The role of abiotic and biotic cues in burrow habitat selection by juvenile crayfish

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ABSTRACT

Environmental cues contain critical information for individuals while searching for mates and suitable habitat. Crayfish have well-developed chemosensory abilities for detecting environmental cues in water; much less is known about these abilities on land. The Devil crayfish (*Cambarus diogenes*) is a burrowing crayfish often found in dense floodplain colonies as adults. Juveniles however are released in surface water and must navigate overland to burrow. Previous work demonstrates juveniles use cues from conspecific adults, and to a lesser extent, soil cues, for burrow site selection. Using mesocosms, we build on this by examining burrowing cues associated with 1) congeneric adults, 2) excavated burrow material and 3) other juveniles. In contrast to conspecific adults, cues provided by congeneric adults did not override cues associated with soil type. Similarly, juveniles burrowed closer to conspecific adult burrow mounds than to congeneric and human-built mounds. Juveniles also showed significant grouping behavior in the absence of all other cues. These results suggest juvenile crayfish integrate multiple terrestrial cues for burrow site selection.

INTRODUCTION

The factors regulating populations are a function of multiple interacting local and regional phenomena broadly explained by adult fecundity and mortality as well as juvenile survivorship and recruitment (Berven 1990, Murdoch 1994, Rodenhouse et al. 1997). Perhaps less obvious, navigation, migration, and other movements are important factors in population regulation as they aid in detection of home ranges, optimal habitats, and mating opportunities (Kamran and Moore 2015). More specifically, success of juveniles finding and occupying critical habitat is essential for population viability; however, complex life cycles can limit successful juvenile recruitment in some species (Wilbur 1980, Berven 1990, Schmidt et al. 2012). Thus, it is important that species use cues from their environment that are reliably effective and capable of discerning true signal from environmental noise and turbulence.

Environmental cues used for navigation, migration, orientation, and homing vary across taxa. For example, desert ants (*Cataglyphis fortis*) use olfactory cues to return to their burrows after foraging (Buehlmann et al. 2012), honey bees (*Apis mellifera*) and several ant species (*Formicidae*) use landmarks associated with previously traveled routes (Menzel et al. 1998, Buhlmann et al. 2011, Holldobler 1974), and juvenile loggerhead sea turtles (*Caretta caretta*) use gravitational cues for migration and feeding (Avens 2004). Additionally, there is evidence that navigating in groups ("many-wrongs principle") is more advantageous than individual navigation due to suppression of individual error by group cohesion (Simons 2004, Codling et al. 2007). Crustaceans use a particularly diverse set of environmental cues to aid in navigation, migration, and orientation, largely due to a wide range in patterns and scales of mobility across species (Zeil and Hemmi 2006, Walls and Layne 2009). Several groups of crustaceans, including amphipods, isopods, and spiny lobsters can orient to the Earth's geomagnetic field (Lohmann and Ernst 2014). Crayfish use a range of chemical and sensory cues to find food, mates, and potential competitors (Thorp and Ammerman 1978, Basil and Sandeman 2000, Keller et al. 2001, Corotto and O'Brien 2002). Recent studies also suggest that crayfish are capable of homing behaviors (Kamran and Moore 2015), but

whether or not this involves a combination of navigational cues and group migration is not yet understood.

Understanding factors driving size and stability of crayfish populations is particularly important because these animals play a key role in aquatic and terrestrial food webs, linking several different trophic levels. They are prey for fish (Stein 1977, Garvey et al. 1994, Adams 2007), birds (Kushlan 1986), and mammals (Englund and Krupa 2000), but also serve as predators, herbivores, detritivores, and scavengers (Heard and Richardson 1995, Nyström et al. 1996, Taylor et al. 2007). They are important geomorphic agents, altering aquatic and terrestrial ecosystems through sediment processing, bioturbation, and burrowing (Statzner et al. 2000, Butler 2002, Creed and Reed 2004, Helms and Creed 2005). Crayfish species vary in their capacity for burrowing and in their ultimate use of burrows (Hobbs 1981, Berrill and Chenoweth 1982, DiStefano et al. 2009). Many burrowing crayfish species tend to exhibit strong spatial clumping, which is partly a function of abiotic factors such as local soil, hydrologic, and geomorphologic conditions (Grow 1982, Loughman et al. 2012, Helms et al. 2013a). However, the degree of connectivity between spatially segregated burrowing populations, and the specific cues and movement patterns used by these animals as they navigate the terrestrial landscape, is largely unknown.

