Analysis and description of burrow structure in four species of freshwater crayfishes (Decapoda: Astacoidea: Cambaridae) using photogrammetry to recreate casts as 3D models

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ABSTRACT

Freshwater crayfishes use burrows for predator avoidance, desiccation prevention, foraging, and brood care. Based on their burrow ecology, crayfishes can be categorized as tertiary, secondary, or primary burrowers, with primary burrowers being semi-terrestrial and highly dependent on burrows for survival; secondary and tertiary burrowers have decreased burrow dependence and complexity. There has been limited investigation into the specifics of burrow structure. We investigated the factors that influence the structure of crayfish burrows including habitat structure and crayfish through the use of burrow casting and photogrammetry to recreate casts as 3D models. Examined species include the primary burrowing Lacunicambarus aff. diogenes Girard, 1852 and Creaserinus fodiens (Cottle, 1863), and the tertiary burrowing Faxonius rusticus (Girard, 1852) and Faxonius propinquus (Girard, 1852). Crayfish burrows (N = 17) were filled with polyester resin and cured burrow casts were excavated, and photogrammetry was used to recreate casts as 3D models. Burrow depth, number and widths of openings, number and widths of chambers, and number of ancillary tunnels were recorded. Differences in burrow features were analyzed by field site/habitat and crayfish species using stepwise linear regression, MANOVAs, and multiple factor analysis (MFA). From stepwise linear regression, “species” was a better predictor than “habitat of burrow structure.” Burrows constructed by L. aff. diogenes were significantly larger and have more chambers than burrows constructed by the other species examined; burrows of C. fodiens were significantly deeper than those of tertiary burrowers. Each species also created a stereotyped burrow shape. The morphometric differences between burrows of primary and tertiary species illustrate the relevance of these informal classifications. Differences between primary and tertiary species may be related to the species social behavior and ecology.

Key Words: 3D modeling, crayfish burrows, Creaserinus, Faxonius, Lacunicambarus, photogrammetry

INTRODUCTION

Burrows are the biogenic structures that result from the active reworking, displacement, and compaction of unconsolidated sediments (Frey, 1973; Kristensen et al., 2012; Dorgan, 2015) in terrestrial and aquatic environments by a large diversity of animals. Burrow access is a prerequisite for activities such as foraging, avoiding predators, and mating by many species (Hansell, 1993). Burrow structure and use are diverse and well-documented among crustaceans (Atkinson & Eastman, 2015). Burrows are important resources for survival under threats of predation and desiccation (Coenen-Stass, 1984; Linsenmair, 1984, 2007; Atkinson & Eastman, 2015) in the desert-dwelling isopod Hemilepistus reaumuri Milne Edwards, 1840. Among decapods, fiddler crabs such as Leptuca pugilator Bosc, 1802 constructs simple burrows that are used as refuges for protection or mating and egg incubation (Christy, 1902; Atkinson & Eastman, 2015). Adult American lobsters...
*Homarus americanus* Milne Edwards, 1837 make U-shaped burrows for courtship cohabitation (Botero & Atema, 1982; Karnolsky 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.

Thalassinidean shrimps, for example, can create one of six different burrow architectures, which may be related to the feeding strategy of the species (Griffis & Suchanek, 1991). Species of the deposit-feeding *Callianassa Leach, 1814* 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).

