ECALS MILESTONE REPORT

ACRCC Framework Item:

2.5.3 Vectors, 2012 Framework
2.6.3 Probabilistic Model, 2013 Framework

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ECALS Project Management Plan Tasks:

1.1.3: Construct conceptual risk model following data accrual and interpretation.
1.5.1: Update the conceptual model following data accrual and interpretation.
3.5.1 Graphical model of eDNA occurrence and persistence in the CAWS.

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Introduction

Asian carp were imported into the United States in the 1970's to control phytoplankton and macrophytes in fish ponds and wastewater treatment lagoons (Kolar *et al.* 2007). Over the past thirty years, these fish have expanded their range within the Mississippi River Basin. Two planktivorous species of Asian carp are of particular concern. Bighead carp (*Hypophthalmicthys noblis*) and silver carp (*H. molitrix*) are highly efficient filter feeders that have caused significant ecological damage in the Mississippi River Basin by undermining food webs and outcompeting native fish populations in the habitats where they become established (Chick and Pegg 2001, Kolar *et al.* 2007). Were these fish were to become established in Lake Michigan, they could harm native fish populations.

Efforts to prevent Asian carp from colonizing Lake Michigan have focused on the Chicago Area Waterway System (CAWS) because it is the principal hydrologic connection between the Mississippi River Basin and Lake Michigan. The Illinois River, a tributary of the Mississippi River, is connected to Lake Michigan via the Chicago Sanitary and Ship Canal (CSSC), which was constructed in the late 1890's to transport sewage from Chicago away from Lake Michigan, the source of the city's drinking water (Changnon *et al.* 1996, MWRD 2008). The leading edge of the Asian carp invasion is presently considered to be at river mile 278 of the Illinois River, at the Dresden Island pool, about 55 miles downstream from Lake Michigan. However, on rare occasions, individual adult fish have been captured and removed from the pool below Lockport Lock and Dam.

Since 2002, the US Army Corps of Engineers (USACE) has operated an electric fish barrier at Romeoville, Illinois, about 35 miles downstream from Lake Michigan. The fish barrier is designed to prevent the Asian carp invasion front from reaching Lake Michigan via the CAWS. Fish that challenge the barrier are stunned by a non-lethal electrical charge. Although the fish barrier greatly reduces the probability that the Asian carp invasion front will advance

toward Lake Michigan via the CAWS, several scenarios under which fish might penetrate or circumvent the barrier may exist (Rasmussen *et al.* 2011) and studies of the barrier's effectiveness are ongoing (ACRCC 2012). There are also other pathways by which the fish might reach waters upstream of the barrier (ACRCC 2012). For example, adult Asian carp are occasionally found in land locked lakes and ponds in the Chicago area. These appear to have been released as fry or fingerlings when lakes and ponds were stocked (ILDNR 2011, USGS 2013).

Over the past several years, a conventional fisheries surveillance program has been implemented in the CAWS to detect the possible presence of bighead and silver carp. This program deploys electrofishing boats and nets at fixed and randomly selected sites to determine the numbers and types of species present. Between 2010 and 2012, monitoring crews logged over 9,600 hours sampling at fixed and randomly selected sites throughout the CAWS upstream of the barrier (MRWG 2013a). No bighead or silver carp have been captured as part of this conventional sampling program. However, there is one reported occurrence of Asian carp in the CAWS. On June 22, 2010, commercial fishermen working in Lake Calumet captured a bighead carp weighing 8.9 kg. The ACRCC's Monitoring and Rapid Response Work Group[1] (MRRWG) poisoned Lake Calumet with the piscicide rotenone on June 23, 2010, but no additional bighead or silver carp were caught (MRRWG 2012).

Between 2009 and 2012, USACE and partner agencies have been collecting water samples from the CAWS and testing for the presence of deoxyribonucleic acid (DNA) specific to bighead and silver carp. Aquatic organisms release DNA into the environment through bodily excretions such as feces, urine, sperm, eggs, and rotting carcasses. This environmental DNA (eDNA) is assumed to degrade quickly. Until recently, it has been assumed that the presence of bighead and silver carp DNA in water samples indicate that a fish has recently been present near the location where the sample was collected. Water samples were collected over the course of 68 sampling events. Of the samples collected within and upstream of the electric fish barrier, bighead carp DNA was detected in 43 of 5,522 water samples tested for bighead carp and silver carp DNA was detected in 236 of 5,503 water samples tested for silver carp (MRWG 2013c).

Positive detections of bighead and silver carp eDNA at a monitoring location during two or more consecutive eDNA monitoring events may, at the discretion of fisheries managers, trigger rapid response actions to remove the fish using fishing gear or poison. Between 2010 and 2012, eleven rapid response actions were undertaken employing an estimated 11,330 man hours. No bighead or silver carp have ever been captured during these rapid response actions or during the course of any other MRRWG sampling activity undertaken above the electric barrier. The use of eDNA evidence as a trigger for rapid response actions was discontinued in 2013 because of the lack of success in capturing the target species and uncertainty about how to interpret eDNA monitoring results (MRWG 2013b).

It is possible that bighead and silver carp are present in the CAWS in very low numbers, and therefore difficult to capture or detect using conventional surveillance methods (Jerde *et al.* 2011). While a very low number of individuals might explain the detection of eDNA and the

[1] In 2013, the Monitoring and Rapid Response Workgroup (MRRWG) was renamed the Monitoring and Response Workgroup (MRWG).

inability to capture or detect the fish, recent studies have also suggested that eDNA evidence should be interpreted carefully (ECALS 2013, Wilcox *et al.* 2013). The detection of eDNA belonging to a particular species in a water body should not, by itself, be taken as proof that a live member of that species is present in that water body because too little is known about factors influencing the distribution of eDNA. For example, it has been shown that eDNA can be transported to the CAWS and released by fish-eating birds, boats, barges, fishing gear and storm sewers (ECALS 2013, MRWG 2013b). Any one or a combination of these sources could provide an alternate explanation for the presence of bighead and silver carp eDNA in the CAWS upstream of the electric fish barrier. Similarly, because eDNA can be difficult to detect at low concentrations, the failure to detect eDNA in a system should not be interpreted as proof that the fish are absent.

Potential actions taken in response to evidence produced by eDNA monitoring in the CAWS have very high costs. If it is concluded that the fish are present, this could lead to costly rapid response actions to remove the fish, the construction of additional barriers, and the closure of navigation routes. However, if it turns out that, in fact, the fish are absent from the CAWS, then these costs could have been avoided. Similarly, an error in concluding that the fish are absent from the CAWS might lead to inaction. However, if it turns out, in fact, that the fish are present and the fish eventually do become established in Lake Michigan, the environmental costs may be high. Total expected costs can be minimized by evaluating the strength of conclusions based on available evidence before taking action. Therefore, the objective of this study is to develop a method that will enable fisheries managers to articulate the strength of conclusions about the source(s) of eDNA detected in the CAWS and the presence of bighead and silver carp above the electric fish barrier.

This interim milestone report summarizes Environmental DNA Calibration Study (ECALS) efforts to understand and interpret eDNA monitoring results. A conceptual model of eDNA occurrence and persistence is developed to identify what factors may be influencing the spatial and temporal pattern of eDNA detections in the CAWS. A second conceptual model is also presented to describe factors influencing the detectability of eDNA using polymerase chain reaction (PCR). The conceptual models are a preliminary step in developing a probabilistic model to facilitate inference from eDNA monitoring results.

The probabilistic modeling objective is to enable natural resource managers to make statements about the relative importance of the potential sources and vectors of eDNA found in monitoring samples and the probability that live fish may be present in the water body where monitoring samples were collected. This report is preliminary. The ideas expressed here will evolve as the various ongoing components of the ECALS study are concluded and the structure of the models will change as the realities of parameterization set in. This report contains absolutely no conclusions. No conclusions are made regarding the relative importance of potential eDNA sources and vectors and no conclusions are made regarding whether or not live Asian carp are present in the CAWS or in Lake Michigan.

This report begins with a brief literature review, the scope of which is to identify other studies that have applied the eDNA methodology as a complement to conventional surveillance methods and highlight emergent issues with the application of that technique. The conceptual

model is introduced and explained. Preliminary plans for parameterization and implementation of the probabilistic model are described. The content of this interim report is preliminary and subject to revision as the ECALS continues an iterative process of developing the insights and tools needed to interpret eDNA monitoring results in the CAWS more effectively.

Literature Review

Darling and Blum (2007) outlined several ways that DNA-based methods *might* be used to monitor invasive species distributions. DNA-based methods are most commonly used to confirm a previously identified specimen or to identify specimens that cannot be otherwise classified because of, for example, a lack of trained personnel or a lack of unique morphological characteristics at a given life stage. In these applications, DNA is extracted directly from a known specimen that provides a point of reference to the original source. Other DNA-based methods use DNA that has been extracted from an environmental sample, such as a sample of soil or water, and there is no specimen, scat, or other evidence that might corroborate the source. These methods include screening for the presence of a target species, quantifying propagule pressure (the number and viability of reproductive units of an invasive species arriving in an area (Stohlgren and Schnase 2006)), and conducting biodiversity surveys (*i.e.*, enumerating all of the species contributing eDNA to an environmental sample). The lack of certainty about where the DNA found in an environmental sample originated and how it arrived at the monitoring location is one of the main challenges of using eDNA based methods.

Several studies have tested the ability of eDNA methods to determine species presence in aquatic environments. Ficetola *et al.* (2008) screened water samples collected from ponds in France to detect the American bullfrog (*Rana catesbeiana = Lithobates catesbeianus*), an invasive species. Goldberg *et al.* (2011) collected water samples from five streams on the Payette National Forest to test for DNA belonging to the Rocky Mountain tailed frog (*Ascaphus montanus*) and the Idaho giant salamander (*Dicamptodon aterrimus*). Foote *et al.* (2012) investigated the feasibility of using eDNA methods to detect harbor porpoise (*Phocena phocena*) in the Baltic Sea. Jerde *et al.* (2011) report the results of monitoring for bighead and silver carp DNA in the CAWS during the period 2009-2010. Jerde *et al.* (2013) document the occurrence of bighead and silver carp DNA in tributaries of the Lake Saint Clair, Lake Erie, and Lake Michigan basins. Thomsen *et al.* (2012a) conducted a biodiversity survey of fish in the Sound of Elsinore, Denmark, using eDNA methods. DeJean *et al.* (2012) compared the sensitivity of eDNA methods to the sensitivity of conventional surveillance methods in a survey of American bullfrogs in freshwater ponds of the Natural Regional Park of Perigord-Limousin, France. Olson *et al.* (2012) used eDNA methods to test for the presence of a salamander, the Eastern Hellbender (*Cryptobranchus a. alleganiensis*), in three streams located in Missouri and Indiana. Wilcox *et al.* (2013) characterized the specificity and sensitivity of an eDNA assay to detect bull trout (*Salmo confluentus*) in Montana streams.

Most eDNA studies, including those listed above, employ conventional polymerase chain reaction (cPCR) to test for the presence of eDNA. The cPCR assay detects the presence of eDNA, but provides no indication of the quantity of DNA in a sample. Quantitative PCR (qPCR) is an alternate assay that provides an estimate of the number of eDNA copies present in

an environmental sample. Takahara *et al.* (2013) used qPCR to evaluate the distribution of bluegill (*Lepomis macrochirus*), an invasive species, in freshwater ponds of Hiroshima Prefecture, Japan. Thomsen *et al.* (2012b) used qPCR in conjunction with cPCR to test freshwater samples obtained from 98 European lakes, ponds, and streams for the presence of DNA associated with six rare species representing different taxonomic groups, including amphibians, fish, mammals, insects, and crustaceans. The authors report positive correlations between eDNA concentrations (molecules / μl) and estimates of animal density based on surveys completed using conventional auditory and visual surveillance methods. Using qPCR, Takahara *et al.* (2012) was able to establish a positive correlation between common carp (*Cyprinus carpio*) biomass and eDNA concentrations in aquaria and controlled ponds.

The primary advantage of qPCR relative to cPCR is that the former provides an estimate of the concentration of an eDNA marker in the environmental sample, which makes it possible to analyze the distribution of target species eDNA at a study site. For example, Takahara *et al.* (2012) used qPCR to analyze the concentration of carp eDNA during the winter season at 21 sites in Iba-naiko Lagoon, Japan. Concentrations were positively correlated with water temperature, which the authors suggest reflects the carp's preference for warmer water. However, there may be tradeoffs in using qPCR rather than cPCR. Both assays can amplify DNA from non-target species that are phylogenetically similar to the target species (Wilcox *et al.* 2013) and in some cases it will be more difficult to use DNA sequencing to verify target (vs. non-target) detection for qPCR results than it is for cPCR results.

