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entitled

Assessing the Spawning Potential of Grass Carp in the Sandusky River Under Varying Conditions

by

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Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Biology

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An Abstract of
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Invasive Grass Carp (*Ctenopharyngodon idella*) have been stocked for decades in the United States for vegetation control. Adults 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. Previous research suggested natural reproduction has occurred in the Sandusky River; hence I sampled ichthyoplankton using bongo net tows and larval light traps June through August 2015 and 2016 to determine if Grass Carp were spawning. I identified and staged eight eggs that were morphologically consistent with Grass Carp. Five eggs were confirmed as Grass Carp using quantitative PCR and DNA sequencing, while three were retained for future analysis. All eggs were collected during high-flow events, either on the day of or 1-2 days following peak flow, supporting a suggestion that high-flow conditions favor Grass Carp spawning. From my egg collection findings, I used hydraulic modeling to estimate the most probable spawning and hatching locations for these eggs. Preliminary model results suggest eggs were most likely released near the hypothesized spawning site near Fremont, Ohio at river km 21.25. Hatch locations were near the mouth of the Sandusky River at Muddy Creek Bay, with the majority of eggs
likely hatching at river km 2.9. These locations will help guide future sampling efforts, inform risk assessments and aid targeted control efforts.
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Preface

Grass Carp are an herbivorous species native to China and Russia (Stanley, 1978). This fish was first introduced to the United States in 1963 for vegetation control, but in 1983, sterile (triploid) individuals began being produced to be used for stocking (Rasmussen, 2011). Adult diploid Grass Carp have been found in all of the Great Lakes except for Lake Superior, but no self-sustaining populations have been found, although naturally reproducing populations are present in the Mississippi River basin (Kocovsky et al., 2012).

Grass Carp can exceed 100 lbs., consuming up to 40% of their body weight in aquatic vegetation each day (van der Lee et al., 2016). Considering their voracious appetite, populations of Grass Carp have the potential to alter aquatic ecosystems by removing large amounts of vegetation. The removal of these plants could result in multiple adverse effects, including reducing native fish rearing locations, invertebrate habitats, as well as causing increased erosion and diminished water quality (Mandrak and Cudmore, 2010). Given the potential negative impacts of this species, there are many control efforts already in place to limit the spread of Grass Carp from self-sustaining populations in the Mississippi River basin, including an electric barrier constructed in the Chicago Area Waterways System, state regulations that prohibit stocking and possession
of live individuals for the food trade, as well as increased surveillance measures by multiple agencies (Cuddington et al., 2013).

Several studies have found that there is the potential for self-sustaining populations to persist in the Great Lakes, and specifically Lake Erie, given the number of fertile (diploid) individuals collected and available spawning habitat (Kocovsky et al., 2012; Wieringa et al., 2016). The Sandusky River, a tributary of Lake Erie, was found to be thermally suitable for spawning to occur (Kocovsky et al., 2012). In 2012, multiple juvenile Grass Carp were collected by a commercial fisherman (Chapman et al., 2013). The otolith microchemistry of these fish indicated that they were most likely produced in the Sandusky River, therefore I focused efforts to confirm the presence of spawning to this location (Chapman et al., 2013).

Here I present the collection of Grass Carp eggs in the Sandusky River, which was the first direct confirmation of Grass Carp spawning in the Great Lakes basin. Additionally, I discuss the importance of high flows for spawning to occur. Following this confirmation, I demonstrate how I developed a methodology to estimate spawning and hatching locations for these naturally spawned eggs using a combination of hydraulic and biological models. These findings can be used by management officials to better inform targeted control efforts for this species as well as fill knowledge gaps for this expanding invasive species in Great Lakes habitats.
Chapter 1

First Direct Confirmation of Grass Carp Spawning in a Great Lakes Tributary


1.1 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 illegal stockings of diploids, and the live fish trade have resulted in the potential for naturally reproducing grass carp populations to establish in 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 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.

### 1.2 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. 1-1). 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. 1-1).

![Figure 1-1: Sampling locations in the Sandusky River in 2014 and 2015. Sites in which eggs were collected are designated with *, while sites where no eggs were collected are marked with °.](image)

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. 1-1). 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. 1-1). 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. 1-1). 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. 1-1). 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’-TGTAAAACGACGGCCAGTTC TACAAACCACAAGACATTGGTAC-3’ and FishR2ac_t1 5’-CAGGAAACAG CTATGACTRACTTCTYGGTGACCAAAGAATCA-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.

1.3 Results

There were two high-flow events that occurred in 2014 when mean daily discharge exceeded 31 m³/s: June 6–12 and June 19–30 (Fig. 1-2). The peak flow of the first event in 2014 was ~98 m³/s while the peak flow of the second event was ~166 m³/s. During the summer of 2015, there were three high-flow events when mean daily discharge exceeded 31 m³/s: June 15–23, June 27–July 4, and July 9–23 (Fig. 1-2). The first event of 2015 (June 15–23) peaked at ~370 m³/s. The second event (June 27–July 4) had a peak flow of ~340 m³/s. The third event was the longest and persisted for 15 days
(July 9–23), with the peak flow of ~320 m³/s. All three events achieved peak flow within five days of exceeding 31 m³/s. The thermal threshold for maturation of 633 ADD15 was reached on June 22, 2014 and June 17, 2015.

Figure 1-2: Mean daily discharge (m³/s) of the Sandusky River from June 3-August 31, 2014 (dashed line) and 2015 (solid line). The high-flow event threshold (31 m³/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.

