

**LIFE HISTORY OF THE VULNERABLE ENDEMIC CRAYFISH  
 CAMBARUS (EREBICAMBARUS) MACULATUS HOBBS AND PFLIEGER, 1988  
 (DECAPODA: ASTACOIDEA: CAMBARIDAE) IN MISSOURI, USA**

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A B S T R A C T

The vulnerable freckled crayfish, *Cambarus maculatus* Hobbs and Pflieger, 1988, is endemic to only one drainage in eastern Missouri, USA, which is impacted by heavy metals mining and adjacent to a rapidly-expanding urban area. We studied populations of *C. maculatus* in two small streams for 25 months to describe annual reproductive cycles, and gather information about fecundity, sex ratio, size at maturity, size-class structure, and growth, capturing a monthly average of more than 50 individuals from each of the two study populations. Information about the density of the species at supplemental sampling streams was also obtained. The species exhibited traits consistent with a *K*-strategist life history: long-lived, slow-growing, with fewer but larger eggs than sympatric crayfish species. Breeding season occurred in mid- to late autumn, potentially extending into early winter. Egg brooding occurred primarily in May. Young of year were first observed in June. We estimated these populations contained four to six size-classes, observed smaller individuals grew faster than larger individuals, and that most became sexually mature in their second year of life. Densities of *C. maculatus* were low relative to several sympatric species of *Orconectes* Cope, 1872. Life history information presented herein will be important for anticipated future conservation efforts.

**KEY WORDS:** crayfish densities, freckled crayfish, life cycle, PIT tags

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INTRODUCTION

Life history information is pivotal to inform conservation decisions and actions to protect species of concern, particularly those that significantly influence ecosystem processes. Specifically, knowledge of species' life history traits provides insights to the timing of crucial events affecting its ecological role (Stearns, 1992; Winemiller and Rose, 1992; Moore et al., 2013), clarifies taxonomic relationships (Moore et al., 2013), and improves sampling protocols and future study design. Crayfishes are recognized as “keystone” organisms or “ecological dominants” in North American freshwater systems (Momot et al., 1978; Momot, 1995; Rabeni et al., 1995; Simberloff, 1998). Knowledge of their life history is therefore an essential component of any crayfish species conservation status review or recovery plan (U.S. Department of Interior and National Oceanic and Atmospheric Administration, 1996; U.S. Department of Interior, 2004).

Freshwater crayfishes are among the most globally threatened taxa (Richman et al., 2015) and the third most threatened taxonomic group in North America (Taylor et al., 2007; NatureServe, 2010), with 48% of the United States and Canadian crayfish species imperiled (Taylor et al., 2007). Crayfishes, however, remain among the most influential organisms in many freshwater communities due to their substantial biomass (Hurny and Wallace, 1987; Rabeni et al.,

1995; Usio and Townsend, 2001), trophic position (Nystrom, 2002), and role as “keystone” or “ecological dominant” species (Creed, 1994; Momot, 1995; Parkyn et al., 1997; Simberloff, 1998). Yet, published life histories exist for only 12% of the 347 U.S. and Canadian crayfish species, and lack of information inhibits conservation (Moore et al., 2013).

Restricted and fragmented ranges of many crayfish species contribute to their imperilment (Taylor et al., 2007; Richman et al., 2015); a narrow range increases vulnerability to human population encroachment and associated physical habitat degradation, habitat loss, water quality degradation, and invasive crayfishes (Taylor et al., 2007; Richman et al., 2015). Recent declines and extirpations have necessitated assessment of the long term conservation status of many narrow-range endemic crayfishes (Kilian et al., 2010; Larson and Olden, 2010; Loughman and Welsh, 2010; DiStefano et al., 2015). In addition to narrow range, extinction risk of North American crayfishes is linked with specific life history traits: low fecundity, small egg and body size, and habitat specialization (Adamowicz and Purvis, 2005; Larson and Olden, 2010). Data on these traits, reproductive behavior, and ecology for U.S. and Canadian crayfishes (particularly those with narrow ranges) are therefore critical in assessing conservation status and provide insight to species vulnerability. This information is relevant to any decision to “list”

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and recover crayfishes under the U.S. Endangered Species Act and Canadian Species at Risk Act, and provide managers with required knowledge for conservation planning and management (U.S. Department of Interior and National Oceanic and Atmospheric Administration, 1996; U.S. Department of Interior, 2004; De Grammont and Cuarón, 2006; Mooers et al., 2010).

*Cambarus maculatus* Hobbs and Pflieger, 1988, the freckled crayfish, is a distinctively colored, “powerfully built” crayfish (Pflieger, 1996), endemic to only the Meramec River drainage of eastern Missouri, one of the smallest reported ranges of all U.S. and Canadian crayfishes (Pflieger, 1996; Taylor et al., 2007). Surveys in the 1970s and 1980s documented its presence in only 12 streams (Pflieger, 1996), although that list has since increased to 19 streams (Missouri Statewide Crayfish Database; Missouri Department of Conservation, 2016). It is considered “vulnerable” (S3; Missouri Natural Heritage Program, 2016) due to its narrow range near the quickly-developing St. Louis metropolitan area, apparent habitat specialization, low local abundance, and unique phylogenetic status (Pflieger, 1996; Crandall, 1998). The species was recommended for upgrading to “rare” (impaired) status in one conservation analysis (Crandall, 1998) and predicted as a future imperiled species in another (Larson and Olden, 2010). The Big River subdrainage (of the Meramec River drainage) has also experienced degraded water quality and instream physical habitat caused by mining activities that adversely affected crayfish populations for approximately 60 river km (Allert et al., 2013). One study in northeastern North America suggested that crayfishes in the genus *Cambarus* Erichson, 1846 are more vulnerable to population losses than those in *Orconectes* Cope, 1872 (Edwards et al., 2009). Despite these concerns, no research on *C. maculatus* has occurred since its description in 1988 (Hobbs and Pflieger, 1988).

Effective conservation strategies for *C. maculatus* require knowledge of its distribution, habitat requirements, population genetics, and life history. Our goal was to examine life history characteristics of *C. maculatus* populations in two streams for two years to account for possible spatial and temporal variation. The objectives were: 1) to describe the life cycles of these populations by collecting data on timing of reproductive-related events, size at sexual maturity, realized fecundity, egg size, and timing of juvenile recruitment; 2) document trait patterns important to population management, including frequency and timing of adult molts, sex ratios, size structure, maximum body size, and longevity and growth; 3) estimate summer densities of multiple populations and relative densities compared to sympatric crayfishes at several stream locations.

## MATERIALS AND METHODS

### Study Sites

We conducted routine monthly sampling to evaluate life cycle characteristics from May 2013 through May 2015 at two stream reaches on two streams in the Meramec River drainage in Washington County, Missouri (Fig. 1); Mill Creek at the Tiff Road crossing in Tiff (X-UTM 0706256, Y-UTM 4210257, Zone 15 N), and Fourche Renault at the Fourche Renault Road crossing west of Latty (X-UTM 0685319, Y-UTM 4207975, Zone 15 N). Mill Creek and Fourche Renault are Ozark streams characterized by moderate gradient, and mostly gravel/pebble surficial substrate, and, compared to many Ozark stream reaches, with relatively high concentrations

of large cobble and small boulders. These two, third-order (Strahler, 1957) stream reaches were 145–200 m long, had alternating riffles, runs and pools, and well-forested riparian corridors. Mill Creek was approximately 11.4 m wide (wetted channel width during June flows), <0.5 m in water depth, and dominated by pebble-sized substrate; Fourche Renault was about 13.5 m wide, <0.5 m deep, and dominated by gravel-sized substrate. Both sites also harbored *Orconectes luteus* (Creaser, 1933) (golden crayfish), *Orconectes medius* (Faxon, 1884) (saddleback crayfish), *Orconectes harrisoni* (Faxon, 1884) (belted crayfish), and *Orconectes punctimanus* (Creaser, 1933) (spot-hand crayfish). *Cambarus diogenes* Girard, 1852 (devil crayfish) was also found at Mill Creek. Catches were dominated by *O. luteus* and *O. medius* at both sites in most months. Two supplemental sampling trips occurred at the Fourche Renault site midway between the March and April 2014 and the April and May 2014 monthly sampling trips to collect more accurate data on timing of oviposition.