TaqMan qPCR, the type of qPCR that will likely be used for eDNA assays, works through interaction between the target DNA and three oligonucleotides: two primers that amplify the DNA and an internal probe with a molecular tag that fluoresces when DNA strands are copied. Because TaqMan qPCR requires a close match between the target DNA (or marker) and three oligonucleotides, as opposed to just the two primers used in cPCR, TaqMan assays can provide an added degree of target DNA specificity relative to what may be possible with cPCR. For example, in cases where eDNA samples contain DNA from closely related species, the two primers may bind to and amplify the DNA of multiple species, but the probe may be specific to the DNA of the target species and provide quantitation for only that target. However, when the qPCR product for such samples is sequenced, amplicons (*i.e.* amplified DNA) from all the related species will be present and will likely confound the sequencing process, preventing validation. Amplicon cloning and sequencing, or next-generation sequencing, could be used to overcome this obstacle, but these are costly and time-consuming processes. With these challenges in mind, exhaustive measures to confirm qPCR marker specificity, along with occasional validation of qPCR results by sequencing, should always be implemented, particularly when significant management, legal, or other consequences will depend on the results of eDNA surveys.

The practice of verifying cPCR and qPCR results by sequencing the DNA that has been amplified during PCR is based on the assumption that marker nucleotide sequences are unique to the target species. However, sequence similarities between target and non-target species are often evaluated using a small sample of individuals, which may or may not come from the geographic region of interest. When the results of eDNA assays may lead to outcomes that have significant management, legal, or other consequences, careful and exhaustive measures would be

taken to characterize both within- and between-species DNA sequence diversity for the selected marker region. ECALS is addressing these issues for Asian carp eDNA makers through careful development and screening of multiple individual Asian carp and several individual samples from Asian-carp related species that occur in the CAWS and Lake Michigan.

The concentration of eDNA present in an aquatic system will be a function of the target species population size, shedding rates, and eDNA degradation rates (Dejean *et al.* 2013, Wilcox *et al.* 2013). Larger populations and higher shedding rates will tend to increase concentrations and higher degradation rates will tend to reduce concentrations. The shedding rate, the rate at which DNA is released from an organism, will vary between species and individuals, and as individual metabolic rates vary with life stage and season (Goldberg *et al.* 2011). In closed systems, such as ponds, eDNA concentrations can be expected to accumulate over time to a level that reflects local hydrodynamics (flows and dilution volumes) as well as a balance between shedding and degradation rates. In open systems, such as streams, rivers, and oceans, higher flows or currents will increase the dilution volume and tend to reduce eDNA concentrations (Goldberg *et al.* 2011, Foote *et al.* 2012, Wilcox *et al.* 2013). Flows and currents entering a study site may also import eDNA that has been released elsewhere either by the target species or by a non-target vector, leading to higher concentrations than might otherwise be observed at the study site (Thomsen *et al.* 2012b, Wilcox *et al.* 2013). ECALS is conducting studies to quantify eDNA shedding rates from bighead and silver carp (Klymus *et al.* 2013).

Most investigators tend to agree that eDNA degrades rapidly in the environment, and this has provided one of the primary justifications for inferring target species presence at the study site where eDNA is detected (Ficetola *et al.* 2008, Jerde *et al.* 2011). Degradation of eDNA occurs by hydrolysis and may be influenced by environmental conditions, such as temperature, pH, microbial activity, and light or ultra-violet radiation. Matsui *et al.* (2001) reported that extracellular DNA fragments up to 400 base pairs (bp) in length can persist for up to one week in lake water at 18 deg. C (Ficetola *et al.* 2008). Thomsen *et al.* (2012a) found that even small eDNA fragments, up to 100 bp in length, degrade beyond detectability within days. Dejean *et al.* (2011) quantified extracellular DNA degradation rates using American bullfrog tadpoles and Siberian sturgeon (*Acipenser baerii*) sub-adults (20 cm); these authors found that DNA could be detected in more than five percent of samples for up to 25 and 17 days, respectively. However, DNA fragments may persist in the environment for very long periods of time. Adsorption to mineral and humic substances protects the DNA from extracellular microbes that would otherwise degrade unbound DNA in solution (Levy-Booth *et al.* 2007). Very cold conditions can also retard degradation. Willerslev *et al.* (2004) report that eDNA may persist for several hundreds of thousands of years in very cold environments (Dejean *et al.* 2011, Thomsen *et al.* 2012). ECALS is investigating how temperature, turbidity, light, pH, and other environmental factors such as adsorption to sediment particles might influence the degradation of bighead and silver carp eDNA.

There are numerous questions about what types of inferences eDNA monitoring can support and how eDNA monitoring studies can be used to inform natural resource management decisions. Two basic inferences are possible. The cPCR assay can be used to infer the presence or absence of a genetic marker in an environmental sample, and an eDNA monitoring program can be used to infer the presence or absence of a target species in the habitat where the

environmental sample was collected. Darling and Mahon (2011) considered potential causes of false positive and false negative conclusions based on the results of eDNA assays using PCR. False positive conclusions are those that infer either the marker or the target species is present when in fact it is not. False negative conclusions are those that infer either the marker or the target species is absent when in fact it is present.

Conceptual Model of eDNA Occurrence and Persistence

The ECALS conceptual model describes factors or variables that are believed to influence the occurrence, persistence, and detectability of eDNA on the outcome of the eDNA surveillance program. The conceptual model is presented in the form of a graph consisting of nodes representing random variables and directed edges between nodes. Random variables are sources of uncertainty in interpreting eDNA monitoring results. Directed edges, or arrows between nodes, signify the influence of one random variable on another random variable in the direction of the edge. Very complex joint probability distributions can be represented by illustrating the dependence and conditional independence relationships between random variables. Although the graphical models presented here are conceptual, they provide a point of departure for developing a fully quantitative probabilistic model for the interpretation of eDNA monitoring data from a monitored water body. Considerable iterative refinement of the conceptual model presented here will be needed before such a transformation can occur.

The conceptual model summarizes knowledge or beliefs about the sources and vectors of eDNA in the CAWS, and the factors influencing eDNA occurrence, persistence, and detectability in a monitored water body. A very general conceptual model of eDNA occurrence, persistence, and detectability is presented in Figure 1 to introduce the concepts. In this version of the conceptual model, the formalities of graphical models are relaxed so that concepts can be introduced briefly in general terms. This should make it easier to follow discussions of a more detailed conceptual model in the next section of this report. The issues raised in the presentation of this preliminary conceptual model will be revisited later in a more detailed conceptual model.

The conceptual model in Figure 1 terminates in three nodes in the lower left hand corner. These nodes represent modes of Asian carp detection in the CAWS: CATCH, SIGHTING, and EDNA ASSAY.[2] One can think of these nodes as binary random variables with potential states *True* or *False*. Catch represents the physical capture of an Asian carp in the CAWS and depends primarily upon the amount and type of CONVENTIONAL SURVEILLANCE employed and the POPULATION of the SPECIES (*Bighead carp* or *Silver carp*) that might be captured. Higher populations and higher levels of CONVENTIONAL SURVEILLANCE should result in higher probabilities of capturing a live Asian carp. SIGHTING refers to the casual observation of an Asian carp that does not result in capture. This is a function primarily of the level of HUMAN ACTIVITY on the waterway (*e.g.*, the amount of NAVIGATION and RECREATION), man hours invested in CONVENTIONAL SURVEILLANCE, and the POPULATION of Asian carp in the monitored water body. The third mode of detection is an EDNA ASSAY. The outcome of an eDNA assay is the a function of the CONCENTRATION of eDNA at the location where the

[2] For clarity in the presentation, the titles of nodes will be capitalized and potential states of random variables represented by these nodes will be italicized throughout this report.

eDNA monitoring sample is collected, characteristics of the eDNA monitoring program (EDNA MONITORING) such as frequency, intensity, and distribution of sample collection efforts, and the SPECIFICITY & SENSITIVITY of the genetic marker that the assay is meant to detect. SPECIFICITY describes how well we know that the genetic marker being tested for is unique to the target species. SENSITIVITY describes how easily the marker being tested for is detected using PCR. WATER QUALITY influences the outcome of an eDNA assay because certain water quality constituents inhibit PCR reactions and may lead to false negative assays.

This conceptual model emphasizes factors influencing CONCENTRATION, the concentration of eDNA in the waterway. The graph contains two nodes representing sources of eDNA: PRIMARY LOAD and SECONDARY LOAD. PRIMARY LOAD is the number of copies of the marker that are released directly from a live fish in the monitored water body. PRIMARY LOAD is a function of the fish POPULATION present in the waterway and the eDNA shedding rate (SHEDDING), which will likely be influenced by SPECIES, the age structure or size distribution (SIZE) of the target species population, and the life-cycle stage or SEASON in which monitoring occurs.

The SECONDARY LOAD node represents all other potential sources of eDNA in the waterway. Known potential sources of secondary load include NAVIGATION, RECREATION, BIRDS, COMBINED SEWERS, CONVENTIONAL SURVEILLANCE, and UPSTREAM sources (ECALS 2013). NAVIGATION captures the potential for barges travelling from below the electric fish barrier to points above the electric fish barrier to inadvertently carry eDNA on their hulls, in bilge water, and in carcasses of fish (especially silver carp) that may have landed on deck. BIRDS captures the potential contribution of eDNA to the waterway when fish-eating birds known to prey on Asian carp such as eagles, cormorants, and pelicans defecate in the water or when runoff transports feces from nearby nesting areas. NAVIGATION and BIRDS are both influenced by a variable called COPKG. COPKG is the eDNA load in one kilogram of fish tissue. This variable is important because the potential contribution of eDNA from NAVIGATION and BIRDS will depend on the quantity of fish carcasses on barges and the quantity of fish in bird diets. RECREATION captures the potential for pleasure boats and fishing boats, such as those participating in Lake Calumet fishing derbies, to transport copies of eDNA markers from carp-infested waters on hulls and in bilge tanks. Asian carp eDNA has also been detected in discharges from combined sewers, which may carry kitchen waste from homes and restaurants where Asian carp are consumed or from fish markets where Asian carp waste may be disposed of in the sewer system (COMBINED SEWERS). CONVENTIONAL SURVEILLANCE is also a potential source of eDNA in the water body because eDNA can be imported to carp-free waters on nets that have previously been used in carp-infested water bodies. Finally, the node UPSTREAM represents the potential for inflows to transport eDNA that has been released into the environment outside the geographic limits of the study area.

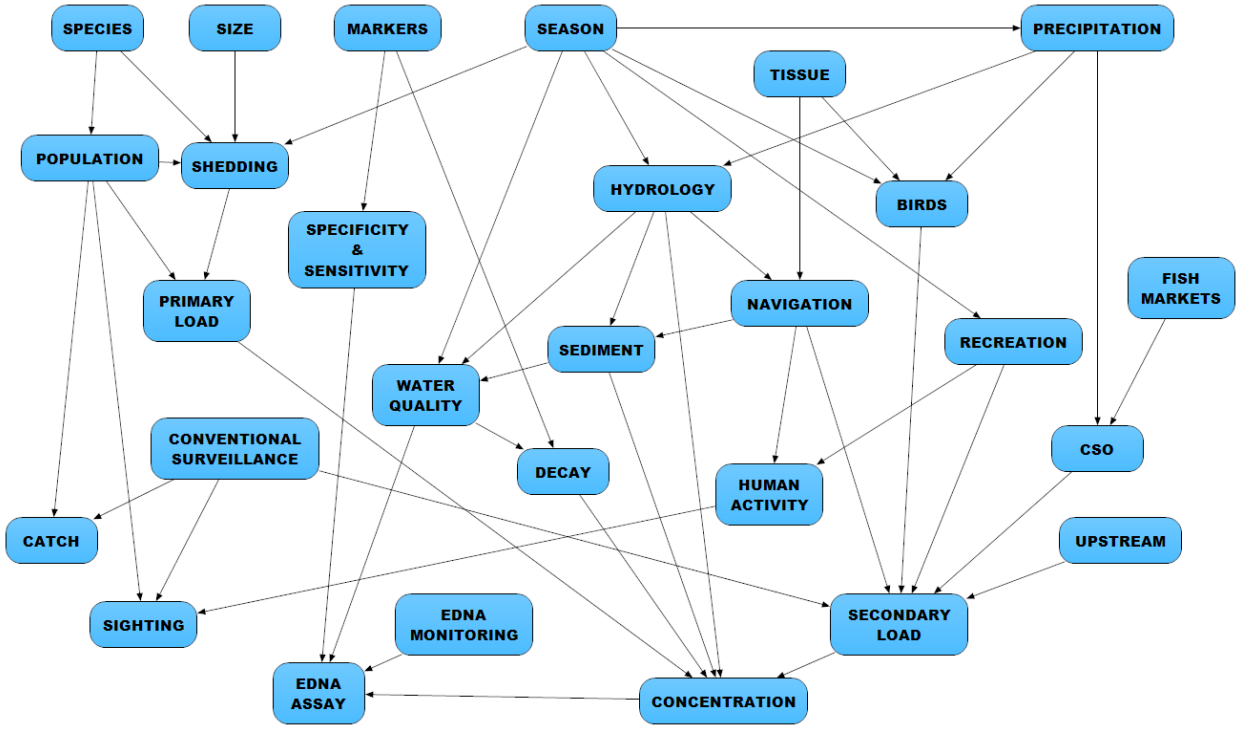

Figure 1: General conceptual model of eDNA occurrence, persistence, and detectability in the CAWS.

