Invasive Carp Reproduction Phenology in Tributaries of the Upper Mississippi River

C. A. Camacho  
Iowa State University

C. J. Sullivan  
Iowa State University

Michael J. Weber  
Iowa State University, mjw@iastate.edu

Clay L. Pierce  
U.S. Geological Survey, cpierce@iastate.edu

Follow this and additional works at: https://lib.dr.iastate.edu/nrem_pubs

Part of the Natural Resources Management and Policy Commons, and the Population Biology Commons

The complete bibliographic information for this item can be found at https://lib.dr.iastate.edu/nrem_pubs/364. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the Natural Resource Ecology and Management at Iowa State University Digital Repository. It has been accepted for inclusion in Natural Resource Ecology and Management Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Invasive Carp Reproduction Phenology in Tributaries of the Upper Mississippi River

Abstract
Invasive Bighead Carp *Hypophthalmichthys nobilis*, Silver Carp *H. molitrix*, and Grass Carp *Ctenopharyngodon idella* are expanding throughout the upper Mississippi River (UMR) basin. Spawning has occurred in the main-stem UMR but could be limited to high-discharge events when dam operations create a free-flowing river. Alternatively, naturally free-flowing tributaries could offer alternative habitat for successful reproduction. Our objectives were to examine temporal and spatial variation in adult gonad development, ichthyoplankton densities, and back-calculated spawn dates of invasive carp collected in three UMR tributaries. We compared ichthyoplankton densities between the upstream and mouth reaches of tributaries and densities between the UMR and adjacent tributaries. Ichthyoplankton samples were collected every 10 d, and adult invasive carp were sampled monthly at nine sites throughout the Des Moines, Skunk, Iowa, Cedar, and Mississippi rivers during April–October 2014 and 2015. Peaks in adult gonadosomatic index, an increase in postspawn females, and ichthyoplankton collections suggested that peak spawning occurred during late May through June, when water temperatures were 18–30°C and channel velocities were at least 0.7 m/s. However, reproduction occurred as late as August in some tributaries. Ichthyoplankton densities were highest in the Des Moines River among tributaries, but tributary densities were similar to the UMR densities, suggesting that both tributaries and main-stem sites provide suitable spawning locations. Invasive carp reproduction in UMR tributaries provides additional sources of potential recruitment for further population expansion upstream in the UMR and should be considered when devising plans for controlling populations along the leading edge of the invasion.

Disciplines
Ecology and Evolutionary Biology | Natural Resources Management and Policy | Population Biology

Comments
Invasive Carp Reproduction Phenology in Tributaries of the Upper Mississippi River

C. A. Camacho,*1 C. J. Sullivan,2 and M. J. Weber

Department of Natural Resource Ecology and Management, Iowa State University, 215 Science Hall II, Ames, Iowa 50011, USA

C. L. Pierce

U.S. Geological Survey, Iowa Cooperative Fish and Wildlife Research Unit, Ames, Iowa 50011, USA

Abstract

Invasive Bighead Carp Hypophthalmichthys nobilis, Silver Carp H. molitrix, and Grass Carp Ctenopharyngodon idella are expanding throughout the upper Mississippi River (UMR) basin. Spawning has occurred in the main-stem UMR but could be limited to high-discharge events when dam operations create a free-flowing river. Alternatively, naturally free-flowing tributaries could offer alternative habitat for successful reproduction. Our objectives were to examine temporal and spatial variation in adult gonad development, ichthyoplankton densities, and back-calculated spawn dates of invasive carp collected in three UMR tributaries. We compared ichthyoplankton densities between the upstream and mouth reaches of tributaries and densities between the UMR and adjacent tributaries. Ichthyoplankton samples were collected every 10 d, and adult invasive carp were sampled monthly at nine sites throughout the Des Moines, Skunk, Iowa, Cedar, and Mississippi rivers during April–October 2014 and 2015. Peaks in adult gonadosomatic index, an increase in postspawn females, and ichthyoplankton collections suggested that peak spawning occurred during late May through June, when water temperatures were 18–30°C and channel velocities were at least 0.7 m/s. However, reproduction occurred as late as August in some tributaries. Ichthyoplankton densities were highest in the Des Moines River among tributaries, but tributary densities were similar to the UMR densities, suggesting that both tributaries and main-stem sites provide suitable spawning locations. Invasive carp reproduction in UMR tributaries provides additional sources of potential recruitment for further population expansion upstream in the UMR and should be considered when devising plans for controlling populations along the leading edge of the invasion.

Ecological communities worldwide are becoming more uniform through the introduction and subsequent establishment of nonnative species (Rahel 2002). At least 83 nonnative fishes have become established in the upper Mississippi River (UMR) basin as a result of dispersal from other basins or by direct introduction from anthropogenic activities (Rasmussen 2002). Silver Carp Hypophthalmichthys molitrix, Bighead Carp H. nobilis, and Grass Carp Ctenopharyngodon idella—collectively referred to as “invasive carp” hereafter—were imported into the United States starting in the 1960s for human consumption and biological control (Guillory and

*Corresponding author: ccamacho0526@hotmail.com
1Present address: Idaho Department of Fish and Game, Nampa Fisheries Research, 1414 East Locust Lane, Nampa, Idaho 83686, USA.
2Present address: Department of Natural Resources and the Environment, University of Connecticut, Storrs, Connecticut 06268, USA.
Received May 8, 2020; accepted July 31, 2020
Gasaway 1978). Population abundance has increased substantially since accidental or deliberate introductions shortly after importation (Irons et al. 2009; Kelly et al. 2011; DeBoer et al. 2018). Due to their high reproductive potential (Shireman and Smith 1983; Kolar et al. 2007) and dispersal ability (Gorbach and Krykhtin 1989; DeGrandchamp et al. 2008; Prechtl et al. 2018) capabilities, these fishes can rapidly expand their distribution and quickly establish abundant populations from a few adult individuals when reproduction is successful (Crawley et al. 1986). Therefore, an understanding of invasive carp reproduction is critical for understanding the mechanisms regulating their population spread and expansion.

