

ECALS MILESTONE REPORT

ACRCC Framework Item: 2.5.3

Milestone: eDNA Deposition from Storm Sewers

Milestone Date: July 2012

Agencies Involved: USACE

Brief Description of Milestone: Determine the potential role of storm sewer outflow as a source of Asian carp eDNA in the Chicago Area waterway System (CAWS). Demonstrate that eDNA can pass through and out of Chicago area storm sewers during high water events. Provide data on how far eDNA may spread and how long eDNA might be detected following a high water event.

How does the accomplishment of the milestone contribute to ECALS and eDNA overall?

Determining the potential for alternative vectors for Asian eDNA to become deposited in the CAWS, such as carp blood and tissue waste from restaurants and fish markets that accumulates in storm sewers, provides critical context for understanding eDNA results and formalizing appropriate action plans.

Was the milestone accomplished? Yes.

We conducted 2 trials to demonstrate that Asian carp eDNA material could potentially travel through storm sewers and be deposited in the CAWS. In the latter of the two trials we also focused on the spread and retention of eDNA signal (positive detections) following an outflow.

Both trials were conducted at the storm sewer drainpipe (sewer outfall 153) just adjacent to the Cermak Rd. Bridge near the intersection of and S. Canal St. and W. Cermak Rd. in Chicago, IL (see Map 1). A manhole on Cermak Rd., situated approximately 75 m from the pipe, was opened and water from a nearby fire hydrant was used to flush the sewer contents towards the CAWS. At a point in each trial, ice from a standard-size cooler that had held fillets of both silver and bighead carp was dumped into the manhole. The ice contained considerable amounts of fish material and blood, though any visible chunks of tissue were removed prior to the ice being dumped. All samples were procured and processed following protocols in the Asian Carp eDNA Quality Assurance Project Plan.

Trial 1 was conducted in November 2011. Ten samples were taken at each stage and sampling point, which included:

1. CAWS, just under and near drainpipe, prior to any activity

2. CAWS, just under and near drainpipe, after flushing storm sewer for 15 minutes and allowing outflow to cease.
3. CAWS, just under drainpipe following dump and flush with Asian carp eDNA material.
4. CAWS, near drainpipe following dump and flush with Asian carp eDNA material.
5. CAWS, just under and in close vicinity of drainpipe 30 minutes after flush with Asian carp eDNA material.

Results of Trial 1 (Fig. 1):

1. One positive sample for bighead carp and 4 from silver carp in the pre-flush batch.
2. Five positive samples for bighead carp and 5 for silver carp in the pre-dump flush batch.
3. Two positive samples for bighead carp and 3 for silver carp in the ice-dump-flush batch taken just under the drainpipe.
4. Four positive samples for bighead carp and 3 for silver carp in the ice-dump-flush batch taken near the drainpipe.
5. Three positive samples for bighead carp and 2 for silver carp in the 30 minutes-post-ice-dump-flush batch taken from under and near proximity to the drainpipe.

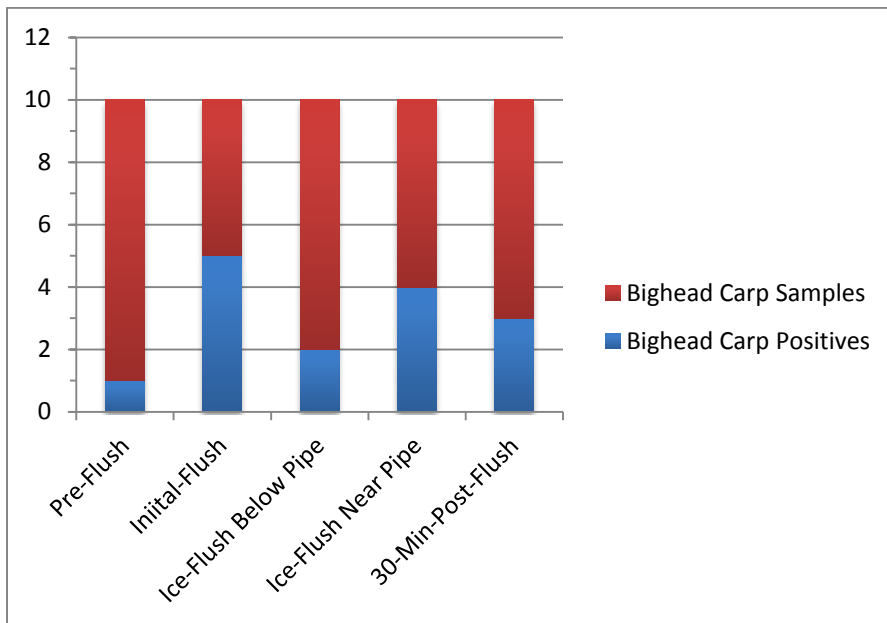


Fig. 1a. Bighead carp results for Trial 1.

