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BENTHIC FORAMINIFERAL DISTRIBUTION INFLUENCED BY RIVERS ON THE NORTHERN GULF OF CADIZ CONTINENTAL SHELF

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Master in Marine and Coastal Systems
Work performed under the supervision of:

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Declaration

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Declaro ser o(a) autor(a) deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída."

x

Olivia Alexander

Declaration of authorship of work

I declare to be the author of this work, which is original and unpublished. Authors and works consulted are duly cited in the text and are included in the list of references.

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Abstract (English)

Benthic foraminifera are widely used as bioindicators due to their sensitivity to environmental stimuli and global distribution. Understanding their ecology is a fundamental factor in interpreting their distribution patterns. This dissertation assesses the distribution of benthic foraminifera as related to rivers on the northern Gulf of Cadiz continental shelf, a highly productive zone with strong fluvial influences. In this study, the distribution of living (stained) and dead benthic foraminifera from six surface sediment samples, collected on the Gulf of Cadiz continental shelf, along two profiles off the Tinto-Odiel and Guadalquivir Rivers, were analyzed. The distribution of the living fauna was compared with the main oceanographic parameters, temperature and salinity, in order to obtain indicator species in present-day shelf environments. 13 species at relatively high abundances (>5%) were identified for the living assemblage. From these, two biofacies were defined and related with their abundances and locations on the shelf. Biofacies I, associated with higher depths (55 m to 91 m), contained *Brizalina spathulata/dilatata*, *Cassidulina laevigata*, *Cassidulina minuta*, *Epistominella vitrea*, *Bulimina elongata*, *Rectuvigerina phlegeri*, and *Bolivina striatula*. Biofacies II, associated with shallower depths (23 m to 39 m) and river influence, contained *Brizalina seminuda*, *Nonionella stella*, *Bolivina ordinaria*, *Textularia earlandi*, *Hopkinsina atlantica*, *Ammonia beccarii/tepida*. These results were compared with samples collected in 2001, which had some differences in the most abundant (>5%) species, relative to those found in 2015. These findings will constitute a baseline for future studies on the ecology of foraminifera and their distribution on the northern Gulf of Cadiz continental shelf.

Key words: Tinto-Odiel River, Guadalquivir River, ecology, bioindicators, Foraminiferal assemblages

Resumo (Português)

Os foraminíferos bentónicos são amplamente utilizados como bioindicadores devido à sua resposta aos estímulos ambientais e à sua distribuição global. Entender a sua ecologia é um fator fundamental para a adequada interpretação de seus padrões de distribuição. Esta dissertação avalia a distribuição de foraminíferos bentónicos no norte do Golfo de Cádis, relacionando-a com a influência dos rios que drenam para a plataforma continental da região, uma zona altamente produtiva e com fortes influências fluviais. Neste estudo, analisou-se a distribuição de foraminíferos bentónicos vivos (corados) e mortos de seis amostras de sedimentos superficiais colhidas na plataforma continental do Golfo de Cádis, ao longo de dois perfis localizados frente aos rios Tinto-Odiel e Guadalquivir. A distribuição da fauna viva foi comparada com os principais parâmetros oceanográficos, temperatura e salinidade, a fim de obter espécies indicadoras de ambientes de plataforma continental atuais. Foram identificadas 13 espécies com abundâncias relativamente altas (>5%) no conjunto de foraminíferos vivos. Estas espécies foram agrupadas em dois conjuntos, de acordo com as suas abundâncias e localizações na plataforma continental. O primeiro grupo constituído pelas espécies *Brizalina spathulata/dilatata*, *Cassidulina laevigata*, *Cassidulina minuta*, *Epistominella vitrea*, *Bulimina elongata*, *Rectuvigerina phlegeri e Bolivina striatula* apresentaram maiores abundâncias em amostras localizadas a maiores profundidades na plataforma. O segundo grupo contendo as espécies *Brizalina seminuda*, *Nonionella stella*, *Bolivina ordinaria*, *Textularia earlandi*, *Hopkinsina atlantica*, *Ammonia beccarii/tepida*, com maiores abundâncias em amostras localizadas a menores profundidades, em áreas influenciadas pelas descargas dos rios. Estes resultados foram comparados com resultados de amostras colhidas em 2001 para determinar as variações, ao longo do tempo, na composição das associações de foraminíferos.

Palavras-chave: Rio Tinto-Odiel, Rio Guadalquivir, Ecologia, Bioindicadores, Associações de foraminíferos

Sumário (Português)

Os foraminíferos bentónicos são protistas com ampla distribuição e que são comumente usados como bioindicadores ambientais ou em reconstrução paleoambiental de ambientes marinhos. A distribuição e a abundância dos foraminíferos bentónicos dependem das condições ambientais circundantes. Estas condições ambientais encontram-se incorporadas nas suas carapaças, que possuem elevada capacidade de fossilização. Essa informação fica preservada no registo sedimentar, tornando-os valiosos indicadores paleoambientais (Corliss, 1985). Os foraminíferos bentónicos podem, também, ser usados para determinar o estado ambiental de uma região. A distribuição e a abundância de foraminíferos bentónicos vivos, ao contrário das faunas mortas, são indicadoras do ambiente no seu estado atual. As faunas vivas refletem as condições ambientais em que eles vivem, enquanto as associações de foraminíferos mortos refletem uma série de processos post-mortem (ex., Murray & Alve, 1999). As associações de foraminíferos mortos podem ser destruídas por desintegração, corrosão, dissolução ou bioturbação (ex., Mackensen & Douglas, 1989; Murray & Alve, 1999). Além de sujeitos a destruição, os foraminíferos podem ser transportados e depositados novamente noutro ambiente, após a sua morte. Compreender a ecologia de foraminíferos bentónicos pode ser útil na reconstrução precisa do passado e na avaliação dos processos atuais que afetam um ambiente. Os objetivos deste estudo são compreender a distribuição das espécies mais abundantes de foraminíferos bentónicos, a sua relação com as distribuições de temperatura e salinidade, e a influência das descargas dos rios Tinto-Odiel e Guadalquivir.

Este estudo foi realizado na plataforma continental do norte do Golfo de Cádis. O Golfo de Cádis é um sistema muito complexo, o que aumenta ainda mais a relevância de entender a ecologia dos foraminíferos na região. O sistema é influenciado pelas descargas de quatro rios principais, o Guadiana, o Piedras, o Tinto-Odiel e o Guadalquivir. Vários outros estudos avaliaram os controles ambientais na distribuição, diversidade e abundância de foraminíferos bentónicos no norte da plataforma continental do Golfo de Cádis (ex., Mendes et al., 2004; 2012). Este estudo foca-se, especificamente, nos parâmetros ambientais associados à influência dos rios no Golfo e seus controles sobre a abundância e distribuição dos foraminíferos bentónicos.

As amostras foram colhidas da porção central da plataforma do norte do Golfo de Cádis, durante o cruzeiro RV *Poseidon*, POS482 CADISED (Cadiz Shelf Sediment Depocentres). Foram selecionados, para este estudo, dois perfis com um total de seis amostras. As amostras de sedimento (~ 50 cm^3) foram colhidas utilizando um Rumohr corer e o primeiro centímetro (0-1 cm) foi preservado numa solução contendo etanol (95%) e Rosa de Bengala (1 g/l). Foram medidos vários parâmetros físico-químicos, incluindo temperatura e salinidade. No laboratório, e após as medições de volume, as amostras de sedimento foram peneiradas. O material maior que 63 µm foi analisado usando uma lupa binocular. Para cada amostra, foram colhidos, identificados e contados, pelo menos, 300 foraminíferos mortos e 100 foraminíferos bentónicos vivos (corados com Rosa de Bengala), com carapaças bem preservadas. Foram calculados para cada amostra, a riqueza específica e outros índices estatísticos de diversidade (ex., índice de Shannon, Fisher-alpha, *evenness*). Efetuaram-se cálculos de densidade populacional e determinou-se a distribuição de espécies de foraminíferos bentónicos com abundância > 5%. Procedeu-se a uma análise grupal (*Cluster Analysis*) para determinar a correlação de espécies relativamente à localização em que foram encontradas (modo Q, agrupamento de estações) e abundância de espécies (modo R, agrupamento de espécies). Os grupos obtidos foram, então, comparados com as características do ambiente para determinar as causas responsáveis. Os parâmetros ambientais usados na comparação foram: salinidade, temperatura, localização relativamente à descarga do rio, densidade, riqueza específica e tipo de sedimento.

A densidade populacional de foraminíferos bentónicos variou entre amostras e entre as associações de foraminíferos vivos (537-5008 ind./10 cm^3) e mortos (1644-17.351 ind./10 cm^3). A densidade máxima da associação de foraminíferos mortos e a mínima de foraminíferos vivos foi registada na amostra 37, colhida a 91 m de profundidade na plataforma adjacente ao Rio Tinto-Odiel. O valor mínimo de densidade da associação de foraminíferos mortos foi registado na amostra 20 (1644 ind./10 cm^3), onde também se registaram valores similares de densidade da associação de vivos (1613 ind./10 cm^3). A densidade máxima da associação de foraminíferos vivos foi registada na amostra 17 (5008 ind./ 10 cm^3). As amostras 20 e 17 foram colhidas na plataforma continental adjacente ao Rio Guadalquivir, a 13 e 39 m de profundidade, respetivamente.

A densidade populacional das associações de foraminíferos vivos e mortos foi comparada para caracterizar as taxas de sedimentação em cada local de amostragem. As taxas de sedimentação mais elevadas foram registadas nas amostras menos profundas, adjacentes ao Rio Guadalquivir, onde foi registada a maior densidade de foraminíferos vivos e similares densidades entre vivos e mortos. As menores taxas de sedimentação ocorreram a 91 m de profundidade, no perfil adjacente ao Tinto-Odiel, onde foram registados as maiores densidades de foraminíferos mortos e as menores de foraminíferos vivos.

Na associação de foraminíferos vivos, foram observadas 13 espécies com abundância > 5%. Duas destas espécies, *Brizalina spathulata/dilatata* (0.95-44.6%) e *Rectuvigerina phlegeri* (0.3-7.7%), ocorreram nas seis amostras analisadas. A espécie *Brizalina seminuda* (0-5.1%) apenas ocorreu nas amostras colhidas na zona adjacente ao Rio Guadalquivir, estando ausente das amostras adjacentes ao Rio Tinto-Odiel. Em contraste, a espécie *Cassidulina minuta* (0-8.3%) apenas foi observada em amostras adjacentes ao Rio Tinto-Odiel e ausente das restantes. No caso da associação de foraminíferos mortos, também foram observadas 13 espécies com abundância > 5%. A maior parte destas espécies foram observadas em todas as amostras analisadas, com exceção das espécies *Ammonia tepida/beccarii*, *Textularia earlandi*, e *Saidovina karreriana* que não ocorreram em uma a três amostras. Em particular, a espécie *S. karreriana* (0-8.65%) com frequência de 50%, apenas foi observada em amostras localizadas na zona adjacente ao Rio Guadalquivir.

A análise de *clusters* foi realizada para avaliar a tendência das 13 espécies de foraminíferos vivas mais abundantes, em relação à sua abundância nas amostras analisadas. As espécies foram agrupadas em dois *clusters*. Foram incluídas no *cluster* I as espécies *spathulata/dilatata*, *Cassidulina laevigata*, *Cassidulina minuta*, *Epistominella vitrea*, *Bulimina elongata*, *Rectuvigerina phlegeri*, e *Bolivina striatula*. No *cluster* II foram incluídas as espécies *Brizalina seminuda*, *Nonionella stella*, *Bolivina ordinaria*, *Textularia earlandi*, *Hopkinsina atlantica*, e *Ammonia beccarii/tepida*. Verificou-se que as espécies do *cluster* I apresentaram as maiores abundâncias em amostras localizadas a maiores profundidades na plataforma continental, enquanto as espécies do *cluster* II, apresentaram as maiores abundâncias em amostras localizadas a menores profundidades, em áreas influenciadas pelas descargas dos rios.

Os dados de salinidade, temperatura e o tipo de sedimento que ocorre nos locais amostrados, foram usados para determinar a influência das descargas dos rios na plataforma continental. A temperatura variou com a profundidade, mas a diferentes magnitudes, dependendo da profundidade e localização da amostra relativamente às desembocaduras dos rios. Para o perfil adjacente ao Rio Guadalquivir, a estação localizada a menor profundidade (23 m) registou a temperatura de 14.9ºC, inferior à temperatura registada na estação menos profunda no perfil adjacente ao Tinto-Odiel (15.1ºC a 39 m). Isto pode refletir a diferença na descarga dos rios e a consequente influência na temperatura da água nas zonas mais próximas da costa. As variações de temperatura observadas estão provavelmente associadas às diferentes localizações das estações relativamente à desembocadura dos dois rios. O tipo de sedimento que constitui as 6 amostras analisadas foi caracterizado como sedimento fino, composto por silte e argila, assim, qualquer variação observada é improvável que esteja relaciona com o tipo de sedimento. As maiores taxas de sedimentação, observadas na zona menos profunda adjacente ao Rio Guadalquivir, indicam uma região de baixa energia hidrodinâmica, que permite a deposição de sedimentos finos provenientes do rio. Além disso, a proporção de fragmentos de foraminíferos bentónicos neste local foi inferior à observada no perfil adjacente ao Tinto-Odiel. A maior proporção de fragmentos no perfil do Tinto-Odiel poderá indicar transporte sedimentar de zonas menos profundas da plataforma.

No geral, verificaram-se várias diferenças entre a composição das associações de foraminíferos vivos, entre 2001 e 2015. Em 2001, as amostras revelaram maior diversidade de ambientes e de habitats. Em comparação com as amostras de 2015, houve mudanças na composição, com três espécies diferentes a apresentarem abundâncias superiores. Por outro lado, foram identificadas espécies em 2015 com abundâncias superiores a 5%, que apresentaram menores abundâncias em 2001. Estas mudanças podem estar relacionadas com alterações ambientais na plataforma continental. No entanto, uma vez que a composição de foraminíferos bentónicos sofre alterações ao longo do tempo (sazonal e anualmente), estas alterações podem também estar relacionadas com diferentes períodos de amostragem (Fevereiro 2001 e Maio 2015).

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1. Introduction

1.1 Motivation

Benthic foraminifera are wide spread protoctists that are commonly used as environmental bioindicators or for paleo reconstruction in marine environments. The distribution and abundance of benthic foraminifera are dependent on their surrounding environmental conditions. These environmental conditions are incorporated into their tests which have a high capacity to fossilize. This valuable information is preserved in the sediment record, making benthic foraminifera valuable paleoenvironmental indicators (Corliss, 1985). Benthic foraminifera can be used to determine the state of the environment. The distribution and abundance of living rather than dead benthic foraminifera are indicative of the environment in its current state. Living faunas reflect the environmental conditions where they live, while dead assemblages reflect a number of post-mortem processes (e.g. Murray & Alve, 1999). Dead assemblages can be destroyed through disintegration, corrosion, dissolution, or bioturbation (e.g. Mackensen & Douglas, 1989; Murray & Alve, 1999). In addition to destruction, assemblages can be transported and re-deposited in another environment after death. Understanding the ecology of benthic foraminifera can be useful in accurate reconstruction of the past and assessment of the current processes acting in an environment.

Environmental conditions at the sediment-water interface can affect the morphological and taxonomic composition of the benthic foraminifera present in the surface sediment (Kaiho, 1994). Parameters such as temperature, salinity, and available organic carbon have been broadly linked to morphological changes in test thickness and specimen size (Boltovskoy et al., 1991). In addition to morphological expression, environmental conditions are expressed in assemblage composition. Studies have shown that the distribution of foraminifera is affected by the sediment type, water temperature, salinity, organic carbon content, turbidity, and oxygen (e.g. Hald and Steinsund, 1992; Mojtahid et al., 2009; Mendes et al., 2012).

It is thus important to determine the current distribution of benthic foraminifera in the surface sediment to identify the ecological processes controlling their distribution. In the Gulf of Cadiz, studies have focused on the distribution of benthic foraminiferal assemblages with organic matter and sediment deposition by rivers on the northern continental shelf. These authors found that the abundance and distribution of the most abundant species of benthic foraminifera within an assemblage were related to water depth, sediment type, river discharge, water temperature, salinity, turbidity and primary productivity. However, few studies have assessed the effects of rivers with different influences (e.g. Tinto-Odiel, Guadalquivir) on assemblage abundance and distribution in the Gulf of Cadiz. It is also important to understand how these distributions and controls may change over time. A greater understanding of the ecology (and forcing parameters) in this region is required to enable the further use of benthic foraminifera as bioindicators.

1.2 Objectives

The objective of this study is to understand the distribution of the most abundant benthic foraminiferal species and their relation with the outflows of two main rivers on the northern Gulf of Cadiz continental shelf. Understanding the impact of changes in salinity and temperature on the living (stained) assemblage composition and density will contribute to obtaining indicator species in present-day shelf environments, which can be used to accurately reconstruct the past. This study also aims to determine the impact of the change in river influence over time, by comparing foraminiferal assemblages with past studies. A concrete understanding of the influence of rivers with different discharges, and their associated effects on temperature, salinity, and sediment distribution, on benthic foraminiferal ecology in the Gulf of Cadiz continental shelf is imperative for proper interpretation of the current and past states of the shelf.

2. State of the Art

Foraminifera are single celled protoctists that can be either pelagic or benthic. Benthic foraminifera are more abundant and can be found in the sediment of both deep and shallow marine environments. There are approximately 4,000 species that compose this phylum, with only 40 species being pelagic. With a high population and global distribution, foraminifera are ideal specimens for studying the environment. To do this, both biological and ecological processes must be understood. A distinct morphological feature of foraminifera is their test. The test can be composed of calcium carbonate or cemented foreign particles (Murray, 2001). Foraminifera with calcium carbonate tests are referred to as calcareous, while those that incorporate cemented foreign particles are referred to as agglutinated. The physical characteristics of their test are dependent on the availability of materials in their environment, such as the quantity of carbonate (Boltovsky et al., 1991). The biology of foraminifera has been the focus of studies since the late 1900s, but their ecology and interactions with the environment have been the focus of more recent research (Murray, 2006).

To understand species-specific interactions with the environment, it is important to understand the biology and reproductive cycles of foraminifera. In addition to this, reproductive cycles are important in understanding regime shifts in the populations. These regime shifts are likely to impact the dominant species found at a particular time in a population. Benthic foraminifera reproduce by a combination of sexual and asexual reproduction (Murray, 2006). This can cause an alternation of generations which can cause phases of population size and stages (Murray, 2006). These forms of reproduction can dictate the dominant species at a specific time, as well as a species ability to cope with a changing environment.

Benthic foraminifera disperse both actively and passively. Active dispersal related to dispersal over small distances and is unlikely to be the cause of dispersal within an environment (Alve, 1999; Murray, 2006). For active dispersal, individual locomotion is used and lacks direction and speed. Passive dispersal is evident at larger dispersal scales and can be observed in multiple ways: 1. Resuspension and advection by currents; 2. Attachment to biota. The ability of a species to disperse and resultant colonization, is important for the interpretation of species found in the sediment. Empty tests are more easily resuspended than living ones, which shows the importance of using stained foraminifera as indicators in an environment rather than their easily dispersed counterparts (Murray, 2006).

Benthic foraminifera utilize a variety of feeding mechanisms and some taxa have been known to utilize more than one of these strategies for survival. Primary production is highest in the euphotic zone in deep waters, while more shallow intertidal zones are home to highly productive sediment-dwelling algae. Benthic foraminifera utilize their reticulopodia for collecting algae. The type of feeding strategy varies widely and is not limited to: herbivory, bactivory, suspension feeding, detritivores, carnivory, omnivory, and parasitism (Murray, 2006).

Foraminifera are found in a wide range of marine environments. Global and local distributions are influenced by the environment. At the species level, foraminifera have been found to colonize habitats based on suitability (Murray, 2001). The distribution of benthic foraminifera has been related to: sediment type, water temperature, salinity, organic carbon content, turbidity, and oxygen (e.g. Hald and Steinsund, 1992; Mojtahid et al., 2009; Mendes et al., 2012). In deep water environments, depth distribution in the sediment has mainly been associated with oxygen and organic carbon flux (Murray, 2006). In shallow environments, such as margins, control of distribution is more dynamic and can depend on chemical, physical, and biological interactions. For example, species diversity has been found to decrease with an increase in chemical pollutants in the Gulf of Gabes, Tunisia (Ayadi et al., 2015). In the Barents Sea, Hald and Steinsund (1992) found that a shift in the abundance of dominant species within an assemblage was related to bottom water temperature.