Invasive carp are pelagophilic spawners that reproduce in open water and broadcast semi-buoyant eggs into the drift. Depending on water temperature, eggs must remain suspended for 24–48 h before larvae hatch, requiring 15–80 km of uninterrupted river flow for successful embryonic development and hatching (Krykhtin and Gorbach 1981; Garcia et al. 2015; Emble et al. 2016). Invasive carp require a combination of hydrological and thermal triggers to initiate spawning during late spring and early summer (Kolar et al. 2007). In their native range within the Yangtze River, China, adult invasive carp use specific spawning grounds near tributary mouths and other areas of turbulent water during periods of rising discharge when water temperatures are between 18°C and 30°C (Kolar et al. 2007). Similar findings have been documented in their nonnative ranges in North America (Deters et al. 2013; Larson et al. 2017). However, deviations from typical spawning conditions related to hydrography (Lohmeyer and Garvey 2009; Deters et al. 2013), spawn timing (Coulter et al. 2016), and spawn location (Hintz et al. 2017) provide evidence of reproductive plasticity.

The UMR is divided into impoundments by a series of 29 lock-and-dam (LD) structures. Some of these dams, such as LD19, are formidable structures that can at times alter the hydrology of the UMR and restrict fish movement (Theiling and Nestler 2010; Tripp et al. 2014). At lower flows, LDs can hold back water and create pool-like conditions that may be unsuitable for invasive carp reproduction (Garvey et al. 2003; Lohmeyer and Garvey 2009; Theiling and Nestler 2010). However, adult invasive carp were able to successfully navigate these obstacles and reproduce in the main-stem UMR under flood conditions when dam lift gates on the UMR were entirely out of the water, thus creating free-flowing, open-river conditions (Lohmeyer and Garvey 2009; Larson et al. 2017). Free-flowing conditions in the UMR vary annually depending on the duration of flood conditions, resulting in inconsistent spawning activity (Lohmeyer and Garvey 2009). Large, free-flowing tributaries connected to the impounded sections of the UMR may provide a source of additional or alternative spawning habitat when main-river conditions are unsuitable. Tributaries are associated with the spawning activity of invasive carp in their native range in the Yangtze River (Yi et al. 1988) and in varying capacities in the Missouri River (Schrank et al. 2001; Deters et al. 2013) and middle Mississippi River (DeGrandchamp et al. 2007; Lohmeyer and Garvey 2009). In their native range, invasive carp only reproduce at a few locations in the main-stem Yangtze River, including mixing waters of large tributary confluences (Yi et al. 1988). In the USA, invasive carp eggs and larvae have been documented at large-tributary confluences in the UMR (Larson et al. 2017) but also at various other locations throughout large (Illinois River; DeGrandchamp et al. 2007) and small (Cache and Wabash rivers; Burr et al. 1996; Coulter et al. 2013) tributaries. Although a few studies have documented invasive carp reproduction in Mississippi River tributaries, little is known about their reproduction phenology in higher reaches of the tributaries. The availability of consistent lotic-like conditions in tributaries compared to the main-stem UMR has implications for understanding the establishment and spread of these fishes throughout other upstream locations in the basin.

To understand the further expansion and success of invasive carp establishment in the UMR, it is essential to evaluate spatial and temporal variation of reproduction in tributaries compared to the UMR. Our objectives were to examine invasive carp reproduction in the impounded UMR and downstream unimpounded reaches of the Des Moines, Skunk, and Iowa rivers (tributaries to the UMR) in southeast Iowa. First, we examined the timing of spawning by using adult gonad development and back-calculated spawn dates from eggs and larvae. Second, we evaluated egg and larval densities to compare the magnitude of reproduction in tributaries relative to the UMR. Identifying areas of reproduction is vital for understanding the extent to which invasive carp could spread beyond their current distribution and for understanding the factors contributing to their spread.

**METHODS**

**Study area.**—The UMR is a large catchment with headwaters in Lake Itasca, Minnesota, and travels 1,070 km to its southernmost point at the confluence with the Missouri River near St. Louis, Missouri. A series of LDs regulates river flow and continual modification to the substrate to sustain a 2.7-m-deep, navigable channel and forms a series of slow-moving impoundments that are more lentic than the historical natural lotic flow regime (Garvey et al. 2003). During periods of low river discharge, water is blocked to maintain the navigable river channel in the impounded area above dams.
Similar to the UMR, the Iowa, Skunk, and Des Moines rivers have been channelized and contain an array of dams, levees, and wing dikes to regulate river flow. Several dams of various sizes exist on these rivers and may be permanent or semi-permanent fish barriers depending on river discharge. Furthermore, each dam disrupts river continuity, thus affecting species that require long stretches of connected habitat for one or more life stages. However, continuous free-flowing stream reaches greater than 60 km exist between dams and the tributary confluences with the UMR. In addition, each river has a spring flood pulse similar to those in other tributaries where invasive carp reproduction has been observed.