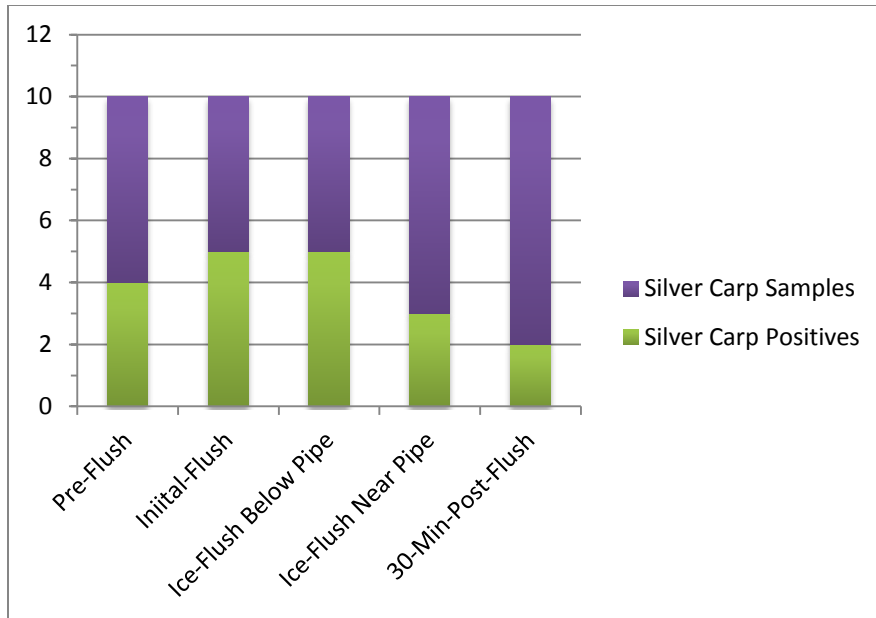


Fig. 1b. Silver carp results for Trial 1.

These results were slightly compromised by potential sample contamination. A negative control for DNA extraction from the pre-dump flush batch showed positive results for both species. This result would indicate that some event, such as genetic material from one sample splashing into the tube containing the negative control (sterile filter paper) or use of a contaminated pipette tip during PCR assembly, likely occurred. It does not mean that each sample, or any other sample, may have been compromised. The fact that two samples showed positive detections for one species and not another indicates that some samples, at least, were not compromised. Additionally, the field blank (= negative control) from the ice-dump-flush batch taken near the drainpipe showed positive results for both species. Here, contamination could have taken place when sampling or further along the processing chain (e.g. extraction or PCR). Again, this result does not mean that all samples from that batch were contaminated, and the fact that 4 samples that tested positive in each species did not test positive for the other species indicates that most samples for this batch were not compromised.

In any case, the detection of several positives at the site prior to any work and the apparent increase in positives following the initial flush were unexpected and of particular interest. It is unknown whether the initial flush carried eDNA with it or simply stirred up eDNA from the CAWS sediment. There was no apparent increase in the number of positive detections following the ice flush. This was likely due to the strong positive eDNA background already in place, especially following the first flush. It may also be a result of testing the CAWS instead of both the CAWS and the water coming out of the pipe.

In order to better test whether eDNA pre-existed in the storm sewer, to better test whether eDNA would flush out of the system, to better understand how long eDNA positives might occur after a flush and how far from the source positive might occur, and, finally, to simply increase sample size, we conducted Trial 2. In Trial 2, we directly collected water flowing out of the drainpipe in order to ascertain if eDNA pre-exists in the sewer and to better determine the degree to which eDNA might flush through the system. We also collected samples from a broader spatial area and over a longer period.

