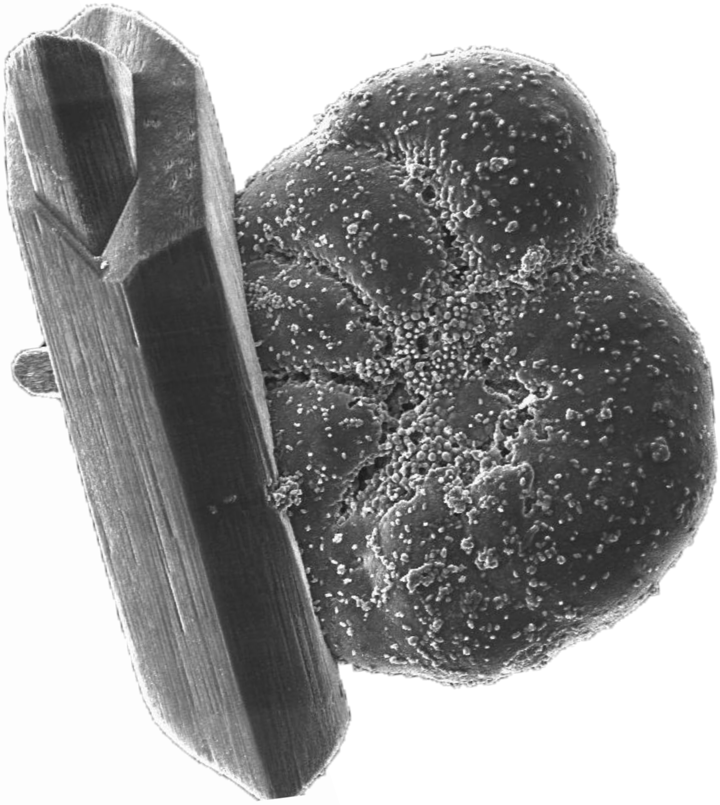
Universidade do Algarve

ECOLOGIC ZONATION MODEL OF THE BENTHIC FORAMINIFERA AND THECAMOEBIANS OF GUADIANA RIVER ESTUARY AND APPLICATION IN PALEOENVIRONMENTAL RECONSTRUCTION

VOLUME I

Dionilde Sarita Graça Camacho da Encarnação



Trabalho efetuado sobre a orientação de:
Doutora Delminda Maria de Jesus Moura
Doutor Tomasz Boski
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Dissertação de Doutoramento em Ciências do Mar

Especialidade em Processos de Ecossistemas Marinhos

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Declaração de autoria de trabalho:

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

“O rio atinge os seus objetivos porque aprendeu a contornar os obstáculos”

Lao Tse

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Abstract

This thesis explores the potential of benthic foraminifera as environmental indicators in the Guadiana River estuary according to an actual perspective, where we study the species distribution patterns and their responses to seasonal environmental changes and a paleoenvironmental perspective, in which the previous information in combination with the knowledge of diagenetic processes is applied in interpreting analogues environments that formed in the estuary during the Holocene.

The variation of environmental parameters in the estuary showed strong dependence on spatial and seasonal gradients, exhibiting prevailing river conditions in winter and marine conditions in summer. The distribution of foraminifera in the Guadiana estuary mirrored the seasonal variation of environmental factors, whose relative importance depended on the proximity the tolerance limits of the species. The elevation proved to be the most important parameter in the distribution of foraminifera by combining the effect of a series of other variables.

Based on the dominant species of living foraminifera and seasonal variations in their relations was possible to define a model of ecological zonation for the Guadiana estuary. Four biocenoses were identified and compared with the fraction of dead and fossil foraminifera. The total assemblage that combines the seasonal and taphonomic effects on modern microfaunas proved to represent reliably the estuary indigenous microfauna. Its application in the interpretation of paleoenvironments recorded over two sedimentary sequences (CM3 and CM5) allowed refining the knowledge on how the estuary has evolved over the past 13 000 cal yr BP.

The pioneering study of communities of thecamoebian and tintinnids suggests that both groups are an asset in studies of environmental monitoring and paleoenvironmental reconstruction. The present work demonstrated that the three indicators analyzed, when integrated in a multi-proxy perspective, increase the potential interpretive which can be applied in paleoenvironmental reconstructions.

Keywords: Estuary, Guadiana, Foraminifera, thecamoebian, tintinnids, Holocene

Modelo de zonação ecológica dos foraminíferos bentónicos e tecamebas do Estuário do Rio Guadiana e sua aplicação na reconstrução paleoambiental

Resumo

A presente tese explora o potencial dos foraminíferos bentónicos como indicadores ambientais no estuário do Rio Guadiana de acordo com duas perspetivas: uma perspetiva atual, em que se estudam os padrões de distribuição das comunidades de foraminíferos bentónicos e as suas respostas às modificações ambientais sazonais, criando-se uma base de dados que sirva de ponto de partida a futuros estudos de monitorização ambiental; e uma perspetiva paleoambiental, em que o conhecimento inerente às comunidades modernas, aliado ao entendimento dos processos tafonómicos, permita interpretar ambientes análogos que se formaram no passado.

Paralelamente ao estudo dos foraminíferos são apresentados dados pioneiros sobre a taxonomia e distribuição das tecamebas e dos tintinídeos no estuário do Guadiana, dois grupos de organismos semelhantes aos foraminíferos mas com exigências ecológicas diferentes. Estes grupos, praticamente desconhecidos para a ciência em Portugal, em particular as tecamebas, são estudados no sentido de avaliar o seu potencial como indicadores ecológicos. O aprofundamento do conhecimento das suas exigências ecológicas aumenta o potencial interpretativo dos ambientes do passado, sobretudo quando esse conhecimento é considerado em conjunto com os foraminíferos.

Os foraminíferos são organismos unicelulares eucariontes, cuja principal característica morfológica é possuírem uma carapaça que, após a morte do organismo, poderá preservar-se no sedimento. A elevada sensibilidade dos foraminíferos em relação a alguns parâmetros ambientais como a frequência de inundação, salinidade, tipo de substrato, disponibilidade de alimento, temperatura e poluição, confere-lhes um grande potencial na caracterização do estado de um ecossistema, permitindo evidenciar precocemente modificações naturais e/ou antrópicas. Os foraminíferos, ao deixarem um registo fóssil nas sequências sedimentares, fornecem informação específica para a reconstrução das características ambientais dos respectivos meios deposicionais. Representam excelentes ferramentas neste género de análise por serem pequenos e abundantes. São amostrados em grandes quantidades em volumes reduzidos de sedimento o que permite executar estudos céleres, significativos e económicos. Para obter reconstruções fidedignas dos paleoambientes é imprescindível conhecer a ecologia e a distribuição das comunidades atuais. Ou seja, com base no princípio do Uniformitarismo, são

as associações de foraminíferos modernos que servem de chave para interpretar as associações fósseis. Por outro lado, o contexto histórico e paleoecológico dos processos naturais, são essenciais na elaboração de diretrizes que nos permitam uma gestão mais eficiente e sustentável do ambiente.

O equilíbrio dinâmico dos estuários tem sido posto à prova de forma crescente pela intervenção antrópica, sobretudo no último milénio. Por possuírem um elevado potencial económico, as zonas costeiras são densamente povoadas, promovendo inúmeras atividades antrópicas que tendem a alterar o frágil balanço na interface oceano/continente. O estuário do Guadiana em particular mantém ainda a sua integridade ambiental, representando a nível europeu uma área de elevada importância ecológica, detendo uma série de estatutos ambientais. No entanto, várias perturbações de origem antrópica têm vindo a alterar o equilíbrio dinâmico do estuário. Várias fontes de poluição atuam ao longo da bacia do Guadiana, sobretudo resultantes da urbanização (descargas de esgotos), agricultura, produção de gado, produção de azeite, etc. A própria morfologia do estuário tem vindo a ser modificada de forma dramática. A construção de dois pontões na desembocadura do estuário inibe a deriva litoral natural, reduzindo a entrada no estuário de sedimentos de origem marinha. A barragem do Alqueva, em funcionamento desde 2002 é, provavelmente, o problema mais premente. Estima-se que o Alqueva, juntamente com outras barragens menores construídas desde os anos 60, retenham aproximadamente 80% do sedimento originário na bacia do Guadiana. A retenção dos sedimentos a montante da barragem reduz o fornecimento de sedimentos finos essenciais ao crescimento dos sapais e inibe a chegada de nutrientes essenciais ao equilíbrio ecológico do estuário. As perturbações do clima terrestre à escala global resultantes da atividade humana aliadas à variabilidade natural da Terra têm levado ao aumento acelerado do nível médio do mar durante o último século. Os efeitos mais marcantes relacionados com esse aumento são as inundações mais frequentes e severas das zonas húmidas e das zonas baixas, por vezes populacionais, a erosão das praias, o aumento da intrusão salina nos rios, nas baías e nos aquíferos.

Nos estuários os foraminíferos atuam como bioindicadores de grande interesse devido aos seus ciclos de vida curtos e à sua reação rápida à mudança ambiental. A sua utilização como indicadores da qualidade do ambiente é recente, tendo-se iniciado nos anos 60 e estando em franca expansão atualmente. Em Portugal a aplicação desta ferramenta diagnóstica é relativamente recente, conhecendo-se um único trabalho publicado na área, desenvolvido por Martins et al. (2010). Neste trabalho, os autores investigam a distribuição das associações de foraminíferos bentónicos nos vários sub-ambientes da Ria de Aveiro, sobretudo nos "pontos quentes” de poluição, relacionados com a introdução de metais pesados resultantes da laboração industrial, atual e passada. Existe ainda um campo muito vasto inexplorado, não só no que respeita à avaliação da qualidade dos ambientes costeiros antropisados, utilizando indicadores alternativos e expeditos como são os foraminíferos, mas também no que respeita à simples caracterização ambiental dos ecossistemas do território nacional ainda considerados prístinos. Esta última aplicação é, na verdade, imprescindível e deve preceder qualquer estudo de qualidade ambiental, uma vez que é importante discernir entre as respostas causadas pelo stress antrópico e as respostas causadas pela mudança natural do ambiente.

A maioria dos estudos ecológicos relacionados com os foraminíferos é realizada com o intuito de elaborar bases de dados contemporâneas através das quais as associações de foraminíferos fósseis possam ser comparadas e interpretadas. Em Portugal, apesar de existir um número significativo de estudos relacionados com foraminíferos (ver Martins et al., 1997 para revisão bibliográfica), apenas alguns, relativamente recentes, estão relacionados com a caracterização ecológica dos estuários. Nalguns destes estudos, a caracterização ambiental é relativamente limitada, realizada com o intuito de encontrar analogias ambientais que permitam interpretações paleo-ambientais e a identificação das associações fósseis (Andrade et al., 2004; Cearreta et al., 2007). Noutros, as comunidades vivas (biocenoses) de foraminíferos bentónicos são analisadas numa perspetiva ecológica, em que a distribuição e a abundância das espécies são minuciosamente relacionadas com os parâmetros espaciais e físico-químicos, de forma a desenvolver bases de dados ecológicas que possam ser aplicadas em futuros estudos de reconstrução paleo-ambiental (Cearreta et al., 2002; Fatela et al., 2009; Fatela and Silva, 1990; Leorri et al., 2010; Moreno et al., 2005). Noutros, ainda, as interpretações paleoecológicas baseiam-se no significado ecológico das próprias associações de foraminíferos fósseis (Alday et al., 2006; Boski et al., 2002; 2008; Delgado et al., 2012; Hilbich et al., 2008). Esta abordagem, apesar de possível, uma vez que o significado ecológico das espécies chave que colonizam os estuários é bem conhecido, descura as alterações pós-deposicionais a que as associações de foraminíferos estão sujeitas. Estas alterações revestem-se de crucial importância na interpretação dos processos de sucessão ecológica (*sensu lato*) à escala geológica e só são consideradas quando se estudam as associações modernas mortas ou totais (vivos e mortos).

Nos anos mais recentes, o estuário do Rio Guadiana tem suscitado o interesse da comunidade científica que estuda os foraminíferos. O primeiro trabalho conhecido executado no interior do estuário foi realizado por Ruiz et al. (1996), cujo principal objetivo foi estudar os padrões de distribuição dos foraminíferos bentónicos e ostracodes modernos, focando-se essencialmente nos processos de transporte com base na dinâmica intermareal, descurando as relações ecológicas e características biológicas das espécies. Mais tarde, os mesmos autores (González et al., 2000; Ruiz et al., 2005) realizaram um novo estudo abrangendo três estuários importantes do Golfo de Cádis, incluindo o Guadiana. Neste último estudo identificaram várias associações contemporâneas de foraminíferos bentónicos e relacionaram as suas distribuições com as características sedimentares dos ambientes, com a salinidade e com o tempo de inundação intertidal. Aplicaram ainda os dados resultantes na reconstrução paleo-geográfica de um importante canal do Rio Tinto.

Também do lado português têm sido realizadas algumas abordagens com o intuito de entender melhor as mudanças geomorfológicas que ocorreram no estuário do Rio Guadiana durante o Holocénico (Boski et al., 2002; 2008). Com base em informação integrada resultante da análise de sequências sedimentares, datações ^{14}C e conteúdos micro e macro-faunísticos, estes autores propuseram a primeira representação cronológica da transgressão marinha holocénica sentida no estuário do Guadiana. Os conteúdos micro-faunísticos aplicados nestes estudos foram previamente analisados por Camacho (1999; 2004), em duas teses não publicadas que incluíram não só a aplicação das paleoassociações de foraminíferos bentónicos na interpretação da evolução holocénica do estuário do Guadiana, mas também do Arade-Boina, o segundo maior sistema estuarino da costa algarvia a seguir ao Guadiana. O sistema estuarino Gilão-Almargem foi também estudado por Boski et al. (2008), mas à semelhança dos estuários do Guadiana e Arade-Boina, não foram recolhidas associações modernas análogas que servissem de comparação às paleoassociações.

A motivação para a presente tese teve em consideração todo o trabalho realizado anteriormente no estuário do Rio Guadiana. O objetivo geral desta tese consiste em atualizar e colmatar a informação pré-existente, criar uma base de dados contemporânea em que futuros estudos de reconstrução paleo-ambiental possam assentar e conhecer as respostas dos foraminíferos às variáveis ambientais, fornecendo um ponto de partida a estudos de monitorização ambiental. De forma a cumprir o objetivo geral, foram prosseguidos vários objetivos específicos nomeadamente:

1. Analisar a variabilidade espacial e sazonal de uma série de parâmetros ambientais ao longo de um gradiente de elevação que foi interpretado como a variação do tempo de exposição às marés (perfil terra-água) e ao longo de um gradiente de salinidade, correspondente ao perfil latitudinal do rio (perfil fluvial-marinho). Com base nos resultados integrados espera-se conhecer o funcionamento do sistema no seu todo e identificar os fatores proeminentes em dois cenários climáticos diferentes (Inverno e Verão);

2. Identificar a variação espaço-sazonal na distribuição das comunidades vivas de foraminíferos bentónicos do estuário do Guadiana e estabelecer, através de análise estatística, as suas relações com os parâmetros ambientais descritos no objetivo anterior. Com este estudo pretende-se aperfeiçoar o valor dos foraminíferos como bioindicadores, fornecer uma base de dados contemporânea que permita futuras inferências na qualidade ambiental e interpretações paleo-ambientais fidedignas à escala regional;
3. Reconstruir a história da evolução holocénica do estuário do Guadiana com base na associação total de foraminíferos modernos, combinando a informação sazonal relativa às biocenoses adquirida no objetivo anterior e a informação relativa aos processos tafonómicos a que as associações faunísticas estão sujeitas após deposição. Para realizar este objetivo foram revisitadas e analisadas duas sondagens geológicas anteriormente realizadas no estuário;
4. Iniciar o estudo das tecamebas e dos tintinídeos (aglutinados) no estuário do Guadiana, avaliando o seu potencial como indicadores de hidrodinâmica em termos espaciais e sazonais e a sua aplicabilidade, individualmente ou em conjunto com outros bioindicadores, em estudos de reconstrução paleo-ambiental;
5. Criar um atlas com a descrição e ilustração de todas as espécies de foraminíferos, tecamebas e tintinídeos encontradas no presente estudo que sirva de base na identificação destes microrganismos em trabalhos futuros.

Durante o ano de 2010 foram realizadas duas campanhas de campo, uma no Inverno e outra no Verão, com o objetivo de aceder às variações das comunidades de foraminíferos e tecamebas nos dois períodos do ano de maior contraste ambiental. A malha de amostragem estendeu-se ao longo de 34 km, desde Alcoutim até à foz do rio Guadiana, abrangendo assim todo o estuário. No estuário médio e alto foi recolhida apenas uma amostra por ponto devido à ausência de zonação marginal. No baixo estuário, onde a zonação ambiental é bem demarcada devido ao efeito mais intenso das marés, foram recolhidas várias amostras ao longo de perfis em cada local de amostragem, normalmente perpendiculares ao curso do rio. A localização exata das amostras prendeu-se com a zonação das espécies halófitas, amostrando as distintas zonas, desde a planície lodosa não vegetada até às zonas mais elevadas do sapal. A delineação dos perfis, com marcação detalhada das coordenadas geográficas (x, y e z), foi efetuada com recurso a um sistema de posicionamento global diferencial (D-GPS) e uma estação total. A zonação halófita e respetivas espécies foram descritas e fotografadas. As amostras para análise da microfauna foram colhidas à superfície do sedimento (~0-1 cm) onde se encontram a maioria dos foraminíferos vivos. Paralelamente foram colhidas amostras de sedimento para análise de matéria orgânica e granulometria. A salinidade, temperatura e oxigénio dissolvido na água, bem como o pH do sedimento foram medidos *in situ* em cada campanha. As amostragens sedimentares e as medições dos parâmetros físico-químicos foram sempre realizadas em período de baixa-mar.

As amostras para análise dos paleoambientes holocénicos do Guadiana foram colhidas de duas sondagens geológicas, CM3 e CM5, realizadas no final dos anos 90 e previamente estudadas por Boski et al. (2002; 2008). A sondagem CM3, localizada no atual sapal de Vila Real de Santo António, atingiu o substrato paleozoico aos 36 m de profundidade. Desta sequência sedimentar foram retiradas 20 amostras para análise microfaunística. A sondagem CM5 localizou-se mais a montante, junto ao canal da Ribeira do Beliche, a cerca de 11 km da linha de costa atual. Esta sondagem atingiu o substrato paleozoico por volta dos 51 m de profundidade e foi sujeita a uma frequência de amostragem mais elevada do que CM3, resultando em 82 amostras para análise da microfauna fóssil. As associações fósseis recuperadas dos dois testemunhos foram analisadas e interpretadas à luz das associações análogas modernas. O enquadramento paleoambiental foi ainda completado com recurso a datações ^{14}C pré-existentes (Boski et al., 2002; 2008; Fletcher et al., 2007; González-Vila et al., 2003) e a descrições sedimentológicas realizadas por Boski et al. (2002; 2008).

As amostras foram processadas em laboratório, o que incluiu etapas como: sub-amostragem, em que a quantidade de sedimento a ser analisada correspondeu ao volume de 20 cc; aplicação do corante Rosa de Bengala para posterior diferenciação entre os indivíduos vivos e os mortos (aplicado só às amostras contemporâneas); lavagem das amostras, em que a fração resultante para análise correspondeu aos sedimentos de granulometria entre 63 e 500 µm; triagem e contagem, em que o número estatisticamente válido para os foraminíferos vivos e para o conjunto tecamebas/tintinídeos total (vivos e mortos) foi de 50 indivíduos, e para foraminíferos mortos e fósseis foi de 100 indivíduos; identificação e reportagem fotográfica, em que, sempre que foi possível, os organismos foram classificados até à espécie e fotografados com recurso a um microscópio eletrónico de varrimento.

Foram realizadas duas campanhas suplementares com o intuito de recolher indivíduos vivos para análise genética em colaboração com o projeto foramBARCODING, sediado na Universidade de Genebra, cujo objetivo é a criação de uma base de dados molecular dos foraminíferos de todo o mundo. Graças a este estudo foi possível identificar geneticamente quatro espécies de foraminíferos bentónicos de estatuto importante no estuário.

Na análise das populações foram calculados vários índices biocenóticos, nomeadamente: a abundância (absoluta e relativa); a densidade faunística (nº de indivíduos por 20 cc de sedimento); a dominância específica, considerando-se dominantes as espécies com uma representação ≥ 20%; a constância das espécies, considerando-se constantes as espécies com ocorrência em ≥ 50% das amostras de valor estatístico; a razão aglutinados/calcários (A/C%), que afere a dominância do tipo de carapaça; a diversidade, através do índice de Shannon (Hs); e o índice de dominância, que afere o stress ambiental da população.

Na análise foram consideradas as seguintes variáveis ambientais: as variáveis espaciais – latitude, longitude e elevação (em relação ao nível médio do mar - NMM); as variáveis químicas – salinidade, carbono orgânico total, carbono inorgânico total, nitrogénio total, a razão carbono/nitrogénio (C/N), o pH do sedimento e o oxigénio dissolvido; e as variáveis físicas – média granulométrica, os conteúdos em areia, silte/argila e gravilha, e temperatura (ºC).

Na análise das relações entre espécies e os parâmetros ambientais, foram utilizadas várias técnicas estatísticas: índices de correlação de Pearson (*r*) e Spearman (*R*); análise de correspondência destendenciada (DCA); análise aglomerativa (cluster analysis); análise multidimensional não-métrica (NMDS); e análise de redundância (RDA).

A variação dos parâmetros ambientais sugere uma dependência significativa dos gradientes espaciais (elevação e distância ao mar) e sazonais, evidenciando condições fluviais prevalecentes no Inverno e condições marinhas no Verão.

Durante o Inverno de 2010, a forte precipitação obrigou à descarga contínua da barragem do Alqueva, promovendo condições hidrodinâmicas excepcionais, que contribuíram para o aumento da deposição de sedimentos mais grosseiros no estuário, sobretudo nas zonas menos elevadas do estuário. Contudo, de uma forma geral, os sedimentos são mal calibrados e com uma assimetria tendencialmente fina, o que corresponde a condições de baixa energia esperadas para ambientes de deposição como são os ambientes da zona marginal intermareal.

No Inverno, não se registam salinidades superiores a 3 ao longo de todo o estuário, exceto nos pontos mais confinados, sujeitos a maior evaporação. No Verão, as águas do estuário apresentam-se melhor misturadas e a intrusão salina é significativa até à zona da Ponte Internacional. A zona de maior salinidade estende-se, aproximadamente, até à latitude da Ribeira do Beliche, a partir da qual, para montante, os valores de salinidade são semelhantes aos observados no Inverno.

As zonas inundadas confinadas estão sujeitas a variações sazonais extremas da salinidade e oxigénio dissolvido. No Inverno, quando o contributo em águas fluviais e pluviais é maior verifica-se salinidades baixas e valores de concentração do oxigénio altas; no Verão, quando a insolação e a evaporação são os fatores mais importantes, a salinidade aumenta e o oxigénio diminui.

A variação sazonal da temperatura das águas superficiais é muito elevada, verificando-se uma diferença de *ca* 14ºC entre as temperaturas máximas registadas no Inverno e no Verão.

Verifica-se uma relação de dependência entre a matéria orgânica, granulometria e pH e a elevação. Nas zonas mais elevadas do sapal onde a hidrodinâmica é atenuada, ocorre deposição diferencial de sedimentos finos. As características coesivas destes sedimentos promovem o aprisionamento de matéria orgânica cuja degradação provoca a diminuição do pH.

A matéria orgânica nos sedimentos do Guadiana caracteriza-se por uma mistura de origem terrestre e aquática autóctone. O material orgânico no estuário médio-alto e nas zonas mais elevadas do sapal no estuário baixo incorporam um maior contributo das fontes terrestres, enquanto que as zonas baixas-médias do estuário marinho experienciam um contributo substancial das fontes aquáticas autóctones.

A distribuição dos foraminíferos bentónicos no estuário do Rio Guadiana espelha a variação sazonal dos fatores ambientais, cuja importância relativa depende da proximidade aos limites de tolerância das espécies. A elevação demonstrou ser o parâmetro mais importante na distribuição dos foraminíferos, já que combina o efeito de uma série de outras variáveis, tais como a matéria orgânica e o conteúdo em finos, que tendem a aumentar quando a elevação aumenta, e o pH do sedimento, o conteúdo em grosseiros, e a temperatura, que tendem a aumentar com a diminuição da elevação. A salinidade integrada no perfil fluvial-marinho revelou-se também importante na distribuição da microfauna. No entanto revelou pouca importância na distribuição das espécies ao longo do gradiente de elevação da zona intertidal, provavelmente devido à elevada osmoresistência reportada para as espécies de sapal.

Ao longo do gradiente de elevação verificou-se uma correlação negativa entre a diversidade e a dominância das espécies. Nas zonas mais elevadas do sapal, onde as condições ambientais são geralmente mais severas, apenas algumas espécies aglutinadas são capazes de sobreviver. Nas zonas mais baixas do sapal, onde o tempo de exposição aérea é menor e as condições ambientais são de uma forma geral mais uniformes, predominam faunas mais diversificadas, maioritariamente compostas por espécies calcárias.

Durante o Inverno, quando os processos fluviais são dominantes, as espécies aglutinadas dos andares mais elevados do sapal proliferam, constituindo mais de 80% do total de indivíduos contados. No Verão, quando as condições marinhas prevalecem, as espécies calcárias tornam-se mais competitivas, aumentando as suas densidades faunísticas e expandindo-se para zonas mais elevadas do sapal e para zonas mais a montante.

As espécies *Miliammina fusca* e *Polysaccammina hyperhalina*, ambas aglutinadas e típicas dos andares médios-baixos de sapal, revelaram um comportamento semelhante às espécies calcárias, exibindo ótimos de ocorrência no Verão. A *Jadammina macrescens*, entre as espécies aglutinadas, e a *Ammonia aberdoveyensis*, entre as espécies calcárias, foram as mais ubíquas e dominantes nas duas estações do ano estudadas.

Com base nas espécies dominantes de foraminíferos vivos e nas variações sazonais das suas relações foi possível definir um modelo de zonação ecológica para o estuário do Guadiana que assenta no reconhecimento de quatro associações de espécies diferentes:

Associação *J. macrescens*: domina as zonas mais elevadas do sapal (entre 1 e 2 metros acima do NMM) ou em zonas mais baixas mas sujeitas a um elevado grau de confinamento. São zonas densamente vegetadas onde os sedimentos são predominantemente finos, o pH é baixo e o conteúdo em matéria orgânica é elevado. A espécie *J. macrescens* é dominante, representando mais de 70% dos indivíduos, associada a *Trochammina inflata*. Ocasionalmente, *Miliammina obliqua*, *Polysaccammina ipohalina* e miliolídeos podem ocorrer em abundâncias significativas;

Associação *J. macrescens* e *T. inflata*: domina as zonas de elevação intermédia, entre 0.1 e 0.6 m acima do NMM, sobretudo em zonas encharcadas (poças intertidais), densamente vegetadas e próximas do mar. Os parâmetros físico-químicos são muito variáveis. Ambas as espécies *J. macrescens* e *T. inflata* são dominantes. Em zonas de menor elevação, a espécie *Discorinopsis aguayoi* pode ocorrer no Inverno e a espécie *A. aberdoveyensis* no Verão;

Associação *M. fusca*: é comum nas zonas de elevação intermédia, entre 0 e 0.75 m acima do NMM, sobretudo a montante do baixo estuário em zonas normalmente não vegetadas. *M. fusca* é a espécie dominante e está associada às espécies secundárias *A. aberdoveyensis* e *Elphidium oceanensis*.

Associação *A. aberdoveyensis*: é comum nas zonas de baixa elevação do baixo estuário, entre -7 e 0.3 m em relação ao NMM, sujeitas a uma forte influência marinha. O sedimento é composto por mais de 50% de areias, ocasionalmente com contributos significativos de bioclastos. A *A. aberdoveyensis* é a espécie dominante, estando associada a *Haynesina germanica*, *P. hyperhalina* e *E. oceanensis*. No Inverno, a *Bolivina ordinaria* é co-dominante com a *A. aberdoveyensis* e a *Asterigerinata mamilla* é também abundante.

O modelo de zonação ecológica dos foraminíferos bentónicos do estuário do Guadiana apresentou maiores semelhanças com os modelos descritos para a zona climática do Mediterrâneo do que com a zona do Atlântico Norte.

A comparação entre as associações de foraminíferos vivos, total e fóssil demonstrou que a associação total, que combina os efeitos sazonais e tafonómicos das microfaunas modernas, representa de forma fidedigna a microfauna indígena do estuário do Guadiana e que a inclusão das espécies exóticas no estudo é uma mais-valia na interpretação das paleoassociações.

O processo tafonómico de dissolução das carapaças calcárias foi observado nas paleo-associações, sobretudo quando episódios de baixas taxas de sedimentação estão associados ao momento de deposição das carapaças. Contudo, o estudo dos sedimentos holocénicos do Guadiana parece indicar que a desintegração diferencial das carapaças aglutinadas representa o maior problema na procura de analogias entre as faunas modernas e as faunas fósseis. Espécies aglutinadas importantes nas biocenoses do Guadiana tais como *Haplophragmoides manilaensis*, *M. fusca*, *M. obliqua*, *P. hyperhalina, P. ipohalina* e *Siphotrochammina* sp., não estão representadas no registo fóssil. Por outro lado, os trocaminídeos e a espécie *Ammobaculites* sp. provaram ser as espécies aglutinadas mais resistentes aos processos tafonómicos.

Através da análise aglomerativa das associações totais (vivos e mortos) de foraminíferos foi possível identificar cinco associações, cada uma com composição faunística diferente, indicativa da sua posição em relação ao nível intertidal e à distância ao mar, com as quais os paleo-ambientes foram interpretados: 1) Associação monoespecífica de *J. macrescens*; e 2) Associação de *J. macrescens* e *T. inflata,* ambas as associações indicativas de um ambiente emergente, em que as taxas de acreção acompanham a subida do NMM; 3) Associação de *A. aberdoveyensis* e *H. germanica,* indicativa de um ambiente submergente, em que a subida do NMM excede as taxas de acreção; 4) Associação de miliolídeos, *A. aberdoveyensis* e espécies exóticas, indicando ambientes energéticos, sujeitos a forte influência marinha; 5) Associação de *M. fusca* e outras espécies aglutinadas, indicando ambientes sujeitos a forte influência fluvial.

A análise das mudanças faunísticas ocorridas ao longo das sequências sedimentares CM3 e CM5, em conjunto com os dados sedimentológicos e cronológicos, permitiram a identificação de quatro fases distintas de evolução do estuário do Guadiana desde *ca* 13 000 cal yr BP:

- ***Fase pré-holocénica:*** teve início antes dos 13 000 cal yr BP e abrangeu o curto evento de clima frio Dryas recente, caracterizando-se por um período de assoreamento do estuário, promovido pelo domínio de processos continentais, sem registo de foraminíferos ou outros indicadores de vida marinha;
- ***Fase de pré-inundação:*** iniciou-se com os primeiros vestígios de forros internos de trocaminídeos e da espécie *T. inflata*, ocorrendo antes de 11 500 cal yr BP, quando o

nível do mar estava a *ca* 45 m de profundidade. Caracteriza-se pela alternância de fases de acreção do sapal em equilíbrio com a subida do NMM (*J. macrescens* e *T. inflata*) e fases de emergência do sapal (presença de *Trochammina* spp. evoluindo para zonas sem foraminíferos);

- ***Transgressão holocénica:*** ocorreu entre *ca* 9300 e 3500 cal yr BP e incluiu duas fases de marcada influência marinha:

  \- *Fase transgressiva inferior* – regista o máximo transgressivo durante o Holocénico, entre *ca* 8000 e 7600 cal yr BP e é caracterizada pela presença de *A. aberdoveyensis*, *H. germanica*, *A. mamilla,* miliolídeos, abundâncias elevadas em espécies exóticas e constância das formas planctónicas. A presença da espécie de águas quentes *Pararotalia* cf. *spinigera* e a evidência de ambientes subtidais na sondagem CM3 sugerem que o estuário do Guadiana experienciou períodos de maior oceanidade e, provavelmente, temperaturas mais elevadas do que o atual;

  \- *Fase transgressiva superior* – regista aos 4400 cal yr BP um curto episódio de renovação marinha caracterizado pela presença de *A. aberdoveyensis*, *H. germanica* e espécies exóticas, interrompendo uma fase mais longa de desaceleração do NMM, caracterizada pela presença de *Trochammina* spp. e *Ammobaculites* sp. Esta fase curta de influência marinha, aparentemente não identificada noutros estudos realizados na zona do Golfo de Cádis, poderá corresponder ao período climático quente reportado para o Sudeste da Península Ibérica, com base em séries temporais palinológicas detalhadas.

- ***Formação das zonas húmidas e planícies de maré:*** de 3500 cal yr BP até ao presente, regista-se uma sequência monótona de sapal alto-médio, caracterizado pela presença contínua de trocaminídeos e *Ammobaculites* sp., sugerindo um ambiente em acreção equilibrada com a subida do NMM.

No estudo pioneiro sobre a distribuição de tecamebas e tintinídeos no estuário do Guadiana identificaram-se 17 espécies de tecamebas (integrando mais de 25 'estirpes') e 3 espécies de tintinídeos. A elevada variabilidade ecofenotípica observada entre as tecamebas revela proximidade e diversidade de ambientes dulciaquícolas no estuário e um elevado potencial científico ainda inexplorado.

Entre as tecamebas, a família Centropyxidae, representada sobretudo pelas espécies *Centropyxis aculeata, Centropyxis arcula* e *Centropyxis constricta*, foi a melhor representada em termos de densidade faunística, integrando mais de 70% do total dos indivíduos contados.

No Inverno, quando a corrente e o nível hidrométrico do rio são mais elevados observa-se um aumento na diversidade das tecamebas e a densidade faunística duplica. Independentemente da estação do ano, as diversidades e densidades de tecamebas mais elevadas são sempre registadas no estuário médio-alto, onde a salinidade é negligenciável. Nestes ambientes, as tecamebas estão associados a espécies de foraminíferos aglutinados tais como: *M. fusca, H. manilaensis, M. obliqua* e *Siphotrochammina* sp. No baixo estuário, junto com as espécies de foraminíferos calcários, ocorre um número significativo de carapaças vazias de tecamebas que tende a diminuir com a aproximação ao mar. Estas ocorrências são mais frequentes nas zonas mais baixas do sapal e relacionam-se com o transporte *post-mortem* que aumenta durante períodos de precipitação elevada no Inverno. No Verão, um aumento significativo das tecamebas vivas no baixo estuário, especialmente localizados na proximidade de zonas com afluência de água doce, poderá indicar que a presença destes organismos no estuário não se deve só a processos estocásticos, mas que algumas espécies são capazes de viver mesmo nas zonas mais marinhas do estuário desde que a salinidade seja diminuída.

Duas espécies de tintinídeos, *Tintinnopsis* cf. *lata* e *Stenosemella ventricosa*, foram acidentalmente incluídas no presente estudo. As suas semelhanças morfológicas com as tecamebas levaram a uma identificação errada no princípio da tarefa de contagem. No entanto, o seu estudo provou ser uma mais-valia, originando informação nova com aplicação em futuros estudos de monitorização paleo-climática e ambiental em estuários. Quando as espécies de tintinídeos aqui estudadas são encontradas na forma fóssil em sedimentos holocénicos sugerem proximidade a lugares de descarga de água doce ou, num sentido mais amplo, zonas salobras de baixas profundidades. A ocorrência de abundâncias elevadas de *S. ventricosa* associadas ao foraminífero calcário *E. oceanensis* pode ser indicativa de períodos climáticos quentes.

O presente estudo demonstrou que os três indicadores aqui abordados, quando relacionados com as condições ambientais oferecem um grande potencial em estudos de monitorização ambiental, bem como em estudos de reconstrução paleoambiental. Este potencial é acrescido se os três grupos de organismos forem analisados numa perspetiva integrada, aumentado assim o leque de cenários ambientais possíveis de aferir numa interpretação paleoambiental. No entanto, muito fica por conhecer, sobretudo no que respeita às tecamebas e aos tintinídeos, ambos pouco conhecidos para a ciência em Portugal e cujo potencial como indicadores ecológicos ficou aqui provado.

Palavras chave: Estuário, Guadiana, foraminíferos, tecamebas, tintinídeos, Holocénico

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Chapter 1

Introduction

Chapter 1

1 – Introduction

1. 1. Study motivation and objectives

During the last century ecological studies of foraminifera gained special importance. Most of these studies aim to provide a contemporary database for comparison and interpretation of fossil foraminifera (Horton and Edwards, 2000). By leaving a microfossil record in the sedimentary sequences, foraminifera enable the reconstruction of the environmental history of a site in the absence of the original physiochemical baseline data (Scott et al., 2001). The quality of paleoecological interpretations depends on how well the modern foraminifera assemblages are known in relation to the ecological conditions controlling their spatial and temporal abundance.