**Adult sampling.**—Adult invasive carp were sampled once per month from April through October in 2014 and 2015 at nine sites in the Des Moines, Skunk, Iowa, Cedar, and Mississippi rivers (Figure 1: Sullivan et al. 2017). Sampling locations were selected based on locations of access points and agency interests and to disperse sampling effort throughout each tributary. At each site, three spatially independent transects were sampled monthly, where one electrofishing survey and one trammel net set were conducted. Within each transect, one 15-min daytime electrofishing transect (Smith-Root; 4–13 A, 100–500-V DC, 25% duty cycle, 25% frequency, 60 pulses/s; with two netters) targeting channel border and backwater habitats was conducted parallel to the shoreline. To effectively electroshock, sampling was conducted using a “standardizing by power” approach (Miranda 2009) wherein electrofishing settings were altered to elicit a “standard response” in carp (see Sullivan et al. 2017). When river conditions allowed, one trammel net (2.4-m-deep inner wall, 1.8-m-deep outer wall, 38.1 m long, 10-cm bar inner mesh with number-9 twine, and a 9.9–12.7-mm foam float line and single lead line) was set in an area of low velocity. One end of the trammel net was anchored to the shore, and the other end was stretched toward the opposite shore or deeper water, restricting fish movement out of the low-velocity area. Trawls were deployed before electrofishing and collected immediately afterward (net deployment duration ranged from 20 to 30 min). The vast majority of adults captured in 2014 (90%) and 2015 (98%) were from the Des Moines River and its confluence with the Mississippi River. Adult invasive carp were captured in the Skunk and Iowa rivers in both years, but total catches during each year were less than 24 fish/river. Species composition was highly skewed toward Silver Carp in both 2014 (90%) and 2015 (90%). Therefore, only Silver Carp in the Des Moines River were evaluated for GSI and gonad classification.

Invasive carp were identified as Bighead, Silver, or Grass carp; measured (mm TL); and weighed (g). Adult hybrids were identified by the presence of deformed gill rakers and were excluded from further analysis ($n = 11$). Each carp was identified as male or female upon visual inspection of the gonads, and gonads from up to 100 females of each species per site during each monthly visit were removed and weighed (g) to calculate the gonadosomatic index (GSI; Crim and Glebe 1990) as

$$
GSI = 100 \times \frac{GW_i}{BW_i},
$$

where $GW = \text{the gonad weight of fish } i$; and $BW = \text{the body weight of fish } i$. Gonads were then classified based on developmental stage (Hunter and Maciewicz 1985; West 1990; Bruch et al. 2001; Colombo et al. 2007; Table 1). Resorption (atresia) usually occurs at the end of the reproductive cycle or when a female is subjected to high stress levels associated with unfavorable conditions (Lubzens et al. 2010). The proportion of all captured male and female invasive carp exhibiting each gonad stage was quantified during each month. All stage 6 females were considered postspawn and were aggregated to determine the proportion of postspawn females caught during each month.

**Ichthyoplankton sampling.**—Ichthyoplankton was sampled every 10 d from April through September during 2014 and 2015 at the same nine sampling sites that were sampled for adult carp (Figure 1). However, additional ichthyoplankton samples were taken approximately 3 km upstream and downstream of the mixing waters of tributary confluences and within the tributary, totaling 19 ichthyoplankton sampling locations. Sampling was not conducted at any site from June 21 to July 20, 2014, due to hazardous river conditions that precluded safe boating. Ichthyoplankton tow (0.5-m-diameter net with 500-μm mesh) were conducted at a constant boat speed relative to the shoreline near the surface for up to 4 min depending on debris load. A General Oceanics Model 2030R flowmeter was mounted in the mouth of the net to estimate the volume (m³) of water filtered during each tow. Three tows were conducted at each site parallel to river flow; the first tow was in the main thalweg for drifting eggs and larvae; the second tow occurred near channel borders, where water velocity was slower than that in the thalweg; and the third tow took place in an adjacent backwater area for mobile larvae. After each tow, ichthyoplankton net contents were rinsed toward the cod end, placed in bottles, and preserved in 95% ethanol.

In the laboratory, eggs and larvae from each tow were separated from debris by at least two individuals on separate occasions until no eggs or larvae were found; the eggs and larvae were stored in 20-mL glass scintillation vials with 95% ethanol. A subset of eggs was identified using genetic analysis. A random subsampling scheme representative of each tow was used to capture the spatiotemporal variation of species assemblages (see Camacho et al. 2010). The proportion of all captured male and female invasive carp exhibiting each gonad stage was quantified during each month. All stage 6 females were considered postspawn and were aggregated to determine the proportion of postspawn females caught during each month.
[2019] for additional subsampling details). We extracted DNA from subsampled eggs by using the Gentra Puregene Tissue Kit (Qiagen, Germantown, Maryland) or the Promega Wizard Genomic DNA Purification Kit (Promega Corp., Madison, Wisconsin) according to the manufacturer’s suggested protocol and stored the samples at −20°C. Polymerase chain reaction was used to amplify portions of the mitochondrial genome corresponding to the cytochrome-\(b\) gene using primers developed by Song et al. (1998) or cytochrome oxidase subunit I using primers developed by Ivanova et al. (2007). Successfully amplified PCR products were sequenced and manually edited in Geneious (Kearse et al. 2012) and compared to the DNA sequences of known invasive carp species for positive identification. Silver Carp and Bighead Carp larvae are difficult to visually distinguish between species and were identified to genus, while Grass Carp larvae were identified to species by using meristic and morphometric characteristics (Chapman 2006; Chapman and George 2011; George and Chapman 2015). To confirm visual identification, 48 larvae collected in 2014 and 21 larvae collected in 2015 that were identified as either Grass Carp or Hypophthalmichthys spp. were genetically analyzed using the same genetic procedure for egg identification. All visual identifications of larvae were accurate based on genetic confirmation.

