THE FORAMINIFERA, SEDIMENT, AND RADIOCARBON SIGNAL IN SHALLOW WATER (<4 M) FROM TWO BERMUDIAN ANCHIALINE CAVES: IMPLICATIONS FOR PALEOENVIRONMENTAL RECONSTRUCTIONS

A Thesis

by

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ABSTRACT

With a high sensitivity to local environmental changes and high preservation potential in coastal sediments, benthic foraminifera are useful proxies of long-term environmental change. However, their ecology and distributions in modern coastal underwater caves remains poorly understood, which hampers their applicability in these environments. It is also unknown if the microfossil processing approach (≥45 µm vs ≥63 µm mesh sieve size) influences resultant foraminiferal assemblages, and if current assumptions regarding calibration of cave-based radiocarbon dates are correct. Shallow water pools (<4 m) in two Bermudian anchialine caves (Deep Blue, Cow Cave) provide a modern analog for the potential benthic foraminifera that may colonize coastal underwater caves during initial inundation by groundwater-level rise. With the presence of a brackish meteoric lens overlaying saline groundwater, the faunal distribution of foraminifera is influenced by changing environmental and hydrographic parameters. However, the role of these environmental parameters on benthic foraminifera in such unique environments is not well studied. In this study we show that: 1) future work in anchialine cave environments can be limited to the ≥63 µm sediment size fraction; 2) the marine reservoir correction cannot be assumed as required for calibrating radiocarbon results from all carbonate samples from anchialine caves, 3) initial inundation of anchialine cave systems produces an assemblage of foraminifera most likely tolerant of brackish-water conditions, assuming a sufficient sediment supply to promote a stratigraphic record.

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Additionally, this work could not have been completed without assistance from my colleagues. Thank you to Jacque Cresswell for collecting both my samples and field data and being my eyes in the field, answering question after question regarding the field sties, as well taxonomic inquiries. To Marissa Hadjuk, I would not have the beautiful SEMs of my foraminifera if it had not been for her guidance and patient teaching. A special thank you to the community of graduate students at the Galveston campus who provide unconditional support and friendship, all of which help make graduate school the unique experience that it has been. Finally, I would like to show my gratitude to Tyler Winkler for technical support for the radiocarbon dates, and more so, his patience, understanding, and support 24/7. Annie Tamalavage also deserves special consideration as she strives to be a ray of sunshine for all graduate students, and who constantly puts others before herself.

CONTRIBUTORS AND FUNDING SOURCES

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The samples analyzed for Section 4 were provided by Professor van Hengstum. The data analyzed in Section 4.4 were prepared by Anne Tamalavage before processing at Baylor University. In Section 4.5 Tyler Winkler collected the data to be processed at the National Ocean Sciences Accelerator Mass Spectrometry facility at the Woods Hole Oceanographic Institute. All other data analysis and work conducted for the thesis was completed by the student under the advisement of Professor van Hengstum.

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1. INTRODUCTION

The physical and biological variables that control benthic foraminifera have been extensively evaluated in the deep-sea, coastal wetlands (marshes, mangroves), and other coastal areas, but there remains a limited understanding on what controls foraminiferal distributions in sinkholes, blueholes, and flooded coastal caves (submarine and anchialine). Previous research indicates that the sediment in coastal caves, sinkholes, and blue holes can preserve records of regional environmental change, which have relevance to broader, paleoclimate and paleoceanographic studies. However, additional information on the modern environmental processes and meiofaunal community patterns in these unique coastal environments is required to better constrain core-based paleoenvironmental reconstructions.

Anchialine environments frequently develop in karst basins (i.e., caves, sinkholes) on carbonate landscapes that are flooded by coastal groundwater (Fig. 1). The hydrography in underwater caves is controlled by local groundwater, and in coastal zones, the local groundwater can be divided into a fresh to brackish water layer (meteoric lens) overlying the saline groundwater below (Beddows et al., 2007; Rowe, 2011; van Hengstum et al., 2010). The differences in physical conditions (temperature, dissolved oxygen, salinity, pH) between water masses are known drivers of benthic meiofaunal community patterns (van Hengstum et al., 2010). In general, salinity in the meteoric lens increases towards the shoreline. In addition, the local groundwater vertically oscillates under local tidal forcing, the extent to which varies on distance from the coast (Beddows et al., 2007; van Hengstum et al., 2010). Cave systems that are proximal to the coastline are further impacted by tidal activity as water from outside the cave is pumped into the underground habitats, by which this process can also transport nutrients and chemicals into the otherwise oxygen and nutrient deficient underwater cave system.

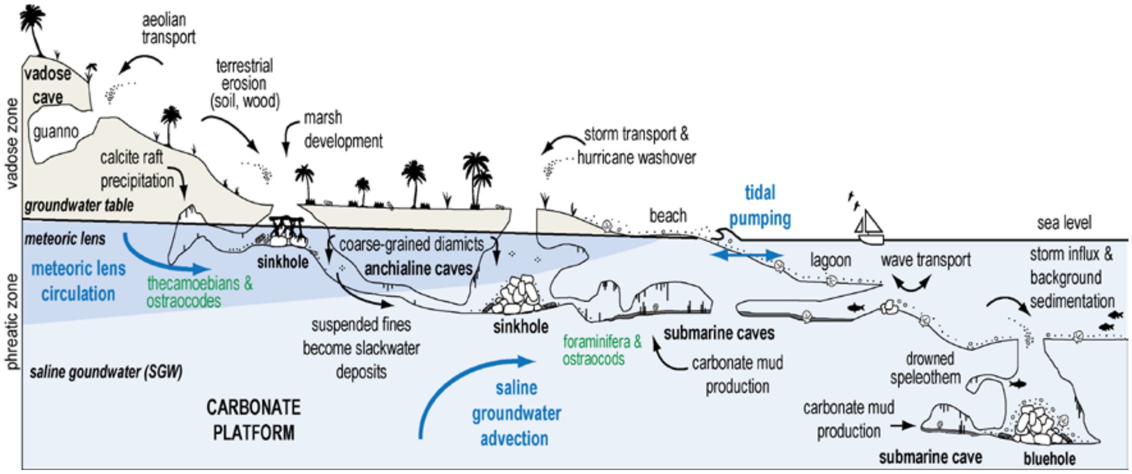


Figure 1. A conceptual model of anchialine environments created by the inundation of coastal caves and sinkholes (adapted after van Hengstum and Scott, 2012).

Few studies currently exist regarding the distribution of benthic foraminifera in anchialine caves systems. Surface samples collected from Green Bay Cave in Bermuda suggest assemblages are controlled by salinity and pH in different groundwater masses, and the influx of organic matter from either the adjacent terrestrial or marine environments (van Hengstum & Scott, 2011a). Samples in the brackish meteoric lens were dominated by the genera *Ammonia, Bolivina, Helenina,* and *Trochammina*, while in the saline groundwater layer, the dominant fauna consisted of *Bolivina* and *Rosalina*. In addition to a shift in dominant taxa, the meteoric lens displays the lowest diversity and faunal densities (i.e., absolute abundance). In Carwash Cave in Mexico, which is primarily flooded by a limnic meteoric lens, the cave system faunal assemblage are currently dominated by testate amoeba (thecamoebians), with only the most euryhaline foraminifera observed (*Paraphysalidia paralica, Trochammina spp.*) (van Hengstum et al., 2009). Previous research has found assemblages of benthic foraminifera in sediment at the contact with the cave's limestone floor (van Hengstum & Scott, 2011a), but it remains poorly understood what benthic foraminiferal assemblage may begin colonizing a coastal cave when it becomes inundated by concomitant groundwater and sea-level rise. By examining additional modern analog assemblages in other anchialine cave systems, it may be possible to discern when benthic foraminifera can begin colonizing a cave environment, and potentially, what environmental variables control their distribution.

Still further, it remains unknown if benthic foraminifera become diminutive in response to living in cave environments, which impacts the microfossil processing techniques for sediment samples from underwater caves. Other organisms are known to have ecological adaptations to living in underwater caves, including loss of skin pigments, eye organs, and body mass (Iliffe & Bishop, 2007). Previous work has used a 45 µm sieve to examine benthic foraminifera in underwater caves (van Hengstum & Scott, 2012), in part because this size fraction can also be simultaneously used to examine other smaller microfossils (e.g. tintinnids, testate amoebae). However, the 45-63 µm sieve sediment fraction includes many smaller and juvenile benthic foraminifera that are difficult to taxonomically identify without individual scanning electron microscopy. In other environments, tests have been conducted to determine the sieve needed to analyze benthic foraminiferal populations. For example, individuals below 125 µm do not need to be examined in order to understand the broader environmental interpretations when studying large, coral reef taxa (Martin & Liddell, 1988, 1989). However, research on arctic benthic foraminifera may be biased or skewed if taxa from the 63-125 µm class is excluded during analysis because the 63 to 125 µm size class make up a considerable proportion of the benthic foraminiferal population (Schröder et al., 1987). It remains worthwhile to test whether a 45 µm or 63 µm sieve is necessary for benthic foraminiferal analysis in anchialine cave environments.

Completing environmental reconstructions in underwater environments has typically employed radiocarbon dating of terrestrial and marine microfossils (e.g. leaves, twigs, microfossils, bivalves). Developing age models with terrestrial plant microfossils is preferred for their simplicity of calibration, as terrestrial plant material was in secular equilibrium with atmospheric radiocarbon production (Ascough et al., 2005). However, in instances where terrestrial plant material is unavailable downcore, one is forced to age carbonate materials (e.g. foraminifera, microfossils). Past studies using foraminifera for radiocarbon dating from anchialine caves have assumed marine reservoir correction of 400 years (Ascough et al., 2005; Stuiver & Polach, 1977) for calibrating conventional radiocarbon ages. However, the validity of this assumption in anchialine cave environments remains unknown.

The objectives of this project are to investigate:

(1) The radiocarbon signature of both carbonate microfossils and terrestrial plant fragments in surface sediment samples from shallow water in anchialine caves.

(2) The relative impact of using the ≥45 um versus ≥63 um sediment fractions for foraminiferal analysis in anchialine caves.

(3) Any differences in faunal distribution between anchialine caves with varying levels of exposure to the adjacent terrestrial surface, and potential linkages between local benthic foraminiferal distributions and sub habitat variability; and

(4) The distribution of benthic foraminifera in different shallow water anchialine cave settings (<4 m) to better understand the timing of foraminiferal colonization of cave habitats during initial inundation.

2. BACKGROUND

Benthic foraminifera are unicellular protists found globally in slightly brackish to marine environments, in shallow coastal to deep-sea environments (Caralp, 1989b; Den Dulk et al., 2000; Dubicka et al., 2015; Duffield et al., 2015; Jeffreys et al., 2015; Ohga & Kitazato, 1997; Scott et al., 2001; van Hengstum & Scott, 2011a). In general, foraminifera are highly sensitive to local environmental conditions (e.g. salinity, temperature, food availability, sediment texture), and changes in these parameters can be reflected in foraminiferal community and biomass (Caralp, 1989b; Dimiza et al., 2016; Duffield et al., 2015; Gooday et al., 1990). Foraminiferal tests have high preservation potential in the sediment long after their death (Ohga & Kitazato, 1997) and consequently, they have become well regarded paleoenvironmental proxies (Caralp, 1989a; du Châtelet et al., 2009; Duffield et al., 2015; van Hengstum et al., 2009). Although many factors influence benthic foraminiferal distributions in natural environments, it is apparent that the relationship between dissolved oxygen concentration and organic carbon plays a critical role (Abu-Zied et al., 2008; Den Dulk et al., 2000; Diz et al., 2004; du Châtelet et al., 2009; Duffield et al., 2015; Jorissen et al., 1995)

In environments with high dissolved oxygen concentrations and low food availability, foraminiferal communities are often dominated by species with a planispiral chamber arrangement that is suggested as primarily reflecting an epifaunal lifestyle (Abu-Zied et al., 2008; Jorissen et al., 1995). Oligotrophic environments have been noted to support higher faunal densities at the sediment surface, with epifaunal individuals consuming particulate organic matter delivered to the benthos and limiting the nutrients delivered to deeper infaunal taxa (Abu-Zied et al., 2008; Duffield et al., 2015). In more eutrophic areas, benthic foraminiferal communities are dominated by foraminifera with bi- or triserially arranged chambers that are considered adaptations to an infaunal life mode. These species are capable of burrowing deeper into the sediment where dissolved oxygen concentration are lower. Typical infaunal genera found in oxygen-poor waters include *Bolivina*, *Hopsinkina*, and *Uvigerina* (Abu-Zied et al., 2008; Den Dulk et al., 2000; Diz et al., 2004).

The quantity or quality of organic matter reaching the benthos can also have a significant impact on benthic foraminifera (Caralp, 1989a; du Châtelet et al., 2009; Duffield et al., 2015). In the open ocean, areas with a higher amount of organic matter flux, allow individuals to grow larger. For example, adult *Melonis barleeanum* grew five times larger than average individuals when the supply of OM was higher. In some instances, the quantity of OM has shown a positive relationship with benthic foraminiferal densities (i.e., absolute abundance) (Caralp, 1989a). In 2009 du Châtelet (2009) noted trends in a Santa Barbara estuary between higher values of organic carbon and higher densities. Similarly, the abundance of *Uvigerina peregrina* increases dramatically following the spring bloom in the Norwegian sea and northeastern Atlantic ocean (Gooday et al., 1990).

Organic matter provenance (terrestrial versus marine) and state of degradation also impacts benthic foraminiferal communities (du Châtelet et al., 2009; Duffield et al., 2015; Talbot & Lærdal, 2000) because differing OM sources have differing nutritional value. For example, benthic foraminifera in the North Atlantic achieved higher-density benthic communities in response to phytoplankton blooms in the overlying surface waters. In areas of high OM influx with minimal degradation or alteration in the water column, the infaunal species *Bulimina exilis* has been known to replace *Melonis barleeanum* as a dominant taxon (Caralp, 1989b; Gooday et al., 1990).

Geochemical signatures such as stable carbon isotopic value ($\delta^{13}C$), and the atomic C:N ratio measured on bulk sedimentary organic matter can serve as indicators of organic matter provenance and quality (Gonneea et al., 2004; Khan et al., 2015). Carbon derived from terrestrial sources will have a $\delta^{13}C$ value of −28‰ to −26‰ (Lamb et al., 2006; O'Leary, 1988; Talbot & Lærdal, 2000), whereas, marine-derived organic matter is more enriched to around −18‰. Atomic C:N ratios are additionally useful in deconstruction of OM source, as a terrestrially dominated sample will have a higher C:N ratio (12 or greater) whereas marine values are conventionally below 10-12 (Talbot & Lærdal, 2000).

Previous investigations indicate that the influence of sediment texture on benthic foraminiferal distributions is uncertain and seemingly geographically variable (Diz et al., 2004; du Châtelet et al., 2009). In 2009, du Châtelet et al. (2009) attributed sediment texture as the main ecological controlling factor in an estuarine marsh in France, where the lowest abundance and diversity occured in areas of coarse texture. Conversely, a study of an embayment in northern Spain revealed coarse sediments as yeilding the highest diversity and abundance, leading the author to suggest coarse sediments as a preferred substrate for benthic communities (Diz et al., 2004; du Châtelet et al., 2009). In a study of Bermudian cave systems (van Hengstum & Scott, 2011a, 2011b), foraminiferal assemblages did accord with sediment textural variability, but these transitions are confounded by simultaenous changes in other environmental variables (organic matter provenance and quantity).