Though the existence of a significant number of works related to foraminifers in Portugal (see Martins, 1997 for review) only few of these are concerned with the characterization of restricted environments such as estuaries and the varied sub-environments that they harbour. In some of these works, the characterization of the modern environment was very superficial, since the main aim was to identify environmental analogies that support paleoenvironmental interpretations and fossil assemblage’s identification (e.g. Andrade et al., 2004; Cearreta et al., 2007). In others, foraminiferal biocenosis are analysed using an ecological approach, where species distribution and abundance are closely related to spatial and physico-chemical environmental parameters in order to develop ecological database for further paleoenvironmental interpretation (e.g. Cearreta et al., 2002; Fatela et al., 2009; Fatela and Silva, 1990; Leorri et al., 2010; Moreno et al., 2005). Other works concerning Holocene paleoenvironmental reconstruction have been based on microfossils alone to interpret fossil sequences (e.g. Alday et al., 2006; Boski et al., 2002; 2008; Delgado et al., 2012; Hilbich et al., 2008). Although the ecological meaning key species in restricted environments is relatively well established, these studies run the risk of failing to account for post-depositional changes, which only modern dead or total (living and dead) assemblages can provide (Duchemin et al. 2005).

In recent years, the Guadiana Estuary has aroused the interest of foraminifera research. The first work was performed by Ruiz et al. (1996), and was concerned with the distribution and ecology of modern benthic foraminifera and ostracoda. They focused essentially on microfauna distribution patterns and transport processes affected by intertidal dynamics, but disregarded biological and ecological perspectives. The same authors (González et al., 2000; Ruiz et al., 2005) subsequently investigated three important estuaries in the Gulf of Cadiz, among them the Guadiana Estuary. Supported by biocenotic indices and multivariate analysis, they were able to identify several modern foraminifera assemblages and relate their distribution to sedimentary environments, granulometry and salinity. The resulting data were also applied in the paleogeographic reconstruction of an important channel of the Tinto River.

On the Portuguese side some approaches have also been made aiming to better understand the geomorphologic changes that occurred in Guadiana Estuary during the Holocene (e.g. Boski et al., 2002; 2008). Based on integrated information from the analysis of sedimentary sequences, ^{14}C dates and micro- and macro-faunistic content, these authors proposed the first chronologic approach to the Holocene marine transgression in the Guadiana Estuary. The micro-faunistic content used in these studies was first analysed by Camacho (1999) and Camacho (2004) (unpublished theses). These studies, in addition to a reconstruction of Holocene estuarine evolution based on foraminifera paleoassemblages from the Guadiana Estuary, also include the first data from the Arade-Boina, the second largest estuarine system in Algarve coast (unpublished data). Another estuarine system from Algarve coast, the Gilão-Almargem, was also studied (Boski et al., 2008), but, like the Guadiana and Arade-Boina systems, no modern analogues were collected and used for comparison.

In the last century, the dynamics equilibrium of estuaries has been increasingly affected by anthropogenic intervention. The particular environmental characteristics of these coastal systems make them very attractive to humankind and, as a consequence, coastal zones are densely populated and hold a high economic potential. Perturbations resulting from human activities allied to the earth’s natural climatic variability have been leading to a global increase in mean sea level (Dias, 1993). The most important effects of sea-level rise are the gradual inundation of wetlands and coastal low lands, erosion of beaches, more frequent and severe flooding, and higher salinity in rivers, bays, aquifers, and wetlands. All of these effects are aggravated in systems strongly controlled by river regulation as is the case of the Guadiana Estuary. Increasing anthropogenic influence in the Guadiana Estuary necessitates the acquisition of scientific information about the estuary’s present condition and long-term monitoring of changes in biochemical and geochemical parameters (Boski, 2004).

Foraminifera can make a substantial contribution to monitoring estuarine environments. They are small and abundant, and easily sampled in small volumes of sediment, enabling statistically reliable and economically attractive studies. Their dependence on estuarine environmental parameters, such as sedimentology, inundation time, salinity, productivity, water quality, etc., can produce responses that can be readily interpreted in terms of environmental evolution.

In Portugal, the use of foraminifera as bioindicators of environmental quality is only in its infancy. To date, there is only one published work on this subject, that of Martins et al. (2010), who investigated benthic foraminifera assemblage distributions in several sub-environments of the Ria de Aveiro, NW Portugal, focusing on pollution ‘hot-spots’ from modern and historic industrial activity. Much remains to be done, not only in the evaluation of the quality of Portuguese restricted environments using alternative and expedient tools such as foraminifera, but also in what concerns the environmental characterization of the still existing well preserved sites.

The present study was conceived with the aim of investigating the present-day distribution patterns of foraminifera in the Guadiana Estuary in order to: i) complement and update earlier information collected by González et al. (2000) and Ruiz et al. (1996; 2005); ii) provide a modern analogue dataset with which reliable paleoenvironmental reconstructions can be made; and iii) to provide a more refined understanding of foraminifera responses to environmental variables, serving as baseline for future monitoring studies in which it is necessary to distinguish between responses due to anthropogenic stress and changes in the natural environment. In order to better fulfil the main aims above described, a series of specific objectives were formulated:

1. To analyse spatial and seasonal variability in a series of environmental parameters along elevational (tidal exposure) and latitudinal (distance to sea) gradients in the Guadiana Estuary; based on the resulting integrated approach, this is expected to improve understanding of the system’s functioning as a whole and to identify the major forcing factors in two different climatic scenarios (winter and summer).
2. To identify the spatial-seasonal variation in the distribution of living benthic foraminiferal assemblages in the Guadiana Estuary and to investigate their relationships with the environmental parameters described in the previous objective; this is expected to improve the use of benthic foraminifera as bioindicators, provide baseline data for future inferences of environmental quality, and to provide a modern analogue dataset for reliable paleoenvironmental interpretations on local and regional scales.

3. To reconstruct the story of the Guadiana Estuary's Holocene evolution based on total assemblage analogues, which combine information on the biocenosis from the previous objective and information on the taphonomic processes that act on assemblages after deposition; to achieve this objective, two boreholes previously drilled in the estuary are revisited and interpreted.
4. To improve knowledge on communities of thecamoebians and tintinnids in the Guadiana Estuary in order to evaluate their potential as indicators of spatial and seasonal hydrodynamics and to evaluate their usefulness as tools in paleoenvironmental reconstructions.
5. To create an Atlas with the description and illustration of all the species of foraminifera, thecamoebians and tintinnids found during the course of the present study, with the aim of providing a species catalogue to aid the identification of these organisms in future works.

1. 2. Structure of the thesis

This thesis is presented in two volumes. Volume I comprises the first 8 chapters and Volume II comprises Chapter 9, which is the Atlas of the thesis, and the Bibliographic References and Appendices sections. Each chapter, except Chapter 1, is organized into Chapter Objectives, a brief Introduction on the discussed subject, Results, Discussion and Conclusions. Chapters 1, 2 and 3 provide base information which supports another 5 independent chapters (4, 5, 6, 7 and 9). Chapter 8 gathers the main conclusions of this thesis:

- Chapter 1 is a general introduction to the thesis. It includes a sub-chapter describing the motivation and objectives of the thesis, as well as the present sub-chapter describing the structure of the thesis;
- Chapter 2 brings together all the field, laboratory and analytical methods applied during the execution of the present thesis, with exception of some specific methods described in the relevant chapters (6 and 9);
- Chapter 3 provides a general description of the Guadiana River, its basin and estuary, along with a detailed characterization of the study sites;
- Chapter 4 analyses the spatial and seasonal variability of environmental parameters in the Guadiana Estuary (objective 1);

- Chapter 5 describes the spatial-seasonal variation in the distribution of living benthic foraminiferal assemblages in the Guadiana Estuary and their relationships with the environmental parameters (objective 2);
- Chapter 6 develops an empirical model that combines seasonal and taphonomic effects and demonstrates its application in the paleoreconstruction of the Guadiana Estuary’s Holocene evolution (objective 3);
- Chapter 7 provides pioneering data on the taxonomy, biocenotic and distributional trends of thecamoebians and two species of tintinnids in the Guadiana Estuary (objective 4);
- Chapter 8 gathers the main conclusions of this thesis;
- Chapter 9 (the ‘Atlas’) presents illustrated reports for almost all the species found in the present study. It includes a brief taxonomic framework, a morphological description accompanied by scanning electron microscope (SEM) and in some cases, stereomicroscope images, species distribution status in the Guadiana Estuary and, for some species, a report with genetic information (objective 5). Although it is the last chapter of the thesis, it is recommended to be the first to be read for those less familiar with foraminifera and thecamoebians.

Along with the Atlas, Volume II comprises the list of Bibliographic References of works consulted and used in the elaboration of this thesis and the Appendices section where data essential to the execution of this thesis but which are sparsely presented or mentioned in the main chapters is compiled, namely: photographs of the study sites and halophytic vegetation, tables with spatial data (x, y and z), tables with biocenotic information on foraminifera, thecamoebians and tintinnids, tables with environmental parameter values, results derived from statistical tests and geochronological data.

Chapter 2

Methodology

Chapter 2

2 - Methodology

2. 1. Chapter objectives

Chapter 2 – *Methodology* was created to bring together all the field, laboratory and analytical methods applied in the execution of the present thesis. The only exceptions are the collection and analysis of boreholes CM3 and CM5 paleosequences, which are dealt with in a sub-chapter of Chapter 6 – *Holocene paleoenvironmental reconstruction of the Guadiana estuary based on modern assemblages of benthic foraminifera*, and the methodologies inherent to the identification and classification of foraminifera and thecamoebians which are documented in Chapter 9 - *Atlas of benthic foraminifera and thecamoebian species from the Guadiana River Estuary*, an independent volume of this thesis.

2. 2. Schedule, logistics and sampling design

The loss of carbonated tests in the samples obtained in one first campaign period (2007 – 2009) meant that all the sampling work had to be repeated. The original plan of replicating data collection on a seasonal basis was abandoned due to time constraints. Considering this, temporal variations in the distribution of benthic foraminifera and thecamoebian communities were assessed based on data from two seasons, winter and summer, which are the periods offering the greatest environmental contrasts.

The study-site location was chosen to encompass a variety of marginal environments in a continent-ocean and intertidal transect, spanning the entire estuary: upper, middle and lower estuary. This approach was designed to capture the maximum spatial variation among species and environmental variables. In order to assess the degree of confinement registered by the microfaunas, some places with a more restricted location in relation to the sea front were also sampled. Twelve different sites were selected and samples were collected at: 6 individual sampling points, along 4 transects (each with 3 sampling points) and along 2 additional transects (each with 4 sampling points: Fig. 3.4 Chapter 3). The transects were placed according to the vertical zonation of halophytic vegetation, with the aim of sampling the distinct zones, from unvegetated mud-flats to the higher levels of the marshlands (all the sites and their particular features were photographed; see Appendix 1).

At each sampling point, two pseudoreplicates were collected (replicates of the same sample) (Hurlbert, 1984) with the aim of avoiding the effect of patchiness (Debenay et al., 2006), or non-uniform distribution of benthic communities (Underwood and Chapman, 2005). Populations exhibit varying degrees of patchiness in response to natural or anthropogenic disturbances, at both small and large scales (Odum, 1997; Underwood and Chapman, 2005).

The exact points of sample collection were marked with a metal landmark, painted yellow, of approximately 40 cm length. This enabled rapid identification and return to the sampling points without the need for a GPS. Sediment sampling and measurement of physical-chemical parameters were all performed during low tide.

Surveys of the halophytic vegetation were carried out during the winter and summer campaigns. The most representative species in sampling points and transects were photographed (Appendix 2) and described (Chapter 3).

Precise coordinates were measured in the winter campaign and elevation survey was carried out during several field trips (see details further).

The winter campaign was carried out over four days (19, 20 and 28 of February and 2 of March 2010), coinciding with an intense precipitation period and continuous water discharge from the Alqueva Dam, resulting in unusually high river flow. These conditions hampered fieldwork such that only 11 sites were sampled during this campaign. In total, 72 samples were collected: 24 superficial (0–1 cm) sediment samples, each one with 2 pseudoreplicates, for the study of benthic foraminifera and thecamoebian biocenosis; 24 superficial (0–2 cm) sediment samples for elemental analysis; and 24 sediment samples (0–5 cm) for sedimentological analysis.

Salinity, temperature, dissolved oxygen, sediment and water pH were measured *in situ* (see details further).

The summer campaign was carried out over two days (11 and 12 of August 2010) during low spring tide. The same sites as in winter were sampled, except that this time it was possible to collect a sample from Alcoutim as well. In total, the summer campaign resulted in 75 samples: 25 superficial (0–1 cm) sediment samples, each one with 2 pseudoreplicates, for the study of benthic foraminifera and thecamoebian biocenosis; 25 superficial (0–2 cm) sediment samples for elemental analysis; and 25 sediment samples (0–5 cm) for sedimentological analysis. Chemical parameters were measured as per the winter campaign.

2. 3. Field methods

2. 3. 1. Samples collection for microfauna analysis

During winter campaign, pseudoreplicates were collected using small PVC cores of 15 cm length and 5 cm diameter. Each core was pressed into the sediment to a depth of 10–15 cm to guarantee the collection of the first 10 cm of sediment (in the present study only the first centimeter of sediment was used; the remaining sedimentary column was stored in a freezer, at –20 ºC, for future studies). The cores were closed on both sides with plastic caps and brought back to the laboratory in a coolbox.

In the summer campaign, pseudoreplicates were collected with a metal spatula, targeting only the first centimeter of sediment.

2. 3. 2. Samples collection for bulk organic matter analysis

At each sampling point, 300 g of sediment was collected from the surface to a maximum depth of 2 cm. The sediment was collected with a soup spoon and stored in labelled glass jars. A layer of aluminium foil was placed over the jar mouth to avoid sediment contact with the plastic cap. Sample jars were transported to the laboratory in a coolbox.

2. 3. 3. Samples collection for sedimentological analysis

For each sampling point 200–300 g of sediment were collected from 0 to 5 cm depth and stored in labeled plastic bags.

2. 3. 4. *In situ* measurement of chemical parameter

2. 3. 4. 1. Sediment – The pH was measured at the sediment surface. A waterproof portable pH-meter, model *spear*, from EUTECH was used. Before each utilization, the device was calibrated with 3 pH standards (4, 7 and 10).

2. 3. 4. 2. Interstitial water – When there was enough water, chemical parameters were measured in the sediment interstitial water. An Ysi 556 MPS handheld multiparameter probe was used to measure pH, temperature and dissolved oxygen. The device was calibrated for all parameters before each use.

2. 3. 4. 3. River water – The pH, salinity, temperature and dissolved oxygen were measured along the river profile during ebb tide in both seasons. Measurements were made as close as possible to the sampling points, which, in the case of the transects, coincides with the lowermost sample.

2. 3. 5. Coordinates and elevation of sampling points and transects

Coordinates and elevation values of the sampling points and transects were obtained with a Real Time Kinematic Global Positioning System (RTK-GPS). This system is composed of a mobile GPS unit that receives position information from GPS constellation satellites along with a communication device (mobile phone) that communicates with a single base station or with a virtual reference station, for obtaining corrections to satellite information. In the Guadiana Estuary field campaigns a Trimble 5800 mobile unit was used. This unit received corrections for recalculating its position from the Portuguese Army Geographical Institute’s virtual reference station service (SERVIR). This system enables the collection of precise coordinates, with errors ranging from millimetres to a few centimetres, after calibration at locations where precise coordinates are known. SERVIR network was calibrated to several landmarks from the Portuguese Geodetic Network (Table I, Appendix 3).

For obtaining very precise coordinates, each sampling point was measured for 60 valid records (1 record per second), meaning that the GPS receptor registered the coordinates of the point 60 times, each measurement with a horizontal error below 1.5 cm and vertical error below 2 cm. Final coordinates for each point are the average of the 60 valid records, with horizontal precision better than 1.5 cm and vertical precision better than 2 cm.

In all, 25 points, 6 geodesic landmarks and 5 of the 6 transects were pinpointed using the RTK-GPS. One transect (VRSA), after several attempts, proved impossible to measure using this equipment due to a signal blackout. A Nikon DTM 310 Total Station was therefore used to measure the transect in VRSA. This device enables the determination of an unknown point in relation to a known point as long as a direct line of sight can be established between the two points. Angles and distances were measured from the total station to the survey points, and the coordinates (northing, easting and elevation) of surveyed points relative to the total station position were calculated using trigonometry and triangulation. The total station was set up over a known point and was within the line-of-sight to another point of known coordinates.

Elevation values were measured in relation to mean sea level (MSL) and hydrographic zero (HZ), the latter being the chart datum used by the Portuguese Hydrographic Institute. The HZ is located below the Extreme Low Water Spring (ELWS), therefore the tide height predictions are always positive (Instituto Hidrográfico, 2009). The MSL is the adopted mean value for water level derived from a series of tide gauge observations of variable duration; it is located approximately 2 meters above HZ (Instituto Hidrográfico, 2009). In this study, all values are expressed relative to MSL.

Other vertical reference levels (see below) were calculated based on the 2010 tide tables for the port of Vila Real de Santo António provided by Instituto Hidrográfico (2009):

- MHW – Mean high water is considered 1 m above MSL (3 m above HZ) and was calculated as the mean value of the mean values of high tide during February and August (2.9 and 3.1 m above HZ, respectively);
- MLW – Mean low water, is considered -1 m below MSL (1 m above HZ) and was calculated as the mean value of the mean values of low tide during February and August (0.9 and 1.1 m above HZ, respectively);
- EHWS – Extreme high water spring, is considered 1.75 m above MSL (3.75 m above HZ) and was calculated as the mean value of the highest tides registered during February and August (3.7 and 3.8 m above HZ, respectively);
- ELWS – Extreme low water spring, is considered -1.65 m below MSL (0.35 above HZ) and was calculated as the mean value of the lowest tides registered during February and August (0.3 and 0.4 m above HZ, respectively).

For all transects, profiles were made based on the distances calculated from the geographic coordinates and elevation, starting from the most distant point from the river water.

2. 4. Laboratory methods

2. 4. 1. Sample preparation for microfauna analysis

2. 4. 1. 1. Sampling for microfauna analysis: Cores were opened and sediment samples taken on the same day or on the day immediately after sampling. The cores were sawn in half longitudinally. A sample of 10 cc of the first centimeter of sediment was collected in a labelled plastic jar. Both halves of each core (pseudoreplicate) were archived in the freezer at –20º C. Each 10 cc pseudoreplicate was processed individually and only after counting and identification the data was combined.

Pseudoreplicates collected during summer were combined in the laboratory. From each pseudoreplicate 10 cc of sediment was collected and mixed in a plastic jar, amounting to a 20 cc sample.

2. 4. 1. 2. Fixing and staining: Immediately after sub-sampling, the samples were fixed and stained, and then left to rest for two days before washing (Scott, personal communication, 2007). Formalin at 4% (100 ml formalin at 38% in 900 ml of distilled water) was used to fixate the foraminiferers protoplasm. In order to neutralize formalin's acidity, which may lead to the dissolution of the carbonated tests, borax (sodium borate) was added to the solution (Eleftheriou and Moore, 2005) at a rate of ¼ of a teaspoon per liter of 4% formalin (Nagorsen and Peterson, 1980).

With the aim of distinguishing living from dead microfauna, the protein stain Rose Bengal was added to the fixing media in a concentration of 1g/L. Rose Bengal adheres to proteins in the protoplasm of the foraminifera, giving it a fuchsia color which enables living tests to be distinguished from dead tests. This method was pioneered by Walton (1952) and is favoured by the majority of benthic marine ecologists and paleoceanographers because it is simple, fast and inexpensive (Bernhard et al., 2006). The available alternatives were not considered adequate for addressing the objectives of this study. Bernhard (2000), in an extensive review, points out that the disadvantages of Rose Bengal include: (1) staining the necrotic cytoplasm of recently dead individuals; (2) staining bacteria or symbiotic algae attached to or located inside the foraminifer test; (3) occasional failure to stain the protoplasm of living organisms; and (4) the stain may be difficult to observe in porcelaneous forms and some agglutinated forms (Bernhard, 2000; Scott et al., 2001). In case of (1) and (2), there is a possibility of overestimating the real number of living individuals; in the case of (3) and (4), underestimation may result. In the present work, the first disadvantage seems irrelevant as the main objective is to identify the living population of benthic foraminifera and thecamoebians typical of each season, in this case winter and summer. Since each season lasts around three months and, during that time, weather conditions evolution is gradual, it is assumed that the foraminifera/thecamoebian population at the time of collection will be substantially alike the living population some days or weeks before and after. To mitigate against the effects of some of the disadvantages mentioned, counting and identification were performed with extra care. Whenever possible, the samples were counted in liquid suspension, which favors the observation and identification of the stained parts, even in porcelaneous and agglutinated tests. Only the individuals with at least three contiguous stained chambers were classed as alive.

2. 4. 1. 3. Washing, sieving and preservation: The fixed sample was poured over a column of three sieves of varying mesh sizes, 500, 63 and 45 µm, resulting in three different fractions for later analysis. Each fraction was carefully washed with running water from a small diameter hose. The sample was considered washed when the percolation water came out clear.

The fraction >500 µm includes material considered extraneous to the observation, counting and identification of microfauna, but can be important for understanding the sedimentary context of the sample. It may contain gravel, bioclasts, vegetal remains (leaves, rhizomes, seeds, etc.), marine meiofauna, insects, algae and, sometimes, some large foraminifera such as *Trochammina inflata*, *Elphidium crispum* and Miliolids.

The 63–500 µm fraction was the target for the study of both foraminifera and thecamoebian. When this fraction was rich in debris, as was commonly the case in the samples from high marsh, an extra decantation was needed to remove excess material. The decantation product was collected and stored in ethanol and later checked for microfauna content. Some lighter tests, such as juvenile forms, inner linings, some individuals of the genus *Jadammina, Deuterammina, Lepideuterammina, Leptohalysis,* etc, and thecamoebians were observed in this material.

The 45–63 µm fraction was initially the target fraction for thecamoebian analysis, but was later discarded for two main reasons: the stereo microscope available for the identification was not powerful enough for their identification and the fraction 63–500 µm proved to be very rich in thecamoebians, with sufficient abundance to achieve statistically valid counts. This fraction was nevertheless described. It was common to find among the thecamoebians some juvenile forms of foraminifera, several species of living and dead diatoms, *Pinus* pollens, spores, and *Pediastrum* green algae, among others.

After washing, each individual fraction was observed in water under a stereo microscope, briefly described and stored in ethanol for further observation and counting.

Samples of the 63–500 µm fraction can be divided into two different groups: sandy samples, usually with few foraminifers, and samples with high content of debris of organic matter usually with large numbers of foraminifera. Two different methods of processing were used for these sample types: microfauna from the first group of samples were concentrated by densimetric floatation in carbon tetrachloride (CCl_4) and samples from the second group were divided using a precision plankton splitter (Scott and Hermelin, 1993).

2. 4. 1. 4. Densimetric floatation in carbon tetrachloride (CCl_4): The aim of this technique is to separate the foraminifera and thecamoebian tests from the rest of the sample using a heavy liquid. It is especially suitable when there is little organic debris, a great quantity of sand and few individual tests. With this technique the samples are observed and counted on dry environment.

Procedure: After washing and preliminary observation, the sandy samples were separated and placed in a greenhouse to dry completely. When dried, the material was handled with gloves in a fume-hood (due to the hazardous nature of CCl_4). For each floatation, the dried sample was poured into a 250 ml measuring cylinder, containing around 50 ml of CCl_4. The lighter material remained on the CCl_4 meniscus, which was then poured through a filter mounted over an Erlenmeyer flask, thus retaining the target material for analysis and the CCl_4 solution for recycling. The paper was folded and left on the fume-hood to dry.

2. 4. 1. 5. Precision splitting: This technique was adopted for samples rich in organic material, like those of the muddy salt marsh, which should not be dried (Scott *et al.*, 2001). It was also useful when great numbers of foraminifera were observed, enabling the sample to be split into several (often 6–8) equal parts with less than 5% error (Scott and Hermelin, 1993).

Procedure: The apparatus consists of a column, 40 cm height, and a base divided in 8 equal parts. The column was filled with water and, immediately before pouring in the sample, the water was agitated to promote homogeneous sedimentation of the material. After one hour of sedimentation, the water in the column was drained out and the column detached from the base. Each of the 8 subsamples was observed individually until a statistically valid minimum count was reached.

2. 4. 1. 6. Sorting and counting: A stereo microscope Olympus SZX7 with a total magnification of 112x was used to count and identify microfauna. A 10/0 brush was used to handle the tests. The brush was also fitted with a syringe needle, replacing the probe traditionally used for sorting. In the majority of the samples all the counted individuals were removed and glued to foraminifera slides. Conventional hair gel was used as glue, offering a very practical and inexpensive solution as it requires no preservatives, is pre-prepared and easily dissolved in water.

In a population of 300 individuals there is a 95% probability of finding all the species that represent at least 1% of the total assemblage of benthic foraminifera (Wright and Hay, 1971 in Fatela, 1995). Counting more than 300 individuals does not increase significantly the reliability of the studies (Phleger, 1960 in Fatela and Taborda, 2002).

Furthermore, it is well known among ecologists and paleoceanographers that counts of 300 individuals are often difficult to achieve, either because counting task is extremely time consuming, or simply because of low concentrations of tests, especially when working with modern and paleo-assemblages from estuaries. Fatela and Taborda (2002) tested the validity of samples with only 100 individuals through the comparison of 4 samples with populations of 100 and 300 individuals. The results show that the most abundant species retain their relative importance whichever count is used. They showed that the probability of ignoring rare species (<5%) using a minimum count of 100 individuals is 0.7%, when compared to a minimum count of 300 individuals. Most foraminiferal studies only analyze the species representing at least 5% of the assemblage because the primary concern is with changes of the more abundant species whose signal is stronger than the uncertainties of counting (Fatela and Taborda, 2002).

In the present study, it was possible to reach ≥300 individuals in total foraminifera assemblage (living + dead) per 20 cc in the majority of samples. Lower counts were obtained from a few winter samples: Aouro (279 individuals), VRSA1 (183) and E2 (126), and some of the summer samples: LAR (111), FO (180) and VRSA1 (144). All the samples were retained for future statistical analysis (Table III and IV in Appendix 4) based on the minimum threshold for statistical significance of 100 individuals (Fatela and Taborda, 2002).

Reaching a statistically valid minimum count was more difficult when considering only foraminifera living tests. Despite the same sampling technique and similar counting effort, there were large discrepancies in faunal densities among samples (15 to 76224 individuals/20cc in winter and 2 to 16576 in summer). Considering both seasons, in 15 of the 49 samples (30.6%) had counts below 100 individuals. To avoid loss of information, an exceptional threshold of 50 live individuals was established concerning statistical significance. Seven of the 49 samples (14% of the total samples) with less than 50 individuals were discarded from the data matrix for statistical analysis (in grey in Table I and II from Appendix 4).

Thecamoebians assemblages were mainly allochthonous, with few living representatives, collected at the same places as foraminifera. Thecamoebian biocenosis study was out of the scope of the present study and only the total assemblage (living + dead per 20 cc of sediment) was analyzed. Usually it was very easy to reach more than 300 individuals but some samples from the upper zones of the marsh were generally impoverished in thecamoebians. For Constancy calculation, only samples with more than 50 individuals were used. The remaining statistics, which included faunistic density (Ni, number of individuals/20 cc of sediment), number of species (S) and multivariate analysis based on absolute frequencies, all samples were included. All these data plus relative frequencies and species constancy can been seen in Table XIV and XV, Appendix 4.

2. 4. 2. Granulometry

The majority of the sediment samples collected along the Guadiana estuary ranged between very fine sands to fine silts (Table I and II, Appendix, 6), although some downstream samples revealed a coarser nature that required different methods. For each season set, the samples were divided into *fine samples* and *mixed samples* according to visible granulometry. Prior to quantitative granulometric analysis, organic matter content was removed with H_2O_2 (33%).

2. 4. 2. 1. Preparation of Fine Samples: After organic matter removal the samples were diluted in a solution of sodium hexametaphosphate (1g/L), which deflocculates the sample. The samples were left to settle for at least 72 h, after which superfluous water was decanted off. The remaining aqueous mud was kept for laser diffraction analysis.

2. 4. 2. 2. Laser diffractometry grain-size analysis: This method is based on the principle that particles of a given size diffract light at a given angle, the angle increasing with decreasing particle size. In this technique, a narrow beam of monochromatic light is passed through a sample cell containing an upward moving suspension of particles. The diffracted light is focused onto detectors, which sense the angular distribution of scattered light intensity from which the size distribution is computed (Ramaswamy and Rao, 2006) on the basis of volume percentages, corresponding to each size fraction.

In the present study, the size distribution of the fine grained samples was measured with a Malvern Mastersizer X laser particle-size analyzer, which operates in an overall size range of 0.1 – 300 microns. Particle-size distributions are calculated by comparing a sample’s scattering pattern with an appropriate optical model using a mathematical inversion process, in this case the Mie Theory (Malvern, 1960–2011) which is the recommended model for samples with particles smaller than 50 µm (ISO13320 – 1999 in Kippax, 2005).

Laser diffraction results can be very accurate if standard operating procedures are used. It is also a superior method when considering speed and ease of use (Malvern, 1960–2011; Ramaswamy and Rao, 2006). Malvern data can be displayed in 11 standard views. In the present study the format *Result 1 – Analysis Sizes* was chosen and gives us: the measurement of 45 size bands (0.313 – 301.68 µm); the *D[4,3]* or volume mean diameter; the *D[3,2]* or surface area mean diameter, also known as the *Sauter* mean; and various standard “percentile” readings from the analysis:

- *D(v, 0.5)* is the particle size for which 50% of the sample is smaller and 50% is larger than this size. This value is also known as the Mass median diameter (MMD).

- *D(v, 0.1)* is the particle size for which 10% of the sample is below this size.
- *D(v, 0.9)* gives a particle size for which 90% of the sample is below this size.

2. 4. 2. 3. Preparation of Mixed samples: For each season set of samples, only 4 samples were considered mixed, i.e. containing both, fine and coarse sediments. A sub-sample of *ca* 100 g was weighed and organic matter removed by H_2O_2. The samples were washed and separated using a 63 µm sieve, resulting in a coarser fraction (> 63 µm) and a finer fraction (< 63 µm). The coarser fraction was dried at 60º C in an oven and weighed. The finer fraction was kept with the resulting washed water and left to settle for at least 72h. After decantation the excess water was drained out.

2. 4. 2. 4. Dry sieving: This technique was applied only to the coarser fraction (> 63 µm) of mixed samples. Dry sieving was carried out using an analytical sieve shaker (Retsch AS 200 base, for 10 minutes at 50% amplitude), with a set of stainless steel sieves (mesh sizes: 63, 90, 125, 180, 250, 355, 500, 710, 1000, 1400, 2000, 2800, and 4000 µm). The quantity of sample retained in each sieve was weighed and converted to percentages of the total sieved weight, which represent the granulometric distribution.

2. 4. 2. 5. Pipette method: This technique based in the Stokes law for silt and clay fractions, was applied only to the finer fraction (< 63 µm) of mixed samples. The quantity of fine material of each dimensional fraction is determined based on its sedimentation velocity, evaluated by aliquot pipette sampling.

After decantation, the mud fraction was suspended in 1000 ml of distilled water plus sodium hexametaphosphate (1g/L) in a measuring cylinder. After homogenization, mass percentages of the silt and clay fractions were measured at combinations of time and depth corresponding to particle diameters: total, 44, 31, 22, 16, 8, 4 and 2 µm (Lewis and McConchie, 1994a).

The weight of each dried subsample is representative of the proportion of the total mud fraction remaining in suspension above a specified depth at a specified time. Thus, each subsample measures the proportion of total mud that is finer than the size that will have settled to the specified depth in the specified time (Lewis and McConchie, 1994b). Once temperature affects the viscosity of water and therefore settling velocities, a correction of the depth of withdrawal must be considered. In the present work, in the withdrawal moment for all samples, the temperature varied between 20.0–20.2º C. For depths of pipette insertion for different temperatures, see Lewis and McConchie, (1994b).

2. 4. 2. 6. Statistical parameters: Statistical analyses of grain size were performed in GRADISTAT, a computer program written in Microsoft Visual Basic, integrated into a Microsoft Excel spreadsheet. The program provides rapid calculations (approximately 50 samples per hour) and accepts results from any of the standard techniques previously described. From the options provided by the software, the method chosen was Folk and Ward measures, based in a Log-normal distribution (geometrically) and expressed in metric units (since the phi (Φ) scale is seldom used among biologists and the results are difficult to visualize) and provides the most robust basis for routine comparisons of sediments with variable composition (Blott and Pye, 2001).

After the input of granulometric data, either in percentages or in mass, the program is able to calculate the most important parameters for sediment description. The statistical parameters are also related to descriptive terms and the mean grain size was described using a modified Udden–Wentworth grade scale (Blott and Pye, 2001) (Table I).

- **Mode(s) (Mo)**, represents the grain-size diameter with the highest frequency in the distribution. Mature sediments and reworked deposits, (e.g. beach and dune sands), are usually unimodal, while immature sediments, with less reworking, are bi- or poli-modal;
- **Mean (M_G)**, gives the medium distribution of particle size and is obtained from a size group instead of a single point;
- **Sorting (σ_G) (standard deviation)** is related to the competence of the different geological processes in selecting a grain of a certain size. Well sorted or well classified sediment refers to sediment with low dispersion in its granulometric values, while poorly sorted sediment has a larger spread around the mean grain size. The sorting value can indicate the energy in an accumulation basin, the textural maturity degree of a deposit and the occurrence of mixed populations;
- **Skewness (Sk_G)**, represents the degree of deviation from a normal distribution. A curve is symmetrical when the mode values, mean and median are all in agreement. In this case, the simple frequency curve will take the classic bell shape, corresponding to a normal Gauss curve when skewness is zero. In practice this seldom occurs because normally there is a deviation toward the coarser or the finer grains, negative or positive skewness, respectively;

Table I – Statistical formulae in the calculation of grain size parameters, descriptive terminology and size scale (adapted from Blott and Pye, 2001).

Mean	Standard deviation
$M_G = \exp \frac{lnP_{16} + lnP_{50} + lnP_{84}}{3}$	$\sigma_G = exp\left(\frac{lnP_{16} - lnP_{84}}{4} + \frac{lnP_5 - lnP_{95}}{6.6}\right)$
Skewness	**Kurtosis**
$Sk_G = \frac{lnP_{16} + lnP_{84} - 2(lnP_{50})}{2(lnP_{84} - lnP_{16})} + \frac{lnP_5 + lnP_{95} - 2(lnP_{50})}{2(lnP_{25} - lnP_5)}$	$KG = \frac{lnP_5 - lnP_{95}}{2.44(lnP_{25} - lnP_{75})}$

Sorting (σ_G)		Skewness (Sk_G)		Kurtosis (K_G)	
Very well sorted	<1.27			Very platykurtic	< 0.67
Well sorted	1.27 – 1.41	Very fine skewed	-0.3 to -1.0	Platykurtic	0.67 – 0.90
Moderately well sorted	1.41 – 1.62	Fine skewed	-0.1 to -0.3	Mesokurtic	0.90 – 1.11
Moderately sorted	1.62 – 2.00	Symmetrical	-0.1 to +0.1	Leptokurtic	1.11 – 1.50
Poorly sorted	2.00 – 4.00	Coarse skewed	+0.1 to +0.3	Very leptokurtic	1.50 – 3.00
Very poorly sorted	4.00 –16.00	Very coarse skewed	+0.3 to +1.0	Extremelyleptokurtic	> 3.00
Extremely poorly sorted	>16.00				

Grain size		Descriptive terminology	
Phi	mm/µm		
-11	2048mm	Very large	Boulders
-10	1024	Large	
-9	512	Medium	
-8	256	Small	
-7	128	Very small	
-6	64	Very coarse	Gravel
-5	32	Coarse	
-4	16	Medium	
-3	8	Fine	
-2	4	Very fine	
-1	2	Very coarse	Sand
0	1	Coarse	
1	500 µm	Medium	
2	250	Fine	
3	125	Very fine	
4	63	Very coarse	Silt
5	31	Coarse	
6	16	Medium	
7	8	Fine	
8	4	Very fine	
9	2	clay	Clay

- **Kurtosis (K_G)**, represents the 'acuteness' of the distribution curve. It should be leptokurtic if it is very acute, mesokurtic if it has a normal distribution, or platykurtic if it is flat;
- A range of cumulative percentile values (the grain size at which a specified percentage of the grains are coarser), namely, D10, D50, D90, D90/D10, D90–D10, D75/D25 and D75–D25.