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 1.1). 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. 1-2). Eggs were collected along a drift distance of approximately 16 km (Fig. 1-1). Seven eggs were collected using bongo nets, while one egg was incidentally caught in a light trap (Table 1.1). The developmental stages of eggs ranged from stage 2 to stage 13 (Table 1.1). 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) m³.

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.
Table 1.1: 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. (1988), corresponding mean daily discharge, and river water temperature measurements for the day of egg collection.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Method</th>
<th>Ballville Dam</th>
<th>Muddy Creek Bay</th>
<th>Dates collected</th>
<th>N eggs</th>
<th>Developmental stage(s)</th>
<th>Mean daily discharge (m³/s)</th>
<th>Water temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>N 41.3566, W 83.1045</td>
<td>Bongo Net</td>
<td>5</td>
<td>20</td>
<td>7/13/2015</td>
<td>1</td>
<td>2</td>
<td>323</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>N 41.3864, W 83.0908</td>
<td>Bongo Net</td>
<td>10</td>
<td>15</td>
<td>6/18/2015</td>
<td>1</td>
<td>8</td>
<td>368</td>
<td>22.8</td>
</tr>
<tr>
<td>B4</td>
<td>N 41.4267, W 83.0503</td>
<td>Light Trap</td>
<td>21</td>
<td>4</td>
<td>7/1/2015</td>
<td>1</td>
<td>13</td>
<td>129</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td>N 41.4267, W 83.0503</td>
<td>Light Trap</td>
<td>21</td>
<td>4</td>
<td>7/1/2015</td>
<td>1</td>
<td>13</td>
<td>129</td>
<td>20.3</td>
</tr>
</tbody>
</table>

1.4 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 \( \sim 165 \text{ m}^3/\text{s} \) 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 \( 280–370 \text{ m}^3/\text{s} \). 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.
Chapter 2

Modeling Framework to Estimate Spawning and Hatching Locations of Pelagically-Spawned Eggs

2.1 Introduction

Identifying spawning and hatching locations is vital to both conserving imperiled fish and controlling invasive fish. If spawning locations in a system are not known, this essential habitat cannot be protected for imperiled species, nor targeted for control of invasive species. Spawning locations are easily identifiable for fish that build nests or attach eggs to specific substrates, such as cobble or vegetation (Balon 1975). However, identifying spawning grounds is difficult for pelagic-spawning species that release semi-buoyant, non-adhesive eggs. Pelagic eggs depend on river flow to remain suspended (Balon 1975) and consequently travel away from their point of origin. Further, as pelagic eggs travel away from spawning grounds, their point of hatching and development through larval stages is even more distant from their natal location. The spatial separation of spawning and hatching locations of fish species with pelagic eggs therefore presents a special problem in terms of conservation or control. Even when eggs are sampled and known to be present in a given system, the collection location does not suffice to determine where and when they were fertilized. Therefore, development of an approach
for estimating locations where spawning and hatching are likely to occur based on collected field data can improve conservation or control of fish species with pelagic eggs.

In freshwater systems, the pelagic-egg reproductive strategy is common in species derived from marine groups and among the highly specious cyprinids (Balon 1975). Some cyprinid species have experienced population declines due to fragmentation in native ranges (Luttrell et al. 1999, Duan et al. 2009), but in contrast, elsewhere they have become increasingly invasive (Kolar et al. 2007). Chinese cyprinid pelagic spawners, *Hypophthalmichthys* spp., *Ctenopharyngodon idella*, and *Mylopharyngodon piceus*, are feared to be declining in their native range due the construction of Three Gorges Dam (Duan et al. 2009), but are considered increasingly threatening in North America as their ranges expand (Kolar et al. 2007). These species were first introduced to the United States in the 1960s and 1970s for biological control of vegetation and aquaculture (Chapman et al. 2013). They have since escaped from introduced locations and established populations in some areas such as the Mississippi River basin, where they have caused declines in native fish populations, as well as caused negative impacts on ecosystem functions (Irons et al. 2007). Chinese carp have continued to expand into the northern and western tributaries of the Mississippi River, causing concern about their potential entry into the Great Lakes, where they may threaten a US$7 billion fishery (Cuddington et al. 2013). One of the Chinese carp species, *Ctenopharyngodon idella* (Grass Carp), is reproducing within the Great Lakes (Embke et al. 2016). Their reproduction was inferred in 2012 with the collection of four juveniles sampled in the Sandusky River (Lake Erie tributary) with otolith microchemistry indicative of Sandusky River origin (Chapman et al. 2013). Direct confirmation of natural reproduction occurred
in 2015 when multiple fertilized and genetically-confirmed Grass Carp eggs were collected in the Sandusky River (Embke et al. 2016). These findings marked the first confirmation of Chinese carp spawning in the Great Lakes basin, increasing the need for further understanding of the spawning of these fishes.