3. STUDY SITES

Bermuda is a carbonate landscape in the North Atlantic (32.31° N, 64.75° W) that has weathered into a mature karst landscape with abundant caves and sinkholes in the oldest geologic formations. These karst features formed from the dissolution of limestone during the late Quaternary (Mylroie et al., 1995; van Hengstum & Scott, 2012) (Fig. 1). Climatologically, Bermuda is considered to have a wet climate (Mylroie et al., 1995), with precipitation rates exceeding that of evaporation. The annual rate of precipitation in Bermuda is 1458 mm of rain (Rowe, 2011). Rainfall is highest in the later part of the year, from August to December, however, rainfall remains consistent year round. Over geologic time, subsurface dissolutional voids are formed as meteoric and marine water penetrates and dissolves the surrounding rock (Mylroie et al., 1995).

Two anchialine caves in Bermuda are the focus of the current study: Deep Blue (32.347, −64.711) and Cow Cave (32.347, −64.710). Cow Cave (CC) is positioned slightly closer to the shoreline with Caste Harbour (170 m away, Fig. 2) relative to Deep Blue (DB, ~250 m). These two caves systems are an ideal location to investigate the relative difference in foraminiferal distributions and test size because they both have shallow, benthic areas that progressively deepen (<4 m), so both areas should be exposed to seasonal, brackish water lens that develops in the caves pools. However, Cow Cave and Deep Blue have differing exposure to sunlight, which likely impacts the local flora (e.g. diatoms), terrestrial sediment flux, and exposure to rain water during a precipitation event.

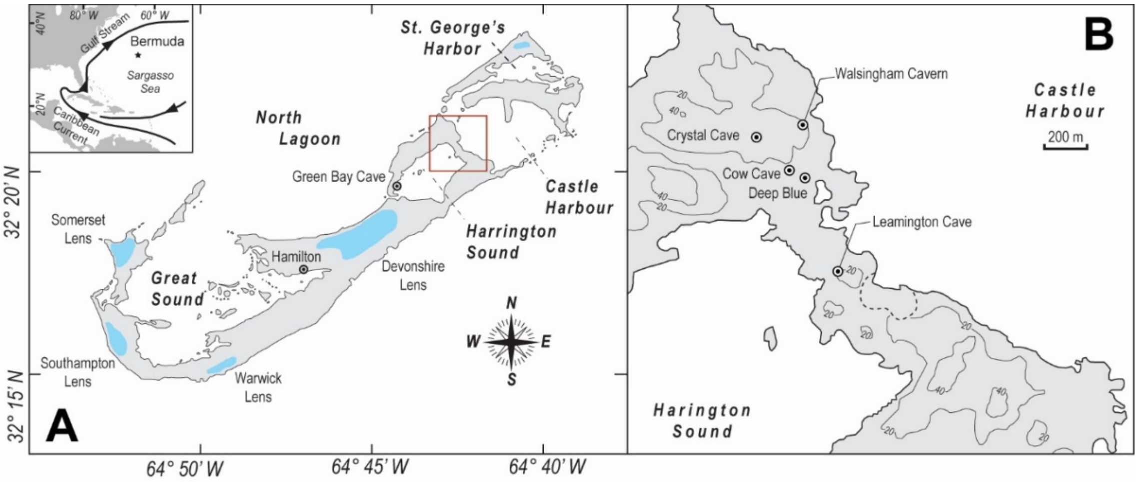


Figure 2. (A) Regional map of Bermuda, with inset (B) depicting the location of Deep Blue and Cow Cave on the isthmus between Castle Harbour and Harrington Sound.

Deep Blue is just one of the entrances (i.e., karst windows) into the spatially extensive Walsingham Cave System, which is one of the largest underwater caves in Bermuda. Deep Blue is located at the base of a large limestone cliff, is directly exposed to a flux of terrestrial sediment and rain water as well as daily indirect sunlight, and the crescent-shaped pool deepens toward the completely inundated Walsingham Cave System (Fig. 3A, 3B). Sediment samples collected from the Deep Blue pool are notably brown in color, with varying quantity of algae and remnant paleosol that is characteristic of the Bermudian landscape.

Cow Cave is the entrance chamber to a horizontally-limited flooded cave system, based on the results from current exploration. Cow Cave has one limited terrestrial access point at one end of the large chamber (Fig. 3C), but otherwise is completely roofed with negligible flux of terrestrial sediment elsewhere in the chamber. Cow Cave is interesting because it has 3 different areas with shallow water (<4 m): (a) Pool 1 (CCP1) is located adjacent to the terrestrial entrance point and exposed to a direct flux of terrestrial sediment and rainwater (Fig. 3C), (b) Pool 2 (CCP2) is physically isolated from CCP1 by rocks but has increasingly deeper areas flooded by groundwater (Fig.

3D), and (c) Pool 3 (CCP3) is the most distal site and removed from direct terrestrial sediment flux, and is a block of limestone that has created a flat habitat in the back of the cave (Fig. 3E, 3F). Sediment from CCP1 is more similar to sediment from Deep Blue: brown in color with a notable presence of some terra rosa paleosol. However, sediment from CCP2 and CCP3 is light beige in color, and distinctively fine-grained carbonate sediments (i.e., micrite).

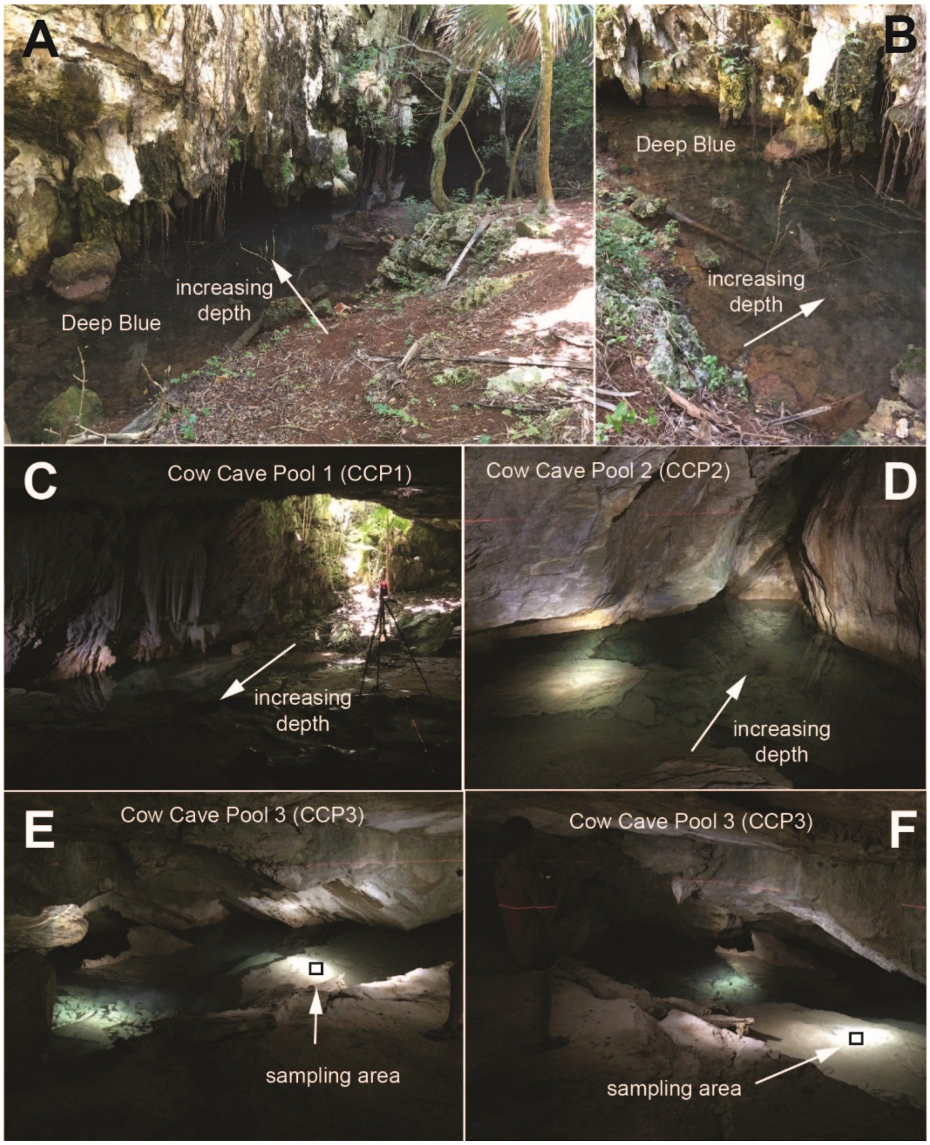


Figure 3. Photographs of Cow Cave and Deep Blue sampling areas. (A, B) Differing views of the crescentic pool known as Deep Blue. (C) The terrestrial entrances into Cow Cave and the sampling area CCP1. (D) The sampling area of CCP2. (E, F) Two different views of sampling area of CCP3. The over-exposed whitish area is related to the abundance of micrite.

4. METHODS

4.1 Sediment collection and field measurements

A total of 27 surface sediment samples (<3 cm depth) were collected from Cow Cave and Deep Blue: CCP1 (*n* = 8), CCP2 (*n* = 9), CCP3 (*n* = 1), and DB (*n* = 9). The samples were collected by free diving from water less than 3.5 m deep, with the water depth at time of collection measured with a collapsible metric ruler, with an uncertainty in depth measurement of ±1 cm.

The shallow sampling depth means that all sediment samples are exposed to the vertical oscillation of the local groundwater table associated with regional tides, and seasonal changes in surface salinity related to local changes in precipitation versus evaporation. Daily hydrographic conditions at each site (Cow Cave vs. Deep Blue), were measured with a YSI EXO1 multiparameter sonde, which was installed for 64 hours in Cow Cave at 1.4 m water depth and 51 hours in Deep Blue at 0.6 m water depth. This instrument continuously measures and logs conductivity (±0.001 mS cm^{-1}), depth (±0.004 m), dissolved oxygen (±0.1 mg L^{-1}), pH (±0.1 pH units), temperature (±0.01°C), and salinity (±0.1 psu). The YSI EXO1 was calibrated with reference pH and conductivity solutions before deployment.

4.2 Foraminiferal processing

Foraminifera were concentrated by wet sieving a 0.63 cm^3-sediment subsample from each station over nested 63-µm and 45-µm mesh sieves, which separated each sample into two different size fractions for foraminiferal analysis. Where necessary, a 250-µm sieve was used to separate large particles (e.g. wood fragments, molluscs), with any foraminifera in this coarse residue picked out and added back to the original sample. One sample from Deep Blue (DB-S7), contained no foraminifera so it was excluded from further analysis, so 26 sample stations were analyzed for foraminifera. A wet splitter (Scott & Hermelin, 1993) was used to achieve a minimum census of ~150 individuals in each size fraction (>45 µm and >63 µm), and individuals were wet picked in Petri dishes and placed onto a labeled 60-box microscope slide for further taxonomic identification.

After taxonomic identification of individuals in each sample, raw species counts were converted into relative abundance, and the corresponding standard error for each species was calculated (Patterson & Fishbein, 1989). To investigate differences between microfossil processing techniques, three databases were created for further analysis: (a) all individuals ≥45 µm, (b) all individuals ≥63 µm, and (c) all individuals sized from 45-63 µm. The first two databases were subject to unconstrained Q-mode cluster analysis using an unweighted paired group averaging algorithm and Euclidean similarity index (all individuals ≥45 µm, all individuals ≥63 µm) (Legendre & Legendre, 1998). The dendrograms produced by this method were compared to dendrograms produced by other algorithms (e.g. Bray-Curtis), however, the Euclidean distance index consistently produced dendrograms of higher cophenetic correlation values. First, the relative abundances used for cluster analysis were first log transformed to minimize the impact of dominant species and more equitably compare community structure (Legendre & Legendre, 1998). Before cluster analysis, if the standard error was greater than the relative abundance for a species in all samples, it was excluded from further analysis. Similarly, if a species was present in only one sample in the cave pool, it was also considered statistically insignificant and warranted exclusion. In total, 133 and 125 taxonomic units were included in the final data matrix for the ≥45 µm and ≥63 µm size fractions, respectively, and analyzed in Q-mode cluster analyses using the free to access software, Paleontological Statistical Software PAST 3.14 (Hammer et al., 2001). Finally, the species richness (*S*) and the Shannon-Weiner (H') diversity index, and proportion of foraminiferal wall structure (i.e., hyaline, porcelaneous, vs. agglutinated taxa) was calculated for each sample station ($n = 26$), which was used to generate summary statistics for each size fraction analyzed and resultant foraminiferal assemblages determined through cluster analysis.

4.3 Foraminiferal taxonomy

Scanning electron microscopy using a Hitachi desktop SEM was used to further confirm benthic foraminiferal taxonomy (Figs. 4, 5, 6, 7, 8, and 9). Throughout this study, approximately 152 taxonomic units were identified. In general, foraminiferal taxonomy followed van Hengstum and Scott (2011), but the taxonomy of several individuals was further improved based upon further examination of new material, and re-examining original micropaleontological slides from Green Bay Cave (*Dentalina communis, Heronallenia craigi*, and *Patellina corrugata)* (Loeblich Jr & Tappan, 1988)

In the order Spirillinida, van Hengstum and Scott (2011) lumped many similar individuals as *Patellina corrugata*, however, upon closer inspection, they can be sub-divided into three genera (*Patellina, Heteropatellina*, and *Patellinoides*) as per Loeblich and Tappan (1988). *Patellina corrugata* is the type species for the genus *Patellina*, which has a test that is hyaline, low conical and planoconvex shape, with all chambers visible on spiral side, and only the last chamber visible on the flattened umbilical side. The periphery of the test is carinate, with each chamber being partitioned by radial septula that run as wide as the chamber. The proloculus is followed by one to three whorls of undivided, tubular chambers. Finally, the umbilical side features an ‘S’ shaped columella.

In contrast, the genus *Heteropatellina* (McCulloch in 1977) is similar in shape to *Patellina* (low planoconvex test, cone-like shape and a keeled periphery), but Heteropatellina has a globular proloculus, earlier chambers that show a spiraled keel, the absence of the ‘S’ shaped columella, and internal chamber partitions. The proloculus itself is followed by a tubular, undivided chamber for one to two whorls, but then transitions to three to four crescent shaped chambers, per whorl, which are broad and low, giving the test a more stunted appearance than *Patellina*. Still further, both *Patellinoides* (Cushman 1933) and *Patellina* share a hyaline, low conical, and planoconvex. The S-shaped columella and all whorls visible on the spiral side with only the final chambers on the flattened, umbilical side. However, notable differences between these genera include the lack of internal partitions of chamber walls, biserially arranged chambers following the proloculus, lack of peripheral keel, and an ovate shape.