Faunal distribution can be regional; a factor that controls the distribution of a species in one region, may not be the controlling factor in another region. For example, Lohmann (1978) found that *Uvigerina peregrina* abundance was inversely correlated with the oxygen content of overlying waters in the Western South Atlantic. A more recent study conducted by Miller and Lohmann (1982), found that *U. peregrina* abundance was controlled by the oxygen level within the sediment in the North Atlantic, specifically within Cape Cod. In the Indian Ocean, *U. peregrina* distribution varies independently of oxygen content (Corliss, 1983). Therefore, the regional variability in controlling factors of foraminifera abundance and distribution is important to take into consideration when utilizing them as bioindicators.

Due to their tendency to colonize habitats based on environmental conditions, benthic foraminifera are widely used as bioindicators. They can be used as indicators of pollution, environmental conditions, and commonly in paleoenvironmental reconstructions. An example of paleoenvironmental applications is the assessment of the temporal formation of geological features as well as the reconstruction of hydrography (e.g. Sierro et al., 1999; Rogerson et al., 2011). To use benthic foraminifera found in the fossil record for the reconstruction of a paleoenvironment, they must be calibrated to regional ecology.

The Gulf of Cadiz is a wide basin in the northeast Atlantic Ocean, between the Iberian Peninsula and northwest African coasts, which is connected to the Strait of Gibraltar (See section 3: Study Area). As the meeting point between the Mediterranean and the North Atlantic Oceans, the Gulf of Cadiz is of particular interest in the area of paleoenvironmental reconstruction (Caralp, 1988; Sierro et al., 1999; Mendes et al., 2010; 2012b; Rogerson et al., 2011). For the proper interpretation of the fossil record, the ecological processes within the Gulf are currently being studied. Schönfeld (2002) studied the influence of local hydrography and sediment facies on the faunal distribution of benthic foraminifera. He found 4 distinct assemblages that were strongly associated with sediment stability. On the northern Gulf of Cadiz continental shelf, several studies have assessed the environmental controls on benthic foraminifera faunal distribution, diversity, and abundance (Mendes et al., 2004; 2012a; 2013).

The controlling factors on species distribution have been associated with water depth (Mendes et al., 2004). At shallow depths, foraminiferal distribution is associated with the hydrodynamic influence of the estuary. In deeper depths, there is an association with hydrodynamic conditions, sediment type, and temperature (Mendes et al., 2004). A more recent study on the distribution of living benthic foraminifera on the northern Gulf of Cadiz continental shelf examined the physio-chemical factors of the region (Mendes et al., 2012). Foraminifera abundance and distribution were associated with water depth, sediment type, river discharge, water temperature, salinity, turbidity, and primary productivity. Four groups were formed based on the distribution of the 26 most abundant species. This study established a baseline description of the living benthic foraminiferal assemblages present in the northern Gulf of Cadiz continental shelf.

The results of Mendes et al. (2012) are based on sediment samples collected in 2001. The collection of samples in 2015, provides a unique opportunity to assess whether there has been a change in foraminiferal distribution and species composition on the northern Gulf of Cadiz continental shelf.

3. Study Area

The focal area of this project is the northern Gulf of Cadiz continental shelf. The northern Gulf of Cadiz spans from the westernmost point of Mainland Portugal (São Vicente Cape) to the Strait of Gibraltar. The northern Gulf of Cadiz continental shelf ranges from less than 5 km wide at the Santa Maria Cape to more than 40 km at the Guadalquivir River (e.g. García-Lafuente et al., 2006).

The study area is influenced by the discharges of four main rivers: the Guadiana, Piedras, Tinto-Odiel, and Guadalquivir (Figure 3.1). The Guadalquivir River is the largest contributor of water to the basin, with an estimated mean water discharge of 164 m^3/s. The Guadiana River discharges less than half that of the Guadalquivir River (75 m^3/s) and the Tinto-Odiel and Piedras have the lower contribution, between 1 and 10 m^3/s (Palanques et al., 1995). The construction of dams in the catchment has greatly decreased the sediment and freshwater supply to the continental shelf (e.g. Morales 1997). In the case of the Guadiana River, the implementation of the Alqueva dam in 2002, and therefore regulation of water flow of the Guadiana river, strongly affected the sediment transport to the shelf (Garel and Ferreira, 2011). Suspended sediment transport into the Guadiana estuary was found to be lower after the implementation of the Alqueva dam, with the potential for impacting the morphological and biological components of the estuary and the adjacent shelf (Garel and Ferreira, 2011). The regulation of freshwater pulses into the estuary also influences salinity, nutrient concentrations, and planktonic communities, which has the effect of regulating primary production within the estuary and along the coastal zone (Chicharo et al, 2006). The major rivers and their estuaries, have social and economic importance for their respective countries. For the Guadalquivir estuary, increased human activities pose a threat to its status (Ruiz et al., 2015). With increased human pressures on the rivers that distribute water into the northern Gulf, it is important to have recent information on the environmental factors that control benthic fauna.

The climate of the Gulf of Cadiz is Mediterranean, with rainfall of less than 600 mm per year (Fernandez Salas et al., 2016). Rainfall maxima occur during winter, which has a seasonal influence on the volume of water that reaches the shelf. Rainfall leads to episodic flooding of the rivers and estuaries, which increases the sediment distributed to the shelf (Morales, 1997). Sediments in the Gulf of Cadiz run in bands parallel to the coast from the inner shelf to the shelf break. The inner is predominantly covered in sandy sediments with some regions of gravel and mud. Mud is associated with the river mouth sand also occurs in a band at the middle shelf (Gonzalez et al., 2004; Fernandez Salas et al., 2016). An important source of sediment to the coast is littoral drift that flows in an eastward direction, transporting approximately 180 x 10^3 m^3/yr (Morales, 1997).

The circulation of this system is highly complex. The shelf is split into two regions based on its shape and resultant oceanographic processes. The eastern region of the shelf, which is the area of focus in this study, is dominated by tidally driven processes. The net circulation is predominantly towards the east (Criado-Aldeanueva et al., 2009). The area is characterized by a mesotidal regime with a mean tidal range of 2 m. The majority of waves approach from the west and southwest. Unlike the western region, the eastern region of the shelf receives nutrients from terrestrial and riverine sources (Navarro and Ruiz, 2006). Upwelling occurs in the western portion off Cape San Vincente, with the presence of cold surface water drawn from depth and pushed off-shore depending on the wind. The upwelling area is reduced during Easterlies and extended during Westerlies, sometimes merging with the upwelling zone adjacent to Cape Santa Maria (Garcia-Lafuente et al., 2006). Upwelling is not a significant form of circulation in the eastern portion of the gulf, where the sample collection for this study was conducted.

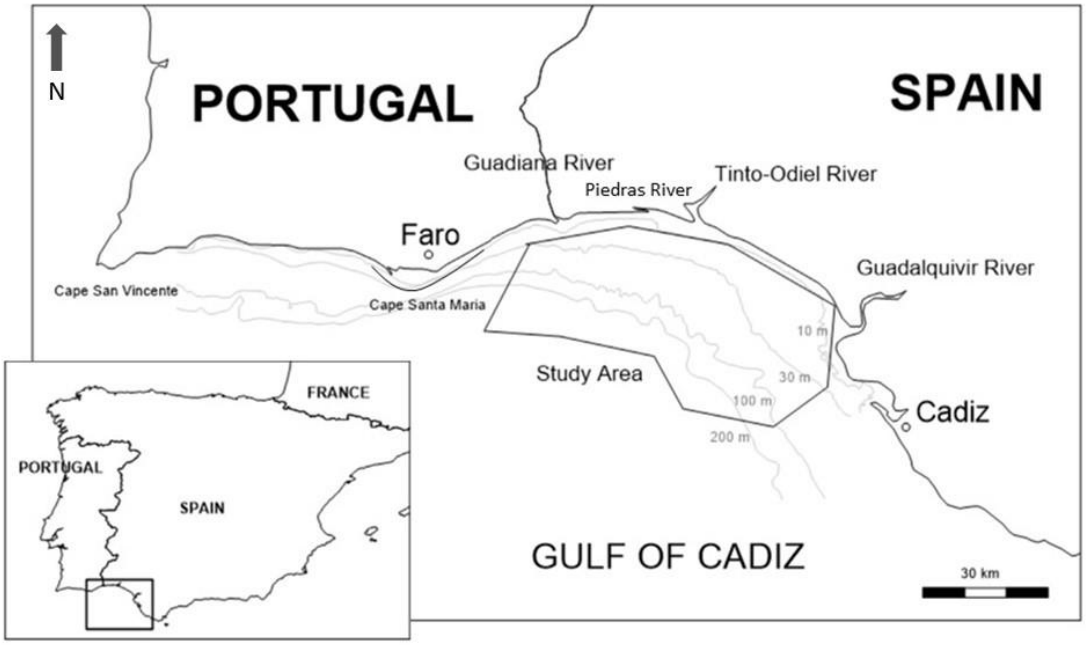


Figure 3.1. Location of the study area on the northern Gulf of Cadiz continental shelf.

4. Methods

4.1 Data Collection

The samples analyzed in this study were collected during the RV *Poseidon* cruise, POS482 CADISED (Cadiz Shelf Sediment Depocentres). The cruise was conducted within the northern Gulf of Cadiz from May 14 - 24 2015, beginning data collection in Faro, Portugal and ending collection in Cadiz, Spain. The objective of this cruise was to reconstruct the formation history and past environmental changes confined in transgressive and highstand sediment depocentres in the Gulf of Cadiz. In addition to sediment samples taken from the depocentres (regions along the shelf with high sediment deposition), other oceanographic data was collected to meet the objectives of the cruise (See: Section 4.1 *In Situ Measurements*).

Sediment Samples

The samples analyzed in this study were collected from the central portion of the northern Gulf of Cadiz Shelf (Figure 3.1). The Rumohr corer, a type of gravity corer that ensures the vertical preservation of the sediment, was used to collect the surface sediment samples. Once the sediment was collected, each sample was preserved in 95% ethanol containing Rose Bengal (1 g/L). This method was chosen based on its global application and low cost (e.g. Murray, 2006; Schönfeld, 2002; Duchemin et al., 2005; Mendes et al., 2012; Grunert et al., 2017). The application of this method has two purposes for treating the sample: Rose Bengal stains the cytoplasm of living and recently deceased foraminifera, while Ethanol preserves the tissues for later analysis (e.g. Barras et al., 2014).

From the samples collected during this cruise, six surface sediment samples of 0-1 cm thickness were selected for this study (Figure 4.1). Each sample had an approximate volume of 50 cm^3. The number of samples chosen was based on the processing time of the samples and time constraints of the project. Two profiles of three samples were selected based on comparable water depth and position relative to river discharges within the northern gulf. The samples range from 23 m to 91 m water depth, both profiles extending from the Guadalquivir and Tinto-Odiel Rivers, in areas expected to be directly influenced by river discharges. Each profile was arranged perpendicular to the coast from the Guadalquivir (Samples: 20 (23 m water depth), 17 (39 m), 14

(90 m)) and the Tinto-Odiel (Samples: 33 (36 m), 36 (55 m), 37 (91 m)) rivers (Figure 4.1;Table I, Appendix I).

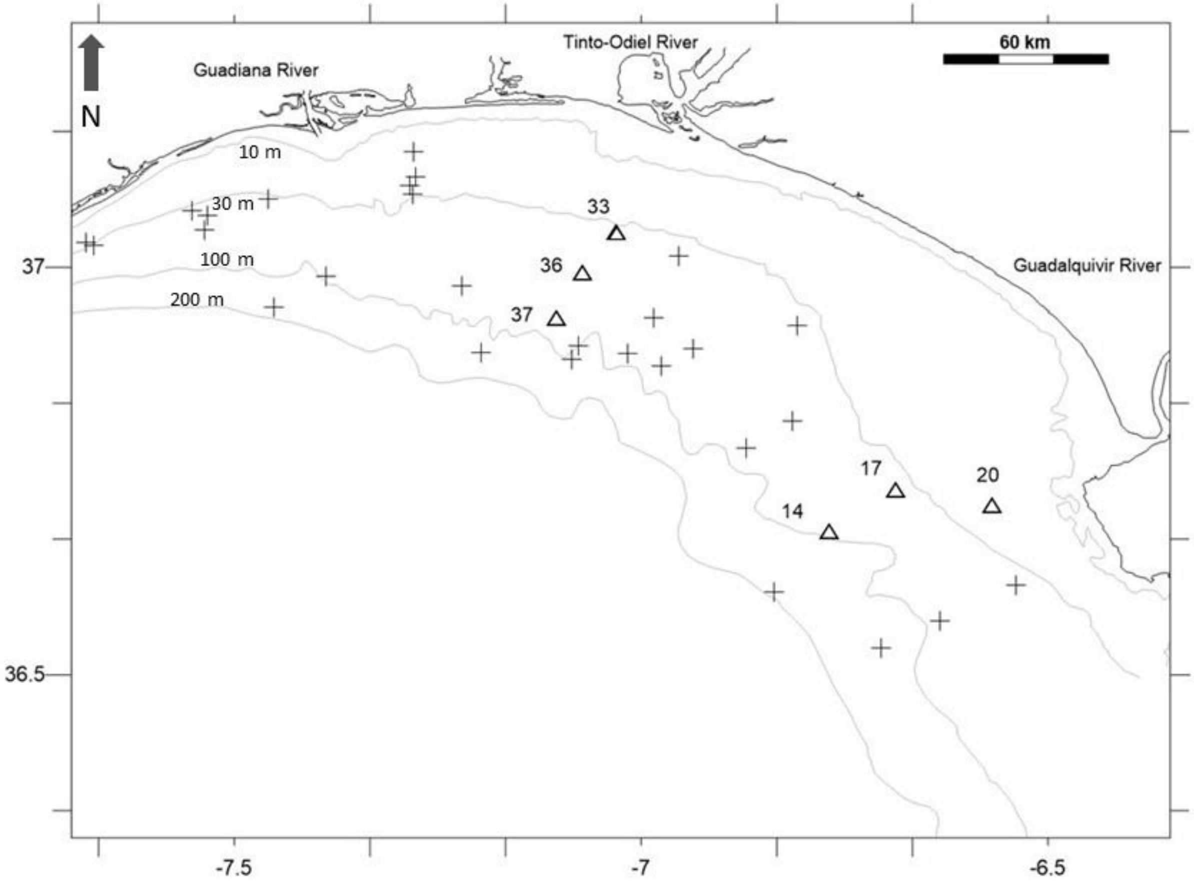


Figure 4.1 Location of the sediment samples collected during the RV *Poseidon* cruise in the northern Gulf of Cadiz. The six samples used for the foraminiferal analysis are represented by triangles. All the stations (triangles and crosses) were used for temperature and salinity measurements.

In Situ Measurements

Multiple physical-chemical parameters were measured during sediment collection, both within the Rumhor corer and in the water column. Surface temperature (^{o}C), near-bottom temperature, and sample temperature were measured at each station. Salinity was also measured in the surface water and at the bottom of the water column. With each reading, the depth (m) was recorded. These parameters were visualized across the entire study region (~36 samples) and compared with the faunal distribution (abundance, density, and species composition) within and between samples (See: Section 5. Results). Near-bottom salinity and temperature were chosen for this analysis due to their previously documented influence on benthic foraminiferal distribution and physiologic responses, as well as the reliability of the sampling technique executed (Mendes et al., 2012). Both temperature and salinity were taken for nearly all of the samples collected in the Gulf during the CADISED POS482 expedition (Lantzsch et al., 2015) and the sampling techniques align with those used in previous studies in the region (Mendes et al., 2004; 2012; Martinez-Garcia et al., 2013). Cruise details and a comprehensive list of the environmental parameters measured on the RV CADISED POS482 cruise used in this study can be viewed in Appendix I (Appendix I, Table I).

4.2 Laboratory Processing

Each sample was processed in the laboratory to obtain accurate measurements of the sediment volume and foraminiferal counts. Sediment samples were prepared in the laboratory before they could be analyzed under the microscope.

Sediment Samples

The total volume of each sample was determined by drawing a line on the sample containers at the top of the settled sediment. Once the samples were emptied into the sieves, the containers were carefully filled with water three times and measured using a graduated cylinder. The three volume measurements were recorded and their average was taken. After the volume was recorded, samples were wet sieved at two different size classes (2000 µm and 63 µm). Sieves were soaked in a tub of Methylene Blue between samples to trace any contamination. The remaining sediment was carefully collected into a graduated cylinder and the final volume was recorded. The ratio of the initial wet volume relative to the after-wash volume allows for the estimation of sand content of the samples. The total sediment volume was used to determine the population density of foraminifera (# of individuals/ 10 cm^3). Wet sieved samples were placed in an oven at 50^oC until dry, before further analysis under a microscope.

Microscope Analysis

Foraminiferal analysis was performed in the laboratory to preserve and count the living and dead assemblages. To determine the abundance of foraminifera in each sample, the samples were viewed under a binocular microscope with a maximum zoom of 140x. Each sample was split into sub samples by use of an Otto micro-splitter. The fraction of each sample picked for both living and dead foraminifera were recorded to calculate density. Each sub sample was spread on a picking tray and viewed under a microscope. Living and dead foraminifera were methodically picked from the tray grids and placed on a cardboard slide for preservation. A minimum of 100 living individuals and 300 dead individuals were picked from each sample. Once picked, the species were identified and counted to determine the relative abundances and indices of diversity. Individuals were identified to the species level using descriptions and pictures within the literature and the World Foraminifera Database (Hayward *et al.*, 2018; Ellis and Messina, 1942; Jones and Brady, 1994; Martins and Gomes, 2004). Those that were not identified to the species level, were assigned to the genus level and given a numeric designation to ensure accurate species counts (Example: *Nonionella sp. 1*). Benthic foraminiferal fragments and intact planktonic foraminiferal tests were counted and recorded to determine their relationship with the distribution of living and dead benthic foraminifera in the study area.

4.3 Data Analysis

To determine the effect of environmental stimuli on the foraminiferal assemblages, species diversity and population density were quantified using the data collected in the laboratory.

Foraminiferal population density (individuals/ 10 cm^3) was calculated for each sample, using the total volume, the fraction of the sample picked in the microscope analysis and the number of individuals counted in each fraction. Species richness and other statistical diversity indices (e.g. Shannon index, Fisher-alpha, evenness) were calculated with the PAST (Palaeontological Statistics) program (Hammer et al., 2001). Sediment type was determined from the literature (Gonzalez et al., 2004) and compared with the foraminiferal distribution results of this study.

The distribution of the most abundant living benthic foraminiferal species (relative abundance >5%) was determined and visualized with distribution maps. These distributions were compared with the most abundant dead benthic foraminiferal species to aid in their interpretation. Distribution maps were drawn for the entire data set (density, richness) as well as for individual species with abundances >5%. To compare these results with the environmental parameters, contour maps were also created for temperature and salinity. All maps (distribution and contour) were computed using Surfer software version 9 (Golden Software, LLC), using the kriging method.

Cluster Analysis was performed to determine species correlation joined by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) relative to the location in which they were found (Q-mode, grouping stations) and species abundance (R-mode, grouping species), by using PAST (Palaeontological Statistics) program (Hammer et al., 2001). The obtain clusters were then compared with the characteristics of the environment to determine causation. Environmental parameters compared were: salinity, temperature, location relative to a river discharge, density, richness, and sediment type.

The results of this study were then compared with those of Mendes et al. (2012) to determine whether there has been a change in species distribution since 2001.

5. Results

From the 6 samples analyzed, approximately 3,315 intact benthic foraminifera were picked from the sediment for this analysis (Table II; Appendix I). Of those picked, 2,240 were empty tests considered dead at the time of collection, ranging from 306 to 513 per sample, while 1,075 individuals were stained tests considered alive at the time of collection, ranging from 101 to 315 per sample. Approximately 410 test fragments were counted during the picking of the dead fraction of the samples (23 to 149 individuals per sample). In addition to fragments, approximately 480 intact planktonic foraminiferal tests were counted with the dead assemblage (49 to 137 individuals per sample).

5.1 Benthic Foraminiferal Density Analysis

Foraminiferal density (number of individuals per 10 cm^3) varied between sample locations and between the living (537-5,008 ind./10 cm^3) and dead (1,643-17,351 ind./10 cm^3) assemblages. Across samples, the density of dead foraminifera was higher when compared to that of the living assemblages (Figure 5.1.1).

