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COMPARISONS OF DISTRIBUTIONS AND ISOTOPIC GEOCHEMISTRY OF BENTHIC FORAMINIFERA FROM SEEP AND NON-SEEP ENVIRONMENTS, OFFSHORE OF COSTA RICA

A thesis

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Master of Science

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by

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Keywords: Benthic foraminifera, Methane seeps, Costa Rica, *Cibicides wuellerstorfi*, Elevated epibenthic ecology

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ABSTRACT

Vertical distribution patterns and stable isotopic geochemistry of benthic foraminifera labeled with CellTracker Green and stained with Rose Bengal were compared at sites of active methane seepage and adjacent non-seep habitats off the Pacific coast of Costa Rica. Sediment cores of bacterial mats from Costa Rica revealed vertical distribution patterns more similar to those seen previously in clam beds, suggesting increased levels of bioturbation compared to non-seep sites. Similar taxa were found at both seep and non-seep sites including: *Chilostomella oolina, Uvigerina peregrina* and *hispida, Cibicides mckannai,* and *Cassidulina braziliensis*. Within active methane seep habitats, elevated substrate such as carbonate rocks, and vestimentiferan tubeworms were examined for living foraminifera. Vestimentiferan tubeworms had highly variable numbers of attached epibenthic foraminifera, dominated by *Cibicides wuellerstorfi* and *Carpenteria monticularis*. Stable carbon isotopic comparisons between epibenthic foraminiferal species of *Cibicides wuellerstorfi* and the vestimentiferan tubeworms on which they reside revealed 10‰ to 30‰ differences between the foraminiferal carbonate and substrate, suggesting that the geochemical signatures of elevated epibenthics were not significantly influenced by the geochemical signature of the substrate on which they reside. This study finds no apparent methane influence on the foraminiferal calcite of elevated epibenthic foraminifera from the three active seep sites studied (Mound 11, Mound 12, and Jaco Scar). This

may be because the elevated epibenthics were not exposed to seep-influenced fluids by inhabiting raised substrates.

This study also provides a quantitative analysis of coiling directions in elevated epibenthic species at seeps, which has never previously been reported. Statistical analysis revealed that there were no significant differences in isotopic composition between sinstral (left) and dextral (right) coiling *Cibicides wuellerstorfi.* The results of this study suggest that coiling direction of elevated epibenthic foraminifera, such as *Cibicides wuellerstorfi* and *Carpenteria monticularis*, is a result of biologic factors rather than environmental influences.

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I would like to dedicate this thesis to Coach Donna J. Newberry. Your legacy will live on in the character, dedication, and mental toughness you instilled in all of your players. Thank you for showing me that nothing in life is free. I would not be where I am today without your lessons.

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INTRODUCTORY AND BACKGROUND MATERIAL

Introduction

Cold hydrocarbon seeps have recently received a great deal of attention from a number of disciplines, including the study of physiological adaptations to high sulfide environments, investigations into slope stability, exploitation as an environmentally sustainable energy resource, and as a causal mechanisms for sudden global climate change (R. Martin *et al.*, 2010). The geochemistry of foraminiferal carbonate has been used to infer information about the organism itself as well as the ambient environment in which the test was precipitated (Katz *et al.*, 2010). Although the isotopic geochemistry of fossil foraminifera has been suggested as a means to assess ancient methane flux (Kennett *et al.,* 2000), questions persist regarding the factors that influence the isotopic composition of foraminiferal carbonate found at seeps (Rathburn *et al.,* 2003; Bernhard *et al.,* 2010). In particular, the causes of the significant disequilibrium between the carbon isotopic signals in foraminiferal carbonate recovered from methane seeps and those of ambient dissolved inorganic carbon remain elusive (e.g. Rathburn *et al.,* 2003; Bernhard *et al.,* 2010). Naturally occurring methane seeps provide a unique opportunity to investigate the influence of methane on foraminiferal ecology and geochemistry.

As part of an ongoing project examining the ecology and biogeochemistry of modern methane seep ecosystems, vertical distribution patterns of living infaunal benthic foraminifera (CellTracker Green labeled or Rose Bengal stained individuals) were compared at sites of active methane seepage and adjacent non-seep habitats off the Pacific coast of Costa Rica. The ecology and stable isotopic compositions of epibenthic foraminifera, those living at or above the sediment water interface, collected from hard substrates at and above the sediment water interface within sites of active seepage were also examined.

Foraminifera

Due to their relatively short life spans, cosmopolitan distribution, and long geologic record, foraminifera have become an important tool in paleoceanographic reconstructions (Boersma, 1978; Jorissen *et al.,* 2007). General characteristics that separate foraminifera from other protists include fine, thread-like pseudopodia and the test (shell) that surrounds the organism (Sen Gupta, 1999). Foraminiferal growth is accomplished in one of two ways. Unilocular foraminifera grow by increasing the size of a single chamber, but more commonly, foraminifera grow through the addition of new chambers (Sen Gupta, 1999). Nearly all foraminifera have a test, which may be organic, composed of agglutinated particles, or secreted as calcium carbonate, depending on the species (Sen Gupta, 1999). Of these varieties of test composition, calcium carbonate tests are more prevalent in the paleorecord due to the greater potential for fossilization.

Benthic foraminifera make up the majority of benthic organism biomass in the most extensive ecosystem on Earth, the deep sea (Thomas, 2007). Because of their sensitivity to perturbations in the environment and global distribution, they are prime candidates for unveiling past climatic conditions. Benthic foraminifera have been used as a proxy for a wide variety of

oceanographic variables including bathymetry, organic flux, temperature, bottom water chemistry, and oxygen concentrations in bottom and pore-waters (Gooday 2003; Jorissen *et al.,* 2007).

Accurate interpretation of a proxy is based on a thorough understanding of all of the variables that can influence the proxy (Jorissen *et al.,* 2007). Thus, the study of modern analogs is essential for calibration of proxies derived from foraminifera. Stable isotopic signatures of benthic foraminifera are used for assessments of paleoceanographic conditions, therefore, it is crucial to understand the factors that influence stable isotopic signatures of foraminifera in the modern ocean. The study of modern methane seeps provides a unique opportunity to study and document the effect methane seepage has on the stable isotopic signatures and ecology of modern benthic foraminifera. Investigation into geochemical influences of foraminiferal carbonate is important because it allows for observations of the influence of methane on foraminiferal carbonate. Large scale methane emissions have been implicated as a mechanism for rapid climate change in the geologic past based on $\delta^{13}C$ records (Kennett *et al.,* 2000; Wefer *et al.,*1994). For these reasons it is vital to understand the environmental factors that contribute to the $\delta^{13}C$ values in the modern ocean.

Background

Below the surface of the ocean, methane seeps can exist in water depths of a few meters to a few thousand meters. At shallow seeps, macrofaunal food sources can be photosynthetically based, but the flux of phytodetritus is not adequate to sustain the large communities of deeper seeps. The hydrocarbons of these seeps can fuel chemosynthesis, a pathway deriving energy from chemical reactions, as the basis of these ecosystems (Levin, 2005). The unique geochemistry of pore-waters at methane seeps results in the colonization of specialized

organisms, adapted to these extreme (high sulfide) environments, which are able to use the methane as their carbon source (Levin *et al.,* 2003). The presence of these specialized organisms, such as vestimentiferan tubeworms, *Beggiatoa* bacterial mats, and vesicomyid clams, have become visual indicators of methane seep areas when seepage is not visible (via bubbles). Although organisms have adapted to life in these extreme environments, no endemic benthic foraminifera have yet been discovered at modern methane seeps (Sen Gupta *et al.*, 1997, 2007; Rathburn *et al.*, 2003; Panieri *et al.*, 2003).

Vestimentiferan tubeworms and carbonate rocks at active seep sites in Costa Rica provide the hard substrates to which a number of organisms become attached to and live on. Vestimentiferan tubeworms, composed of a soft bodied worm residing in a chitinous tube, are among the largest worms on this planet, some reaching over 3 meters in length (Boetius, 2005). In the study area, vestimentiferan tubeworms vary in size from tens of centimeters to meters in length. On the seafloor these tubeworms are generally found in "bushes", some of which can be as large as a small car.

Study Area

Converging plates off the Pacific coast of Costa Rica have resulted in subduction erosion, a process which destroys continental crust and returns it to the mantle, as the Cocos plate is squeezed by the overlying Caribbean plate subducting at a rate of about 88mm/yr (see Figure 1; Tryon *et al.*, 2010; Sahling *et al.,* 2008; Kukowski and Oncken 2006). As these massive plates collide, the underlying sedimentary material is compacted and the liquids that comprised the pore-waters concentrate and ultimately seep to the sediment water interface. These types of seeps are often referred to as "cold seeps" because the seeping fluids are comparable in temperature to

those of the surrounding waters (Levin, 2005; Sahling *et al.,* 2008). These seeping liquids can be enriched with hydrocarbons such as methane.

Sampled locations in this study reside off the Pacific coast of Costa Rica (Figure 1). Three main seep structures in the area were sampled (Mound11, Mound 12, and Jaco Scar; see Figures 2-4). Mound 11 and 12 are mud extrusions, formed as a result of seep activity (Mau, 2004). Jaco Scar is a steeply sloped scar feature resulting from the subduction of a seamount (Ranero *et al.,* 2008). These sampled areas are all at a depth of ~1,000m with an average bottom water oxygen level of 0.73mL/L (32.56μM) and temperatures ~4°C (Figure 4). Three pushcores were taken in bacterial mats at Mound 12 at depths ranging from 995-1001m (AD4511 TC2, AD4586 TC4, AD4587 TC2). Nearby non-seep samples were also taken to compare with active seep sites and were collected from 1 to 1.5km from active seep areas. These nearby non-seep environments are similar to the seep sites in depth, temperature, and bottom water oxygen concentration with the only difference between seep and non-seep sites being methane activity. Four non-seep sites were sampled using an Oceans Insturments multicore (MC1 TC7, MC2 TC 2, MC3 TC2, MC4 TC2). Isotopic data was obtained from attached elevated epibenthic foraminifera collected from several tubeworms collected from Mound 11, Mound 12, and Jaco Scar. Core designations and locations are provided in Table 1.

Objectives

The objectives of this study are the following:

1. Compare, for the first time, elevated epibenthic foraminiferal carbonate geochemistry with that of tubeworms on which they were living.

2. Provide one of the few quantitative data sets of elevated epibenthic foraminifera (species elevated above the sediment water interface attached to hard substrates) living in methane seep habitats;
3. Investigate, for the first time, coiling direction of elevated epibenthic seep foraminifera;
4. Compare foraminiferal abundances on vestimentiferan tubeworms at seep sites;
5. Investigate differences and similarities between two methods used to distinguish living foraminifera (CTG and RB).

This study provides one of the few data sets of $\delta^{13}C$ values from elevated epibenthic species attached to tubeworms at sites of active methane seepage. It also investigates the possibility of transfer of $\delta^{13}C$ values from the substrate directly to foraminiferal calcite as suggested by Mackensen *et al.* (2006). Finally, results from this study provide important clues about the factors that control the distribution and isotopic biogeochemistry of foraminifera on hard substrates and within sediments at seeps.

Microhabitats

Live benthic foraminifera have been found from elevated substrates above the sediment water interface to a depth of 15cm within the sediment (Corliss and Emerson, 1990). Microhabitat preference labels have been assigned based on the tendency of some foraminiferal species to occupy a certain microhabitat niche (Jorissen, 1999). Microhabitats are subenvironments within the habitat. Variations can exist in chemical and physical characteristics between microhabitats, thereby influencing the species of foraminifera that are able or likely to inhabit specific microhabitats (Jorissen, 1999).

In order to determine microhabitat preference, it must first determine how to describe the

interval at which a microhabitat preference exists. Jorissen *et al.* (1995) suggested the average living depth (ALD) of foraminifera could help quantify depth distribution patterns. ALD is defined as:

$$ALD_x = \sum_{i=1,x} \frac{n_i d_i}{N}$$

Where x is the depth of the deepest layer, n_i the number of foraminifera of a species in the ith sediment layer. d_i is the depth midpoint of the ith layer, and N is the total number of specimens in all layers. This formula facilitates comparisons between species living within seafloor sediments.

A number of microhabitat preference categories have been proposed. To describe vertical distribution patterns of benthic foraminiferal taxa typical microhabitat categories are assigned, most definitions of which include the terms “epifaunal”, “shallow infaunal”, and “deep infaunal” (Jorissen, 1999). Epifaunal species are living individuals that occur on the surface and within the top 1cm of sediment (Jorissen 1999). Due to the difficulty and uncertainty of sampling only the sediment-water interface, Buzas *et al.* (1993) argues that the term “epifaunal” be reserved only for those species inhabiting an elevated habitat (Jorissen *et al.,* 1995). For the purposes of this study, foraminifera found attached to rocks and vestimentiferan tubeworms will be referred to as “elevated epibenthics”. Species inhabiting the 0-1cm interval are referred to as “epifaunal”, but the remainder of species occupying the environments below the 0-1cm depth are termed “infaunal” and further divided into “shallow” and “deep” infaunal, as has been previously described (Jorissen *et al.,* 1995; Jorissen 1999). Corliss and Emerson (1990), described “shallow infaunal” species as those able to live from 0-2cm in the sediment, followed by “intermediate infaunal” species residing in the 1-4cm interval (Rathburn and Corliss, 1994), and “deep infaunal” species found below 4cm. Infaunal foraminifera can migrate within the sediments in

response to changes in microhabitat conditions (Corliss and Emerson,1990; Jorissen, 1999). Depth categories merely reflect preferences for typical characteristics of environments at depths within marine sediments. For the purpose of this study "shallow infaunal" will refer to those able to inhabit the 0-2cm interval and "deep infaunal" as those able to inhabit sediments below 2cm (Figure 5).

Jorissen (1999) classified microhabitat categories based on vertical distribution patterns. In this system, the vertical distribution pattern found in a sample should fit into one of four type categories (Figure 6). A Type A profile exhibits a population maximum near the sediment surface. Type B profiles exhibit fairly stable populations in the upper part (several centimeters) of the sediment column followed by a fairly sharp decline in deeper layers. Type C profiles exhibit one or more subsurface maxima. Type D profiles possess irregular patterns with a surface maximum and one or more subsurface maxima.

Benthic foraminiferal abundances are influenced by a large number of variables. A strong correlation between foraminifera and a single variable is typically only found under extreme conditions where a single variable is the limiting parameter (Thomas, 2007). While variables such as sediment grain size and temperature can influence foraminiferal distributions, the availability of oxygen and food are thought to be primary influences on the character and distribution of benthic foraminifera (e.g., Corliss and Emerson, 1990; Jorissen *et al.,* 2007). The influences of oxygen and food availability (carbon flux) are intricately tied together and difficult to separate. Several studies of living faunas indicate that food supply is of prime importance and that oxygen concentrations becomes a major factor only where high organic input leads to oxygen depletion (Sen Gupta *et al.,* 1993; Gooday, 1994; Thomas, 2000). Corliss and Emerson (1990) inferred the zonation of microhabitats as the result of a response to a chemical or physical

gradient. Corliss and Emerson (1990) observed a correlation between habitat and the oxic layer, indicating that the species-specific microhabitat preference occurs at or near the lower limit of the oxic layer. Jorrisen *et al.* (1995) incorporated the ideas of Corliss and Emerson (1990) into the "TROX model" (trophic conditions and oxygen concentrations) wherein the interactions between carbon flux, oxygen, and microhabitat are visually displayed. Zwaan *et al.* (1999) modified this concept in the TROX II model by allowing the model to account for additional variables such as competition for labile organic matter and other geochemical changes. These models illustrate that oxygen is a controlling factor only where it is absent or in limited supply. Where there is oxic bottom water and an oxic surface layer of sediment, oxygen is no longer a limiting factor, and other variables, such as food availability, become the limiting factor (Murray, 2006).

Rathburn and Corliss (1994) examined foraminifera from the Sulu Sea, where dissolved oxygen was uniform despite changes in water depth, and organic input was variable. Rathburn and Corliss (1994) showed that foraminiferal assemblages changed as a result of organic carbon flux and discourage the use of foraminiferal assemblages to determine bottom oxygen conditions because oxygen concentrations may not be the limiting factor. Differences in the degradation state of organic carbon are utilized by different species of foraminifera. Therefore, the type of organic carbon available can also produce variations in assemblage patterns and these effects cannot be determined without direct correlations between components of organic carbon and fauna (Jorissen *et al.,* 2007).

Stable Isotopes of Benthic Foraminiferal Carbonate

Isotopic and elemental analyses of the carbonate tests of foraminifera are commonly used for the interpretation of past ocean conditions. By determining the isotopic composition of

foraminiferal tests and understanding the relationship between surrounding conditions and these foraminiferal isotopic values, environmental conditions at the time of calcification can be evaluated. Geochemical signatures derived from the tests of foraminifera have been used as proxies for some aspects of past environments, and as with any proxy, calibration is required. Therefore, studies of living foraminifera and the factors that influence their distribution and test geochemistry help create more accurate proxies through an understanding of the relationships between environmental variables and foraminiferal abundances, assemblages, and geochemical signatures (Sen Gupta, 1999).

The phrase "vital effects" refers to the biological characteristics of an organism that influences the fractionation of isotopes or selection of elements that may be incorporated into the test (Sen Gupta, 1999). The term "vital effect" can be applied to a variety of organisms and encompasses different biological attributes for different organisms, as well as species. This biologic constraint can be different between species and therefore, the vital effect of specific species must be known for interspecies comparisons (Sen Gupta, 1999).

Carbon isotopic values obtained from benthic foraminiferal tests may be influenced by direct factors, such as vital effect, and indirect influences, including organic carbon flux and accumulation, microhabitat, and bottom water chemistry, including the presence of methane (Rathburn *et al.,* 1996). McCorkle *et al.* (1990; 1997) examined the stable isotopic signatures of benthic foraminifera from different microhabitats, and found that pore-water geochemistry influences the $\delta^{13}C$ value of tests. The carbon isotopic composition of foraminiferal calcite of many species of benthic foraminifera is strongly influenced by the $\delta^{13}C$ of the water they live in (bottom and pore water DIC; McCorkle *et al.,* 1990). In general, the deeper (in the sediments) dwelling foraminiferal species, are more depleted in ^{13}C relative to their more shallow dwelling

counterparts (McCorkle *et al.,* 1990). Microhabitat preference can be influenced by ambient conditions such as organic carbon flux at the sediment water interface and oxygenation in the benthic environment (McCorkle *et al.,* 1990, 1997; Filipsson *et al*., 2004; Corliss *et al*., 2006; Fontainer *et al*., 2008). Within a given region, each foraminiferal species typically has a relatively narrow range of $\delta^{13}C$ values (e.g., McCorkle *et al.,* 1990, 1997; Rathburn *et al.,* 1996). Rathburn *et al.* (2000) observed that even species with a broad sediment depth range tend to have low variability in $\delta^{13}C$ values between individuals regardless of the sediment depth where the individual was collected. This raises questions as to whether the pore-waters are the sole influence on the geochemistry of foraminiferal carbonate or if species-specific influences are also present.

Carbon isotopic values of pore-water DIC and foraminiferal carbonate often decrease with depth (McCorkle *et al.,* 1990). It is thought that foraminifera use the DIC of the surrounding pore-waters as source of carbon, thus pore-water DIC can be a direct influence on foraminiferal $\delta^{13}C$ values (Rathburn *et al.,* 2003). Due to this relationship, influences on pore-water DIC therefore can influence $\delta^{13}C$ values of foraminifera, allowing for ambient conditions, along with biologic factors and ecological preferences to be either direct or indirect influences (Rathburn *et al.,* 2003). At areas of methane seepage, seep activity levels and the dispersal of methane rich fluids through the sediments create a highly variable environment, perhaps creating highly variable pore-water DIC. Differences in the composition of chemosynthetic communities have been attributed to fluid flow rate (Sahling *et al.,* 2008). In general, vesicomyid clams are associated with lower rates of seepage than bacterial mats (Mau *et al.,* 2006). In general, fluid flow near Mounds 11 and 12 in the study area is low compared to other seep structures and has been estimated to have flow rates on the order of 0.1 to 2 cm a-1 (Ranero *et al.,* 2008).

The $\delta^{13}C$ Disequilibrium Problem at Seeps

The study of foraminifera at modern seeps allows researchers the unique opportunity to study and compare foraminiferal abundance, distribution, and geochemistry across a natural gradient of seepage activity from methane-influenced environments to surrounding non-seep areas. The use of benthic foraminifera to discern past methane activity has been proposed, attempted, and debated (Wefer *et al* 1994; Kennett *et al.,* 2000; Stott *et al.,* 2002). Modern seep foraminifera continue to be studied in an attempt to better understand the extent to which methane release influences the geochemistry of foraminiferal carbonate (e.g., Martin *et al.,* 2010; Bernhard *et al.,* 2010; Geiskes *et al.,* 2011).

Methane has a very light $\delta^{13}C$ signature (e.g., -50‰ and -62‰ offshore Costa Rica; Mau, 2004) and has been suggested to influence the $\delta^{13}C$ values of benthic foraminiferal carbonate around active seeps (Rathburn *et al.,* 2003; Mackensen *et al.,* 2006). Kennett *et al.* (2000) hypothesized that oxidation of methane in pore-waters is the mechanism responsible for transferring negative $\delta^{13}C$ values to DIC and, in turn, to the biogenic carbonate of foraminifera. However, modern studies suggest that surrounding waters saturated in isotopically light methane do not typically result in extremely negative $\delta^{13}C$ values of foraminiferal tests (Rathburn *et al.,* 2003). Differences between the $\delta^{13}C$ values of foraminiferal tests and those of methane seep pore-water DIC enveloping them (deemed "disequilibrium") has been addressed in several studies (e.g., Rathburn *et al.,* 2003; Torres *et al.*, 2003; Bernhard *et al.*, 2010). This disequilibrium raises concern about the reliability of foraminifera as recorders of methane release events, and the use of benthic foraminiferal $\delta^{13}C$ values to infer these events through geologic time.

Several explanations for the observed disequilibrium have been presented over the past few years. Torres *et al.* (2003) hypothesized that the disequilibrium that occurs between $\delta^{13}C_{DIC}$ and foraminiferal carbonate at seeps results from test calcification only during periods when there is little or intermittent methane release. If calcification does not occur during times of active seepage, foraminiferal carbonate would not record the effects of methane. However, this hypothesis suggests that foraminifera are not really living in seep conditions, but merely surviving in a dormant state until non-seep conditions return. This hypothesis also implies that foraminifera do not reproduce until these inactive, or intermittently active time periods. These ideas bring into question the extent or duration of seep inactivity necessary for foraminifera to come out of the dormant state, calcify new chambers, and possibly even reproduce.

Rathburn *et al.* (2003) suggests that geochemical variability within a seep may cause the greater variability seen in benthic foraminiferal $\delta^{13}C$ values. This presence of nano-environments would explain the greater variability, but suggests that in order for foraminifera to generate non-seep $\delta^{13}C$ values, there needs to be small areas where pore-waters are very different from the average seep pore-water geochemistry through dispersal patterns of seeping fluids, or through fine-scale biogeochemical processes.

The suggestion by Torres *et al.* (2003) that the isotopic disequilibrium at seeps may be due to a lack of calcification during active periods was taken one step further by Bernhard *et al.* (2010) who proposed that most foraminifera found at seeps are transported in or were in place before seep activity began in the area. Bernhard *et al.* (2010) suggested that most foraminifera do not survive in active seep sites, and those that survive are only able to calcify a last chamber in seep conditions. The isotopic signal of the single chamber of new "seep calcite" would be diluted by the "non-seep calcite" (all previous chambers). Bernhard *et al.* (2010) estimated that should

the “seep calcite” comprise only the final chamber (up to 10-20% of the total foraminiferal carbonate) isotopic analysis of the whole test would produce a $\delta^{13}C$ value that roughly coincides with isotopic results of some of the lowest values for seep specimens observed in their data set (-1.6 to -3.2‰).

According to Bernhard *et al.* (2010), cellular ultrastructural analyses indicate that many foraminifera found at seeps are dead or dying. Bernhard *et al.* (2010) suggests that the presence of Rose Bengal staining in these seep specimens indicate that although bacteria have invaded specimens, cytoplasm has not degraded to the point where the specimens would not stain. In this scenario, when unsuitable seep conditions persist, foraminifera die and may be invaded by bacteria but still appear as living foraminifera when stained with Rose Bengal (a vital stain). Although this idea accounts for the $\delta^{13}C$ disequilibrium and lack of endemic foraminifera, it suggests that the decay rates of benthic foraminiferal protoplasm are very slow, that the vertical distribution patterns of foraminifera observed at seeps result entirely from bioturbation, and that there is constant influx of specimens from outside seeps.

Seep Foraminiferal Ecology

To date, no endemic foraminifera have been found at methane seeps and may be due to the attraction of foraminifera to high food availability environments which require no adaptations beyond those needed for life in organic rich, reducing environments (Gooday and Rathburn, 1999; Rathburn *et al.,* 2000). This observation is supported by whale falls and turbidite deposits in which opportunistic species that typically thrive in low oxygen, organically rich environments have also failed to produce any endemic benthic foraminifera (Gooday and Rathburn, 1999).