Uniserial taxa attributed to *Dentalina communis* in Green Bay Cave were originally lumped by van Hengstum and Scott (2011). The genus *Dentalina*, as first described by Risso in 1826, specifically applies to elongate calcareous tests that are arcuate in shape with a uniserial arrangement of cylindrical chambers. However, the genus as described by Leoblich and Tappan (1986) has longitudinal costae along the entire test. Upon further examining of individuals in Green Bay Cave, Deep Blue, and Cow Cave, the individuals in question are better designated to the genus *Laevidentalina*, which has a calcareous test remaining elongate and arcuate, showing horizontal to oblique sutures, and chambers that are uniserial, inflated and elongate.

Lastly, specimens found in the Cow Cave and Deep Blue samples were identified as *Heronallenita* sp. and *Heronallenia* sp. following Loeblich and Tappan (1988). Samples containing *Heronallenita* in the Green Bay Cave samples were reexamined and noted to be misidentified, with corrections in nomenclature from *Heronallenita craigi* to *Heronallenia craigi*. *Heronallenita* has a calcareous test, trochospiral arrangement with enlarging, hemispherical shaped chambers separated by deep sutures on the spiral side that also exhibit up to four flange-like ornamentations per chamber. Flanges on spiral side turning inwards from the periphery of the chambers on the spiral side, across the chambers on the umbilical side reaching in towards the umbilicus. On the umbilical side, the umbilicus is wide and open, with radial striae running perpendicular to umbilicus and a rounded periphery.

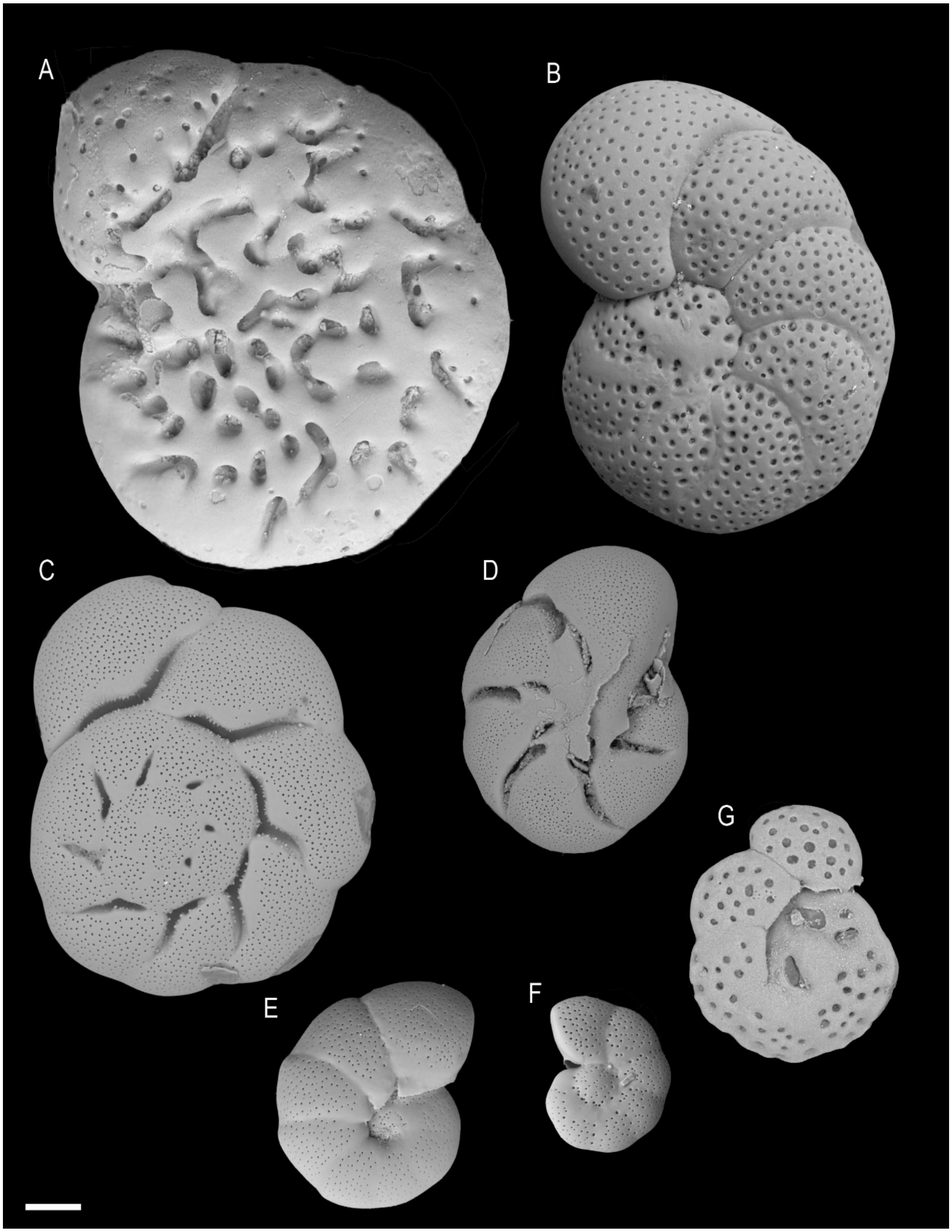


Figure 4. A, B *Trichohyalus aguayoi* Bermúdez, 1935. C, D *Helenina anderseni* (Warren, 1957). E, F *Melonis barleeanum* (Williamson, 1858). G *Cibicides lobatulus* (Walker and Jacob, 1798). Scale bar = 50 µm.

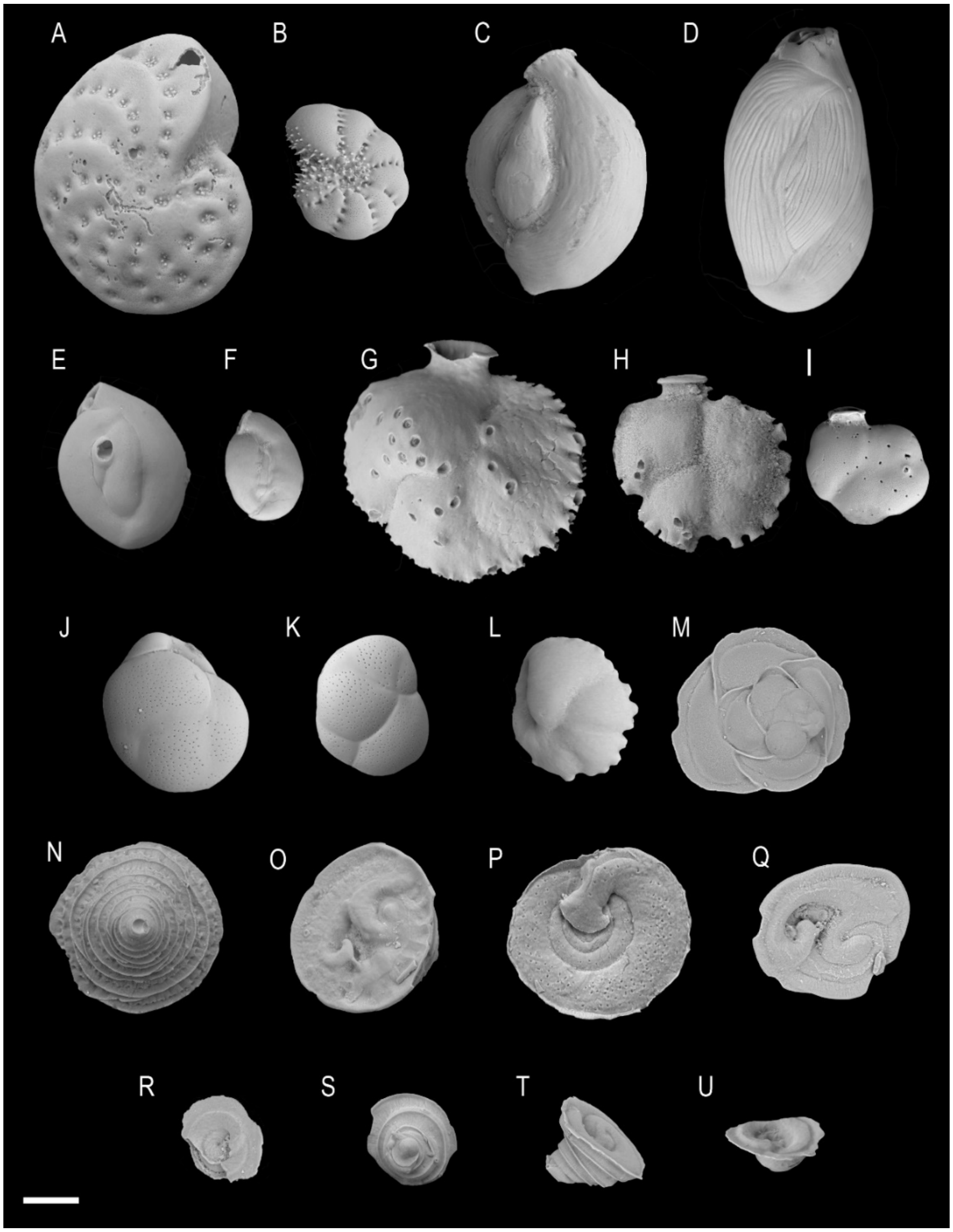


Figure 5. A *Elphidium* sp. de Montfort, 1808. B *Elphidium norvangi* Buzas, Smith and Beam, 1977. C *Quinqueloculina candiana* d’Orbigny, 1839a. D *Quinqueloculina laevigata* d'Orbigny, 1826. E, F *Quinqueloculina seminulum* (Linné, 1758). G, *H Siphonina reticulata* (Czjzek, 1848). I *Siphonina temblorensis* Garrison, 1959. J, K *Globocassidulina subglobosa* (Brady, 1881). L *Epistominella pulchella* (Husezima & Maruhasi, 1944). M *Unknown rotalid*. N, O *Patellina corrugata* Williamson, 1858. P *Mychostomina revertens* (Rhumbler, 1906). Q *Patellinoides sp*. Cushman, 1933a. R-U *Heteropatellina sp*. McCulloch, 1977. Scale bar = 50 µm.

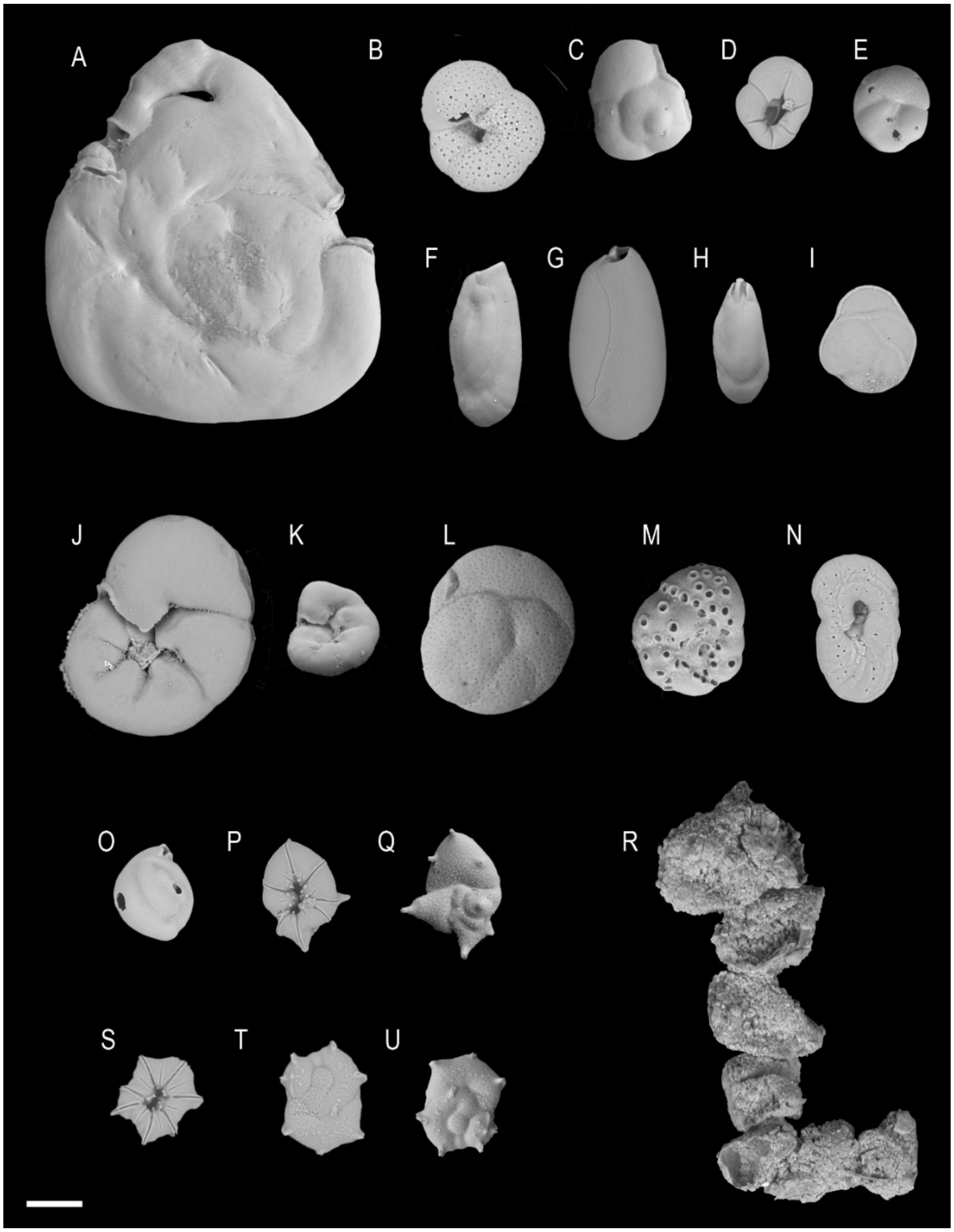


Figure 6. A *Opthalmidium inconstans* (Brady, 1879). B, C *Metarotaliella simplex* (Grell, 1979). D, E *Metarotaliella* sp. Grell, 1962. F *Quinqueloculina bosciana* d'Orbigny, 1839a. G, H *Triloculina oblonga* (Montagu, 1803). I *Rosalina williamsoni* (Parr, 1932). J, K Ro*salina globularis* d'Orbigny, 1826. L *Rosalina* sp. d'Orbigny, 1826. M *Svratkina australiensis* (Chapman, Parr, and Collins, 1934). N *Heronallenia craigi* Chapman and Parr, 1931. O *Miliolinella circularis* (Bornemann, 1855). P, Q *Rotaliella arctica* (Scott & Vilks, 1991). R *Polysaccamina ipohalina* (Scott, 1976). S-U *Heronallenita* sp. (Seiglie and Bermúdez, 1965. Scale bar = 50 µm.