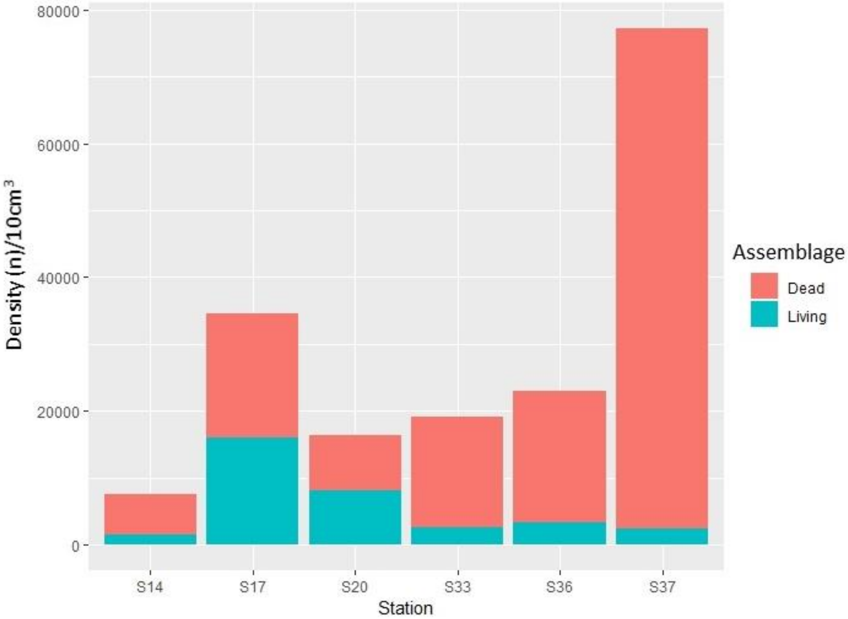
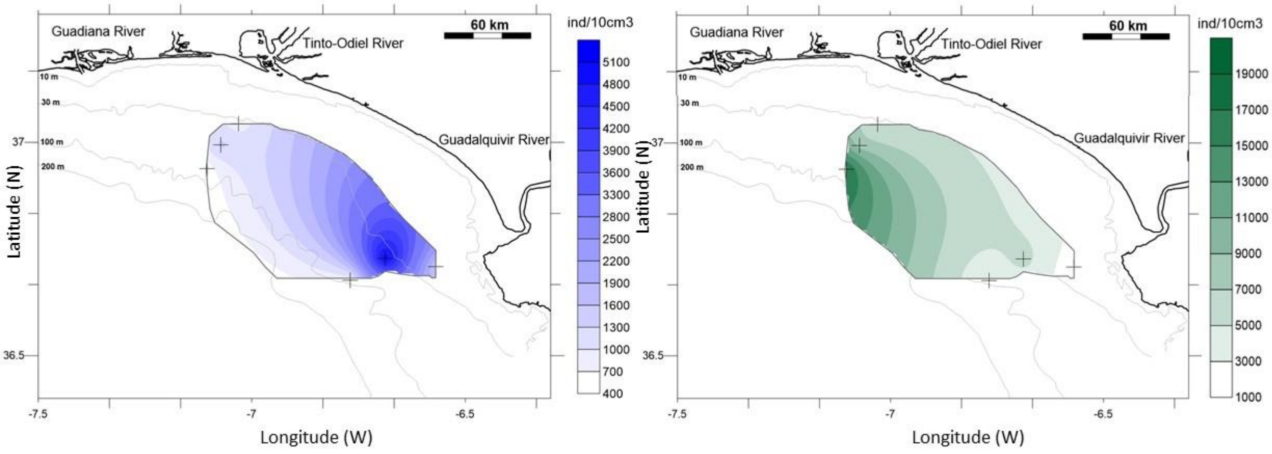


Figure 5.1.1 Foraminiferal density in number of individuals (n) per 10cm^3 across the 6 sampling sites in the study area (S14, S17 and S20 profile off the Guadalquivir River; S33, S36 and S37 profile off the Tinto-Odiel River).

The highest density value of the dead (17,351 ind./10cm^3) and the lowest of the living (537 ind./10cm^3) assemblages, occurred at sample 37, collected at 91 m water depth off Tinto-Odiel River (Figure 5.1.1). This sample had the greatest difference between dead and living assemblages, with the dead assemblages having a density 32 times that of the living. The lowest dead foraminiferal density occurred in sample 20 (1,644 ind./10 cm^3), with similar density values of the living assemblage (1,613 ind./10 cm^3). This sample was collected off Guadalquivir River at 23 m water depth, the closest sample to the Guadalquivir River mouth. The highest density values of the living assemblages were recorded at sample 17 (5,008 ind./ 10 cm^3), collected at 39 m water depth, also in the vicinity of the Guadalquivir River.

Dead foraminiferal density shows a positive linear association with depth, as depth increases, so does the density, with the exception of sample 14, located off the Guadalquivir River at 90 m water depth, where the density is 2,960 ind./10cm^3 (Figure 5.1.2). There is no significant association between living foraminiferal density and depth.



(A) Living (B) Dead

Figure 5.1.2 Interpolated density distribution of living (A) and dead (B) benthic foraminifera obtained from the 6 analyzed samples in the study area.

Similar trends were observed between living (537 to 1,387 ind./10cm^3) and dead (5,211 to 17,351 ind./10cm^3) assemblages within the Tinto Odiel sampling profile (Figure 5.1.3). For the dead assemblages, density increased with depth, with the lowest density adjacent to the Tinto Odiel

River outflow at 36 m water depth (5,211 ind./10cm^3). The living assemblages showed parabolic trends, with the lowest density (537 ind./10cm^3) observed farthest from the outflow (Sample 37; 91 m), reaching a peak (1387 ind./10cm^3) as it approaches the outflow (Sample 36; 55 m), and then declining to 831 ind./10cm^3 at the shallowest sampling depth of the profile (Sample 33; 36 m). Between the two river profiles, densities of the dead foraminifera were overall higher adjacent to the Tinto-Odiel outflow.

Within the Guadalquivir profile, the lowest density of the living assemblage (774 ind./10cm^3) is observed farthest from the outflow at sample 14, collected at 90 m water depth. The density of the living assemblage was the highest at sample 17 (5008 ind./10cm^3), collected 39 m water depth respectively. The living foraminiferal density closest to the outflow of the Guadalquivir was 1612 ind./10cm^3, which was also the shallowest sample at approximately 23 m water depth.

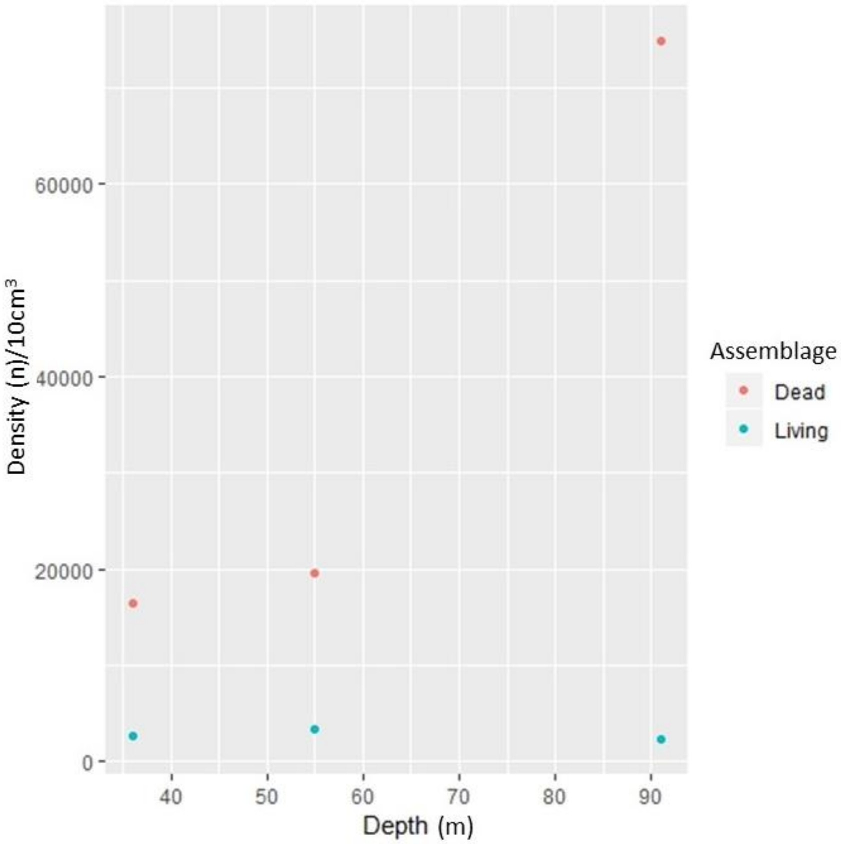


Figure 5.1.3. Density of the living and dead foraminifera in 3 samples off of the Tinto-Odiel river outflow.

There is an observable trend in the Tinto-Odiel sample profile between the dead and living foraminiferal density. As depth increases, so does the difference between the living and dead density. There is an increase in the proportion of dead tests found in the sample with depth, although the living density stays relatively similar between samples.

5.2 Diversity Indices

There is no clear association between depth and the diversity indices (richness, Shannon, Fisher-alpha, and evenness) in the living assemblage. Species richness (number of species in each sample), varied from 16 to 25 species and showed distinct differences between profiles (Figure 5.2.1). When analyzed separately, richness in the Guadalquivir profile did not change with depth, with 25 species composing all living samples. In the Tinto-Odiel profile, the highest richness was found at a depth of 55 m (25 species), and the lowest richness was found at 91 m (16 species).

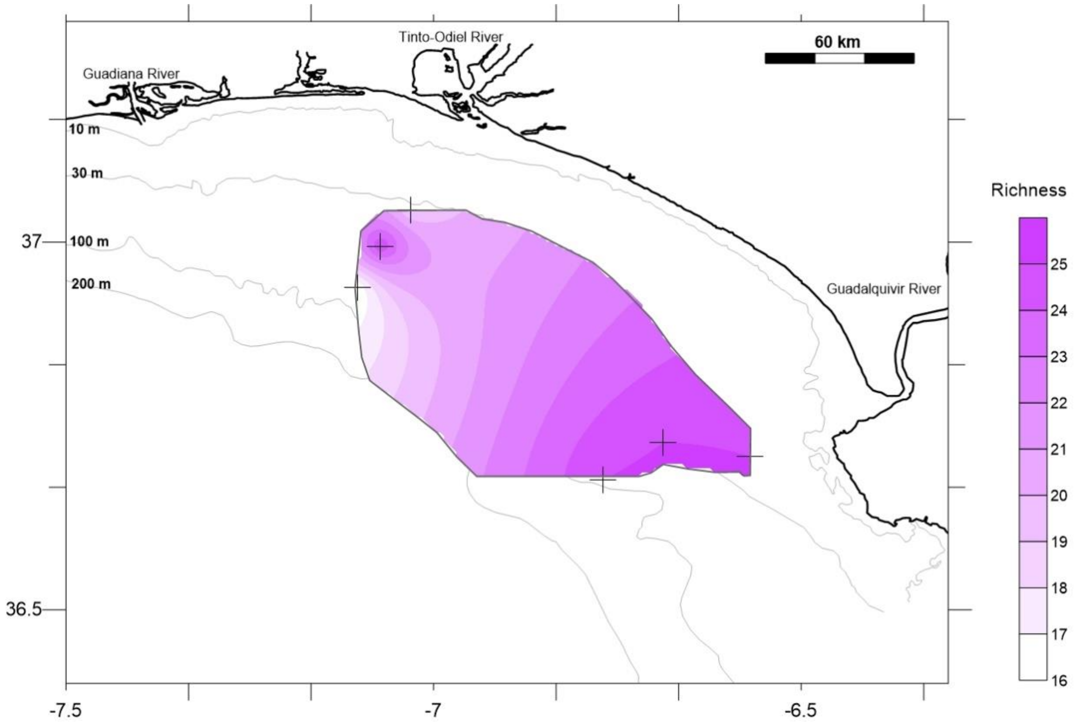


Figure 5.2.1 Distribution of species richness for the living assemblage interpolated from the 6 samples collected in the northern Gulf of Cadiz.

Shannon (H) and Fisher Alpha (a) diversity indices indicate the highest species diversity is found in sample 36, collected at 55 m water depth at the Tinto Odiel outflow (H = 2.8, a = 10.4) (Figure 5.2.2). This is also where the highest richness is found. The two indices are not in agreement on the location of the sample with the lowest diversity, which will be discussed in section 6.1. For the Shannon index, the lowest diversity was obtained at 40 m water depth (H =

2.1). For Fisher-alpha, the lowest diversity was obtained at a depth of 36 m (a = 5). Both the highest and lowest diversities were reported in the Tinto-Odiel profile.

Evenness ranges from 0.34 to 0.65. For all depths, the Tinto-Odiel had the highest evenness of the two profiles (range 0.49 to 0.65). The highest evenness was obtained at 55 m water depth (Tinto-Odiel; 0.65) and the lowest evenness at 39 m water depth (Guadalquivir; 0.34). The lowest evenness was reported in the Guadalquivir profile.

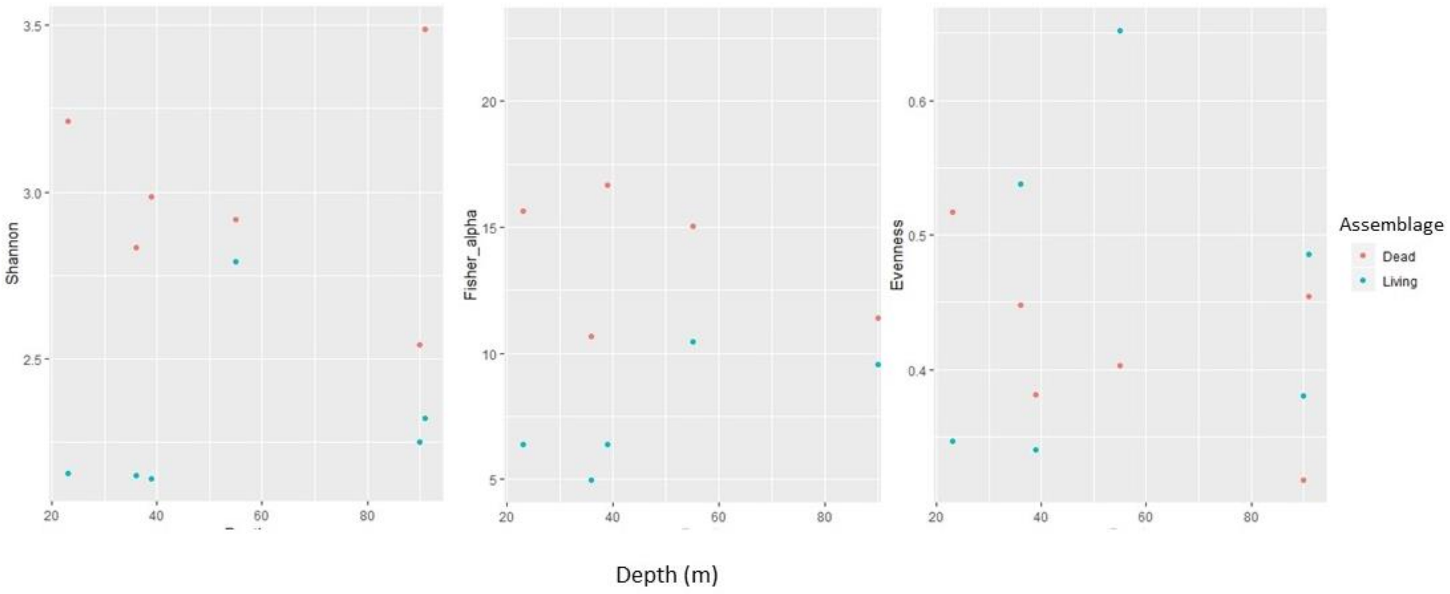


Figure 5.2.2 Diversity indices for the living and dead foraminifera within the 6 samples.

Although the focus of this study is to assess those assemblages that were alive at time of collection for an accurate assessment of the environment at that time, it is important to note the state of the dead assemblage. The species found in the dead assemblage are valuable indicators of post mortem processes and seasonality.

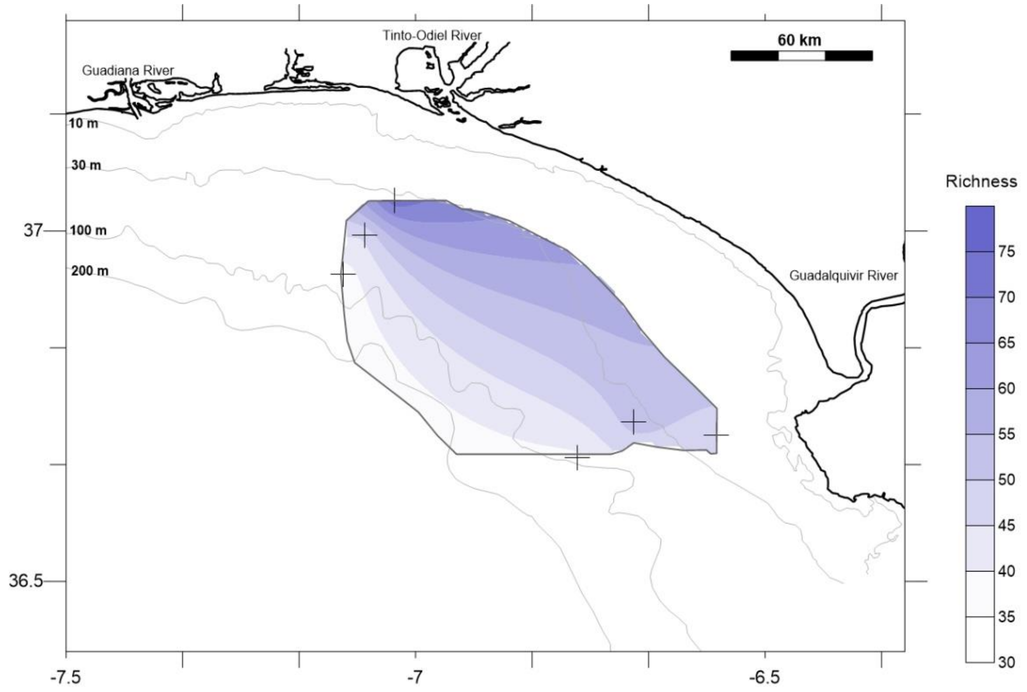


Figure 5.2.3 Richness of the dead assemblage interpolated from the six samples collected in the northern Gulf of Cadiz.

The dead assemblage had a richness that varied between 38 and 73 species (Figure 5.2.3). Unlike the living assemblage, richness did vary between samples within the Guadalquivir profile. The highest richness was obtained at 36 m water depth, closest to the Tinto-Odiel river outflow. The lowest richness was found in the same profile, at 91 m water depth. The lowest richness in the dead assemblage coincides with the lowest richness in the living assemblage, at sample 37 (91 m).

Richness is not clearly associated with density for both living and dead assemblages (Figure 5.2.4). This shows that an increase in species is not associated with an increase in foraminifera, which indicates independence. Due to this, they can be compared and interpreted as independent variables. In addition to general density and diversity statistics, it is important to understand what species compose these samples and how they are distributed.

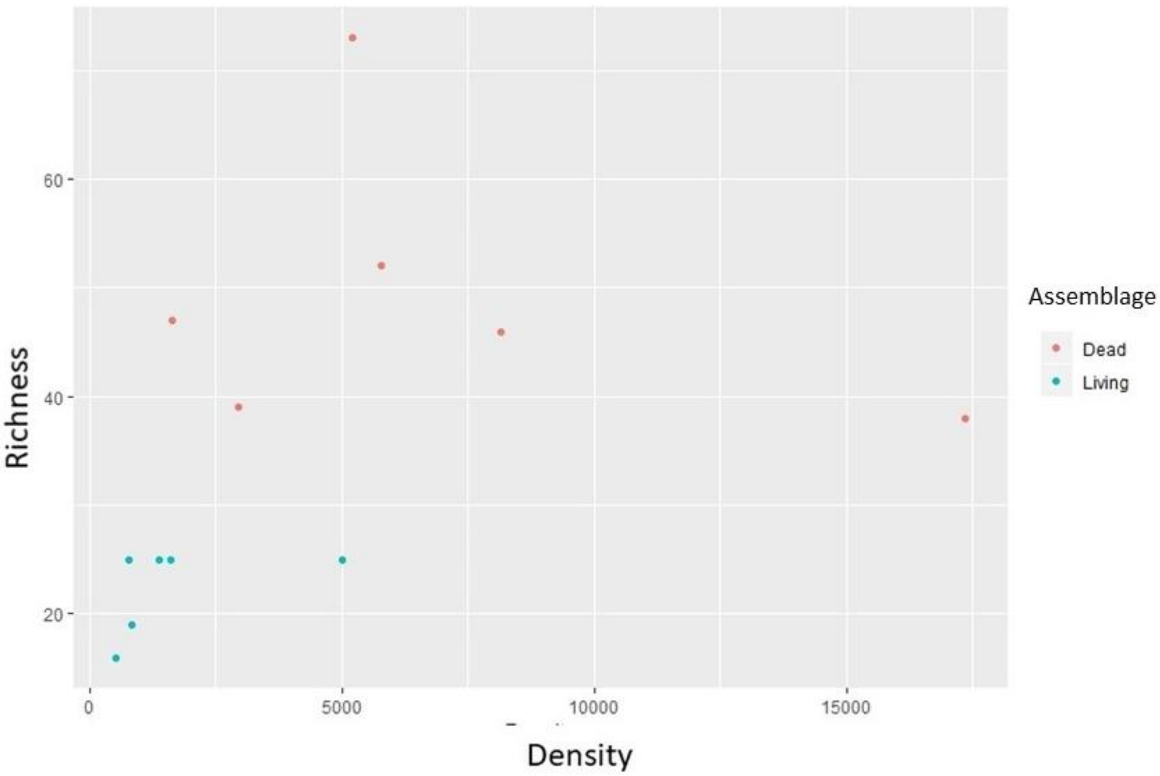


Figure 5.2.4 Richness plotted by density for both the living and dead assemblages.

