

Research Article

Egg size variability in an establishing population of invasive silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844)

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Abstract

Reproductive investment (e.g., egg size) is generally critical for the successful establishment of invasive species, with high variability often positively influencing success. Silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) are highly successful invasive fish on a global scale and threaten biodiversity in a wide range of freshwater habitats. However, factors influencing egg size variability in silver carp are not fully understood. We collected drifting silver carp eggs from the Wabash River, Indiana (USA) throughout the 2012 and 2013 spawning seasons to examine egg diameter variation was related to environmental factors and across time. The Wabash River is largely unregulated with few backwater habitats, and the resident silver carp population is relatively new. As a result, the Wabash River offers the opportunity to observe an expanding silver carp population in a river with a comparatively “natural” flow regime. Egg size was positively correlated with both embryo total length and yolk sac volume, indicating larger silver carp eggs contain larger, better-provisioned embryos at a given developmental stage. Eggs collected in 2013 were significantly larger than those collected in 2012 despite a decline in mean length of mature females. Relationships between egg diameter and environmental factors appear complex, with relationships varying between years. Silver carp eggs displayed high variability in diameter, with a mean coefficient of variation of 9.5%. This high variation may reflect a diverse population of spawning females, but it may also constitute a reproductive strategy to facilitate egg transport across habitats and potentially reduce intraspecific competition. Such reproductive plasticity in silver carp and other invasive species is likely to be key for achieving reproductive success in newly invaded ecosystems despite the species’ naivety with novel and unpredictable environmental conditions therein.

Key words: parental investment, Asian carp, aquatic invasive species, maternal effects, bet-hedging, plasticity, coefficient of variation

Introduction

Invasive species can have considerable detrimental impacts as they invade, establish, and spread through newly invaded ecosystems. However, many non-native species fail to establish and spread when introduced. Numerous characteristics have been hypothesized to contribute to the success of invasive fishes, many of which are related to life history. For example, large body size (Marchetti et al. 2004; Vila-Gispert et al. 2005; Ribiero et al. 2008), protracted or variable spawning periods (Russell et al. 2012), high fecundity (Vila-Gispert et al. 2005; MacInnis and Corkum 2000; Charlebois et al. 2001), and high levels of phenotypic plasticity (MacInnis and Corkum 2000;

Russell et al. 2012; Coulter et al. 2013) have all been proposed as contributors to the success of invasive species. These characteristics are often used in risk assessments to determine regulations; therefore, further examination of these factors will provide valuable information for the management of existing invasive populations and the prevention of future invasions (Kolar and Lodge 2002).

For an invasive species to establish, successful reproduction and recruitment must occur. Therefore, examination of patterns and trends in reproductive investment is warranted. Reproductive investment in fishes can be examined based on egg size, which is related to provisioning of the egg (Moodie et al. 1989) and ultimately impacts embryo (Beacham and Murray 1990; Johnston 1997)

and larval survival (Bagenal 1969; Hutchings 1991; Johnston 1997; Berkeley et al. 2004). Egg size is generally considered an optimization of egg number and size with respect to maternal fitness (Beacham and Murray 1990; Smith and Fretwell 1974; Crean and Marshall 2009), which can be influenced by environmental conditions and resource availability (Hutchings 1991). Variable environments may lead to the production of fewer, larger eggs or increased within-clutch egg size variation (Einum and Fleming 2004; Crean and Marshall 2009; Marshall et al. 2008). Life history strategy may also impact egg size (Winemiller and Rose 1992). For example, species with pelagic eggs appear to exhibit less variation in egg size than demersally-spawning species, presumably because the offspring of pelagic eggs face less intense post-hatch resource competition, and therefore are selected to be consistently small so as to maximize maternal fecundity and reproductive success (Einum and Fleming 2002). The timing of spawning can also influence egg size, with eggs spawned later in the season being smaller than those spawned earlier (Bagenal 1971; Bengtson et al. 1987; Beacham and Murray 1993; but see Beacham et al. 1988). Overall, these sources of variation can contribute a high degree of both intra- and inter-individual variation in egg sizes produced by a population.

Despite the myriad sources of variation and complex trade-offs involved that can drive egg size variation, egg size appears to be well-conserved within many iteroparous fish populations. Coefficients of variation (CV) in egg size are typically reported as between 3 and 9% among species with diverse life histories and habitats (Rinchar and Kestemont 1996; Einum and Fleming 2002; Johnston and Leggett 2002). Therefore, egg size in most species may reflect an adaptation of the egg size-egg number trade-off to local environmental conditions to maximize maternal fitness and yield relatively little within-population egg size variation (Einum and Fleming 2002). However, all of these studies have focused on egg size variation within the native range of a species. Invasive species, especially those in recently colonized habitats, may not have experienced their new environment for sufficient time to adapt and maximize reproductive efficiency, and the few studies that examined offspring size variation in invasive species have been primarily limited to plants (e.g., Buckley et al. 2003). Thus, the role egg size variation may play in promoting invasion success as invading species respond to novel environments and varying selection pressures

remains unclear, even though shifts in offspring size may influence reproductive success and ultimate establishment of invasive populations.

Silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) have successfully invaded a variety of freshwater ecosystems. Many global rivers have become highly regulated (e.g. locks, dams), providing large areas of low to no velocity habitats more consistent with the lentic habitats of adult silver carp in their native range compared to the often swiftly moving waters of unregulated rivers. In contrast, the Wabash River, IN, USA, is largely unregulated, and while silver carp were first reported in this watershed at the same time as in the Illinois River, USA, they occur at comparatively lower densities and are likely still establishing (Stuck et al. 2015). Because it is largely unregulated, the Wabash River presents a unique set of environmental constraints for silver carp, especially because slackwater habitats are scarce. Understanding the suite of responses exhibited by silver carp (e.g., reproductive ecology) that contribute to their successful establishment in the Wabash River can help to better predict the range of unregulated rivers in the Laurentian Great Lakes basin and other global freshwaters that may be vulnerable to invasion and establishment.