All of the more than 600 globally documented species of freshwater crayfishes have the ability to burrow, regardless of geography or habitat (Hobbs, 1981; Berrill & Chenoweth, 1982; Grandall & De Grave, 2017). Although all crayfish burrow, the form and function of burrows vary across species. Such variation is captured in a classification of crayfishes based on burrow ecology. Hobbs (1942, 1981) proposed categorizing crayfishes into tertiary, secondary, and primary burrowing species. Tertiary burrowing crayfishes 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 crayfishes 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 can be connected to the water table (Fitzpatrick, 1975; Hobbs, 1981). Secondary burrowers will leave their burrows and travel to nearby open water during the rainy season (Hobbs, 1981). Secondary burrows function primarily as protection from desiccation, but there are additional benefits of predator avoidance and food provision. Primary burrowing crayfishes are semi-terrestrial, inhabiting clay substrates near floodplains, marshes, or swamps (Hobbs & Hart, 1959; Hobbs, 1981). These burrows do not connect to open water or the water table. Primary burrows are not attached to open water or the water table and 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 present in the structure of crayfish burrows. Research addressing crayfish burrows, however, 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). The recreation of burrows and the digitization has also been hampered by poor modeling compounds, remote and isolated field conditions that limit excavation. Given that a large number of animals, particularly crayfishes, spend a significant portion of their time below ground, the need to perform a more detailed analysis on burrow structure and the environmental factors that limit or contribute to burrow structures is needed.

Crayfish burrows have more recently been studied by creating casts of burrows to investigate their tri-dimensional structure (Grows & Richardson, 1988; Welch et al., 2008; Noto & Buckley, 2010; Atkinson & Eastman, 2015). In spite of the increasing precision of burrow descriptions, there has been no work investigating the factors that influence burrow structure. Our work aimed to investigate the factors that influence burrow structure, specifically elucidating the role that habitat/substrate and the type of species play in burrow structure through the examination of burrows from tertiary and primary burrowing species at different sites. We hypothesized that tertiary and primary burrowers have significantly different burrow structures and that primary burrowers have deeper and more complex burrows. A second hypothesis is that soil structure (composition) has the largest influence on burrow structure and that burrows found in clay or silt environments are more complex than those in sandy sites.

### MATERIALS AND METHODS

#### Study sites

Four sites were selected based on the presence of crayfish populations and burrows (Supplementary material Table S1; Dennis Albert, personal communication) with the aim of comparing as many species as possible. At each site, burrows were selected for study based on evidence of recent activity. Active burrows were identified by the presence of crayfishes at burrowing openings or via a Shekar NTS100R digital endoscope camera (Shekar, Zhengzhou, China) in the burrow. When crayfishes 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 entrance characteristics are indicators of recent burrowing activity (Thoma & Armitage, 2000).

The four study sites were characterized based on burrow substrate. The substrate at Bowling Green State University’s Ecology Research Station, western end of Lake Erie, OH, USA, is loam and burrows were terrestrial. Burrows were fully aquatic at Trail’s End Bay on Lake Michigan (the tip of the lower peninsula of Michigan, USA), which is characterized by clay and sand substrate on a coastal floodplain. Burrows were also aquatic at Carp Lake River with a substrate of a mixture of clay, sand, and cobble, and at Grapevine Point on Douglas Lake, Cheboygan county, MI, USA, a sandy shoal with algal shelves.

#### Crayfish species

Four species of crayfishes were collected across the different habitats. The species include the primary burrowing *Lacunicambarus aff. diogenes* (Girard, 1852) and *Craweisurus fodiens* (Cottle, 1863), and the tertiary burrowing *Paxiurus rusticus* (Girard, 1832) and *Paxiurus proximins* (Girard, 1852; Glon et al., 2018). A note on the species classification: *L. diogenes* we will refer to the *Lacunicambarus* species as *Lacunicambarus aff. diogenes* (Mael Glon, personal communication).

#### Collection of specimens

Seventeen active burrows were selected for study. Live crayfishes 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 state as well as carapace length and width (cm) and chela length and width (cm) were also measured. Crayfishes that could not be extracted from burrows were cured in cast material (N = 5). We identified species based on the local population in the case of active burrows that did not have visible individuals in burrows or in the cured casts (N = 4).

#### Creation of casts

Casts were made in the field by mixing 15 ml methyl-ethyl ketone peroxide catalyst (MEK-P 925 Clear) (FiberGlass Coatings, St. Petersburg, FL, USA) with 0.95 l polyester boatyard resin (FiberGlass Coatings). 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 the displacement of water from burrows (Rowden & Jones, 1995; Ziebis et al., 1996; Rudnick et al., 2005; Noro & Buckup, 2010). A funnel was used to directly pour resin onto the inner walls of the burrow openings to minimize trapped air and water in the cured cast. 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 of the surrounding burrow openings. Burrows were in water approximately 1 m deep at GVP. 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 plastic bottles. The resin was left to cure for +48 hours in all burrows.