Egg and larval development (Chapman and George 2011; George and Chapman 2015) and water temperature at the time of collection were used to determine the time (h) since fertilization for each egg and larva according to the cumulative thermal units equations provided by George and Chapman (2013, 2015). The spawning date of eggs and larvae was then estimated as

\[
\text{Spawning date} = \frac{TC_i - TF_i}{C0},
\]

where \(TC_i\) = time and date of capture of egg or larval fish \(i\); and \(TF_i\) = time since fertilization of egg or larval fish \(i\) (Deters et al. 2013). Grass Carp and Hypophthalmichthys spp. egg and larval densities were calculated as the number per 100 m\(^3\) of water sampled for each tow. Egg densities were extrapolated from genetic results as

\[
\text{Egg density} = \frac{SP_i \times T}{W} \times 100,
\]

where \(SP_i\) = the frequency of eggs genetically identified as species \(i\); \(SP\) = the frequency of eggs from all species genetically identified; \(T\) = total number of eggs caught; and \(W\) = the volume of water sampled during each tow, calculated from the flowmeter mounted at the mouth of the ichthyoplankton net. Larval densities were calculated as...
Larval density = \( \frac{L_i}{W} \times 100 \),

where \( L_i \) = the frequency of larvae visually identified as species \( i \). Ichthyoplankton densities in water samples were calculated as

\[
\text{Ichthyoplankton density} = \left( \frac{\sum \text{SP} \times T}{W} \right) + \frac{L_i}{100}.
\]

Environmental variables.—Surface water temperature was measured with an ExtStick II conductivity meter (Extech Instruments, Nashua, New Hampshire) in the thalweg at each site and date. Temperature data were not available for every day of the year. Linear regression using field-collected water temperature from each site and mean daily water temperature from the nearest U.S. Geological Survey gauging station within each river was performed to predict mean daily water temperature at each site (Table 2). Mean daily temperature data from the Iowa River gauging station were used to model the adjacent Skunk River because a station with temperature data was not available.

Mean daily discharge data for 2014 and 2015 were collected from the U.S. Geological Survey gauging station nearest to each sampling site (Table 3). General hydrologic trends 24 and 48 h prior to each spawning date were calculated as the difference in mean daily discharge. However, a rising limb of discharge may not be a key diagnostic to commence spawning for the invaded Mississippi River basin (Coulter et al. 2016) as in other areas (Kolar et al. 2007). Furthermore, differences in river depth, width, and gradient at each site varied, making it difficult to conduct meaningful comparisons using discharge data. Alternatively, a specific velocity requirement of 0.7 m/s for invasive carp spawning has been proposed (Abdusamadov 1987; Kocovsky et al. 2012). Thus, paired field measurements of discharge and velocity (U.S. Geological Survey 2016) from each sampling site were converted to mean daily channel velocity to provide a relative unit for comparing all sites.

Spatiotemporal variation of spawning.—Female GSI and gonad classification data for each tributary during each month were averaged into an upriver section (three sites in the Des Moines and Skunk rivers and four sites in the Iowa River) and mouth section (two sites for each tributary). Female GSI for each month during 2014 and 2015 was evaluated using a generalized linear model with Tukey’s honestly significant difference test to evaluate differences between the upriver and mouth sections during each month within each year. Data were nonnormal, and generalized linear models are not constrained by normality (Hubert and Fabrizio 2007). Pearson’s product-moment correlation coefficient was used to determine whether female GSI for the upriver and mouth sections during each year was related to the proportion of postspawn females.

Both eggs and larvae are important to determine the magnitude of spawning, especially since egg incubation times can be short (1–2 d). Ichthyoplankton densities were averaged across the three tows (one per habitat) for each site. For each tributary, densities were averaged across sites on each date into an upriver section (three sites in the Des Moines and Skunk rivers and four sites in the Iowa River) and a mouth section (two sites for each tributary). The main-stem Mississippi River site upstream of each confluence was not included because it did not receive eggs or larvae from the nearby tributary. Spatiotemporal variation in mean

### Table 1. Description of gonad development stages used to classify male and female invasive carp.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No ovaries visible</td>
<td>No testes visible</td>
</tr>
<tr>
<td>2</td>
<td>Ovaries thread-like in size</td>
<td>Testes thread-like in size</td>
</tr>
<tr>
<td>3</td>
<td>Small ovaries and oocytes</td>
<td>Small testes without folds</td>
</tr>
<tr>
<td>4</td>
<td>Large ovaries full of mature greenish-olive oocytes</td>
<td>Large testes with folds</td>
</tr>
<tr>
<td>5</td>
<td>Gametes released with light pressure applied to the abdomen</td>
<td>Gametes released with light pressure applied to the abdomen</td>
</tr>
<tr>
<td>6</td>
<td>Ovaries whitish in color and slightly opaque; few (&lt;100) mature oocytes</td>
<td>Gametes not released with light pressure applied to the abdomen; large testes with folds and substantially smaller on the posterior and anterior ends</td>
</tr>
<tr>
<td>7</td>
<td>Ovaries whitish in color, are gelatinous in texture, and contain various amounts of deteriorated and indistinguishable oocyte material</td>
<td>N/A</td>
</tr>
</tbody>
</table>
log_{10}(x + 1)-transformed ichthyoplankton densities was then compared within and among tributaries using repeated-measures ANOVA to compare differences between sections, among tributaries, and between years as well as their interactions. A Bonferroni-adjusted pairwise comparison was used to evaluate ichthyoplankton density differences when main effects or the interaction were significant.

Repeated-measures ANOVA was also used to test for differences in mean log_{10}(x + 1)-transformed ichthyoplankton densities between tributaries and the mainstem Mississippi River at each tributary confluence. For each confluence, habitat tows from each site (tributary mouth, main-stem Mississippi River downstream of the confluence, and main-stem Mississippi River upstream of the confluence) and date were averaged (i.e., 3 tows-site^{-1}-date^{-1}). Tributary site, river, year, and their interactions were tested. Sampling sessions at each tributary during each year were included as random effects to account for temporal variation at each tributary. A Bonferroni-adjusted pairwise comparison was used to compare density differences when main effects were significant. All statistical analyses were performed in R version 3.2.0 (R Core Team 2015) with a significance level $\alpha$ of 0.05.

