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First direct confirmation of grass carp spawning in a Great Lakes tributary

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ABSTRACT

Grass carp (*Ctenopharyngodon idella*), an invasive species of Asian carp, has been stocked for many decades in the United States for vegetation control. Adult individuals have been found in all of the Great Lakes except Lake Superior, but no self-sustaining populations have yet been identified in Great Lakes tributaries. In 2012, a commercial fisherman caught four juvenile diploid grass carp in the Sandusky River, a major tributary to Lake Erie. Otolith microchemistry and the capture location of these fish permitted the conclusion that they were most likely produced in the Sandusky River. Due to this finding, we sampled ichthyoplankton using paired bongo net tows and larval light traps during June–August of 2014 and 2015 to determine if grass carp are spawning in the Sandusky River. From the samples collected in 2015, we identified and staged eight eggs that were morphologically consistent with grass carp. Five eggs were confirmed as grass carp using quantitative Polymerase Chain Reaction for a grass carp-specific marker, while the remaining three were retained for future analysis. Our finding confirms that grass carp are naturally spawning in this Great Lakes tributary. All eggs were collected during high-flow events, either on the day of peak flow or 1–2 days following peak flow, supporting an earlier suggestion that high flow conditions favor grass carp spawning. The next principal goal is to identify the spawning and hatch location(s) for the Sandusky River. Predicting locations and conditions where grass carp spawning is most probable may aid targeted management efforts.

Introduction

Multiple species of invasive Asian carp have been monitored for potential range expansion into Great Lakes watersheds for years and are considered threats to ecological function of the lakes (Mills et al., 1993). Grass carp (*Ctenopharyngodon idella*) differ from other potential invaders because after their import to the United States in 1963, triploid individuals have been widely stocked for vegetation control since 1983 (Rasmussen, 2011). These triploid fish are intended to be functionally sterile and therefore incapable of founding naturally reproducing populations (Zajicek et al., 2011). Stocking of triploid individuals has been legally approved in multiple states, including Ohio (Chapman et al., 2013). Nevertheless, errors in the production of triploid individuals, illegal stockings of diploids, and the live fish trade have resulted in the potential for naturally reproducing grass carp populations to establish in unplanned locations (Wittmann et al., 2014). Adult grass carp individuals have been found in all of the Great Lakes except Lake Superior, but no self-sustaining populations have yet been verified in Great Lakes tributaries (Kocovsky et al., 2012).

In 2012, a commercial fisherman caught four juvenile diploid grass carp in the Sandusky River, a major tributary to Lake Erie (Chapman et al., 2013). Otolith microchemistry indicated that these fish were most likely produced in the Sandusky River due to the elevated strontium:calcium ratio distinctive of the Sandusky River (Chapman et al., 2013). Based on the age of these fish, it was established that all individuals were most likely spawned during a high-flow event occurring July 23–29, 2011 (Chapman et al., 2013). Multiple studies have found that the Sandusky River would be a suitable spawning and recruitment habitat for grass carp based on hydraulic characteristics (channel velocity, shear velocity, and temperature) and undammed river length (Garcia et al., 2015; Kocovsky et al., 2012; Murphy and Jackson, 2013). Therefore, we focused sampling efforts on the Sandusky River to determine if there was evidence of naturally spawning populations.

Grass carp are thought to require large, turbid rivers for reproduction (Stanley et al., 1978). In China, the native range for grass carp, spawning is correlated with high-flow events (Duan et al., 2009; Tan et al., 2010). This correlation has been found to exist in the non-native

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range in the United States, with mass spawning events occurring primarily on the rising portion of significant peaks in the hydrograph (Chapman et al., 2013). Spawning during high-flow events may be adaptive due to increased turbulence. Grass carp spawn near the surface and hatching success is greatest when their semi-pelagic eggs remain in suspension in the water column before hatching (George et al., 2015). Additionally, laboratory and field measurements have shown that Asian carp spawning success declines at temperatures below 18 °C, thus this temperature is considered to be the minimum thermal threshold for spawning (Kolar et al., 2005). Following egg hatching, larvae swim vertically while drifting downstream until gas bladder inflation (George and Chapman, 2015). They then actively swim from the fast-flowing channel into still backwater areas where they mature (George and Chapman, 2015). The Sandusky River is turbid, experiences high-flow events, and exceeds the thermal minimum for spawning and development, and is therefore suitable for grass carp reproduction.