Vertical distribution patterns of infaunal foraminifera at sites of active methane seepage in the Pacific have shown infaunal populations similar to those observed in non-seep habitats

(Rathburn *et al.,* 2003; Rathburn *et al.,* 2000). Rathburn *et al.* (2000) determined there to be no significant differences in foraminiferal densities between seep and nearby non-seep sites off the coast of California. Rathburn *et al.* (2000) also sampled clam beds and reported a density maximum at 2.5-3cm for several infaunal species. Rathburn *et al.* (2003) sampled both clam beds and a bacterial mat in Monterey Bay and found that clam beds contained slightly higher densities than bacterial mats, and the vertical distribution patterns of foraminifera in clam beds show appreciable infaunal densities within the sediments from 1.5 cm to 4 cm (Rathburn *et al.,* 2003). Geiskes *et al.* (2011) also observed appreciable densities and infaunal distributions within clam bed and bacterial mat habitats at Monterey Bay.

Heinz *et al.* (2008) conducted one of the few studies to investigate foraminifera in the area off the Pacific coast of Costa Rica. The samples collected from Mound 12 by Heinz *et al.* (2008) were located on the northern flank of the mound and were deemed to be from inactive areas (e.g., no sulfide smell, presence of bubbles, or seep fauna; Heinz *et al.,* 2008). Heinz *et al.* (2008)'s samples were collected from depths ~1,000m and bottom water oxygen concentrations of about 0.5mL/L. Heinz *et al*. (2008) found vertical distributions with infaunal maxima residing within the top 2cm. Dominant taxa in Heinz *et al,* (2008) include *Cassidulina, Bolivina, Bulimina, Uvigerina, Chilostomella*.

Elevated Epibenthic Foraminifera

Of particular interest are elevated epibenthic foraminifera that have colonized worm tubes at seep sites. *Cibicides wuellerstorfi,* an epibenthic foraminifera, is commonly used in paleoceanographic studies because it is known to precipitate its test in isotopic equilibrium with bottom-water $\delta^{13}C$ (Sen Gupta, 1999).

The attachment of elevated epibenthic foraminifera such as *Cibicides wuellerstorfi* to tubeworms at active seepage sites has previously been observed (Mackensen *et al.,* 2006; Sen Gupta, 2007; Lobegier and Sen Gupta, 2008). Sen Gupta (2007) observed 12 species of epibenthic foraminifera attached to *Lamellibrachia luymesi* and *Escarpia laminate* tubeworms at active hydrocarbon seep environments in the Gulf of Mexico. The foraminifera were attached by protoplasm, or an organic adhesive, indicating that the foraminifera were alive during collection. Sen Gupta (2007) argued that for much of tubeworm growth, water above the base of the tube is oxic and almost sulfide-free, thus providing an environment in which many epibenthic foraminifera can settle, creating a mechanism for escaping the anoxic or sulfidic environments below. If Sen Gupta (2007)'s hypothesis is correct and elevated epibenthic foraminifera are able to escape methane influenced bottom waters, it might be expected that $\delta^{13}C$ of foraminiferal carbonate would not be influenced by the presence of methane. Non-seep *C. wuellerstorfi* $\delta^{13}C$ values typically range from 0‰ to 0.5‰ (Eberwein *et al.,* 2006). Mackensen *et al.* (2006) analyzed the carbon isotopic values of epibenthic foraminifera attached to pogonophoran tubeworms off the Norwegian margin and found negative $\delta^{13}C$ values of -0.03‰ to -3.8‰. Suggestive of a strong influence of the low $\delta^{13}C_{DIC}$ in which they reside. The study by Mackensen *et al.* (2006) shows that *C. wuellerstorfi* on the Norwegian Margin were living above the sediment-water interface and exposed to methane-influenced fluids.

Mackensen *et al.* (2006) suggested that the negative $\delta^{13}C$ values of the epibenthic foraminifera might not be obtained directly from the $\delta^{13}C_{DIC}$, but perhaps from the incorporation of depleted $\delta^{13}C$ methanotrophic bacteria, or from the tubeworm (substrate) on which the foraminifera reside. If the carbon isotopes of the foraminifera positively correlate with those of tubeworms then it seems reasonable to assume that both are being influenced by the same factor

or that the foraminifera are influenced by the tubeworm itself. If the carbon isotopes do not positively correlate with the tubeworms then it can be assumed that the foraminifera are not influenced exclusively by the tubeworms or factors that influence tubeworm isotopic geochemistry.

CHAPTER 2

THE ISOTOPIC GEOCHEMISTRY OF *CIBICIDES WUELLERSTORFI* AND *CARPENTERIA MONTICULARIS* LIVING ON VESTIMENTIFERAN TUBEWORMS AT METHANE SEEPS ON THE COSTA RICAN MARGIN

Introduction

Some benthic foraminifera are able to live attached to substrates that extend above the seafloor, including biogenic structures (Lutze and Theil, 1989), and can be the dominant organism colonizing elevated substrates in the deep sea (Beaulieu, 2001). It is thought that elevated biogenic structures provide hard substrata and act as habitat islands to which organisms are able to attach, perhaps providing advantages in feeding, or as a refuge from inhospitable bottom conditions (Sen Gupta, 2007; Beaulieu 2001).

Sites of active or intermittent methane seepage are often dominated by chemosynthetic based communities with inhabitants such as vestimentiferan tubeworms, *Beggiatoa* bacterial mats, and *Calyptogena* clams (Levin *et al.,* 2005). Vestimentiferan tubeworms and carbonate rocks at active seep sites provide hard substrates on which a number of organisms live. Vestimentiferan tubeworms, composed of a soft bodied annelid residing in a chitinous tube, are among the largest annelids on this planet, some reaching over three meters in length (Boetius, 2005). On the Costa Rican margin, vestimentiferan tubeworms vary in size from tens of

centimeters to meters in length. On the seafloor these tubeworms are often found in clumps or bushes, some of which can be as large as a small car. Sen Gupta *et al.* (2007) observed *Cibicides wuellerstorfi* attached to vestimentiferan tubeworms at seeps in the Gulf of Mexico. Often at sites of active methane seepage, anaerobic oxidation of methane (AOM) can produce high sulfide environments, which are toxic to most organisms (Levin *et al.,* 2005). Sen Gupta *et al.* (2007) hypothesized that the upward migration of elevated epibenthics may provide a means by which foraminifera are able to escape conditions at the sediment water interface.

Geochemical analyses of foraminiferal carbonate has been used to infer information about the environment in which the test was precipitated. Stable isotopic analyses of elevated epibenthic foraminifera have been of interest for paleoceanographic reconstructions because these taxa are surrounded by bottom water and not subject to the potential variability of pore-water geochemistry (Sen Gupta, 1999; Corliss and Rathburn, submitted). Some studies (Dickens *et al.*, 1995, Kennett *et al.,* 2000) have attempted to use the $\delta^{13}C$ composition of benthic foraminiferal carbonate as an indicator of marine methane release in the geologic past. Observations of foraminiferal geochemistry at modern seep environments allow for an analysis of the relationship between the $\delta^{13}C$ of foraminiferal carbonate, ecological distribution, and seepage.

Coiling

Coiling patterns of foraminifera results, in part, from the tendency of the organism to begin formation of its test in the sinistral (left) or dextral (right) direction (Nigam and Rao, 1989). Coiling patterns of planktonic foraminifera have been used as stratigraphic tools in paleoceanographic studies (Sen Gupta, 1999). Despite the major implications of coiling direction in planktonics, very little attention has been paid to the coiling directions of benthic foraminifera,

especially deep-sea species. Due to the difficulty of culturing deep-sea species, a great deal of information regarding their life cycle still remains unknown (Sen Gupta, 1999). Researchers have identified two prominent mechanisms that control benthic foraminiferal coiling direction; heritability (genetically derived) and influence by environmental conditions such as temperature (Collins 1990; Nigam and Rao,1989). Coiling direction, therefore, may have possible applications in establishing phylogenetic and reproductive relationships among species, and as a proxy for seafloor conditions, such as temperature (Galeotti, 2002). It has also been suggested that coiling directions may be genetically inherited, yet still have the potential to be influenced by environmental conditions (Nigam and Rao, 1989). Observations of coiling directions of elevated epibenthic foraminifera from the unique environments of methane seeps may help identify factors that influence isotope variability within a foraminiferal species, and provide clues about the influence of environmental conditions on coiling direction.

Study Area

Off the Pacific coast of Costa Rica the Cocos Plate subducts under the Caribbean Plate at a rate of ~88mm/yr (Tryon *et al.*, 2010; Sahling *et al.,* 2008; Kukowski and Oncken, 2006). As these plates collide, the underlying sedimentary material is compacted and liquids comprising the pore-waters concentrate, ultimately seeping to the sediment-water interface (Sahling *et al.,* 2008). Cold seep habitats result when the seeping fluids are comparable in temperature to those of the surrounding waters (Levin, 2005; Sahling *et al.,* 2008). Around the world along continental slopes, mud extrusions can be common features. Offshore Costa Rica and Nicaragua, 81 mud extrusions have been discovered (Mau, 2004).

This study focuses on samples collected from Costa Rican seeps from two mud extrusions (Mound 11 and Mound 12), and Jaco Scar, a prominent structure created by the

subduction of a seamount (Figure 1-4). As the Cocos Plate subducts under the Caribbean Plate, topographic features, such as seamounts, can cause residual structures such as Jaco Scar. These residual features can result in deep sedimentary structures that can trap and accumulate methane, which can then be discharged at rates comparable to mud extrusions and result in continuous venting over time (Mau, 2004).

Samples are collected from water depths of 995 to 1001 meters with an average bottom water oxygen level of 0.73mL/L (32.56μM) and temperatures of ~4°C (Table 1). The study area is colonized by chemosynthetic communities including *Calyptogena* clams, *Lamellibrachia* tubeworms (a species of vestimentiferan tubeworm), and *Beggiatoa* bacterial mats. Water column samples taken directly above the various faunal assemblages at Mound 12 showed that generally regardless of chemosynthetic assemblage, methane is seeping through these areas with the highest volumes emitted from areas covered by bacterial mats (Mau, 2004). The output of methane at Mounds 11 (5.1-17.5x 10^3mol/yr) and 12 (15.5-52.5x10^3mol/yr) are lower than the reported outputs from mud extrusions found in the Norwegian Sea (Ginsburg *et al.,* 1999) and Mediterranean Sea (Kopf & Behrmann, 2000). These differences in output possibly result from the effect of anaerobic oxidation of methane or structural differences. Methane output at Jaco Scar is more comparable to that of Hydrate Ridge off the coast of Oregon (3.4-4.0x 10^6mol/yr; Mau, 2004).

Materials and Methods

In February and March of 2009 (AT15-44) and January of 2010 (AT15-59) aboard the *R/V Atlantis* the deep submergence vehicle *ALVIN* was used to obtain tubecores of sediment and hard substrate samples off the Pacific coast of Costa Rica. *ALVIN*'s manipulator arm facilitated the recovery of vestimentiferan tubeworms and carbonate rocks. Samples were placed in a

covered biobox, allowing for the substrate to be isolated and protected for transport to the surface. It is altogether possible that foraminifera were lost during the collection and/or shaken from the tubes during transport, making counts a conservative estimate of populations. Shortly after the recovery of the samples, hard substrates were examined using microscopes onboard the *R/V Atlantis* and living foraminifera were manually removed and placed in seawater-filled Petri dishes. Some specimens were observed moving around in the Petri dishes. Foraminifera were then identified and frozen at -80°C onboard the ship and kept frozen. In the lab at Indiana State University, pristine specimens were selected for isotopic analysis, and these individuals were ultrasonically cleaned and rinsed with Milli-Q water. These specimens were sent to the University of Florida for stable carbon and oxygen isotope analyses where foraminiferal carbonate was acidified at 73° with anhydrous phosphoric acid in a Kiel III device connected to a Finnigan MAT 252 isotope ratio mass spectrometer. Data are reported relative to the PDB (PeeDee Belemnite) standard. Most foraminifera were large enough to be analysed individually, but when necessary, some specimens were paired with another individual (Table 2). For this study, 275 individual foraminifera were analyzed for stable isotopic composition from which 147 values were reported (Table 2).

Photographs were taken of each individual prior to isotopic analysis. These photographs allowed for comparison between the coiling direction and isotopic value of individual specimens. Any foraminiferal isotope values generated by multiple specimens with pairing of more than one coiling direction were not included in the coiling/isotope analysis. Coiling direction was confirmed for 83 individuals with coupled isotopic analysis.

Comparisons are made between the isotopic values of 30 elevated epibenthic foraminifera and the 11 tubeworms on which they resided. Small portions of tissue and tube were

sampled from the tube and corresponding tissues of designated vestimentiferan individuals. Tissues and tubes were then placed into pre-weighed tin boats and stored frozen at -80ºC. Containers were cleaned via combustion and methanol cleaned utensils were used to prevent cross-contamination of samples. All samples were cleaned as thoroughly as possible by removing external/non-tissue sources of carbon by rinsing the sample with distilled or Milli-Q water. Contaminate carbonate from tubes (i.e. mollusc shells) was removed by hand. Tissues were rinsed, dried, and ground to a fine powder prior to isotopic analysis to insure homogenization and complete combustion. Samples were combusted in a Costech Elemental Analyzer (Valencia, CA) then analyzed using a GV isoprime mass spectrometer (Manchester, UK).

To compare the stable $\delta^{18}O$ composition of foraminiferal calcite between sites, the $\delta^{18}O$ of foraminiferal calcite (reported in PeeDee Belemnite standard, $\delta^{18}O_{(e.c.,PDB)}$) and estimated bottom water $\delta^{18}O$ values (reported in Standard Mean Ocean Water, $\delta^{18}O_{(b.w.,SMOW)}$) were used to calculate temperature. Bottom water $\delta^{18}O_{(b.w.,SMOW)}$ was estimated from Craig and Gordon (1965). Calculations of bottom water temperatures (which are converted to degrees Celsius) are based on this equation (see calculations in Appendix A). The formula used to calculate temperature (equation (1)) is based on the equation found in Friedman and O'Neil (1977). The formula used to convert foraminiferal calcite $\delta^{18}O_{(ecPDB)}$ values to $\delta^{18}O_{(ecSMOW)}$ was done with equation (2) and can be found Rathburn *et al.* (1996).

(1) $T=\sqrt{((2.78x103)/\ln((1000+ \delta^{18}O_{(e.c.,SMOW)})/(\delta^{18}O_{(b.w.,SMOW)}+1000)+(2.89/10^3))}$

(2) $\delta^{18}O_{(e.c.,SMOW)}=((\delta^{18}O_{(e.c.,PDB)}+29.94)/0.97006)$

Bottom-water $\delta^{13}C$ values in this region are estimated using geochemical data repositories such as World Ocean Circulations Experiment (WOCE), GEOSECS, and Levitus *et al.* (1994). In the eastern Pacific Ocean, bottom-water $\delta^{13}C$ values at depths of about 1,000 to 1,700m water depth are estimated to be -0.02‰ from section P19C (Stations 387 and 378) of the WOCE Atlas.

Results

Foraminiferal distributions on vestimentiferan tubeworms were highly variable, but typically dominated by *Cibicides wuellerstorfi* (Schwager, 1866; also placed under *Fontbotia, Planulina*, or *Cibicides*; see Sen Gupta 1989) and *Carpenteria monticularis* (Carter, 1877). Foraminifera were often found near the end of the tubeworm (furthest from the sediment). The paucity of foraminifera found attached near the base of the worm tube may be due to the fact that foraminifera do not inhabit these areas, or that they were lost during recovery (*ALVIN's* manipulator arms typically grabbed tubeworm clumps near the base). Difficulties in the manual removal of foraminifera from the tubeworms suggest that individuals were capable of maintaining their attachment to the tubes, and the attached individuals of this study represent the elevated epibenthic population.

Statistical analysis using *Statistica*© were performed to compare isotopic values of foraminiferal calcite (*Cibicides wuellerstorfi* and *Carpenteria monticularis*) from different locations in the study area. A Shapiro-Wilk normality test revealed that only two (Jaco Scar $\delta^{13}C$ and Mound 12 $\delta^{18}O$ isotope) of the six data groups (Mound 11 $\delta^{13}C$, Mound 12 $\delta^{13}C$, Jaco Scar $\delta^{13}C$, Mound 11 $\delta^{18}O$, Mound 12 $\delta^{18}O$, and Jaco Scar $\delta^{18}O$) to be normally distributed. Because comparisons are made between normally and data that was not normally distributed, non-parametric analyses (Kruskal-Wallis test) were used.

Carbon Isotopes

Stable carbon isotopic values from foraminiferal carbonate of *Cibicides wuellerstorfi* from the Costa Rican margin ranged from 0.83‰ to -1.39‰ with an average of 0.06‰. Comparisons of these values with conspecifics included previous reports from cold seeps show comparable values (Figure 8). There was a statistically significantly difference in foraminiferal $\delta^{13}C$ values between Mound 11 and Mound 12 ($H=17.7$, $p<0.00$). There was no statistical significance between foraminiferal carbon isotopic values between Jaco Scar and Mound 12 ($H=17.7$, $p=0.18$). There was a significant statistical difference between foraminiferal $\delta^{13}C$ values between Mound 11 and Jaco Scar ($H=17.7$, $p=0.04$). Analysis of bottom water DIC $\delta^{13}C$ values of bottom-water samples taken near tubeworm sites will reveal the relationship between elevated epibenthic $\delta^{13}C$ values and bottom-water DIC $\delta^{13}C$ in which they reside.

Oxygen Isotopes

A total of 91 stable isotopic ($\delta^{13}C$ and $\delta^{18}O$) values were analyzed from elevated epibenthic foraminifera from the three sites. Oxygen isotope values of *Cibicides wuellerstorfi* ranged from 1.59‰ to 2.94‰, with an average of 2.30‰. This range is slightly greater than the range of oxygen isotopic values (2.41‰ to 2.72‰) previously reported (e.g. McCorkel *et al.,* 1997; Fontanier *et al.,* 2006; Figure 8). There was a significant statistical difference between oxygen isotopic values from Mound 11 and those from Mound 12 ($H=48.13913$, $p<0.00$; Figure 7b). There was a significant statistical difference between $\delta^{18}O$ values between Jaco Scar and Mound 12 ($H=48.13913$, $p=0.04$; Figure 7b). There was a significant statistical difference between oxygen isotopic values between Mound 11 and Jaco Scar ($H=48.13913$, $p<0.00$; Figure 7b).

These bottom-water values may not be representative of bottom-waters that exist at seeps, however, estimated bottom-water values (-0.02‰) of the WOCE Atlas are only 0.08‰ from the average $\delta^{13}C$ observed from elevated epibenthic foraminiferal calcite (average 0.06‰) and fall within the $\delta^{13}C$ range of variability of *Cibicides wuellerstorfi*. The difference between isotopic values between sites was determined and used to calculated expected temperature change based on the estimation that 0.23‰ difference in $\delta^{18}O$ would result in a 1°C water temperature change (Katz *et al.,* 2010).

Coiling Direction Isotopes

A total of 83 individual elevated epibenthic foraminifera were used in comparisons of coiling direction and stable isotope values. Dextral (right) coiling of *Cibicides wuellerstorfi* constituted 66% of the 61 individuals and only 32% of the 22 individuals of *Carpenteria monticularis*. A Kruskal-Wallis test revealed that there were no statistical differences between carbon isotopes of left and right coiling directions ($H=0.84$, $p=0.36$). There were no statistical differences between oxygen isotopes of left and right coiling directions ($H=0.67$, $p=0.41$).

Cibicides wuellerstorfi and Tubeworm Isotopes

Vestimentiferan soft tissue as well as their tubes were sampled and compared to the $\delta^{13}C$ values of the tests of several foraminifera residing on these tubes. Comparisons of $\delta^{13}C$ values of vestimentiferan tissues and tubes show a 10‰ to 30‰ difference compared to *C. wuellerstorfi* (Figure 9). No relationship is evident between the $\delta^{13}C$ values of foraminiferal carbonate and those of the tubeworm on which it resides.

Discussion

Elevated Epibenthic Foraminifera Stable Isotope Values

This study provides new information about elevated epibenthic taxa and addresses potential influences of foraminiferal geochemistry at sites of active methane seepage. Costa Rican vestimentiferan tubeworms had highly variable numbers of attached epibenthic foraminifera (from 0 to 52 agglutinated and calcareous individuals), dominated by *Cibicides wuellerstorfi*. It is possible that epibenthic metazoans influence the abundance and distribution of foraminifera on tubeworms through displacement and/or destroying of foraminifera. At this point, however, no comparative studies between foraminifera and metazoan abundances have been undertaken for methane seep epibiota. Comparisons between metazoan abundances and foraminiferal epibenthic populations in this study are planned when metazoan data from the study area become available.

Individuals selected for isotopic analysis may represent a sample bias, as larger individuals were preferentially chosen for individual isotopic analysis. Carbon isotopic values of *Cibicides wuellerstorfi* collected from vestimentiferan tubeworms in this study are not as negative (0.83‰ to -1.39‰) as those reported in other studies (such as Mackensen *et al.,* 2006; -0.03‰ to -3.29‰). Pogonophoran tubeworms observed in the Norwegian and Alaskan margins possessed maximum lengths of only tens of centimeters (Mackensen *et al.,* 2006; Rathburn *et al.,* 2009). Therefore, if seep activity is present, it could surround the elevated epibenthics residing only centimeters above the sediment-water interface and may be evidenced by the depleted $\delta^{13}C$ values of *Cibicides wuellerstorfi* tests as seen in Mackensen *et al.* (2006). Foraminiferal calcite of *C. wuellerstorfi* attached to corals and pogonophoran tubeworms collected on the Alaskan margin resulted in $\delta^{13}C$ values (range of 0.40‰ to 0.16‰ and an average of 0.27‰; Rathburn *et*

al., 2009, unpublished data) similar to non-seeps. Although individuals from the Alaskan margin resided close to the sediment-water interface, the lack of depleted $\delta^{13}C$ values in epibenthics may result from reduced/absence of methane seep activity as evidenced by the paucity of living pogonophorans within tubes at this site. Vestimentiferan tubeworms collected in Costa Rica ranged from 1 to 3m in length. The long lengths of Costa Rican tubeworms allows for the possibility that the epibenthic foraminifera, which are typically found near the tops of the tubeworms, may not be bathed in methane-influenced bottom water.

The presence of $\delta^{13}C$ values typical of non-seep foraminifera at active seep sites of Costa Rica may be a result of:

1. Little to no seep activity resulting in reduced methane-influenced fluids during elevated epibenthic calcification.
2. Foraminifera residing and precipitating calcite in bottom-waters not influenced by methane (e.g. well above the sediment water interface thereby escaping seep bottom waters).
3. Foraminifera obtaining $\delta^{13}C$ values from a source other than their surrounding bottom-waters or substrate (e.g. food).
4. The presence of ^{13}C-depleted methane does not influence foraminiferal $\delta^{13}C$ values of *Cibicides wuellerstrofi*.

Vestimentiferan tubeworms require seep activity to survive and therefore it is less likely that seep activity in the Costa Rican sample sites is substantially low, however, lower levels of seep activity may explain the non-seep $\delta^{13}C$ values obtained from *Cibicides wuellerstrofi* from the Alaskan margin. The long length (1 to 3m) of Costa Rican vestimentiferan tubeworms may allow attached elevated epibenthic foraminifera to reside in bottom waters that are not influenced

by methane. The 10‰ to 30‰ differences between foraminiferal calcite and vestimentiferan tubeworms of this study (Figure 9) suggest that foraminiferal $\delta^{13}C$ values are not directly influenced by the substrate on which they reside. If methane is capable of influencing foraminiferal calcite, as was observed by Mackensen *et al.* (2006), then a lack of activity during epibenthic calcification, or escaping influences bottom-waters (via long tubeworms) may contribute to the perceived disequilibrium in this study.

Elevated Epibenthic Foraminifera and Tubeworms

Epibenthic $\delta^{13}C$ values from this study are similar to those of non-seep conspecifics (Figure 8). This together with the 10‰ to 30‰ difference between isotopic composition of epibenthic living foraminiferal calcite and the tubeworm on which they reside suggests that the tubeworm substrate on which epibenthic foraminifera attach does not influence the $\delta^{13}C$ signatures of foraminifera. The study by Mackensen *et al.* (2006) also indicates that *C. wuellerstorfi* on the Norwegian margin are living above the sediment-water interface despite the obvious presence of methane-influenced fluids. For epibenthic foraminifera that have more negative $\delta^{13}C$ values at seeps than at non-seeps (e.g. Mackensen *et al.,* 2006), carbon isotopic signatures are likely to be influenced by their food, as suggested by Mackensen *et al.* (2006), or directly from the negative $\delta^{13}C_{DIC}$ of their surrounding water, or some as of yet unknown mechanism.