RESULTS

Adult Sampling

Overall, 578 female and 481 male Silver Carp were collected in 2014 and 1,110 females and 907 males were collected in 2015 from the Des Moines River and confluence for reproductive assessment. Silver Carp GSI was higher in the upriver section than in the mouth during June 2014 ($P < 0.01$) but was similar between the two sections across all other months (Figure 2). In 2015, Silver Carp GSI was significantly higher at the mouth than in the upriver section during April ($P = 0.03$), June ($P < 0.01$), and September ($P = 0.04$; Figure 2). Gonad development of female Silver Carp in the Des Moines River varied by year and river section. In the upriver section during 2014, females exhibited ovaries with immature oocytes (stage 3) and fully mature oocytes (stage 4) in April and May, leading up to the first fully spawned ovaries (stage 6) starting in June (Figure 3). The vast majority of females captured in each month after June were postspawn (Figure 2). However, some females in August and September were observed with immature oocytes similar to females captured during spring. Resorption was observed in late fall but at low proportions. Female Silver Carp in the mouth section were comprised of mainly stage 4 fish in April and
stage 3 fish in May, but females with mature oocytes were captured from April through September (Figure 3). Postspawn females were first observed in June and comprised the highest proportion of females in July, September, and October (Figure 2). Two females with thread-like ovaries (stage 2) were observed in the mouth section during 2014. Immature Silver Carp without visible gonads (stage 1) and ripe females releasing eggs (stage 5) were not captured in either river section. During 2015, stage 2 female Silver Carp were sampled in every month and made up the vast majority of fish in all months except April and May, when mainly stage 4 females were captured (Figure 3). Ripe females releasing eggs and postspawn females were first captured in May. However, the proportion of postspawn females remained low throughout the year (Figure 2). Females in the mouth section during each month spanned from immature (stage 2) to postspawn (stage 6). However, females releasing eggs were only found in April and May. Only one immature (stage 1) Silver Carp was captured during the study.

The proportion of postspawn females captured during each month varied by year and section (Figure 2). In both years, the proportion of postspawn females was bimodal for each section. The initial peak was 1 month earlier in 2015 compared to 2014. The proportion of postspawn females in the upriver section during 2015 had the lowest peak of any section in both years and remained low throughout sampling. The proportion of postspawn females was negatively related to GSI in the upriver section during 2014 (0.05) and 2015 (0.29). There was no significant relationship between GSI and the proportion of postspawn females in the upriver section during 2015 (r = -0.46, t5 = -1.71, P = 0.29).

**Ichthyoplankton Sampling**

In total, 10,205 eggs were collected from May 5 to September 26, 2014, and 5,929 eggs were collected from April 23 to September 25, 2015. Invasive carp accounted

---

<table>
<thead>
<tr>
<th>USGS station number</th>
<th>USGS station description</th>
<th>Log–log linear regression</th>
<th>Calculated discharge (m³/s) for 0.7-m/s velocity</th>
<th>Corresponding sampling site</th>
</tr>
</thead>
<tbody>
<tr>
<td>5488500</td>
<td>Des Moines River near Tracy, Iowa</td>
<td>$V = 0.3069 \times \log_e(D) - 0.6527$</td>
<td>0.88</td>
<td>82</td>
</tr>
<tr>
<td>5489500</td>
<td>Des Moines River at Ottumwa, Iowa</td>
<td>$V = 0.2655 \times \log_e(D) - 0.5524$</td>
<td>0.85</td>
<td>112</td>
</tr>
<tr>
<td>5490500</td>
<td>Des Moines River at Keosauqua, Iowa</td>
<td>$V = 0.356 \times \log_e(D) - 0.8811$</td>
<td>0.85</td>
<td>85</td>
</tr>
<tr>
<td>5473400</td>
<td>Cedar Creek near Oakland Mills, Iowa</td>
<td>$V = 0.155 \times \log_e(D) + 0.2068$</td>
<td>0.78</td>
<td>24</td>
</tr>
<tr>
<td>5473065</td>
<td>Skunk River at Merrimac, Iowa</td>
<td>$V = 0.1247 \times \log_e(D) + 0.2446$</td>
<td>0.65</td>
<td>39</td>
</tr>
<tr>
<td>5474000</td>
<td>Skunk River at Augusta, Iowa</td>
<td>$V = 0.2133 \times \log_e(D) - 0.0202$</td>
<td>0.85</td>
<td>29</td>
</tr>
<tr>
<td>5465000</td>
<td>Cedar River near Conesville, Iowa</td>
<td>$V = 0.1887 \times \log_e(D) - 0.231$</td>
<td>0.64</td>
<td>139</td>
</tr>
<tr>
<td>5455500</td>
<td>English River at Kalona, Iowa</td>
<td>$V = 0.1514 \times \log_e(D) + 0.1401$</td>
<td>0.69</td>
<td>40</td>
</tr>
<tr>
<td>5455700</td>
<td>Iowa River near Lone Tree, Iowa</td>
<td>$V = 0.1741 \times \log_e(D) - 0.0739$</td>
<td>0.92</td>
<td>85</td>
</tr>
<tr>
<td>5465700</td>
<td>Iowa River at Oakville, Iowa</td>
<td>$V = 0.2929 \times \log_e(D) - 0.9156$</td>
<td>0.87</td>
<td>249</td>
</tr>
<tr>
<td>5420500</td>
<td>Mississippi River at Clinton, Iowa</td>
<td>$V = 0.4291 \times \log_e(D) - 2.5948$</td>
<td>0.96</td>
<td>1,938</td>
</tr>
</tbody>
</table>
for 338 of 805 genetically identified eggs in 2014 (42%), resulting in densities of up to 5,473 eggs/100 m$^3$. In 2015, 209 of 774 eggs were genetically identified as invasive carp (27%), resulting in densities of up to 1,516 eggs/100 m$^3$. Additionally, 43,194 larvae were collected from May 5 to September 27, 2014, and 30,419 larvae were collected from April 22 to September 25, 2015. Invasive carp accounted for a total of 1,860 larvae in 2014 (4%), with a maximum density of 3,251 larvae/100 m$^3$, and an additional 578 invasive carp larvae were captured in 2015 (2%), with a maximum density of 441 larvae/100 m$^3$.

