Appendix A. Protocol application

March 2024

The purpose of this appendix is to provide examples as templates to assist with preparation of an event description (pages 1-14). Actual event descriptions that have been processed through the invasive fishes communications protocol are listed in a summary table at the end of document; please refer to Appendix D for the final event descriptions. This document is subject to periodic revision (note date above).

Section 1. Examples of protocol application

Summary

Scenario #1 - URGENT event: New invasive species unexpectedly collected by a signatory agency during *routine fish sampling* in a Great Lake.

- Hypothetical scenario, suggests actions under all five steps, a timeline that might generally apply for all event scenarios, and a timeline for this scenario specifically (p. 2-3).

Scenario #2 - Important event: New evidence of spread by invasive fish already in a Great Lake, from targeted sampling for that species.

- Hypothetical scenario demonstrates how key terms may be used in talking points (p. 4).

Scenario #3 - Routine event: Plans for a coordinated project to slow the spread of an invasive fish through removals.

- Hypothetical scenario of both a management and research application (p. 5).

Scenario #4 - Important Event: Silver carp eDNA in Sandusky Bay, Ohio.

- Hypothetical scenario but similar to real-world event (p. 6-7).

Scenario #5 - Important Event: Northern snakehead in Lake Ontario watershed, New York.

- Real-world, pre-protocol, scenario from 2018, provided by NYSDEC with supporting materials (p. 8-12).

Scenario #6 - Important Event: Larval grass carp in Maumee River, USGS

- Real-world, pre-protocol, scenario from 2018 (p. 13-14).

<u>Scenario #1 - URGENT Event:</u> New invasive species unexpectedly collected by a signatory agency during fish sampling in a Great Lake.

Step 1: Internal protocol is initiated by the responsible agency. At a minimum, the following occurs:

- ***Lead person identified***: field crew leader or other designated person assumes responsibility for handling of event, including all communications while afield
- ***Specimen handling***: specimen is preliminarily identified, if possible, measured, weighed, externally inspected, photographed, stored/preserved for professional vouchering
- ***Recording of sampling details***: sampling information (location, date/time, gear, conditions, specimen description, crew members, public involvement, etc.) is logged while afield
- ***Internal communication***: lead person contacts immediate supervisor, ASAP via cell phone; additional communication follows agency protocols to determine course of action.
- ***External communication:*** designated person of responsible agency contacts designated contact from the management agency with jurisdiction over sampling area (if not the collecting agency); additional external communication occurs according to agency protocols.

Step 2: Responsible agency prepares event description, procures internal approval for distribution to Plan groups; responsible agency may share event information confidentially with management agency for input concomitantly with implementation of internal process.

Step 3: Responsible agency initiates formal consultation with Plan groups by emailing approved event description to lake committee members (see example). All information and subsequent communications among Plan groups remains confidential. The 24-h response period begins with the time of the sent email by the Responsible agency.

Step 4: Comments from Plan groups are provided to Responsible agency within 24 h of the received email.

Step 5: Responsible agency review comments, issues final talking points to Plan groups within 24 h of the received email.

<u>Suggested General Timelines</u>

Timing	Draft talking points to Lake Committee (Steps 1-2)	Final talking points to Plan groups (Steps 3-5)
URGENT	**<u>≤</u> 12 hours**	**<u>≤</u> 24 hours**
Important	**<u>≤</u> 3 business days**	**<u>≤</u> 5 business days**
Routine	**<u>≤</u> 5 business days**	**<u>≤</u> 10 business days**

Hypothetical Application to Scenario #1:

Steps 1 - 2

0 hr: event occurs
< 1 hr: specimen identified/processed afield, designated contact informed
< 2 hrs: agency administrators informed, event description preparation initiated
< 12 hrs: event description approved and emailed to lake committee members

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Steps 3 - 5

0 hr: event description received by lake committee members via email
< 12 hrs: lake committee members seek clarification from responsible agency, develop lake committee comments on draft talking points with GLFC assistance as needed, send comments to responsible agency, CLC, CGLFA, GLFC
< 16 hrs: comments from all Plan groups emailed to responsible agency
< 24 hrs: final talking points from responsible agency distributed to designated contacts of all agencies.

