The influence of soil type, congeneric cues, and floodplain connectivity on the local distribution of the devil crayfish (*Cambarus diogenes* Girard)

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Abstract. Burrowing crayfish are potentially important ecological links between terrestrial and aquatic systems, but little is known about what drives their local distribution patterns. We investigated potential mechanisms underlying the patchy local distribution of the devil crayfish (*Cambarus diogenes* Girard) in a stream floodplain ecosystem of the coastal plain of Alabama. We used a series of field surveys and laboratory trials to: 1) quantify local distributions and soil-type associations, 2) identify soil preferences of juveniles and adults, 3) examine the effects of biotic (adult occupancy) and abiotic (soil compaction) factors on juvenile burrowing preferences, and 4) investigate the role of floodplain connectivity on burrow density. Juvenile crayfish were more abundant in sandy streamside soils, whereas adults were more abundant in clay-based floodplain soils. In laboratory preference trials in artificial burrowing chambers, adults and juveniles showed affinity for floodplain over streamside soils, a result suggesting that factors other than soil preference influence field patterns. When juveniles were presented with situations where adults were established, they initiated burrows in association with the adult burrows regardless of soil type. Soil compaction did not influence juvenile burrowing preference, but did result in smaller burrows in laboratory trials. Adult burrow density in the floodplain was negatively related to stream bank angle and top-of-bank height. Juvenile *C. diogenes* took longer to climb banks with high bank angles than those with low bank angles, a result suggesting increased risk of predation and desiccation associated with disconnected floodplain conditions. Local distribution of *C. diogenes* burrows in stream/floodplain systems appears to be a product of interacting physical and biological factors rather than of soil-type preferences. Stream–floodplain connectivity may affect recruitment and population viability of burrowing crayfish.

Key words: burrowing crayfish, artificial burrowing chamber, shelter competition, habitat preference, stream incision.

Crayfish link organisms and influence processes in aquatic systems. They are prevalent predators, herbivores, detritivores, and scavengers (Heard and Richardson 1995, Nyström et al. 1996, Taylor et al. 2007) and are important prey for other consumers, partic-

ularly fish (Stein 1977, Garvey et al. 1994, Adams 2007), birds (Kushlan 1979), and mammals (Englund and Krupa 2000). Crayfish often are considered geomorphic agents. They physically modify their environment through sediment processing, bioturbation, and burrowing, which in turn, can alter community composition (Statzner et al. 2000, Creed and Reed 2004, Helms and Creed 2005, Welch et al. 2008). Crayfish also are hosts to annelid (Branchiobdellida) and arthropod (Ostracoda) ectosymbionts (Alderman and Polglase 1988). Therefore, the "pluralistic role" of crayfish (sensu Momot 1995) may greatly influence the distribution of other organisms and general ecosystem processes, with potentially far-reaching effects in aquatic and terrestrial systems.

All organisms require habitats or behaviors that facilitate feeding, reproduction, and use of refugia. Burrowing into benthic or terrestrial substrates is one such adaptation used by many crayfishes. Most, if not all, crayfishes are capable of burrowing to some degree (Hobbs 1981, Berrill and Chenoweth 1982, DiStefano et al. 2009), and many spend most of their lives in semiterrestrial habitats. Burrow morphology of crayfish can range from single blind tubes in lake and stream bottoms (tertiary burrowers), to relatively simple burrows near the water's edge (secondary burrowers), to extensive subterranean labyrinths far-removed from surface water (primary burrowers), with some overlap in these categories for some species (Hobbs 1981, Hasiotis 1993, Welch et al. 2008). Burrowing activities can mix and disturb local soils, increase soil habitat complexity (Robertson and Johnson 2004, Welch et al. 2008) and respiration (Richardson 1983, Stone 1993), and provide refugia for other organisms (Pintor and Soluk 2006, Loughman 2010). Therefore, the distribution and abundance of primary and secondary burrowing crayfishes (burrowers) can have broad ecological implications. Burrowers are often associated with a high water table (Hobbs 1981) and specific soil types (Grow and Merchant 1980, Loughman et al. 2012, Helms et al. 2013), but factors influencing their distribution and abundance are poorly understood.

Some investigators have documented life histories or distributions of individual burrowers (e.g., Johnston and Figiel 1997, Loughman 2010, Helms et al. 2013), but little is known about their general biology and ecological associations (Taylor et al. 2007, Guiasu 2009, Skelton 2010). Furthermore, ~¼ of the threatened or endangered crayfish species in North America are primary burrowers (Taylor et al. 2007). Subterranean behavior combined with rarity presents unique challenges when studying these organisms, but knowledge of their basic biology, ecology, and behavior is central for effective population management and conservation.

The devil crayfish (*Cambarus diogenes*), a primary/secondary burrower, is widely distributed throughout eastern North America (Pflieger 1996). Large populations generally occur in low-lying woodland and former woodland areas along rivers, streams, and ponds. In these habitats, individual *C. diogenes* often construct an elaborate burrow complex with multiple openings, which sometimes is topped with a chimney (Tarr 1884, Hobbs and Hart 1959, Grow and Merchant 1980, Grow 1981, 1982). Burrows often contain hypoxic/anoxic water and are usually constructed in clay-dominated soils to a depth of 15 cm to 5 m, depending upon groundwater conditions (Grow and Merchant 1980). In marshes and swamps, burrow construction by adults and juveniles generally follows a predictable behavioral pattern with a preference for and superior ability to burrow in fine-grained over coarse-grained soils (Grow 1981, 1982). However, whether these patterns hold for populations in stream/floodplain systems with varying degrees of floodplain connectivity and stream incision is unknown.

No detailed study on the reproductive biology of *C. diogenes* has been published, but several lines of evidence suggest that one strategy used by this species involves mating in the burrow or open water from autumn through early spring. Ovigerous females then release their young in open water from early spring through early summer (Hobbs 1981, Pflieger 1996, BH, unpublished data). Streamside burrows of juveniles often are abundant by mid-summer in temperate areas (Pflieger 1996, BH, unpublished data). Presumably, these individuals ultimately migrate to the floodplain habitat where adult burrows occur.