Due to their voracious appetite and large adult size, grass carp have the ability to alter vegetation structure, thus affecting native communities of fishes and invertebrates, as well as water quality (Mandrak and Cudmore, 2010). Possible specific detrimental effects resulting from the removal of submerged macrophytes include the reduction of critical spawning and recruitment areas for native fishes, decreased mitigation of nonpoint source pollution, and increased turbidity and shoreline erosion (Chapman et al., 2013; Wilson et al., 2014). The Great Lakes have fisheries valued at more than $7 billion annually and provide drinking water for 40 million people, and these ecosystem services could be damaged by grass carp (Cuddington et al., 2013; Wilson et al., 2014). Therefore, early detection and a rapid management response are necessary to prevent detrimental effects of grass carp to the Great Lakes basin.

As a principal step in determining the threat of grass carp in the Great Lakes, it is necessary to verify that naturally reproducing populations exist. Here we report on the sampling efforts we undertook in the Sandusky River during the summers of 2014 and 2015 for the presence of grass carp spawning. We targeted high-flow events in the main channel to detect eggs and slow-water areas for larvae. In addition to the first documented evidence of spawning, we aimed to provide information that can aid targeted management efforts.

Methods

The Sandusky River is the third largest tributary to the western basin of Lake Erie, flowing for approximately 215 km into the lake at Sandusky Bay (Fig. 1). There are six dams on the Sandusky River, the downstream-most at Ballville, approximately 25 km from the mouth at Muddy Creek Bay. Ballville Dam is impassable; hence the primary study area is the length from Ballville Dam to Muddy Creek Bay. Some areas of this portion nearest Fremont, Ohio are ~1 m deep, with the majority of the river ~5–6 m deep during low-flow conditions. For this portion of the Sandusky River, width varies between ~32 and 160 m, but at our sampling locations ranged between ~80 and 120 m wide.

To determine if grass carp eggs were present in the stretch of the Sandusky River below Ballville Dam, we sampled ichthyoplankton during June–August of 2014 (pilot study) and 2015 (full sampling implemented). We hypothesized that spawning might occur approximately 1 km downstream of the Ballville Dam, in Fremont, Ohio due to the characteristic turbulent water and shallow depths of this reach (Kocovsky et al., 2012). Asian carp eggs are semi-buoyant and it is thought that they need to remain suspended in order to hatch (Stanley et al., 1978). In the Sandusky River, Asian carp eggs have an increased probability of settling beyond ~15–16 km of the spawning site (Garcia et al., 2013). Therefore, the area we sampled included sites extending a total of ~10 km downstream of Fremont, Ohio to 11 km upstream of Muddy Creek Bay (Fig. 1).