Scenario #1 (continued): **New invasive species unexpectedly collected by a signatory agency during fish sampling in a Great Lake.**

Event Description Form

[x] **Draft** [ ] **Final**

[x] **URGENT** [ ] **Important** [ ] **Routine**

Species: *name*

Location: near tributary to Great Lake; lat/long coordinates are xxxxxx/xxxxxx

Event time/duration: date(s)

Responsible agency: signatory agency

Contact person/e-mail: designated by signatory agency

Type: [X] **Unexpected** [ ] **Planned**

Information category: [X] **Population status** [ ] **Impacts**

Activity:

[ ] **Management**	[ ] **Research**
[ ] prevention	[ ] population status
[ ] surveillance	[ ] ecological impacts
[ ] response	[ ] fishery impacts
[ ] suppression	[ ] tools/techniques
[ ] control	[ ] other

Activity boxes were not marked because this was an unexpected event during non-AIS sampling. Had it been an AIS survey, the **management** and **surveillance** boxes would be checked. See Terminology/Definitions (p 3-4) in the Protocol.

Talking Points (bullets):

- An invasive [*name*] was unexpectedly collected during a routine fish community assessment survey by the agency, the first record of [*name*] arrival in this Great Lake.
- Specimen measured xxx mm (TL), weighed xxxx g at capture (include photo if possible)
- Field identification was made by agency biologists; confirmation and further examination of the specimen is being conducted at/by xxxxxxxxxxxxxx.
- Native to *wherever*, the [*name*] was introduced to North America via *likely pathway*
- The [*name*] is a nearshore predator/omnivore/ etc., potentially able to impact local fish communities if they establish an abundant population in the lake.
- Agency is leading investigations to determine how fish entered this lake and feasible management options to guard against impacts from this invader.

Supporting information: (attach additional files or links as necessary)

http://www.gsgp.org/news/great-lakes-st-lawrence-governors-premiers-add-five-least-wanted-ais/
https://www.invasivespeciesinfo.gov/

<u>Scenario #2</u>: New evidence of spread by invasive fish already in a Great Lake, from targeted sampling in a new area for that species.

Event Description Form

[x] **Draft** [ ] **Final**

[ ] **URGENT** [x] **Important** [ ] **Routine**

Species: *name*

Location: somewhere in a Great Lake; lat/long coordinates are xxxxxx/xxxxxx

Event time/duration: date(s)

<u>Responsible agency:</u> signatory agency

Contact person/e-mail: designated by signatory agency

Type: [ ] **Unexpected** [x] **Planned**

Information category: [X] **Population status** [ ] **Impacts**

Activity: [X] **<u>Management</u>** [ ] **<u>Research</u>**

[ ] prevention	[ ] population status
[X] surveillance	[ ] ecological impacts
[ ] response	[ ] fishery impacts
[ ] suppression	[ ] tools/techniques
[ ] control	[ ] other

Defined terms have been highlighted to demonstrate potential usage below

Talking Points (bullets):

- An invasive [*name*] was collected during annual targeted ***surveillance*** to detect its possible ***spread*** to an area of the lake where it has not been previously detected.
- Specimen(s) measured xxx mm (TL), weighed xxxx g at capture (include photo if possible)
- The [*name*] arrived in this lake in xxxx; population appears to be [*increasing/stable/decreasing*] since then based on ***surveillance*** with evidence of [***survival/reproduction***]
- It is believed to have ***spread*** via natural population expansion and fish movement.
- The time lags between **arrival** and **establishment** provide important information to managers as they undertake adaptive ***response*** efforts and consider feasible options for ***suppression or control***.
- Native to [*wherever*], the [*name*] was introduced to North America via [*likely pathway*]
- The [*name*] is a nearshore [*predator/omnivore/etc*.], potentially able to ***impact*** local fish communities, particularly after ***establishment*** in the lake.
- Studies are planned to assess potential ***ecological consequences*** from the expanding population of [*name*] on the native fish community.