2. 4. 3. Bulk organic matter analysis:

Analysis of total organic carbon (TOC), total inorganic carbon (TIC) and total nitrogen (TN) were performed in the Faculty of Sciences and Technology, University of the Algarve, using gas chromatography (GC). According to CSIRO (2000), this method offers more reliable means for analyzing organic matter (OM) content in sediments than the method of weight loss on ignition (LOI), which can seriously overestimate OM content. A total of 49 samples collected in the same places as the microfauna samples were subjected to elemental analysis. Prior to the analysis the samples were lyophilized in a Thermo Savant MicroModulyo Bench-model freeze dryer and were finely ground and homogenized in an agate 7 Planetary Micromill Pulverisette from Fritsch. Two aliquots of about 20 mg were packed into pewter and silver cups. The aliquots packed in silver cups were decarbonated by using 25% HCl prior to analysis in an EA1108 Carlo Erba C/H/N/S elemental analyzer. The pewter cup aliquots were directly analyzed without any pre-treatment. Both groups of cups were pinched closed before instrumental analysis. TN and TOC content were obtained from direct instrumental reading of a decarbonated sample and TIC content was calculated from the difference between the total carbon (TC) reading of an untreated sample and TOC. C/N ratio was also calculated due to its usefulness as an organic matter (OM) proxy (Meyers, 1994). C/N ratios have been used to distinguish between OM inputs in estuaries, since autochthonous marine organisms rich in protein material have C/N values ranging from 4 to 10, much lower than terrestrial plants (>20) (Meyers, 1994). Nitrogen is also present in inorganic form (IN), mostly as ammonium bound between the lattices of clay minerals, and can be a significant fraction of the TN in marine sediments (Zhiyang and Guodong, 2011). The separation of total organic nitrogen (TON) from bulk sediments is difficult because of the intimate association between OM and inert clay minerals (Zhiyang and Guodong, 2011). In the present work this separation was not made. As a consequence, low values of organic carbon (OC) may have relatively low C/N ratios because the measured N includes both organic and inorganic forms (Stein, 1991; Meyers, 1997). According to Meyers (1997), in sediments having OM concentrations (< 0.3%) the proportion of IN can sometimes be a large fraction of the residual nitrogen and C/N ratios based on residual nitrogen could be artifactually depressed. In the present study only 2 samples collected during winter had OC values lower than 0.3%.

2. 5. Microfauna population data analysis

2. 5. 1. *Incertae sedis*

Due to uncertainties in identifying some of the living and dead forms of the following genera, their taxonomy was not defined to species level: *Amphycoryna*, *Ammobaculites, Ammotium*, *Ammovertelina, Bulimina*, *Cassidulina*, *Discorbis*, *Epistominella, Eponides, Fissurina, Glabratella, Globulina, Haplophragmoides, Neocornorbina, Nodosaria*, *Nonion, Nonionella*, *Oolina*, *Portatrochammina*, *Rosalina*, *Rotalia*, *Stainforthia*, *Hopkinsina*, *Trifarina, Uvigerina* and *Valvulineria*. This problem is most evident among porcelaneous forms, for which ten species were recognized but left in *incertae sedis*. In the Atlas some names are suggested. The presence of very small, broken and dissolved tests necessitated the creation of artificial indeterminate groups, namely: calcareous indeterminate, agglutinated indeterminate, calcareous linings and miliolids. The first three groups were used in calculations of quantity indexes but not in quality indexes. The miliolids group was the only artificial (including more than one species) group used in statistical analyses because it was very abundant in some samples, for instance in LEZ2 (winter) where it represents more than 82% of the total living species, and because its ecological significance is known to a certain extent. Identification to a lower taxonomic level than the order was impossible because of the very tiny sizes (juvenile forms).

Planktic foraminifers were absent among the living communities but were present in the total assemblage (living + dead individuals). These forms were rare and generally very small. They were combined in an artificial group named Planktic.

The range of sizes in which thecamoebians varies, the rarity and the fragility of the test of some groups, hampered the identification of a few specimens to species level. Some species of the following genera were left in open nomenclature: *Arcella, Cyclopyxis, Lesquereusia* and *Pyxidicula*. A plexus of very small-sized individuals were included in an artificial group named Indeterminate.

2. 5. 2. Abundance

The absolute abundance of a species is the total number of individuals of the same species of the total counted individuals in a sample. Is the first data resulting from the sorting and counting of a sample, providing the basis for calculation of relative abundance. The latter expresses the importance of each species in the whole sample and is calculated according to the following equation:

$$Aspi = xspi * 100/Ni$$

Where *Aspi* is the relative abundance of species *i*, *xspi* is the number of individuals of species *i* in the sample and *Ni* is the total number of individuals in the sample.

2. 5. 3. Faunistic density

In the present work, faunistic density expresses the number of individuals (Ni) per 20 cc of sediment. This value was determined for the living, total (living + dead) and fossil assemblages of benthic foraminifera, total assemblage of thecamoebians and total assemblage of tintinnids.

2. 5. 4. Specific dominance

This is the trend that some species show in comprising the majority of identified individuals in the sample and is usually evaluated as the percentage of the species in the sample (Boltovskoy and Totah, 1985). There is no standard threshold value for dominance (Boltovskoy and Totah, 1985) although the later authors propose 10% as the critical value for dominance recognition.

In the present work specific dominance was divided in 4 classes of dominance:

Dominant: > 20%; common: 10–20%; accessory: 5–10%; and rare: < 5%

2. 5. 5. Species constancy

The constancy of a species expresses the persistence of its presence in a certain sample set. The constancy of the most representative species was evaluated on spatial and temporal scales and was calculated in percentages using the expression suggested by Dajoz (1978):

$$C = n/N * 100$$

where n is the number of samples where the species occurs and N is the total of samples collected.

Species were considered constant when occurring in more than 50% of the samples (Dajoz, 1978) with statistical meaning.

2. 5. 6. Agglutinated/calcareous ratio (A/C %)

Agglutinated foraminifera, which have the least demand for carbonate as a shell or wall material, are most abundant under hyposaline conditions, typical of marginal marine environments, and below the carbonate compensation depth (CCD). The miliolids are largely confined to hypersaline conditions (tropical and shallow water) and hyaline foraminifera are found everywhere above the CCD, especially in shelf and bathyal deposits (Douglas, 1979). The transition from brackish to a normal marine near-shore fauna is generally marked by increases in the proportion of calcareous species (Sen Gupta, 1999).

Moreover, the $CaCO_3$ availability increases with increasing temperature and salinity (Buzas, 1989). Thus the ratio of agglutinated to calcareous species (A/C%) is proxy for salinity (Douglas, 1979) and $CaCO_3$ availability.

In the present work the A/C (%) ratio was calculated for each sample according to the following equation:

$$A/C\,(\%) = A * 100/Ni$$

Where A is the total number of agglutinated tests and Ni is the total number of individuals.

2. 5. 7. Diversity

One method of analyzing taxonomic diversity is the application of indexes, which may be ratios or other mathematical expressions that quantify relations between species and importance (Odum, 1997). Diversity has two components: the number of species or ‘richness’ and the homogeneity of species distribution or ‘evenness’, described by the species’ relative abundances (Lin, 1992; Magurren, 1991).

2. 5. 7. 1. Shannon Index of Diversity (Hs): The Shannon Index of Diversity (*Hs*), also called Shannon-Wiener information function (Buzas, 1979), was the diversity index adopted in the present study. It has the advantage of considering the relative abundances of species. It is not dependent on a mathematical model and allows the comparison of diversity between samples of different sizes (Odum, 1997). The *Hs* value is essentially determined by the relative abundances of common species and is little affected by the contribution of rare species (Gibson and Buzas, 1973). The Shannon index assumes that all the individuals are randomly sampled from an indefinitely large population and that all species are represented in the sample (Pielou, 1975 in Magurren, 1991). Its formula is:

$$Hs = -\sum pi * \ln pi$$

Where *pi* = ni/N is the proportion of the different species *i* in the sample, N is the total number of individuals in the sample, *Hs* is the diversity and *s* is the total number of species *i* (Magurren, 1991).

Shannon diversity is highest when all the species in an assemblage present the same relative abundance: *Hs* = ln*S*. *Hs* usually vary between 1.5 and 3.5 and seldom exceed 4.5 (Margalef, 1972 in Magurren 1991). Shannon diversity indexes were calculated in the software for multivariate analysis PRIMER-E, (Plymouth Routines in Multivariate Ecological Research) version 5.2.0 (2001) for Windows.

2. 5. 8. Dominance index (c)

In nature, the degree of dominance is concentrated in one, several or many species and can be expressed by an index that sums each species' importance in relation to the community as a whole (Odum, 1997). In communities from extreme environments (e.g. desert, tundra, etc.) the controlling influence is divided among a small number of species. Thus, the dominance index will be higher the more stressed the environment (Odum, 1997). This index can be calculated as follows:

$$c = \sum \left({}^{ni}/_{N} \right)^2$$

Where *c* is the "dominance concentration";
ni is the importance value for each species (number of individuals); and
N is the total importance values (Odum, 1997).

2. 5. 9. Multivariate analysis

2. 5. 9. 1. Exploratory statistics:

Species elimination: Non-constant species with abundances lower than 5% were eliminated from the original data matrix. Rare species can represent a problem for multivariate analysis. The similarity between rare species has low statistical significance. In most cases, these species occur in isolation, are distributed more or less randomly, and may lead to extreme values of (dis)similarity that, if left in the matrix, can distort de overall classification. Likewise, indeterminate taxa were eliminated from the original data matrix. The inclusion of these groups can bias the overall ecological scenario. In the present work, 26 rare species and 3 indeterminate groups were eliminated from the living foraminifera assemblage matrix for winter sampling, resulting in a matrix with only 18 species (rows). From the equivalent summer matrix, 29 rare species and 3 indeterminate groups were eliminated, resulting in a matrix with only 15 species. Although this seems a remarkable reduction, the species considered valid (>5%) always represent more than 81% of the total taxa in winter (mean: 93.1%/sample) and more than 82.7% in summer (mean: 93.6%/sample).

In the thecamoebians winter data matrix, 8 rare species and 1 indeterminate group were eliminated, resulting in a matrix of 10 species. From the equivalent summer matrix 9 rare species and 1 indeterminate group were eliminated, resulting in a matrix of 8 species. The species that remained in the winter and summer data matrices represented usually more than 90% of the total taxa (mean in winter: 92.1%; mean in summer: 94.7%).

See procedure for paleoassemblages in Chapter 6, item *6. 2. 2. 2*.

Standardization and transformation: Response variables (species) were expressed in relative abundances, a common standardization for samples with much skewed faunistic densities. The assumptions of normal analysis of variance must be satisfied so that parametric statistical methods may be applied to benthic data (Downing, 1979). In the present study, the number of samples is relatively small and foraminifera distribution density curves vary widely. In such cases, a data transformation is recommended (Downing, 1979) to remove correlation between the variance and the mean (in ecological data variance often increases with the mean). Transformation reduces the effect of skewness in the distribution. Square-root ($\sqrt{y}$) transformation was chosen as it allows not only the dominant species contribute to similarity, but also species of intermediate abundance play a part (Clarke and Warwick, 1994).

Explanatory variables: Also called predictors or independent variables, they are the environmental variables and represent the knowledge that we have about our samples and that can be used to predict the values of the response variables (Leps and Smilauer, 2003), which, in the present case, are species abundances. Autocorrelation is a very general property of ecological variables and, indeed, of most natural variables observed over time series (temporal autocorrelation) or over geographic space (spatial autocorrelation). Because it indicates lack of independence among the observations, autocorrelation creates problems when attempting to use tests of statistical significance that require independence of the observations (Legendre and Legendre, 1998). In the present work, three sets of environmental variables were considered for multivariate analysis: spatial variables: Latitude (Lat), Longitude (Long) and elevation above MSL (Elev); chemical variables: salinity (Sal), total organic carbon (TOC), total inorganic carbon (TIC), carbon/nitrogen ratio (C/N), sediment pH (pH_{sed}), dissolved oxygen (DO); physical variables: granulometric mean (Gmean), sand, mud, gravel and temperature in Celsius degrees (T). The autocorrelation degree between environmental variables was inferred through Pearson (r) and Spearman (R) correlation coefficients and through forward selection in redundancy analysis, provided by the multivariate analysis software (see further on).

Gradient length: When using ordination methods, it is necessary to first decide which response model (species response to environment gradients being estimated) should be used. Two models are frequently used: linear response and unimodal response. When the covered portion of the environmental gradient is short, most of the species will have their distributions truncated, and estimates of species optima will be biased. The longer the axis, the more correctly species optima will be estimated. It is expected that, the more homogeneous the data, the shorter the gradient (Leps and Smilauer, 2003). To estimate heterogeneity in community composition, Leps and Smilauer (2003) suggest performing a detrended correspondence analysis (DCA) for indirect gradient analysis, which gives the gradient length for each ordination axis. If the gradient length is larger than 4 standard deviations (SD), then unimodal methods are more appropriate. If the gradient length is shorter than 3 SD, a linear method is a better choice. In the range between 3 and 4 SD, both types of ordination methods work reasonably well. DCA analysis was performed on both winter and summer living foraminifera datasets and to thecamoebian, tintinnid and foraminifera total assemblage dataset, using CANOCO for Windows, version 4.56 (ter Braak and Smilauer, 1997–2009).

Final data set: The present dataset for winter foraminifera living assemblages consists of two data matrices: a biotic matrix of 19 samples and 18 species and an abiotic matrix of 19 samples and 14 environmental variables. For summer, the biotic matrix includes 23 samples and 15 species and the abiotic matrix has 23 samples and 14 environmental variables. The data set used in multivariate analysis of thecamoebian, tintinnid and foraminifera total assemblages consists in two data matrices: a biotic matrix of 47 samples (winter + summer) and 33 species (23 foraminifera, 8 thecamoebians and 2 tintinnids) and an abiotic matrix of 47 samples and 10 environmental variables.

2. 5. 9. 2. Similarity: Before multivariate techniques were applied (clustering and non-metric multidimentional scaling), similarity matrices between the samples (Q-mode) were calculated. There are many ways of defining similarity, each giving a different weight to different aspects of the community (Clarke and Warwick, 1994). The main concern in choosing a suitable similarity coefficient for the present data was to rise above the double zero problem. In species datasets it is common to have a large quantity of zeros in the matrix. The existence of doubles zeros is not desirable when determining similarity, as it may be interpreted as an indication of resemblance among samples (Borcard et al., 2011; Clarke and Warwick, 1994). This is easily understandable if one consider the example of Field et al. (1982): “Taking account of joint absences has the effect of saying that estuarine and abyssal samples are similar because both lack outershelf species”.

Bray-Curtis Similarity Coefficient: Of the numerous similarity coefficients suggested in the literature, the Bray-Curtis Coefficient offers the most balanced compromise for count data that have been moderately (e.g. √y) transformed (Clarke and Warwick, 1994). It is not affected by joint absences and is therefore sufficiently robust for marine data, yet the commoner species are generally given greater weight than the rare ones (Field et al., 1982). According to Field et al. (1982), the Bray-Curtis measure has the formula:

$$\delta_{jk} = \frac{\sum_{i=1}^{s} \left| Y_{ij} - Y_{ik} \right|}{\sum_{i=1}^{s} \left(Y_{ij} + Y_{ik} \right)}$$

This may be transformed into a similarity coefficient by adding the complement:

$$S_{jk} = 100\left(1 - \delta_{jk}\right)$$

Where δ_{jk} – measures the dissimilarity between the *jth* and *kth* samples summed over all species and ranges from 0 (identical scores for all species) to 1 (no species in common);

y_{ij} – score for the *ith* species in the *jth* sample;

y_{ik} – score for the *ith* species in the *kth* sample;

S_{jk} – measures the similarity between *j* and *k* (Clarke and Warwick, 1994).

In the present study, similarity measures in Q mode were performed using Bray-Curtis similarity coefficient in √y-transformed data.

2. 5. 9. 3. Classification analysis: The aim of classification is to obtain groups of objects (samples or species) that are internally homogeneous and distinct from the other groups. Homogeneity can be interpreted as representing similar ecological behavior, as reflected in the similarity of species distributions (Leps and Smilauer, 2003).

Agglomerative hierarchical classification (cluster analysis): The aim of this method is to form a hierarchical classification (groups containing sub-groups), usually represented as a dendrogram. First, a similarity matrix is calculated for all pairs of objects and then those objects are clustered through an algorithm so that, after each clustering, the newly formed group is considered to be an object and the similarities of the remaining objects to the newly formed group are recalculated (Leps and Smilauer, 2003).

Agglomerative method: The chosen method was UPGMA (Unweighted Pair Group Using Arithmethic Avarages), also known as Group Average Linkage (Leps and Smilauer, 2003; Clarke and Warwick, 1994), which joins 2 groups of samples together at the average level of similarity between all members of one group and all members of the other (Field et al., 1982). According to Gauch (1982), this method maximizes the relation between the original symmetric matrix and the final dendrogram. Krebs (1999) gives the UPGMA algorithm as the following formula:

$$S_{JK} = \frac{1}{t_J t_K (\sum S_{JK})}$$

Where S_{JK} – measures the similarity between the clusters *J* and *K*;

t_J – is the number of samples in cluster *J* 'Σ 1≤;

t_K – is number of samples in cluster *K* 'Σ 2≤.

Cluster analyses, with Group Average Linkage, was applied to the Q mode symmetrical matrices to produce cluster dendrograms. All steps were performed in PRIMER-E, version 5.2.0 (2001) for Windows.

2. 5. 9. 4. Ordination techniques: Contrary to most clustering techniques, which aim at revealing discontinuities in the data, ordination mainly displays gradients (Legendre and Legendre, 1998). The aim of ordination methods is to represent the data in a reduced number of orthogonal axes, constructed in such a way that they represent, in decreasing order, the main trends in the data (Legendre and Legendre, 1998).

Non-metric Multidimentional Scaling (NMDS): In conjunction with cluster analysis, this ordination method was applied to highlight the gradient and individual relationships that are lost in dendrogram representations. Among indirect gradient analysis methods, NMDS is one of the best rated by scientists because it is simple, flexible and has few assumptions (Clarke and Warwick, 1994; Field et al., 1982). It analyses a matrix of dissimilarities between the samples and aims to find a configuration of these samples in a k-dimensional ordination space, where k is determined a priori, so that their distances in ordination space correspond to dissimilarities. A statistical termed 'stress' is designed to measure the 'lack of fit' between distances in ordination space and dissimilarities and, according to Leps and Smilauer, (2003), is calculated by the following formula:

$$stress = \Sigma\left[d_{ij} - f(\delta_{ij})\right]^2$$

Where d_{ij} is the distance between sample points in the ordination diagram;
δ_{ij} is the dissimilarity in the original matrix of distances calculated from the data;
f() is a non-metric monotonous transformation.

Thus, the 'correspondence' is defined in a non-metric way, so that the method reproduces the general rank-ordering of dissimilarities (not exactly the dissimilarities values) (Leps and Smilauer, 2003).

NMDS analysis was performed using WinKyst (a separate Windows program which provides NMDS and works with CANOCO, see below) and the same symmetrical matrices as in cluster analyses (Species data √-transformed and a Bray-Curtis similarity coefficient). The final solution was presented through a principal components analysis (PCA) in CANOCO for

Windows, version 4.56 (ter Braak and Smilauer, 1997–2009) and the final diagrams were plotted using CanoDraw version 4.14 (Smilauer, 1999–2009).

Redundancy Analysis (RDA): DCA showed gradient lengths to be < 4 SD for all datasets analyzed (see results of Chapter 5 and 7), indicating that most species responses can be approximated with a linear model. RDA is a constrained form of the linear ordination method of PCA and was chosen to explore the species-environment relationships. The aim of constrained ordination, also known as direct gradient analysis, is to find the variability in species composition that can be explained by the measured environmental variables (Leps and Smilauer, 2003). In RDA, as in other constrained methods, the ordination seeks the axes that are best explained by a linear combination of explanatory variables (Borcard, 1992). The number of constrained axes cannot exceed the number of environmental variables, such that when there is only one environmental variable, only the first ordination axis is constrained (Zuur et al., 2007). In the present study, partial RDA was also performed. In partial analysis, the variability in the species composition explained by the covariables is subtracted and a constrained ordination is performed on the residual variability (Leps and Smilauer, 2003). RDA and partial RDA were performed with a focus on inter-species correlations. The species scores were divided by a standard deviation, where the length of each species arrow on the ordination diagram expresses how well the values of that species are approximated. This representation corresponds to a correlation biplot (Legendre and Legendre, 1998) and the abundance of individual species is transformed to a comparable scale (Leps and Smilauer, 2003).

Important steps during RDA:

- *Monte Carlo permutation test:* Tests the significance of the relationship between environmental variables and species composition. The null hypothesis (H_0) states that there is no effect of environmental variables on species composition. The rejection of the H_0 means that at least one of the variables has some effect on the species composition. The meaning of this test is analogous to an overall ANOVA on the total model in a multivariate regression (Leps and Smilauer, 1999). The test randomly re-shuffles (permutes) the samples in the environmental data table, while keeping the species data intact. Any combination of the species and environmental data obtained in that way is as probable as the 'true' data set, if the H_0 were true (Leps and Smilauer, 2003). A Monte Carlo Permutation Test was used to test the significance of the environmental variables when using forward selection (manual and automatic) and to test the global model significance without forward selection, with a maximum number of 499 permutations under reduced model (CANOCO default and the safest choice in relation to type I errors – H_0 incorrectly rejected) (ter Braak and Smilauer, 1997–2009).

- *Strength and intercorrelation between environmental variables:* Explanatory environmental variables are usually correlated, and thus colinear, which may bias model parameter estimation (Lobo et al., 2002). A preliminary examination of the strength of each environmental variable and inter-set correlations between the variables and the ordination axes was performed through a constrained RDA, plotted as a simple ordination diagram in CanoDraw.

- *Forward selection:* This was used for ranking environmental variables according to importance for determining the species data or for reducing the initially large set of environmental variables. The variables were selected automatically and manually. In automatic selection, the K best variables were selected sequentially on the basis of maximum extra fit (ter Braak and Smilauer, 1997–2009) and the marginal and conditional effects of the variables were provided. The marginal effect is the independent effect of each environmental variable in the dataset and the conditional effect is the effect that each variable brings in addition to all the variables already selected (Leps and Smilauer, 2003). In manual selection, the model begins empty and the environmental variables are selected, one by one, for inclusion in the model. At the same time, it is possible to test the statistical significance of the variable, helping to decide whether to include it in the model or not. The same is possible for the remaining variables in order of extra variance that each of them will explain when included in the model. The selection is over as soon as the 'best' among the remaining variables is not significant (ter Braak and Smilauer, 1997–2009; Leps and Smilauer, 2003).

- *Variance inflation factors (VIFs):* As a final screening measure, the VIFs were inspected from the log file for each performed RDA. The VIF value measures how much of the variance of the canonical coefficients is inflated by the presence of correlations among explanatory variables (Legendre and Legendre, 1998), with high VIFs indicating multi-colinearity among environmental variables (ter Braak and

Smilauer, 2002). VIFs with a value > 20 indicate that the environmental variable is nearly perfectly correlated with other environmental variables and should be removed from the analysis (ter Braak and Smilauer, 2002).

- *Variance partitioning:* Any two explanatory variables that are correlated share part of their effect on the species data. This concept forms the basis of the variance partitioning procedure (Borcard et al., 1992). When partitioning variation is used, independent variables are grouped into sets representing broad factors. In this context, variation partitioning is more suitable than analyzing the individual contributions of regressors via their partial correlation coefficients (Peres-Neto et al., 2006). The use of partial RDAs, where some of the environmental variables are treated as covariables, allows the determination of covariance among the groups of the most important variables and, thus, identification of the groups exerting the most influence on species distribution.

- *Correlation biplot interpretation:* In linear methods, species and environmental variables, usually, are represented by arrows and samples by symbols. Vectors show the correlations between an environmental factor and the two ordination axes, where the vector length indicates the strength of this correlation. Vectors pointing in the same direction are highly correlated, orthogonal vectors are not correlated, and vectors in opposing directions are negatively correlated. The ranking of species or sample relative to each environmental variable is determining by projecting the symbol (samples) or arrow tip (species) perpendicularly to the environmental vector. To know the abundance of a species on a determinate sample, the sample symbol is projected in the species arrow. Samples projecting further from zero in the direction of the arrow are predicted to have above-average abundances, while the sample points projecting in the opposite direction are predicted to have below-average values (Leps and Smilauer, 2003). In the RDA performed under the present study, the species arrow bodies are erased and sometimes the tip of the arrow is replaced by a symbol, only due esthetical proposes. The interpretation based on biplot rule is exactly the same.

2. 5. 10. Correlations

2. 5. 10. 1. Normality: The normality of the distributions of the environmental and ecological variables was tested through Shapiro-Wilks W test in STATISTICA, version 7.0

(StatSoft Inc., 2004). In this test, the null hypothesis (H_0) states that the data follow a normal distribution. Where *p*-values were less than a rejection level of 0.05 (95% confidence), the null hypothesis was rejected and the data were assumed *not* to be normally distributed. This step was crucial to verify the possibility of using Pearson Correlation Coefficient, which is the most sensitive and widely used but, the test of significance is based on the assumption that the distribution is normal (StatSoft Inc., 2004).

Results of normality tests on all environmental and ecological variables are presented in Table I, Appendix 9. Of the 47 environmental and ecological variables used on correlations only few were normally distributed. For this reason correlation coefficients in this study were calculated using a Spearman's Rank Correlation coefficient, which does not require data to be normally distributed. Nevertheless, Pearson's Correlation Coefficient was also calculated when both variables were normally distributed and those results were preferable in relation to Spearman R results.

2. 5. 10. 2. Pearson Correlation Coefficient (r): Also known as linear or product-moment correlation, this determines the extent to which values of two variables are "proportional" to each other. The value of the correlation does not depend on the specific measurement units used and assumes that the two variables are measured on at least interval scales. The test of significance is based on the assumption that the distribution of the residual values (i.e. the deviations from the regression line) for the dependent variable y, follows the normal distribution, and that the variability of the residual values is the same for all values of the independent variable x.

2. 5. 10. 3. Spearman Rank Correlation (R): Spearman R is a nonparametric measure of statistical dependence between two variables, that can be thought of as the regular Pearson (r) in terms of the proportion of variability, except that Spearman R is computed from ranks of the measurements and not the actual values (StatSoft Inc., 2004).

The correlation coefficients can range from -1.00 to +1.00. The value of -1.00 represents a perfect negative correlation while a value of +1.00 represents a perfect positive correlation. A value of 0.00 represents a lack of correlation. In the present study intermediate values of Pearson r and Spearman R were classified has weak when < 0.5, moderate from 0.5 to 0.7, relatively strong from 0.7 to 0.9 and strong when > 0.9.

2. 6. Design and image improvement

Several of the illustrations in the present thesis were created in Adobe illustrator (Ai) CS5, version 15.0.0. This software was used to improve the maps created using ArcMap, graphs created using STATISTICA, CanoDraw, PRIMER-E and Psimpoll.

Chapter 3

Study area

Chapter 3

3 – Study area

3. 1. Chapter objectives

In this chapter a general description of the Guadiana River, its basin and estuary is presented, alongside a detailed characterization of the study sites. The information contained herein is derived from reference works and data provided primarily by Portuguese and Spanish scientific public entities, such as the Portuguese Ministry of the Environment (Ministério do Ambiente), Spanish Guadiana Hydrographic Confederation (chguadiana.es - Confederación Hidrográfica del Guadiana), Portuguese Hydrographic Institute (IH), Portuguese National Water Institute (INAG), Portuguese National System of Hydrological Resources (SNIRH), Portuguese Institute for Nature Conservation and Biodiversity (ICNB) and Ramsar - Scientific and Technical Review Panel. Every effort was made to gather data from the Guadiana River and Estuary after 2002, when the Alqueva Dam was commissioned. Nevertheless, the majority of works concerning the Guadiana are based in pre-Alqueva data, even those published after 2002.

Description of the study sites and their halophytic vegetation was made from direct observations and data collection during field trips (see photographs in Appendices 1 and 2).

3. 2. Geomorphological and hydrological characteristics

The Guadiana Estuary is one of the most important mesotidal fluvio-marine systems of the south-western Iberian Peninsula (Morales, 1997; Morales et al., 2006). The river rises in Lagoas de Ruidera in Spain, at 1700 m altitude, and runs 810 km until reaching the Atlantic Ocean, between the towns of Vila Real de Santo António, in Portugal and Ayamonte, in Spain. The Portuguese stretch of the river is 260 km, of which 110 km delimit the Spanish border (Ministério do Ambiente, 1999). Located at 37º and 40º N and 2º and 8º W, its catchment area covers 66 889 km^2, of which approximately one fifth (11 525 km^2) is in Portugal (Brandão and Rodrigues, 2000) (Fig. 3.1), with a mean altitude of 237 meters (Farinha and Trindade, 1994).

At the Portuguese side, the river bed cuts into the Hercynian basement, consisting of Carboniferous schist and greywackes, and follows a N-S course defined during the Quaternary (Vidal et al., 1993).

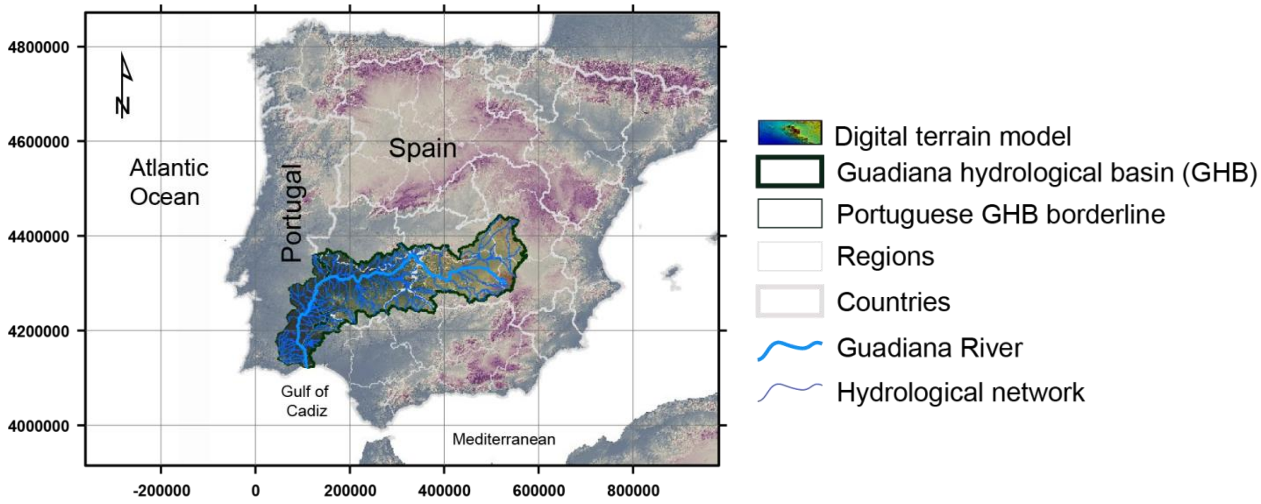

Figure 3.1. Geographical context of the Guadiana River basin in the Iberian Peninsula (Europe). Adapted from chguadiana.es (2012). Coordinate system: Datum ETRS89 UTM Zone 30N.

The estuary is perpendicular to the coast and extends some 79 km inland, beginning 7 km upstream of Mértola and debouching into the North Atlantic at Vila Real de Santo António (Lopes et al., 2003). The estuary's shape is regular with an average width of 200 m at Mértola, progressively increasing to 600 m in the mouth (Lopes et al., 2003) where it cuts into Pliocene sands (Morales, 1995; Morales et al., 2006), forming a wide, funnel-shaped estuary infilled with post-glacial sediments (Boski et al., 2006). Today, the estuary is in an advanced state of sediment infilling, with the formation of a delta at its mouth caused by the interaction of coastal sedimentation processes and a relatively stable sea level (Morales et al., 2006). The Portuguese shore of the estuary comprises a littoral spit with large dunes rising up to 20 m high. On the seaward side of the spit, the gentle sloping coast has a high rate of sedimentation giving rise to lateral growth of the spit in addition to swash bars. On the landward part of the spit, a large saltmarsh area drains directly into the main estuarine channel (Plater and Kirby, 2006; Morales et al., 2006). The Spanish coast consists of a succession of old barrier islands separated by wide marshes. The area comprises: (a) a barrier island, tidal inlets, flood tidal deltas, washover fans, barrier islands, spits, beaches; and (b) a back-barrier area with a bypassing estuary channel, lateral tidal bars, lagoons, tidal creek network, active channel margins, tidal flats and saltmarshes (Plater and Kirby, 2006).

Based on geological, topographical, hydrological and climacteric characteristics the estuary can be divided in three zones (Boski et al., 2006) (Fig. 3.2):

- Upper estuary – located between Mértola and Álamo, this area is subject to salinity variations, such that the water can be either fresh or brackish. Although a tidal influence is present, this area is clearly fluvial;
- Middle estuary – comprises the part of the estuary between Álamo and the mouth of the Beliche Rivulet. This is an area of salinity transition, although a major control is exerted by the tides. Most of the sediments in this area have a fluvial genesis;
- Lower estuary – also called “maritime estuary” with salinity having similar values as sea water. This comprises the widest part of the estuary, located between the Beliche Rivulet and the Guadiana mouth. In this area the tide and wave actions are strong, with considerable sediment movement.

According to recent data provided by the Portuguese Hydrographic Institute (IH, 2012) the mean channel depth varies from 7 meters in the upper part of the estuary to 6.8 meters in the middle part and 4.5 meters in the lower part (Fig. 3.2). The lower part is especially affected by sedimentation that has been occurring since the Alqueva Dam was commissioned in 2002 (Morales, 2008 in ecosfera.publico.pt). Alqueva is the most recent dam to be built on the Guadiana, with the aim of providing water for irrigation of the Portuguese interior. This dam, the largest man-made lake in Europe (Plater and Kirby, 2006), has caused a major decline in water and sediment supply from river to estuary. According to Dias et al. (2004), the volume of impounded water that will never reach the estuary and coastal area is 13 000 hm^3/year. The construction of successive dams along the Guadiana since the 1960s has increased sediment retention, with 75% of the Guadiana catchment (Morales, 1997; Morales et al., 2006) under some form of regulation prior to 2002, increasing to 81% post-Alqueva (Rocha et al., 2002; Dias et al., 2004). Before Alqueva Dam was commissioned, the hydrological regime of the Guadiana River was characterized by irregular discharges on both seasonal and inter-annual scales as a consequence of its dry Mediterranean climate and the poor permeability of its hydrological basin rocks. During periods of intense rainfall (deluges), the Guadiana flow could exceed 10 000 m^3/s, contrasting with negligible flow during summer (INAG, 2002). With the growing flow regulation, the deluges and occasional floods are now captured for irrigation purposes and only the surplus is drained out for the estuary. Years of high precipitation are thus transformed into ‘normal’ drainage years, with the exception of years with extraordinarily high precipitation and/or sequences of wet years.

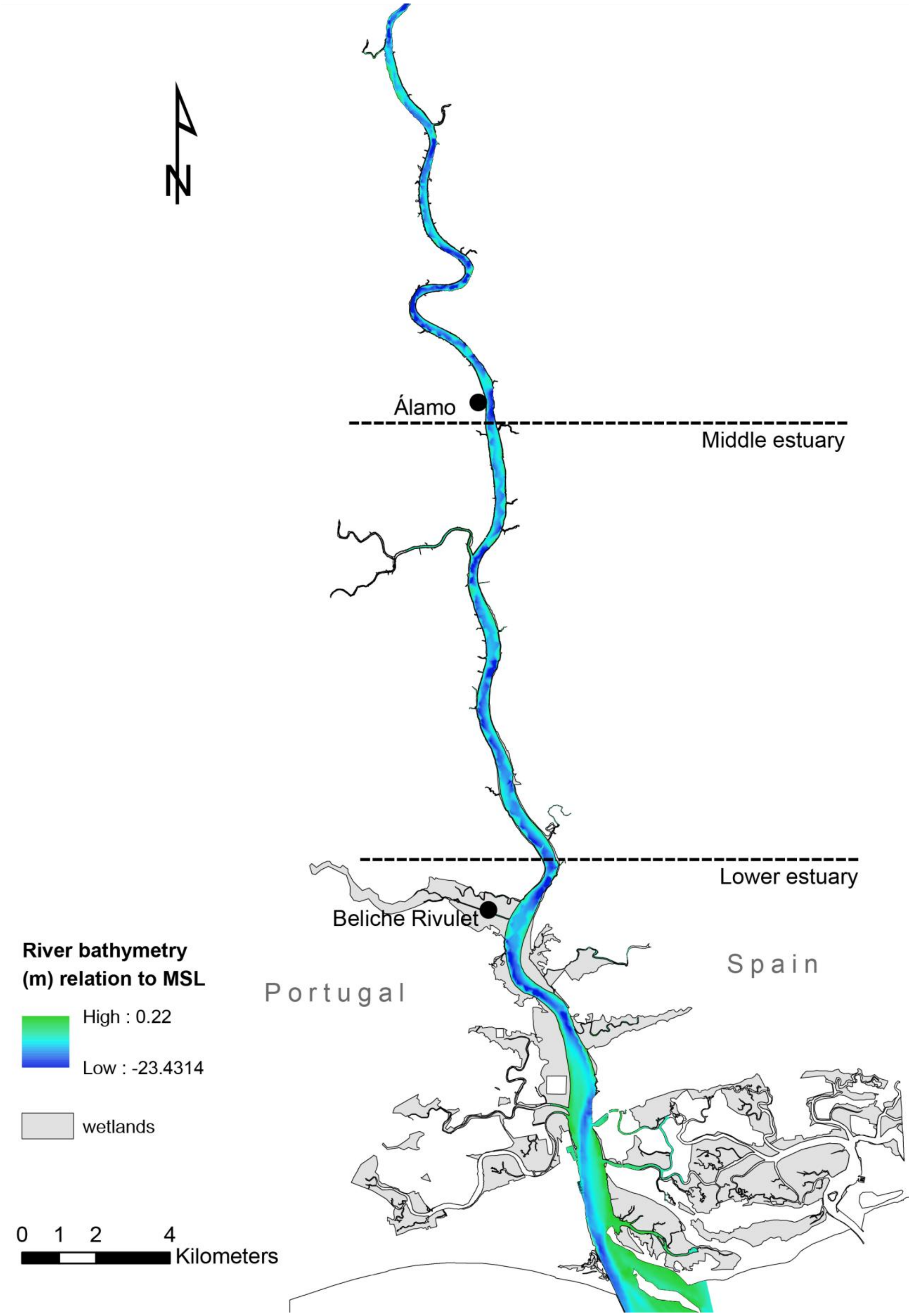

Figure 3.2. Guadiana River estuary - spatial division and bathymetry.

