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The contribution of double-crested cormorants (*Phalacrocorax auritus*) to silver carp (*Hypophthalmichthys molitrix*) DNA loads in the Chicago Area Waterway System

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ABSTRACT

Waterfowl and colonial waterbirds can have significant impacts on water quality in lakes and reservoirs by depositing feces that contribute to nitrogen and phosphorus loads. Piscivorous birds can also contribute the DNA of prey species to a water body. Here, we develop and apply a loading model to estimate the number of silver carp (*Hypophthalmichthys molitrix*) DNA target marker copies that are potentially deposited by nesting double-crested cormorants (*Phalacrocorax auritus*) in the Chicago Area Waterway System (CAWS). The model assumes a conservative breeding population estimate ranging between 6000 and 8000 cormorants distributed among three large colonies in the Chicago metropolitan area. The model also assumes that cormorants are distributing feces randomly throughout the CAWS in proportion to the amount of time spent at each location. Results show that cormorants may be contributing 2.6 to 113 target marker copies/m^2/day if birds are spending 22% of their time on open water and 6.4 to 291 target marker copies/m^2/day if birds are spending 56% of their time on open water. Over the entire CAWS, cormorants may contribute tens of millions to billions of silver carp DNA copies each day. These target marker loads may be contributing to positive detections of silver carp environmental DNA (eDNA) in the CAWS. This study does not address other potential sources of silver carp genetic material in the CAWS, including live fish, and provides no indication as to whether or not the loads attributed to cormorants are large or small in relation to these other potential sources.

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Introduction

Fecal waste deposited by waterfowl and colonial waterbirds can have a significant impact on nutrient levels in lakes and reservoirs (Manny et al., 1994; Scherer et al., 1995; Hahn et al., 2007; Gwiazda et al., 2010; Klimaszyk et al., 2014). Birds may deposit feces directly into water bodies and accumulations of fecal material under communal roosts may leech into adjoining water bodies through runoff or erosion (Gwiazda et al., 2010; Klimaszyk and Rzymski, 2013). Researchers have developed nutrient loading models to analyze and understand the impact of waterfowl and waterbird populations on water quality (Manny et al., 1994; Scherer et al., 1995; Hahn et al., 2007). In most cases, the nutrient load contributed by birds through fecal deposition is considered minimal compared to other sources of nutrients entering the system (Murphy et al., 1984; Hoyer and Canfield, 1994; Scherer et al., 1995), or minimal at the landscape scale, with potential impacts at the local scale (Hahn et al., 2007). Nevertheless, in some situations, nutrient loading by birds may result in high nitrogen (N) and phosphorous (P) concentrations (Manny et al., 1994; Scherer et al., 1995; Hahn et al., 2007), contribute to associated algal blooms (Manny et al., 1994), exacerbate cadmium and lead pollution (Mathis and Kevern, 1975) and increase coliform bacteria loadings (Klimaszyk and Rzymski, 2013).

Waterbird populations can contribute the genetic material of prey species to waterbodies in the same way that they contribute nutrients (Merkes et al., 2014; Guilfoyle et al., 2017a). The genetic material is allochthonous if it is transported from one water body to another by means other than a live fish. Allochthonous DNA is a source of false positive error in environmental DNA monitoring studies, which attempt to document the presence of an aquatic species by detecting their genetic material in water samples (Darling and Mahon, 2011; Guilfoyle et al., 2017a). It is important to understand sources of error in eDNA studies because false positive errors can lead to faulty environmental management decisions that are costly and have negative environmental outcomes (Merkes et al., 2014; Guilfoyle et al., 2017a).

Natural resource managers are conducting eDNA studies to monitor for the presence of two invasive species of carp in the Chicago Area Waterway System (CAWS). These two species, bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*), have expanded their ranges north in the Mississippi and Illinois Rivers and are now approximately 115 km southwest of Lake Michigan (United States Fish and Wildlife Service, 2015). The probability that these fish will become established in the CAWS and Lake Michigan is reduced by

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the presence of an Electric Dispersal Barrier at Romeoville, Illinois, approximately 50 km downstream from Lake Michigan. If these fish were to become established upstream of the barrier, they would negatively impact the ecology of the Great Lakes and threaten commercial and recreational fishing opportunities (Zhang et al., 2016). Monitoring is being conducted upstream of the barrier to determine whether or not the fish may have penetrated the barrier and to inform decisions about the need to control or eradicate the fish.