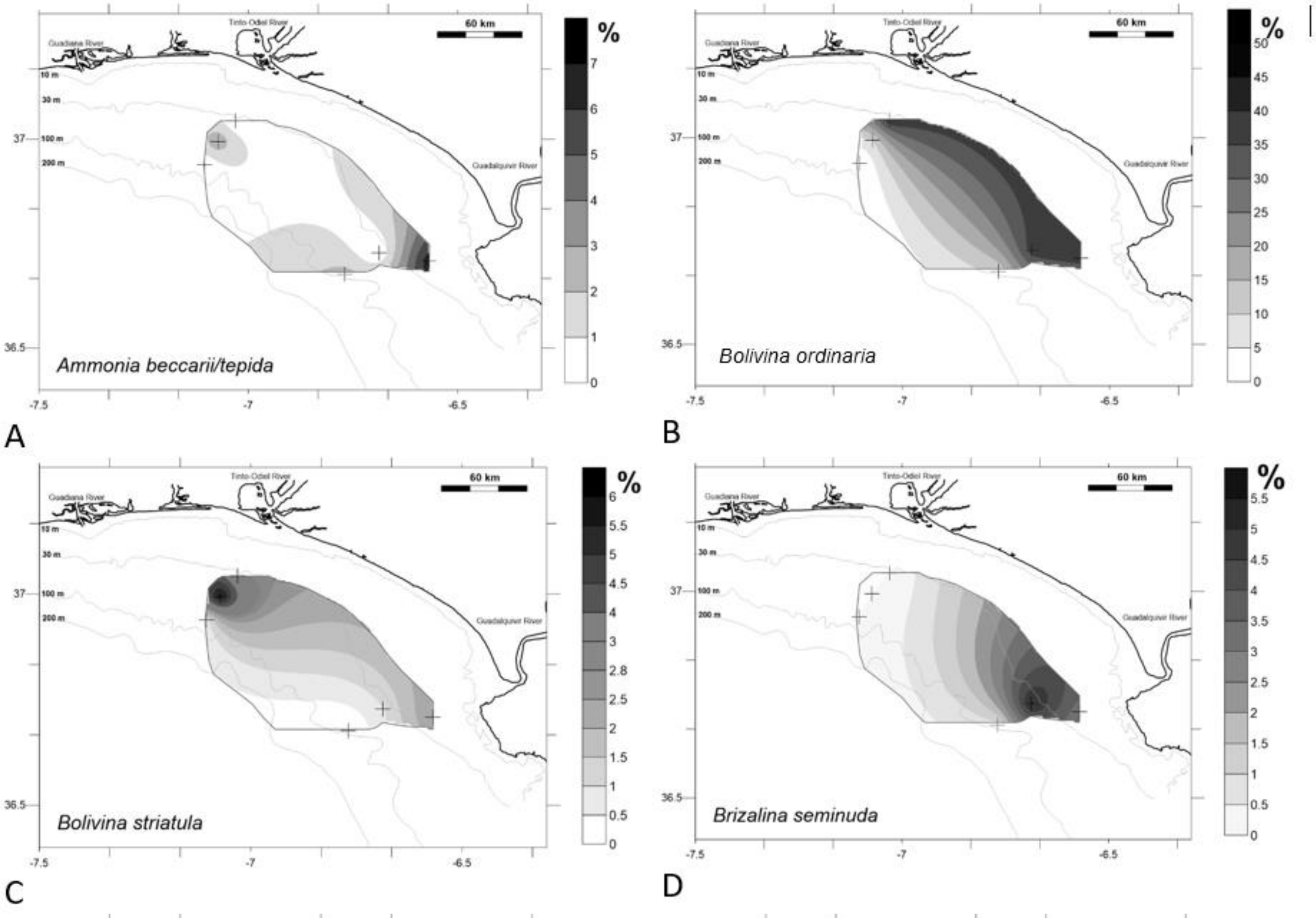
5.3 Species Composition

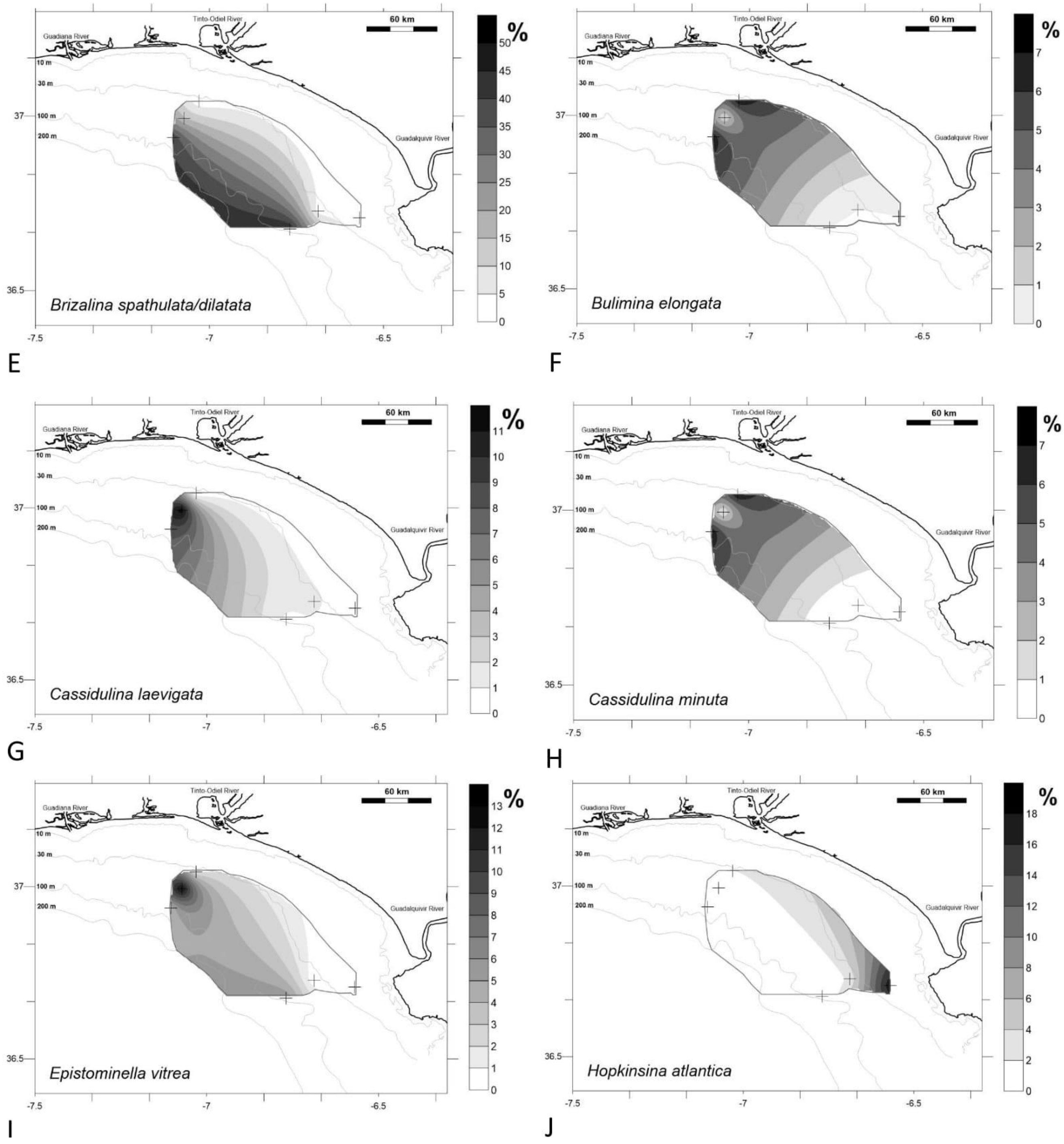
A total of 134 *taxa* were identified during this analysis. For the living assemblage, 57 *taxa* were separated, of which, 43 were identified to the species level and 14 to the genus level. A total of 13 species showed a relative abundance >5%, in at least in one analyzed sample. The most abundant species were: *Ammonia beccarii/tepida, Bolivina ordinaria, Bolivina striatula, Brizalina seminuda, Brizalina spathulata/dilatata, Bulimina elongata, Cassidulina laevigata, Cassidulina minuta, Epistominella vitrea, Hopkinsina atlantica, Nonionella stella, Rectuvigerina phlegeri,* and *Textularia earlandi*. Two of these species, *B. spathulata/dilatata* (0.95-44.6%) and *R. phlegeri* (0.3-7.7%), were present in all six of the analyzed samples. Both species reach their minimum abundances at the same water depth (23 m), closest to the Guadalquivir River.

Initial analysis shows patterns in species composition of each sample and within the profiles (Figure 5.3.1). These patterns are influenced by the interpolation methods used due to the sample size, and must therefore be interpreted and applied carefully. *Brizalina spathulata/dilatata* has the highest maximum abundance of the 6 samples (Figure 5.3.1 E). *Brizalina seminuda* (0-5.1 %, frequency 50 %) is present only in the samples adjacent to the Guadalquivir River and it is absent from the samples adjacent to the Tinto-Odiel River (Figure 5.3.1 D). In contrast, *C. minuta* (0-8.3%, frequency 50 %) is present in the samples adjacent to the Tinto-Odiel River, and absent from the others (Figure 5.3.1 H). This species reaches a maximum abundance of 8.3 % at 91 m water depth, the location with the lowest number of species present (7 most abundant species are present). *Rectuvigerina phlegeri* (0-6.7%) reaches a maximum abundance at mid-depth in both profiles (39 m and 55 m) (Figure 5.3.1 L). Although *A. beccarii/tepida* (0-6.7%) is only absent at mid-depth (0%, 39 m) in the Guadalquivir profile, it shows an opposite trend in the Tinto-Odiel profile, where it is only present at mid-depth (2.9%, 55 m) (Figure 5.3.1 A).

For the Guadalquivir profile, *A. beccarii/tepida* and *H. atlantica* (0-17.5%) have their highest abundance at 23 m water depth (Figure 5.3.1, A & J). *Bulimina elongata* (0-6.9%), *C. laevigata* (0-11.5%), *C. minuta,* and *E. vitrea* (0-5.8%), are all absent at this depth (Figure 5.3.1, F, H, & I). In the Guadalquivir profile, at 39 m water depth, *B. seminuda*, *N. stella* (0-21.4%), *R. phlegeri,* and *T. earlandi* (0-6.7%), reach their maximum abundances (Figure 5.3.1, D, K, L, & M). *Ammonia beccarii/tepida, B. elongata*, and *C. minuta*, are absent at this location. At a depth of 90 m, *B. spathulata/dilatata* reaches a maximum abundance. *B. striatula* (0-5.8%) (Figure 5.3.1, C), *B. elongata*, *C. minuta, and H. atlantica* are all absent at this location. This sample is the only location where *B. striatula* is absent in the living assemblage.

In the Tinto-Odiel profile, at a sampling depth of 36 m, *B. ordinaria* (0-39.6%) and *B. elongata* are at maximum abundances. Comparatively, *A. beccarii/tepida*, *B. seminuda*, and *C. laevigata* (Figure 5.3.1, G) are absent at this depth. *B. striatula*, *C. laevigata*, and *E. vitrea* reach their maximum abundances at 55 m. A depth at which *B. seminuda* and *H. atlantica* are absent. As stated previously, at 91 m, only 54 % of the most abundant species (>5%) are present (*A. beccarii/tepida, B. ordinaria, B. seminuda, H. atlantica*, *N. stella*, and *T. earlandi*). At this depth, only *C. minuta* reaches its maximum abundance.




Tinto-Odiel River
Guadiana River
Guadalquivir River
60 km
10 m
30 m
100 m
200 m
37
36.5
-7.5
-7
-6.5
%
Brizalina spathulata/dilatata
0
5
10
15
20
25
30
35
40
45
50
E
Bulimina elongata
0
1
2
3
4
5
6
7
F
Cassidulina laevigata
0
1
2
3
4
5
6
7
8
9
10
11
G
Cassidulina minuta
0
1
2
3
4
5
6
7
H
Epistominella vitrea
0
1
2
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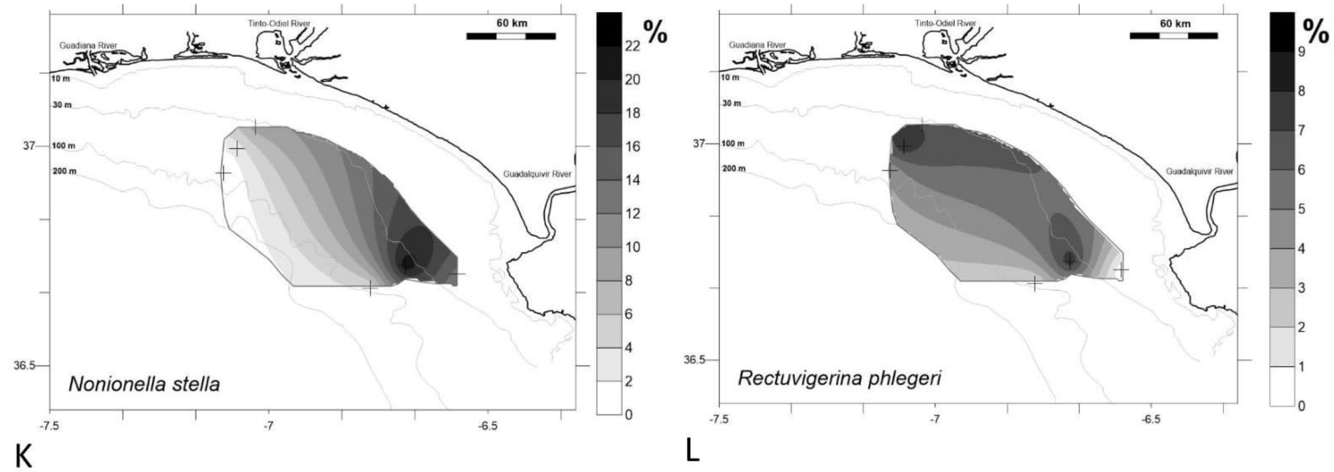

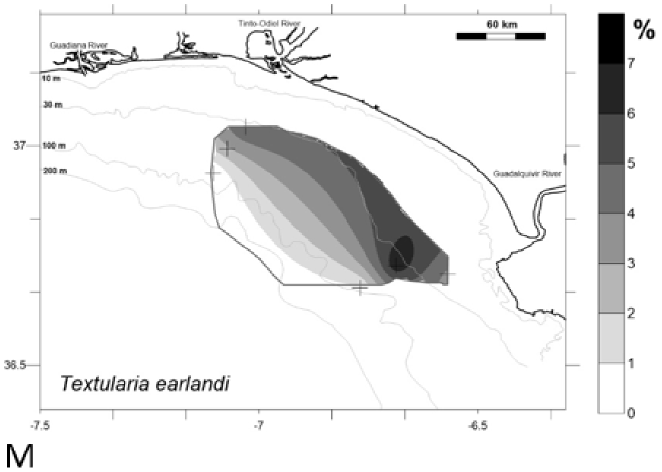

Figure 5.3.1. Distribution of the 13 living benthic foraminiferal species with relative abundance >5%, in at least one analyzed sample: A. *Ammonia beccarii/tepida* B. *Bolivina ordinaria* C. *Bolivina striatula* D. *Brizalina seminuda* E. *Brizalina spathulata/dilatata* F. *Bulimina elongata* G. *Cassidulina laevigata* H. *Cassidulina minuta* I. *Epistominella vitrea* J. *Hopkinsina atlantica* K. *Nonionella stella* L. *Rectuvigerina phlegeri* M. *Textularia earlandi*.

For the dead assemblage, 131 *taxa* were sorted, of which 68 were identified to the species level and 63 were identified to their genus level. For the dead faunas, a total of 13 species were also found with relative abundance >5%, in at least one analyzed sample. Although present in the living assemblage, *B. striatula* (0-2.8%), *B. seminuda* (0-1.9%), and *B. elongate* (0-3%) showed abundances lower than 5% in the dead assemblage. For the distribution maps of all 13 species in the dead assemblage, please see Appendix II, Figure I.

Three species were found with relative abundances >5% in the dead assemblage, but showed lower abundances in the living assemblage: *Bulimina marginata* (Dead: 0.62-7.7%; Living: 0-3%), *Nonionella sp. 1* (Dead: 1.2-7.7%; Living: 0-4%), and *Saidovina karreriana* (Dead: 0-8.65%, Living: 0) (Figure 5.3.3). The majority of these species were found in all samples, with the exception of *A. tepida/beccarii*, *T. earlandi,* and *S. karreriana,* which were absent from one to three samples. In particular, *S. karreriana* had a frequency of 50 % and was only found in the samples adjacent to the Guadalquivir River. The sample taken from 91 m water depth in the Tinto-Odiel profile had the lowest number of species present, the same as the pattern seen in the living assemblage.

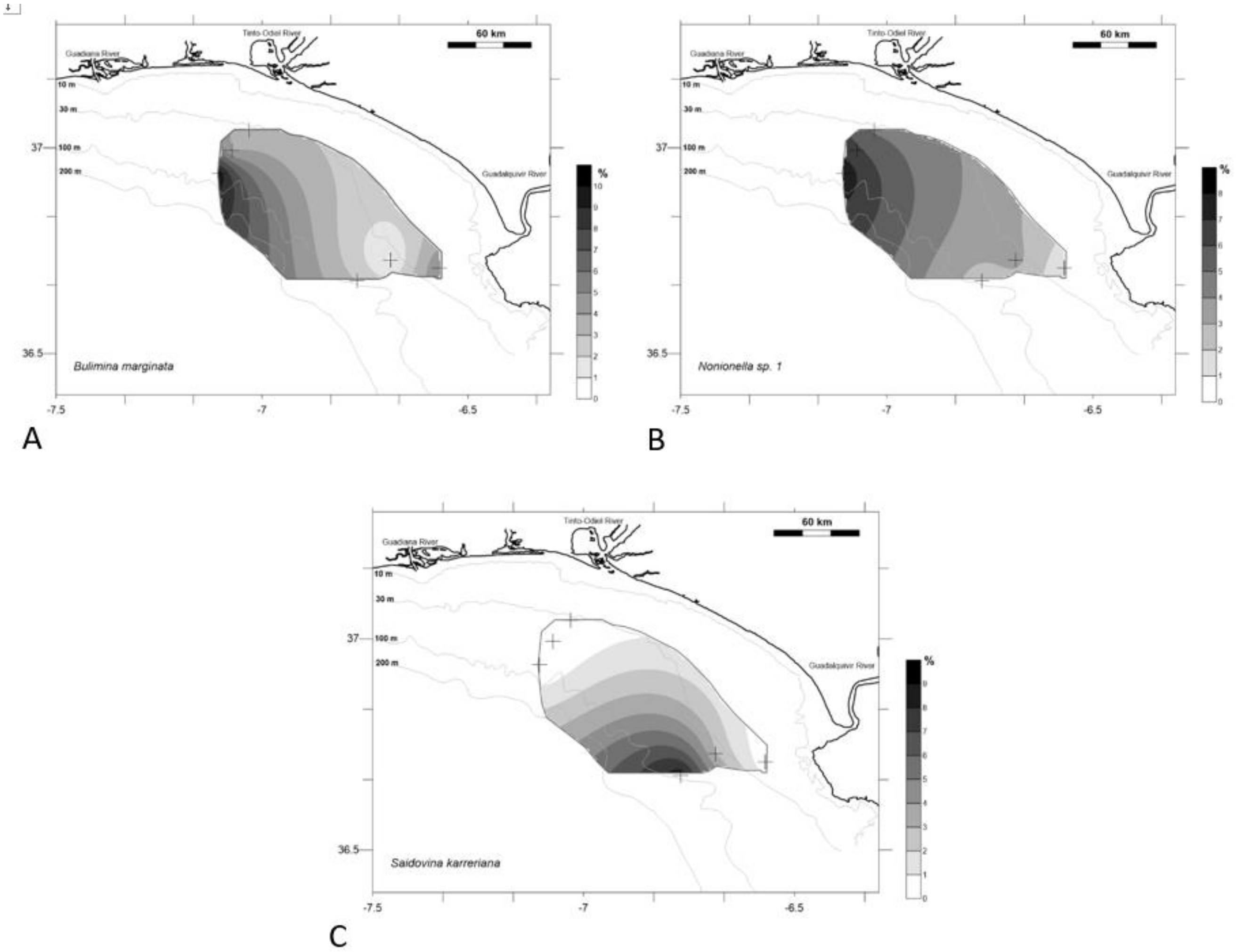

Figure 5.3.3 Distribution of the three species that showed abundances >5% in the dead assemblage and lower abundances in the living assemblage: (A) *B. marginata*, (B) *Nonionella sp. 1*, (C) *S. karreriana.*

The distributions of the dead assemblages were similar to those of the living when each abundant (>5%) species was compared. Higher dead abundances and similar distributions between assemblages were found for *A. beccarii/tepida*, *E. vitrea*, and *T. earlandi*. Higher living abundances were observed for *B. spathulata/dilatata*, *H. atlantica*, and *R. phlegeri*. For *H. atlantica*, higher abundances of the dead assemblage were shifted deeper, although the shape of the contour stayed the same. For *R. phlegeri*, the distribution of the dead assemblage (Figure 5.3.4 A) did not match the distribution of the living assemblage (Figure 5.3.4 B).