During informal collections in streams and floodplains in eastern Alabama (Lee, Macon, Chambers, and Tallapoosa counties), we observed several distributional patterns of *C. diogenes* burrows, laterally from the stream and longitudinally along the stream/floodplain corridor. Adult burrows were most abundant in clay–loam floodplain soils, an observation that was not surprising. Juvenile burrows were most abundant in sandy streamside soils, an observation that contradicted published soil preferences (Grow and Merchant 1980). We also observed longitudinal patchiness in the distribution of adult *C. diogenes* burrows in flood plains.

Our goal was to assess reasons for the disparate or patchy distributions of adult and juvenile *C. diogenes* in a stream floodplain forest. We addressed this goal with a series of field surveys and laboratory experiments designed to: 1) quantify local distributions of adult and juveniles and their soil-type associations, 2) identify soil preferences of juveniles and adults, 3) examine the relative importance of biotic (adult occupancy) and abiotic (soil compaction) factors on juvenile burrowing preferences, and 4) investigate the role of floodplain connectivity on adult burrow density.

Methods

Field studies

Study site.—All field studies were done in a 1-km reach of Choctafaula Creek and its adjacent floodplain in Tuskegee National Forest, Macon County, Alabama

(USA). Choctafaula Creek is a 3rd-order, low-gradient, sandy-bottom, coastal plain stream in the Tallapoosa River Basin with an extensive floodplain forest dominated by hardwood trees. Extensive collections in this particular flood plain revealed that nearly all active terrestrial and streamside burrows encountered were occupied by *C. diogenes* (BH, unpublished data). Bank conditions in the study reach ranged from extreme incision and apparently low floodplain connectivity, probably consequent to historical intense agriculture, to areas of relatively low incision and apparently high floodplain connectivity.

Burrow surveys.—We quantified lateral variation in burrow density relative to distance from the stream edge by surveying adult and juvenile *C. diogenes* burrows in a series of transects from September to October 2010. Transects (*n* = 12) were parallel to stream flow, 4 m wide (except for transect 1, which was 2 m wide), and 50 m long. Transects ran from water's edge to 50 m into the flood plain. Thus, we effectively surveyed 2400 m². We counted all crayfish burrows showing evidence of recent activity (presence of chimney, fresh mud, cleared opening) in each transect and recorded its position and diameter. We compared the number of crayfish burrows among transects and regressed the diameter of crayfish burrow openings against distance from stream to test for a relationship between crayfish size and distance from stream.

Floodplain connectivity surveys.—To quantify longitudinal variation in burrow density in relation to floodplain connectivity, we established 18 transects (2 m wide × 30 m long) perpendicular to stream flow originating at the top of the bank and extending into the flood plain. We attempted to capture the range of apparent floodplain connectivity across the entire 1-km study reach. Therefore, we spaced transects throughout the reach. We cleared each transect of organic debris with a gas-powered leaf blower and counted all burrows, measured their diameters, and classified them as active or inactive based on the presence of disturbed soil at the burrow entrance. Inactive crayfish burrows were distinguished from spider burrows by the presence of silk linings or trapdoors associated with the latter. At the origin of each transect, we estimated 2 proxies for floodplain connectivity, top-of-bank height and maximum bank angle. Top-of-bank height was the vertical distance from the water's edge to the top of the bank, and maximum bank angle was calculated by measuring the vertical distance and horizontal height from the water's edge to any obvious break in bank slope and then calculating the pertinent angle with trigonometry. This procedure was repeated for each slope break

(using the previous slope break as a point of origin) until top of bank was reached. We used the maximum angle calculated as maximum bank angle. We used simple regression to relate the number of burrows/transect (active and total) to top-of-bank height and maximum bank angle.

Laboratory experiments

Study animals.—We collected all laboratory test animals between August and October 2011, held them in live wells, and used them within 3 wk of capture. Based on previous studies with other species (e.g., DiStefano et al. 1991, Helms et al. 2013), we identified juvenile crayfish as individuals with a carapace length (CL) ≤20 mm and adults as individuals with CL ≥~30 mm. We captured all juveniles (CL 13–19 mm) and most adults (CL 28–40 mm) from Choctafaula Creek. We collected additional adults from several local sites within the Tallapoosa drainage. We used each individual only once. However, given the difficulty of excavating and obtaining animals, we were unable to control for sex effects in 1 trial (soil preference, see below). For all other trials, we controlled for sex by using even numbers of males and females (adult occupancy, floodplain connectivity, see below) or only males when insufficient females could obtained (soil compaction, see below). We deposited all experimental animals remaining after laboratory trials in the Auburn University Museum of Natural History.

