Zebra mussel maturation and seasonal gametogenesis in Marion Reservoir, Kansas, USA

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Abstract
Based on its native distribution and temperature constraints, the invasive zebra mussel (*Dreissena polymorpha*) was not expected to colonize southern portions of the U.S., but it has now spread from the Laurentian Great Lakes to the Gulf of Mexico. Temperature is critical in zebra mussel reproduction, yet no studies have compared gametogenesis in the cooler north vs. the warmer south. We studied zebra mussel seasonal gametogenesis in Marion Reservoir, Kansas, histologically, examining monthly gonad development and categorizing mussels into one of five stages: resting, early development, late development, mature, or reabsorbing. We also histologically examined multiple size classes to determine size at maturity, and measured juveniles on artificial substrates in the reservoir to determine time to maturity. Adults were mature March–August (at 7.8–34.6°C), compared to mid-June–September (at 18–24°C) in the Great Lakes. Sixty percent of zebra mussels were mature at 5 mm; 100% were mature at 7 mm, compared to 7.5–10 mm in the northern U.S. and 5–12 mm in Europe. Zebra mussels reached maturity within 4 weeks compared to 5 weeks in the Great Lakes and Europe. We conclude that zebra mussels in Kansas matured faster, at a smaller size, and that there could be multiple generations within one year, possibly contributing to greater spread across the region.

Key words: zebra mussel, *Dreissena polymorpha*, gametogenesis, growth, maturity, reproduction, hermaphrodite

Introduction
The zebra mussel (*Mollusca, Bivalvia, Dreissena polymorpha* (Pallas, 1771)) is an aquatic nuisance species introduced from Eastern Europe into the Laurentian Great Lakes where it was discovered in 1988 and from which it has rapidly spread (Herbert et al. 1989; Griffiths et al. 1991). Although it was once thought that the species would not colonize the lower Mississippi River Basin region of the United States due to warm water temperatures (Strayer 1990), zebra mussels have now spread past New Orleans (Allen et al. 1999) to river mile 10 near Venice, Louisiana (U.S. Geological Survey 2013). Zebra mussels have detrimental impacts on industries, recreation, and native species, including unionids and fishes (Williams et al. 1993; Ludyanskiy et al. 1993; MacIsaac 1996; Minchin et al. 2002; Lalaguna and Marco 2008). There is need to understand the life history and ecology of these biofouling and aquatic nuisance species so that more effective management methods can be developed and further spread can be controlled. To gain a greater understanding of zebra mussels and their potential distributive limits, scientists must develop extensive knowledge of their biology, including reproduction (Wacker and von Elert 2003).

Investigators have studied reproduction and seasonal progression of gametogenesis in zebra mussels in Europe and North America (e.g. Borcherding 1991; Wang and Denson 1995; Claxton and Mackie 1998; Karataev et al. 1998; Vailati et al. 2001; Juhel et al. 2003; Lucy 2006; Churchill 2013), and Nichols (1996) noted that minor environmental changes such as calcium, pH, and suspended nutrients can lead to substantial differences in timing of gamete production. Many studies (e.g., Kirpichenko 1964; Stanczykowska 1977; Borcherding...
1991; Garton and Haag 1993; Nichols 1996; Ram et al. 1996; Karataev et al. 1998; Lucy 2006; Churchill 2013) have shown that temperature is a major factor controlling gametogenesis and spawning in zebra mussels.

In both Europe and North America, limited spawning can begin at 12°C (McMahon 1996) but 15°C is generally cited as the temperature at which spawning commences (Kirpichenko 1964; Stanczykowska 1977; Karataev et al. 1998; Lucy 2006). Zebra mussel reproduction and gametogenesis varies from year to year based on temperature (Garton and Haag 1993; Lucy 2006).

In Europe, zebra mussel maturity typically begins in May or June, ends in August or September, and is followed by a resting stage in September (Borcherding 1991; Bacchetta et al. 2001; Lucy 2006). Gametogenesis is resumed between late November and February, when gametes develop, until water temperatures rise to >12°C and spawning begins (Borcherding 1991; Bacchetta et al. 2001; Juhel et al. 2003; Mantecccia et al. 2003). In Lake Erie, zebra mussels typically reach maturity in mid to late June (Haag and Garton 1992; Garton and Haag 1993; Claxton and Mackie 1998) and cease in September (Gist et al. 1997). In the Erie Canal, New York, zebra mussels typically reach maturity in June, are spent by August or September, and resume gamete development in November (Wang and Denson 1995). In cooling reservoirs and under laboratory conditions with suitable water temperatures (12–24°C), spawning has been documented year round (Stanczykowska 1977; Nichols 1993; Nichols 1996), or reproduction can begin earlier in spring and last longer into fall than in unheated lakes (Lewandowski and Ejsmont-Karabin 1983).