Three other factors contribute to the occurrence, persistence, and detectability of eDNA. These include DECAY, HYDROLOGY, and SEDIMENT. While several studies have shown that eDNA decays rapidly in the environment, there is also evidence that eDNA can persist for long time periods when adsorbed to sediment or in cold temperatures. If eDNA decay is a random process that occurs as the bonds between nucleotides become broken, rendering the marker non-detectable by an eDNA assay, decay rates may, in part, be a function of marker length. Genetic markers consisting of a larger number of nucleotide bonds may have a higher probability of becoming cleaved. This explains the relationship between DECAY and MARKERS, which describes the characteristics of the eDNA marker that is being tested for in the assay. Decay rates may also be influenced by WATER QUALITY. Not much is known about how water quality may influence decay rates, but factors such as pH, water temperature, microbial content, and turbidity may be important.

The HYDROLOGY node represents all variables related to the movement and flow of water in space and time, including hydrography, flow, velocity, stream geometry, hydrological residence time and age of water. HYDROLOGY has an important and complex influence on CONCENTRATION. For example, hydrologic variables such as stream geometry and flow will influence eDNA concentrations by altering the dilution volume, the travel time through the system, and the age of water in the system. Higher flows will tend to reduce eDNA concentrations by increasing the dilution volume and reducing the time that eDNA particles remain in the system. Slower moving water may be less likely to contain eDNA that has been

imported from outside the system because its residence time in the system is longer, providing more opportunities for eDNA to degrade before it is detected. HYDROLOGY influences WATER QUALITY, NAVIGATION, and SEDIMENT. The effects of HYDROLOGY on WATER QUALITY may be similar to those summarized for eDNA concentrations. While sufficient flows must be available to support NAVIGATION, the importance of this relationship for understanding eDNA occurrence, persistence, and detection in the CAWS is unclear. The importance of the influence of HYDROLOGY on SEDIMENT is more obvious. Higher flows tend to reduce sediment deposition rates and increase shear stress, inducing resuspension of settled sediments and increasing suspended sediment concentrations in the water column.

The SEDIMENT nodes represents all variables related to the suspension, deposition, and resuspension of sediment, the adsorption of eDNA to sediment particles, and the potential accumulation and burial of eDNA in the sediment layer. Higher flows tend to keep sediment particles in suspension, reduce net-deposition, increase scour, and increase suspended sediment concentrations, increasing the opportunity for eDNA particles to become sorbed to sediment. Once sorbed to sediment particles, eDNA may no longer be susceptible to degradation in the same way that it would be if it were in solution. Sorbed eDNA may also have a tendency to settle out of the water column and become buried in the sediment layer. Thus, the sediment layer may become a reservoir for eDNA. The influence of NAVIGATION on SEDIMENT is similar to that of HYDROLOGY. Barge traffic may tend to increase scour, leading to higher concentrations of suspended sediment in the water column. Overall, the net effect of SEDIMENT on CONCENTRATION is ambiguous. It may be that eDNA concentrations are reduced when eDNA settles out of the water column, but may also be that eDNA concentrations are increased during periods of resuspension if eDNA particles in the sediment layer are resuspended into the water column.

SEASON has a potentially large influence over several variables in the conceptual model. The patterns of PRECIPITATION and its influence on HYDROLOGY may vary by SEASON. SEASON may also influence SHEDDING, BIRDS, and WATER QUALITY. For example, Asian carp may shed more eDNA during those seasons when their metabolic rates are highest or during mating season when sperm and eggs are disseminated in the water column. SEASON influences BIRDS because piscivorous birds may be more active during some seasons of the year than others, or may migrate during fall and winter months. WATER QUALITY and HYDROLOGY are influenced by SEASON because inflows from Lake Michigan into the CAWS are less likely to occur during winter months than at other times of the year; therefore, water in the system may be more likely to originate at sewer and wastewater treatment plant outfalls where total dissolved solids concentrations are believed to be higher than in Lake Michigan.

The conceptual model in Figure 1 has been introduced to briefly describe the range and complexity of issues that should be considered in interpreting eDNA monitoring results. Several of the nodes represent active areas of research within ECALS, including SHEDDING, MARKERS, SPECIFICITY & SENSITIVITY, HYDROLOGY, BIRDS, CSO, DECAY, and EDNA ASSAY. Others such as CONVENTIONAL SURVEILLANCE and EDNA MONITORING represent ongoing efforts of multiple federal and state agencies involved in ACRCC efforts. ECALS will draw on these and other available sources in developing and

parameterizing a probabilistic model for inference from eDNA monitoring data. While much progress has been made in understanding the issues described here, insights are continuously emerging to inform development of the probabilistic model. As new insights emerge, the conceptual model will be updated and refined to reflect emerging knowledge.

A Refined Conceptual Model

In this section of the report, a more refined version of the conceptual model is presented. The conceptual model is presented in three parts. The first part focuses on hydrologic influences within the CAWS, which determines eDNA transport. The second part focuses on the occurrence and persistence of eDNA in the CAWS. The third part focuses on the eDNA assay and the detectability of eDNA. While the concepts and ideas described in the more general version of the conceptual model are preserved, each node of the graphs presented here represents a well-defined variable within the system that can be quantified in some way. However, there may be no direct mapping of the variables described here to the nodes described above. Preliminary plans for quantification of the variables are also described. The conceptual model should be considered a "work in progress" that is subject to revision rather than an end product. As noted above, considerable refinements of the conceptual model may be needed before it can be transformed into a probabilistic model for interpretation of eDNA monitoring results.

1. Hydrologic Influences in the CAWS

The backbone of the conceptual model of eDNA occurrence and persistence in the CAWS is a graph that describes hydrologic influences among reaches, or stream segments, that make up the CAWS.[3] Nodes in Figure 2 represent the concentration of eDNA (copies/L) in each reach. The edges between nodes indicate the direction of the dominant hydrologic influence between reaches and the flow of eDNA. Development of this hydrologic map of the CAWS is based primarily on information contained in MWRD (2008) and on a detailed hydrologic grid developed by ECALS for the purpose of hydrodynamic modeling to support interpretation of eDNA monitoring results. A map of the CAWS is provided in Figure 3 to assist in locating the reaches.

In Figure 2, the CAWS main stem is divided into 24 variable length reaches. Each reach of the CAWS main stem between Lake Michigan and Dresden Lock and Dam is represented by a three or four digit code that is defined in Table 1. A three digit code is used for main stem reaches. These are defined in Table 1. A four-digit code is used to reference the 37 boat slips and harbors that form backwaters along the canal. Boat slips and harbors are labeled by appending a single letter to the three- digit code beginning at the top of the reach and proceeding to the bottom of the reach in order of occurrence. Tributaries and backwaters to the left of the main stem in Figure 2 are on the north or west bank of the CAWS, those on the right are on the south or east bank.

[3] Hydrologic influences illustrated in Figure 2 represent ECALS current understanding of the system. ECALS is currently modeling hydrodynamics in the CAWS to validate this understanding.

Hydrologic influence in Figure 2 flows from upstream to downstream in direction of the edges, from Lake Michigan (LMI) towards Dresden Lock and Dam, at the base of CR8. There are four major inflows to the CAWS. These include Lake Michigan (LMI), which contributes flow at three locations in the CAWS, the North Branch of the Chicago River (NBC), the Grand Calumet River (GCR) and the South Branch of the Little Calumet River (LCR). Flows from Lake Michigan enter the system at the Wilmette pump station, the Chicago Lock and Dam, and at the head of the Calumet River. Other sources of inflow include Bubbly Creek (BCR) and Lake Calumet (LKC), but these contribute flows to the system only after rainfall events. Water reclamation plants also contribute flow to the system. Edges between main stem nodes and backwaters are typically in the direction of main stem to the backwater. This reflects our current hypothesis that net DNA transport is from the main stem into stagnant boat slips and harbors. However, boat slips and harbors may also concentrate surface runoff and sewer discharges from the Chicago Area to the CAWS following rain events that periodically reverse the direction of DNA transport toward the main stem.

The load of eDNA at each node in the system originates either from an upstream node or from primary and secondary sources within the reach. Primary sources, secondary sources other than upstream loads, and other factors influencing the occurrence and persistence of eDNA in the waterway are not represented in this part of the conceptual model, but are discussed in the following section.

SYMBOL	DESCRIPTION
LMI	Lake Michigan (LMI).
NSC	North Shore Channel (NSC) from the Wilmette Pump Station to the North Branch of the Chicago River (NBC).
NBC	North Branch of the Chicago River (NBC) upstream of its confluence with NSC .
CR1	North Branch of the Chicago River (CR1) below the confluence of NBC and NSC to the South Branch of the Chicago River (CR2).
CRM	Chicago Sanitary and Ship Canal (CSSC) from LMI at the Chicago River Controlling Works (CRCW) to its confluence with CR2.
CR2	CSSC from the confluence of the North Branch of the Chicago River (CR1) and CRM to the upstream boundary of CR3.
BCR	Bubbly Creek (BCR), a canal extending south from the main stem of the CSSC at MXZ to its terminus, 1.3 miles upstream.
MXZ	A mixing zone at the base of Bubbly Creek that separates BCR, CR2, and CR3.
CR3	CSSC from MXZ to a point just upstream of Stickney Water Reclamation Plant (WRP).
CR4	CSSC from Stickney WRP to its confluence with the Cal-Sag Channel (CRE).
CR5	CSSC from the confluence of CR4 and CRE to the upstream boundary of the electric fish barrier.
FBA	CSSC between the upstream and downstream boundaries of the electric fish barrier (FBA).
CR6	CSSC from the downstream boundary of FBA to Lockport Lock and Dam.
CR7	Illinois River from Lockport Lock and Dam to Brandon Road Lock and Dam.
CR8	Illinois River from Brandon Road Lock and Dam to Dresden Lock and Dam.
CRA	Calumet River from Lake Michigan to the canal linking the Calumet River to Lake Calumet.
CLK	The canal linking the Calumet River to Lake Calumet.
LKC	Lake Calumet.
CRB	A mixing zone at the confluence of CRA and CLK.
CRC	Little Calumet River from the base of its confluence with CLK and CRA to its confluence with the Grand Calumet River (GCR).
CRD	Little Calumet River from its confluence with GCR to the South Branch of the Little Calumet River (LCR).
CRE	Cal-Sag Canal from the South Branch of the Little Calumet River to CR5
GCR	Grand Calumet River (GCR).
LCR	South Branch of the Little Calumet River (LCR).