Silver carp exhibit life history traits, such as high fecundity and rapid growth that have been identified as potential contributing factors to their rapid spread and high abundances (Kolar et al. 2007). Silver carp also appear to exhibit a high degree of plasticity in a variety of traits, such as reproductive requirements (i.e., spawning site selection, watershed size), which may contribute to their invasion success in many ecosystems (Kolar et al. 2007; Coulter et al. 2013; Deters et al. 2013). One potentially important component for reproductive success may relate to egg size. Relatively little is known about silver carp egg size and variation, especially *in situ* and in non-native ecosystems. Previous studies have examined silver carp eggs in laboratory settings (Chapman and George 2011; George and Chapman 2013) or directly from the fish (Schrank and Guy 2002; Williamson and Garvey 2005). While *in situ* collection of eggs results in limitations because eggs are not associated with individual females, it does allow for population level generalizations of eggs produced. As indicated by the variability in egg size found within silver carp ovaries, they likely spawn on multiple occasions (Aliyev 1976; Freeze and Crawford 1983), and so knowledge of their egg size variability through time may be

vital in understanding their invasion success. For instance, high size variability in spawned eggs could act as a bet-hedging mechanism to improve the likelihood of producing eggs appropriately sized to match unpredictable offspring environments.

We investigated variation in silver carp egg diameter based on eggs collected *in situ* throughout the spawning seasons in 2012 and 2013 in the Wabash River. First, egg diameter was correlated with embryo total length and yolk sac volume to determine whether larger eggs contained larger, better-provisioned embryos that may be more likely to survive post-hatching. This relationship has been observed in other species, but not in silver carp. Second, we evaluated trends in egg diameter and egg diameter variation within and between years and in relation to environmental variables. Finally, 2012 and 2013 were very different years hydrologically, allowing for comparison of egg diameter under two different climate-based environmental regimes. Gaining a better understanding of egg size variation can provide insight into the reasons why silver carp exhibit high levels of success as invaders in novel environments.

Methods

Silver carp eggs were collected at least weekly from May 8th – August 28th, 2012, and May 7th – September 10th, 2013 from the Wabash River. The Wabash River contains a single mainstem dam and over 662 km of free-flowing water below the dam. The watershed is 85236 km² and is dominated by agricultural land uses (~66% watershed area). Sampling began prior to spawning and continued until no eggs were collected for three weeks which was considered the end of spawning. Eggs were collected using paired bongo nets with a mesh size of 500 µm at Wabash River km 499 and River km 521 near Lafayette, IN, USA. Bongo nets were deployed from the bow of a 4.9 m boat piloted downstream in reverse at ~8 kmh⁻¹ and towed for ~5 min. Boat speed was adjusted to ensure that the net was not collecting material suspended by the boat and that the net remained submerged below the water surface. Tows were done in triplicate (except: day of year [DOY] 139 in 2012 on which 6 tows were done; DOY 155 in 2013 on which subsamples were taken from a single tow due to a high volume of eggs) and collected samples were placed in watertight containers and taken back to the lab where the eggs were sorted from debris. To evaluate the importance of river flow rate,

discharge data were gathered from a nearby United States Geological Survey (USGS) gage (#003335500) using the water level measured at noon. Discharge (m³ sec⁻¹) data were not available for 2012 prior to DOY 205 sampling date and were therefore estimated based on the regression of water depth with discharge for the remainder of 2012 as discharge = 42.16 × water depth (m) + 12.98 (R² = 0.90). Water temperature (°C) was also measured *in situ* prior to each bongo net sampling event.

Up to 10 eggs were randomly selected from each bongo net pull (10 eggs per pull resulted in 30–60 eggs measured for diameter per sampling event, a portion of which were silver carp), photographed, and measured to the nearest 0.001 mm in diameter (Nikon SMZ 1500, Tokyo, Japan; Nikon Imaging Software Elements D). Photographs were taken within ~1.5 hr of collection. Eggs were checked for viability and staged based on established developmental stages for silver carp (Yi et al. 2006; Chapman and George 2011). In these developmental stages, stage 31 is hatching. Additionally, embryo total length (mm) and yolk volume (mm³) were measured from photographs of embryos later identified as silver carp (see below) at the three most common developmental stages (17, 24, and 29) to examine the relationship between egg provisioning, embryo size, and egg size. Only embryos positioned in photographs such that both total length and yolk sac dimensions were clearly visible and measurable were used for this analysis. Total length was measured along the midline of the embryo (Figure 1). Yolk sacs of silver carp change shape with development; therefore, yolk sac dimensions were measured two different ways. For yolk sacs of stage 17 and 24 embryos, two perpendicular measurements were taken and averaged to account for yolk sacs not being perfectly spherical. Four measurements were taken of stage 29 embryo yolks: two perpendicular measurements across the anterior yolk sac and two measurements across the posterior portion of the yolk sac. Yolk sac volume at stages 17 and 24 was calculated as the volume of a sphere. The stage 29 anterior portion of the yolk sac was also calculated as a sphere. The posterior portion of stage 29 yolk sac was calculated as a cylinder and total yolk sac volume at this stage was quantified as the anterior and posterior volumes combined.

After photographing, measured eggs were frozen in liquid nitrogen and stored at -80 °C until testing was performed to verify that eggs used in further analysis were from silver carp.