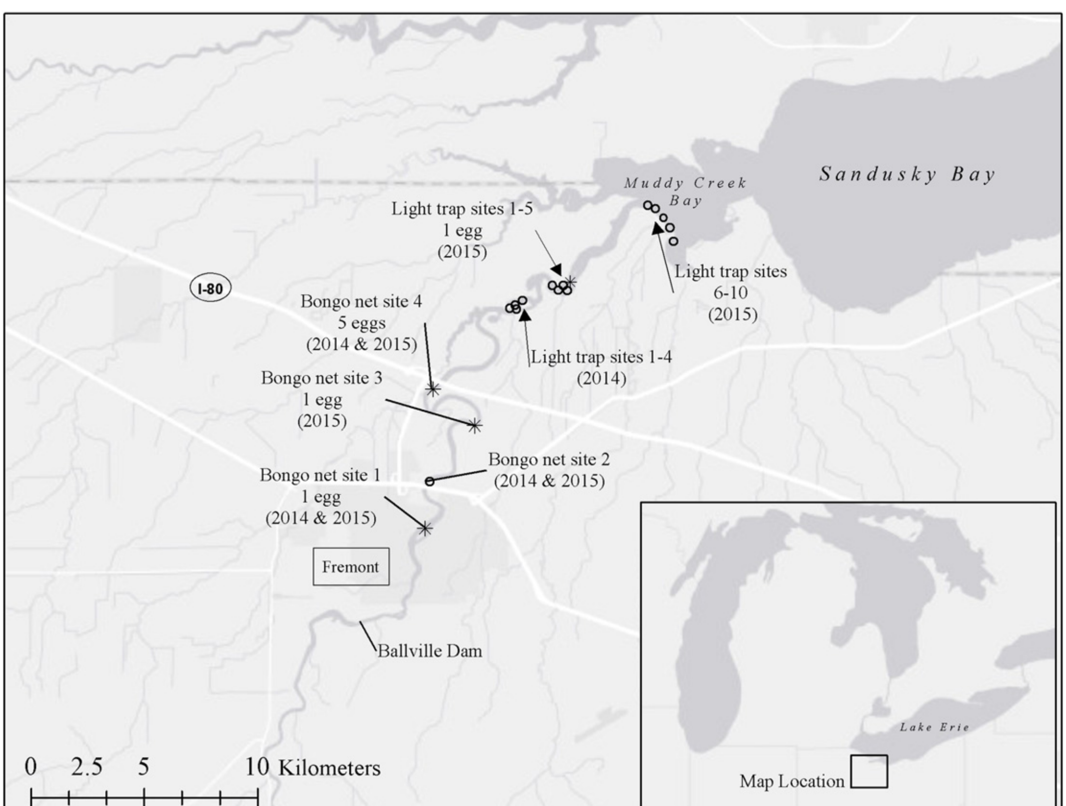

Fig. 1. Sampling locations in the Sandusky River in 2014 and 2015. Sites where eggs were collected are designated with *, while sites where no eggs were collected are marked with °.

During June–August of 2014, we conducted a pilot study to establish methods for sampling the Sandusky River for grass carp ichthyoplankton. Three sites were sampled on a weekly basis regardless of flow conditions (Fig. 1). Each sample was collected with a bongo net (0.5 m diameter each and 500 μm mesh). The nets were deployed during the day in a fixed position from the bow of a small boat (4 m) while the boat was held stationary against the current. The net was fished for 5 min. We fished just below the surface due to shallow water at our upstream-most site and to avoid snags. We estimated sample volume using General Oceanics 2030R flow meters placed in each of the net openings. In 2015, we sampled four sites once a week, except during high-flow events when sampling was increased to three times a week (Fig. 1). We intensified sampling during high-flow events to increase the likelihood that we would capture eggs if grass carp spawned. At each of the sites, we sampled two points separated by a minimum width of 15 m.

Additionally, during July and August of 2014 and 2015, we sampled larval fish using quadrafoil light traps constructed of polycarbonate as designed by Aquatic Research Instruments (for complete description and photo examples, see http://www.aquaticresearch.com/aquatic_invertebrate_light_traps.htm). Light traps were deployed in backwater areas approximately 6 km upstream of and at the mouth of the Sandusky River in Muddy Creek Bay. These sites were selected based on the distance from the hypothesized spawning site and because they were slow-flowing, vegetated areas characteristic of grass carp rearing habitat (Stanley et al., 1978). In the preliminary 2014 study, light trap sampling was conducted at night on four dates in the Sandusky River (Fig. 1). In 2015, light traps were deployed once weekly at night at five sites in the Sandusky River and five sites in Muddy Creek Bay (Fig. 1). During both years, light traps were set for 1 h no earlier than one half hour post-sunset. At each site, three light traps were set in one of three habitat types: vegetation, wood, or open water. In both years, four replicates of traps for each habitat type were fished each night. We identified and enumerated the eggs and larvae from collected samples following Auer (1982) and Yi et al. (2006). Egg stages were classified following Yi et al. (2006).

Grass carp are thought to require high-flow events for spawning, therefore we needed to determine when high-flow events were occurring. We monitored mean daily river discharge (water volume/day) provided by the USGS National WaterWatch website (http://waterwatch.usgs.gov/index.php?r=oh&id=ww_current) at the National Stream Quality Accounting Network Station 04198000 located in Fremont, Ohio, 9 km upstream of the first bongo net site. We considered a high-flow event to occur when river discharge exceeded approximately 31 m^3/s, because this corresponds to the flow when most Asian carp eggs will remain suspended in the Sandusky River (Murphy and Jackson, 2013).