The 40th parallel north was historically the southern distribution limit of zebra mussels in Europe (Nichols 1996); however, recently zebra mussels spread past this line in Spain (Lalaguna and Marco 2008). Given the historical range, the 40th parallel, which forms the border between Kansas and Nebraska, is also a point of interest in the United States. The spread of zebra mussels past the 40th parallel to the lower Mississippi River by 1991, three years after their discovery in the Great Lakes (Allen et al. 1999), demonstrated their capability of colonizing areas with water temperatures that are harmful or lethal to Eastern European populations (McMahon and Tsou 1990; Karataev et al. 1998). Zebra mussels are still rapidly spreading throughout the U.S., despite national attempts to stop them (U.S. Geological Survey 2013).

As with spawning, zebra mussel growth rates are positively correlated with water temperature (Nichols 1996), unless temperatures exceed 30°C (McMahon and Tsou 1990; McMahon 1996; Karataev et al. 1998). In Europe and the Great Lakes, zebra mussels may or may not be reproductive within their first year, depending on settling time, temperature, and calcium constraints that limit growth (Mackie 1991; Sprung 1992). Time to maturity determines how fast young cohorts can become reproductive and contribute to the breeding population (Caswell 1982), and zebra mussels can mature faster in warmer climates than they do in Europe and the Great Lakes (Mackie and Schloesser 1996; Nichols 1996). In Kansas, temperature and calcium levels are favorable for zebra mussel shell growth (Whittier et al. 2008), yet it is unknown how long it takes Kansas individuals to mature. Growth rates are important to managers because the size of zebra mussels is more detrimental to industries than their sheer numbers – larger mussels clog pipes faster than numerous small mussels, reducing the effectiveness of dewatering (a common management practice) (Stice 1997).

Borcherding (1991) and Vailati et al. (2001) noted that very few studies have been conducted on the size and age at which zebra mussels reach sexual maturity, and even fewer studies have been conducted on populations south of the 40th parallel north. Research on northern populations (U.S. and Europe) has shown that zebra mussels become sexually mature at shell lengths of 5–12 mm (Stanczykowska 1977; Afanasyev and Protasov 1988 [5–5.5 mm]; Borcherding 1991 [7–8 mm]; Garton and Haag 1993 [8–9 mm]; Wang and Denson 1995 [7.5–10]; Mackie and Schloesser 1996 [8–10 mm]; Nichols 1996 [≥ 5 mm, range 5–12]; Vailati et al. 2001 [≥ 5 mm for males, ≥ 6 mm for females]). Mackie (1991) determined that zebra mussels in the Great Lakes reach maturity in approximately 5 weeks (at 8 mm) after settlement, but later observed settled juveniles can have a maximum grow rate of 0.5 mm per day (Mackie 1993). In Europe, zebra mussels can grow 0.5 mm per day, but the growing season tends to be shorter than in North America (Sprung 1992; Neumann et al. 1993). Shell growth stops in early fall and resumes in spring in Europe (Neumann et al. 1993), however in the lower Mississippi River, zebra mussels grow less in summer and more in winter (Allen et al. 1999).

Zebra mussel reproduction studies have been performed by sampling veligers (Claxton and Mackie 1998), juvenile recruitment (Lucy 2006),
and histology (Juhel et al. 2003). Many state and provincial programs, including that of Kansas, rely almost exclusively on veligers to monitor zebra mussel reproduction and population densities (J. Goeckler, Kansas Department of Wildlife, Parks and Tourism, pers. comm.). However, this may be an inconsistent method because water currents, wind, and daily vertical migration of plankton make collection of larvae inconsistent (Nichols 1996; Claxton and Mackie 1998). Therefore, we chose to examine zebra mussels histologically and compare our findings to maturation patterns from northern U.S. and European studies, as well as with veliger sampling from Kansas Department of Wildlife, Parks and Tourism.

To gain additional knowledge of zebra mussel maturation south of the 40th parallel north, we examined seasonal variation in gametogenesis, size at maturity, and time to maturity in Marion Reservoir, Kansas, U.S. We predicted that, due to environmental conditions in Kansas differing from those of Europe and the Great Lakes region, timing of gametogenesis, size at maturity, and time to maturity would differ from that previously reported in the literature. Specifically, we predicted that these zebra mussels would develop mature gametes earlier in the year, mature at a smaller size, and mature faster than those in more northern populations in Europe and the Great Lakes.