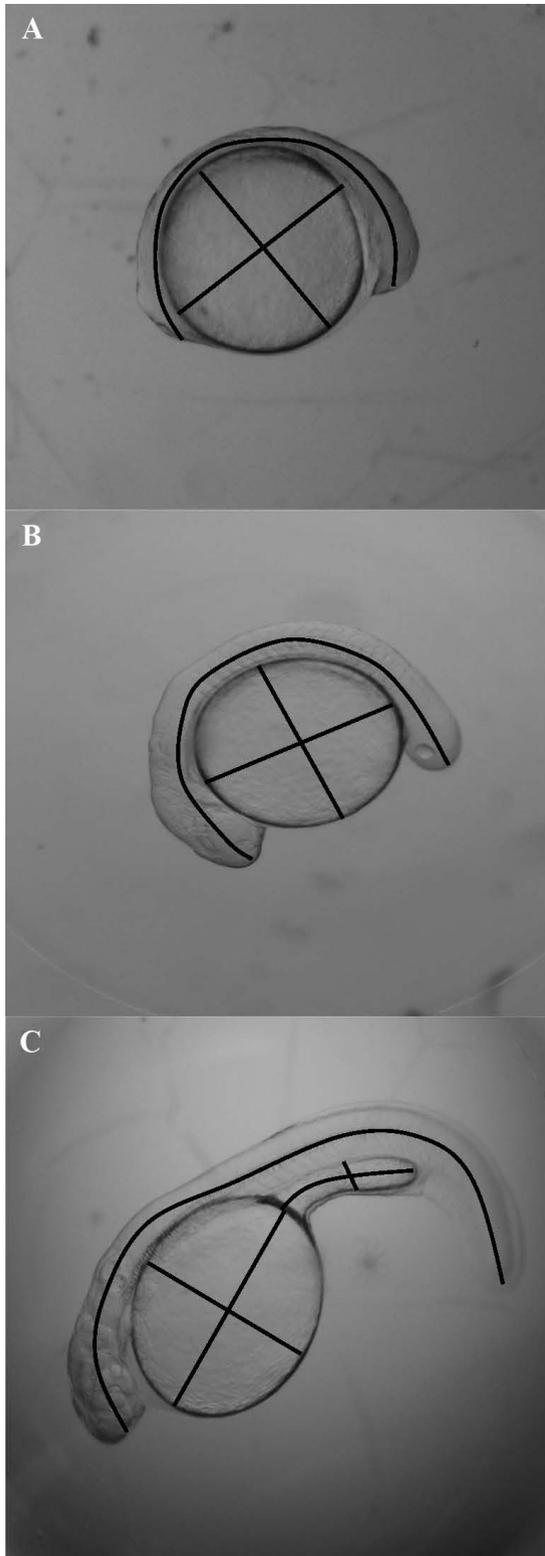


Figure 1. Measurements taken of silver carp embryo total length (mm) and yolk size for embryo developmental stages (A) 17, (B) 24, and (C) 29. Black lines indicate measurement locations. Photographs by A.W. Lenaerts and A.A. Coulter.

DNA was extracted from eggs according to manufacturer's instructions using QIAamp mini DNA kits (Qiagen Inc., Valencia, California, USA). Extracted DNA was tested with polymerase chain reaction (PCR; CPX96, Bio-Rad laboratories, Inc., Hercules, California, USA) using 50 ng genomic DNA. Each sample was tested in duplicate with three established primers (Mia et al. 2005; Hmo1, Hmo3, and Hmo11). Negative controls using nuclease free water were included in each run. Reactions totalled 20 μl and consisted of 5 μM each of forward and reverse primers, 50 ng μL^{-1} template DNA, SensiMix (Bioline USA, Inc., Tauton, Massachusetts, USA) and nuclease-free water. Conditions for PCR were as follows: 94°C for 2.5 min, 50°C for 45 s, and 72°C for 1 min for 45 amplification cycles with an extension period of 72°C for 10 minutes. Results of PCR were visualized with gel electrophoresis on a 2% agarose gel run for 75 min at 3.8 V cm^{-1} . Results of electrophoresis were interpreted using established basepair sizes (Mia et al. 2005). Only individuals exhibiting silver carp genes at all three loci were included in analyses.

Yolk sac volume and embryo total length were correlated with egg diameter for each of the three developmental stages examined separately using Pearson correlations. Differences in the distributions of individual egg diameters were evaluated between years by grouping eggs into 0.2 mm bins and using a χ^2 goodness-of-fit test for significantly different distributions. For all subsequent tests to evaluate differences in egg diameter and egg diameter variation among years and in relation to the environment, egg diameters were averaged by developmental stage for each collection date because eggs collected on the same day were potentially not independent observations (i.e., some number of eggs could have been produced by a single individual). Egg diameter variation was quantified as the coefficient of variation (CV) of all eggs collected on each date within each developmental stage. Egg diameter CV was only determined for dates and developmental stages where at least three egg diameters were available. Mean egg diameters between years were compared with a Mann-Whitney's U test. T-tests were used to compare mean embryo developmental stage, egg diameter CV, water temperature, and discharge between years. To examine the impacts of the environmental variables on egg diameter and CV, linear models of DOY, discharge, and water temperature were utilized. Strong Pearson correlations ($r > 0.6$) between these environmental variables

prevented the creation of a larger model including all variables; rather, each environmental variable was used as a continuous explanatory variable in a model that also included developmental stage as a categorical variable and the interaction between stage and the environmental variable. Data from 2012 and 2013 were run separately because of extreme differences in conditions (e.g., discharge) between years.

To characterize the spawning population, silver carp were collected between April and November of 2012 and 2013 as part of an ongoing study using a combination of techniques. Electrofishing was conducted as described in Coulter and Goforth (2011) at Wabash River km 499. Gill nets (12.7 cm bar mesh and 2.54 cm to 12.7 cm scientific mesh, 30.5 m in length) were set for 0.5–1.5 hrs. Hook-and-line and overnight sets of fyke nets were also used. Total length (cm) of mature females was measured and compared between years using a t-test. All analyses were run in R (v. 2.14.1) with an $\alpha = 0.05$.