Isotopic Comparisons Between Sites

Statistical differences in isotopic compositions exist between sites (Figure 7). To determine if these statistical differences are caused by temperature differences, bottom water temperature was calculated using the equation from Friedman and O'Neil (1977). Bottom water temperatures calculated from average $\delta^{18}O$ of foraminiferal calcite values resulted in ~3°C

deviation from measured bottom water temperatures (Table 3). Organismal vital effect cannot be invoked to explain this offset because *Cibicides wuellerstorfi* is known to precipitate its calcite in equilibrium with bottom waters (Sen Gupta, 1999). Salinity measurements from CTD casts during the two cruises did not indicate any significant differences in salinity between sites (Table 1). Temperature variability was observed at Jaco Scar during the 2009 expedition and may explain some of the oxygen isotopic variability seen between Mound 11, Mound 12, and Jaco Scar (Levin *et al.,* in prep). Stable oxygen isotopic composition of *C. wuellerstorfi* in Costa Rica are slightly more variable (1.59‰ to 2.95‰) than those previously seen (2.41‰ to 2.63‰ in McCorkle *et al.,* 1997; 2.50 to 2.72 ‰ in Fontanier *et al.,* 2008) and support the idea that variable bottom water $\delta^{18}O$ values account for the differences observed between calculated and measured bottom water temperatures. The degradation of carbonate rocks (3.2‰ to 7.44‰ reported by Geiskes *et al.,* 2005) may contribute to variations in bottom water $\delta^{18}O$. Bottom water temperature differences and variations in bottom water $\delta^{18}O$ via carbonate disolution would be expected to result in even more variable $\delta^{18}O$ values between sites. However, temperature $\delta^{18}O$ variations in bottom water cannot account for the constant 3°C seen at all three sites. It was expected that temperature differences would be reflected in foraminiferal calcite $\delta^{18}O$ values between Mound 11 and Jaco Scar, as well as between Mound 12 and Jaco Scar, but not between Mound 11 and Mound 12. Differences between calculated bottom water temperatures and measured temperatures ranged from 0.2 to 0.5°C.

Statistical differences in the carbon isotopic composition exist between Mound 11 and Mound 12 and Mound 11 and Jaco Scar, but not between Jaco Scar and Mound 12. This difference in stable carbon isotopes between sites might result from methane seep variations between sites. Several studies have published fluid estimates for the study area. The most recent

data published by Furi *et al*. (2010) combines estimated methane fluxes from Mau (2006) with their observations to create a carbon mass balance for the area. According to Furi *et al.* (2010), Mound 11 shows low salinity and altered fluid chemistry relative to seawater. Mound 12 was measured to have highly variable flow rates, with water chemistry more similar to that of seawater (Furi *et al.,* 2010). Variable seepage activity measured by Furi *et al.* (2010) may explain the different bottom water chemistry of Mound 12. Despite the variable flow at Mound 12, Mound 12 emits more methane per year than Mound 11 (according to estimates reported in Mau, 2006). The possibility that Mound 12 is emitting large amounts of methane at variable intervals supports the concept that Mound 12 is older and possibly in the waning portion of its cycle. Variable levels of activity would allow for increased rates of methane oxidation resulting in the observed dissolved carbon and sulfide characteristics observed at Mound 12 (Tryon *et al.,* 2010; Furi *et al.,* 2010). This mixing may dilute the $\delta^{13}C$ of the bottom-water and contribute to isotopic differences between foraminiferal carbonate from different sites. The statistical significance observed in stable carbon and oxygen isotopes of foraminiferal calcite between the three areas (Mound 11, Mound 12, and Jaco Scar) corroborates the idea that varying seepage between sites may be recorded in foraminiferal calcite of the elevated epibenthics.

Coiling

This study provides the first coiling direction observations of living foraminifera from sites of active methane seepage. There were no statistical differences between isotopic values of sinstral (left) and dextral (right) *Cibicides wuellerstorfi,* indicating that factors contributing to coiling direction do not also influence stable isotopic composition of the test.

It has been proposed that coiling direction of benthic foraminifera is a result of reproduction or is influenced by environmental factors, such as temperature (Nigam and Rao,

1989). Collins (1990) found a strong association between dextrally coiled *Bulimina* and warm bottom water temperature. Although the warm bottom waters of Collins' (1990) study correlated with dominance in dextrally coiling individuals, cold bottom waters did not show any correlation with sinstral coiling. In this study, *Cibicides wuellerstorfi* dominantly coiled in the dextral direction. Based on the relationship between temperature and coiling direction suggested by Collins (1990), dominance of dextrally coiled individuals is indicative of exposure to warm (~17°C to ~13°C) temperatures. However, the bottom water temperatures of our study area (2°C to 4°C) fall into what Collins (1990) would describe as "cold" (~4°C to ~3°C) bottom water temperatures. The high percentage of dextrally coiled *Cibicides wuellerstorfi* from the cold waters of the Costa Rican margin do not support the idea that coiling direction is uniformly indicative of bottom water temperature. However, differences in coiling direction of co-occurring species suggest that biological differences between epibenthic species and not environmental factors are primary influences of coiling direction in these species. These findings suggest that coiling direction of these deep-sea species may not be applied as measurements of environmental influences.

Conclusions

Elevated epibenthic foraminifera from this study do not have depleted $\delta^{13}C$ values suggestive of the presence of methane (compared to the more depleted values of Mackensen *et al.,* 2006). These results are important because they illustrate that foraminifera inhabiting active areas of methane seepage have the potential to retain a non-seep $\delta^{13}C$ value. Therefore caution must be taken when attempting to apply methane signatures of elevated epifauna to the geologic record. Stable carbon and oxygen isotopic composition are statistically different between sites (Mound 11, Mound 12, and Jaco Scar), possibly reflecting variations in bottom water

composition $\delta^{13}C$ and $\delta^{18}O$ between sites. Further analysis of bottom water concentrations will allow for a more quantitative analysis of variations between sites and allow for quantification of any disequilibrium that may exist between foraminiferal calcite and bottom-water $\delta^{13}C$. Comparisons of $\delta^{13}C$ values between coiling directions of *Cibicides wuellerstrofi* were statistically similar, suggesting that whatever factors influence coiling direction are not also influencing stable isotopic composition. This observation lends support to the hypothesis that coiling direction of *Cibicides wuellerstorfi* and *Carpenteria monticularis* result from biologic rather than environmental factors. The relationship between coiling direction and bottom water temperatures described by Collins (1990) is not observed in elevated epibenthic foraminifera from vestimentiferan worms from seeps off Costa Rica, indicating that the coiling direction of these species of elevated epibenthic foraminifera cannot be used to assess temperature.

CHAPTER 3

COMPARISONS OF THE ECOLOGY AND VERTICAL DISTRIBUTION PATTERNS OF BENTHIC FORAMINIFERA LIVING IN SEEP AND NON-SEEP HABITATS ON THE COSTA RICAN MARGIN

Introduction

The ecology and vertical distribution of benthic foraminifera at sites of active methane seepage and adjacent non-seep sites have been documented in a number of regions, including the Eel River Margin (Rathburn *et al.,* 2000), Monterey Bay (Rathburn *et al.,* 2003; Bernhard *et al.,* 2001), Hydrate Ridge (Hill *et al.,* 2004; Torres *et al.,* 2003), the Gulf of Mexico (Sen Gupta *et al.,* 1997; Sen Gupta 2007; Lobegier *et al.,* 2008), Hakon Mosby mud volcano on the Barents Sea continental slope (Mackensen *et al.,* 2006; Wollenburg *et al.,* 2009), and the Santa Barbara Channel (Hill *et al.,* 2003). To date, no endemic foraminiferal species have been found in seep environments. The study of modern, naturally occurring methane seeps provides the opportunity to observe how active seepage influences the ecology of living foraminifera.

The objectives of this study were to investigate differences and similarities between two methods used to distinguish living foraminifera (CellTracker Green (CTG) labeled or Rose Bengal (RB) stained individuals), and to compare the ecology of benthic foraminifera at seeps off Costa Rica with other seeps in the Pacific. Vertical distribution patterns and species

percentages of living infaunal benthic foraminifera (>150μm) stained with RB or labeled with CTG were compared at sites of active methane seepage off the Pacific coast of Costa Rica. The ecology, densities, and vertical distribution patterns of benthic foraminifera have been well documented at other sites of active seepage in the Pacific and provide a means for comparison with Costa Rican seep foraminiferal populations.

Study Area

All sediment samples reported in this study were collected on or around Mound 12 off the coast of Costa Rica (Figures 1-2). Mound 12 is a mud extrusion with a height of 30 meters, elongated in the northeast-southwest direction with a diameter of about 1 to 1.6 km (Mau, 2004). Samples were collected from water depths of ~1000m, with average bottom water oxygen concentrations of 0.73mL/L (32.56μM) and temperatures of ~ 4°C. The top of the mound is probably the oldest portion of the study area, as indicated by large fractured carbonates that sealed venting (Mau, 2004). The youngest area of the mound is located in the southwest section where bacterial mats in soft sediments are abundant (Mau, 2004). Chemosynthetic communities including *Calyptogena* clams, and vestimentiferan tubeworms typically colonize seeps of the Costa Rican margin. Bacterial mats were very common.

Materials and Methods

The *R/V Atlantis* and the deep submergence vehicle, *ALVIN,* were used for cruises in February and March of 2009 (AT15-44) and January of 2010 (AT15-59) off the Pacific coast of Costa Rica to obtain tubecore samples from active seep bacterial mats (Figure 1-2). An Ocean Instruments multicore was used to obtain sediment samples from non-seep sites from 1 to 1.5km from the active seep sites. There was no indication that these off-seep samples were active, for example, there was no sulfur odor or the presence of carbonates that are common in active areas.

All cores were sectioned into one-centimeter sections at the 0-1cm interval, half-centimeter sections for 1½ -2, 2-2 ½, 2½-3 centimeter intervals, and one-centimeter sections below 3cm to at least 5cm (in some cases 10cm). To distinguish living or recently living specimens from dead individuals, RB stain and CTG labeling were used. Core samples designated for RB staining were then preserved in 4% buffered formaldehyde (diluted 37% formaldehyde by a factor of approximately 10 and buffered with Mule Team Borax©) onboard and stained with RB in the lab at Indiana State University. When stained, 65mL of RB solution (1g/L 4% formaldehyde) was added to each sample and allowed to stain for at least a week. Select cores were labeled using CTG onboard the ship shortly after arrival on deck. CTG was added to each of these samples (60μL for one-centimeter sections and 30μL for half-centimeter sections), which were then incubated at ambient seafloor temperatures of 2°C for 12 hours before preservation with 4% buffered formaldehyde and kept dark until further processing back at the lab (methodology described in Bernhard *et al.,* 2006).

Sediment volumes were determined in the lab following the volumetric procedures outlined in Rathburn and Corliss (1994). Samples from the top 3cm were wet-sieved with 63 and 150μm mesh sieves and the 63 to 150μm fraction was preserved and stored for subsequent analysis. The >150μm fraction was wet-split for feasible working sizes, then wet-picked for living foraminifera (average of 412 individuals examined per core). Specimens were placed on microslides, identified, and counted.

RB samples were examined using a Nikon SMZ-1500 epifluorescence stereomicroscope. Once the sample was picked for CTG individuals, it was then subsequently stained with RB and picked. This allows for the comparison of these samples using both methods. Subsequent RB staining assumes that any CTG labeled individuals will also be stained with RB. This is a fair

assumption as CTG labeling requires that the cell actively take up the label, producing more conservative live foraminiferal counts (Bernhard *et al.*, 2006). In this study, both techniques were used to compare the results of the techniques.

RB staining has been the stain of choice among paleoceanographers and benthic ecologists for most community-scale studies because it is inexpensive, and easy to use, in addition to the availability of extensive existing data sets (Murray and Bowser, 2000; Rathburn *et al.,* 2003; Bernhard *et al.,* 2006). RB is a non-vital stain that adheres to cytoplasm (Bernhard *et al.,* 2006). It has been argued that RB's lack of specificity in staining living vs. dead tissues results in overestimation of living populations due to protoplasm retention and test invasion by bacteria (Bernhard, 2000: Bernhard *et al.,* 2006). It has been estimated that the protoplasm of foraminifera can exist from days to years after the death of the individual (Corliss and Emerson, 1990). This retention of protoplasm and the presence of an organic internal lining in some species may result in the staining of non-living individuals (Rathburn *et al.,* 2003; Bernhard *et al.,* 2006). Invasion of tests by bacteria or other organisms may also result in a false positive identification of living individuals using RB stain (Bernhard *et al.,* 2006). Despite these potential complications, a conservative approach when distinguishing between stained and unstained individuals seems to yield reasonable and adequate resolution of deep-sea foraminiferal populations (Altenbach and Sarnthein, 1989; Murray and Bowser, 2000).

CellTracker Green 5-chloromethylfluorescein diacetate (CellTrackerTM Green CMFDA; Molecular Probes, Invitrogen Detection Technologies) is a vital fluorogenic probe developed to stain living cells (Bernhard *et al.,* 2006). It is thought that CTG is a more conservative labeling technique than RB. After the probe passes across the cell membrane, the cell will begin to fluoresce after it interacts with esterases, which are lacking in cells that have been dead long

enough (e.g. hours to days; Bernhard *et al.,* 2006). Although CTG is thought to be a more conservative estimator of living individuals, the methodology required for the technique requires more processing time on the ship and is much more expensive than RB (Murray and Bowser, 2000). In order for living cells to fluoresce with CTG, samples need to be incubated at ambient seafloor conditions for a period of time which may vary based on where the samples are from (see Bernhard *et al.,* 2006 for a discussion).

Results

The following results are based on data from the >150μm size fraction of the top 3cm from 7 pushcores. Three pushcores were collected in bacterial mats in three different active areas of water depths between 995-1001m (AD4511 TC2, AD4586 TC4, AD4587 TC2), and four multicore tubes were collected from a range of 1 to 1.5km away from the seep samples in nearby non-seep sites (MC1 TC7, MC2 TC 2, MC3 TC2, MC4 TC2) (Figure 1-2; Table 1).

Seep foraminiferal distribution patterns

Although vertical distribution patterns of the three-seep sites vary, they all show substantial infaunal populations. Total foraminifera/50cc counts show infaunal maxima between 1½-2½cm in AD4511 (Figure 10c), 1-1 ½cm in AD4587 (Figure 10b), and two separate maxima in AD4586 (Figure 10a) with the more substantial maximum occurring at 2-2½cm, and the second at 0-1cm. AD4511 had a total density of 1128/50cc and all species showed greater infaunal abundances deeper in the core. *Chilostomella oolina* (Schawger, 1878; see Phleger et al., 1953, Plate 10, Fig. 18) showed an infaunal maximum at 1½-2cm, while *Uvigerina peregrina* (Cushman, 1923; see Resig, 1981, Plate 2, Fig. 5) and *Cibicides mckannai* (Galloway and Wissler, 1927; see Phleger, 1964, Plate 3, Figs. 26, 27) had maximum densities at 2-2½cm. AD4586 had a total density of 271/50cc and *Chilostomella oolina* showed highest densities at 1-

1½cm and *Uvigerina peregrina* and *Cibicides mckannai* showed highest densities at 2-2½cm. AD4587 had a total density of 180/50cc with species maxima appearing shallower than the other two pushcores. Infaunal maxima of *Chilostomella oolina* and *Uvigerina peregrina* occurred at 1-1½cm, and *Cibicides mckannai* showed highest densities at 1½-2cm. The average non-seep density was 926/50cc. Distribution patterns of total agglutinated and calcareous plots mimic each other as can be seen in Figure 11.

Non-seep foraminiferal distribution patterns

Infaunal maxima of most non-seep foraminifera occur closer to the sediment-water interface (Rathburn *et al.,* 2003). Non-seep vertical distribution patterns of this study show infaunal maxima closer to the sediment-water interface. Vertical distribution patterns of non-seep cores collected from off-seep Mound 12 appear to be very similar to one another. In core MC1 (Figure 10d), infaunal population maxima of *Chilostomella oolina* occurred at 1½-2cm, *Uvigerina peregrina* at 2½ -3cm, *Cibicides mckannai* 1½-2cm, and *Cassidulina braziliensis* at 0-1cm. In core MC1, which had a total density of 742/50cc, distributions of *Uvigerina hispida* appeared shallower than *Uvigerina peregrina*, with an infaunal maximum at 0-1cm. Core MC2, which had a total density of 703/50cc (Figure 10e), had the same infaunal maxima as MC1 in *Chilostomella oolina* (1½-2cm), *Cibicides mckannai* (1½-2cm), and *Cassidulina braziliensis* (0-1cm), but unlike MC1, *Uvigerina peregrina* in MC2 displayed two infaunal maxima at 0-1cm and 1½ -2cm. MC3, possessed total density of 389/50cc (Figure 10f), and had the same infaunal maxima as MC1 and MC2 in *Cibicides mckannai* (1-1½cm). Unlike the infaunal maxima of MC1 and MC2, *Chilostomella oolina* in MC3 possessed double maxima at 0-1cm and 2½ -3cm. *Uvigerina peregrina* and *Uvigerina hispida* of MC3 possessed a shallow infaunal maxima (0-1cm) more similar to the infaunal maximum seen in *Uvigerina hispida* in MC1. *Cassidulina*

braziliensis of MC3 also presented slightly deeper (1-1½cm) than MC1 and MC2. MC4, total density of 1870/50cc (Figure 10g), showed *Chilostomella oolina, Cibicides mckannai,* and *Cassidulina braziliensis* all showed highest densities at 1½-2cm. This depth is consistent in all cores for *Cibicides mckannai,* and the same in MC1 and MC2 (not MC3) for *Chilostomella oolina*. The infaunal maximum of *Cassidulina braziliensis* of MC4 is the deepest of all the cores. MC4 also has an infaunal maxima of *Uvigerina peregrina* and *Uvigerina hispida* at 1-1½cm which is dissimilar from all other cores. The average seep density was 526/50cc and the average non-seep density 926/50cc. Total infaunal distributions of total non-seep agglutinated and calcareous individuals mimic each other in as can be seen in Figure 10.

Seep and Non-seep Comparisons

Vertical distributions of the six most abundant species, average vertical distributions were plotted, and average living depth (ALD) was calculated for non-seep and seep cores (Figures 10-11, Table 3). Averages and standard deviations instead of totals, were examined due to differences in the number of cores analyzed for non-seep and seep cores. Bacterial mat (seep) abundances were highest at depths of 2-2½cm and 1½-2cm intervals while the highest abundances in non-seeps occurred in the 0-1cm and 1-1½cm intervals. A two tailed t-test was used to statistically compare total densities between seep nearby non-seep sites. The test reveled statistical similarity between the total densities of seep and non-seep cores ($p=0.78$; Table 4). Agglutinated maxima coincided with the maxima in calcareous foraminifera and total maxima in both the seep and non-seep averages (Figure 11). Average species vertical distribution patterns in seep foraminifera show most species increasing with depth to 3cm (one interesting exception to be discussed later is *Chilostomella oolina*). Average species vertical distribution patterns in non-seep foraminifera showed a significant reduction in most species below 2cm. Of the six most

abundant species found at both seep and non-seep sites, average living depth calculations of all species (Table 3) revealed deeper average living depths at seeps than non-seeps. The one exception to this general trend of deeper ALD at seeps was *Chilostomella oolina*, where distribution patterns are very similar between seep and non-seep environments and ALD is deeper at non-seeps (Table 3).

CellTracker Green compared to Rose Bengal

The top three most abundant species (*Chilostomella oolina, Cibicides mckannai*, and *Uvigerina peregrina*) from both CTG labeled and RB stained samples were averaged from the three seep cores (AD4511, AD4586, AD4587; Figure 12) and total percent abundances were compared between staining techniques (Figure13). All RB data include CTG labeled individuals based on the assumption that CTG labeled individuals would have stained with RB. *Chilostomella oolina* comprised 51% of 0-1cm, 43% of 1-1½cm, and 65% 1 ½ -2cm of samples labeled with CTG. RB stained *Chilostomella oolina* comprised 17% of 0-1cm, 15% of 1-1½cm, and 15% 1½ -2cm. *Cibicides mckannai* comprised 25% of 0-1cm, 22% of 1-1½cm, and 6% 1½ -2cm of samples labeled with CTG. RB stained *Cibicides mckannai* comprised 14% of 0-1cm, 15% of 1-1½cm, and 13% 1½ -2cm. *Uvigerina peregrina* comprised 7% of 0-1cm, 5% of 1-1½cm, and 0% 1½ -2cm of samples labeled with CTG. RB stained *Uvigerina peregrina* comprised 11% of 0-1cm, 15% of 1-1½cm, and 11% 1½ -2cm. Total density averages of the three cores show *Chilostomella oolina* comprises 54% of CTG labeled individuals and 71% of RB individuals. *Cibicides mckannai* comprises 16% of CTG labeled and 63% of RB individuals. And *Uvigerina peregrina* comprises only 3% of CTG labeled individuals and 55% of RB individuals.

Discussion

No endemic foraminifera have yet been discovered at seep sites; however, similar to other studies, taxa that are cosmopolitan and important in paleoceanographic studies were abundant at active sites of seepage and inactive sites near Costa Rica (Rathburn *et al.,* 2000, 2003; Hill *et al.,* 2004). These abundant taxa included *Bolivina*, *Cassidulina, Cibicides, Chilostomella*, and *Uvigerina.* Bacterial mats in Costa Rica possess vertical distribution patterns that are not typical of bacterial mats previously reported from the Pacific. The similarity of these distribution patterns to those of clam beds from Monterey Bay suggests increased biologic activity, or bioturbation, as a contributor to these atypical distributions.

CellTracker Green compared to Rose Bengal

Comparisons of two methods of differentiating living from non-living foraminifera allow for quantification of the potential for over or under-labeling/staining, allowing for more accurate foraminiferal population identification in the future. As expected, abundances of CTG labeled individuals (Table 4) are much lower than RB stained individuals (Figure 12 Table 4). This pattern has been observed in studies of non-seep environments (Bernhard *et al.,* 2006) and may result from overstaining by RB, underlabeling of CTG (e.g. death of individuals before incubation) or a combination. Despite density differences, both methods showed the presence of substantial infaunal populations at seep sites (Figure 12).

This comparison of total percent abundance of species stained with CTG and labeled with RB at seeps is one of the first of its kind. Comparing percent abundance of the three most abundant species of CTG labeled and RB stained foraminiferal species allows for a quantitative assessment of the two labeling techniques and will illustrate the potential for over or under-labeling/staining between them (Figure 13). As can be seen in Figure 13, variations exist within

percent abundances between the two labeling techniques. Percent abundances of CTG are always higher for *Chilostomella ollina*, and are higher in 0-1 and 1-1½cm for *Cibicides mckannai,* but RB are higher for *Uvigerina peregrina*. These differences in labeling and staining activity illustrate the possibility of over/under-labeling/staining to exist between techniques. It is assumed that CTG is a conservative labeling technique due to the requirement of an actively metabolizing cell (Bernhard *et al.,* 2006). Possible reasons for the differences between species include the following:

1. A higher percentage of *C. oolina* and *C. mckannai* may be able to survive the trip from the seafloor better than *U. peregrina* and more are alive to be labeled with CTG (understaining of CTG).
2. *U. peregrina* protoplasm degrades slower than that of *C. oolina* and *C. mckannai*, so more recently living specimens of *U. peregrina* stain with RB, and reducing relative percentages of *C. oolina* and *C. mckannai.* Burial and preservation of foraminiferal protoplasm may result in RB stained individuals (overstaining by RB).
3. At the time of collection, more *C. oolina* and *C. mckannai* were alive in the seeps than *U. peregrina* (accurate labeling by CTG; seasonal differences in *U. peregrin*a populations, leaving more recently dead specimens).
4. Metabolic differences between species may cause differences with the uptake of CTG.

Seep and Non-seep Comparisons

Heinz *et al.* (2008) reported benthic foraminiferal distribution of non-seep areas off the Pacific coast of Nicaragua and Costa Rica. Direct comparisons of the results of Heinz *et al.* (2008) with this study are difficult due to differences in methodology. Heinz *et al.* (2008) dried

and re-wet samples prior to picking stained individuals, while this study kept samples wet before and during picking. Heinz *et al.* (2008) split samples into 63–125µm and >125 µm size fractions while this study used >150µm. Despite differences in methodologies, vertical distribution patterns of Heinz *et al.* (2008) are similar to those of non-seep sites of this study. This is expected as Heinz *et al.* (2008) indicated that all cores used in the study showed no indication (e.g., sulfur smell, chemosynthetic organisms) of seepage activity. At Mound 12, Heinz *et al.* (2008) reported stained foraminifera as deep as 5cm with density maxima occurring near the surface and decreasing with sediment depth, a similar pattern to that observed in the non-seep samples of this study (Figures 10d-g and 11b and d).

Several other seep sites in the Pacific have also been examined for benthic foraminiferal vertical distribution patterns (Rathburn *et al.,* 2000; Rathburn *et al.,* 2003; Torres *et al.,* 2003) with some showing substantial infaunal populations. Vertical distribution patterns from clam beds off the coast of California produced density maxima at 2.5-3cm for several species and were attributed to a favorable infaunal microenvironment (Rathburn *et al.,* 2000). Rathburn *et al.* (2003) sampled both clam beds and a bacterial mat in Monterey Bay and found that clam beds contained slightly higher foraminiferal densities than bacterial mats (Table 4). Rathburn *et al.* (2003) noted that these densities were within the range of non-seep environments reported in previous studies of the Pacific margin at ~1000m water depth. Densities of seep sites from this study were variable, yet were not statistically different than the nearby non-seep densities (Table 4). Average seep and non-seep densities show even less variability with 449/50cc at seeps and 926/50cc at nearby non-seeps. At Hydrate Ridge (Torres *et al.,* 2003) and Blake Ridge (Panieri *et al.,* 2008), densities of living benthic foraminifera were higher at seep sites compared to non-seep sites, while Bernhard *et al.* (2001) observed lower densities at seep sites than non-seep sites

in Monterey Bay (average 33/cm^2 Seep vs. 80/cm^2 Non-seep in 0-1cm). The results of this study are consistent with those suggested by Rathburn *et al.* (2003); in general, seep foraminiferal densities are similar to those of non-seep environments.

