DESCRIPTION OF BURROW STRUCTURE FOR FOUR CRAYFISH SPECIES (DECAPODA: ASTACOIDEA: CAMBARIDAE)

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ABSTRACT

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Crayfish use burrows for predator avoidance, desiccation prevention, foraging, and brood care. Based on their burrow ecology, species of crayfish can be categorized as tertiary, secondary, or primary burrowers. Primary burrowers are semi-terrestrial and highly dependent on burrows for survival. Secondary and tertiary burrowers have decreased burrow dependence and complexity. While most literature provides information about the function of burrows, there has been limited investigation into specifics of burrow structure. The purpose of this study is to describe burrow structure of four crayfish species: the primary burrowing species *Lacunicambarus diogenes* Girard, 1852 and *Creaserinus fodiens* Cottle, 1863, and the tertiary burrowing species *Faxonius rusticus* Girard, 1852 and *Faxonius propinquus* Girard, 1852. In the field, 17 crayfish burrows were filled with polyester resin. Cured burrow casts were excavated, and photogrammetry was used to recreate casts as 3-D models. Burrow depth, number and widths of openings, number and widths of chambers, and number of branches were recorded. Multiple factor analysis (MFA) was performed on burrow casts, with response variables separated into two groups: soil particle classification and burrow characteristics. In regard to variance in data, 33.1% was explained by dimension one (burrow size), and 20.9% was explained by dimension two (fine-grained sediment). On the MFA plot, 95% confidence interval ellipses for *F. rusticus* and *F. propinquus* overlapped, with both ellipses to the left of the vertical axis and below the horizontal axis. The *C. fodiens* ellipse was to the left of the vertical axis and above the horizontal axis. The *L. diogenes* ellipse was to the right of the vertical axis, and the mean was above the horizontal axis. Each species was also found to create a stereotyped burrow shape. This study contributes to the growing body of work on crayfish burrow structure, which has implications for future work on crayfish biology.

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INTRODUCTION

Burrows are the biogenic structures that result from organisms' active reworking, displacement, and compaction of unconsolidated sediments (Frey, 1973; Kristensen *et al.*, 2012; Dorgan, 2015). Burrows are constructed in terrestrial and aquatic environments by a large diversity of animals, such as earthworms (Quillin, 2000), ants (Robinson, 2014), tortoises (Smith *et al.*, 2005), and mice (Hamilton, 1938). For many species, burrow access is a prerequisite for life activities such as foraging, avoiding predators, and mating. For predatory dune-burrowing spiders, for example, burrows are important resources for accessing food (Lubin & Henschel, 1990). When being stalked by a predator, rats retreat to their burrows for safety (Blanchard & Blanchard, 1989). For myrmecophilous ants, such as *Acromyrmex* leaf-cutting ants, burrows (i.e., nests) are important community structures for colony functions such as brood care, including the cultivation of larval food sources (Lopes *et al.*, 2005). Among burrowing organisms, one taxon of animals that has a high reliance on burrows is the crustaceans.

Among crustaceans, burrow structure and use are diverse and well-documented (Atkinson & Eastman, 2015). For the desert-dwelling isopod *Hemilepistus reaumuri* Milne Edwards, 1840, burrows are important resources for family survival under threats of predation and desiccation (Coenen-Stass, 1984; Linsenmair, 1984; Linsenmair 2007; Atkinson & Eastman, 2015). In decapods, the sand fiddler crab *Leptuca pugilator* Bosc, 1802 constructs simple burrows that are used as refuges for protection or mating and egg incubation (Christy, 1982; Atkinson & Eastman, 2015). Adult American lobsters *Homarus americanus* Milne Edwards, 1837, make U-shaped burrows for courtship cohabitation (Botero & Atema, 1982; Karnofsky *et al.*, 1989; Bushmann & Atema, 1997). In these examples, species morphology, ecology, and lifestyle are very different, and these differences are reflected in burrow structure and use. These differences between burrows, however, can also be seen in more closely-related, morphologically similar species.

In thalassinidean shrimps, for example, species can create one of six different burrow architectures, which may be related to the feeding strategy of the species (Griffis & Suchanek, 1991). Deposit-feeding *Callianassa* Leach, 1814 species construct Y-shaped burrows with spiral shafts and large chambers (Griffis & Suchanek, 1991; Ziebis *et al.*, 1996). The complex structure of these burrows allows for the cultivation of microbial communities on the seagrass-lined burrow walls, providing a food source for residents (Abed-Navandi & Dworschak, 2005). Comparatively, *Axius serratus* Stimpson, 1852 construct deep burrows with multiple chambers, which are utilized for storing macrophytes and other food materials that individuals catch drifting near burrow openings (Pemberton *et al.*, 1976; Griffis & Suchanek, 1991). For filter-feeding *Upogebia* Leach, 1814 species, shallow, U-shaped burrows facilitate the one-way flow of water for capturing suspended food particles (Griffis & Suchanek, 1991; Nickell & Atkinson, 1995). Similar to the thalassinids, crayfishes are another group of decapods that are dependent on burrows for survival.