### Life Cycle Field Sampling

Diel sampling typically occurred during the middle of every study month. Kick-seining (1.5 m-wide seines, 3 mm Delta mesh) and hand collections (turning large rocks) throughout rocky habitats were conducted by 3–4 workers for 5–6 h at each site to collect as many *C. maculatus* as possible, but with a minimum goal of 50 individuals. Water temperature was recorded continuously at both sites using remote Tidbit v2 temperature loggers (Onset Computer Corporation, Cape Cod, MA, USA) as an additional means to evaluate life cycle patterns. A large flood swept away both loggers at Mill Creek in April 2015, resulting in a loss of one month’s data.

Data recorded for captured *C. maculatus* included sex, carapace length (CL, from tip of rostrum to the posterior border of the thoracic region, to nearest 0.1 mm), evidence of recent molting (judged by softness of exoskeleton or very clean and slippery exoskeleton; Muck et al., 2002a, b), male reproductive form (Form I = reproductively active, Form II = reproductively inactive or immature; Riggert et al., 1999), and indications of female reproductive activity such as presence of ovarian or pleopodal eggs, hatchlings, active glair glands around the base of pleopods and/or on uropods (Stephens, 1952), mucilaginous sperm plugs in the annulus ventralis indicating successful mating (Andrews, 1904; Fielder, 1972), and copulation. All processed crayfish were then released throughout the study reach.

Realized fecundity (Corey, 1987; Stechey and Somers, 1995) (hereafter “fecundity”) was estimated by counting numbers of pleopodal (external) eggs in the field for 19 ovigerous (carrying pleopodal eggs) females (14 and 5 from Mill Creek and Fourche Renault, respectively) captured in May of 2013 and 8 ovigerous females (6 and 2, respectively) from May 2014, whose clutches appeared to be intact (not damaged during our collection). All ovigerous females were transported to the laboratory where eggs were removed, counted again, and mean egg diameters (to nearest 0.1 mm) were estimated (Muck et al., 2002a) based on measurements of 10 of the roundest eggs. After determining that field egg count estimates were 18% lower than laboratory egg counts, we used and reported only laboratory counts of pleopodal eggs. No females bearing attached hatchlings were found in the study.

### Growth and Longevity

Multiple approaches were employed to examine the growth of *C. maculatus*. We developed models to predict weight from CL for males and females for each population. Predictive models were developed based on CL and mass (weighed on digital scale to nearest 0.01 g, following drying on absorbent towels) data from 43 male and 42 female (Mill Creek), and 46 male and 42 female (Fourche Renault) individuals sampled during October 2013.

We also attempted to assess growth rate of PIT-tagged crayfish *in situ*. On 22 October 2012, 92 *C. maculatus* (20.6–42.8 mm CL) were captured, processed, and tagged as described in Westhoff and Sievert (2013) with individually-coded PIT-tags (Biomark model TXP148511B, 8.5 mm long × 2.12 mm in diameter, weighing 0.067 g) at the Fourche Renault site. Approximately one (3–4 October 2013) and two (6 October 2014) years later we scanned the entire study reach for tags over a 6–8 h period using a portable antenna and transceiver (Biomark model FS2001F-ISO). Tagged crayfish were processed at each capture, including CL measurements. We also scanned all captured *C. maculatus* for tags during all routine monthly life cycle sampling at this site. Data were intended to monitor growth and supplement size structure and longevity analyses.

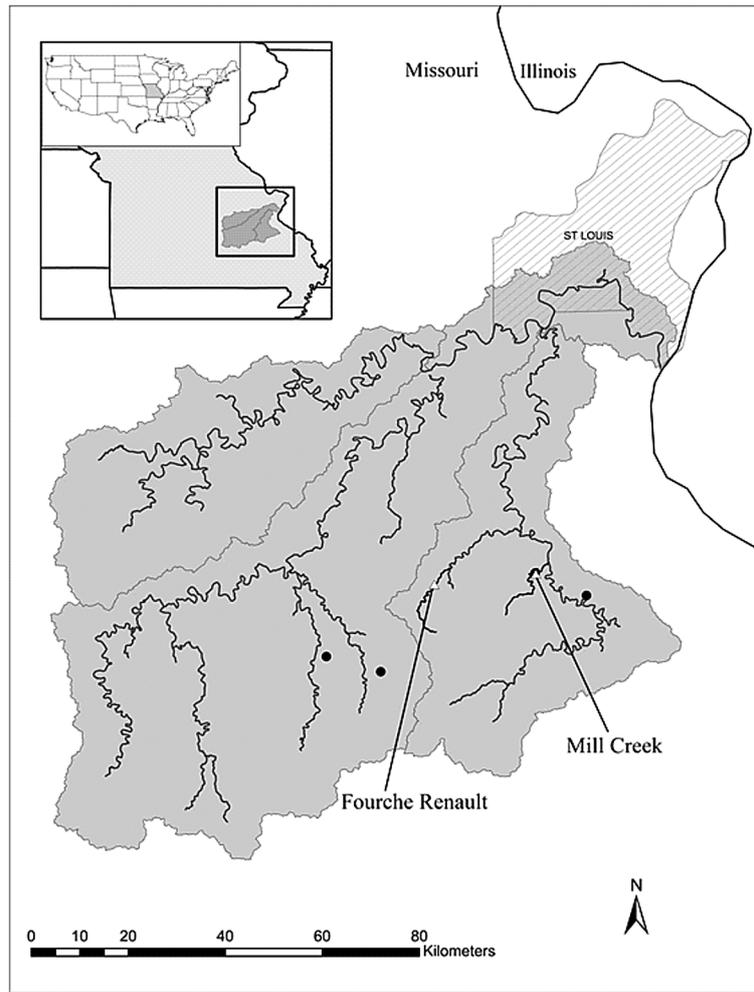


Fig. 1. Geographic range (gray shading) of *Cambarus maculatus* (from Pflieger, 1996; Missouri Statewide Crayfish Database, Missouri Department of Conservation, 2016) in MO, USA. White triangles indicate locations of Mill Creek and Fourche Renault study streams; black dots indicate locations of Shoal Creek, Hazel Creek, Shibolet Branch and Coonville Creek supplemental density sampling sites (from West to East).