The discovery of Grass Carp eggs in the Sandusky River created a need to determine the location of spawning so management agencies could monitor this area or possibly implement control actions. While general characteristics of preferred spawning habitat is known for Grass Carp based in their native ranges, similar information is lacking for many of their introduced locations. Based on their native range preferences, they target shallow, fast-flowing waters with coarse substrate (Stanley et al. 1978), but current knowledge gaps related to accurate estimates of the spatial location of spawning for this species in the Great Lakes limits the ability to monitor or control this invasive species. Therefore, to estimate the most likely spawning location in the Sandusky River I employed the Fluvial Egg Drift Simulator (FluEgg; Garcia et al. 2013, Garcia et al. 2015), a model that tracks pelagically-spawned egg movements through time and space. The FluEgg model was developed to estimate the travel and dispersal patterns of Chinese carp eggs and larvae from a known fertilization location to determine where they would hatch prior to falling out of suspension. Chinese carp eggs that fall from the water column in unsuitable habitat have a diminished likelihood of hatching (George et al. 2015). Based on an assumed spawning location and hydraulic analysis (Murphy and Jackson 2013), the FluEgg model predicts that the Sandusky River is likely suitable for Chinese carp reproduction (Murphy and Jackson 2013). However, the FluEgg model was developed to track egg movements based on a known fertilization location, and does not take into
account additional information collected from the field, such as egg collection location or age. Therefore, this model needed to be adapted to incorporate real-time data to back-track the most likely origin of the eggs in the river and then use that location to predict the hatching success of the eggs.

Here I developed a novel application of FluEgg that uses developmental stage of fertilized eggs of Grass Carp captured in the Sandusky River in combination with hydraulic characteristics to estimate the most probable spawning location(s). This approach employs the histogram density estimation method (Silverman 1986) to iteratively build a probability distribution for each date an egg was collected, which are then combined to calculate the most probable spawning location. I then used those results to estimate hatching locations, weighting each hatching location by the probability of its spawning location. The innovative methodology I have developed can be broadly applied to inform management efforts specific to pelagic spawners, including actions aimed at invasive species as well as to address conservation needs of imperiled native pelagic spawners.

2.2 Methods

The Sandusky River, the third largest tributary to the Western Basin of Lake Erie, flows for ~215 km into the lake at Sandusky Bay. The downstream-most dam on the river (Ballville Dam), is impassable and lays ~25 km from the mouth of Muddy Creek Bay, which opens into Sandusky Bay. Therefore, the study area encompassed the river length from Ballville Dam to Muddy Creek Bay. Upstream reaches are ~1 m deep, with the majority of the river ~5–6 m deep during low-flow conditions. In this portion of the
Sandusky River, width varies between ~32 and 160 m, but at the sampling locations widths ranged between ~80 and 120 m (Fig. 2-1). During May-August, discharges fluctuate between ~1 m$^3$/s to ~35 m$^3$/s, but can exceed 400 m$^3$/s during high-flow events. Water temperatures during May-August typically do not exceed 28°C, but can fall to as low as 18°C during high discharges.

I sampled for eggs June – August of 2015 and 2016 using a bongo net (0.5 m diameter each and 500 μm mesh) deployed for 5 min in a fixed position just below the surface of the water against the current from the bow of a stationary boat. Although I sampled for eggs in 2015 and 2016, I only collected Grass Carp eggs in 2015. Additionally, I sampled for larval Grass Carp using quadrafoil light traps once weekly in 2015 and 2016. Further detail on field sampling can be found in Embke et al. (2016).

The FluEgg model requires water temperature measurements to track the growth and buoyancy changes of eggs over time. Therefore, during the summer of 2015, 20 HOBO© temperature loggers were deployed at 10 sites along a distance of ~17 km (Fig. 2-1). Two loggers were secured by rope to a cinderblock at two depths at each site to determine if there was a difference in water temperature at differing depths in the water column. The first logger was fixed 0.5 m from the bottom of the river. The second was secured one meter above the first logger. Temperature readings were recorded every 15 minutes. Water depth measurements were taken at each site on the day of deployment as well as every egg sampling date to record the depth of the loggers in the river, with an average depth of 1.5 m below the surface for the shallowest logger. I deployed the loggers in the river on June 29, 2015 and retrieved them on August 31, 2015. Based on
findings from 2015, there was no difference in temperature at differing water column depths and as such I did not deploy temperature loggers in 2016.

Figure 2-1: HOBO© temperature logger sampling locations (o) in the Sandusky River for 2015. Egg collection sites are indicated (*) with site labels corresponding to Table 2.1.
To inform the hydraulic conditions set as the foundation in the FluEgg model, I used discharge and temperature data provided by the U.S. Geological Survey (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 near Fremont, Ohio. I collected one Grass Carp egg prior to the deployment of temperature loggers, therefore I used water temperature from the USGS stream gauge as the modeling input for this date. This stream gauge was also used to monitor mean daily river discharge (water volume/day). Measurements were available every 30 minutes, therefore I used the discharge measurement that was taken at the time closest to when the egg was collected (Table 2.1).

Eggs were identified as probable Grass Carp eggs based on gross morphology (size and lack of oil globule; Yi et al. 1988). They were then verified genetically (Embke et al. 2016). I determined their developmental stage following Yi et al. (1988; Table 2.1) to estimate how long the egg had been drifting, which I used as the simulation time required as a FluEgg input. Developmental stage and water temperature from the nearest HOBO© logger site permitted me to estimate the age of each egg according to George and Chapman (2015):

\[ t = \frac{\text{CTU}}{(T_c - 13.5)} \]  \hspace{1cm} (1)