**Spatiotemporal Variation of Spawning**

Temporal distributions of invasive carp eggs in the Des Moines River differed between upriver and mouth sections. No eggs were caught in the upriver section of the Des Moines River during 2014, whereas egg densities were highest for Silver and Grass carp in the mouth during late May (Figure 4). Peak egg densities in 2015 were captured at the end of May in the upriver and mouth sections of the Des Moines River. In general, the first observation of invasive carp eggs coincided with the peak egg density for a given section in the Des Moines River except in the mouth section during 2015, when Silver Carp eggs were captured 20 d prior to the peak in egg density. Bighead Carp eggs were only collected in the upriver section of the Des Moines River during 2015.

Similar to eggs, temporal distributions of larvae differed between upstream and mouth river sections and between years in the Des Moines River. Furthermore, peak larval densities were lower than egg densities. In 2014, a single larval Grass Carp was caught in the upriver section during the August 30 sampling session. Grass Carp and *Hypophthalmichthys* larvae were captured during two consecutive sampling sessions (May 26 and June 3, 2014) in the mouth section, and additional Grass Carp larvae were captured during the June 11 sampling session (Figure 5). *Hypophthalmichthys* larvae were captured in all three sampling sessions during June 2015 in the upriver section and were captured in all sampling sessions from May 31 to July 30, 2015, in the mouth section, except for the July 20 session (Figure 5). A single peak in larval Silver Carp densities occurred on June 10 in the upriver section, and two similar peaks occurred in the mouth section during June 29 and July 30, 2015. Grass Carp larvae were captured on the same sampling sessions as Silver Carp except for the last session, during which Silver Carp were captured in both sections. Grass Carp densities peaked on June 24 in the upriver section and on May 31 in the mouth during 2015. Grass Carp larval densities were generally lower than Silver Carp densities for each session when both species were captured.

In the Skunk River, invasive carp eggs were captured in the upriver section during 2015 and in the mouth...
section during both 2014 and 2015, but densities were less than 2 eggs/100 m³. No invasive carp eggs were captured in the upriver section during 2014. The Skunk River mouth in 2014 had a peak density of less than 1 Grass Carp egg/100 m³ on June 18, with additional Grass, Bighead, and Silver Carp eggs captured on May 26 (Figure 4). During 2015, Silver Carp eggs were captured in the upriver section of the Skunk River on June 24 and July 20 (Figure 4). In the mouth section, Silver Carp eggs were captured on June 24 and Grass Carp eggs were only captured on May 20 (Figure 4).

Invasive carp larvae were only captured in the upstream section of the Skunk River during 2015 despite eggs being caught in the Skunk River mouth section during both years (Figure 5). The highest larval densities occurred during the May 26, 2014, session in the mouth, when 565 Hypophthalmichthys larvae/100 m³ were captured (Figure 5). Larvae were also captured at the mouth during two other sessions in 2014 (June 3 and 18) but at substantially lower densities. Grass Carp and Hypophthalmichthys larvae were captured in 2015 at the mouth on May 20 and June 24 but at low densities (<5 larvae/100 m³).

Invasive carp eggs were captured in the mouth of the Iowa River during 2014 and 2015 and in the upriver section during 2015. No invasive carp eggs were captured in the upriver section of the Iowa River during 2014, and Grass Carp eggs were not captured in the Iowa River during 2015. Silver, Grass, and Bighead Carp egg densities peaked on the June 18, 2014 sampling session in the mouth (Figure 4). Silver Carp eggs were also captured on June 11, and Grass Carp eggs were also captured on May 26 and June 3 (Figure 4). Bighead Carp eggs were not captured at any other time in either section during 2014.
or 2015. Silver Carp eggs were caught in the upriver section on May 31 and June 29, 2015, and in the mouth section on July 10, 2015 (Figure 4).

Similar to eggs, invasive carp larvae in the Iowa River were not captured in the upriver section during 2014 (Figure 5). A single Grass Carp larva was captured in the upriver section on June 10, 2015. *Hypophthalmichthys* larvae were captured in the mouth section on multiple sampling dates in 2014 with a peak on June 18, and a single sampling date on May 20, 2015. Grass Carp larvae were only captured on June 18, 2014, but had the highest density in the Iowa River mouth during both 2014 and 2015 (Figure 5).

Ichthyoplankton (eggs and larvae) densities were significantly different among rivers ($F_{1, 156} = 3.276, P = 0.04$; Figure 6). Densities in the Des Moines River were higher than those in the Iowa River ($t = 2.32, P = 0.02$) and Skunk River ($t = 2.03, P = 0.04$). Comparisons of ichthyoplankton densities at river confluences resulted in a significant interaction between river and year ($F_{2, 246} = 3.388, P = 0.04$). In the Des Moines River, densities at the mouth were higher in 2015 than in 2014 ($t = 2.92, P < 0.01$; Figure 7). None of the other comparisons was significant ($P > 0.05$).