Materials and methods

Study site

We studied zebra mussels in Marion Reservoir, Marion Co., Kansas, USA 38°23’23”N 97°05’27”W (Figure 1), an impoundment of the Cottonwood River in the upper Neosho River basin. Zebra mussel presence was confirmed in this reservoir in 2008, and the population is well-established (Smith 2013). Marion Reservoir has an approximate surface area of 24.9 km², a shoreline of 96.5 km, and a maximum depth at conservation pool of 15 m (Kansas Department of Wildlife, Parks and Tourism 2013).

Field data collection

Zebra mussels for histological study were collected from rocks, sticks, logs, and debris to which mussels were attached. All mussels were collected from a cove in the lower third of the reservoir (Figure 1) at ~1m depth to ensure they had undergone the same approximate temperature regime (Claxton and Mackie 1998; Mantecca et al. 2003) and immediately preserved in Bouin’s Fixative Solution (Thermo Fisher Scientific Inc., Fremont, CA) (Vailati et al. 2001). Zebra mussels were collected between the 21st and 28th of each month from July 2011 to July 2012.
Temperature monitoring

We recorded reservoir water temperatures with iButton™ (Maxim Integrated Products, Sunnyvale, CA) thermo-sensors placed in a perforated PVC pipe that allowed water to pass through. The iButtonsTM were programmed to record temperatures twice a day, at 0750 h and 1950 h, from July 2011 to July 2012, to provide approximate daily maxima and minima. Data from sensors was uploaded to a personal computer after 13 months and retrieved with iButton Viewer (Embedded Data Systems, LLC, Lawrenceburg, KY).

Seasonal variation in gametogenesis

Adults ≥15 mm in length (measured from umbo to the posterior margin of the shell) were used to analyze seasonal gametogenesis because of their relative ease of processing, and because they were certain to be mature (Sprung 1992). We randomly collected at least 25 adult mussels monthly for examination. Mussels were removed from substrates by using a scalpel to cut the byssal threads, rinsed in lake water to remove debris, and immediately fixed with Bouin’s Fixative Solution.

We examined gametogenesis via standard histological procedures (Vailati et al. 2001). We stored all collected specimens in Bouin’s Fixative Solution for at least 3 days. The visceral sac was removed with a scalpel and forceps, and washed in an ascending isopropyl alcohol series of 50%, 70%, 80%, two washes of 95%, and two washes of 100%, for at least 30 min each, but no longer than 8 h, to dehydrate the specimens (Sheehan and Hrapchak 1987). After dehydration, visceral sacs were cleared with Histo-Clear™ (Thermo Fisher Scientific Inc., Fremont, CA) for at least 1 h but no longer than 3 h, and placed into liquid (melted) paraffin (Paraplast Plus, Thermo Fisher Scientific Inc., Fremont, CA) in a paraffin oven (Model 4, Precision Scientific Co., Chicago, IL) and left overnight. The oven temperature was set at 60°C, just above the melting point of paraffin (56°C), so as not to scorch the specimens or damage the structure of the paraffin (Humason 1962). This step was repeated with fresh paraffin to ensure that no Histo-Clear™ remained in the samples. The paraffin-infiltrated visceral sacs were then placed in containers of folded aluminum foil, filled with melted paraffin, and placed on a differential slide warmer, which allowed the paraffin to cool slowly. Once the paraffin cooled and a film developed on top, the foil containers containing the specimens embedded in paraffin were submerged into a cold-water bath at 10–15°C. Cooled paraffin blocks were trimmed and squared for sectioning, and cut to a thickness of 10 µm (Vailati et al. 2001) by a rotary microtome (Model 820, Spencer Scientific Corporation, Derry, NH). Three sections each were sliced from the anterior, middle, and posterior portions of each mussel, increasing the chance that if gonads were small or underdeveloped, they would be seen in at least one section of the visceral sac (Mantecca et al. 2003).