When precipitation is near the mean value, the necessity for keeping strategic water reserves means that the majority of the flow is captured, giving rise to the effects of a typical drought year (Dias et al., 2001). With artificial regulation, river flow is still dependent of climatic variability, but the effects of wet years are diminished (extreme flood episodes are controlled) and the effects of drought years are exacerbated. Since the Alqueva Dam was commissioned, freshwater discharge into the estuary has remained low throughout the year (generally < 20 m^3 s^{-1}, i.e. the discharge corresponding to a dry summer prior to the dam's construction) (Garel and Ferreira, 2011).

Considering river inflow and rainfall data provided by the SNIRH (1995–2012) for 2010, the sampling year for the present study, it is clear that major discharges coincide with periods of higher precipitation (Fig. 3.3). The year 2010 had a very wet winter, with strong discharge periods, especially during March, when maximum discharge values of 2111.3 m^3 s^{-1} were registered in Ponte Quintos, a hydrographic station downstream of Alqueva and Pedrogão (the last dam on the river's main course). All the fresh water flowing into the estuary passes by this station, except the water from small tributaries located further south (Ferreira, personal communication, 2012). The 2010 discharge period coincided with the winter campaign (19, 20 and 28 of February and 2 of March 2010), which caused several difficulties in the sampling. The summer was typically dry, with no rain and negligible discharge.

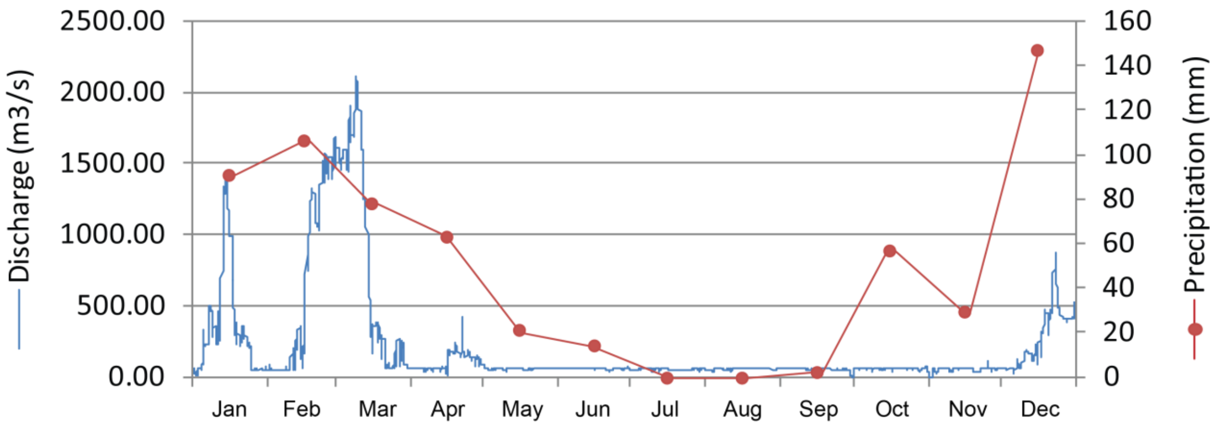

Figure 3.3. Daily discharge values during 2010 registered at Ponte Quintos hydrographic station (26L/01H) and monthly precipitation values during the same year at Reguengos meteorological station (23L/01G) (SNIRH, 2012).

Below Mértola, on the Portuguese side, there are 7 major tributaries flowing into the Guadiana estuary: the Oeiras, Carreiras, Vascão, Alcoutim, Foupana, Odeleite and Beliche rivulets, as well as the river Chança, located on the Spanish side (Dias et al., 2001). Chança, Odeleite and Beliche are controlled by dams constructed for irrigation and water supply that only release water during flood periods. The other tributaries are governed by strong seasonality, being dry during summer and “arriving” (as the locals say) during periods of high rainfall.

Maximum turbidity is observed, depending on tides, between 20–35 km upstream of the mouth, between Foz de Odeleite and Alcoutim (Dias et al., 2001; 2004). Well mixed (during spring tides) and partially stratified conditions (during neaps) alternate during periods of low river flows; highly stratified conditions are observed during periods of high freshwater inflows (Garel et al. 2009; 2011).

Together with the Guadalquivir River, the Guadiana River is the main sediment supplier to the adjacent Gulf of Cadiz margin (Lobo et al., 2003; 2005). There are no published data on the Guadiana’s real sediment supply, although there are estimates of the average suspended load (57.90×10^4 m^3/yr) and bed-load (43.96×10^4 m^3/yr) for the period between 1946 and 1990 (Morales, 1995; Morales et al., 2006).

3. 3. Climatic Characteristics

The Guadiana basin has Mediterranean climatic characteristics, with hot, dry summers, strong insolation and high evapotranspiration. The winters are relatively rigorous in the more elevated zones (north, northeast) and become less harsh as one moves downstream (Ministério do Ambiente, 1999). The annual average temperature is approximately 16º C in almost all the basin. In the warmer months (July and August), the air average temperature varies between 24º C near the sea and 28º C near the river source (Ciudad Real). In the coolest month (January), the air average temperature is around 9º C in the Spanish basin and 11º C near the sea (Ministério do Ambiente, 1999).

Data for rainfall and river discharges for the Guadiana River basin show a strong link with North Atlantic Oscillation (NAO) index patterns (Dias et al., 2004). The NAO is the dominant mode of winter climate variability in the North Atlantic region, ranging from central North America to Europe and even into northern Asia. A negative NAO index (dry conditions in the northern latitudes) usually results in more rainfall (in the southern latitudes of Europe), and subsequent flooding in the river basin during winter months (Dias et al., 2004). The interannual distribution of precipitation is extremely irregular, with 386 to 422 mm in dry years and 722 to 766 mm in wet years. In what concerns the monthly precipitation distribution, the entire basin is affected by an aestival period characterized by an almost total lack of rain. On average, more than 80% of the total annual precipitation falls between October and April (Ministério do Ambiente, 1999) (Fig. 3.3).

According to data series collected during 1954 and 1963 in the meteorological station of Vila Real de Santo António, the prevailing winds come from the north and southwest, followed by less frequent winds from the southeast (Morales, 1995).

3. 4. Oceanographic characteristics

The Guadiana estuarine system is semi-diurnal and mesotidal (Morales et al., 2006) with tidal influence as far upstream as Pomarão (Mértola) (Barros and Candeias, 1998) for river flows in the order of 10 m^3 s^{-1} (homogeneous or weakly stratified conditions) during spring tides (Oliveira et al., 2006). The mean tidal range at the river mouth is approximately 2 m, reaching 3.4 m during spring tides (IH, 1998). The lag between maximum water level amplitude and peak currents is about 2 h throughout the entire estuary (Garel et al., 2009; 2011).

Tidal waves along the coast move from east to west, producing slow velocity currents (flood is 0.4 m/s to the west and ebb is 0.3 m/s to the east during a mean spring tide). In the estuary, these waves propagate following a synchronic model, generating stronger currents than along the coast (floods can reach 0.80 m/s and ebb can reach 0.90 m/s during a mean spring tide) (Morales, 1997; Morales et al., 2006). The falling tide is appreciably longer than the rising tide in the main channel (flood duration 5 h 35 min and ebb duration 6 h 50 min) (Morales, 1997). Along the coast, south-westerly waves are associated primarily with swell from the Atlantic Ocean (75%) and prevail in relation to south-easterly waves, which are generally more energetic (Morales, 1997).

The equatorial North Atlantic Ocean current and the current generated at the connection between the Mediterranean Sea and Atlantic Ocean (Strait of Gibraltar) are the main flows acting in the Gulf of Cadiz. The Mediterranean current is denser and colder, and consequently only circulates at depth. It causes a deficit in Alborán Sea promoting the emergence of compensatory superficial currents which circulates in opposite directions (Morales, 1995). Stevenson (1977) (cited in Morales, 1995) studied these currents and found a circulation cell in the center of the Gulf of Cadiz moving in clockwise direction. Between this cell and the neighbouring coastlines he found another current that sweeps along the coast from Huelva in the east to Monte Gordo in the west and is dependent on atmospheric circulation.

The wave system results in a net eastward littoral drift which, according to several authors (see Dias et al., 2004), is estimated to be 150 000-300 000 m^3/year in the area of the Guadiana mouth. Accretion of drifting sands in the proximity of the main channel mouth, which is continuously reworked by wave action, has led to the formation of highly mobile washover banks and to rapid migration of estuarine channels (Boski et al., 2002). Borrego et al. (2002) suggest that both currents entering the estuary (flood, ebb and fluvial currents) and flood/ebb tidal currents parallel to the shoreline are the main processes driving the modern configuration of the estuarine mouth.

3. 5. Ecological characteristics

The Guadiana river basin is the fourth largest on the Iberian Peninsula (Dias et al., 2004). In a European context, the river and its tributaries represent an important area in terms of endemism and biological communities of interest. The Lower Guadiana River is listed as a Wetland of International Importance (Ramsar, 2009) and is included in the Natura 2000 Network. Its extensive marshlands were declared a Natural Reserve in 1975 due to their high biological and ecological value. It is an important site for ornithological reproduction, covered by several international protection statutes (ICNB, 2007). The estuary is also an important nursery for several fish, mollusk and crustacean species. Around 22 species of migratory fresh-water fish live in the river and tributaries, of which 9 are Iberian endemics and 3 are restricted to the Guadiana basin (ICNB, 2006).

The Estuary represents a rich wetland zone, where salt-marshes dominate and salt pans, lagoons, tidal creeks, intertidal flats, barrier islands and sandy spits and many other habitats of high ecological value are found (Boski et al., 2008). The wetland is noteworthy for its halophytic salt-marsh communities (Ramsar, 2009), which carry out an essential role in the functioning and maintaining of healthy ecosystem status (Simonson, 2007). In estuaries, halophytic vegetation plays an essential role in sedimentation processes. It provides abundant vegetal detritus that is transported by tidal currents and becomes trapped in the vegetation itself, forming a dense debris sheet which retards currents and promotes the retention of suspended sediments (Costa, 2001). Sedimentation processes increase after the establishment of *Spartina maritima* meadows, which are a pioneer species capable of colonizing very low elevation levels and tolerate long periods of oxygen deprivation (Costa, 2001). Sediment entrapment by *Spartina* elevates the marsh surface, which induces a number of changes in soil characteristics, and the subsequent replacement of *Spartina* by other species (ALFA, 2004; Sánches et al., 2001).

Recently, an inventory of plant species was compiled from field observations and published records and a library of photographs of plants species and communities was developed (Simonson, 2007). These are available via the GUADID application (Boski et al., 2006). During this study 420 species were inventoried, including several of conservation importance. A large part of the Guadiana Estuary area is dominated by plants of the family Chenopodiaceae and other halophytes, in particular the succulent shrubs *Arthrocnemum macrostachyum* and *Sarcocornia* spp. A typical vertical zonation was found in many places, from the lowermost colonies of the Cord-grass *Spartina maritima*, frequently submerged in tidal waters at the edge of creeks, passing through *Sarcocornia* and Sea Purslane *Halimione portucaloides* in the middle-marsh zone, to the plants of the upper marsh, such as *Arthrocnemum*, Shrubby Sea-blite *Suaeda vera* and Shrubby Sea Lavender *Limoniastrum monopetalum*, which also form a distinct community on top of the earthen banks that retain salt pans and fish farms (Simonson, 2007).

Juncus maritimus is common in *Sarcocornia fruticosa* meadow communities whenever freshwater aquifers are present; *J. maritimus* and *Juncus acutus* occur in the halophytic strip located upstream where saltwater effects are first detected; and *Juncus subulatus* occurs in abandoned saltpans or in marsh depressions susceptible to brackish water accumulation during winter (ALFA, 2004).

A large proportion of the marshes is affected by the highly competitive exotic grass *Spartina densiflora*, which covers more than 1000 ha of the salt-marshes (Simonson, 2007). *S. densiflora* is a South American cord-grass that has become established in the salt-marshes of SW Europe, as well as parts of NW Africa and SW North America. It was probably introduced accidentally to Iberia by the lumber trade as early as the 16th century, and has become invasive in the estuaries of the Gulf of Cadiz (Simonson, 2007; Mateos et al., 2008). Today, the invasion of this cord-grass is a conservation problem, especially in Odiel and Guadiana salt-marshes. The major concern is the possibility of exclusion of other species and even whole communities by competition for space (ALFA, 2004).

Besides wetlands, the Guadiana estuary region also incorporates agricultural land, pine woods, Mediterranean shrublands and cork oak plantations. All these habitats support plant and animal communities that not only increase the estuary's natural value, but also make it one of Europe's most 'pristine' estuarine environments (Boski et al., 2006).

Nevertheless, at present the Guadiana River basin constitutes a system that has been significantly altered from its natural state (Dias et al., 2004). Several pollution sources exist in the Guadiana Estuary area, mainly resulting from urbanization (sewage discharges), agriculture (fertilizers, pesticides, and herbicides), cattle breeding and olive oil production (Ministério do Ambiente, 1999; Wolanski et al., 2006). The estuary morphology itself has been heavily influenced by the construction of two jetties in 1974, one on the western side of the estuary mouth with a length of 2090 m and another (submerged breakwater) on the eastern side with a length of 900 m. Construction of these jetties led to significant inhibition of westward littoral drift, thus reducing sand supply to the estuary and/or altering its rhythm (Dias et al., 2004). However, the major problem in the Guadiana Estuary seems to be related with recent changes in river flow due to river regulation, particularly the construction of Alqueva Dam, which is expected to affect the estuarine biological and non-biological factors, both in the short and long term (Chícharo et al., 2006a). According to Chícharo (2008 in Ecosfera.publico.pt), one of the major concerns is the retention of fine sediments behind the dam, depriving the marshlands of sediment and nutrient supply. The sediments that do reach the marshlands will be coarser and not suitable for the current vegetation, and without vegetation the avifauna could be compromised.

Well-flushed estuaries are intrinsically more robust than poorly flushed systems (Wolanski et al., 2006). As a result, environmental degradation in the Guadiana is most often apparent during periods of reduced freshwater inflows, e.g. during drought or when human activities reduce the freshwater flow (Wolanski et al., 2006). Reduction in sand supply from river basins to the oceans may cause large-scale coastal erosion and alteration of sedimentation patterns on the shelves, with consequences not only for natural habitats but also for the economy (Dias et al., 2004).

3. 6. Holocene estuary evolution

Modern estuaries formed when the sea level rose at the end of the last glaciation. Estuaries respond to sea-level rise by infilling with sediment derived from both the riverine inflow and from coastal processes (Wolanski, 2007). This infilling may occur in various ways, according to the specific characteristics of the river valleys, with the space of accommodation being one of the most important factors. The Guadiana River has a very narrow and incised paleovalley when compared with the other river valleys along the coast of the Gulf of Cadiz (Boski et al., 2002). These characteristics promoted the deposition of a thick sedimentary column and provided an excellent sedimentary record during the most recent postglacial transgression, enabling a better understanding of the processes involved in the formation of the estuary. Several studies concerning the sedimentary infilling of the estuarine valleys in the Spanish portion of Gulf of Cadiz have been published. The Holocene sedimentary sequences of Guadalete River Estuary were studied by Dabrio et al. (1995) and Goy et al. (1996), and the geochemical signature of sedimentary sequences of Tinto River Estuary was studied by Borrego et al. (2004). Dabrio et al. (2000) and Lario et al. (2002) presented a regional synthesis based on material collected from boreholes and drains in the estuaries of the Tinto-Odiel, Guadalete, San Pedro and Guadalquivir Rivers. More recently, Zazo et al. (2008) presented a synthesis of coastal responses to climate changes on the Atlantic-Mediterranean margin during the last 15 ky. On the Portuguese side of the Guadiana, Boski et al. (2002 and 2008) studied the postglacial sedimentary infill of the estuary. Based on sedimentary features, ^{14}C dates and foraminifera paleo-assemblages in five boreholes drilled in the estuarine sediments, these authors proposed the first chronological model for post-glacial marine transgression in the Guadiana Estuary. According to Boski et al. (2008), the estuary's formation began relatively early, *ca* 13 000 cal yr BP, and was marked by two distinct phases of sea-level rise: an accelerated phase, during which the estuary was infilled by clay sediments at a rate of 7 mm yr^{-1} in the period between 13 000 to 7500 cal yr BP (approximately 11 000 to 6500 ^{14}C yr BP) and a second phase of slower sea-level rise with predominantly sandy sedimentation at a rate of 0.9 mm yr^{-1}within an estuary enclosed by sand spits, from 7500 cal yr BP to the present. Dabrio et al. (2000) and Lario et al. (2002) suggested 6500 yr BP as the age of maximum flooding. After this time, the rate of eustatic rise decreased markedly and the rate of sedimentation in the estuaries surpassed the rate of sea-level rise. Estuarine filling followed a two-fold pattern governed by the progressive change from vertical accretion to lateral (centripetal) progradation. During the highstand phase, the Guadiana Estuary witnessed the emergence and recycling of barrier islands and spits until approximately 3000 yr BP, after which a phase of back-barrier wetland formation began, with infilling and dune development (Kirby and Plater, 2006). The coastline reached a position close to present one approximately 200 years ago (Morales, 1997).

Lateglacial and Holocene climatic changes in the area around the Guadiana Estuary were studied by Fletcher et al. (2007), who analysed palynological evidence in a 48-m sediment sequence (CM5 borehole) dated by AMS-radiocarbon. According to the author 5 main phases were identified: (1) a moist, temperate Lateglacial interstadial (Allerød chronozone) from the base of the record at *ca* 13 090 cal BP to *ca* 12 900 cal BP; (2) an arid, cold, continental

Younger Dryas phase between *ca* 12 900 cal BP and *ca* 11 790 cal BP; (3) a warm, dry early-Holocene phase between *ca* 11 790 cal BP and *ca* 9000 cal BP; (4) generally warm, moist, oceanic conditions from *ca* 9000 cal BP to *ca* 5000 cal BP; and (5) a return to warm, dry conditions after *ca* 5000 cal BP. In addition to the principal record of forest/shrubland dynamics, the record of xerophytic taxa indicates a series of Holocene episodes of increased aridity that the authors associate with Atlantic sea-surface coolings and atmospheric reorganization.

3. 7. Study sites location and characterization

3. 7. 1. Study site locations

The majority of the study sites were located on the Portuguese side of the Guadiana Estuary, except *Espanha ETAR* and *Espanha*, which were located in the Spanish side (Fig. 3.4). The sampling points and transects were named according to the names of the localities where they were collected or according to neighboring landmarks. *Alcoutim*, a small historic village, was the name given to the northernmost site (37º28'29.91" N and 7º28'14.31" W), approximately at 34 km distance from the sea. The *Lagoa* site, which is a small lagoon at the mouth of the river, was the southernmost site (37º10'24.03" N and 7º24'24.36" W) (geographic coordinates for all points appear in Table I, Appendix 3).

The *Laranjeiras* site was located in the upper estuary. In the middle estuary there were two sites, *Foz de Odeleite* and *Almada d'Ouro*. *Foz de Odeleite* is the mouth of Odeleite rivulet. In the lower estuary, where the diversity of sub-environments was higher due to tidal influence, the sampling effort was increased, with eight different sites: *Foz do Beliche*, *Ponte Internacional*, *Lezíria*, *Sapal de Castro Marim*, *VRSA*, *Espanha ETAR*, *Espanha* and *Lagoa*.

In middle and upper estuary, due to the lack of intertidal zonation, only one sample was collected per site at *Alcoutim (AL)*, *Laranjeiras (LAR)*, *Foz de Odeleite (FO)* and *Almada d'Ouro (Aouro)* sites. In the lower estuary, except for the *Espanha ETAR* (EE) and *Sapal de Castro Marim* (SCM) sites in which only one sample was collected, all the remaining sites were sampled along transects.

In most of cases, these transects ran perpendicularly to the river, resulting in 4 transects of 3 samples each at *Foz do Beliche (FB)*, *Lezíria (LEZ)*, *Espanha (E)* and *Lagoa (LG)* sites and 2 transects of 4 samples at *Ponte Internacional (PI)* and *Vila Real de Santo António (VRSA).* SCM and LEZ were the most distant sites from the river.

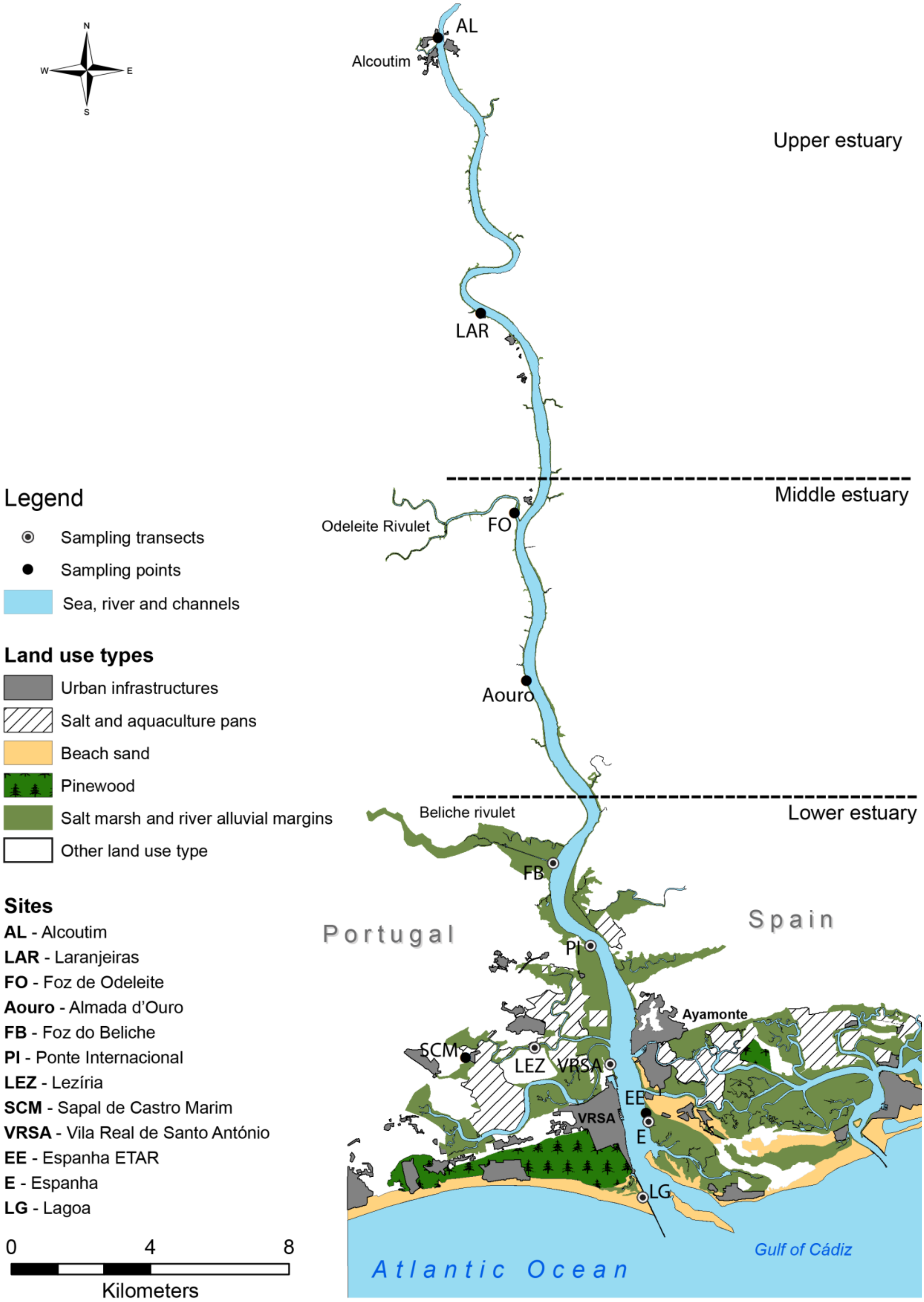

Figure 3.4. Map of Guadiana River Estuary with site location.

Transects were sampled according to the vertical zonation in the halophytic vegetation, trying to sample the main distinct zones, from non vegetated mud-flats to the higher levels of the marshlands.

3. 7. 2. Topography, morphology and halophytic zonation

The geographical and topographic data (x, y and z) appears in Appendix 3. All the sites and exact sampling points were photographed (Appendix 1) together with halophyte vegetation along the transects (Appendix 2).

Figure 3.5. Google Earth image (June, 2007 survey) of the southernmost site LG. Colored points mark the exact location of the measured d-GPS points; sampling points are in red.

In the southernmost transect LG, 8 points were measured in a total distance of 16.23 m of profile perpendicular to the lagoon, with starting point where the halophytic meadows become homogenize until the low-tide limit, where the last point was measured (Fig. 3.5). The LG transect was located in the margin of a small shallow lagoon that evolved over a littoral spit with transverse progradation and high rates of sedimentation (Morales et al., 2006). The lagoon is separated from the sea eastwards by the Portuguese jetty and southwards by a sand spit that continues to grow seawards. Although the tides do not reach it directly, the lagoon waters are frequently renewed due to a connection to sea through a tidal channel (parallel to the jetty in the Fig 3.5). The margins of the lagoon are densely covered by halophytic vegetation which presents a very smooth zonation due to the terrain flatness. The lagoon exhibited signs of eutrophication manifested by the presence of a filamentous seaweed (locally known as 'barbas', Fig. 1, Appendix 2). However, the coexistence of *Ruppia maritima* seagrass, typical of brackish to saline running waters and common in lagoons and shallow channels (Bolibar, 2010), indicates some hydrodynamism inside the lagoon.

Three points were sampled on the LG transect: LG1, LG2 and LG3 (Fig. 3.6; Figs. 5-7 in Appendix 1). From the lowest (inside the lagoon) to the highest point, elevation rises by only 0.54 m. LG1 is situated at 0.397 m above MSL and was collected in between a dense meadow of the invasive cord-grass *Spartina densiflora*; LG2, at 0.167 m, is located in a narrow belt of the succulent shrub *Sarcocornia perennis* ssp. *perennis* with upper contact with *S. densiflora* and lower contact with the limit of mean low water level. LG3 was located inside the lagoon at – 0.09 m depth. In the surroundings of the transect was common to find occasionally *Suaeda maritima* in the low marsh zone and *Haliminone portucaloides* in the middle marsh. In the high marsh zone, which comprises the external belt of the lagoon marshlands, the succulent shrubs *Arthrocnemum macrostachyum* and *Sarcocornia perennis* ssp. *alpini* were the most common species.

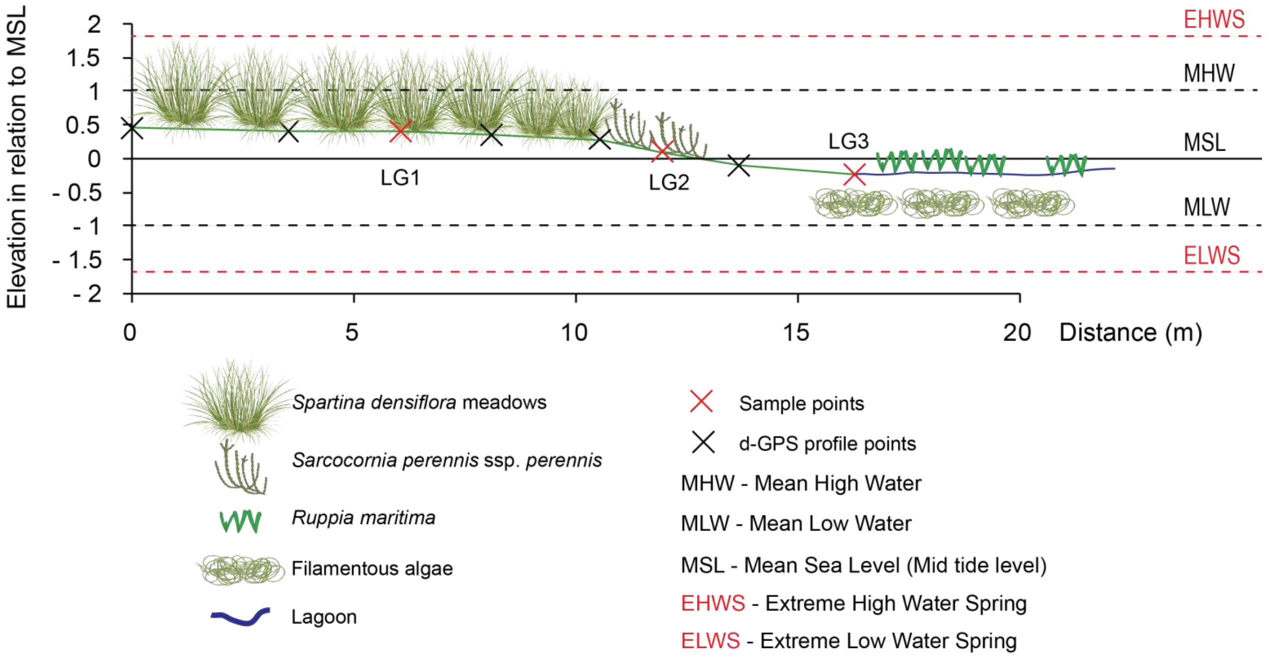

Figure 3.6. Halophytic characterization along the topographic profile LG.

Transect E was the only one surveyed on the Spanish side, with 32 points measured along 280.74 m of profile perpendicular to the river (Fig. 3.7). The site was located on the margin of a wide area of natural dendritic marshlands between the coast of Ayamonte and the 'Punta de la Espada' sand bank. The profile started at the margin of a natural tidal channel, crossed the marshlands and ended near the limit of low tide in an oyster bed.

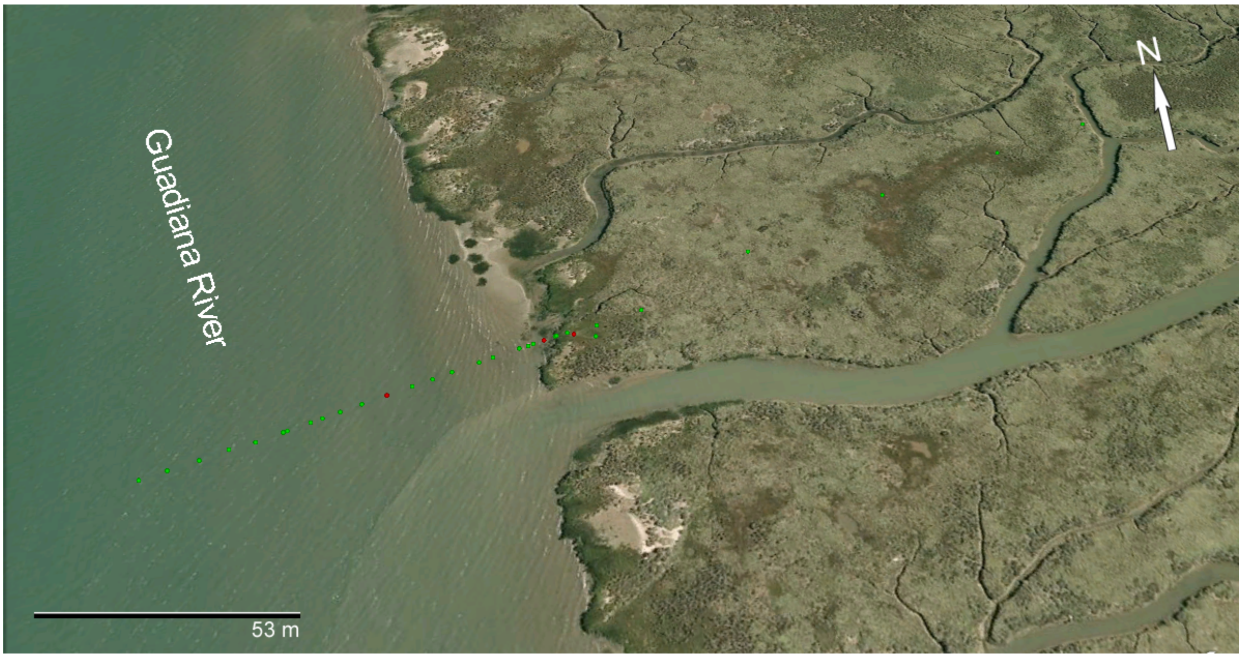

Figure 3.7. Google Earth image (June, 2007 survey) of the transect E, located on the Spanish side. Colored points mark the exact location of the measured d-GPS points; sampling points are in red.

Three points were sampled on transect E: E1, E2 and E3 (Fig. 3.8; Figs. 9-11 in Appendix 1).

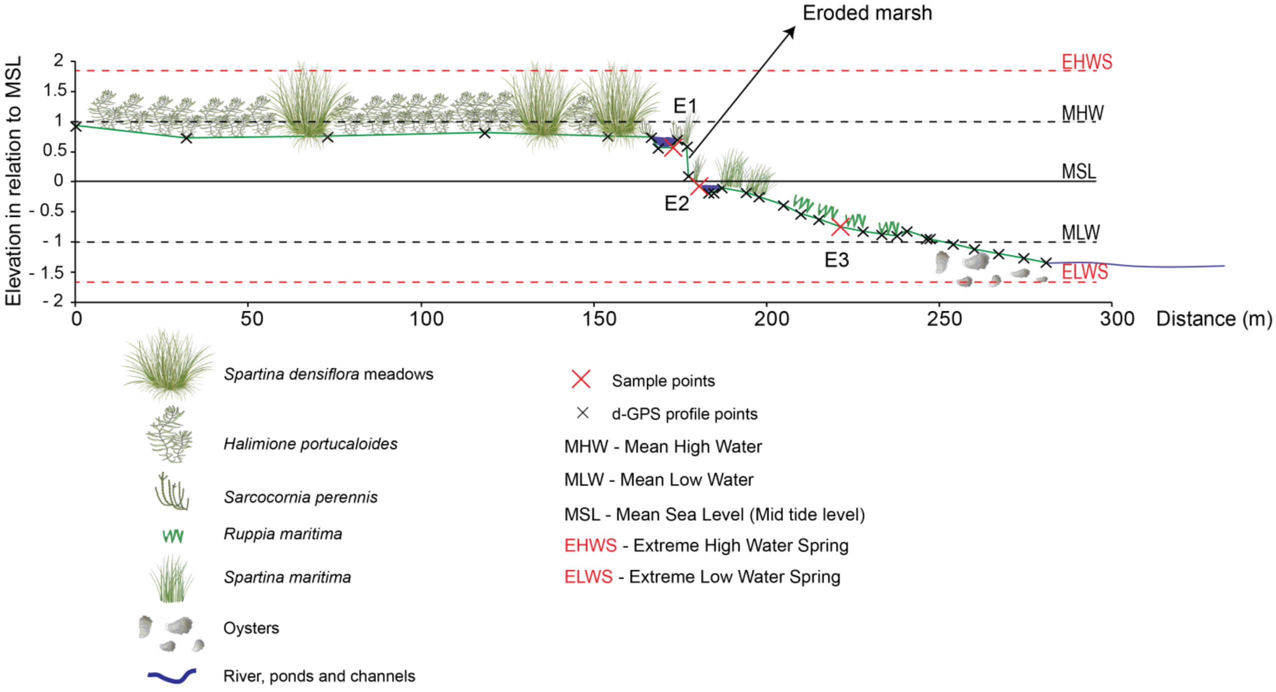

Figure 3.8. Halophytic characterization along the topographic profile E.

From the lowest to the highest point the elevation rises 1.38 m. The slope was steep due to marked erosion on the margin between E1 and E2. Point E1 was situated at 0.57 m elevation and was on the margin of a small tidal pond surrounded by halophytic vegetation, with a predominance of *Sarcocornia perennis* and some tufts of *Spartina maritima* on the river side, and *S. perennis, S. densiflora* and *H. portucaloides* on the inland side. Further inland there was an alternation between these three species and *S. desinflora* in the transition zone between the higher stands dominated by *H. portucaloides* and the lowermost, saturated stands dominated by *S. perennis.* E2 was collected at – 0.06 m depth at the base of the eroded marsh layer where, in between some remains of the layer colonized by *S. maritima,* it was possible to see the uncovered sand spit base. Immediately below, towards the river, there was a shallow rill with a great quantity of bivalve shells. E3 was collected at – 0.74 m depth in the mud margin scattered with *R. maritima* seagrass.