Trial 2 was conducted in June 2012. The following samples were taken:

1. 20 samples -- 5 near drainpipe, 15 from grid downstream from drainpipe and bridge (Fig. 2)
2. 20 samples from drainpipe during initial flush.
3. 20 samples from drainpipe during ice-flush.
4. 20 samples post-1-hour after cessation of flow from ice-flush -- 5 near drainpipe, 15 from grid downstream from drainpipe and bridge
5. 20 samples post-1-day after ice-flush -- 5 near drainpipe, 15 from grid downstream from drainpipe and bridge
6. 20 samples post-1-week from ice-flush -- 5 near drainpipe, 15 from grid downstream from drainpipe and bridge
7. 20 samples post-2-weeks from ice-flush -- 5 near drainpipe, 15 from grid downstream from drainpipe and bridge

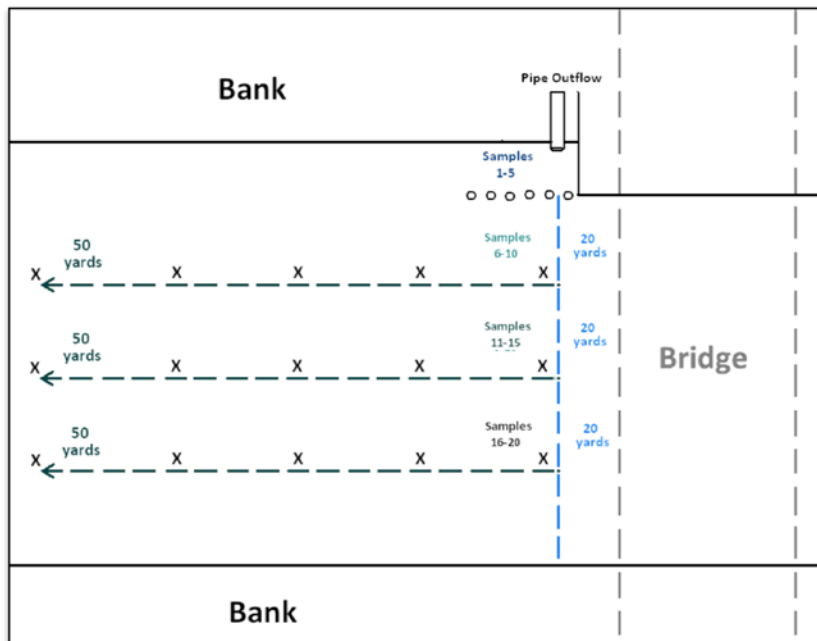


Fig. 2. Sampling grid for the pre-flush, 1-Hr-Post-Ice-Flush, and later sampling.

Results of Trial 2 (Fig. 3):

1. One positive detection for silver carp eDNA at furthest point from drainpipe (sample point 5 on left descending bank).
2. Nine positive detections for silver carp eDNA out of drainpipe during initial storm sewer flush
3. Twenty positive eDNA detections for both bighead and silver carp, separately, out of drainpipe during ice-flush
4. Two positive detections for silver carp, including one near drainpipe and one at far bank (sample 4 on left descending bank) one hour after the ice-flush ceased flowing from the drainpipe.
5. Three positive detections for silver carp at one day after ice-flush, with 2 near drainpipe and one downstream of drainpipe on same bank.
6. No positive eDNA detections for either species 1 week after ice-flush.
7. The post-1-week samples were not processed due to lack of eDNA in post-1-week samples.

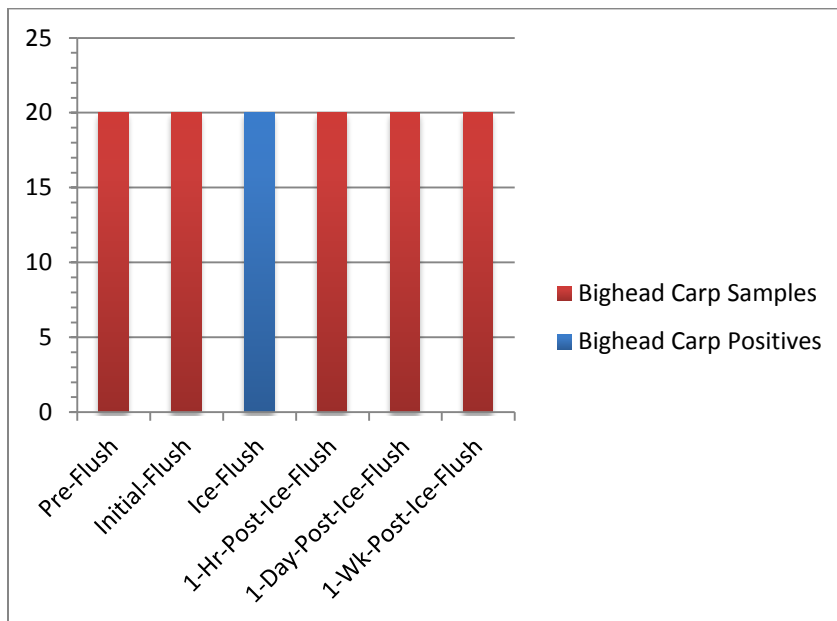


Fig. 3a. Bighead carp results for Trial 2.