### Densities

We sampled four additional *C. maculatus* populations from 30 July to 6 August 2013 to estimate summer densities and provide quantitative data about the abundance/rarity and preliminary information on the diurnal habitat use of the species in relation to other crayfishes. Results of a pilot search for streams harboring *C. maculatus* during summer of 2012 were used to select these populations. Study reaches measuring about 200 m long were located on Hazel Creek (type locality; Hobbs and Pflieger, 1988) at Palmer Road and Hazel Creek Campground (X-UTM 0674277, Y-UTM 4190031, Zone 15 N), Coonville Creek at St. Francis State Park (X-UTM 0716681, Y-UTM 4205752, Zone 15 N), Shoal Creek at Little Shoal Creek Road (X-UTM 0663051, Y-UTM 4193150, Zone 15 N), and Shibolet Branch at the Tiff Lane railroad bridge (X-UTM 0706092, Y-UTM 4210396, Zone 15 N). We attempted to locate four distinct high current velocity (HCV, riffles) and four low current velocity (LCV, runs/pools) habitat units < 0.75 m in water depth at each site to provide data from a range of habitats. Sampling site distribution was as follows: Shibolet Branch, 4 HCV and 3 LCV units; Coonville Creek, 4 HCV and 4 LCV units; Shoal Creek, 3 HCV and 2 LCV units; Hazel Creek, 3 HCV and 3 LCV units. Upstream and downstream bounds of habitat units were flagged and GPS points recorded, and their lengths and wetted widths (at randomly located transects) were measured with a measuring tape to determine unit area. Fifty 1 m<sup>2</sup> samples per site were allocated proportionately among the habitat units, based on total areas of each habitat type (HCV vs. LCV), and located randomly within those units, insuring that

no sample occurred within 1 m of any other (to prevent disturbing other potential sampling areas). At each sampling site, we obtained the 1 m<sup>2</sup> kick seine samples using a 1.5 m wide, 1.5 m high seine (3 mm Delta mesh) and a 1 m<sup>2</sup> pvc quadrat frame following Allert et al. (2013). Recorded data for each sample included sample number, habitat unit/type, presence or absence of boulder substrate (>250 mm diameter, modified Wentworth scale; Bovee and Milhous, 1978), and the number of each species.

### Data Analysis

We focused life cycle analyses largely on adult *C. maculatus*, so we defined and established size (CL) break points or “thresholds” between juveniles and adults. We used the smallest crayfish exhibiting sexual maturity (females indicating reproductive activity and Form I males) within each population (Payne and Price, 1983) as that threshold. Descriptive statistics and frequency histograms summarized temporal life cycle changes. We used CL-frequency histograms (RStudio v 0.99.447; RStudio, 2015) including all sizes of crayfish to examine seasonal changes in size-class structure.

We used plotted CL-frequency histograms to estimate size classes. Separation between classes was agreed upon by two independent observers and assessed as the lowest point between two parabolic shapes, a method providing at least 80% accuracy for species of *Orconectes* (Momot, 1967; DiStefano et al., 2013). We determined that interannual variation in size structure was small, thus months were pooled across years to facilitate visual delineation of size classes. Final analysis relied primarily on data from May and July, secondly on data from other months, and thirdly on annual growth data from a few PIT-tagged individuals. May and July were

selected because young-of-year (YOY, age 0) were generally not recruited to our sampling until after May, and July represented the first month when large numbers of YOY readily appeared in our samples for both sites in both years. These months thus provided the strongest contrast in size classes.

Linear regression was used to explore the relationship between CL and laboratory counts of pleopodal eggs (Microsoft Excel). We compared realized fecundity between the two populations using ANCOVA (SAS version 9.4), which accounted for CL of females (a few females were removed as outliers because many eggs were inadvertently lost during capture). Potential egg size (diameter) differences between populations were examined with *t*-tests (two-sample, assuming unequal variance; Microsoft Excel). We also examined for a potential relationship between egg size and CL using linear regression (Microsoft Excel). Sex ratios (M:F) of captured crayfish were calculated for each season of the year and tested (Microsoft Excel) for differences from the null hypothesis of an equal (1:1) ratio with Chi-Square analysis (Larson and Magoulick, 2011). We developed four separate regression models to determine CL-weight relations in SAS (version 9.4). All CL and weight data were first  $\log_{10}$  transformed. The first two models combined sites, but separated males and females. The other two models combined sexes, but separated the two sites. We then compared regression coefficients (slope and intercept estimates) by looking for overlap in 95% confidence intervals (Pope and Kruse, 2007).

Relative densities of *C. maculatus* were compared to sympatric species and among the two habitat unit types using means and 95% confidence intervals. A two-sample *t*-test assuming unequal variances tested the hypothesis of no differences in sampled mean *C. maculatus* densities based on presence/absence of boulders. Statistical significance was evaluated using  $\alpha = 0.05$  throughout. Unless otherwise stated, discussion comparisons made to “congeners” refer to reports of only stream-dwelling species of *Cambarus*.

## RESULTS

We found that sampling for *C. maculatus* required more effort than might be expended for other Ozark crayfishes, but, when located, they were easily recognizable. We sampled 1310 and 1470 from Mill Creek and Fourche Renault, respectively. All individuals conformed to the previously noted color pattern featuring a yellowish-tan background with conspicuous black spots or “freckles” over all dorsal surfaces (Hobbs and Pflieger, 1988; Pflieger, 1996), but we noted substantial variation in the tone of the yellowish-tan color and the degree of “freckling.”

### Annual Reproductive Cycle

We collected *C. maculatus* individuals with monthly means  $\pm$  SE of  $56 \pm 5$  and  $64 \pm 6$  at Mill Creek and Fourche Renault, with generally the lowest numbers in winter and the second year of study (monthly means  $71.3 \pm 6.4$  and  $50.5 \pm 4.3$  for first vs. second year). We observed reproductively active (Form I) males in both populations and both years for much of the year (mid-September to mid-May). Males in both populations began molting to Form I in September with increasing numbers in October (Fig. 2). Relative proportion of Form I to Form II males was high (Form I/Form II ratio = 0.35) from October through April, peaking in October (0.56) in Fourche Renault and in December (0.83) for Mill Creek (although preliminary project sampling at Mill Creek

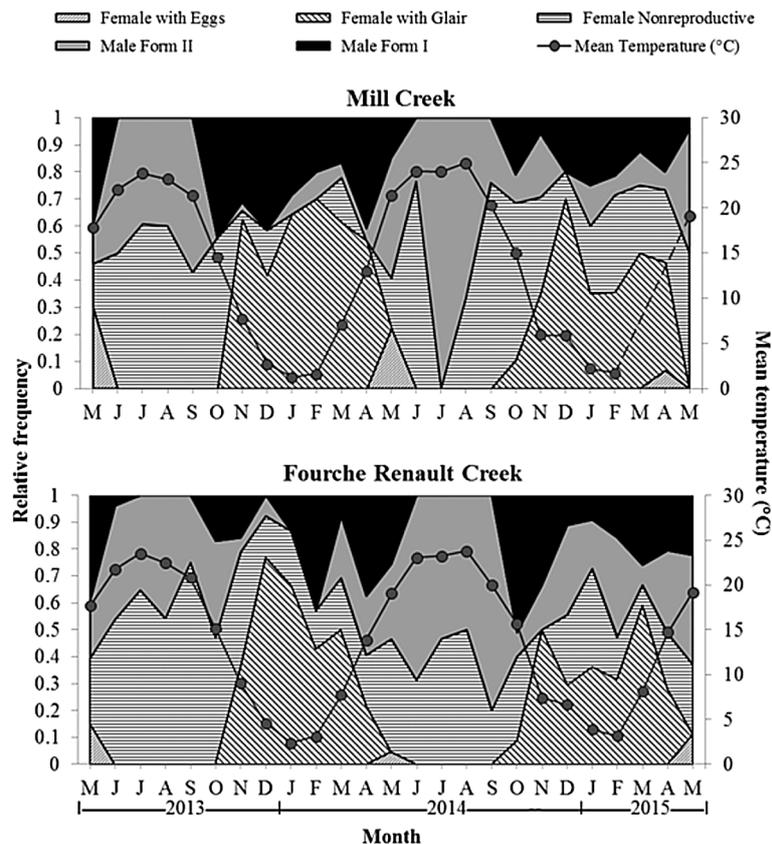


Fig. 2. Reproductive state of mature (adult) male and female *Cambarus maculatus* in Mill Creek and Fourche Renault, MO, USA. Percentage occurrence is the percentage of crayfish in a reproductive state from May 2013 to May 2015 relative to all mature individuals found in that sampling period. Mature *C. maculatus* were all individuals that had reached the minimum size of sexual maturity (see Table 1). Dashed line for mean size of temperature profile in Mill Creek indicates data loss during March and April 2015 due to a flood.

in 2012 resulted in the highest observed male Form I/Form II ratio also in October). The only copulation we observed took place in a collection bucket on October 16, 2013 at Fourche Renault. Form I males during October to April averaged  $32.0 \pm 0.5$  and  $31.0 \pm 0.3$  mm CL for Mill Creek and Fourche Renault populations (Table 1), whereas Form II males (juveniles and adult-sized combined) averaged  $16.3 \pm 0.3$  and  $18.1 \pm 0.3$  mm CL, respectively, and appeared to be YOY and Age 1  $\pm$  crayfish.