Of the over 600 globally documented crayfish species, all have the ability to burrow, regardless of geography or habitat (Hobbs, 1981; Berrill & Chenoweth, 1982; Crandall & De Grave, 2017). Although all crayfish burrow, burrow form and function are variable across species. The variation in species burrows is captured in a classification of crayfish based on burrow ecology. Hobbs (1942) and Hobbs (1981) proposed categorizing crayfish into tertiary, secondary, and primary burrowing species. Tertiary burrowing crayfish are species that live in open water, such as lakes, ponds, rivers, and streams (Hobbs & Hart, 1959; Hobbs, 1981). These species shelter under rocks and logs but are capable of constructing shallow, simple burrows within the water body. Tertiary burrowers rely on burrows for predator avoidance, desiccation prevention during droughts, and protection during estivation. Secondary burrowing crayfish do not live in open water, but instead spend the majority of their lives in their burrows (Hobbs & Hart, 1959; Hobbs, 1981). These species are found primarily in peatlands and clay habitats, and burrows may or may not be connected to the water table (Fitzpatrick, 1975; Hobbs, 1981). During the rainy season, secondary burrowers will leave their burrows and travel to nearby open water (Hobbs, 1981). Secondary burrows function primarily as protection from desiccation, but there are additional benefits of predator avoidance and food provision. Primary burrowing crayfish are semi-terrestrial species that inhabit clay substrates near floodplains, marshes, or swamps (Hobbs & Hart, 1959; Hobbs, 1981). These burrows do not connect to open water or the water table (Hobbs & Hart, 1959; Hobbs, 1981). Because primary burrows are not attached to open water or the water table, these burrows receive water from floodplains and surface runoff (Hasiotis & Mitchell, 1993). Primary burrowers spend the majority of their lives in their burrows, except while foraging or mating (Hobbs & Hart, 1959; Hobbs, 1981).

The differences in habitat and burrow ecology between species are also found in the structure of crayfish burrows. However, research addressing crayfish burrows has been limited by the difficulties presented in studying fossorial species. Many studies about crayfish burrowing have been dependent on field observations, with early investigations of burrow structure relying solely on the excavation (i.e., destruction) of burrows in the field (Tarr, 1884; Grow, 1981). More recently, crayfish burrows have been studied by creating casts of burrows to investigate their 3-dimensional structure (Growns & Richardson, 1988; Noro & Buckup, 2010; Atkinson & Eastman, 2015). This work aims to expand on the current literature of crayfish burrows through the investigation of burrow structure and substrate for two tertiary and two primary burrowing species.

METHODS

Study sites

Four study sites were selected based on presence of crayfish populations and burrows (Table 1; Dennis Albert, personal communication) with the aim of identifying as many species as possible for comparison. At each site, burrows were selected for study based on evidence of recent activity. Active burrows were identified by the presence of crayfish at burrowing openings or via a Shekar NTS100R digital endoscope camera (Shekar, China) in the burrow. When crayfish were not directly observable ($N = 7$), active burrows were identified by the presence of either a wet opening or chimney (for terrestrial burrows), or smoothed entrance walls (for both aquatic and terrestrial burrows). These burrow entrance characteristics are indicators of recent burrowing activity (Thoma & Armitage, 2008).

The four study sites were characterized based on burrow substrate. Bowling Green State University's Ecology Research Station (ERS) substrate is loam. Burrows located at ERS were terrestrial. Trail's End Bay (TEB) is characterized by clay and sand substrate on a coastal floodplain. Burrows were fully aquatic. Carp Lake River (CLR) substrate is a mixture of clay, sand, and cobble. Burrows were aquatic. Grapevine Point (GVP) is a sandy shoal with algal shelves. Burrows at GVP were aquatic.

Organism collection

Seventeen active burrows were selected for study. Live crayfish were extracted from eight burrows by hand ($N = 5$) or with yabby pumps made of PVC pipe ($N = 3$). Species, life stage, sex, and reproductive form as well as carapace length and width (cm) and chelae length and width (cm) were also measured. Crayfish that could not be extracted from burrows were cured in cast material (*N* = 5). For active burrows that did not have visible crayfish in the burrow or in the cured cast (*N* = 4), species was determined based on local population.