The depth between the top of the cast and the ground surface was recorded after cure (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 bare saw and pocket knives. Excavated casts were transported to the laboratory at the University of Michigan Biological Station 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**

We took soil samples from the surrounding the terminal chamber. Collected 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, Amazon.com) 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 following a standard particle size analysis (Pansu & Gautheryrou, 2007) using sieves (Wentworth, 1922).

**Photogrammetry**

3D models were created from photographs of casts by means of 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**

3D cast meshes (file format.obj) were imported into MeshLab (version 2016.12, www.meshlab.net) 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 spatial dimensions are scaled by the ratio of the cast measurement to the digital mesh measurement. We used MeshLab’s Compute Geometric Measures tool after scaling to calculate mesh surface area (cm²) and volume (cm³).

Definitions of the measured burrow characteristics are given in Supplementary material Table S2. Burrow depth was measured by laying the burrow cast on a flat surface and measuring the length from the top of the burrow openings to the deepest point of the terminal chamber (Fig 1). Widths of chambers, openings, and shafts were measured with calipers at the points with the widest diameter. The number of openings, tunnels, and chambers were recorded.

![Figure 1. Model of burrow made by Lucanuambars aff. digenes at Ecological Research Station, Bowling Green State University, indicating burrow characteristics. D, depth; S, shaft; O, openings; A, ancillary tunnels; C, chambers.](image)

**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 - μ)/σ). “Crayfish species” was used as the supporting variable. 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 (> 0.5 mm), 60 (> 0.25 mm), 120 (> 0.125 mm), 230 (> 0.63 mm), and silt (< 0.63 mm). 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 ancillary tunnels, shaft width, and depth.

To provide a more quantitative analysis, all data analysis was performed with MacOSX R statistical software “stats” package (version 3.4.2) (R Core Team, 2019). A 4 × 4 factorial Multivariate Analysis of Variance (MANOVA) was run, where the first factor was study site (ERS, TEB, CLR, GVP) and the second factor was crayfish species (C. fodiens, F. propinquus, F. rusticus, L. aff. diogenes), to determine differences of site and species on number and widths of burrow openings, branches, chambers, as well as burrow depth, volume, and surface area.

Response variable ~ study site + crayfish species + site * species

Significance level for MANOVA was set to 0.05. Tukey honestly significant difference (HSD) post hoc analysis was used to determine differences within statistically significant factors.

Burrow characteristics were also analyzed by stepwise regression R function “step” to obtain Akaike Information Criterion (AIC) values. As with MANOVA analysis, the R “stats” package was used (version 3.4.2) (R Core Team, 2019). Predicting factors were study site and crayfish species. Step function was performed
in the forward direction with null model predictor set as the integer “1.” Significance level was set as 0.05. The predicting factors with the lowest AIC value were recorded for each burrow characteristic (Table 1).

**RESULTS**

**Methods improvement**

The advancement of material science (resin types) certainly improved the rigidity of the burrows and made excavation easier than what appears in previous literature. One of the most significant difficulties of excavation occurs during the digging process. In many cases, the burrows were under water, roots, and in murky water. So, the use of visual cues to guide excavation was impossible. The feel of the solidified burrow therefore often determined when and where digging occurred. The use of small implements, garden gloves with ‘claws’, and small saws helped removed the burrows intact. Finally, the most exciting part of this work is the development of photogrammetry techniques to digitize burrow structures. This method provides access to precision data about burrow structures for quantitative analysis. Finally, these digitized files allow the burrows to be printed using 3D printers. We have tested printing materials that dissolve in water which could open up the possibility of using real burrow structures in a repeated fashion to test many different ideas around burrow ecology.