Sampling point EE was located 265 m north of transect E and was collected at – 0.74 m depth (Fig. 3.9; Fig. 12 in Appendix 1) in the muddy margin of the main river channel near the Ayamonte Sewage Treatment Plant (STP) outfall (Fig. 13, Appendix 1). It was selected to infer the possible effects of nutrient loads from the effluent. The salinity was always low and there was a great concentration of bivalve shells around the STP outfall (Fig. 14, Appendix 1).

Figure 3.9. Google Earth image (June, 2007 survey) of the site EE with a red dot marking the place of sample collection. Ayamonte Sewage Treatment Plant and its outfall are highlighted.

On the VRSA transect, the only one surveyed using a total station, 51 points were measured along 259.4 m of profile perpendicular to the river (Fig. 3.10). The transect was located 350 m north of the VRSA Port on the river margin, where a clear marsh zonation was visible (see

Appendix 1). The transect starting point was located at the landward edge of a tide-protected lagoon and the last measured point was in the mud-flats, at the limit of low tide.

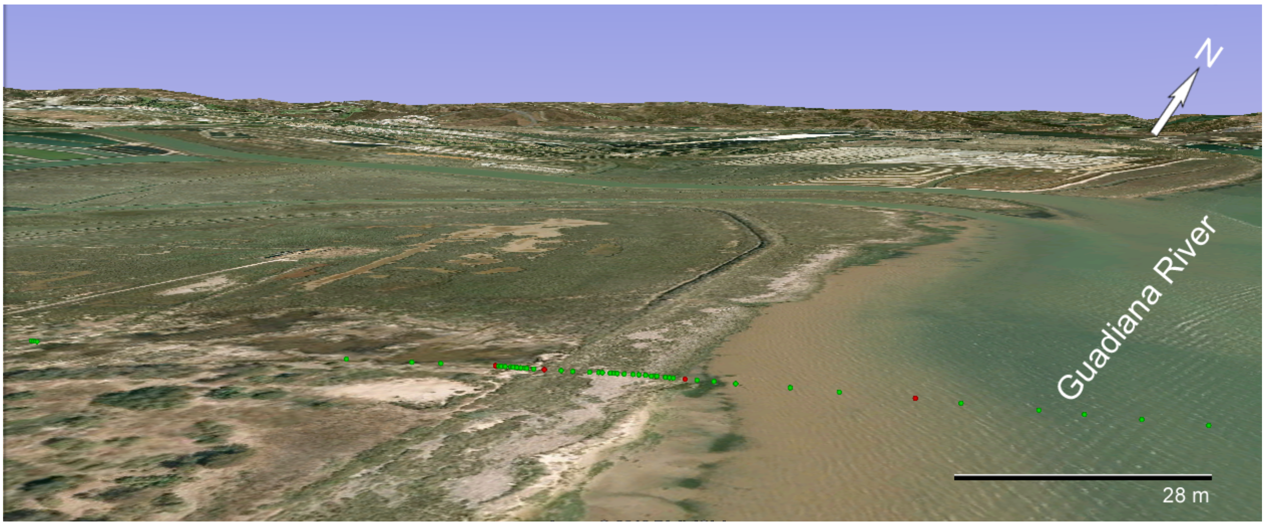

Figure 3.10. Google Earth image (June, 2007 survey) of VRSA site. Colored points mark the exact location of the measured d-GPS points; sampling points are in red.

Four points were collected in VRSA transect: VRSA1, VRSA2, VRSA3 and VRSA4 (Fig. 3.11; Figs. 16-21 in Appendix 1). From the lowest to highest point the elevation rises 1.68 m. The slope was especially steep in the zone where the salt-marsh is being eroded, between VRSA 2 and 3 (Fig. 23, Appendix 1). The point VRSA1 was collected 1.21 m above MSL at the margin of a tide-protected pool with eutrophication indicated by the presence of a condensed mat of filamentous seaweed approximately 1 cm thick covering the sediment. The surrounding sediments were very coarse, suggesting episodes of overwash or possibly artificial (anthropogenic) sediment remobilization. Further inland the profile follows the pool for 124.5 m, bordered by terrestrial vegetation. Towards the river, VRSA1 is separated from the other sampling points by an artificial sand bank covered in halophytic vegetation (Fig. 3.11). At the inner base of the bank, right near the edge of the pool, there were *Sarcocornia perennis* ssp. *alpini* and *Suaeda vera*. A little further up bank there were *Sarcocornia fruticosa*, *Limoniastrum monopetalum*, among other middle to high marsh species. The presence of *S. vera* and *L. monopetalum* suggests that only equinoctial tides can breach the sand bank and reach the pool directly. On the other side of the sand bank there was a dense *S. densiflora* meadow where VRSA2 was collected at 1.03 m above MSL. The *S. densiflora* meadow abruptly ends at the edge of the eroded marsh (Fig. 3.11). VRSA3 was collected at the next stand colonized by the cord-grass *S. maritima* at 0.29 m above MSL. VRSA4, the last sampling point, was collected at – 0.47 m depth in a meadow of *R. maritima*.

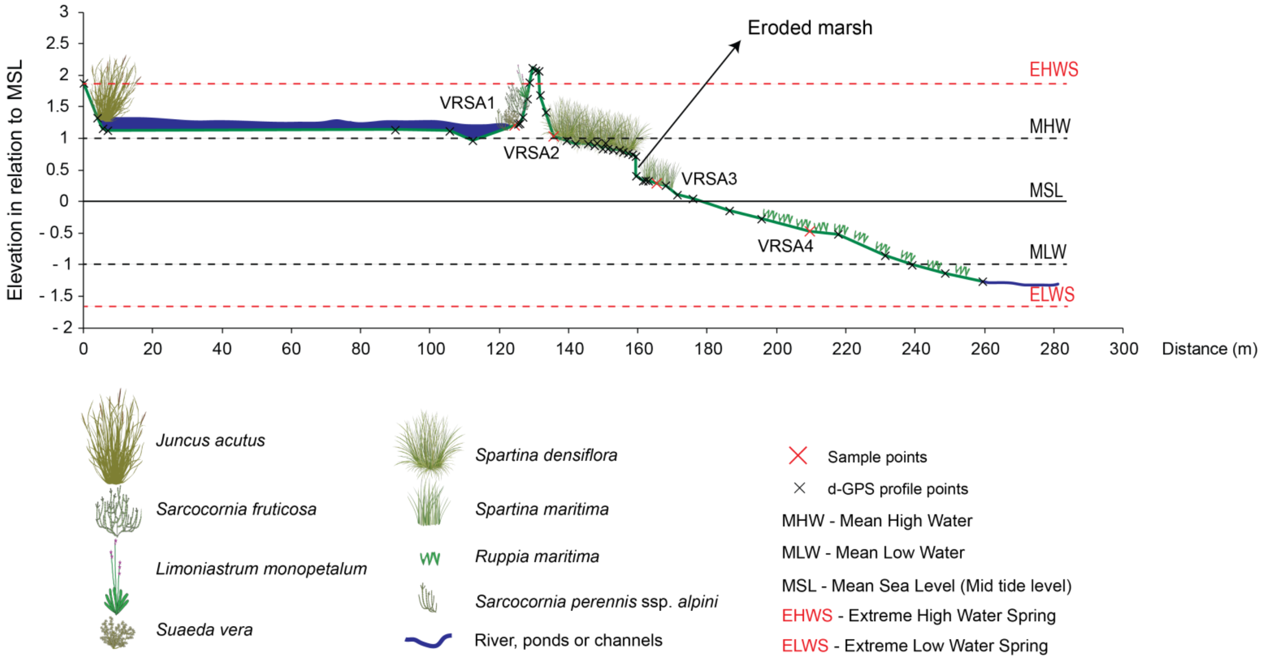

Figure 3.11. Halophytic characterization along the topographic profile VRSA.

The sampling point SCM had the most landward location, *ca* 4200 m from the river edge. It was collected at 1.96 m above MSL in the margin of an active saltworks pond colonized mainly by *S. perennis* ssp. *alpini* and few *S. vera* (Fig. 3.12).

Figure 3.12. Google Earth image (June, 2007 survey) of the site SCM with a red dot marking the place of sample collection.

The LEZ transect was located in the south margin of the Lezíria inlet, a brackish water arm that feeds an extensive area of saltpans and other aquacultural activities. Along the LEZ transect, 15 points were measured on a 24.5 m profile perpendicular to the inlet (Fig. 3.13) and parallel to the river channel. The starting point was located in the limit of the marshlands and the last point was at the edge of the inlet channel, at the limit of low tide.

Figure 3.13. Google Earth image (June, 2007 survey) of the site LEZ. Colored points mark the exact location of the measured d-GPS points; sampling points are in red.

Three sampling points were located on the LEZ transect: LEZ1, LEZ 2 and LEZ3 (Fig. 3.14; Figs. 28-30, Appendix 1). The elevation change along the transect was 1.12 m.

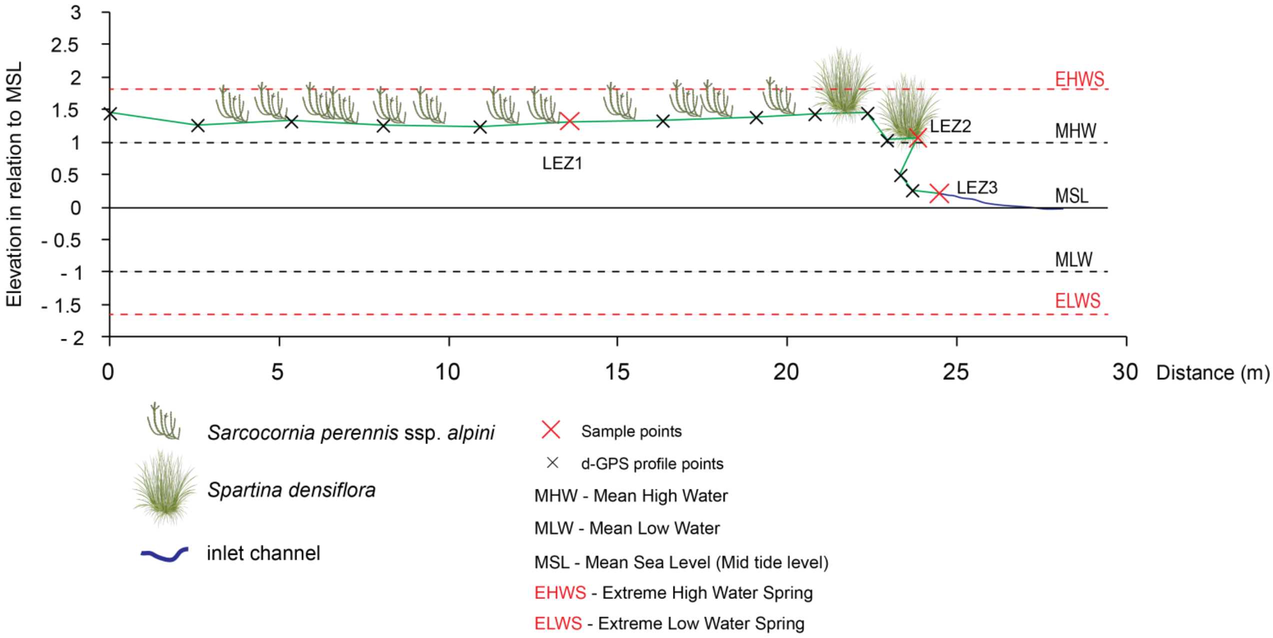

Figure 3.14. Halophytic characterization along the topographic profile LEZ.

LEZ1 was collected at 1.32 m, approximately in the middle of the inlet margin colonized by *S. perennis* ssp. *alpini*. The terrain and the vegetation were much degraded due to the recent trampling by cattle and tractors. LEZ2 was collected on a marginal ledge colonized exclusively by *S. densiflora*, at 1.06 m above MSL. LEZ3 was collected in the inlet's non-vegetated mud margin exposed during low tide at 0.2 m above MSL.

The PI transect was collected from the margin of the river main channel 168 m north of the International Bridge. Thirty points were measured along a 52.5-m transect running perpendicularly to the river through well-zoned marshland (Fig. 3.15; Fig. 32, Appendix 1). The starting point was in the middle of mixed high-marsh halophytic meadows, coinciding with the first sampling point and separated from the remaining points by an artificial tidal channel followed by a sand bank. The last measured point was in mud-flats at the limit of low tide.

Figure 3.15. Google Earth image (June, 2007 survey) of the PI site. Colored points mark the exact location of the measured d-GPS points; sampling points are in red.

PI transect included 4 sampling points: PI1, PI2, PI3 and PI4 (Fig. 3.16; Figs. 32-35, Appendix 1). From the lowest to the highest point the elevation rises 1.18 m. PI1 was collected only during the summer campaign. It was located 1.2 m above MSL in the middle/high marsh, where a great diversity of halophytic species was observed. From the collection point towards the river, the most abundant species was *H. portucaloides*. Further inland *H. portucaloides* was replaced by *Salicornia ramosissima* and, to a lesser extent, by *Arthrocnemum macrostachyum* (see Appendix 2). PI2 was collected in the middle of a defined *S. densiflora* meadow at 1.2 m above MSL. In the stand immediately below, PI3 was collected in a patch of *S. maritima* at 0.61 m above MSL. PI4 was collected on the non-vegetated mud-flats at MSL.

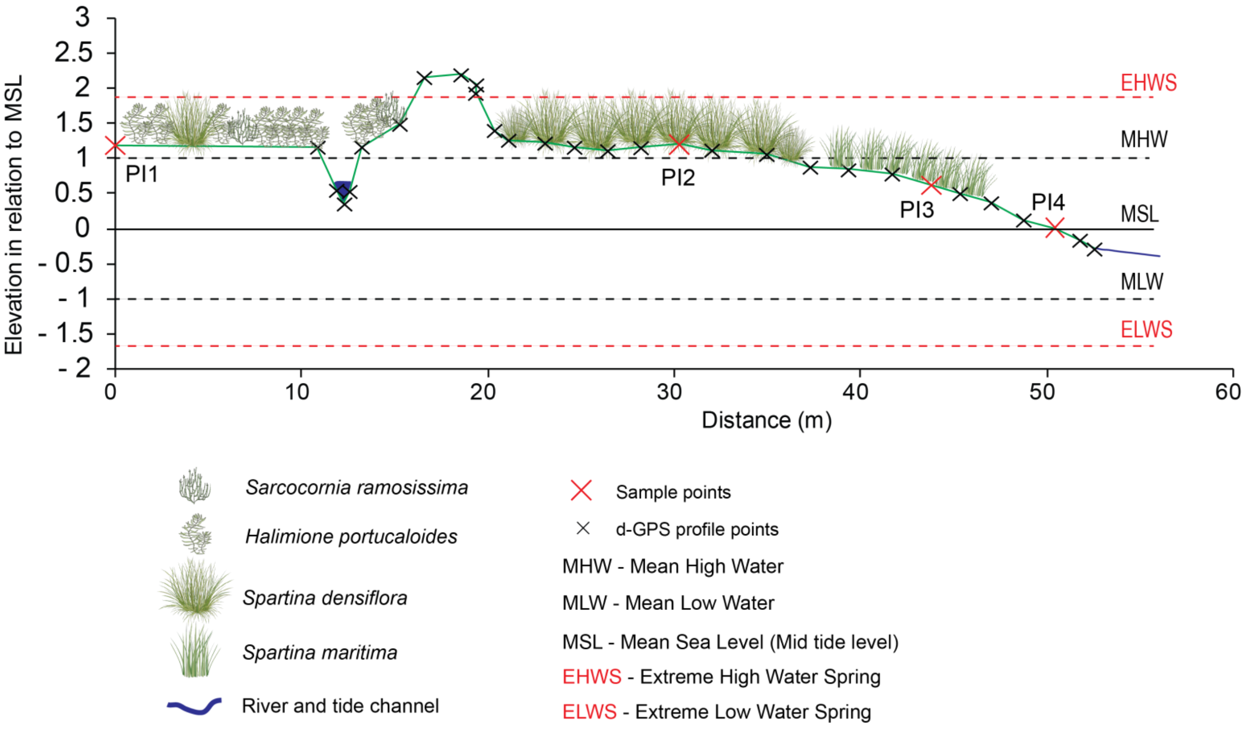

Figure 3.16. Halophytic characterization along the topographic profile PI.

FB was the northernmost transect and was located at the mouth of the Beliche rivulet, the last significant tributary of the Guadiana, draining a basin of about 125 km^2 (Boski et al., 2008). Thirty-two points were measured along the 26-m profile running parallel to the river channel on the south side of the wide alluvial plain of the Beliche rivulet (Fig. 3.17).

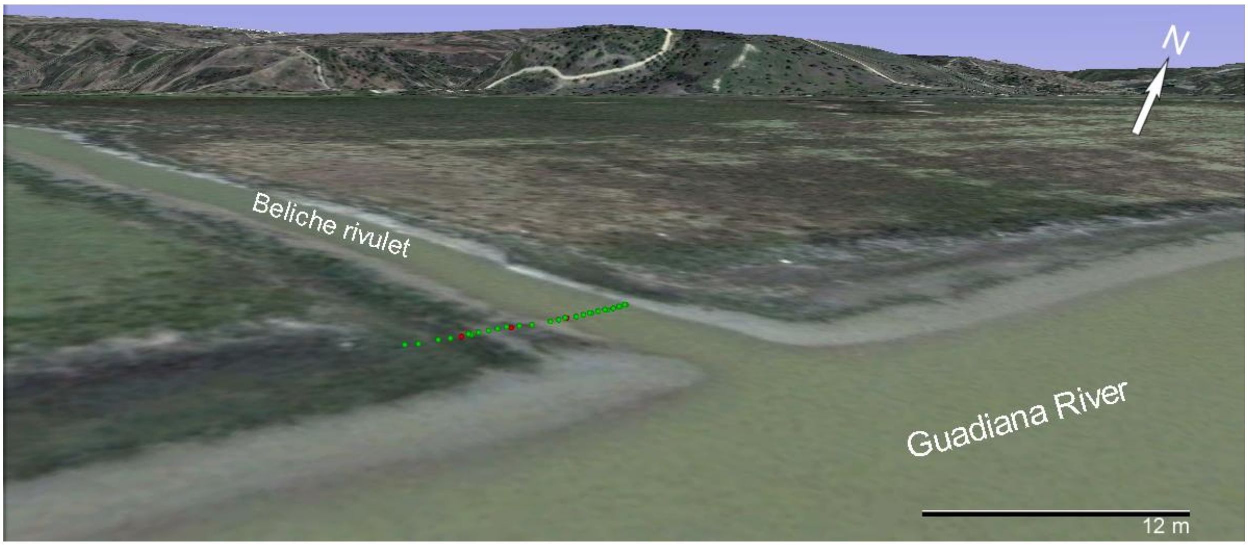

Figure 3.17. Google Earth image (May, 2011 survey) of the FB site. Colored points mark the exact location of the measured d-GPS points; sampling points are in red.

The starting point was in a high marsh zone dominated by *S. fruticosa* and the last point was in the middle of the rivulet channel, which empties during extreme low spring tide. The transect included 3 sampling points: FB1, FB2 and FB3 (Fig. 3.18; Figs. 42-44, Appendix 1). The elevation difference between these points is 0.96 m. FB1 was collected in a homogenous zone of *S. fruticosa* at 1.04 m above MSL. The sediment was hard and very dry and the surrounding environment showed signs of cattle trampling. FB2 was collected in an *S. densiflora* scattered meadow, with saturated, soft sediments at 0.75 m above MSL. FB3 was collected in the soft, non-vegetated mud-flats, at the margin of the rivulet channel at 0.47 m above MSL. FB was the last transect of the sampling transect series. Further upstream only four sites were sampled, each with a single sampling point.

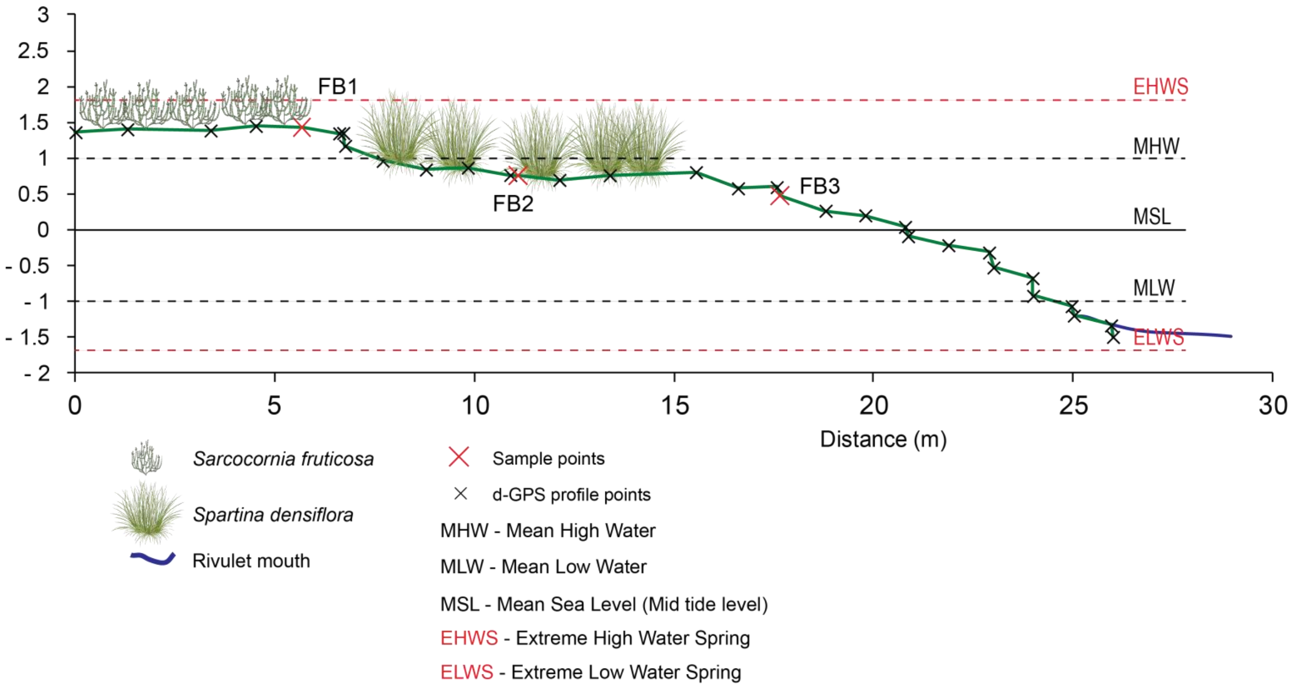

Figure 3.18. Halophytic characterization along the topographic profile FB.

Aouro site was located at the edge of the main river channel, at 0.53 m above MSL in a narrow non-vegetated mudflat belt exposed during low tide (Fig. 3.19).

At this point there was no marked zonation in the vegetation. From the non-vegetated mudflat the margin evolves to a higher area colonized by freshwater vegetation like rushes (*Juncus*), canes (*Arundo donax*), grasses (Bermuda buttercups, clovers, etc.), shrubs and trees.

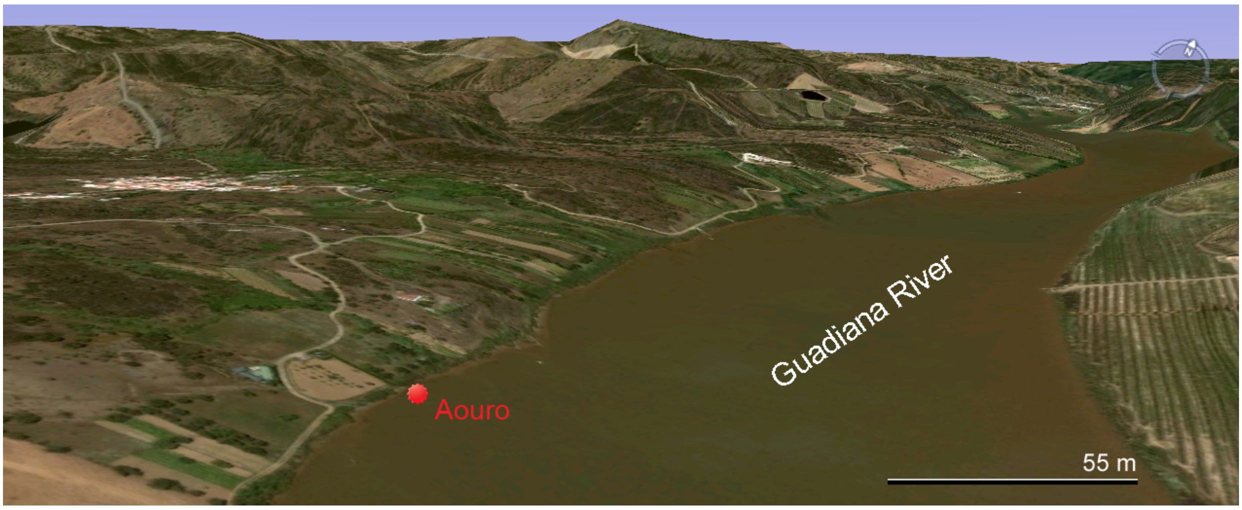

Figure 3.19. Google Earth image (June, 2011survey) of the site Aouro with red dot marking the place of sample collection.

The FO site was located at the southern edge of the Odeleite rivulet mouth near the village of Foz de Odeleite (Fig. 3.20). Like Aouro, here there was no marked zonation in the vegetation, which was essentially terrestrial. The sample was collected at 0.33 m above MSL in a meadow of *Elymus* cf. *repens*, a hygrophilous grass typical of permanent watercourses (ALFA, 2004; Fig. 22 and 23, Appendix 2). A dense fringe of *Juncus inflexus* separated the *Elymus* cf. *repens* meadow from the terrestrial vegetation.

Figure 3.20. Google Earth image (June, 2011survey) of the site FO with a red dot marking the place of sample collection.

The LAR site was on the margin of the main river channel at 0.31 m above MSL near the village of Laranjeiras (Fig. 3.21). The site was very similar to FO in terms of vegetation, although less vegetation on account of its more exposed location.

Figure 3.21. Google Earth image (June, 2011survey) of the site LAR with a red dot marking the place of sample collection.

AL was the northernmost and the last site surveyed, with one sample collected on the margin of the main river channel near a boat mooring at MSL (0.013 m) (Fig. 3.22). The mud-flat was colonized by *Elymus* cf. *repens* and was sampled only in summer for thecamoebian analysis.

Figure 3.22. Google Earth image (June, 2011survey) of the site AL with a red dot marking the place of sample collection.

Chapter 4

Environmental characterization of Guadiana River Estuary

Chapter 4

4 – Environmental characterization of Guadiana River Estuary

4. 1. Chapter objectives

In the present chapter, the spatial and seasonal variability of environmental parameters in the Guadiana Estuary are analyzed. Sediment grain-size, pH and bulk organic matter proxies, such as total organic carbon (TOC), total inorganic carbon (TIC), total nitrogen (TN) and C/N ratio (organic carbon/total nitrogen), were analyzed in all superficial sediment samples from the winter and summer campaigns. Physico-chemical parameters of the water - salinity, oxygen saturation and temperature - were analyzed from *in situ* measurements at sampling points with sufficient water depth for the multiparameter probe. All parameters are represented graphically according to elevational (tidal exposure) and latitudinal (distance to sea) gradients, except those measured in the water, which are represented only latitudinally. The parameters were cross-correlated to highlight its main relationships. This integrated perspective is expected to provide an understanding of how the estuarine system works as a whole and to identify the main forcing factors in two different climatic scenarios.

The data arising from this environmental characterization will be related through multivariate analysis with the benthic foraminifera and thecamoebians communities, in Chapter 5 and 7, respectively. The environmental variables will be then evaluated in terms of their influence on species composition, in order to identify which drive in the major distributional trends within each season.

4. 2. Sedimentological parameters

Grain-size analysis is an essential tool for classifying sedimentary environments (Blott and Pye, 2001). In a broad sense the grain size of siliciclastic sediments reflects the hydraulic energy of the environment: coarser sediments are transported and deposited by faster-flowing currents than finer sediments, which tend to accumulate in quieter waters (Tucker, 1995). In estuaries, clayey sediments are mainly carried in suspension within the water column, with the finest sediments being deposited at the landward limit of tidal excursion. In marshlands this effect is increased by a buffer of dense vegetation (Sen Gupta, 1999c). Sands are mainly carried along and very close to the river bottom (Wolanski, 2007) and its degree of sorting reflects the depositional process (Tucker, 1995).

The sedimentary dynamics in the Guadiana Estuary is essentially dominated by tidal forcing during low river flow and freshwater discharge during flood events (Machado et al., 2007). Coarse sediments (sand and gravel) are common along the entire estuary but are mainly associated with the deepest and most dynamic zones, where the flux is more intense. On both river margins there is prevalence of muddy sediments, as well as in some shallower and less hydrodynamic zones (Dias et al., 2001). Machado et al. (2007) found some differences in the content of suspended sediment according to the tide and river flow conditions. Under low-runoff conditions, the suspended sediment concentration is lower than during periods of heavy rain (flood events) and is mainly related with the incursion of saline waters into the estuary. The main minerals in suspension are phyllosilicates, quartz and plagioclases. Illite, kaolinite, chlorite, smectite and irregular illite-smectite mixed-layers are the main clay minerals (Machado et al., 2007).

The effects of grain-size on foraminifera density and diversity have rarely been studied, but the few works that have made these comparisons show that grain-size is a determinant factor in foraminifera distribution. Debenay et al. (2001) correlates the presence of bolivinids with fine sediments (silts and clay); Diz et al. (2004) suggest that very coarse sediments provide a favorable substrate for living foraminifera; Châtelet et al. (2008) showed that the grain-size (among other physico-chemical parameters studied) was the parameter which limited most the density and diversity of foraminifera. In the Guadiana Estuary, Ruiz et al. (2005) found a relationship between *Jadammina macrescens* and *Trochammina inflata* (both agglutinated foraminifers) and sediments with significant percentage of silts, and a further relationship between *Quinqueloculina seminulum* and *Elphidium crispum* and the sandy sediments of the subtidal channels located near the mouth, which present normal marine conditions. On the other hand, they considered *Ammonia tepida* as insensitive to grain-size distribution.

Clay sediments are usually considered to be an indirect factor influencing the distribution of foraminifera. Their high surface area, plasticity, adhesiveness, absorbency and adsorption of clay minerals make them excellent retainers of organic and inorganic compounds (Gomes, 1988). Nonetheless, clay content has been more broadly considered in studies where the aim is to analyze the effects of pollutants on foraminifera communities, especially inorganic pollutants (metallic cations). However, some attempts at relating the clay content to foraminifera preferences have already be made. For instance, Châtelet et al. (2008) associate the occurrence of *Miliammina fusca* with sediments enriched with smectite, suggesting that this mineral may play an important role in cementation during test construction.

4. 2. 1. Results and discussion

The main sedimentological analysis was based on the division of the sediments into three groups according to particle size: gravel, sand and mud (i.e. silt and clay) (Folk, 1954). Other sedimentological parameters, such as granulometric mean, sorting, skewness and kurtosis, were also inferred and are summarized in Table II, according to the elevation at which the samples were collected. The individual values for all the sedimentological parameters appear in Tables I, II, III and IV of Appendix 6.

In general, no significant differences were found between sediment grain-size distribution in winter and summer (Fig. 4.1). Only a few samples exhibit some differences (e.g. LAR), attributed to local changes or small deviations in the sampling area. In both seasons, a gradient from finer sediments in the upper elevations (from 1–2 m above MSL) to coarser sediments in the lowest elevations was observed (middle elevations, ranging from 0–1 m above MSL and lower elevations, ranging from -1–0 relative to MSL). To verify this trend, the relationship between elevation (MSL) and the three sedimentary classes was evaluated through the Spearman Rank Correlation coefficient (*R*) (Table I). Although slightly stronger in summer, in both seasons there was a moderate positive relationship between elevation and mud content, and a moderate negative relationship with sand content.

With the exception of sample VRSA1, which is distinctly different from the other samples of the upper elevations, the remaining samples are characterized by more than 90% mud content, of which 63 to 80% are silts. VRSA1 sample was collected on the margin of a eutrophic pond separated from the sea by a sand bank of possible anthropogenic origin. VRSA1 is characterized by more than 92% sand, 5-7% mud and a negligible percentage of gravel.

The middle elevations samples comprise all the samples collected in the middle and upper estuary and in some sites of the lower estuary, represented by two samples (FB, PI and LG). In what concerns samples from the upper elevations, those from the middle elevations have a coarser nature, with a mud content varying from 9 to 94% in winter (E1 and LG1, respectively; mean 69.5%) and from 15 to 98% in summer (LAR and LG1, respectively; mean 69%). At sites with two samples in the middle elevations, the one in the most elevated position has a finer nature than the lower location, but are still coarser than the upper elevations samples.

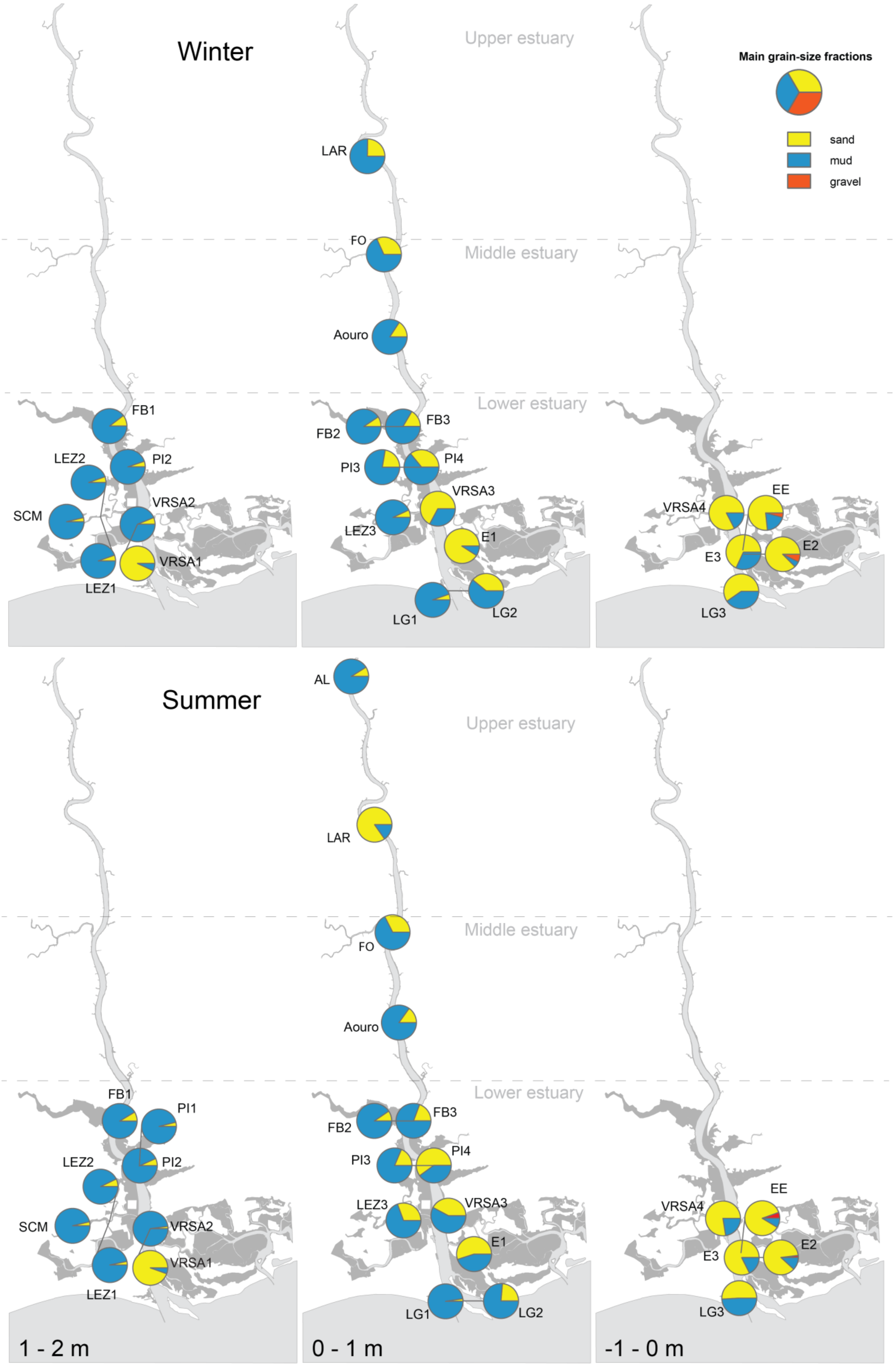

Figure 4.1. Diagrams showing the variation in the content of mud, sand and gravel along a north/south and elevation gradients during winter and summer.

