

APPENDIX 2

RESEARCH AND TECHNOLOGIES POTENTIALLY USEFUL FOR CONTROLLING THE SPREAD OF ASIAN CARP (INDIVIDUAL PROJECT DESCRIPTIONS)

1.1 Field Deployment of Carbon Dioxide Barrier to Deter Asian Carp

Large-scale pond trials to assess behavioral responses of Bighead Carp and Silver Carp to CO₂ were completed, along with the behavioral response of non-target fishes (Bigmouth Buffalo, Channel Catfish, Paddlefish and Yellow Perch). Results from these studies are currently being summarized and prepared for publication. Studies to assess the effectiveness of CO₂ as a barrier by creating a miniature Brandon Road Lock and Dam in the lab have been completed by USACE and USGS. The Brandon Road site has been proposed by USACE as a potential location for using CO₂ as a barrier to Asian carp. Results suggest that both Silver Carp and Bighead Carp avoid areas that have CO₂ concentrations at levels documented by scientists. Large-scale CO₂ field trials were completed at Hansen Material Services backwater site near Morris, Illinois. This was the first study that demonstrates the application of CO₂ into an area comparable to the approach channel at Branden Road Lock and Dam. Scientists will continue to work on establishing a minimum concentration needed for an effective CO₂ barrier and to identify the most efficient way to inject CO₂ into the water to create a barrier to Asian carp.

1.2 Developing Targeted Micro-Particle Control Systems for Asian Carp

USGS scientists have been developing a targeted toxin delivery system in the form of a coated micro-particle with high specificity for Bighead and Silver Carp to control or limit Asian carp while minimizing potential impacts on native species. Antimycin was identified as the toxin to which the Bighead and Silver Carp were most sensitive and USGS continued to test how long it remained in the water after initial application. Scientists conducted testing to determine the dose of toxin that would need to be in the microparticle for it to be lethal to Silver Carp and Bighead Carp when eaten; and conducted studies to evaluate non-target species response to the microparticle. USGS worked with the IL DNR, FWS, and other partners to identify potential field sites along IL R. for microparticle field trials. USGS and partners will then begin to assess fish and invertebrate populations at the field site before microparticle testing. Scientists also conducted initial studies in USGS ponds to assess the use of food attractants (e.g. algae) in combination with the application of microparticles. Asian carp are highly attracted to algae as a food source so this strategy is meant to more effectively draw the fish to the area of application. Additional testing will be needed to finalize methods. Laboratory trials were also completed that demonstrated the species specific capability of microparticles to deliver antimycin to Asian carp when mixed with native fish (to avoid harming native species), and completed preliminary analysis of food availability by season and habitat type to inform microparticle development and application.

1.3 Registration of Asian Carp Control Technologies (CO2 as a Barrier and Microparticles)

Specific registration processes must be followed to obtain approval for the use of CO2 and microparticles as control agents in the environment. The USGS is providing support for these activities to the USFWS through the compilation of data and reports for submission to (the U.S. Environmental Protection Agency (EPA) or other regulatory agencies, identification of required data to attain chemical registration, coordination of experimental use permits and other regulatory support as needed to attain and maintain chemical registrations of tools to control Asian carp. The USGS is also developing specific data required to attain registration of microparticles to control Asian carp including studies to describe product chemistry, physical/chemical properties and toxicity studies. The USGS has initiated discussions with EPA regarding the regulatory framework for research applications of CO2 in open water applications and as a barrier. A CO2 injection system has been manufactured that will allow for a large-scale open-water treatment of CO2. The USGS also completed the physiological testing on the response of native mussels to CO2 under the guidance of Section 7-ESA consultation by the USFWS. USGS will continue to provide support to USFWS as standard operating procedures and safety and training protocols are developed for use of CO2 in the field to control Asian carp.

1.4 Use of Seismic technology to divert Asian Carp and Seismic Monitoring for Asian Carp Watergun Deployment

Initial testing of waterguns in USGS research ponds and in the field indicated that water gun operation altered Asian carp behavior though a few carp did pass within/near the water guns during operation, suggesting that it may not create a complete barrier to carp movement. In 2015, USGS conducted a more complete field evaluation of the use of water guns as a barrier to Asian carp movement at a backwater area in Morris, IL. The watergun array design included 4 water guns (as compared to 2 in the past) and operated at shorter intervals between discharges than previous testing. Acoustic telemetry tags were implanted in Bighead and Silver Carp enabling scientists to monitor fish behavior with and without water gun operation. Though analysis is preliminary, the operation of the water guns did appear to alter the behavior of Bighead Carp and Silver Carp. However, a number of fish still crossed the barrier with and without water gun operation. At this point, given the body of data available, the operation of water guns does not appear to create a complete barrier to deter fish movement. Based on this, scientists are placing additional focus on the use of complex sound to deter Asian carp.

