Environmental DNA Calibration Study
Interim Technical Review
Executive Summary

The Environmental DNA Calibration Study (ECALS) is a three-year study to improve the understanding and interpretation of the detection of Asian carp DNA in environmental samples (eDNA). eDNA surveillance programs seek to detect the presence of genetic material (DNA in cells sloughed off in slime, feces, urine, etc.) extracted from water samples; the detection of genetic material is linked to the possible presence of Asian carp. The study involves collaboration between the U.S. Army Corps of Engineers, the U.S. Geological Survey, and the U.S. Fish and Wildlife Service. ECALS addresses three major Action Items from the Asian Carp Regional Coordinating Committee (ACRCC) Asian Carp Control Strategy Framework, of which results to date are presented below. Initial ECALS efforts focused on eDNA vectors whereas marker development and calibration experiments will receive greater attention in 2013.

Asian Carp eDNA Vectors (alternative sources of DNA)

In addition to DNA shed by live Asian carp, vectors of Asian carp eDNA could transfer eDNA into the Chicago Area Waterway System (CAWS). To integrate what has been learned through ECALS and other ACRCC studies, a conceptual model is being developed to provide a structured visualization of the potential eDNA inputs (e.g. presence of a live fish vs. vectors of eDNA) as well as the factors or variables that influence release, transport, persistence, and detection of eDNA in the CAWS. ECALS is investigating several potential eDNA vectors:

Storm Sewers. Asian carp carcasses are transported on ice brought to Chicago-area fish markets. That ice and associated body fluids are dumped into storm gutters and street drains. Because fish may be displayed on ice at these markets during the day, change out of melting ice (potentially multiple times during the day) may supply additional amounts of ice/ice water containing Asian carp fluid/tissue into the storm sewer system. The ECALS Team executed trials in fall 2011, summer 2012, and fall 2012 to demonstrate that ice from ice chests holding Asian carp carcasses could be a source of eDNA in the CAWS.

- Two points of particular interest have been observed – One, Asian carp eDNA was detected in sewers prior to our trials (perhaps originating in fish markets) and two, eDNA that was deposited into storm sewers during experimental trials largely dissipated in receiving waters (CAWS) within a day. Whether the eDNA signal was lost due to degradation, dilution, or downstream flow is unclear.

Fertilizers. Wild-caught Asian carp are used to develop fertilizer for commercial and residential uses. The team tested two brands of fertilizer that contain Asian carp as part of the fertilizer formula for the presence of detectable eDNA (using current markers).

- There were no eDNA detections in assays of small volumes of either brand of fertilizer. Tests of larger volumes or more samples of fertilizer may be needed to completely rule out this potential vector. Currently, the team is searching for more information on the role of Asian carp in producing these fertilizers and for additional brands of fertilizer that may contain Asian carp DNA.

Fisheries gear. Gear (boats, nets) used by natural resources agencies, contract fishermen and/ or recreational anglers may be exposed to Asian carp DNA in waters where carp are present then moved into

the CAWS where some Asian carp DNA could be sloughed off into the water. The potential for these sources to harbor eDNA and result in a positive eDNA detection was evaluated in fall 2012.

- Vessel hulls have considerable amounts of adhering DNA, which can persist for days and is not removed by overland transport.
- Adhering DNA also does not appear to be completely or quickly washed off of boats moving through the water. Thus, vessel hulls can be vectors for DNA movement.
- Nets appear to be sources of very large amounts of eDNA but require confirmation and quantification of DNA associated with nets through an additional sampling trial.

Bird Transport and Deposition of eDNA. Given the assumption that eDNA is deposited by piscivorous (fish-eating) birds, ECALS has focused on the amount of eDNA in a bird fecal sample, degradation, and piscivorous bird feeding and movement patterns in the Chicago region.