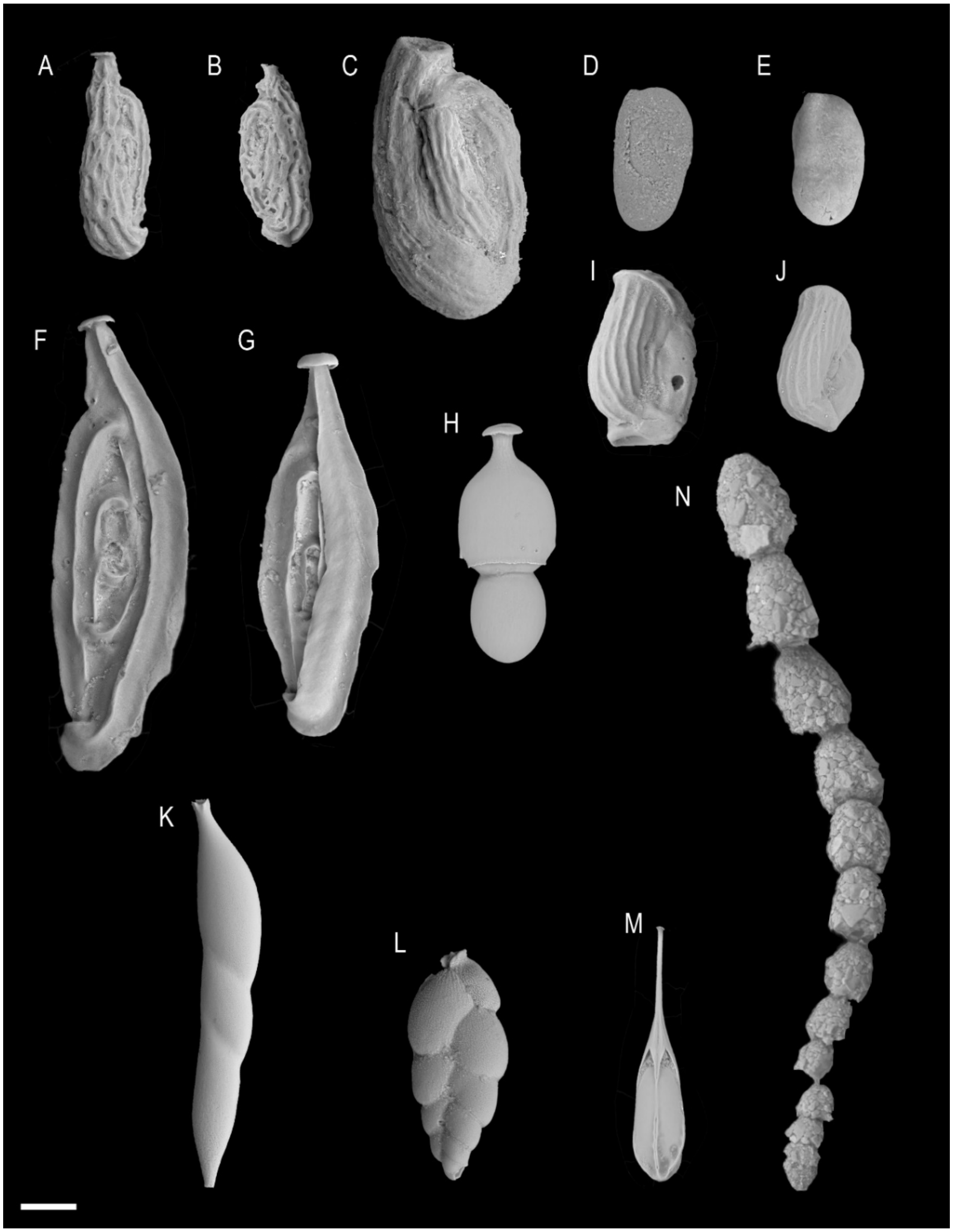


Figure 7. A, B *Quinqueloculina subpoeyana* Cushman, 1922. C *Quinqueloculina tenagos* Parker et al., 1953. D, E *Miliammina fusca* (Brady, 1870). F, G *Spirophthalmidium emaciatum* Haynes & Adams, 1973. H *Lagenolingulina* sp. McCulloch, 1977. I, J *Articulina pacifica* Cushman, 1944. K *Laevidentalina communis* (Leoblich and Tappan, 1986). L *Hopkinsina pacifica* Cushman, 1933b. M *Lagenosolenia sp*. McCulloch, 1977. N *Reophax scottii* Chaster, 1892. Scale bar = 50 µm.

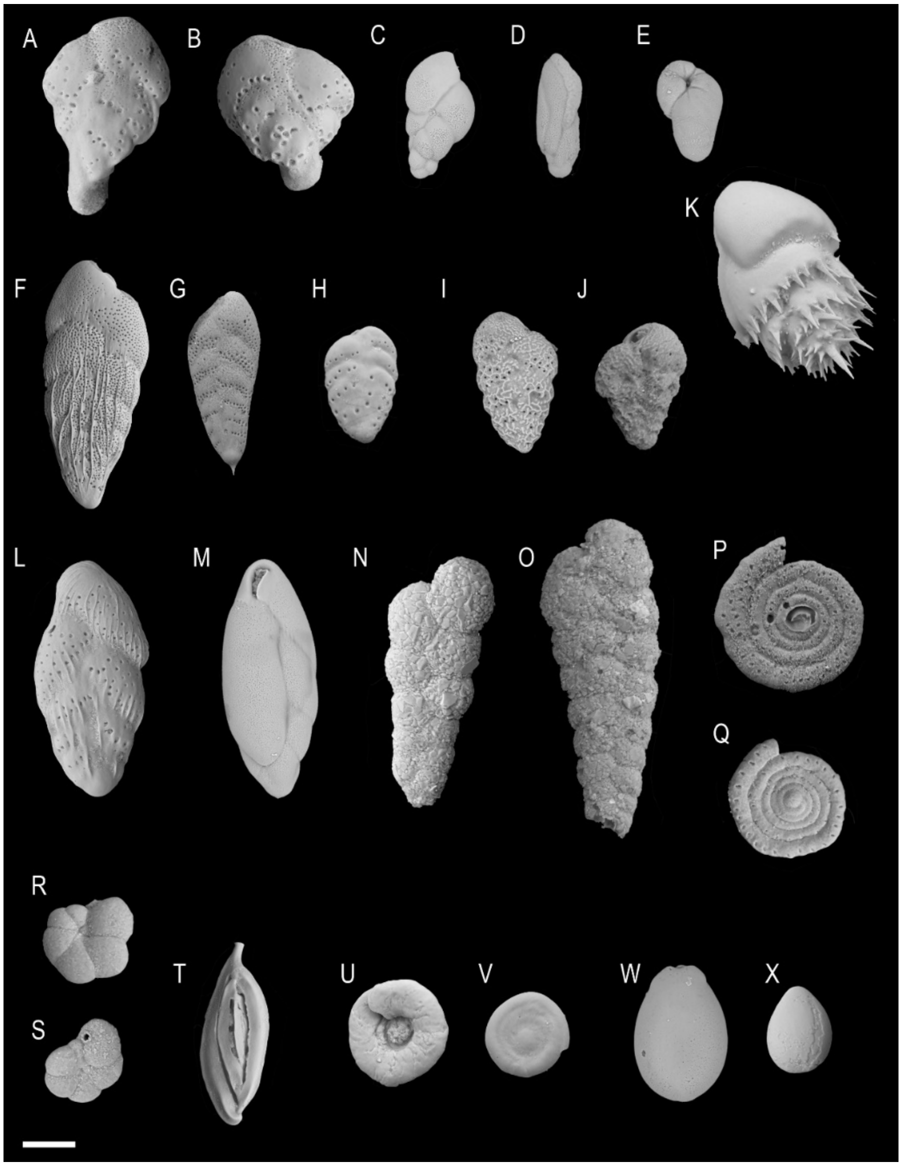


Figure 8. A, B *Bolivina tortuosa* Brady, 1881. C *Bulimina consectata* (McCulloch, 1977). D, E *Buliminella elegantissima* D'Orbigny, 1839b. F *Bolivina striatula* Cushman, 1922. G *Bolivina psuedopunctata* Höglund, 1947. H *Bolivina paula* Cushman and Cahill, 1932 (in Cushman and Ponton, 1932). I, J *Bolivina variabilis* (Williamson, 1858). K *Bulimina marginata* D'Orbigny, 1826. L *Loxostomum mayori* (Cushman, 1922). M *Fursenkoina fusiformis* Loeblich & Tappan, 1961. N, O *Textularia earlandi* Parker, 1952. P, Q *Spirillina vivipara* Ehrenberg, 1843. R, S *Labrospira evoluta* (Natland, 1938). T *Sigmoilina tenuis* (Czjzek, 1848). U, V *Cyclogyra involvens* (Reuss, 1850). W, X *Fissurina* sp. Reuss, 1850. Scale bar = 50 µm.

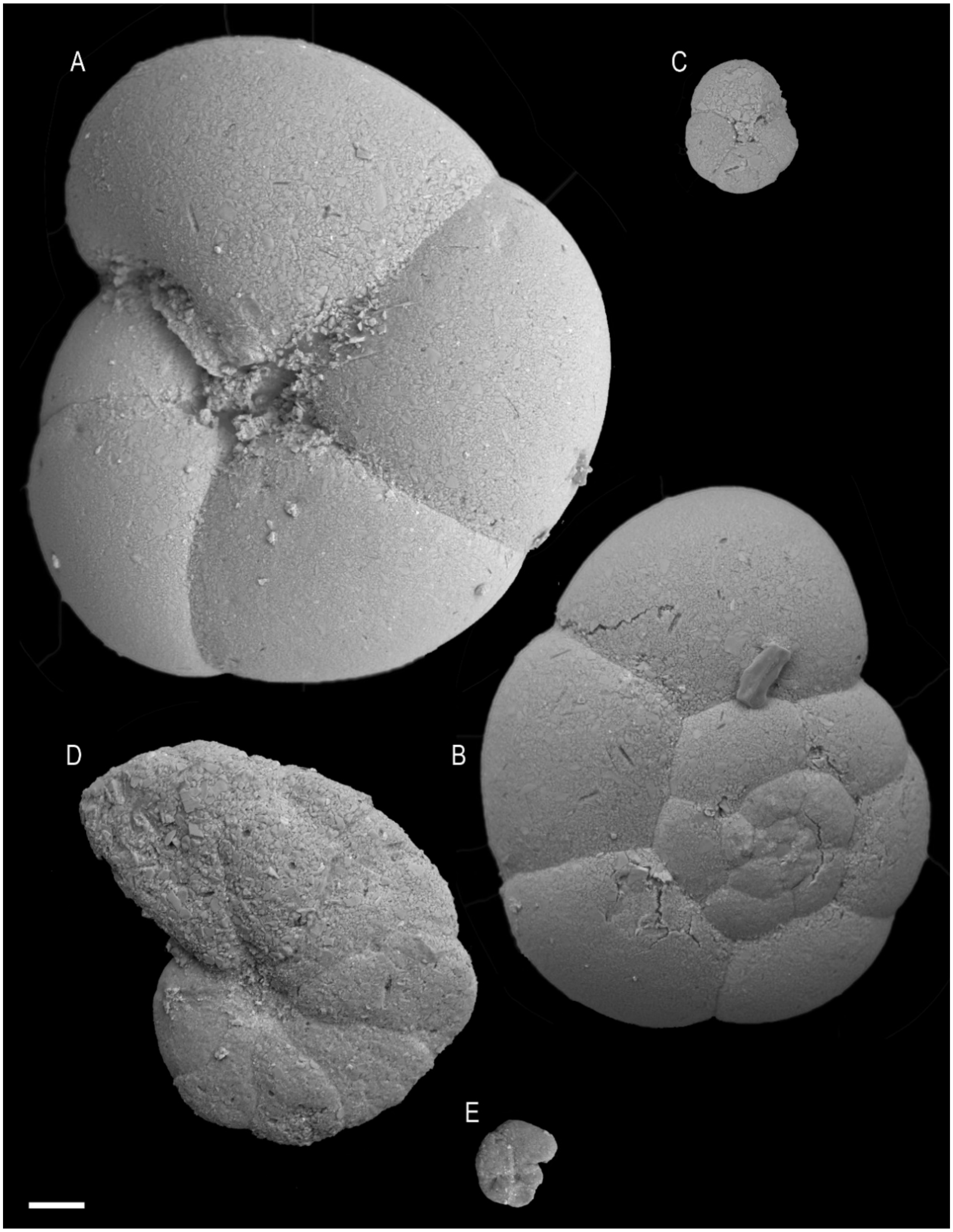


Figure 9. A, B *Trochammina inflata* (Montagu, 1808). C, D *Jadammina macresens* (Brady, 1870). E *Trochammina quadriloba* Höglund, 1947. Scale bar = 50 µm.

4.4 Sediment analysis (texture and organic matter content)

Textural analysis was completed on a Malvern Mastersizer 2000G Hydro model laser particle size analyzer, which measures particle sizes ranging between 0.02-2000 µm. Sediment sub-samples were placed into test tubes, suspended in deionized water and a 5.5 mg L^{-1} solution of sodium hexametaphosphate ($NaPO_3$) dispersant, and homogenized in the test tube with a vortex mixer. Standards were run after every third sample to ensure acceptable background levels and instrument cleanliness. Cleaning cycles were run twice in between each sample, as well as three times after each standard, again to ensure readings were accurate and that sediment grains were not accumulating in the system.

Organic matter quantity was estimated at each sampling station site (*n* = 26), the bulk organic matter content for each sample station was estimated using standard loss-on-ignition (LOI) procedure (Heiri et al., 2001). Sediment sub-samples were placed in a clean, dry, labeled crucible to be weighed. The crucibles were first placed in a drying oven at 80°C for 24 hours to remove moisture, and weighed once again, before being ignited in a muffle furnace at 550°C for 4.5 hours. The final weight was obtained and used to calculate the estimate the organic matter content for each sample as a percent.

To help determine the provenance of organic matter at each sample station (*n* = 26), the stable carbon isotopic value ($\delta^{13}C_{org}$) and C:N ratio of bulk sediment in each sample was measured. For the total carbon and nitrogen values, approximately 100 mg of sample sediment was weighed into tin capsules. To obtain isotopic values, ~5 mg of sample was weighed using a microbalance and acidified with approximately 8 mL of 1.0M HCl. After 12 hours of acidification, the remaining HCl was removed and samples were stored in a drying oven until desiccated. The dried sample was then weighed into silver capsules using a microbalance and sent to Baylor University for isotopic ratio determination on an isotope ratio mass spectrometer (IRMS).