Soil-preference trials.—We tested for soil preferences of juvenile and adult *C. diogenes* in acrylic artificial burrowing chambers (ABCs) that were 30 cm high × 46 cm long × 5 cm wide (Stoeckel et al. 2011; Fig. 1A–D). We used field-excavated streamside and floodplain soils from the survey area where juvenile and adult crayfish burrows had occurred as burrowing substrates. We created standard test soil by sieving soils through an ~1-cm mesh screen to break up large conglomerates and remove roots and rocks. We set aside three ~500 g subsamples of soil for particle-size analysis and sent a representative soil sample to an analytical laboratory for measurement of elemental composition (Auburn University Soil Testing Laboratory, Auburn, Alabama) to assess gross soil differences between collection sites. We dried samples for particle-size analysis for 24 h at 60°C, weighed, pulverized with a mortar and pestle, and poured them through a sieve-stack containing 2-, 1-, 0.5-, 0.25-, 0.1-, and 0.05-mm sieves and a pan (Soil Survey Division Staff 1993). The mass of the soil fraction retained on each sieve was divided by the dry mass of the entire sample and multiplied by 100 to estimate %
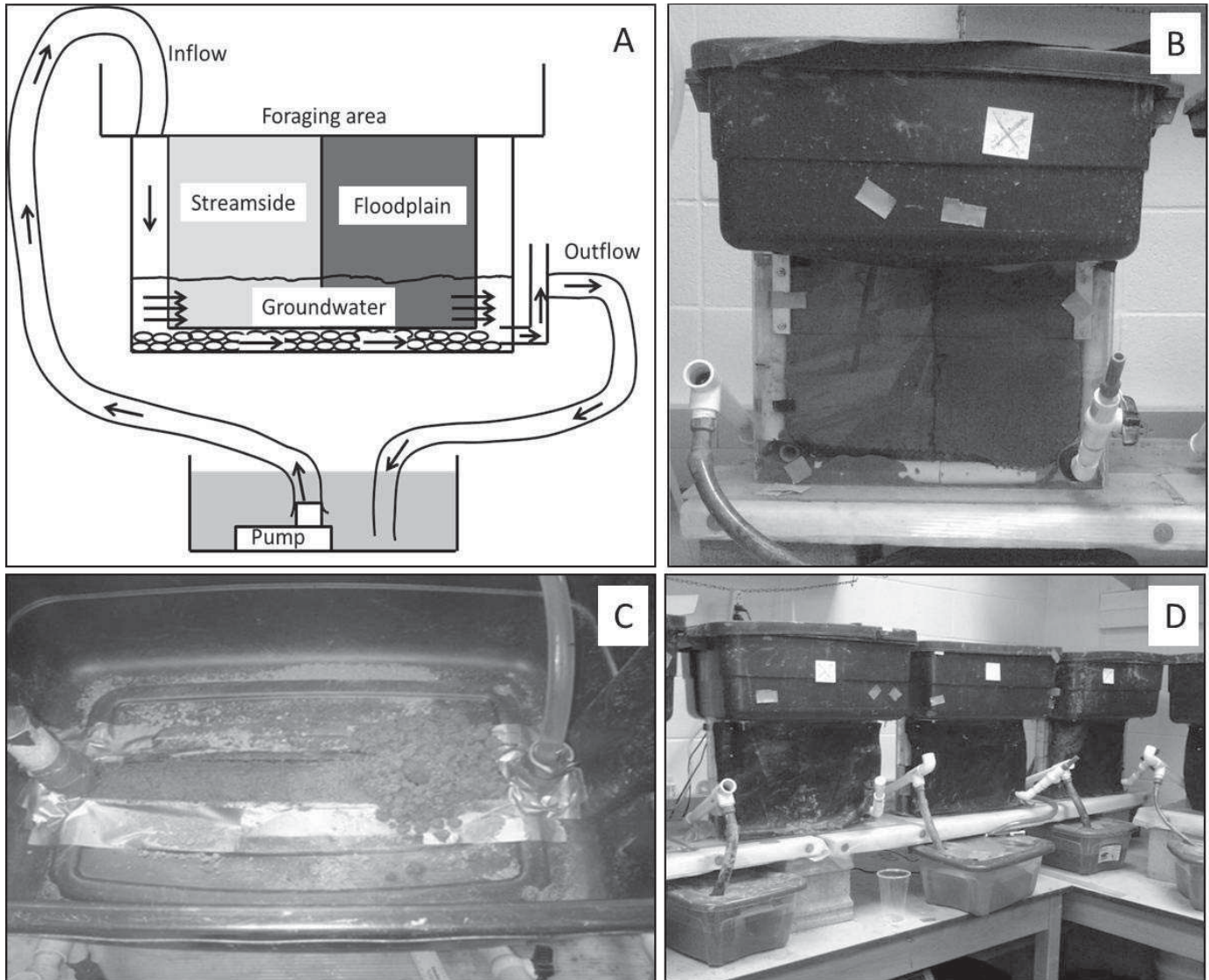
Fig. 1. Schematic diagram of an artificial burrowing chamber (ABC) (A), side view (B), top view (C), and array (D). Chambers were constructed of acrylic sheets and polyvinyl chloride (PVC) pipe. Chamber dimensions are 30 cm high × 46 cm long × 5 cm wide. Arrows in panel A show direction of water flow.

sand (coarse particles in sieves) and clay/silt (fine particles in pan).

For each trial, we filled ½ of each of 10 ABCs with floodplain soil and the other ½ with streamside sand. We alternated soil assignments between sides in the 10 ABCs (Fig. 1A). We raised groundwater levels in the ABCs overnight to saturate soils completely and then lowered levels to 15 cm below the surface for 24 h before adding crayfish (Stoeckel et al. 2011). All sides of each ABC were covered with black plastic sheeting to simulate subterranean darkness during burrowing activity (Stoeckel et al. 2011) (Fig. 1B). At the start of a trial, we placed 1 crayfish on the soil midline of an ABC, closed the lid, and allowed the animal to

burrow. We noted the soil selected for burrow initiation and measured burrow shape and vertical cross-sectional area from digitized daily tracings (ImageJ, version 1.47; US National Institutes of Health, Bethesda, Maryland). We ran 5 adult and 5 juvenile trials concurrently and a subsequent set of 3 adult and 3 juvenile trials within 1 wk, for a total of 8 replicate trials per age group. For each age group, we used Pearson's χ^2 test to test for departures from random in initial soil selection and paired t-tests to test for differences in total and below-water burrow area between soil types after 7 d.

Adult occupancy trials.—To determine if occupancy of adult burrows influenced juvenile substrate selec-

tion, we used 2 sequential trials (10 ABCs per trial, $n = 20$) similar to soil preference trials, except that we placed 2 crayfish (1 adult and 1 juvenile) in each ABC. First, we added an adult crayfish to each ABC and constrained the burrow ~10 cm from the midline in the streamside soil (streamside treatment) or in floodplain soil (floodplain treatment) side by covering the adjacent soil type in each ABC with a strip of black 6-mil plastic sheeting. Within 24 h of burrow construction by the adult, we removed the sheeting, placed a juvenile at the mid-line in the foraging chamber, and closed the top lid. To test for a relationship between the locations of the juvenile and adult burrows, we measured the perpendicular distance-from-center (DFC) of a juvenile burrow in each ABC. We treated distances moved into the streamside soil as negative and those into the floodplain soil as positive. We used a general linear model to test for effects of treatment, sex, and treatment × sex interactions on DFC after 48 h.