Cast creation

In the field, 15 ml methyl-ethyl ketone peroxide catalyst (MEK-P 925 Clear) (FiberGlass Coatings, Inc., St. Petersburg, FL, USA) was mixed with 0.95 L polyester boatyard resin (FiberGlass Coatings, Inc., St. Petersburg, FL, USA). Polyester resin was chosen as the casting material because the resin is able to cure in aquatic environments and has a greater density than water, which aids in displacement of water from the burrow (Rowden & Jones, 1995; Ziebis *et al.*, 1996; Rudnick *et al.*, 2005; Noro & Buckup, 2010). To minimize trapped air and water in the cured cast, a funnel was used to directly pour resin onto burrow openings' inner walls. Resin was mixed and poured into burrows in 0.95 l batches to allow for slow-pouring. Resin was poured until the level of liquid resin in the burrow was level with the substrate surrounding burrow openings. At GVP, burrows were in water approximately 1 m deep. For ease of pouring resin, 0.5 l of resin was mixed in empty 0.7 l sports-cap plastic bottles. Divers used snorkel gear and dive weights to identify active burrows, and resin was squirted into burrows from the plastic bottle. For all burrows, resin was left to cure for 48+ hours.

After cure, depth between the top of cast and ground surface was recorded (*N* = 6). All casts were excavated using garden trowels, garden spades, and weeder hand tools. Any roots present were cut from around the cast using a bear saw and pocket knives. Excavated casts were transported to the lab for removal of soil and debris with running water and a wire grill brush. To remove remaining particles, casts were placed in a crayfish housing tank so that crayfishes could graze on detritus and other organic tissue on the cast.

Soil characterization

After casts were completely excavated, soil samples were taken from the area immediately surrounding the terminal chamber. Collected soil samples were characterized by color and particle size. For color, a small pad of moist, non-glistening soil was held behind pages of Munsell Soil Book of Colors (X-Rite, M50215B) and matched to a color chip (Munsell Color). Soil samples were categorized by particle size by Hubbard particle classification using a standard soil sieve set (ASTM E11). Soil and water were added into the top-most sieve, and the set was shaken until particles settled. Percent of sample in each sieve was recorded (Wentworth, 1922).

Photogrammetry

To digitize burrow casts, 3-D models were created from photographs of casts, a process referred to as photogrammetry. Casts were photographed on a white turntable in front of a white poster board backdrop using a Samsung Galaxy S9 (Samsung Electronics, Suwon, South Korea) smartphone 12MP rear-facing camera set to “Auto” with the High Dynamic Range (HDR) setting. Casts were rotated, and 49 to 426 photos were taken for each cast. The number of photographs taken was dependent on burrow size and complexity. Images were imported to AgiSoft PhotoScanPro (version 1.4.4) and processed according to Dietrich (2015), excluding georeferencing steps. Final meshes of digitized casts were exported in .obj format.

Cast data collection

Three-dimensional cast meshes (file format .obj) were imported into MeshLab (version 2016.12) mesh processing software. The MeshLab tape measure tool was used to measure the size of a mesh feature corresponding to a feature of the burrow cast. Digital meshes were scaled to the size of the cast using MeshLab's "Transform: Scale, Normalize" function, where the axes are scaled by the ratio of the cast measurement to the digital mesh measurement. After scaling, MeshLab's "Compute Geometric Measures" tool was used to calculate mesh surface area (cm^2) and volume (cm^3).

Definitions of measured burrow characteristics can be found in Table 2. Burrow depth was measured by laying the burrow cast down on a flat surface and measuring the length from the top of burrow openings to the deepest point of the terminal chamber (Fig. 1). Widths of chambers, openings, and shafts were measured with calipers at the point with the widest diameter. Number of openings, tunnels, and chambers were counted.

Statistical analysis

A multiple factor analysis (MFA) was performed on all 17 burrows using the FactorMineR and factoextra functions in the statistical program R (Le *et al.*, 2008; Kassambara & Mundt, 2017; R Core Team, 2019). All quantitative data loaded in the principal component analysis (PCA) was Z-normalized using the mean (μ) and standard deviation (σ) of each value ($Z = (X - \mu)/\sigma$). Crayfish species was used as the supporting variable in the MFA. The response variables were separated into two groups: soil particle classification and burrow characteristics. The soil particle classification group consisted of seven factor variables describing the percent of the soil sample in each sediment sieve: 10 (particles > 2 mm), 18 (> 1 mm), 35 (> 500 μm), 60 (> 250 μm), 120 (> 125 μm), 230 (> 63 μm), and silt (< 63 μm). The burrow characteristics group included nine factor variables: volume, surface area, number of openings, openings average width, number of chambers, chambers average width, number of branches, shaft width, and depth.

RESULTS

Variance in data

From the MFA, dimensions one and two explained 54% of variance in data (Table 3, Table 4). Specifically, dimension one explained 33.1% (eigenvalue = 1.61), and dimension two explained 20.9% (eigenvalue = 1.01). The remaining four dimensions explained 46% of the variance, with no single dimension accounting for more than 15%.