Cambarus diogenes (Devil crayfish) is a burrowing crayfish widely-distributed alongside streams, rivers and ponds across Eastern North America (Grow 1981, Taylor et al. 2007, Helms et al. 2013a). It inhabits flood plains and seasonally flooded woodland areas, constructing elaborate burrow systems usually topped with well-constructed earthen mounds ("chimneys" see also Grow 1982, Helms et al. 2013a). Female *C. diogenes* generally leave terrestrial burrows and enter nearby surface waters to release their young during winter and early spring (Hobbs 1981, Pflieger and Dryden 1996). After a period of development and growth in surface waters, juveniles return to terrestrial habitats. Juvenile burrows are abundant in stream margins by mid-summer with adult burrows active in the floodplain early spring through fall (Pflieger and Dryden 1996, Helms et al. 2013a). Thus accurate navigation from an aquatic environment to terrestrial burrowing habitat containing suitable soil, groundwater, and foraging

conditions, as well as potential mates, is necessary to maintain population viability. Previous studies with *C. diogenes* suggest that isolated juveniles prefer clayey, floodplain soils over sandy, streamside soils for burrowing; in the presence of conspecific adults however, juveniles prefer adult burrowing locations irrespective of soil type (Grow 1982, Helms et al. 2013a). In this study, we investigate further potential drivers of observed burrowing distributions by addressing the following questions:

1) Are juveniles attracted to burrows near congeneric adults in the same manner as previously demonstrated for conspecific adults?

2) Do adult burrow mounds serve as an important cue for juvenile site selection?

3) Do juveniles display group behavior when searching for burrow sites?

Prior studies using *Fallicambarus fodiens* (Trepanier and Dunham 1999, Punzalen et al. 2001) collectively have shown chimneys act as cues for conspecifics and adults can discriminate conspecific-built and human-built chimneys. Building on these findings, we predicted that *C. diogenes* juveniles would burrow in areas with congeneric adult chimneys over areas that contained no chimney cue, and when provided a choice, juveniles would burrow near conspecific adult chimneys over congeneric chimneys. We also predicted that, based on prior studies of conspecific tolerances of burrowing crayfish (Dalosto et al. 2013, Helms et al. 2013b), juveniles would group when selecting sites for initial burrows.

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METHODS

Study Animals

All crayfishes used were collected from tributaries of the Tallapoosa River basin in east Alabama, USA. Adult *C. diogenes* (mean carapace length (CL) 36.45 mm ± 3.15 SD) were collected via burrow excavation in the flood plains of Choctafaula Creek and Odum Creek, Macon County, AL. *Cambarus striatus* (Ambiguous crayfish) and *C. latimanus* (Variable crayfish), closely- related species often

occurring in the same open water and burrowing habitats as *C. diogenes* (MC *personal observation*), were used as congeneric outgroups in study trials. Although both *C. latimanus* and *C. striatus* are recognized species, they often strongly overlap in habitat and physical characters (Bouchard 1978, Hobbs 1981), thus were considered ecological equivalents for this effort. Adult *C. striatus* (CL 43.61 mm ± 5.13) were collected from Odum Creek and adult *C. latimanus* (CL = CL 33.40 mm ± 6.02) were collected from Choctafaula Creek in spring 2013. Juvenile *C. diogenes* were hatched from a berried female collected from Odum Creek and reared in a flow-through pond water trough until they had attained an average carapace length of 16.77 mm ± 1.04, sexes indistinguishable. Adults were housed separately from juveniles in species-specific flow-through tanks containing PVC refugia prior to experimentation at the South Auburn Fisheries Research Station, Auburn, AL.

Experiment 1: Effects of congeneric adults on site selection

We used artificial burrowing chambers (ABCs, Figure 1, Stoeckel et al. 2011) to test whether presence of a congeneric adult influenced juvenile burrow site selection in a similar manner as reported in Helms et al. (2013a) for conspecific adults. Chambers measured 30 cm H x 46 cm L x 5 cm W. They were laterally bisected with a removable plastic divider, filled with clayey floodplain soils in one-half and sandy streamside soils in the other half, and the plastic divider subsequently removed (Figure 1). A single adult *C. latimanus* was placed in each of 10 chambers and constrained to burrow in either streamside or floodplain soils (5 chambers per soil designation). After the adults burrowed (usually within 24 h), one juvenile *C. diogenes* was placed on the center line and allowed to burrow (Figure 1). After juveniles burrowed (usually within 8 hrs), we recorded soil type of the juvenile burrow location and used Pearson's X^2 test to test for departures from random across all chambers.

Experiment 2: Effect of soil mound source on juvenile burrow location

To test whether the source of burrow mounds influenced location of juvenile burrows, we exposed juvenile *C. diogenes* simultaneously to mounds constructed by a conspecific adult, a congeneric

adult (*C. striatus)*, and a human. Adult crayfish mounds were obtained by allowing 10 *C. diogenes* and 10 *C. striatus* to burrow separately in individual 18.9 L buckets filled 22 cm deep with moistened, common test soil mixed from a clayey-loam collected on the grounds of the South Auburn University Fisheries Research Station. Adults were randomly selected for each bucket and placed in a center thumb-depression, covered, and allowed to burrow. Excavated material consisted of amorphous piles rather than carefully constructed chimneys. After 48 h, species-specific excavations were shaped into similar, semi-circular mounds, keeping form and texture similar between crayfish species. Similar human-built mounds were constructed from the common test soil mixture that had no contact from test animals (Figure 2). We wore separate nitrile gloves for handling mounds and filling buckets to avoid cross-contamination of potential chemical cues.