Table 1: Nodes representing the main stem of the CAWS

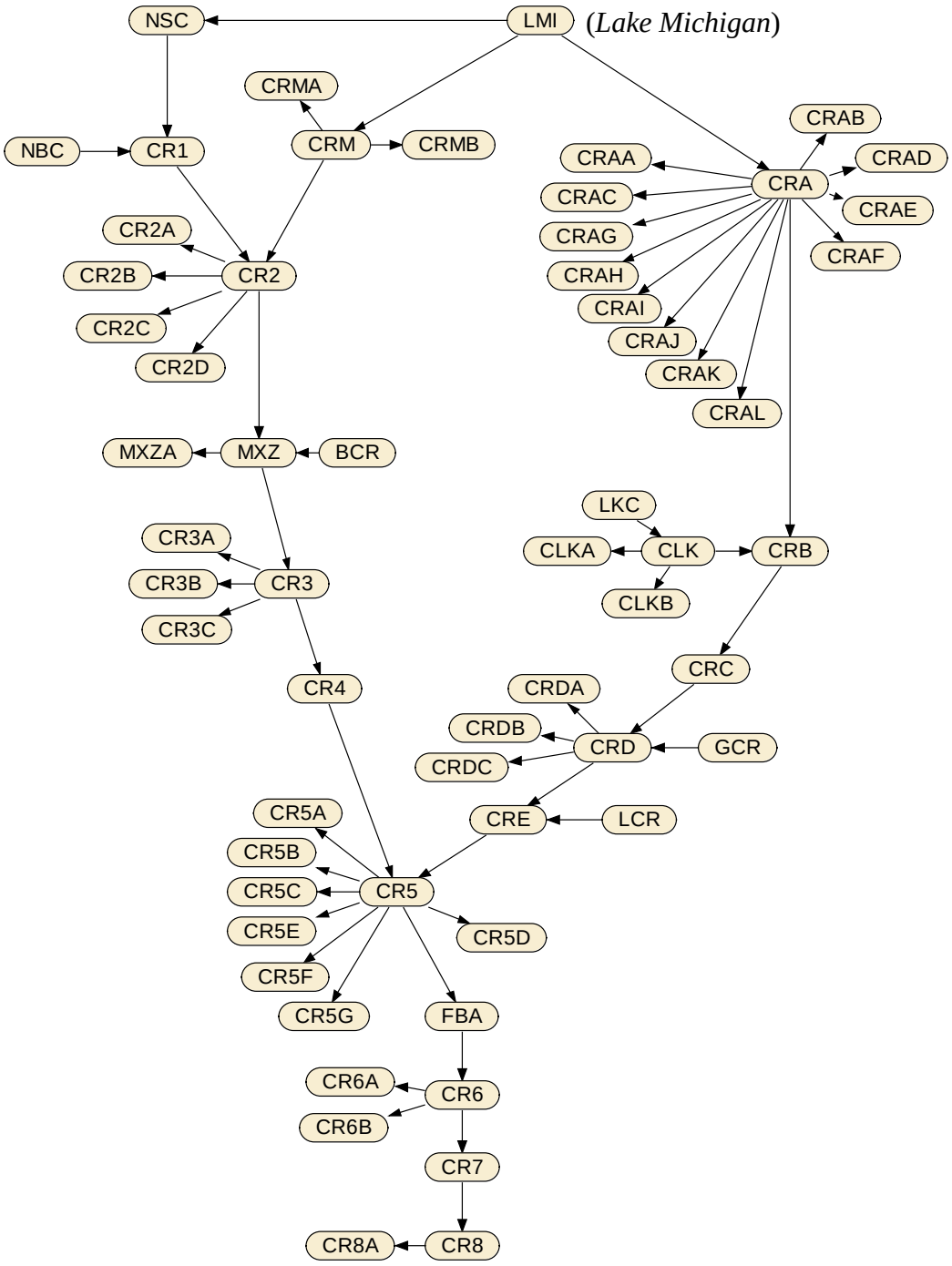

Figure 2: Representation of the main stem of the CAWS.

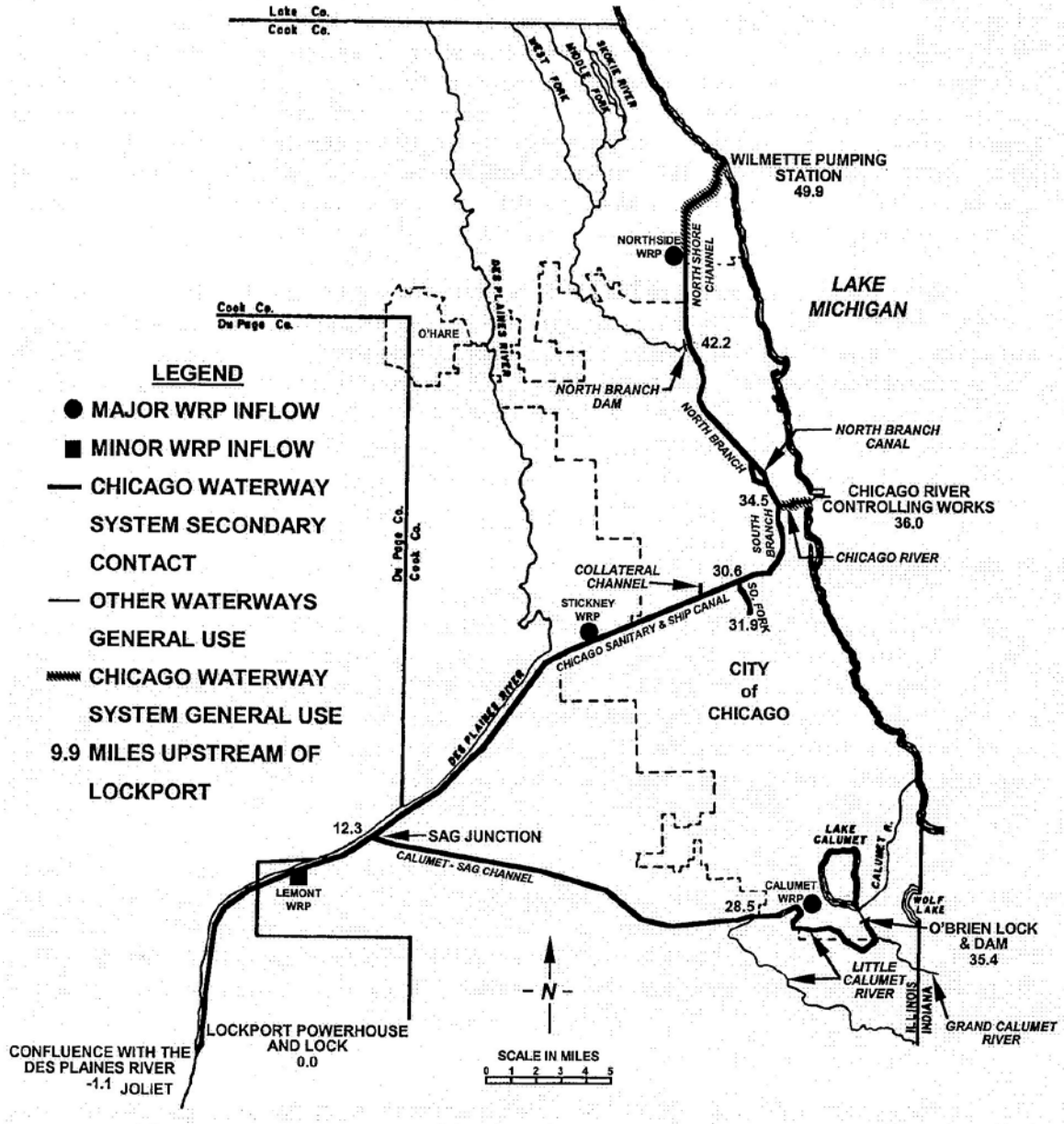

Figure 3: Map of the Chicago Area Waterway System illustrating major features of the CAWS between Lake Michigan and Lockport Lock and Dam (MRWD 2008). Source: Metropolitan Water Reclamation District.

2. Occurrence and Persistence of eDNA in the CAWS

The second part of the conceptual model describes the occurrence and persistence of eDNA in the CAWS, which is some function of how much eDNA is released into the system, when and where it is released, how it is transported, and how quickly it degrades. The conceptual model is presented in Figure 4 for a single reach. Each node represents a random variable that is a source of uncertainty in estimating either the concentration of eDNA in that reach or the outcomes of conventional surveillance (capture or sighting of an Asian carp). Each node in the conceptual model is labeled with a brief title. Random variables are described in the text and defined in Appendix A.

The conceptual model is presented in Figure 4. There are three terminal nodes in the network: VIS, CATCH, and CSEG. VIS describes the visual detection of a fish. CATCH describes the capture of an Asian carp in the course of conventional surveillance. Either one of these events could resolve the question about whether or not bighead or silver carp are present in the waterway; although there might still be uncertainty about the source of eDNA detected in monitoring samples. CSEG is the average daily concentration of eDNA in the reach (copies/L). The CSEG node provides a conceptual link between the graph of eDNA occurrence and persistence and the graph representing hydrologic influences in the CAWS (Figure 2) because the random variables are identically defined in both graphs. The graphs have been developed this way to facilitate inference in each reach.

CSEG is a function of the degradation rate (K, day^{-1}), residence time of water within the reach (RES, days), flow (QSEG, m^3/day), primary eDNA load (LOAD1, copies/day) and secondary eDNA load (LOAD2, copies/day). The primary load is generated by live fish in that reach; therefore, LOAD1 depends upon the biomass of the target species (BIOM, kg) and the unit shedding rate (SHED, copies/kg/day). The unit shedding rate depends upon the species and size distribution of the population. Therefore, SHED is a function of SPECIES and fish length (FLEN, mm), which is a proxy for the age or size structure of the population within the reach. FLEN is related to biomass by a length-weight function for the target species. As described for the general conceptual model (Figure 1), LOAD2 is the sum of all eDNA loads to the reach other than those from live Asian carp. Secondary sources of eDNA load may include upstream load (UPL, copies/day), bird load (BIL, copies/day), combined sewer loads (CSOL, copies/day), recreational boat load (RBL, copies/day), commercial boat load (CBL, copies/day), and commercial net load (CNL, copies/day).

Sources of secondary load (LOAD2) include UPL, BIL, CSOL, RBL, CBL, and CNL. UPL is the combined load from all upstream reaches of the waterway that may contribute eDNA to the reach of interest. Potential sources of BIL include eDNA in excrement from birds that may be flying over or otherwise in contact with the waterway (DROP, copies/day) and eDNA in excrement from birds that may be carried in surface runoff from rookeries or high density nesting sites near the waterway (ROOK, copies/day). We distinguish these two sources because DROP is a distributed source and ROOK is a point source. DROP is a function of bird density in the reach (BDTY, birds/day), the amount of target species ingested (INGEST kg/day), the rate of assimilation (ASSIM, day^{-1}) and respiration (RESP, day^{-1}), all of which vary with bird species

(BIRDS). INGEST depends on BIRDS (bird species) and SPECIES (fish species) because bighead and silver carp may constitute a lesser or greater fraction of the diet of some birds species than others, either because of differences in the availability of bighead or silver carp for consumption or difference in the preference of different birds species for bighead and silver carp. BDTY is likely to vary by SEASON because birds are more likely to be less active during winter months. ROOK is a function of the number of birds nesting at the rookery (RSIZE, birds/day), SEASON, and precipitation (PRECIP, mm/day). ROOK is influenced by DROP because similar biological processes control the rate of eDNA deposition at rookeries as elsewhere.

CSOL represents the eDNA load from combined sewers that discharge to the reach. These discharges may contain eDNA generated by households or restaurants where Asian carp are consumed or fish markets were Asian carp are processed and sold. The volume of discharges from combined sewers tends to increase with the amount of precipitation (PRECIP), the amount of Asian carp sold (SALES, kg/day) and consumed and fraction of fish that are discarded as waste (WMKT, kg/day). Waste may be disposed of either through the wastewater system or directly to storm sewers. For example, ice contaminated with fish slime and scales may be dumped into storm sewers directly (ECALS 2013). Commercial fishing nets used to help control Asian carp populations below the electric fish barrier are another potential secondary source of eDNA in the CAWS. Fishing gear can become a vector of eDNA if it becomes contaminated with eDNA and is later used to carry out planned intensive surveillance, fixed and random site monitoring, or rapid response actions above the electric fish barrier. CNL (copies/day) is the eDNA load from commercial fishing gear (especially fishing nets). The CNL will vary with gear type (GTYPE), level of fishing effort (EFFORT, units), and the unit load of fishing gear (UNL, copies/unit/day). UNL depends on GTYPE and PUSE, which is a variable describing the extent to which fishing gear has been previously used in waters known to be infested with bighead and silver carp.

Recreational and commercial boaters may unintentionally distribute eDNA from carp infested waters to waters that have not yet been infested. BTRAF (boats/day) is the amount of commercial boat and barge traffic in a reach. Only the fraction of commercial boat and barge traffic (UPFRACT) travelling from below the electric fish barrier to locations above the barrier is believed to carry eDNA on hulls (BHULLS, copies/m^2), in bilge water ((BBILGE, copies/L), or in fish carcasses that can sometimes be transported on the barge deck (CARC copies/kg). The contribution from barges is dependent on the concentration of eDNA in waters where the barge became contaminated with eDNA of the target species, the surface area of barge hulls (BSAREA, m^2), the volume of bilge water taken on in contaminated waters, and the frequency with which boats and barges inadvertently transport target species carcasses across the barrier. Similar processes are involved when recreational boats transport eDNA, but it seems much less likely that recreational boats might carry target species carcasses on deck. The distribution of recreational boats in the CAWS (RTRAF boats/day) may differ substantially from the distribution of commercial boat and barge traffic (BTRAF) with recreational boat activity more likely to occur closer to Lake Calumet and the entrances to Lake Michigan.