4.5 Radiocarbon

Four samples from surface sediment in CCP2 (Fig. 3D) were submitted for radiocarbon dating to the Woods Hole Oceanographic Institution (WHOI) National

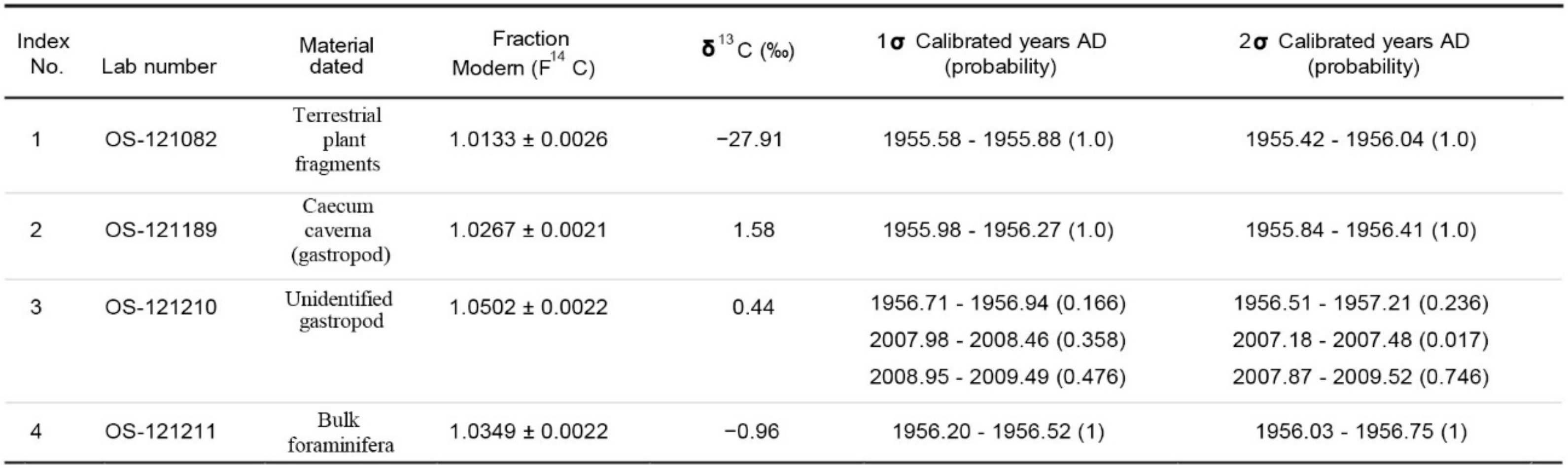
Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility. Three carbonate samples were analyzed to measure the impact of a possible reservoir or hard water effect on the biogenic carbonate: multiple shells of the gastropod *Caecum caverna* shell, a marine bivalve shell, and a bulk microfossil sample of benthic foraminifera (primarily *Quinqueloculina* spp.). A sample was also submitted of terrestrial organic matter fragments. Given all samples contained a fraction modern ($F^{14}C$) >1.000 (discussed further below), which is indicative of the samples secreting their carbonate since 1950 CE, the measured radiocarbon dates were calibrated with the post-bomb curve of (Hua et al., 2013). Traditionally, the marine reservoir correction is determined using 14C ages of marine samples collected from a known date, preceding nuclear weapons typically as part of a previous study or museum collection (Reimer & Reimer, 2001). However, underwater cave diving was not prevalent until the 1970's (Exley, 1986), as such, there is likely no samples collected from a submerged cave system prior to nuclear weapons testing.

5. RESULTS

5.1 Radiocarbon

All samples submitted for radiocarbon contain more ^{14}C than the modern reference sample and post-date nuclear weapons testing in the 1950's (Table 1). This indicates that the radiocarbon content of the groundwater where the carbonate materials were precipitated is in secular equilibrium with atmospheric radiocarbon production. These results also indicate that terrestrial organic matter fragments have the potential to become incorporated into cave sediment relatively quickly after death.

Table 1. Radiocarbon dates collected from terrestrial material and carbonate microfossils.



Index No.	Lab number	Material dated	Fraction Modern ($F^{14}C$)	$\delta^{13}C$ (‰)	1σ Calibrated years AD (probability)	2σ Calibrated years AD (probability)
1	OS-121082	Terrestrial plant fragments	1.0133 ± 0.0026	−27.91	1955.58 - 1955.88 (1.0)	1955.42 - 1956.04 (1.0)
2	OS-121189	Caecum caverna (gastropod)	1.0267 ± 0.0021	1.58	1955.98 - 1956.27 (1.0)	1955.84 - 1956.41 (1.0)
3	OS-121210	Unidentified gastropod	1.0502 ± 0.0022	0.44	1956.71 - 1956.94 (0.166) 2007.98 - 2008.46 (0.358) 2008.95 - 2009.49 (0.476)	1956.51 - 1957.21 (0.236) 2007.18 - 2007.48 (0.017) 2007.87 - 2009.52 (0.746)
4	OS-121211	Bulk foraminifera	1.0349 ± 0.0022	−0.96	1956.20 - 1956.52 (1)	1956.03 - 1956.75 (1)

5.2 Hydrographic conditions

A 36-hour monitoring period is insufficient to capture the full spectrum of environmental parameters potentially experienced by the shallow benthos of the two caves. Indeed, the development of a shallow brackish water meteoric lens (<1 m) is dependent upon regional weather, and brackish water lenses were not observed at the sampling sites during the monitoring interval. However, 36 hours does provide some insight into the hydrographic similarities and differences between the two caves (Fig. 10). The variability in water depth recorded by the sensor in each caves during the 36-hour monitoring period indicates that the local groundwater table in each cave is impacted by tidal action. At Deep Blue, which is located further inland the sensor was mounted at 0.6 m water depth, it experienced 38.8 cm of water depth change during a tidal cycle. At Cow Cave, which is more proximal to the shoreline, the sensor was mounted at 1.5 m water depth and experienced 48.4 cm of water depth change during a tidal cycle. Assuming negligible change in atmospheric pressure during the monitoring intervals, the ~10 cm difference is likely due to the attenuation of tidal action with increasing distance from the shoreline (Martin et al., 2012).

During the interval of time where monitoring occurred, changes in local benthic hydrographic conditions were also linked to tidal cycles, with overall hydrographic conditions more variable at Cow Cave than Deep Blue. In Cow Cave, temperature varied 2.7°C, with warmer temperatures measured with increased water depth, which suggests that warmer water is brought to the cave during high tides. Salinity in Cow Cave maintained an oceanic value of ~39 psu, experiencing mild changes with water levels as could be expected with the incoming flux of saline ocean water. Dissolved oxygen concentration (DO) varied, oscillating between 3.5 mg L^{-1} (sub-oxic) and 6.8 mg L^{-1} (well oxygenated). DO shows correlation to water levels, whereby DO increased when water levels are highest like salinity and temperature. The pH also oscillated in Cow Cave between 7.48 to 7.97 pH units. The pH values approached normal values for the surface of the ocean (~8.0 mg L^{-1}) during high water levels. It was during low water levels that pH drops to acidic conditions, a pH value of <7.9 may detrimentally impact both diversity and density of benthic foraminifera (Uthicke et al., 2013).

The conditions observed in Deep Blue at an even shallower depth (0.6 m) remained more constant relative to Cow Cave. Being 80 m further inland, one would expect a higher potential for a shallow (<50 cm) brackish water meteoric lens to develop, which was not observed during the fieldwork. The temperature values in Deep Blue are relatively constant, between 26.6 to 27.0°C, and overall cooler temperature than Cow Cave (27.2 to 29.9°C). The salinity of ~37 psu in Deep Blue was less than Cow Cave (~39 psu). Deep Blue can be considered a low-oxic environment, with dissolved oxygen values relatively constant ~3.4 mg L^{-1}, which is considerably lower than values recorded in Cow Cave (6.8 mg L^{-1}). The pH in the shallow water of Deep Blue varies between 7.6 and 7.8. Neither cave site had a developed brackish meteoric lens during the period of observation in 2015.

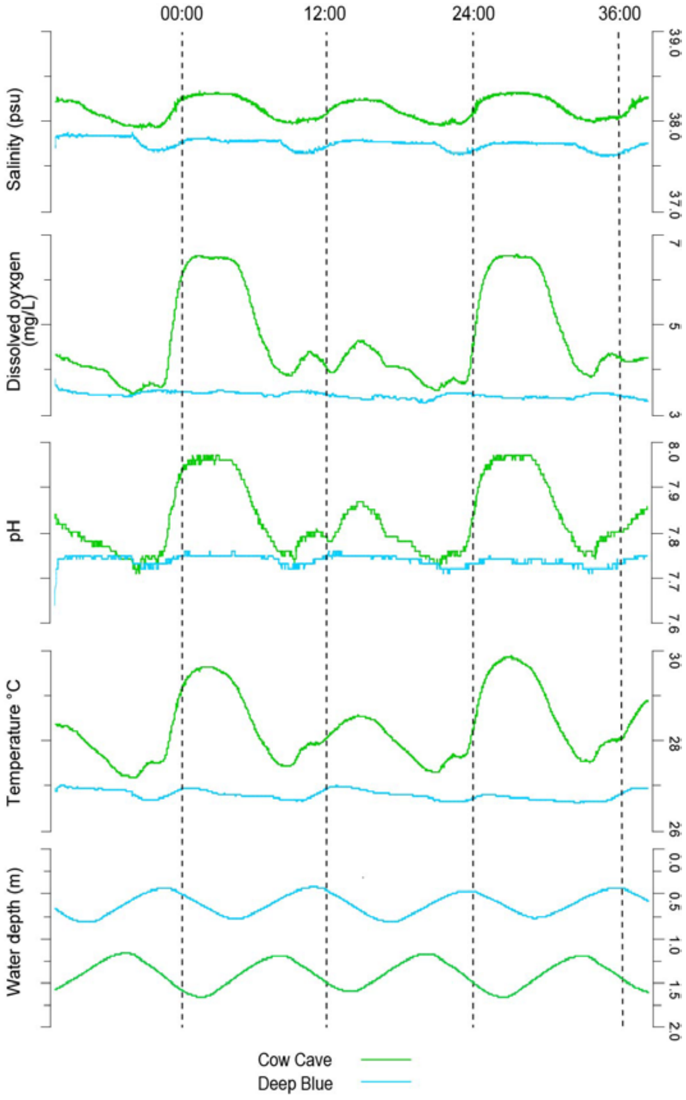


Figure 10. 36 hours of hydrographic variability in Deep Blue and Cow Cave. The sensor was positioned at an average depth of 0.6 m in Deep Blue (1:44 pm on 19 August, to 2:02 pm on August 21 2015), and an average depth of 1.5 m in Cow Cave (1:44 pm on 19 August, to 2:02 pm on August 21 2015).

5.3 Impact of microfossil processing technique on foraminiferal assemblages

Based on the dendrograms produced by Q-mode cluster analysis on the >45 µm and the >63 µm assemblages, there is no substantial change in the resultant foraminiferal assemblages by including the smaller sieve size (Fig. 11). In both dendrograms, samples from Deep Blue and most stations from CCP1 grouped together, versus an alternate group that includes CCP3, 2 samples from CCP1 (CCP1-S8, CCP1-S7), and CCP2 (Figs. 11A, 11B).

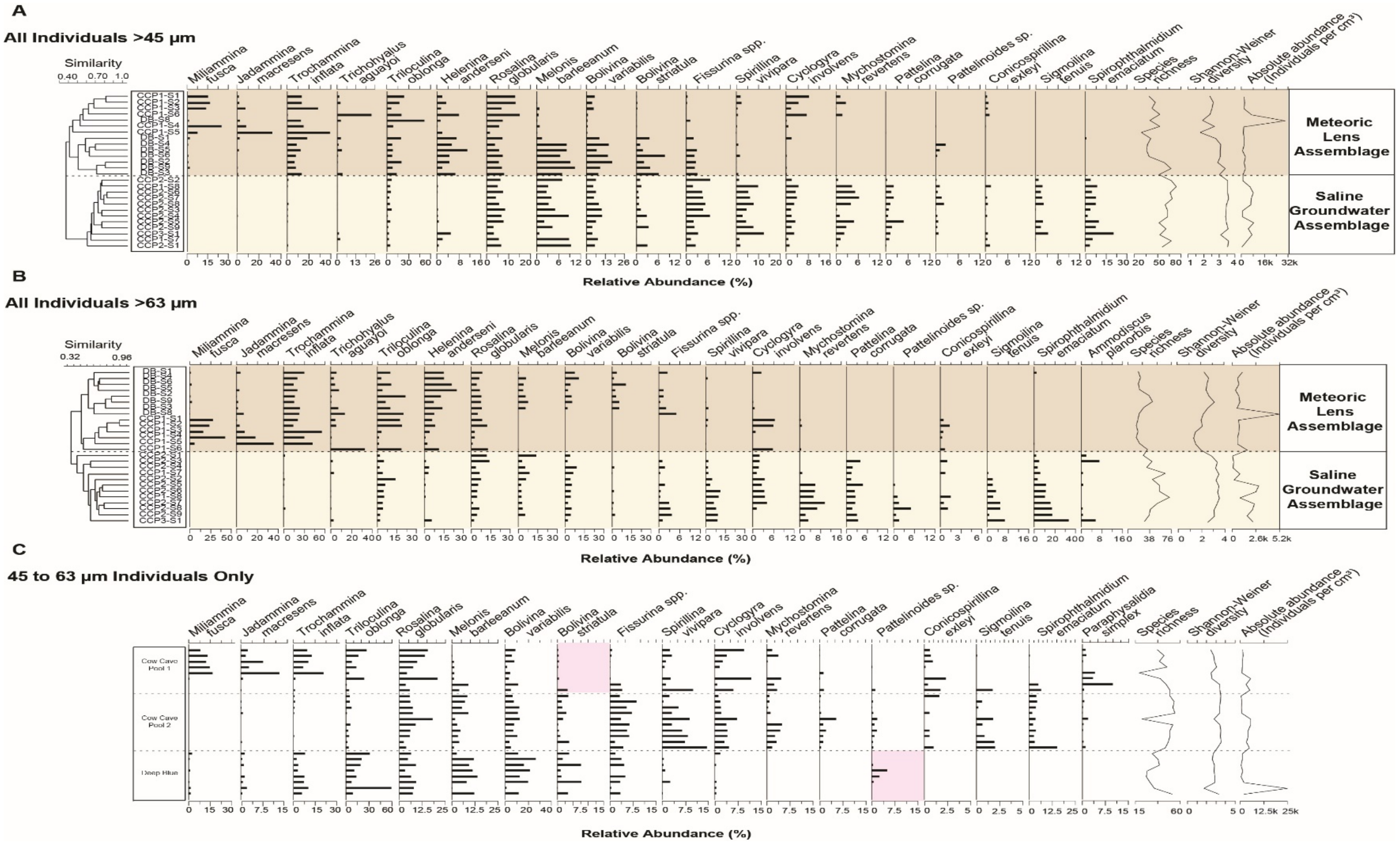


Figure 11. Detailed foraminiferal results from Cow Cave and Deep Blue illustrating the relative abundance of common species, absolute abundance, and Shannon-Weiner Diversity Index (H´). A, B: Dendrograms produced by Q-mode cluster on the >45 µm and >63 µm size fraction. Both dendrograms can be divided into the Meteoric Lens Assemblage and Saline Groundwater Assemblage, which contain the same sampling stations. C: Foraminifera present in the 45-63 µm size fraction only, with the pink-colored box highlighting taxa that re only present within this size fraction.