Glass microscope slides were placed on a slide warmer at 52°C and treated with Haupt’s Gelatin Fixative (Humason 1962) for 24 h before sectioned tissue was applied, which aided adherence to the slide. Sections of tissue were floated on drops of warm water (52°C) placed on the slides to allow compression from slicing to relax. Slides were dried on the slide warmer overnight (12 h or until dry) and then treated in a series of baths to remove the paraffin and rehydrate the tissue for staining. The bath series consisted of 10 min in Histo-Clear, 5–10 min each in 100%, 75%, 50%, and 25% isopropyl alcohol, and then 10 min to full rehydration in distilled H2O. Sections were stained with Mayer’s Hematoxylin (Thermo Fisher Scientific Inc., Fremont, CA) for 3 min, washed in running tap water for 3 min, placed in Scott’s Bluing Solution (Humason 1962) for 3 min, and rinsed again in tap water for 3–5 min. Sections were counter-stained with alcoholic eosin (Thermo Fisher Scientific Inc., Fremont, CA) for approximately 8 min, rinsed in distilled H2O for 2 sec and placed in 75% and 95% isopropyl alcohol for 1 min each before dehydrating completely in 100% isopropyl alcohol for 5–10 min. Slides were mounted with glass coverslips and Eukitt mounting medium (Thermo Fisher Scientific Inc., Fremont, CA) (Vailati et al. 2001) and examined under an Olympus BX 51 light microscope (Olympus America Inc., Center Valley, PA) equipped with a digital camera and a calibrated eyepiece. We used a modified version of the gametogenic index of Juhel et al. (2003) to classify mussels into one of five stages: resting, early development, late development, mature, or reabsorbing (Table 1).

Size at maturity

To determine the size at which Marion Reservoir zebra mussels reached sexual maturity, in July 2011, we collected at least 20 mussels from each of 12 size classes (3–14 mm), a range that extended
Table 1. Gametogenic stages in zebra mussels (modified from Juhel et al. 2003).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>Connective tissue present in gonad. Follicles empty. May see slight redevelopment of unidentified gametes.</td>
<td>Ovaries slack and empty. May contain sporadic remaining ova. Many blood cells observed within the ovaries’ interstitial tissue.</td>
</tr>
<tr>
<td>Early Development</td>
<td>Round tubule. Thick germinal epithelium and a few germinal cells in center of lobes.</td>
<td>Ovaries small with oocytes in early maturation. Central lumina lined completely by germinal epithelium. Haemocytes observed in central lumen and ovaries’ interstitial tissue.</td>
</tr>
<tr>
<td>Late Development</td>
<td>Tubule completely filled by reproductive cells in different maturational stages. Small mature cells present in center of tubule, large germinal epithelium at periphery.</td>
<td>Ovaries swollen, containing many ova and few oocytes &gt; 40 µm. Germinal epithelia no longer active, forming discontinuous layers, often with one germ cell lining central lumina.</td>
</tr>
<tr>
<td>Mature</td>
<td>Small, mature spermatozoa found in center of follicle; large germinal cells found at periphery. Spermatozoa tails visible in center of tubule.</td>
<td>Stalk-like (pedunculated) oocytes present. Mature oocytes observed in connective tissue. Ovaries large but showing signs of becoming slack.</td>
</tr>
<tr>
<td>Reabsorbing</td>
<td>Connective tissue present in gonad. Triangular-shaped follicles. Many haemocytes present in tubules, generally surrounding residual spermatozoa.</td>
<td>Many haemocytes observed in ovaries’ interstitial and connective tissues. Ovaries slack, with signs of tissue destruction. Oocytes staining darkly (basophilic).</td>
</tr>
</tbody>
</table>

±2 mm beyond previously known maturity lengths (5–12 mm) (Stanczykowska 1977; Garton and Haag 1993; Nichols 1996). The same histological procedures used to examine seasonal variation were used to assess sexual maturity (Vailati et al. 2001), however for mussels smaller than 6 mm we sectioned the entire mussel because they were so small that the gonads were difficult to isolate by themselves. Following Vailati et al. (2001), we classified mussels as being in the mature stage if oocytes were ≥ 40 µm or spermatozoa had a flagellum. We examined the 4–7 mm size classes first because we expected to find mature mussels within these groups, and once we discovered a size class with 100% maturity, we assumed that all larger mussels were also sexually mature (Vailati et al. 2001).

**Time to maturity**

To determine zebra mussel time to maturity in Marion Reservoir, we conducted growth experiments in which four PVC colonization substrates were placed in the reservoir between the 22nd and 28th of each month from March to June 2012. During those months, we checked previously deployed substrates for settlement and growth (for example, in May we checked and measured mussels on all four substrates from March and all four from April). Colonization substrates were made of 5 cm-diameter white PVC piping (Cresline Plastic Pipe Co., Inc., Evansville, IN) cut to 25 cm lengths. The pipe was cut lengthwise and halves held together by hose clamps that could be opened to observe zebra mussels. The PVC substrates were placed at approximately 1 m depth from the surface of the water (Wainman et al. 1996), and suspended by a 6.4 mm-diameter aircraft cable from the underside of a dock. Zebra mussels on the internal surface only were used and shell length was measured from umbo to the posterior margin of the shell with a ruler and rounded down to the nearest millimeter. We found this to be more accurate than calipers since the delicate margin of the shell would break from slight pressure of the calipers.