Once released into the water column, eDNA will be distributed within the waterway by hydrologic forces that are a function of stream geometry (depth (DSEG, m), width (WSEG, m), length (LSEG, m)), and flow (QSEG, m^3/day). Inflows to CAWS reaches come either from

upstream reaches (QUPSEG, m^3/day), wastewater reclamation plants (QWRP m^3/day), CSOQ discharges (CSOQ m^3/day), or runoff (QROF m^3/day). Surface runoff is a function of drainage area (DAREA m^2) and precipitation. The occurrence of eDNA in the reach also depends upon the degradation rate and residence time of water. Degradation may be influenced by the length of the marker (BP, base pairs) and environmental conditions such as water temperature (WTEMP, deg C) and ultra-violet light (LIGHT). Other factors such as pH and microbial activity may also be important, but are not represented in this graph.

The eDNA in the water column (CSEG) may either be free in solution or adsorbed to suspended sediment particles (SUSP, copies/mg TSS). The fraction adsorbed to sediment depends upon the concentration of suspended sediment (TSS, mg/L), and the adsorption rate (ADS, day^{-1}). SUSP is also influenced by QSEG because flows will affect both the concentration of eDNA and TSS in the water column. If eDNA is adsorbed to suspended sediment, it may tend to settle out of the water column and become stored in sediment where it is unavailable for capture and detection by an eDNA monitoring program. The load of eDNA stored in sediment layer (SED) depends upon the net sediment deposition rate (NETDEP, day^{-1}), which is the difference between the deposition rate (DEP, day^{-1}) and the resuspension rate (RESUSP, day^{-1}). Resuspension may occur as a result of high water velocities (USEG, m/day) or barge traffic (BTRAF, barges/day) and the amount resuspension that occurs may depend on the sediment grain size distribution (GSIZE, μm) and other sediment characteristics.

Asian carp might also be detected in the CAWS by casual observation (VIS) or conventional surveillance (CATCH). VIS is a binary node that takes the state *True* if a bighead or silver carp has been sighted or *False* if no bighead or silver carp has been sighted in the reach. VIS depends on whether an individual sighting a bighead or silver carp reports the sighting (VISRPT) and the degree of credibility of the report (VISCRED). The probability of visual detection increases with sighting opportunity (SOPP) and the size of the fish population (POP). Opportunities for casual observation of bighead or silver carp are a function of the level of commercial and recreational activity and conventional surveillance in the reach and fish behavior, which will vary by species. CATCH is also a binary node that takes the value *True* if an Asian carp has been captured and *False* if no Asian carp has been captured in the course of conventional monitoring efforts in the reach. This is a function of the catchability of the species given the population number (POP), the population size distribution (FLEN), and the unit effort expended on conventional monitoring (UEFFORT). UEFFORT is a function of gear type employed in monitoring and the level of fishing effort (EFFORT).

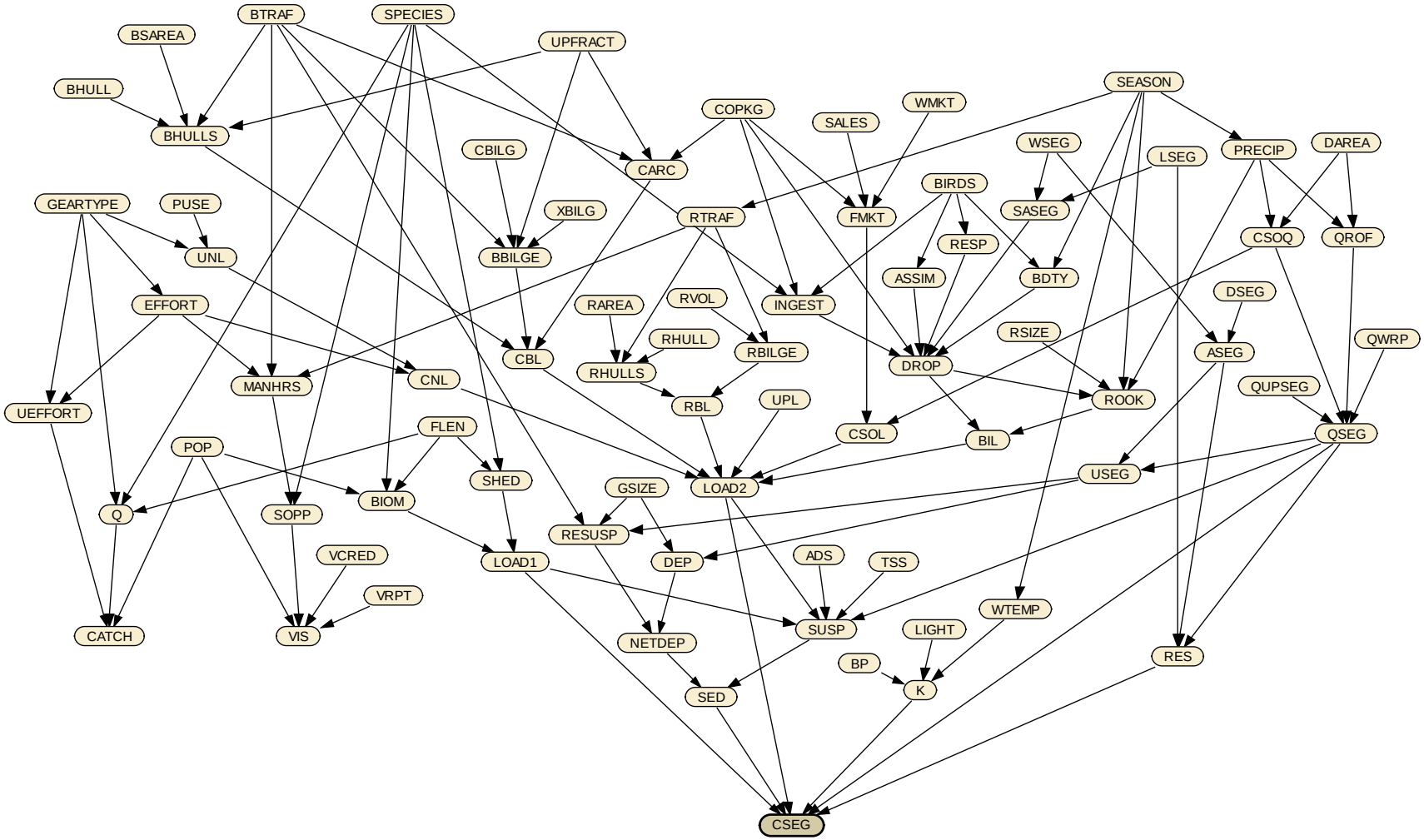

Figure 4: Conceptual model of eDNA occurrence and persistence in a single reach of the CAWS.

3. Detectability of eDNA in the CAWS

Asian carp eDNA is detected in monitoring samples from the CAWS using PCR, which is capable of detecting very small quantities of a genetic marker in an environmental sample. Sample collection and analysis procedures were originally developed at the University of Notre Dame with funding from USACE (USACE 2012). An independent peer review of the eDNA methodology by Environmental Protection Agency's (EPA's) Great Lakes National Program Office in 2009 assessed the reliability of analytical procedures at the University of Notre Dame. The review expressed confidence in the methodology and procedures. The EPA review did not address interpretation of eDNA monitoring results in regards to the presence or absence, proximity, or abundance of carp in the study area (Blume *et al.* 2010). USACE subsequently contracted with Battelle Memorial Institute for a second independent peer review of the eDNA methodology. The review found that the eDNA methodology was sound in principle and presented several advantages over conventional surveillance methods. However, it also identified some key limitations of the approach. In particular, the review concluded that detection of eDNA does not provide conclusive proof of species presence and does not provide information on the size or age of individuals or the size of a population, if present (BMI 2012).

Sample collection and analysis procedures are described in the Quality Assurance Product Plan (QAPP) and, with a few exceptions, follow those developed by the University of Notre Dame (USACE 2012). Samples of water (usually two liters) are collected from the CAWS and filtered through one or more 1.5 micron glass fiber filters. Filters are then shipped on ice to a laboratory where the eDNA is extracted from the filter paper using a MoBio Power Water DNA Isolation Kit ® and separated from non-DNA extracts by centrifugation. A 100 µl elution containing the sample is then stored at -20 deg. C for PCR. PCR is an iterative process of heating and cooling the sample to denature the eDNA and amplify a genetic marker that is specific to the target species. Theoretically, the concentration of a target species marker will double each time the sample is heated and cooled. Samples can be analyzed using two types of PCR: cPCR and qPCR. The cPCR assay is strictly a test for the presence or absence of the marker. Samples testing positive for the genetic marker using the cPCR assay are sequenced to confirm that they come from the target species. The qPCR assay (referring, again, to TaqMan-style qPCR) detects the presence of the marker and also provides an estimate of the concentration; however, samples testing positive using qPCR are assumed to be specific to the target species and are typically not sequence confirmed. This assumption, however, will require rigorous testing and occasional verification. Currently, only the cPCR assay is included in the QAPP as an approved technique for Asian carp eDNA monitoring studies in the CAWS. The graph for detectability of eDNA (Figure 5) terminates in two nodes (cPCR and qPCR) that describe the outcomes of the PCR assays. The cPCR and qPCR assays are performed on separate 1 µl aliquots drawn from a 100 µl elution that is produced using the extraction procedure.

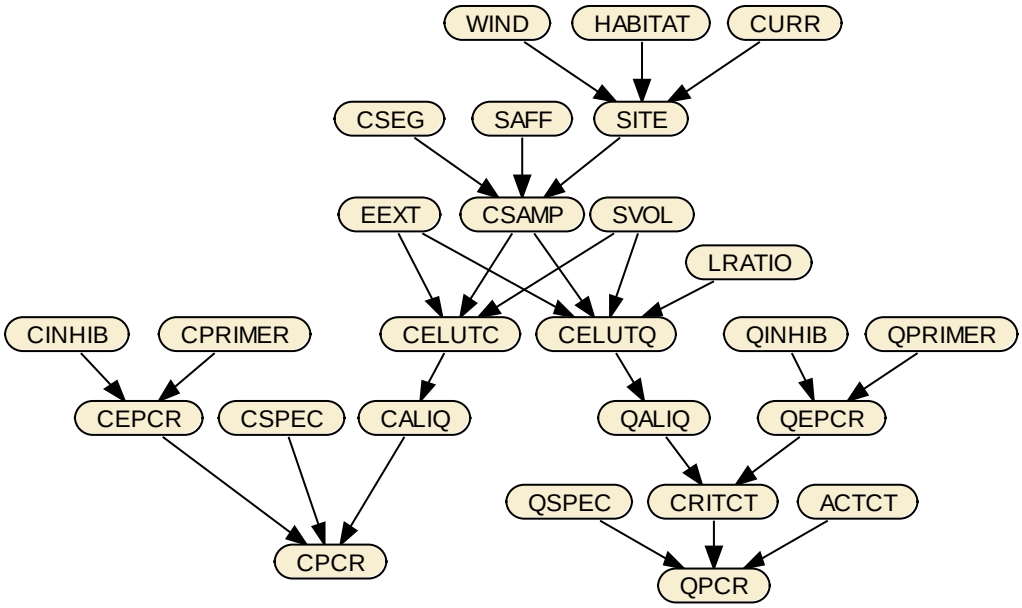

Figure 5: Conceptual model of eDNA detectability using PCR.

The CPCR node can take one of two possible states, *Negative* if eDNA is not detected or *Positive* if eDNA is detected. In cPCR, DNA fragments other than the target marker can sometimes produce fluorescent bands that may indicate a positive test result. Therefore, the DNA from all positive cPCR assays must be sequenced and sequences are evaluated using BLAST searches in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Benson *et al*. 2011) to assess how closely the DNA fragment matches the target species. The outcome of a confirmed positive cPCR test result may remain uncertain if there are questions about target marker specificity. This uncertainty is represented by the variable CSPEC, which is the degree of belief in the specificity of the target marker. The outcome of the cPCR assay depends on the concentration in the aliquot drawn for the assay (CALIQ, copies/µl), cPCR marker sensitivity, and PCR efficiency (EPCR). The cPCR marker sensitivity is the probability of detecting the marker given its concentration. PCR efficiency (EPCR) is difficult to measure in cPCR, but is the ratio of the amount of DNA that is detected and the amount of DNA that should be detected. This is primarily a function of the level of inhibition (CINHIB) and primer quality (CPRIMER). CINHIB is the level of PCR inhibition present in the aliquot. Algae, bile salts and humic acid are just a few of the known substances that, if present, can inhibit PCR reactions (Alaeddini 2012). CPRIMER is the ability of the cPCR primer to locate and bind to the genetic marker. This is important because different primers exhibit differences in their ability to bind to a target.