Vertical distribution patterns of foraminifera in clam beds from Rathburn *et al.* (2003) show appreciable infaunal abundances within the sediments from 1.5 cm to 4 cm. Bacterial mats in Monterey Bay have been reported to have infaunal maxima near the surface and densities decreasing deeper in the sediment (Rathburn *et al.,* 2003; Geiskes *et al.,* 2011). Clam beds showed increasing abundance of several species, including *Uvigerina peregrina*, in the 1½ to 2cm interval with substantial populations as deep as 4cm (Rathburn *et al.,* 2003). Infaunal densities of *Uvigerina peregrina* were evident in bacterial mats from Costa Rica (AD4587 *Uvigerina peregrina* at 1-1½cm; AD4586 *Uvigerina peregrina* at 2-2½cm; AD4511 *Uvigerina peregrina* at 2-2½cm). Costa Rican seep distributions present an unusual pattern of typically deep infaunal species residing at shallower depths than their shallower infaunal counterparts (McCorkle et al., 1990; Rathburn and Corliss, 1994). Within sediments from seeps, *Uvigerina peregrina*, a typically shallow infaunal species, tends to be found in shallower sediment depths than *Chilostomella oolina*, a deep infaunal species (e.g. McCorkle et al., 1990; Rathburn and Corliss, 1994). Rathburn *et al.* (2003) also noticed this unusual pattern of *U. peregrina* consistently deeper than a typically deeper infaunal species (*Globobulimina pacifica*) at clam beds but not in bacteria mats at Monterey Bay. It is not known what is driving this inverted microhabitat preference at these bacteria mats in Costa Rica, but vertical distribution patterns may result from bioturbation creating suitable microhabitats deeper within the sediment (Loubere *et al.,* 1995). Distribution patterns of the nearby non-seep samples show *U. peregrina* maxima within a more typical microhabitat, shallower than *C. oolina* (MC1 *Uvigerina peregrina*

at 2½ -3cm, MC2 *Uvigerina peregrina* maxima at 0-1cm and 1½ -2cm, MC3 *Uvigerina peregrina* at 0-1cm, MC4 *Uvigerina peregrina* at 1-1½cm). Geiskes *et al.* (2011) also observed appreciable densities and infaunal distribution within clam bed habitats from Monterey Bay, in which vertical distribution patterns revealed infaunal species able to live deeper within clam beds than bacterial mats likely due to the greater biologic activity that extended more hospitable conditions deeper into the sediments. Vertical distribution patterns from bacterial mats of Costa Rica resemble those of clam beds observed by Gieskes *et al.* (2011) rather than previously observed bacterial mats (Rathburn *et al*., 2003).

Seep clams bioturbate sediments, creating heterogeneous distributions of oxygen and organic materials deeper within the sediments. This may allow shallow infaunal species to inhabit deeper sediments, creating this unique pattern of deep infaunal species above typically shallower sediment counterparts. Rathburn *et al.* (2003) observed these unique distributions in clam beds, and the vertical distribution patterns from this study appear similar to these distributions, suggesting a similar mechanism. However, significant bioturbation is not typical in dense bacterial mats, which are often associated with the lowest oxygen penetration and highest sulphide concentrations at seeps (Levin *et al.,* 2005). Nevertheless, large polychaete worms were observed in bacterial mat cores processed in this study. The presence increased organismal activity in more sparce Costa Rican bacterial mats may create deeper microhabitats suitable for shallow infaunal taxa, and explain the substantial infaunal foraminiferal populations and unique vertical distribution patterns more commonly seen in clam beds.

Conclusions

Vertical distribution patterns of Costa Rican bacterial mats reveal an atypical distribution of typically shallow infaunal species deeper than their typically deep infaunal counterparts. This

pattern has been observed previously in clam beds and may be explained by polychaete bioturbation. No significant differences were observed between seep and non-seep densities or species assemblages. The results of this study demonstrate that foraminiferal ecology, distribution, and densities are similar between seep and nearby non-seep environments in the Costa Rican margin.

Comparisons between CTG and RB suggest the potential for over and/or under staining/labeling by both techniques. Some species may be better able to survive between collection and incubation than others, differences in protoplasm degradation rates between species, differences in the species present at the time of collection, and metabolic differences between species causing differences with the uptake of CTG, or a combination of the suggested mechanisms may contribute to the difference between the two techniques.

CHAPTER 4

SUMMARY

Distribution patterns of foraminifera living in Costa Rican bacteria mats are different from those reported from bacterial mats in other regions (Rathburn *et al.,* 2000; Rathburn *et al.,* 2003). Substantial infaunal populations and distribution patterns were more reminiscent of clam beds than bacteria mats (Rathburn *et al.,* 2003). The causes of differences in vertical distribution patterns of these Costa Rican bacterial mats likely the result of polychaete bioturbation, and analyses of foraminiferal and macrofaunal interactions are needed to confirm this hypothesis as the cause of these atypical vertical distribution patterns. Previous studies of seep sites in the Pacific have reported variable foraminiferal densities between seep and non-seep sites (Rathburn *et al.,* 2000; Bernhard *et al.,* 2001; Rathburn *et al.,* 2003; Torres *et al.,* 2003; Panieri *et al.,* 2008). Foraminiferal densities from this study suggest that no significant difference in foraminiferal densities existed between seep and non-seep sites on the Costa Rican margin. Total percent abundances of foraminifera using two different techniques for identifying living/recently living foraminifera suggest the potential for over and under labeling/staining.

Statistical analyses revealed statistical differences between $\delta^{18}O$ compositions of *Cibicides wuellerstorfi* from the three active seep sites (Mound 11, Mound 12, and Jaco Scar). However, these bottom water temperatures calculated from *Cibicides wuellerstorfi* calcite

yielded temperatures ~3°C different from the measured values, suggesting influences other than temperature. It is known that *Cibicides wuellerstorfi* precipitates its calcite in equilibrium with bottom waters; therefore the $\delta^{18}O$ values obtained from these *Cibicides wuellerstorfi* would be expected to be representative of bottom water values and comparisons of foraminiferal carbonate geochemistry with geochemistry of the bottom waters will determine if the calculated temperatures are an artificial or true disequilibrium. This disequilibrium may be explained by variations in bottom waters $\delta^{18}O$. This study finds no apparent methane influence on the foraminiferal calcite of elevated epibenthic foraminifera from all three active seep sites (Mound 11, Mound 12, and Jaco Scar). This may be because the elevated epibenthics were not exposed to seep-influenced fluids by inhabiting raised substrate. Stable carbon isotopic comparisons of foraminiferal carbonate of elevated epibenthics and the substrate on which they were found suggest that elevated epibenthics do not obtain stable isotopic values of the tests directly from their substrate.

This study provided a first look at coiling direction of elevated epibenthic foraminifera from sites of active methane seepage. Statistical analysis revealed no differences in stable isotopic composition between dextrally and sinstrally coiled *Cibicides wuellerstorfi*. *Cibicides wuellerstorfi* demonstrated a dominance of dextral coiling, while *Carpenteria monticularis* was observed to have a dominance of sinstral coiling. Differences in coiling direction of these two co-occurring species suggest that the primary influence of coiling direction in these species are biological differences rather than environmental factors and suggest that the relationship between ambient temperature and coiling direction seen by Collins (1990) does not apply to these species of elevated epibenthic species. The results of this study suggest that biologic factors rather than

environmental factors influence the coiling direction of *Cibicides wuellerstorfi* and *Carpenteria monticularis*.

FIGURES

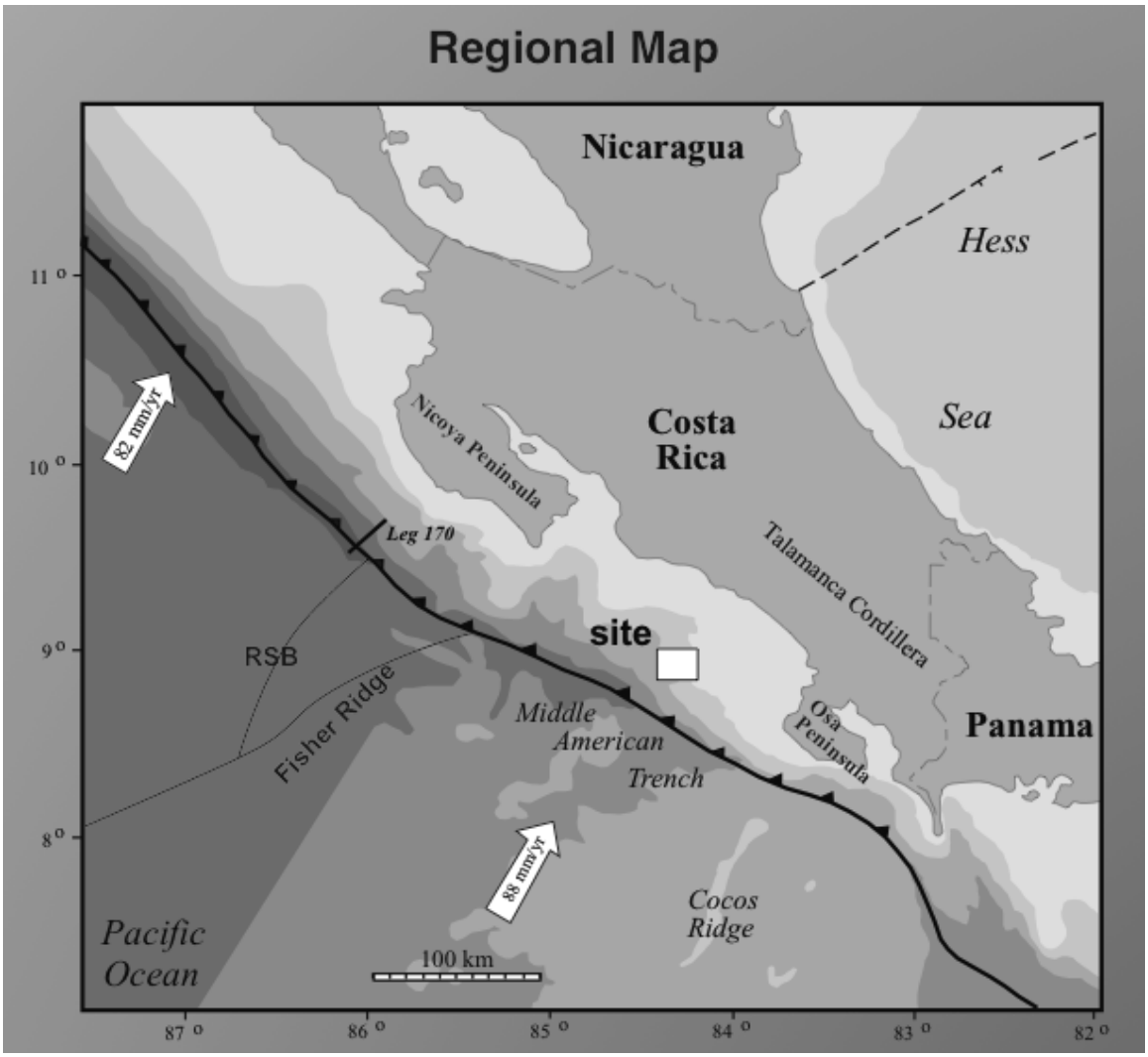


Figure 1. Costa Rica regional map modified from Tryon *et al.,* 2010. Samples analyzed in this study were taken from seep and non-seep areas off the Pacific coast of Costa Rica. Three active seep sites, designated Mound 11, Mound 12, and Jaco Scar were sampled and lie within the white square on the map. In this region the Cocos Plate is subducting under the Caribbean Plate at a rate of 88 mm/yr. these subduction processes are resulting in these seep habitats off the coast of Costa Rica. Map area encompasses 7° to 12°N and 82° to 88°W.

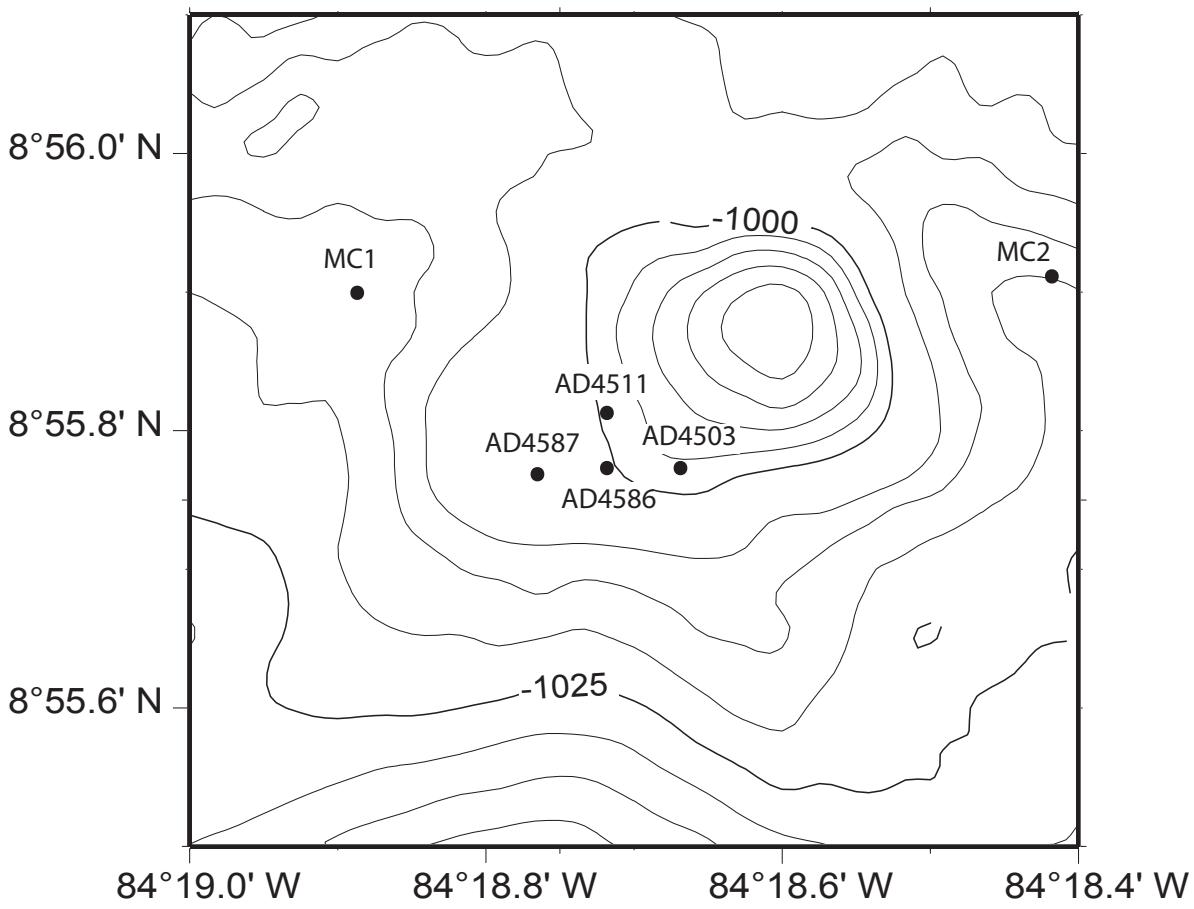


Figure 2. Mound 12 sample area map (modified from Mau, 2004). This study analyzed sediment cores from bacterial mats at AD4511, AD4586, and AD4587. Adjacent non-seep cores were taken in the area and include MC1, MC2, MC3 (not pictured), and MC4 (not pictured). Tubeworms examined for elevated epibenthic foraminifera from Mound 12 were collected at AD4586, AD4587, and AD4503.

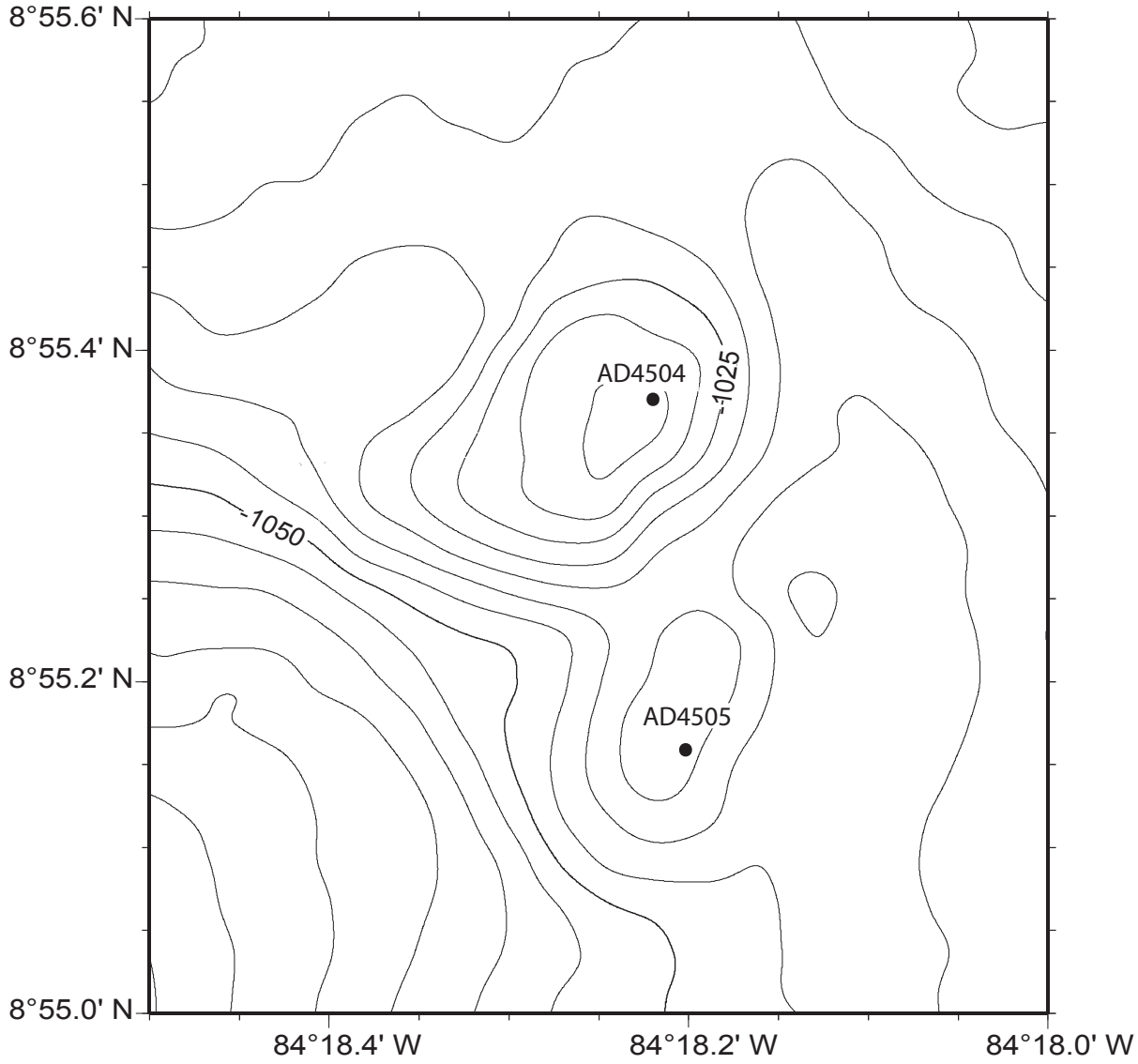


Figure 3. Mound 11 sample area map (modified from Mau, 2004). Tubeworms examined for elevated epibenthic foraminifera from Mound 11 were collected at AD4504, and AD4505 shown in this figure.

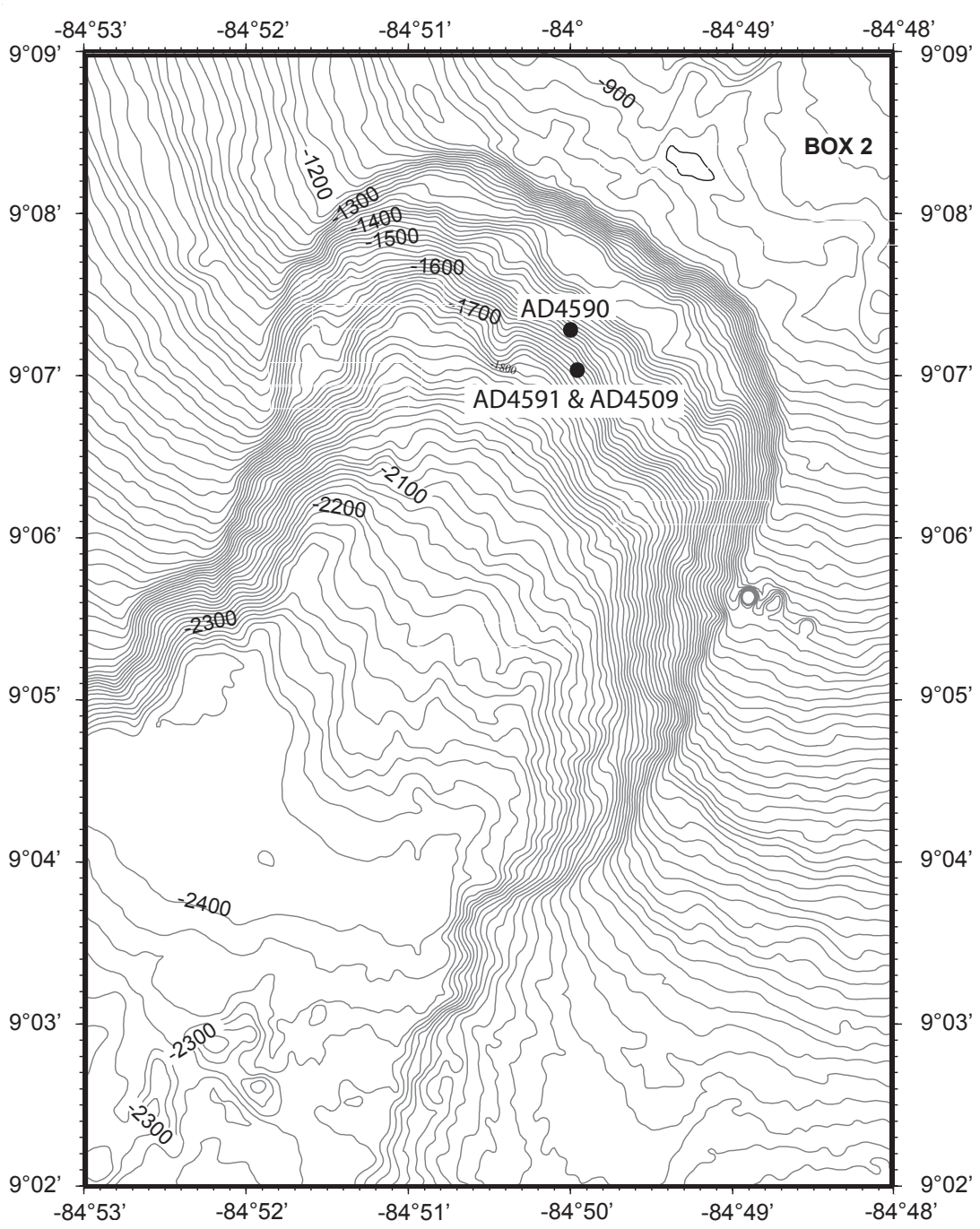


Figure 4. Jaco Scar sample map area (modified from Mau, 2004). Tubeworms examined for elevated epibenthic foraminifera from Jaco Scar were collected at AD4509, and AD4590 shown in this figure.

