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Imaging bioturbation in supratidal carbonates: non-invasive field techniques enhance neoichnological and zoogeomorphological research, San Salvador, The Bahamas

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ABSTRACT - A case study in unconsolidated carbonates on San Salvador Island, The Bahamas, utilized high-frequency (800 MHz) georadar imaging to augment existing methodologies (burrow counts and measurements, casting) in brachyuran bioturbation research (Ocypode quadrata *and* Gecarcinus lateralis), *and as part of a new dataset characterizing blue land crab* (Cardisoma guanhumi) *burrows. Non-invasive techniques such as ground-penetrating radar (GPR) can complement traditional field surveys aimed at quantifying mesoscale bioturbation in modern settings. These methods can establish diagnostic features for tracemaker identification and refine existing ichnofacies models. Drone-mounted aerial coverage provided the first high-resolution images of the micro-topography and large burrow openings of* Cardisoma *in supratidal muddy sands. Measurements of 20 burrows (minimum length, entrance diameter, and spoil mound size) were complemented by endoscopic camera observations (burrow fill, large bioglyphs, and occupants). Extensive 2D transects and quasi-3D georadar grids not only reveal characteristic subsurface interfaces (open vs. filled burrow, water table, saltwater), but also serve as an archive of bulk in situ sedimentary characteristics of the bioturbated substrate. Signal resolution in dry carbonate sand (~ 4 cm) was sufficient to differentiate and measure known burrow structures. Our study demonstrates that co-located and georeferenced aerial, geophysical, and ground-based databases will allow rapid and effective assessment of the spatial distribution and gross geometry of comparable biogenic structures in a variety of environments and substrates.*

RIASSUNTO - [Rappresentare la bioturbazione per immagini in carbonati sopratidali: tecniche non invasive da campo migliorano la ricerca neoicnologica e zoogeomorfologica, San Salvador, Isole Bahamas] - *Le tecniche geofisiche non invasive e di telerilevamento stanno sempre più integrando le indagini neoicnologiche tradizionali. Questi metodi possono stabilire caratteristiche diagnostiche subsuperficiali per identificare i produttori delle tracce, caratterizzare eterogeneità di strato su larga scala e affinare i modelli esistenti di icnofacies. In questo studio sono state impiegate tecniche di imaging basate su georadar ad alta frequenza (800 MHz) sui carbonati non consolidati dell'isola di San Salvador, Bahamas, al fine di migliorare il risultato delle metodologie tradizionali (conteggi e misurazione di tane, calchi) nello studio della bioturbazione di brachiuri* (Ocypode quadrata, Gecarcinus lateralis, Cardisoma guanhumi).

Un drone è stato impiegato per rilevare sabbie fangose sopratidali, restituendo le prime fotografie ad alta risoluzione della microtopografia e delle grandi aperture delle tane di Cardisoma. *Le misurazioni di 20 tane (lunghezza minima, diametro dell'entrata e dimensioni del mound) sono state integrate con osservazioni endoscopiche (riempimento della tana, bioglifi di grande dimensioni e occupanti). I transetti 2D ed il georadar (quasi-3D) rivelano non solo le caratteristiche interfacce subsuperficiali (tane aperte o chiuse, tavola d'acqua, acqua salata) ma forniscono anche un archivio in situ delle caratteristiche del substrato bioturbato. La risoluzione del segnale in sabbia carbonatica secca (~ 4 cm) è sufficiente a distinguere e misurare le tane conosciute, permettendo di riconoscere anche alcune caratteristiche architetturali (ad es. tunnel, camere) e calcolare il diametro delle tane. L'obiettivo futuro è quello di estendere questi approcci a substrati litificati. Il nostro studio dimostra l'utilità di database con misurazioni georeferenziate (aeree, geofisiche e a terra), riferite alla stessa area. Infatti, essi consentiranno una valutazione rapida ed efficace della distribuzione spaziale e della geometria di strutture biogeniche comparabili a quelle presentate in questo studio, anche in ambienti e substrati diversi.*

INTRODUCTION

Traditional methods of neoichnological field and laboratory research should be continuously refined to offer effective means of characterizing modern traces, thereby allowing for accurate interpretation of similar features in the geological record. Accurate observations in modern settings, linking tracemakers to certain environmental and habitat ranges, enhance the utility of ancient traces to paleo-environmental interpretation (Curran & Martin, 2003; Rodriguez-Tovar et al., 2014; Seike & Curran, 2014). For burrowing organisms, field measurements (burrow counts, length, depth, inclination), casting, and trenching have provided datasets of burrow morphology and distribution (Seike & Curran, 2014). However, the geometry and spatial distribution of some subsurface biogenic structures (e.g., decapod burrows in supratidal carbonates), their patterns as linked systems, and the geomorphic changes they generate, are poorly known or are addressed at limited scales. This hampers