Table I - Spearman’s Rank Correlation (*R*) between spatial variables: elevation in relation to MSL and latitude and the three main grain-size fractions: gravel, sand and mud in winter and summer samples. (*R*) is indicated for each pairwise, in **bold** only when significant (** for $p < 0.01$ and * for $p < 0.05$).

	winter		Summer	
	Elev MSL	Latitude	Elev MSL	Latitude
gravel	-0.15	-0.39	-0.15	**-0.40***
sand	**-0.60****	-0.36	**-0.64****	-0.19
mud	**0.62****	0.37	**0.65****	0.19

Figure 4.2 illustrates the variation of the content of mud, sand and gravel along an elevational profile, using the PI site during summer as an example.

Samples collected in the lower elevations were the coarsest of all, with sand content ranging from 60 to 87% in winter and from 51 to 86% in summer. In some of these samples the gravel content was significant, especially on the Spanish side, where it sometimes reaches 7% of total sediment. However, this gravel component results essentially from the contribution of bioclasts, which were very abundant within the samples in question. Among the samples of lower elevations, LG3 had the most consistent content in terms of mud and sand in both seasons (40/60% mud/sand in winter and 49/51% mud/sand in summer), due to its protected location in relation to hydrodynamic action.

The trend of increasing grain size from the upper elevations to lower elevations was observed in every transect of both seasons, although only illustrated for the PI site during summer. The sub-environments in the upper elevations were rich in muddy sediments, mostly silts, which are transported in suspension by the river. The finer sediment fraction is deposited in the upper zones where the hydrodynamics were lower (only the slack waters of the flood peak) and attenuated by the buffering effect of the dense halophytic vegetation. Sedimentation of the finer content contributes to the extension of the muddy margins of the river that, despite their apparent fragility, are resistant to erosion due to the high yield strength and viscosity of the sediments, promoted by high organic matter content. Middle elevation sub-environments had a more balanced content of mud and sands due to their location in the most hydrodynamic intertidal zone, subject to the daily rise and fall of tides. The lower elevations had an increased content of sand and gravel due to their proximity to the bypassing subtidal zone. According to Morales et al. (2006) and Dias et al. (2001), this is the deepest zone of the river channel in which bedload transportation is dominant, and only coarser sediments are present.

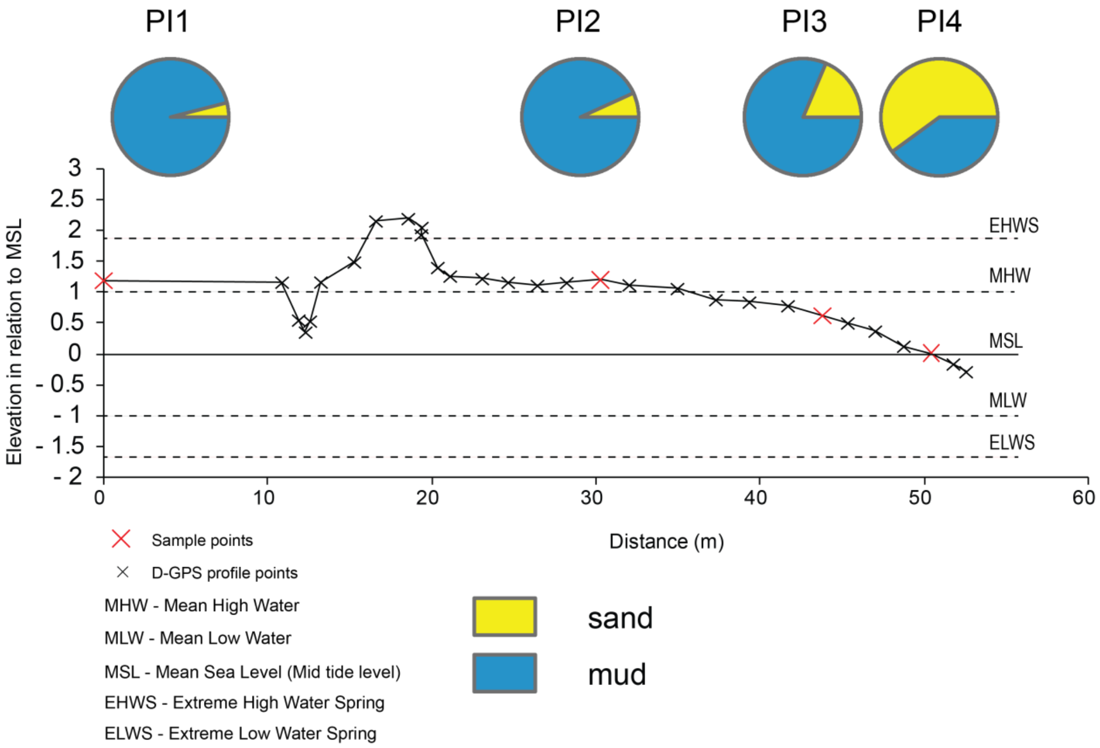

Figure 4.2. Variation in mud and sand content along an elevational profile at the PI site during summer.

Although weaker, the same gradient from finer to coarser sediments was found in the north/south gradient in winter samples (samples from middle-upper estuary have a mean value of 76% mud content; 67% in the lower estuary). The same trend was not observed in summer, when the mud content in the samples of the middle-upper estuary and of the lower estuary were similar (65 and 71% mud content, respectively). No significant relationship was found between latitude and mud or sand content. Nevertheless, a positive trend was observed for mud and a negative trend observed for sand, for both seasons. A weak but significant negative relationship was found between latitude and gravel (Table I). This lack of significant relationships between latitude and sediment type could be explained by the data considered for correlation, which comprises all the samples on the elevational gradient, including upper elevation samples that, as mentioned previously, had a very fine nature. In a bedrock controlled estuary such as the Guadiana, a longitudinal sediment-size gradient is expectable, due to the energetic textural bipolarity (river × tidal/sea energy), in which fluvial sediment is reworked from the river to the sea by the ebb current and marine sediment is introduced into the estuary by the flood current (Morales et al., 2006). Seaward-flowing currents dominate throughout the Guadiana Estuary during periods of significant freshwater discharge (spring freshets), in relation with the narrow and confined channel morphology. This mechanism supplies coarse-grained sediment to the system from the upstream river and governs net seaward sediment-transport within the estuary and exportation to the nearshore at a yearly to centennial scale (Garel et al., 2009). These fluvial sediments are mixed with marine sediments in the lower part of the estuarine channel during tidal cycles, where active sedimentation takes place on the lateral tidal bars (Morales et al., 1997; 2006). This process is most pronounced on the left margin (Spanish side), where the channel is shallower (probably with lower velocity currents) and high accretion is visible. This could explain the incidence of gravel-bioclast deposits in samples from the Spanish side, especially in summer when the hydrodynamic processes are predominantly sea-dominated (Machado et al., 2007) and a significant negative relationship was found between latitude and gravel content.

According to the Folk and Ward (1957) method, the samples in the upper elevations in both seasons were classified as fine to medium silt, except for VRSA1 which was classified as medium sand in winter and fine sand in summer (Table II; see GRADISTAT Tables III and IV in Appendix 6 for further details). The sediments were poorly sorted, symmetrical and mesokurtic, except for VRSA1, which exhibited very leptokurtic sediment distributions.

Table II – Synthesis of the main sedimentological parameters for the samples of winter and summer according to an elevational gradient (upper elevations: 1-2 m; middle elevations: 0–1 m; and lower elevations: -1–0 m).

Season	**Winter**			**Summer**		
Elev MSL (m)	**1 - 2**	**0 - 1**	**- 1 - 0**	**1 - 2**	**0 - 1**	**- 1 - 0**
Granulometric mean (μm)	fine silt to medium sand	fine silt to medium sand	very fine sand to fine sand	fine silt to fine sand	fine silt to fine sand	very coarse silt to fine sand
max	278.16	287.97	219.97	201.99	141.83	248.50
min	6.63	5.68	66.66	6.44	5.81	41.50
mean	46.23	42.30	125.41	31.68	29.17	138.65
SD	102.28	79.74	62.07	68.83	36.25	78.17
Sorting	poorly sorted	very poorly sorted	very poorly sorted	poorly sorted	very poorly sorted	very poorly sorted
max	4.32	9.32	8.93	4.27	7.27	6.82
min	1.90	1.98	2.31	1.70	2.54	2.58
mean	3.67	5.17	4.56	3.59	4.94	4.10
SD	0.81	1.72	2.57	0.82	1.21	1.67
Skewness	symmetrical	very fine skewed to coarse skewed	very fine skewed	symmetrical	fine skewed	very fine skewed
max	-0.01	0.19	0.16	-0.03	0.01	-0.01
min	-0.11	-0.62	-0.64	-0.14	-0.61	-0.61
mean	-0.06	-0.08	-0.35	-0.09	-0.21	-0.43
SD	0.03	0.21	0.35	0.04	0.22	0.24
Kurtosis	leptokurtic	mesokurtic	very leptokurtic	mesokurtic	mesokurtic	very leptokurtic
max	1.61	1.87	2.88	1.53	2.42	3.23
min	1	0.68	0.74	0.96	0.75	0.69
mean	1.12	1.02	1.87	1.07	1.04	1.80
SD	0.22	0.33	0.85	0.19	0.43	0.94

In the middle elevations samples, the sediments ranged from fine silt to medium sand. LG1 (winter and summer) and E1 (winter), respectively, were very poorly sorted and had a mesokurtic, very fine skewed to symmetrical distribution, while sample LG2 in summer had a coarsely skewed distribution.

Sediments in the lower elevations ranged from very coarse silt to fine sand and were very poorly sorted. The sediment distribution was very fine skewed and very leptokurtic.

Overall, it seems that during winter there was a slight increase in the proportion of coarser sediments in the estuary, especially in the middle-lower elevations of the lower estuary. This slight difference could be related to the presence of alternating layers of muddy and sandy sediments on the river banks, which Dias et al. (2001) observed and attributed to seasonally contrasting hydrodynamic situations.

All sites had poorly to very poorly sorted sediments, suggesting weak energy when compared to the deep channel of the estuary in which the sands are well sorted due to the high energy and influence of fluvial currents (Morales et al., 2006). Indeed, these authors identified two hydrodynamic zones in the estuarine channel: the shallow portion of the channel where active deposition of sediments occurs (which includes all the sampling points in the present work) and the deepest portion of the channel (subtidal) where bedload transportation occurs (not sampled in the present work).

4. 3. Organic matter

Estuarine zones are good environments for studying the origins, pathways and fates of sedimentary organic materials due to the rapid accumulation of fine sediments (González-Vila et al., 2003). In coastal estuarine sediments, organic carbon compounds are provided to biological communities from three main sources: detritus of photoautotrophic biota, components of vascular plants and anthropogenic inputs (González-Vila et al., 2003). Each of these sources supplies dissolved organic matter (OM) which feeds bacteria and thus enters, or is recycled into, the particulate food chain (CSIRO, 2000; Loubere and Fariduddin, 1999). Once in the sediment, organic material decomposition is the key to the continuity of the biogeochemical cycle of carbon and other associated elements (N, P, S, etc.) (Nuño, 2008). Organic matter, being a reduced form of carbon and hence at an elevated free energy state, is intrinsically unstable in oxidizing environments. Degradation of OM is rapid in the photic zone, continues during the sinking of organic particles, and is intense in the bioturbated surface layer of sediments (Meyers, 1997). Typically no more than a few percent of the original amount of primarily produced OM survives remineralization to CO_2 and becomes buried in the sediments (Meyers, 1997; Wakeham and Canuel, 2006). The characterization of OM is of great interest in establishing the possible origins and sources of sedimentary materials. Allochthonous sources of particulate matter include not only marine and riverine inputs, but also direct domestic, industrial and runoff inputs from the land into the estuary. Autochthonous particles are due to primary production by phytoplankton, microphytobenthos, higher plants and chemoautotrophic producers (Middelburg and Niewenhuize, 1997). Due to its usefulness, C/N ratios determined from elemental analysis have been widely used as an OM proxy (Meyers, 1994). The C/N ratio for actively growing marine phytoplankton is 5.7, and values in the range 6-9 are typically reported for autochthonous marine-derived OM, whereas values greater than 12 are usually found in terrestrially derived OM (Bordovskiy 1965, cited in CSIRO, 2000).

Although it is recognized that organic carbon (OC) availability is important to benthic foraminifera in shallow waters, it has been difficult to assess its exact effect. This is because OC flux is not usually directly measured in foraminiferal studies (OC content of sediments is only an indirect proxy for labile organic carbon flux), and because food availability is often covariant with many other ecological factors that are important for foraminifera (Loubere and Fariduddin, 1999).

In estuaries, the effects of pollution on microfaunistic communities have been widely studied. The majority of the works inferring the relation between OM and foraminifera focus on their response to anthropogenic inputs: sewage effluents (e.g. Topping et al., 2006; Ward et al., 2003), industry (e.g. Frontalini et al., 2009; Martins et al., 2010) and aquaculture (e.g. Schafer et al., 1995), among others. However, the study of OM inputs in natural environments can be hampered due to the effects on the surrounding physico-chemical environment (sediment properties, sedimentation rate, oxygen concentration, etc.), which can obscure the foraminiferal response to those inputs (Loubere and Fariduddin, 1999; Ward et al., 2003). The results of recent microcosm experiments suggest that the food sources and diet of two of the most common foraminifers of temperate estuarine zones (*Ammonia beccarii* and *Haynesina germanica*) are uninfluenced by sewage particulate organic matter (POM) (Topping et al., 2006; Ward et al., 2003). The foraminifera do not benefit directly from sewage-POM but from the enhancement in bacteria/microalgal biomass which blooms in response to sewage-POM input (Topping et al., 2006; Ward et al., 2003), thus corroborating the concept that foraminifera respond mostly to the flux of labile material (Loubere and Fariduddin, 1999), i.e. matter that is easily broken down by chemical and microbiological pathways (CSIRO, 2000). In estuaries, most labile matter consists of detrital particles and their associated microorganisms (phytoplankton and benthic microalgae), which provide the basic food sources for primary consumers like benthic foraminifera. In the Guadiana, when the river flow is low (spring and summer or when the natural flow is artificially retained), there is low bioavailability of silica (Si), promoting blooms of toxic phytoplankton (cyanobacteria) and damaging other phytoplanktonic species (Rocha et al., 2002). The bioavailability of nutrients, especially Si, is enhanced with higher inputs of river freshwater (rainy seasons) and promotes the bloom of diatoms, which are the preferred food source for healthy foodwebs (Dias et al., 2001; Rocha et al., 2002).

Benthic fluxes in inorganic carbon are the result of a balance between the release of inorganic carbon to pore waters and the dissolution–precipitation of calcium carbonate (Ortega et al., 2005). Until a few years ago, the contribution of $CaCO_3$ dissolution to benthic flux of inorganic carbon was not considered significant. Over the past decade, however, many researchers have come to attribute a large part of the benthic flux of inorganic carbon to $CaCO_3$ dissolution processes (see Ortega et al., 2005). $CaCO_3$ availability is higher in summer, when temperature and salinity are higher (Buzas, 1989). Greiner (1974) presents evidence that $CaCO_3$ availability controls the distribution of shell types, suggesting a gradient in estuaries, where environments with little availability would be dominated by agglutinated foraminifera, intermediate availability by hyaline foraminifera and high availability by porcelaneous foraminifera (miliolids).

4. 3. 1. Results and discussion

4. 3. 1. 1. Total Organic Carbon (TOC): In Guadiana Estuary sediment samples, TOC values were highly variable (0.2 to 9%) and relatively high when compared to other estuaries such as Land van Saaftinghe salt-marshes, Netherlands, with 3.36 to 4.21% (Nieuwenhuize et al., 1994), the Pearl River Estuary, China, with 0.06 to 1.02% (Hu et al., 2006), the Yangtze salt-marshes, China, with 0.1 to 0.7% (Zhou et al., 2007) and other coastal depositional environments (Nieuwenhuize et al., 1994). The Guadiana values are, however, similar to those of the Huon Estuary, Tasmania (0.2 to 9.6%, CSIRO, 2000) and the river-dominated estuary of Winyah Bay, SC, USA (0.05 to 7.62%, Goñi et al., 2003). The high variability in TOC content in Guadiana sediments reflects the great diversity of the sampled sub-environments, which are subject to a multiplicity of physico-chemical conditions, among which, TOC sources, hydrodynamics and topography play major roles.

Figure 4.3. Variation in TOC content along a north/south and elevation gradients during winter and summer.

No major differences were found between TOC concentration in winter and summer, varying from 0.2 to 8.2% in winter and from 0.3 to 9% in summer. Nevertheless, some differences in TOC distribution during the different seasons were noted. Although not evident in Fig. 4.3, the mean value of TOC content decreases gradually from the upper elevations to the lower elevations in both seasons (mean TOC values in winter: 1–2 m: 4.1%; 0–1 m: 2.7%; and -1–0 m: 0.81%; mean TOC values in summer: 1– 2 m: 3.1%; 0–1 m: 2.9%; and -1–0 m: 1.9%).

This pattern was especially evident in winter, where Spearman *R* indicates a moderate positive relationship between Elevation and TOC (Table III). In summer this relationship was weaker and insignificant. The decreasing TOC gradient from upper elevations to lower elevations is strongly related to tidal circulation patterns (Suguio, 2003), which promote marked differences in vegetation zonation, water circulation velocities and, consequently, sediment sorting. Hence, one would expect to find the highest TOC value in the most elevated areas, where the hydrodynamics are lower, vegetation is abundant and the sediments are finer and more cohesive. The TOC content of the sediments was inferred through its relationship with mud content (Table III) and a relatively strong positive relationship was found in winter and a moderate relationship in summer. The opposite trend was found for sands (inversely proportional to mud content), which are more abundant in the lower elevations of the intertidal zone where the water circulation is more efficient, enabling the resuspension and oxidation of the deposited organic matter. This relationship has been observed in other estuarine environments around the world (CSIRO, 2000; Goñi et al., 2003; Zhou et al., 2007). The relationship between TOC and elevation may be enhanced during winter due to stronger hydrodynamics in the estuary, promoting more efficient sorting of both organic matter and sediments.

No TOC content gradation was visible along the north/south gradient (Fig. 4.3). In general, the values were relatively low (varying from 0.2 to 3.2%) from upper to lower estuary, with some exceptions at the LG site in both seasons (5.6% in winter and 8.1% in summer) and the FO site in summer (9% - the highest TOC value for the entire estuary). In both seasons, Spearman’s *R* indicates no significant relationship between Latitude and TOC (Table III). Middelburg and Herman (2007) also found TOC concentrations to be uniform along a salinity gradient in the Sado Estuary and other turbid estuaries.

Table III - Spearman’s Rank Correlation (*R*) between elevation (MSL), latitude, mud and the organic matter and carbonates proxies in winter and summer samples. (*R*) is indicated for each pairwise, in **bold** only when significant (** for $p < 0.01$ and * for $p < 0.05$).

	Winter						Summer					
	Elev MSL	Lat	mud	TOC	TIC	TN	Elev MSL	Lat	mud	TOC	TIC	TN
TOC	**0.55****	0.10	**0.78****				0.26	0.06	**0.60****			
TIC	-0.01	**-0.52****	0.04	0.32			0.11	**-0.44***	0.02	0.23		
TN	**0.47***	-0.01	**0.75****	**0.96****	0.33		0.23	0.04	**0.57****	**0.98****	0.21	
C/N	**0.43***	**0.71****	**0.50***	0.39	-0.33	0.21	-0.04	0.19	-0.24	-0.19	0.08	-0.35

4. 3. 1. 2. Total Nitrogen (TN): No major seasonal differences were found in TN concentrations, varying from 0.02 to 1.1% in winter (VRSA4 and LG1, respectively) and from 0.03 to 0.9% in summer (E2 and LG1, respectively) (Fig. 4.4). Both of these ranges are higher and more variable than those found in other estuarine systems such as Land van Saaftinghe saltmarshes, Netherlands, with 0.28 to 0.34% (Nieuwenhuize et al., 1994), Huon Estuary, Tasmania, with 0.01 to 0.74% (CSIRO, 2000), Winyah Bay, SC, USA, with 0.01 to 0.4% (Goñi et al., 200), Yangtze salt-marshes, China, with 0.014 to 0.078% (Zhou et al., 2007). Like TOC, TN is positively correlated with elevation during winter, although weakly. The same trend was found for summer, but was even weaker and statistically insignificant (Table III) (mean TN values in winter: 1–2 m: 0.4%; 0–1 m: 0.3%; and -1–0 m: 0.1%; mean TN values in summer: 1–2 m: 0.33%; 0–1 m: 0.32%; and -1–0 m: 0.23%). The weak relationship between TN and Elev during summer could be influenced by the seasonal opposite trends observed along the VRSA transect in particular, with TN concentrations decreasing from upper to lower elevations in winter and increasing in summer (Fig. 4.4).

TN exhibited a statistically insignificant negative relationship with Latitude in both seasons (Table III). TN concentration, which is considered indicative of the contribution of aquatic flora (González-Vila et al., 2003), although in much lower concentrations, was strongly proportional to TOC concentration in both seasons (R > 0.96). This good correlation supports the model that organic matter in sediments is largely controlled by mixing of aquatic and terrestrial end members. This relationship was also found in other estuarine systems (Goñi et al., 2003; Hu et al., 2005; Middelburg and Herman, 2007; Zhou et al., 2007).

TN was positively correlated with mud-rich sediments in both seasons, with a stronger relationship observed in winter (Table III).

Figure 4.4. Variation in TN content along a north/south and elevation gradients during winter and summer.

4. 3. 1. 3. Organic Carbon/Nitrogen ratio (C/N): The strong relationship between TOC and TN observed in both seasons caused C/N values to be quite similar along the estuary, ranging from 6 to 12 in the majority of the samples (Fig. 4.5). This suggests that the organic matter in the Guadiana Estuary results primarily from inputs of autochthonous fluvial and allochthonous marine sources (6-9), sometimes mixed with terrigenous organic matter (9-12). Only few samples had C/N values greater than 12, among which it is noteworthy that the FB1 site in winter reached 15.3, the highest C/N value for the entire estuary. The only value lower than 6 was registered at the E2 site in winter, but could be somehow biased due to its TOC value lower than 0.3%, which, according to Meyers (1997), is the minimum necessary to reliably calculate C/N.

There was an observable decrease in C/N from the upper to lower elevations in winter (Fig. 4.5) (mean C/N values in winter: 1–2 m: 10; 0–1 m: 9; and -1–0 m: 7.4). This is also visible through Spearman *R* correlation between Elev and C/N, in which these two variables had a positive, although weak, relationship. In summer the same trend was not evident, and there is even a slight increase from upper to lower elevations (mean C/N values in summer: 1–2 m: 9; 0–1 m: 9.4; and -1–0 m: 10.2). This inverse trend in the elevation gradient in both seasons lead to a positive correlation between C/N and muddy sediments in winter and a negative, non significant, correlation in summer (Table III), when high C/N values were found in sandy samples of E and EE sites. According to Meyers (1997), different sediment size-fractions typically have different C/N ratios, with coarse sediment fractions containing a larger proportion of intact land-plant debris than fine fractions and thereby having elevated C/N ratios. Furthermore, fine sediment fractions contain a larger proportion of clay minerals, which have both large surface areas and negative surface charges. Clays therefore adsorb ammonia well, and this can depress their C/N ratios due to the uptake of inorganic nitrogen (Meyers, 1997). These general trends explain the opposing trends observed in the present work. In wet seasons, the hydrodynamics are stronger, promoting more efficient transport of particulate organic matter out of the estuary (Oliveira et al., 2006). In dry seasons (summer), hydrodynamic processes are weakened and the residence time of the particulate organic detritus in the lower elevations is increased, leading to temporarily increased C/N ratios. Nevertheless, the elevated C/N ratios in the lower elevations during summer, especially in the sites in the Spanish margin (E and EE) could be related to the proximity of the Ayamonte Sewage Treatment Plant, which could be contributing with allochthonous biomass enriched in cellulose.

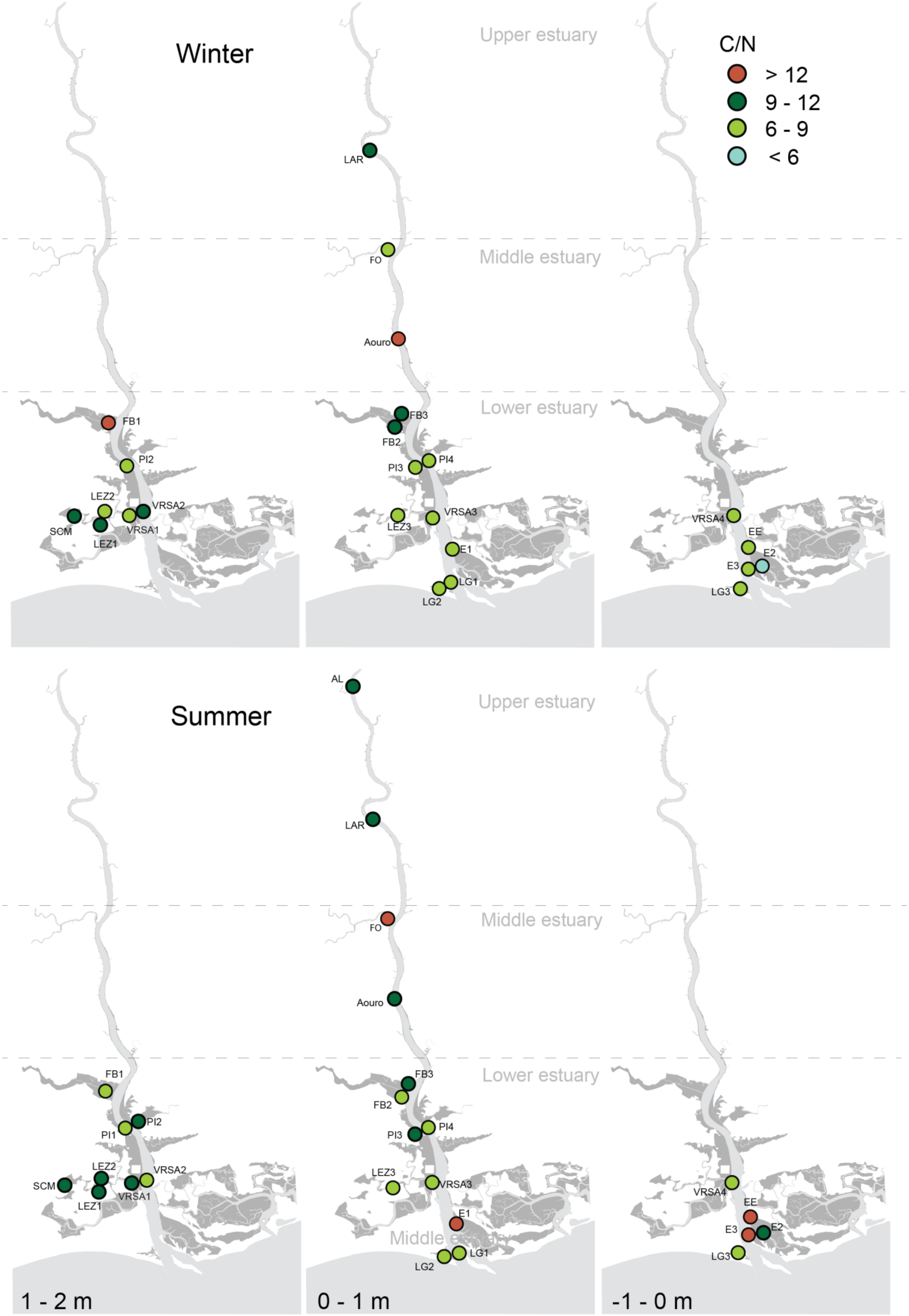

Figure 4.5. Variation in C/N along a north/south and elevation gradients during winter and summer.

C/N had a positive and relatively strong relationship with latitude in winter (R = 0.71), with the highest values in the northernmost samples, but a much weaker, non significant, relationship in summer, when C/N values were much more heterogeneous throughout the estuary. Despite these seasonal differences, a consistent picture emerges of organic material being derived largely from terrestrial sources in the middle-upper estuary and upper elevations of the lower estuary, whereas there is a substantial input of autochthonous marine material in the middle-lower elevations of the lower estuary.

The C/N ratio was not significantly correlated with any of the other organic matter proxies (Table III).

4. 3. 1. 4. Total Inorganic Carbon (TIC): Carbonate content and indirect availability of $CaCO_3$ in the Guadiana Estuary were inferred from TIC variations (Fig. 4.6). No major differences were found in TIC concentration between winter and summer, varying from 0 to 2% in winter (E1 and LG1, respectively) and 0 to 1.2% in summer (PI1 and LG1, respectively). Both these ranges were more variable than, but comparable to, other estuarine sediments (1.2 to 1.4%), but were much lower than the values found for deeper depositional coastal environments (1.2 to 9.96%) (Nieuwenhuize et al., 1994).

No TIC gradient was found in relation to elevation (mean TIC values in winter: 1–2 m: 0.4%; 0–1 m: 0.32%; and -1–0 m: 0.56%; mean TIC values in summer: 1–2 m: 0.33%; 0–1 m: 0.3%; and -1–0 m: 0.4%). There was no correlation between TIC and Elev (Table III). This is also in accordance with microscopic observations, which yielded low counts of autochthonous (stained) meiofauna in the upper and middle elevations (although some samples of the upper elevations exhibit large numbers of gastropods). In the lower elevations, both autochthonous meiofauna (stained juvenile bivalvia, ostracoda and foraminifera) and reworked bioclastic facies contributed to TIC. In any case, the middle elevations samples had the lowest TIC concentrations in either season, especially if the LG site was not included (mean winter value: 0.11% and mean summer value: 0.16%). In the middle elevations, both samples from the LG site (small lagoon) exhibited an opposite trend in relation to the remaining samples of the same group and presented the highest TIC values for the entire estuary. Dyer (1990) showed that bioclastic materials in estuaries have various origins, resulting from the local fauna or sediment transport. In the present work, the highest TIC values were found in the least hydrodynamic environments, which are more suitable for fauna fixation, and in the lower elevations, where autochthonous infaunal organisms are found alongside bioclastic reworked material, which

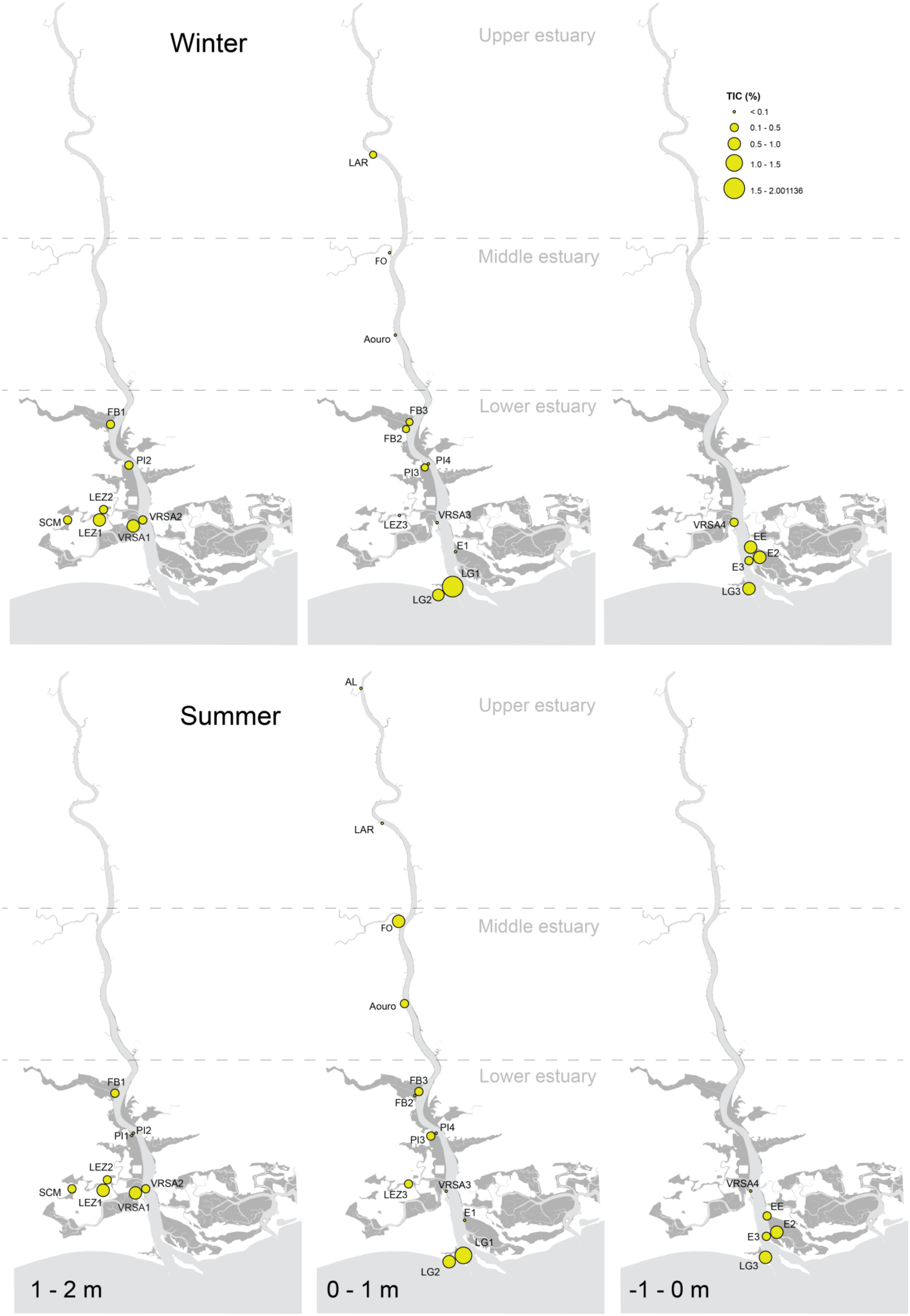

Figure 4.6. Variation in TIC content along a north/south and elevation gradients during winter and summer.

accumulates in sorted deposits under the moderate hydrodynamic conditions of the intertidal lower elevations.

TIC variation on a north-south gradient (Fig. 4.6) suggests a gradation from the upper-middle to lower estuary, where the highest TIC percentages are concentrated. This relationship was also confirmed through the Spearman *R* correlation coefficient (Table III) between Latitude and TIC in winter and summer, in which the two variables expressed a negative relationship: moderate in winter and weak in summer. This relationship is expected if pH differences in river and sea waters are considered along a salinity gradient. Carbonates, in particular calcite, precipitate in alkaline waters (pH > 7, typical of seawater) whereas calcite dissolves in acidic water (pH < 6, typical of freshwater) (Suguio, 2003). Indeed, a moderate positive relationship was found between TIC and salinity (Table V), although only significant in winter.

Relationships between TIC, latitude and salinity could be attenuated during summer due to higher availability of $CaCO_3$ during this season, when temperature and salinity are higher (Buzas, 1989).

The relationship between TIC and sediments (mud content) was also evaluated, but no statistical relationship was found between these variables in either season (Table III). This is not surprising due to the presence of bioclasts in both muddy and sandy sediment fractions.

4. 4. Water chemical parameters and sediment pH

Salinity, together with depth and temperature, are considered to be the most important factors in controlling the abundance and distribution of foraminifera (Lin, 1992). Although foraminifera can inhabit environments with salinities ranging from 0.5 to 57 (Lin, 1992) the majority are adapted to normal marine salinities (about 35), in which the most diverse assemblages are found (Armstrong and Brasier, 2005). The low salinity of brackish lagoons and marshes favors low-diversity assemblages of agglutinated foraminifera and certain hyaline forms (e.g. *Ammonia* and *Elphidium*) (Armstrong and Brasier, 2005). In general, littoral foraminifera are well adapted to strong salinity oscillations (Debenay et al., 1996) but their abundance tends to increase from low salinities (0.5) to typical sea water salinities (35-37). In waters with salinities higher than sea water, the number of species and standing-crops decreases abruptly (Lin, 1992).

In alluvial estuaries such as the Guadiana, the longitudinal distribution of salinity depends on river inflow, varying from stratified (riverine estuarine) conditions when high river discharges occur, resulting in a saline wedge close to the mouth, to well mixed (marine estuarine) conditions during low flows, resulting in upstream salt intrusion (Savenije, 2005). During river low flows, the Guadiana Estuary is dominated by tidal dynamics, causing a strong vertical mixing in salinity during spring tides and a pronounced saline intrusion during neap tides (Rocha et al., 2002). Two principal water masses are identifiable: a freshwater mass which circulates above and a saline water mass which circulates at the bottom and which is present even during low tides (Dias et al., 2001). The depth and extension of those water masses depends on the tidal phase. Independently of the tides, an asymmetry in the distribution of water masses is observed, with more salt water circulating on the left margin (Spanish) and more fluvial waters circulating on the right margin of the Guadiana Estuary (Dias et al., 2001). In marshlands, pore-water salinity depends on several factors, one of which is elevation. In the often-submerged lower saltmarsh, salinity is relatively constant and close to that of the flooding water. In the upper marsh, however, the pore-water salinity can be extremely variable. Episodes of tidal flooding are infrequent, and, in the interim, rainfall may cause a reduction of pore-water salinity. On the other hand, during periods of desiccation, salinity may increase significantly through evaporation leading to the formation of a saline crust at the surface (Sen Gupta, 1999c) (see also picture nº 40 in Appendix 1).