Results

A total of 315 eggs in 2012 and 239 eggs in 2013 were measured, tested, and verified as silver carp (Table 1; Figure 2). Eggs were collected on 19 days in 2012 and 15 days in 2013, spanning 112 and 126 total days, respectively, with two – five developmental stages observed per sampling date. Photographs with embryos appropriately positioned for measuring both total length and yolk sac dimensions were available for 26 eggs at stage 17, 31 eggs at stage 24, and 24 eggs at stage 31. Based on these measurements, silver carp egg diameter was significantly positively correlated with both embryo total length and yolk sac volume for all three developmental stages (Figure 3). Mean embryo stage was 26 ± 1.11 standard error (SE) (formation of eye lens) in 2012 and 23 ± 1.36 SE (appearance of otic capsule) in 2013, and the lowest developmental stage eggs collected in each year was 14. Egg diameter was not correlated with developmental stage in 2012 ($r = 0.208$, $p = 0.394$) but was negatively correlated in 2013 ($r = -0.654$, $p = 0.013$). Mean egg diameter was different between 2012 and 2013 ($W = 379$, $p = 0.006$) with means of 2.90 ± 0.04 SE mm ($n = 73$) and 3.25 ± 0.07 SE mm ($n = 61$), respectively. Mature females collected showed the opposite trend, with significantly smaller total length in 2013 (65.9 ± 0.94 SE cm, $n = 123$) than in 2012 (70.4 ± 1.0 SE cm, $n = 21$; $T = 3.23$, $df = 62$, $p = 0.002$).

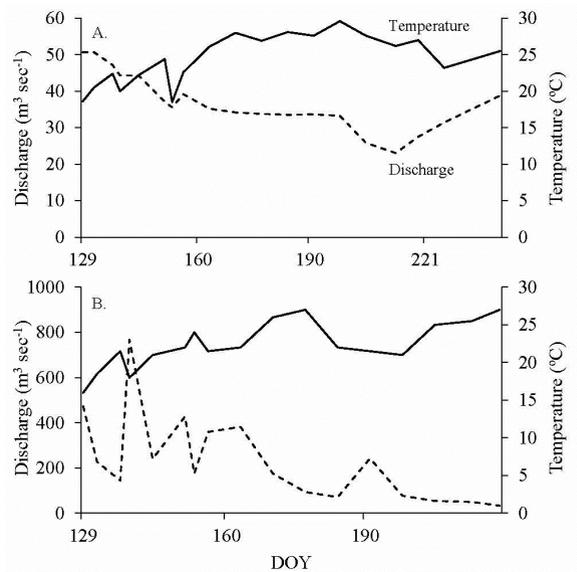


Figure 2. Water temperature and discharge in the Wabash River, IN, USA, over the study periods in (A) 2012 and (B) 2013.

The distribution of egg diameters also differed significantly between 2012 and 2013, again indicating that larger eggs were more commonly collected in 2013 (Figure 4; $\chi^2 = 102.75$, $df = 11$, $p \leq 0.001$). However, egg diameter CV was not significantly different between years ($t = 1.05$, $df = 89$, $p = 0.295$) and averaged $9.52\% \pm 0.59$ SE ($n = 92$) across both years. Mean water temperature was not significantly different between years ($t = 1.97$, $df = 33$, $p = 0.057$) and averaged $23.4^\circ\text{C} \pm 0.78$ SE. Discharge was significantly higher in 2013 ($235.1 \text{ m}^3 \text{ sec}^{-1} \pm 47.3$ SE) than in 2012 ($36.8 \text{ m}^3 \text{ sec}^{-1} \pm 1.8$ SE; $t = -4.19$, $df = 16$, $p = 0.001$).

There were no significant relationships between egg diameter and environmental variables in 2012, and egg size CV was never significantly related to environmental variables in either year (Table 2). Developmental stage was never a significant predictor of egg diameter or egg size CV, although there was a slight decline in egg diameter observed with stage in 2013. Discharge and DOY, but not temperature, were significantly negatively related to egg diameter in 2013 (Figure 5). These significant models including discharge and DOY explained about 33% and 45% of variation in egg diameter in 2013, respectively.

Discussion

Reproductive success is a critical step in biological invasions. In fishes, larger, better-provisioned embryos typically exhibit better survival in adverse

Table 1. Mean daily egg diameter, stage and CV determined for silver carp eggs collected from the Wabash River, IN, across the 2012 and 2013 spawning seasons. Egg diameter CV was not calculated for days with too few eggs. Mean stage was not calculated for days when photographs were not available to determine stage. Silver carp egg diameters were averaged by stage then by day of year (DOY).

Year	DOY	# Eggs	Mean Diameter ±SE (mm)	Stage	CV	Temperature (°C)	Discharge (m ³ sec ⁻¹)
2012	129	5	2.91 ±0.12	16.8 ±0.20	9.33	18.6	50.7
	132	5	3.11 ±0.14	16.8 ±0.25	9.99	20.5	50.7
	137	26	2.99 ±0.07	22.2 ±0.49	12.63	22.4	47.2
	139	54	2.90 ±0.05	22.1 ±0.32	13.06	20.0	44.3
	144	21	2.79 ±0.08	23.6 ±0.47	13.83	22.2	44.3
	151	20	2.76 ±0.05	27.9 ±0.32	8.07	24.4	37.1
	153	23	2.68 ±0.05	16.5 ±0.24	9.59	18.5	35.6
	156	16	2.89 ±0.07	24.3 ±0.48	10.88	22.6	39.2
	163	16	2.73 ±0.11	29.0 ±0.28	16.16	26.1	35.2
	170	19	2.85 ±0.11	29.2 ±0.23	16.81	28.0	34.2
	177	18	2.61 ±0.06	29.6 ±0.12	9.95	26.9	33.8
	184	14	3.12 ±0.12	29.5 ±0.19	14.61	28.1	33.5
	191	10	2.71 ±0.14	28.9 ±0.48	16.79	27.6	33.7
	198	5	3.19 ±0.22	28.7 ±0.88	15.32	29.6	33.3
	205	10	3.48 ±0.18	29.8 ±0.37	16.21	27.6	25.8
	213	12	2.68 ±0.14	29.6 ±0.16	18.07	26.2	23.1
	219	9	3.74 ±0.12	29.5 ±0.18	9.70	27.0	27.5
	226	21	2.73 ±0.08	26.7 ±0.40	13.81	23.2	31.4
	241	11	2.87 ±0.12	29.7 ±0.14	13.53	25.5	38.8
	2013	127	3	3.38 ±0.25	-	10.64	16.0
133		1	3.01	-	-	18.5	228.0
140		14	3.76 ±0.18	20.1 ±0.65	17.98	21.5	144.4
155		26	4.61 ±0.08	15.0 ±0.67	8.60	18.0	767.4
161		24	3.52 ±0.09	17.6 ±0.35	12.87	21.0	240.7
170		15	3.42 ±0.10	20.5 ±0.61	11.73	22.0	427.6
177		18	3.10 ±0.05	24.4 ±0.23	7.50	24.0	175.3
182		7	2.63 ±0.17	16.5 ±0.50	16.91	21.5	359.6
192		19	3.34 ±0.09	21.0 ±0.45	11.54	22.0	382.3
196		15	3.49 ±0.12	27.9 ±0.27	13.86	26.0	175.3
203		19	2.98 ±0.07	28.4 ±0.23	10.02	27.0	93.7
210		15	2.99 ±0.10	24.3 ±0.18	13.19	22.0	71.6
217		1	4.34	-	-	21.5	122.1
226		11	2.86 ±0.12	23.8 ±0.75	13.59	21.0	93.7
233		24	3.00 ±0.08	29.3 ±0.18	13.58	25.0	71.6
241		23	2.91 ±0.07	29.8 ±0.10	10.78	25.5	242.7
253		5	3.09 ±0.05	30.0 ±0.31	3.64	27.0	77.3