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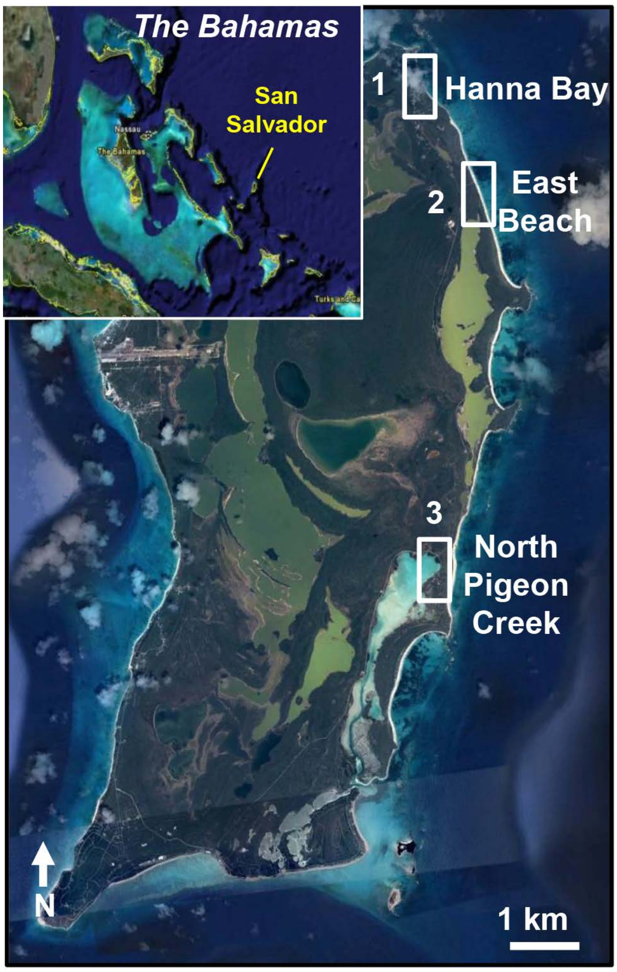

Fig. 1 - Location of the study area (2006 Terrametrics images of the Bahamian Archipelago and San Salvador Island). See text for descriptions of the three field study sites along the east coast of the island.

recognition of tracemakers and, importantly, obscures the impact of burrow systems on primary bedding, from slight disturbance to thorough reworking. A strategy to extrapolate from point-source measurements and labor-intensive casting to habitat/reservoir scale is through the use of geophysical and remote-sensing technology. These methods complement traditional datasets, providing spatial models that accurately reflect a broader scale of the subsurface and subaerial effects of burrowing. In this fieldwork, a combination of high-frequency ground-penetrating radar (GPR) and drone-mounted high-definition photography enhanced understanding of mesoscale bioturbation structures such as decapod burrows. Additionally, these techniques had the advantage of being non-invasive (e.g., drone) or minimally invasive (e.g., GPR, borescope cameras). For example, subsurface probes, such as a simple video/camera borescope, quickly collected burrow data (inclination, depth, gross shape, commensal organisms) without invasive and labor-intensive casting.

Researchers have utilized these techniques to complement traditional fieldwork in various disciplines, including archaeology, ecology, and forensics (Hirano et al., 2009; Conyers, 2015; Widodo et al., 2016). A growing number of studies has employed georadar imaging to identify animal burrows as part of conservation studies or infrastructure analysis (Di Prinzio et al., 2010; Chlaib et al., 2014; Jayawickreme et al., 2014; Swinbourne et al., 2015). Utilizing field geophysical techniques to characterize bioturbation is a relatively new application in ichnology (Stott, 1996; Buynevich et al., 2009; Buynevich, 2011; Buynevich et al., 2014). These methods, when used as a comprehensive assessment of bioturbation intensity, have implications for achieving accurate palaeoenvironmental interpretation.

The subsurface architecture of large terrestrial (supratidal) burrows produced primarily by decapod crustaceans has not been addressed from a neoichnological point of view, especially in carbonate settings (Curran & White, 1991). Because carbonate platforms hold approximately 50% of the world's hydrocarbons, extensive research into its marine ichnology has received the bulk of attention to better understand the ichnofabric (bioturbation intensity) effect on reservoir properties (Knaust et al., 2012). To date there is a lack of neoichnological and ichnological studies of ecologically important carbonate supratidal biotopes. In addition, trace fossil content in all supratidal facies has been described as "generally devoid of burrows" (Gingras & MacEachern, 2012) while Desjardins et al. (2012, p. 535) state that there is "… a remarkable dearth of (ichnological) studies in ancient (supratidal) deposits", resulting in an under-developed ichnological classification in these facies.

The goal of this paper is to present an integrated approach for characterizing decapod bioturbation in modern supratidal carbonates using three species from San Salvador Island, The Bahamas (Fig. 1). Non-invasive field techniques, combined with ground-truthed datasets, are used to assess their value as tools for neoichnological analysis (Fig. 2). This includes: 1) rapid assessment of the vertical and lateral extent of biogenic structures; 2) characterization of burrow type and morphology, and 3) assessment of subsurface disturbances and subaerial sediment transfer.