Dimension one

Dimension one explained 33.1% of the variance in the data set (eigenvalue = 1.61, Table 3, Fig. 2). The burrow characteristics group had the largest contribution to dimension one (51.34%). Within the burrow characteristics, the surface area variable contributed the most (10.10%) with high quality of representation ($\cos^2 = 0.74$). Volume, number of chambers, and average chamber width also contributed 9.73%, 9.06%, and 7.87% respectively to the dimension with high quality of representation (volume $\cos^2 = 0.71$, number of chambers $\cos^2 = 0.66$, average chamber width $\cos^2 = 0.58$). The soil particle classification group also contributed to dimension one (48.65%). Soils of particle sizes 10 and 18 each contributed 21.59% and 10.28% respectively to dimension one within the soil particle classification factor. Of these soil particle classification factor variables, particle size 10 had high quality of representation ($\cos^2 = 0.85$). Particle size 18 had moderate quality of representation ($\cos^2 = 0.40$). Due to the high $\cos^2$ values of burrow characteristics variables of surface area, volume, number of chambers, and average chamber width, dimension one can be described as the burrow size dimension.

Dimension two

Dimension two explained 20.9% of the variance in the data (eigenvalue = 1.01, Table 4, Fig. 3). The soil particle classification group was the greatest contributor to dimension two

(71.48%). Soils of particle sizes 230, 60, silt, and 120 contributed to this dimension (27.51%, 16.76%, 13.75%, and 6.67% respectively). Particle size 230 had a high quality of representation in dimension two ($\cos^2 = 0.68$), while the other contributing particle sizes had moderate quality of representation in the dimension (60 $\cos^2 = 0.41$, silt $\cos^2 = 0.34$, 120 $\cos^2 = 0.16$). The burrow characteristics variables group also contributed to the dimension (28.51%). The burrow depth and average opening width variables contributed 12.52% and 4.96% respectively) with a low quality of representation (depth $\cos^2 = 0.58$, average opening width $\cos^2 = 0.03$). Due to the high quality and large contribution of the soil particle classification variables of size 230, 60, silt, and 120 particles, dimension two can be described as a fine-grained sediment axis.

MFA plot

The 95% confidence ellipses for the tertiary burrowing species, *F. rusticus* and *F. propinquus*, show significant overlap (Fig. 4). The ellipses for both of these species are located to the left of the vertical axis, with the ellipse for *F. rusticus* bisected by the horizontal axis. The means for both of these species are located below the horizontal axis. The position of the means and ellipses for *F. rusticus* and *F. propinquus* suggest these tertiary burrowing species have similar responses to dimension one (burrow size) and dimension two (fine-grained sediment).

Ellipses for both primary burrowing species *C. fodiens* and *L. diogenes* do not overlap with any other ellipses (Fig. 4). The ellipse for *L. diogenes* is to the right of the vertical axis. The ellipse also bisects the horizontal axis, with the mean just above the horizontal axis. The position of the *L. diogenes* ellipse suggests that this species is different from other species in regard to dimension one (burrow size). The ellipse and mean for *C. fodiens* are to the left of the vertical axis and above the horizontal axis. This position suggests that differences between *C. fodiens* and the other species are related to dimension one (burrow size) and dimension two (fine-grained soil).

Species burrow structure

Primary and tertiary burrowing species have different burrow structures. Burrows constructed by the primary burrowing species *L. diogenes* are deeper than those of the tertiary burrowing species *F. propinquus*. Burrows made by *C. fodiens*, the other primary burrowing species examined, are deeper than burrows of both the tertiary burrowing species, *F. propinquus* and *F. rusticus*. Furthermore, *L. diogenes* burrows are larger (in total surface area, total volume, and chamber width) and have more chambers than all other species.

Burrows constructed by the tertiary burrowing species *F. rusticus* were characterized by a simple tube-shaped burrow oriented horizontally. Burrows from the second tertiary burrowing species examined, *F. propinquus*, were also shallow and oriented horizontally. Unlike *F. rusticus*, burrows by *F. propinquus* have horizontally extending branches with two or more openings.

In contrast to the more simplified tertiary burrows, primary burrowers had more vertically oriented burrows. Burrows by *C. fodiens* had a stereotyped “J” shape with one or two openings. Burrows by *C. fodiens* can be described as “J-shaped” because of their simple, tube-like structure that extends vertically into the substrate with a terminal chamber that appears to double back toward the surface. Of all the species examined in this study, *L. diogenes* burrows were the largest and most structurally varied. Burrows made by *L. diogenes* were horizontally or vertically oriented, with multiple openings, branches, and chambers.

DISCUSSION

The position of each species on the MFA plot illustrates the differences in burrow structure between species, particularly as the distributions relate to the burrower categories of primary and tertiary (Fig. 4). The relatively larger size of *L. diogenes* burrows is illustrated by the position of this species' 95% confidence ellipse, which is located to the right of the vertical (burrow size) axis. All other species are located to the left of the vertical axis, meaning that *C. fodiens*, *F. rusticus*, and *F. propinquus* have burrows that are of a different size than those made by *L. diogenes*. From the burrow casts and 3-D models, *L. diogenes* burrows are bigger than burrows constructed by the other three species.

