Comments .....	8
3.5 IEPR Panel Teleconference .....	8
3.6 Preparation of Final Panel Comments .....	8
4. PANEL DESCRIPTION .....	9
5. SUMMARY OF FINAL PANEL COMMENTS .....	14
6. REFERENCES .....	18
Appendix A Final Panel Comments on the eDNA Science and Methodology	
Appendix B. Final Charge to the Independent External Peer Review Panel on the eDNA Science and Methodology	
Appendix C. Revisions Made to the Final Independent External Peer Review Report on Environmental DNA (eDNA) Science and Methodology	

## LIST OF TABLES

Table ES-1. Overview of 8 Final Panel Comments Identified by the eDNA Science and Methodology IEPR Panel.....	iii
Table 1. eDNA Science and Methodology IEPR Schedule .....	4
Table 2. eDNA IEPR Panel: Technical Criteria and Areas of Expertise .....	11
Table 3. Overview of 8 Final Panel Comments Identified by the eDNA Science and Methodology IEPR Panel.....	17

## LIST OF ACRONYMS

ANSBAP	Aquatic Nuisance Species Barrier Advisory Panel
CAWS	Chicago Area Waterway System
CSSC	Chicago Sanitary and Ship Canal
DNA	Deoxyribonucleic Acid
DrChecks	Design Review and Checking System
eDNA	Environmental DNA
ERDC	Engineer Research and Development Center
IEPR	Independent External Peer Review
mtDNA	mitochondrial DNA
nucDNA	nuclear DNA
NTP	Notice to Proceed
OMB	Office of Management and Budget
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
SOP	standard operating procedure
UCD	University of California at Davis
UND	University of Notre Dame
USACE	United States Army Corps of Engineers
UV	ultraviolet

# 1. INTRODUCTION

State and federal agencies and stakeholders have been working together to stop the spread of nuisance fish between the Mississippi River Basin and the Great Lakes since the late 1990s. To do this, they have constructed a series of electrical barriers near Romeoville, Illinois. These barriers are a non-lethal deterrent to fish that do not interfere with water flow and minimize impact to navigation in the Chicago Sanitary and Ship Canal (a man-made waterway that provides a direct hydraulic connection between Lake Michigan and the Mississippi River Basin). Without intervention, invasive species such as the Asian carp can transfer between the basins, competing with native species for food, living space, and spawning areas with potential major negative impact to the economy and the environment.

An interagency group of fisheries scientists developed an Asian carp monitoring program to determine the level of threat to the Great Lakes by attempting to identify the leading edge of the invasion of Asian carp. As part of this plan, a team from the University of Notre Dame (UND) led by Dr. David Lodge began testing water samples for the presence of DNA of bighead and silver carp (collectively referred to as Asian carp) in the Chicago Area Waterway System (CAWS) using a technique called the environmental DNA (eDNA) method in the spring of 2009. In the summer of 2009 they found DNA fragments closer to the barrier than had been detected earlier, indicating that Asian carp may be closer to the Great Lakes than previously thought. In November 2009, Asian carp eDNA was detected above the fish barrier, and on June 22, 2010, a live bighead carp was captured in Lake Calumet. Even if additional Asian carp are above the fish barrier, it is not known how many fish may be present. It is critical that the barrier continue to be operated effectively to minimize the potential that a sustaining population of Asian carp becomes established above the barrier.

All fish, including Asian carp, release DNA into the environment naturally as mucoidal secretions (slime), feces, and urine. These substances and the DNA within them slowly degrade in the environment, but can be collected in water samples if caught soon enough. Water samples are filtered and the DNA is collected and analyzed for the presence or absence of Asian carp using an eDNA method developed by researchers at UND. The process of testing eDNA is not instantaneous, but DNA can be held in suspension and transported. The presence of eDNA is detected by extracting and amplifying short fragments of the shed DNA from the water sample. In contrast to other surveillance methods, the eDNA method does not rely on direct observation of Asian carp to evaluate presence.

The objective of using the eDNA method, the only available analytical tool, is to use the method as a screening and early detection indicator. Laboratory and field studies using eDNA methods indicate that Asian carps can be detected in two liter water samples from sites where Asian carp have been captured through electrofishing.

The objective of the work described here was to conduct an Independent External Peer Review (IEPR) of the eDNA science and methodology in accordance with procedures described in the Department of the Army, U.S. Army Corps of Engineers Engineer (USACE) Circular *Civil Works Review Policy* (EC No. 1165-2-209) (USACE, 2010), USACE CECW-CP memorandum

*Peer Review Process* (USACE, 2007), and Office of Management and Budget (OMB) bulletin *Final Information Quality Bulletin for Peer Review* (OMB, 2004). Battelle, as a 501(c)(3) non-profit science and technology organization with experience in establishing and administering peer review panels, was engaged to coordinate the IEPR of the eDNA science and methodology. Independent, objective peer review is regarded as a critical element in ensuring the reliability of scientific analyses.

This final report details the IEPR process, describes the IEPR panel members and their selection, and summarizes the Final Panel Comments of the IEPR Panel on the existing genetic and population ecology analyses contained in the eDNA science and methodology. Detailed information on the Final Panel Comments is provided in Appendix A.

When USACE learned of the eDNA research, USACE evaluated the use of eDNA testing to help determine the possible location of Asian carp. USACE viewed it as an emerging technology still in the research stage. It had never been applied in the field before in a water body like the CAWS. Nor had it undergone independent scientific studies or peer reviews of the type that the Corps would normally require before applying a technology which would inform management decisions. USACE decided to use eDNA in the CAWS despite the uncertainties associated with research that had not been fully tested and despite results that would leave many questions unanswered. Subsequently, USACE decided to commission this peer review to enhance understanding of the meaning of eDNA results. This report documents the methods and results associated with conducting the peer review of the eDNA technology.

## **2. PURPOSE OF THE IEPR**

To ensure that USACE documents are supported by the best scientific and technical information, USACE has implemented a peer review process that uses IEPR to complement the Agency Technical Review, as described in USACE (2010) and USACE (2007).

In general, the purpose of peer review is to strengthen the quality and credibility of the USACE decision documents in support of its Civil Works program. IEPR provides an independent assessment of the economic, engineering, and environmental analysis of the project study. In particular, the IEPR addresses the technical soundness of the project study's assumptions, methods, analyses, and calculations and identifies the need for additional data or analyses to make a good decision regarding implementation of alternatives and recommendations. The IEPR of the eDNA science and methodology reviewed the scientific ideas, methods, and evaluations and did not include an assessment of management decisions or actions.

In this case, the IEPR of the eDNA science and methodology was conducted and managed using contract support from Battelle, which is an Outside Eligible Organization under Section 501(c)(3) of the U.S. Internal Revenue Code with experience conducting IEPRs for USACE.

## **3. METHODS**

This section describes the method followed in selecting the members for the IEPR Panel (the Panel) and in planning and conducting the IEPR. The IEPR was conducted following procedures

described by USACE (2010) and in accordance with USACE (2007) and OMB (2004) guidance. Supplemental guidance on evaluation for conflicts of interest was obtained from the *Policy on Committee Composition and Balance and Conflicts of Interest for Committees Used in the Development of Reports* (The National Academies, 2003).

### **3.1 Planning and Schedule**

After receiving the notice to proceed (NTP), Battelle held a kick-off meeting with USACE to review the preliminary/suggested schedule, discuss the IEPR process, and address any questions regarding the scope (e.g., clarify expertise areas needed for panel members). Any revisions to the schedule were submitted as part of the final Work Plan.

Table 1 defines the schedule followed in executing the IEPR. Due dates for milestones and deliverables are based on the NTP date of April 13, 2010.

**Table 1. eDNA Science and Methodology IEPR Schedule**

<b>TASK</b>	<b>ACTION</b>	<b>DUE DATE</b>
<b>1</b>	<b>Notice to Proceed (NTP)</b>	April 13, 2010
	<b>Review documents available</b>	May 18, 2010
	*Submit draft Work Plan	June 2, 2010
	USACE provide comments on draft Work Plan	June 9, 2010
	Teleconference (if necessary)	June 9, 2010
	*Submit final Work Plan	June 14, 2010
<b>2</b>	Battelle requests input from USACE on the conflict of interest (COI) questionnaire	May 25, 2010
	USACE provides comments on COI	May 27, 2010
	*Submit list of selected panel members	June 18, 2010
	USACE provides comments on selected panel members	June 23, 2010
	Complete subcontracts for panel members	July 8, 2010
<b>3</b>	*Submit draft charge (combined with draft Work Plan – Task 1)	June 2, 2010
	USACE provides comments on draft charge	June 9, 2010
	*Submit final charge (combined with final Work Plan – Task 1)	June 14, 2010
	USACE approves final charge	June 15, 2010
<b>4</b>	USACE/Battelle kick-off teleconference	May 18, 2010
	Review documents sent to Panel	July 9, 2010
	Battelle/Panel kick-off teleconference	July 12, 2010
	USACE/Battelle/Panel kick-off meeting at University of Notre Dame	July 22, 2010
<b>5</b>	Battelle convenes teleconference with Panel, USACE, and UND to answer any clarifying questions	July 29, 2010
	Panel completes review	August 19, 2010
	Convene panel review teleconference	August 30, 2010
	Panel provides draft FPCs to Battelle	September 8, 2010
<b>6</b>	*Submit final IEPR report	September 24, 2010
<b>7</b>	Input FPCs to DrChecks and Battelle provides FPC response template to USACE	September 28, 2010
	USACE provides draft Evaluator responses and clarifying questions to Battelle	October 26, 2010
	FPC Teleconference between Battelle, Panel, and USACE to discuss FPCs, draft responses and clarifying questions	November 29, 2010
	USACE inputs final Evaluator responses in DrChecks	December 13, 2010
	Battelle inputs BackCheck responses in DrChecks	December 28, 2010
	*Battelle submits pdf printout of DrChecks project file	December 29, 2010
	Project Closeout	March 7, 2011

\* Deliverable

Note that the work items listed in Task 7 occur after the submission of this report. Battelle will enter eight Final Panel Comments developed by the Panel into USACE's Design Review and Checking System (DrChecks), a Web-based software system for documenting and sharing comments on reports and design documents, so that USACE can review and respond to them.

USACE will provide responses (Evaluator Responses) to the Final Panel Comments, and the Panel will respond (BackCheck Responses) to the Evaluator Responses. All USACE and Panel responses will be documented by Battelle.

### **3.2 Identification and Selection of IEPR Panel Members**

The candidates for the Panel were evaluated based on their technical expertise in the following key areas: genetics and population ecology. These areas correspond to the technical content of the eDNA science and methodology and overall scope of the eDNA science and methodology project.

Battelle initially identified more than 27 candidates for the Panel, evaluated their technical expertise, and inquired about potential conflicts of interest. Of these, Battelle chose six of the most qualified candidates and confirmed their interest and availability. Of the six candidates, four were proposed for the final Panel and two were proposed as backup reviewers. Information about the candidate panel members, including brief biographical information, highest level of education attained, and years of experience, was provided to USACE for feedback. Battelle made the final selection of panel members according to the selection criteria described in the Work Plan.