As for pH, it is an indicator of acidity in water and sediments and is a very important environmental factor in controlling foraminifera distribution. Microbial respiration of organic carbon produces CO_2 which, in solution, is acidic. Since the sedimentation of organic material tends to be associated with relatively quiescent environments, and the scope for oxygenation of sediments through physical disturbance or diffusion is consequently reduced, anaerobic conditions tend to be associated more with silts and clays than sands (Bale and Kenny, 2005). Foraminifera with agglutinated tests are resistant to reduced environments, where the oxygen concentration and pH are low. Thus, the agglutinated forms are common in the sub-surface of upper elevations paralic environments and, after death, their tests are preserved in the sediment, contributing to the fossil record. The same is not true for calcareous foraminifera, which can live in acidic environments, but do not fossilize in the sediment due to rapid calcite dissolution (Scott et al., 2001).

Based on a recent and long dataset (hourly sampling intervals, from March 2008 to February 2010) provided by the SIMPATICO system (a non-permanent environmental monitoring station deployed in the mouth of Guadiana Estuary), the pH of the estuarine water varies from 7.75 to 8.25. Temporal variability is predominantly driven by tides, increasing during ebb and decreasing during flood (Garel and Ferreira, 2011). As far as we know, no data on sediment pH (other than that presented in the current study) is available for the post-Alqueva period.

As mentioned, temperature is one of the most important environmental factors in foraminifera distribution. Foraminifera live at temperatures between 1º and 50º (Boersma, 1998; Lin, 1992), but each species is adapted to a certain range of temperature conditions, the most critical being the range in which successful reproduction can take place (Armstrong and Brasier, 2005). Primitive forms, with agglutinated tests and non-calcareous cements, are typical of cold waters. In temperate waters the assemblages are more diverse and in the tropics the forms are essentially calcareous, with large and highly ornamented tests (Seyve, 1990).

Based on data from the SIMPATICO buoy, Garel and Ferreira (2011) found that the temperature signal for the Guadiana Estuary has two distinct seasons, delimited by the 18º C isotherm: winter temperatures varying from 12 to 18º C, increasing during flood and decreasing during ebb; and summer temperatures varying from 18 to 24º C, increasing during ebb and decreasing during flood.

Oxygen dissolved in the water has two main sources: directly from the atmosphere and resulting from microbial and aquatic plant photosynthesis. The most widespread dysoxic and microxic (< 1 ml/l) marine/brackish environment is the subsurface sediment (Bernhard and Sen Gupta, 1999). Since most aerobic, benthic organisms live near the sediment-water interface, oxygen consumption is high in this habitat. At a particular sedimentary depth, depending primarily on respiration rates and organism abundance, oxygen demand will exceed supply and sediments will become anoxic. The top of the anoxic zone is typically within the upper decimeters of sediment, even when overlying waters are well-aerated (Bernhard and Sen Gupta, 1999). Oxygen availability is not an indispensable factor in foraminifera distribution because several benthic foraminifera from various water depths inhabit oxygen-poor, anoxic and even sulfidic conditions (Bernhard and Sen Gupta, 1999). Nevertheless, oxygen depletion is widely regarded as a stress factor, causing a decrease in standing crops and species diversity. It may also dwarf the size of individuals, due to decreasing metabolic rates and may lead to the proliferation of pioneer and opportunistic species (Boltovskoy and Wright, 1976; Duijnstee et al., 2004).

In the Guadiana Estuary, Garel and Ferreira (2011) found a negative correlation between dissolved oxygen (DO) and temperature because of the greater solubility of oxygen in colder water. In winter, DO varies from 7.5 to 10 mg/l and in summer from 6 to 7.5 mg/l. Both temperature and DO exhibit a strong diurnal component due to daily insolation.

4. 4. 1. Results and discussion

In the present work, only data on sediment pH (pH_{sed}) is presented for all of the samples because it was measured directly from the sediment using a probe for solids (see Chapter 2). Salinity (Sal), temperature (T) and dissolved oxygen (DO) were measured only in locations with sufficient interstitial water for the probe to collect measurements without breaking. This reduced significantly the number of measurements taken per site. Graphical interpretation of the three aforementioned parameters will therefore focus on the site locations along a north/south profile, without the previous division into three elevation levels (Fig. 4.7). Interpretation of pH_{sed} variation is based on the same graphical presentation as the previous diagrams (Fig. 4.8). In both seasons, water parameters were always measured during low-tide periods in the superficial waters near the low-tide level. In samples located in isolated lagoons, such as SCM, VRSA1, E1 and LG3, measurements were taken inside the water bodies. Results on Sal, T, pH_{sed} and DO for winter and summer are given in Table I, Appendix 8.

4. 4. 1. 1. Salinity (Sal): Sal values were very different in winter and summer, especially in the lower estuary, where major differences were visible. In winter, Sal varied within a range of very low values along the entire estuary: 0.13 to 3, for FO and VRSA4, respectively. Only SCM (saltworks pond) and LG3 (salty lagoon - the southernmost site) presented higher Sal values, 14.3 and 27.8, respectively. In summer, no major differences were found in the upper and middle estuary, in which values remained lower than 1 for all samples. However, from the FB site towards the mouth, Sal tended to increase gradually, starting from 2.8 at FB and ending in 36 at LG3 (Fig. 4.7).

Correlation analysis supports this negative relationship between salinity and latitude, which was stronger in summer (R = - 0.85) (Table IV). These observations are in accordance with those made by other authors (Dias et al., 2001; Garel et al., 2009; Rocha et al., 2002), who identified a salt wedge in the lower estuary during winter with a dominance of freshwater during high river flows, as in the case of the present winter campaign.

During summer, the river flows are almost nil and the estuary is dominated by tidal effects, causing strong vertical mixing of salinity at spring tides and strong saline intrusion during neap tides (Rocha et al., 2002). The lengthening of the intrusion depends on tidal phase (Dias et al., 2001) and, in the present work, salt intrusion was marked up until the PI site (International Bridge) with 9.3 during a low spring-tide phase.

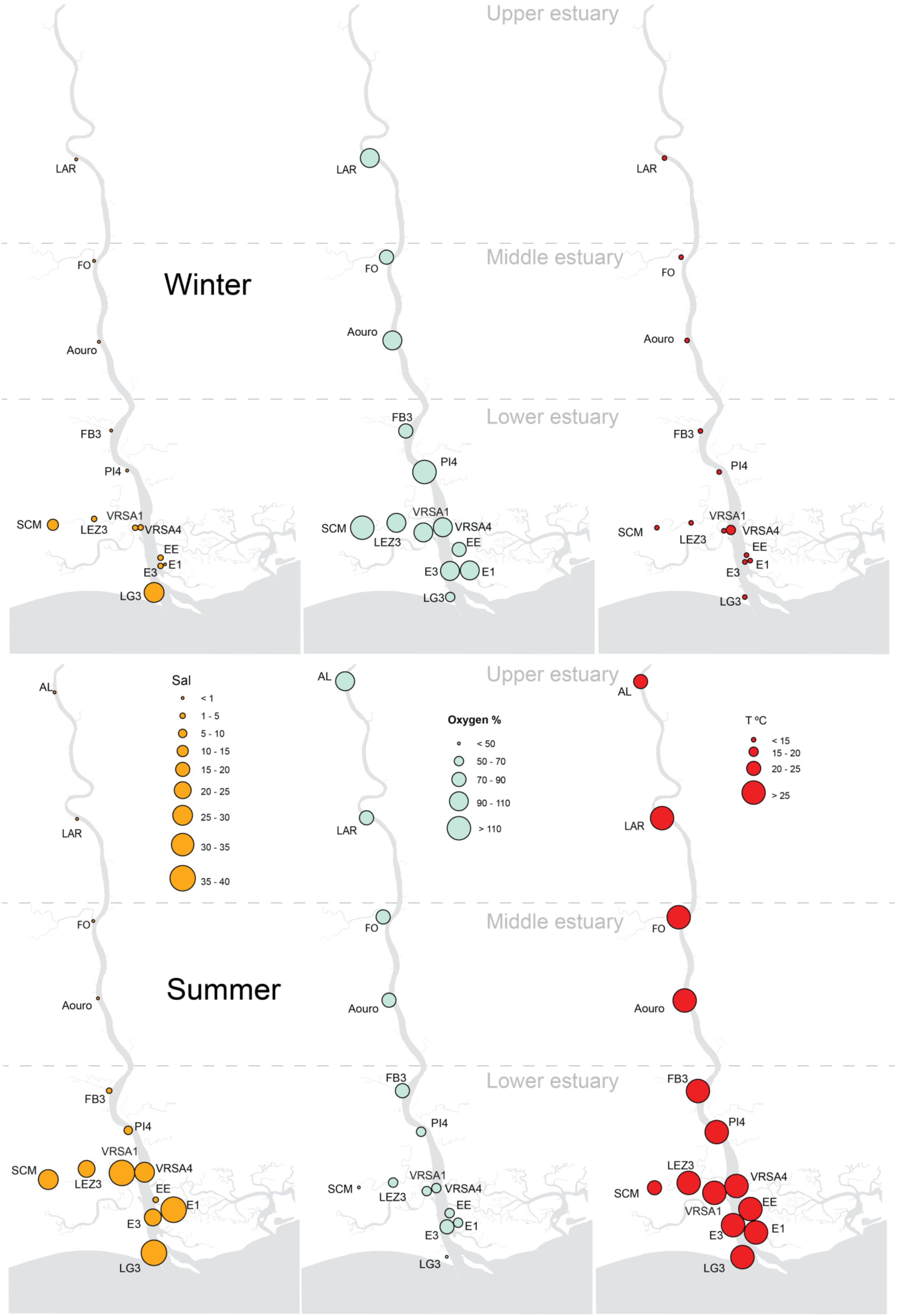

Figure 4.7. Variation of salinity, in yellow, Oxygen (%), in blue, and Temperature (ºC), in red, along a north/south gradient during winter and summer.

In summer, all the confined sites (SCM, VRSA1, E1 and LG3) exhibited high salinity values, varying from 26 to 36, at SCM and LG3, respectively. Site EE retained a low Sal value (4) due to continuous freshwater flow from the Ayamonte Sewage Treatment Plant. This freshwater flux had probably influenced salinity in the southern areas of the estuary, since E3 also registers a lower Sal value than the sample immediately above it on the north/south gradient (VRSA4).

No significant relationship was found between elevation and salinity, although a positive trend was observed and was slightly stronger in summer (Table IV). This may relate to the more elevated position of the most confined sites, which are more susceptible to evaporation effects, leading to increasing summer salinities. Nevertheless, the high Sal values in the upper elevations of the marshlands could be related to, or enhanced by, the metabolism of halophytic marsh plants that excrete salt excess (Phleger and Bradshaw, 1966).

No significant relationship was found between salinity and sediment type. Nevertheless, opposite trends were identified for the two seasons, with salinity correlating positively with mud in winter and negatively in summer (Table IV).

4. 4. 1. 2. Dissolved Oxygen (DO): The DO signal exhibited great differences between the two seasons. In winter the values of DO were higher, varying from 69 to 115% (at LG3 and SCM, respectively), and lower in summer, when it varied from 35 to 92% (at SCM and AL, respectively). These differences were more evident in the lower estuary, where higher discrepancies were found between winter and summer: in winter DO mean value was 95% and in summer 61.6%. The most extreme cases were found at the confined sites, where the renewal of water in winter is frequent, either by rain or by maritime agitation, keeping DO levels high, while in summer the absence or reduced occurrence of rain, floods and storms, allied with high insolation and evaporation values, lead to DO depletion: SCM 115% and 35%; VRSA1 94.5% and 65%; E1 106% and 68.6%; and LG3 69% and 44.4%, for winter and summer respectively. These environmental differences promoted a positive correlation between elevation and DO in winter and negative in summer (Table IV).

In both seasons there was an increasing oxygen gradient from the lower estuary to middle-upper estuary, most evident in summer (Fig. 4.7), although not significant (Table IV). Thus, DO exhibited an opposing trend to salinity, which was also more evident in summer (R = - 0.7; Fig. Table IV).

No significant relationship was found between DO and sediment type.

Table IV - Spearman's Rank Correlation (*R*) or Pearson Correlation Coefficient (*r*) between elevation (MSL), latitude, mud and the and salinity (Sal), Oxygen (DO%), Temperature (TºC) and pH of the sediment (pH_{sed}) in winter and summer samples. According to the normality of the variable, (*R*) or (*r*) is indicated for each pairwise, in **bold** only when significant (** for $p < 0.01$ and * for $p < 0.05$).

	Winter						Summer					
	Elev MSL	Lat	mud	Sal	DO	T	Elev MSL	Lat	mud	Sal	DO	T
Sal	-0.32	**-0.74****	-0.19				0.03	**-0.85****	-0.21			
DO	0.50	0.33	0.29	-0.17			-034	0.50	-0.08	**-0.70****		
T	**-0.56***	-0.48	**-0.72****	0.15	-0.33		-0.22	-0.38	-0.53	0.23	0.02	
pH_{sed}	-0.21	-0.19	**-0.53****	0.22	-0.14	**0.59***	0.18	-0.23	**-0.56****	0.19	-0.10	-0.01

4. 4. 1. 3. Temperature (T): Like Sal and DO, the T signal is quite different in winter and summer (Fig. 4.7). In winter the values varied between 12.4 and 15.7 ºC at LEZ3 and VRSA4, respectively, whereas in summer they varied between 20.2 and 29.6 ºC at SCM and E3, respectively. In winter the values are in accordance with the range proposed by Garel and Ferreira (2011), but in summer, except for SCM with 20.2 ºC, the values are always above the maximum threshold of 24 ºC suggested by those authors, with more than a 5º C difference in some cases (e.g. E3 with 29.6º C). These higher values could be related to differences in measurement depth, which in the current case was always less than 50 cm, where the water tends to be warmer.

Although not clearly evident in Fig. 4.7, there was a moderate negative relationship between T and elevation in both seasons, but only significant in winter (Pearson *r* = - 0.56; both variables are normally distributed) (Table IV). The same trend was found with T and mud content in both seasons, but also only significant in winter (R = - 0.72). The most elevated sampling locations were those in the middle to upper estuary and those located in the small, confined water bodies, such VRSA1, E1 and SCM. These observations are in agreement with Garel and Ferreira (2011), who found major differences between continental estuarine waters and offshore waters during winter, with lower temperatures upstream and higher temperatures offshore. During summer they found almost no difference between the two. Of the samples located in small confined water-bodies, only SCM exhibited a lower temperature, equivalent to those found in the middle to upper samples. This could be related to the greater depth of this water body which may keep the waters colder.

Temperature correlated negatively with both TOC and TN, relatively strongly in winter and moderately in summer (Table V). On the other hand, TOC and TN were covariable and were positively correlated with Elev and especially with mud content. Despite the strength and significance of the relationship between T, TOC and TN, it seems that these variables are independent and that their inverse relationship results from T relationships with Elev and mud, which exert some control over TOC and TN content.

Although no significant relationship was found between T and latitude (Table IV), T tends to decrease towards north (mean T value in winter: middle to upper estuary = 13.3 ºC; lower estuary = 13.7 ºC; mean T value in summer: middle to upper estuary = 25.9 ºC; lower estuary = 26.6 ºC).

No significant relationship was found between T and salinity (R < 0.23) in either season. Temperature relationship with DO exhibited opposing trends in winter (decreasing) and similar trends in summer (both increasing). These relationships, although not significant, were perceptible on the distribution map (Fig. 4.7), and seem to be in agreement with Garel and Ferreira's (2011) data, which shows opposite trends in the same tidal phase throughout the different seasons. In other words, during the ebb phase, T decreased and oxygen increased in winter, whilst T increased and oxygen decreased in summer.

Table V - Spearman's Rank Correlation (*R*) between physical-chemical parameters: Sal, DO, T and pH_{sed} and the chemical parameters related to organic and inorganic matter content: TOC, TIC, TN and C/N in winter and summer samples. (*R*) is indicated for each pairwise, in **bold** only when significant (** for $p < 0.01$ and * for $p < 0.05$).

	Winter				Summer			
	Sal	DO	T	pH_{sed}	Sal	DO	T	pH_{sed}
TOC	-0.09	0.12	**-0.72****	**-0.52****	0.02	-0.26	**-0.56***	**-0.40***
TIC	**0.58***	-0.52	0.11	0.03	0.30	-0.33	-0.05	0.37
TN	0.05	-0.04	**-0.76****	**-0.51***	0.03	-0.29	**-0.55***	**-0.43***
C/N	-0.52	0.48	-0.24	-0.27	-0.34	0.43	0.09	**0.41***

4. 4. 1. 4. Sediment pH (pH_{sed}): No major differences were found between the pH_{sed} during winter and summer, varying from 6.2 to 7.8 in winter (at LEZ3 and VRSA1, respectively), and from 6 to 8 in summer (at PI2 and VRSA1, respectively; Fig. 4.8). The pH_{sed} values were not as high as would be expected of seawater (7.4 to 8.4), nor as low as river water (*ca*. 5) (Suguio, 2003). In sediment, pH reflects a mixture of both saltwater and freshwater inputs, as well as the quantity of organic matter present. Marsh sediments are rich in organic matter, especially that provided by the halophytic vegetation, which, along with bioactivity, promotes reducing conditions (Suguio, 2003).

Although exhibiting similar ranges of variation in both seasons, pH_{sed} values were slightly higher in winter: (mean pH_{sed} values in winter: 1–2 m: 7.3; 0–1 m: 7.1; and -1–0 m: 7.6; mean pH_{sed} values in summer: 1–2 m: 6.8; 0–1 m: 6.8; and -1–0 m: 7.1).

Figure 4.8. Sediment pH variation along a north/south and elevation gradients during winter and summer.

Phleger and Bradshaw (1966) found a positive relationship between pH_{sed} and oxygen in a diurnal cycle, being strongest during daytime, when marsh plants are producing oxygen, and during flood tide, when high pH and high-oxygen water is being introduced from the sea. In the present work, DO values were highest during winter, although no significant relationship was found between pH_{sed} and DO and the correlation between the two was negative (Table IV). Garel and Ferreira (2011) found the same trend as Phleger and Bradshaw (1966) between water pH and oxygen when the diurnal and tidal factors are the major forcing factors. However this trend is broken down when river flow plays a part. For periods of high freshwater (oxygen-saturated) discharge, oxygen increases substantially and pH values decrease abruptly (see Garel and Ferreira, 2011).

There was not an obvious pH gradient in relation to elevation, although the lowest values were always found in the middle to upper elevations and the highest values in the lower elevations. Once again, the upper elevations sample VRSA1 was an outlier, having the highest pH in both seasons.

No significant relationships were observed between pH_{sed} and spatial and physico-chemical parameters in either season, except for T in winter. The highest winter temperatures were found in the lower estuary samples suggesting lower pH values for the northernmost samples, where freshwater influence is higher (Fig. 4.7). Opposing seasonal trends were found between pH and some of the parameters (elevation, salinity and temperature), which could be related to pH sensitivity to diurnal, tidal and seasonal variations.

During both seasons there was a moderate negative correlation between pH_{sed} and mud content (Table IV). This is somehow expected due to the yield strength and viscosity of cohesive sediments (Sornin, 1983), which promotes the entrapment of organic matter that in turn is decomposed by microbial activity increasing the reduction potential (Bale and Kenny, 2005). Indeed, a negative relationship was found between TOC, TN and pH_{sed} (Table V), which proved to be stronger in winter.

4. 5. Conclusions

Knowledge of environmental factors, and the way in which they interact with each other, is essential for understanding how a system works as a whole and for evaluating the environmental determinants of fauna and flora population distributions. During the past decade, a considerable amount of scientific effort has been concentrated on the Guadiana Estuary to build up this knowledge base, although focusing mainly on aquatic communities and hence, water quality (Chícharo et al., 2006a and b; Cravo et al., 2006; Domingues et al., 2005, 2007, 2012; Faria et al., 2006; Morais et al. 2009; Rocha et al., 2002;), with no extension to the intertidal margins where significant biological activity and biodiversity are also known to exist.

The present work provides an analysis of a series of physico–chemical parameters from the sediments and waters of the intertidal margins of the Guadiana Estuary (i.e. between – 0.74 and 1.96 meters in relation to MSL, extending 34 km from the coast to Alcoutim). Along with the marginal vegetation (analyzed in the previous chapter), sedimentological characteristics, bulk organic matter properties and sediment-water physico-chemical parameters were studied in terms of spatial and seasonal variability.

Variation in environmental parameters was found to be very dependent on distance to sea and elevation energy gradients, which in turn are dependent on the different seasonal periods, with riverine conditions prevailing during winter and marine and tidal conditions prevailing during summer.

Sediments along the marginal intertidal area vary from fine silt to medium sand, with increasing average grain-size from the upper elevations to the lower elevations. Upper elevations are characterized majorly by silts and lower elevations are mainly sand, often with significant content in bioclasts. Sediments are generally poorly sorted, with a symmetrical to very finely skewed distribution, in accordance with the low-energy conditions typical of the deposition areas. During winter, when the river flow is highest, a slight increase in coarser sediments is observed, especially in the less elevated areas of the lower estuary where the hydrodynamics play a greater role.

Organic matter concentrations in the sediment are very variable, reflecting the diversity of sub-environments, each of which is subject to a multiplicity of physico-chemical conditions, whit organic matter production, hydrodynamics and topography playing the leading roles. There is a positive correlation between organic matter, elevation and mud content, with the most elevated and less hydrodynamic zones of the marshland having higher concentrations of TOC and TN entrapped in the fine, cohesive sediments.

Organic matter in the Guadiana Estuary’s sediments is largely controlled by a mixing of aquatic and terrestrial sources. Organic material in the middle-upper estuary and upper elevations of the lower estuary incorporates a higher contribution from terrestrial sources while there is a substantial input of autochthonous aquatic sources in the middle-lower elevations of the lower estuary.

TIC (carbonate) concentrations are higher in both elevation end-members, with the intertidal upper elevations receiving a major contribution from autochthonous (stained) epifauna, and the intertidal lower elevations receiving a contribution from autochthonous (stained) infauna, as well as allochthonous, reworked material. TIC concentration increases from the continental to marine end-member. The strong relationship between TIC, latitude and salinity observed in winter is attenuated during summer, which may be explained by higher $CaCO_2$ availability under higher temperatures and salinity.

Salinity data indicate the existence of a salt wedge near the estuary mouth during winter and a well mixed lower estuary during summer. During winter, except for two land-locked sites, salinity never exceeded 3 along the estuary. In summer, at low spring tide and negligible river flow, salt intrusion is detected significantly upstream to the International Bridge. The salinity turning-point is located near the Beliche rivulet mouth; upstream of this point salinity values are similar to those observed in winter; downstream it increases gradually to typical seawater values.

Confined zones are subject to extreme seasonal variations in salinity and oxygen, with lower salinities and high oxygen during winter, when the fluvial and rain waters are the major contributors, and higher salinities and lower oxygen during summer, when high insolation and evaporation are the major drivers. A slightly increasing gradient of oxygen saturation is observed from the lower to the upper estuary, especially during summer when the values are generally lower and less homogeneous.

Superficial water temperatures varied greatly in the different seasons, from 12.4 to 15.7 ºC in winter and from 20.2 to 29.6 ºC in summer. An inverse correlation is observed between temperature, elevation and latitude, with decreasing temperature values towards the upper elevations and towards the north. Temperature’s relationship with oxygen is seasonally-controlled, with decreasing temperatures and increasing oxygen levels in winter and increasing temperatures and decreasing oxygen in summer.

There is no major difference between sediment pH in winter and summer. In sediments, pH reflects a mixture of both saltwater and freshwater inputs, as well as the quantity of organic matter present. Although tenuous, there is a decreasing trend in sediment pH with increasing elevation and latitude. This trend is reinforced by the significant inverse relationship between sediment pH and mud content and organic matter content.

Environmental parameters that are strongly dependent on hydrodynamics, such as sediments and organic/inorganic matter distribution, experience little seasonal variation and are better correlated with spatial parameters. Conversely, water parameters, which are directly dependent on seasonality, exhibit higher seasonal variation but weak relationships with spatial parameters.

Chapter 5

Seasonal variations in benthic foraminifera in the Guadiana Estuary

Chapter 5

5 - Seasonal variations in benthic foraminifera in the Guadiana Estuary

5. 1. Chapter objectives

The general aim of this chapter is to identify the spatial-seasonal variation in the distribution of living benthic foraminiferal assemblages in the Guadiana Estuary and to establish the statistical relationships with the environmental parameters. This study is expected to improve the use of benthic foraminifera as bioindicators, at least on a regional scale, and to provide the base-line data for the final model used in the paleoenvironmental interpretation, presented in Chapter 6. Prior to the presentation and discussion of the results, a brief introduction of the importance of foraminifera as bioindicators is presented.

To achieve the general aim of the present chapter, a series of superficial sediment samples were collected along a north–south gradient (river–sea) and an elevation gradient (intertidal gradient) in winter and summer seasons. Benthic foraminifera diversity, density, dominance and test-type in these samples were then compared and correlated statistically. Environmental parameters collected from the same sites and seasons were analyzed in detail in Chapter 4 and these same parameters are related to living foraminifera distribution in the present Chapter. Through canonical ordination (RDA and partial RDA) the environmental variables were evaluated in terms of their influence on species composition. The most important variables were identified for each season. Both seasons were compared and a distribution model is proposed.

5. 2. Benthic foraminifera as bioindicators in paralic environments

Based on the uniformitarism principle stated by Hutton (1726–1797), foraminifera have been widely used as paleoindicators (Lowe and Walker, 1997). Their usefulness in stratigraphy was gradually realized and they quickly attained a place in Applied Science as stratigraphical index fossils, widely used in petroleum exploration. Foraminifera are also important in paleoclimate, paleo-sea-level, paleo-depth and paleoceanographic studies and, recently, in absolute age determinations using oxygen isotopes (Albani et al., 2001).

Foraminifera offer some advantages over many other environmental proxies because they leave a permanent microfossil record in sedimentary sequences, enabling the reconstruction of the environmental history of a site in the absence of the original physiochemical baseline data (Scott et al., 2001).

Most of the ecological studies of foraminifera have been carried out with the aim of providing a contemporary database with which fossil foraminifera can be compared and interpreted (Cann et al., 2000; Cearreta, 1998; Duleba et al., 1999; Edwards and Horton, 2000; Gerdes and Waterman, 2003; Gustafsson and Nordberg, 2001; Li et al., 2000; Thomas and Varekamp, 1991; Wang et al., 1985). Salt marsh foraminifera, in particular, are useful tools for Holocene sea-level rise studies (Scott and Medioli, 1978; 1980).

Historical and paleoecological perspectives of natural processes are essential to provide complementary guidelines for more efficient and sustainable management of the environment. Statistical studies, based on the distribution of benthic foraminifera in the marine and estuarine environments, have also shown that these organisms can be successfully used to identify various ecological provinces, to detect environmental stress conditions and to monitor pollution (Albani et al., 2001). In estuaries, foraminifers act as bioindicators of great interest because they have short life cycles and react quickly to changes (Debenay et al., 2000), enabling ecosystem conditions to be described and monitored for natural or human-induced modifications (Bladin, 1986). Being small and abundant, foraminifers are found in great quantities in small sediment volumes, enabling statistically reliable and economically attractive studies (Scott et al., 2001).

The use of foraminifers in the characterization of modern environments and pollution monitoring is recent but has been increasing significantly in the last few decades (Frontalini et al., 2009). The first studies concerning the effects of pollution in foraminifera distribution started at the end of the 1950s (Bergamin et al., 2009). The use of benthic foraminifera as bioindicators of environmental quality may be investigated in terms of population density and diversity, assemblage structure, reproduction capability, test morphology, including size (dwarfism), prolocular morphology, ultrastructure, pyritization, abnormality, and chemistry of the test (Frontalini et al., 2009). In Portugal, the only known study on this subject is the work of Martins et al. (2010) that investigates benthic foraminifera assemblage distribution in several sub-environments of the Ria de Aveiro, NW Portugal, especially in the pollution ‘hot-spots’ related to modern and historic industrial activity.

Before using foraminiferal assemblages as pollution indicators in paralic environments, a precise understanding of their response to environmental variables is necessary in order to distinguish between responses due to anthropogenic stress and due to changes in the natural environment (Debenay et al., 2000). This requirement is particularly critical in estuaries and coastal lagoons that are subject to a complex interaction of numerous physico-chemical natural parameters, which present space and time variability, and because these environments are heavily exposed to a number of chemicals, including industrial pollutants, agricultural pesticides and other human impacts (Debenay, 1995; 2000; Debenay et al., 2000).

The faunistic zonation model proposed by Scott and Medioli (1980) has been widely used in Quaternary sea-level studies (e.g. Thomas and Varekamp, 1991; Varekamp et al., 1992; Nydick et al., 1995). This model indicates that different associations of species occupy well-defined vertical zones in relation to mean sea level (MSL), roughly coinciding with marsh vegetation zones, each of which is influenced by aerial exposure between tidal cycles.

More recently, Debenay et al. (2000) have also demonstrate that the presence or absence of vegetation as an important parameter controlling foraminifera distribution, alongside with sediment type, temperature and salinity. Moreover, these authors defend that there is a general trend in the East Atlantic Temperate Estuaries for foraminifera assemblages to change landward, with highly diverse assemblages dominated by calcareous forms occupying niches near the open sea, followed by oligospecific assemblages with dominant agglutinated species, and ultimately by monospecific populations in further inland sites. On the other hand, in a study performed in the Great Marshes of Massachusetts, De Rijk (1995) and De Rijk and Troelstra (1997) stated that foraminifera distribution was mainly related to salinity variations resulting from the influence of freshwater seepage and infiltration of sea water and rain water. Under these conditions, these authors suggest that foraminifera distribution is controlled not only by elevation but especially by spatial and temporal variations of a number of environmental variables, especially salinity. They conclude that there is no unique model with worldwide applicability that can be related solely to elevation.

In conclusion, in estuaries, especially in meso to micro-tidal temperate environments, a great diversity of stresses disturb the living communities and cause significant variability in the assemblages of benthic microfauna. As a result, the composition of benthic foraminifera assemblages reflects the complex interaction between biotic and abiotic parameters and their multiple changes in space and time (Debenay et al., 2000).

5. 3. Results

For the analysis of benthic living foraminifera distribution, 24 samples of superficial sediment were collected during a winter campaign and 25 during a summer campaign. All samples were projected using ArcView software and divided in terms of:

- Elevation (intertidal) gradient - with 3 graphics: upper elevations corresponding to samples collected at 1–2 meters above MSL; middle elevations corresponding to samples collected at 0–1 meters above MSL; and lower elevations corresponding to samples collected at -1–0 meters in relation to MSL;
- North–south gradient – in each graph, the division between lower, middle and upper estuary is marked (see Chapter 2 for more details);

Between 1–2 m, 7 samples were collected in winter, mostly in the upper part of the lower estuary: sites FB1, PI2, LEZ1, LEZ2, SCM, VRSA1 and VRSA2. In summer, the same samples were collected as in winter plus a PI1, to add up to 8 samples. Between 0–1 m, 12 samples were collected, spanning the entire estuary: LAR, FO, Aouro, FB2, FB3, PI3, PI4, LEZ3, VRSA3, E1, LG1 and LG2. At the lower elevations, between -1–0 m, 5 samples were collected, mainly in the lower part of the lower estuary: VRSA4, EE, E2, E3 and LG3.

Only Ni (the estimated number of individuals per 20 cc of sediment) and S (number of species) were analyzed and described for all samples in winter and summer. For calculating the remaining metrics, only statistically valid samples were considered. Of the 24 samples collected in winter, 19 were statistically valid and used as a basis for the final winter distribution model. Of the 25 samples collected in summer, 23 were statistically valid. Lacking the minimum of 50 living individuals required for statistical analysis, the samples LAR, Aouro, LEZ3, VRSA1 and E2 were eliminated from the original winter matrix and the samples LAR and VRSA1 from the original summer matrix (columns in grey in Table I and II, Appendix 4).

5. 3. 1. Faunistic density (Ni)

Ni was analyzed for all samples in winter and summer (Table I and II, Appendix 4). Its variation amongst sites is expressed graphically using ArcView software (Fig. 5.1).

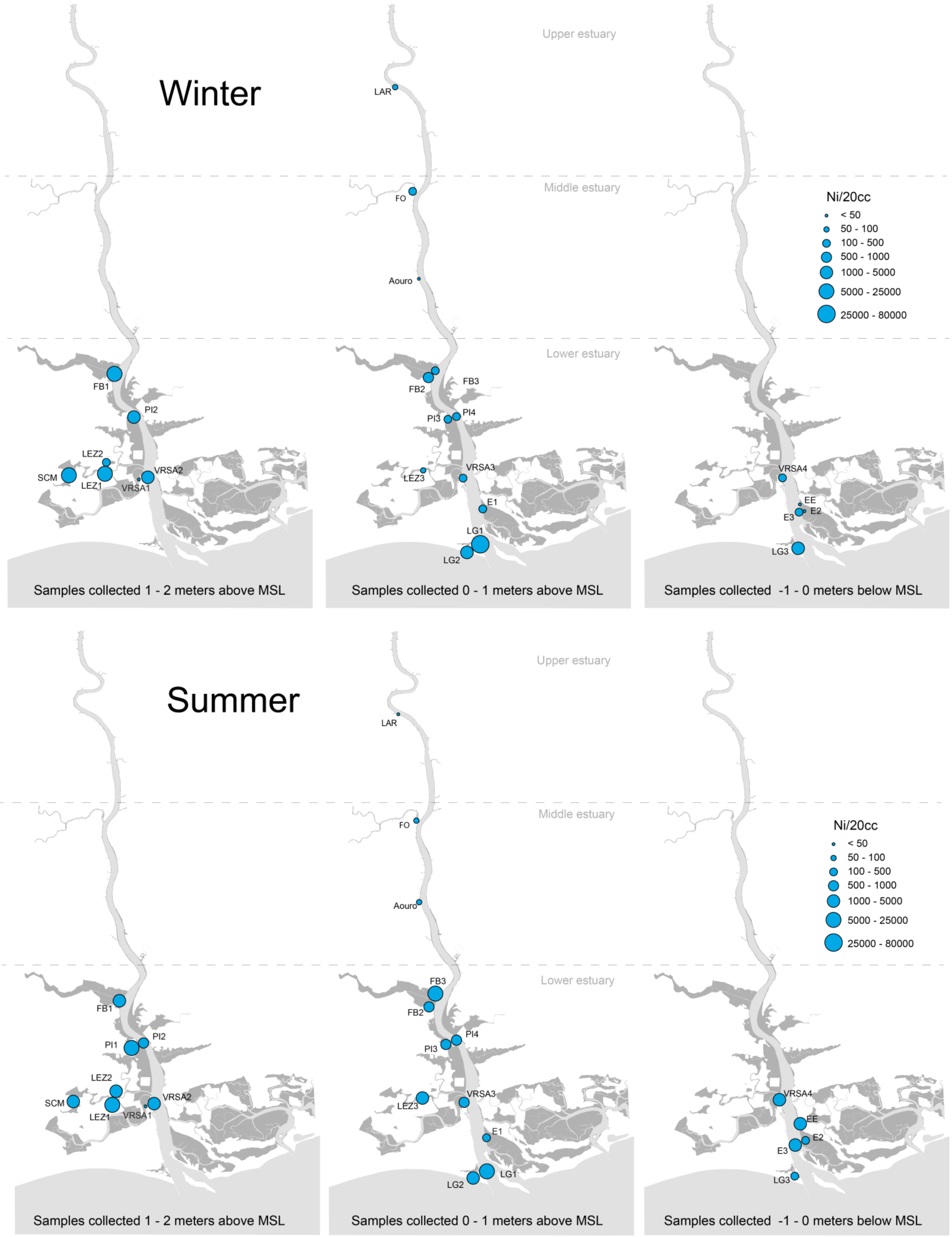

Figure 5.1. Variation of the number of individuals per 20 cc of sediment (Ni) along a north-south and elevation gradients in winter and summer.

Winter: In winter there was a clear decreasing trend in Ni with decreasing elevation. In the upper elevations, Ni varied from 17 to 21 424, at VRSA1 and FB1, respectively (mean value of 6079 individuals/sample); in the middle elevations, Ni varied from 24 to 76 224, at Aouro and LG1, respectively (mean value of 6770 individuals/20 cc); and in the lower elevations, Ni varied from 15 to 2200, at E2 and LG3, respectively (mean value of 591 individuals/20 cc). The mean Ni value in the middle elevations is probably overestimated due to an extreme Ni value (76 224 individuals/20 cc) in the LG1 sample.