Back-calculated ages of invasive carp ichthyoplankton ranged from 2 to 40 h for eggs and from 24 to 68 h for larvae, except one larva in the Des Moines River, for which age was estimated at 188 h. Estimated spawning dates from back-calculated ages ranged from May 25 to August 25, 2014, and from May 11 to August 10, 2015,

![FIGURE 4. Egg densities (mean ± SE) of Bighead, Silver, and Grass carp in the upriver and mouth sections of the Iowa, Skunk, and Des Moines rivers during 2014 (left panels) and 2015 (right panels). Asterisks represent sessions in which sampling did not occur due to hazardous river conditions.](image-url)
across all sites. In 2014, the August 25 spawn date was from a single Grass Carp larva caught in the upriver section of the Des Moines River at Eddyville. No other spawn dates were estimated in August for the Des Moines River during 2014 (Appendix Figures A.1, A.2). However, spawning in August occurred in the upriver section of the Skunk River during 2015 (Figure A.3)—more than a month after the first spawning event and 11 d after the last spawning event at the Skunk River mouth. Estimated spawning dates for sites in the upriver section of the Iowa River were between early June and early July (Figure A.4). Mean daily channel velocity and temperature were above the minimum threshold of 18°C and 0.7 m/s in all sites, sections, and rivers during both years. Mean daily channel velocity on each spawning date ranged from 0.87 to 1.21 m/s when water temperatures were 19.1–26.5°C in 2014 and from 0.68 to 1.65 m/s when water temperatures were 18.8–27.6°C in 2015.

FIGURE 5. Larval densities (mean ± SE) of Hypophthalmichthys spp. (Bighead and Silver carp) and Grass Carp in the upriver and mouth sections of the Iowa, Skunk, and Des Moines rivers during 2014 (left panels) and 2015 (right panels). Because Bighead Carp and Silver Carp larvae are difficult to visually distinguish due to their morphological similarities, they were combined into a single genus classification. Asterisks represent sessions in which sampling did not occur due to hazardous river conditions.
DISCUSSION

Determining whether invasive carp in the UMR and its tributaries are established is dependent upon the documentation of successful reproduction. Invasive carp prefer turbulent waters of river confluences for spawning in their native range (Yi et al. 2006) and in the middle Mississippi River (Shrank et al. 2001). Similarly, Bighead, Silver, and Grass Carp eggs and larvae were collected in the main-stem UMR in association with tributary confluences. Similar invasive carp reproduction has been documented in association with our study tributaries (Larson et al. 2017) and other tributary confluences (Burr et al. 1996; DeGrandchamp et al. 2007; Coulter et al. 2013). However, eggs and larvae were also collected more than 40 km upstream from the Des Moines, Iowa, and Skunk River confluences, indicating that reproduction is not limited to the main-stem Mississippi River and adjacent tributary mouths.

Invasive carp can successfully reproduce in the UMR and major tributaries when conditions are favorable for spawning. Adults can travel long distances and have increased movement during the spawning season (DeGrandchamp et al. 2008). In the Des Moines River, adult abundances vary but are highest during May and June (Sullivan et al. 2017), coinciding with egg presence, high GSI levels, and few females with postspawn status, and are lowest in July and August, coinciding with egg absence, low GSI levels, and females mostly in postspawn status. Adult movements related to spawning can have a significant effect on ichthyoplankton densities. For example, the highest density of invasive carp ichthyoplankton was detected in the UMR main stem upstream of the Des Moines River confluence during a large spawning event observed on May 27, 2014, whereas very few ichthyoplankton were caught within the Des Moines River (6 river kilometers away) on the same date. The following year, the Des Moines River had the highest ichthyoplankton densities, whereas very few eggs or larvae were captured in the UMR upstream of the confluence. The ability of adults to move in search of spawning habitat highlights
that localized spawning may be highly variable on an annual basis, but reproduction in a larger spatial context may be more consistent.

In 2014, the UMR and tributaries experienced a major flooding event that likely influenced where invasive carp reproduced. Rising discharge has been suggested as a spawning trigger (Kolar et al. 2007), and the crest in the UMR on July 3, 2014, was the 12th-highest flood level on record. This large pulse returned the UMR into a continuously free-flowing river system that was adequate for invasive carp spawning by cresting many of the low-head portions of the LDs and forcing dam gates to be fully raised. Furthermore, flood waters inundated much of the adjacent floodplain, providing larval invasive carp with an abundance of backwater area for rearing. Unfortunately, unsafe working conditions and logistical difficulties made it necessary to halt sampling operations during much of the flooding event, which likely excluded a major spawning event from our data set. However, adult GSI levels peaked as the Mississippi River water levels were increasing in June before the flood and were at the lowest levels in July, immediately after the flood. Additionally, eggs and larvae were collected elsewhere in the UMR main stem during this flood event (Larson et al. 2017). Both findings suggest that this high-magnitude flood pulse created favorable spawning conditions throughout the UMR.

Invasive carp reproduction generally occurs in late spring to early summer (Kolar et al. 2007). However, collections of mixed size-classes of larvae (Galat et al. 2004), gonadal maturation (Schrank and Guy 2002; Papoulias et al. 2006), and eggs collected in the Wabash River, Indiana (Coulter et al. 2013, 2016), provide evidence that spawning can be protracted through fall. The persistence of ripe, unspent, and partially spent ovaries in adult females observed as late as October and the back-
calculated spawn dates of eggs and larvae ranging from May through August indicate that invasive carp exhibit protracted spawning in the tributaries of the UMR. Although eggs and larvae were collected in the late summer and fall, peak densities generally occurred during late May and June, coinciding with a decline in adult female GSI and an increase in postspawn females. It is currently unknown whether adults are able to spawn multiple times in a season, but bimodal distributions of intraovarian egg maturation suggest that it is possible for females (Schrank and Guy 2002). Partially spent, ripe ovaries observed in this study also suggest that females may not release all eggs in a single spawning event and could spawn multiple times if conditions are adequate. However, various stages of egg resorption from partially spent females were also observed, indicating that females may not release all of the eggs that are produced in a given year. It is unknown whether egg resorption is due to environmental stressors or a lack of favorable spawning conditions after the initial spawning event. Furthermore, spawning events occurring late in the season limit the time larvae have for growth and storing energy reserves prior to winter, potentially resulting in higher mortality than larvae that hatch during the spring (Freeze and Crawford 1983).