The four proposed primary reviewers constituted the final Panel. The remaining candidates were not proposed for a variety of reasons, including lack of availability, disclosed conflicts of interest, or lack of the precise technical expertise required.

The candidates were screened for the following potential exclusion criteria or conflicts of interest.<sup>1</sup> Participation in previous USACE technical peer review committees and other technical review panel experience was also considered.

- Involvement by you or your firm in any part of Environmental DNA (eDNA) Science and Methodology Study, including
  - Laboratory Audit Report, Lodge Laboratory, Center for Aquatic Conservation, Department of Biological Sciences University of Notre Dame, dated February 5, 1010, US Environmental Protection Agency, Great Lakes National Program Office and National Exposure Research Laboratory, Office of Research and Development.
  - University of Notre Dame sampling and laboratory testing procedures and protocols

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<sup>1</sup> Battelle evaluated whether scientists in universities and consulting firms that are receiving USACE-funding have sufficient independence from USACE to be appropriate peer reviewers. See OMB (2004, p. 18), "...when a scientist is awarded a government research grant through an investigator-initiated, peer-reviewed competition, there generally should be no question as to that scientist's ability to offer independent scientific advice to the agency on other projects. This contrasts, for example, to a situation in which a scientist has a consulting or contractual arrangement with the agency or office sponsoring a peer review. Likewise, when the agency and a researcher work together (e.g., through a cooperative agreement) to design or implement a study, there is less independence from the agency. Furthermore, if a scientist has repeatedly served as a reviewer for the same agency, some may question whether that scientist is sufficiently independent from the agency to be employed as a peer reviewer on agency-sponsored projects."

- Involvement by you or your firm<sup>2</sup> in any work related to:
  - USACE navigation projects in the Great Lakes and Upper Mississippi River system, including the Chicago Sanitary and Ship Canal (CSSC) or the Calumet-Saganashkee (Cal-Sag) Channel;
  - the Aquatic Nuisance Species Dispersal Barrier on the CSSC, Aquatic Nuisance Species Study, Great Lakes and Mississippi River Interbasin Study, Aquatic Nuisance Species Barrier Advisory Panel or related groups;
  - the litigation on closing navigation between the Great Lakes and the Illinois River.
- Involvement by you or your firm<sup>2</sup> in any invasive species or Environmental DNA studies in the Mississippi River Basin and Great Lakes areas.
- Involvement by you or your firm<sup>2</sup> in the conceptual or actual design of any studies for the Environmental DNA (eDNA) Science and Methodology Study.
- Current employment by the U.S. Army Corps of Engineers (USACE).
- Involvement with paid or unpaid expert testimony related to the Environmental DNA (eDNA) Science and Methodology Study.
- Current or previous employment or affiliation with the non-Federal sponsors or any of the following cooperating Federal, State, County, local and regional agencies, environmental organizations, and interested groups: following Federal, State, County, local and regional agencies, environmental organizations, and interested groups: U.S. Environmental Protection Agency, U.S. Fish and Wildlife Service, U.S. National Marine Fisheries Service, Chicago Area Waterway System Team, Illinois Department of Natural Resources, Metropolitan Water Reclamation District of Greater Chicago, United States Coast Guard, Great Lakes Fishery Commission, University of Notre Dame, or Asian Carp Working Group (for pay or pro bono).
- Past, current, pending, or future interests (financial or otherwise) by you, your spouse or children related to the Environmental DNA (eDNA) Science and Methodology Study, including interest in related contracts or awards from USACE.
- Current personal involvement with other USACE projects, including whether involvement was to author any manuals or guidance documents for USACE. If yes, provide titles of documents or description of project, dates, and location (USACE district, division, Headquarters, Engineering Research and Development Center [ERDC], etc.), and position/role. Please highlight and discuss in greater detail any projects that are specifically with the Chicago District.
- Current firm<sup>2</sup> involvement with other USACE projects, specifically those projects/contracts that are with the Chicago District. If yes, provide title/description, dates, and location (USACE district, division, Headquarters, ERDC, etc.), and position/role.
- Any previous employment by the USACE as a direct employee or contractor (either as an individual or through your firm<sup>2</sup>) within the last 10 years, notably if those

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<sup>2</sup> Includes any joint ventures in which your firm is involved.

projects/contracts are with the Chicago District. If yes, provide title/description, dates employed, and place of employment (district, division, Headquarters, ERDC, etc.), and position/role.

- Previous experience conducting technical peer reviews. If yes, please highlight and discuss any technical reviews concerning:
  - genetics
  - fisheries science
  - population ecologyand include the client/agency and duration of review (approximate dates).
- A significant portion (i.e., greater than 50%) of personal or firm<sup>2</sup> revenues within the last 3 years came from USACE contracts.
- Participation in relevant prior Federal studies/programs relevant to this project.
- Participation in relevant prior non-Federal studies/programs relevant to this project.
- Any publicly documented statement (including, for example, advocating for or discouraging against) related to the Environmental DNA (eDNA) Science and Methodology Study.
- Is there any past, present or future activity, relationship or interest (financial or otherwise) that could make it appear that you would be unable to provide unbiased services on this project? If so, please describe:

In selecting the final members of the Panel from the list of candidates, Battelle chose experts who best fit the expertise areas and had no conflicts of interest. The four final reviewers were either affiliated with academic institutions or consulting companies or were independent engineering consultants. Battelle established subcontracts with the panel members when they indicated their willingness to participate and confirmed the absence of conflicts of interest through a signed Conflict of Interest form. Although the panel was disclosed to USACE, Battelle made the final decision on selecting the panel. Section 4 of this report provides names and biographical information on the panel members.

Prior to beginning their review and within 2 days of their subcontracts being finalized, all members of the Panel attended a kick-off meeting via teleconference planned and facilitated by Battelle in order to review the IEPR process, the schedule, communication, and other pertinent information for the Panel.

### **3.3 Preparation of the Charge and Conduct of the IEPR**

Battelle drafted a preliminary charge document, including specific charge questions and discussion points. The charge was prepared by Battelle to assist USACE in the development of the charge questions that guided the peer review, according to guidance provided in USACE (2010) and OMB (2004). The draft charge was submitted to USACE for evaluation as part of the draft Work Plan. USACE provided comments and revisions to the draft charge, which were used to produce the final charge. The final charge was submitted to USACE for approval. In addition to a list of six charge questions/discussion points, the final charge included general guidance for the Panel on the conduct of the peer review (provided in Appendix B of this final report).

Battelle planned and facilitated a final kick-off meeting via teleconference during which USACE presented project details to the Panel. Before the meeting, the IEPR Panel received an electronic version of the eDNA science and methodology documents and the final charge. In addition, an in-person meeting to discuss the eDNA science and methodology was held at UND on July 22 and 23, 2010. Other than the teleconference and the in-person meeting, there was no direct communication between the Panel and USACE during the peer review process.

A full list of the documents reviewed by the Panel is provided in Appendix B of this report. The Panel was instructed to address the charge questions/discussion points within a comment-response form provided by Battelle.

### **3.4 Review of Individual Comments**

The Panel produced approximately 24 individual comments in response to the charge questions/discussion points. Battelle reviewed the comments to identify overall recurring themes, areas of potential conflict, and other overall impressions. As a result of the review, Battelle was able to summarize the 24 comments into a preliminary list of 8 overall comments and discussion points. Each panel member's individual comments were shared with the full Panel in a merged individual comments table.

### **3.5 IEPR Panel Teleconference**

Battelle facilitated a 4-hour teleconference with the Panel so that the panel experts, many of whom are from diverse scientific backgrounds, could exchange technical information. The main goal of the teleconference was to identify which issues should be carried forward as Final Panel Comments in the IEPR report and decide which panel member would serve as the lead author for the development of each Final Panel Comment. This information exchange ensured that the final IEPR report would accurately represent the Panel's assessment of the project, including any conflicting opinions. The Panel engaged in a thorough discussion of the overall positive and negative comments, added any missing issues of high-level importance to the findings, and merged any related individual comments. In addition, Battelle confirmed each Final Panel Comment's level of significance to the Panel.

At the end of these discussions, the Panel identified eight comments and discussion points that should be brought forward as Final Panel Comments.

### **3.6 Preparation of Final Panel Comments**

Following the teleconference, Battelle prepared a summary memorandum for the Panel documenting each Final Panel Comment (organized by level of significance). The memorandum provided the following detailed guidance on the approach and format to be used to develop the Final Panel Comments for the eDNA science and methodology IEPR:

- **Lead Responsibility:** For each Final Panel Comment, one Panel member was identified as the lead author responsible for coordinating the development of the Final Panel Comment and submitting it to Battelle. Battelle modified lead assignments at the direction of the Panel. To assist each lead in the development of the Final Panel Comments, Battelle distributed the merged individual comments table, a summary

detailing each draft final comment statement, an example Final Panel Comment following the four-part structure described below, and templates for the preparation of each Final Panel Comment.

- Directive to the Lead: Each lead was encouraged to communicate directly with other IEPR panel members as needed and to contribute to a particular Final Panel Comment. If a significant comment was identified that was not covered by one of the original Final Panel Comments, the appropriate lead was instructed to draft a new Final Panel Comment.
- Format for Final Comments: Each Final Panel Comment was presented as part of a four-part structure:
  1. Comment Statement (succinct summary statement of concern)
  2. Basis for Comment (details regarding the concern)
  3. Significance (high, medium, low; see description below)
  4. Recommendation for Resolution (see description below).
- Criteria for Significance: The following were used as criteria for assigning a significance level to each Final Panel Comment:
  1. High: Describes a fundamental problem with the methodology that could affect the recommendation or justification of the methodology for the intended purpose.
  2. Medium: Affects the completeness or understanding of the reports/methodology.
  3. Low: Affects the technical quality of the reports, but will not affect the recommendation of the methodology for the intended purpose.
- Guidance for Developing the Recommendation: The recommendation was to include specific actions that the USACE should consider to resolve the Final Panel Comment (e.g., suggestions on how and where to incorporate data into the analysis, how and where to address insufficiencies, areas where additional documentation is needed).

At the end of this process, eight Final Panel Comments were prepared and assembled. Battelle reviewed and edited the Final Panel Comments for clarity, consistency with the comment statement, and adherence to guidance on the Panel's overall charge, which included ensuring that there were no comments regarding either the appropriateness of the selected alternative or USACE policy. There was no direct communication between the Panel and USACE during the preparation of the Final Panel Comments. The Final Panel Comments are presented in Appendix A of this report.

## **4. PANEL DESCRIPTION**

Candidates for the Panel were identified using Battelle's Peer Reviewer Database, targeted Internet searches using key words (e.g., technical area, geographic region), searches of websites of universities or other compiled expert sites, and referrals. Battelle prepared a draft list of primary and backup candidate panel members (which were screened for availability, technical background, and conflicts of interest), and provided it to USACE for feedback. Battelle made the final selection of panel members.

An overview of the credentials of the final four primary members of the Panel and their qualifications in relation to the technical evaluation criteria is presented in Table 2. More detailed biographical information regarding each panel member and his or her area of technical expertise is presented in the text that follows the table.