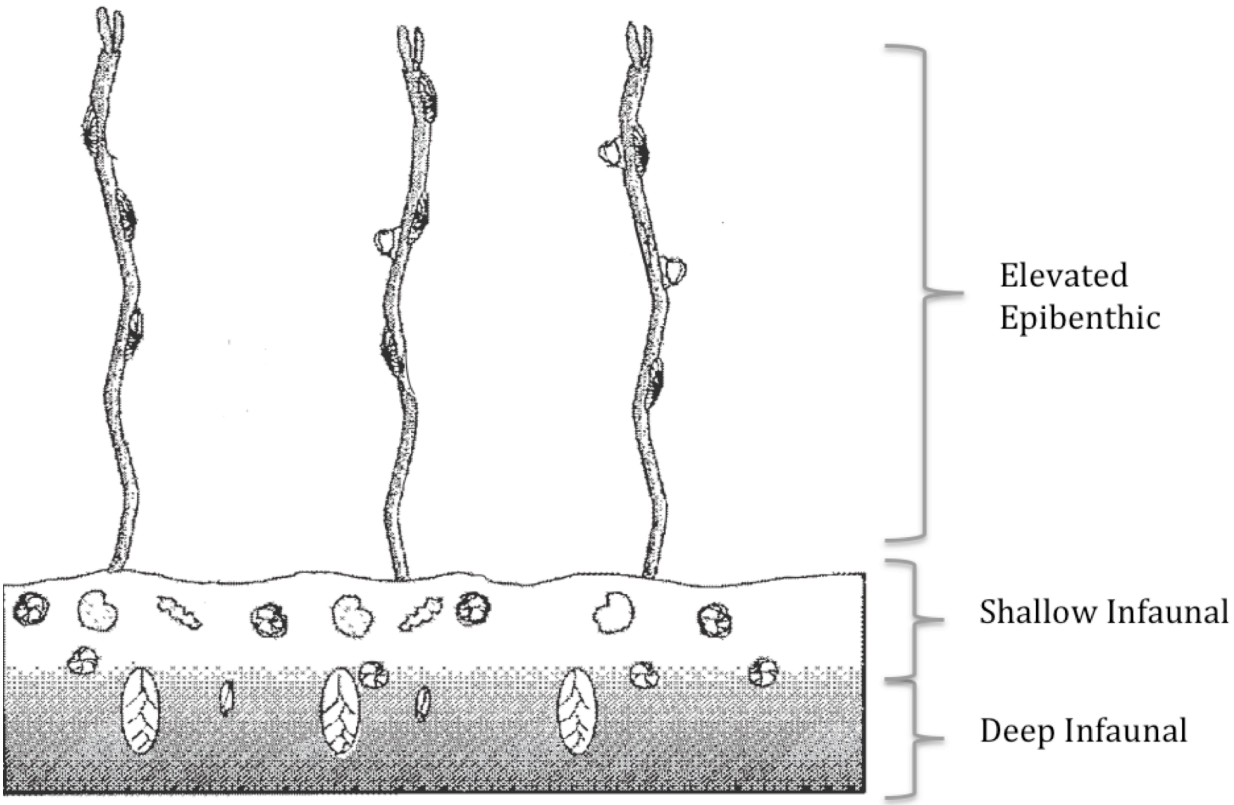


Figure 5. Microhabitat definitions used in this study. Individuals attached to vestimentiferan tubeworms are raised above the sediment- water interface and are referred to as elevated epibenthic. Shallow infaunal are those capable of living within the sediments from 0-2cm and deep infaunal are those that are capable of living within the sediments at depths greater than 2cm. This diagram modified from Wollenburg *et al.* (2009).

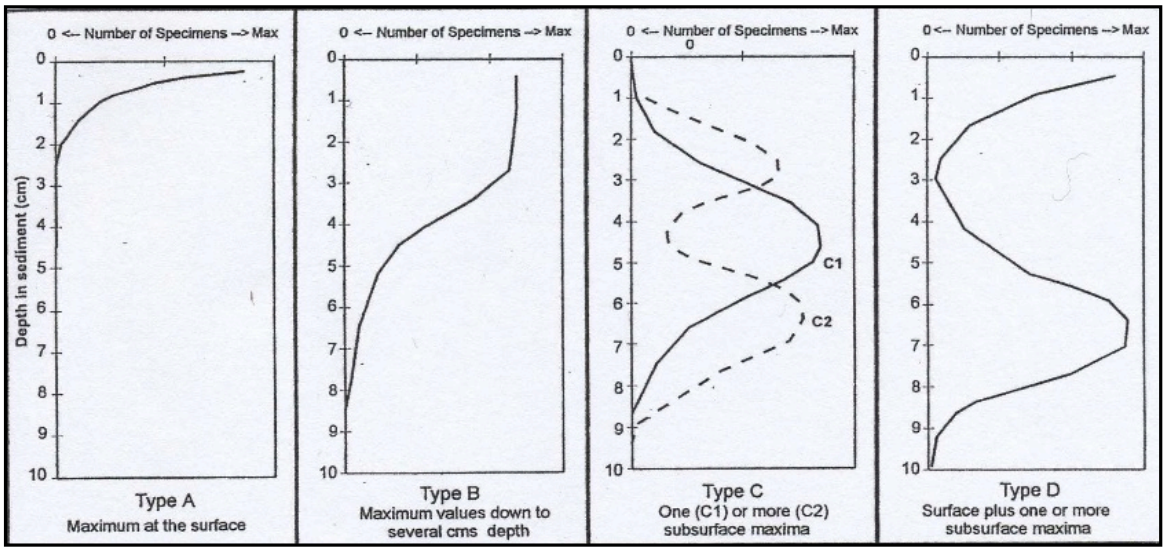
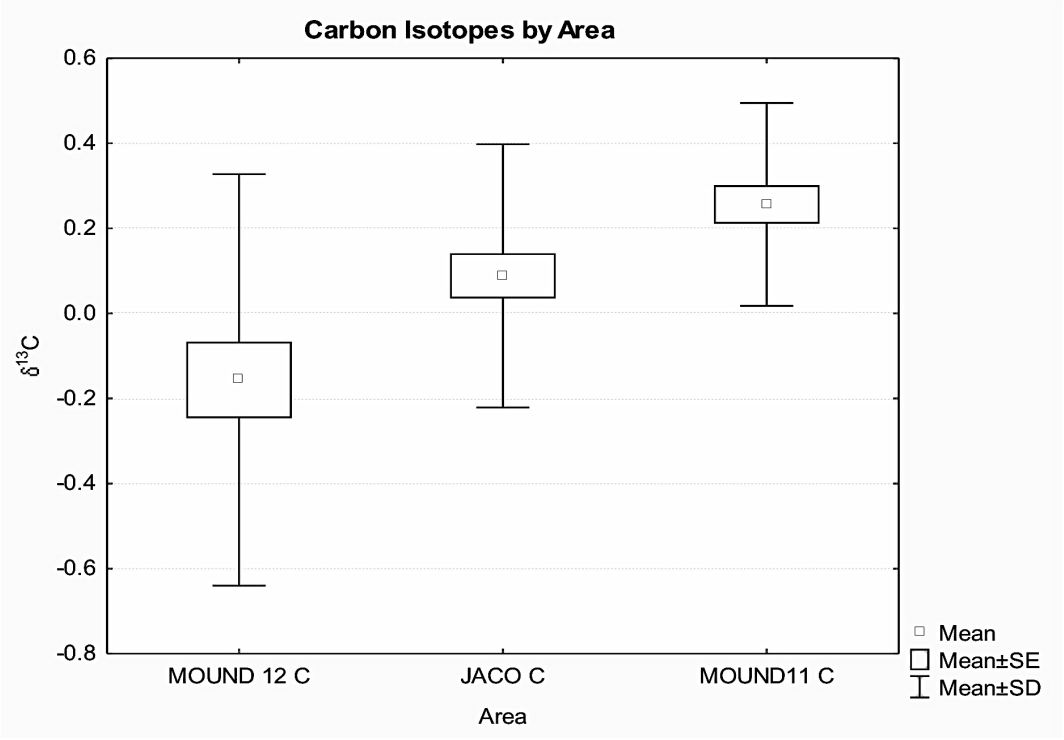


Figure 6. Vertical distribution patterns of foraminiferal assemblages to group them into categories. Figure taken from Sen Gupta (1999) based on Jorissen (1999).

7a.



7b.

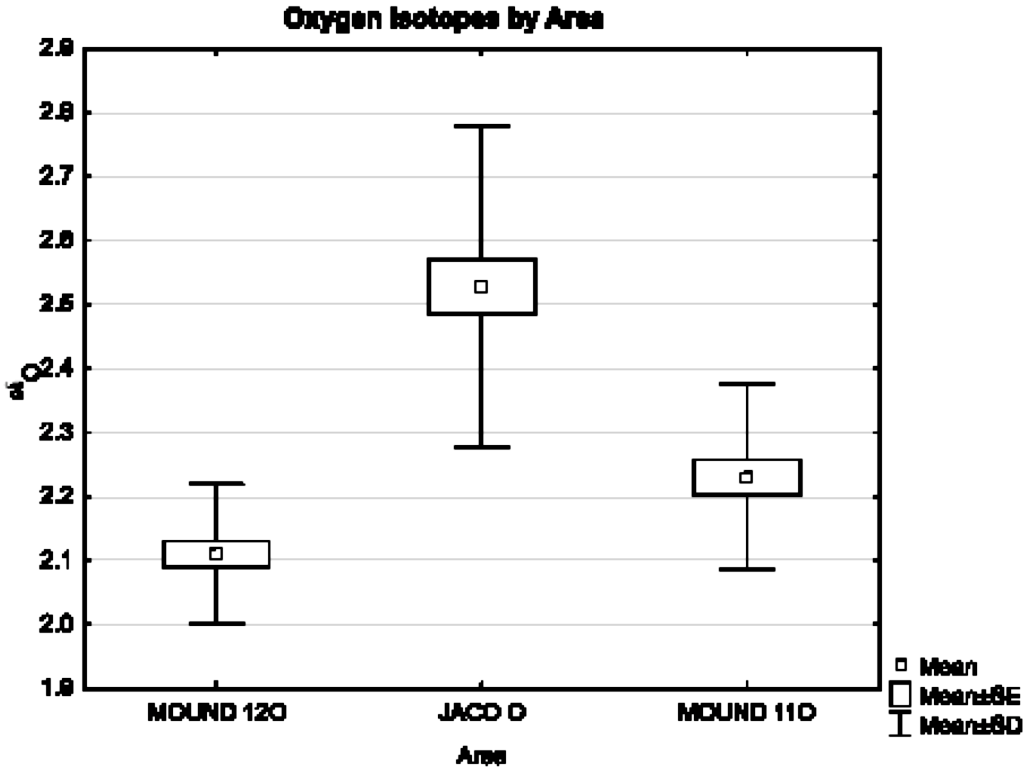


Figure 7. Box plots illustrating mean, standard deviation and standard error of stable isotopic composition of foraminiferal calcite between areas (Mound 11, Mound 12, and Jaco Scar). a: Carbon Isotopes b: Oxygen Isotopes

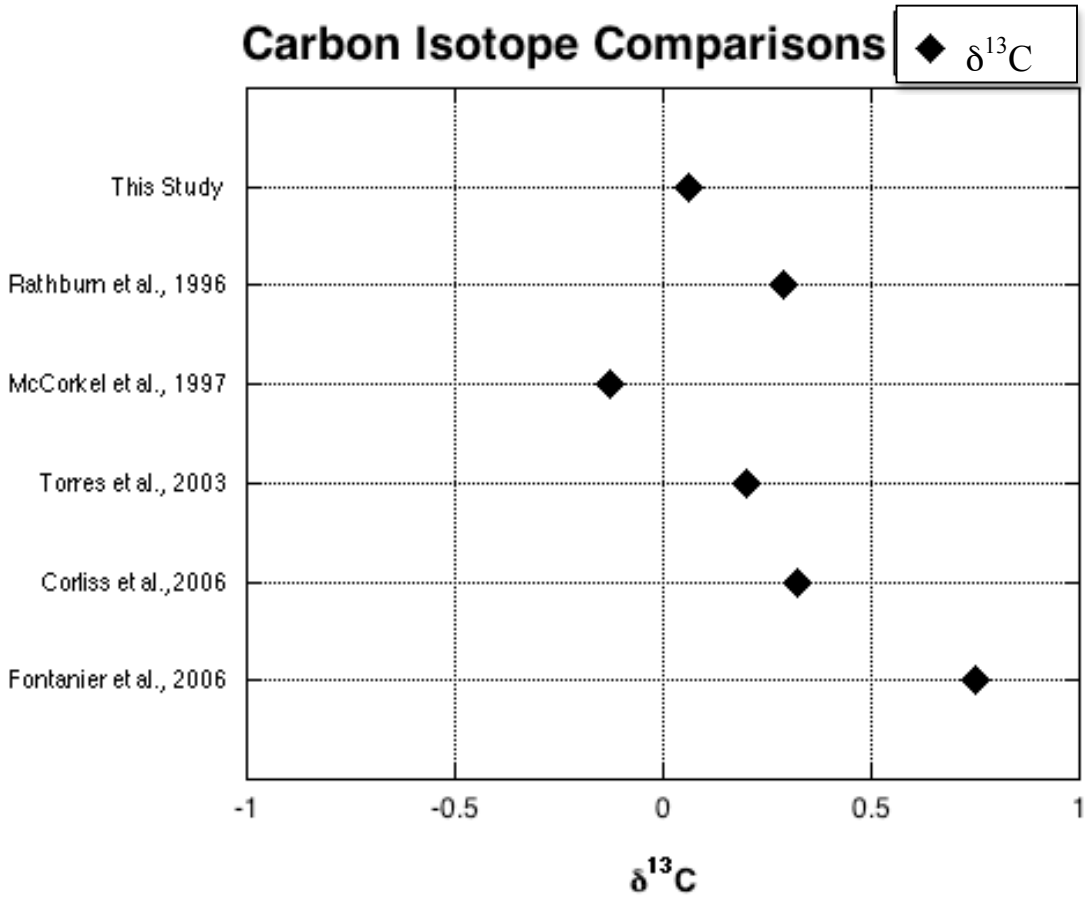


Figure 8. Average carbon isotopic values from seep *Cibicides wuellerstorfi* compared to non-seep *Cibicides wuellerstorfi* from previous studies. Typically carbon isotopic values of non-seep *Cibicides wuellerstrofi* range.

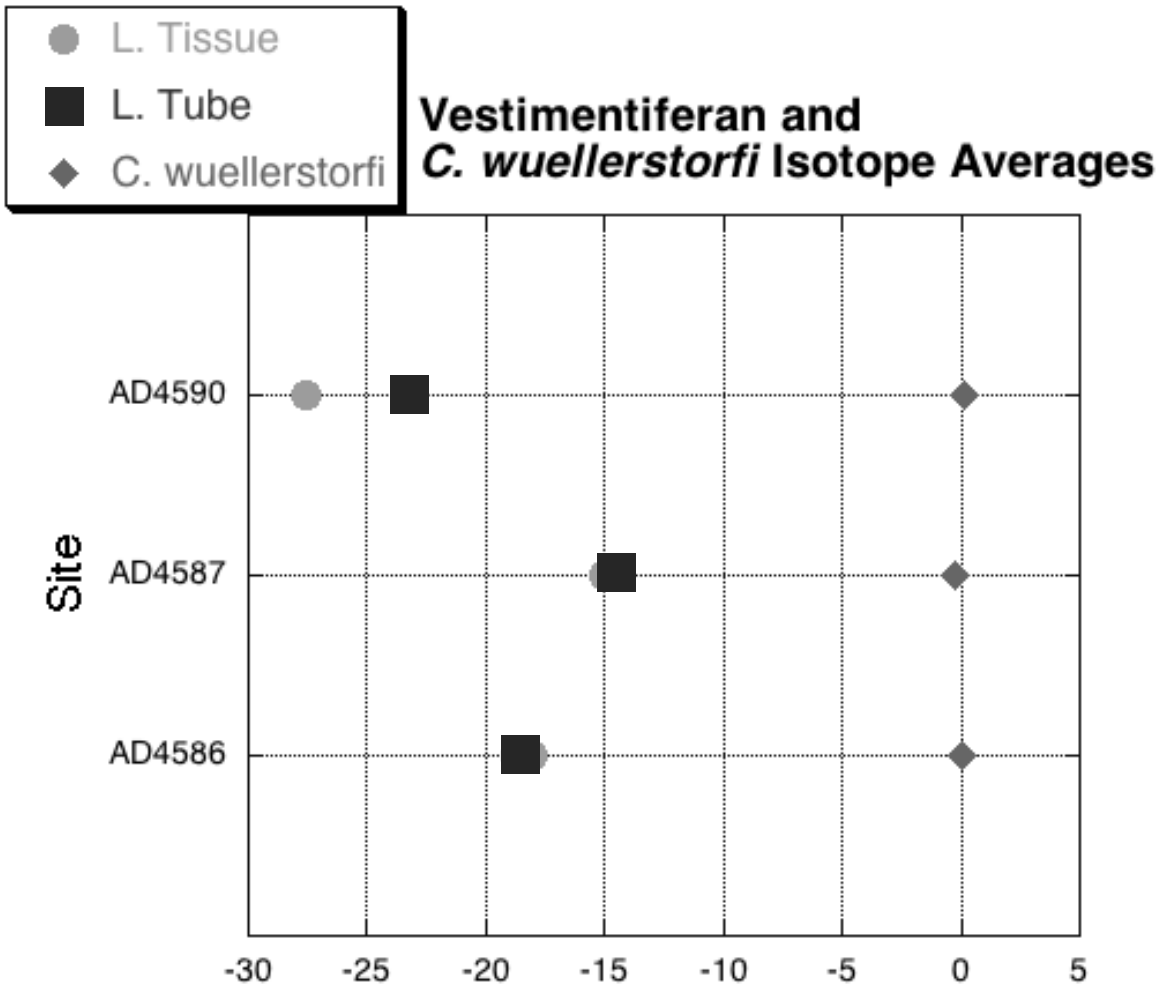


Figure 9. $\delta^{13}C$ values of foraminiferal calcite and the substrate on which it was attached. $\delta^{13}C$ of elevated epibenthic species *Cibicides wuellerstorfi* (diamond), vestimentiferan tissue (circle), and vestimentiferan tube (circle). *Cibicides wuellerstrofi* show $\delta^{13}C$ values 10 to 30‰ less depleted than that of vestimentiferan on which it resides.

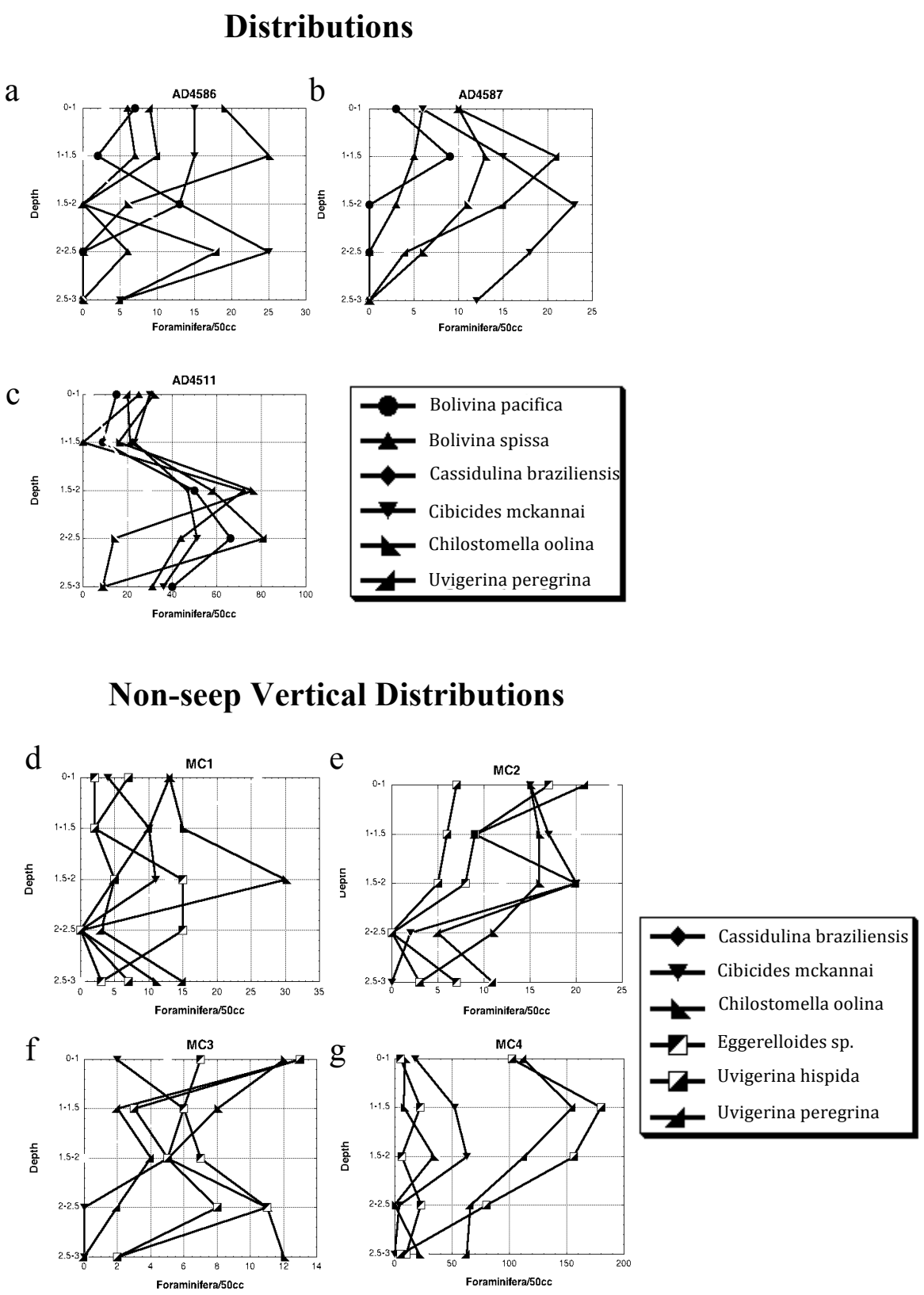


Figure 10. Vertical distribution patterns of each core analyzed in this study. Seep cores (a-c) and non-seep cores (d-g).

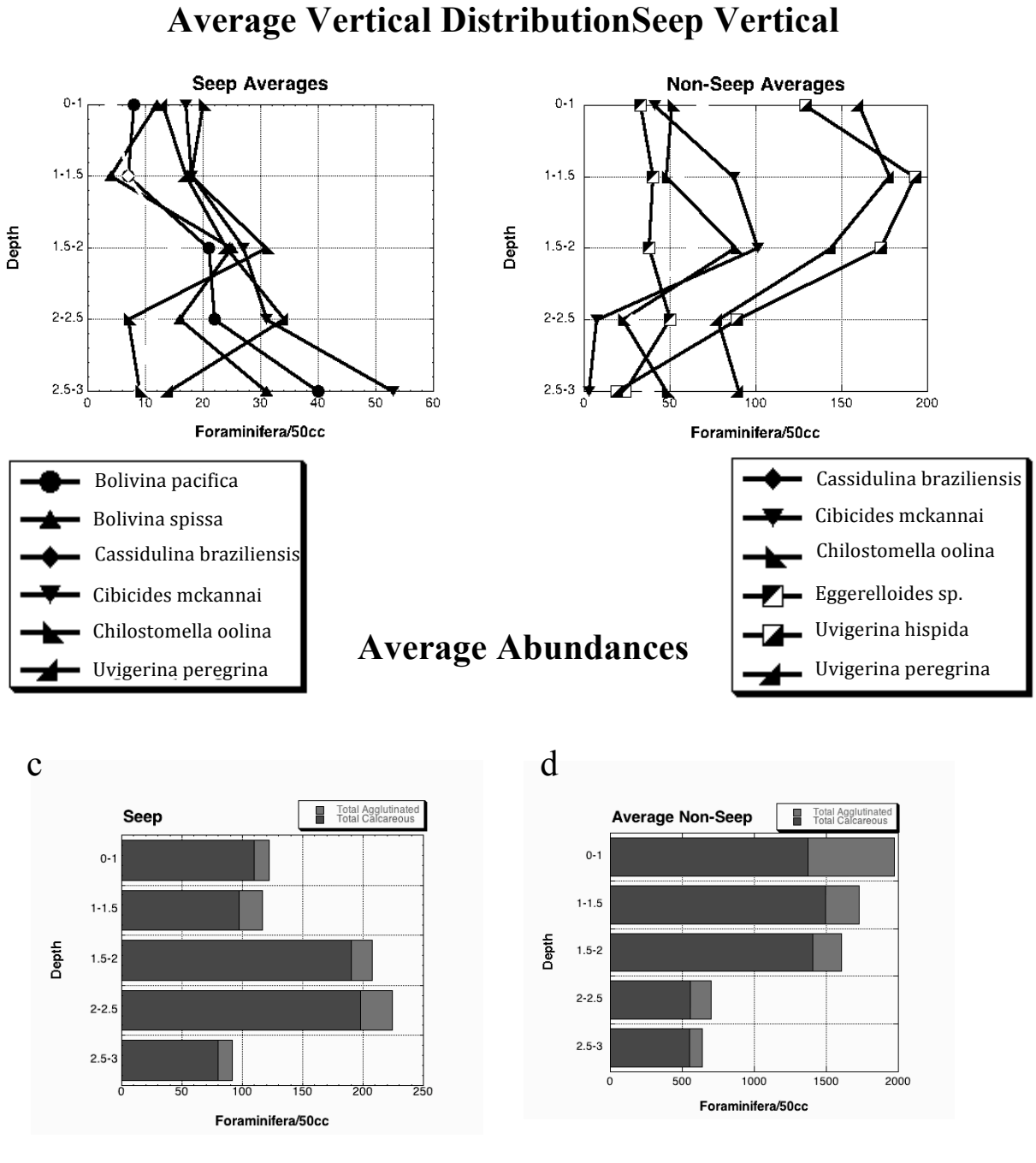


Figure 11. Average vertical distributions pattern of seep (a) and non-seep (b) sites illustrate the depth similarities and differences between specific species (such as *Uvigerina peregrina* and *Chilostomella oolina*). Average abundances (c-d) clearly show infaunal populations at a greater depth in seep sites when compared to non-seep sites.