Invasive carp reproduction is governed by a suite of environmental factors, including water temperature, mean velocity, and hydrological trends (Kolar et al. 2007). Water temperatures during the spawning events documented here were within the optimal spawning temperatures of 18–30°C (Kolar et al. 2007). Additionally, mean water velocities on all spawning dates were greater than or equal to 0.7 m/s (Kocovsky et al. 2012). A rising hydrologic trend may be required for spawning, where rising water levels create more turbulent conditions to keep eggs suspended in the drift (Kolar et al. 2007). However, spawning occurred on both the rising and falling limbs of the hydrograph 24 and 48 h prior to spawning. Furthermore, invasive carp spawn in the heavily regulated Karakum Canal, Turkmenistan, where water levels do not change (Kolar et al. 2007), and they spawn in the Wabash River during static base flow conditions. Thus, a rising hydrograph may not be required but may assist in inducing spawning activity (Kocovsky et al. 2012; Deters et al. 2013).

For more than a decade, managers have enacted various methods to control the spread and subsequent establishment of invasive carp (Conover et al. 2007). For the UMR, managers could alter dam operations to reduce the duration and magnitude of free-flowing conditions in the UMR; however, free-flowing conditions in tributaries still provide adequate spawning habitat. Adults could migrate to spawn in tributaries instead of using the UMR, potentially negating any reduction in spawning within the UMR. Furthermore, reducing adult migrants from downstream reaches by placing deterrents such as broadband sound (Vetter et al. 2017) or bubble curtains (Zielinski and Sorensen 2016) in the locks of LD19 would not eliminate recruitment upstream of the dam. Otolith microchemistry of adult invasive carp captured upstream of LD19 suggests that recruitment is not limited to migrants from downstream of LD19 and that spawning in the tributaries (Skunk and Iowa rivers) evaluated here has led to successful recruitment of adults (Whitledge et al. 2019). Juvenile carp older than the onset of squamation were not captured during this study despite the capture of eggs and larvae in both years. However, numerous age-0 Silver, Bighead, and Grass carp were captured in a Skunk River backwater near the mouth during August 2018 (M. J. Weber, unpublished data), providing evidence that larvae produced in these areas can survive and inhabit these sites when conditions are suitable. Determining the linkages between larval and juvenile life stages as well as juvenile rearing habitat is a critical missing piece in understanding the life cycle of invasive carp in the UMR. It is also critical to determine where and the conditions under which spawning leads to recruitment so that targeted removal efforts can be most effective at reducing population growth. To control invasive carp in the UMR, a multifaceted approach must be utilized that addresses not only recruitment from the immigration of adults but also local recruitment from main-stem and tributary spawning.

ACKNOWLEDGMENTS
This project was funded by the Iowa Department of Natural Resources, U.S. Fish and Wildlife Service and Iowa State University. This study was performed in accordance with Protocol 7-13-7599-I approved by the Institutional Animal Care and Use Committee at Iowa State University, and animals were collected under Iowa State Permit SC1037. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. We thank Alison Coulter and two anonymous reviewers for comments that improved the manuscript. There is no conflict of interest declared in this article.

ORCID
C. A. Camacho, https://orcid.org/0000-0001-9828-1987
C. J. Sullivan, https://orcid.org/0000-0001-7214-3789
M. J. Weber, https://orcid.org/0000-0003-0430-3087
C. L. Pierce, https://orcid.org/0000-0001-5088-5431

REFERENCES


FIGURE A.1. Estimated water temperature, channel velocity, and invasive carp spawning dates for the sampling sites at Eddyville (DSM-EDD; top panels), Cliffland (DSM-CLF; middle panels), and Keosauqua (DSM-KQA; bottom panels) in the upriver section of the Des Moines River during 2014 (right panels) and 2015 (left panels). The horizontal black line represents the minimum water temperature or minimum velocity required for spawning.
FIGURE A.2. Estimated water temperature, channel velocity, and invasive carp spawning dates for the sites at the Des Moines River mouth (DSM-MTH; top panels) and the Mississippi River site downstream of the Des Moines River confluence (UMR-DND; bottom panels) in the downriver section of the Des Moines River during 2014 (right panels) and 2015 (left panels). The horizontal black line represents the minimum water temperature or minimum velocity required for spawning.
FIGURE A.3. Estimated water temperature, channel velocity, and invasive carp spawning dates for the Skunk River site downstream of the Cedar Creek confluence (SKK-DNC; top panels) in the upriver section, the Skunk River mouth (SKK-MTH; middle panels), and the Mississippi River site downstream of the Skunk River confluence (UMR-DNS; bottom panels) during 2014 (right panels) and 2015 (left panels). The horizontal black line represents the minimum water temperature or minimum velocity required for spawning.
FIGURE A.4. Estimated water temperature, channel velocity, and invasive carp spawning dates for the Iowa River sites at Conesville (IAR-CON) and downstream of the English River (IAR-DNE) in the upriver section; and the Iowa River mouth (IAR-MTH) and the Mississippi River site downstream of the Iowa River confluence (UMR-DNI) in the downriver section during 2014 (right panels) and 2015 (left panels). The horizontal black line represents the minimum water temperature or minimum velocity required for spawning.