Double-crested cormorants (*Phalacrocorax auritus*), hereafter cormorant(s), are known to feed on silver carp and are one potential source of allochthonous silver carp DNA. Presently, cormorants are not known to feed on bighead carp (Guilfoyle et al., 2017a). Our objectives in this paper are: 1) to develop a nutrient loading model that can estimate, to an order of magnitude, how much silver carp DNA cormorants might be contributing to the Chicago Area Waterway System (CAWS) each day; 2) to gather the information needed to parameterize that model for the 2009 to 2012 breeding seasons; and 3) to compare this result with one described by Schultz et al. (2014), who used a Bayesian Markov chain Monte Carlo (MCMC) simulation to estimate how many copies of the genetic marker sources other than live fish might be contributing to the CAWS. The output of the model is highly uncertain, but it suggests that, during the breeding season, the contribution of cormorants to the load of the silver carp genetic marker in the CAWS may be on the order of tens to hundreds of copies per meter^2 per day.

Methods

The potential contribution of cormorants to the load of silver carp genetic marker in the CAWS is assessed using a nutrient loading model approach. In this section, we describe the model and its parameterization. The model is expressed using the following equation:

$$Z = \sum_j N_j \cdot b_j \cdot c \cdot d_j \cdot e \cdot f$$

The output, Z, is the sum of silver carp genetic marker copies deposited each day by all $j = \{1, 2, \ldots, J\}$ breeding colonies that may be defecating in the water body. N_j is the number of cormorants in breeding colony j that show evidence of silver carp in their diet. It is assumed that cormorants will distribute their feces randomly throughout their home range in proportion to the amount of time spent at each location. Therefore, the amount of feces deposited in the CAWS is proportional to the fraction of water surface area in the home range of colony j that is the CAWS, d_j, and the fraction of time cormorants spend on open water, c. The amount of genetic material deposited in the CAWS is also proportional to the amount of feces produced each day, f, and the concentration of the genetic marker in the feces, e. Finally, we consider what fraction of each colony's diet consists of silver carp, b_j. Most of the inputs to this model are highly uncertain, so they are treated as random variables and uncertainty in the output of the nutrient loading model is obtained by Monte Carlo simulation. The following subsections describe how each input variable is defined and parameterized.

Number of cormorants with evidence of carp in their diet (N_j)

N_j is a binomially distributed random variable representing the number of breeding cormorants in colony j that show evidence of silver carp in their diet. The distribution is defined by the parameters n and p, where n is equal to the population in a breeding colony and p is equal to the fraction of cormorants that contain genetic material from silver carp in their gastrointestinal tract. The value of p, 0.47, is based on the results of a study that tested throat and cloacal swabs from cormorants at Bakers Lake (Fig. 1) for evidence of silver carp in their diet (Guilfoyle et al., 2017a). The population in each breeding colony is estimated from field surveys. Bird populations are inherently variable, and surveys can be very uncertain. Therefore, we used a uniform probability distribution with lower and upper bounds equal to the lower and upper bounds of population estimates to represent uncertainty in the n parameter of the binomial distribution. However, no such bounds were available for the Baker's Lake colony, so we used a point estimate of that population.

The cormorant population in the Chicago metropolitan area is largely confined to three large breeding colonies, Baker's Lake near Barrington, Illinois, Lake Renwick near Plainfield, Illinois, and the ArcelorMittal Steel Mill, in East Chicago, Indiana (Fig. 1). The Baker's Lake colony typically supports about 1000 adult birds, and between 2009 and 2012, the number of breeding adults at Lake Renwick ranged from approximately 1200 to 1600 birds (Doug Stotz, The Field Museum Chicago, personnel communication, 2013). The Indiana Division of Fish and Wildlife makes regular cormorant nest counts at the ArcelorMittal Steel Mill colony site. Based on nest counts taken during the 2009 to 2012 breeding seasons, the number of adults in the ArcelorMittal Steel Mill colony was between 3582 and 5600 (John Castrale, Biologist, Indiana Division of Fish and Wildlife, 2013).