STUDY SITE AND TRACEMAKERS

San Salvador Island, the outermost in the Bahamian archipelago (Fig. 1), was selected as an ideal field location due to its long history of intensive and thorough geological and ecological research, and particularly its pioneering carbonate ichnological research (Curran & White, 1991; Curran & Williams, 1997; Martin, 2006; Curran, 2007). There are many other factors that make this small tropical island a natural laboratory for our study: 1) relatively simple habitat constraints of the three studied decapods; 2) existing datasets describing burrow attributes; 3) the observable zoogeomorphological effects of the tracemakers, and 4) the homogeneous carbonate substrate (mostly skeletal sand) that serves as a background for anomalies associated with biogenic activity.

The dominant agents of bioturbation in marginal-marine carbonate substrates are crustaceans that occupy adjacent intertidal to supratidal zones (Curran & Martin, 2003; Seike & Curran, 2014). In particular, three brachyuran decapods (land crabs) inhabit three separate

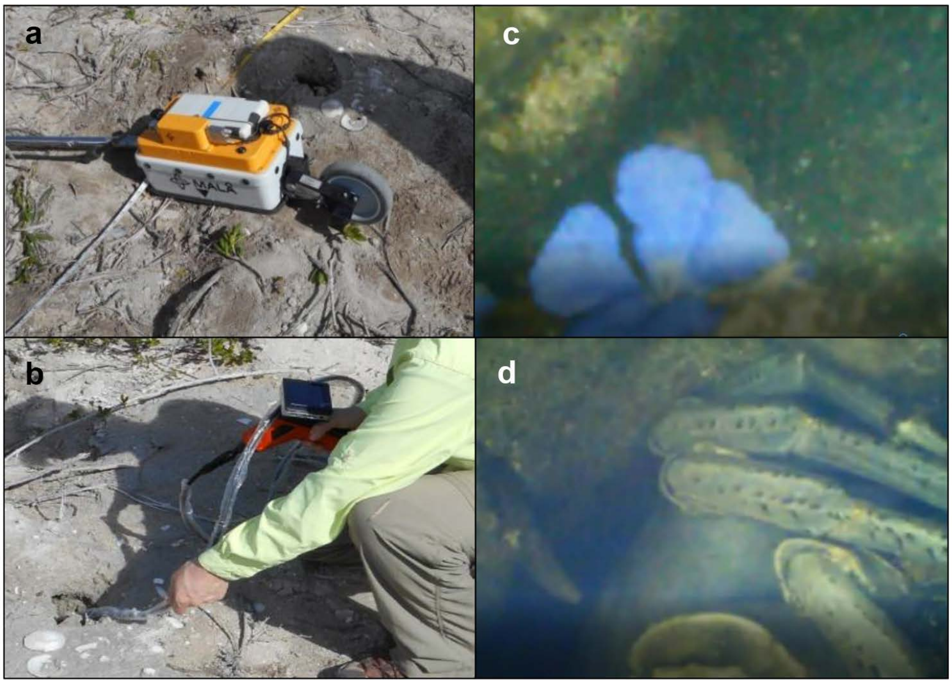

Fig. 2 - Field photos of (a) 800 MHz MALÅ GPR and (b) borescope (both taken at Site 3). Borescope images: c) periwinkle flower in a blackback crab burrow (Site 1) and d) blue land crab deep in tree roots (Site 3).

biotopes within this transitional zone, with little to no habitat overlap. The blackback land crab (*Gecarcinus lateralis* Freminville, 1835) occupies the upper vegetated biotope dominated by dunes. Its ubiquitous burrows were only recently studied in detail, with several examples of Holocene, subfossil remains of chelae and burrows discovered to date (Locatelli, 2013; Seike & Curran, 2014). Their unbranched tunnels are variably shaped and gently inclined (~ 30°).

The ghost crab (*Ocypode quadrata* Fabricius, 1787) is geographically wide-ranging and, in Bahamian sandy carbonate environments, this species inhabits the narrow intertidal beach/unvegetated dune environment. It is known for its steeply inclined (~ 60°) J- and Y- shaped shafts (some > 1 m long; Seike & Curran, 2014). *Psilonichnus upsilon* (Fursich,1981), the holotype for such ghost crab burrows, occurs in Quaternary deposits on San Salvador and forms the basis for the *Psilonichnus* ichnofacies (Frey et al., 1984). Morphological differences between *G. lateralis* and *O. quadrata* burrows were highlighted with the recent discovery on San Salvador of poorly lithified *G. lateralis* burrows (Seike & Curran, 2014).

The blue land crab (*Cardisoma guanhumi* Latreille, 1825), the largest of the three decapods, is confined to the mangroves and tidal creeks of the supratidal zone, directly abutting the habitat of *G. lateralis. Cardisoma guanhumi* excavates wide, inclined tunnels to the water table, moving sediment and altering the landscape (Wolcott, 1988). Therefore, it meets the definition of a landscape engineer (Kinlaw & Grasmueck, 2012) in tropical supratidal environments due to its numerous, extensive surficial (spoil mounds) and subsurface (burrow galleries) structures. To date, only a handful of biological studies have focused on this habitat (Herreid & Gifford, 1963; Semple & Albrecht, 2015) and only Shinn (1968) has briefly addressed *Cardisoma* burrow morphology from a geological perspective.