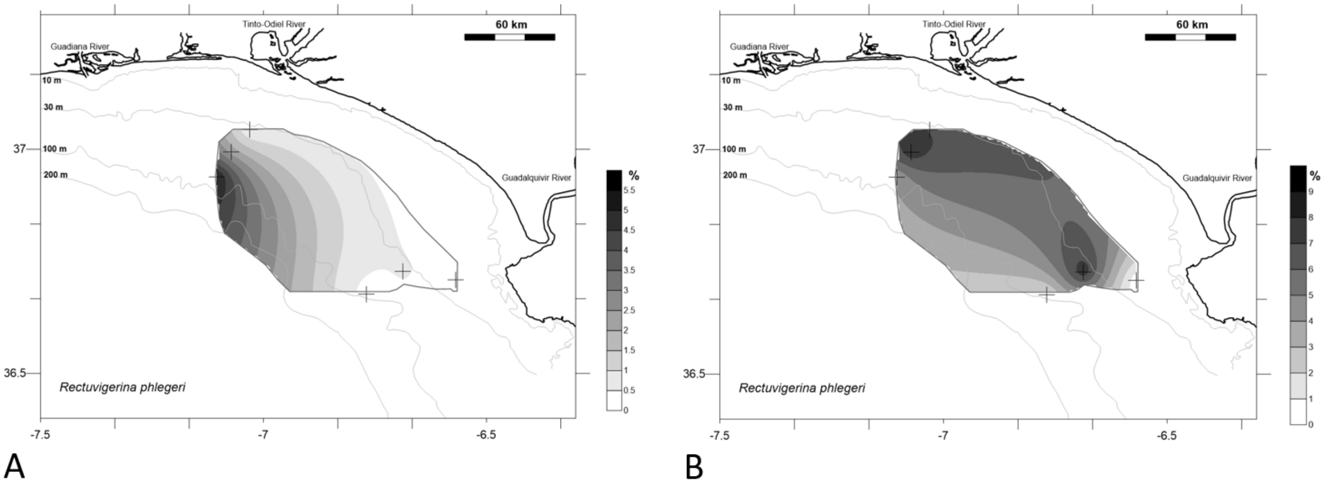

Figure 5.3.4 Distribution of *R. phlegeri* abundance in the (A) dead and (B) living assemblage.

In the Guadalquivir profile, there is an increase in abundance at mid-depth for both living and dead assemblages of *R. phlegeri*, with the living assemblage having a higher abundance. There is also a peak in the Tinto-Odiel profile that occurs at mid-depth in the living assemblage (39 m) and deeper (91 m) in the dead assemblage. Living abundance is higher, relative to the dead assemblage, at shallower depths in the Tinto-Odiel profile.

Similar abundances between the living and dead assemblages were found for *C. laevigata*, *C. minuta*, and *N. stella*. Although the relative abundance of *B. ordinaria* was similar to that observed in the living assemblage, the distribution of the dead assemblage was different, with the highest abundance at 90 m water depth, where few living individuals were found (Figure 5.3.5).

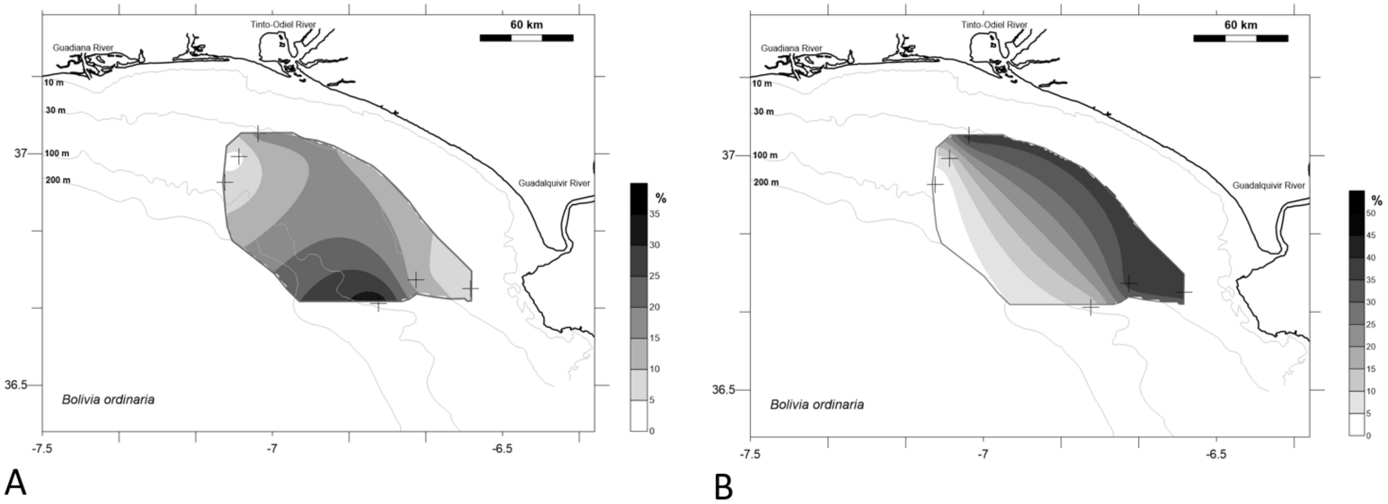

Figure 5.3.5 Distribution of *B. ordinaria* abundance for the dead (A) and living (B) assemblages.

5.4 Environmental Parameters

Temperature and salinity are not linearly correlated with depth across the 6 samples. However, within each profile, both parameters have similar patterns with depth. Salinity is low at the shallowest stations, experiences a peak at mid depth, and is lowest at the deepest samples. Temperature is the highest at the shallowest station and decreases with depth within each profile (Figure 5.4.1).

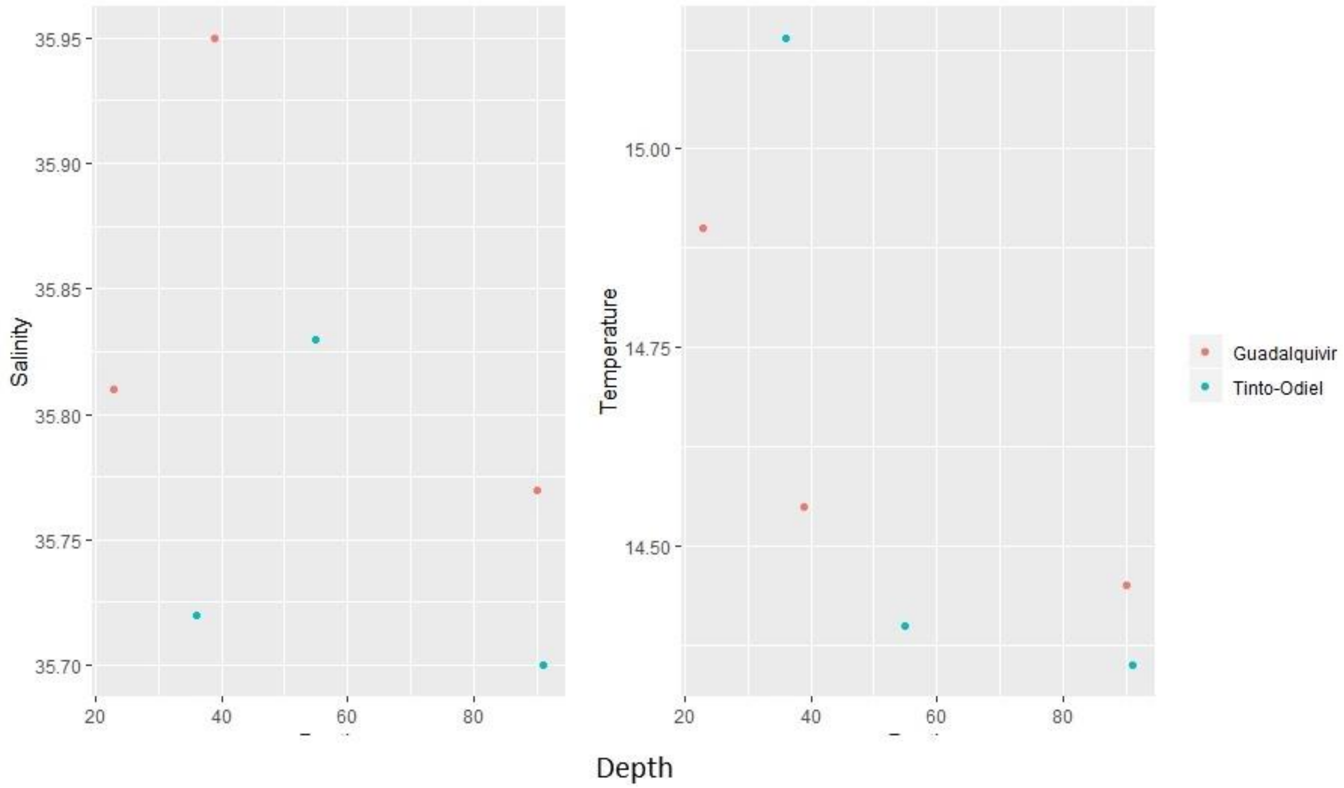

Figure 5.4.1 Near-bottom temperature and salinity measurements by depth obtained in the same location of the 6 samples analyzed for benthic foraminiferal assemblages. The two profiles are distinguished by colours (pink = Guadalquivir profile samples; blue = Tinto-Odiel profile samples).

Salinity and temperature distributions were computed to compare with the living foraminiferal distributions on the shelf, in order to determine species specific distribution patterns related to environmental stimuli. Near-bottom salinity varied from 34.3 to 36.1 across the entire sampling region (Figure 5.4.2). Salinity varied slightly in the 6 samples addressed in this study and ranged from 35.7 to 36. The lowest salinity value obtained in the study samples was 35.7, off the Tinto-Odiel at 91 m water depth.

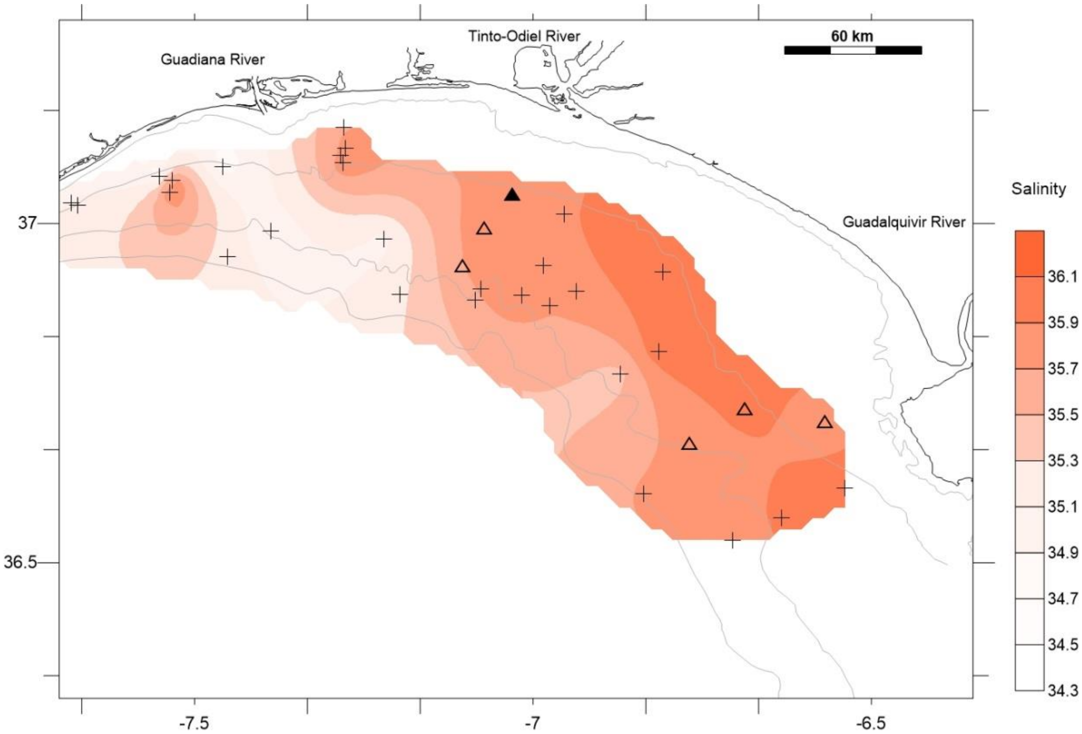

Figure 5.4.2 Near-bottom salinity interpolated from 34 samples collected in the northern Gulf of Cadiz.

Near-bottom temperature varied from 13.6 to 16°C across the 34 samples (Figure 5.4.3). Within the 6 samples analyzed in this study, temperature did vary between samples and profiles (14.4 to 15.2°C). For the Tinto-Odiel profile, temperature is highest at the shallowest depth (36 m, 14.9°C) and decreases slightly as depth increases (change of 0.3°C). For the Guadalquivir profile, temperature is the same at 39 and 90 m depths (14.4°C), and higher at 23 m water depth (15.2°C).

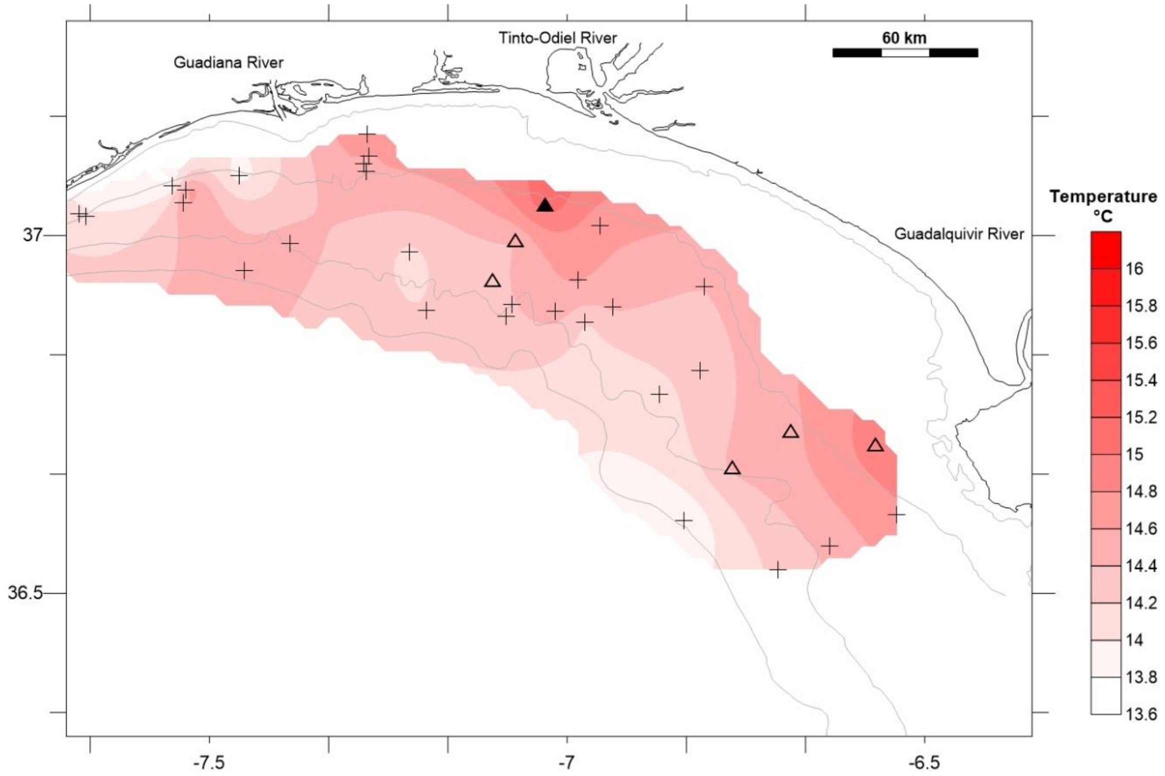

Figure 5.4.3. Near-bottom temperature interpolated from 34 samples collected in the northern Gulf of Cadiz.

5.5 Cluster Analysis

R-mode cluster analyses, with a cophenetic coefficient of 0.82 (Correlation method, joined by UPGMA) produced two distinct clusters by using the 13 living species with abundance >5%, (Figure 5.5.1 A).

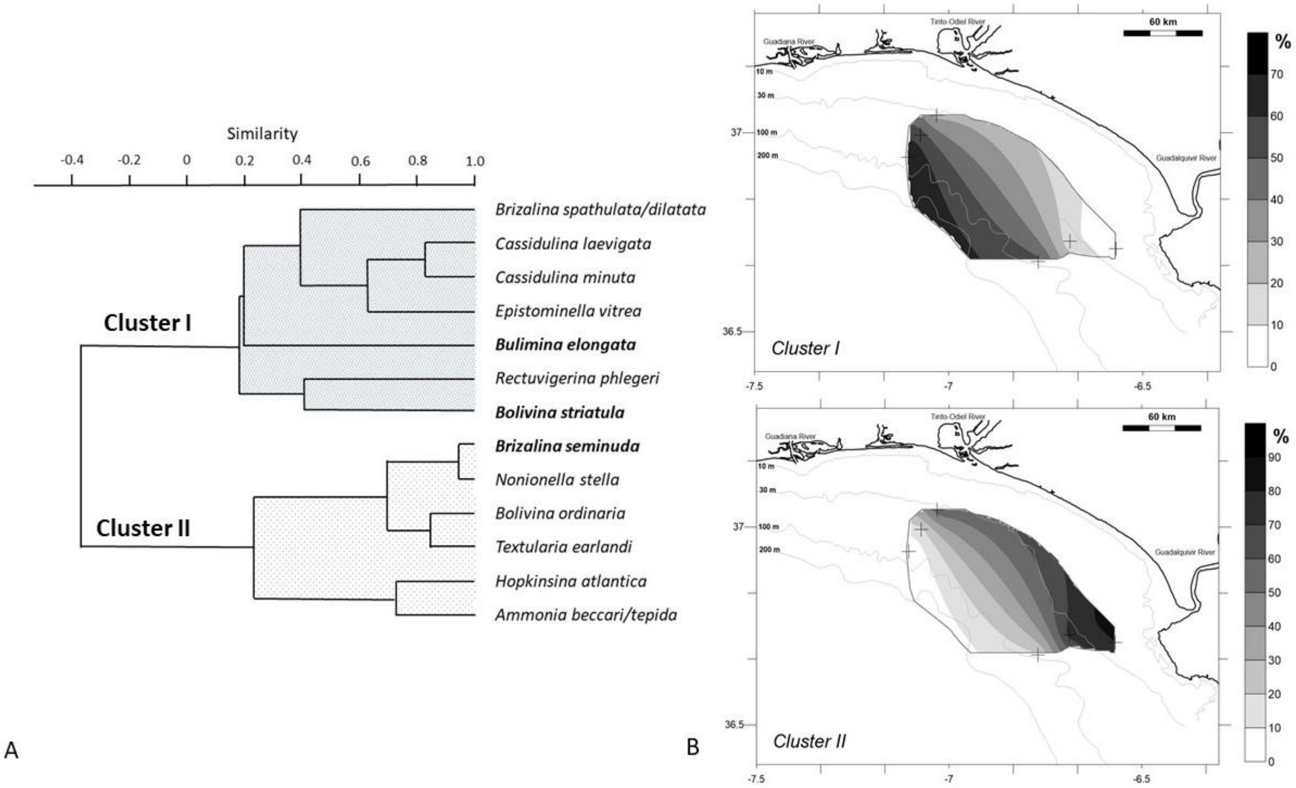

Figure 5.5.1 R-mode cluster analysis (correlation method joined by UPGMA) using the 13 most common living species, with an abundance > 5%. A. Dendrogram with two distinct clusters. B. Distribution of the two clusters recognized in the study region. Species names highlighted in bold indicate abundances >5% only in the living assemblage.