**Table 2. eDNA IEPR Panel: Technical Criteria and Areas of Expertise**

	May	Allendorf	Harrell	Hayes
<b>Genetics (two experts needed)</b>	<b>X</b>	<b>X</b>		
Minimum of 8 years experience in performing population genetic analyses of non-domestic species, including at least 4 years as lead investigator	<b>X</b>	<b>X</b>		
Familiar with noninvasive or environmental sampling of genetic material	<b>X</b>	<b>X</b>		
Familiar with standard laboratory genetics, including DNA extraction, polymerase chain reaction(PCR), and DNA sequencing	<b>X</b>	<b>X</b>		
Familiar with use of mitochondrial DNA (mtDNA) loci as genetic markers	<b>X</b>	<b>X</b>		
Experience is desired in aquatic systems, fish ecology, and genetics	<b>X</b>	<b>X</b>		
At least five relevant peer-reviewed publications	<b>X</b>	<b>X</b>		
Familiar with large, complex civil works projects with high public and interagency interests	<b>X</b>	<b>X</b>		
Ph.D. degree in genetics or associated field (e.g., biology, ecology, zoology)	<b>X</b>	<b>X</b>		
<b>Population Ecology (two experts needed)</b>			<b>X</b>	<b>X</b>
Minimum of 8 years experience in performing studies of population ecology in non-domesticated fish, including at least 4 years as lead investigator			<b>X</b>	<b>X</b>
Demonstrated experience in fish ecology and behavior			<b>X</b>	<b>X</b>
Familiar with fish ecology and behavior in both riverine and lake systems			<b>X</b>	<b>X</b>
Experience in some or all of the following areas is desired:				
Invasive species biology			<b>X</b>	<b>X</b>
Fish response to human activities and structures			<b>X</b>	<b>X</b>
Trophic ecology of fish			<b>X</b>	<b>X</b>
Reproductive ecology and behavior of fish				<b>X</b>
At least five relevant peer-reviewed publications			<b>X</b>	<b>X</b>
Familiar with large, complex civil works projects with high public and interagency interests			<b>X</b>	
Ph.D. in population ecology or associated field (e.g., biology, ecology, zoology)			<b>X</b>	<b>X</b>

### **Bernie May**

**Role:** This panel member was chosen primarily for his genetics experience and expertise.

**Affiliation:** University of California at Davis (UCD)

**Dr. Bernie May** is an adjunct professor in the Department of Animal Science at UCD and, for the past 15 years, has been the Director of UCD's Genomic Variation Laboratory. He earned his Ph.D. in genetics from The Pennsylvania State University in 1980. For over 30 years, Dr. May has conducted genetic analyses of a wide diversity of organisms, having spent 14 years prior to joining UCD as the Director of Cornell University's Laboratory for Ecological and Evolutionary Genetics. His primary research interests include genomic structure, population analysis, mixed stock analysis, genomic manipulation, effects of non-indigenous species, and isolate identification. He has worked on over 100 taxa (including a variety of fish species) and in a variety of ecosystems (riverine, marine, lacustrine, and terrestrial systems). Many of his research projects have involved non-invasive or environmental sampling of genetic material (fish fins, blood samples, feathers, wing membranes, saliva, fecal matter, and hair). All of Dr. May's laboratory projects involve DNA extraction and polymerase chain reaction (PCR), and many of them also utilized DNA sequencing, including "Characterization of 24 Microsatellite Loci in Delta Smelt, *Hypomesus transpacificus*, and Their Cross-species Amplification in Two Other Smelt Species of the *Osmeridae* Family" and "Six Diagnostic SNP Markers for Detecting Introgression Between Cutthroat and Rainbow Trout". Dr. May's research also includes using mitochondrial DNA (mtDNA) as genetic markers, as described in "Mitochondrial DNA Haplotype Diversity in Apparent XY-Female Fall-and Spring-Run Chinook Salmon in California's Central Valley" and "Mitochondrial DNA Variation Among Lake Trout (*Salvelinus namaycush*) Strains Stocked into Lake Ontario". Within the past few years, Dr. May has been a principal investigator on approximately 33 research grant projects, including developing new microsatellite genetic markers for white sturgeon; researching the genetic population structure of the threatened delta smelt; and using genetic techniques to detect Mississippi silverside predation on larval delta smelt. He has published over 150 papers in top peer-reviewed journals and has served as a reviewer for approximately 58 journals or organizations. He is a member of the American Fisheries Society and the American Association for the Advancement of Science.

### **Fred Allendorf**

**Role:** This panel member was chosen primarily for his genetics experience and expertise.

**Affiliation:** University of Montana

**Dr. Fred Allendorf** is a Regents professor at the University of Montana in the biological sciences department, as well as a professorial research fellow at the Victoria University of Wellington in New Zealand. He earned his Ph.D. in fisheries and genetics from the University of Washington in 1975. He has over 39 years of experience in fish population genetics. Over the past 30 years, he has received nearly \$4 million in major research grants, including a grant for a study on the detection of trout species by PCR amplification of DNA from stream water, a grant to direct a working group's research on developing genetic monitoring tools, and a grant to study whether mutations in mitochondrial DNA affect population viability. His experience with PCR includes research on genetic variation in coho salmon detected by PCR amplification of growth hormone gene introns. Dr. Allendorf is familiar with noninvasive sampling of genetic material, including recent work on bison fecal matter in Yellowstone National Park. He is currently on the

editorial boards of Conservation Biology and the New Zealand Journal of Marine and Freshwater Research and has been an associate editor for Conservation Genetics and Molecular Biology and Evolution. From 1999 to 2004, he was a member of the National Science Foundation's Invasive Species Collaboratory and was the editor for a special section of "Conservation Biology: Population Biology of Invasive Species" (February 2003). In 2007, he co-authored the book Conservation and the Genetics of Populations and has published over 100 peer-reviewed publications relevant to fish population genetics, including "Concordance of Nuclear and Mitochondrial DNA Markers in Detecting a Founder Event in Lake Clark Sockeye Salmon", "Genetic Basis of Variation in Morphological and Life History Traits of Pink Salmon (*Oncorhynchus gorbuscha*)", and "Concordance of Genetic Divergence Among Sockeye Salmon Populations at Allozyme, Nuclear DNA, and mtDNA Markers". Dr. Allendorf is a member of the Society for the Study of Evolution and the Society for Conservation Biology. He is also a former President of the American Genetic Association.

### **Reginal Harrell**

**Role:** This panel member was chosen primarily for his population ecology experience and expertise.

**Affiliation:** University of Maryland

**Dr. Reginal Harrell** is professor and administrator of Fisheries and Wildlife Sciences and Extension Specialist at the University of Maryland Department of Environmental Science and Technology. He earned his Ph.D. in biology/ecology from the University of South Carolina in 1984 and is a Certified Fisheries Scientist of the American Fisheries Society and a Fellow of the American Institute of Fisheries Research Biologists. He has over 30 years of experience as a fisheries biologist at the South Carolina Department of Natural Resources and the University of Maryland system, conducting research on the management, life history aspects, natural hybridization, and recruitment success of fishes in freshwater and estuarine systems (American eels and striped bass in coastal and Chesapeake Bay watersheds). He is experienced as a lead investigator in research, aquaculture, biology and genetics, fisheries and wildlife management, and population ecology in non-domesticated fish, having been involved in over \$11 million in research grants and contracts. He is experienced in fish ecology and behavior, having studied and assessed the age, growth, and sex differentiation in American eels in South Carolina rivers. His knowledge of invasive species biology is demonstrated by his research into the impact of the exotic aquatic vegetation *Hydrilla* on habitat utilization of fishes (especially with respect to its impact on species diversity and richness in the Potomac River and the upper Chesapeake Bay). His knowledge of fish response to human activities and structures is associated with his evaluation of natural and anthropogenic stressors on the health of native fish populations, including currently evaluating the impact of capture on stress-induced spawning failure on Atlantic sturgeon. Dr. Harrell is experienced in the trophic ecology of fish, having examined the trophic interaction of trout in South Carolina/North Carolina mountain streams. He has authored over 240 publications, abstract, and reports (119 of them peer-reviewed), including "Behavioral Observations of Striped Bass (*Morone saxatilis*) on the Spawning grounds of the Choptank and Nanitoke Rivers, MD, USA" and "DNA Evidence for Genetic Purity of Captive and Domestic Striped Bass Broodstock". He is familiar with large, complex civil works projects due to his involvement with the USACE rediversion project on the Santee-Cooper system in South Carolina as well as his appointment to and involvement with the Governor of Maryland's white

paper councils on fisheries-related issues. Dr. Harrell has been appointed to several state and federal task forces and committees, including Director of the US Department of Agriculture Northeast Regional Aquaculture Center.

**Daniel Hayes**

**Role:** This panel member was chosen primarily for his population ecology experience and expertise.

**Affiliation:** Michigan State University

**Dr. Daniel Hayes** is professor in the Department of Fisheries and Wildlife at Michigan State University, specializing in fish habitat and population dynamics, community interaction in aquatic and marine ecosystems, and the impact of invasive species. He earned his Ph.D. in fisheries and wildlife from Michigan State University in 1990. He has over 20 years of experience in fish population ecology, with more than 16 years as faculty member and lead principal investigator on multiple projects studying population ecology in non-domesticated fish. He is experienced in fish ecology and behavior and in invasive species biology, due in part to his studies of “Balancing Aquatic Habitat Fragmentation and Control of Invasive Species; Enhancing Selective Fish Passage at Sea Lamprey Control Barriers”. Dr. Hayes is also familiar with fish ecology and behavior in both riverine and lake systems, having studied yellow perch in Saginaw Bay, Lake Huron, and ecological health studies in temperate warm-water stream fish communities. He is experienced in the study of fish response to human barriers through his research of “Low-head Lamprey Barrier Effects on Stream Habitat and Fish Communities of Great Lakes Basin,” and his knowledge of trophic ecology of fish is demonstrated through his research of “Food Habitats of Coexisting Salmonines Above and Below Stronach Dam in the Pine River, MI”. His research into studies such as his “Evidence of Walleye Spawning in Maumee Bay, Lake Erie” have led to his experience in the research of reproductive ecology of fish. He has authored more than 200 publications, technical papers, and presentations, with 60 peer-reviewed publications, including “Linking Fish Population Dynamics to Habitat Conditions: Insights From the Application of a Process-Oriented Approach to Several Great Lake Species”. He has participated on past national review panels for such programs as the Gulf States Marine Fishery Commission and the Environmental Protection Agency STAR Aquatic Systems Ecology Fellowship. He also served as an expert witness for several high-profile environmental legal cases. He received a Special Accomplishment Award from the National Marine Fisheries Service and the Albert S. Hazzard Award from the Michigan Chapter American Fisheries Society. He is a member of the American Fisheries Society, Technical Advisor to the Michigan Department of Natural Resources, and past board member of the Technical Experts of the Great Lakes Fishery Commission (1996-2001).