We assessed thermal suitability for spawning by calculating dates on which published thermal thresholds for adult maturation were achieved. Grass carp are believed to require 633 annual degree-days greater than 15 °C (ADD15) to reach spawning maturation (Gorbach and Krykhtin, 1980), which we calculated using mean daily water temperature (°C) taken at 1.5 m (5 ft) below low water datum (LWD) of Lake Erie. We used data from the NOAA monitoring station 9063079 located in Marblehead, Ohio near Sandusky Bay accessed from the Tides and Currents website (http://tidesandcurrents.noaa.gov/stationhome.html?id=9063079#sensor, accessed 12/27/2015). Dates of achievement of thermal thresholds were compared to dates of high-flow events to determine if thermal thresholds were met prior to high-flow events.

A subset of eggs that were identified as possible grass carp based on morphological characteristics was verified by genetic testing. Eggs for genetic testing were preserved in 70% ethanol. DNA was extracted with an AutoGen 245 system (AutoGen, Inc.) according to the manufacturer's protocol. Putative grass carp egg DNA samples were first screened with quantitative Polymerase Chain Reaction (qPCR) using a primer-probe set directed against an 83-bp portion of the mitochondrial cytochrome oxidase I (COI) gene as described, with modifications (Wilson et al., 2014). No contamination was observed in any of the qPCR runs.

Samples positive for grass carp mitochondrial DNA in the qPCR assay were verified by DNA sequencing of a 655-bp portion of the COI gene. Silver carp, bighead carp, and grass carp genomic DNA samples were amplified and sequenced alongside the egg samples as negative and positive controls. Primers FishF1ac_t1 5′-TGTAAAACGACGGCCAGTTCTACAAACCACAAAGACATTGGTAC-3′ and FishR2ac_t1 5′-CAGGAAACAGCTATGACTARACTTCYGGGTGACCAAAGAATCA-3′ were used for amplification and sequencing. The primer sequences were modified from previously published universal primers for DNA barcoding in fish (Ivanova et al., 2007). Assembled sequences were identified by Basic Local Alignment Search Tool (BLAST) against the GenBank non-redundant database.

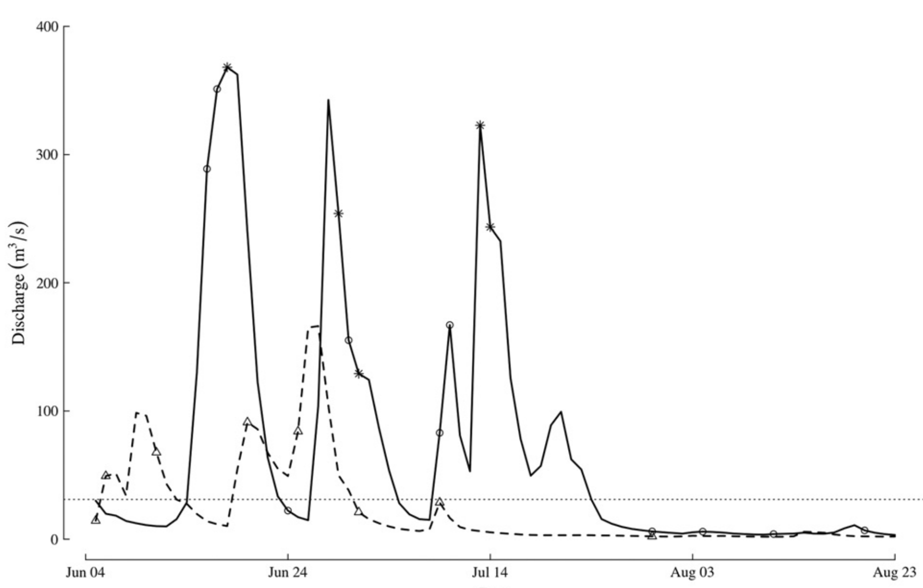

Fig. 2. Mean daily discharge (m^3/s) of the Sandusky River from June 3 to August 31, 2014 (dashed line) and 2015 (solid line). The high-flow event threshold (31 m^3/s) is shown (dotted line). Dates when ichthyoplankton were sampled in 2015 (o) and in 2014 (Δ) as well as dates when eggs were collected (*) are illustrated.

Table 1
Site locations of bongo net and light trap sampling where grass carp eggs were captured with corresponding distance from Ballville Dam and Muddy Creek Bay, number of eggs collected and their developmental stages according to Yi et al. (2006), corresponding mean daily discharge, and river water temperature measurements for the day of egg collection.