Fraction of the cormorant diet that is invasive carp (b)

The random variable b is the fraction of cormorant diet that is invasive carp. Since there are no data on the fraction of invasive carp in the diet of cormorants, we assume a uniform distribution for the variable with lower and upper bounds 0 and 1, respectively. Under this distribution, the diet of an individual cormorant may vary between 0% carp to 100% carp. The expected value of the random variable is 0.5, which may greatly overstate the fraction of silver carp in the diet of cormorants.

Fraction of time cormorants spend on open water during daylight hours in the breeding season (c)

It is assumed that birds are defecating randomly during the day, during daylight and non-daylight hours. During non-daylight hours, cormorants are roosting in colonies away from the CAWS and are not defecating in the CAWS. Dorr et al. (2014) report that in the daily time budget of cormorants, at least 36% of daylight hours are spent on water and at most 93% of daylight hours are spent on water. There are, on average, about 14.5 h of daylight each day during the breeding season, which is 60.4% of a 24-hour day. Thus, we estimate that cormorants might be spending as little as 22% of their time on open water during daylight hours in the breeding season and at most 56% of their time. Uncertainty in this variable is addressed by estimating a lower and an upper bound on the number of copies of silver carp DNA contributed to the CAWS by cormorants.

Fraction of water surface area in home range j that is the CAWS (d_j)

The variable d_j is the fraction of total water surface area within the home range of colony j that is the CAWS. The proportion of open water surface area that incorporates the CAWS in a cormorant's breeding territory was estimated using 95% convex polygons of breeding territories from nine cormorants captured at Baker's Lake (Fig. 1). Breeding territories from these cormorants were determined by use of Sirtrack® Argos Satellite Platform Transmitting Terminal (PTT) Harness Transmitters (model: K3H174A KiwiSat 303) (Guilfoyle et al., 2017b), and were combined to form one large polygon over the Baker's Lake colony site (Fig. 1). For the purposes of this model, the breeding territories of these nine birds are assumed to approximate the local breeding territories for all cormorants nesting in the region. The territories of cormorants from the other two colony sites was estimated by taking 25 random radial measurements from the Baker's Lake polygon (in km) and using the average radial distance to create a circular polygon around the Lake Renwick and ArcelorMittal Steel Mill colony sites (Fig. 1). Using ArcGIS (ver. 10.2), open water habitats within each polygon for small lakes, Lake Michigan, and the CAWS (Table 1) were calculated. Generally, cormorants rarely forage in large open water areas and are usually