where \( t \) = time since post-fertilization in hours, \( T_c \) = water temperature at nearest HOBO© logger site in °C, 13.5 = thermal minimum in °C (George and Chapman 2015), and CTU = cumulative thermal units (as defined by stage of the egg).
<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Method</th>
<th>N eggs</th>
<th>Distance (km) from Ballville Dam</th>
<th>Dates collected</th>
<th>Time egg(s) collected</th>
<th>Developmental stage(s)</th>
<th>Cumulative Thermal Units</th>
<th>Age post-fertilization (hours)</th>
<th>Mean discharge for egg growth (m³/s)</th>
<th>Water temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>N 41.3566, W 83.1045</td>
<td>Bongo Net</td>
<td>1</td>
<td>5</td>
<td>7/13/2015</td>
<td>11:34</td>
<td>2</td>
<td>7.8</td>
<td>1.2</td>
<td>365</td>
<td>19.77</td>
</tr>
<tr>
<td>B3</td>
<td>N 41.3864, W 83.0908</td>
<td>Bongo Net</td>
<td>1</td>
<td>10</td>
<td>6/18/2015</td>
<td>11:30</td>
<td>8</td>
<td>32.5</td>
<td>3.5</td>
<td>365</td>
<td>22.80</td>
</tr>
<tr>
<td>B4</td>
<td>N 41.3972, W 83.1026</td>
<td>Bongo Net</td>
<td>1</td>
<td>4</td>
<td>6/29/2015, 7/14/2015</td>
<td>14:22, 12:07</td>
<td>9, 10, 10, 12</td>
<td>49.9, 40.1, 49.9, 49.9, 64.6</td>
<td>8.2, 5.2, 6.4, 6.4, 8.3</td>
<td>265, 236</td>
<td>19.58, 21.25</td>
</tr>
<tr>
<td>LT2</td>
<td>N 41.4267, W 83.0503</td>
<td>Light Trap</td>
<td>1</td>
<td>21</td>
<td>7/1/2015</td>
<td>22:40</td>
<td>13</td>
<td>73.5</td>
<td>10.7</td>
<td>132</td>
<td>20.34</td>
</tr>
</tbody>
</table>

Table 2.1: Egg collection date, time, and location, number of eggs, discharge, distance from Ballville Dam, stage, cumulative thermal unit (CTU) taken from George and Chapman (2015), water temperature, and estimated age of egg(s).
2.2.1 Modeling Egg Transport

Modeling egg transport was a multi-step procedure first involving modeling hydraulic conditions in the river during events when eggs were captured, then using those model results as inputs for the FluEgg model to predict the movement of eggs. The approach I developed has four components: (1) establishing hydraulic characteristics of the river at the time of egg collection, (2) iteratively running simulations in FluEgg to model egg transport, (3) calculating the most probable spawning locations, then (4) using those locations to calculate the most probable hatching locations (Fig. 2-2).

1. Simulate hydraulic conditions in river at time of egg collection (HEC-RAS)
2. Simulate egg transport (FluEgg)
3. Calculate probability distribution of spawning locations
4. Use spawning location prediction to calculate probability distributions of hatching locations

Figure 2-2. Diagram illustrating the modeling process developed to estimate the spawning and hatching locations of collected Grass Carp eggs.

2.2.2 Simulating Hydraulic Characteristics of River at the Time of Egg Collection

To track the movement of eggs in a river, FluEgg requires fluvial environmental and hydraulic state variables defined by the user in one-dimensional discrete cells of the
study river (Garcia et al. 2013). Cell dimensions are defined by the cell hydraulic width, length, and water depth (Garcia et al. 2013). Necessary input variables include:
cumulative distance from impassable barrier (km), water depth (m), discharge (m³/s),
velocity magnitude (m/s), shear velocity (m/s), and water temperature (°C). Data for all
of these parameters, except water temperature, can be measured in the field during a
spawning event when eggs are in the drift or simulated using a hydraulic model
implemented in the Hydrologic Engineering Centers River Analysis System (HEC-RAS)
5.0 software developed by the United States Army Corps of Engineers Hydrologic
Engineering Center (U.S. Army Corps of Engineers 2016). Using a HEC-RAS model of
the lower Sandusky River developed by Stantec (2011), I generated one-dimensional
steady flow simulations to define hydraulic scenarios of the Sandusky River at the time of
egg collection as field measurements were not available. It was necessary to run
individual HEC-RAS simulations for each flow and temperature regime an egg was
collected (i.e. date; n=5) to determine the most realistic hydraulic conditions for the
Sandusky River at that time.

To achieve the most realistic egg transport outputs, I set the simulation conditions
in HEC-RAS to match the measured conditions for water temperature and discharge for
the date and time of the collected eggs. The downstream boundary condition was set to
normal depth with a slope of 0.000005. After performing simulations with varying
boundary conditions, I manually modified the slope so that HEC-RAS derived outputs
agreed with previously measured conditions in the Sandusky River from Murphy and
Jackson (2013). HEC-RAS results were used as the river input data for FluEgg
simulations.
2.2.3 Simulating Egg Transport to Determine the Most Probable Spawning Location(s)

I developed a modeling framework to estimate the likely spawning location for each date on which an egg was collected using a Bayesian approach in combination with the histogram density estimation method (Silverman 1986). Input variables required by FluEgg were water temperature (°C), species being modeled (options include Bighead Carp, Grass Carp, and Silver Carp), number of eggs per simulation, duration (in hours) of the simulation, and spawning location. Because I only sampled eggs at the surface of the water, water temperatures from the nearest HOBO© temperature logger location nearest the surface, or when not available from the USGS gauge at Ballville Dam, were used as the inputs for FluEgg for each simulation. The duration of the simulation was determined by the age of the egg, in hours (Table 2.1). The likely travel distance of the egg is estimated using a FluEgg-based Monte Carlo simulation.