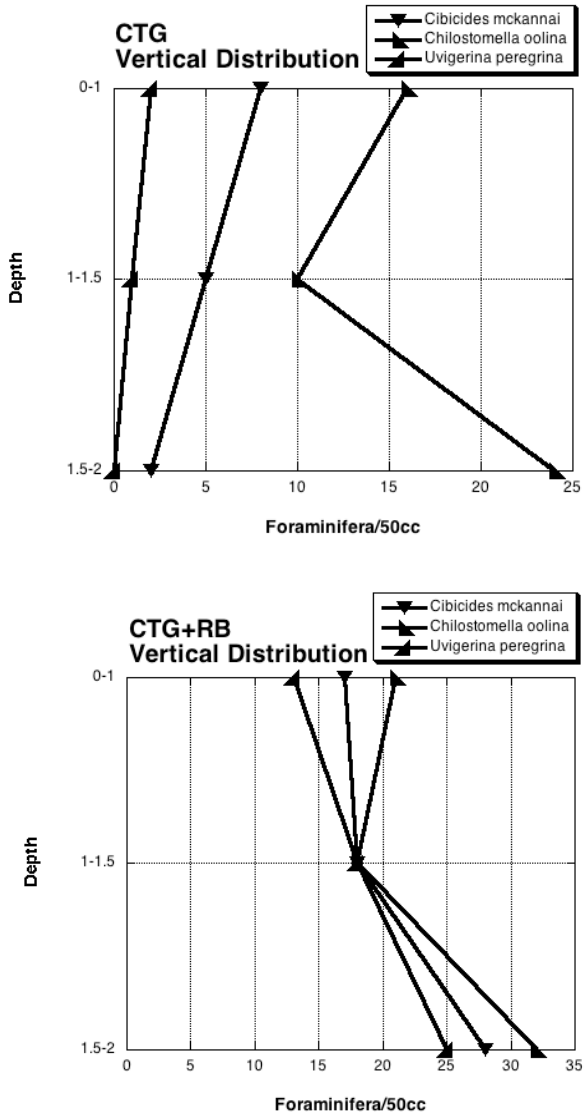


Figure 12. Average vertical distributions pattern of the three most abundant species of CellTracker Green (CTG) labeled and Rose Bengal (CTG+RB) stained show that despite density differences, both vital recognition methods showed the presence of substantial infaunal populations at seep sites

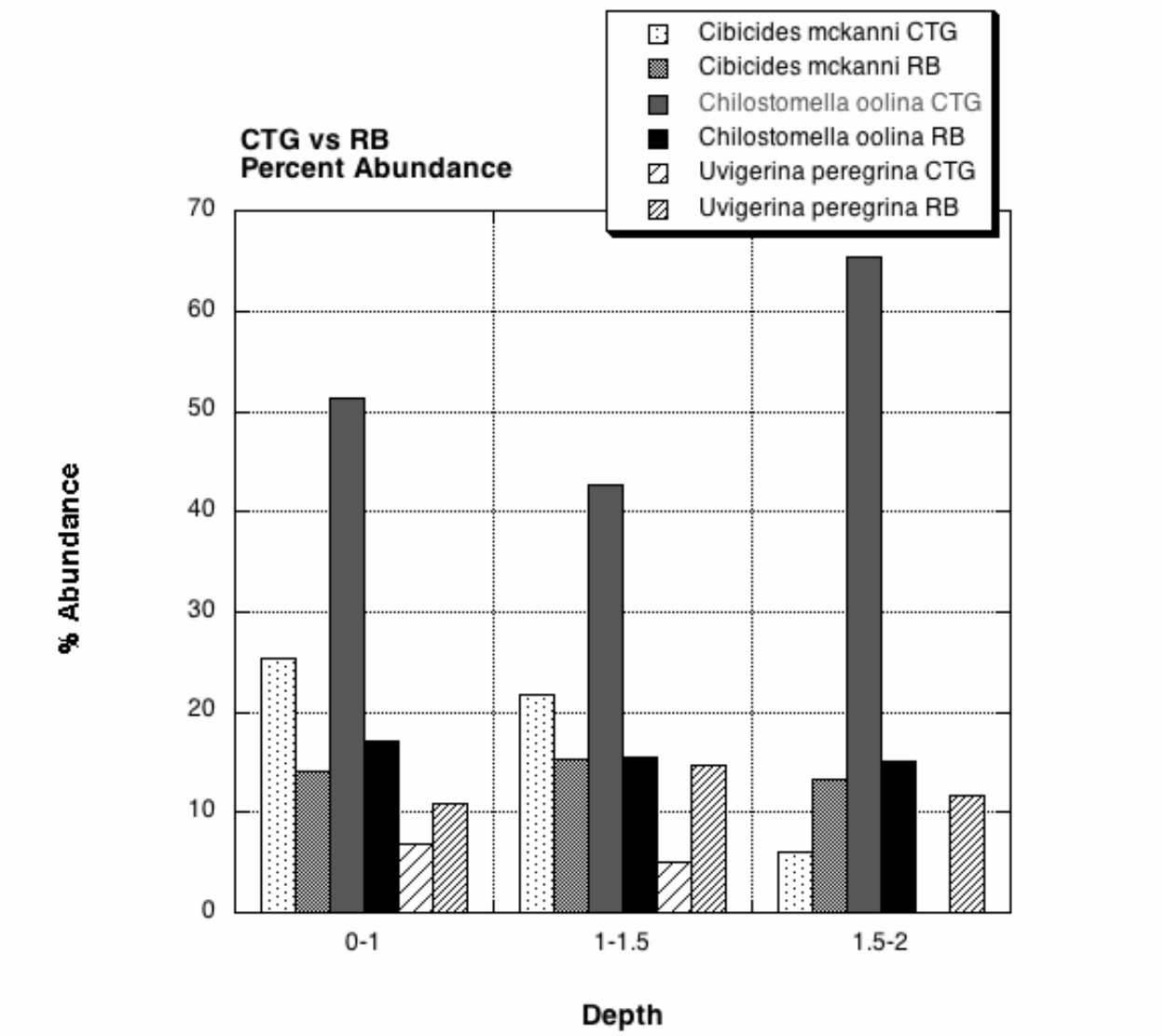


Figure 13. Total percentage of the top three most abundant species labeled with CTG vs. those stained with RB (RB+CTG) show differences in the two techniques. These differences in labeled and stained populations may be a result of survivorship, differences in protoplasm degradation between species, and/or metabolic differences between species can cause differences in the uptake of CTG.

Table 1
Geographic and bottom water conditions of sediment cores used for ecological analysis on the Costa Rican margin.

Dive No.	Core No.	Habitat	Depth (m)	BW Oxygen (mL/L)	BW Temp (C)	BW Sal	Lat(N)	Long(W)
AD4511	TC2	Bacterial Mat White	995	0.52	4.9	34.58	8 55.833	84.18.744
AD4586	TC4	Bacterial Mat Grey	997	0.77	4.6	34.58	8 55.790	84 18.722
AD4587	TC2	Bacterial Mat	1001	0.76	4.5		8 55.767	84 18.779
MC1	7	Off-seep	995	0.77	4.6	34.58	8 55.951	84 18.948
MC2	2	Off-seep	988	0.77	4.6	34.58	8 55.999	84 18.443
MC3	2	Off-seep	1008	0.77	4.5	34.58	8 55.498	84 18.497
MC4	2	Off-seep	1031	0.81	4.4	34.58	8 55.249	84 18.097

Table 2
a.Estimated and measured bottom water temperatures. Using estimates of bottom water $\delta^{18}O_{SMOW}$ from P19C of the WOCE Atlas in combination with $\delta^{18}O$ of foraminiferal carbonate to calculate bottom water temperatures. Calculations were completed using an equation modified from Friedman and O'Neil (1977).

	Calcite $\delta^{18}O_{PDB}$	**Calcite $\delta^{18}O_{SMOW}$**	**Bottom Water $\delta^{18}O_{SMOW}$**	**Temp Kelvin**	**Temp C**	**Temp C Measured**	**Difference**
Mound 11	2.23	33.16	0.12	280.63	7.48	4.40	3.08
Mound 12	2.11	33.04	0.12	281.10	7.95	4.60	3.35
Jaco Scar	2.53	33.47	0.12	279.47	6.32	2.60	3.72

Table 3.
Average Living Depth (ALD) calculations by Jorissen *et al.* (1995). ALD allows for a quantification of depth distribution patterns of infaunal foraminifera. See Appendix A for calculations. Calculations were made for average seep, average non-seep, average CTG, and average RB (RB+CTG).

Species	Seep (cm)	Non-seep (cm)	CTG (cm)	RB (cm)
Bolivina pacifica	2.10	--	0.50	1.36
Bolivina spissa	2.00	--	1.44	1.33
Cassidulina braziliensis	1.87	1.50	1.75	1.42
Cibicides mckannai	2.01	1.39	0.94	1.27
Chilostomella oolina	1.49	1.64	1.25	1.25
Eggerelloides sp	--	1.69	--	--
Uvigerina hispida	--	1.43	--	--
Uvigerina peregrina	1.81	1.50	0.78	1.29

Table 4
Density comparisons of seep and non-seep samples in this study as well as Rathburn *et al.* (2003). Those stained with RB are labeled RB+CTG, based on the assumption that all individuals labeled with CTG would have stained with RB.

Study	Technique	Core Designation	Density per 50cc	Environment
This Study	CTG	AD4511	158	Bacterial mat
This Study	CTG	AD4586	91	Bacterial mat
This Study	CTG	AD4587	29	Bacterial mat
This Study	RB+CTG	AD4511	1428	Bacterial mat
This Study	RB+CTG	AD4586	549	Bacterial mat
This Study	RB+CTG	AD4587	398	Bacterial mat
This Study	RB	MC1	742	Off-seep
This Study	RB	MC2	703	Off-seep
This Study	RB	MC3	389	Off-seep
This Study	RB	MC4	1870	Off-seep
Rathburn 2003	RB	TC 31	1382	Clam Bed
Rathburn 2003	RB	TC 30	703	Clam Bed
Rathburn 2003	RB	TC 67	282	Bacterial mat

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APPENDIX A: ALD Calculations

Average Living Depth Calculations: Calculations were used to determine average living depth of infaunal foraminifera from seep and nearby non-seep habitats. Data are reported in Table 4. The average living depth equation used for these calculations was originally described by Jorisen *et al.,* 1995 and is the following:

$$ALD_x = \sum_{i=1,x} \frac{n_i d_i}{N}$$

Where x is the depth of the deepest layer, n_i the number of foraminifera of a species in the ith sediment layer. d_i is the depth midpoint of the ith layer, and N is the total number of specimens in all layers. This formula facilitates comparisons between species living within seafloor sediments.

NON-SEEP AVERAGES: Non-seep averages are averaged species counts of MC1, MC2, MC3, and MC4 from 0-3cm.

Cassidulina braziliensis

ALD_5=∑((70x0.5/256)+(56x1.25/256)+(64x1.75/256)+(29x2.25/256)+(38x2.75/256))=1.50

Cibicides mckanni

ALD_5=∑((41x0.5/241)+(88_x1.25/241)+(101x1.75/241)+(8x2.25/241)+(4x2.75/241))=1.39

Chilostomella oolina

ALD_5=∑((51x0.5/259)+(49x1.25/259)+(88x1.75/259)+(23x2.25/259)+(49x2.75/259))=1.64

Eggereloides sp.

$ALD_5=\sum((33x0.5/187)+(40x1.25/187)+(38x1.75/187)+(51x2.25/187)+(25x2.75/187))=1.69$

Uvigerina hispida

$ALD_5=\sum((130x0.5/606)+(193x1.25/606)+(174x1.75/606)+(90x2.25/606)+(19x2.75/606))=1.43$

Uvigerina peregrina

$ALD_5=\sum((160x0.5/652)+(179x1.25/652)+(145x1.75/652)+(79x2.25/652)+(90x2.75/652))=1.50$

SEEP AVEREAGES: Seep averages are the average species counts of AD4511, AD4586, and AD4587 from 0-3cm.

Bolivina pacifica

$ALD_5=\sum((9x0.5/100)+(7x1.25/100)+(21x1.75/100)+(22x2.25/100)+(41x2.75/100))=2.10$

Bolivina spissa

$ALD_5=\sum((12x0.5/91)+(4x1.25/91)+(26x1.75/91)+(17x2.25/91)+(32x2.75/91))=2.00$

Cassidulina brazilinensis

$ALD_5=\sum((4x0.5/49)+(8x1.25/49)+(16x1.75/49)+(12x2.25/49)+(9x2.75/49))=1.87$

Cibicides mckanni

$ALD_5=\sum((17x0.5/149)+(18x1.25/149)+(28x1.75/149)+(32x2.25/149)+(54x2.75/149))=2.01$

Chilostomella oolina

$ALD_5=\sum((21x0.5/87)+(18x1.25/87)+(32x1.75/87)(7x2.25/87)+(9x2.75/87))=1.49$

Uvigerina peregrina

$ALD_5=\sum((13x0.5/104)+(18x1.25/104)+(25x1.75/104)+(35x2.25/104)+(14x2.75/104))=1.81$

CTG AVERAGES: CTG averages are the average species counts of individuals labeled with CTG from AD4511, AD4586, and AD4587 from 0-2cm.
Bolivina pacifica

$ALD_5=\sum((2x0.5/2)+(0x1.25/2)+(0x1.75/2))=0.50$

Bolivina spissa

$ALD_5=\sum((1x0.5/3)+(0x1.25/3)+(2x1.75/3))= 1.44$

Cassidulina braziliensis

$ALD_5=\sum((0x0.5/5)+(0x1.25/5)+(5x1.75/5)) = 1.75$

Cibicides mckanni

$ALD_5=\sum((8x0.5/15)+(5x1.25/15)+(2x1.75/15))= 0.94$

Chilostomella oolina

$ALD_5=\sum((16x0.5/51)+(10x1.25/51)+(24x1.75/51))=1.25$

Uvigerina peregrina

$ALD_5=\sum((2x0.5/3)+(1x1.25/3)+(0x1.75/3))=0.78$

CTG+RB AVERAGES: CTG+RB averages are the average species counts of individuals labeled with CTG and stained with RB from AD4511, AD4586, and AD4587 from 0-2cm.

Bolivina pacifica

$ALD_3=\sum((9x0.5/37)+(7x1.25/37)+(21x1.75/37))=1.36$

Bolivina spissa

$ALD_3=\sum((12x0.5/42)+(4x1.25/42)+(26x1.75/42))=1.33$

Cassidulina braziliensis

$ALD_3=\sum((4x0.5/28)+(8x1.25/28)+(16x1.75/28))= 1.42$

Cibicides mckanni

$ALD_3=\sum((17x0.5/63)+(18x1.25/63)+(28x1.75/63))=1.27$

Chilostomella oolina

$ALD_3=\sum((21x0.5/71)+(18x1.25/71)+(32x1.75/71))=1.25$

Uvigerina peregrina

$ALD_3=\sum((13x0.5/55)+(18x1.25/55)+(25x1.75/55))=1.29$

Conversion of Mg/yr to mol/year for Mau, 2004 methane emissions data.

$56Mg/yr=(5.6x10^7 g/yr)/(16.042)=3.4x10^6 mol/yr$

$65Mg/yr=(6.5x10^7 g/yr)/(16.042)=4.0x10^6 mol/yr$

APPENDIX B: Bottom Water Calculations

Bottom water oxygen calculations: Bottom water calculations were determined using an equation modified from Friedman and O'Neil (1977). First stable isotopic values obtained form foraminiferal calcite were converted from PDB to SMOW using the following equation:

$\delta^{18}O_{(ecSMOW)}=((\delta^{18}O_{(ecPDB)}+29.94)/0.97006)$.

Bottom water temperature (Kelvin) was then calculated using the formula below:

$T=\sqrt{((2.78x103)/\ln((1000+ \delta^{18}O_{(ecSMOW)})/(\delta^{18}O_{(ecSMOW)}+1000)+(2.89/10^3))}$

Calculations are as follows:

Mound 11	Calcite $\delta^{18}O_{PDB}$	Calcite $\delta^{18}O_{SMOW}$	BW $\delta^{18}O_{PDB}$	BW $\delta^{18}O_{SMOW}$	Calc Temp (Kelvin)	Calc Temp C	Meas Temp C	Diff
High	2.75	33.70	-29.85	0.12	278.60	5.45		1.05
Low	2.00	32.93	-29.85	0.12	281.55	8.40	4.40	4.00
Average	2.23	33.16	-29.85	0.12	280.63	7.48		3.08
Mound 12								
High	2.29	33.23	-29.85	0.12	280.39	7.24		2.64
Low	1.83	32.75	-29.85	0.12	282.22	9.07	4.60	4.47
Average	2.11	33.04	-29.85	0.12	281.10	7.95		3.35
Jaco Scar								
High	2.94	33.90	-29.85	0.12	277.87	4.72		2.12
Low	1.59	32.51	-29.85	0.12	283.19	10.04	2.60	7.44
Average	2.53	33.47	-29.85	0.12	279.47	6.32		3.72

APPENDIX C: Stable Isotope Data

Including number of individuals run, location of collection, dive number, and stable isotopic values. Specimens were sent to Dr. Jon Martin at the University of Florida for stable carbon and oxygen isotope analyses. Foraminiferal carbonate was acidified at 73° with anhydrous phosphoric acid in a Kiel III device connected to a Finnigan MAT 252 isotope ratio mass spectrometer. Data are reported relative to the PDB (PeeDee Belemnite) standard.

Species	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VPDB}$	Location	Dive No.	No. Individuals Analyzed
Cibicides wuellerstorfi	0.44	2.23	Mound 11	AD4504	1
Cibicides wuellerstorfi	0.41	2.23	Mound 11	AD4504	2
Cibicides wuellerstorfi	0.36	2.25	Mound 11	AD4504	1
Cibicides wuellerstorfi	0.61	2.24	Mound 11	AD4504	1
Cibicides wuellerstorfi	0.34	2.27	Mound 11	AD4504	1
Cibicides wuellerstorfi	0.56	2.18	Mound 11	AD4504	1
Cibicides wuellerstorfi	0.24	2.29	Mound 11	AD4504	3
Cibicides wuellerstorfi	0.44	2.15	Mound 11	AD4504	3
Cibicides wuellerstorfi	0.17	2.25	Mound 11	AD4504	2
Cibicides wuellerstorfi	0.39	2.36	Mound 11	AD4504	2
Cibicides wuellerstorfi	0.41	2.37	Mound 11	AD4504	3
Cibicides wuellerstorfi	-0.02	2.28	Mound 11	AD4504	3
Cibicides wuellerstorfi	0.47	2.29	Mound 11	AD4504	3
Carpenteria monticularis	0.23	2.23	Mound 11	AD4504	2
Carpenteria monticularis	0.35	2.14	Mound 11	AD4504	2
Carpenteria monticularis	0.37	2.27	Mound 11	AD4504	3
Carpenteria monticularis	0.42	2.09	Mound 11	AD4504	3
Carpenteria monticularis	0.28	2.25	Mound 11	AD4504	3
Cibicides wuellerstorfi	0.45	2.05	Mound 11	AD4504	2
Cibicides wuellerstorfi	0.32	2.08	Mound 11	AD4504	1
Cibicides wuellerstorfi	0.42	2.07	Mound 11	AD4504	1
Cibicides wuellerstorfi	-0.02	2.15	Mound 11	AD4504	2
Cibicides wuellerstorfi	0.11	2.10	Mound 11	AD4504	1
Cibicides wuellerstorfi	0.33	2.01	Mound 11	AD4504	2
Cibicides wuellerstorfi	0.19	2.26	Mound 11	AD4504	1

Species	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VPDB}$	Location	Dive No.	No. Individuals Analyzed
Cibicides wuellerstorfi	-0.42	2.00	Mound 11	AD4505	1
Carpenteria monticularis	0.00	2.38	Mound 11	AD4505	2
Carpenteria monticularis	-0.11	2.36	Mound 11	AD4505	1
Carpenteria monticularis	0.06	2.29	Mound 11	AD4505	1
Cibicides wuellerstorfi	0.00	2.26	Mound 11	AD4505	1
Cibicides wuellerstorfi	-0.05	2.25	Mound 11	AD4505	3
Cibicides wuellerstorfi	-0.19	2.27	Mound 11	AD4505	3
Carpenteria monticularis	0.33	1.95	Mound 11	AD4505	2
Carpenteria monticularis	-0.29	2.29	Mound 11	AD4505	2
Carpenteria monticularis	-0.13	2.29	Mound 11	AD4505	2
Cibicides wuellerstorfi	0.19	2.19	Mound 11	AD4505	2
Cibicides wuellerstorfi	0.21	2.24	Mound 11	AD4505	1
Cibicides wuellerstorfi	0.44	2.39	Mound 11	AD4505	2
Cibicides wuellerstorfi	0.38	2.75	Mound 11	AD4505	2
Carpenteria monticularis	0.00	2.15	Mound 11	AD4505	2
Carpenteria monticularis	0.34	2.31	Mound 11	AD4505	2
Carpenteria monticularis	0.32	2.24	Mound 11	AD4505	1
Carpenteria monticularis	0.26	2.29	Mound 11	AD4505	2
Carpenteria monticularis	-0.54	1.87	Mound 11	AD4505	2
Cibicides wuellerstorfi	0.27	2.09	Mound 12	AD4503	2
Cibicides wuellerstorfi	-1.16	2.13	Mound 12	AD4503	2
Cibicides wuellerstorfi	0.12	2.28	Mound 12	AD4503	2
Cibicides wuellerstorfi	0.18	2.22	Mound 12	AD4586	2
Cibicides wuellerstorfi	0.42	2.16	Mound 12	AD4586	1
Cibicides wuellerstorfi	0.25	2.07	Mound 12	AD4586	2
Cibicides wuellerstorfi	0.12	2.24	Mound 12	AD4586	2
Cibicides wuellerstorfi	0.10	1.93	Mound 12	AD4586	2
Cibicides wuellerstorfi	0.32	2.18	Mound 12	AD4586	2
Cibicides wuellerstorfi	-0.02	2.01	Mound 12	AD4586	2
Cibicides wuellerstorfi	0.05	2.13	Mound 12	AD4586	2
Cibicides wuellerstorfi	-0.13	2.22	Mound 12	AD4586	2
Cibicides wuellerstorfi	-1.39	2.08	Mound 12	AD4586	2
Cibicides wuellerstorfi	0.25	2.08	Mound 12	AD4586	1
Cibicides wuellerstorfi	0.18	2.16	Mound 12	AD4586	3
Cibicides wuellerstorfi	-1.16	2.07	Mound 12	AD4586	1
Cibicides wuellerstorfi	-0.26	2.12	Mound 12	AD4586	1
Cibicides wuellerstorfi	0.00	1.83	Mound 12	AD4586	2
Cibicides wuellerstorfi	-0.50	2.00	Mound 12	AD4587	2
Cibicides wuellerstorfi	0.28	2.24	Mound 12	AD4587	3
Cibicides wuellerstorfi	0.06	2.18	Mound 12	AD4587	2
Cibicides wuellerstorfi	-0.03	2.23	Mound 12	AD4587	2
Cibicides wuellerstorfi	-0.35	2.05	Mound 12	AD4587	3
Cibicides wuellerstorfi	-0.27	2.10	Mound 12	AD4587	3
Cibicides wuellerstorfi	-0.98	1.92	Mound 12	AD4587	3
Cibicides wuellerstorfi	-0.75	2.01	Mound 12	AD4587	2
Cibicides wuellerstorfi	0.08	2.20	Mound 12	AD4587	3
Cibicides wuellerstorfi	0.14	2.29	Mound 12	AD4587	3

Species	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VPDB}$	Location	Dive No.	No. Individuals Analyzed
Cibicides wuellerstorfi	0.10	2.12	Mound 12	AD4587	3
Cibicides wuellerstorfi	-0.13	2.14	Mound 12	AD4587	2
Cibicides wuellerstorfi	-0.35	2.54	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	0.07	2.53	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	0.27	2.94	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	0.26	2.46	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	-0.58	2.37	Jaco Scar	AD4590	1
Cibicides wuellerstorfi	0.29	2.76	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	0.83	1.59	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	-0.24	2.40	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	0.04	2.23	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	0.15	2.65	Jaco Scar	AD4590	1
Cibicides wuellerstorfi	-0.13	2.65	Jaco Scar	AD4590	1
Cibicides wuellerstorfi	0.23	2.73	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	0.21	2.65	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	-0.02	2.64	Jaco Scar	AD4590	2
Cibicides wuellerstorfi	-0.10	2.74	Jaco Scar	AD4590	1
Cibicides wuellerstorfi	0.05	2.61	Jaco Scar	AD4590	1
Cibicides wuellerstorfi	0.36	2.65	Jaco Scar	AD4590	1
Cibicides wuellerstorfi	0.05	2.65	Jaco Scar	AD4590	1
Cibicides wuellerstorfi	0.06	2.67	Jaco Scar	AD4591	2
Cibicides wuellerstorfi	-0.51	2.73	Jaco Scar	AD4591	2
Cibicides wuellerstorfi	0.08	2.76	Jaco Scar	AD4591	2
Cibicides wuellerstorfi	-0.76	2.68	Jaco Scar	AD4591	2
Cibicides wuellerstorfi	0.13	2.61	Jaco Scar	AD4591	3
Cibicides wuellerstorfi	0.24	2.59	Jaco Scar	AD4591	3
Cibicides wuellerstorfi	0.11	2.55	Jaco Scar	AD4591	3
Cibicides wuellerstorfi	-0.06	2.71	Jaco Scar	AD4591	3
Cibicides wuellerstorfi	0.20	2.66	Jaco Scar	AD4591	3
Cibicides wuellerstorfi	0.24	2.58	Jaco Scar	AD4591	3
Cibicides wuellerstorfi	0.39	2.27	Jaco Scar	AD4509	2
Cibicides wuellerstorfi	0.11	2.37	Jaco Scar	AD4509	1
Cibicides wuellerstorfi	0.36	2.09	Jaco Scar	AD4509	1
Cibicides wuellerstorfi	0.45	2.25	Jaco Scar	AD4509	2
Cibicides wuellerstorfi	0.38	2.31	Jaco Scar	AD4509	2
Cibicides wuellerstorfi	0.20	2.29	Jaco Scar	AD4509	2
Carpenteria monticularis	0.24	2.12		AD4506	1
Carpenteria monticularis	0.33	2.25		AD4506	2
Carpenteria monticularis	0.33	2.51		AD4506	1
Carpenteria monticularis	0.22	2.45		AD4506	2
Carpenteria monticularis	0.25	2.26		AD4506	1
Carpenteria monticularis	0.12	2.27		AD4506	2
Carpenteria monticularis	0.05	2.41		AD4506	1
Carpenteria monticularis	0.16	2.28		AD4506	1
Carpenteria monticularis	-0.33	2.53		AD4506	2
Carpenteria monticularis	0.30	2.34		AD4506	2
Cibicides wuellerstorfi	0.17	2.36		AD4506	2

Species	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VPDB}$	Location	Dive No.	No. Individuals Analyzed
Carpenteria monticularis	0.17	2.42		AD4506	2
Carpenteria monticularis	0.21	2.29		AD4506	2
Carpenteria monticularis	0.22	2.21		AD4506	2
Carpenteria monticularis	0.26	2.32		AD4506	2
Cibicides wuellerstorfi	0.20	2.34		AD4507	2
Cibicides wuellerstorfi	0.58	2.77		AD4507	2
Cibicides wuellerstorfi	0.42	2.53		AD4507	2
Cibicides wuellerstorfi	0.01	2.65		AD4507	2
Cibicides wuellerstorfi	0.11	2.67		AD4508	2
Cibicides wuellerstorfi	0.25	2.56		AD4508	2
Cibicides wuellerstorfi	-0.03	2.31		AD4508	3
Cibicides wuellerstorfi	-0.82	2.32		AD4508	1
Cibicides wuellerstorfi	-0.54	2.56		AD4508	2
Cibicides wuellerstorfi	0.15	2.50		AD4508	2
Cibicides wuellerstorfi	0.12	2.57		AD4508	2
Cibicides wuellerstorfi	-0.90	2.31		AD4508	2
Cibicides wuellerstorfi	0.31	2.54		AD4508	2
Cibicides wuellerstorfi	0.20	2.50		AD4508	3
Cibicides wuellerstorfi	0.17	2.36		AD4508	3
Cibicides wuellerstorfi	0.32	2.41		AD4508	3
Cibicides wuellerstorfi	0.26	2.45		AD4508	2
Cibicides wuellerstorfi	0.17	2.36		AD4508	1
Cibicides wuellerstorfi	-0.73	2.24		AD4508	2
Cibicides wuellerstorfi	-0.23	2.47		AD4508	1
Cibicides wuellerstorfi	-0.07	2.62		AD4513	1
Cibicides wuellerstorfi	0.02	2.05		AD4513	2

Data used for foraminiferal isotopic comparisons between sites.