**Variance in data**

Dimensions one and two from the MFA explained 54% of variance in the data (Supplementary material Tables S3, S4). 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; Fig 2 and Supplementary material Table S3). 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 average chamber width also contributed 9.73%, and average chamber width also contributed 9.73%, and 9.06%, and 7.87%, respectively, to the dimension with high quality of representation (cos² = 0.74). Volume, number of chambers, and average chamber width also contributed 9.73%, 9.06%, and 7.87%, respectively, to dimension two within 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 cos² = 0.53, average opening width cos² = 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.

**Dimension two**

Dimension two explained 20.9% of the variance in the data (eigenvalue = 1.01; Fig 3 and Supplementary material Table S4). 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%, 15.75%, and 6.67% respectively). Particle size 230 had a high quality of representation in dimension two (cos² = 0.68), while the other contributing particle sizes had moderate quality of representation in the dimension (60 cos² = 0.41, silt cos² = 0.34, 120 cos² = 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 cos² = 0.53, average opening width cos² = 0.03). Due to the high quality and large contribution of the soil particle classification dimensions 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

![Figure 2](image-url)
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 (depth of burrow and opening width); black bars soil particle classification variables (soil sieve sizes 60, 120 230, and silt). 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.

similar responses to dimension one (burrow size) and dimension two (fine-grained sediment).

Ellipses for both primary burrowing species C. fodiens and L. aff. diogenes do not overlap with any other ellipses (Fig. 4). The ellipse for L. aff. 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. aff. diogenes ellipse suggests that this species is different from other species examined 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).

Burrow structure

Primary and tertiary burrowing species have different burrow structures. Burrows constructed by the primary burrowing species L. aff. diogenes (Supplementary material Figs. S5, S6) are deeper than those of the tertiary burrowing species E. propinquus (Supplementary material Figs. S7, S8). Burrows made by C. fodiens (Supplementary material Figs. S9, S10), the other primary burrowing species examined, are deeper than burrows of both the tertiary burrowing species, E. propinquus and F. rusticus (Supplementary material Figs. S11, S12). Furthermore, L. aff. diogenes burrows are larger (in total surface area, total volume, and chamber width) and have more chambers than all other species examined.

Burrows constructed by the tertiary burrowing species F. rusticus were characterized by a simple tube-shaped burrow oriented horizontally (Supplementary material Figs. S11, S12). Burrows from the second tertiary burrowing species examined, E. propinquus, were also shallow and oriented horizontally. Unlike F. rusticus, burrows by E. propinquus have horizontally extending ancillary tunnels with two or more openings (Supplementary material Figs. S7, S8).

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 (Supplementary material Figs. S9, S10). 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, L. aff. diogenes burrows were the largest and most structurally varied. Burrows made by this species were horizontally or vertically oriented, with multiple openings, ancillary tunnels, and chambers.

Quantitative analysis

Burrow structure by species. Burrow structure was significantly correlated with species (Supplementary material Table S1) ($F_{(3, 13, 0.05)} = 4.01, P < 0.001$). Specifically, there were differences in the number of chambers ($F_{(3, 13, 0.05)} = 6.85, P = 0.01$), volume ($F_{(3, 13, 0.05)} = 16.24, P < 0.001$), surface area ($F_{(3, 13, 0.05)} = 16.37, P < 0.001$), depth ($F_{(3, 13, 0.05)} = 12.13, P < 0.001$), shaft widths ($F_{(3, 13, 0.05)} = 6.73, P = 0.01$), openings widths ($F_{(3, 13, 0.05)} = 4.87, P < 0.03$), and chamber widths ($F_{(3, 13, 0.05)} = 5.27, P = 0.01$). Burrows constructed by the primary burrowing L. aff. diogenes were larger and had more chambers than both tertiary burrowing species (E. propinquus and F. rusticus) and from the other primary burrowing species (C. fodiens).