<u>Scenario #3</u>: Plans for a coordinated project to slow the spread of an invasive fish through removals.

Event Description Form

[x] **Draft** [ ] **Final**

[ ] **URGENT** [ ] **Important** [x] **Routine**

Species: *name*

Location: somewhere in a Great Lake; lat/long coordinates are xxxxxx/xxxxxx

Event time/duration: date(s)

<u>Responsible agency:</u> signatory agency

Contact person/e-mail: designated by signatory agency

Type: [ ] **Unexpected** [x] **Planned**

Information category: [X] **Population status** [ ] **Impacts**

Activity: [X] **<u>Management</u>** [x] **<u>Research</u>**

[ ] prevention	[ ] population status
[ ] surveillance	[ ] ecological impacts
[x] response	[ ] fishery impacts
[ ] suppression	[x] tools/techniques
[ ] control	[ ] other

This example demonstrates a hypothetical fish removal effort that uses various gears/techniques to adaptively remove fish over several days, potentially qualifying as both management and research.

Talking Points (bullets):

- ***Surveillance*** indicates that invasive [*name*] are increasing in abundance in [*specific area of a lake*] and are likely to ***spread*** to other areas of the lake.
- Studies show that [*name*] tend to aggregate in [*specific area*] during [*season*], affording an opportunity to efficiently capture and remove as many fish as possible.
- Several agencies will be working collaboratively to increase collection effort as part of a coordinated interagency adaptive ***response*** effort.
- Various collection techniques will be used to determine the most effective means for capturing these fish.
- Additional ***surveillance*** will be conducted to evaluation of the effectiveness of this effort.

Supporting information: (attach additional files or links as necessary)

- Response/management plans, if available

Scenario #4: Silver carp eDNA in Sandusky Bay, Ohio

Event Description Form

NOTE: This information is confidential, not for distribution or use beyond intended audiences.

[X] **Draft** [ ] **Final**

[ ] **URGENT** [X] **Important** [ ] **Routine**

Species: Silver Carp and Bighead Carp eDNA

Location: Sandusky Bay and Maumee Bay, Lake Erie

Event time/duration: April 22, 2019

Responsible agency: Ohio DNR Division of Wildlife

Contact person/e-mail: John Navarro john.navarro@dnr.state.oh.us

Type: [ ] **Unexpected** [X] **Planned**

Information category: [X] **Population status** [ ] **Impacts**

Activity:

[X] **Management**	[ ] **Research**
[ ] prevention	[ ] population status
[X] surveillance	[ ] ecological impacts
[ ] response	[ ] fishery impacts
[ ] suppression	[ ] tools/techniques
[ ] control	[ ] other

This example demonstrates lengthier, more descriptive talking points than in previous scenarios.

Talking Points (bullets):

- The Ohio Department of Natural Resources (ODNR), Michigan Department of Natural Resources (MDNR), and U.S. Fish and Wildlife Service (USFWS) are collaborating to assess the current status of bighead and silver carp within western Lake Erie bays and select tributaries.
- Laboratory results received earlier this month indicated the presence of Asian carp environmental DNA (eDNA) in 6 of the 417 water samples collected in April 2019. Four samples from Sandusky Bay, in Ohio waters, tested positive for bighead carp eDNA, while two samples from north Maumee Bay, in Michigan waters, were positive for silver carp eDNA.
- The findings indicate the presence of genetic material left behind by the species, such as scales, excrement or mucous, but not the establishment of Asian carp in Lake Erie. Bighead and Silver Carp eDNA can come from other sources (ex. bird droppings, boats and

equipment from infested waters) and is not a positive indication of the presence of live fish but is an indication that fish may be present.