Compaction trials.—To evaluate whether soil compaction influenced juvenile burrowing substrate preference and burrow size, we conducted 2 sequential trials (10 ABCs per trial, $n = 20$) similar to preference trials except that soil density was manipulated in ½ of the chambers. We filled 5 ABCs loosely with the 2 soil types (streamside and floodplain), and we compacted the soils added to 5 ABCs by forcefully pressing a horizontal piece of PVC pipe on the surface at 5-cm depth intervals as the chamber was filled. We used similar compacting effort across ABCs, and we measured wet-soil compaction with a pocket soil penetrometer (H-4200; Humboldt Mfg. Company, Schiller Park, Illinois). We arranged ABCs so compaction treatments (loose and compact) were alternated. We noted the soil selected for burrow initiation and measured burrow shape and vertical cross-sectional area from digitized daily tracings. For each soil treatment, we used Pearson's χ^2 test to test for departures from random in initial soil selection. We tested for effects of soil compaction, soil type, and a compaction × type interaction on total burrow area after 5 d with a split-plot design using a general linear model (Neter et al. 1996).

Connectivity trials.—We tested the influence of bank angle on juvenile movements in laboratory trials. We simulated banks with plastic ramps that were anchored in and extended out of a common holding tank. We constructed each ramp from 5 cm × 7.6 cm × 1-m-long plastic downspout material lined with plastic mesh for traction and with an observation groove cut lengthwise down the center. We supported the ramps with a polyvinyl chloride (PVC) pipe frame. We angled ramps at either 45° or 80° to
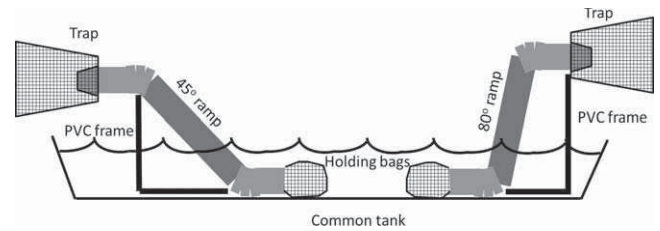

FIG. 2. Schematic diagram of the plastic ramps used in the bank angle experiment. Each ramp was constructed of 5 cm × 7.6 cm × 1-m-long plastic downspout material and was supported by a polyvinyl chloride-pipe frame angled at either 45° or 80°.

correspond to observed bank angles in the field surveys. At the top of each ramp, a funnel trap captured the crayfish as it escaped (Fig. 2). For each trial, we placed a juvenile crayfish in a mesh holding bag at the bottom of the ramp and allowed it to climb out over a 5-d period. We checked the bag every 12 h. We measured the time needed for each juvenile to reach the top of each ramp, and assessed the effects of bank angle, sex, and the bank angle × sex interaction on days to climb with a general linear model.

Results

Burrow survey

We found 166 burrows in the 2400-m^2 study area. Nearly all burrows (164) were confined to the first 6 transects, representing 1100 m^2, or ~1 burrow/7 m^2 (0.14/m^2) for this area of concentration. Burrow density was highest along 2-m-wide transect 1 (0.55/m^2); however, no burrows were found in transect 2 (Fig. 3). Burrow density increased steadily from transect 3 (0.04/m^2) to transect 6 (0.24/m^2). From there, burrow density sharply declined (0.01/m^2) along transect 7 (22–26 m from stream's edge), and burrows were absent from all subsequent transects (Fig. 3). Burrow diameter increased with distance from the stream and was best described by the regression $y = 20.29(x)^{0.189}$ ($R^2 = 0.917$, $p < 0.01$), with the smallest burrows occurring in transect 1 (mean = 19.8 mm). Burrows increased in size from 29.7 to 37.5 mm in transects 3 to 7 (Fig. 3).

Floodplain-connectivity surveys

Stream banks and crayfish burrow density varied considerably along transects. Maximum bank angle ranged from 35° to 115° and averaged 78°, and top-of-bank height ranged from 1.6 to 4.0 m and averaged 2.9 m. We observed 102 burrows in the survey, but <½ (49) showed signs of recent activity. The total number of burrows/transect ranged from 0 to 17
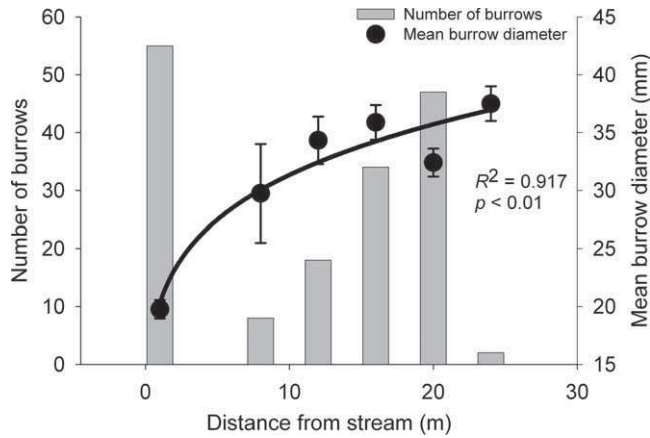
Fig. 3. Number of *Cambarus diogenes* burrows and mean (±1 SD) burrow diameter as a function of transect distance from steam margin. Each bar reflects the number of burrows observed on a given transect parallel to the stream margin. Regression results are for the relationship between burrow diameter and transect distance from stream margin.

(0–0.28 burrows/m^2) and averaged 5.4 (0.9 burrows/m^2). The number of active burrows/transect ranged from 0 to 10 (0–0.17 burrows/m^2) and averaged 2.6 (0.04 burrows/m^2). Total and active burrows per transect were negatively related to maximum bank angle (total burrows: $R^2 = 0.413$, $p < 0.01$; active burrows: $R^2 = 0.422$, $p < 0.01$; Fig. 4A, B) and to top-of-bank height (total burrows: $R^2 = 0.593$, $p < 0.01$; active burrows: $R^2 = 0.442$, $p = 0.01$; Fig. 4C, D).