Using FluEgg, I estimated the likelihood of an egg spawned at location \(x\) (measured in meters (m) from Ballville dam) being collected at a distance of \(y\) (m from Ballville dam). I approximated this likelihood by using a Monte-Carlo simulation, where a large number of simulated eggs were released at the known spawning location (\(x\)) and FluEgg tracked their drifting distances for a given time (determined by the collected egg’s age). Using the histogram density estimation method, I estimated the probability density function of the drift distance:

\[
 f(y|x)
\]  

where \(y\) = location of a collected egg measured by the drift distance (m from Ballville Dam) and \(x\) = spawning location relative to Ballville Dam (m). This probability distribution did not satisfy my objective. After collecting an egg, I knew that this
particular egg drifted a distance of \( y^* \), from which I wanted to learn about the most likely spawning location \( x \). That is, I aimed to estimate:

\[
f(x|y^*)
\]

where \( x = \) (unknown) spawning location relative to Ballville Dam (m), and \( y^* = \) collection location of the egg(s) as drift distance (m) from Ballville Dam. Based on the Bayes Theorem, I had:

\[
f(x|y^*) \propto f(x)f(y^*|x)
\]

where \( f(x) \) represents my knowledge of the likely spawning location prior to observing the egg. Initially, because I did not have information regarding the likelihood of spawning locations, I assumed that all spawning locations were equally likely, therefore:

\[
f(x) \propto 1
\]

Consequently:

\[
f(x|y^*) \propto f(y^*|x)
\]

Although similar at first glance, the right-hand-side of equation (4) is not the same as the quantity in equation (2). In equation (2), the spawning location \( x \) is fixed and known, while the drift distance \( y^* \) on the right-hand-side of equation (4) is known and fixed. I quantified equation (4) numerically by estimating the density function of equation (2) at a number of spawning locations and evaluated the density values at \( y^* \) for each of the densities. These density values are numerical estimations of the right-hand-side of equation (4). Normalizing these likelihood values with respect to the likely spawning locations, I obtained the estimated probability distribution of the spawning location (the left-hand-side of equation (4)).
This process was repeated for each of the five collection dates (i.e., flow and temperature regimes; Table 2.1). I then multiplied all of the probability distributions for each collection date (n=5) to calculate the most probable spawning location for all dates:

$$f(x|y_{i=1,5}) \propto \prod_{i=1}^{5} f(y_{i}^{*}|x)$$

(7)

2.2.4 Details of FluEgg-Based Monte Carlo Simulations to Estimate Spawning Locations

For each FluEgg simulation run, I simulated the transport of 5,000 eggs starting from several hypothetical spawning locations ($x_n$; Fig. 3) downstream of Ballville Dam for the duration estimated by the collected egg(s) on that date. The 5,000 estimated travel distances represent a random sample of the travel distance. Using the histogram density estimation method (Silverman 1986), I estimated the probability distribution of the travel distance (measured as distance from Ballville Dam). After a simulation was complete, I used post-processing tools within FluEgg to calculate the percentage of eggs that passed the collection location $y^*$ at the sampling depth and time (corresponding to the age of the collected egg; Fig. 2-3A). The density of the location (i.e. percentage of eggs) where the egg was collected was the likelihood of the hypothetical location being the actual spawning location, represented as $f(x_n|y^*)$ (Fig. 2-3A).

I repeated simulating egg movements at 100 m increments downstream of the impassable barrier until the percentage of eggs at the collection location was zero (i.e., the entire egg plume had passed the collection location; Fig. 2-3C). I chose units of 100 m as I believed this level of resolution was reasonable given the biology of the species and would provide sufficient information for management efforts. This resulted in a likelihood distribution of the eggs illustrating the most likely spawning locations for that temperature and flow regime (i.e., date). I then calculated a probability distribution from
the likelihood distribution by binning likelihoods every 500 m and calculating the area of
the single bin over the total area of the curve. This resulted in a normalized probability
distribution of the most probable spawning locations for that flow and temperature
regime, with each bin midpoint corresponding to a spawning location probability.

Figure 2-3. Diagram illustrating the iterative modeling process developed to calculate the
most likely spawning location for varying input spawning locations ($x_1, x_2, x_3$) and a
known egg collection location ($y^*$). Symbols ($\bigtriangleup$, $\bigstar$, $\blacklozenge$) denote the intersection of the
longitudinal distribution of the simulated eggs at a given time (corresponding to the age
of the egg, in hours). This intersection corresponds to the likelihood of the input
spawning location being the origin for the collected egg: $f(x_n | y^*)$.  

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2.2.5 Simulating Egg Transport to Calculate the Most Probable Hatching Locations

Once I estimated the likely spawning location, I used the result to estimate the most probable hatching locations for the collected eggs as described by:

\[ f(z|y^*) \] (8)

where \( z = \) hatching location and \( y^* = \) collection location, both described by the drift distance (m) from Ballville Dam. What I was able to estimate using FluEgg was the distribution of likely hatching location \( z \) given the spawning location \( x \), that is:

\[ f(z|x, y^*) \] (9)

where \( z = \) hatching location, \( x = \) spawning location, and \( y^* = \) locations where eggs were collected, all described by the drift distance (m) from Ballville Dam. The predictive distribution of the likely hatching distance is then:

\[ f(z|y^*) = \int_x f(x|y^*)f(z|x,y^*)dx \] (10)

Numerically, this integral can be approximated using the previously estimated spawning location distribution (equation (7)). Specifically, for each likely spawning location (i.e., the midpoint of each histogram bar of the spawning location probability distribution) I estimated the likely hatching location distribution (\( f(z|x,y^*) \)) using FluEgg. The weighted average of these densities was the predictive distribution of hatching location. The weights were the respective densities of the selected spawning location (\( f(x|y^*) \)).