We ran trials in ten 120-cm diameter (375 cm circumference, 11,309 cm^2 total area) plastic-pool mesocosms, filled with 10.2 cm of test soil (not exposed to either species). Each mesocosm was divided into 3 equal sections (‘1’, ‘2’, or ‘3’) with a 1.25 cm diameter hole created 7.62 cm from the outside edge of each section (Figure 2) to represent an adult burrow. A *C. diogenes* mound, a similarly-sized *C. striatus* mound, and a human-built mound were randomly assigned a section in each mesocosm and placed around the corresponding ‘burrow’ hole. We assigned six randomly selected juveniles to each mesocosm, and placed them in the center inside an inverted cup. After an hour acclimation, the cup was removed and mesocosms were covered to maintain dark, humid conditions. After 16 h, mesocosms were uncovered and we counted and marked the position of each juvenile using metal pins. Since some juveniles did not burrow, we marked both the total number of individuals and the total number of burrowed individuals and used these as separate and combined response variables. Two trials were run within 5 d with randomly selected animals (total mesocosm n=10). A generalized linear mixed model with Poisson error distribution was used to compare mean juvenile abundance and occupied burrow abundance among the three soil mound treatments. The model included treatment (*C. diogenes, C. striatus,* or human mound) as a fixed effect and mesocosm and run as random effects. To determine if

there was an overall treatment effect, we used a likelihood ratio test to compare an intercept-only model (+ random effects) to a fixed effects model (+ random effects), which was followed with a post-hoc Tukey's test if significant.

Experiment 3: Grouping behavior and site selection

To test whether juvenile *C. diogenes* exhibit grouping tendencies when selecting sites for burrowing, we allowed 6 juveniles to burrow in plastic pool mesocosms as above, except without adults or chimneys present (i.e., no adult or physical cues). For each trial, we added juveniles to the center of the mesocosm and marked their distributions (total and burrowed) as above. All burrowed and non-burrowed individuals were marked as above and photographed with a Canon EOS Rebel T5 digital SLR camera. All images were captured perpendicular to the center of the mesocosm at a fixed height (2m) with a ruler added to the field of view for scale. Distances between each burrow were calculated in ImageJ software (Abramoff et al., 2004). From these calculated distances, nearest neighbor analysis (Clark and Evans 1954) was performed to distinguish patterns of distribution. For each mesocosm, the mean observed nearest neighbor distance (r_o) was divided by the expected mean distance under a random pattern of distribution (r_e, given by $1/\sqrt{d}$, density) to give R, a measure of departure from random. Distributions are random when R=1, dispersed when R>1, and clumped when R<1 (Clark and Evans 1954). Statistical significance for each trial was determined by standard z scores. See Clark and Evans (1954) and Cade (1981) for calculation details. All analyses were performed in R (R Development Core Team 2016).

RESULTS

Experiment 1: Effects of congeneric adults on site selection

All *C. diogenes* tested survived soil preference trials and burrowed within 48 h. All juveniles chose floodplain soils for burrowing. For ABCs containing a *C. latimanus* adult burrowing in streamside soil, 100% of *C. diogenes* juveniles selected the floodplain soils (χ^2= 5.0, P = 0.025). For ABCs

containing a *C. latimanus* adult burrowing in floodplain soil, 100% of *C. diogenes* juveniles selected the floodplain soils (χ^2= 5.0, P = 0.025).

Experiment 2: Effect of soil mound source on juvenile burrow location

In general, juvenile crayfishes tended to aggregate near soil mounds constructed by adult crayfish and avoid mounds constructed by humans. Of the 60 *C. diogenes* juveniles tested for burrow mound preference, 36 burrowed within the 16-h trial. Based on the likelihood ratio test, there was a significant overall treatment effect (X^2 = 7.342, df = 2, p = 0.026) on the total number of juveniles (burrowed + non-burrowed). Specifically, there was a significant effect in sections containing *C. diogenes* mounds (average number of juveniles ± SE = 2.9 ± 0.29, Z = 2.57, p = 0.0101) but not in sections with *C. striatus* mounds (1.8 ± 0.36, Z = 1.246, p = 0.2127) or human-built mounds (1.2 ± 0.12, Z = 0.632, p = 0.5277). Tukey's post hoc test revealed significantly more juveniles in *C. diogenes* sections than in human-built sections (p = 0.027, Fig 3). Of the 36 burrowed juveniles, there was also an overall significant treatment effect (X^2 = 14.69, df = 2, p<0.001), with specific significant effects for the *C. diogenes* section (2.3 ± 0.26, Z = 3.09, p = 0.001) but not the *C. striatus* (0.8 ± 0.30, Z = 0.824, p = 0.409) or human-built (0.5 ± 0.18, Z = -1.55, p = 0.121) sections. Similarly, Tukey's post hoc test revealed that there were significantly more juveniles burrowed in the *C. diogenes* section than in the human-built (p = 0.005) and *C. striatus* (p = 0.026) sections (Figure 3).