Soil-preference trials

Floodplain test soils contained moderate amounts of sand (mean = 62.8%) with relatively high concentrations of Ca (247 mg/kg), K (18 mg/kg), Mg (155 mg/kg), and Fe (206 mg/kg). In contrast, streamside test soils were mostly sand (mean = 98.4%) with lower concentrations of Ca (137 mg/kg), K (15 mg/kg), Mg (39 mg/kg), and Fe (60 mg/kg). Crayfish burrowed readily in the ABCs, and generally maintained their position slightly below the groundwater line in their burrow when not actively excavating. The point of burrow initiation was readily identified by a surface opening topped with a constructed chimney in the foraging arena. Neither adults ($\chi^2 = 0.143$, $p = 0.71$) nor juveniles ($\chi^2 = 1.28$, $p = 0.26$) showed a soil preference for burrow initiation. All crayfish except 1 juvenile burrowed to the ground water within 24 h of the beginning of a trial. Total burrow area did not differ between the 2 trial periods for juveniles ($F_{1,7} = 0.92$, $p = 0.38$) or adults ($F_{1,7} = 3.78$, $p = 0.11$). After 7 d, juvenile burrow area was larger in floodplain than streamside soils (paired $t_7 =$

-2.6, $p = 0.04$; Fig. 5A). Adult burrow areas tended to be larger in floodplain than streamside soils (paired $t_7 = -2.27$, $p = 0.06$; Fig. 5A). The underwater portions of juvenile (paired $t_7 = 3.48$, $p = 0.01$) and adult (paired $t_7 = 2.79$, $p = 0.03$) burrows were more extensive in floodplain than in streamside soils (Fig. 5B).

Adult-occupancy trials

All adult crayfish burrowed into their respective soil treatments within 48 h of being put in the ABCs. Juveniles usually initiated construction within 24 h, and all 20 burrowed within 48 h, and ultimately survived the experiment. In all cases, juveniles initiated their burrows in the soil type containing the adult burrow as evidenced by their general soil selection and the mean DFC of the burrow entrance. Juveniles generally made their burrows near the base of the chimney topping the adult burrow. Two juveniles were observed in the adult's burrow, but most constructed their own small burrow near the adult. DFC of juvenile burrows differed between soil treatments ($F_{1,19} = 98.89$, $p < 0.01$). DFC of juvenile burrows was greater in streamside soil than in floodplain soil (Fig. 6). Sex ($F_{1,19} = 2.14$, $p = 0.16$) and the treatment × sex interaction ($F_{1,19} = 1.31$, $p = 0.27$) did not influence DFC.

Compaction trials

Physical compression of soils resulted in different compressive loads for the 2 compaction treatments and soil types. Mean loads were 0.17 ± 0.08 kg/cm^2 (SD) for loose streamside soil and 0.08 ± 0.04 kg/cm^2 for loose floodplain soil. Mean loads were 0.23 ± 0.05 kg/cm^2 for compacted streamside soil (26% increase in compressive load) and 0.11 ± 0.02 kg/cm^2 for compacted floodplain soils (27% increase). Juveniles in all ABCs burrowed readily within 48 h of introduction. Soil selection for burrow initiation did not differ from random in the compacted ($\chi^2 = 1.8$, $p = 0.18$) or loose ($\chi^2 = 1.8$, $p = 0.18$) treatments. However, after 5 d of burrowing activity, burrows were larger in loose than in compacted soils ($F_{1,19} = 5.15$, $p = 0.05$) and tended to be larger in floodplain than in streamside soils ($F_{1,19} = 4.56$, $p = 0.06$) (Fig. 7). The soil type × compaction interaction effect was not significant (Fig. 7).

Connectivity trials

Ten of 36 crayfish escaped and 1 died before scaling the ramp during the bank angle ramp trials. These crayfish were excluded from analyses. After 5 d, 19 of
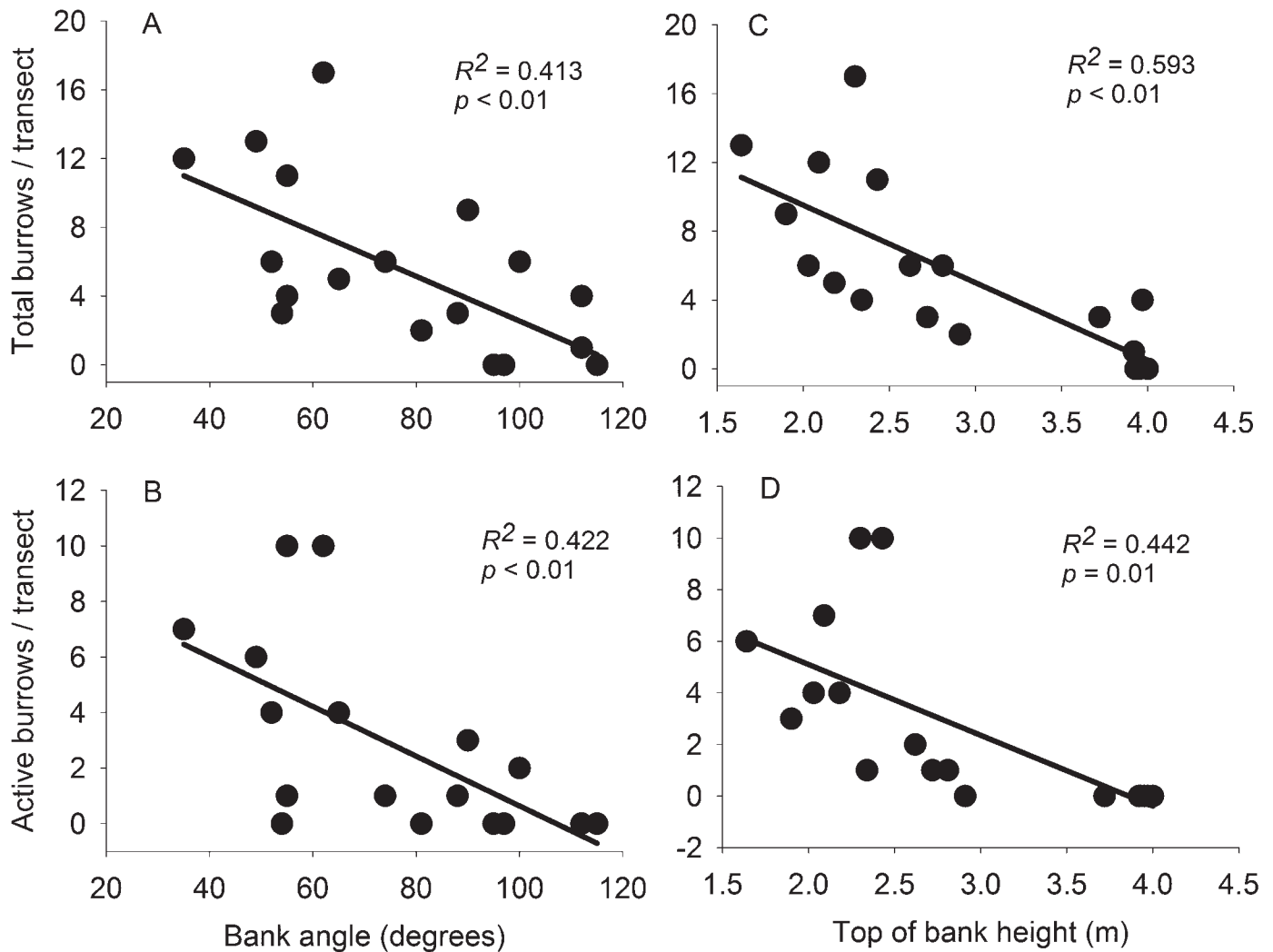
FIG. 4.   Regressions between total (A) and active (B) *Cambarus diogenes* burrows per transect (perpendicular to the stream) and bank angle and total (C) and active (D) burrows per transect and top-of-bank height in the floodplain connectivity surveys.