2.2.6 Details of FluEgg-Based Monte Carlo Simulations to Estimate Hatching Locations

FluEgg is dependent on temperature and flow regimes, therefore I simulated the longitudinal distribution of eggs in the river at hatching time for each of the five dates. At the completion of the simulation, I identified the percentage of eggs (i.e., likelihood) at a location at hatching time. I weighted the hatching location likelihoods based on the
densities of the respective input spawning sites by multiplying the hatching likelihoods by the corresponding spawning location from the probability distribution calculated in equation (7). I then summed the weighted likelihoods for each hatching location to determine the range of locations in which eggs may have hatched in the river for that date. This process was repeated for each of the five collection dates to produce five hatching location probability distributions.

Lastly, FluEgg was used to simulate the developmental stage of larvae when they exited the river. I did this by setting the simulation length in FluEgg to the time it takes to reach gas bladder inflation. If the larvae did not reach gas bladder inflation while in the river, the simulation duration when larvae exit the river was used to calculate cumulative thermal units, and therefore stage, using equation (1) and the table presented in George and Chapman (2015). In the equation, $t$ was set to the total simulation length when larvae exited the river, and $T_c$ was the corresponding water temperature for the simulation.

2.3 Results

In 2015, discharge varied dramatically throughout the sampling period: 2.86-368.11 m$^3$/s (Fig. 2-4). When eggs were collected, discharges were between 129 m$^3$/s and 368 m$^3$/s (Fig. 2-4, Table 2.1), greatly exceeding the high-flow threshold required for Grass Carp eggs to remain suspended [31 m$^3$/s for the Sandusky River established by Murphy and Jackson (2013)]. In 2016, discharge was lower and less variable, 0.85-36.81 m$^3$/s with a mean discharge of 5.51 m$^3$/s, remaining below the high-flow threshold for the entire sampling period, except for one day (June 27, 2016) when flow reached 36.81 m$^3$/s (Fig. 2-4). No eggs were collected in 2016.
Seven of eight eggs collected were captured in bongo net tows, while one egg was incidentally captured in a light trap. The developmental stages of eggs ranged from stage 2 to stage 13 (Table 2.1). Corresponding calculated cumulative thermal units varied between 7.8 and 73.5, resulting in a range of post-fertilization ages from 1.2 to 10.7 hours (Table 2.1). On dates when eggs were collected and HOBO® temperature readings were available, the maximum difference between HOBO® temperature observations and the temperature readings from the USGS gauge was 0.58°C, with an average difference of 0.43°C. This difference would not have made a large biological difference to egg development after only a couple of hours, consequently I used the data from the USGS gauge for the egg when HOBO® loggers were not available. During 2015, water
temperatures from HOBO© temperature loggers varied between 18.30°C and 33.84°C. Eggs were only collected when temperatures were between 19.58°C and 21.25°C (Table 2.1).

The spawning site probability distribution identified that the most likely spawning site was near Fremont, Ohio (Fig. 2-5). The peak location at rkm 21.75 had a probability of 39%, which was included in the range of probabilities exceeding 5% from rkm 22.75-20.75 (Fig. 2-5). Downstream from this location, the egg collected on July 1, 2015 indicated a downstream spawning site from rkm 7.25-5.75, but probabilities did not exceed 1% for this range (Fig. 2-5). Hatching site location was less variable despite the range of probable spawning locations. All eggs most likely would have hatched in the river close to the mouth at Muddy Creek Bay, with all hatching site locations exceeding 5% probability between rkm 4.1-1 (Fig. 2-6). The hatching location of the earliest egg collected on June 18, 2015 had probabilities greater than 5% spanning rkm 2.5-1.4, with a peak of 27.3% at rkm 1.4 (Fig. 2-6). The egg collected on June 29, 2015 had a similar hatching site range with probabilities greater than 5% from rkm 2.1-1, and a peak probability of 41.1% at rkm 1.4 (Fig. 2-6). The egg incidentally captured in the light trap had less variation in hatching locations, with locations exceeding 5% from rkm 2.9-2.5, and a peak probability of 49.5% at rkm 2.9 (Fig. 2-6). The egg collected on July 13, 2015 had the largest range of potential hatching locations, with probabilities greater than 5% from rkm 4.1-2.5 and a peak of 41.7% at rkm 2.9 (Fig. 2-6). The eggs collected on July 14, 2015 were estimated to have hatched near the mouth at Muddy Creek Bay, with probabilities greater than 5% from rkm 2.5-1.4 and a peak probability of 24.2% at rkm
1.4 (Fig. 2-6). When reaching the bay, all larvae were estimated to be between or near stages 31 (hatching) and 32 (pectoral fin bud).

Figure 2-5. Probability distribution of the most likely spawning locations (river km) for all 5 dates on which eggs were collected in the Sandusky River.