Experiment 3: Grouping behavior and site selection

Of the 60 juveniles tested in the grouping trials, 9 were observed on the surface after 16 h. The number of burrowed crayfish in each mesocosm ranged from 3 – 6 (Table 1). In many instances, multiple crayfish occupied the same burrow, thus their nearest neighbor distance was 0. Overall, observed mean nearest neighbor distance ranged from 12.71 – 32.08 (Table 1.), and R values for all trials were < 1, indicating clumped distributions. Based on standard z scores, 9 of the 10 trials were significant at p =

0.05, with the only trial not significantly clumped having a reduced number (N = 3) of individuals burrowing (Table 1).

DISCUSSION

Navigation by animals to a target, such as suitable habitat, is influenced by a suite of environmental, idiothetic, and learned cues. To various degrees depending on the organism, these cues integrate multiple sensory processing systems (olfaction, chemosensory, vision, etc.) with the ultimate outcomes influenced by potential interactions and synergies between these systems (Knaden and Graham 2016). Our experiments support earlier studies (Grow 1982, Helms et al. 2013a) and suggest the importance of environmental cue integration, in this case soil substrate composition, conspecific adults, and group behavior, for habitat selection and recruitment of juvenile burrowing crayfish.

Juvenile burrowing crayfish face a particular challenge when transitioning between aquatic and terrestrial environments. They must find suitable burrowing habitat while avoiding desiccation, predation, and other migratory risks. Accordingly, although burrowing + non-burrowing individuals showed strong differences between treatments, there were stronger species-specific effects of burrow-mound and group cues when we considered only the individuals that burrowed. We consider these burrowing individuals to be the individuals that ultimately would have survived desiccation and predation in a natural setting. However, suitable habitat is not the only consideration. Crayfish must burrow in close enough proximity to conspecifics if they are to find mates and successfully reproduce, which requires recognition and processing of a combination of cues associated with habitat and conspecifics. *C. diogenes* appear to be able to identify specific substrate types. They also appear to respond to cues provided from burrowed adults of the same species. In the absence of conspecifics, *C. diogenes* prefer fine (e.g. clay, silt) to coarse (e.g. sand) particle soils (Grow and Merchant 1980). However, when adult

conspecifics are present, juveniles burrow in close proximity to adults regardless of soil type (Helms et al. 2013a).

Several other crayfish species burrow in the same soil types as *C. diogenes* (Hobbs 1981), thus one may expect juvenile *C. diogenes* to burrow in close proximity to congenerics if they were simply using the presence of crayfish burrows as an indicator of habitat suitability. However, in our study, using the same experimental apparatus as Helms et al (2013a), juvenile *C. diogenes* consistently chose to burrow in fine, clayey soils regardless of the location of congeneric burrows. Together this suggests that attraction to conspecific burrows is driven by the integration of abiotic and species-specific biotic cues and that certain cues override others, as seen in the path integration and visual cues encountered by other species (e.g., Wehner et al. 1996). Further, since associating with conspecifics appears to trump burrowing in preferential soils, juvenile site selection in these crayfish may be more related to the benefits of intraspecific interactions like reproduction rather than habitat preference.

Species-specific detection has been demonstrated previously for crayfishes in aquatic environments where water is an effective medium for dispersal of chemical signals. Juvenile *Orconectes sanbornii*, *O. virilis*, and *Procambarus clarkii* can discriminate between brooding and non-brooding individuals using chemical stimuli, with cues likely being species-specific (Little 1975). Several species demonstrate conspecific, parent-offspring and/or kin recognition based on dispersed chemical cues (Dunham and Oh 1992, Levi et al. 1999, Mathews 2011). Chemical communication in general is prevalent in crayfish mating (Fero et al. 2007, Berry and Breithaupt 2010), agonistic interactions (Breithaupt and Eger 2002), and foraging (Wolf et al. 2004). Our results suggest that the cues used by *C. diogenes* can be detected in terrestrial as well as aquatic environments; however, cue recognition among crayfish in terrestrial environments has received little attention, and how they navigate following a transition to terrestrial habitat is not well known. Wehner (1987) postulated that physical, physiological, and behavioral components of a sensory system correspond to the environment carrying the most relevant signal ('matched filters'). Multiple lines of evidence from this study and others (e.g., Keller et al. 2001)

suggest that crayfish have such adaptations, but specifically how they differ in burrowing species is not well known.