The onset of visible female glair development was consistent between populations, beginning in November 2013, but a month earlier (October) in 2014 (Fig. 2). Presence of visible active glair glands peaked in Mill Creek in January 2013 and December 2014 when the percentage of mature-sized females with glair (relative to all adults, both sexes) was 64% and 70%, respectively; visible glair peaked in Fourche Renault in December 2013 (77%) and March (59%) of 2015. Ovarian (internal) eggs were first observed in October and visible through April. Ninety-nine percent of crayfish with visible glair observed from November–April 2014 also had ovarian eggs. No sperm plugs were found on any female *C. maculatus*. Only 32 ovigerous females were observed in these populations during our study: 22 in Mill Creek, 10 in Fourche Renault. All ovigerous females were observed in May, except for one individual in April 2014, when we also found ovigerous *O. luteus*, *O. harrisoni*, and *O. medius*.

*Cambarus maculatus* YOY were first observed in June (5–7 mm CLs) at both sites in both years. Most (72%) June 2013 samples and 42% of June 2014 samples consisted of YOY, suggesting recruitment occurred slightly later in 2014. Most recruitment was completed to both populations by July (both years), when only about 3% of all *C. maculatus* captured were <7 mm CL.

### Molting

Molting patterns for *C. maculatus* were more observable with greater sample sizes in 2013 than in 2014 (Fig. 3). There appeared to be a synchronous late summer/early autumn molt among crayfish of both sexes at both sites that occurred just before and as water temperatures decreased in autumn; this trend was most apparent in 2013 when 20–31% of captured crayfish showed signs of recent molting. At that time, we saw many recently-molted males larger than YOY (12–21 mm CL) that had not molted to Form I for the mating season. We observed another molting period in late spring/early summer that apparently followed recruitment of YOY, as water temperatures warmed to above 20°C. We observed no molting activity at either site during either year in December–April, when water temperatures were below 14°C.

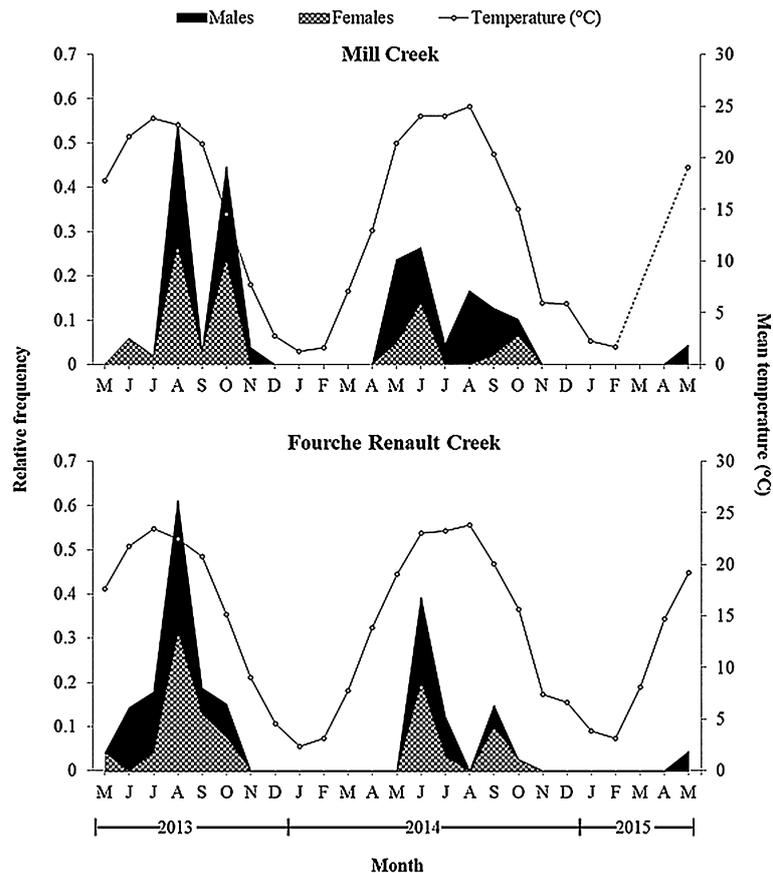


Fig. 3. Monthly molting activity of *Cambarus maculatus* (both sexes, all ages) in Mill Creek (top) and Fourche Renault (bottom), MO, USA, with superimposed mean monthly water temperatures (in °C). Data points represent proportion of recently molted crayfish collected relative to all crayfish collected for each sex during that sampling period.

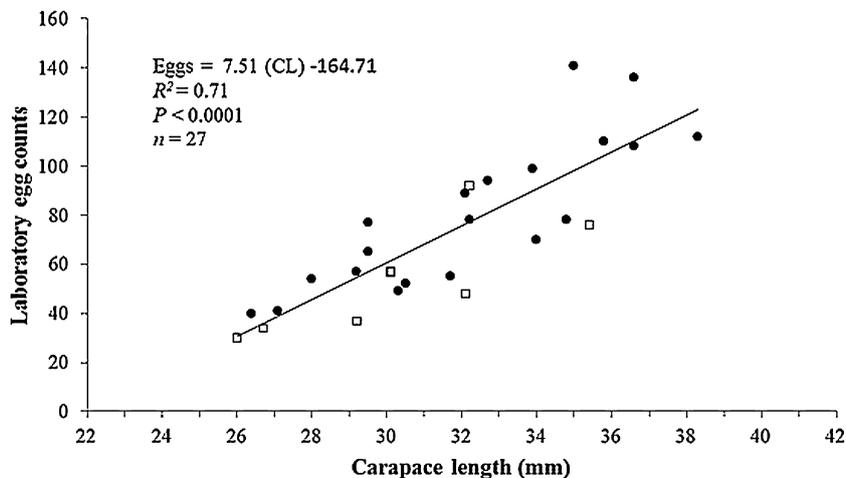


Fig. 4. Relationship between carapace length (mm) of *Cambarus maculatus* and realized fecundity (number of external abdominal eggs counted in laboratory).  $N = 27$  female individuals: 20 individuals from Mill Creek (solid circles); 7 from Fourche Renault (open squares).

### Fecundity

Ovigerous *C. maculatus* females (populations combined) ranged in size from 26.4–42.4 mm CL (Table 1) with means  $\pm$  SE of  $32.6 \pm 0.8$  and  $31.0 \pm 1.0$  mm for Mill Creek and Fourche Renault. Females carried 30–141 pleopodal eggs (laboratory egg counts,  $\bar{X} = 71 \pm 5.4$ ; Fig. 4). The overall ANCOVA was significant ( $F_{(2,24)} = 34.0$ ,  $P < 0.0001$ ) and indicated that CL was a significant predictor of realized fecundity ( $F = 53.8$ ,  $P < 0.001$ ), and the two populations did not exhibit significantly different fecundity ( $F = 3.07$ ,  $P = 0.09$ ) when CL was used as a covariate. Mill Creek females carried more eggs ( $\bar{X} = 80 \pm 4.5$  (95% CI),  $n = 20$ ) than Fourche Renault females ( $\bar{X} = 53 \pm 5.9$ ,  $n = 7$ ), but we saw no difference between 2013 ( $\bar{X} = 72 \pm 14$ ,  $n = 13$ ) and 2014 ( $\bar{X} = 80 \pm 23$ ,  $n = 10$ ) with sites pooled. Linear regression indicated a positive relationship between the total number of pleopodal eggs and CL (Eggs =  $7.5(\text{CL}) - 164.7$ ;  $P < 0.0001$ ,  $R^2 = 0.71$ ). Mean egg size among both populations ranged from 2.4–3.3 mm (Mill Creek  $\bar{X} = 2.9$ , Fourche Renault  $\bar{X} = 2.9$ ), with no difference between populations ( $t = -0.96156$ ,  $df = 15$ ,  $P = 0.35$ ). Egg size was not related to female's CL ( $P = 0.31$ ,  $R^2 = 0.04$ ).