Figure 2-6. Probability distributions of the most likely hatching locations (river km) for all 5 dates on which eggs were collected in the Sandusky River. Lines are designated by the dates on which egg(s) were collected, along with the flow measurement for that date.
2.4 Discussion

I was able to use field data on pelagic eggs, a hydraulic transport model (FluEgg) and a novel probabilistic modeling approach to estimate the locations where Grass Carp are spawning and where larvae are likely maturing in the Sandusky River. The Sandusky River is the first confirmed location for reproduction of any species of Chinese Carp in the Great Lakes basin (Embke et al. 2016). Therefore, determining the most likely location for spawning in this tributary is crucial for monitoring and implementing potential control actions for Grass Carp. Further, this approach is directly transferable to other species of Chinese carp that may invade the Great Lakes (Garcia et al. 2013, Kolar et al. 2007). Additionally, the approach I developed can be used to identify critical spawning habitat for imperiled cyprinid species with similar semi-buoyant eggs. The modeling framework I developed is a novel application of existing models that incorporates real-time data to inform management efforts, whether for control or restoration.

Using simulations, I identified the most probable spawning location for Grass Carp using information from collected eggs (Fig. 2-5), which encompassed habitat consistent with spawning sites in Grass Carp’s native range. Specifically, habitat characteristics included channelized segments of the river that have high berms covered in large boulders on both sides to protect the city of Fremont, Ohio from erosion during high flows. Flow in this reach is particularly fast and turbulent. These model results further support the idea based on native spawning locations that Grass Carp target shallow, fast-moving water and coarse substrate for spawning (Stanley et al. 1978). The spawning site further downstream identified by the single egg collected on July 1, 2015 is
surprising in that this area has very different habitat traits, being much deeper and more turbid with a silty bottom. Although I was able to stage this egg, indicating it was viable and developing normally, incidentally collecting the egg in a light trap and much further downstream than would have been expected was surprising. As this egg was collected on the descending limb of a high-flow event, this egg could have been released by a fish that was attracted to the river later than other individuals. Although it is unclear what the exact cue is for Grass Carp spawning, it is possible that spawning fish release pheromones that could attract other fish to the spawning location, as is the case for other riverine spawners (Johnsen and Hasler 1980, Vrieze and Sorensen 2001). I collected an egg two days prior (June 29, 2015) on the peak of that high-flow event, therefore spawning had already occurred in the river and cues may have been present. Thus, it is not implausible that a fish could have spawned in the predicted location given the lower flows and higher temperatures, but it is not likely given the low probabilities estimated from the modeling results. Overall, the modeling results support that Grass Carp in the Sandusky River utilize similar habitat as they do in their native range for spawning.

From the hatching location simulations, I was able to estimate that all eggs would have hatched in the river (Fig. 2-6), likely resulting in increased survival and therefore higher recruitment potential. There are drastic changes in habitat between the river and the bay. Flows are slower, depths are shallower, and areas are siltier in the bay, and therefore eggs would likely fall out of suspension when reaching the bay (Garcia et al. 2013, Murphy and Jackson 2013). Eggs that fall out of suspension have diminished hatching and survival rates (George et al. 2015), however George et al. (2015) demonstrated in laboratory settings that eggs covered in sediment can still survive and
hatch. Although the eggs I collected would have hatched in the river, when they reached Muddy Creek Bay the larvae were predicted to be at hatching stage or the following pectoral fin bud stage, both of which precede gas bladder inflation. Larval Grass Carp are known to be able to vertically swim immediately following hatching (Stanley et al. 1978), but because these stages are prior to gas bladder inflation, they may not have been able to control horizontal movement and therefore ended up in the bay. Therefore, larval maturation zones are likely in Muddy Creek Bay or even Sandusky Bay, thus tailoring actions for larvae in these locations would be advised.

The modeling approach used in this study has resulted in further information for researchers and management agencies, however there are limitations given the potentially high levels of uncertainty of the models used. For example, the hydraulic modeling process used the steady-state HEC-RAS outputs that were created using limited calibration data from the study system during low flow conditions and as such may not represent the most realistic conditions as inputs for FluEgg. Additionally, flow conditions depend on the interaction of streamflow coming downstream and the seiche intrusion from the lake into the Sandusky River. The steady-state HEC-RAS model I used does not consider lake seiche events or changes in water surface elevation in the downstream boundary condition caused by lake seiche events that could have influenced the drifting time of eggs as well as larvae. Seiche events are thought to play a smaller role when flows are higher, especially during those high-flow events when eggs were collected in 2015; although unlikely to have a large effect on my results, they could influence similar analyses done during lower flow conditions by increasing the development period for drifting eggs and larvae. Overall, while there is uncertainty associated with modeling the
hydraulic conditions at the time of egg collection, it is the best solution currently available for estimating egg movement in the Sandusky River, as well as for other riverine systems with pelagic spawners. For future work, following the calibration and validation of the unsteady-state HEC-RAS model, simulations should be modified to refine the most realistic locations given updated hydraulic conditions.

The lack of spawning evidence in low-flow years, 2014 (Embke et al. 2016) and 2016, in combination with the collection of eggs in a high-flow year, 2015, indicate a link between spawning and high flows in the Sandusky River (Fig. 2-4). Although there are limitations to egg collections given the restricted gear type, sampling depth, and lack of knowledge regarding egg “catchability,” Chinese carp species are known to spawn during high discharge events in their native ranges (Lin 1935, Stanley et al. 1978) as well as in some areas of the United States, such as the Mississippi River basin (Chapman et al. 2013). Elsewhere in the world where Chinese carps have invaded, including the Wabash River (Coulter et al. 2013), canals in Italy (Milardi et al. 2015), and the Kara-Kum canal in Turkmenistan (Aliyev 1976), these fish have spawned regardless of increased flows.