The function of the soil mounds associated with crayfish burrows has been pondered for decades (Abbott 1884). Several hypotheses exist as to why these are constructed, ranging from regulation of airflow (Hobbs 1981), species recognition (Trepanier and Dunham 1999, Punzalan et al. 2001), or simply discarded material from burrow enlargement (Walls and Layne 2009). Our study supports the hypothesis that mounds function, at least in part, in species recognition. By associating burrow entrances with a constructed mound, the location of favorable habitat and presence of conspecifics is advertised. Previous studies show that crayfish mounds can attract conspecifics. *Fallicambarus fodiens* individuals are more likely to burrow near a conspecific-constructed chimney than a human-constructed one, and they prefer mud saturated with water exposed to a con-specific over mud saturated with distilled water (Punzalan et al. 2001). Further, *F. fodiens* and *Orconectes rusticus* have been shown to be capable of homing behaviors and that visual cues are unnecessary for success (Kamran and Moore 2015). Our results confirm and extend these findings as we measured juvenile burrowing preference in an additional species (*C. diogenes*) and in association with 3 contrasting cue sources: human-built (tactile/visual cue only), congeneric (tactile/visual cue and general crayfish cue), and conspecific-constructed mounds (tactile/visual cue and species-specific cue). Juveniles navigated toward, and were more likely to burrow near, conspecific mounds over human-built and congeneric mounds, even when burrow complexes were unoccupied. This preference for burrowing near conspecific mounds, in the absence of conspecific occupants, suggests that site selection cues are species-specific and provided from the mound itself. Although not specifically tested for in this study, these cues are likely to be chemical, rather than visually or texturally based, as demonstrated for *F. fodiens* (Punzalen et al. 2001). During burrow enlargement and mound construction for *C. diogenes*, soil particles are formed meticulously into pellets with the third maxillipeds (Grow 1981). These appendages are proximate to the nephropore, the excretory point of the green gland and source of many chemical cues associated with urine in crayfishes (Breithaupt 2002). In

our surface mesocosm study, experimental mounds were constructed such that width, height, and texture were standardized among treatments. If cues were entirely visual or tactile, we should have observed no preference for burrowing near conspecific mounds over human-built mounds, as both treatments were visually and texturally similar.

Crayfish are generally territorial with a propensity for agonistic, aggressive behaviors, thus showing low tolerance for other individuals (Gydemo et al. 1990, Figler et al. 1995, Fero and Moore 2008). However, recent studies suggest that burrowing crayfish may be an exception as they show a level of conspecific tolerance usually not found in surface-dwelling species. High concentrations of individuals in burrowing habitats, the occupancy of single burrows by multiple individuals, and a lack of an escalated sequence of agonistic behaviors (Norrocky 1991, Hamr and Richardson 1994, Guiasu et al. 2005, Dalosto et al. 2013, Helms et al. 2013b) suggest a high level of conspecific tolerance. Similarly, we found juvenile *C. diogenes* to associate with both conspecific juveniles and adults. However, whether these observed associations reflect complex social structure or merely tolerance is unknown. It is likely that there is selective advantage to tolerating other individuals when exhibiting burrowing habits and burrowers may be released from the typical limiting resources experienced by open-water dwellers (Dalosto et al. 2013). Given the relatively specific environmental conditions required by burrowing crayfishes, such tolerance may be one of the mechanisms that have allowed them to persist in semi-terrestrial environments. However, burrows are a resource of high value and of considerable physiological cost to construct (Richardson 2007), so increased agonistic interaction may be expected. Conclusive evidence of compelling reasons as to why burrowing species show increased tolerance and reduced agonist behavior remain elusive.

Despite the territorial and agonistic behaviors typically observed in crayfishes, benefits of grouping are numerous. Quite often, organisms in groups can more effectively avoid predation (Hamilton 1971), forage (Crook 1960), consume less oxygen thus conserve energy (Ritz et al. 2001), and have increased mate accessibility (Dobson and Poole 1998) over solitary individuals. Juvenile aggregation in

particular has been observed in many invertebrate species (e.g., spiny lobsters, see Butler et al. 1999, Dolan and Butler 2006; and porcelain crabs, see Jensen 1991). This aggregation often coincides with optimal seasonal dispersal conditions suggesting timing is particularly important for vulnerable juveniles (Rasa 1995, Palaoro et al. 2016). However, navigational error can be pervasive as a result of a limitation of cues, errors in interpretation and integration of cues, and environmental turbulence (Codling et al. 2007). Although it may seem like a daunting task for juvenile crayfish to emerge from aquatic habitats and traverse a forest floor to burrow, there is evidence that group movement can improve an individual's ability to align and reach a target direction by the suppression of individual error by interactions and group cohesion (Simons 2004). Further, aggregation is predicted to be more prevalent in turbulent or dangerous environments where the efficacy of navigational cues is limited (Simons 2004). This 'many-wrongs principle' has been shown in migratory birds (Bergman and Donner 1964), fish schooling in a turbulent environment (Grunbaum 1998, Codling et al. 2007), and the foraging patterns of Bornean bearded pigs (*Sus barbatus*, Hancock et al. 2006). Whether this phenomenon explains our results is speculative; however, our observed patterns of aggregation suggest that there is some benefit to grouping, particularly for juveniles searching for potential burrowing sites. Although grouping was observed in our trials, some caution should be used in broad interpretation of our results, as juveniles used for grouping trials were reared from the same female. As such there could be a maternal imprint or some other brood-related influence on their behaviors. Further directed studies are needed to elucidate the true relationship between juvenile crayfish group size, relatedness, and navigational success.