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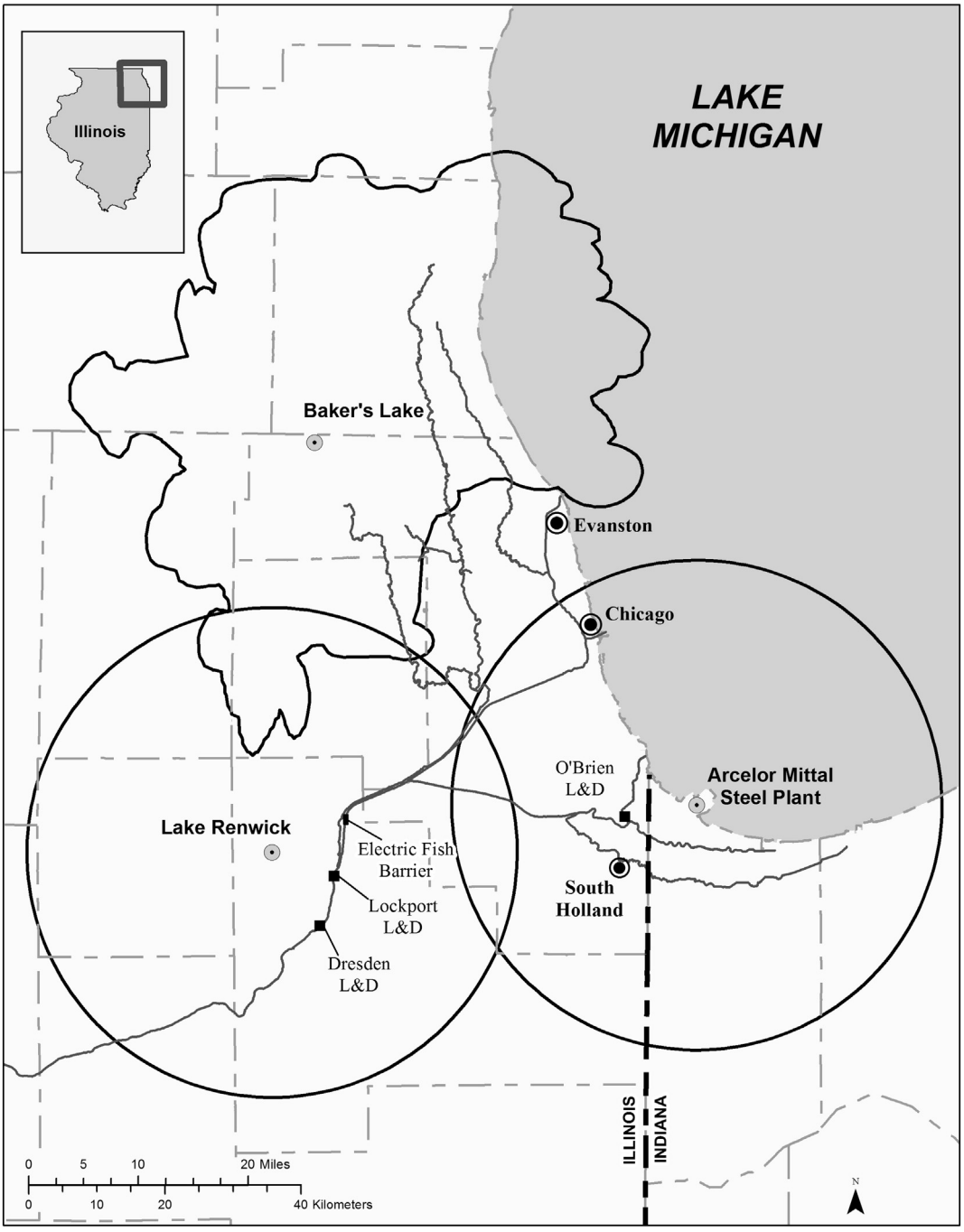

Fig. 1. The Chicago metropolitan area showing the location and breeding territories of the three cormorant colonies: Baker's Lake, Lake Renwick, and the ArcelorMittal Steel Mill, and locations of the Electric Fish Barrier and several locks and dams (L&D) in the Chicago Area Waterway System.

focused along coastal areas (Dorr et al., 2014). Therefore, the proportion of the open water that is Lake Michigan was removed from open water habitat available in cormorant territories from all three colonies (Table 1).

Copies of target marker in unit dry weight of bird feces (gram^{-1}) (e)

The random variable *e* is the number of copies of the target genetic marker in unit dry weight of bird feces (gram^{-1}). The value of this variable is based on a study that quantified the amount of bighead and silver carp DNA in fecal samples taken from four captive Bald Eagles (*Haliaeetus leucocephalus*) that were fed a diet consisting exclusively of bigheaded carp (Jon Amberg, United States Geological Survey,

Table 1
Open water surface area (ha) and total territory area (ha) for three cormorant colonies. The total territory area for Bakers Lake colony was estimated by combining the 95% convex polygons of breeding territory for nine birds. The mean radial distance of the Baker's Lake polygon was used to estimate circular territories for the Lake Renwick and ArcelorMittal Steel Mill colonies.