The MFA plot also shows that species may be influenced by the presence of fine-grained sediment (Fig. 4). The ellipse of *C. fodiens* is located above the horizontal (fine-grained sediment) axis, which is different from the ellipses of all other species. In relation to the *C. fodiens* ellipse, the *F. propinquus* ellipse is below the horizontal axis, indicating that this species burrows in areas that have a different proportion of fine-grained sediment. *F. rusticus*' ellipse overlaps the ellipse of *F. propinquus* but is bisected by the horizontal axis. This location of the *F. rusticus* ellipse suggests that these two species burrow in similarly fine-grained sediments. The bisection of the *L. diogenes* ellipse by the horizontal axis illustrates that this species burrows in sediments of varied particle sizes.

The burrow structure and sediment align with the burrowing nomenclature of each of these species. The two tertiary species have similar burrow structures to each other as represented by the MFA plot (Fig. 4). Yet, the two primary burrowers occupy different spatial positions within this analysis (Fig. 4). In *F. rusticus*, populations are primarily found in lakes and flowing streams with gravel and cobble substrate (Jezerinac, 1982; Corey, 1988). In their rocky habitat, *F. rusticus* do not rely on self-made burrows as the sole source of physical protection from predators and competitors. Often, individuals find shelter under rocks and logs (Langlois, 1935). Individuals frequently leave burrows and shelters to forage on detritus, macrophytes, and small invertebrates (Willman *et al.*, 1994; Bergman & Moore, 2003; Klocker & Strayer, 2004). *F. rusticus* also exhibit low shelter fidelity, which is beneficial for species that frequently leave to forage and may need quick access to a shelter for protection from predators and conspecifics (Kershner & Lodge, 1995; Martin & Moore, 2008). With this low shelter fidelity, *F. rusticus* is an aggressive species that engages in frequent agonistic interactions over access to resources, including shelters (Capelli & Munjal, 1982; Hazlett *et al.*, 1992; Bergman & Moore, 2003; Martin & Moore, 2007). As such, there is frequent turnover of shelter residence and ownership (Martin & Moore, 2007; Klar & Crowley, 2012). In this system, where there is frequent turnover in burrow and shelter ownership, the creation of simple burrows by *F. rusticus* is an energetically efficient strategy.

Similar to *F. rusticus*, *F. propinquus* is found in lakes or streams with gravel, cobble or sand substrate (Van Deventer, 1937; Bovbjerg, 1952; Stein & Magnuson, 1976; Jezerinac, 1982). While both species share aspects of foraging ecology, *F. propinquus* displays more sheltering behavior and less aggression when threatened by the presence of predators or aggressive heterospecifics (Engle, 1926; Stein & Magnuson, 1976; Hazlett *et al.*, 1992). Burrows by *F. propinquus* have horizontally extending branches with two or more openings, which may be important for this species' sheltering behaviors. These findings are consistent with early field observations that *F. propinquus* individuals create shallow, superficial burrows (Harris, 1903; Engle, 1926; Van Deventer, 1937; Bovbjerg, 1952).

As a primary burrowing species, individuals of *F. fodiens* spend much of their lives in their burrows and rely on their subterranean habitat for food, including plant roots and seeds as well as small macroinvertebrates and larvae (Bovbjerg, 1952). For this semi-terrestrial species, the burrow's terminal J-shaped chamber is well-suited to collecting and holding surface runoff and preventing desiccation, particularly for a species that inhabits seasonally flooded areas (Norrocky, 1991; Loughman *et al.*, 2012). Burrows for this species are also important for reproduction as they serve as refuges for mating and brood care, as is evidenced by the cohabitation of reproductive males and juveniles with reproductive and ovigerous females (Norrocky, 1991; Trépanier & Dunham, 1999).

The variation in *L. diogenes* burrow structure is consistent with observations by Grow (1981) and Girard (1852). Similar to the other primary burrowing species *C. fodiens*, *L. diogenes* is found in seasonal floodplains and relies on burrows for protection from desiccation and predators as well as for access to subterranean food sources (Tarr, 1884; Helms *et al.*, 2013). Burrow chambers for this species are thought to be used for food storage or as retreats during dry periods (Grow & Merchant, 1980; Grow, 1981). Similar to *C. fodiens*, *L. diogenes* burrows are important for reproduction and brood care (Girard, 1852). For *L. diogenes*, juveniles actively participate in burrow maintenance and practice burrowing in the safe burrow environment (Helms *et al.*, 2013). During this period, juveniles also depend on burrows as they forage on macroinvertebrates and organic matter in the substrate (Helms *et al.*, 2013). This high dependence on burrows for both juvenile and adult *L. diogenes* may account for the differences in burrow size and number of chambers between species.