## 5. SUMMARY OF FINAL PANEL COMMENTS

USACE guidance (2010) states the final report will contain the Panel's “assessment of the adequacy and acceptability of the economic, engineering, and environmental methods, models, and analyses used.” However, for the eDNA IEPR, the Panel focused on the environmental aspects of the eDNA science and methodology; no economic or engineering assessment was conducted. The Panel generally agreed on its assessment of the adequacy and acceptability of the environmental methods, models, and analyses used in the eDNA documents. Disagreements

among panel members existed regarding the levels of significance for Final Panel Comments 1 and 2. For each of these Final Panel Comments, three panel members thought it warranted a “high” level of significance, while one panel member thought a “medium” level of significance was appropriate.

When it was apparent that a consensus would not be reached on the levels of significance for these two Final Panel Comments, Battelle recommended that the opinion of the majority of the Panel be the deciding factor and the Panel agreed. For Final Panel Comment 1, the panel member who thought it should be a “medium” level of significance thought that while distinguishing silver or bighead carp from hybrids of these species may be important in many contexts, it is not essential in all situations. In particular, if passage of fish above the barrier in the CAWS is of concern, distinguishing a hybrid from “pure” silver or bighead carp would not be critical. For Final Panel Comment 2, the panel member who thought it should be a “medium” level of significance thought that the concerns raised in that comment were already addressed as much as possible by the methodology itself and he did not think that the recommendations for resolution would resolve the issue.

The following statements summarize the Panel’s findings, which are described in the Final Panel Comments and are discussed in more detail in Appendix A.

**Genetics:** The overall methodology to detect eDNA in the water column and attribute that eDNA in the form of mtDNA back to silver and bighead maternal lineages is sound. However, opportunities to estimate the number of fish contributing DNA to a positive sample, quantify levels of silver and bighead carp DNA in the water samples, rule out alternate alternate pathways to the occurrence of silver and bighead carp DNA in CAWS, address the awareness of the possible presence of hybrids of these two species, and use more sophisticated DNA detection protocols have been missed or overlooked. It is surprising that the available mtDNA sequences were not examined for intraspecific variation, and that the limitations of the mtDNA data alone to deal with the problem of potential hybrids were not presented.

**Population Ecology:** The eDNA methodology has great potential to complement traditional fishery sampling methods. Strengths of the method include the ability to rapidly collect water samples for analysis from a large geographic area with minimal cost. Also, the method is potentially more sensitive at detecting bighead and silver carp in environments typical of the CAWS than traditional fishery methods. Key limitations of the method, however, are that the location of fish contributing eDNA to a water sample is not known with certainty, and there is the potential for transport of eDNA over substantial distances or across barriers. Also, because sampling is seasonal with no sampling during the colder months (especially preceding spring spawning runs) when the fish’s susceptibility to the electrical barriers is at its lowest physiologically, there may be significant upstream movement that is being discounted inappropriately due to unfounded assumptions. Finally, not using the eDNA technology as a tool to screen ichthyoplankton collections misses a great opportunity to determine if successful spawning and hatch is occurring above the barrier system.

**Alternative Surveillance Methods:** In addition to these overall genetics and population ecology findings, the following paragraphs include the Panel’s evaluation of whether any alternative

surveillance methods exist that might more accurately or precisely detect the location of the invasion front of Asian carp in the CAWS.

The question of whether a feasible alternative exists to the eDNA methodology that is more reliable and precise needs to be answered in at least two parts. Firstly, the precision of a sampling program depends not only on the measure methods (e.g., eDNA vs. electrofishing), but also on the sample size. Thus, a comparison of electrofishing (for example) with the eDNA methodology would require additional information such as the sample size (or total cost) allocated to each method. Further, there is insufficient information on the “catchability” of Asian carp in any gear to allow for an accurate comparison.

The question of whether an alternative exists (emphasis on the word an) implies a view that either one method or another should be employed in a sampling program, when in fact, various methods may in fact complement one another. Some of the advantages of the eDNA methodology include:

- sample collection can occur over a large spatial area very rapidly
- the cost per sample is relatively low compared to traditional fishery methods
- dispersal of eDNA within the water allows for the eDNA sample to integrate fish presence over a larger area than traditional fishery methods where capture of a fish depends on gear being present at the exact location and time as an Asian carp

In the Panel’s opinion, no other single method provides this suite of advantages offered by eDNA samples.

Some of the limitations of the eDNA method include:

- detection of eDNA does not provide conclusive proof of the physical presence of a live fish at a given location in space and time
- eDNA detections do not provide information on the size or age (for example) of individuals present
- as currently implemented using mitochondrial DNA, the method cannot distinguish between pure silver or bighead carp and their hybrids, nor can it specify the gender of individuals caught
- there is a time delay inherent between water sample collection and processing for eDNA, and thus detection is not immediate as it would be with traditional fish sampling gears

Because of these limitations, a sampling program using traditional fish sampling methods is likely required to provide all of the information needed to make critical management decisions. The eDNA method thus provides a useful complement to traditional fish sampling gears, and can greatly improve the efficiency of a sampling program for Asian carp.

Table 3 lists the eight Final Panel Comment statements by level of significance.

**Table 3. Overview of 8 Final Panel Comments Identified by the eDNA Science and Methodology IEPR Panel**

<b>Significance – High</b>	
1	The current mitochondrial DNA (mtDNA) methodology cannot distinguish pure silver or bighead carp from hybrids of the two species.
2	The eDNA methodology does not unequivocally indicate the physical presence of live bighead or silver carp.
<b>Significance – Medium</b>	
3	The assumption that the eDNA methodology is of limited use in the winter months should be evaluated.
4	The sampling design used is not statistically based.
5	The eDNA methodology should be used to screen ichthyoplankton and egg samples to provide a means to identify sites with successful reproduction.
6	The current eDNA methodology should be modified to estimate the number of individual fish contributing eDNA to a positive sample.
7	The current PCR methodology should be changed to a quantitative PCR (qPCR) approach to estimate the quantity of silver and bighead DNA in a sample and to speed detection of eDNA in water samples.
8	The production, movement, and degradation of eDNA in the system should be evaluated.

## 6. REFERENCES

- ANSBAP Monitoring Subgroup (2009). Chicago Sanitary and Ship Canal Aquatic Nuisance Species Barrier Asian Carp Monitoring Plan. Aquatic Nuisance Species Barrier Advisory Panel.
- Arya, M., I.S. Shergill, M. Williamson, L. Gommersall, N. Arya, and H.R. Patel (2005). Basic principles of real-time quantitative PCR. *Expert Review of Molecular Diagnostics*. 5:209-219.
- Asian Carp Regional Coordinating Committee (undated). Monitoring and Rapid Response Plan for Asian Carp in the Upper Illinois River and Chicago Area Waterway System.
- Blume, L., M. Vazquez, J. Darling, and J.S. Chandler (2010). Laboratory Audit Report. Lodge Laboratory, Center for Aquatic Conservation, Department of Biological Sciences, University of Notre Dame.
- Green, B. W., and R. O. Smitherman (1984). Relative growth, survival and harvestability of bighead carp, silver carp, and their reciprocal hybrids. *Aquaculture* 37:87-95.
- Hedmark, E., and H. Ellegren (2006). A test of the multiplex pre-amplification approach in microsatellite genotyping of wolverine faecal DNA. *Conservation Genetics* 7:289-293.
- Jerde, C., M. Barnes, J. McNulty, A. Mahon, W.L. Chadderton, and D. Lodge (2010). Draft Final Report: Aquatic Invasive Species Risk Assessment for the Chicago Sanitary and Ship Canal. Center for Aquatic Conservation, University of Notre Dame.
- Kelso, W.E., and D.A. Rutherford (1996). Collection, preservation, and identification of fish eggs and larvae. *Fisheries Techniques*. B.R. Murphy and D.W. Willis, eds. American Fisheries Society, Bethesda, MD. 2<sup>nd</sup> edition, pp. 255-302.
- Kolar, K.S., D.C. Chapman, W.R. Courtenay Jr., C.M. Housel, J.D. Williams, and D.P. Jennings (2005). Asian carps of the genus *Hypophthalmichthys* (Pisces, Cyprinidae)—a biological synopsis and environmental risk assessment. U.S. Fish and Wildlife Service.
- Lamer, J. T., C. R. Dolan, J. L. Petersen, J.H. Chick, J. M. Epifanio (in press). Introgressive hybridization between bighead and silver carp in the Mississippi and Illinois Rivers. *North American Journal of Fisheries Management*.
- Lodge, D. (2009). Declaration of David M. Lodge in the cases of State of Wisconsin, et al., the State of Michigan, and the State of New York v. State of Illinois and Metropolitan Sanitary District of Greater Chicago, et al. United States Supreme Court. January 4, 2009.
- Mahon, A. R., A. Rohly, M. Budny, E. Elgin, C. L. Jerde, W. L. Chadderton, and D. M. Lodge (2010). Environmental DNA Monitoring and Surveillance: Standard Operation Procedures. Report to the United States Army Corps of Engineers, Environmental Laboratories, Cooperative

Environmental Studies Unit, Vicksburg, Mississippi. CESU agreement #W912HZ-08-2-0014, modification P00007.

OMB (2004). Final Information Quality Bulletin for Peer Review. Executive Office of the President, Office of Management and Budget, Washington, DC. Memorandum M-05-03. December 16.

Peterson, S.A., N.S. Urquhart, and E.B. Welch (1999). Sample representativeness: a must for reliable regional estimates of lake condition. *Environ. Sci. Technol.* 33: 1559-1565.

Piggott, M.P., E. Bellemain, P. Taberlet, and A.C. Taylor (2004). A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. *Conservation Genetics* 5:417-420.

Reynolds, J.B. (1996). Electrofishing. *Fisheries Techniques*. B.R. Murphy and D.W. Willis, eds. American Fisheries Society, Bethesda, MD. 2<sup>nd</sup> edition, pp. 221-253.

The National Academies (2003). Policy on Committee Composition and Balance and Conflicts of Interest for Committees Used in the Development of Reports. The National Academies (National Academy of Science, National Academy of Engineering, Institute of Medicine, National Research Council). May 12.

USACE (2007). Peer Review Process. Department of the Army, US Army Corps of Engineers, Washington, DC. CECW-CP Memorandum. March 30.

USACE (2010). Water Resources Policies and Authorities: Civil Works Review Policy. Department of the Army, US Army Corps of Engineers, Washington, DC. Engineer Circular (EC) No. 1165-2-209. January 31.