1.5 Use of Complex Sound to Alter Behavior of Asian Carp

USGS and University of Minnesota Duluth (UMD) scientists recently studied Silver Carp reaction to sound in study ponds at USGS in LaCrosse, WI. The researchers found that Silver Carp reacted strongly to complex noises such as underwater recordings of boat motors, consistently swimming away up to 37 times in succession. Pure tones, which sound like a dial tone, were also tested but scientists found that fish adjusted to the pure tones, never swimming away more than two consecutive times. The results are published in the journal *Biological Invasions*. Results from this study have provided the foundation for the UMD, USGS, and Illinois Natural History Survey to conduct field trials in 2016, testing the efficacy of complex noise as a Silver Carp control tool in the Illinois River. Results of the testing will guide the

development of recommendations for management agencies on operating conditions to establish deterrent barriers or mobile herding systems.

1.6 Field evaluation of chemical attractants to control Asian carp and development of protocols for field verification of response

Laboratory and field studies, including underwater video, conducted by USGS and by independent researchers have consistently confirmed that an algal food stimulus is highly attractive to Asian carp and can increase the abundance of fish in areas where Asian Carp have been conditioned to the food. Initial testing was conducted in 2015 to evaluate the use of the algal attractant in combination with the toxic microparticle application in USGS study ponds to ensure the ingestion of toxic microparticles by the fish. Next steps will include characterizing the feeding response of the fish to the microparticles and documenting distress and mortality at the feeding stations. Scientists also collaborated with the IL DNR to test the use of algal attractants in combination with netting, electroshocking, and other techniques to determine if it increased Asian carp harvest. In addition, tests confirmed that carp can be conditioned to come to feeding stations and that such strong attraction can be used to facilitate their capture. Attraction to the feeding stations was enhanced through the use of sound which drew the fish in from greater distances than the algal attractant alone.

1.7 Integrated Pest Management (IPM)

These collaborative efforts which include USGS, IL DNR, Southern Illinois University, and other partners are considered the field evaluation component of the various control tools and technologies being developed for Asian carp control. Many potential control tools, including CO₂, feeding attractants, microparticles and commercial Asian carp harvest are being evaluated to determine if integrating these tools increases the ability to reduce Asian carp populations. The first of such efforts is described in a report published in 2015 by USGS on IPM operation. As another part of the IPM, initial CO₂ field tests were conducted in a backwater area of the Illinois River to determine if CO₂ could be used as a barrier. An injection system was deployed to disseminate the CO₂ across the middle of the channel, into the water. Levels of carbon dioxide, pH, and other water quality parameters were measured continuously throughout the study and analysis of testing results is underway. USGS scientists also completed laboratory trials that demonstrated the efficacy of microparticles to deliver antimycin to Asian carp when mixed with native fish, and completed preliminary analysis of food availability by season and habitat type to inform microparticle development and application. The final year of field work for a fish telemetry study on emerging populations of Asian carp was completed to inform contracting fishing efforts and the application of developing controls tools such as CO₂, sound, and microparticles).

1.8 Assessing life history traits of Asian carp in established and emerging populations to identify and characterize vulnerabilities that can be exploited for control

A better understanding of life history traits and population dynamics of Asian carp in areas with established (e.g., Illinois River) and emerging populations is necessary to identify vulnerabilities that can

be exploited for control strategies using an IPM approach. In particular, a better understanding of seasonal movements, habitat use, predators, and behaviors of adult and juvenile Asian carp and native fish is needed for safe and effective application of species-specific toxicants, selective removal by fishing, predator stocking, and habitat manipulation and barriers. In 2015, USGS scientists learned much more about the developmental rate and behavior of Asian carp from egg to larval stages. This biological egg and larval data has been incorporated into and enhanced the FluEgg model which is a tool being used to predict Asian carp spawning locations and what conditions are required for eggs and larval fish to survive and grow. Scientists also completed analysis and summary of data on feeding behavior and recruitment constraint (i.e., juvenile habitat, adult spawning requirements, and predation) studies on the emerging population in the Upper Mississippi River; and initiated planning to transition life history studies to the established population of Asian carp in the Illinois River Basin with a focus on controlling the leading (upstream) edge of the population, thus reducing propagule pressure on existing and future barriers.

1.9 Improving Molecular Techniques for Monitoring, Biomass Estimation, and Correlation with Live Fish

Early detection is a vital part of managing any invasive species, including invasive Asian carp. Significant efforts have been made to detect Bighead and Silver Carp at low abundances and identify their invasion front using traditional capture gear which Asian carp have been found to avoid. An alternative method for monitoring Asian carp is the detection of molecular signals such as eDNA in water samples. Applications of eDNA technology under development in 2015 include estimation of the amount of fish in an area and methods for determination of the amount of time since eDNA was shed from a live fish. Refinements to eDNA methodology are also being pursued to improve detection sensitivity, prevent false negatives caused by PCR inhibition, increase cost-effectiveness, and decrease time between sampling and results.