Our study sheds light on the burrowing site selection cues used by a common species of burrowing crayfish and provides insight on recruitment strategies used by burrowing crayfish in general. Our experiments suggest that juvenile *C. diogenes* integrate cues from the soil, locally burrowed adults, and each other for transitioning from semi-terrestrial habitats to preferred burrowing sites in the flood plain. Other studies have shown that burrowing crayfish have a stronger homing capability than non-burrowing crayfish (Kamran and Moore 2015), although the mechanisms for this capacity remain elusive.

Accumulating data suggest that burrowing crayfish are unique in that they have a semi-communal, shared-effort existence with a high tolerance of conspecifics, a strong chemosensory ability to detect volatile and water-soluble odor cues, and the ability to use these cues in conjunction with soil composition and juvenile aggregation to locate optimal burrowing habitat. Such traits may allow for continued recruitment in a heterogeneous environment.

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Tables

Table 1. Nearest neighbor analysis for experiment #3 (group navigation). N = number of juveniles that burrowed, d = density of individuals in mesocosm (per cm^2), r_0 = observed distance (cm), r_e = expected distance, R = r_0/r_e, and Z = standard z score. All R values are <1, indicating clumped distributions. Z values that are bold are statistically significant.

Trial	N	d	r_0	r_e	R	z
1	6	0.00053	17.89	43.42	0.41	**-2.26**
2	4	0.00035	17.48	53.17	0.33	**-2.04**
3	5	0.00044	16.37	47.56	0.34	**-2.57**
4	5	0.00044	32.08	47.56	0.67	**-6.71**
5	5	0.00044	22.39	47.56	0.47	**-2.49**
6	3	0.00027	24.55	61.40	0.40	-1.50
7	5	0.00044	19.07	47.56	0.40	**-2.35**
8	6	0.00053	13.39	43.42	0.31	**-3.92**
9	6	0.00053	18.05	43.42	0.42	**-2.92**
10	6	0.00053	12.71	43.42	0.29	**-2.42**

Figure Legend

Figure 1. A schematic and photograph of an artificial burrowing chamber (ABC) used to manipulate soil preferences and congeneric cues. Arrows in schematic denote water flow. The ABC was constructed of acrylic, was 30 cm H x 46 cm L x 5 cm W and filled with streamside soil on one side and floodplain soil on the other. Adult crayfish were constrained to burrow in one soil treatment, and juveniles were placed on soil midline and allowed to burrow.

Figure 2. Artificial burrow and mound layout in mesocosm experiments. Mesocosms were divided into sections (highlighted in white), each containing a treatment mound (*C. diogenes*, *C. striatus*, or human-built). Treatment mounds were arranged as similarly as possible. White markers indicate location of juveniles (burrowed or on surface) when cover removed.

Figure 3. Mean number (+ SE) of juveniles per mesocosm section containing different chimney treatments. Top panel refer to all individuals (burrowed + surface), whereas bottom panel is mean number of juveniles that burrowed only. Letters above bars denote significant difference according to post hoc Tukey's test.

Figure 1.