Given that samples from Deep Blue and CCP1 generally form an assemblage from areas shallower than 2.0 m water depth (Fig. 12), this assemblage is hereafter referred to as the Meteoric Lens Assemblage. Indeed, no brackish water was measured at these sample stations during the fieldwork, but it is known to the author that this is not always the case (discussed further below). The samples from deeper localities (all samples from CCP2, CCP3, and CCP1-S8, CCP1-S7) are referred to as the Saline Groundwater Assemblage. The proportion of foraminifera with different test structures is also similar for both microfossil processing approaches. In both the ≥45 µm and ≥63 µm fraction, the meteoric lens assemblage has a higher proportion of individuals with an agglutinated test, versus a higher proportion of hyaline and porcelaneous wall types in the Saline Groundwater Assemblage. In fact, many samples from the Saline Groundwater Assemblage had <10% proportion of agglutinated taxa (Fig. 13).

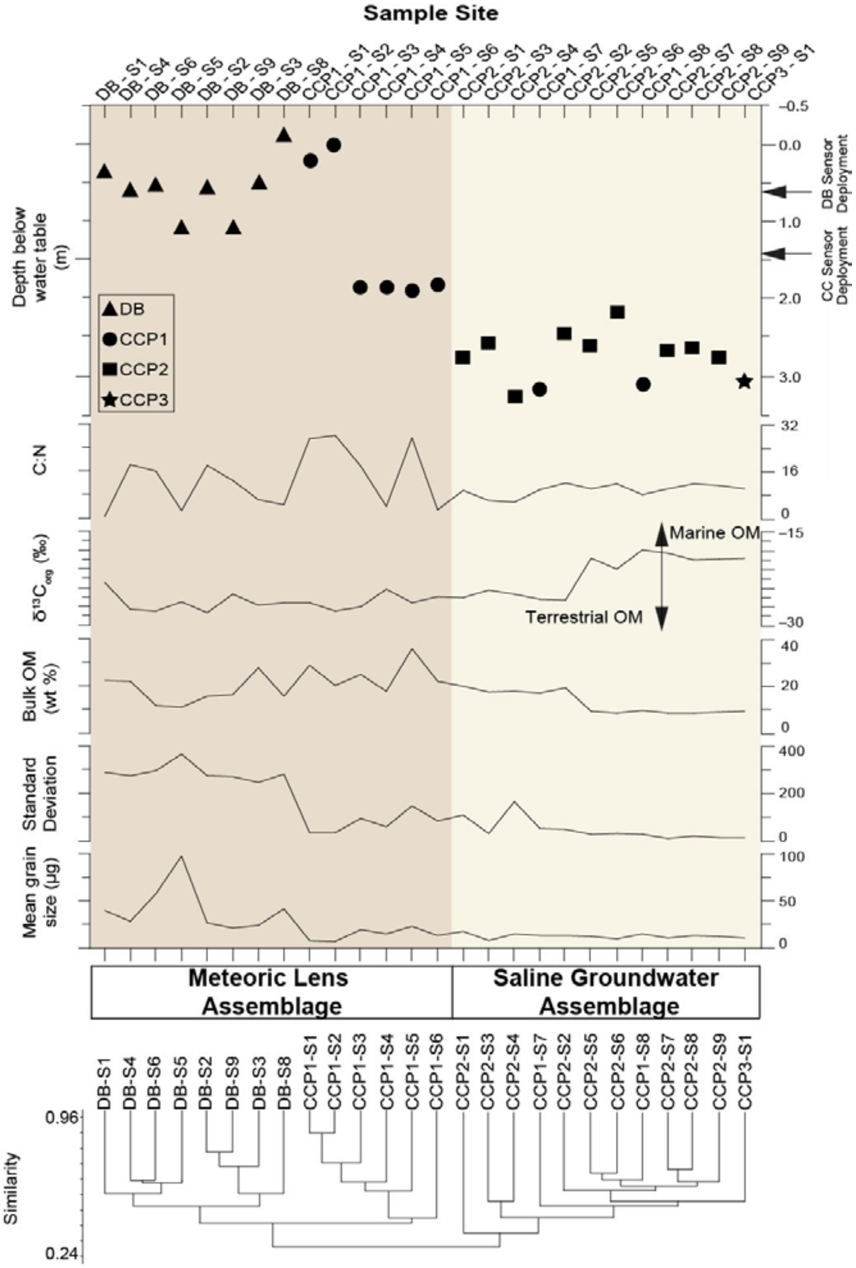


Figure 12. Depth below the water table for each sample in Cow Cave and Deep Blue, organized by grouping in Q-mode dendrogram alongside corresponding textural conditions including: C:N, $\delta^{13}C_{org}$, bulk OM, sediment mean grain size and standard deviation. Arrows indicate deployment of sensor probe in each pool. The samples can be split into two groups: Meteoric Lens Assemblage and Saline Groundwater Assemblage, with notable shifts in isotopic signatures from terrestrial to marine OM.

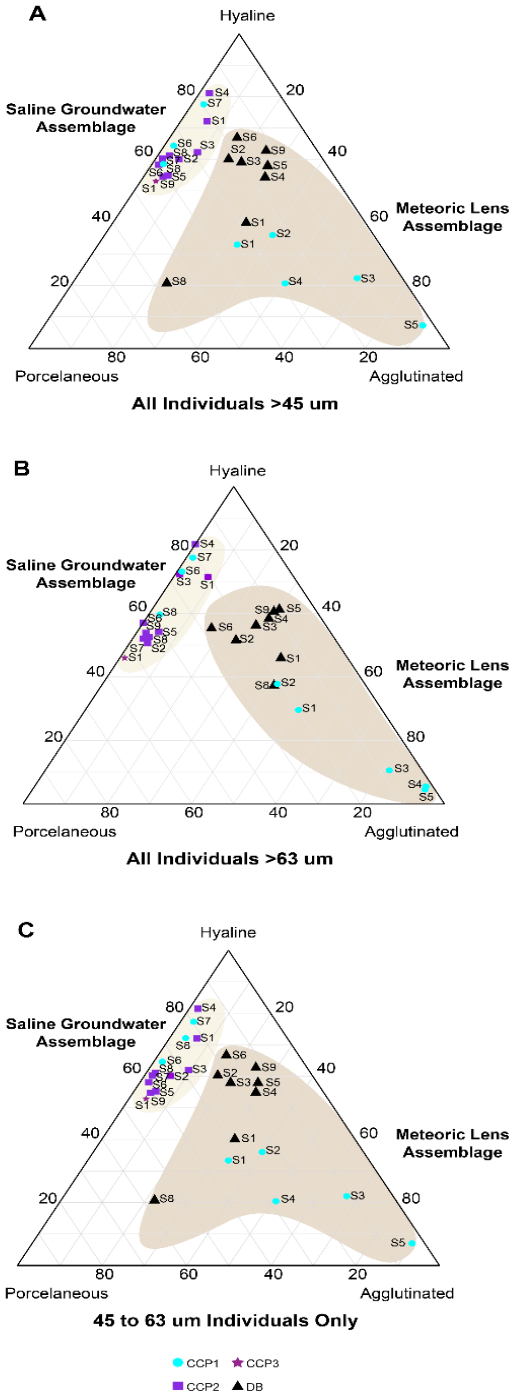
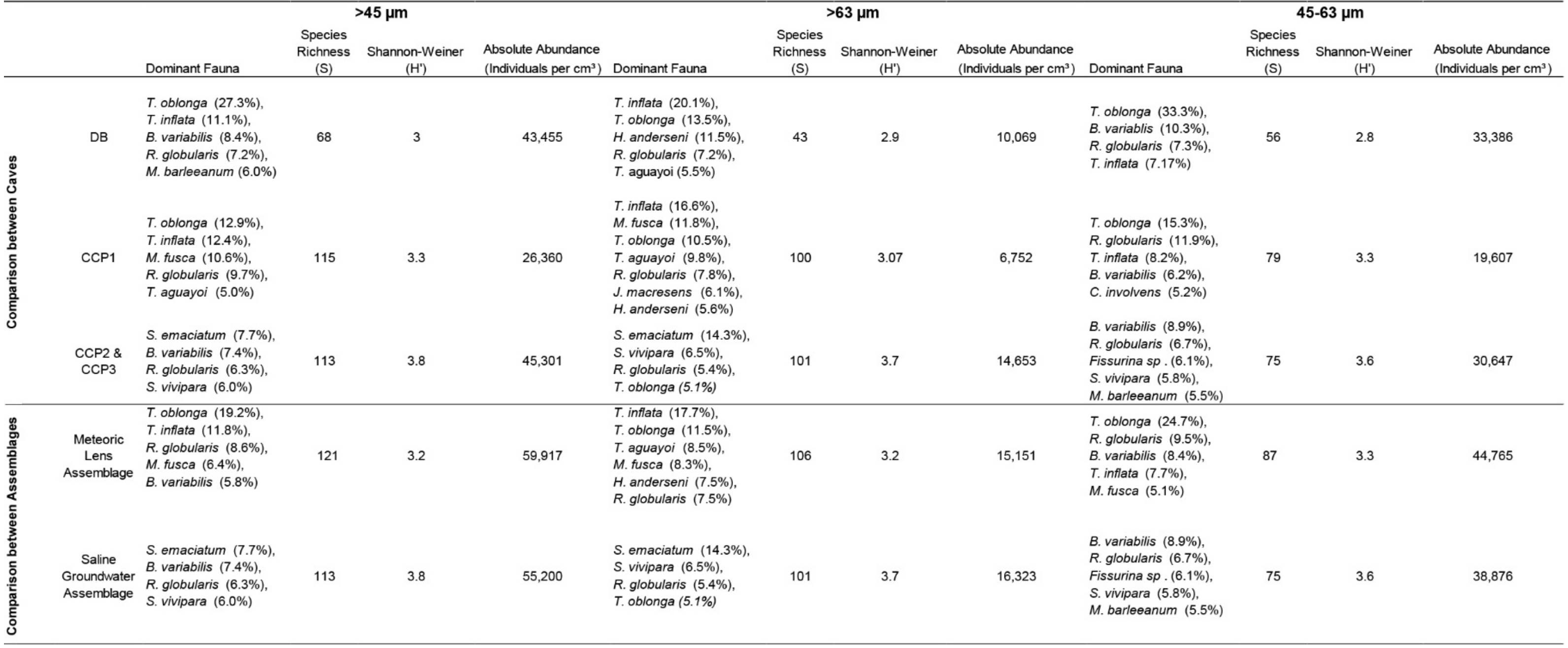


Figure 13. Ternary plots of foraminiferal wall type in all samples from Cow Cave and Deep Blue (n = 26). Colored ellipses correspond to assemblages identified by Q-mode cluster analysis.

The primary difference observed by analyzing the smaller size fraction of benthic foraminifera is an increase in faunal density (absolute abundance), and subtle increases in species richness (*S*) and diversity (H') by including the smaller size fraction (≥45 µm). This is generally observed when comparing the different cave sites (Cow Cave versus Deep Blue) and assemblages (Meteoric Lens versus Saline Groundwater). Between the ≥45 µm and ≥63 µm sizes in Cow Cave, species richness is higher in the ≥45 µm fraction (Table 2). Analyzing only the ≥63 µm size fraction, a small number of taxonomic units are not addressed in CC. The diversity between size fractions remains relatively unchanged in both Cow Cave and Deep Blue (Table 2). The Shannon-Weiner diversity value for the ≥63 µm size fraction for CCP1 and CCP2/3 is 3.1 and 3.7, respectively. Compared to the larger size fraction, the difference in the ≥45 µm values of 3.3 and 3.8 are negligible. Similarly to CC, the overall species richness in Deep Blue is less in the ≥63 µm than in the ≥45 µm however only by ~10 taxonomic units. A total of 68 taxonomic units are identified by using the ≥45 µm fraction, however, if only the ≥63 µm was analyzed the species richness would decrease by 25 taxonomic units. By including the species present in the 45-63 µm size range, the overall species richness for each pool is higher by 12-25 taxonomic units than if only the ≥63 µm group was analyzed. The exclusion of numerous taxonomic units may bias paleoenvironmental interpretations but the foraminifera that are excluded tend mostly to be juvenile individuals of taxa also represented in the ≥63 µm size fraction. Generally, larger sized foraminifera have a higher fecundity rate than smaller individuals, producing proportionally more offspring, potentially leading to an influx of juvenile individuals of a larger size class (Murray, 2014). When comparing the ≥45 µm and ≥63 µm sizes in the Meteoric Lens Assemblage the diversity index scores is the same (H' = 3.2), while faunal density is highest in the ≥45 µm fraction at 121 taxonomic units compared to 106 in the ≥63 µm size fraction. In the Saline Groundwater Assemblage, diversity scores change only slightly from the ≥45 µm to ≥63 µm size fraction (H' = 3.8; H' = 3.7), with a small decrease of 12 taxonomic units (*S* = 113; *S* = 101).

The choice of utilizing the ≥45 µm does not result in any major changes to the inter-pool comparisons between caves or between assemblages. In the ≥63 µm, and ≥45 µm the overall trend in faunal density is that CCP2 and CCP3 contain the lowest density, with Deep Blue having intermediate values, and CCP1 containing the highest abundance (Table 2). While this difference may result in an initial conclusion that Deep Blue contributes the highest density, the fact that the ≥45 µm fraction yields the same conclusion as the ≥63 µm dismisses this. Whether the 45-63 µm size fraction is included or not, the end result will remain the same, with CCP1 having the highest density of benthic foraminifera, and CCP2 and CCP3 having the lowest. species. In Deep Blue, this happens with *Helenina anderseni* and *Trichohyalus aguayoi*. In CCP1, *H. anderseni* and *Jadammina macresens* are excluded, while in CCP2 and CCP3, *Triloculina oblonga* is no longer considered dominant. Whether to include the 45-63 µm size fraction or not, either decision will result in a slight change to the species considered dominant in each pool. When looking at the Meteoric Lens Assemblage, faunal density drops greatly from 59, 917 to 15, 151 individuals per cm^3 between the ≥45 µm and ≥63 µm size fractions respectively. Though the density is nearly halved the ≥63 µm contains the same dominant fauna as the ≥45 µm, including dominant species such as: *T. oblonga, Trochammina inflata, Rosalina globularis,* and *Miliammina fusca*. The Saline Groundwater Assemblage also shares a decrease in faunal density in the ≥63 µm size fraction (16, 323 individuals per cm^3) versus the ≥45 µm (55, 200 individuals per cm^3). Despite the change in density, the dominant fauna remains the same between the two size fractions with *Spirophthalmidium emaciatum, Spirillina vivipara,* and *R. globularis* present as the dominant fauna in the ≥45 µm and ≥63 µm size fraction. Comparing the Meteoric Lens Assemblage to the Saline Groundwater Assemblage, the inclusion of the ≥45 µm sediment size fraction does increase the faunal density, but does not alter the final faunal analysis and resultant environmental interpretations.

Table 2. Diversity, species richness, and dominant fauna in three sediment size fractions comparing Cow Cave and Deep Blue and the meteoric lens and saline groundwater assemblages.