			Distance (km) from						
Site	Location	Method	Ballville Dam	Muddy Creek Bay	Dates collected	N eggs	Developmental stage(s)	Mean daily discharge (m^3/s)	Water temperature (°C)
B1	N 41.3566, W 83.1045	Bongo Net	5	20	7/13/2015	1	2	323	19.8
B3	N 41.3864, W 83.0908	Bongo Net	10	15	6/18/2015	1	8	368	22.8
B4	N 41.3972, W 83.1026	Bongo Net	14	11	6/29/2015	1	10	254	19.5
					7/14/2015	4	9, 10, 10, 12	244	21.1
LT2	N 41.4267, W 83.0503	Light Trap	21	4	7/1/2015	1	13	129	20.3

Results

There were two high-flow events that occurred in 2014 when mean daily discharge exceeded 31 m^3/s: June 6–12 and June 19–30 (Fig. 2). The peak flow of the first event in 2014 was ~98 m^3/s while the peak flow of the second event was ~166 m^3/s. During the summer of 2015, there were three high-flow events when mean daily discharge exceeded 31 m^3/s: June 15–23, June 27–July 4, and July 9–23 (Fig. 2). The first event of 2015 (June 15–23) peaked at ~370 m^3/s. The second event (June 27–July 4) had a peak flow of ~340 m^3/s. The third event was the longest and persisted for 15 days (July 9–23), with the peak flow of ~320 m^3/s. All three events achieved peak flow within five days of exceeding 31 m^3/s. The thermal threshold for maturation of 633 ADD15 was reached on June 22, 2014 and June 17, 2015.

Success of egg capture varied between years. In 2014 there were no eggs collected that were morphologically consistent with grass carp. In 2015 we identified and staged eight potential grass carp eggs on five dates (Table 1). All eggs were morphologically consistent with grass carp in that the embryo lacked an oil globule and was surrounded by a large transparent membrane (Yi et al., 2006). Five eggs were confirmed as grass carp using qPCR for a grass carp-specific marker (Wilson et al., 2014). The remaining three eggs, one from August 13 and two from August 14, were retained for future analysis. All eggs were collected during high-flow events, either on the day of peak flow or 1–2 days following peak flow (Fig. 2). Eggs were collected along a drift distance of approximately 16 km (Fig. 1). Seven eggs were collected using bongo nets, while one egg was incidentally caught in a light trap (Table 1). The developmental stages of eggs ranged from stage 2 to stage 13 (Table 1). There were no larval grass carp individuals captured in light traps either year, but a total of 2266 larval fish were collected. The mean sample volume filtered was 29.23 +/− 11.22 (sd) m^3.

Of the five eggs that tested positive for the grass carp-specific qPCR marker, four were further tested by DNA sequencing. The remaining egg was damaged during transport and yielded insufficient DNA for the sequencing procedure. Sequencing yielded 655 bp corresponding to base pairs 51–705 of the mitochondrial cytochrome c oxidase subunit I (COI) protein-coding sequence. The four sequenced egg samples (GenBank accession numbers KX060554, KX060555, KX060556, KX060557) were identical to each other and to the grass carp genomic DNA positive control. Searches of the GenBank non-redundant database with BLAST supported grass carp as the closest match to the sequenced eggs, with 99%–100% sequence identity to sequences identified as grass carp or grass carp hybrids.

Discussion

This is the first direct confirmation of spawning of grass carp in a Great Lakes tributary. Eggs were confirmed as grass carp by morphology and by two independent genetic methods, qPCR and sequencing of the DNA barcode portion of the COI gene. Thus, the eggs have been identified as grass carp to a very high degree of certainty.

All eggs were collected during high-flow events, either on the day of peak flow or 1–2 days following peak flow. This finding supports an earlier suggestion (Chapman et al., 2013) that high-flow conditions favor grass carp spawning. This pattern is consistent with Lin (1935), who reported that high magnitude increases in flow were required to trigger grass carp spawning in Chinese rivers. Although high flows were associated with spawning evidence collected in 2015, others have demonstrated that non-native populations of Asian carps have successfully spawned despite only low-magnitude changes in flow (Aliyev, 1976; Coulter et al., 2013). In the Kara-Kum Canal in Turkmenistan, several species of Asian carp, including grass carp, spawn without discernable flow changes (Aliyev, 1976). Additionally, in the Wabash River, bighead carp and silver carp, which have very similar spawning requirements as grass carp, have spawned regardless of flow increases (Coulter et al., 2013, Deters et al., 2013). Although our sampling was more intense during high-flow events, we did sample during low flows. Collectively, the weight of the evidence suggests high magnitude increases in flow are conducive, but may not be necessary, for grass carp spawning.