Some of these systems, such as the Wabash River and Kara-Kum canal, have fewer blockages than other systems where there is a strong association between high flows and spawning. Therefore, perhaps the lack of relationship between high flows and successful spawning is a result of the lack of fragmented habitat. In the fragmented Sandusky River, the connection between high flows and spawning evidence could provide further information to management agencies to indicate when spawning will occur.

Although I observed a wide range of water temperatures in the Sandusky River during the sampling season, I only collected eggs during a narrow range of temperatures
between 19.58°C and 21.25°C. This range exceeds the spawning threshold of 18°C established in the literature for Chinese carp populations in their native range (Kolar et al. 2007, Duan et al. 2009). In some invaded areas such as the Mississippi River basin, evidence of spawning has not been collected at water temperatures below 18°C (DeGrandchamp et al. 2007, Kolar et al. 2007, Deters et al. 2013). Contrarily in the Wabash River, eggs have been collected at temperatures below 18°C, although these temperatures may have been temporarily lowered due to precipitation events (Coulter et al. 2016). My results corroborate the threshold established in the literature, but overall indicate that the water temperature requirement for spawning may be secondary to the stronger link between high flows and spawning.

This modeling framework can currently be utilized to determine spawning and hatching locations of Chinese carp species, whether for population restoration within their declining native range or for population management within their expanding invasive range. Identifying the specific locations these fish are utilizing for critical early life history stages is imperative to understanding how populations will fare in different habitats under varying conditions. The approach I developed allows for the use of real-time data to inform spawning and hatching location estimations to best focus management efforts and conserve systems of interest.

In addition to utilizing this approach to estimate spawning locations for Chinese carp species, there is potential to apply it to all pelagic, riverine spawners. River modifications have been associated with the declines of multiple imperiled pelagic spawners, such as *Macrhybobsis* spp., *Notropis girardi*, and *Platygobio gracilis* throughout the Mississippi River basin and Great Plains (Perkin and Gido 2011). If
required data on species-specific egg buoyancy and hydraulic data is available, this methodology has the ability to inform the locations that provide habitat for critical reproductive events, which could then be highlighted for conservation and inform the recovery efforts of riverine species.

Spawning locations provide key information to researchers about the reproductive requirements of species and to agencies about how best to manage populations for restoration or control. While identifying these locations can be difficult for species that release pelagic, semi-buoyant eggs, I developed a novel modeling framework that can estimate the most likely spawning and hatching locations for these species, many of which are imperiled or invasive. This approach incorporates real-time field data to inform the otherwise abstract modeling process, thus providing researchers and managers with additional information to protect imperiled fish or control invasive fish.
Chapter 3

Summary

Through researching the spawning potential of Grass Carp in the Sandusky River, I have established that this species is utilizing the Great Lakes basin for natural reproduction with the collection of multiple eggs in 2015. Although I sampled for larval Grass Carp using light traps in 2015 and 2016, no direct evidence of recruitment was collected. From the eggs collected in 2015, I developed a methodology that incorporated real-time field data into a hydraulic model to estimate the most probable spawning and hatching locations for the eggs. Two spawning locations were identified, with the majority of eggs originating from the hypothesized spawning location near Fremont, OH. All eggs were predicted to hatch in the river, not far upstream from the mouth at Muddy Creek Bay. These areas were then communicated to management officials to target their action locations in the Sandusky River. This modeling methodology can also be applied to other systems and species of invasive Asian Carps to garner as much information as possible about the spawning requirements of these species.

In an effort to understand the environmental conditions that favor spawning, I compared the collection times of eggs to abiotic conditions, primarily focusing on river discharge. All eggs were collected either the day of peak flow or 1-2 days following the
peak, indicating that at least for the Sandusky River, high-flow events favor spawning. This connection is supported by the literature, wherein Grass Carp target high-flow events in their native (China and Russia) and non-native (Mississippi River basin) ranges (Duan et al., 2009; Tan et al., 2010; Chapman et al., 2013). My research emphasizes the importance of flows in affecting the spawning ability of these fish, as I did not collect any evidence of spawning when high-flow conditions were not met in 2015 and 2016. Others described how an invasive species with a low population can become large populations given favorable environmental conditions (Walsh et al., 2016). This could be the current setting occurring in Lake Erie, as more diploid individuals are being captured (Wieringa et al., 2016) and the severity and frequency of high-flow events in the Sandusky River are increasing, indicating that there is the potential for population expansion for this species.

Looking forward, potential avenues to explore would include long term monitoring of conditions that favor spawning in an effort to understand the abiotic factors that are necessary for spawning to occur. It has been established that land use changes, specifically increased agriculture, results in susceptibility to rapid hydraulic changes (Fausch et al., 2002). These swift fluctuations could provide more suitable spawning conditions for invasive carp.

This work has numerous applications beyond understanding the spawning requirements of Grass Carp. The established methodologies can be applied to other invasive carp species, as well as all pelagic spawners, including those threatened or endangered species encountering issues with habitat fragmentation and landscape changes. Given the concern regarding the adverse impacts large populations of Grass Carp could have on Great Lakes ecosystems, it is imperative to further understand the
biology, distribution, and interactions of these fish through further research and adaptive management efforts.
References