### Sex Ratios

Total sex ratios (populations and years combined) of sampled *C. maculatus* were significantly skewed toward females (M:F = 0.84;  $\chi^2 = 21.8$ ,  $P < 0.001$ ); males dominated samples during only one month (May 2014) at one site (Mill Creek). We captured more females than males at Mill Creek during winter (M:F = 0.76;  $\chi^2 = 3.8$ ,  $P = 0.052$ ), and Fourche Renault during winter (M:F = 0.58;  $\chi^2 = 17.1$ ,  $P < 0.001$ ) and summer (M:F = 0.72;  $\chi^2 = 12.6$ ,  $P < 0.001$ ); no other seasons' ratios differed statistically from 1:1 at either site.

### Size at Maturity

It appeared that some *C. maculatus* individuals had the potential to reproduce in their second year (Table 1, Fig. 5). We encountered one female with glair (14.4 mm CL)

and one Form I male (17.7 mm CL) that we considered abnormally small to be sexually mature. We therefore excluded these outliers and used the CL of the next smallest individuals exhibiting signs of reproductive activity as the sexual maturity breakpoints for *C. maculatus*. Females were considered mature at 21.5 and 23.5 mm CL, and males at 23.1 and 22.3 mm in Mill Creek and Fourche Renault, respectively. We also saw several individuals of both sexes lacking signs of sexual maturity but measuring up to 25–26 mm CL during the mating season.

### Size-Class Structure

The CL of *C. maculatus* individuals ranged from 5.1–49.4 mm and 6.6–43.0 mm in Mill Creek and Fourche Renault (Table 1, Fig. 5). The May CL-frequency distributions (Fig. 5), prior to recruitment of YOY, indicated substantial variation in growth among the previous year's YOY at both sites. Some individuals had not grown past 10 mm CL, whereas others had grown past 17 or 18 mm CL. July CL-frequency distributions differed from May distributions. July distributions (with confirmation from other months) suggested the presence of four to six size classes. The peak of the second size class at that time appeared to be about 19–21 mm CL, and the peak of the third size class was 26–28 mm CL.

### Longevity and Growth Analyses

All four linear regressions of weight and CL were statistically significant ( $P < 0.001$ ) with high correlation coefficients ( $R^2 > 0.99$ ). Regression coefficient 95% confidence intervals for sexes did not overlap indicating statistical difference between males and females; males were heavier than females at CLs greater than 14 mm. Regression equations were  $\log_{10}(\text{weight}) = 3.33 \times \log_{10}(\text{CL}) - 3.94$  for males, and  $\log_{10}(\text{weight}) = 3.24 \times \log_{10}(\text{CL}) - 3.84$  for females. There was not a significant difference between populations.

Growth and longevity analyses using PIT-tagged crayfish were hampered by a lack of recaptured tagged crayfish. The October 2013 search produced only seven tagged live crayfish (6 females, 1 male) and eight tags that were no longer

Table 1. Carapace lengths (CL) of various life history stages for *Cambarus maculatus* in Mill Creek and Fourche Renault, MO, USA. Data provided are mean CL (mm, to nearest 0.1)  $\pm$  standard error (SE), and range. Adult females were designated as those larger than the smallest to show signs of reproductive activity; Fourche Renault > 23.5 mm CL, Mill Creek > 21.5 mm CL. Adult males were designated as those larger than the smallest Form I males; Fourche Renault > 22.3 mm CL, Mill Creek > 23.1 mm CL. Some “juveniles” are reported to have CLs that are greater than the smallest size to show signs of sexual maturity because these “larger” juveniles were measured in spring, prior to recruitment of the next YOY-class.

Life cycle stage	Mill Creek (CL $\pm$ SE (range))	<i>N</i>	Fourche Renault (CL $\pm$ SE (range))	<i>N</i>
All individuals	20.1 $\pm$ 0.3 (5.1-49.4)	1310	21.0 $\pm$ 0.2 (6.6-43.0)	1470
All adult females	30.2 $\pm$ 0.3 (21.5-49.4)	302	29.5 $\pm$ 0.2 (23.5-41.3)	322
Non-reproductive females (adult-sized)	28.3 $\pm$ 0.4 (21.5-49.4)	167	28.6 $\pm$ 0.3 (23.5-39.8)	206
Females with glair	32.7 $\pm$ 0.4 (21.5-48.0)	113	31.4 $\pm$ 0.4 (23.5-41.3)	106
Ovigerous females	32.6 $\pm$ 0.8 (26.4-42.4)	22	31.0 $\pm$ 1.0 (26.5-36.0)	10
Females with hatchlings	—	0	—	0
All adult males	30.1 $\pm$ 0.2 (23.1-44.1)	196	28.6 $\pm$ 0.3 (22.3-43.0)	285
Form I males	32.0 $\pm$ 0.5 (23.1-42.5)	92	31.0 $\pm$ 0.3 (22.3-43.0)	118
Form II males (adult-sized)	28.4 $\pm$ 0.4 (23.1-44.1)	104	26.9 $\pm$ 0.3 (22.3-40.8)	167
All juveniles	13.8 $\pm$ 0.2 (5.1-23.0)	812	15.3 $\pm$ 0.1 (6.6-23.4)	863

implanted in crayfish (recovered in stream sediment). The October 2014 search produced no tagged live crayfish and six tags (although only four recovered). Scanning captured crayfish during monthly samplings produced four tagged crayfish during the first year, but no useful information. No 2015 search was conducted based upon previous poor recapture success. Despite the low tag returns, we made two observations. Among the seven recovered crayfish, we observed high variation in the range of observed growth during one year (0.5-6.1 mm CL) (Fig. 6), and the amount of growth appeared to decrease with age/size. The four largest individuals grew <1.5 mm CL over one year, and the three smallest crayfish grew 4-6.1 mm CL.

#### Densities

We obtained relative density data on *C. maculatus* and four sympatric species: *O. harrisoni*, *O. luteus*, *O. medius*, and *O. punctimanus* at the four supplemental study streams,

although we did not capture all species at all streams. Two additional species were captured, but at such low frequencies that we eliminated them from the analysis; four *Orconectes hylas* (Faxon, 1890) (woodland crayfish) individuals were collected from only Coonville Creek, and three *Orconectes virilis* (Hagen, 1870) (virile crayfish) individuals were collected from Hazel Creek.