- Initial surveys began this week and are focusing on the collection of water samples for eDNA analysis. Electroshocking and netting survey efforts will also be conducted starting next week. The eDNA surveys will occur in the Sandusky River and Bay, and the Maumee River and Bay. Samples will be collected in the areas where positive eDNA samples were collected in 2019 and at additional locations believed to provide suitable bighead and silver carp habitat.
- MDNR and ODNR requested assistance from the USFWS to develop and implement this assessment effort. The USFWS is contributing significant technical and logistical expertise, as well as personnel, survey equipment and vessels. The USFWS will analyze the collected eDNA water samples.
- Since 2010, numerous have partnered to collect water samples from Great Lakes basin waters, including southern Lake Michigan, western Lake Erie and tributary streams of lakes Michigan and Erie. The collaborative early-detection Asian carp surveillance program is funded by the USFWS with a federal Great Lakes Restoration Initiative grant.
- Asian carp, including bighead and silver carp, pose a significant threat to the Great Lakes ecosystem, the $7 billion dollar fishery, and other economic interests dependent on the Great Lakes and its tributaries. Silver and bighead carp are likely to compete with native and recreational fish species and are known to quickly reproduce. Anglers are urged to become familiar with the identification of Asian carp, including both adults and juveniles, as the spread of juvenile Asian carp through the use of live bait buckets has been identified as a potential point of entry into Great Lakes waters.
- MDNR and ODNR are committed to the conservation, protection, management, use and enjoyment of the region's natural and cultural resources for current and future generations. The mission of the U.S. Fish and Wildlife Service is working with others to conserve, protect and enhance fish, wildlife, plants and their habitats for the continuing benefit of the American people.

Supporting information:

- USFWS Bighead & Silver Carp eDNA Early Detection Results: https://www.fws.gov/midwest/fisheries/eDNA/results/ohio/2017-06-06/2017-06-06.html

Scenario #5: Northern Snakehead in Lake Ontario watershed, New York

Event Description Form

NOTE: This information is confidential, not for distribution or use beyond intended audiences.

☐ **Draft** ☒ **Final**

☐ **URGENT** ☒ **Important** ☐ **Routine**

Species: *Channa argus*

Location: Oswego River, (Oswego Co., NY; tributary to Lake Ontario's east end)

Event time/duration: May XX, 2018

Responsible agency: NYS Department of Environmental Conservation (NYSDEC)

Contact person/e-mail: Steve Hurst, Chief, Bureau of Fisheries steve.hurst@dec.ny.gov

Type: ☒ **Unexpected** ☐ **Planned**

Information category: ☒ **Population status** ☐ **Impacts**

Activity:

☒ **Management**	☐ **Research**
☐ prevention	☐ population status
☒ surveillance	☐ ecological impacts
☐ response	☐ fishery impacts
☐ suppression	☐ tools/techniques
☐ control	☐ other

This example demonstrates a real-world event.

Talking Points (bullets):

- As previously reported, the Nature Conservancy reported to NYSDEC positive eDNA detections for Northern snakehead (NSH) at several locations in the Oswego River during experimental testing in 2017.
- Those samples were re-analyzed, along with new samples collected in 2018, by Christopher B. Rees and Meredith L. Bartron at the USFWS Northeast Fishery Center, Lamar, Pennsylvania. All tests results were negative (see report below).
- NYSDEC will continue to work with the U.S. Fish and Wildlife Service to periodically test Oswego River water samples for NSH eDNA.
- A formal announcement regarding this event is not warranted.

