2017

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Movement of *Hypophthalmichthys* DNA in the Illinois River Watershed by the Double-Crested Cormorant (*Phalacrocorax auritus*)

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Abstract.—Paired throat and cloacal swabs, along with feather samples, from nesting Double-crested Cormorants (*Phalacrocorax auritus*) at two sites in Illinois, USA, were tested for presence of invasive bigheaded carp (*Hypophthalmichthys* spp.) DNA. We also used DNA from the feather calamus to determine cormorant sex. Throat and cloacal swabs from cormorants at both locations tested positive for DNA from silver carp (*H. molitrix*), but none tested positive for bighead carp (*H. nobilis*). *Hypophthalmichthys* DNA was not detected on feathers. There were no significant differences among positive *Hypophthalmichthys* DNA detection frequencies between cormorant sexes. To our knowledge, this is the first demonstration of silver carp as part of the Double-crested Cormorant diet in North America. *Hypophthalmichthys* are major invasive species of concern in this region, the detection of water-borne environmental DNA of *Hypophthalmichthys* is an important monitoring tool, and the potential movement of DNA via piscivorous birds may have significant implications for interpreting environmental DNA monitoring data.

Key words.—DNA, Double-crested Cormorant, eDNA, feathers, fecal deposition, *Hypophthalmichthys, Phalacrocorax auritus*, silver carp, swab samples.

Invasive bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*) pose serious ecological and economic threats to aquatic communities in North America (Zhang et al. 2016). In the North American Great Lakes Region, invasive *Hypophthalmichthys* are of major concern as populations of both species continue to spread upstream in the Illinois River drainage. Adult *Hypophthalmichthys* have been regularly detected about 65 km southwest of Lake Michigan in the Dresden Cooling Lake, adults and juveniles have recently been observed at Starved Rock Lock and Dam about 130 km from Lake Michigan, but the main invasion front with a large spawning population in the Illinois River is about 180 km away, just north of Peoria, Illinois, USA (U.S. Fish and Wildlife Service 2015). In an effort to prevent the spread of *Hypophthalmichthys* into the Great Lakes, the Electric Dispersal Barrier was constructed about 135 km upstream of the invasion front near Romeoville, Illinois. As part of tracking the spread of *Hypophthalmichthys* in the Illinois River drainage and to verify the effectiveness of the Electric Dispersal Barrier, a large-scale environmental DNA (eDNA) monitoring program has been established to test water samples in the region for *Hypophthalmichthys* eDNA (Jerde et al. 2011; Schultz and Lance 2015). Dozens of positive *Hypophthalmichthys* eDNA results have been detected in the Chicago Area Waterway System; however, standard fish community sampling efforts above the Electric Dispersal Barrier have yielded only one adult *H. noblis* and no *H. moltrix* since 2009 (Merkes et al. 2014). The potential for *Hypophthalmichthys* DNA to be moved from location to location within the system by piscivorous birds, including the abundant Double-crested Cormorant (*Phalacrocorax auritus*; hereafter, cormorant) is a potentially important factor in interpreting the results from eDNA monitoring (Merkes et al. 2014).

To that end, we captured nesting cormorants along the Illinois River and used DNA testing of throat and cloacal swabs, as well as feather samples, to determine if cormorants included *Hypophthalmichthys* in their diets or could transfer carp DNA in their feces or on their feathers.
**Methods**

**Study Area**

From 25-29 May 2012, we captured 15 nesting cormorants at Baker’s Lake (42° 08′ 42.3″ N, 88° 07′ 28.5″ W) in Barrington, Illinois. Baker’s Lake is a suburban lake managed by the Forest Preserve of Cook County (River Forest, Illinois) and is located about 170 km northeast of the *Hypophthalmichthys* invasion front and about 60 km north of the Electric Dispersal Barrier (Fig. 1). Ongoing monitoring for *Hypophthalmichthys* has found evidence that the invasion front is in the process of moving upstream near the Starved Rock Pool.
DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit following manufacturer’s guidelines. DNA samples were amplified using PCR primers 2550F (5’-GTTACTGATTGCTGCTACGAGA-3’) and 2718R (5’-ATTGAAATGCCTGCTTGT-3’) and a touch-down thermocycler protocol described by Fridolfsson and Ellegren (1999). PCR reactions consisted of 2.5 µL 10x buffer (including Mg), 0.5 µL each of dNTP solution and primers (10 mM concentration), 0.2 µL of 5PRIME Taq polymerase (1 U; 5 Prime, Inc.), 3 µL of DNA extract, and 17.8 µL of water for a total reaction volume of 25 µL. PCR products were separated on 2% agarose gels containing ethidium bromide and visualized under ultraviolet light to identify females (two bands) and males (single band). This procedure was used on 29 of 30 captured cormorants. For one bird, which escaped prior to feather collection, we used cormorant DNA extracted from the throat/cloacal swabs. Tissue samples from four male and two female cormorant specimens of known sex (necroscopic gonadal inspection) were acquired to validate the sex determination technique.

Statistical Analysis

We used a Fischer’s Exact Test to determine any differences among sexes in the frequencies of positive Hypophthalmichthys DNA found in the cormorant throat and cloacal swabs. All analyses were performed using PROC FREQ (SAS Institute, Inc. 2010).