We captured *C. maculatus* at three of the streams (with the exception of Coonville Creek, despite detecting the species there during pilot sampling), and estimated mean densities of typically <1/m<sup>2</sup> (Fig. 7). Densities for high and low velocity habitat units, respectively, were as follows: Hazel Creek, 0.1 and 0.3/m<sup>2</sup>; Shibboleth Branch, 0.4 and 0.8/m<sup>2</sup>; Shoal Creek, 0.9 and 1.0/m<sup>2</sup> (SE for all means presented in Fig. 7). Densities of *C. maculatus* did not differ significantly between high velocity (0.20  $\pm$  0.15/m<sup>2</sup>) and low velocity (0.26  $\pm$  0.13/m<sup>2</sup>) habitat units, although we captured greater absolute numbers of them in low velocity units at all three

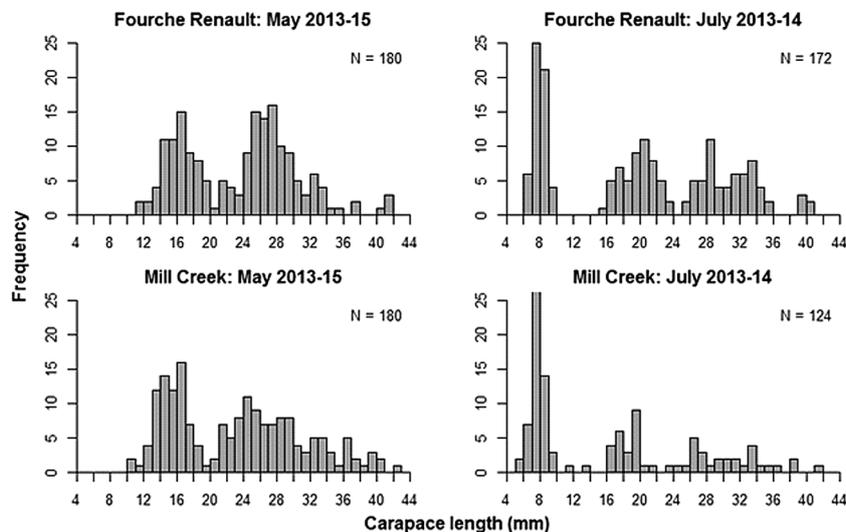


Fig. 5. Example of Fourche Renault (top panels) and Mill Creek (bottom panels) population size structure of *Cambarus maculatus* as depicted by carapace length frequency distribution histograms from May 2013-2015 and July 2013-2014 (years pooled). Sample size for each location indicated in upper right corner of each panel. Cutoff bar at 7 mm CL in the “Mill Creek: July 2013-14” panel extends to 42 individuals.

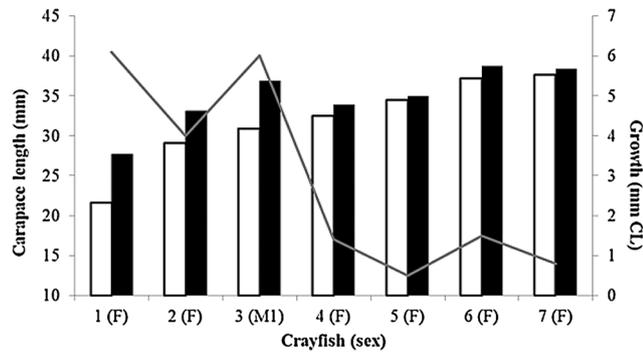


Fig. 6. Growth of six female and one Form I male PIT-tagged *Cambarus maculatus* recaptured one year following tagging in Fourche Renault, MO, USA. Open bars represent carapace lengths (CL in mm) of individuals when tagged (October 2012, open bars) and solid (black) bars represent CL of the same individuals when recaptured one year later. Superimposed line represents the total growth (mm CL, right axis) of each individual crayfish during that year.

streams. We captured a greater density of *C. maculatus* in samples containing boulder substrate ( $0.84/\text{m}^2$ ) than those without boulders ( $0.16/\text{m}^2$ ) ( $N = 200$  samples,  $t = -3.25$ ,  $P = 0.0015$ ).

## DISCUSSION

More sampling effort was required to capture fewer *C. maculatus* as our study progressed, illustrated by lower sample sizes during the second year. This was probably due to continuous (monthly) disturbance of the sampling reaches, in particular, dislodging and turning the targeted large cobble and small boulders where we sought the species. It appeared that *C. maculatus* individuals initially found in our study reaches either relocated to up- or downstream reaches in response to our disturbance, or that

we indirectly caused their mortality by perhaps increasing their vulnerability to predation. The observed decreasing sample sizes could have also resulted from several high flow events that occurred during the study. Our experience suggests that *C. maculatus* populations could be vulnerable to instream physical habitat disturbance.

## Annual Reproductive Cycle

The timing of the annual reproductive cycle events of *C. maculatus* was more similar between Mill Creek and Fourche Renault populations than we expected. The most apparent differences between populations were in the relative proportions of the populations exhibiting the various life stages during some months rather than temporal variations between populations. For example, during December 2014, we observed about half (proportionately) as many females showing glair at Fourche Renault as at Mill Creek. Such observations could be attributable to lower sample sizes than real biological differences, since we captured less than our target 50 individuals per site during some months, especially later in the study. Other slight inter-population differences could be attributed to minimal temperature differences even if the temperature profiles of the sites were very similar (Fig. 2), variation in other environmental factors such as precipitation/hydrologic patterns, or perhaps temporal differences in our sampling (i.e., inability to sample both sites on the same day each month). DiStefano et al. (2013) noted the importance of life history study designs like in the present study that simultaneously sample multiple locations/populations to account for spatio-temporal variation and to provide more robust data sets. Several studies using this approach have also noted such variability (Corey, 1988; DiStefano et al., 2002, 2013), while others have not (Muck et al., 2002a, b; Larson and Magoulick, 2008).

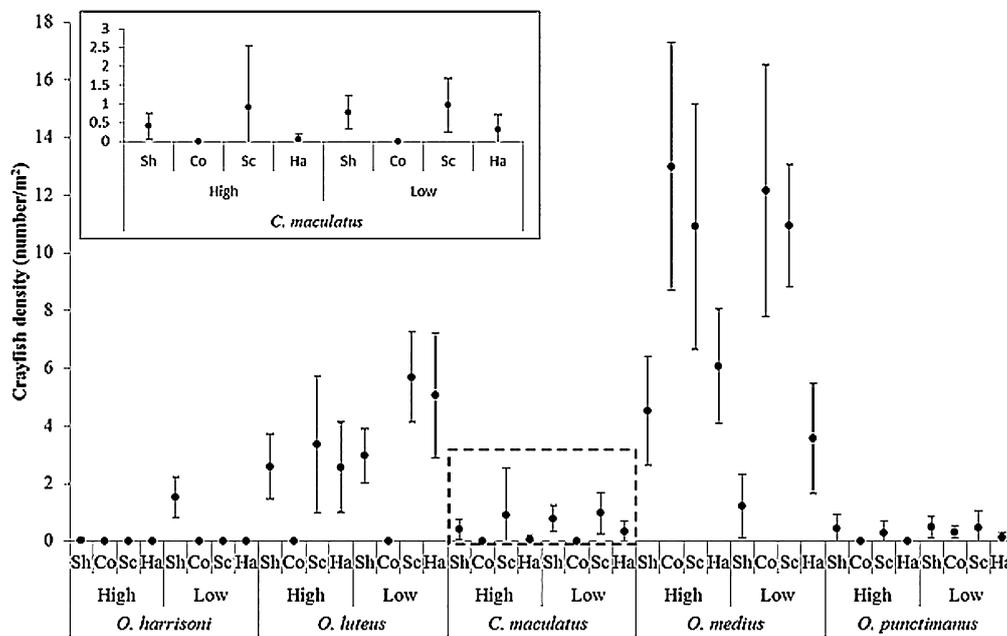


Fig. 7. Estimated mean densities (crayfish/ $\text{m}^2 \pm 95\%$  confidence intervals) of *Cambarus maculatus* and four sympatric species in high- and low-velocity habitats at four supplemental Meramec River drainage stream sites, MO, USA during 30 July-6 August 2013. Sh, Shibboleth Branch; Co, Coonville Creek; Sc, Shoal Creek; Ha, Hazel Creek.