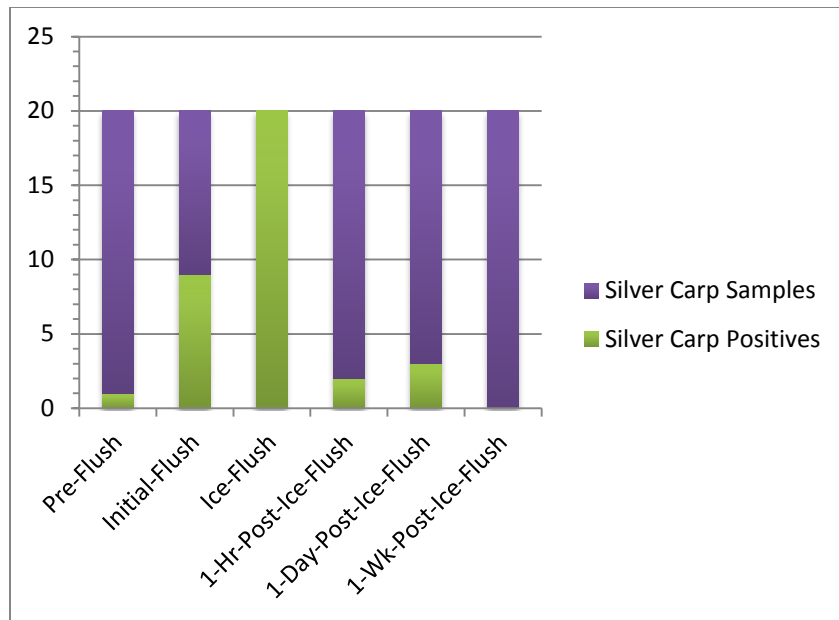


Fig. 3b. Silver carp results for Trial 2.

As with Trial 1, there was a surprising amount of eDNA already in the storm sewer. This might indicate that Asian carp genetic material (blood, body parts, etc.) are commonly discharged into storm sewers. This material likely enters the food chain and eDNA might be spread by scavengers (e.g. rats) within the storm sewer network. The degree to which the presence of Asian carp eDNA in storm sewers might be particularly linked to storm sewers in areas with Asian fish markets and restaurants is unknown. The drainpipe we tested does run through an area with such markets and restaurants.

There was a highly significant pulse of eDNA for both species after ice with Asian carp genetic material was dumped into the system. This is a fairly simple demonstration of the potential for this type of event to occur. The storm sewer path from the manhole to the drainpipe was somewhat complex, including at least, a large reservoir that had to be filled before water could run out. It took approximately 30 minutes for water to travel the 75 m from manhole to drainpipe.

The eDNA from the ice-flush either was quickly diluted to levels where it could not longer be detected, as exhibited by low detection levels at the 1-Hr-Post-Ice-Flush samples, or it was fairly rapidly carried away for water currents. We make the supposition, however, that the eDNA persisted at least a day after deposition – though it cannot be ruled out that another source of Asian carp eDNA might have accounted for the fairly weak 1-Hr-Post-Ice-Flush and later results.

The relative strength of the silver carp eDNA “signal” compared to that of the bighead carp can likely be explained, likely in large part, by the fact that in-house tests of the markers used for the species, as well as published information (Jerde et al. 2011), shows the bighead carp marker to be two orders of magnitude less sensitive to eDNA than the silver carp marker. Additionally, the bighead carp marker is over 100 DNA base pairs longer than the silver carp marker and may be more susceptible to degradation, though the potential difference in degradation rates associated with such length difference are currently unknown. Planned ECALS studies will help answer this question.

Trials 1 and 2 indicate that Asian carp eDNA can exist within storm sewers and be subsequently flushed into the CAWS. The extent to which this source of eDNA results in positive detections during monitoring is unknown. Upcoming studies of eDNA degradation, marker sensitivity, and proposed particle flow modeling and risk assessment work units will utilize this data, along with other vector studies, to more completely understand and act on the context of positive eDNA detections.