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**APPENDIX A**

**Final Panel Comments**

**on the**

**eDNA Science and Methodology**

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<b>Final Panel Comment 1:</b>
<b>The current mitochondrial DNA (mtDNA) methodology cannot distinguish pure silver or bighead carp from hybrids of the two species.</b>
<b>Basis for Comment:</b>
<p>The methods presented in the standard operating procedures (SOP) document (Mahon et al., 2010) include sufficient quality control and species specificity for polymerase chain reaction (PCR) and sequence analysis to ensure that the “confirmed positives” do indeed detect silver and bighead carp-specific mtDNA present in Chicago Area Waterway System (CAWS) water samples (see also Blume et al., 2010).</p> <p>However, the environmental (eDNA) procedure based on mtDNA alone can misidentify hybrids between these two species as either pure silver or bighead carp. It has long been established in the literature that hybrids between these two species are viable (Green and Smitherman, 1984). A recent examination of 120 presumptive silver and bighead carp from the Illinois and Mississippi River by Lamer et al. (in press) revealed 22.5% to be first-generation (F<sub>1</sub>) or post-F<sub>1</sub> hybrids between silver and bighead carp.</p>
<b>Significance – High:</b>
The inability of the current mtDNA SOP to distinguish silver or bighead carp from hybrids of the two species weakens the conclusions from “positive” water samples.
<b>Recommendations for Resolution:</b>
<p>To resolve these concerns, the methodology would need to include:</p> <ol style="list-style-type: none"> <li>1. A protocol for nuclear markers that would differentiate hybrid individuals from pure-species individuals.</li> <li>2. Efforts to use nuclear markers in “positive” water samples to differentiate pure silver and bighead carp from hybrids of the two species.</li> <li>3. An examination of downstream populations for the amount and pattern of hybridization.</li> </ol>

**Literature Cited:**

Blume, L., M. Vazquez, J. Darling, and J.S. Chandler (2010). Laboratory Audit Report. Lodge Laboratory, Center for Aquatic Conservation, Department of Biological Sciences, University of Notre Dame.

Green, B. W., and R. O. Smitherman (1984). Relative growth, survival and harvestability of bighead carp, silver carp, and their reciprocal hybrids. *Aquaculture* 37:87-95.

Lamer, J.T., C.R. Dolan, J.L. Petersen, J.H. Chick, and J.M. Epifanio (in press). Introgressive hybridization between bighead and silver carp in the Mississippi and Illinois Rivers. *North American Journal of Fisheries Management*.

Mahon, A. R., A. Rohly, M. Budny, E. Elgin, C. L. Jerde, W. L. Chadderton, and D. M. Lodge (2010). Environmental DNA Monitoring and Surveillance: Standard Operation Procedures. Report to the United States Army Corps of Engineers, Environmental Laboratories, Cooperative

Environmental Studies Unit, Vicksburg, Mississippi. CESU agreement #W912HZ-08-2-0014, modification P00007.

<b>Final Panel Comment 2:</b>
<b>The eDNA methodology does not unequivocally indicate the physical presence of live bighead or silver carp.</b>
<b>Basis for Comment:</b>
<p>The methods presented in the SOP document (Mahon et al., 2010) include sufficient quality control and species specificity for PCR and sequence analysis to ensure that the “confirmed positives” do indeed detect bighead and silver carp-specific mtDNA present in CAWS water samples (see also Blume et al., 2010).</p> <p>However, detection of species-specific mtDNA does not unequivocally indicate the physical presence of live bighead or silver carp. The confidence in interpreting that eDNA indicates live fish in the vicinity of a water sample can be increased by lowering the probability that “confirmed positive” results are due to other sources. Fundamentally, the question is whether the eDNA comes from live carp in CAWS or from other sources. As stated in item 41, p. 20 of Lodge (2009),</p> <p style="padding-left: 40px;">“Alternative explanations for the presence of eDNA include i) sewage treatment effluent from humans that had consumed bighead or silver carp or discarded fish waste, ii) deposition of excrement by seagulls or other birds that many have consumed silver or bighead carp tissue at other locations, iii) humans discarding one or more carcasses of bighead or silver carp directly into the waterway, and iv) transport and release by barges of water containing eDNA”.</p> <p>Arguments in item 42 are convincing for the exclusion of sewage treatment effluent as an alternate source: “...the spatial pattern of positive results (Figure 2) is not consistent with sewage treatment outfall(s) as a source(s)”; however, no tests have been done on birds eating carp, carp carcasses deposited into the water, or barge ballast water to exclude these alternate pathways for the presence of carp eDNA in the system. An additional source of eDNA mentioned in discussions between the Panel and the University of Notre Dame (UND) might be artificial ponds with bighead or silver carp draining into CAWS.</p>
<b>Significance – High:</b>
<p>Alternate pathways for the source of the eDNA in CAWS are not covered in the review documents. Not addressing alternate pathways seriously weakens any arguments that attempt to explain what the detection of “positives” in water samples implies. Testing coupled with considered logic would lower the probability that these alternate pathways are possible sources of the eDNA.</p>
<b>Recommendations for Resolution:</b>
<p>To resolve these concerns, the methodology would need to include the following:</p> <ol style="list-style-type: none"> <li>1. Tests of barge water for eDNA of bighead and silver carp to determine the likelihood that this source could contribute to observed positive samples.</li> <li>2. Tests of the duration of positive detections at locations downstream of bighead and silver carp carcasses placed into CAWS and monitored until no positives are detected. Sampling design should include both spatial and temporal components.</li> </ol>

3. Tests of the excrement of birds that have been fed fresh bighead and silver carp to determine the likelihood that this source could contribute to observed positive samples.
4. Tests of ponds draining into CAWS for bighead and silver carp eDNA.

**Literature Cited:**

Blume, L., M. Vazquez, J. Darling, and J.S. Chandler (2010). Laboratory Audit Report. Lodge Laboratory, Center for Aquatic Conservation, Department of Biological Sciences, University of Notre Dame.

Lodge, D. (2009). Declaration of David M. Lodge in the cases of State of Wisconsin, et al., the State of Michigan, and the State of New York v. State of Illinois and Metropolitan Sanitary District of Greater Chicago, et al. United States Supreme Court. January 4, 2009.

Mahon, A.R., A. Rohly, M. Budny, E. Elgin, C.L. Jerde, W.L. Chadderton, and D.M. Lodge (2010). Environmental DNA Monitoring and Surveillance: Standard Operation Procedures. Report to the United States Army Corps of Engineers, Environmental Laboratories, Cooperative Environmental Studies Unit, Vicksburg, Mississippi. CESU agreement #W912HZ-08-2-0014, modification P00007.

**Final Panel Comment 3:**

**The assumption that the eDNA methodology is of limited use in the winter months should be evaluated.**

**Basis for Comment:**

According to the Lodge Supreme Court Affidavit (Lodge, 2009), sampling during the winter months above the barrier did not occur because of its limited utility. This conclusion was reached under the assumption that fish activities decline with decreasing temperature and that therefore the probability of detecting eDNA when fish are actually present would be lower than the rest of the year. While in general this assumption has value, the evidence from supplemental reports indicates that the bighead and silver carp in the Missouri River are active in winter, with movement not slowing down until temperatures reach  $<4^{\circ}\text{C}$  (Kolar et al., 2005). Similarly the January 29, 2010 Interim Report showed that two of five positive samples for silver carp were collected on December 8, 2009 (no temperature was given).

According to Kolar et al. (2005), the bighead carp tend to move back and forth between tributaries during this time, while the silver carp tend to stay within pools. If winter movement is not curtailed until temperatures are at or below  $4^{\circ}\text{C}$ , significant upstream movement due to normal behavior may be missed, especially for the bighead carp. In addition, Kolar et al. (2005) indicate that both carp species feed at temperatures as low as  $2.5^{\circ}\text{C}$ . If the eDNA source is fecal material, even with lowered metabolism it is logical to assume that a source of DNA will be present at temperatures at least as low as  $2.5^{\circ}\text{C}$ .

Likewise, one of the triggers to migration is the onset of spawning. Kolar et al. (2005) clearly state that the onset of spawning triggers movement and that movement is upstream of tributaries. Therefore, during late winter and early spring months when temperature and photoperiod trigger spawning migrations, a critical window of fish movement resulting in an advance of the invasion front can be missed if eDNA sampling is not occurring.

Not sampling during the winter and early spring months does not take advantage of behavioral traits of the fish. It also misses a window of the fish potentially crossing the barriers because of reduced efficacy of the electrical barrier during colder months, especially given the fact that electrofishing (i.e., electrical current) efficacy decreases with fish size, large scales, and lower metabolism associated with colder waters (Reynolds, 1996).

**Significance – Medium:**

The lack of sampling during winter and early spring months impacts the completeness of the eDNA methodology by missing a window of sampling when fish still could be reasonably active and the efficacy of the electrical barriers will be at its lowest point.

**Recommendations for Resolution:**

To resolve these concerns, the methodology would need to include:

1. A determination as to whether temperature affects eDNA detection and, if so, at what temperature is the technique ineffective.
2. A continuation of sampling protocols for eDNA during winter and early spring months.
3. Use of the documented natural behavior of the fish (i.e., tributary movement for bighead and pool aggregation for silver carp) to establish an appropriate sampling strategy.

**Literature Cited:**

Kolar, K.S., D.C. Chapman, W.R. Courtenay Jr., C.M. Housel, J.D. Williams, and D.P. Jennings (2005). Asian carps of the genus *Hypophthalmichthys* (Pisces, Cyprinidae)—a biological synopsis and environmental risk assessment. U.S. Fish and Wildlife Service.

Lodge, D. (2009). Declaration of David M. Lodge in the cases of State of Wisconsin, et al., the State of Michigan, and the State of New York v. State of Illinois and Metropolitan Sanitary District of Greater Chicago, et al. United States Supreme Court. January 4, 2009.

Reynolds, J.B. (1996). Electrofishing. *Fisheries Techniques*. B.R. Murphy and D.W. Willis, eds. American Fisheries Society, Bethesda, MD. 2<sup>nd</sup> edition, pp. 221-253.

**Final Panel Comment 4:**

The sampling design used is not statistically based.

**Basis for Comment:**

The reliability of eDNA evidence for indicating the physical presence of bighead and silver carp in the system as a whole depends on the sampling design used as well as the probability of detection for individual samples. The overall sampling plan is not clearly described in any of the primary review documents. A large number of eDNA samples (>1,000) have been processed to date, but the results presented in the interim sampling reports indicate that sampling to date has been a mix of directed sampling, sampling to answer specific research or management questions, opportunistic sampling, and to some extent some sample collection based on random site selection. One of the supplemental review documents (Asian Carp Regional Coordinating Committee) provides a substantial amount of detail on sampling plans; however, it does not specify sample sizes or sampling allocation for eDNA samples or other gear types, and it does not clearly state the goal(s) relative to the sampling being proposed. Although not all sampling programs need to be statistically based, it is not possible to evaluate the precision (e.g., standard error) or accuracy (i.e., degree of potential bias) of results of the sampling program's results as a whole without such a design (e.g., Peterson et al., 1999).

Some of the sampling designs that are design-unbiased and are commonly used in fishery or ecological investigations include simple random sampling, stratified random sampling, and systematic sampling. More sophisticated spatial sampling designs, as well as model-based sampling designs, are also commonly used to improve precision of estimated means. Such designs should be considered relative to the question being posed. Furthermore, such designs provide the benefit that the properties (e.g., precision, accuracy, overall reliability) of the results of the sampling program can be evaluated in a formal statistical framework.

The details on how replicate samples (i.e., samples collected from essentially the same time and place) were collected and analyzed were not provided in the primary review documents. The Panel presumes that replicate PCR analysis of individual water samples was conducted to increase the probability of detection or to confirm samples testing positive (i.e., to confirm presumptive positives). The Panel notes, however, that assays on replicate water samples do not provide independent samples and would need to be handled with an appropriate statistical method (e.g., a cluster sampling estimator).