**Veliger abundance**

We conducted a post-hoc comparison in an effort to determine whether our histological data was congruent with veliger data from Marion Reservoir collected by the Kansas Department of Wildlife Parks, and Tourism (KDWPT). The KDWPT veliger data were collected monthly April–October 2011 and April–October 2012. In each sampling period, three veliger samples were taken in the middle of the down-lake portion of the reservoir with a 63-µm Wisconsin plankton net (Wildlife Supply Company, Yulee, FL) via vertical tows from a boat, using distance towed to calculate water volume sampled.
Results

Seasonal gametogenesis

We histologically examined gametogenesis in 325 adult zebra mussels (150 males, 149 females, 25 indistinguishable, and 1 hermaphrodite). Male and female zebra mussels showed similar patterns of gametogenesis; however, there was slight variation in gametogenic development during March and April, when the majority of females were in late development stage but most males were in mature stage (Figure 2).

For males, March was the first month of maturity, with 92% (11 of 12) in mature stage, and August was the last month, with 31% mature, 46% reabsorbing, and 23% resting \((n=4, 6, 3, \text{ respectively})\) (Figures 2, 3). From May through July, 98% (48 of 49) of males were mature, with only one in reabsorbing stage in July 2011. In September, 100% of mussels \((n=25)\) observed were in resting stage, completely lacking gametes and therefore indistinguishable in terms of gender. From October to January, 98% (51 of 52) of males were in early development, with one in resting stage in October. In February, one of 13 males was in late development and the other 12 were in early development; two males were observed in late development February–March. Mature spermatocytes were first observed in males at daily minima, and 100% of gonads were maxima between 7.8–15.5°C mature between 17.0 and 34.6°C.

For females, March was also the first month of maturity, with 8% in mature stage, 69% in late development, and 23% in early development \((n=1, 9, 3, \text{ respectively})\) (Figures 2, 4). By April, 50% \((n=7)\) were mature and 50% \((n=7)\) were in

![Figure 2. Frequency of gametogenic stages in male and female adult (≥ 15 mm) zebra mussels in Marion Reservoir, Kansas, July 2011–July 2012. Black line represents mean daily water temperature. Numbers above bars represent sample size each month (n=25 total male and female; February hermaphrodite not included). Asterisk indicates gender indeterminable; resting mussels lacked gametes, and all mussels were in resting stage in September.](image-url)
Figure 3. Stages of zebra mussel spermatogenesis in Marion Reservoir, Kansas, July 2011–July 2012, at 400x. A: resting; B: early development; C: late development; D: mature; E: reabsorbing.

Figure 4. Stages of zebra mussel oogenesis in Marion Reservoir, Kansas, July 2011–July 2012, at 100x. A: resting; B: early development; C: late development; D: mature; E: reabsorbing.
late development. From May–July, 98% (50 of 51) of females were mature, with only one in a reabsorbing stage in July 2011. August was the last month of maturity, with 8% mature, 58% reabsorbing, and 33% resting (n=1, 7, 4, respectively). In September, 100% of mussels (n=25) were in resting stage, completely lacking gametes, making gender indistinguishable. From October to January, 100% of females (n=48), were in early development. In February, 18% were in late development while 82% were in early development (n=2, 9, respectively). Unlike males, which largely lacked an observable late development stage, females were seen in late development from February to April. As with males, mature oocytes were first observed between 7.8–15.5°C, and 100% of gonads were mature between 17.0 and 34.6°C.

Water temperature

The highest water temperature recorded was 34.6°C in July 2011, and the lowest was 0.6°C in January 2012 (not apparent in Figure 2 which illustrates mean of daily high and low temperature). Water temperatures gradually fell after July 2011, and were consistently below 12°C between November 2011 and March 2012. The minimum and maximum temperatures of each gametogenic stage in both genders occurred at approximately: 7.8–34.6°C for mature, 15–30°C for resting, 0.5–12°C for early development, 5–22°C for late development, and 17–34.6°C for reabsorbing (Figure 2).

Size at maturity

None of the 4-mm size class mussels we examined (n=20) were mature (Table 2). We were unable to determine gender or stage in nine of these mussels, however four were identifiable as females in late development stage with well-developed oocytes of 20–35 μm, and two were females in early development. Five identifiable males were much less developed, but showed beginning signs of spermatogenesis, with germinal cells present. In the 5-mm size class (n=20), five males and seven females were mature. Large portions of the testes were in late development in five immature males, but the five mature males possessed fully developed testes with mature gametes. In the seven mature females, there were large numbers of 20–30 μm oocytes, but each female contained numerous oocytes ≥ 40 μm, and therefore were classified as mature. In three immature mussels, gender was unidentifiable. In the 6-mm size class (n=20), 11 females were mature, seven males were mature, and two mussels lacked distinguishable gonads. In the 7-mm size class (n=20), all 11 males and nine females were mature (Table 2).