Cluster I consisted of 7 species whose abundance ranged from 0 to 70 % across the 6 stations, showing the higher abundances in the deeper analyzed samples (Figure 5.5.1 B). Cluster II consisted of the remaining 6 species, whose abundance ranged from 0 to 90% across the 6 stations, which show the higher abundances at shallow depths.

Two clusters were also obtained for the 6 stations by using Q-mode cluster analyses (Bray-Curtis measure joined by UPGMA), with a coefficient of 0.92 (Figure 5.5.2 A). There is a clear association between depth and living species abundance based on this analysis. Stations 36 (55 m), 37 (91 m), and 14 (90 m) located at higher depths composed Cluster A. Cluster B showed a correlation between species abundance at the shallow stations 20 (23 m), 33 (36 m), and 17 (39 m).

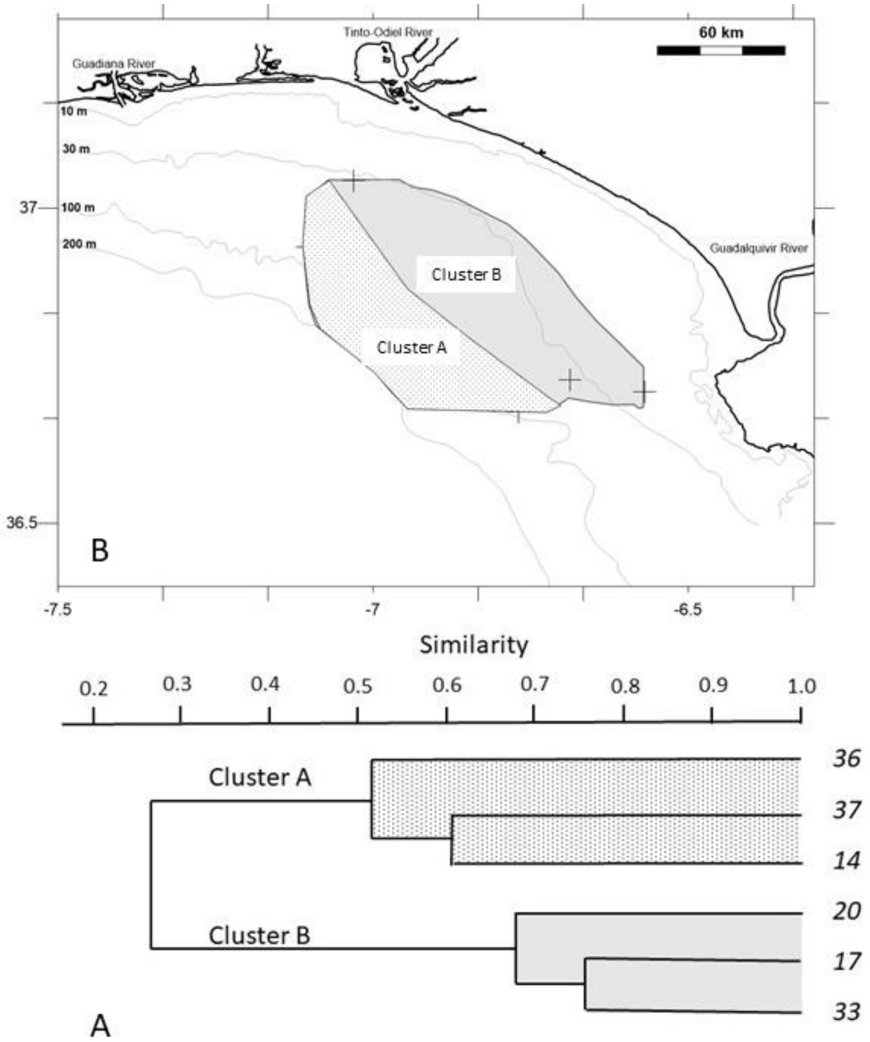

Figure 5.5.2 Q-mode cluster analysis (Bray-Curtis measure joined by UPGMA) of the samples with living species abundance >5%. A. Dendrogram with two distinct clusters. B. Distribution of the two clusters in the study region.

Two clusters were formed by using the 13 most abundance species (>5%) in the dead assemblage (Cophenetic coefficient = 0.91). The peak abundance of species grouped in Cluster I is at station 17 (39 m), with a maximum abundance of 80 % (Figure 5.5.3). This peak is at mid-depth in the Guadalquivir profile. In cluster II the peak of abundance is recorded at the shallowest station 20 (23 m). Both peaks of abundance were recorded in the profile off the Guadalquivir River.

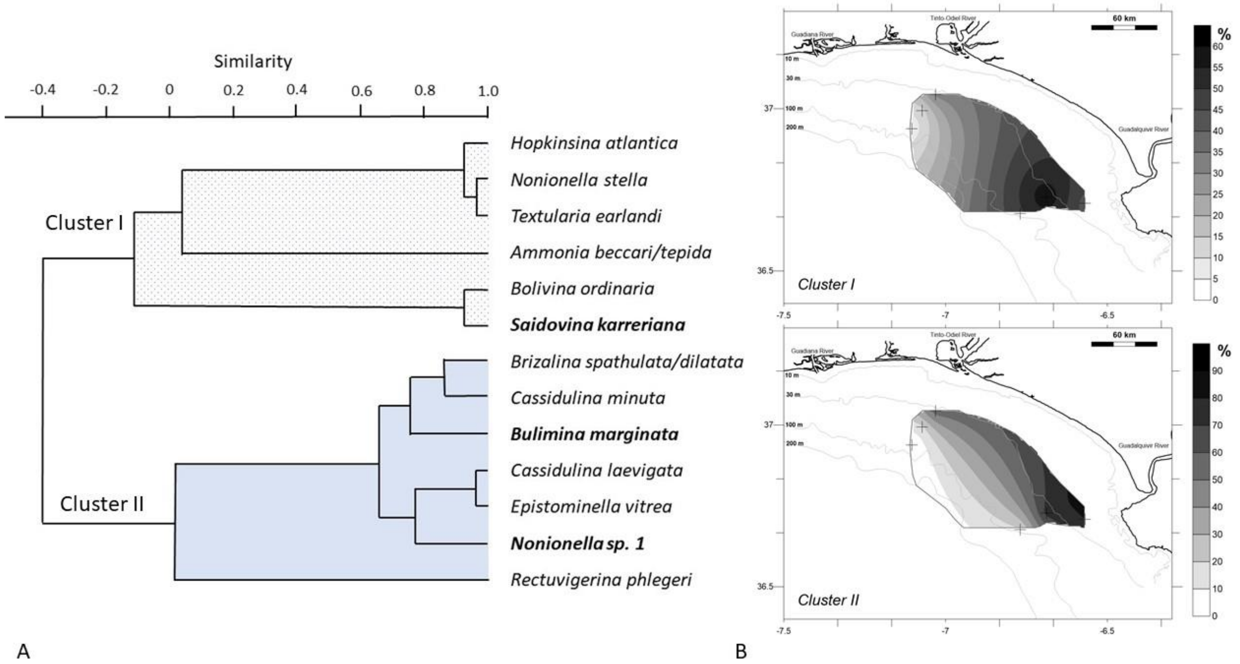

Figure 5.5.3 R-mode cluster analysis (correlation method joined by UPGMA) using the 13 dead species, with an abundance > 5%. A. Dendrogram with two distinct clusters. B. Distribution of the two clusters in the study region. Species names highlighted in bold indicate abundances >5% in the dead assemblage only.

Q-mode cluster analyses (Bray-Curtis measure joined by UPGMA) using the samples with abundance >5% of dead species, a cophenetic coefficient of 0.88, produced two clusters. Cluster A is composed of 4 stations at varying depth: 14 (90 m), 33 (36 m), 36 (55 m), and 37 (91 m). Cluster A contained all 3 stations from the Tinto-Odiel profile and the deepest station from the Guadalquivir profile (Figure 5.5.4). Cluster B is composed of the two stations closest to the Guadalquivir outflow.

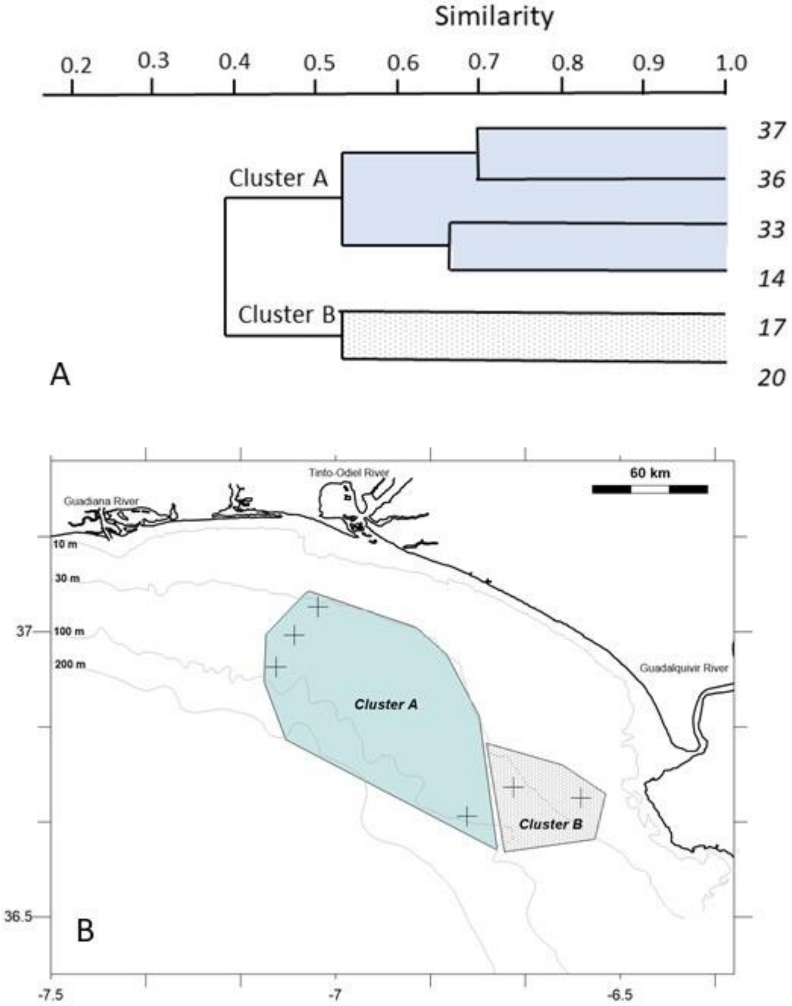

Figure 5.5.4 Q-mode cluster analysis (Bray-Curtis measure joined by UPGMA) of the samples with dead species abundance >5%. A. Dendrogram with two distinct clusters. B. Distribution of the two clusters in the study region.

5.6 Fragment Analysis

Well preserved planktonic and benthic foraminiferal tests were collected during the dead foraminiferal microscopic analysis. In addition to the well preserved benthic foraminiferal tests, fragments were also counted. The proportions of the test types were plotted for each sample (Figure 5.6.1). All samples were dominated by well-preserved benthic foraminiferal tests (65-78%). Planktonic tests were at their lowest abundance at the shallow samples, increasing with distance from the coast. The abundance of fragments (4-21%) was highest at 36 m water depth in front of the Tinto-Odiel profile. The lowest proportion of fragments was found at 91 m, in the Guadalquivir profile. Overall, the Tinto-Odiel profile has more fragments than the Guadalquivir profile. The Guadalquivir has a notable change in fragments with depth, as depth increases, the proportion of fragments decreases (11% to 4%, 23 m to 91 m).

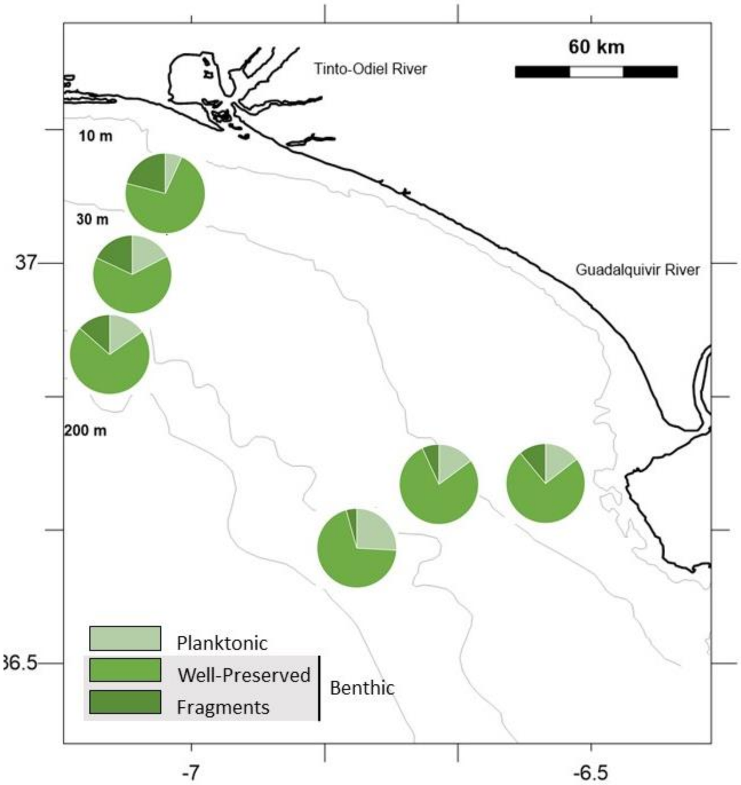

Figure 5.6.1 Proportion of dead planktonic and benthic (well-preserved & fragments) foraminifera tests picked in the microscopic analysis of the dead assemblage for the 6 study samples.

6. Discussion

Both living and dead foraminiferal assemblages show trends in their distributions that can be associated with the environmental parameters. These results give us preliminary insights into the effects of the Guadalquivir and Tinto-Odiel river discharges on foraminiferal distributions on the northern Gulf of Cadiz.

6.1 Foraminiferal density and the diversity of living and dead assemblages

Since the living assemblage represents current processes and the dead assemblage represents processes over generations, it was expected to have differences between these assemblages. Previous studies have found differences between living and dead assemblages in near-shore environments (Alve and Murray, 1997; Mendes et al., 2013). This study showed similar patterns. The dead assemblage had a higher Fisher-Alpha and species richness when compared to the living counterpart. These results are consistent with those found in the region (Mendes et al., 2013). In addition to this, the dead assemblage had an overall higher density than that of the living assemblage. These results show that the living assemblage is closely related to the dead assemblage.

There is no linear trend between density values and depth of the entire sample set (6 samples) in the living assemblage, which is consistent with previous results in this region (Mendes et al., 2012). For the dead assemblage, density increases with depth adjacent to the Tinto-Odiel (Figure 5.1.2). By comparing the densities of the living and dead assemblages, there is an opportunity to understand the sedimentation at each station. From the coast (36 m) to the deepest sample (91 m) there is a linear increase in the difference between living and dead assemblages, adjacent to the Tinto-Odiel. Higher sedimentation rates were also observed at shallow depths off the Guadalquivir, with similar densities of the living and dead assemblages. The deepest sample has the largest proportion of tests recorded but shows similar living densities when compared with those found in the Guadalquivir profile at a similar depth (90 m). This difference in dead foraminifera can be attributed to a low sedimentation rate. Dead foraminiferal tests that are collected, are an accumulation of many generations. When the local sedimentation rate is high, it is expected that the tests are buried to levels deeper than 1 cm. In comparison, the living foraminiferal individuals will continue to inhabit the surficial layer. Based on this, sedimentation rates can be inferred from a comparison between the living and dead densities. When the densities of the two assemblages are similar in magnitude, such as those found adjacent to the Guadalquivir river, the local sedimentation rate is high.

The living foraminiferal densities measured in the six samples were consistent with other studies in the Gulf of Cadiz, as well as Western Europe. In the Gulf of Cadiz, Mendes et al. (2012) reported densities ranging from 14 to 1173 individuals/10cm^3 for the living assemblage, while this assessment had similar densities ranging from 531 to 1612 ind./10cm^3 (Figure 5.1.2). Although these results fall into the range reported in the Gulf of Lions (Mediterranean Sea), living benthic foraminiferal densities reach a much lower density maximum there (56-515 ind./10cm^3) (Motjahid et al., 2009). In the Bay of Biscay, reported living densities are also consistently lower, with a minimum of 280 ind./10cm^3 and a maximum of 760 ind./10cm^3 (Duchemin et al., 2005).

Density of the living assemblage is the highest (5,008 ind./10 cm^3) at mid-depth in the vicinity of the Guadalquivir profile, which coincides with the highest salinity (36.1). The lowest salinity (35.7) is also associated with the lowest density of the living assemblage (537 ind./10 cm^3) recorded off the Tinto-Odiel at 91 m water depth (Figures 5.1.2 and 5.4.2). These results suggest that density may be controlled by salinity, however, due to the slight variation of salinity across the samples, this is likely placed in the error of the measurement. Due to this, dependence can not be analyzed against this factor. The control on density could be further explored and supported with a larger sample size that includes samples with a higher salinity gradient, such as those found near the Guadiana and at deeper sites (> 200 m). Diversity indices (Shannon and Fisher Alpha) varied greatly between sites but were not associated with river influence or density. Diversities were typical for this type of environment (Murray 2006). Richness did not vary between sites in the Guadalquivir profile, even when density reached its peak (Figure 5.2.1).

Based on the results, there are no significant trends associating density and diversity. Richness and density are not associated across the 6 samples. Although the link between variables is not apparent between stations, density and diversity indices may be associated with the distributions of the clusters and species-specific distributions in the study region. Diversity indices (Fisher-alpha, Shannon) were not in agreement in the living assemblage, which is likely due to their calculation. Fisher-alpha is based on a logarithmic calculation that predicted the number of species that one individual represents (and then two individuals, three individuals etc.). In comparison, the Shannon index is based on proportions and uses the natural logarithm (Murray 2006). Richness was not linearly correlated with density, which shows that they were independent in these samples. An increase in density is therefore not associated with an increase in the number of species found. Shannon and Fisher Alpha diversity indices neither increases or decreases with density.

6.2 River influence on the shelf

The influence of river drainage into the Gulf of Cadiz has been well studied, and is known to impact the fine sediment flux, primary productivity through nutrient loading, oxygen, salinity, and temperature in the near-shore waters. The differences in magnitude of the Tinto-Odiel and Guadalquivir river discharges should result in different temperature and salinity measurements between profiles. The Guadalquivir brings the highest amount of fresh water to the coast relative to the other four rivers (Tinto-Odiel, Guadiana, Piedras) (Morales 1997).

Temperature and salinity changed with depth but at different magnitudes depending on station depth and location relative to the two river outflows. If temperature was simply dependent on depth, the temperature would be expected to reach its highest at the shallowest depth and decrease as depth increased. For the Guadalquivir profile, the shallowest depth (23 m) has a temperature of 14.9^{o}C, which is less than the shallowest temperature of the Tinto-Odiel profile (15.1^{o}C, 39 m). This may reflect the difference in river discharge and the consequential influence on the temperature of the near-shore water. Although the shallowest station off the Guadalquivir has a lower temperature than the Tinto-Odiel, the entire profile of the latter changes more drastically over a shorter depth gradient (Figure 5.4.1). The temperature in the Tinto-Odiel profile changes by approximately 0.7 ^{o}C over an increase in water depth of 52 m. These characteristics of temperature change are likely associated with the difference in their locations relative to the two rivers.

Salinity was more variable at smaller magnitudes across the 6 stations and is not clearly linked to the locations relative to the river discharges. Salinity was relatively uniform, with a peak at mid-depth in the Guadalquivir profile. As mentioned previously, salinity showed some association with foraminiferal density. Due to the possibility of the obtained values being in the error of the instrument, salinity will be considered uniform in these samples. When compared to other studies conducted in this region, it can be seen that salinity near these two rivers stays uniform until approximately 200 m water depth, at which point it drops significantly (Mendes et al., 2012). Salinity is also clearly much lower in front of the Guadiana River outflow, which is expected due to the effects of upwelling in this location (Mendes et al., 2012). The differences in salinity and temperature are also reflected in the changes in foraminiferal distributions in the northern Gulf of Cadiz.

The distribution of fresh water to the Gulf experiences seasonal fluxes and although the salinity did not show a significant association with river location, it may be more evident in seasons with higher riverine inputs. Fresh water is also less dense, so water distributed into the Gulf is likely to be in the upper layers of the water column, while these measurements were taken near the bottom, where the water is denser.

For all 6 stations, the sediment characteristics are the same, when compared to those outlined by Gonzalez et al. (2004). These authors classified the sediment as mud, composed of silt and clay, which occur in a mud belt in the study area. As a habitat for colonization, mud is much more difficult for foraminifera to colonize, with lower oxygen and nutrient levels (Murray 2006). Since the sediment for all 6 stations is composed of silt and clay, any variation observed is unlikely to be due to sediment type. In Gonzalez et al. (2004), there is variation in sediment across the region, with sediment types ranging from gravelly mud and sand off of the Tinto-Odiel at shallow depths (10 m to 30 m) and uniform mud off of the Guadalquivir River. Although these differences in sediment type exist in the region, the samples used in this study are only located in the mud-belt. This is helpful in distinguishing between differences in species distributions, while controlling for sediment type.