Species	**$\delta^{13}C_{VPDB}$**	**$\delta^{18}O_{VPBD}$**	**Location**
Cibicides wuellerstorfi	-0.42	2.00	Mound 11
Cibicides wuellerstorfi	-0.19	2.27	Mound 11
Cibicides wuellerstorfi	-0.05	2.25	Mound 11
Cibicides wuellerstorfi	-0.02	2.28	Mound 11
Cibicides wuellerstorfi	-0.02	2.15	Mound 11
Cibicides wuellerstorfi	0.00	2.26	Mound 11
Cibicides wuellerstorfi	0.11	2.10	Mound 11
Cibicides wuellerstorfi	0.17	2.25	Mound 11
Cibicides wuellerstorfi	0.19	2.19	Mound 11
Cibicides wuellerstorfi	0.19	2.26	Mound 11
Cibicides wuellerstorfi	0.21	2.24	Mound 11
Cibicides wuellerstorfi	0.24	2.29	Mound 11
Cibicides wuellerstorfi	0.32	2.08	Mound 11
Cibicides wuellerstorfi	0.33	2.01	Mound 11
Cibicides wuellerstorfi	0.34	2.27	Mound 11
Cibicides wuellerstorfi	0.36	2.25	Mound 11
Cibicides wuellerstorfi	0.38	2.75	Mound 11
Cibicides wuellerstorfi	0.39	2.36	Mound 11
Cibicides wuellerstorfi	0.41	2.23	Mound 11
Cibicides wuellerstorfi	0.41	2.37	Mound 11
Cibicides wuellerstorfi	0.42	2.07	Mound 11
Cibicides wuellerstorfi	0.44	2.23	Mound 11
Cibicides wuellerstorfi	0.44	2.39	Mound 11
Cibicides wuellerstorfi	0.44	2.15	Mound 11
Cibicides wuellerstorfi	0.45	2.05	Mound 11
Cibicides wuellerstorfi	0.47	2.29	Mound 11
Cibicides wuellerstorfi	0.56	2.18	Mound 11
Cibicides wuellerstorfi	0.61	2.24	Mound 11
Cibicides wuellerstorfi	-1.39	2.08	Mound 12
Cibicides wuellerstorfi	-1.16	2.07	Mound 12
Cibicides wuellerstorfi	-1.16	2.13	Mound 12
Cibicides wuellerstorfi	-0.98	1.92	Mound 12
Cibicides wuellerstorfi	-0.75	2.01	Mound 12
Cibicides wuellerstorfi	-0.50	2.00	Mound 12
Cibicides wuellerstorfi	-0.35	2.05	Mound 12
Cibicides wuellerstorfi	-0.27	2.10	Mound 12
Cibicides wuellerstorfi	-0.26	2.12	Mound 12
Cibicides wuellerstorfi	-0.13	2.22	Mound 12
Cibicides wuellerstorfi	-0.13	2.14	Mound 12

Species	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VPBD}$	Location
Cibicides wuellerstorfi	-0.03	2.23	Mound 12
Cibicides wuellerstorfi	-0.02	2.01	Mound 12
Cibicides wuellerstorfi	0.00	1.83	Mound 12
Cibicides wuellerstorfi	0.05	2.13	Mound 12
Cibicides wuellerstorfi	0.06	2.18	Mound 12
Cibicides wuellerstorfi	0.08	2.20	Mound 12
Cibicides wuellerstorfi	0.10	1.93	Mound 12
Cibicides wuellerstorfi	0.10	2.12	Mound 12
Cibicides wuellerstorfi	0.12	2.28	Mound 12
Cibicides wuellerstorfi	0.12	2.24	Mound 12
Cibicides wuellerstorfi	0.14	2.29	Mound 12
Cibicides wuellerstorfi	0.18	2.16	Mound 12
Cibicides wuellerstorfi	0.25	2.08	Mound 12
Cibicides wuellerstorfi	0.25	2.07	Mound 12
Cibicides wuellerstorfi	0.28	2.24	Mound 12
Cibicides wuellerstorfi	0.32	2.18	Mound 12
Cibicides wuellerstorfi	0.42	2.16	Mound 12
Cibicides wuellerstorfi	0.27	2.09	Mound 12
Cibicides wuellerstorfi	-0.76	2.68	Jaco Scar
Cibicides wuellerstorfi	-0.58	2.37	Jaco Scar
Cibicides wuellerstorfi	-0.51	2.73	Jaco Scar
Cibicides wuellerstorfi	-0.35	2.54	Jaco Scar
Cibicides wuellerstorfi	-0.24	2.40	Jaco Scar
Cibicides wuellerstorfi	-0.13	2.65	Jaco Scar
Cibicides wuellerstorfi	-0.10	2.74	Jaco Scar
Cibicides wuellerstorfi	-0.06	2.71	Jaco Scar
Cibicides wuellerstorfi	-0.02	2.64	Jaco Scar
Cibicides wuellerstorfi	0.04	2.23	Jaco Scar
Cibicides wuellerstorfi	0.05	2.61	Jaco Scar
Cibicides wuellerstorfi	0.05	2.65	Jaco Scar
Cibicides wuellerstorfi	0.06	2.67	Jaco Scar
Cibicides wuellerstorfi	0.07	2.53	Jaco Scar
Cibicides wuellerstorfi	0.08	2.76	Jaco Scar
Cibicides wuellerstorfi	0.11	2.37	Jaco Scar
Cibicides wuellerstorfi	0.11	2.55	Jaco Scar
Cibicides wuellerstorfi	0.13	2.61	Jaco Scar
Cibicides wuellerstorfi	0.15	2.65	Jaco Scar
Cibicides wuellerstorfi	0.20	2.29	Jaco Scar
Cibicides wuellerstorfi	0.20	2.66	Jaco Scar
Cibicides wuellerstorfi	0.21	2.65	Jaco Scar
Cibicides wuellerstorfi	0.23	2.73	Jaco Scar
Cibicides wuellerstorfi	0.24	2.59	Jaco Scar

Species	**$\delta^{13}C_{VPDB}$**	**$\delta^{18}O_{VPBD}$**	**Location**
Cibicides wuellerstorfi	0.24	2.58	Jaco Scar
Cibicides wuellerstorfi	0.26	2.46	Jaco Scar
Cibicides wuellerstorfi	0.27	2.94	Jaco Scar
Cibicides wuellerstorfi	0.29	2.76	Jaco Scar
Cibicides wuellerstorfi	0.36	2.09	Jaco Scar
Cibicides wuellerstorfi	0.36	2.65	Jaco Scar
Cibicides wuellerstorfi	0.38	2.31	Jaco Scar
Cibicides wuellerstorfi	0.39	2.27	Jaco Scar
Cibicides wuellerstorfi	0.45	2.25	Jaco Scar
Cibicides wuellerstorfi	0.83	1.59	Jaco Scar

APPENDIX D: Average Vertical Distributions and Seep/Non-seep Averages

Seep Average Vertical Distribution

CTG+RB Species	#/50cc 0-1cm	#/50cc 1-1.5cm	#/50cc 1.5-2cm	#/50cc 2-2.5cm	#/50cc 2.5-3cm	Totals
Bolivina pacifica	8.72	7.21	21.41	22.22	40.91	100.47
Bolivina spissa	12.44	4.42	25.52	16.90	31.82	91.10
Cassidulina braziliensis	4.34	7.81	15.77	11.95	9.09	48.96
Cibicides mckannai	17.13	18.06	27.89	31.68	53.86	148.63
Chilostomella oolina	20.97	18.38	31.52	7.21	9.09	87.17
Uvigerina peregrina	13.27	17.53	24.52	34.93	14.09	104.33

Seep Averages

CTG+RB	#/50cc 0-1cm	#/50cc 1-1.5cm	#/50cc 1.5-2cm	#/50cc 2-2.5cm	#/50cc 2.5-3cm
Totals	122.25	118.75	209.04	224.67	92.78
Total Agglutinated	12.22	20.89	18.08	26.38	12.42
Total Calcarous	110.03	97.85	190.96	198.29	80.35

Non-seep Average Vertical Distribution

RB Species	#/50cc 0-1cm	#/50cc 1-1.5cm	#/50cc 1.5-2cm	#/50cc 2-2.5cm	#/50cc 2.5-3cm	Totals
Cassidulina braziliensis	69.83	55.59	63.90	28.53	37.85	255.70
Cibicides mckannai	41.07	87.59	101.30	7.54	3.85	241.35
Chilostomella oolina	51.13	48.85	88.10	22.54	48.63	259.25
Eggerelloides sp.	33.31	40.44	38.08	50.62	24.50	186.96
Uvigerina hispida	129.99	193.10	173.73	89.52	19.24	605.59
Uvigerina peregrina	160.14	178.65	144.55	78.93	90.08	652.34

Non-seep Averages

RB	#/50cc 0-1cm	#/50cc 1-1.5cm	#/50cc 1.5-2cm	#/50cc 2-2.5cm	#/50cc 2.5-3cm
Totals	1133.97	927.63	891.96	374.52	368.33
Total Agglutinated	365.37	129.17	125.16	80.90	54.90
Total Calcareous	768.59	798.46	766.80	293.63	313.43

APPENDIX E: Stained and Labeled Foraminiferal Counts

AT15-44	AD4511 #/50cc	TC#2 #/50cc	CTG #/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm
Original Sediment Volume	40	21	13.75
Sample split	No Split	No Split	No Split
Species			
Ammobaculites agglutinans	0.00	0.00	0.00
Ammtium planissimum	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00
Bolivina alata	0.00	0.00	3.64
Bolivina costata	0.00	0.00	0.00
Bolivina pacifica	0.00	0.00	0.00
Bolivina plicata	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00
Bolivina spissa	1.25	0.00	0.00
Bulimina mexicana	0.00	0.00	0.00
Bulimina pacifica	0.00	0.00	0.00
Cancris carmenensis	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00
Cassidulina braziliensis	0.00	0.00	0.00
Cassidulina carinata	0.00	0.00	0.00
Cassidulina tumida	0.00	0.00	0.00
Cibicides mckanni	16.25	7.14	0.00
Cibicides wuellerstorfi	2.50	4.76	0.00
Chilostomella oolina	25.00	9.52	65.45
Cyclammina cancellata	0.00	0.00	0.00
Cyclammina trullissata	0.00	0.00	0.00
Cystammina?	0.00	0.00	0.00
Dentalina gottifera	0.00	0.00	0.00
Eggerelloides sp.	0.00	0.00	0.00
Epistominella sp	0.00	0.00	0.00
Epodides sp.	1.25	0.00	0.00
Globobulimina hoeglundi	0.00	0.00	0.00
Globobulimina spinifera	0.00	0.00	0.00
Globocassidulina subglobosa	2.50	0.00	0.00
Gyroidina multilocula	0.00	0.00	0.00

Haplaphragomoides rotulatum	0.00	0.00	0.00
Haplaphragomoides sp	0.00	0.00	0.00
Hoeglundina elegans	0.00	0.00	0.00
Lenticulina sp	0.00	0.00	0.00
Martinonella sp	0.00	0.00	0.00
Melonis affinis	0.00	0.00	0.00
Nonionella sp	1.25	0.00	3.64
Oridosalis umbonatus	0.00	0.00	0.00
Pullenia bulloides	1.25	0.00	0.00
Pullenia subcarinata	0.00	0.00	0.00
Reophax dentaliniformis	0.00	0.00	0.00
Reophax hispidulus	0.00	0.00	0.00
Reophax horridus	0.00	0.00	0.00
Reophax sp	0.00	0.00	0.00
Trochammina	0.00	0.00	0.00
Uvigerina hispida	0.00	0.00	0.00
Uvigerina peregrina	1.25	0.00	0.00
Valvulinera sp	1.25	0.00	3.64
Valvulinera oblonga	0.00	0.00	0.00
Unknown	0.00	7.14	0.00
Total agglutinates	0.00	0.00	0.00
Total calcareous	53.75	28.57	76.36
Total foraminifera	53.75	28.57	76.36

AT15-44	AD4511 #/50cc	TC#2 #/50cc	CTG+RB #/50cc	#/50cc	#/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm	2-2.5cm	2.5-3cm
Original Sediment Volume	40	21	13.75	35	25
Sample split	No Split	No Split	No Split	¼ split	¼ split
Species					
Ammobaculites agglutinans	0.00	0.00	0.00	0.00	0.00
Ammtium planissimum	0.00	0.00	0.00	7.41	0.00
Angulogerina carinata	0.00	0.00	0.00	0.00	0.00
Bolivina alata	2.50	2.38	32.73	7.41	9.09
Bolivina costata	0.00	0.00	0.00	0.00	0.00
Bolivina pacifica	15.00	9.52	50.91	66.67	40.91
Bolivina plicata	0.00	0.00	0.00	0.00	0.00
Bolivina seminuda	0.00	4.76	0.00	0.00	0.00
Bolivina spissa	25.00	0.00	72.73	44.44	31.82
Bulimina mexicana	0.00	0.00	14.55	0.00	4.55
Bulimina pacifica	0.00	0.00	0.00	0.00	0.00
Cancris carmenensis	0.00	0.00	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00	0.00	0.00
Cassidulina braziliensis	3.75	11.90	29.09	14.81	9.09
Cassidulina carinata	5.00	7.14	7.27	0.00	0.00
Cassidulina tumida	0.00	0.00	0.00	0.00	0.00
Cibicides mckanni	30.00	23.81	47.27	51.85	36.36
Cibicides wuellerstorfi	2.50	4.76	0.00	0.00	4.55
Chilostomella oolina	32.50	16.67	76.36	14.81	9.09
Cyclammina cancellata	0.00	0.00	0.00	0.00	0.00
Cyclammina trullissata	0.00	0.00	0.00	0.00	0.00
Cystammina?	2.50	0.00	0.00	0.00	4.55
Dentalina gottifera	1.25	0.00	0.00	0.00	0.00
Eggerelloides sp.	0.00	11.90	40.00	44.44	18.18
Epistominella sp	0.00	0.00	0.00	0.00	0.00
Epodides sp.	1.25	0.00	0.00	0.00	0.00
Globobulimina hoeglundi	6.25	0.00	7.27	22.22	9.09
Globobulimina spinifera	1.25	0.00	0.00	7.41	0.00
Globobulimina pacifica	0.00	0.00	0.00	7.41	0.00
Globocassidulina subglobosa	8.75	0.00	7.27	14.81	0.00
Gyroidina multilocula	0.00	0.00	0.00	0.00	0.00
Haplaphragomoides rotulatum	0.00	0.00	3.64	0.00	0.00
Haplaphragomoides sp	2.50	0.00	3.64	0.00	0.00
Hoeglundina elegans	0.00	0.00	0.00	0.00	0.00
Lenticulina sp	0.00	0.00	0.00	0.00	0.00

Martinonella sp	1.25	0.00	3.64	0.00	0.00
Melonis affinis	0.00	0.00	0.00	0.00	0.00
Nonionella sp	1.25	0.00	3.64	0.00	0.00
Oridosalis umbonatus	3.75	0.00	0.00	7.41	4.55
Pullenia bulloides	1.25	0.00	0.00	0.00	0.00
Pullenia subcarinata	0.00	0.00	0.00	0.00	0.00
Reophax dentaliniformis	0.00	0.00	0.00	0.00	0.00
Reophax hispidulus	0.00	0.00	0.00	7.41	0.00
Reophax horridus	0.00	0.00	0.00	0.00	0.00
Reophax sp	0.00	0.00	0.00	0.00	0.00
Trochammina	0.00	0.00	0.00	0.00	4.55
Uvigerina hispida	10.00	0.00	7.27	7.41	0.00
Uvigerina peregrina	20.00	21.43	58.18	81.48	9.09
Valvulinera sp	1.25	0.00	7.27	22.22	0.00
Valvulinera oblonga	0.00	0.00	0.00	7.41	0.00
Unknown	0.00	7.14	3.64	14.81	4.55
Total agglutinates	6.25	11.90	50.91	59.26	27.27
Total calcareous	172.50	109.52	425.45	392.59	172.73
Total foraminifera	178.75	121.43	476.36	451.85	200.00

AT15-59	AD4586 #/50cc	TC#4 #/50cc	CTG #/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm
Original Sediment Volume	33	20	15
Sample split	No Split	No Split	No Split
Species			
Ammobaculites agglutinans	0.00	0.00	0.00
Ammtium planissimum	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00
Bolivina alata	0.00	2.50	0.00
Bolivina costata	0.00	0.00	0.00
Bolivina pacifica	0.00	0.00	0.00
Bolivina plicata	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00
Bolivina sp	0.00	0.00	0.00
Bulimina mexicana	0.00	0.00	0.00
Bulimina sp? (tenuata?)	0.00	0.00	0.00
Cancris carmenensis	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00
Cassidulina braziliensis	0.00	0.00	6.67
Cassidulina carinata	0.00	0.00	0.00
Cassidulina tumida	0.00	0.00	0.00
Cibicides mckanni	7.58	5.00	6.67
Cibicides wuellerstorfi	0.00	2.50	0.00
Chilostomella oolina	15.15	20.00	3.33
Cyclammina cancellata	0.00	0.00	0.00
Cyclammina trullissata	0.00	0.00	0.00
Cystammina?	0.00	0.00	0.00
Dentalina gottifera	0.00	0.00	0.00
Eggerelloides sp.	0.00	0.00	0.00
Epistominella sp	0.00	0.00	0.00
Globobulimina hoeglundi	1.52	0.00	3.33
Globobulimina pacifica??	0.00	0.00	0.00
Globobulimina spinifera	0.00	0.00	0.00
Globocassidulina subglobosa	1.52	2.50	3.33
Gyroidina multilocula	0.00	0.00	0.00
Haplaphragomoides rotulatum	0.00	0.00	0.00
Haplaphragomoides sp	0.00	0.00	0.00
Hoeglundina elegans	1.52	0.00	0.00
Lenticulina sp	0.00	0.00	0.00
Martinonella sp	0.00	0.00	0.00
Melonis affinis	0.00	0.00	0.00

Nonionella sp	0.00	0.00	0.00
Oridosalis umbonatus	0.00	0.00	0.00
Pullenia bulloides	0.00	0.00	0.00
Pullenia subcarinata	0.00	0.00	0.00
Reophax dentaliniformis	0.00	2.50	0.00
Reophax hispidulus	0.00	0.00	0.00
Reophax horridus	0.00	0.00	0.00
Reophax sp	0.00	0.00	0.00
Trochammina	0.00	0.00	0.00
Uvigerina hispida	0.00	0.00	0.00
Uvigerina peregrina	3.03	2.50	0.00
Valvulinera glabra	0.00	0.00	0.00
Valvulinera oblonga	0.00	0.00	0.00
Unknown	0.00	0.00	0.00
Total agglutinates	0.00	2.50	0.00
Total calcareous	30.30	35.00	23.33
Total foraminifera	30.30	37.50	23.33

AT15-59	AD4586	TC#4	CTG		
	#/50cc	#/50cc	#/50cc	#/50cc	#/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm	2-2.5cm	2.5-3cm
Original Sediment Volume	33	20	15	16	20
Sample split	No Split	No Split	No Split	1/2split	1/2split
Species					
Ammobaculites agglutinans	0.00	0.00	0.00	0.00	0.00
Ammtium planissimum	1.52	0.00	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00	0.00	0.00
Bolivina alata	4.55	2.50	0.00	0.00	0.00
Bolivina costata	0.00	0.00	0.00	0.00	0.00
Bolivina pacifica	7.58	2.50	13.33	0.00	0.00
Bolivina plicata	0.00	0.00	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00	0.00	0.00
Bolivina sp	6.06	7.50	0.00	6.25	0.00
Bulimina mexicana	0.00	0.00	0.00	6.25	0.00
Bulimina sp? (tenuata?)	0.00	0.00	0.00	0.00	0.00
Cancris carmenensis	0.00	0.00	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00	0.00	0.00
Cassidulina braziliensis	3.03	0.00	6.67	18.75	0.00
Cassidulina carinata	3.03	0.00	3.33	6.25	0.00
Cassidulina tumida	0.00	0.00	0.00	0.00	0.00
Cibicides mckanni	15.15	15.00	13.33	25.00	5.00
Cibicides wuellerstorfi	1.52	2.50	0.00	6.25	10.00
Chilostomella oolina	19.70	25.00	6.67	0.00	0.00
Cyclammina cancellata	0.00	0.00	0.00	0.00	0.00
Cyclammina trullissata	3.03	0.00	0.00	0.00	0.00
Cystammina?	0.00	0.00	0.00	0.00	0.00
Dentalina gottifera	0.00	0.00	0.00	0.00	0.00
Eggerelloides sp.	12.12	17.50	0.00	6.25	10.00
Epistominella sp	0.00	0.00	0.00	0.00	0.00
Globobulimina hoeglundi	7.58	2.50	16.67	6.25	0.00
Globobulimina pacifica??	0.00	0.00	0.00	25.00	10.00
Globobulimina spinifera	1.52	0.00	0.00	0.00	0.00
Globocassidulina subglobosa	9.09	5.00	3.33	18.75	0.00
Gyroidina multilocula	0.00	0.00	0.00	0.00	0.00
Haplaphragomoides rotulatum	0.00	0.00	0.00	0.00	0.00
Haplaphragomoides sp	0.00	0.00	0.00	0.00	0.00
Hoeglundina elegans	1.52	0.00	0.00	6.25	0.00
Lenticulina sp	0.00	0.00	0.00	0.00	0.00
Martinonella sp	3.03	0.00	3.33	0.00	0.00
Melonis affinis	0.00	0.00	0.00	0.00	0.00

Nonionella sp	0.00	0.00	0.00	0.00	0.00
Oridosalis umbonatus	0.00	0.00	3.33	6.25	0.00
Pullenia bulloides	0.00	0.00	0.00	0.00	0.00
Pullenia subcarinata	0.00	0.00	0.00	0.00	0.00
Reophax dentaliniformis	0.00	2.50	0.00	0.00	0.00
Reophax hispidulus	0.00	0.00	0.00	0.00	0.00
Reophax horridus	0.00	0.00	0.00	0.00	0.00
Reophax sp	0.00	0.00	0.00	0.00	0.00
Trochammina	0.00	0.00	0.00	0.00	0.00
Uvigerina hispida	3.03	0.00	0.00	0.00	5.00
Uvigerina peregrina	9.09	10.00	0.00	18.75	5.00
Valvulinera glabra	0.00	0.00	0.00	0.00	0.00
Valvulinera oblonga	0.00	0.00	0.00	0.00	0.00
Unknown					
Total agglutinates	19.70	20.00	3.33	6.25	10.00
Total calcareous	92.42	72.50	66.67	150.00	35.00
Total foraminifera	112.12	92.50	70.00	156.25	45.00