Colony	Total water surface area (ha)				Total territory area (ha)
	Lake Michigan	Small lakes and streams	CAWS	Total water surface area	
Baker's Lake	80,357	10,418	0	90,775	412,023
Lake Renwick	0	4464	2619	7083	409,155
ArcelorMittal Steel Mill	155,573	1085	1389	158,047	409,155

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unpublished data). Metabolic processes for carnivorous birds are generally similar (Hahn et al., 2007); therefore, we used these results to estimate the quantity of silver carp genetic markers in cormorant feces by fitting a lognormal distribution to these data using the method of moments. The distribution has a mean of 42,016 copies/g and a standard deviation of 28,442 copies/g.

Amount of feces (dry weight) produced by one bird in one day (grams/day) (f)

The random variable f is the amount of feces (dry weight) produced by one cormorant (grams/day). Twelve captive cormorants fed diets of invasive carp had an average dry fecal weight per day of 71.6 g (n = 12, SD = 8.50) (D.T. King, United States Department of Agriculture, National Wildlife Research Center, unpublished data). We used a lognormal probability distribution to represent uncertainty in this value. The distribution has a mean of 71.6 g and a standard deviation equal to 8.50 g.

Results

The output of the model is a probability distribution characterizing uncertainty in the number of copies of the silver carp genetic marker deposited by cormorants defecating in the CAWS each day. The 90% confidence interval on the load of silver carp DNA attributable to cormorants ranges from 1.1×10^7 to 0.5×10^9 copies/day. We divide this number by the surface area of the CAWS, 12.72 km^2, to obtain the result in copies/m^2/day (Fig. 2). If cormorants are using open water habitat in the CAWS 22% of the time, the 90% confidence interval on the silver carp DNA loading rate ranges from 2.6 to 113 copies/m^2/day (Fig. 2). However, if cormorants are using the open water habitat in the CAWS 56% of the time, then the 90% confidence interval ranges from 8.4×10^7 to 3.7×10^9 copies/day or 6.6 to 291 copies/m^2/day (Fig. 2).

Discussion

Since 2009, state and federal agencies have been implementing a two-pronged monitoring effort to determine whether silver carp are present in the CAWS. The effort consists of an eDNA monitoring program to document the presence of silver carp DNA in water samples, and a conventional fish sampling effort aimed at capturing a live specimen. The results of that effort have been ambiguous. While a small fraction of water samples have continuously tested positive for the presence of silver carp eDNA (Merkes et al., 2014; Schultz et al., 2014), no live silver carp were captured in the CAWS until June 22, 2017, when one four-year old silver carp weighing 3.6 kg was captured just downstream of T.J. O'Brien Lock and Dam. Several explanations for this result have been proposed, one of which is that there are sources of carp eDNA in the CAWS other than live fish. Studies have detected silver carp eDNA in throat and cloacal swabs taken from cormorants nesting in the Chicago metropolitan area (Guilfoyle et al., 2017a). Therefore, cormorants are one possible source of silver carp DNA in the CAWS. Currently, there are no known populations of silver carp near the three large cormorant nesting colonies in the Chicago metropolitan area. In order to find a source of silver carp, these birds would have to travel south of the Electrical Dispersal Barrier along the Illinois River or west to the Mississippi River (U.S. Fish and Wildlife Service, 2015). Satellite tagged cormorants were observed to travel distances sufficient to forage on silver carp; however, the birds maintained locations north and east of Chicago and largely outside the known range of silver carp (Guilfoyle et al., 2017a, 2017b). Additional research is needed to determine where these cormorants are foraging on silver carp.