This work investigated burrow structure and habitat in four species of crayfish. Both tertiary burrowing species (*F. rusticus* and *F. propinquus*) create simple, shallow burrows. The primary burrowing species *C. fodiens* constructs deep, j-shaped burrows. Finally, the primary burrowing species *L. diogenes* constructs burrows that are larger than all other species. These differences in burrow structure between tertiary and primary species coincide with differences in ecology and social behavior, as burrows serve as valued resources for protection, foraging, and reproduction. Continuing the investigation of crayfish burrow structure will allow for greater understanding of these species' life histories, sociality, and ecology.

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APPENDIX A – TABLES

Table 1. Coordinates, substrate types, species, and number of burrows from each field site. Soil classification based on particle size distribution in sediment sieves and Wentworth (1922). ERS, Ecological Research Station; TEB, Trail's End Bay; CLR, Carp Lake River; GVP, Grapevine Point.

Site	**Coordinates**	**Soil classification**	**Species found (burrows)**
ERS	-83.62776247, 41.39587068	loam	*L. diogenes* (1), *C. fodiens* (3)
TEB	-84.80699684, 45.74503179	clay, sand	*L. diogenes* (3)
CLR	-84.82565897, 45.73121821	clay, sand, cobble	*F. propinquus* (3), *F. rusticus* (4)
GVP	-84.68095647, 45.56939619	sand	*F. propinquus* (3)

Table 2. Definitions of measured burrow characteristics.

Characteristic	**Definition**
Volume	Total volume of mesh of digitized burrow cast (cm^3) as calculated by MeshLab 3-D triangular mesh processing software
Surface area	Total surface area of mesh of digitized burrow cast (cm^2), including area across openings, as calculated using MeshLab 3-D triangular mesh processing software
Depth	Vertical depth of entire burrow from entrance to terminal chamber of burrow cast, as measured perpendicular to surface level; includes any top depth of burrow that was not filled with casting material
Openings	Where shaft of burrow terminates at the substrate surface; may be used by crayfish as an entrance or as a point for sediment removal
Shaft	Main tunnel of burrow, attached to one or more openings; usually oriented vertically; may have branches
Branch	Burrow tunnels off-shooting from shaft; may be oriented vertically or horizontally
Chamber	Area of tunnel widened for crayfish brood care, feeding, or protection; may or may not be located on the terminal end of a tunnel

Table 3. Summarized contributions of dimension one and factors to the multiple factor analysis (MFA).

Dimension 1
Eigenvalue = 1.61; Contribution = 33.1%

Factor	**Contribution (%)**	**Quality ($\cos^2$)**
Burrow characteristics	51.34	
Volume	9.73	0.71
Surface area	10.10	0.74
Number of openings	5.60	0.41
Openings average width	0.78	0.06
Number of chambers	9.06	0.66
Chambers average width	7.87	0.58
Number of branches	3.76	0.28
Shaft width	3.76	0.28
Depth	0.68	0.05
Soil particle sizes	48.65	
10 (> 2 mm)	21.59	0.85
18 (> 1 mm)	10.28	0.40
35 (> 500 µm)	0.63	0.02
60 (> 250 µm)	7.59	0.30
120 (> 125 µm)	4.03	0.16
230 (> 63 µm)	2.20	0.09
Silt (< 63 µm)	2.33	0.09

Table 4. Summarized contributions of dimension two and factors to the multiple factor analysis (MFA).

Dimension 2
Eigenvalue = 1.01; Contribution = 20.9%

Factor	Contribution (%)	Quality ($\cos^2$)
Burrow characteristics	28.51	
Volume	2.13	0.10
Surface area	1.63	0.08
Number of openings	0.05	0.01
Openings average width	4.96	0.23
Number of chambers	0.15	0.01
Chambers average width	0.56	0.03
Number of branches	2.71	0.13
Shaft width	3.80	0.18
Depth	12.52	0.58
Soil particle sizes	71.48	
10 (> 2 mm)	0.30	0.01
18 (> 1 mm)	4.77	0.12
35 (> 500 µm)	1.72	0.04
60 (> 250 µm)	16.76	0.41
120 (> 125 µm)	6.67	0.16
230 (> 63 µm)	27.51	0.68
Silt (< 63 µm)	13.75	0.34

APPENDIX B – FIGURES

Figure 1. Model of burrow made by *Lacunicambarus diogenes* at ERS, indicating burrow characteristics. D, depth; S, shaft; O, openings; B, branches; C, chambers.