- Piscivorous birds have the capacity to be a direct vector of Asian carp DNA or to contaminate fomites (e.g. barges, boats) with Asian carp DNA via fecal deposits.
- Silver carp DNA was detected in fecal samples collected from piscivorous birds offered one to three meals of silver carp.
- Silver carp DNA could be amplified from bird fecal samples collected up to 1 week following consumption of a silver carp meal.
- Silver carp DNA in fecal material deposited on metal sheets persisted for 30 days under ambient environmental conditions despite exposure to temperatures exceeding 60°C (140°F).
- Satellite-tagged double-crested cormorants exhibited large variation in daily and seasonal bird movement, with some birds staying close to tagging locations and others traveling as far as Canada or the Gulf Coast. Additional work will examine available records of the frequencies of observation for other piscivorous birds in the CAWS region.
- Throat and cloacal swabs taken at the time of satellite-tagging resulted in silver carp DNA detection in 13 of 15 cormorants (positive carp DNA from: 3 throat only, 4 cloaca only, and 6 both throat and cloaca) from a rookery near Peoria, IL and 7 of 15 cormorants (positive carp DNA from 6 throat only and 1 both throat and cloaca) from a rookery near Baker's Lake (within the CAWS), showing evidence of Asian carp consumption.

Fish Carcasses. Since biologists had reported the presence of dead Asian carp on decks of barges above the U.S. Army Corps of Engineers Electric Dispersal Barrier in the Chicago Sanitary and Shipping Canal (CSSC) and slime from those decaying carp trailing down the sides of barges to the water line, concerns have existed regarding the capacity of fomites (objects that carry DNA) like barges to transport Asian carp DNA (in the form of carcasses or slime) from areas where Asian carp are present to areas where they are not present or abundant. The goal of this study was to assess whether Asian carp carcasses or residual slime on fomites such as barges or boats could be responsible for the presence of Asian carp DNA in waters where Asian carp are not present.

Silver carp eDNA can be detected for at least 18 days when the surfaces of carp carcasses or water that had flowed over those carcasses were sampled. Samples from Asian carp slime coat that had been placed on metal surfaces also showed intact Asian carp DNA, but disappeared by day 18. These trials indicate that carcasses, or rain or other run-off from surfaces where Asian carp carcasses or slime residue reside, can be a source of eDNA entering a system.

Barge Transport of Carcasses. Guidelines for vessel operators were developed in May 2012 by USACE, ILDNR, and USEPA for vessels that enter the CAWS through four lock and dams (Dresden Island, Brandon Road, Lockport, and TJ O'Brien). The guidelines outline the protocol for vessels that may be carrying dead silver or bighead carp carcasses (and potentially depositing them on the upstream side of the barrier) and require that lock staff document these occurrences, verify the species, and ensure removal before the vessel crosses.

- During the 2012 shipping season, there were three reported incidents concerning a total of five Asian carp carcasses on vessels. On 10 April, two silver carp were found on the deck of a tow at Lockport; on 12 April one silver carp was found on a barge at the mouth of the Calumet River; and on 8 June two separate barges locking upstream at Brandon Road each reported having one silver carp on deck.

Sediments. The potential for sediments both within the CAWS and outside of the CAWS to sequester and/or transport eDNA was investigated.

- Five of 13 stream bank samples taken approximately 105 km downstream of Lockport on the Illinois River tested positive for silver carp DNA; bighead carp DNA was not detected.
- Sediment samples (n=28) were collected from Lake Peoria dredged materials being off-loaded at the old US Steel site near Calumet Harbor. Eleven samples tested positive for silver carp DNA, and one sample tested positive for bighead carp DNA.
- Additional surface sediment samples were collected in November 2012 from Lake Calumet and Lockport Pool for eDNA sorption studies that are presently underway.

Asian Carp eDNA Genetic Marker Development

The current eDNA markers for both bighead and silver carp are comprised of short segments of the mitochondrial DNA control region (or "D-loop") and primarily provide information on presence/absence of that DNA in a sample. The team's aim is to develop a suite of different markers that provide different capabilities, including 1) improved detection probabilities by increasing the number of markers simultaneously assayed, 2) more efficient processing by reducing background non-target PCR amplification, 3) real-time quantitative PCR estimates of DNA abundance (qPCR has added benefit of increased efficiency by eliminating gel electrophoresis and reducing or eliminating the need for sequencing), 4) data on allelic variability (or "polymorphism") to a degree that will allow at least broad estimation or corroboration of Asian carp abundance, and 5) some indication of the nature or time since deposition of an eDNA sample.