Supporting information: (see appended document)

Supporting information:

Northern Snakehead May 2018 eDNA Analysis Results:
Seneca River and Oswego River
Report prepared by:
Christopher B. Rees and Meredith L. Bartron, USFWS Northeast Fishery Center, Lamar, Pennsylvania, June 27th, 2018
Report prepared for:
Dave Adams, New York Department of Environmental Conservation, Albany, New York
Sandra Keppner and Theodore Lewis, USFWS Lower Great Lakes Fish and Wildlife Conservation Office, Basom, New York

Background
Northern snakehead (*Channa argus*) are an invasive species of concern for many state and federal agency early detection programs in the Great Lakes region. The New York State Department of Environmental Conservation (NYSDEC), U.S. Fish and Wildlife Service (USFWS), and other partners have been focusing survey efforts to assess the presence of Northern snakehead through traditional gear and environmental DNA (eDNA) methods in various locations throughout the Oswego River, New York. Although the majority of eDNA sampling has resulted in negative samples with a couple of exceptions, a purported capture of a live Northern snakehead occurred in spring of 2018 on the Seneca River, New York downstream of Cayuga Lake, New York. In an effort to determine potential Northern snakehead eDNA distribution within the Oswego River and/or Seneca River, and to aid in deployment of traditional fisheries gear to locate live individuals, sampling was conducted in the spring of 2018 and analysis of eDNA samples by the USFWS Northeast Fishery Center Conservation Genetics Lab was requested from NYSDEC. The overall objective of our analyses was to evaluate environmental samples from the Oswego River and Seneca River collected in May 2018 at sixty high priority sites using two validated quantitative PCR markers (*Casey* and *Egan Taqman*) to test for presence of Northern snakehead DNA.

Methods
Sample collection
Samples were collected from the Oswego River and Seneca River, New York on May 30th, 2018. Sites selected for sampling were areas where previous eDNA positive detections for Northern snakehead have been reported, areas where purported Northern snakehead live captures have occurred (by recreational fishermen), or areas that contain desirable habitat for Northern snakehead (see Figures 1a – 1e). All 60 water samples collected were 1L in volume and processed by the USFWS Lower Great Lakes Fish and Wildlife Conservation Office, Basom, New York (LGLFWCO). Water samples were captured by grab sampling surface water, placed in a cooler with wet ice, filtered with 1.5 µM borosilicate glass fiber filters on-site in a dedicated mobile eDNA sampling trailer (Table 1), and placed at -20°C until delivery to the analysis laboratory. USFWS Northeast Fishery Center Conservation Genetics Lab, Lamar, Pennsylvania (NEFC) received the filter samples on May 31st, 2018 and they were immediately stored at -80°C until DNA extractions were carried out.

DNA Extractions
All filters received from the LGLFWCO May 2018 sampling were extracted in two separate extraction batches on June 6th and 7th, 2018. The filters were extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Corporation, Valencia, California) using a modified protocol. The filter extraction protocol followed that of the U.S. Fish and Wildlife Service eDNA Quality Assurance Project Plan (QAPP) for filter samples (U.S. Fish and Wildlife Service, 2016). Briefly, filters were transferred individually from sample

tubes to a Qiagen Lyse and Spin Column (Qiagen Corporation, Valencia, California) containing 375 µl Buffer ATL and 25 µl Proteinase K using a clean set of nitrile gloves and laboratory consumables. Gloves and consumables were replaced in between each filter transfer. Once all filters were transferred to the Lyse and Spin Columns, samples were incubated at 56˚C in the Buffer ATL:Proteinase K mixture according to the QAPP (U.S. Fish and Wildlife Service, 2016). Filter digestions were then centrifuged at 16,000 x g for 1 minute, filters remaining in Lyse and Spin Columns archived at -80˚C, and remaining eluate mixed with 375 µl Buffer AL and 375 µl 100% ethanol. All remaining steps of the DNA extraction followed the manufacturer's protocol. In addition to the environmental filter samples extracted, NEFC also included several extraction negative controls (only elution buffer added or blank filter) and positive extraction controls (Northern snakehead fin clip DNA pipetted on the filter and co-extracted at the same time as all other filters). During the elution step of DNA extractions, all samples were eluted with 200ul of Buffer AE. In cases where multiple filters were needed to filter the sampled river water, filters were extracted individually, eluted with 200ul of Buffer AE, then pooled into the same DNA extraction vial. All samples were extracted in a dedicated DNA extraction room with mechanical controls/hoods to maintain a clean, contamination-free work environment. Samples were stored at -20°C until quantitative PCR analysis.