the remaining 25 crayfish had climbed the ramp ($45°$: $n = 13$, $80°$: $n = 6$). Crayfish in the $45°$ treatment on average needed significantly less time to reach the top than those in the $80°$ treatment (mean = 1.4 and 3.0 d, respectively; $F_{1,24} = 5.59$, $p = 0.03$; Fig. 8). Time to reach the top did not differ between males and females ($F_{1,24} = 3.40$, $p = 0.08$), and the angle × sex interaction effect was not significant ($F_{1,24} = 0.04$, $p = 0.84$).

Discussion

We used surveys and manipulative experiments to elucidate some potential mechanisms explaining the spatial distribution of *C. diogenes* burrows. Because of the fossorial nature of burrowing crayfishes, little is known about their natural history and environmental controls, and our study is one of a growing few that

have specifically examined life-history components or ecological patterns of burrowing crayfishes (Grow and Merchant 1980, Norrocky 1991, Johnston and Figiel 1997, Welch et al. 2008, Loughman 2010, Loughman et al. 2012).

Burrow distribution: space, soils, and congeners

We observed strong disparities in the spatial distribution of *C. diogenes* burrows in a stream/floodplain system and habitat segregation between adults and juveniles. Burrows were abundant in the predominantly sandy soils along stream margins but density decreased noticeably on the top of the shoulder of stream banks. This decrease was followed by an increase into loamy floodplain soils and, ultimately, burrows were absent >30 m of the stream's edge. *Cambarus diogenes* burrows often have
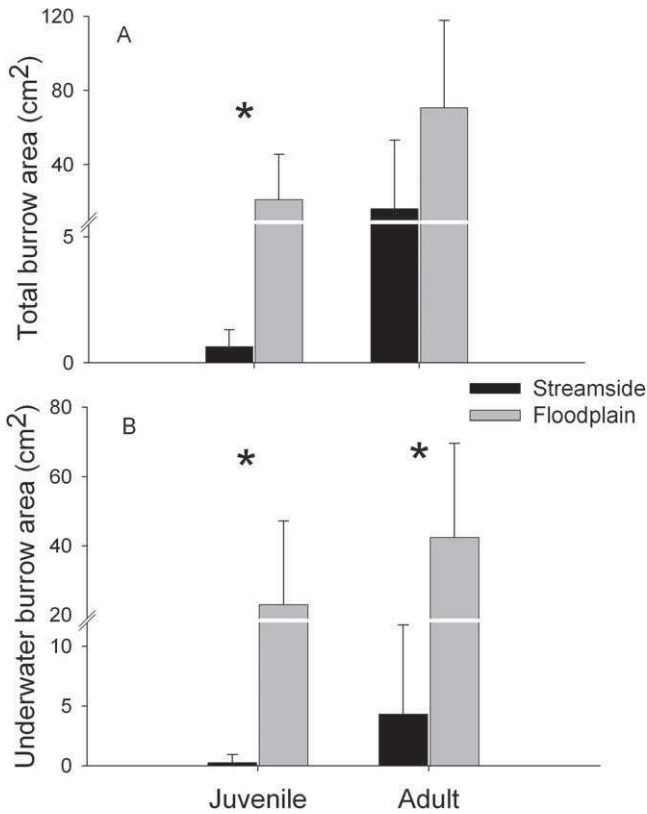
Fig. 5. Mean (±1 SD) total burrow area (A) and area of burrow below groundwater (B) constructed in floodplain and streamside soils by juvenile and adult *Cambarus diogenes* in the soil-preference trials. Asterisks denote significant differences ($p \leq 0.05$) in total burrow area between the 2 soil types.

Fig. 6. Mean (±1 SD) distance of juvenile *Cambarus diogenes* burrow opening from the midline of the ABC for streamside and floodplain treatments. All ABC's were half-filled with streamside and half-filled with floodplain soils collected from field sites. Streamside treatments had an adult burrowed in the streamside soils, floodplain treatments had an adult burrowed in the floodplain soils. Hashed lines represent the average distance from center (DFC) of the adult burrow opening. Streamside distances were arbitrarily scored as negative for comparison purposes.

multiple openings, and we did not document how many animals were in a given burrow. Our focus on active burrows alleviated some of the uncertainty in crayfish density, but our surveys should be considered a relative measure of density.