**Significance – Medium:**

Without a clear description of the sampling design or the statistical basis of the design, it is not possible to assess the precision and accuracy, and thus the statistical reliability, of the sampling program as a whole.

**Recommendations for Resolution:**

To resolve these concerns, the methodology would need to include:

1. A clarification of the goals of the sampling program to set the proper context for evaluating the sampling design.
2. A clearer description of the sampling program, with an emphasis on the overall design, sample sizes by time and location, the method used to statistically handle replicate samples, and the method used to estimate measures of variability (e.g., standard error). The rationale for the sampling design should also be included, and if a non-statistical sampling design is used, this choice should be justified.
3. A report of measures of variability where appropriate (e.g., proportion of samples testing positive).

**Literature Cited:**

Asian Carp Regional Coordinating Committee (undated). Monitoring and Rapid Response Plan for Asian Carp in the Upper Illinois River and Chicago Area Waterway System.

Peterson, S.A., N.S. Urquhart, and E.B. Welch (1999). Sample representativeness: a must for reliable regional estimates of lake condition. *Environ. Sci. Technol.* 33: 1559-1565.

**Final Panel Comment 5:**

**The eDNA methodology should be used to screen ichthyoplankton and egg samples to provide a means to identify sites with successful reproduction.**

**Basis for Comment:**

One of the points recognized in the Blume et al. report (2010) was that additional research would increase the utility of the eDNA methodology for surveillance and monitoring. Clearly from the Chicago Sanitary and Ship Canal (CSSC) *Aquatic Nuisance Species Barrier Asian Carp Monitoring Plan* (Aquatic Nuisance Species Barrier Advisory Panel [ANSBAP] Monitoring Subgroup, 2009), the purpose of monitoring is to identify location and upstream movement of bighead and silver carp in relation to the species barrier using the best tools and technologies available. Likewise, in the Final Charge Guidance document provided to the Panel, it was stated that it is important to know how many fish may be present to determine if it is likely that a sustaining population of bighead and silver carp will become established above the barrier. The Panel perceives that, given the success of detecting and segregating eDNA into species-specific fragments, the utility of this technology could be greatly enhanced to determine the significance of clarifying what is meant by determining an “invasion front” and to determine the presence of a self-sustaining population above the barrier.

One of the traditional tools used by fisheries biologists is ichthyoplankton sampling (Kelso and Rutherford, 1996). The *Monitoring and Rapid Response Plan for Asian Carp in the Upper Illinois River and Chicago Area Waterway System* (Asian Carp Regional Coordinating Committee, undated) recognized the value of this important sampling information. Yet, the ANSBAP Monitoring Subgroup (2009) discounted this sampling methodology as not being useful due to the processing time and resource limitations in collecting and sorting. The Panel recognizes that using the eDNA methodology could significantly reduce the processing time and resource limitations of ichthyoplankton samples by splitting samples and analyzing for the presence of bighead and/or silver carp DNA. If a sample was found to contain bighead and/or silver carp DNA, then that particular sample could be evaluated by the more traditional means to assess the development stage of the egg/larvae and with appropriate data collection (i.e., water temperature and flow rates) calculate spawning reaches of the CSSC system above the barriers where spawning and/or hatch is occurring. Identifying successful reproduction would be the first and crucial step in determining if a self-sustaining population exists above the barrier.

**Significance – Medium:**

Insufficient sampling and/or insufficient evidence of spawning success upstream of the barriers do not allow for determining whether a self-sustaining population of bighead and/or silver carp is present or whether positive eDNA samples collected thus far are only reflective of fish DNA presence.

**Recommendations for Resolution:**

To resolve these concerns, the methodology would need to include:

1. An ichthyoplankton sampling protocol during the spring of the year when bighead and silver carp are known to spawn.
2. A provision for the preservation of collections in a fixative that will not compromise the DNA or ability to qualify and quantify egg/larval development from a sample, if warranted.
3. The incorporation of nuclear DNA (nucDNA) markers to estimate numbers of spawners contributing to collected eggs/larvae.

**Literature Cited:**

ANSBAP Monitoring Subgroup (2009). Chicago Sanitary and Ship Canal Aquatic Nuisance Species Barrier Asian Carp Monitoring Plan. Aquatic Nuisance Species Barrier Advisory Panel.

Asian Carp Regional Coordinating Committee (undated). Monitoring and Rapid Response Plan for Asian Carp in the Upper Illinois River and Chicago Area Waterway System.

Blume, L., M. Vazques, J. Darling, and J.S. Chandler (2010). Laboratory Audit Report. Lodge Laboratory, Center for Aquatic Conservation, Department of Biological Sciences, University of Notre Dame.

Kelso, W.E., and D.A. Rutherford (1996). Collection, preservation, and identification of fish eggs and larvae. *Fisheries Techniques*. B.R. Murphy and D.W. Willis, eds. American Fisheries Society, Bethesda, MD. 2<sup>nd</sup> edition, pp. 255-302.

**Final Panel Comment 6:**

The current eDNA methodology should be modified to estimate the number of individual fish contributing eDNA to a positive sample.

**Basis for Comment:**

The current eDNA procedure detects the presence of silver or bighead carp DNA in a water sample. The results have been used as an indication of bighead and silver carp presence or absence in the CAWS.

The last two sentences of the second paragraph of the Final Charge Guidance to the Peer Reviewers indicate that knowing how many fish are present is important in order to determine if it is likely that a sustaining population of bighead and silver carp will become established above the barrier. The eDNA procedures should be further developed to estimate the number of bighead and silver carp contributing to the detected DNA.

Information presented at the July 22, 2010 meeting at UND indicated that mtDNA sequences had been aligned and compared for intraspecific variation and that two different sequences were discovered. Because mtDNA is haploid, this indicates that DNA had been detected from least two different individuals.

**Significance – Medium:**

The completeness of the eDNA methodology could be improved by including estimates of the number of fish in a water sample. This would provide valuable information about whether a sustaining population of bighead or silver carp is established above the barrier.

**Recommendations for Resolution:**

To resolve these concerns, the methodology would need to include:

1. An estimate of the number of fish present. The easiest way to do this is to remove the diagnostic PCR fragment bands of mtDNA from the gel and sequence them. They then could be aligned and examined for intraspecific variation.
2. The use of another region (or regions) of mtDNA other than that used for identifying species. This could be determined either by a literature search and/or by sequencing the entire mtDNA control region, which tends to be highly polymorphic in many species. The literature search should include the published literature, GenBank, and contacting labs known to be working on one or both of these species. Other regions of mtDNA could also be sequenced for intraspecific variation.
3. The collection of baseline data from both species. Many individual samples (perhaps 50) should be taken from the populations of each species below the barrier. These should then be screened for intraspecific genetic variation at mtDNA. Sequencing all positive eDNA samples and comparing them to this baseline information would make it possible to estimate the minimum number of individuals present in the eDNA samples.
4. The development of a suite of nucDNA markers to estimate the number of individuals present in the eDNA samples. mtDNA provides very limited information because it is a maternally inherited haploid marker. Ideally, a set of microsatellites would be developed to do this analysis because the genotype of each individual is potentially unique. There are some technical issues to overcome to make the nucDNA approach possible. First, there is roughly 100 to 1,000 times more mtDNA in a sample than

nucDNA. Therefore, there might not be enough nucDNA present for this approach. A pre-amplification process prior to PCR might make it possible to PCR nuclear markers from eDNA (Piggott et al., 2004; Hedmark and Ellegren, 2006). Second, a positive eDNA sample could contain DNA from more than one individual. This could make it difficult to identify the genotypes of separate individuals, though still permitting the recognition of samples with two to five individuals present. This approach will require the use of acrylamide gels in either slab or capillary formats.

5. The development of a new set of microsatellites especially designed for the low amounts of DNA present in eDNA, if the available microsatellite primer sequences do not work. Such special microsatellites would have small fragment size and the primer sequences could be especially designed to be sensitive with small amounts of DNA. As with using mtDNA to estimate the number of individuals from eDNA, data from populations of both species below the barriers would be needed as baseline information.

#### **Literature Cited:**

Hedmark, E., and H. Ellegren (2006). A test of the multiplex pre-amplification approach in microsatellite genotyping of wolverine faecal DNA. *Conservation Genetics* 7:289-293.

Piggott, M.P., E. Bellemain, P. Taberlet, and A.C. Taylor (2004). A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. *Conservation Genetics* 5:417-420.

<b>Final Panel Comment 7:</b>
The current PCR methodology should be changed to a quantitative PCR (qPCR) approach to estimate the quantity of silver and bighead DNA in a sample and to speed detection of eDNA in water samples.
<b>Basis for Comment:</b>
Currently eight replicate PCRs are done on the DNA extraction from each water sample, and these amplification products are run out on agarose gels, as outlined in the SOP document (Mahon et al., 2010). If any of these replicates are “positive” for the presence of silver or bighead carp DNA, then the sample is amplified and run out on a gel 16 more times.
Quantification of silver and bighead mtDNA in the water sample is not discussed. Although it is likely that higher proportions of “positives” in the replicates reflect greater concentrations of silver and bighead mtDNA (e.g., eight “positives” versus one “positive”), the current methodology does not provide a quantitative measure of the amount of silver and bighead carp DNA present.
The current detection methodology of running the PCR products on agarose gels containing ethidium bromide and imaging the DNA with an ultraviolet (UV) transilluminator is a relatively slow method and is not as sensitive as newer methods that use lasers to detect fluorescence.
<b>Significance – Medium:</b>
The completeness of the eDNA methodology would benefit from the quantification of silver and/or bighead carp-specific eDNA in the water sample. The current SOPs also require the use of agarose gels and extraction of the DNA from the gel, adding to the time to process samples.
<b>Recommendations for Resolution:</b>
To resolve these concerns, the methodology would need to include: <ol style="list-style-type: none"> <li>1. A protocol for qPCR (qPCR, Ayra et al., 2005) in the SOPs substituted for the current PCR protocol. This protocol would include TaqMan assays specific for silver and for bighead carp mtDNA. qPCR permits the quantification of DNA in the sample because it monitors the amplification each cycle (real-time amplification), not just at the end of PCR as currently practiced using agarose gels. The “positives” could then be repeated 16 times and sent directly for sequencing. This alternate process removes the use of agarose gels to detect the presence of silver or bighead carp mtDNA and the current approach of excising the DNA band from the gel for sequencing, significantly reducing the time to process samples. Finally, a qPCR approach can detect lower concentrations of amplification products.</li> </ol>

**Literature Cited:**

Arya, M., I.S. Shergill, M. Williamson, L. Gommersall, N. Arya, and H.R. Patel (2005). Basic principles of real-time quantitative PCR. *Expert Review of Molecular Diagnostics*. 5:209-219.