- Asian carp specimens from across North America (10 silver carp populations, 12 bighead carp populations) and Asia (3 populations each, silver and bighead carp) were acquired, and DNA sequencing was performed using a next-generation DNA sequencer. As a result, complete mitochondrial DNA sequences for 33 bighead and 25 silver carp from 9 North American locations were obtained.
- Genetic material for numerous non-target fish species occurring in the CAWS was procured and, along with existing data on aquatic species DNA residing in GenBank, is being used to test new markers to ensure that they are specific to silver and/or bighead carp.
- For presence/absence markers, the team is testing 12 trial markers that potentially could be used to selectively detect silver carp, testing 11 for bighead carp, and testing 17 that could potentially amplify both Asian carp species to the exclusion of all other species. Expectations are that most will be eliminated, as DNA segments that correspond to eDNA markers that would have both

absolute specificity for a target species and high detectability (in large part a function of being relatively more numerous in cells than other DNA segments, like mitochondrial DNA) are rare.

Asian Carp eDNA Increased Efficiency and Calibration Studies

Increasing Efficiency

Presently, the time from field sampling to analytical results for eDNA can take as long as two weeks. Even before laboratory analysis, several hours of very intensive fieldwork followed by laborious sample filtering is required. ECALS is evaluating ways to reduce time and effort for this process. Identification of the most cost and time-efficient extraction approach and most robust cross-platform quantitative PCR (qPCR) approach will benefit future monitoring efforts.

- Tissue grinding using a higher-throughput bead-beater instrument demonstrated no significant difference in apparent DNA yield or quality compared to the Quality Assurance Project Plan (QAPP) method, and could replace the longer vortexer-based step in the existing protocol.
- Comparison of different DNA extraction kits suggests that different extraction kits may yield different quantities of amplifiable DNA, and that different extraction kits may have varying susceptibilities to environmental inhibitors.
- A comparison of different sampling methods (filtration, centrifugation, sieve cloth) has been hampered by difficult field conditions and equipment contamination. Refined protocols and additional fieldwork are planned for 2013.
- A comparison of sampling from different depths in the CAWS water column yielded more positive eDNA hits (7 of 15 samples) for surface samples than for mid-column samples (0 of 15 samples) or bottom-depth samples (1 of 15 samples).

Calibration Studies

Calibration studies seek to examine eDNA release (i.e. shedding) rates and degradation rates under laboratory conditions to inform hydrodynamic modeling of how deposited eDNA may be distributed by water flow in the CAWS. The team has designed experiments to determine how fish size, number, behavior, as well as water temperature and diet influence eDNA loading (or shedding) by Asian carp. We will also investigate sperm as a source of eDNA over time in static water conditions.

Loading Studies

- Preliminary studies show that eDNA shedding rates are consistent over different water-flow rates. Currently, one experiment assessing effects of temperature on shedding rates of silver carp sub-adults has been completed. The team found no effect of temperature on shedding rates.
- Preliminary studies of eDNA from sperm in water showed that eDNA was detectable for at least 17 days.
- Results of these studies will provide information necessary to determine the degree to which qPCR can be used to determine abundance or biomass of bighead and silver carp from eDNA samples. Eight of the 12 designed studies (examining effects of temperature, biomass and diet; and sperm degradation) have been completed.
- However, some trials are incomplete because PCR inhibitors have prevented DNA quantitation using qPCR. The team observed that the greatest PCR inhibition is associated with samples from tanks that were fed algae and is currently working to find ways to reduce PCR inhibitors without reducing the sensitivity of the qPCR assay for these lab-based samples. However, it has become

apparent that a more accurate measurement of inhibition and a more extensive survey of methods for avoiding or removing inhibitors will be necessary, especially for the processing of field samples that are likely to have many more (and more diverse) PCR inhibitors.

Hydrodynamic Model

- The hydrodynamic grid for the area to be modeled has been completed, and protocols have been established to enable passing of information back and forth between the hydrodynamic and eDNA transport (i.e. water quality) parts of the model. Results from other ECALS investigations (e.g. eDNA degradation studies) will be incorporated into the eDNA transport model when available. 3D simulations of the hydrodynamics of the barrier area are also underway, and the electrical field modeling is in preliminary development at present.