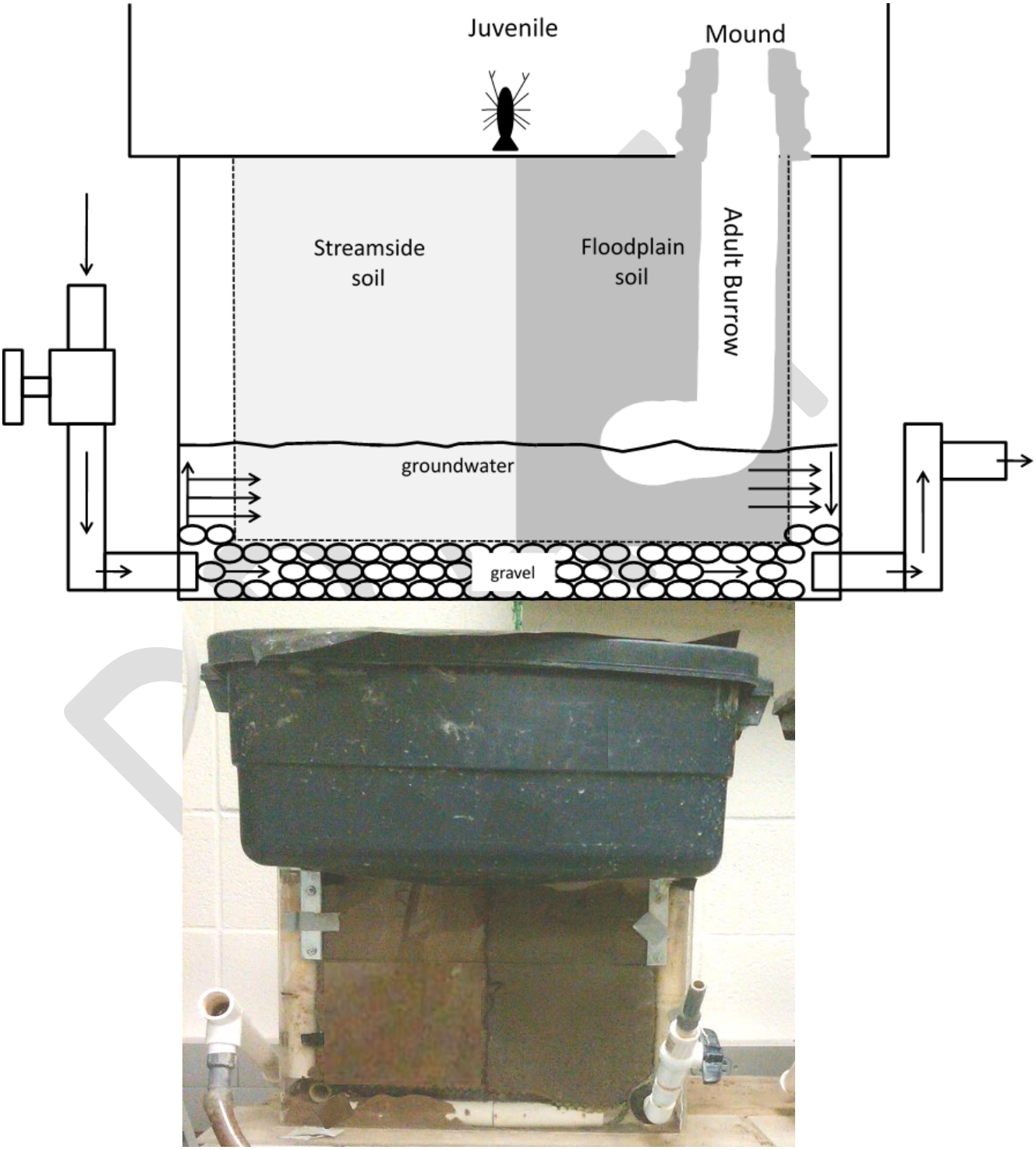

Juvenile
Mound
Streamside soil
Floodplain soil
Adult Burrow
groundwater
gravel

Figure 2.