Burrows constructed by L. aff. diogenes had more chambers than burrows of all other species examined (C. fodiens $P < 0.01$, E. propinquus $P < 0.01$, F. rusticus $P < 0.01$). Burrows of L. aff. diogenes were also larger than those of the other species regarding burrow volume (C. fodiens $P < 0.03$, E. propinquus $P < 0.01$, F. rusticus $P < 0.01$), surface area ($P = 0.02$, burrows $P < 0.001$, chamber widths ($P < 0.001$), and widths of burrow chambers ($P = 0.02$, $P < 0.03$), chamber widths ($F_{(3, 13, 0.05)} = 5.27, P = 0.01$). Burrows of L. diogenes were significantly deeper than in E. propinquus ($P < 0.03$). The other primary burrowing species, C. fodiens, had significantly deeper burrows than both tertiary burrowing species (E. propinquus $P < 0.001$, F. rusticus $P < 0.01$).

Shaft width of the tertiary burrowing species E. propinquus was significantly smaller than those of L. aff. diogenes ($P < 0.01$) and F. rusticus ($P = 0.02$). Widths of the openings of F. rusticus burrows were only significantly different from burrows constructed by C. fodiens ($P = 0.02$).

Burrow structure by site. Site was also shown to have a significant influence on burrow structure ($F_{(3, 13, 0.05)} = 4.63, P < 0.001$). As with species, there are significant differences between number of burrow chambers ($F_{(3, 13, 0.05)} = 6.37, P < 0.01$), burrow volume ($F_{(3, 13, 0.05)} = 9.44, P > 0.001$), surface area ($F_{(3, 13, 0.05)} = 10.29, P < 0.001$), depth ($F_{(3, 13, 0.05)} = 40.81, P < 0.001$), and shaft widths ($F_{(3, 13, 0.05)} = 8.27, P > 0.001$).

Burrows at the Ecological Research Station (ERS) were significantly deeper than burrows from all other sites (Carp Lake River (CLR) $P < 0.001$, Grapevine Point (GVP) $P < 0.001$, Trail’s End Bay (TEB) $P < 0.001$).

Burrows at TEB had significantly greater volume and surface area than those at CLR (volume $P > 0.001$, surface area $P > 0.001$) and GVP (volume $P > 0.001$, surface area $P > 0.001$). In addition to the greater volume and surface area, TEB burrows were also significantly deeper than those at GVP ($P = 0.04$). Burrows at TEB also had significantly more chambers than burrows located at CLR ($P > 0.001$).

Burrows at GVP had significantly smaller shaft widths than burrows from all other sites (CLR $P = 0.01$, ERS $P = 0.04$, TEB $P > 0.001$).

Stepwise regression

The stepwise regression (Table 3) revealed that the type of species alone is the most accurate predictor of the number of burrow openings, number of burrow chambers, burrow volume, surface area, and chambers widths, whereas the study site is the most accurate predictor of burrow depth. The species plus the study site
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 (*F. propinquus* and *F. rusticus*). The closed black squares represent the mean coordinates for primary burrowing species (*C. fodiens* and *L. aff. diogenes*). The black and gray outlines are 95% confidence ellipses centered on the mean of each treatment. Gray ellipses represent tertiary burrowing species; black ellipses 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.
predict burrow opening widths, and the study site plus species predict shaft widths. Neither species nor the study site accurately predict the number of branches.

**DISCUSSION**

We found significant differences in burrow structure and that such structure is more closely related to the crayfish species than to the study site (i.e., habitat). Primary and tertiary burrowing species have significantly different burrow structures. Burrows constructed by the primary burrowing species *L. aff. diogenes* (Supplementary material Figs. S5, S6) are deeper than those of the tertiary burrowing species *F. propinquus* (Supplementary material Figs. S7, S8). Burrows made by *C. fodiens* (Supplementary material Figs. S9, S10), the other primary burrowing species examined, are deeper than burrows of both the tertiary burrowing species, *F. propinquus* and *E. rutilus* (Supplementary material Figs. S11, S12). Furthermore, burrows of *L. aff. diogenes* are larger (in total surface area, total volume, and chamber width) and have more chambers than all other species examined. The morphometric differences between burrows of primary and tertiary burrowers illustrate the ecological relevance of this informal classification. In addition to the statistical analysis run on individual variables of burrows, through examination of complete burrow casts, we establish that each species constructs stereotyped burrow forms, which could be related to the microhabitat of these species.