The QPCR node describes the outcome of the qPCR assay and is the concentration of the target marker in an aliquot withdrawn from the elution. Because qPCR and cPCR utilize different markers, and qPCR markers are generally shorter than cPCR markers, cPCR and qPCR markers may tend to be present in different concentrations that reflect differences in degradation rates that depend on marker length. If so, the ratio of the two marker concentrations may vary with the age

of the DNA and the ratio of the lengths of the markers (LRATIO). The concentration of a qPCR marker is estimated using TaqMan PCR (Holland *et al.* 1991), which is a qPCR method designed to increase specificity of the PCR reaction. A fluorescently labeled probe designed to bind to the target marker is added to the aliquot. As the DNA fragment is copied by enzymes, the probe is cleaved from the template and a fluorescent signal is emitted. The number of copies of the eDNA fragment doubles with every PCR reaction cycle, producing a logarithmic increase in the intensity of the fluorescent signal over time. If no signal is reached after 40 qPCR cycles, the sample is presumed negative. An estimate of the number of eDNA copies in an aliquot is obtained by measuring the amount of fluorescence produced following each PCR cycle and comparing this to the level of fluorescence produced at each cycle by a dilution series of known DNA marker concentrations. The CT value, the number of qPCR cycles required to achieve a critical level of fluorescence, increases as the concentration of eDNA in the aliquot decreases. At very low concentrations of eDNA, qPCR may produce false negatives (failing to detect target species eDNA when it is actually present) because the concentration of DNA in the sample is below the detection limit for the qPCR marker system.

In Figure 5, the qPCR node depends on the critical CT value (CRITCT, cycles), the actual CT value observed during the assay (ACTCT, cycles), and the specificity of the qPCR marker (QSPEC). The CRITCT node is parameterized by running a set of standards containing known concentrations of the marker. CRITCT is uncertain because, when creating a set of standard fluorescent curves, the number of cycles needed to exceed critical fluorescence at a given concentration will vary. This depends, in part, on the efficiency of the qPCR reaction (EPCR), which is a function of the level of inhibition (QINHIB) and primer quality (QPRIMER). ACTCT is uncertain because at least three aliquots from the elution with unknown copy number are used in each qPCR assay and these may produce fluorescence at different CT values, either because of the random effects associated with the process that produces fluorescence or because of differences in the concentration of a marker among the aliquots. QSPEC is similar to the node CSPEC and represents the degree of belief in the specificity of the qPCR marker.

Each monitoring sample from the CAWS is processed and reduced to a single 100 µl elution of unknown concentration. One possibility would be to assume that the elution is an homogenous solution. However, eDNA markers are discrete particles and aliquots extracted from an elution may contain a variable copy number. For example, at concentrations less than 100 copies/µl, the number of aliquots potentially drawn from an elution exceed the copy number and some aliquots will contain no copies of the marker. The CALIQ and QALIC nodes describe the uncertain concentration (copies / µl) of cPCR and qPCR markers in an aliquot, respectively. Aliquot concentrations depend on the elution concentration and are influenced by the processes involved with extracting aliquots from the elution.

Marker concentrations in an elution depend on sample volume (SVOL, liters), the concentration of the marker in the water sample drawn from the CAWS (CSAMP, copies/L), and the extraction efficiency (EEXT). Water samples are typically two liters. All else equal, larger water samples should contain a larger number of markers than smaller water samples. The node CSAMP is the unknown concentration of a marker in the monitoring sample. This depends on concentration of the marker in the reach (CSEG, copies/L) and the distribution of eDNA in the water column. After collection, the content of water samples are collected on glass fiber filters

and the eDNA is then extracted from the filter. Higher extraction efficiencies will result in higher elution concentrations.

There are significant questions about how eDNA is distributed in the water column, and this may influence the concentration of the sample. For example, one hypothesis is that eDNA has a strong surface affinity (SAFF) and this has been the rationale for collecting water samples from the surface of the water column. There are arguments both for and against this hypothesis. The primary argument for this hypothesis has been that eDNA is associated with fish feces that have a tendency to float and may therefore be associated with scum that accumulates on the water surface. The primary argument against this hypothesis is that a large fraction of fish feces do not float and fecal matter may disintegrate rapidly, leaving small particles of free DNA in solution to become mixed in the water column. SAFF is a partition coefficient describing the propensity of eDNA to be in the surface layer of the water column. High values of SAFF indicate that eDNA has a strong propensity to be in the surface layer, values of SAFF close to one suggest that eDNA is equally distributed in all layers of the water column, and values of SAFF less than one suggest eDNA is more likely not to be in the surface layer.

The node labeled SITE is another partition coefficient. If eDNA is associated with scum on the water surface, then its distribution on the water surface may be influenced by wind and currents. For example, during windy days, there may be a tendency for surface scum to accumulate along the banks or in backwaters. Similarly, currents may tend to be stronger in the center of the canal than along the edges, making it more likely that surface scum, and possibly eDNA, will be found along the banks rather than toward the center of the canal. The SITE variable is a function of wind speed at the time of sampling (WIND, kmh), currents at the location the sample is taken (CURR, m/s), and a qualitative variable describing habitat characteristics (HABITAT), such as along the bank, in a backwater, or mid-stream.

Preliminary Plans for Parameterization of Network Nodes

The conceptual models presented above represent interdependencies among random variables that influence the outcome of eDNA assays. These graphs can be converted to probabilistic models by parameterizing network nodes. Parameterization proceeds by defining a discrete domain for each random variable that is a mutually exclusive and collectively exhaustive set of potential random variable states (variables that are naturally continuous must be discretized). A random variable whose state does not depend directly upon the state of another random variable (e.g., has no edges directed toward it from another node) is defined by a probability table that gives, for every potential state, the probability of that state. All other variables are defined by conditional probability tables (CPTs) that define the probability of the random variable state for every possible combination of parent node states (Koller and Friedman 2009). Prior uncertainty in the value of each variable may be based on existing data, external model outputs, functions of parent node variables, or engineering judgment. In this study, a strong preference will be given to using data, external models, and functions. Engineering judgment will be used only as a last resort. In general, preference will be given to databases developed during the course of ECALS or through other ACRCC efforts, or through published studies. Additional laboratory or field experiments may be needed to construct the CPTs.

In this section of the report, we provide a more detailed definition for each node and, where information is presently available, summarize the plans for parameterization of each node. Each node of the conceptual model of eDNA occurrence and persistence in the CAWS (Figure 4) is defined in Appendix A, Table A.1. Each node of the conceptual model of eDNA detectability (Figure 5) is defined in Appendix A, Table A.2. Preliminary plans for parameterizing each node are described following the definition. Where CPTs are derived from existing models, ECALS will use the outputs of one of two models. A Curvilinear-grid Hydrodynamics 3D (CH3D) model is being developed to simulate hydrodynamics in the CAWS for the period 2009-2012. Twenty main stem reaches between Lake Michigan and Dresden Lock and Dam, as described in Table 1, are represented in the model. The CH3D model includes the four tributary reaches (LMI, NBC, GCR, LCR) as open-flow boundaries. Outputs of CH3D will support implementation of ECALS' CE-QUAL-ICM model, which will be used to simulate the fate and transport of eDNA markers and coincident water quality constituents. Details regarding calculations or model runs are not provided in this summary.

Conclusion

This interim milestone report has described a conceptual model that will serve as a point of departure for developing the probabilistic model. ECALS vision is to develop a probabilistic model that will enable fisheries managers to interpret eDNA monitoring data more effectively so that appropriate management actions can be taken in response to eDNA monitoring results. The probabilistic model is designed to estimate: 1) the probability that each of the potential sources and vectors of eDNA in the CAWS is, in fact, the actual source of eDNA detected in monitoring samples; and 2) the probability that bighead or silver carp are present in the CAWS given the evidence from eDNA monitoring results and other lines of evidence.

ECALS has identified several design principles to help guide development of the probabilistic model: 1) the model should be available for real-time implementation so that results can be obtained as soon as possible after eDNA monitoring results become available; 2) the cost associated with implementing the model should be minimal; 3) the analytical procedure should be accessible to a trained technician at the Master's level; 4) the analysis should be transparent and credible to support decision making; 5) the model should require little or no updating in the near term; and 6) the model should be transferable to other locations on the Asian carp invasion front with a minimum level of effort. The extent to which each of these goals can be successfully met is unclear because the model is still under development. As ECALS finalizes development plans, these principles will be considered.

APPENDIX A: Definitions of Random Variable Nodes

Variable	Description
ADS	Adsorption rate of eDNA marker to suspended sediment. This variable is defined as a discretized continuous random variable between 0 and 1. The information is being developed through ECALS studies on how eDNA interacts with sediment.
ASEG	Cross-sectional area of the reach (m^2). This is a discretized random variable greater than or equal to 0. This information will be developed from information about stream geometry and hydrology as represented in ECALS CH3D model of the CAWS.
ASSIM	Assimilation rate of birds (kcal/day). This variable is defined as a discretized continuous random variable. This information will be developed by consulting peer-reviewed literature sources.
BBILGE	The number of eDNA markers imported in commercial boat and barge bilge water (copies/day). This variable is defined as a discretized continuous random variable greater than or equal to 0. This variable is calculated from BSAREA, BTRAF, UPFRACT and CBILGE.
BDTY	Bird density in the reach (m^{-2}). This variable is defined as a discretized continuous random variable greater than or equal to 0. The Cornell Lab of Ornithology's eBIRD Database (http://ebird.org/content/ebird/) contains voluntary reports of bird sightings and provides one potential source of information about the relative densities of birds along the CAWS.
BHULL	The number of eDNA markers attached per unit area of commercial boat and barge hulls (copies/m^2). This variable is defined as a discretized random variable greater than or equal to 0. This node is parameterized using results of ECALS studies of eDNA on commercial boat and barge hulls.
BHULLS	The number of eDNA markers imported on commercial boat and barge hulls (copies/day). This variable is defined as a discretized random variable greater than or equal to 0. The variable is calculated from BSAREA, BHULL, and BTRAF using information about the rate at which eDNA markers may be washed off hulls in transit. ECALS studies suggest that eDNA adhering to commercial boat and barge hulls is not easily washed off during transit.
BIL	Load of eDNA markers contributed to the reach by birds (copies/day). This variable is a discretized continuous random variable greater than or equal to one. This variable is calculated from DROP and ROOK.
BIOM	Biomass of the supposed population of Asian carp in the reach (kg). This variable is a discretized continuous random variable greater than 0. It is calculated from POP, FLEN and a species-specific length-weight relationship.
BIRDS	Piscivorous bird species composition in the CAWS. This is a discrete random variable with at least four possible states (*Cormorants*, *Bald eagles*, *Pelicans*, *Other*). This information is based on reported bird sightings in the CAWS.
BP	Length of the genetic marker in nucleotide base pairs (bp). This variable is a constant given knowledge of which genetic marker for Asian carp is being tracked in the CAWS.
BSAREA	Surface area of commercial barges and boats (m^2/day). This variable is a discretized continuous random variable. The information is based on knowledge of commercial boat and barge characteristics in the waterway.
BTRAF	The number of commercial boats and barges transiting through a segment of the CAWS on any given day (boats and barges/day). This is a discretized continuous random variable. The information is based on records of commercial boat and barge movements.
CARC	Load of eDNA marker contributed from carcasses on barge decks (copies/day). This is a discretized continuous random variable greater than or equal to 0. The information is based on reports of carcasses on barge decks at USACE operated locks and dams on the waterway.
CATCH	The probability that a fish from the supposed population within the reach is caught. This is a discretized continuous random variable greater than or equal to 0. The variable is calculated form UEFFORT, POP, and Q.
CBILGE	Concentration of eDNA in bilge water of commercial boats and barges travelling upstream in the CAWS (copies/L). This variable is defined as a discretized continuous random variable greater than or equal to 0. ECALS presently has limited information on this variable.