environmental conditions and would therefore be expected to yield higher reproductive success. More specifically, embryo size and yolk sac volume are positively linked to larval survival, swimming capacity, and starvation resistance in many fish species (e.g., Rice et al. 1987; Berkeley et al. 2004; Andree et al. 2014). Although post-hatching impacts were not specifically tested in this study, it is likely that larger silver carp eggs also result in better surviving offspring since these eggs also contained larger, better provisioned embryos. However, larger embryos may also be energetically more costly to produce compared to smaller embryos. Therefore, offspring size likely represents a trade-off between quality and quantity of eggs produced.

It is possible that establishing invasive fishes produce variably sized offspring as a bet-hedging

strategy to ensure reproductive success in invaded ecosystems where local environmental conditions may depart substantially from those encountered in the species' native ranges. Indeed, we found drifting silver carp egg diameter CV was relatively high, while mean egg diameter varied significantly between years. Silver carp egg size CV appeared to be largely unrelated to environmental variables or developmental stage. In addition, egg diameter was unrelated to environmental variables in 2012, but negatively related to DOY and discharge in 2013. Annual differences in silver carp egg diameter and its relationship to the environment between years may have resulted from several factors. For instance, 2012 was a severe drought year in the Midwestern United States, which both reduced the range of discharge observed (therefore