Results

Of the 15 cormorants captured at Baker’s Lake, seven birds (47%) had positive results for H. molitrix DNA from the cloacal and/or throat swabs (one bird had positive detections from both cloacal and throat swabs and six had positive results from only throat swabs) (Table 1). Of the 15 cormorants captured at the Emiquon Preserve, 13 (87%) showed positive results for H. molitrix DNA in cloacal and/or throat swabs (six birds had positive detections from both cloacal and throat swabs, four had positive results from only cloacal swabs and three had positive results from only throat swabs) (Table 1). No H. nobilis DNA was detected in any sample. No Hypophthalmichthys DNA was detected on any of the feather samples. Genetic sexing procedures determined that sex ratios were similar at Baker’s Lake (seven males, eight females) and at the Emiquon Preserve (nine males, six females) (Table 1). Frequencies of positive Hypophthalmichthys DNA detections in the throat and cloacal swabs were not significantly different among sexes (F = 0.88, P > 0.05).
Hypophthalmichthys DNA was found on both throat and cloacal swabs taken from nesting cormorants, demonstrating for the first time that North American populations of this bird species are predators of the invasive carp. The significant portion of sampled birds from both the Baker’s Lake and Emiquon Preserve colonies that tested positive for Hypophthalmichthys DNA (47% and 87%, respectively) suggests that this fish is a common prey species for cormorants. These results indicate that cormorants have the capacity to move invasive carp DNA into the Chicago Area Waterway System from other regions. We consider the movement of a target species’ DNA beyond the known distribution by predators as an “allochthonous eDNA” result to distinguish from other false positive results (e.g., positive results arising from detection of DNA from a non-target species). The detection of Hypophthalmichthys in the diets of cormorants nesting at Baker’s Lake is particularly interesting as there are no known populations near the colony site, and the leading edge of the Hypophthalmichthys invasion front is about 115 to 170 km to the southwest. The cormorants at Baker’s Lake would have to fly south of the Electric Dispersal Barrier in the Illinois River, or west to the Mississippi River, to forage on smaller-sized Hypophthalmichthys (U.S. Fish and Wildlife Service 2015). While cormorants at Baker’s Lake do make such
long daily foraging movements during the breeding season, satellite-tagged cormorants were observed to maintain locations north and east of the Electric Dispersal Barrier and largely outside of the known range of *Hypophthalmichthys* (M. P. Guilfoyle, unpubl. data); therefore, the source of carp found in the diets of these cormorants remains unknown.

It is also possible that rather than actively transporting *Hypophthalmichthys* DNA via fecal deposition, cormorants swimming or diving in aquatic habitats where *Hypophthalmichthys* occur may transfer the DNA when fish slime (Merkes *et al.* 2014) or other biological films that contain the carp’s DNA adhere to their feathers. However, we could find no evidence of *Hypophthalmichthys* DNA on cormorant feathers. It would also seem unlikely that feathers of other waterbirds, including thousands of ducks and geese in the region, could act to transfer *Hypophthalmichthys* DNA.

More research is needed to comprehensively assess the role of the cormorant and other piscivorous birds as vectors of *Hypophthalmichthys* DNA in the Chicago Area Waterway System. Since it is now known that *Hypophthalmichthys* DNA can be detected in fecal material (Merkes *et al.* 2014), establishing a standardized approach for collecting swab samples from feces-covered nests at large nesting colonies in the Chicago metropolitan area could be a cost-effective approach to determining a timeline for the incorporation of *Hypothal-michthys* in the diets of nesting cormorants while also assessing the proportion of invasive carp in the diets of cormorants (Symondson 2002; Barrett *et al.* 2007). Understanding the role of highly vagile predators on the movement of DNA and their proportional contributions within a system could improve the utility and power of eDNA monitoring efforts. For example, a better understanding of secondary sources of *Hypophthalmichthys* DNA in the Chicago Area Waterway System, including the role of piscivorous birds, may permit the capability to more effectively model, interpret and distinguish patterns of positive eDNA results arising from secondary sources rather than the actual presence of *Hypophthalmichthys*. Such capability could provide a better assessment tool for determining the current effectiveness of the Electric Dispersal Barrier and other control efforts for preventing *Hypophthalmichthys* from invading the Great Lakes.

Acknowledgments

This study was part of the Environmental DNA Calibration Study funded by the U.S. Environmental Protection Agency Great Lakes Restoration Initiative (https://www.glri.us) through the Asian Carp Regional Coordinating Committee (http://www.asiancarp.us) and the U.S. Army Corps of Engineers. Kelly Baerwaldt (currently with the U.S. Fish and Wildlife Service) was program manager during the Environmental DNA Calibration Study. We are grateful for access to cormorant colonies by personnel at The Nature Conservancy’s Emiquon Preserve, and Chris Anchor (Wildlife Biologist, Forest Preserve District of Cook County, Illinois) and Paul Fioranelli (U.S. Department of Agriculture, National Wildlife Research Center) assisted in the capture of the cormorants. The U.S. Department of Agriculture, National Wildlife Research Center assisted in the capture of the cormorants. The U.S. Department of Agriculture, National Wildlife Research Center, provided tissue samples from cormorants to validate sex determination. Mr. Scott Beckerman and Mr. Travis Guerrant (U.S. Department of Agriculture, Wildlife Services) aided with State of Illinois permits. All capture and handling of cormorants was approved by the U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center’s Institutional Animal Care and Use Committee and attending veterinarian (Quality Assurance Protocol 2003). Cormorant capture efforts were conducted under authority of U.S. Geological Survey Federal Banding Permit #23700 and Illinois Department of Natural Resources Scientific Collecting Permit #NH12.5003. All applicable ethical guidelines for the use of birds in research have been followed including those presented in the Ornithological Council’s “Guidelines to the Use of Wild Birds in Research.”

Literature Cited