AT15-59	AD4587 #/50cc	TC#2 #/50cc	CTG #/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm
Original Sediment Volume	56	26	13
Sample split	No Split	No Split	No Split
Species			
Ammobaculites agglutinans	0.00	0.00	0.00
Ammtium planissimum	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00
Bolivina alata	0.00	0.00	0.00
Bolivina costata	0.00	0.00	0.00
Bolivina pacifica	1.79	0.00	0.00
Bolivina plicata	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00
Bolivina sp	0.00	0.00	3.85
Bulimina mexicana	0.00	0.00	0.00
Bulimina sp? (tenuata?)	0.00	0.00	0.00
Cancris carmenensis	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00
Cassidulina braziliensis	0.00	0.00	3.85
Cassidulina carinata	0.00	0.00	0.00
Cassidulina tumida	0.00	0.00	0.00
Cibicides mckanni	0.00	3.85	0.00
Cibicides wuellerstorfi	0.00	0.00	0.00
Chilostomella oolina	8.04	1.92	3.85
Cyclammina cancellata	0.00	0.00	0.00
Cyclammina trullissata	0.00	0.00	0.00
Cystammina?	0.00	0.00	0.00
Dentalina gottifera	0.00	0.00	0.00
Eggerelloides sp.	0.00	0.00	0.00
Epistominella sp	0.00	0.00	0.00
Globobulimina hoeglundi	0.00	0.00	0.00
Globobulimina spinifera	0.00	0.00	0.00
Globobulumina pacifica??	0.00	0.00	0.00
Globocassidulina subglobosa	0.00	0.00	0.00
Gyroidina multilocula	0.00	0.00	0.00
Haplaphragomoides rotulatum	0.00	0.00	0.00
Haplaphragomoides sp	0.00	0.00	0.00
Hoeglundina elegans	0.00	0.00	0.00
Lenticulina sp	0.00	0.00	0.00
Martinonella sp	0.00	0.00	0.00
Melonis affinis	0.00	0.00	0.00

Nonionella sp	0.00	0.00	0.00
Oridosalis umbonatus	0.00	0.00	0.00
Pullenia bulloides	0.00	0.00	0.00
Pullenia subcarinata	0.00	0.00	0.00
Reophax dentaliniformis	0.00	0.00	0.00
Reophax hispidulus	0.00	0.00	0.00
Reophax horridus	0.00	0.00	0.00
Reophax sp.	0.00	0.00	0.00
Trochammina	0.00	0.00	0.00
Uvigerina hispida	0.00	0.00	0.00
Uvigerina peregrina	0.00	0.00	0.00
Valvulinera glabra	0.00	1.92	0.00
Valvulinera oblonga	0.00	0.00	0.00
Unknown	0.00	0.00	0.00
Total agglutinates	0.00	0.00	0.00
Total calcareous	9.82	7.69	11.54
Total foraminifera	9.82	7.69	11.54

AT15-59	AD4587	TC#2	CTG+RB		
	#/50cc	#/50cc	#/50cc	#/50cc	#/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm	2-2.5cm	2.5-3cm
Original Sediment Volume	56	26	13	11	24
Sample split	No Split	No Split	No Split	1/2split	1/2split
Species					
Ammobaculites agglutinans	0.00	1.92	0.00	0.00	0.00
Ammtium planissimum	0.00	0.00	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00	0.00	0.00
Bolivina alata	0.89	1.92	0.00	0.00	0.00
Bolivina costata	0.00	0.00	0.00	0.00	0.00
Bolivina pacifica	3.57	9.62	0.00	0.00	0.00
Bolivina plicata	0.00	0.00	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00	0.00	0.00
Bolivina sp	6.25	5.77	3.85	0.00	0.00
Bulimina mexicana	1.79	3.85	0.00	2.27	0.00
Bulimina sp? (tenuata?)	0.00	0.00	0.00	4.55	0.00
Cancris carmenensis	0.00	0.00	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00	0.00	0.00
Cassidulina braziliensis	6.25	11.54	11.54	2.27	0.00
Cassidulina carinata	3.57	1.92	0.00	2.27	4.17
Cassidulina tumida	0.00	0.00	7.69	0.00	0.00
Cibicides mckanni	6.25	15.38	23.08	18.18	12.50
Cibicides wuellerstorfi	2.68	1.92	0.00	0.00	4.17
Chilostomella oolina	10.71	13.46	11.54	6.82	0.00
Cyclammina cancellata	0.00	0.00	0.00	0.00	0.00
Cyclammina trullissata	1.79	0.00	0.00	0.00	0.00
Cystammina?	0.00	0.00	0.00	0.00	0.00
Dentalina gottifera	0.00	0.00	0.00	0.00	0.00
Eggerelloides sp.	7.14	21.15	0.00	9.09	0.00
Epistominella sp	0.00	0.00	0.00	0.00	0.00
Globobulimina hoeglundi	0.00	3.85	0.00	0.00	0.00
Globobulimina spinifera	0.00	0.00	0.00	2.27	0.00
Globobulumina pacifica??	0.00	0.00	0.00	2.27	8.33
Globocassidulina subglobosa	5.36	11.54	3.85	2.27	4.17
Gyroidina multilocula	0.00	0.00	3.85	0.00	0.00
Haplaphragomoides rotulatum	0.00	3.85	0.00	2.27	0.00
Haplaphragomoides sp	0.00	0.00	0.00	2.27	0.00
Hoeglundina elegans	0.00	0.00	0.00	0.00	0.00

Lenticulina sp	0.00	0.00	0.00	0.00	0.00
Martinonella sp	0.00	1.92	0.00	0.00	0.00
Melonis affinis	0.00	0.00	0.00	0.00	0.00
Nonionella sp	0.00	3.85	0.00	0.00	0.00
Oridosalis umbonatus	1.79	0.00	0.00	2.27	0.00
Pullenia bulloides	0.00	0.00	0.00	0.00	0.00
Pullenia subcarinata	0.00	0.00	0.00	0.00	0.00
Reophax dentaliniformis	0.00	0.00	0.00	0.00	0.00
Reophax hispidulus	0.00	0.00	0.00	0.00	0.00
Reophax horridus	0.00	0.00	0.00	0.00	0.00
Reophax sp	0.89	0.00	0.00	0.00	0.00
Trochammina	0.89	1.92	0.00	0.00	0.00
Uvigerina hispida	2.68	3.85	0.00	2.27	0.00
Uvigerina peregrina	10.71	21.15	15.38	4.55	0.00
Valvulinera glabra	1.79	1.92	0.00	0.00	0.00
Valvulinera oblonga	0.89	0.00	0.00	0.00	0.00
Unknown	0.00	0.00	0.00	0.00	0.00
Total agglutinates	10.71	30.77	0.00	13.64	0.00
Total calcareous	65.18	111.54	80.77	52.27	33.33
Total foraminifera	75.89	142.31	80.77	65.91	33.33

AT15-59	MC1	#7	RB		
	#/50cc	#/50cc	#/50cc	#/50cc	#/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm	2-2.5cm	2.5-3cm
Original Sediment Volume	67	41	26	26	26
Sample split	1/2split	No Split	No Split	1/2split	1/2split
Species					
Ammobaculites agglutinans	0.00	0.00	0.00	0.00	0.00
Ammtium planissimum	0.00	0.00	1.92	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00	0.00	0.00
Bolivina alata	0.00	1.22	0.00	3.85	0.00
Bolivina costata	0.00	0.00	0.00	0.00	0.00
Bolivina pacifica	0.00	0.00	0.00	0.00	0.00
Bolivina plicata	0.00	0.00	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00	0.00	0.00
Bolivina sp	1.49	1.22	1.92	0.00	0.00
Bulimina mexicana	16.42	1.22	1.92	7.69	7.69
Bulimina sp? (tenuata?)	1.49	0.00	0.00	0.00	0.00
Cancris carmenensis	0.00	0.00	0.00	0.00	0.00
Cancris inflatus	0.00	1.22	0.00	0.00	0.00
Cassidulina braziliensis	26.87	14.63	13.46	3.85	0.00
Cassidulina carinata	29.85	8.54	5.77	0.00	0.00
Cassidulina tumida	0.00	0.00	0.00	0.00	0.00
Cibicides mckanni	4.48	10.98	11.54	0.00	3.85
Cibicides wuellerstorfi	0.00	0.00	0.00	0.00	0.00
Chilostomella oolina	13.43	15.85	30.77	0.00	11.54
Cyclammina cancellata	4.48	0.00	1.92	0.00	3.85
Cyclammina trullissata	2.99	0.00	3.85	0.00	0.00
Cystammina?	2.99	1.22	0.00	0.00	7.69
Dentalina gottifera	0.00	0.00	0.00	0.00	0.00
Eggerelloides sp.	2.99	2.44	15.38	15.38	3.85
Epistominella sp	0.00	0.00	0.00	0.00	0.00
Eponides sp.	1.49	0.00	0.00	0.00	0.00
Globobulimina hoeglundi	7.46	7.32	9.62	3.85	7.69
Globobulimina spinifera	0.00	4.88	7.69	0.00	0.00
Globocassidulina subglobosa	14.93	8.54	5.77	3.85	15.38
Gyroidina multilocula	0.00	1.22	0.00	0.00	0.00
Haplaphragomoides rotulatum	2.99	4.88	5.77	0.00	0.00
Haplaphragomoides sp	1.49	1.22	0.00	0.00	0.00
Hoeglundina elegans	1.49	3.66	1.92	0.00	0.00
Lenticulina sp	0.00	0.00	0.00	0.00	0.00
Martinonella sp	5.97	0.00	0.00	0.00	0.00
Melonis affinis	0.00	0.00	0.00	0.00	0.00

Nonionella sp	2.99	2.44	0.00	0.00	0.00
Oridosalis umbonatus	14.93	7.32	17.31	0.00	0.00
Pullenia bulloides	0.00	0.00	0.00	0.00	0.00
Pullenia subcarinata	0.00	0.00	1.92	0.00	0.00
Reophax dentaliniformis	5.97	7.32	9.62	3.85	3.85
Reophax hispidulus	49.25	1.22	5.77	0.00	0.00
Reophax horridus	29.85	1.22	1.92	0.00	0.00
Reophax sp	2.99	0.00	0.00	0.00	0.00
Trochammina	1.49	2.44	1.92	0.00	0.00
Uvigerina hispida	7.46	2.44	5.77	0.00	7.69
Uvigerina peregrina	13.43	10.98	5.77	3.85	15.38
Valvulinera glabra	1.49	0.00	5.77	0.00	0.00
Valvulinera oblonga	0.00	0.00	0.00	0.00	0.00
Unknown	19.40	1.22	1.92	3.85	7.69
Total agglutinates	125.37	23.17	48.08	19.23	19.23
Total calcareous	167.16	103.66	128.85	30.77	76.92
Total foraminifera	292.54	126.83	176.92	50.00	96.15

AT15-59	MC2	#2	RB		
	#/50cc	#/50cc	#/50cc	#/50cc	#/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm	2-2.5cm	2.5-3cm
Original Sediment Volume	70	31	36	36	26
Sample split	1/2split	No Split	No Split	1/2split	1/2split
Species					
Ammobaculites agglutinans	0.00	0.00	0.00	0.00	0.00
Ammtium planissimum	1.43	0.00	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00	0.00	0.00
Bolivina alata	0.00	3.23	4.17	0.00	0.00
Bolivina costata	0.00	0.00	0.00	0.00	0.00
Bolivina pacifica	0.00	0.00	0.00	0.00	0.00
Bolivina plicata	0.00	0.00	0.00	0.00	0.00
Bolivina seminuda	0.00	1.61	1.39	0.00	0.00
Bolivina spissa	1.43	0.00	1.39	0.00	0.00
Bulimina mexicana	17.14	0.00	1.39	0.00	0.00
Bulimina sp? (tenuata?)	0.00	0.00	0.00	0.00	3.85
Cancris carmenensis	0.00	0.00	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00	0.00	0.00
Cassidulina braziliensis	24.29	20.97	12.50	2.78	11.54
Cassidulina carinata	7.14	11.29	6.94	0.00	3.85
Cassidulina tumida	0.00	0.00	0.00	0.00	0.00
Cibicides mckanni	15.71	17.74	20.83	2.78	0.00
Cibicides wuellerstorfi	1.43	4.84	2.78	0.00	0.00
Chilostomella oolina	15.71	16.13	16.67	11.11	3.85
Cyclammina cancellata	5.71	1.61	1.39	0.00	3.85
Cyclammina trullissata	0.00	1.61	1.39	0.00	0.00
Cystammina?	4.29	3.23	4.17	0.00	3.85
Dentalina gottifera	0.00	0.00	0.00	0.00	0.00
Eggerelloides sp.	17.14	9.68	8.33	0.00	7.69
Epistominella sp	0.00	0.00	0.00	0.00	0.00
Epodides ?	0.00	0.00	0.00	0.00	0.00
Globobulimina hoeglundi	5.71	8.06	2.78	2.78	7.69
Globobulimina spinifera	2.86	3.23	1.39	0.00	0.00
Globocassidulina subglobosa	20.00	17.74	5.56	2.78	7.69
Gyroidina multilocula	0.00	0.00	0.00	0.00	0.00
Haplaphragomoides rotulatum	4.29	0.00	1.39	2.78	0.00
Haplaphragomoides sp	1.43	3.23	0.00	0.00	0.00
Hoeglundina elegans	1.43	0.00	1.39	0.00	0.00
Lenticulina sp	0.00	0.00	0.00	0.00	0.00
Martinonella sp	5.71	0.00	0.00	0.00	0.00
Melonis affinis	0.00	0.00	0.00	0.00	0.00

Nonionella sp	1.43	0.00	0.00	0.00	0.00
Oridosalis umbonatus	11.43	1.61	9.72	2.78	3.85
Pullenia bulloides	4.29	0.00	4.17	2.78	0.00
Pullenia subcarinata	0.00	0.00	0.00	0.00	0.00
Reophax dentaliniformis	21.43	3.23	6.94	2.78	0.00
Reophax hispidulus	0.00	11.29	4.17	2.78	0.00
Reophax horridus	0.00	0.00	4.17	0.00	0.00
Reophax sp	4.29	4.84	1.39	0.00	0.00
Trochammina	15.71	4.84	1.39	0.00	0.00
Uvigerina hispida	7.14	6.45	5.56	0.00	3.85
Uvigerina peregrina	21.43	9.68	20.83	5.56	11.54
Valvulinera sp	1.43	0.00	1.39	0.00	0.00
Valvulinera oblonga	0.00	0.00	1.39	0.00	0.00
Unknown	14.29	1.61	4.17	0.00	3.85
Total agglutinates	82.86	45.16	38.89	8.33	15.38
Total calcareous	172.86	122.58	122.22	33.33	61.54
Total foraminifera	255.71	167.74	161.11	41.67	76.92

AT15-59	MC3	#2	RB		
	#/50cc	#/50cc	#/50cc	#/50cc	#/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm	2-2.5cm	2.5-3cm
Original Sediment Volume	91	41	51	35	41
Sample split	1/2split	No Split	No Split	1/2split	1/2split
Species					
Ammobaculites agglutinans	0.00	0.00	0.00	0.00	0.00
Ammtium planissimum	0.00	0.00	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00	0.00	0.00
Bolivina alata	2.20	0.00	0.00	0.00	0.00
Bolivina costata	0.00	0.00	0.00	0.00	0.00
Bolivina pacifica	0.00	0.00	0.00	0.00	0.00
Bolivina plicata	0.00	0.00	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00	0.00	0.00
Bolivina spissa	1.10	0.00	2.94	5.71	0.00
Bulimina mexicana	5.49	1.22	1.96	0.00	0.00
Bulimina sp? (tenuata?)	0.00	0.00	0.00	0.00	2.44
Cancris carmenensis	0.00	0.00	0.00	0.00	0.00
Cancris inflatus	1.10	0.00	0.00	0.00	0.00
Cassidulina braziliensis	5.49	6.10	0.98	2.86	0.00
Cassidulina carinata	9.89	3.66	0.00	0.00	0.00
Cassidulina tumida	0.00	0.00	0.00	0.00	0.00
Cibicides mckanni	2.20	6.10	5.88	0.00	0.00
Cibicides wuellerstorfi	0.00	0.00	0.00	0.00	0.00
Chilostomella oolina	12.09	8.54	5.88	11.43	12.20
Cyclammina cancellata	8.79	0.00	1.96	0.00	0.00
Cyclammina trullissata	0.00	0.00	0.00	0.00	0.00
Cystammina?	1.10	0.00	0.00	0.00	2.44
Dentalina gottifera	0.00	0.00	0.00	0.00	0.00
Eggerelloides sp.	7.69	6.10	7.84	11.43	2.44
Epistominella sp	0.00	0.00	0.00	0.00	0.00
Epodides ?	0.00	0.00	2.94	0.00	0.00
Globobulimina hoeglundi	2.20	0.00	0.00	2.86	0.00
Globobulimina spinifera	1.10	4.88	0.00	0.00	0.00
Globocassidulina subglobosa	10.99	3.66	0.00	0.00	0.00
Gyroidina multilocula	0.00	1.22	0.00	0.00	0.00
Haplaphragomoides rotulatum	2.20	0.00	1.96	0.00	0.00
Haplaphragomoides sp	6.59	0.00	0.00	0.00	0.00
Hoeglundina elegans	2.20	0.00	0.98	0.00	0.00
Lenticulina sp	0.00	0.00	0.00	0.00	0.00
Martinonella sp	9.89	0.00	2.94	0.00	0.00
Melonis affinis	0.00	0.00	0.00	0.00	0.00

Nonionella sp	0.00	0.00	0.00	0.00	0.00
Oridosalis umbonatus	4.40	4.88	0.98	0.00	0.00
Pullenia bulloides	1.10	0.00	1.96	0.00	0.00
Pullenia subcarinata	1.10	1.22	0.00	0.00	0.00
Reophax dentaliniformis	5.49	3.66	2.94	5.71	4.88
Reophax hispidulus	30.77	4.88	0.00	2.86	0.00
Reophax horridus	1.10	6.10	0.98	0.00	0.00
Reophax sp	3.30	1.22	0.00	0.00	0.00
Trochammina	3.30	0.00	0.00	0.00	0.00
Uvigerina hispida	13.19	3.66	5.88	8.57	2.44
Uvigerina peregrina	13.19	2.44	4.90	2.86	0.00
Valvulinera glabra	0.00	2.44	0.00	0.00	0.00
Valvulinera sp	0.00	0.00	0.00	0.00	0.00
Valvulinera oblonga	0.00	0.00	0.00	0.00	0.00
Unknown	0.00	0.00	0.00	0.00	0.00
	4.40	0.00	0.00	2.86	2.44
Total agglutinates	80.22	21.95	18.63	20.00	9.76
Total calcareous	93.41	50.00	35.29	34.29	17.07
Total foraminifera	173.63	71.95	53.92	54.29	26.83

AT15-59	MC4	#2	RB		
	#/50cc	#/50cc	#/50cc	#/50cc	#/50cc
Interval	0-1cm	1-1.5cm	1.5-2cm	2-2.5cm	2.5-3cm
Original Sediment Volume	91	36	46	42	38
Sample split	1/2split	1/2split	1/2split	1/4split	1/4split
Species					
Ammobaculites agglutinans	0.00	0.00	0.00	0.00	0.00
Ammtium planissimum	2.20	2.78	0.00	0.00	0.00
Angulogerina carinata	0.00	0.00	0.00	0.00	0.00
Bolivina alata	3.30	0.00	0.00	0.00	0.00
Bolivina costata	0.00	0.00	0.00	0.00	0.00
Bolivina pacifica	0.00	0.00	0.00	0.00	0.00
Bolivina plicata	0.00	0.00	0.00	0.00	0.00
Bolivina seminuda	0.00	0.00	0.00	0.00	0.00
Bolivina spissa	4.40	0.00	4.35	0.00	5.26
Bulimina mexicana	15.38	16.67	6.52	4.76	0.00
Bulimina sp? (tenuata?)	0.00	0.00	0.00	0.00	0.00
Cancris carmenensis	0.00	0.00	0.00	0.00	0.00
Cancris inflatus	0.00	0.00	0.00	0.00	0.00
Cassidulina braziliensis	13.19	13.89	36.96	19.05	26.32
Cassidulina carinata	6.59	5.56	6.52	0.00	0.00
Cassidulina tumida	0.00	0.00	2.17	4.76	0.00
Cibicides mckanni	18.68	52.78	63.04	4.76	0.00
Cibicides wuellerstorfi	0.00	0.00	4.35	0.00	0.00
Chilostomella oolina	9.89	8.33	34.78	0.00	21.05
Cyclammina cancellata	6.59	2.78	0.00	0.00	0.00
Cyclammina trullissata	1.10	0.00	2.17	0.00	0.00
Cystammina?	0.00	0.00	0.00	4.76	0.00
Dentalina gottifera	0.00	0.00	0.00	0.00	0.00
Eggerelloides sp.	5.49	22.22	6.52	23.81	10.53
Epistominella sp	0.00	2.78	0.00	0.00	0.00
Epodides ?	1.10	0.00	0.00	0.00	0.00
Globobulimina hoeglundi	2.20	13.89	8.70	9.52	10.53
Globobulimina spinifera	1.10	2.78	4.35	0.00	5.26
Globocassidulina subglobosa	18.68	27.78	15.22	0.00	5.26
Gyroidina multilocula	1.10	0.00	0.00	0.00	0.00
Haplaphragomoides rotulatum	3.30	0.00	4.35	0.00	0.00
Haplaphragomoides sp	0.00	2.78	0.00	0.00	0.00
Hoeglundina elegans	2.20	8.33	0.00	0.00	0.00
Lenticulina sp	0.00	0.00	0.00	0.00	0.00
Martinonella sp	8.79	2.78	4.35	4.76	0.00
Melonis affinis	0.00	0.00	0.00	0.00	0.00

Nonionella sp	0.00	0.00	0.00	0.00	0.00
Oridosalis umbonatus	8.79	19.44	10.87	4.76	5.26
Pullenia bulloides	1.10	2.78	0.00	0.00	0.00
Pullenia subcarinata	0.00	0.00	0.00	0.00	0.00
Reophax dentaliniformis	1.10	0.00	0.00	0.00	0.00
Reophax hispidulus	31.87	0.00	0.00	0.00	0.00
Reophax horridus	10.99	2.78	0.00	0.00	0.00
Reophax sp	2.20	0.00	2.17	0.00	0.00
Trochammina	3.30	2.78	0.00	0.00	0.00
Uvigerina hispida	102.20	180.56	156.52	80.95	5.26
Uvigerina peregrina	112.09	155.56	113.04	66.67	63.16
Valvulinera glabra	6.59	0.00	0.00	0.00	0.00
Valvulinera sp	0.00	0.00	0.00	0.00	0.00
Valvulinera oblonga	0.00	0.00	0.00	0.00	0.00
Unknown	0.00	0.00	0.00	0.00	0.00
	6.59	11.11	13.04	0.00	10.53
Total agglutinates	76.92	38.89	19.57	33.33	10.53
Total calcareous	335.16	522.22	480.43	195.24	157.89
Total foraminifera	412.09	561.11	500.00	228.57	168.42

APPENDIX F: Stable Isotopic Data of Substrate and Foraminifera

Figure 9 plots average vestimentiferan tube, tissue, and *Cibicides wuellerstorfi* values. This appendix includes all the data and a table of the averages.

Dive	Vestimentiferan Tissue $\delta^{13}C$	Vestimentiferan Tube $\delta^{13}C$	*Cibicides wuellerstorfi* $\delta^{13}C$
4586	-14.87	-15.83	-0.13
4586			-1.39
4586			0.25
4586	-18.98	-19.15	0.25
4586			0.12
4586	-19.72	-20.93	0.10
4586			0.32
4586			-0.02
4586			0.05
4586	-18.69	-18.82	0.18
4586			0.42
4587	-15.25	-11.98	-0.35
4587			-0.27
4587			-0.98
4587	-14.04	-13.90	-0.50
4587			0.28
4587	-15.79	-16.31	-0.75
4587			0.08
4587			0.14
4587			0.10
4587	-15.15	-16.44	0.06
4587			-0.03
4590	-16.91	-17.07	-0.24
4590			0.04
4590	-44.81	-28.62	0.07
4590			0.27
4590			0.26
4590	-20.97	-24.20	-0.58
4590			0.29
4590			0.83

Average values. These data were used to create Figure 9.

Dive	Vestimentiferan Tissue $\delta^{13}C$	No. Tissues Averaged	Vestimentiferan Tube $\delta^{13}C$	No. Tubes Aver	*Cibicides wuellerstorfi* $\delta^{13}C$	No. *C. wuellerstorfi* Averaged
4586	-18.07	4	-18.68	4	0.01	11
4587	-15.06	4	-14.66	4	-0.20	11
4590	-27.56	3	-23.30	3	0.12	8