6.3 Species distribution and the environment

Cluster analysis identified two major biofacies based on the distributions of the 13 most abundant living species (Figure 5.5.1). Biofacies I included the species from cluster I: *B. spathulata/dilatata, C. laevigata, C. minuta*, *E. vitrea, B. elongata, R. phlegeri,* and *B. striatula*, which showed the higher abundances at higher depths of the study area (55 m to 91 m), corresponding to cluster A (Figure 5.5.2). All of these species are associated with silt-clay muds and low-oxygen environments. *Brizalinia spathulata* and *B. dilatata* have been grouped in this study due to their similarities in morphology and similar characteristic (Murray 2006). For accurate comparison, literature referencing either species will be used, since they have similar distributions and may be misclassified in other studies. *Brizalina spathulata/dilatata* are free living benthic foraminifera that are typically found at a range of depths. For example, B. spathulata/dilatata have been found in shallow depths, between 20 and 50 m (Murray, 2006). On the continental shelf, north of the Nazare canyon, *B. spathulata/dilatata* and *C. laevigata* were found to be dominant in silt-clay sediments at higher depths (80-100 m) (Guerreiro et al., 2009). In this study, *B. spathulata/dilatata* and *C. laevigata* also inhabit silt-clay muds at similar depths (55 m to 91 m) to those found north of the Nazare canyon, which can be due to the mud-belt existing in this area of the continental shelf (e.g. Gonzalez et al., 2004).

C. laevigata is an infaunal species that is is typically influenced by sediment type rather than other environmental parameters such as oxygen availability (Murray 2006). *C. laevigata* was found at high abundance on the Basque shelf, generally resistant to hypoxic environments and abundant in habitats with a high sand-silt percentage (Martinez-Garcia et al, 2013). *B. spathulata/dilatata* is also found in high abundance in habitats colonized by *C. laevigata* on the Basque shelf. *E. vitrea* is an opportunistic infaunal species which is abundant in regions with high food availability (Murray 2006). Rivers influence the nutrients that reach the shelf and increase the primary production in the coastal zone. *E. vitrea* abundance has been found to change seasonally, with a >10 % abundance for 1 to 2 months in marginal marine environments (Murray 2006). In the Adriatic Sea, *E. vitrea* was associated with low oxygen content and temperature (Duijninstee et al., 2004). It is interesting to note that this study grouped *B. dilatata*, *B. spathulata* and *B. seminuda* together in their assessment. Although this may be the case in the Adriatic Sea, *B. seminuda* does not have a similar distribution to the other two species in the northern Gulf of Cadiz, which should be noted in future studies of this region.

B. elongata showed similar distributions to *B. spathulata/dilatata*, *B. striatula*, and *E. vitrea* in the North Sea (Murray 2006). Both *B. elongata* and *R. phlegeri* were also associated with silt-clay sediments in the Pozzuoli Gulf, Naples (Magno et al., 2012). In addition to sediment type controlling the distribution of *B. elongata*, this species was also found to inhabit areas with high organic matter and low oxygen (Eichler et al., 2003). *B. striatula* was also found at high abundances in these conditions. *C. minuta* was not previously found in high abundances at these stations, although the species inhabited deeper (> 95 m) depth near the Guadiana River (Mendes et al., 2004; 2012).

Biofacies II included species from cluster II: *B. seminuda, N. stella, B. ordinaria, T. earlandi, H. atlantica,* and *A. beccarii/tepida,* which have high abundances at shallow depths (23 m to 39 m), corresponding to Cluster B. All of these species are associated with silt-clay, low oxygen environments. The rivers influence the nutrient levels in the coastal waters which increase primary production, and consequently increase the organic matter decomposing in the sediment. Distribution maps show the highest abundance of this assemblage at the shallowest region (23 m) in front of the Guadalquivir River. This cluster also extends to the influence of the Tinto-Odiel River. Based on this distribution, this cluster is likely influenced by river discharge in this region.

Bolivina seminuda is morphologically similar to the other *Brizalina* species addressed in this study and has previously been characterized as being an intermediary form between *B. dilatata/spathulata* and *B. striatula* (Duijnstee et al., 2004). *B. seminuda* has been associated with high silt-clay fractions (Magno et al., 2012). *Ammonia beccarii* and *A. tepida* were grouped for similar reasons as *B. spathulata/dilatata. Ammonia beccarii/tepida* are commonly found in intertidal zones and can range as far north as the Oslo fjords (Murray 2006). This species has previously been found in high abundances in this region (Mendes et al., 2012), and in similar sediments (Murray 2006). This species has also been associated with near-shore environments in the Adriatic Sea (Jorissen, 1986). *Ammonia beccarii* experiences facultative alternation of generations due to its use of sexual and asexual reproduction (Goldstein and Moodey, 1993). In Spain, *A. beccarii* and *Haynesina germanica* alternate in their reproductive cycles and tend to switch dominance in a population (Murray, 2006). This implies that *H. germanica* should be found at higher abundances in the dead assemblage, but this species was only found at low abundances with a frequency of 25%. This association is likely apparent in environments where both species are found in much higher abundances. Although the link between reproductive cycles and abundance was not evident in this study, *H. germanica* tests may not be present or may have been impacted by post-mortem processes, such as dissolving or eroding.

It is possible that the distinction between clusters is caused by a difference of energy and local hydrodynamics. The entire study area may be affected by increased organic matter and low oxygen conditions. Biofacies II is associated with higher riverine influence, since they are located adjacent to the river outflows. The stations more adjacent to the rivers should have higher flow energy and were observed to have differences in sedimentation rates. Sedimentation was higher adjacent to the Guadalquivir and lower adjacent to the Tinto-Odiel. *Textularia earlandi*, the only agglutinated form found in this assemblage, has been associated with environments that have low sedimentation rates (Snyder et al., 1990). Based on this, *T. earlandi* would be expected in higher abundances at higher depths, in environments that are less influences by the rivers. This species has been characterized as opportunistic, which may explain its association with Biofacies II.

6.4 Fragments as indicators

To further understand the hydrodynamics and post-mortem transport of foraminifera in the study region, fragments of dead benthic foraminifera were counted and compared with intact ones, as well as the intact tests of planktonic foraminifera. The proportion of fragments were highest at the Tinto-Odiel outflow, which may be associated with near-shore sediment characteristics. Off the Tinto-Odiel, there are coarser sediments until approximately 30 m water depth. Although this depth was not sampled during this study, it is likely that the high hydrodynamic energy in this location causes the resuspension and off-shore transportation of fragment tests into the study area. The highest sedimentation rate was observed adjacent to the Guadalquivir and the proportion of fragments found here support this location as being a low hydrodynamic environment. This low energy environment may allow the deposition of mud sediments and a lower number of broken tests. In addition to this, within each profile, the proportion of fragments decreased with depth. In combination with the increase of dead foraminifera with depth adjacent to the Tinto-Odiel, the decrease in fragments shows that there is an overall decrease in disturbance of the sediment with distance from the coast, and the Tinto-Odiel outflow. Mendes et al. (2004) conducted a similar analysis that assessed the fragments found adjacent to the Guadiana River to use as indicators for post mortem transport. They found similar results to this study, with fragments decreasing with distance from the coast. This change in the proportion of fragments was attributed to occasional wave energy and the seasonal flooding of the river during winter.

In addition to dead foraminiferal test fragments, the pattern of planktonic tests was similar to those found previously (Mendes et al., 2004). Planktonic tests increased in proportion with depth. Since planktonic species live in the water column, an increase in depth should mark an increase in deposition after depth (Mendes et al., 2004; Murray, 2006).

6.5 Temporal changes in foraminiferal assemblages (2001 to 2015)

Seasonal changes in benthic foraminiferal compositions have been found and well studied in a number of environments (Murray, 2006; Mendes, 2012). These changes in composition are directly associated with the environmental parameters that change over these short periods of time. With increased human activities in the region, it is important to understand how the environment may change over longer periods of time (Ruiz et al., 2015). It is also important to understand the validity of bioindicators in a specific environment, based on when they were studied. To determine whether there has been a change in benthic foraminiferal distributions in the study area, the results were compared to those found by Mendes et al. (2012). In February 2001, 47 surficial samples in the study region were collected and a foraminiferal analysis was conducted (Mendes et al., 2012). To determine changes in distribution and composition, the closest approximate stations were compared to those addressed in this study (Figure 6.7.1). Across the entire study region (Guadiana to Guadalquivir), four clusters were formed based on the environmental conditions on the shelf. The comparable samples closely match those in the Guadalquivir sampling profile. The samples used for comparison in the Tinto-Odiel profile were less perpendicular to the coast and may explain the differences noted over the 14-year period.

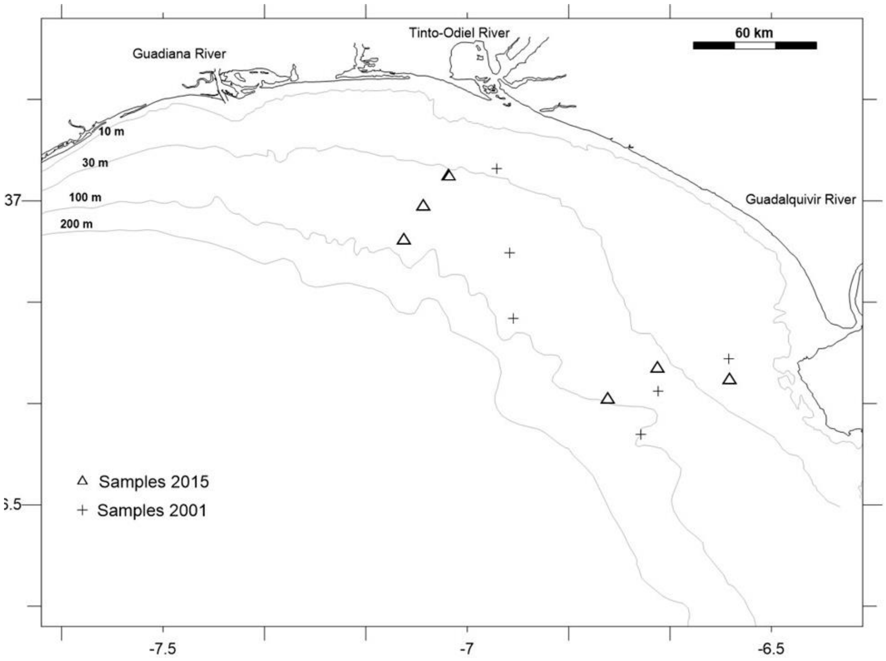

Figure 6.7.1 Closest approximate sample locations for comparison between the 2001 and 2015 foraminiferal distributions in the northern Gulf of Cadiz.

In 2001, there was a maximum density of living foraminifera near the Tinto-Odiel river outflow (28 m), while the maximum in this study occurred at 23 m water depth adjacent to the Guadalquivir river outflow. They found a decrease in density seaward near the Tinto-Odiel outflow, with 14 individuals/10cm^3 at 87 m water depth. Overall, the densities of living foraminifera occurred at similar magnitudes, although densities in 2015 were higher, with a range of 537-5008 ind./10 cm^3 in 2015 and 14-1173 ind./10 cm^3 in 2001. The 2001 samples were taken over a larger region than those collected in 2015, which may explain this discrepancy. In 2001 species richness decreased with sampling depth. This trend was not evident in the 2015 samples. Diversity (H) in 2015 had a smaller range (2.1-2.8) compared to 2001 (1.1-3.2), which is expected over a larger area.

In the six similarly located samples in 2001, twelve species were found at an abundance greater than 5% in at least one sample. Of these twelve, three were not found in the 2015 samples at high abundances: *Bulimina aculeata*, *Eggerelloides scaber,* and *Elphidium excavatum*. Therefore in 2015, five new species with high abundances were found: *B. striatula, B. seminuda, C. laevigata, C. minuta,* and *T. earlandi*. *B. striatula, C. laevigata,* and *C. minuta* were found in cluster I at depths 23-39 m. *B. seminuda* and *T. earlandi* were found in cluster II at depths 55 – 91 m. In 2001, each cluster was compared with the sediment characteristics of the shelf. In 2015, all samples were collected from locations with similar sediment types. *B. ordinaria, H. atlantica, A. beccarii/tepida, B. elongata, E. vitrea, R. phlegeri, B. elongata, B. striatula,* and *B. spathulata* were found in regions with sandy mud to mud type sediments.

Salinity was not associated with density in 2001 but there was only a small variation in salinity adjacent to the Tinto-Odiel and Guadalquivir rivers in the 2001 study. It is interesting to note that salinity remains uniform until approximately 200 m water depth, where it increases. In this study, samples extended to an offshore depth of only 91 m.

Overall, there are multiple differences in assemblage composition between 2001 and 2015. In 2001, the samples were more diverse in environments and habitats. From the samples compared with 2015, there were changes in composition, with three additional species present at high abundances that were previously present at low abundances. Conversely, 5 species were found in 2015 at high abundances that were not found at high abundances in 2001. This may be due to the changing shelf environment, or to the sampling period. Foraminiferal composition changes temporally (seasonally, yearly) and these changes could be attributed to this.

6.6 Improvements and future studies

The results of this study are a preliminary assessment of riverine influence on the benthic foraminiferal composition and distribution on the northern Gulf of Cadiz continental shelf. There were some limitations to this work that can be used for the improvement of future studies. To improve future works, a wider sampling area with different gradients of salinity, sediment type, and temperature should be considered. Direct river measurements should be compared to aid in the interpretation of this assessment. These measurements were taken, but require analysis and comparison to other results and discharge values to be useful, which was unfortunately not in the scope of this project. As stated previously, it is likely that any differences in salinity were negligible, since they were within the error of the instrument used. River flow changes seasonally which results in seasonal influences on the shelf. During a period of flooding, it is expected that more sediment would enter the coastal region, possibly altering the environment and changing the interactions between species. For this study, time and resources were limited which resulted in a smaller than ideal sampling set. Future studies should explore the association between density and salinity for the entire sampling area and the sedimentation rates seen to be associated with the living and dead assemblages.

7. Conclusion

The analysis of live benthic foraminifera is important for the construction of the processes that compose the current environment. Benthic foraminiferal distributions in the northern Gulf of Cadiz have been well studied in the recent past. The objective of this study was to determine the influence of two major rivers, the Tinto-Odiel and the Guadalquivir, on the environment and foraminiferal distribution in the Gulf. Benthic foraminiferal distribution was found to be associated with depth and sample location relative to the river. Two general biofacies were formed from the living foraminifera collected, based on depth and distance from the coast. Both biofacies were associated with mud-type sediments. Biofacies I, associated with cluster A (55 m to 91 m) was composed of: *B. spathulata/dilatata, C. laevigata, C. minuta, E. vitrea, B. elongata, R. phlegeri,* and *B. striatula.* Biofacies II, associated with cluster B (23 m to 39 m), was composed of: *B. seminuda, N. stella, B. ordinaria, T. earlandi, H. atlantica,* and *A. beccarii/tepida*. This biofacies was associated with river influence. The Guadalquivir River had the greatest impact on sedimentation rates based on the dead foraminiferal distribution and fragments. In addition to this, shifts in foraminiferal composition and distributions have been identified over a 14-year period. These results provide a preliminary understanding of the distributions of dead and living benthic foraminifera in the Gulf of Cadiz, which will aid in the reconstruction of past environments and the understanding present environmental conditions in the northern Gulf of Cadiz.

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Appendix I: Tables

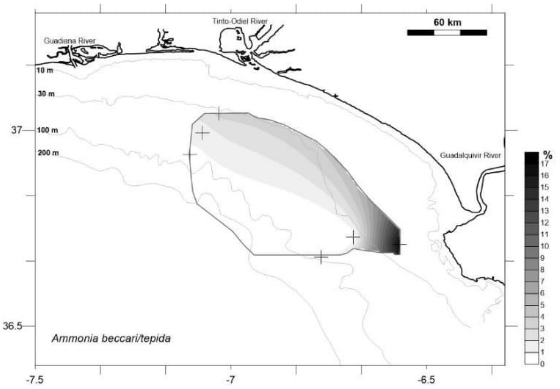
Table I. Meaurements taken during the RV Poseidon CADISED POS 482 cruise at all stations. Measurements were taken in the surficial water of the sample, which represents the near-bottom measurement. Sediment samples were retrieved with a rumhor core.

Station	Longitude	Latitude	Temperature (ºC)	Salinity
19501	37.0262833	7.673033333	14.02	35.13
19502	0:46:15	47.906		
19504	36.8880333	14.724	14.2	34.96
19505	36.8631167	14.803	15.98	34.39
19506	36.9439	60.984	14.01	35.03
19507	36.94845	60.755	14.67	34.84
19508	37.0700333	40.083	13.77	34.88
19509	37.0833833	34.498	13.9	34.81
19510	36.9892167	30.208	14.47	34.99
19511	36.9767	20.19	14.16	35.05
19512	36.8114667	54.877	14.28	36.04
19513	82.716	58.257	14.15	35.44
19514	76.584	52.14	14.45	35.77
19515	88.729	64.475	14.17	35.72
19516	89.989	62.156	14.44	35.82
19517	79.643	47.262	14.55	35.95
19518	69.974	43.984	14.45	35.93
19519	72.606	38.34	14.77	35.99
19520	78.519	40.168	14.9	35.81
19521	91.707	54.501	14.4	36.03
19522	45.487	23.779	14.84	35.66
19523	43.684	23.664	14.55	35.79
19524	43.036	24.037	14.62	35.69
19525	42.348	23.842	14.58	35.85
19526	40.83	38.952	14.73	35.77
19527	39.742	39.174	14.54	35.85
19528	89.245	12.137	-	-
19529	90.183	11.595	-	-
19530	89.735	18.766	-	-
19531	-	-	-	-
19532	93.069	34.063	-	-
19533	39.608	8.839	15.14	35.72
19534	37.842	63.229	14.66	35.88
19535	92.243	65.078	14.62	35.81
19536	95.651	11.335	14.4	35.83
19537	92.276	13.239	14.35	35.7
19538	89.68	7.986	14.53	35.85
19539	72.068	56.209	13.8	35.73
19540	67.999	48.318	14.25	35.81

Table II. Results of the foraminiferal analysis.