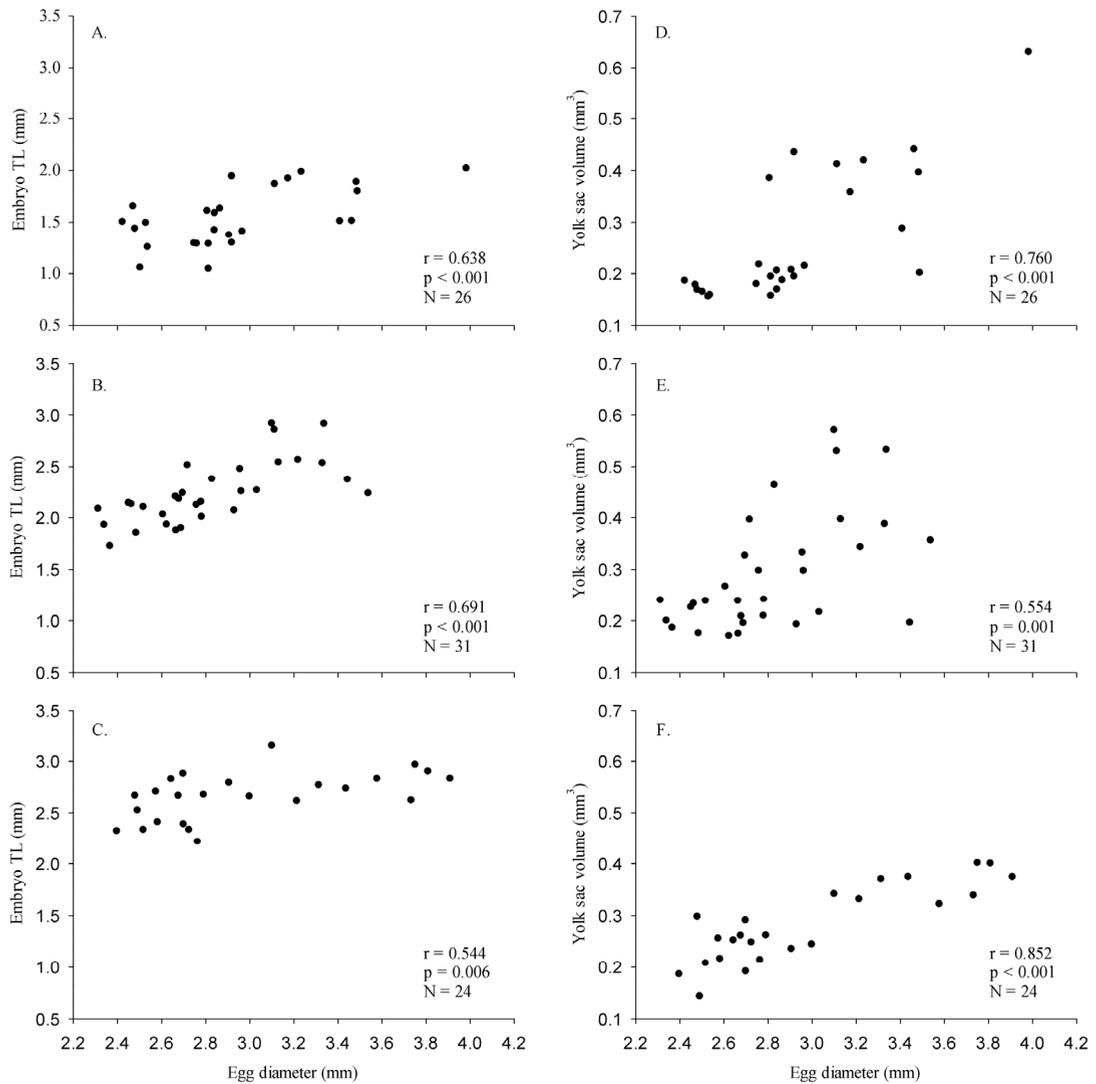


Figure 3. Correlations of silver carp egg diameter with embryo total length and embryo yolk sac volume. Egg diameter was significantly correlated with embryo total length for all three developmental stages examined: (A) 17, (B) 24, and (C) 29. Egg diameter was also significantly correlated with yolk sac volumes across all three stages (D) 17, (E) 24, and (F) 29.

reducing statistical power to discern relationships with egg characteristics), and may also have represented harsh conditions for female silver carp, reducing the numbers or phenotypes of adults that spawned or altering spawning conditions. Additional research into levels of plasticity in egg traits under exposure to different environmental conditions could reveal potentially important patterns behind these observations. Whatever the cause, these results suggest high interannual levels of variability in silver carp egg size, both in general and in relation to environmental conditions,

and egg size CV overall, which may influence their reproductive success in variable environments.

Environmental variables generally accounted for small portions (<50%) of the observed within-year variation in silver carp egg diameter in our study, with DOY and discharge being the only significant relationships in 2013. Laboratory experiments have demonstrated that simply increasing water temperature can result in larger diameter silver carp eggs (Chapman and George 2011; George and Chapman 2013), although we detected no significant relationship between egg diameter and

Table 2. Coefficients and results of linear models examining the impacts of discharge, water temperature and day of year (DOY) as well as year and how they impact egg diameter (mm) and egg coefficient of variation (CV). Significance values (p) for model parameters are displayed below each coefficient.

Model	Variable	Stage	Stage*Variable	df	F	p	R ²
2012							
Egg size		1.301	1.187	0.498	29,43	0.882	< 0.001
	Gage height (m)	<i>0.260</i>	<i>0.318</i>	<i>0.914</i>			
Egg size		1.900	1.375	0.921	29,43	1.190	0.297
	Water temperature (°C)	<i>0.175</i>	<i>0.203</i>	<i>0.540</i>			
Egg size		2.680	1.301	0.091	29,43	1.130	0.352
	DOY	<i>0.109</i>	<i>0.241</i>	<i>0.648</i>			
Egg CV		1.909	1.925	0.762	24,27	1.488	0.159
	Gage height (m)	<i>0.178</i>	<i>0.070</i>	<i>0.651</i>			
Egg CV		10.646	1.780	0.582	24,27	1.700	0.092
	Water temperature (°C)	<i>0.003</i>	<i>0.096</i>	<i>0.800</i>			
Egg CV		4.983	1.636	0.533	24,27	1.362	0.218
	DOY	<i>0.034</i>	<i>0.132</i>	<i>0.838</i>			
2013							
Egg size		29.763	1.191	0.821	33,27	1.888	0.047
	Gage height (m)	< <i>0.0001</i>	<i>0.334</i>	<i>0.684</i>			
Egg size		14.898	1.307	0.529	33,27	1.365	0.167
	Water temperature (°C)	<i>0.001</i>	<i>0.260</i>	<i>0.901</i>			
Egg size		26.802	1.894	1.585	33,27	2.508	0.008
	DOY	< <i>0.0001</i>	<i>0.067</i>	<i>0.144</i>			
Egg CV		0.684	1.597	1.837	26,13	1.645	0.174
	Gage height (m)	<i>0.423</i>	<i>0.200</i>	<i>0.154</i>			
Egg CV		0.048	0.570	0.761	26,13	0.61	0.863
	Water temperature (°C)	<i>0.829</i>	<i>0.860</i>	<i>0.641</i>			
Egg CV		0.156	0.841	1.835	26,13	1.159	0.403
	DOY	<i>0.699</i>	<i>0.633</i>	<i>0.155</i>			

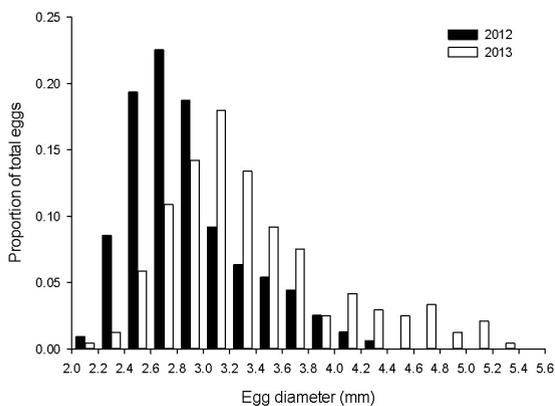


Figure 4. Diameter distributions of silver carp eggs collected across two spawning seasons in the Wabash River, IN, USA. Proportion of eggs collected in 2012 and 2013, respectively, within 0.2 mm size bins. Distribution of egg diameters were different between 2012 and 2013 ($\chi^2 = 102.747$, $df = 11$, $p \leq 0.001$).

water temperature in our study. In fact, we observed the opposite trend between years, with 2012 tending to have higher water temperatures and smaller drifting egg sizes. Observed drifting egg sizes were therefore likely influenced by other, more complex factors. For example, female condition and ambient environmental factors can influence energy allocated by females for ova development, thus ultimately affecting egg provisioning and size (e.g., Sharpe et al. 2012; Muir et al. 2014). In this case, smaller, less provisioned eggs may have facilitated egg transport by allowing these eggs to remain entrained in the dramatically lower flows during the drought in 2012. However, it was impossible for us to determine whether the prevalence of smaller eggs was due to preferential production of smaller eggs by female silver carp or a result of larger diameter eggs settling out in upstream areas before they were vulnerable to our sampling gears. Moreover, we cannot discount changes in the spawning population as factors influencing drifting egg size (e.g., females from different age/size classes preferentially spawning