Northern snakehead assays

Two fluorescent qPCR probe-based markers were used for the detection of Northern snakehead DNA. One marker was developed by Dr. James W. Casey, Cornell University, New York ("*Casey* marker", pers.communication, unpublished). The second marker was based on a marker developed by Egan (Egan et al. report to EPA) and modified by Chris Rees, NEFC, by adding an internal probe in order to use as a qPCR TaqMan® assay ("*Egan TaqMan®"*). The qPCR probe for each primer-probe assay was a TaqMan® MGB (Minor Groove Binder, Applied Biosystems™, Waltham, Massachusetts) probe labeled on the 5' end with 6-FAM. Both of these probe-based markers were recently carried through validation protocols at NEFC (Rees and Bartron, 2018) and have been shown to be both highly specific and sensitive in the amplification of Northern snakehead DNA.

qPCR reactions were run in 20µl volumes and included 17µl of master mix/primer/probe mixture and 3µl of DNA template. All qPCR reactions were analyzed on an ABI ViiA7 PCR Thermalcycler (Applied Biosystems™, Waltham, MA). Reaction concentrations and cycling conditions for qPCR analysis for all samples analyzed using the Northern snakehead markers can be found in Rees and Bartron (2018). Both Northern snakehead qPCR markers (*Casey* and *Egan TaqMan*) were used to evaluate the field-collected samples from New York from the sampling effort in May 2018. Each of the environmental samples was analyzed in octet reactions (8 PCR replicates per field sample) for the two Northern snakehead qPCR markers used.

Inhibition tests

Following qPCR analysis of all environmental samples, NEFC tested for the presence of PCR inhibition by running triplicate PCR reactions for each of the field samples by using the TaqMan® Exogenous IPC (Internal Positive Control) Reagents Kit (Applied Biosystems™, Waltham, MA, USA). qPCR IPC reactions were run in 20µl volumes and included 17µl of master mix/primer/probe mixture and 3µl of DNA template. Cycling conditions were based on manufacturer's recommendations and carried out for 40 cycles.

Positive Scoring Criteria

A cycle threshold (*Ct*) of 40 was chosen as the cutoff threshold for all primer-probe sets in this study. Therefore, only samples with *Ct* values that were 40 or less in at least one of the PCR replicates were considered positive. Cycle threshold cutoff is entirely dependent on the efficiency of the primer and

probe set and as a result will vary among different qPCR assays. In general, assay completion at > 40 cycles is suspect because of the implied low efficiency (Burns and Valdivia, 2008; Bustin et al., 2009) and as *Ct* increases, the likelihood of false positives also increases due to thermal or random probe cleavage, amplicon artifacts, or primer dimers (Caraguel et al., 2011). More importantly, initial testing of the primers and probe of the Northern snakehead assays tested in this study (Rees and Bartron, 2018) demonstrated high assay efficiency (*E* = 100% ± 5%) with replicated 10 copy gBlock standard reactions resulting in *Ct*=36. Therefore, amplification of dilute samples (approaching concentrations of just 1 copy) should be detected between a *Ct* of 36 and 40.

Results

Environmental Samples

All 60 environmental samples collected in the Oswego River and/or Seneca River were negative for the presence of Northern snakehead DNA using both the *Casey* marker as well as the *Egan TaqMan* marker (Table 2). Both positive extraction controls amplified in all 8 PCR replicates for each Northern snakehead marker, and combined with the expected performance of negative controls, amplifications for the field samples were of high integrity.