Appearance of Form I males in October (both populations, both years), females with glair in November (both populations, 2013) or December (both populations, 2014), and one population in October, following no such signs during summer, suggest a mid- to late-autumn breeding season as stream temperatures decreased below 20°C. This distinct breeding season contrasts with what was suggested for some congeners, at least at northern North American climates (Guiaşu, 2002). Breeding possibly extended into early winter as suggested by continuing proportional increases of Form I males and females with glair. Autumn transition to breeding form was similar to reports for several congeners, both at northern (Hamr and Berrill, 1985) and southern (Camp et al., 2011) North American climates, but also in other Ozark streams (Larson and Magoulick, 2011). One congener in nearby Kentucky exhibited a similar pattern, but shifted to slightly later in the year (Prins, 1968). Alternatively, other studies reported distinctly different patterns featuring Form I male congeners and females with glair during summer months (Hamr and Berrill, 1985; DiStefano, 1987; Corey, 1990; Jones and Eversole, 2011).

Female *C. maculatus* never showed the mucilaginous sperm plugs that appear to confirm the timing of breeding season for many species of *Orconectes* in North America (Andrews, 1904; Fielder, 1972; Riggert et al., 1999). This was consistent with congeners as sperm plugs were not mentioned in any of the twelve *Cambarus* life history studies we reviewed. The continued presence of Form I males and female *C. maculatus* with active glair glands and developing ovarian eggs through winter into early spring, followed by external oviposition in spring was observed in only one Ozark congener that was previously studied, *Cambarus hubbsi* Creaser, 1931 (Hubbs' crayfish) (Larson and Magoulick, 2011). This pattern also supports what Hobbs and Pflieger (1988) reported; 18 of 20 male *C. maculatus* collected from October-April were Form I, whereas five of six males collected from July-September were Form II. Other congeners exhibited a similar pattern (Smart, 1962; Dennard et al., 2009), although some showed oviposition occurring slightly later, possibly due to cooler water temperatures associated with regional climate differences (Hamr and Berrill, 1985) or a spring branch stream (Prins, 1968).

Recruitment of YOY *C. maculatus* to the population occurred earlier in the year than reported for most congeners, but later than at least two and possibly three sympatric *Orconectes* species. The Ozark *C. hubbsi* recruited earlier (May; Larson and Magoulick, 2011), *Cambarus halli* Hobbs, 1968 (slackwater crayfish) also recruited in June, but most congeners recruited in mid- to late summer (Smart, 1962; Hamr and Berrill, 1985), autumn (Prins, 1968; DiStefano, 1987; Corey, 1990), or possibly winter (Jones and Eversole, 2011).

Nearly all (31 of 32) observations of ovigerous females in our populations over three spring seasons (2013-2015) occurred in May, when water temperatures averaged 17-21°C. A total of 26% and 12.5% of all sampled females were ovigerous during May at Mill Creek and Fourche Renault. May is also when Hobbs and Pflieger (1988) reported ovigerous *C. maculatus*. Unfortunately, we never observed females bearing hatchlings, but our data suggested

this occurred in late May to early June. Some congeners exhibited similar spring egg-bearing seasons (Smart, 1962; Dennard et al., 2009; Larson and Magoulick, 2011), whereas others reported later or less-defined periods for berried females (Prins, 1968; Hamr and Berrill, 1985; DiStefano, 1987; Corey, 1990; Robison and Leeds, 1996; Jones and Eversole, 2011).

The realized fecundity of crayfishes is variable both among and within species and is influenced by species and individual female size, species morphology, environmental conditions (and thus, latitude), system productivity, egg extrusion and attachment process, and egg size (Momot, 1984; Reynolds, 2002). Some congeners were categorized as *K*-selected species, producing smaller numbers of larger eggs (Guiaşu, 2002; Reynolds, 2002). Our data suggest that *C. maculatus* generally fits that description, with a range of pleopodal eggs (30-141) similar to species such as *Cambarus robustus* Girard, 1852 (big water crayfish) (Corey, 1990) and *Cambarus bartonii bartonii* Fabricius, 1798 (Appalachian Brook crayfish). Fecundity of *C. maculatus* was greater than the Ozark congener *C. hubbsi* (see Larson and Magoulick, 2011), *C. longulus* Girard, 1852 (Atlantic Slope crayfish) (Smart, 1962), and the northern populations of *C. b. bartonii* (see Hamr and Berrill, 1985), but lower than *C. elkensis* Jezerinac and Stocker, 1993 (Elk River crayfish) (Jones and Eversole, 2011), *C. halli* (see Dennard et al., 2009), *C. tenebrosus* Hay, 1902 (cavespring crayfish) (Prins, 1968), and *C. sciotensis* Rhoades, 1944 (Teays River crayfish) (Jezerinac et al., 1995). Such comparisons are made with caution due to probable variation associated with latitude/climate, local environmental conditions, stream productivity, and commonly small sample sizes.

Fecundity of *C. maculatus* is probably also related to its relatively large egg size ( $\bar{X} = 2.9$  mm, range = 2.4-3.3 mm) (Reynolds, 2002). Those we studied were larger than the previously reported values for the species (based on only three females; Hobbs and Pflieger, 1988; Pflieger, 1996), and for all (Hamr and Berrill, 1985; Corey, 1990; Jezerinac et al., 1995; Dennard et al., 2009) but one (Prins, 1968) congener; the *C. maculatus* egg size range extended to a larger size than all congeners. Egg sizes were also larger than reports for all species of *Orconectes* for which we found data (Prins, 1968; Payne and Price, 1983; Muck, 1996; Muck et al., 2002a, b; Reynolds, 2002; Adams, 2008; DiStefano et al., 2013), and even some species of *Procambarus* Ortmann, 1905 and *Pacifastacus* Bott, 1950 (Reynolds, 2002).

The CLs of ovigerous *C. maculatus* were similar between sites and similar to those reported for the species by Hobbs and Pflieger (1988). Few studies report CLs for ovigerous congeners, but our size range was relatively large, generally spanning sizes greater than for *C. hubbsi* (see Larson and Magoulick, 2011) and somewhat smaller than *C. robustus* (see Hamr and Berrill, 1985). *Cambarus maculatus* appears to produce young over a span of at least three to four age classes, which is characteristic of what have been termed *K*-selected species or those at higher latitudes (Momot, 1991).

Crayfish fecundity is generally proportional to body size (Reynolds, 2002), as was evident for *C. maculatus* in our study (Fig. 4). Our correlation coefficient was not as high as some previous studies (Reynolds, 2002), but

variation is introduced by environmental factors or the transfer and attachment of the eggs from the ovaries to the pleopod (Reynolds, 2002). Similar positive relationships were reported for four congeners (Hamr and Berrill, 1985; Corey, 1990; Dennard et al., 2009; Jones and Eversole, 2011).

### Molting

Two fairly synchronous molting periods involving both sexes, in late spring/early summer and again in autumn (Fig. 3) were observed for *C. maculatus*. Two distinct molting periods were also observed for *C. elkensis* (see Jones and Eversole, 2011), *C. halli* (see Dennard et al., 2009), *C. hubbsi* (see Larson and Magoulick, 2011), and *C. longulus* (see Smart, 1962), whereas other congeners exhibited one extended molting season (Hamr and Berrill, 1985; DiStefano, 1987) or no synchronicity (Prins, 1968). Corey (1990) reported different molting periods for Age I and II in contrast to Age III *C. robustus*.