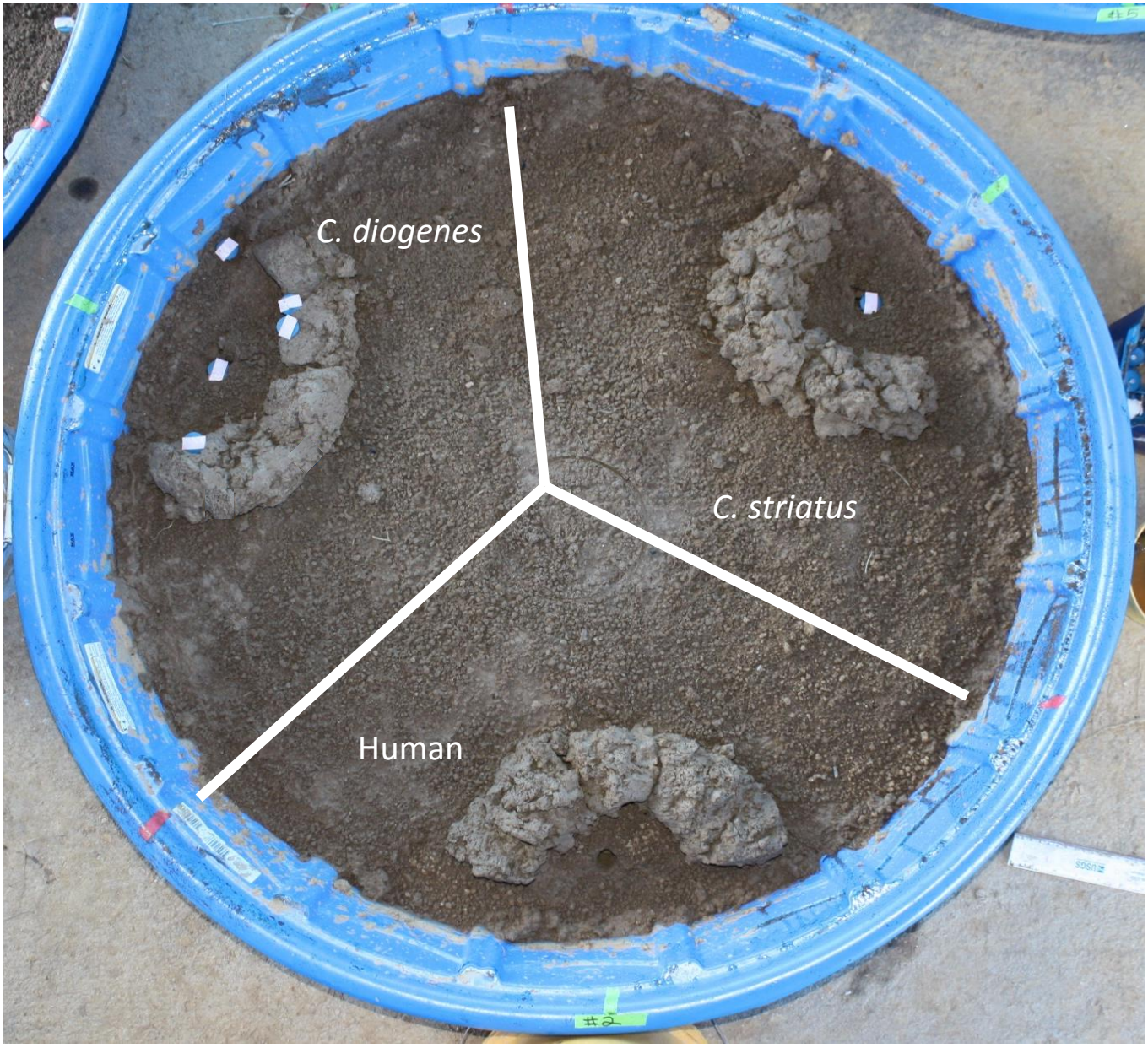
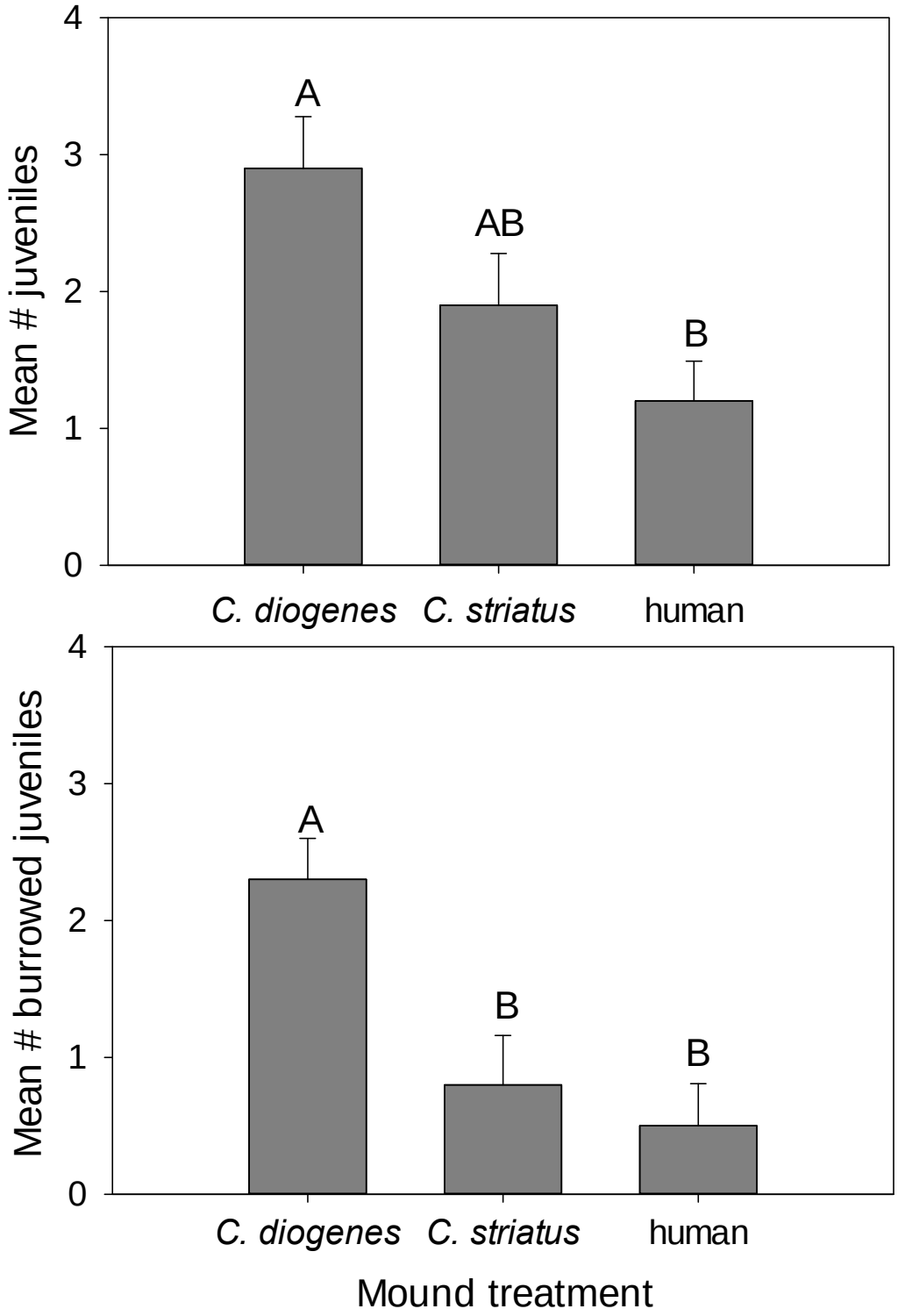


Figure 3.


Mean # juveniles
4
3
2
1
0
A
AB
B
C. diogenes
C. striatus
human
Mean # burrowed juveniles
4
3
2
1
0
A
B
B
C. diogenes
C. striatus
human
Mound treatment