Future Work: An additional suite of markers has been developed to determine the size of DNA collected in samples. These markers have been designed to amplify various lengths of DNA within the same portion of carp DNA. It has been hypothesized that DNA is fragmented into shorter sequences over time, thus by using markers of various lengths, one might be able to infer recency of deposition of the carp DNA. By using these markers, it will allow for the determination of degradation and fragmentation of the Asian carp DNA collected during storm sewer studies. Information from this additional testing will be important in development of appropriately sized markers that will allow for minimizing the rate of positive detections from sources other than actual fish.

Acknowledgements: USACE would like to thank the Metropolitan Water Reclamation District of Greater Chicago, specifically Tom Minarik, and the City of Chicago for their assistance with the sewer access, traffic control, and flushing for these experiments.



Exhibit 1. Cooler with large pieces of bighead and silver carp tissue on ice.



Exhibit 2. After sewer was flushed, fish pieces were removed from the cooler and the ice was dumped into sewer.



Exhibit 3. Hose from fire hydrant flushing sewer



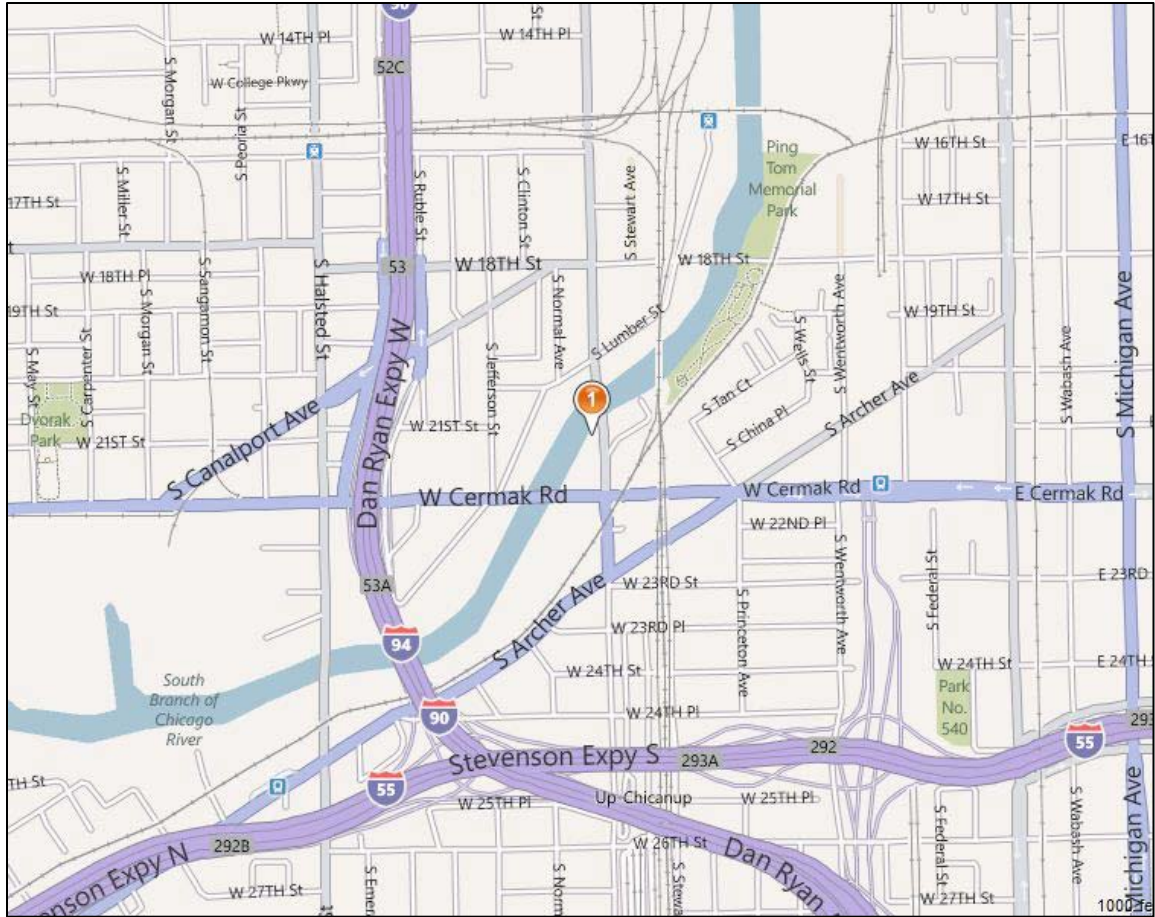
Exhibit 4. Observed water flow from outfall after flush



Exhibit 5. USACE technician sampling water from outfall.



Exhibit 6. USACE technician sampling water from outfall.



Map 1. Location of storm sewer used for both trials.