Burrow diameter and abundance patterns showed that juveniles generally clustered near the stream's edge and adults in the floodplain. This spatial complementarity supports the general model that young crayfish are released in open water and gradually emerge to the floodplain habitat (Hobbs 1981, Pflieger 1996). This strategy differs from that used by other burrowing species (Loughman 2010, Helms et al. 2013). Moreover, some other accounts of *C. diogenes* in the mid-Atlantic area describe adult females as releasing and raising their young in the burrow (Grow 1982). *Cambarus diogenes* occupies a variety of habitats and probably is a species complex across its range (Breinholt et al. 2012), so variation in reproductive strategies may reflect the polyphyletic nature of this species. More studies coupling phylo-
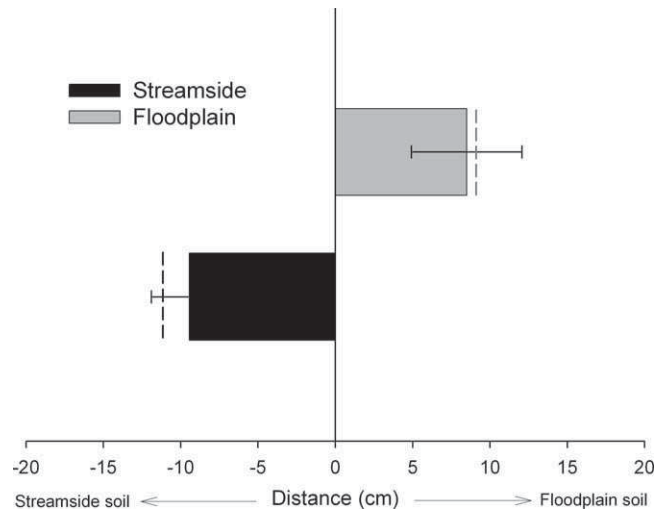
genetics and behavioral ecology are needed to tease apart potential variation in habitat use and life-history strategies within the *C. diogenes* species complex.

The preponderance of juveniles in sandy streamside soils in the field was surprising because it is counter to relationships observed elsewhere where juveniles appear to prefer fine-grained clay soils in the field and laboratory (Grow and Merchant 1980, Grow 1982). This preference has been attributed to the malleability of fine-grained soils and the crayfish's inability to handle and excavate coarse-grained soils properly, and Grow (1982) speculated that the
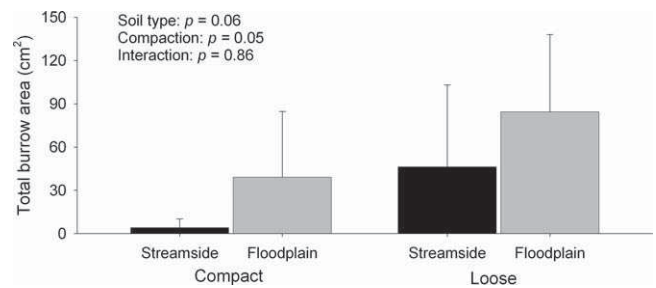

Fig. 7. Mean (±1 SD) total burrow area for juvenile *Cambarus diogenes* in compact and loose streamside and floodplain soils. Inset denotes *p*-values of factors in the general linear model associated with the experiment.
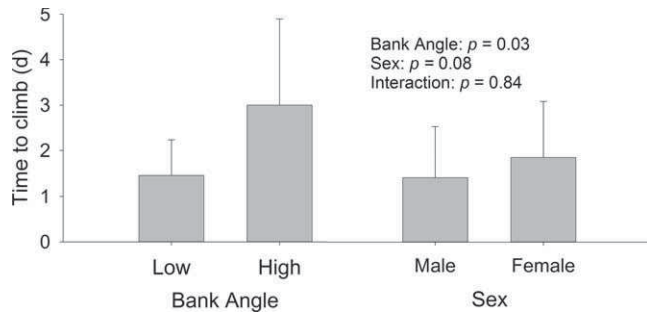
FIG. 8. Mean (±1 SD) time required for male and female juvenile *Cambarus diogenes* to climb ramps in the low (45°) and high (80°) bank-angle treatments. Inset denotes *p*-values of factors in the general linear model associated with the experiment.

preference had a genetic basis. Our laboratory experiments support these prior observations and experiments. Both juvenile and adult animals burrowed more extensively in floodplain than streamside soils. This phenomenon was most obvious in the underwater portion of the burrows, where the most direct contact with physicochemical environment (immersion in dissolved solids) occurs and where structural integrity of the burrow is most important to the animal. The distance *C. diogenes* must travel to reach floodplain soils in the field is considerably larger than the distance they had to travel in the ABCs. Increased travel distance probably increases the risk to individuals of mortality from predators and desiccation. The contradiction between our laboratory preference trials and field observations of burrow density suggests that influences other than soil-type preferences account for the spatial segregation of *C. diogenes* adults and juveniles along Choctafaula Creek.

We found no evidence to suggest that adult presence in an existing burrow would exclude juveniles from preferred soil type. In fact, in every case, juveniles selected the side of the ABC already occupied by the adult, regardless of soil type. Burrows often were constructed at the base of the adult chimney and, on occasion, juveniles were observed in the same burrow as adults. That juveniles were not consumed and often were permitted to burrow alongside adults suggests a level of tolerance between life stages. Juveniles burrowing in close proximity to conspecific adults also has been observed for *Fallicambarus fodiens* (Trepanier and Dunham 1999, Punzalan et al. 2001), and juveniles of *Fallicambarus gordoni* (Johnston and Figiel 1997), *Cambarus dubius* (Loughman 2010), and *Cambarus harti* (Helms et al. 2013) have been observed sharing burrows with adults. Collectively, these results suggest some selective advantage to tolerance among conspecifics in and around burrowers, but they are counter to the agonistic behaviors generally observed between conspecific surface-water-dwelling crayfishes (Bovbjerg 1953, Capelli and Hamilton 1984, Bergman and Moore 2003). Existing burrows may attract new individuals to a presumed optimal habitat and could affect the ultimate distribution of burrows across multiple species. However, this explanation does not address the distribution of burrows observed in the field. Burrow openings in the floodplain did vary in size, and we could not account for juveniles sharing adult-sized burrows, but virtually all juvenile burrows were found streamside and not associated with adults in the flood plain. Thus, even though recognition of conspecific burrows seems to occur, other environmental factors appear to constrain juvenile–adult aggregation.