1.10 Development of a Rapid and quantitative genetic-based Asian carp Detection Method

The USGS recently verified a new technology for detecting Asian carp in water samples. The technology uses a portable hand-held detection device that detects eDNA of Bighead and Silver Carp in water samples. Using the hand-held kit significantly reduces the time between sample collection and results compared to typical laboratory-based analysis which generally require several days to obtain. Developing portable, rapid, and reliable methods and kits such as this one will improve the detection of invasive species and pathogens and will improve the ability of resource managers to make timely decisions to prevent, contain, and control invasive species and pathogens. Future efforts will continue the evaluation of the method and kit under field conditions, including fish shipments that might contain invasive carps, and developing procedures and information needed to allow conservation officers and law enforcement agencies.

1.11 Use of Acoustic video and side-scan technology to determine behavior of Asian carps, especially net avoidance behavior

Asian carp are believed to have highly developed net avoidance behavior, which inhibits control methods based on harvest. The goal of the project is to assess the behavior of Asian carp in relation to different gear types and propose gear modifications that might enhance harvest efficiency. Several types of nets and gear were included in the study including hoop, gill, and trammel nets. Dual-Frequency Identification SONAR (DIDSON) underwater video was used to record carp behaviors at nets on the Mississippi and Des Moines rivers, and other data collection for fish behavior in relation to hoop nets was completed. Gill and trammel nets were recorded with the DIDSON on the Missouri River, Cedar Creek, Lamine River, and Blackwater River in Missouri. Despite several delays due to rain, a large portion of the field work necessary for the project was completed. Optimum DIDSON camera settings and modifications for turbid waters were determined.

1.12 Hydraulic and Water Quality Evaluation of Asian Carp Habitat in the Upper Illinois River and their Impacts on Asian Carp movement

This project was conducted to determine natural and managed controlling factor(s) that contribute to the stalled migration of Bighead and Silver Carp within the pools of the Illinois River, and how one or more of these factors could be used to prevent future migration or to reduce Bighead and Silver Carp populations. Sharp contrasts in habitat, flow conditions, water quality, and food supply between these pools and the CAWS may be factors that are keeping Asian carp from moving further upstream. In 2015 USGS collected, analyzed, and began evaluation of critical hydraulic and water quality data to look for specific chemistry or flow conditions that Asian carp may be avoiding. This information can give managers insight into how habitat may be altered to deter Asian carp from spreading into new areas. The FluEgg and other flow models were also used to simulate water flow and movement of particles to determine which rivers may pose a greater probability for the establishment of sustainable populations; and to better understand and predict how carp eggs and larvae move through the river. This work is being conducted in collaboration with IL DNR, USFWS, USACE, and other partners.

1.13 Grass Carp

USGS research on Grass Carp is diverse, focusing primarily on understanding biology and hydrologic factors related to biology. As knowledge on biology and hydrologic drivers expands we are building the base of knowledge for managers to formulate potential control and management strategies and tactics under the IPM framework. Ongoing biological and hydrological studies have focused on documenting spawning of Grass Carp in the Sandusky River in Ohio and quantifying hydrologic conditions necessary and sufficient for spawning. Collaborators include Ohio DNR, Michigan DNR, University of Toledo, Bowling Green State University, University of Illinois, USFWS, Department of Fisheries and Oceans Canada. During June and July 2015 sampling, Grass Carp eggs were found in the Sandusky River on 5 different sampling dates during 3 separate high-flow events. Five eggs, at least one from each high-flow event, were confirmed as Grass Carp using specific genetic analyses that target Grass Carp. These discoveries confirmed directly that Grass Carp are using the Sandusky River for spawning. Understanding the extent of the Grass Carp invasion and whether the population is self-sustaining or expanding is critical to providing managers information they need to address their control in the Great

Lakes. USGS scientists have already begun to determine Grass Carp spawning habitat and investigate egg survival concurrently with studies on Bighead Carp and Silver Carp.

1.14 Interim barrier (Brandon Road Lock and Dam)

The use of carbon dioxide barriers and other technologies have been proposed for use at the Brandon Road Lock and Dam (BRLD) on the Illinois River in to help prevent the movement of Asian carp from the Mississippi River Basin to the Great Lakes Basin. In 2015, USGS conducted assessments of hydrologic and water chemistry conditions and changes in river flow and currents associated with the river and lock and dam operations. This information is helping the USACE identify possible impacts of these technologies on or near their structures, how potential new barrier technologies could be used at the BRLD, and provides background data needed to design control barriers in ways that will minimize impact on barge and vessel movement through the lock and approaches. The scope of this work will continue to be dynamic based on input from other cooperators and stakeholders such as the USACE, USFWS, and IL DNR. USGS is prepared to continue to address the data, research, and technology needs identified as these efforts progress.

1.14 Wabash/Maumee Hydrologic Support to Prevent Interbasin Transfer of Asian Carp

USGS continued to operate streamflow, temperature, and water level gages as well as a webcam at Eagle Marsh in Ft. Wayne, Indiana to monitor flow conditions that may lead to flooding in the area and at the temporary fence currently in place to prevent movement of adult Asian carp. These efforts support USACE, IN DNR, and other partners in preventing movement of Asian carp from the Wabash River into the Great Lakes Basin. The stream data has been used to help determine the location for a permanent barrier currently being constructed by the USACE and will provide additional data once the barrier is in place.