To characterize and distinguish the spatial arrangement and subsurface burrow patterns of the three decapods, we used an integrated approach of non-invasive imaging followed by intensive ground-truthing at specific sites along the eastern side of San Salvador Island (Fig. 1). Hanna Bay site (Site 1; see Fig. 1) is a classic Bahamian strata type with a variety of fossil and modern traces. Our study focused on a 2.5 x 2.5 m vegetated backdune area, where a series of parallel geophysical surveys were conducted to investigate high density *G. lateralis* burrows. Site 2 (East Beach) includes both a shore-normal dune transect (*G. lateralis* georadar imaging) and an upper berm *O. quadrata* habitat (imaging and casting). Site 3 (North Pigeon Creek) is home to both *G. lateralis* and *C. guanhumi*, with the latter being the focus of drone surveys, geophysical and endoscope imaging, and casting. Below is a description of each imaging method and selected examples.

NON-INVASIVE TECHNIQUES

Small Unmanned Aerial Vehicles (SUAVs) or Drones

In the initial phase of this field investigation, low-altitude (40-50 m) drone-mounted camera surveys were collected using a Phantom 3 Advanced with 12 megapixels, f/2.8 lens and a 94-degree field of view to get high-resolution images of Hanna Bay and North Pigeon Creek (Sites 1 and 3; Fig. 3). The team, led by researchers from the Smith College Spatial Analysis Lab, programmed an autonomous flight plan that generated an orthomosaic image with a 2-cm ground sampling distance.

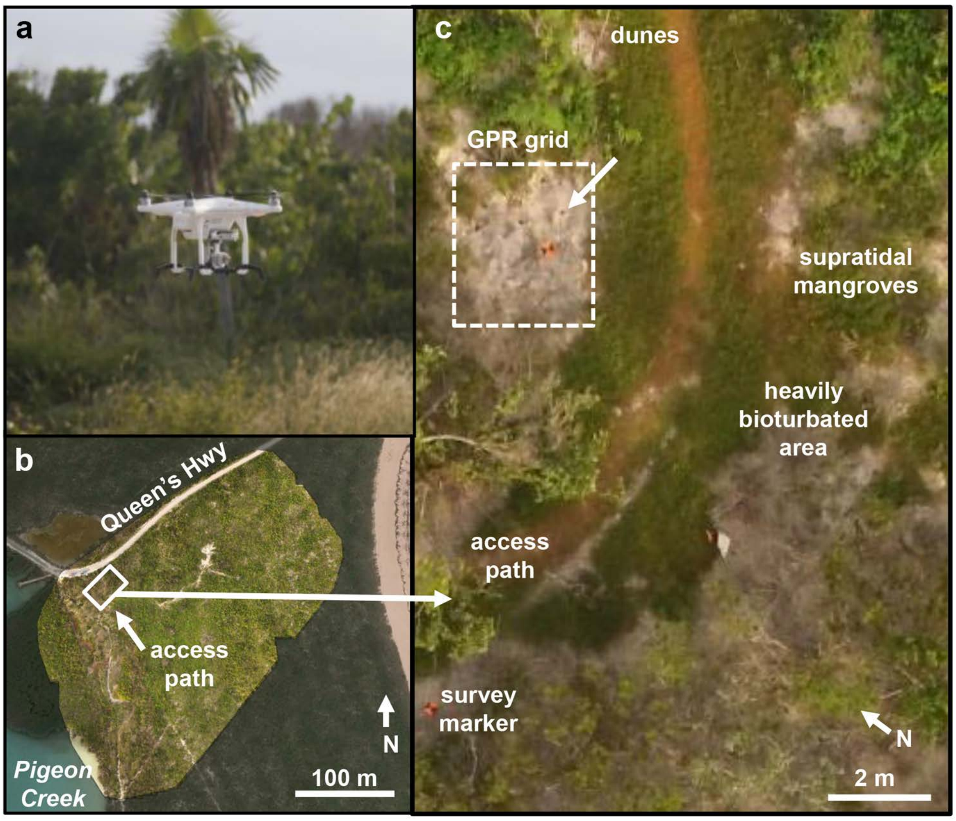

Fig. 3 - Examples of multiple methods at North Pigeon Creek (Site 3). a) Small Unmanned Aerial Vehicle (SUAV). This drone took rapid continuous photos, which give (b) a high-resolution mosaic. c) Individual *C. guanhumi* burrows (arrows) can be seen from 40 m altitude.

Coordinates were measured with a mapping-grade GPS unit for five ground control markers that were distributed through the survey area. The ground control enabled the orthomosaic image to be georeferenced to within 1 m of its true location.