Time to maturity

No mussels of mature size were observed on any colonization substrate until July. Colonization substrates checked in July (substrates placed in March, May, and June – which had no juveniles from previous months) were used to determine how long it took the mussels to reach a length consistent with maturity. Mussels (n=110) were 3–9 mm after one month or less of growth.

<table>
<thead>
<tr>
<th>Size Class</th>
<th>Number Mature</th>
<th>Number Immature</th>
<th>% Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mm</td>
<td>0 0</td>
<td>7 4 9</td>
<td>0</td>
</tr>
<tr>
<td>5 mm</td>
<td>5 7</td>
<td>5 0 3</td>
<td>60</td>
</tr>
<tr>
<td>6 mm</td>
<td>7 11</td>
<td>0 0 2</td>
<td>90</td>
</tr>
<tr>
<td>7 mm</td>
<td>11 9</td>
<td>0 0 0</td>
<td>100</td>
</tr>
</tbody>
</table>
Zebra mussel gametogenesis

(Figure 5). The majority of mussels (n=69, 63%) were 5–7 mm, with 6% (n=7) ≥ 8 mm. Thus, given that 100% of zebra mussels examined from our size at maturity analysis were mature at 7 mm, it is clear that this species reached maturity within one month in Marion Reservoir. The growth of some individuals (n=3, 3%) to 9 mm within one month indicates a growth rate of at least 0.29 mm d⁻¹ over a 31-day period.

Sex ratio
Of the 325 adult zebra mussels examined, 150 were male, 149 were female, 1 was hermaphroditic, and 25 were in resting stage and unidentifiable as male or female. There was no significant difference in sex ratio (χ²=0.0027, df=1, p= 0.95). The hermaphroditic mussel (Figure 6) was collected on 23 February 2012, and was in the early development stage. This mussel was predominantly male, but had developing oocytes in the same vesicles as the testis. Oocytes were observed in all three histological sections of the middle portion of the mussel, but there were no signs of oocytes in the anterior or posterior sections.

Veliger abundance
Veligers were first detected in April 2012 (0.01 L⁻¹) and May 2011 (1.14 L⁻¹) and were present in low numbers throughout the summer, with peaks occurring in July 2011 (14.18 L⁻¹) and August 2012 (1.80 L⁻¹) (Figure 7).

Discussion
Seasonal gametogenesis
All zebra mussels examined from September were in resting stage, and gender was not identifiable due to the lack of gametes. This same pattern of gametogenesis has been observed in Germany (Borcherding 1991) and Ireland (Juhel et al. 2003). In Kansas, gametogenesis resumed in October, and mussels remained in early development stage until February. In the northern U.S., in New York (Wang and Denson 1995) and the Great Lakes (Gist et al. 1997), early development was observed from November–January, but in Ireland (Juhel et al. 2003) early development did not resume until December. In our study, both males and females began to enter late development by February. Northern populations typically enter late development stage as early as March in the Netherlands and New York (Antheunisse 1963; Wang and Denson 1995), and as late as June in the Great Lakes (Gist et al. 1997), with most populations in the Great Lakes in late development in May (Haag and Garton 1992; Claxton and Mackie 1998). By March in Kansas, 92% of males had entered mature stage, while 69% of females were still in late development. In April, 91% of males and 50% of females were mature, and by May 100% of males and females were mature. Both males and females had mature individuals from March to August (Table 3). This is one to two months longer for the presence of mature gametes.
Figure 7. Mean (±1 SD) veliger densities (L⁻¹) from three plankton tows, compared to stages of male and female zebra mussel gametogenesis in Marion Reservoir, Kansas, 2011–2012. Black line represents veliger density. Numbers above bars represent sample size each month (n=25 total male and female; February hermaphrodite not included). Asterisk indicates gender indeterminable; resting mussels lacked gametes, and all mussels were in resting stage in September. Veliger densities include samples from months in which histological analysis was not conducted.

than in or near the Great Lakes, where maturity typically starts in June or July and ends in August or September (Haag and Garton 1992; Garton and Haag 1993; Wang and Denson 1995; Claxton and Mackie 1998) (Table 3). It is also longer than in Europe where zebra mussels are mature from May or June–August (Bacchetta et al. 2001; Mantecca et al. 2003) (Table 3). In Ireland, Juhel et al. (2003) observed mature females from March–August; however, males were not mature until May (33%). Therefore, it appears that zebra mussels in Marion Reservoir underwent gametogenesis over a shorter period than mussels north of the 40th parallel, possibly allowing for a longer spawning season, which could contribute to greater annual reproduction and thereby facilitate the spread of zebra mussels.