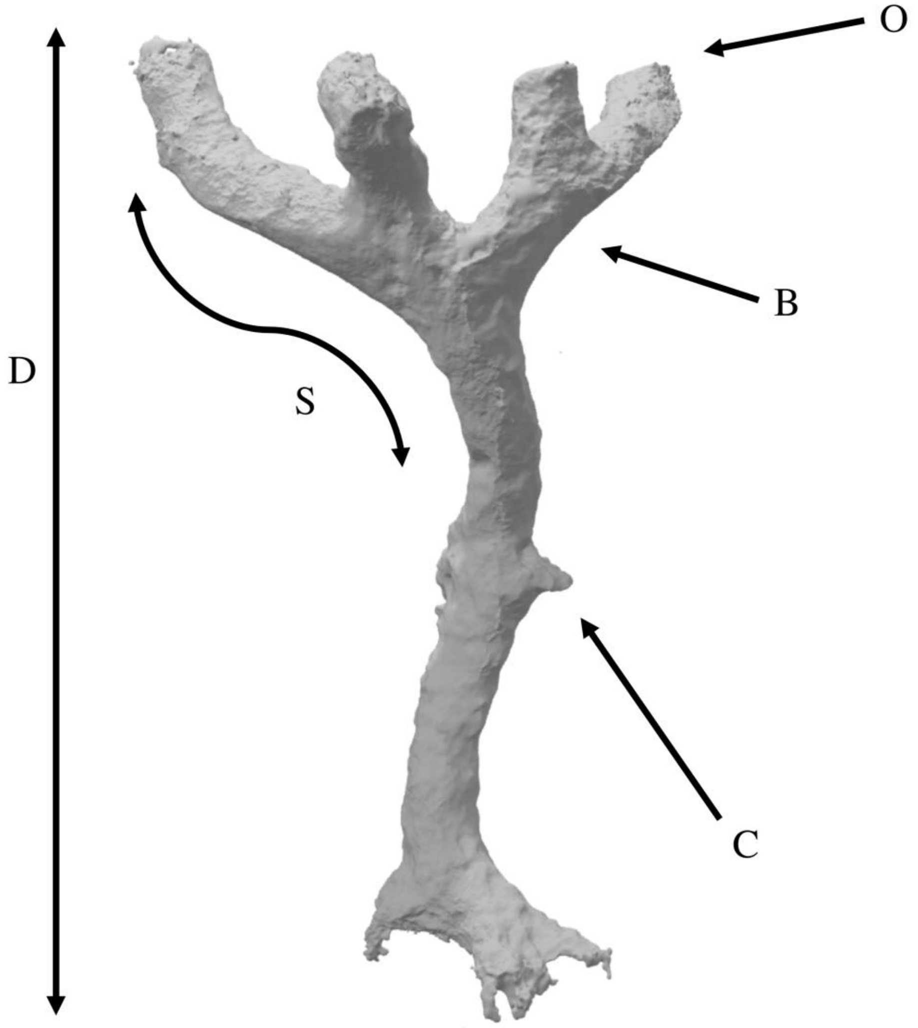

Figure 2. Contribution of variables to dimension one. Each bar shows the percent contribution of a variable to dimension one. Gray bars are burrow characteristics variables. Black bars are soil particle classification variables. The dashed line indicates the expected average contribution of each of the six variables if all variables were equally contributing to the dimension (6.25%). Bars that exceed the dashed line are important contributors to dimension one.