Mahon, A.R., A. Rohly, M. Budny, E. Elgin, C.L. Jerde, W.L. Chadderton, and D.M. Lodge (2010). *Environmental DNA Monitoring and Surveillance: Standard Operation Procedures*. Report to the United States Army Corps of Engineers, Environmental Laboratories, Cooperative

Environmental Studies Unit, Vicksburg, Mississippi. CESU agreement #W912HZ-08-2-0014, modification P00007.

**Final Panel Comment 8:**

**The production, movement, and degradation of eDNA in the system should be evaluated.**

**Basis for Comment:**

The identification of the length of river contributing eDNA to water collected at a specific point in space and time depends on the production, degradation, and transport dynamics of bighead and silver carp DNA in the environment. These dynamics are currently unknown and were not discussed in the primary review documents provided. Thus, while the exact location of a water sample can be known, the point of origin (i.e., one or more fish producing feces, mucus, urine, or other sources of eDNA) could vary substantially depending on these factors.

The transport dynamics of eDNA are likely to be complex and highly site- and time-specific. Because of this, characterizing water transport at a sufficient spatial and temporal resolution to provide precise interpretation of individual eDNA samples would likely be cost prohibitive. Multiple factors contribute to the concentration of eDNA at a particular point in space at a given time, and the amount of eDNA is likely to be spatially and temporally heterogeneous. The first of these factors is the amount of eDNA shed per fish per unit time, as well as the number of fish present. Essentially, this defines the total amount of eDNA production per unit time. Second, the rate of degradation of eDNA is unknown. This defines the rate of loss of eDNA from the system as a whole per unit time. The final factor is the transport dynamics of water containing eDNA at a site. Together, all of these factors define the spatial-temporal dynamics of eDNA in the system and provide the connection between the physical presence of fish in the system and the interpretation of eDNA sampling results. Without knowledge of the dynamics of eDNA, it is not possible to know how far from a sample point any eDNA is likely to be detected.

Preliminary results of degradation experiments with common carp DNA are reported in the Lodge affidavit (Lodge, 2009) and in Jerde et al. (2010) and suggest that degradation to non-detectable levels occurs in 6 to 48 hours. This preliminary work needs further clarification of the methodology and expansion to include silver and bighead carp DNA in natural environmental conditions.

To the Panel's knowledge, other studies have not yet completed a full evaluation of the dynamics of eDNA in natural systems. Such an evaluation would greatly enhance the ability to interpret the results of an individual eDNA sample as well as the results of the sampling program as a whole.

**Significance – Medium:**

The lack of documentation and understanding of the dynamics of eDNA in natural systems limits the interpretability of sampling results.

**Recommendations for Resolution:**

To resolve these concerns, the methodology would need to include:

1. The production rates of various sources of eDNA (e.g., feces, mucus, urine). If the methodology can be used to quantify the amount of DNA in a sample, the production rate could be determined from captive fish. Because the production rate likely depends on the size of fish, water temperature, and possibly the diet of fish (among other

factors), some type of factorial design would be a useful approach.

2. Degradation rates of DNA from direct DNA quantification measures or qPCR using an exponential model of decay. Degradation rates likely depend on a variety of factors, such as water temperature, UV light exposure, and water chemistry; as above, a factorial design would be a useful first step. Even if quantitative measures cannot be implemented, the amount of time that eDNA persists at a detectable level could still be determined. An expansion of the UND laboratory experiment reported above should be carried out upon bighead and silver carp DNA detections using the primers developed for these species under laboratory and “natural” conditions (i.e., with the complex microbiotic community present in CAWS water samples).
3. General maps of water flow and velocity within the CAWS to broadly scale the area likely contributing to positive eDNA samples (i.e., is the eDNA likely to have come from less than 100 meters in distance, 500 to 1,000 meters in distance, or farther?). Feces, mucus, and urine sample dilution should be modeled under these water conditions.

#### **Literature Cited:**

Jerde, C., M. Barnes, J. McNulty, A. Mahon, W.L. Chadderton, and D. Lodge (2010). Draft Final Report: Aquatic Invasive Species Risk Assessment for the Chicago Sanitary and Ship Canal. Center for Aquatic Conservation, University of Notre Dame.

Lodge, D. (2009). Declaration of David M. Lodge in the cases of State of Wisconsin, et al., the State of Michigan, and the State of New York v. State of Illinois and Metropolitan Sanitary District of Greater Chicago, et al. United States Supreme Court. January 4, 2009.

**APPENDIX B**

**Final Charge to the Independent External Peer Review Panel**

**on the**

**Environmental DNA (eDNA) Science and Methodology**

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**Final Charge Guidance and Questions to the Peer Reviewers  
for the  
Environmental DNA (eDNA) Science and Methodology**

**BACKGROUND**

State and federal agencies and stakeholders have been working together to stop the spread of nuisance fish between the Mississippi River Basin and the Great Lakes since the late 1990s. To do this, they have constructed a series of electrical barriers near Romeoville, Illinois. These barriers are a non-lethal deterrent to fish that do not interfere with water flow and minimize impact to navigation in the Chicago Sanitary and Ship Canal (a man-made waterway that provides a direct hydraulic connection between Lake Michigan and the Mississippi River Basin). Without intervention, invasive species such as the Asian carp can transfer between the basins, competing with native species for food, living space, and spawning areas with potential major negative impact to the economy and the environment.

An interagency group of fisheries scientists developed an Asian carp monitoring program to determine the level of threat to the Great Lakes by attempting to identify the leading edge of the invasion of Asian carp. As part of this plan, a team from the University of Notre Dame (UND) led by Dr. David Lodge began testing water samples for the presence of DNA of bighead and silver carp (collectively referred to as Asian carp) in the Chicago Area Waterway System (CAWS) using a technique called the environmental DNA (eDNA) method in the spring of 2009. In the summer of 2009 they found DNA fragments closer to the barrier than had been detected earlier, indicating that Asian carp may be closer to the Great Lakes than previously thought. In November 2009, Asian carp eDNA was detected above the fish barrier, but no live or dead Asian carp has been captured to date in this stretch of the waterway. Even if Asian carp are above the fish barrier, it is not known how many fish may be present. It is critical that the barrier continue to be operated effectively to minimize the potential that a sustaining population of Asian carp becomes established above the barrier.

All fish, including Asian carp, release DNA into the environment naturally as mucoidal secretions (slime), feces, and urine. These substances and the DNA within them slowly degrade in the environment, but can be collected in water samples if caught soon enough. Water samples are filtered and the DNA is collected and analyzed for the presence or absence of Asian carp using an eDNA method developed by researchers at UND. The process of testing eDNA is not instantaneous, but DNA can be held in suspension and transported. The presence of eDNA is detected by extracting and amplifying short fragments of the shed DNA from the water sample. In contrast to other surveillance methods, the eDNA method does not rely on direct observation of Asian carp to evaluate presence.

The objective of using the eDNA method, the only available analytical tool, is to use the method as a screening and early detection indicator. Laboratory and field studies using eDNA methods indicate that Asian carps can be detected in two liter water samples from sites where Asian carp have been captured through electrofishing.

## OBJECTIVES

The objective of this work is to conduct an independent external peer review (IEPR) of the eDNA science and methodology in accordance with the Department of the Army, U.S. Army Corps of Engineers, Water Resources Policies and Authorities' *Civil Works Review Policy* (EC 1165-2-209) dated January 31, 2010 and the Office of Management and Budget's *Final Information Quality Bulletin for Peer Review* released December 16, 2004.

Peer review is one of the important procedures used to ensure that the quality of published information meets the standards of the scientific and technical community. Peer review typically evaluates the clarity of hypotheses, validity of the research design, quality of data collection procedures, robustness of the methods employed, appropriateness of the methods for the hypotheses being tested, extent to which the conclusions follow from the analysis, and strengths and limitations of the overall product.

The purpose of this IEPR is to assess the adequacy and acceptability of the environmental methods, data, and analyses used for the eDNA science and methodology. The panel members will identify, examine, and comment upon the assumptions underlying the analyses as well as evaluate the soundness of analytic methods, and will evaluate whether the interpretations of analyses and conclusions are technically sound and reasonable, provide effective review in term of both usefulness of results and of credibility, and have the flexibility to bring important issues to the attention of decision makers. The IEPR will be limited to technical review and will not involve policy review. The IEPR will be conducted by subject matter experts (i.e., IEPR panel members) with extensive experience in genetics and population ecology relevant to the project.

The panel members will be "charged" with responding to specific technical questions as well as providing a broad technical evaluation of the eDNA science and methodology used to detect the presence of Asian carp in the CAWS. The panel members may also offer opinions as to whether there are sufficient analyses upon which to base the ability to implement the eDNA analyses in the context described in the review documents.

## DOCUMENTS PROVIDED

The following is a list of documents and reference materials that will be provided for the review. **The documents and files presented in bold font are those which are to be reviewed.** All other documents are provided for reference.

- **Laboratory Audit Report, Lodge Laboratory, Center for Aquatic Conservation, Department of Biological Sciences, University of Notre Dame, dated February 5, 2010. U.S. Environmental Protection Agency, Great Lakes National Program Office and National Exposure Research Laboratory, Office of Research and Development**
- **26 interim sampling reports on eDNA results**
- **Environmental DNA Monitoring and Surveillance: Standard Operating Procedures, Center for Aquatic Conservation, Department of Biological Sciences, University of Notre Dame, dated May 10, 2010.**

- Monitoring and Rapid Response Plan for Asian Carp in the Upper Illinois River and Chicago Area Waterway System
- Asian Carp Literature Review
- Asian Carp of the Genus *Hypophthalmichthys* (Pisces, Cyprinidae) – A Biological Synopsis and Environmental Risk Assessment. Cindy Kolar, Duane Chapman, Walter Courtenay, Jr., Christine Housel, James Williams, and Dawn Jennings. Dated April 12, 2005. Report to U.S. Fish and Wildlife Service.
- Chicago Sanitary and Ship Canal Aquatic Nuisance Species Barrier Asian Carp Monitoring Plan. Dated October 2009
- Beja-Pereira, A., R. Oliveira, P.C. Alves, M.K. Schwartz, and G. Luikart. Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources* 9, 1279-1301.
- Risk Reduction Study Fact Sheet: Environmental DNA (eDNA). Center for Aquatic Conservation, Department of Biological Sciences, University of Notre Dame.
- Ficetola, G.F., C. Miaud, F. Pompanon, and P. Taberlet. Species detection using environmental DNA from water samples. *Biology Letters* 4, 423-425.
- Management and Control Plan for Bighead, Black, Grass, and Silver Carps in the United States. Asian Carp Working Group. Dated November 2007.
- Audit Report: Lodge Laboratory, Center for Aquatic Conservation, University of Notre Dame. Dated January 2010.
- Supreme Court affidavit of David M. Lodge
- Response to request for information to USACE from David M. Lodge, Christopher L. Jerde, W. Lindsay Chadderton, and Andrea R. Mahon.
- Risk Assessment for Asian Carps in Canada. Nicholas Mandrak and Becky Cudmore. Fisheries and Oceans Canada. Research Document 2004/103.
- Draft Final Report: Aquatic Invasive Species Risk Assessment for the Chicago Sanitary and Ship Canal. Christopher Jerde, Matthew Barnes, Joanna McNulty, Andrew Mahon, W. Lindsay Chadderton, and David M. Lodge.
- Dispersal Barrier Efficacy Study. Interim I—Dispersal Barrier Bypass Risk Reduction Study and Integrated Environmental Assessment. Final Report. Dated January 2010
- USACE guidance *Civil Works Review Policy* (EC 1165-2-209) dated January 31, 2010
- CECW-CP Memorandum dated March 31, 2007
- Office of Management and Budget's *Final Information Quality Bulletin for Peer Review* released December 16, 2004.