Results - On San Salvador, drone flights identified clearings and burrow clusters in variably vegetated environments that were then utilized for geophysical ground surveys. In aerial images along north Pigeon Creek (Site 3), *C. guanhumi* burrows (average opening diameter: 8 cm) and microtopography (average excavated mound height: 12 cm) are visible from 40 m height in clearings of the mangrove forest (Fig. 3c). An orthomosaic and Digital Surface Model were created from individual geo-tagged drone images using ArcMap. Due to successful flights and data acquisition, these maps and derivative digital surface models were used to measure microtopographic elevation changes in areas of intense blue crab bioturbation, permitting remote measurements of sediment volumes moved by these landscape engineers.

Remarks - Smaller clustered burrows of *G. lateralis* are visible on larger-scale images, with dense vegetation being the key limiting factor. Isolated *O. quadrata* burrow openings are too small (< 4 cm) for drone imaging at this altitude, although flight altitude can be optimized for detecting these smaller structures on open beaches and washover fans. When collected by drones, aerial imagery by photographic means is often higher resolution than satellite images (i.e., those viewed in Google Earth), allowing recognition and measurement of subtle surface features such as burrows (Perlmutter et al., 2016). In addition, this technique is significantly more cost effective than airborne LIDAR in areas of minimal vegetation cover.

Borescope Camera

An Explorer PremiumTM wireless inspection camera with a 3.5" LCD recordable monitor and a 2-m flexible optical fiber cable (endoscope) was utilized in the field to measure minimum burrow length, to assess gross burrow geometry, and to capture video and photo (640 x 480 pixels, AVI format stills) images of burrow interiors (Fig. 2). This inexpensive and portable instrument allowed rapid in situ examination of undisturbed burrow sculpture and content (vegetation, sediment, burrowers and commensal organisms) at a resolution not achievable by geophysical methods and with minimal invasiveness compared to traditional field techniques (Semple & Albrecht, 2015).

Results - At Sites 1 and 3, a small number of *G. lateralis* and *O. quadrata* burrows was scoped to test the viability of the method to acquire data typically gathered by casting. The endoscope was then utilized to complement ongoing development of a new dataset for *C. guanhumi* burrows, providing minimum length, inclination, and camera observations of interiors.

Remarks - Difficulty in maneuvering the scope through tight bends, as well as debris obscuring the image, were the primary issues with obtaining clear pictures of the entire burrow. Despite this, organics (flowers, seeds, roots) and commensal organisms were seen, and the cable accurately captured the gross shape and minimum length of the main tunnels. This technique has promise as a viable field tool for subsurface neoichnology, but due to the above limitations, requires operator practice to ensure optimal results.

Near-surface Geophysics: Ground-Penetrating Radar (GPR)

In unsaturated and freshwater-dominated media, high-frequency (500-1200 MHz) georadar surveys provide high-resolution images of the shallow subsurface, making it a practical geophysical tool for the analysis of burrow attributes and enclosing matrix. As with all geophysical techniques, the proper frequency is required to successfully balance the requirements of a GPR survey, with a tradeoff between penetration depth and feature resolution of images. High-frequency GPR limits depth of penetration, but allows for clearer resolution of small features in the near subsurface (Jol & Bristow, 2003). Decapod burrows on San Salvador are ideal for these mesoscale neoichnological GPR studies, with diameters (> 3-4 cm) that can be differentiated in images and depths (0.2-1.0 m) within the range of high-frequency penetration in carbonates (Buynevich et al., 2014). In addition, the carbonate substrate provides a homogenous background that allows for a clear signal response from biogenic structures.

GPR TECHNIQUE - The GPR antenna (Fig. 2a) transmits high-frequency electromagnetic (EM) waves into the shallow subsurface, with densely spaced pulses providing a continuous image. EM waves reflect off objects or other interfaces as a response to contrasting dielectric properties of the substrate and targets, are captured by a receiver, and presented as a two-dimensional image in real time (Jol & Bristow, 2003). Subsurface boundary changes, from bedding scale to point sources, are displayed as high-amplitude reflectors of the EM wave (anomalies) due to differences from the surrounding substrate in texture, lithology, density, porosity and water content. Depending on the trajectory of the survey line relative to the target, the response will be a discontinuous reflection (e.g., longitudinal burrow section) or a hyperbolic diffraction that represents a point source (e.g., a clast or a cross-section of a burrow; the hyperbola apex is the actual object depth). Two-way travel time of the EM signal is converted to depth using signal velocity, which is obtained in situ or by using empirical values from analogous substrates. For our study sites velocity was obtained by measuring the return time from electromagnetic signal scatter off burrow structures to the receiver (hyperbola fitting), and varied slightly between subenvironments (~ 9-11 cm/ns). Since signal velocity varies as a function of innate substrate properties, this variation in velocity between sediment types can be a crucial factor in distinguishing different environments.