Depending on location, the duration of zebra mussel spawning differs from a brief concentrated event to an extended one. Zebra mussels in many northern locations, including the Great Lakes, have brief spawning periods because temperatures are unsuitable for most of the year (Claxton and Mackie 1988; Gist et al. 1997). However, in power plant cooling reservoirs where temperature conditions remain > 12°C, spawning can persist over most or all of the year (Lewandowski and Ejsmont-Karabin 1983; Afanasyev and Protasov 1988). In areas where zebra mussels spawn year-round, most gametogenic stages are present at any given time (Lewandowski and Ejsmont-Karabin 1983; Mantecca et al. 2003), while in areas with short spawning seasons, only one or two gametogenic stages are present concurrently (Claxton and Mackie 1988; Gist et al. 1997). In Germany and Ireland, zebra mussel gametogenesis is non-synchronous, with most gametogenic stages being present throughout the year (Borcherding 1991;
Zebra mussel gametogenesis

Table 3. Zebra mussel maturity, size at maturity, and time to maturity in Europe, northern U.S., and Kansas.*

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Months With Mature Gametes</th>
<th>Months With Mature Gametes</th>
<th>Size at Maturity (mm)</th>
<th>Time to Maturity (Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe (N52° 48.83’ – N45° 42’)</td>
<td>3 May–Aug./Sept.</td>
<td>5–12</td>
<td>6-7</td>
<td></td>
</tr>
<tr>
<td>Northern U.S. (N42° 56’ – N41° 28’)</td>
<td>3 June–Sept.</td>
<td>7.5–10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Kansas (N38° 21’)</td>
<td>5 Mar.–Aug.</td>
<td>5–7</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>


Juhel et al. (2003). In Marion Reservoir, we observed that males and females were synchronous in gametogenesis during 10 months of the year, with only one or two gametogenic stages usually present. March and April were the two months in which we observed males and females in different stages; males became mature first and females followed. Highly coordinated spawning has also been observed in zebra mussels in Italy (Mantecca et al. 2003; Binelli et al. 2004) and rivers in the northern U.S. (Wang and Denson 1995); however, in these populations, gametogenesis begins in females before males.

Size at maturity

Zebra mussel size at maturity varies greatly among locations, ranging 5–12 mm (Nichols 1996) (Table 3). We examined mussels 4–7 mm for maturity, and found no 4-mm mussels mature (Table 2). In the 5-mm size class, 60% of the mussels examined were mature, and in the 7-mm size class 100% were mature (Table 2). In Italy, male zebra mussels mature at 5 mm but females do not mature until 6 mm (Vailati et al. 2001), and in a Ukrainian thermal reservoir, 77% of zebra mussels were mature at 5–5.5 mm (Afanasyev and Protasov 1998). In Ireland, zebra mussels mature at 6 mm (Juhel et al. 2003), in Germany, they mature at 7–9 mm (Walz 1973; Borcherding 1991; Sprung 1992; Jantz and Neumann 1998), and in the Great Lakes region of the U.S. they mature at 7.5–10 mm (Wang and Denson 1995; Mackie and Schloesser 1996). Zebra mussels may mature at slightly different sizes due to their physical condition and health, which affect development of sufficient energy reserves for reproduction.

Vailati et al. (2001) found that male zebra mussels mature before females, which could be expected because zebra mussels with a smaller body size are less able to store energy for reproduction, and sperm production requires less energy than oocyte production. However, we observed that females appeared to mature and had more developed sex cells at a smaller size than males (Table 2). Females developing before males has also been observed in New York (Wang and Denson 1995) and Ireland (Juhel et al. 2003).