Variable	Description
CBL	Load of eDNA marker contributed from commercial boat hulls (copies/day). This variable is defined as a discretized continuous random variable. The variable is calculated from BHULLS, BBILGE, and CARC.
CHULL	Number of eDNA markers per unit area of commercial boat and barge hull surface area (copies/m^2). This variable is defined as a discretized continuous random variable. The information is obtained from ECALS studies on eDNA attached to commercial boat and barge hulls.
CNL	Load of eDNA originating from commercial fishing nets (copies/day). This is a discretized continuous random variable. The variable is calculated from EFFORT and UNL.
COPKG	Copies of eDNA marker per kilogram of fish tissue (copies/kg). This variable is a discretized continuous random variable with lower and upper bounds to be determined. The value is calculated based on information about the eDNA content of fish tissue.
CSEG	Concentration of an eDNA marker (copies/L) in a reach. CPTs can be calculated or derived from the outputs of ECALS eDNA fate and transport model in the CAWS.
CSOL	Load of eDNA markers contributed to the reach from combined sewer outfalls (copies/day). This variable is a discretized continuous random variable greater than or equal to 0. The variable is calculated from CSOQ and FMKT.
CSOQ	Reach inflows from CSO discharges (m^3/day). This variable is a discretized continuous random variable greater than or equal to 0. The variable is estimated from PRECIP and DAREA using relationships developed by MWRD and incorporated into ECALS CH3D model of the CAWS.
DAREA	Drainage area to the reach (m^2). This is a discretized continuous random variable greater than or equal to 0. The information is based on data provided by MWRD and used in developing ECALS CH3D model of the CAWS.
DEP	Deposition rate of sediment from the water column to the sediment layer (day^{-1}). This is a discretized continuous random variable greater than or equal to 0. The value is calculated from GSIZE.
DROP	Quantity of eDNA marker excreted per day (copies/day). This variable is a discretized continuous random variable greater than or equal to 0. The value is calculated from INGEST, ASSIM, RESP, SASEG, and BDTY.
DSEG	Depth of the reach (m). This is a discretized random variable greater than or equal to 0. This information will be developed from information about stream geometry and hydrology as represented in ECALS CH3D model of the CAWS.
EFFORT	Level of fishing effort by gear type. This variable is a discretized continuous ransom variable greater than or equal to 0. The information is based on records of fishing effort for conventional surveillance in the CAWS.
FLEN	Size distribution (mm) of the supposed population in the reach. This variable is a discretized continuous random variable greater than or equal to 0.
FMKT	Load of eDNA markers contributed to CSOs by fish markets (copies/day). This variable is a discretized continuous random variable greater than or equal to one. The value is calculated from COPKG, SALES and WMKT.
GEARTYPE	Gear type used during conventional surveillance (e.g., Fixed and random site monitoring, Planned intensive surveillance, and Rapid response actions). A discrete random variable representing types of fishing gear used in the course of conventional surveillance (*Electrofishing*, *Gill nets*, *Fyke nets*, *Trawl nets*, etc.).
GSIZE	Sediment grain size distribution (μm). This variable is a discretized continuous random variable representing sediment grain sizes in the CAWS. This probability table is developed from available data on sediment size in the CAWS.
INGEST	Quantity of food ingested by birds per day (kcal/day). This is a discretized continuous random variable greater than or equal to 0. This information will be developed by consulting peer-reviewed literature sources.
K	Degradation rate of eDNA marker (day^{-1}). This is a discretized continuous random variable between 0 and 1. The CPT for this variable is developed based on ECALS degradation studies.

Variable	Description
LIGHT	The amount of light, UV radiation, microbial activity, or other factors influencing degradation rates in the CAWS. Estimated from external data sources. This is a discretized continuous random variable greater than or equal to 0. The probability table for this variable is developed based on results of ECALS degradation studies.
LOAD1	Load of an eDNA marker originating from a live fish within the reach (copies/day). This variable is a discretized continuous random variable greater than or equal to 0. This value is calculated from SHED and BIOM.
LOAD2	Load of an eDNA marker originating from secondary sources within the reach (copies/day). This variable is a discretized continuous random variable greater than or equal to 0. This value is calculated from CNL, CBL, RBL, UPL, CSOL, and BIL.
LSEG	Length of the reach (m). The information is based on data provided by MWRD and used in developing ECALS CH3D model of the CAWS.
MANHRS	Man hours of conventional surveillance (electro-fishing and netting) (hours). This variable is a discretized continuous random variable greater than or equal to 0. The information is based on records of fishing effort for conventional surveillance in the CAWS.
MPCAL	eDNA marker per calorie of bird tissue by Asian carp species (copies/kcal). This variable is a discretized continuous random variable greater than or equal to 0. The CPT for this variable is developed from external sources in the peer reviewed literature and CAWS databases.
NETDEP	Net deposition rate of eDNA to the sediment layer (day^{-1}). This variable is a discretized continuous random variable greater than or equal to 0. The value is calculated from DEP and RESUSP.
POP	Number of individuals in the supposed bighead or silver carp population in the reach. This variable is a discretized continuous random variable greater than or equal to 0.
PRECIP	Precipitation (mm/day). This variable is a discretized continuous random variable greater than or equal to 0. The probability table is developed from National Weather Service records from the Chicago area during the period coincident with the hydrologic modeling period.
PUSE	The extent to which fishing gear has been used previously in waters where bighead and silver carp are present.
Q	Probability that a single fish of a given species and length is caught using a specific gear type. This variable is a discretized continuous random variable between 0 and 1. Catchability is a difficult quantity to measure, but can be related to fishing mortality and fishing effort.
QROF	Surface runoff to the reach (m^3/day). This variable is a discretized continuous random variable greater than or equal to 0. The CPT is estimated from CH3D outputs.
QSEG	Flow in the stream segment (m3/day). This variable is a discretized continuous random variable greater than or equal to 0. The CPT is estimated from CH3D outputs.
QUPSEG	Inflows to the reach from all upstream reaches (m^3/day). This variable is a discretized continuous random variable greater than or equal to 0. The value is calculated from the QSEG node for upstream reaches.
QWRP	Inflows to the reach from water reclamation plants (WRP) (m^3/day). This variable is a discretized continuous random variable greater than or equal to 0. The CPT is estimated from CH3D outputs.
RAREA	Recreational boat hull area (m^2). This variable is a discretized continuous random variable greater than or equal to 0. The node is parameterized using external data sources documenting fleet characteristics in the CAWS.
RBILGE	The number of eDNA markers imported in recreational bilge water (copies/day). This variable is a discretized continuous random variable greater than or equal to 0. This value is calculated from RTRAF and RVOL.
RBL	Load of eDNA marker imported by recreational fishing vessels (copies/day). This variable is a discretized continuous random variable greater than or equal to 0. The value is calculated from RHULLS and RBILGE.
RES	Residence time of water in the reach (day). This variable is a discretized continuous random variable greater than or equal to 0. This node is parameterized using the outputs of ECALS CH3D model for the CAWS.
RESP	Respiration rate of birds (kcal/day). This variable is a discretized continuous random variable greater than or equal to 0. This node is parameterized by using external peer-reviewed literature sources.

Variable	Description
RESUSP	Resuspension rate of sediment from the sediment layer to the water column (day^{-1}). This variable is a discretized continuous random variable greater than or equal to 0. This node is calculated from BTRAF, GSIZE, and USEG.
RHULL	The number of eDNA markers attached per unit area of recreational boat hulls (copies/m^2). This variable is a discretized continuous random variable greater than or equal to 0. The value is calculated from RHULL, RTRAF and RAREA.
RHULLS	The number of eDNA markers imported on recreational boat hulls (copies/day). This variable is a discretized continuous random variable greater than or equal to 0. The value is calculated from RHULL, RTRAF and RAREA.
ROOK	The number of eDNA markers contributed by bird feces runoff from known bird rookeries. This variable is a discretized continuous random variable greater than or equal to 0. The value is calculated from DROP and RSIZE.
RSIZE	Population of birds roosting at the rookery. This variable is a discretized continuous random variable greater than or equal to 0. Estimated from external data sources.
RTRAF	Recreational boat traffic (boats/day). This variable is a discretized continuous random variable greater than or equal to 0. Estimated from external data sources.
RVOL	Recreational boat bilge or ballast tank volume (m^3). This is a discretized continuous random variable greater than or equal to 0. The probability table will be estimated from data on recreational boat characteristics.
SALES	Wet weight of silver and bighead carp sold by fish markets (kg). This is a discretized continuous random variable greater than or equal to 0. External data sources.
SASEG	Surface area of the reach (m^2). This is a discretized continuous random variable greater than or equal to 0. This value is calculated from WSEG and LSEG as reported in CH3D stream geometry.
SEASON	Season of the year (*Winter*, *Spring*, *Summer*, *Fall*).
SED	The number of eDNA markers stored in the sediment layer (copies/day). This is a discretized continuous random variable greater than or equal to 0. This value is calculated from NETDEP and SUSP.
SHED	The shedding rate from live fish in the reach (copies/kg/day). This is a discretized continuous random variable greater than or equal to 0. This value is estimated from ECALS studies of eDNA shedding rates.
SOPP	The opportunity available for casual sighting of Asian carp in the reach (man hours/day). This is a discretized continuous random variable greater than or equal to 0. This information is developed based on information about the types and levels of activity in each reach.
SPECIES	Target Asian carp species (*Bighead carp*, *Silver carp*).
SUSP	The number of eDNA markers adsorbed to suspended sediment (copies / mg TSS). This is a discretized continuous random variable greater than or equal to 0. This value is calculated from LOAD1, LOAD2, ADS, TSS, and QSEG.
TSS	Total suspended sediment concentration (mg/L). This is a discretized continuous random variable greater than or equal to 0. This value is estimated from external databases.
UEFFORT	Unit effort calculated for gear type and reported effort. This is a discretized continuous random variable greater than or equal to 0. This value is based on records of conventional surveillance in CAWS reaches.
UNL	The number of eDNA markers per unit length of fishing gear (commercial nets) by gear type (copies/m^2). This information is developed from ECALS studies on the level of gear contamination.
UPFRACT	Fraction of commercial barge and boat traffic headed upstream. External data sources.
UPL	The number of eDNA marker contributed by flow from upstream reaches (copies/day). This is a discretized continuous random variable greater than or equal to 0. This value can be derived from CE-QUAL-ICM outputs or calculated.
USEG	Velocity of water in the reach (m/s). This is a discretized continuous random variable greater than or equal to 0. The value is estimated using CH3D outputs.
VCRED	Credibility given to the reported sighting of an Asian carp. This is a discretized continuous random variable between 0 and 1. This value reflects the degree of belief in the truth of a report.
VIS	A discrete variable indicating that an Asian carp has been sighted in the reach (*True, False*).

Variable	Description
VRPT	A discrete variable indicating that a report stating that an Asian carp has been sighted in the reach has been received (*True*, *False*).
WMKT	Fraction of fish market sales discarded to the sewer system. This is a discretized continuous random variable between 0 and 1. This information is based on external data sources.
WSEG	Width of the reach (m). This is a discretized continuous random variable greater than or equal to 0. Estimated from CH3D outputs.
WTEMP	Water temperature (deg. C) in the reach. This is a discretized continuous random variable greater than or equal to 0. Estimated from external data sources.
XBILG	The exchange of commercial boat and barge bilge water during transit or while tied up at dock (L/barge/day). ECALS presently has little information about where and how commercial boats and barges exchange bilge water. This information will be developed by consulting industry experts.

Table A.1: Description of variables in the conceptual model of eDNA occurrence and persistence. This information is preliminary and subject to change.