All additional extraction and PCR negative and/or positive controls performed as expected in both assays.

Inhibition Tests

PCR inhibition was absent for 58 or the 60 environmental samples collected in this effort. Mean cycle threshold (*Ct*) values from 58 replicates for environmental samples during the internal positive control tests was *Ct* of 26.83 (SD ± 0.07). Various criteria exist as to the appropriate threshold, but general guidelines are that an increase in *Ct* of at least 2 from the mean *Ct* for all internal positive control reactions indicates that PCR inhibition is a problem in a reaction.

For two samples, IPC tests demonstrated slight inhibition for OSW14 (mean *Ct* = 28.31, increase of 1.48 *Ct* over the mean) and major inhibition for OSW59 (mean *Ct* = 32.95, increase of 6.12 *Ct* over the mean). To reduce the potential of inhibition, both samples were purified using the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Irving, CA, USA). After sample clean-up, IPC tests demonstrated both samples were free of inhibition (no deviation from the average *Ct*). These samples were then re-screened for the presence of Northern snakehead DNA in 8 additional octet reactions for each marker (*Casey* and *Egan TaqMan*) and both were found to be negative.

Summary

Using the two primer-probe assays, the *Casey* marker and the modified *Egan TaqMan* marker, Northern snakehead DNA was not detected on any of the filters provided to the NEFC by LGLFWCO from the Oswego River and Seneca River in May 2018. After PCR inhibitor clean-up in two samples (OSW14 and OSW59), PCR inhibition was not detected in any of the field samples tested, therefore lack of amplification of Northern snakehead DNA was not due PCR inhibitors limiting amplification in the samples. Similarly, because all positive and negative control samples performed as expected, lack of detection of Northern snakehead DNA is interpreted to reflect no Northern snakehead DNA was present in the filtered water samples from the Oswego River or Seneca River included in this analysis.

Acknowledgements

This work was made possible by Sandra Keppner, Ted Lewis, and crew from the USFWS Lower Great Lakes Fish and Wildlife Conservation Office, Rob Williams and crew of SLELO-PRISM/TNC, Dave Lemon

and crew of NYSDEC, and Greg Cocquyt of NYSDEC Region 7 Fisheries. Thank you to Dr. Jim Casey for providing his primer and probe sequences.

References

Burns, M., and Valdivia, H. (2008). Modelling the limit of detection in real-time quantitative PCR. Eur. Food Res. Technol. *226*, 1513–1524.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., et al. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin. Chem. *55*, 611–622.

Caraguel, C.G.B., Stryhn, H., Gagné, N., Dohoo, I.R., and Hammell, K.L. (2011). Selection of a Cutoff Value for Real-Time Polymerase Chain Reaction Results to Fit a Diagnostic Purpose: Analytical and Epidemiologic Approaches. J. Vet. Diagn. Invest. *23*, 2–15.

Egan, S., D. Lodge, M. Pfrender, J. Feder, C. Jerde, S. Ruggiero, C. Tanner, C. Gantz, D. Erickson. Improving e-DNA-based surveillance programs for high risk potentially invasive species. Final report to EPA, EPA-R5-GL2012-1. 85 pg.

Rees, C.B. and Bartron, M.L. (2018). Northern Snakehead Fall 2017 eDNA Sampling Results for Oswego River, New York. Report to New York State Department of Environmental Conservation. U.S. Fish and Wildlife Service (2016). Quality Assurance Project Plan

Scenario #6: Larval grass carp in Maumee River, USGS

Event Description Form

NOTE: This information is confidential, not for distribution or use beyond intended audiences.