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

Burrow structure and sediment align with the burrowing nomenclature of each of these species. The two tertiary species have similar burrow structures as shown by the MFA plot (Fig. 4). Yet, the two primary burrowers occupy different spatial positions within this analysis (Fig. 4). Populations of *F. rutilus* are primarily found in lakes and flowing streams with gravel and cobble substrate (Jezernic, 1982; Corey, 1988). In their rocky habitat, *F. rutilus* does not rely on self-made burrows as the sole source of physical protection from predators and competitors, with individuals often finding shelter under rocks and logs (Langlois, 1935). Individuals frequently leave burrows and shelters to forage on detritus, macrophytes, and small invertebrates (Willman, 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). *Faxonius rutilus* also exhibits 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). A consequence of this low-shelter fidelity, individuals of *F. rutilus* are aggressive and engage in frequent agonistic interactions over access to resources, including shelters (Capelli & Munjal, 1982; Hazlett et al., 1992; Bergman & Moore, 2003; Martin & Moore, 2007). The building of simple burrows by *F. rutilus* is an energetically efficient strategy that results from the frequent turnover of shelter residence and ownership (Martin & Moore, 2007; Klar & Crowley, 2012).

As in *F. rutilus*, *F. propinquus* is found in lakes or streams with gravel, cobble or sand substrates (Van Deventer, 1937; Bavnborg, 1952; Stein & Magnuson, 1976; Jezernic, 1982). While both species share a similar foraging ecology, *F. propinquus* displays a stronger sheltering behavior and a reduced aggressiveness when threatened by the presence of predators or aggressive heterospecifics (Engle, 1926; Stein & Magnuson, 1976; Hazlett et al., 1992). Burrows by *F. propinquus* have horizontal ancillary tunnels with two or more openings (Supplementary Figs. S07–S08), which may be important for its sheltering behavior. These findings are consistent with early field observations that individuals of *F. propinquus* create shallow, superficial burrows (Harris, 1905; Engle, 1926; Van Deventer, 1937; Bavnborg, 1952).

As a primary burrowing species, individuals of *F. rutilus* 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 terminal J-shaped chamber of its burrows is well-suited for collecting and holding surface runoff and preventing desiccation, particularly for a species that inhabits seasonally flooded areas (Norrocky, 1991; Loughman et al., 2012). Burrows of this species are also important for reproduction as they serve as refuges for mating and brood care, as evidenced by the co-habituation of reproductive males and juveniles with reproductive and ovigerous females (Norrocky, 1991; Trépanier & Dunham, 1999). Yet, the ovigerous females of some species, *C. fodiens*, do leave burrows and use temporary surface waters, so the importance of burrows, even for ovigerous females, may be only temporary (Loughman & Simon, 2011).

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

The 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. Particular aspects of burrow structure (ancillary tunnels, size, and depth) are certainly due to the burrowing species themselves, but environment influences, such as soil composition, are also important. An outcome of this work is the development of a method for the analysis of underground structures in an effort to understand the ecological significance of these structures. Continuing the future investigations on the crayfish burrow structure of crayfishes should allow for greater understanding of the life histories, social behavior, and ecology of these ecologically significant species.

**SUPPLEMENTARY MATERIAL**

Supplementary material is available at *Journal of Crustacean Biology* online.

Supplementary material Table S1. Coordinates, substrate types, species, and number of burrows sampled from each field site.

Supplementary material Table S2. Definitions of measured burrow characteristics.

Supplementary material Table S3. Summarized contributions of dimensions and factors to the multiple factor analysis (MFA).
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Supplementary material Table S4. Summarized contributions of dimension two and factors to the multiple factor analysis (MFA).

Supplementary material Figs. S5–S12. 3D burrow models.

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BURROW STRUCTURE OF FOUR CRAYFISHES