		>45 µm				**>63 µm**				**45-63 µm**			
		Dominant Fauna	Species Richness (S)	Shannon-Weiner (H')	Absolute Abundance (Individuals per cm³)	Dominant Fauna	Species Richness (S)	Shannon-Weiner (H')	Absolute Abundance (Individuals per cm³)	Dominant Fauna	Species Richness (S)	Shannon-Weiner (H')	Absolute Abundance (Individuals per cm³)
Comparison between Caves	DB	*T. oblonga* (27.3%), *T. inflata* (11.1%), *B. variabilis* (8.4%), *R. globularis* (7.2%), *M. barleeanum* (6.0%)	68	3	43,455	*T. inflata* (20.1%), *T. oblonga* (13.5%), *H. anderseni* (11.5%), *R. globularis* (7.2%), *T.* aguayoi (5.5%)	43	2.9	10,069	*T. oblonga* (33.3%), *B. variablis* (10.3%), *R. globularis* (7.3%), *T. inflata* (7.17%)	56	2.8	33,386
	CCP1	*T. oblonga* (12.9%), *T. inflata* (12.4%), *M. fusca* (10.6%), *R. globularis* (9.7%), *T. aguayoi* (5.0%)	115	3.3	26,360	*T. inflata* (16.6%), *M. fusca* (11.8%), *T. oblonga* (10.5%), *T. aguayoi* (9.8%), *R. globularis* (7.8%), *J. macresens* (6.1%), *H. anderseni* (5.6%)	100	3.07	6,752	*T. oblonga* (15.3%), *R. globularis* (11.9%), *T. inflata* (8.2%), *B. variabilis* (6.2%), *C. involvens* (5.2%)	79	3.3	19,607
	CCP2 & CCP3	*S. emaciatum* (7.7%), *B. variabilis* (7.4%), *R. globularis* (6.3%), *S. vivipara* (6.0%)	113	3.8	45,301	*S. emaciatum* (14.3%), *S. vivipara* (6.5%), *R. globularis* (5.4%), *T. oblonga (5.1%)*	101	3.7	14,653	*B. variabilis* (8.9%), *R. globularis* (6.7%), *Fissurina sp* . (6.1%), *S. vivipara* (5.8%), *M. barleeanum* (5.5%)	75	3.6	30,647
Comparison between Assemblages	Meteoric Lens Assemblage	*T. oblonga* (19.2%), *T. inflata* (11.8%), *R. globularis* (8.6%), *M. fusca* (6.4%), *B. variabilis* (5.8%)	121	3.2	59,917	*T. inflata* (17.7%), *T. oblonga* (11.5%), *T. aguayoi* (8.5%), *M. fusca* (8.3%), *H. anderseni* (7.5%), *R. globularis* (7.5%)	106	3.2	15,151	*T. oblonga* (24.7%), *R. globularis* (9.5%), *B. variabilis* (8.4%), *T. inflata* (7.7%), *M. fusca* (5.1%)	87	3.3	44,765
	Saline Groundwater Assemblage	*S. emaciatum* (7.7%), *B. variabilis* (7.4%), *R. globularis* (6.3%), *S. vivipara* (6.0%)	113	3.8	55,200	*S. emaciatum* (14.3%), *S. vivipara* (6.5%), *R. globularis* (5.4%), *T. oblonga (5.1%)*	101	3.7	16,323	*B. variabilis* (8.9%), *R. globularis* (6.7%), *Fissurina sp* . (6.1%), *S. vivipara* (5.8%), *M. barleeanum* (5.5%)	75	3.6	38,876

5.4 Cave specific differences in foraminiferal distributions in the >63 µm fractions

Given the similarity of results between the foraminiferal processing techniques (≥45 µm versus ≥63 µm), the ≥63 µm size fraction will be used to describe differences in foraminiferal distributions between each cave. Foraminiferal distributions in Cow Cave are diverse, with different taxa towards the opening with the terrestrial surface (Fig. 11). In CCP1, closest to the terrestrial environment, Shannon-Weiner Diversity index scores range from 2.1 to 3.6, lowest at the shallow localities (H' = 1.4 at 1.8 m water depth) and increases with depth (H' = 3.6 at 3.1 m water depth). Diversity generally correlates with the isotopic signature of organic matter in each sample, with higher diversity in samples with organic matter containing a higher proportion of marine-derived material in the deeper areas. And, faunal density is generally inversely correlated with diversity (577 individuals per cm^3 at H' = 1.4 versus 826 individuals per cm^3 at H' = 3.6). Faunal density in CCP1 ranged from 577 to 1,661 individuals per cm^3 and Species Richness of CCP1 ranged from 14 (CCP1-S2, CCP1-S4) to 71 (CCP1-S8). In total, 100 taxonomic units were identified throughout all samples in CCP1, with a cumulative diversity of H' = 3.1. Of the taxonomic units identified, the most common species in CCP1 included: *Rosalina globularis, Triloculina oblonga, Trochammina inflata*, and *Miliammina fusca*. Both *T. inflata* and *M. fusca* are agglutinated species commonly associated with areas of high terrestrial signals, including coastal salt marshes. The dominance of agglutinated species is expected considering CCP1 is predominantly high bulk OM content, with a terrestrial, enriched $\delta^{13}C_{org}$ signature, open to the influence of terrestrial events.

In CCP2 and CCP3, a total of 101 taxonomic units were identified in the ≥63 µm fraction with a diversity index of H' = 3.7. Within these two sampling areas, the species richness and diversity are similar in each sample (*S* range: 26 (CCP2-S1) to 54 (CCP2-S5, CCP2-S6; H' = 2.6 (CCP3-S1) to 3.6 (CCP2-S5)), while the faunal density varied greatly (77 (CCP2-S1) to 2895 (CCP2-S5) individuals per cm^3). Though Species Richness between CCP1 and CCP2 & CCP3 was similar, the community composition was quite different. In CCP2 and CCP3, the dominant fauna was hyaline and porcelaneous species, such as *Rosalina globularis, Spirophthalmidium emaciatum, Bolivina variabilis,* and *Spirillina vivipara*. The species *B. variabilis* is an infaunal taxa that is often an indicator species for areas with lower concentrations of dissolved oxygen, while *S. emaciatum* and *S. vivipara* are common in Bermudian anchialine caves.

The samples of Deep Blue displayed low values for diversity, species richness, and abundance, and these characteristics were similar throughout the pool. In total, only 43 taxonomic units were identified for the Deep Blue samples, the dominant species being: *Triloculina oblonga, Trochammina inflata, Helenina anderseni, Rosalina globularis,* and *Melonis barleeanum.* The two most dominant species, *T. oblonga* and *T. inflata,* are the same dominant species associated with CCP1. The Species Richness ranged from 18 (DB-S1, DB-S6) to only 42 (DB-S9) taxonomic units. The diversity index score for Deep Blue was 2.9, however values ranged from 2.2 (DB-S8) and as high as 3.2 (DB-S9). Faunal density in Deep Blue also varied greatly between samples ranging from 350 (DB-S5) to 5,196 (DB-S8) individuals per cm^3.

The proportion of foraminiferal test structure between Cow Cave and Deep Blue was variable, and seemed to correlate with the source of organic material utilized by the benthic foraminifera (Fig. 13). In CCP1, the overall ratio of foraminiferal tests is dominated by hyaline species, 40:24:36, however within the pool there were key distinctions. At the entrance to the pool, samples that were exposed to a more depleted $\delta^{13}C_{org}$ signature displayed high proportions of agglutinated species upwards of 90% (CCP1-S5). However, with a change to a marine $\delta^{13}C_{org}$ signature, agglutinated species were almost nonexistent, with a domination of hyaline and porcelaneous species. CCP2 and CCP3, were consistently devoid of agglutinated species with an average ratio of 63:33:4. Hyaline species remained the dominant wall type throughout both pools, usually comprising 50-80% of the composition. Deep Blue contained wall type ratios similar to all the Cow Cave pools, with an overall ratio of 53:23:24 indicating dominance by hyaline species with more agglutinated species than found in CCP2 or CCP3. As both Deep Blue and CCP1 have more depleted $\delta^{13}C_{org}$ values and receive increased amounts of bulk OM, a higher percentage of agglutinated species would be expected. CCP1 and Deep Blue share a higher proportion of agglutinated fauna (Fig. 13), and both represent shallow water samples collected from a depth ≤2 m that are likely influenced by rainfall (Fig.11).

5.5 Sedimentary characteristics (texture, organic matter, $\delta^{13}C_{org}$, and C:N)

The substrate of each pool of Cow Cave differs in terms of silt:sand:clay ratio and mean grain size, which reflects the dominant local sedimentary processes in the cave (authigenic carbonate production, influx from terrestrial erosion). The majority of the sediment in CCP1 belongs to the silt size class, with an average percent silt value of 68.2%. The coarsest sample within CCP1 is CCP1-S5 with a mean grain size of 24.8 µm and mean standard deviation of 154.5 µm (Fig. 4). CCP1 has an average grain size of 16.1µm and a range of 9.3 µm to 24.8 µm, with a mean bulk organic matter content of 22.6 % (range: 10.1% (CCP1-P1C8) to 36.4% (CCP1-S5)). The stable carbon isotopic ratio varies from −17.82‰ (CCP1-S8) to −27.53‰ (CCP1-S2), with a mean isotopic value of −24.90‰. Organic carbon ranged from 2.66% (CCP1-S6) to 16.3% (CCP1-S3), with a mean %C_{org} of 7.17%. Similarly to %C_{org}, the C:N values in CCP1 were variable with a mean value of 16.09 (range: 3.41 to 28.73).

Samples from CCP2 and CCP3 that are located more distally into the cave have a similar, less variable sediment texture than CCP1. Still dominated by silt-sized particles (mean silt = 71.5% and 77.7%), the grain size in CCP2 ranged from 9.7 µm to 19.6 µm, with the average grain size of 14.3 µm and a standard deviation of 57.9 µm. CCP3 has a similar value for both mean grain size and standard deviation as CCP2, at 12.5 µm and a standard deviation of 20.4 µm. Both pools share similar bulk organic matter content with average values of 13.7% (CCP2) and 10.1% (CCP3). Bulk organic matter in CCP2 varies from 9.0% (CCP2-S6) to 20.5% (CCP2-1). Stable carbon isotopic ratios vary from −25.40‰ (CCP2-S1) to −18.32‰ (CCP2-S7), with a mean value of −21.88 ‰. CCP3 has a $\delta^{13}C_{org}$ value of −19.12‰, which is indicative of a higher proportion of marine-derived organic matter becoming deposited at the sample site. CCP3 and CCP3 have comparatively lower C:N and %C_{org} values than CCP1. In CCP2, the mean C:N is 10.23 (range: 6.04 (CCP2-S4) to 12.43 (CCP2-S2)) compared to the C:N value of 10.57 in

CCP3. The %C_{org} content in CCP2 samples range from 4.01% (CCP2-S3) to 7.21% (CCP2-S6) in CCP2 with a mean 5.40% compared to a mean percentage of 5.87% in CCP3.

Sediment texture in Deep Blue is more variable with a higher bulk organic matter content than Cow Cave. Samples in Deep Blue have a mean grain size of 43.8 µm with a mean standard deviation of 293.4 µm, with a mean bulk organic matter content of 18.5% (range: 28.3% (DB-S3) to 11.6% (DB-S5)). The coarsest sediment was present in DB-S5 (mean particle size: 99.4 µm; standard deviation: 371.6 µm). Sediment samples from Deep Blue also have a variable range of stable carbon isotopic ratio (mean = −26.10‰). The $\delta^{13}C_{org}$ ratio of most samples from Deep Blue range from −27.68‰ (DB-S2) to −22.96 ‰ (DB-S1), which is indicative of predominantly terrestrially-derived organic matter. However, DB-S1 and DB-S9 seem to have a slightly higher proportion of marine-derived OM, with $\delta^{13}C_{org}$ values of −22.96‰ and −24.77‰ respectively. The C:N values in Deep Blue are variable with a mean C:N of 10.38 (range: 18.7 (DB-S4) to 1.14 (DB-S1)), which is indicative of a mixed organic matter signal. Values of %C_{org} in Deep Blue vary with a mean %C_{org} of 4.39% and range from 7.88 (DB-S2) to 1.53% (DB-S5).

6. DISCUSSION

6.1 Implications for calibrating radiocarbon dates from anchialine caves

Terrestrial plant materials remain undoubtly the best choice for developing downcore age models on sediment cores from anchialine caves. The terrestrial plant fragments present in the surface sediment samples from Cow Cave indicate that terrestrial material can effectively become part of the sediment record within a short time of the plants death, as they are less than 50 years old (Table 1). However, the sediment sample from Cow Cave is adjacent to the terrestrial surface where a terrestrial plant macrofossil is likely to become eroded into the cave and become part of the sediment record in a short time span.

However, not all cave depositional histories (van Hengstum & Scott, 2012) and cave geometries (Collins et al., 2015) may promote the deposition of terrestrial plant material in the sediment record. In cases where sedimentation has persisted during marine intervals, alternate material may be required for radiocarbon dating. For example, van Hengstum et al. (2011, 2016) dated carbonate microfossils (ostracodes and foraminifera) and molluscs (bivalves and gastropods) from cave sediment records that accumulated in marine anchialine conditions, and the standard marine reservoir correction was applied in order to calibrate these dates. The modern age dates of the carbonate samples from Cow Cave indicate that the microfossils living in the shallow waters (<4 m) in this locality are in secular equilibrium with atmospheric ^{14}C variability. Therefore, these results indicate that cannot be assumed that the marine reservoir correction can be equally applied to all marine-derived carbonate samples from anchialine caves. Indeed, these results equally do not imply that carbonate samples will not be impacted by a marine reservoir correction, just that core samples will be required to assess any site-specific marine reservoir correction (i.e., paired terrestrial and marine samples). Otherwise, an additional ~400 years of uncertainty exists with these marine carbonate samples.

6.2 Relative impact of using the >45 µm versus >63 µm sediment fractions

Based on the results from this study, the additional time commitment required to analyze the 45-63 µm size fraction from anchialine caves is unnecessary because similar assemblages can be produced by analyzing only the >63 µm size fraction (Fig. 11). The diversity of Cow Cave and Deep Blue does not differ greatly when the >45 µm individuals are considered (Table 2), but diversity typically does increase if the smaller size fraction is included (Table 2). This means that the 45-63 µm assemblage contains many juveniles or smaller microspheric individuals, and omitting these smaller-sized individuals will not bias the resultant paleoecological interpretations (i.e., groundwater salinity or dissolved oxygen levels). Similarly, a high proportion of juveniles or smaller microspheric individuals promotes a higher faunal density when the >45 µm fraction is analyzed. Generally, larger sized foraminifera have a higher fecundity rate than smaller taxa, producing proportionally more offspring, potentially leading to an influx of juvenile individuals in the ≥45 µm fraction (Murray, 2014). In this study there were two instances of species present in the 45-63 µm fraction, that were never identified in the ≥63 µm fraction: *Bolivina striatula* and *Patellinoides sp.*. However, unless the research question is focused on inherently small or rare species, the trends in dominant species are nearly identical in using both size fractions.