Sample ID	Condition	Sample Volume (ml)	# of individuals	Density (ind./10cm3)	Richness	Shannon (H)	Fisher-alpha	Evenness
19514	Living	20	121	774	25	2.253	9.565	0.3806
19514	Dead	20	370	2960	39	2.542	11.39	0.3176
19517	Living	32	313	5008	25	2.141	6.391	0.3403
19517	Dead	32	361	5776	52	2.987	16.66	0.3811
19520	Living	50	315	1613	25	2.159	6.378	0.3466
19520	Dead	50	321	1643	47	3.212	15.64	0.5171
19533	Living	31.5	101	831	19	2.152	4.958	0.5378
19533	Dead	31.5	513	5211	73	2.834	10.66	0.4476
19536	Living	43	104	1387	25	2.792	10.44	0.6523
19536	Dead	43	306	8160	46	2.919	15.02	0.4027
19537	Living	24	120	537	16	2.322	8.062	0.4853
19537	Dead	24	366	17351	38	3.488	22.81	0.4546

Appendix II: Figures



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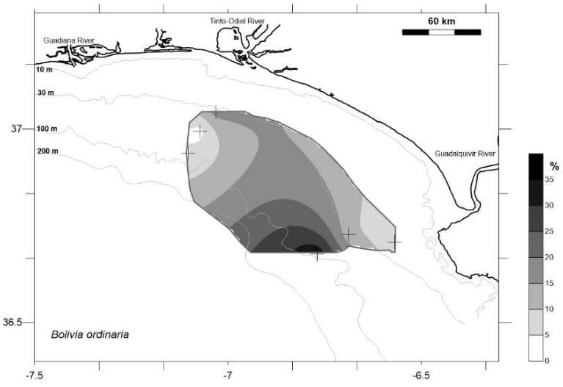

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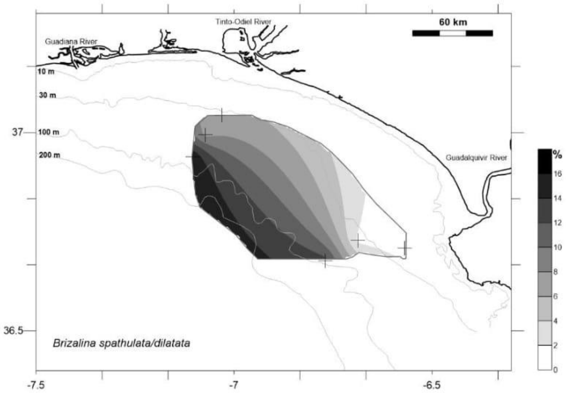

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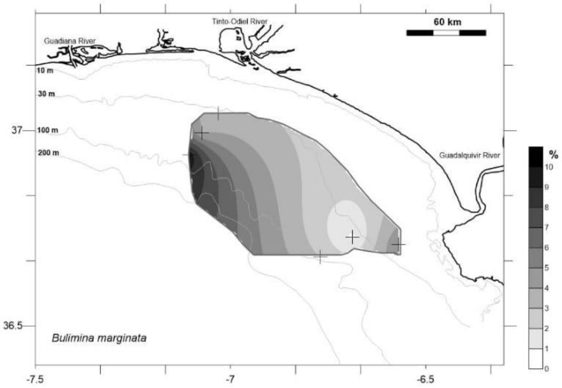

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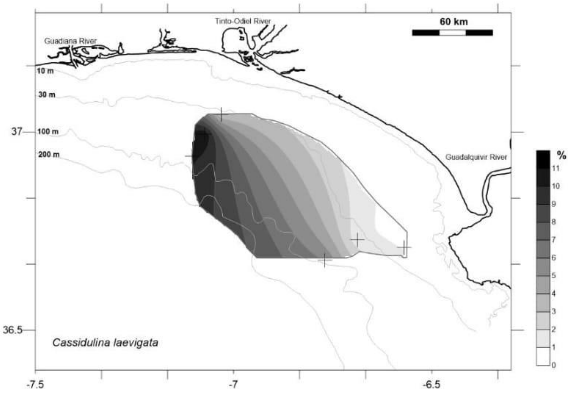

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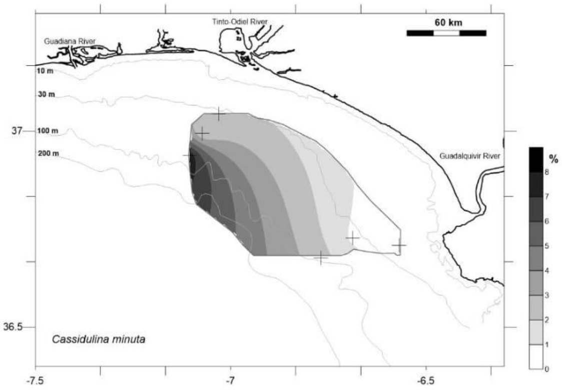

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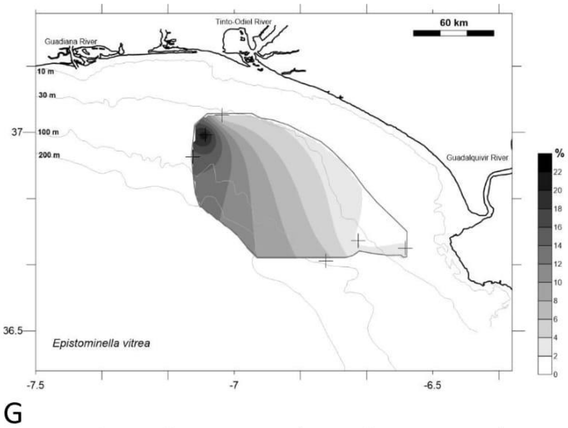
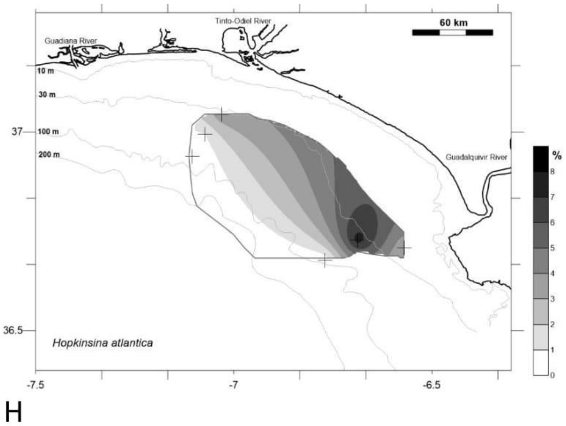
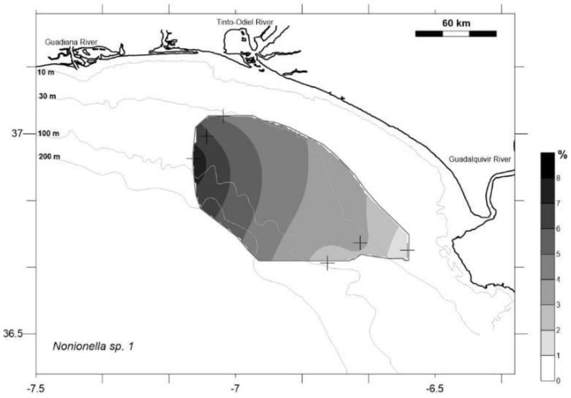

Tinto-Odiel River
60 km
Guadiana River
10 m
30 m
37
100 m
200 m
Guadalquivir River
36.5
Epistominella vitrea
-7.5
-7
-6.5
%
22
20
18
16
14
12
10
8
6
4
2
0

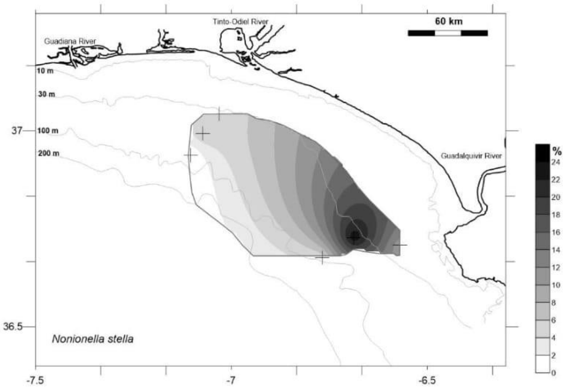
G


Tinto-Odiel River
60 km
Guadiana River
10 m
30 m
37
100 m
200 m
Guadalquivir River
36.5
Hopkinsina atlantica
-7.5
-7
-6.5
%
8
7
6
5
4
3
2
1
0

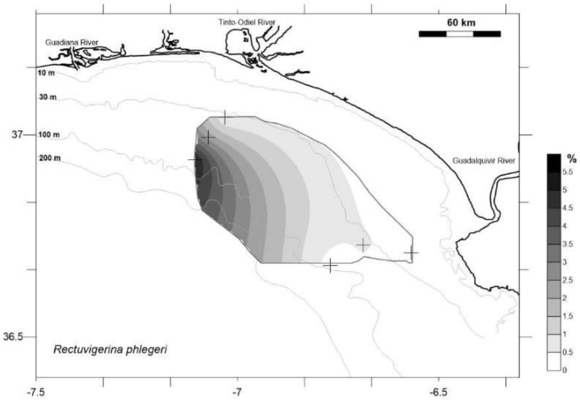
H


Tinto-Odiel River
60 km
Guadiana River
10 m
30 m
37
100 m
200 m
Guadalquivir River
36.5
Nonionella sp. 1
-7.5
-7
-6.5
%
8
7
6
5
4
3
2
1
0

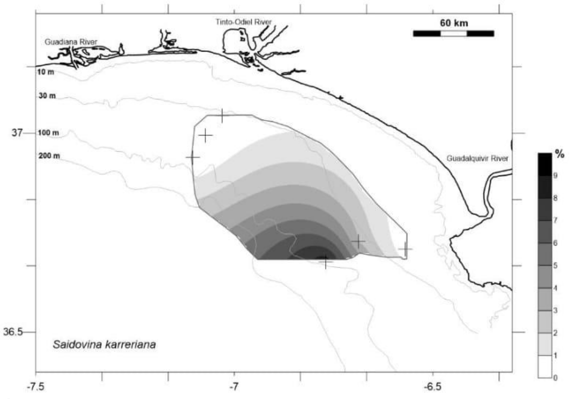
I


Tinto-Odiel River
60 km
Guadiana River
10 m
30 m
37
100 m
200 m
Guadalquivir River
36.5
Nonionella stella
-7.5
-7
-6.5
%
24
22
20
18
16
14
12
10
8
6
4
2
0

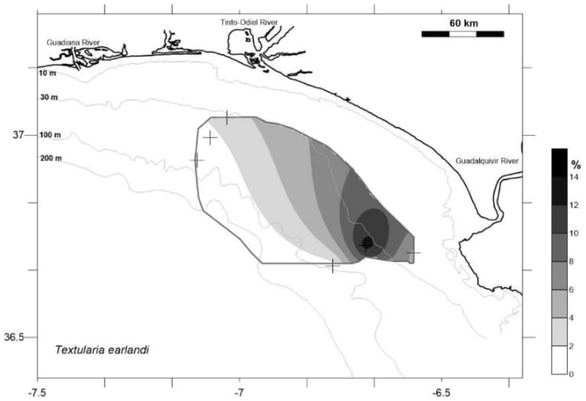
J


Tinto-Odiel River
60 km
Guadiana River
10 m
30 m
37
100 m
200 m
Guadalquivir River
36.5
Rectuvigerina phlegeri
-7.5
-7
-6.5
%
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0

K


Tinto-Odiel River
60 km
Guadiana River
10 m
30 m
37
100 m
200 m
Guadalquivir River
36.5
Saidovina karreriana
-7.5
-7
-6.5
%
9
8
7
6
5
4
3
2
1
0

L



M

Figure I. Distribution of the 13 dead benthic foraminiferal species with relative abundance >5%, in at least one analyzed sample: A. *Ammonia beccari/tepida* B. *Bolivina ordinaria* C. *Brizalina spathulata/dilatata* D. *Bulimina marginata* E. *Cassidulina laevigata* F. *Cassidulina minuta* G. *Epistominella vitrea* H. *Hopkinsina atlantica* I. *Nonionella sp. 1* J. *Nonionella stella* K. *Rectuvigerina phlegeri* L. *Saidovina karreriana* M. *Textularia earlandi*.

Appendix III: Taxonomy

This appendix contains the taxonomy of benthic foraminifers addressed during this study. This taxonomic list is comprised of the most abundant (>5%) living and dead benthic foraminiferal species found in this study. Foraminifera are Protoctists, and their higher level taxonomy is currently being evaluated (Hayward et al., 2018). This taxonomy follows those outlined by Loeblich and Tappan (1987), with high level classifications following those found in the World Foraminiferal Datbase (Hayward et al., 2018), which have been updated with genetic research from multiple publications.

I have presented the taxonomy, beginning at the Superfamily, listed alphabetically along the text. The largest font is Superfamily, and with descending order, the font size decreases (from superfamily to species). Multiple species may comprise a Superfamily and have been incorporated at their taxonomic level alphabetically. The species presented in bold is the name used in this work, which is followed by examples from the literature. A link has been provided under each example for more information about this species.

Phylum: Foraminifera

Superfamily: ***Buliminoidea*** Jones*, 1875*

Family: ***Buliminidae*** Jones, 1875

Genus: ***Bulimina*** d'Orbigny, 1826

Species:
Bulimina elongata d’Orbigny, 1846

Bulimina elongata, Jones, 1994, p. 54, pl. 50, figs. 3-4

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Bulimina elongata* d'Orbigny, 1846. Accessed at:
http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=933974

Species:
***Bulimina marginata* d’Orbigny, 1826**

Bulimina marginata, Jones, 1994, p. 55, pl. 51, figs. 3-5
Bulimina marginata, Martins and Gomes, 2004, p. 148-150, fig. 2.83

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Bulimina marginata* d'Orbigny, 1826. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113042

Family: ***Siphogenerinoididae*** Saidova, 1981

Subfamily: ***Tubulogenerininae*** Saidova, 1981

Genus: ***Rectuvigerina*** Mathews, 1945

Species:
Rectuvigerina phlegeri Le Calvez, 1959

Rectuvigerina phlegeri, Martins and Gomes, 2004, p. 138-139, fig. 2.77

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Rectuvigerina phlegeri* Le Calvez, 1959. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113755

Superfamily: ***Discorbinelloidea*** *Sigal, 1952*

Family: ***Pseudoparrellidae*** Voloshinova, 1952

Subfamily: ***Pseudoparrellinae*** Voloshinova, 1952

Genus: ***Epistominella*** Husezima & Maruhasi, 1944

Species:
Epistominella vitrea Parker, 1953

Epistominella vitrea, Martins and Gomes, 2004, p. 199-200, fig. 2.118.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Epistominella vitrea* Parker, 1953. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113340

Superfamily: ***Nonionoidea*** *Schultze, 1854*

Family: ***Nonionidae*** Schultze, 1854

Subfamily: ***Nonioninae*** Schultze, 1854

Genus: ***Nonionella*** Rhumbler, 1949

Species:
Nanionella sp. 1

Species:
Nanionella stella **Cushman and Moyer, 1930**

Nonionella stella, Martins and Gomes, 2004, p. 229-230, fig. 2.136.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Nonionella stella* Cushman & Moyer, 1930. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113604

Superfamily: ***Rotalioidea*** Ehrenberg, 1839

Family: ***Ammoniidae*** Saidova, 1981

Subfamily: ***Ammoniinae*** Saidovina, 1981

Genus: ***Ammonia*** Brünnich, 1771

Species:
Ammonia beccarii / tepida (Linnaeus, 1758; Cushman, 1926)

Ammonia beccarii, Martins and Gomes, p. 253-256, fig. 2.150

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Ammonia beccarii* (Linnaeus, 1758). Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=112849

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Ammonia beccarii subsp. Tepida* (Cushman, 1926). Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=812466

Superfamily: ***Serioidea*** Holzmann & Pawlowski, 2017

Family: ***Bolivinitidae*** Cushman, 1927

Sub-Family: ***Bolivinitinae*** Cushman, 1927

Genus: ***Bolivina*** d'Orbigny, 1839

Species:
Bolivina ordinaria Phleger & Parker, 1952

Bolivina ordinaria, Martins and Gomes, 2004, p. 90-91, fig. 2.53.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Bolivina ordinaria* Phleger & Parker, 1952. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=112978

Species:
Bolivina striatula Cushman, 1922

Bolivina striatula, Martins and Gomes, 2004, p. 100-101, fig. 2.57.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Bolivina striatula* Cushman, 1922. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=112989

Genus: ***Brizalina*** Costa, 1856

Species:
Brizalina seminuda Cushman, 1911

Brizalina seminuda, Chiji & Lopez, 1968, p. 135, plate X 16.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Brizalina seminuda* (Cushman, 1911). Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=849406

Species:
Brizalina spathulata/dilatata (Williamson, 1858; Reuss, 1850)

Brizalina dilatata, Martins and Gomes, 2004, p. 88, fig. 2.51

Brizalina spathulata, Martins and Gomes, 2004, p. 108-110, figs. 2.61 and 2.62.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Brizalina dilatata* (Reuss, 1850). Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113003

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Brizalina spathulata*(Williamson, 1858). Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113008

Genus: ***Saidovina*** Haman, 1984

Species:
Saidovina karreriana Brady, 1881

Saidovina karreriana, Jones, 1994, p. 59, pl. 53, figs. 19-21.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Saidovina karreriana* (Brady, 1881). Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=466355

Family: ***Cassidulinidae*** d’Orbigny 1839

Subfamily: ***Cassidulininae*** d'Orbigny, 1839

Genus: ***Cassidulina*** d'Orbigny, 1826

Species:
***Cassidulina laevigata* d’Orbigny, 1826**

Cassidulina laevigata, Mendes et al, 2012, p. 23, fig. 3.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Cassidulina laevigata*d'Orbigny, 1826. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113077

Species:
***Cassidulina minuta* Cushman, 1933**

Cassidulina minuta,, Martins and Gomes, 2004, p. 123, fig. 2.69.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Cassidulina minuta* Cushman, 1933. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=113078

Superfamily: ***Textularoidea*** *Ehrenberg, 1838*

Family: ***Textulariidae*** Ehrenberg, 1838

Subfamily: ***Textulariinae*** Ehrenberg, 1838

Genus: ***Textularia*** Defrance, 1824

Species:

Textularia earlandi Parker, 1952

Textularia earlandi, Chiji & Lopez, 1968, p. 127, plate VI, 10.

Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Textularia earlandi* Parker, 1952. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=114273

Superfamily: ***Turrilinoidea*** *Cushman, 1927*

Family: ***Stainforthiidae*** Reiss, 1963

Genus: ***Hopkinsina*** Howe & Wallace, 1933

Species:
Hopkinsina atlantica Cushman, 1944

Hopkinsina atlantica, Mendes et al, 2012, p. 23, fig. 3.

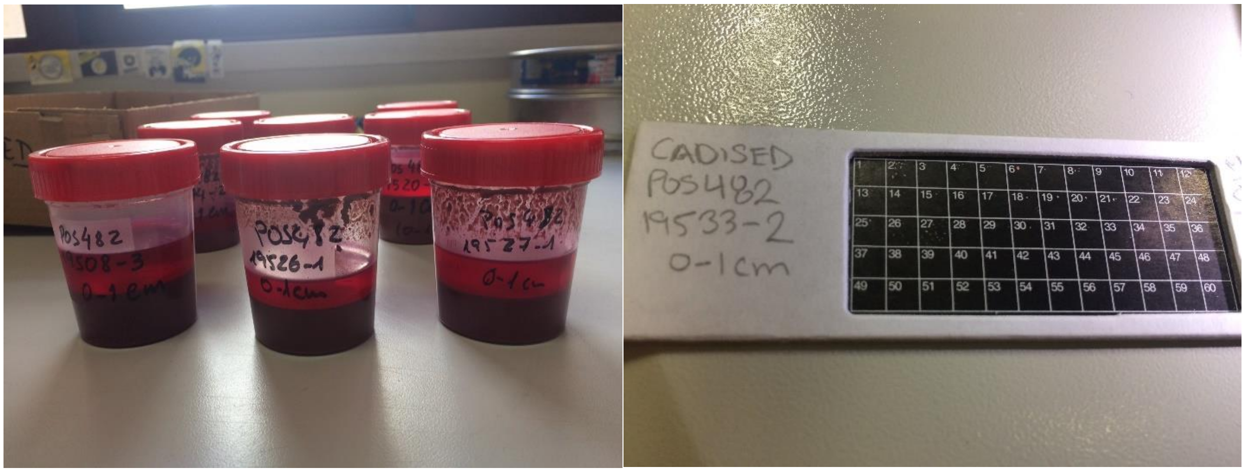
Hayward, B.W.; Le Coze, F.; Gross, O. (2018). World Foraminifera Database. *Hopkinsina atlantica*Cushman, 1944. Accessed at: http://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=582285

Appendix IV: Photos of materials and methods

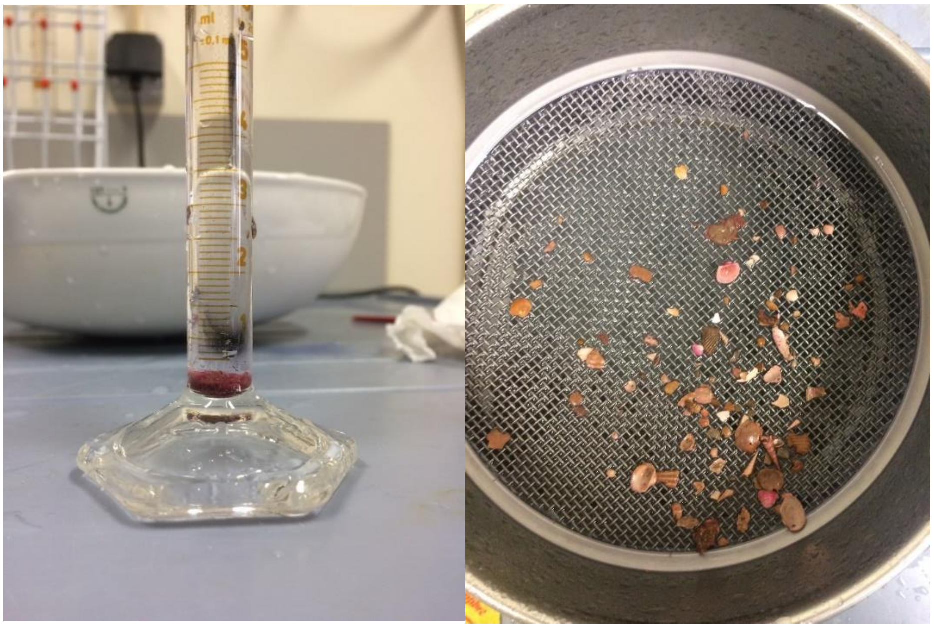
CADISED RV POSEIDON (POS482) CRUISE

RV POSEIDON in which the CADISED (POS482) scientific campaign took place.

Measurements of the environmental parameters and collected sediment Rumohr cores



Sediment samples and the prepared cardboard slide for the foraminiferal analysis.



Sieving of a sample and volume measurement

Foraminiferal Analysis

Microscope and labelled cardboard slides.

Close image of initial species separation (*Rectuvigerina phlegeri*)