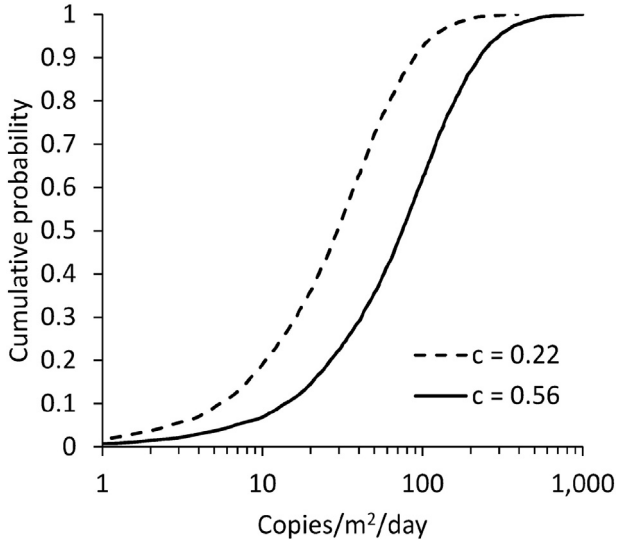

Fig. 2. Estimated contribution of cormorants to the load of the silver carp genetic marker in the Chicago Area Waterway System (copies/m^2/day). The factor c is the fraction of time cormorants spend on open water during daylight hours in the breeding season.

The model estimates how much silver carp DNA target marker cormorants may be contributing to the CAWS. The estimate reflects knowledge and assumptions about the life-history of cormorants, such as daily time budgets and their regional dietary habits, and captures the uncertainty in these assumptions. Very little is known about some model parameters. For example, very little is known about the fraction of silver carp in the cormorant's diet. A uniform distribution between 0 and 1 is used to describe this uncertainty, which suggests that, on average, silver carp comprise half the diet of the cormorant population. Because it is not clear where the cormorants might be foraging for silver carp, this estimate may very well overstate the amount of silver carp in the diet of cormorants. If so, the potential contribution of cormorants to DNA loads will have been overestimated. Estimates of DNA target marker load are strictly proportional to this parameter, so these estimates can be scaled to represent smaller fractions of silver carp in cormorant diet.

The model described in this paper is based on the assumption that cormorants defecate randomly during the day and distribute their feces in proportion to the amount of time spent at each location. However, Hutchinson (1950) indicates that cormorants appear especially prone to deposit the greater part of their droppings near nesting colonies. This behavior provides a supply of guano for use in nest construction. If the distribution of feces is non-random, the results obtained using this model would overestimate the amount of silver carp eDNA cormorants contribute to the CAWS because no nesting colonies are located in open water areas of the CAWS. This model also does not account for non-breeding cormorants, including northward migratory cormorants during spring migration. Large numbers of cormorants are known to pass through the Chicago area during spring migration and may also be contributing silver carp DNA to the CAWS (Guilfoyle et al., 2017b).

This model represents an initial attempt to estimate the contribution of cormorants to silver carp DNA loads in the CAWS. It provides no information about other potential sources of the silver carp genetic marker in the CAWS, including the presence of live fish, and no indication as to whether or not the loads attributed to cormorants are large or small in relation to these other potential sources. Additional research will be needed to more comprehensively assess the role of piscivorous birds (Guilfoyle et al., 2017a, 2017b) and other secondary sources of silver carp DNA in the CAWS. For example, Schultz et al. (2014) used Markov chain Monte Carlo (MCMC) simulation to estimate how much of the silver carp target marker each potential source of allochthonous DNA would need to contribute in order to explain estimated target marker concentrations in the CAWS assuming that no live fish were present. This analysis indicated that combined sewer overflows and commercial

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navigation were most likely to be the largest sources of allochthonous DNA in the CAWS. Piscivorous birds were among the smaller sources of allochthonous DNA, contributing an expected 2524 copies/m^2/day, with a 90% confidence interval ranging from 145 to 7324 copies/m^2/day. The estimates generated in this paper, which are based on a nutrient loading model approach, tend to be lower, but the upper percentiles of the upper bound distribution overlap the lower percentiles of the MCMC estimates, suggesting that these two independent estimates provide some degree of mutual support for one another.

Overall, the results of this study indicate that piscivorous birds are one potentially important source of allochthonous silver carp DNA in the CAWS. However, more research will be needed to understand fully the role of cormorants and other piscivorous birds as sources of silver carp DNA in the CAWS. For example, these research efforts should determine the amount and sources of silver carp in the diets of cormorants in the Chicago metropolitan area, as well as overall abundance, habitat use and distribution of these birds in the CAWS during the breeding season. Additional research will also be needed to determine the extent to which sources of allochthonous DNA might be influencing the results of eDNA monitoring efforts.

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