In the case of decapod bioturbation, substrate changes captured by the radar can exist between the matrix and burrow fill or void space (Kopcznski et al., 2015). The increase in the EM wave velocity as it moves from sediment to air through a void (empty burrow) causes the reflected waveform to show reverse polarity (positive to negative peak amplitudes) relative to the initial ground wave. In addition, truncation can be seen in the radargram at some points where bioturbation interrupts the background bedding. These changes in the radargram, when observed against the background matrix, provide

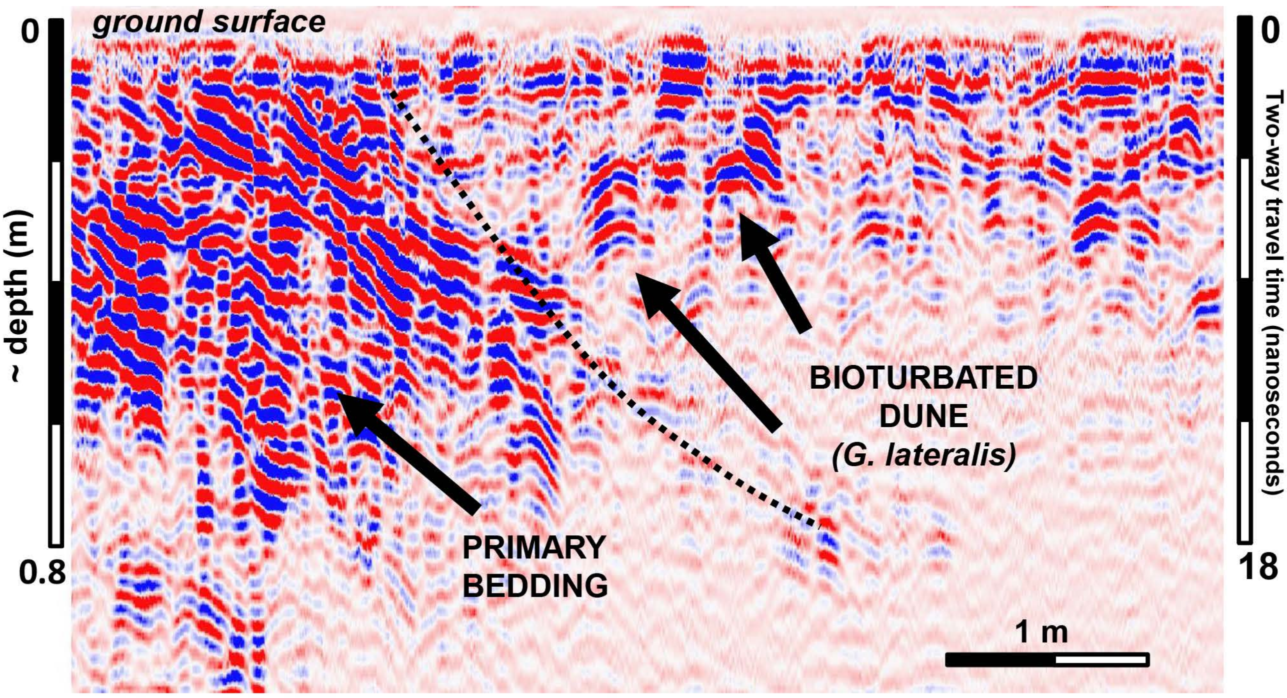

Fig. 4 - Dune transect at East Beach (Site 2): 2D georadar image shows an example of disturbance of primary bedding by a dense occurrence of *G. lateralis* burrows (seen as point source hyperbolics in the radar image) on a vegetated dune. In contrast, the dune bedding on the left is largely undisturbed.

data about gross burrow morphology as well as lateral patterns and extensive bedding disruption caused by closely spaced burrow assemblages.

Field surveys - Two- and three-dimensional GPR surveys were conducted at three primary field sites (East Beach, Hanna Bay, and North Pigeon Creek). These sites encompass all three decapod habitats, ensuring a substantial collection of each burrow type. More than 1.5 km of georadar surveys were collected using a MALÅ Geoscience radar (Fig. 2a) with a monostatic 800 MHz antenna. Survey lines ranged from 2-250 min length, with trace spacing of two mm and a range window optimized for penetration and resolution (200-512 samples). In addition, closely spaced (7-14 cm spacing, 2.5 x 2.5 m) grid surveys were collected for each burrow type. Depending on the spacing of the line surveys within the grid, these can be post-processed to give either true or pseudo-3D images using advanced software. For *G. lateralis* and *C. guanhumi*, these grids were established in areas of dense burrow occurrence and reflect their typical lateral arrangements. *Ocypode quadrata* burrows can be spatially proximal, but never form dense clusters. In this case, single burrow structures were imaged. The results of 3D visualization are beyond the scope of this paper. Most 2D surveys were designed to maximize imaging of tunnel structures, and to capture a large sample of both open and partially collapsed burrows (Fig. 4). Orientation of the GPR unit relative to burrows was recorded for accurate designation of both transverse and longitudinal orientation in the radar images.