### Size-Class Structure and Sexual Maturity

*Cambarus maculatus* is a medium-sized crayfish relative to its congeners, with a maximum size of nearly 50 mm CL (Table 1). Other species of *Cambarus* were smaller (Smart, 1962; Dennard et al., 2009; Camp et al., 2011; Larson and Magoulick, 2011) and larger (Prins, 1968; Jones and Eversole, 2011), although inter-specific size differences among populations of some species (Hamr and Berrill, 1985; DiStefano, 1987; Corey, 1990) confound such comparisons. Our estimate of four to six size classes is in the reported range of 3-7 classes observed for congeners. It further suggests this genus is longer-lived than many North American species of *Orconectes*, including several from Ozarks streams that were reported with 2-4 age classes (Boyd and Page, 1978; Payne and Price, 1983; Corey, 1988; Mitchell and Smock, 1991; Muck, 1996; Muck et al., 2002a, b; DiStefano et al., 2013). The ability to accurately report the maximum size and age structure of a species is contingent on effectively sampling all occupied habitats (Muck et al., 2002a). It is therefore possible that our size estimates are conservative because we did not sample deeper pools, where large *C. maculatus* have been observed (Hobbs and Pflieger, 1988). Our data suggest *Cambarus maculatus* becomes sexually mature in its second year of life, which is similar to several congeners that become sexually mature in or after their second year, in contrast to several *Orconectes* species in the Ozarks region that can attain maturity in their first year (Muck, 1996; Muck et al., 2002a, b; DiStefano et al., 2012).

### Growth

Observed growth of *C. maculatus* conformed to that exhibited by several other temperate crayfishes. Growth varies among populations, even those in close proximity (e.g., *Cambarus robustus* Hamr and Berrill, 1985; *Orconectes propinquus* Corey, 1988), depending upon several environmental factors (Reynolds, 2002; Seiler and Turner, 2004), but especially water temperature (Brett, 1979). Mean monthly summer water temperatures ranged 0-25°C, but between-site patterns were similar (Fig. 2). Growth appeared

to cease when stream temperatures dropped below approximately 10°C (generally November-March or early April). The relatively small number of crayfish that we PIT-tagged combined with poor recapture success (7%) of tagged crayfish during our yearly scanning events limited inference from these data. It is doubtful that tag-related mortality was a factor since tagging was limited to larger crayfish (Black et al., 2010; Westhoff and Sievert, 2013). Despite our small amount of tag-related data, there are indications that growth slowed as an individual aged. This pattern has been observed in other crustaceans (Price and Payne, 1984; Frisch, 2007) and could be further investigated in crayfishes with ossicular growth marks and following von Bertalanffy growth equations (Leland et al., 2015).

Observed differences in CL-weight relation slope coefficients between sexes was mostly due to heavier males among individuals greater than 14 mm CL. This difference is not uncommon for crayfishes, as older females direct more energy reserves into reproduction, rather than growth (Mason, 1974; Flint, 1975; Reynolds, 2002). Greater weights of males might be partly attributable to sexual dimorphism as illustrated by heavier chelae that typically constitute between 35-50% of total weight of Form I males (Stein, 1976). This is common in cambarid crayfishes, although differences can be offset by other secondary sexual characteristics such as wider abdomens in many females (Flint, 1975).

### Densities

Population densities of *C. maculatus* appear typically low relative to many Ozark stream crayfish species (Riggert et al., 1999; DiStefano et al., 2002, 2003, 2009; Flinders and Magoulick, 2007). These crayfishes have among the highest reported densities anywhere (Momot et al., 1978; Nyström, 2002; DiStefano et al., 2003), but *C. maculatus* densities (typically <1/m<sup>2</sup>) from several streams were low in comparison to sympatric species. Densities of our two study populations were not estimated, but raw data suggest they were only the third or fourth most abundant species in most monthly samples. More effort was routinely required to obtain adequate *C. maculatus* sample sizes than for previous life history studies of crayfishes in Ozark streams (Riggert et al., 1999; DiStefano et al., 2002, 2013). Engelbert et al. (2016) reported similarly low densities of *C. maculatus*, which was found to be somewhat common in the Meramec River drainage (present at 8 of 12 sites), but one of the least locally abundant species at individual sites (generally lower densities than 5 of 7 native species). Crandall (1998) reported the species to have a narrow distribution and restricted habitat specificity, while "locally abundant" in the Meramec River, but reported no density estimates; Pflieger (1996) reported it to be "far less abundant" than sympatric crayfish species.

It can be speculated that low *C. maculatus* densities in generally productive Ozark streams with higher densities of sympatric species of *Orconectes* are related to both physical habitat and biotic factors. Our data indicating a relationship between *C. maculatus* and large rock substrate, combined with numerous field observations by us and others (Pflieger, 1996; Crandall, 1998) suggest that *C. maculatus* is a patchily-distributed habitat specialist that potentially relies upon large cobble to boulder-sized substrate for shelter.

This was observed for all size classes of the species. The relatively low densities of the species might be constrained by the rarity of large cobble and boulder substrate (relative to smaller sizes) in many Meramec River drainage streams (Allert et al., 2009; Engelbert, 2013). This apparent habitat specificity and observed somewhat reclusive behavior, similar to *C. hubbsi*, might also be manifestations of its relative *K*-selected life history strategy (Larson and Magoulick, 2011). A successful life history strategy implies occupation of preferred habitat that includes adequate food, low predation pressure, and minimal energy expenditure (Rabeni, 1985). Perhaps *C. maculatus* is occupying the only specific micro-habitat conducive to its life history strategy, in contrast with sympatric species of *Orconectes* that are able to exploit a wider range of habitats under the same climatic and environmental conditions. A comprehensive study of *C. maculatus* habitat use would help clarify these uncertainties.

### Conclusions

Comprehensive life history studies have been conducted on less than 10% of the known species of *Cambarus*, and fewer still on stream-dwelling species of the genus (Moore et al., 2013). Those few studies suggest that annual life cycle patterns vary temporally among stream-dwelling *Cambarus* species, and are likely related to climatic and environmental variables. Our study helps address the lack of data for the genus, and in particular presents comprehensive data on one of the few stream-dwelling members of the genus west of the Mississippi River (Guiasu, 2002; Larson and Magoulick, 2011).

*Cambarus maculatus* exhibited patterns generally consistent with other species on the *K* side of the *r*- and *K*-selection strategy continuum (Stearns, 1976; Adams, 1980). The species has fewer but larger eggs with reproductive output partitioned over 3-4 years, and appears to be longer-lived, slower-growing, and reaches sexual maturity later, relative to other Ozarks Ecoregion crayfishes (Price and Payne, 1984; Muck, 1996; DiStefano et al., 2002, 2013; Muck et al., 2002a, b). This includes the four sympatric species of *Orconectes*, some of whose members exhibit noteworthy life history and ecological differences (see Rabeni, 1985; Muck, 1996; Muck et al., 2002a; DiStefano et al., 2003).

Concern about the conservation of this “vulnerable” species (Missouri Natural Heritage Program, 2016) is based upon its narrow geographical range near the rapidly-developing St. Louis metropolitan area, restricted habitat specificity, mining activities in one sub-basin of its range (Allert et al., 2013), and the recently reported presence of an invasive crayfish species in its drainage (DiStefano et al., 2015). Disturbance created by our sampling may have been enough to adversely affect abundance of the species at our sites, which does not bode well for its persistence under multiple combined threats. Extinction risk in crayfishes was associated with small body size and habitat specialization (Adamowicz and Purvis, 2005); *C. maculatus* is a medium-size species, but an apparent habitat specialist. Larson and Olden (2010) predicted it to be an “impaired” species in one trait analysis model primarily due to its small range. Conversely, another of their models that did not incorporate range size, but did include fecundity and egg size, predicted the species to remain “secure” (Larson

and Olden, 2010). Our study indicates that *C. maculatus* uses a life history strategy that is different from sympatric species in its narrow range, suggesting that “one size-fits-all” management/conservation strategies for Meramec River drainage crayfish could fail to provide the habitat characteristics necessary for a diverse and persistent crayfish fauna. The paucity of basic data on *C. maculatus* must be addressed before appropriate analyses can be conducted concerning its conservation status and potential conservation strategies. We propose more comprehensive study of its distribution within the Meramec River drainage, its habitat associations and specificity, and its population genetics throughout its range.

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