We found no evidence that compaction influences soil selection by juveniles. However, burrow area was smaller in more compact soils, a result suggesting that compaction does influence burrowing ability. This result may partially explain the observed distribution of juveniles because streamside sediments are deposited more regularly and are likely to be less compact than floodplain sediments. Furthermore, streamside sediments are close to the water and have a shallower water table than floodplain habitat. Water depth in streamside burrows ranged from 2 to 26 cm, whereas floodplain burrows often were dry at depths >1 m into the soil (BH, unpublished data). A possible explanation for the observed segregation between adult and juvenile burrows in the field is that juveniles minimize their risk of desiccation by burrowing quickly in comparatively loose substrate. This hypothesis is consistent with work published by Dorn and Volin (2009), who showed the importance of substrate type and bulk density on seasonal desiccation potential in *Procambarus* species, and March and Robson (2006), who showed that density of parastacid crayfish burrows decreased with soil compaction in floodplain soils in Australia. However, the soil type that carried the greatest risk for desiccation in the study by Dorn and Volin (2009) was sand, which was the type of soil used in the field by juveniles in our study. One key factor that differed between our study and the study by Dorn and Volin (2009) is the shallow water table of near-stream habitats of juvenile burrowers in our study vs the deeper water table associated with adult burrowers in the study by Dorn and Volin (2009). Avoiding exposure and reaching the water table are critical for burrowing crayfish, and an increased ability for juveniles to burrow effectively in loose streamside soils with a high water table may

explain the apparent lack of juveniles in floodplain habitats. These physical constraints may override any soil preferences.

The importance of floodplain connectivity

Floodplains are highly productive and heterogeneous habitats that support a high level of biodiversity because of their dynamic and variable connection with open water (Junk et al. 1989, Opperman et al. 2010). However, many flood plains have become disconnected from their respective rivers and streams as a result of past and present anthropogenic influences, such as agriculture (Tockner and Stanford 2002). This disconnection often takes the form of stream incision and subsequent reduction in floodplain groundwater levels (Galay 1983, Amoros and Bornette 2002). *Cambarus diogenes* spends the early stages of its life in open water and streamside burrows, and progresses to floodplain habitats as an adult, so floodplain connectivity probably is critical for population persistence. Our results support this notion. Densities of total and active burrows were highest where the top-of-bank height and the maximum bank angle were low (where connectivity was high).

Many investigators have reported that reduced floodplain connectivity or increased channel incision strongly influences fish, amphibian, reptile, and macroinvertebrate assemblage structure (e.g., Shields et al. 1994, Tockner et al. 1999, Ward et al. 1999, Bodie et al. 2000). Assemblage changes resulting from decreased connectivity/increased incision often can be attributed to habitat alterations (quite often homogenization) and the specific ecological requirements and life histories of local species (Amoros and Bornette 2002). Multiple factors could explain why fewer *C. diogenes* burrows were found on higher/ steeper banks. Stream incision can cause groundwater levels to decrease and can alter riparian vegetation or soil conditions. In-stream habitat associated with incised banks often is homogenized (Shields et al. 1994) and, given that adult *C. diogenes* use these habitats for part of the season, this homogenization could decrease local abundances. However, caution should be used when inferring stream condition from estimates of incision because this characteristic is only one of many indicators of geomorphic degradation and may have little bearing on critical in-stream habitat (Duncan et al. 2009).

Stream incision may influence juvenile recruitment directly via physical constraints. This idea is supported by the results of our bank angle manipulation. Juveniles needed nearly 2 d longer to climb 80° banks than 45° banks. Whether our laboratory results can be extrapolated to the field is debatable, but steeper banks appear to prolong exposure of vulnerable juveniles to increased risk of predation and desiccation (Dorn and Volin 2009). We were unable to test specifically for bank-height effects because of constraints in the laboratory, but we think it likely that higher banks take longer to climb than lower banks. Thus, the near-bank condition may be crucial for maintaining juvenile recruitment into the flood plain.

Changes in the abundances of crayfishes may have far-reaching effects in floodplain ecosystems. Burrowing by *Procambarus rogersi* influences soil mixing and subsurface water flow in a Florida coastal terrace (Stone 1993). Along the Roanoke River in North Carolina, crayfish created fine-scale spatial heterogeneity in the floodplain soil. Soils in areas with burrows had considerably higher clay content than soils in areas with no crayfish burrows (Butler 2002). *Parastacoides tasmanicus* can influence the respiration rate in soils by improving gas exchange deep into the soil, which can potentially influence root and fungal growth in Tasmania (Richardson 1983). The broader effects of *C. diogenes* in the habitats we studied are not known, but their habits are similar to those of crayfishes in other studies, so the potential for significant ecosystem-level effects is likely to be high. We did not test for ecosystem-level effects, but our results suggest that broader influences of *C. diogenes* are likely to be highest in connected flood plains with low bank heights and low bank angles.

Our study shed considerable light on mechanisms that influence the local distribution of a widespread burrower, *C. diogenes*. Our data suggest that multiple factors beyond soil type affect where these animals burrow. Juveniles are attracted to conspecific adult burrows, and adults tolerate nonrelated juvenile conspecifics. This attraction/tolerance may help explain the patchy, aggregated nature of crayfish burrows in the floodplain. Stream-bank condition and floodplain connectivity also may contribute to patchy distributions by affecting the ability of juveniles and adults to migrate to the flood plain. To some degree, these details of *C. diogenes* life history and burrowing behavior may be valuable for efforts aimed at conserving rare burrowing crayfish because their life history and burrowing behavior may be similar to that of *C. diogenes*. The abundance of crayfish in a flood plain can provide an indication of connectivity and might be useful as a biological metric that could be used in conjunction with traditional geomorphic metrics to gauge degradation and restoration efforts (Duncan et al. 2009). Thus, information gained from our study provides insight for guiding

management plans for primary burrowing crayfish and floodplain ecosystems.

Acknowledgements

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