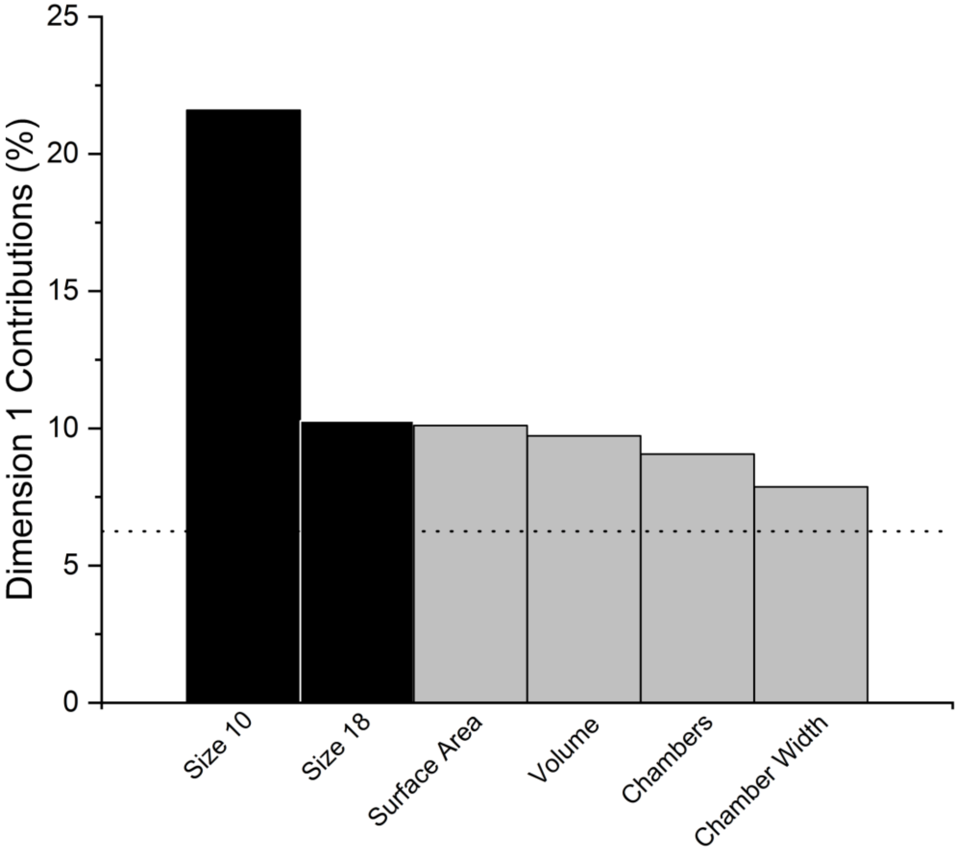

Figure 3. Contribution of variables to dimension two. Each bar shows the percent contribution of a variable to dimension two. Gray bars are burrow characteristics variables. Black bars are soil particle classification variables. The dashed line indicates the expected average contribution of each of the six variables if all variables were equally contributing to the dimension (6.25%). Bars that exceed the dashed line are important contributors to dimension two.

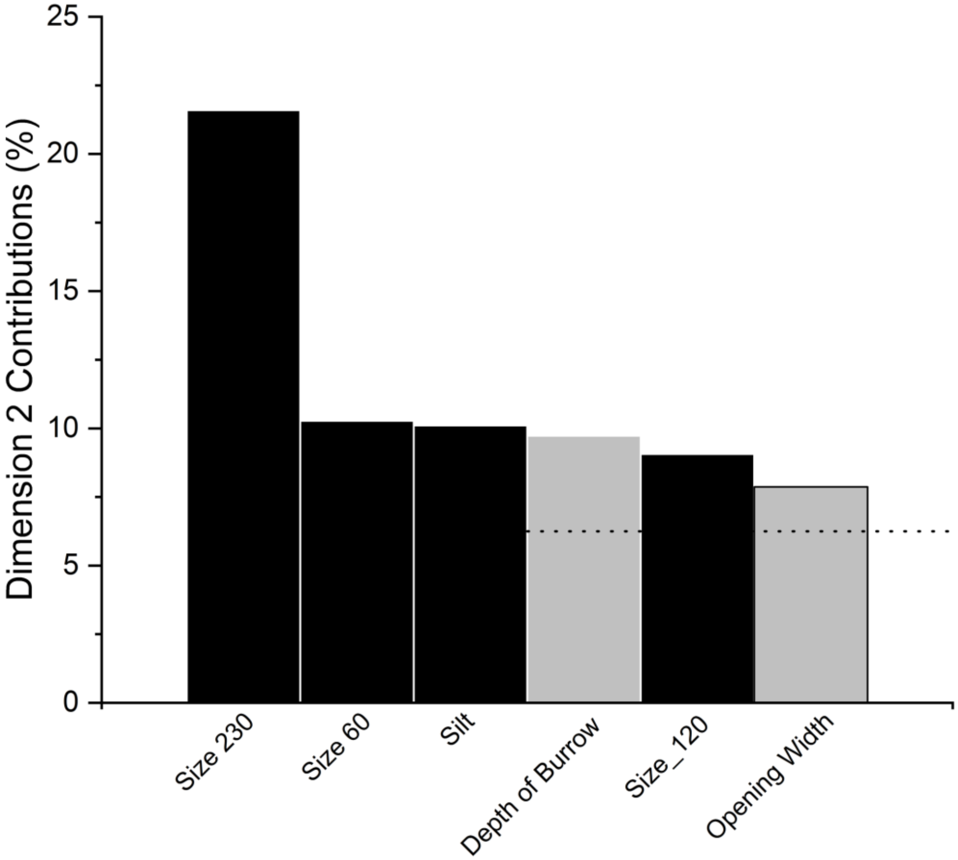

Figure 4. MFA factor map. Individual burrows are plotted as dots along the two main dimensions of the MFA. The open gray squares represent the mean coordinates for tertiary burrowing species. The closed black squares represent the mean coordinates for primary burrowing species. The black and gray outlines are 95% confidence ellipses centered on the mean of each treatment. Gray ellipses represent tertiary burrowing species. Black ellipses represent primary burrowing species. Dimension one summarizes the burrow characteristics of surface area, volume, number of chambers, and average chamber width. Percent of soil particle sizes 10 and 18 are also included in dimension one. Dimension two summarizes the soil particle classification for particle sizes 230, 60, silt, and 120. Burrow depth and average opening width are also included in dimension two.