[X] **Draft** [ ] **Final**

[ ] **URGENT** [X] **Important** [ ] **Routine**

Species: Grass Carp (larvae)

Location: Maumee River, 280 Bridge and near Brenner's Marina

Event time/duration: 13 and 26 June 2018

Responsible agency: US Geological Survey

Contact person/e-mail: Patrick Kočovský (pkocovsky@usgs.gov)

Type: [x] **Unexpected** [ ] **Planned**

Information category: [x] **Population status** [ ] **Impacts**

Activity:

[ ] **Management**	[x] **Research**
[ ] prevention	[x] population status
[ ] surveillance	[ ] ecological impacts
[ ] response	[ ] fishery impacts
[ ] suppression	[ ] tools/techniques
[ ] control	[ ] other

This example demonstrates a real-world event.

Talking Points (bullets):

- A University of Toledo Crew funded by and collaborating with the US Geological Survey captured 6 fish suspected of being larval Grass Carp in separate surveys conducted on 13 June and 26 June during suspected spawning events. Fertilized eggs were captured on the same dates. Samples were preserved in the field. Processing of the samples containing the suspected larvae was completed in late December.
- Suspected larvae were genetically confirmed as Grass Carp at the USGS UMESC lab in La Crosse, WI in early February. The delay between finding the larvae and genetic confirmation was due to the lapse in federal appropriations.
- These are the first larval Grass Carp captured in a Great Lakes River, confirming natural reproduction is occurring in the Maumee River.
- Grass Carp have been known to spawn in the Maumee River since 2017 when the first genetically-confirmed eggs were collected.

- This finding does not permit any conclusions or change our understanding of Grass Carp in the Lake Erie system (previous otolith microchemistry evidence linked fertile, naturally-reproduced Grass Carp to the Maumee River).
- USGS continues to work with Ohio DNR and other Federal, Provincial, and State agencies to develop control methods.

Supporting information: (attach additional files or links as necessary)

Summary Table of Actual Event Descriptions

#	YEAR	EVENT TYPE	SPECIES	LIFE STAGE	LOCATION	RESPONSIBLE AGENCY	COMMENT
1	2019	important	grass carp	egg	Sandusky R., OH	USGS	new knowledge
2	2019	important	grass carp	adult	Sandusky R., OH	ODNR	removal
3	2019	important	ruffe	adult	St. Marys R., MI	USFWS	range expansion
4	2019	important	tubenose goby	adult	Cheboygan R., MI	USFWS	range expansion
5	2019	routine	grass carp	adult	Cuyahoga R., OH	ODNR	removal
6	2019	important	silver carp	eDNA	Sandusky R., OH	ODNR	new '+' detection
7	2020	important	grass carp	adult	Tittawabassee R, MI	MDNR	1st known diploid
8	2020	important	grass carp	adult	Jordan Harbour, ON	DFO	1st at location
9	2021	important	grass carp	adult	St. Joseph, Galien R, MI	MDNR	two fish, bow kills
10	2021	important	bighead carp	eDNA	Sandusky R., OH	ODNR	one '+' sample
11	2021	important	grass carp	adult	Little Calumet R, IN	IDNR	bow kill
12	2021	important	grass carp	adult	Milwaukee R, WI	WDNR	1st since 2015
13	2021	important	grass carp	adult	Muskegon Lake, MI	MDNR	1st known diploid
14	2021	important	bighead carp	eDNA	Milwaukee R, WI	WDNR	new '+' detection
15	2021	important	bighead carp	eDNA	Milwaukee R, WI	WDNR	2nd '+' detection
16	2021	routine	grass carp	adults	Huron, Grand R, OH	ODNR	1st known diploids
17	2021	important	e.b. killifish	adults	Lake Michigan, WI	WDNR	new knowledge
18	2022	important	silver carp	eDNA	Presque Isle, PA	PFBC	new '+' detection
19	2023	important	grass carp	egg	Huron R, OH	ODNR	new knowledge
20	2023	important	silver carp	eDNA	Maumee R, OH	ODNR	new knowledge
21	2023	routine	grass carp	eDNA	Presque Isle, PA	PFBC	rare occurrence
22	2023	important	silver carp	eDNA	St Joseph R, MI	MDNR	new '+' detection