Radar processing - The raw radar images were post-processed in 2D using MATGPR v. 3.5 and Radexplorer v. 7, and in 3D using GPR Slice. To improve image quality and clarity, post-processing steps were prioritized to maximize the clarity of near-surface (< 1 m) features and minimize the strong initial ground-air wave, the first wave emitted from the transmitter that is immediately captured by the receiver before subsequent EM waves can enter the ground. This initial wave gives a powerful reflection, which can impede recognition of very near surface disturbances and features such as burrow entrances. For standard protocol of post-processing steps (gain functions, filtering, etc.) see Cassidy (2009). In this study, bandpass filters and depth-dependent amplitude functions were optimized based on the size and attitude of biogenic structures. No topographic correction was necessary for short sections containing burrows. Near-surface anomalies were then cross-referenced with field notes to distinguish bioturbation structures from physical and other biogenic structures (roots, clasts).

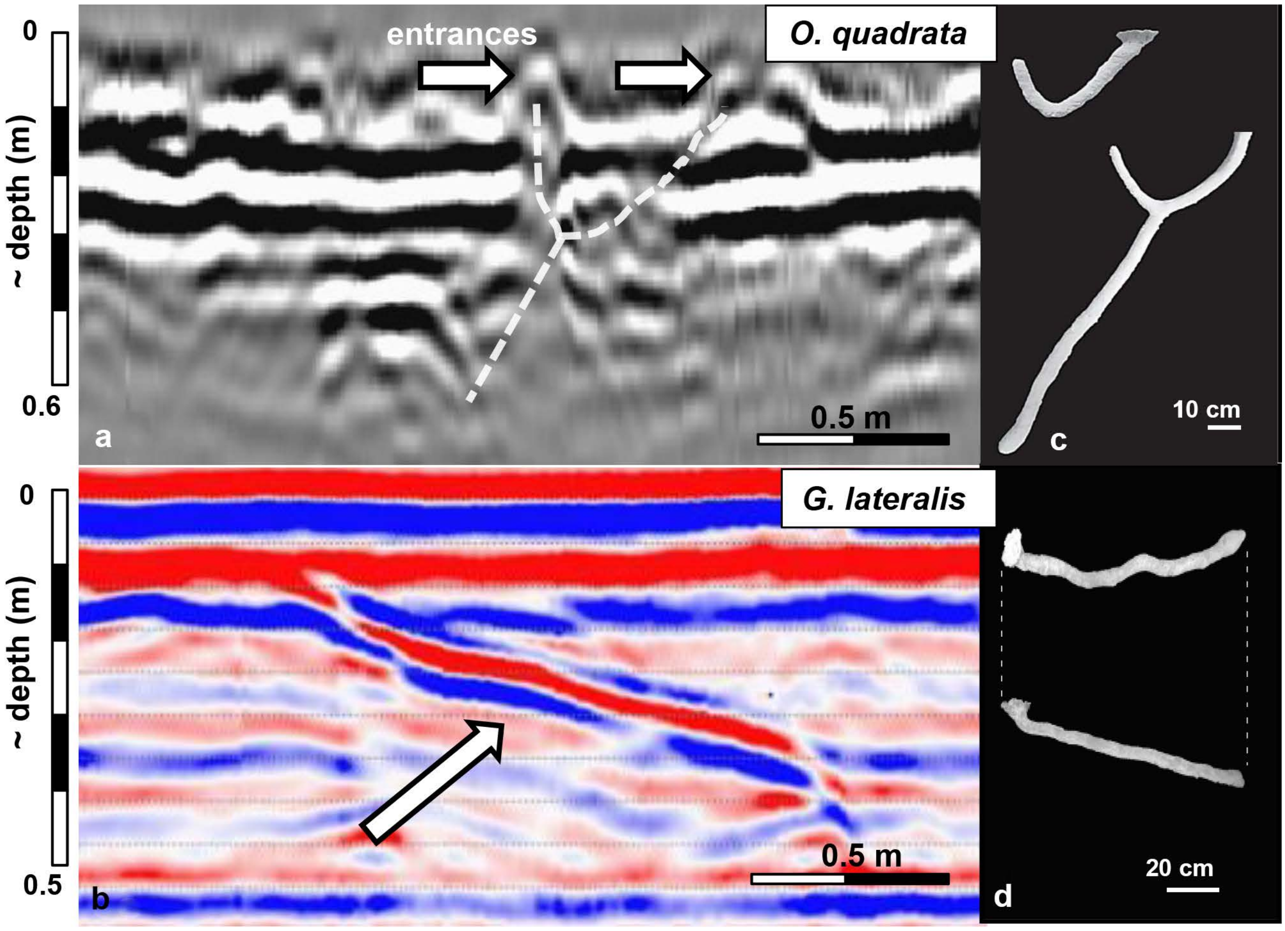

Fig. 5 - Post-processed GPR image of an (a) *O. quadrata* burrow, distinguishable as disturbed bedding (Site 2, berm). b) In contrast, a *G. lateralis* tunnel has a gentler inclination, with polarity reversal indicating void space (Site 2, dunes). c-d) Casts from Seike & Curran (2014) show the typical geometry of the two burrow types.