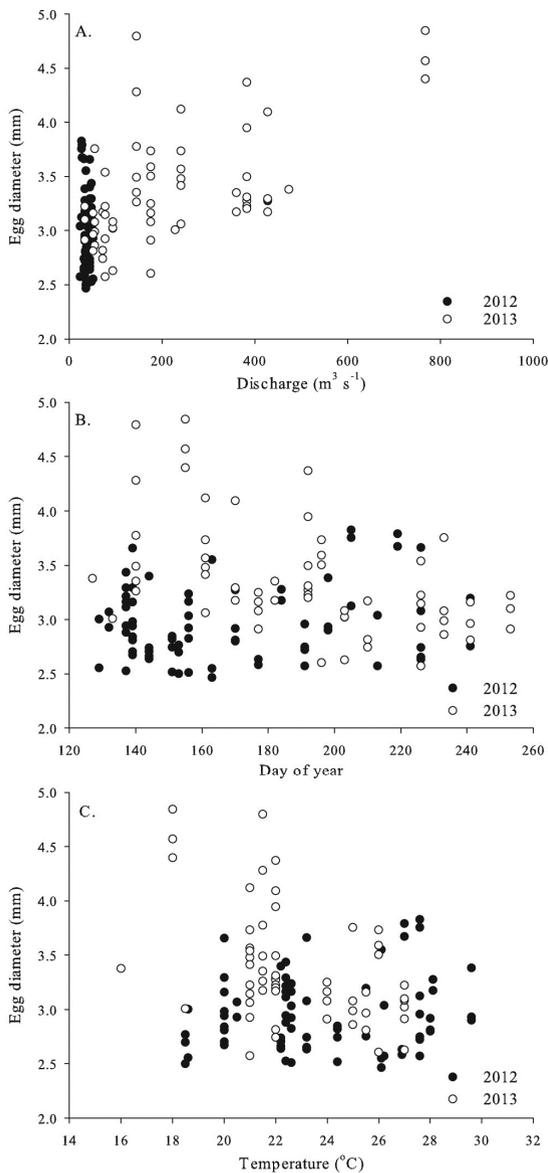


Figure 5. Comparisons of silver carp egg diameter to environmental variables showing trends in egg diameter in relation to (A) discharge ($\text{m}^3 \text{sec}^{-1}$), (B) day of year (DOY), and (C) water temperature ($^{\circ}\text{C}$). For 2013 only, discharge and DOY were significant in linear models (Table 2).

at different times or under differing environmental conditions). Regardless, there do appear to be environmental influences on silver carp egg size, although the exact nature of these relationships remains unclear.

Established relationships between temperature and egg development and maturity indicate the potential for temperature trends to result in differences in spawning timing, as younger

individuals matured later in the summer and thus relate DOY to egg size. However, egg size did not consistently change in a manner that would support this assumption. Moreover, correlations between our environmental variables and their covariation between years make it difficult to directly assess their relative importance to variation in egg size. For instance, egg diameter was significantly negatively related to DOY in 2013, which could have resulted from more rapid development of embryos later in the spawning season when later developmental stage eggs were more commonly collected. 2013 also exhibited a slight decline in egg diameter related to stage, as had previously been observed in laboratory studies (George and Chapman 2013), which may have also influence the observed temporal trend. Additionally, discharge explained over 30% of the variation in drifting egg size. Increased flows are known to trigger spawning behaviour in silver carp (Abdusamadov 1987), and increased discharge may provide better spawning conditions by decreasing the likelihood that drifting eggs will settle out of the water column. Moreover, increased discharge is consistent with spawning conditions to which these fish are adapted in their native range, and this environmental cue may be widely recognized by silver carp as a time of ideal spawning conditions. Larger or older females, with potentially larger eggs, may reproduce primarily during high flows, which could increase mean egg size. Discharge may also determine how differently sized eggs are distributed in the water column, which may, in turn, have impacted the egg sizes collected using our sampling techniques. Two years of data make this difficult to ascertain. However, a comparison of egg sizes collected from different depths showed no significant difference in egg size with depth in May of 2012 (A. Coulter, unpublished data). Changing water levels may also result in changes in water quality parameters, including water hardness. Water hardness is known to impact silver carp egg size, with increasing hardness resulting in smaller eggs (Gonzal et al. 1987; Rach et al. 2010). However, no water hardness values were available for our analysis. Regardless of the potential mechanism, it is clear that egg size is related to discharge, although the specific basis for this relationship needs further study.

Egg diameter CV did not change within or between years and was high regardless of year or environmental conditions. The ranges of egg diameter within a day were often 1–2 mm. This CV is similar to variation observed previously in silver carp where egg sizes ranged from 3.2–6.4 mm

(from eggs > 4 hrs post-fertilization, 100% water hardened; George and Chapman 2013) depending on temperature and trial and from 4.9–5.6 mm in their native range (Yi et al. 2006), although CV was not calculated in these studies. Because CV did not appear to vary temporally, egg diameter CV may thus be high both within and among individuals. Based on this study, the source of this high CV (i.e., within or among females) is indeterminate, but evidence does exist that CV within a silver carp female may be high. Silver carp eggs collected from a single female and allowed to develop at either 19.6 or 22.5°C displayed a mean CV of 8.1% in water-hardened eggs (Chapman and George 2011; George and Chapman 2013), although these CV estimates may be artificially raised due to the varying developmental stages included in the average. Additionally, eggs pooled from two silver carp females in two additional studies displayed CVs between 5.5 and 6.5% depending on temperature and trial (Chapman and George 2011; George and Chapman 2013), indicating that high within female egg size CV may be contributing to the high egg size CV observed in this study. The CV detected in this study appears to be generally high compared to those reported for silver carp and other species. Most estimates of CV in other fishes range from 3–9% (e.g., Johnston and Leggett 2002), with significant variability across species and life histories. Thus, invasive silver carp appear to exhibit high variation in egg size, which may have important implications for their invasion success.