The dominant species in both size fractions of the Meteoric Lens Assemblage (CCP1 and Deep Blue) are: *Miliammina fusca*, *Trochammina inflata* and *Triloculina* oblonga (Table 2). This dendrograms. Based on the relative abundance of all species within Cow Cave and Deep Blue, the dendrograms group CCP1 and Deep Blue together using the faunal matrix of ≥63 µm fraction and ≥45 µm fraction. Including the 45-63 µm size fraction did not change the interpretations regarding similarity between cave pools, just as its inclusion did not benefit diversity indices. The choice to ignore the smaller foraminiferal tests also does not bias the proportion of foraminiferal wall structure represented in the final assemblages (Fig. 13). Regardless of sediment size fraction analyzed (i.e., ≥45 µm or ≥63 µm fraction), CCP1 and Deep Blue consistently grouped together as the Meteoric Lens Assemblage with a predominance of brackish-water tolerant species. The Saline Groundwater Assemblage consistently emerges as CCP1-S7, CCP1-S8, CCP2, and CCP3 group together, sharing a dominance of hyaline and porcelaneous taxa.

6.3 Potential environmental drivers of foraminiferal distributions

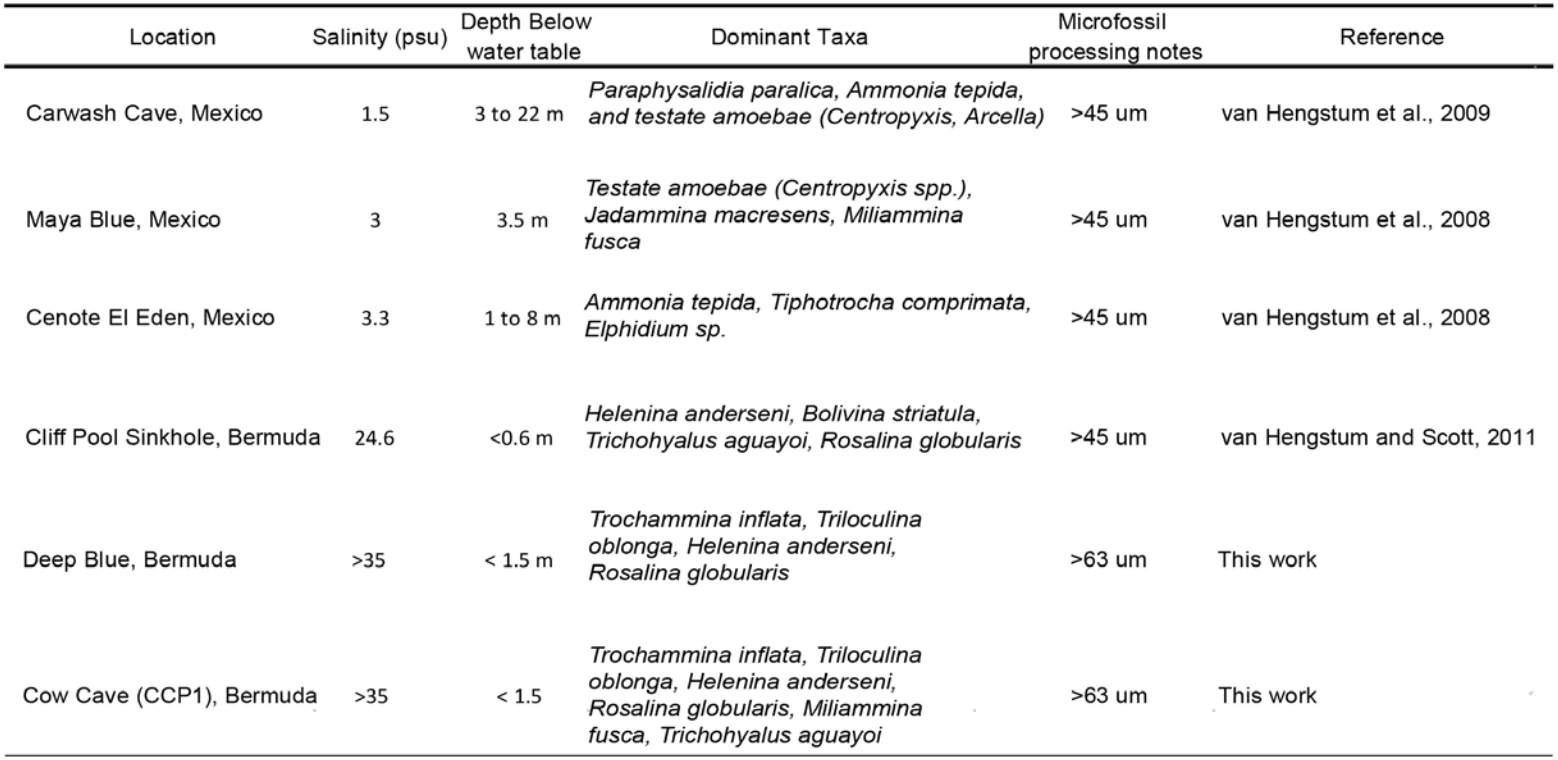
It is interesting that CCP1 is more similar Deep Blue (Meteoric Lens Assemblage) than either CCP2 or CCP3, which are <20 m away, especially considering the differences in hydrographic conditions overall between the two different caves. During tidal cycles, Cow Cave experiences a greater fluctuation in temperature, pH, salinity, and dissolved oxygen relative to Deep Blue, whereas conditions at Deep Blue are more hydrographically stable (Fig. 10). The range in temperature in both pools is within the tolerances of most tropical species of benthic foraminifera, and is likely not the controlling factor. Dissolved oxygen is an ecologically significant variability for foraminifera (Duffield et al., 2015; Jorissen et al., 1995), and the Meteoric Lens Assemblage in Cow Cave and Deep Blue are in water with a dissolved oxygen level of 4.7 ± 1.0 mg L^{-1}, and 3.5 ± 0.07 mg L^{-1}, respectively. These sites have a similar dominant fauna (*Triloculina oblonga*, *Trochammina inflata*, *Rosalina globularis*), but species richness at CCP1 (*S* = 100) doubles Deep Blue (*S* = 43) where *Miliammina fusca* and *Jadammina macrescens* are also dominant (Table 2). These species are commonly associated with brackish water conditions (e.g. coastal marshes) in coastal environments along the Eastern and Western United States and Canada, as well as noted in Japan (Murray et al., 2011; Scott et al., 1996). So although dissolved oxygen is likely impacting the diversity of the benthic foraminiferal community at the two sites, the dominant taxa in the Meteoric Lens Assemblage (Deep Blue and CCP1) are all tolerant of brackish water. Low pH can also have detrimental impacts on the benthic foraminiferal population (Uthicke et al., 2013), which given the lower pH at Deep Blue is likely creating additional stress on the benthic foraminifera. However, if pH was the primary factor driving foraminiferal assemblages, then one would expect all samples from Cow Cave to plot together on the dendrograms as this locality experiences pH at least tidally close to oceanic conditions (pH ~8) (Fig. 11). As such, it would be expected that if hydrographic variables at each site (dissolved oxygen, pH, and temperature) were controlling the foraminiferal assemblages, then all sample stations from Cow Cave would plot together on the resultant dendrograms.

However, CCP1 and Deep Blue both have in common (a) sample locations that are shallower in the coastal aquifer (groundwater, Fig. 12), and (b) an are positioned to more likely to receive a higher degree of influence from the adjacent terrestrial surface based on the caves geometry (e.g. rainfall flushing and terrestrial sediment supply). Both CCP1 and Deep Blue were obtained from depths where the influence of rain water could directly influence salinity and pH with meteorological values. Intense rainfall events could produce a ‘hosing affect’, whereby increased quantities of terrestrial OM and rainwater is delivered to the foraminiferal habitat. In contrast, the Saline Groundwater Assemblage (CCP1-S7, CCP1-S8, CCP2, and CCP3) are all located deeper in the coastal groundwater or further away from terrestrial entrances and likely do not experience the same impact of rainfall. Therefore, CCP2 and CCP3 cannot be expected to have the same textural, geochemical, or hydrographic variation as CCP1 and Deep Blue. It is not surprising then that the two samples from deeper in the coastal aquifer (CCP1-S7, CCP1-S8) are more similar to the deeper samples of the Saline Groundwater Assemblage.

6.4 Can shallow-water benthic foraminifera document flooding of anchialine caves?

Clearly, benthic foraminifera living in anchialine environments are capable of distinguishing the difference between the two primary subsurface water masses in carbonate coastal aquifers. However, benthic foraminifera and other microfossils (e.g. testate amoebae, ostracodes) appear to more aptly differentiate the salinity of the meteoric lens, rather than the thickness of the meteoric lens itself. The faunal distribution of Cow Cave and Deep Blue have resulted in groups of species related to depth in the coastal aquifer. First, a Meteoric Lens assemblage was recovered in areas < 2 m deep in the aquifer and dominated by agglutinated and brackish-tolerant species in areas likely influenced by increased flux of rain water. These taxa include *Miliammina fusca, Jadammina macresens, Trochammina inflata, Triloculina oblonga, Helenina anderseni* and small densities of *Bolivina spp.*. Second, a saline Groundwater Assemblage dominated by hyaline and porcelaneous taxa was recovered from water depths ≥2 m. In these areas (≥2 m), there is a faunal shift to porcelaneous and hyaline species, including *Spirillina vivipara*, *Cyclogyra involvens, Mychostomina revertens, Patellina corrugata, Sigmoilina tenuis* and *Spirophthalmidium emaciatum*. Elsewhere, meteoric lenses that are thicker and more limnic also have benthic foraminiferal assemblages, but the diversity of benthic foraminifera decreases as the meteoric lens becomes fresher (Table 3). As such, benthic foraminifera of core based reconstructions of anchialine environments will be better indicators of salinity, and moreover the specific water mass that was previously flooding a cave, than a specific water depth. Therefore, when a cave system first begins to flood, one would expect to find a community of benthic foraminifera living in a meteoric lens, potentially similar to the assemblage in this study if the paleo meteoric lens was thin and only seasonally brackish. However, if the paleo meteoric lens was fresher, one would potentially expect to find assemblages with decreased diversity and potentially testate amoebae. With continued sea-level rise, the cave benthos may potentially become inundated by saline groundwater, which should cause assemblage to shift away from the dominance of brackish-tolerant taxa.

Table 3. Comparison of dominant benthic foraminifera and testate amoebae recovered from habitat flooded by the meteoric lens in previous work (Carwash Cave: van Hengstum et al., 2009, Maya Blue, El Eden: van Hengstum et al., 2008, Cliff Pool Sinkhole: van Hengstum et al., 2009, Maya Blue, El Eden: van Hengstum et al., 2008, Cliff Pool Sinkhole: van Hengstum and Scott, 2011).



Location	Salinity (psu)	Depth Below water table	Dominant Taxa	Microfossil processing notes	Reference
Carwash Cave, Mexico	1.5	3 to 22 m	*Paraphysalidia paralica, Ammonia tepida, and testate amoebae (Centropyxis, Arcella)*	>45 um	van Hengstum et al., 2009
Maya Blue, Mexico	3	3.5 m	*Testate amoebae (Centropyxis spp.), Jadammina macresens, Miliammina fusca*	>45 um	van Hengstum et al., 2008
Cenote El Eden, Mexico	3.3	1 to 8 m	*Ammonia tepida, Tiphotrocha comprimata, Elphidium sp.*	>45 um	van Hengstum et al., 2008
Cliff Pool Sinkhole, Bermuda	24.6	<0.6 m	*Helenina anderseni, Bolivina striatula, Trichohyalus aguayoi, Rosalina globularis*	>45 um	van Hengstum and Scott, 2011
Deep Blue, Bermuda	>35	< 1.5 m	*Trochammina inflata, Triloculina oblonga, Helenina anderseni, Rosalina globularis*	>63 um	This work
Cow Cave (CCP1), Bermuda	>35	< 1.5	*Trochammina inflata, Triloculina oblonga, Helenina anderseni, Rosalina globularis, Miliammina fusca, Trichohyalus aguayoi*	>63 um	This work

6.5 Future work

In future studies, the influence of %OM could be improved upon through more refined methods of determination, as compared to overly broad LOI analysis. While LOI can provide an idea of bulk organic matter, obtaining $\delta^{15}N$ values would allow for increased accuracy in terms of C:N ratio as well as a better representation of the marine organic matter contributing to total organic matter values. Perhaps the contribution of $\delta^{15}N$ will prove an important variable in those samples lacking terrestrial organic matter influx, such as Cow Cave Pools 2 and 3. In this sense, an analysis of the feeding behavior of foraminifera on phytoplankton and algae would also shed light on the role of feeding on foraminiferal species distribution, and possibly the still unexplained environmental link to absolute abundance. Broader still is the possible influence of sunlight attenuation throughout the cave pools and the possible role this may play on the degradation of organic matter as it travels through the water column to the benthic community, or on potential diatom distributions. Additionally, the presence or absence of sunlight will be an important consideration if choosing to factor in the feeding habits of herbivorous foraminifera on photosynthetic algae. It may be of benefit in future studies to introduce a bathymetric aspect which includes flow meters so that circulation within cave pools can captured. The way in which nutrients cycle throughout the pool may be a function of bottom topography and flow, providing certain areas with more or less organic matter, especially in areas where sunlight and terrestrial organic matter is limited. Finally, although it was only touched on in this study, we now know that the pH of these anchialine cave pools can reach harmful levels for calcareous fauna (e.g. foraminifera) (Uthicke et al., 2013). Variations in the pH value of marine environments below a pH of 7.9 has the potential to decrease diversity and faunal density. Further investigation may clarify to what extent pH influences these shallow water assemblages in Cow Cave and Deep Blue, and additionally shed light onto the limits of ocean acidification of benthic foraminifera in such environments. As the issue of ocean acidification becomes more of a concern in the world's oceans, it would be advantageous to explore this idea in a unique setting.

7. CONCLUSION

1. Radiocarbon dating of terrestrial and marine material from Cow Cave, Bermuda, yielded modern age dates. These results indicate that the marine reservoir correction is not always necessary in the calibration of radiocarbon dates from marine settings in anchialine environments.
2. The use of the 45-63 μm size fraction is unnecessary in the anchialine cave systems of Bermuda, and possible other cave systems as well. The inclusion of this size fraction does not impact diversity, abundance, or the overall ecological interpretations. As identification of individuals below 63μm is difficult and time consuming, there is no scientific advantage to justify the effort expended.
3. The shallow benthic foraminifera analyzed in this study segregate into two groups: (a) a Meteoric Assemblage of individuals inhabiting shallow waters (<2 m), (b) a Saline Groundwater Assemblage of individuals inhabiting deeper waters (≥2 m). Depth below the water table, quality and quantity of organic matter, and environmental variables associated with rainfall are the largest contributors to faunal distributions in Cow Cave and Deep Blue. However, shallow water areas (≤2 m) are vulnerable to the influence of rainfall washing in larger grain sizes, increased terrestrial OM, and changes to hydrographic conditions (e.g. salinity and pH). The fauna associated with these areas are predominantly brackish-tolerant species (e.g. *T. oblonga, T. inflata,* and *M. fusca*).
4. This study indicates that subfossil benthic foraminifera can be used to help differentiate benthic habitats in anchialine cave environments that are flooded by the meteoric lens versus saline groundwater.

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