## SCHEDULE

TASK	ACTION	DUE DATE
<b>Conduct Peer Review</b>	Review documents sent to Panel	7/9/2010
	Battelle/Panel kick-off teleconference	7/12/2010
	USACE/Battelle/Panel kick-off meeting at University of Notre Dame	7/22/2010
	Panel completes review	8/19/2010
<b>Prepare Final Panel Comments and Final IEPR Report</b>	Battelle provides Panel merged individual comments and talking points for panel review teleconference	8/26/2010
	Convene panel review teleconference	8/30/2010
	Battelle provides Final Panel Comments (FPC) directive to Panel	8/31/2010
	Panel provides draft FPCs to Battelle	9/8/2010
	Battelle provides feedback to Panel on draft FPCs; Panel provides revised draft FPCs per Battelle feedback	ongoing
	FPCs finalized	9/15/2010
	Battelle provides final IEPR report to Panel for review	9/17/2010
	Panel provides comments on final IEPR report	9/21/2010
	*Submit final IEPR report	9/24/2010
<b>Comment/ Response Process</b>	Input FPCs to DrChecks and Battelle provides FPC response template to USACE	9/28/2010
	USACE provides draft Evaluator responses and clarifying questions to Battelle	10/8/2010
	Battelle provides Panel the draft Evaluator responses and clarifying questions	10/13/2010
	Panel provides Battelle with draft BackCheck responses	10/18/2010
	Teleconference with Battelle and Panel to discuss Panel's draft Backcheck responses	10/18/2010
	FPC Teleconference between Battelle, Panel, and USACE to discuss FPCs, draft responses and clarifying questions	10/25/2010
	USACE inputs final Evaluator responses in DrChecks	11/8/2010
	Battelle provides Evaluator responses to Panel	11/12/2010
	Panel provides Battelle with BackCheck responses	11/17/2010
	Battelle inputs BackCheck responses in DrChecks	11/23/2010
	*Battelle submits pdf printout of DrChecks project file	11/24/2010

\* Deliverable

## CHARGE FOR PEER REVIEW

Members of this peer review panel are asked to determine whether the technical approach and scientific rationale presented in the eDNA science and methodology is effective and accurate and if the application and interpretation of the methodology is appropriate. The panel members will evaluate whether the analyses are technically sound and reasonable, provide effective review in terms of both usefulness of results and of credibility, and have the flexibility to bring important issues to the attention of decision makers. The panel members are **not** being asked whether they would have conducted the work in a similar manner.

Specific questions for the panel members (by report section or Appendix) are included in the general charge guidance, which is provided below.

### General Charge Guidance

Please answer the scientific and technical questions listed below and conduct a broad overview of the eDNA science and methodology. Please focus on your areas of expertise and technical knowledge. Please feel free to make any relevant and appropriate comment on any of the documents you were asked to review. In addition, please note the following guidance. Note that the panel will be asked to provide an overall statement related to 2 below per USACE guidance (EC 1165-2-209; Appendix D).

1. Your response to the charge questions should not be limited to a “yes” or “no.” Please provide complete, descriptive answers to fully explain your response.
2. Assess the adequacy and acceptability of the assumptions, data, methodology, and analyses presented in the eDNA review documents.
  - a. Identify, explain, and comment on the assumptions that underlie the analyses, as well as evaluate the soundness of methods.
  - b. Evaluate whether the interpretations of analysis are reasonable
  - c. If appropriate, offer opinions as to whether there are sufficient analyses upon which to base the ability to implement the methodology.

Please **do not** comment on or make recommendations on policy issues and decision making. Comments should be provided based on your professional judgment, **not** the legality of the document.

1. If desired, panel members can contact one another. However, panel members **should not** contact anyone who is or was involved in the project, prepared the subject documents, or was part of the USACE Agency Technical Review.
2. Please contact the Battelle deputy project manager (Corey Wisneski, [wisneskic@battelle.org](mailto:wisneskic@battelle.org)) or project manager (Karen Johnson-Young, [johnson-youngk@battelle.org](mailto:johnson-youngk@battelle.org)) for requests or additional information.
3. In case of media contact, notify the Battelle project manager immediately.

4. Your name will appear as one of the panelists in the peer review. Your comments will be included in the Final IEPR Report, but will remain anonymous.

**Please submit your comments in electronic form to Corey Wisneski, [wisneski@battelle.org](mailto:wisneski@battelle.org), no later than August 9 2010, 10 pm EDT.**

**Independent External Peer Review  
Environmental DNA (eDNA) Science and Methodology**

**Final Charge Questions**

1. Does the eDNA method effectively and reliably detect the genetic presence of bighead and silver carp in water samples collected from the CAWS?
2. Is the eDNA method an effective, accurate, and reliable indicator for detecting the physical presence of bighead and silver carp in the CAWS?
3. Are there limitations/questions remaining about what eDNA indicates? If so, what are they and how should they be answered?
4. Do the SOPs for sample collection in the field, filtration, DNA isolation, amplification and electrophoresis provide appropriate assurances that the eDNA results are technically reliable?"
5. What is the most important concern you have with the documents or the eDNA methodology that was not covered in your answers to the questions above?
6. Do any alternative surveillance methods exist that might more accurately or precisely detect the location of the invasion front of Asian carps in the CAWS?

## **APPENDIX C**

### **Revisions Made to the Final Independent External Peer Review Report on Environmental DNA (eDNA) Science and Methodology**

The Final Independent External Peer Review Report for the Environmental DNA (eDNA) Science and Methodology, which was originally submitted on September 24, 2010, has been revised as described below to include clarifications and additional background information that was not available when the original Final Report was submitted. None of the changes detailed below represent revisions to the IEPR Panel’s opinion or the results of the IEPR.

**1. Page i (Executive Summary) and Page 1 (Section 1).**

- In the sentence “In November 2009, Asian carp eDNA was detected above the fish barrier, but no live or dead Asian carp has been captured to date in this stretch of the waterway”, the phrase “. . . but no live or dead Asian carp has been captured to date in this stretch of the waterway” has been removed.
- To reflect the live bighead carp that was captured in Lake Calumet on June 22, 2010, the following revision was made to this sentence and the one following it (italicized font indicates changes to the original text): “In November 2009, Asian carp eDNA was detected above the fish barrier, *and on June 22, 2010, a live bighead carp was captured in Lake Calumet.* Even if *additional* Asian carp are above the fish barrier, it is not known how many fish may be present.”

**2. Page iii (Executive Summary) and Page 14 (Section 5).**

- The sentence “Slight disagreements among panel members existed regarding the levels of significance for Final Panel Comments 1 and 2” has been revised to read “Disagreements among panel members existed regarding the levels of significance for Final Panel Comments 1 and 2.”

**3. Page iii (Executive Summary) and Page 15 (Section 5).**

- The sentence “There are also some problems with the interpretation of the genetic data.” has been removed because it was redundant.

**4. Page iv (Executive Summary) and Page 15 (Section 5).**

- The following paragraphs have been added to two locations of the report to further describe the Panel’s findings.

**“Alternative Surveillance Methods:** In addition to these overall genetics and population ecology findings, the following paragraphs include the Panel’s evaluation of whether any alternative surveillance methods exist that might more accurately or precisely detect the location of the invasion front of Asian carp in the CAWS.

The question of whether a feasible alternative exists to the eDNA methodology that is more reliable and precise needs to be answered in at least two parts. Firstly, the precision of a sampling program depends not only on the measure methods (e.g., eDNA vs. electrofishing), but also on the sample size. Thus, a comparison of electrofishing (for example) with the eDNA methodology would require additional information such as the sample size (or total cost) allocated to each method. Further, there is insufficient information on the “catchability” of Asian carp in any gear to allow for an accurate comparison.

The question of whether an alternative exists (emphasis on the word an) implies a view that either one method or another should be employed in a sampling program, when in fact, various methods may in fact complement one another. Some of the advantages of the eDNA methodology include:

- sample collection can occur over a large spatial area very rapidly
- the cost per sample is relatively low compared to traditional fishery methods
- dispersal of eDNA within the water allows for the eDNA sample to integrate fish presence over a larger area than traditional fishery methods where capture of a fish depends on gear being present at the exact location and time as an Asian carp

In the Panel's opinion, no other single method provides this suite of advantages offered by eDNA samples.

Some of the limitations of the eDNA method include:

- detection of eDNA does not provide conclusive proof of the physical presence of a live fish at a given location in space and time
- eDNA detections do not provide information on the size or age (for example) of individuals present
- as currently implemented using mitochondrial DNA, the method cannot distinguish between pure silver or bighead carp and their hybrids, nor can it specify the gender of individuals caught
- there is a time delay inherent between water sample collection and processing for eDNA, and thus detection is not immediate as it would be with traditional fish sampling gears

Because of these limitations, a sampling program using traditional fish sampling methods is likely required to provide all of the information needed to make critical management decisions. The eDNA method thus provides a useful complement to traditional fish sampling gears, and can greatly improve the efficiency of a sampling program for Asian carp.”

#### **5. Page 2 (Section 1).**

- The following paragraph has been added to Section 1 to explain at what point in the eDNA development and evaluation process the Panel conducted the IEPR.

“When the Corps learned of the eDNA research, the Corps evaluated the use of eDNA testing to help determine the possible location of Asian carp. The Corps viewed it as an emerging technology still in the research stage. It had never been applied in the field before in a water body like the CAWS. Nor had it undergone independent scientific studies or peer reviews of the type that the Corps would normally require before applying a technology which would inform management decisions. The Corps decided to use eDNA in the CAWS despite the uncertainties associated with research that had not been fully tested and despite results that would leave many questions unanswered. Subsequently, the Corps decided to commission this peer review to enhance understanding of the meaning of eDNA results. This report documents the methods and results associated with conducting the peer review of the eDNA technology.”

**6. Page 2 (Section 2).**

- The following sentence has been added to Section 2: “The IEPR of the eDNA science and methodology reviewed the scientific ideas, methods, and evaluations and did not include an assessment of management decisions or actions.”

**7. Page 9 (Section 3.6).**

- To further clarify the level of significance (LOS) definitions to be more customized for this particular IEPR, the LOS definitions in the Final Report (provided in Section 3.6) have been changed to read the following (italicized font indicates changes to the original text):
  - High: Describes a fundamental problem with the *methodology* that could affect the recommendation or justification of the *methodology for the intended purpose*.
  - Medium: Affects the completeness or understanding of the reports/*methodology*.
  - Low: Affects the technical quality of the reports, but will not affect the recommendation of the *methodology for the intended purpose*.