Many species exhibiting lower egg size CV have been established for extended periods of time in the areas where they were studied, and egg sizes of these species may be locally adapted to optimize reproductive fitness over a long term. High CV in silver carp may reflect the relatively short duration of their presence in the Wabash River and other Midwestern rivers, and it may decrease through time as they become locally adapted. On the other hand, selection pressures on different traits can vary through time, resulting in species with higher diversity in life history traits to be more successful across multiple generations (Greene et al. 2010); thus, high variability in egg size may benefit invasive species. Another possible factor that could contribute to egg size CV is the range in sizes of spawning females. Larger females tend to produce larger eggs (Wu and Tan 2000 in George and Chapman 2013), and if many females of varying sizes are reproducing, egg size CV would be greater as the

population of spawning females becomes more diverse in age. Egg size would also influence the suspension of eggs in the water column, thereby impacting survival during development (Chapman and George 2011; George and Chapman 2013) and the distance eggs may travel downstream. Therefore, greater variability in egg sizes could result in more widely distributed offspring of more varied sizes, ultimately minimizing intraspecific competition and improving the probability that at least some offspring survive each year. Thus, the chance of poor year classes is reduced and the likelihood of the successful dispersal and establishment of silver carp is increased as a result of the production of a wide range of egg sizes, regardless of the ultimate causes of this variation.

While *in situ* collection of eggs made it impossible to assess any specific maternal effects such as the length or age of spawning females, maternal effects can play a critical role in producing phenotypic variation in offspring size and fitness (Capinera 1979; Crump 1981; Charpentier et al. 2011; Rollinson 2013). However, the *in situ* collection of silver carp eggs does allow for an examination of the eggs produced by a naturally spawning silver carp population, as opposed to individuals that have been induced to spawn or have spawned in artificial experiments (e.g., Chapman and George 2011; George and Chapman 2013). Bighead carp *H. nobilis* display a positive linear relationship between egg size and female length (Schrank and Guy 2002), and smaller silver carp may also produce smaller eggs (Wu and Tan 2000 in George and Chapman 2013). Mean total length of mature females in the Wabash River declined from 2012 to 2013 (Coulter 2015), which should have produced a decline in mean egg size. However, the opposite trend was observed. It is also unknown how many females were producing eggs on a given collection date, which may influence egg size variation. Several spawning events have been documented in the Wabash River where many fish with acoustic telemetry transmitters moved on apparent spawning runs, meaning it is unlikely that eggs within one day were only coming from one female (Coulter and Goforth 2011). Whether the eggs collected all came from a few or many females, the CV is still high compared to values reported for other fishes representing a wide range of life history strategies. This study has attempted to minimize the impact of number of spawning females by averaging egg diameter by stage on a daily basis, but maternal effects likely impacted egg diameter as well. The lack of a consistent

relationship between egg diameter and developmental stage, and the non-significance of stage in linear models, suggests that eggs collected during this study were already water hardened (i.e., eggs did not expand with age), and so development should not greatly impact our assessments of egg diameter.

Overall, silver carp egg diameter was consistently smaller compared to other systems where diameter is usually ~4 mm (Kolar et al. 2007). A suite of factors may contribute to the small egg size such as the relatively new population of invaded silver carp where smaller individuals may compose a greater proportion of reproducing females compared to established populations. Differences in water quality (e.g., hardness) among ecosystems may also contribute to the differences in egg size. Smaller egg diameters were produced in the drought conditions of 2012, which lacked the rises in hydrograph thought to trigger spawning (Duan et al. 2009); in contrast, the 2013 hydrograph had substantial rises and eggs were larger. Silver carp may have allocated less reproductive effort, or have been in poorer spawning condition, during a relatively 'poor' year in 2012, thereby producing smaller eggs. A potentially similar mechanism has been observed in other fish species where perceived poor conditions (i.e., high density, predator abundance) result in the production of smaller eggs or larvae (e.g., McCormick 2006; Mukherjee et al. 2014). Alternatively, some species have been found to increase their reproductive investment in poor or adverse conditions (e.g., Hutchings 1991; Mérona et al. 2009) or show no change at all (e.g., Tarkan et al. 2007). Therefore, silver carp egg size may vary interannually in response to environmental effects or may reflect a response by females to produce eggs that match the flow conditions under which spawning occurs, given the importance of remaining suspended for silver carp embryos. However, we may only speculate on potential causes of this variation in the current study; further observations spanning multiple years and a variety of environmental conditions may help to clarify the causal mechanisms underlying egg size variability in silver carp. Finally, we did not determine whether egg size influenced embryo survival or eventual recruitment, so we cannot assume that small eggs were less viable than large eggs, although both larval and yolk sac size were positively correlated with egg size across multiple developmental stages. Therefore, we are unable to determine whether the production of smaller eggs does indeed reflect lower reproductive

investment and reduced recruitment under what may be considered to be suboptimal conditions (e.g., low flow). Additional research in this area could elucidate the mechanisms driving these patterns, which may have important implications for silver carp reproductive success in novel riverine habitats.

Silver carp's ability to invade novel environments has been attributed, in part, to its high fecundity, protracted spawning, and rapid growth. Results of this study suggest that high levels of egg size variation may also contribute to the success of silver carp, improving chances of successful recruitment in novel ecosystems. Such variation may indicate differences in age, size, or condition of spawning females in addition to responses to local environmental conditions. Thus, potential bet-hedging may benefit invasive species in their invasion of novel and variable environments through high levels of intrapopulation egg size CV. This study also supports previous work that has demonstrated high levels variability in a newly established population. Additionally, invasive species that engage in bet-hedging strategies may have increased invasion success, especially in variable environments.

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