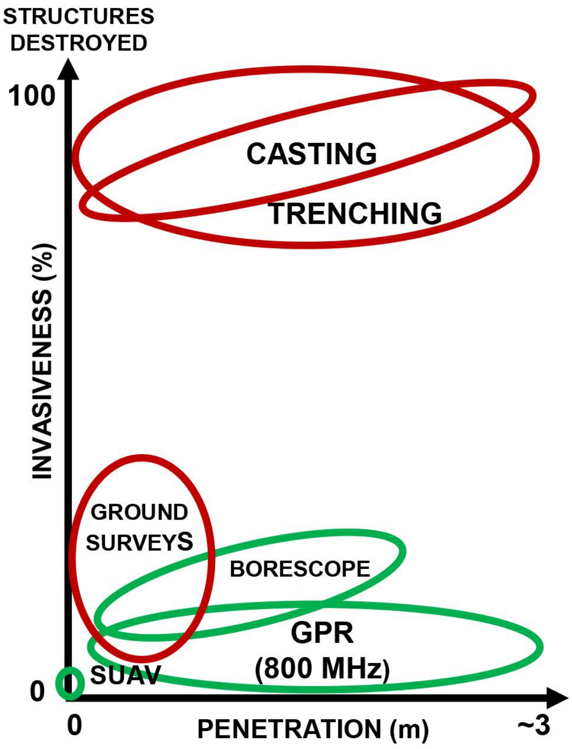

Fig. 6 - Generalized relationship between relative degree of invasiveness and penetration of field techniques used in this study (solid lines for methods discussed in this paper). Note the general positive correlation between disturbance and penetration, with GPR being an exception.

Results - As an example of recognizing and mapping bioturbation, Fig. 4 shows a clear distinction between aeolian bedding and a section of coastal dune bioturbated by *G. lateralis*. Fig. 5 shows detailed images of *G. lateralis* and *O. quadrata*, easily distinguishable by contrasting characteristics and similarity of reflection geometry to burrow casts recently made by Seike & Curran (2014). EM signal attenuation by clay minerals and salt content (Jol & Bristow, 2003) make the characterization *C. guanhumi* burrows challenging in areas where burrows reach the saline water table in muddy sands. However, their large size makes them most suitable for the suite of non-invasive techniques presented here, and new georadar signature attributes for the blue crab will be combined with ongoing ground survey datasets to present a complete picture of its subsurface patterns.

Remarks - A growing database of known burrow images was generated and will ultimately address two fundamental aspects: 1) mapping and quantification of bioturbation intensity, and 2) diagnostic attributes of decapod burrows that distinguish them from each other and other subsurface anomalies. When analyzed together, a combination of burrow attributes (e.g., depth, inclination angle), substrate characteristics (EM signal speed, presence of bedding, signal attenuation), and radar signatures (reverse polarity), provided the most accurate means of distinguishing among burrows and burrowers in georadar records.

SUMMARY AND IMPLICATIONS

Our study demonstrates that, both separately and in concert, three imaging techniques (drone low-altitude aerial surveys, borescope camera imagery, and GPR imaging) used in geological and ecological studies can be successfully applied to ichnological and zoogeomorphological research. These remote sensing and geophysical techniques require equipment that is relatively inexpensive and highly portable.

Drone imaging is a rapid and non-invasive means of identifying large burrow openings and associated spoil mounds in supratidal settings, with potential extension to intertidal and shallow subtidal environments. A portable borescope camera allows rapid assessment of burrow length and general geometry, while providing video and still photography. Successful borescope imaging depends on the circumstances of each particular setup (e.g., burrow complexity, sediment type).

Ground-penetrating radar images reveal patterns that allow differentiation of burrow structures from each other (Fig. 5). Diagnostic radar reflection patterns show promise for allowing recognition of burrow type in geophysical field surveys, as well as buried and/or lithified structures at depth. Understanding and recognizing these structures provides a context for accurate identification of disruption of background bedding and undisturbed substrate that can be attributed to bioturbation over large spatial and temporal scales.

Ground-penetrating radar stands out as the most effective means of imaging the gross subsurface characteristics of burrows (depth, diameter, inclination, extent) and the enclosing matrix (the sum of lithological characteristics determining water retention structure). As with any geophysical technique, knowledge of the study area (vegetation, sediment characteristics, degree of lithification, water table depth, groundwater salinity) is vital for accurate interpretations of georadar records. Additionally, for neoichnological applications, GPR antenna frequency must be selected to allow both detection and resolution of biogenic structures, while maintaining reasonable penetration depth. Whereas the invasiveness (degree of disturbance) of most methods increases with the increasing penetration required to characterize biogenic structures, GPR has the advantage of being minimally invasive while achieving the penetration necessary to detect and resolve key subsurface anomalies (Fig. 6).

Data acquisition can cover large areas rapidly, and data processing, while requiring more expertise, can be learned with extensive practice. Groundtruth, where permitted and feasible, is always required to confirm the nature of imaged biogenic structures, however only a few targeted excavations may be sufficient as a basis for interpreting geophysical datasets. Ultimately, to complement traditional field techniques, georadar and drone imaging will serve as powerful neoichnological tools for characterizing bioturbation and identifying biogenic structures in both modern and ancient successions.

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