No evidence of spawning was found in 2014 although the conclusions we can draw from the pilot study are limited given the restricted sampling effort. It is possible that we did not detect eggs that were in fact present, but it is also possible that eggs were not present. The lack of evidence may have been related to cooler temperatures or insufficient flow events. In 2014, the 633 ADD15 believed to be required for grass carp to mature was not reached until June 22, 16 days after the first high-flow event and during the second high-flow event. Conversely in 2015, ADD15 reached 633 on June 17, five days earlier than in 2014 and during the ascending limb of that high-flow event. If the thermal thresholds for maturation are accurate and if the temperatures at the Marblehead station accurately reflect the thermal environment experienced by grass carp, then this would permit one to conclude spawning probably did not occur in 2014. This would explain why we did not sample eggs. Either of these conditions may be false. We agree with Cooke (2015), who argued that the methods used to determine thermal thresholds for grass carp maturation were unclear, and that the importance of thermal thresholds and what those thresholds might be are not yet well established. Insufficient flow events may have also limited spawning potential. The highest-magnitude flow event in 2014 achieved a peak flow of ~165 m^3/s after a gradual increase over multiple days. Conversely, the high-flow event in 2011 that most likely produced diploid juveniles previously found in the Sandusky River (Chapman et al., 2013) and the three events in 2015 during which eggs were collected all persisted for at least seven days and had rapid, substantial increases in flow, resulting in peak flows that were 280–370 m^3/s. It is also possible that both flow and temperature provide proximal spawning cues, but the factors that drive spawning behavior of grass carp are not well understood.

No grass carp larvae were collected in 2015 sampling, despite the capture of eggs. Three scenarios may explain the observed results: 1) grass carp eggs did not survive to the larval stage in the Sandusky River in 2015; 2) larval grass carp were present where we sampled, but we did not detect them; or 3) we did not sample where larvae were present. Previous efforts demonstrated that grass carp produced in the Sandusky River could survive to at least age 1.5 years (Chapman et al., 2013). If grass carp larvae were present but went undetected, future studies should aim to increase the detection probability by improving sampling design, including timing, location, effort, and sampling equipment (e.g., light source, intensity and wavelength used in light traps, and net design). Detection probability of larval fish of other species varies widely in other tributaries to Lake Erie and can depend on density of larvae and the life-history characteristics of the target species (Pritt et al., 2014). Through further sampling, we may be able to better assess the recruitment potential of grass carp, which is a critical step in determining the threat this species poses to the Great Lakes.

There is a great need for additional spawning assessments in Great Lakes tributaries as indicated by our findings. There are considerable knowledge gaps regarding the distribution, quantity, behavior, and physiological requirements of grass carp in the Great Lakes. Specifically in the Sandusky River, continuing sampling for eggs during high and low flows is necessary to clarify the relationship between flow and spawning potential. The earliest stage egg (stage 2) was collected furthest upstream while the oldest stage egg (stage 13) was collected at the downstream-most point. All eggs followed this sequence, with longer sampling distance from Ballville Dam corresponding to older eggs, indicating an upstream spawning location. Future hydrologic modeling efforts using the FluEgg fluvial drift simulation model (Garcia et al., 2015) can be used to project where eggs were spawned and where larvae will hatch to help guide sampling and control actions. Furthermore, continued sampling of larvae is necessary to determine the hatching and recruitment potential of larvae. Other sampling efforts, such as electro-fishing, are planned to identify evidence of recruitment. The presence of eggs in this tributary emphasizes the urgency for expanded sampling of early life stages of grass carp in other tributaries.

Our sampling protocol proved effective in determining the presence of grass carp spawning in Great Lakes tributaries, but it can be improved. For example, sampling more frequently during high-flow events or more thoroughly sampling the water column might increase the probability of capturing eggs. Female grass carp can release over 1 million eggs; that we captured only eight suggests we sampled the periphery of the egg plume. This protocol can be used in other similar systems to determine whether grass carp are reproducing in those locations. Identifying the distribution of grass carp in the Great Lakes is a crucial first step to informing management options in controlling or eliminating this invasive species.

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