Variable	Description
ACTCT	The CT-value at which fluorescence exceeds critical fluorescence during the qPCR assay. This variable is defined as a discretized continuous random variable greater than or equal to 0. The CPT for this variable is based on the results of the cPCR assay with three replicates.
CALIQ	Concentration of the cPCR marker in the aliquot withdrawn for a PCR assay (copies/µl). This variable is defined as a discretized continuous random variable greater than or equal to 0. This variable is calculated as a function of CELUTC.
CELUTC	Concentration of the cPCR marker in the elutriate (copies/µl). This variable is defined as a discretized continuous random variable greater than or equal to 0. This variable is calculated from EEXT, SVOL, and CSAMP.
CELUTQ	Concentration of the qPCR marker in the elutriate (copies/µl). This variable is defined as a discretized continuous random variable greater than or equal to 0. This variable is calculated from EEXT, SVOL, LRATIO, and CSAMP.
CEPCR	Efficiency of the cPCR reaction, which is the ratio of the number of copies detected in an aliquot and the number of copies present in the aliquot. This is a discretized continuous random variable between 0 and 1. The CPT is constructed by running the assay on a bank of standards with known concentrations.
CINHIB	The fraction of cPCR markers that cannot be detected because of the presence of one or more inhibitors. This is a discretized continuous random variable between 0 and 1. The CPT is constructed from standards run to characterize inhibition in water samples taken from the CAWS.
CPCR	A binary variable describing the outcome of the cPCR assay (*True*, *False*). This variable is the detection sensitivity (probability of detection given the concentration) and is adjusted for beliefs about the specificity of the marker (CSPEC) and the efficiency of the PCR reaction (CEPCR).
CPRIMER	The ease with which a cPCR primer binds to the target marker.
CRITCT	The CT-value at which fluorescence should exceed critical fluorescence during the qPCR assay given prior knowledge of the concentration. This node is parameterized by running a bank of standards with known concentration.
CSAMP	Concentration of the target eDNA marker in the monitoring sample taken from the CAWS (copies/L). This variable is defined as a discretized continuous random variable greater than or equal to 0. This value is calculated from CSEG, SAFF, and SITE.
CSEG	Concentration of the target eDNA marker in the reach (copies/L). This variable is defined as a discretized continuous random variable greater than or equal to 0. This value is based on outputs of the CE-QUAL-ICM model
CSPEC	Specificity of the cPCR marker. This is a discretized continuous random variable between 0 and 1. The variable represents the degree of belief in the uniqueness of the target marker to the target species.
CURR	A variable characterizing flow conditions at the site where the monitoring sample was collected (*Backwater, Bank, Mid-Channel*).
EEXT	Efficiency with which eDNA markers are extracted from the sample to the elutriate. This is a discretized continuous random variable between 0 and 1. The node is parameterized by running a set of experiments to assess the extraction efficiency in the laboratory doing the assay.
HABITAT	A variable characterizing habitat at the location where the monitoring sample was collected.
LRATIO	Ratio of the length of the cPCR marker to the length of the qPCR marker. This is a discretized variable between 0 and 1. The value is calculated from the lengths of the cPCR and qPCR markers.
QALIQ	Concentration of the qPCR marker in the aliquot withdrawn for the qPCR assay. This variable is defined as a discretized continuous random variable greater than or equal to 0. This variable is calculated as a function of CELUTQ.
QEPCR	Efficiency of the qPCR reaction, which is the ratio of the number of copies detected in an aliquot and the number of copies present in the aliquot. This is a discretized continuous random variable between 0 and 1. The CPT is constructed from standards run to characterize the efficiency of the assay.
QINHIB	The fraction of qPCR markers that cannot be detected because of the presence of one or more inhibitors. This is a discretized continuous random variable between 0 and 1. The CPT is constructed from standards run to characterize inhibition in water samples taken from the CAWS.

QPCR	The concentration of the qPCR marker in the aliquot withdrawn for the assay (copies/μl). This variable is defined as a discretized continuous random variable greater than or equal to 0. This variable is calculated from CRITCT, ACTCT, and QSPEC.
QSPEC	Specificity of the qPCR marker. This is a discretized continuous random variable between 0 and 1. The variable represents the degree of belief in the uniqueness of the target marker to the target species.
QPRIMER	The ease with which a qPCR primer binds to the target marker.
SAFF	A variable describing the surface affinity of eDNA. The fraction of the eDNA in a water body that is located at the surface. This variable captures the belief that there is a tendency for eDNA to be located on the surface rather than mid-water. This is a continuous random variable between 0 and 1.
SITE	A variable that describes how much more likely it is that eDNA would be encountered at the site within the reach where the monitoring sample was collected than elsewhere within the reach.
SVOL	The volume of the monitoring sample (L).
WIND	Wind conditions at the time the sample was collected. If eDNA has a high surface affinity, eDNA may be blown across the surface and have a tendency to collect in backwaters and along banks.

Table A.2: Description of variables in the conceptual model of eDNA detectability.

Literature Cited

Alaeddini, R., 2012, Forensic implications of PCR inhibition – A review, *Forensic Science International: Genetics* 6(2012): 297-305

Asian Carp Regional Coordinating Committee (ACRCC), 2012, FY 2012 Asian Carp Control Strategy Framework, http://www.asiancarp.us/documents/2012Framework.pdf (Accessed July 16, 2013), February, p. 165.

Battelle Memorial Institute (BMI), 2010, Revised Final Independent External Peer Review Report Environmental DNA (eDNA) Science and Methodology, Report to the US Army Corps of Engineers, Rock Island District, December 7, Unpublished Report (http://www.usace.army.mil/Portals/2/docs/civilworks/Project%20Planning/eDNA_review.pdf) Accessed August 26, 2012.

Benson, D.A., M. Cavanaugh, K. Clark, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, and E.W. Sayers, 2011, GenBank, *Nucleic Acids Research* 39(Database Issue):D32-D37.

Blume, L., J. Darling, M. Vazquez, and J.S. Chandler, 2010. Laboratory Audit Report: Lodge Laboratory, Department of Biological Sciences, University of Notre Dame, Report by the Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL, February 5, (http://people.cst.cmich.edu/mahon2a/MahonLab/Publications_files/2011%20Blume%20et%20al%20EPA%20Audit%20Report%20of%20eDNA%20process%205%20Feb%202009.pdf), Accessed August 26, 2013.

Changnon, S.A. and J.M. Changnon, 1996, History of the Chicago diversion and future implications, *Journal of Great Lakes Research*, 22(1996): 100-118.

Chick, J.H., and M.A. Pegg, 2001, Invasive carp in the Mississippi River, *Science* 292: 2250-2251.

Darling, J.A. and M.J. Blum, 2007, DNA-based methods for monitoring invasive species: A review and prospectus, *Biological Invasions* 9(2007): 751-765.

Darling, J.A. and A.R. Mahon. 2011. From molecules to management: Adopting DNA-based methods for monitoring biological invasions in aquatic environments, *Environmental Research* 111(7): 978-988.

Dejean, T., A. Valentini, A. Duparc, S. Pellier-Cuit, F. Pompanon, P. Taberlet and C. Miaud, 2011, Persistence of environmental DNA in freshwater ecosystems, *PLoS ONE* 6(8):e23398. Doi: 10.371/journal.poine.0023398

Environmental DNA Calibration Study (ECALS), 2013, Environmental DNA Calibration Study Interim Technical Review Report, US Army Corps of Engineers, January, http://www.asiancarp.us/documents/ECALS_INTERIM.pdf, Accessed August 27, 2013.

Ficetola, G.F., C. Miaud, F. Pompanon and P. Taberlet, 2008, Species detection using environmental DNA from water samples, *Biology Letters*, 4(2008): 423-425.

Foote, A.D., P.F. Thomsen, S. Sveegaard, M. Wahlberg, J. Kielgast, L.A. Kyhn, A.B. Salling, A.Galatius, L. Orlando, M.T.B. Gilbert, 2012, Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals, *PLoS ONE* 7(8):e41781, doi:10.1371/journal.pone.0041781.

Goldberg, C.S., D.S. Pilliod, R.S. Arkle, and L.P. Waits, 2011, Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders, *PLoS ONE* 6(7): e22746, doi: 10.1371/journal.pone.0022746.

Holland, P.M., R.D. Abramson, R. Watson, and D.H. Gefland, 1991, Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of *Thermus aquaticus* DNA polymerase, *Proceedings of the National Academy of Sciences, USA*, 88(1991): 7276-7280.

Illinois Department of Natural Resources (ILDNR), 2011, *Bighead Carp in Illinois Urban Fishing Ponds*, Unnumbered report prepared by the ILDNR Aquatic Nuisance Species Program, http://asiancarp.us/documents/BigheadCarpinIllinoisUrbanFishingPonds.pdf, Accessed November 27, 2011.

Jerde, C.L., A.R. Mahon, W.L. Chadderton, and D.M. Lodge, 2011, "Sight unseen" detection of environmental DNA for surveillance of aquatic organisms at low densities, *Conservation Letters* 4: 150-157.

Jerde, C.L., W.L. Chadderton, A.R. Mahon, M.A. Renshaw, J. Corush, M.I. Budny, S. Mysorekar, and D.M. Lodge, 2013, Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program, *Canadian Journal of Fisheries and Aquatic Science* 70(4): 522-526.

Klymus, K., C. Richter, D. Chapman, and C. Paukert, 2013, ECALS: Loading Studies Interim Report July 2013, Interim Milestone Report to Asian Carp Regional Coordinating Committee, Unpublished, p. 21.

Kolar, C.S., D.C. Chapman, W.R. Courtenay Jr., C.M. Housel, J.D. Williams, and D.P. Jennings, 2007, Bighead Carps: A Biological Synopsis and Environmental Risk Assessment, Special Publication No. 33, American Fisheries Society, Bethesda, MD, p. 204.

Koller, D., and N. Friedman, 2009, *Probabilistic Graphical Models: Principles and Techniques*, MIT Press, Boston, MA, p. 1231.

Levy-Booth, D.J., R.G. Campbell, R.H. Gulden, M.M. Hart, J.R. Powell, J.N. Klironomos, K.P. Pauls, C.J. Swanton, J.T. Trevors, K.E. Dunfield, 2007, Cycling of extracellular DNA in the soil environment, *Soil Biology & Biochemistry* 39(2007): 2977-2991.

Lodge, D.M., C.R. Turner, C.L. Jerde, M.A. Barnes, W.L. Chadderton, S.P. Egan, J.L. Feder, A.R. Mahon, M.E. Pfrender, 2012, Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA, *Molecular Ecology* 21(2012): 2555-2558.

Matsui, M., Honjo, M., and Z. Kawabata, 2001, Estimation of the fate of dissolved DNA in thermally stratified lake water from the stability of exogenous plasmid DNA, *Aquatic Microbial Ecology* 26(2001): 95-102.

Metropolitan Water Reclamation District of Greater Chicago (MWRD), 2008, *Description of the Chicago Waterway System for the use Attainability Analysis*, MWRD Research and Development Department Report 08-15R, March, p.23.

Monitoring and Response Work Group (MRRWG), 2012, 2011 Asian Carp Monitoring and Rapid Response Plan Interim Summary Reports, Asian Carp Regional Coordinating Committee, April.

Monitoring and Response Work Group (MRWG), 2013a, 2012 Asian Carp Monitoring and Rapid Response Plan Interim Summary Reports, Asian Carp Regional Coordinating Committee, April.

Monitoring and Response Work Group (MRWG), 2013b, Monitoring and Response Plan for Asian Carp in the Upper Illinois River and Chicago Area Waterway System, Asian Carp Regional Coordinating Committee, May.

Monitoring and Response Work Group (MRWG), 2013c, MRRWG Environmental DNA Database, Excel Spreadsheet, unpublished.

Olson, Z. H., J.T. Briggler, R.N. Williams, 2012, An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water, *Wildlife Research* 39(2012): 629-636,

Rasmussen, J. L, H.A. Regier, and R.E. Sparks, 2011, Dividing the waters: The case for hydrologic separation of the North American Great Lakes and Mississippi River Basins, *Journal of Great Lakes Research* 37(3): 588-592.

Stohlgren, T.J., and J.L. Schnase, Risk analysis for biological hazards: What we need to know about invasive species, *Risk Analysis* 26(1): 163-173.

Takahara, T., T. Minamoto, H. Yamanaka, H. Doi, and K. Zen'ichiro, 2012, Estimation of fish biomass using environmental DNA, *PLoS ONE* 7(4): e35868 doi: 10.1371/journal poine.0035868.

Takahara, T. T. Minamoto, H. Doi, 2013, Using environmental DNA to estimate the distribution of an invasive fish species in ponds, *PLoS ONE* 8(2): e56584. doi: 10.1371/journal.pone.0056584.

Thomsen, P.F., J. Kielgast, L.L. Iversen, P.R. Moller, M. Rasmussen, and E. Willerslev, 2012a, Detection of a diverse marine fish fauna using environmental DNA from seawater samples, *PLoS ONE* 7(8):e41732, doi:10.1371/journal.pone.0041732.

Thomsen, P.F., J. Kielgast, L.L. Iversen, C. Wiuf, M. Rasmussen, M.T.P. Gilbert, l. Orlando, and E. Willerslev, 2012b, Monitoring endangered freshwater biodiversity using environmental DNA, *Molecular Ecology* 21(2012): 2565-2573.

US Army Corps of Engineers, 2012, Quality Assurance Project Plan: eDNA Monitoring of Invasive Asian Carp in the Chicago Area Waterway System, USACE Chicago District Chicago, May (http://www.asiancarp.us/documents/USACE-eDNA-QAPP.pdf) Accessed August 26, 2013.

US Geological Survey, 2013, Non-Indigenous Aquatic Species Database (http://nas.er.usgs.gov/) (accessed August 9, 2013).

Wilcox, T.M., K.S. McKelvey, M.K. Young, S.F. Jane, W.H. Lowe, A.R. Whiteley, and M.K. Schwartz, 2013, Robust detection fo rare species using environmental DNA: The importance of primer specificity, *PLoS ONE* 8(3): e59520. Doi:10:1371/journal.pone.0059520.

Willerslev, E., Hansen, A.J., Poinar, H.N., 2004, Isolation of nucleic acids and cultures from fossil ice and permafrost, *Trends in Ecology & Evolution* 19(2004):141-147.