Time to maturity

The time it takes for zebra mussels to grow to a mature size is based on local factors, such as temperature, food availability, and calcium concentration (Nichols 1996). In the lower Mississippi River basin in the southern U.S., zebra mussel growth is retarded by extreme water temperatures in summer but is comparable to that of northern populations over an entire year (Allen et al. 1999). In cold climates, zebra mussel growth is much slower than in more temperate climates, and these mussels grow seasonally with little growth in winter (Nichols 1996). In northeastern Poland, zebra mussels may not mature until two years after settlement (Stanczykowska 1977) and in parts of Germany they do not mature until one year after settlement (Sprung 1992), but in most places they mature within their first year. In other locations in Germany, zebra mussels mature in 6–7 weeks (Borcherding 1991) to 1 year (Walz 1973) (Table 3). In the northern U.S., zebra mussels mature in 5 weeks (Mackie 1991) to 1 year (Mackie and Schloesser 1996). In our study, zebra mussels matured in less than 1 month, with some reaching 9 mm within 4 weeks. Such rapid growth is probably due to favorable conditions in Marion Reservoir. Thus, zebra mussels that settle early in the year can likely reproduce within the same season in Kansas, and young of the year can likely contribute to the reproductive population. With veligers appearing in the water column as early as April, a 4-week time to maturity, and an additional 1–5 weeks for veliger settlement (Sprung 1989; Neumann et al.
1993; Lucy 2006), there is the possibility of multiple generations per year in Kansas. Such rapid turnover could result in faster spread of zebra mussels across Kansas and the southern U.S.

**Sex ratio and hermaphroditism**

The sex ratio of zebra mussels in Marion Reservoir was 1:1. In Italy, Mantecca et al. (2003) also found a balanced sex ratio. Studies in the Great Lakes (Nichols 1993) and New York (Wang and Denson 1995) showed that females were predominant. In Ireland, females appeared to expend more energy during spawning than males, resulting in a die-off over winter, causing the sex ratio to change drastically (Juhel et al. 2003). In Kansas, it seems that males and females had an equal probability of surviving summer and winter, and that spawning effort did not negatively impact one gender disproportionately.

Hermaphrodite zebra mussels are rare, with most field studies finding none (Wang and Denson 1995; Juhel et al. 2003; Mantecca et al. 2003) or one (Binelli et al. 2004), although one laboratory study found 8% of mussels to be hermaphroditic (Nichols 1993). The single hermaphroditic zebra mussel found during our study was predominantly male, with a few oocytes intermixed with spermatocytes. Antheunisse (1963) noted that the oocytes and spermatocytes in a hermaphrodite are usually found in separate vesicles, but on occasion can be found in the same vesicle. The individual in our study contained oocytes and spermatocytes in the same vesicles. Only the middle section of the gonad contained oocytes; none were located in the anterior or posterior. This is not consistent with Antheunisse’s (1963) suggestion that hermaphroditism begins in the anterior portion of the female zebra mussel gonad since our mussel was predominantly male and we only observed female oocytes in the middle section. We collected the hermaphrodite in February when it was in early development, and we are unable to say whether the oocytes or spermatocytes would have continued to develop or been fertile, or whether this mussel could fertilize itself.

**Seasonal gametogenesis vs. veliger abundance**

We observed mature zebra mussels histologically one month (March 2012) before any veligers were found in the reservoir (0.01 veligers L⁻¹ in April 2012) (Figure 7). In 2012, the veliger density peaked in August, following the observed peak presence of mature gametocytes (Figure 7). Because veligers were present in the water column following months when we observed mature gametes in adults, it appears that both our data on gametocyte maturity and veliger sampling conducted by KDWPT were effective in determining the presence of spawning zebra mussels in this well-established population.

Veligers were first detected in Marion Reservoir in April 2012 and May 2011, the same months of first appearance as in Lake Texoma, Texas (Churchill 2013); however, they were not detected until late May or June in Lake Erie (Garton and Haag 1993). In 2012, our veliger peak occurred in August; in 2011, the peak occurred in July, and many mussels were observed in reabsorbing stage in August. In Lake Erie, peak veliger density occurs in late July or August (Garton and Haag 1993), but in Lake Texoma peak density occurs in late May or June (Churchill 2013). Thus, the zebra mussels in Marion Reservoir, Kansas, were more similar to northern populations (Great Lakes) in terms of date of peak veliger density, but more similar to southern populations (Lake Texoma) in date of first detection.

**Summary**

We determined that in Marion Reservoir, Kansas, zebra mussels developed mature gametes from March–August, matured as small as 5 mm, and reached that length in 4 weeks or less. Given the small length at maturity and rapid growth to that size, we demonstrated the possibility of multiple generations of zebra mussels in one year. This could lead to a prolific spread of zebra mussels in Kansas and other warm regions, increasing the possible negative effects on aquatic ecosystems and industries that depend on water resources. We are unaware of any histological studies undertaken south of Kansas; based on the gametogenesis and veliger comparisons from Europe, Lake Erie, Lake Texoma, and Marion Reservoir, it would be instructive to compare maturation in more southern populations.

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