In the middle elevations samples, it was also possible to observe a decrease in Ni from the lower estuary to middle and upper estuary. In the middle to upper estuary samples, Ni varied from 24 to 313 individuals/20 cc, at Aouro and FO, respectively (mean: 133 individuals/20 cc), and in the lower estuary Ni varied from 72 to 76 224 individuals/20 cc, at LEZ3 and LG1, respectively (mean: 8982 individuals/20 cc).

Summer: In summer there was a trend of decreasing Ni with decreasing elevation. Ni values in the upper elevations varied from 2 to 16 576 individuals, at VRSA1 and PI1 respectively (mean: 5224 individuals/20 cc); in the middle elevations, Ni varied from 31 to 6768 individuals, at LAR and LG1, respectively (mean: 1756 individuals/20 cc); and in the lower elevations, Ni varied from 332 (at E2 and LG3) to 2072 (at VRSA4; mean: 956 individuals/20 cc).

The middle elevations samples also displayed an obvious decrease in Ni from the lower estuary to middle and upper estuary. In the middle to upper estuary, Ni varied from 31 to 83 individuals/20 cc, at LAR and FO, respectively (mean: 63 individuals/20 cc), and in the lower estuary Ni varied from 286 to 6768 individuals/20 cc, at E1 and LG1, respectively (mean: 2320 individuals/20 cc).

5. 3. 2. Number of species (S)

S was analyzed for all samples in winter and summer (Table I and II, Appendix 4) and its variation among sites was expressed graphically using ArcView software (Fig. 5.2).

Winter: Along the elevation gradient, S exhibited an opposing trend to Ni, but the same trend in what concerns the latitudinal gradient. The number of species increased from the upper to lower elevations and decreased from downstream (lower estuary) to upstream samples (middle and upper estuary).

Figure 5.2. Variation of the number of species/sample (S) according to the north-south and to the elevation gradients, in winter and summer.

In the upper elevations, S varied from 2 to 11 species, at FB1 and VRSA2, respectively (mean: 5 species/sample); in the middle elevations, S varied from 5 to 20 species, at LG1 and FB3, respectively (mean: 11 species/sample); and in the lower elevations, S varied from 6 to 21 species, at E2 and LG3, respectively (mean: 13 species/sample). According to the north–south gradient and considering only the samples collected between 0–1 m, S varied from 6 to 14 species, at LAR and FO, respectively (mean: 9 species/sample) in the middle to upper estuary, and from 5 to 20, at LG1 and FB3, respectively (mean: 12 species/sample) in the lower estuary.

Summer: As in winter, S exhibited an opposing trend to Ni along the elevation gradient, but the same trend in what concerns the north-south gradient. The number of species increased from the upper to lower elevations and decreased from lower estuary to middle and upper estuary. In the upper elevations, S varied from 1 (at FB1 and VRSA1) to 13 species (at VRSA2; mean: 4 species/sample); in the middle elevations, S varied from 4 to 19 species, at LAR and FB2, respectively (mean: 12 species/sample); and, in the lower elevations, S varied from 13 to 16 species, at E2 and VRSA4, respectively (mean: 14 species/sample). Following the north-south gradient and considering only the samples collected between 0–1 meters above MSL (i.e. the samples that span the entire estuary), S varied from 4 to 14 species in the middle to upper estuary, at LAR and Aouro, respectively (mean: 9 species/sample), and from 8 to 19 species in the lower estuary, at LG1 and FB2, respectively (mean: 13 species/sample).

5. 3. 3. Shannon diversity (Hs)

Diversity (Hs) was calculated for all statistically valid samples collected in both seasons (see Table I and II, Appendix 5) and its variation among sites was expressed graphically using ArcView software (Fig. 5.3).

Winter: Hs values varied from 0.04 at LEZ1 (upper elevations) to 2.34 at EE (lower elevations). These extremes are representative of the general trend of increasing Hs with decreasing elevation.

Summer: A decrease in Hs with decreasing elevation was observed, but the middle and lower elevations had the same Hs mean value. In the upper elevations, Hs values varied from 0 to 1.5 at FB1 and LEZ2, respectively (mean: 0.56), whereas in the middle elevations Hs varied from 0.66 to 2.2 at LG1 and Aouro, respectively (mean: 1.57) and, in the lower elevations, Hs varied from 1.31 to 1.87 at EE and LG3, respectively (mean: 1.57). In contrast to S, no decrease was observed in Hs with increasing latitude.

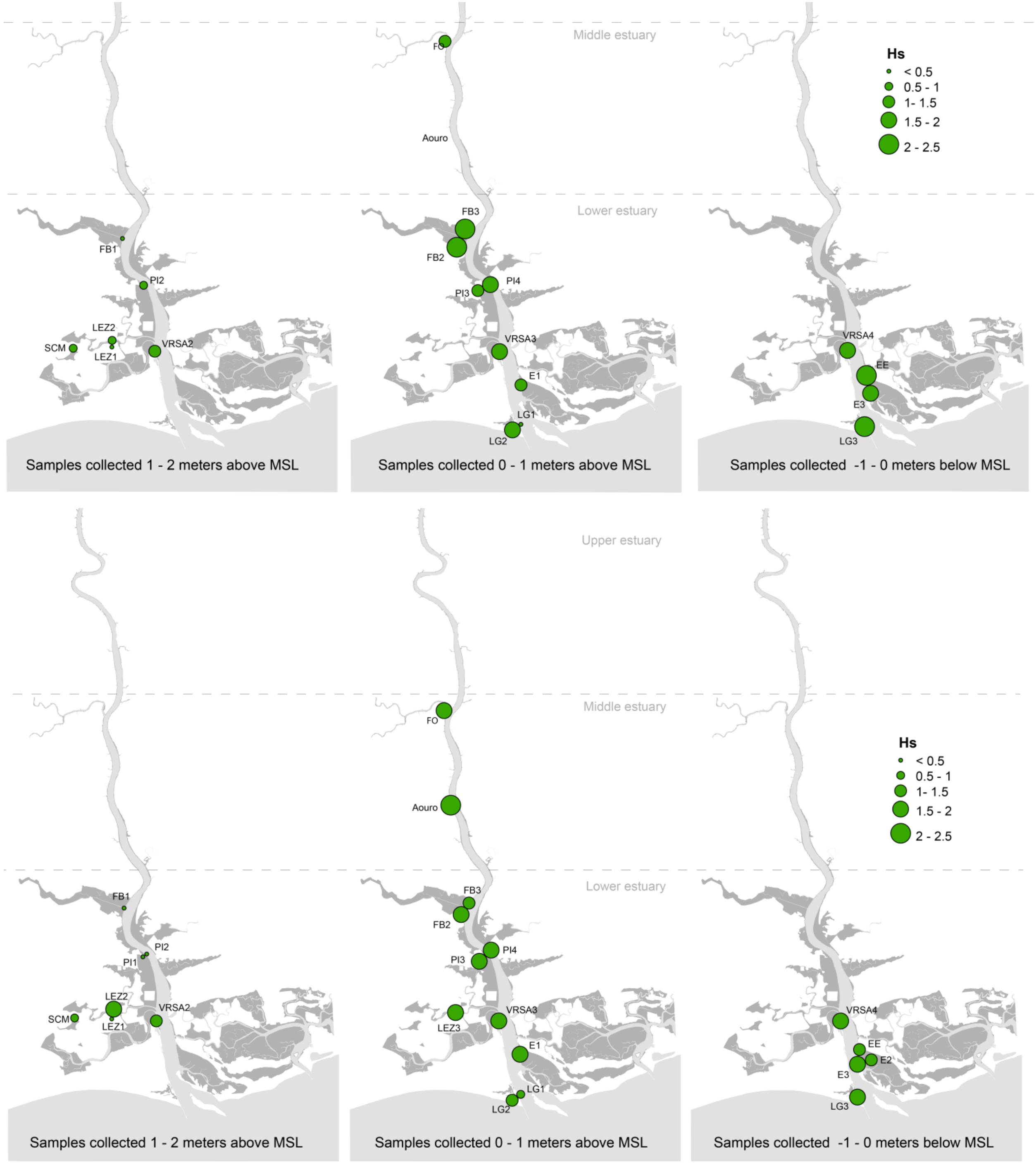

Figure 5.3. Variation of diversity (Hs) along a north-south and elevation gradients, in winter and summer.

5. 3. 4. Species abundance and dominance

Relative abundances were calculated for all living taxa in all samples (see Table I and II, Appendix 4).

Winter: In the winter samples, 42 species of living benthic foraminifera were counted and identified, belonging to at least 27 genera, at least 19 families and 8 orders (Table I).

Table I – Taxonomic context of the 42 living species occurring in winter (agglutinated taxa in **bold**).

Orders	Families	Genus	Species
Astrorhizida	**Polysaccamminidae**	***Polysaccammina***	***Polysaccammina hyperhalina***
Buliminida	Bolivinidae Buliminellidae	*Bolivina* *Buliminella*	*Bolivina ordinaria* *Bolivina variabilis* *Buliminella elegantissima*
Lagenida	Ellipsolagenidae	*Fissurina*	*Fissurina* sp.
Lituolida	**Ammodiscidae** **Haplophragmoididae** **Hormosinidae** **Lituolidae** **Rzehakinidae**	***Ammobaculites*** ***Ammotium*** ***Ammovertelina*** ***Haplophragmoides*** ***Leptohalysis*** ***Miliammina*** ***Reophax***	***Ammobaculites* sp.** ***Ammotium cassis*** ***Ammotium salsum*** ***Ammovertelina* sp.** ***Haplophragmoides* sp.** ***Leptohalysis scottii*** ***Miliammina fusca*** ***Miliammina obliqua*** ***Reophax nana***
Miliolida	Incertae familiae Cornospiridae	*Incertae generis* *Cornuspira*	Miliolid sp:1, 2, 3, 4, 6, 8, 9 and 10 *Cornuspira involvens*
Rotaliida	Asterigerinatidae Elphidiidae Heleninidae Nonionidae Rotalidae	*Ammonia* *Asterigerinata* *Cribroelphidium* *Elphidium* *Haynesina* *Helenina*	*Ammonia aberdoveyensis* *Aterigerinata mamilla* *Elphidium poeyanum* *Elphidium excavatum* *Elphidium oceanensis* *Elphidium excavatum williamsoni* *Elphidium gerthi* *Haynesina depressula* *Haynesina germanica* *Helenina anderseni*
Textulariida	**Textulariidae** **Valvulamminidae**	***Discorinopsis*** ***Textularia***	***Discorinopsis aguayoi*** ***Textularia earlandi***
Trochamminida	**Trochamminidae**	***Arenoparrella*** ***Jadammina*** ***Lepidodeuterammina*** ***Portatrochammina*** ***Tiphotrocha*** ***Trochammina***	***Arenoparrella mexicana*** ***Jadammina macrescens*** ***Lepidodeuterammina ochracea*** ***Lepidodeuterammina plymouthensis*** ***Portatrochammina* sp.** ***Tiphotrocha comprimata*** ***Trochammina inflata***

The calcareous orders (Buliminida, Lagenida, Miliolida and Rotaliida) were the best represented, comprising 54.8% of the total species identified. Of those, 21.4% corresponded to porcelaneous species, represented in the order Miliolida. The orders comprising agglutinated species (Astrorhizida, Lituolida, Textulariida and Trochamminida) represented the remaining 45.2% of the total number of occurring species.

Analyzing the relative abundances of all taxa found in winter samples, 80.8% were agglutinated and 19.2% were calcareous, the latter comprising 6.7% of porcelaneous individuals.

Of the 42 living species identified in the winter samples, only 8 were classified as dominants, representing more than 20% of the total individuals in at least one sample, namely: *Ammonia aberdoveyensis*, *Bolivina ordinaria*, *Haynesina germanica*, Miliolid sp3, miliolids, *Miliammina fusca*, *Trochammina inflata* and *Jadammina macrescens*. Five were classified as common species, representing 10–20% of the total individuals in at least one sample, namely: *Polysaccammina hyperhalina*, *Asterigerinata mamilla*, *Discorinopsis aguayoi*, *Elphidium oceanensis* and *Miliammina obliqua*

The distribution of the common to dominant species during winter is represented in Figure 5.4. According to the division of samples by elevation it is clear that the upper elevations were almost exclusively colonized by the agglutinated species *J. macrescens*. In two of these samples, LEZ2 and VRSA2, there was a marked presence of porcelaneous species, especially in LEZ2, where this group represents 82.3% of the total individuals.

In the middle elevations, the faunal composition become more diverse, especially in samples located on the river margin. At the most confined sites (E1, located in the margin of a tidal pond, and LG2 and LG3, located in the margin of a small lagoon), *J. macrescens* still dominated, together with *T. inflata*. *A. aberdoveyensis* and *H. germanica*, having its optimum representation in the samples collected in the middle to lower elevations of the lower estuary.

A. aberdoveyensis exhibited a more euryhaline behavior than *H. germanica*, being present with considerable percentages in the northernmost sample (FO), as well as in the lagoonal environment (LG3) which occupies the southernmost position. The agglutinated species *Miliammina fusca* had its optimum spanning from the upper reaches of the lower estuary to the middle estuary, at samples of lower to middle elevations. *Bolivina ordinaria* is common in the middle elevations but had its optimum in the lower elevations, occurring together with *A. aberdoveyensis* and *H. germanica*. *Polysaccammina hyperhalina* occurred mainly in the lower elevations. It is worth pointing out that the porcelaneous forms, represented here by the Miliolids group and Miliolid sp3, only occurred in environments subject to some degree of confinement, such as the samples located in the upper elevations and in the small lagoon site (LG).

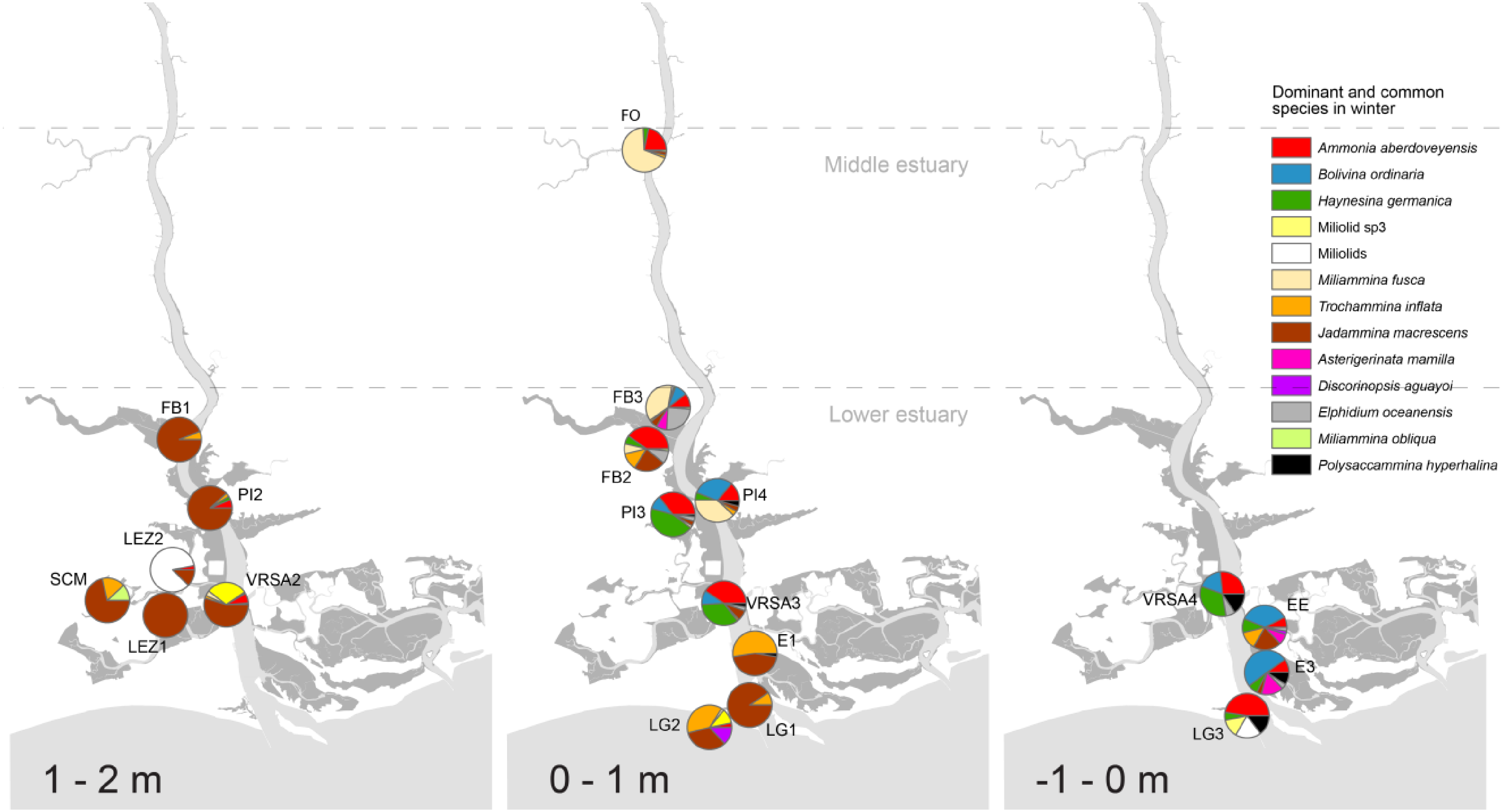

Figure 5.4. Distribution patterns of the common to dominant species in the samples collected in winter along a north-south and elevation gradients.

Summer: In the summer samples, 43 species of living benthic foraminifera were counted and identified, belonging to at least 30 genera, at least 21 families and 8 orders (Table II). Calcareous and agglutinated orders represent approximately the same proportion of occurring species: 49% calcareous and 51% agglutinated. From the calcareous taxa, 11.6% correspond to porcelaneous species of the Order Miliolida.

Analyzing the relative abundances of all taxa found in the summer samples, 41.6% were calcareous and 58.4% were agglutinated. Of the calcareous, 2.7% are porcelaneous individuals.

Of the 43 living species identified in summer, 8 were classified as dominants, representing more than 20% of the total individuals in at least one sample, namely: *A. aberdoveyensis*, *E. oceanensis, H. germanica, M. fusca, P. hyperhalina, Polysaccammina ipohalina, T. inflata* and *J. macrescens*. Only *Siphotrochammina* sp. was classified as a common species, representing 12% of the total individuals in one sample.

The distribution of the common to dominant species during summer is represented in Fig. 5.5. According to the division of samples by different levels of elevation, it is possible to see that the upper elevations were almost exclusively colonized by the agglutinated species, *J. macrescens*. The exceptions were samples SCM, LEZ2 and VRSA2, where other species had significant abundances. At SCM, *J. macrescens* shares dominance with *P. ipohalina*, the latter representing more than 20% of the individuals and was exclusive to that sample, suggesting specific environmental conditions. At LEZ2 and VRSA2, both samples located in the lowermost position within the upper elevations (1.06 and 1.03 meters above MSL), *J. macrescens* lost prominence to other species such as *P. hyperhalina*, *M. fusca*, *A. aberdoveyensis* and *E. oceanensis*, suggesting that the elevation thresholds for the latter species were above the 1 m level.

In the middle elevations there was a shift toward more diversified faunas, especially in samples located on the channel margin, where *J. macrescens* loses dominance. At FB2, E1, LG2 and LG3, which are more confined locations, *J. macrescens* is still prominent, sharing dominance with *T. inflata*, *A. aberdoveyensis* and *M. fusca*. When considering foraminiferal distribution along the samples collected in the middle elevations, the dominance of *M. fusca* stands out. This species had its optimum in the middle elevations of the upper reaches of the lower estuary to the middle estuary and is associated with *A. aberdoveyensis* and *E. oceanensis*. In the middle elevations, it is important to point out that the species *Siphotrochammina sp*., which was common in the dead assemblages of the northern samples (see Chapter 7), was found alive only in the Aouro sample, but with significant abundance.

Table II – Taxonomic context of the 43 living species occurring in summer (agglutinated taxa in **bold**).

Orders	Families	Genus	Species
Astrorhizida	**Polysaccamminidae**	***Polysaccammina***	***Polysaccammina hyperhalina*** ***Polysaccammina ipohalina***
Buliminida	Bolivinidae	*Bolivina*	*Bolivina ordinaria* *Bolivina variabilis*
Lituolida	**Ammodiscidae** **Haplophragmoididae** **Hormosinidae** **Lituolidae** **Rzehakinidae**	***Ammobaculites*** ***Ammotium*** ***Ammovertelina*** ***Haplophragmoides*** ***Leptohalysis*** ***Miliammina*** ***Reophax***	***Ammobaculites exigus*** ***Ammobaculites*** **sp.** ***Ammotium cassis*** ***Ammotium salsum*** ***Ammovertelina*** **sp.** ***Haplophragmoides*** **sp.** ***Leptohalysis scottii*** ***Miliammina fusca*** ***Miliammina obliqua*** ***Reophax nana***
Miliolida	Incertae familiae Cornuspiridae	*Incertae generis* *Cornuspira*	Miliolid sp1, 3, 4 and 5 *Cornuspira involvens*
Rotaliida	Asterigerinatidae Discorbidae Elphidiidae Heleninidae Nonionidae Rosalinidae Rotalidae	*Ammonia* *Asterigerinata* *Discorbis* *Elphidium* *Haynesina* *Helenina* *Nonion* *Rosalina*	*Ammonia aberdoveyensis* *Aterigerinata mamilla* *Discorbis* sp. *Elphidium advenum* *Elphidium excavatum* *Elphidium williamsoni* *Elphidium gerthi* *Elphidium oceanensis* *Haynesina depressula* *Haynesina germanica* *Helenina anderseni* *Nonion* sp. *Rosalina* sp.
Robertinida	Ceratobuliminidae	*Lamarckina*	*Lamarckina haliotidea*
Textulariida	**Eggerellidae** **Textulariidae** **Valvulamminidae**	***Discorinopsis*** ***Eggerelloides*** ***Textularia***	***Discorinopsis aguayoi*** ***Eggerelloides scaber*** ***Textularia earlandi***
Trochamminida	**Trochamminidae**	***Arenoparrella*** ***Deuterammina*** ***Jadammina*** ***Lepidodeuterammina*** ***Siphotrochammina*** ***Tiphotrocha*** ***Trochammina***	***Arenoparrella mexicana*** ***Deuterammina eddystonensis*** ***Jadammina macrescens*** ***Lepidodeuterammina ochracea*** ***Siphotrochammina*** **sp.** ***Tiphotrocha comprimata*** ***Trochammina inflata***

A. aberdoveyensis had its optimum in the samples collected in the lower elevations, where it was dominant. Of all species, it was the most euryhaline, being present with considerable percentages along the north/south gradient and in the most confined environments of the middle elevations, as well as in some samples of the upper elevations. It was closely associated with *H. germanica*, especially in the lower elevations, and with *E. oceanensis* in both middle and lower elevations.

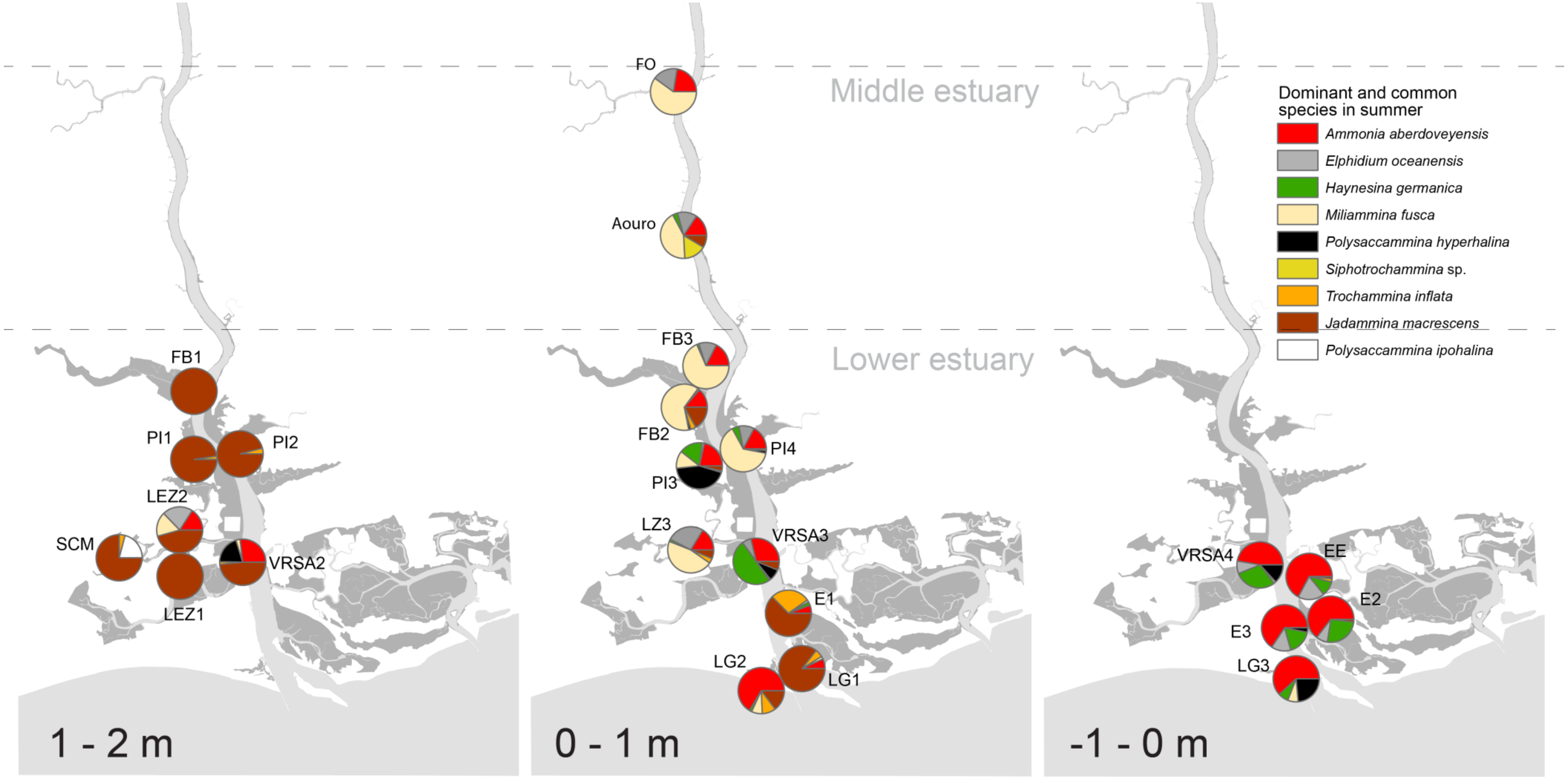

Figure 5.5. Distribution patterns of the common to dominant species in the samples collected in summer along a north-south and elevation gradients.

Polysaccammina hyperhalina, which was somehow restricted to the lower elevations in winter, seems to have a patchy distribution in summer, more dependent on the site location than on the height of the sample.

5. 3. 5. Species constancy

Winter: Of all species occurred in the winter samples, only *A. aberdoveyensis*, *H. germanica*, *J. macrescens*, *T. inflata*, *M. fusca* and *P. hyperhalina* were constant (present in more than 50% of the samples). The most constant species was *J. macrescens*, present in 94.7% of the samples. *P. hyperhalina* was the least constant of these species, being present in only 52.6% of the samples. Constancy values for the remaining species can be consulted in Table I, Appendix 4.

Summer: Of all the species occurred in summer, only the dominant species *A. aberdoveyensis*, *H. germanica*, *E. oceanensis*, *J. macrescens*, *T. inflata* and *M. fusca* and the accessory species *Textularia earlandi*, were constant. Of these, the most constant species was again *J. macrescens*, present in 82.6% of the samples. Inversely, *T. earlandi* was the least constant, being present in only 56.5% of the samples. Constancy values of the remaining species can be consulted in Table II, Appendix 4.

5. 3. 6. Agglutinated/Calcareous ratio (A/C%)

A/C % was calculated for all samples (see Table I and II, Appendix 5) and its variation among sites is expressed graphically using ArcView software (Fig. 5.6).

Winter: A general trend of decreasing A/C% with decreasing elevation was observed. A/C% was highest in the upper elevations, where some samples were exclusively colonized by agglutinated species (A/C% = 100). Towards the lower elevations, the agglutinated forms are successively replaced by more diversified calcareous assemblages.

The lack of statistically valid samples in the middle and upper estuary originated some difficulties in inferring the A/C% variation according to the north–south gradient.

Summer: A general trend of decreasing A/C% with decreasing elevation was observed. A/C% was higher in the upper elevations, where the majority of the samples were exclusively colonized by agglutinated species (A/C% = 100). Towards the lower elevations the agglutinated forms were successively replaced by more diversified calcareous assemblages and A/C% drops to 62 and 11%, mean values for middle and lower elevations, respectively.

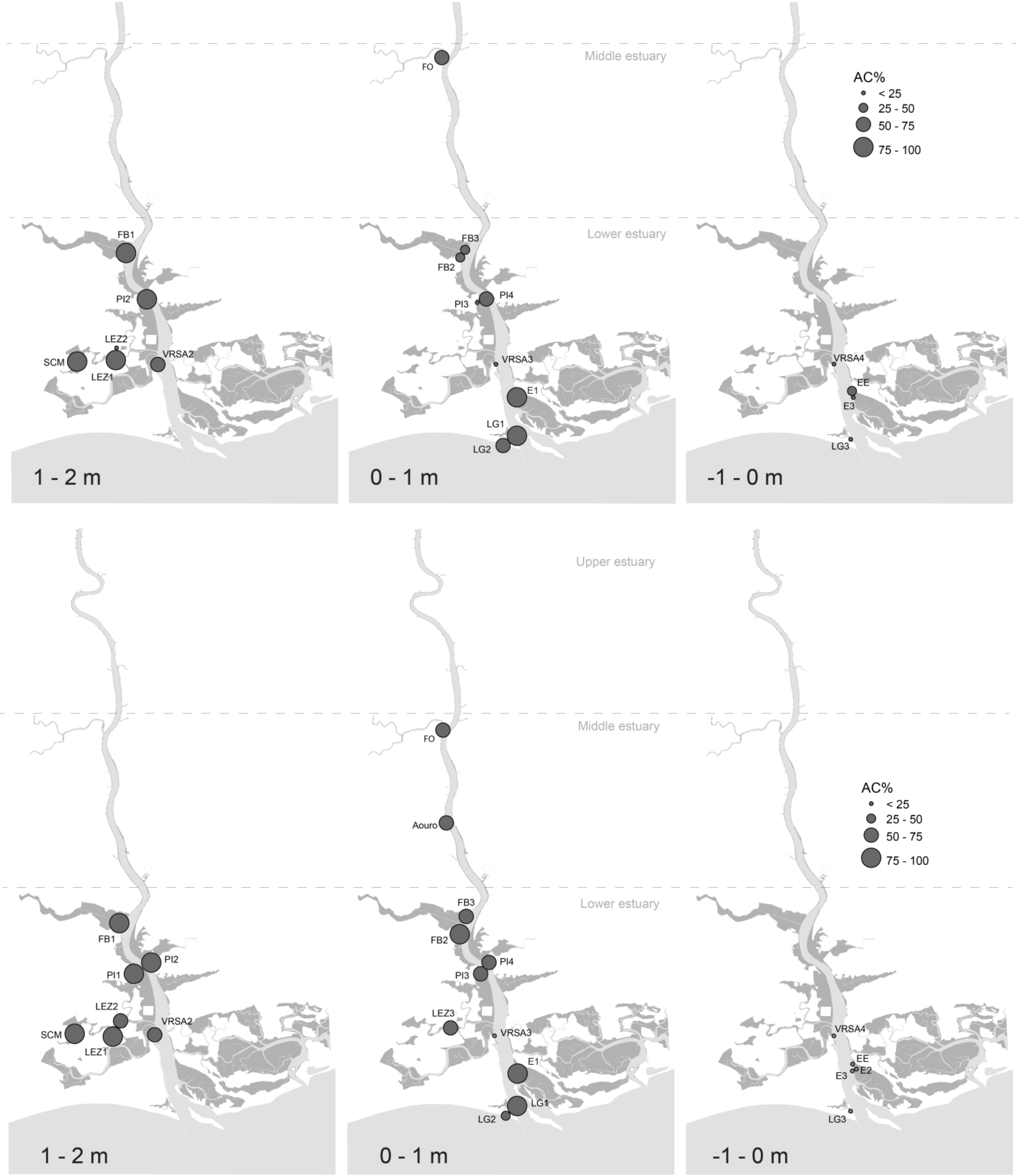

Figure 5.6. Variation of agglutinated/calcareous ratio (A/C%) in the statistical valid samples collected along north-south and elevation gradients in winter and summer.

5. 3. 7. Dominance index (c)

(c) was calculated for all samples (see Table I and II, Appendix 5) and its variation among sites is expressed graphically using ArcView software (Fig. 5.7).

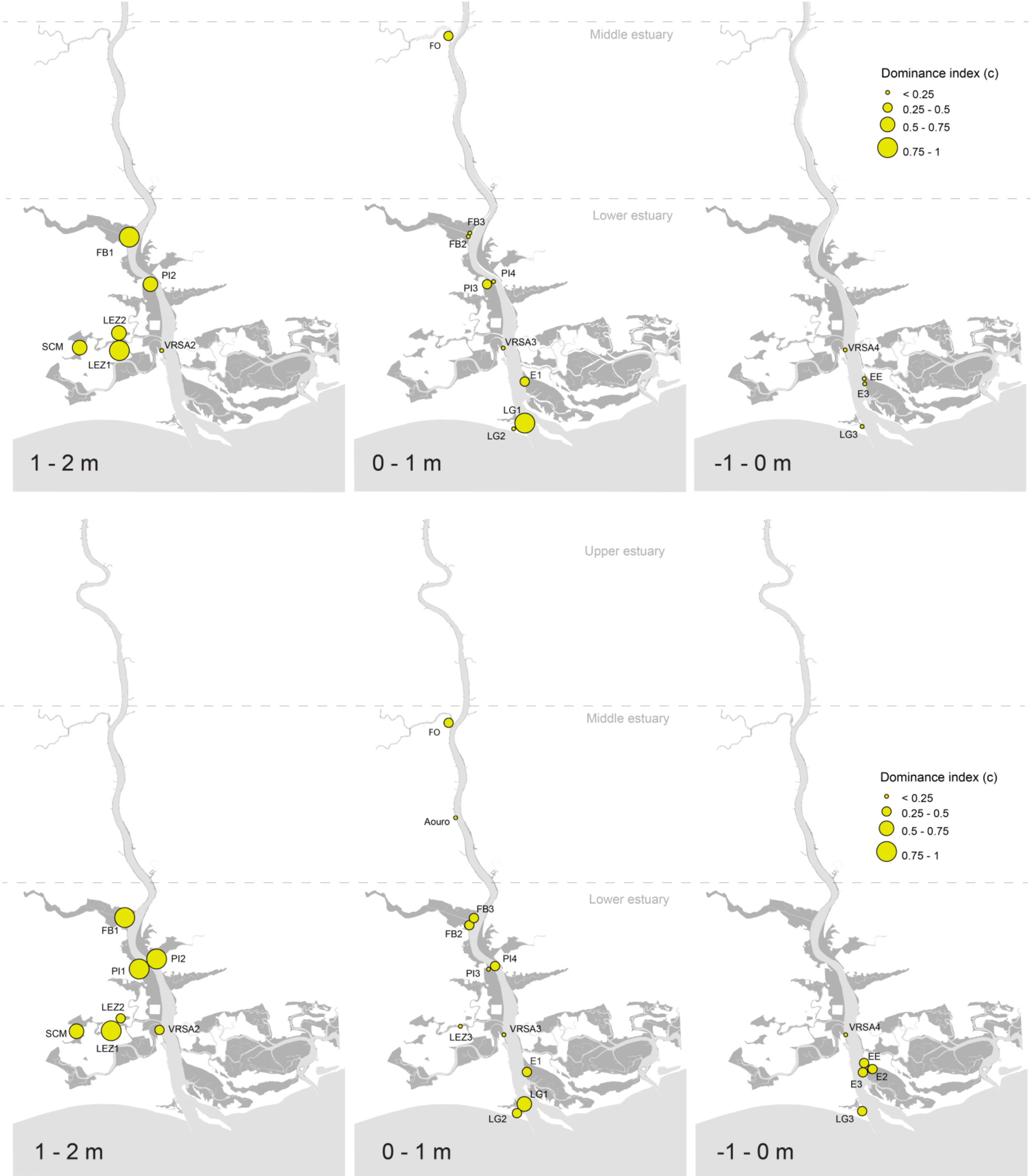

Figure 5.7. Variation of dominance index (c) in the statistical valid samples collected along north-south and elevation gradients in winter and summer.

For both seasons, the highest (c) values were found in the upper elevations, where a few agglutinated species dominated. Towards the lower elevations, where the environment is frequently inundated by the tides, (c) strongly decreased as the number of species increased, suggesting higher environmental stress levels in the areas less exposed to tidal effects.

5. 3. 8. Correlation between spatial variables and ecological indices

In order to look for generalized trends within the benthic foraminifera communities along different seasons, correlation coefficients were measured between spatial and ecological variables and among the ecological variables themselves (Fig. 5.8).

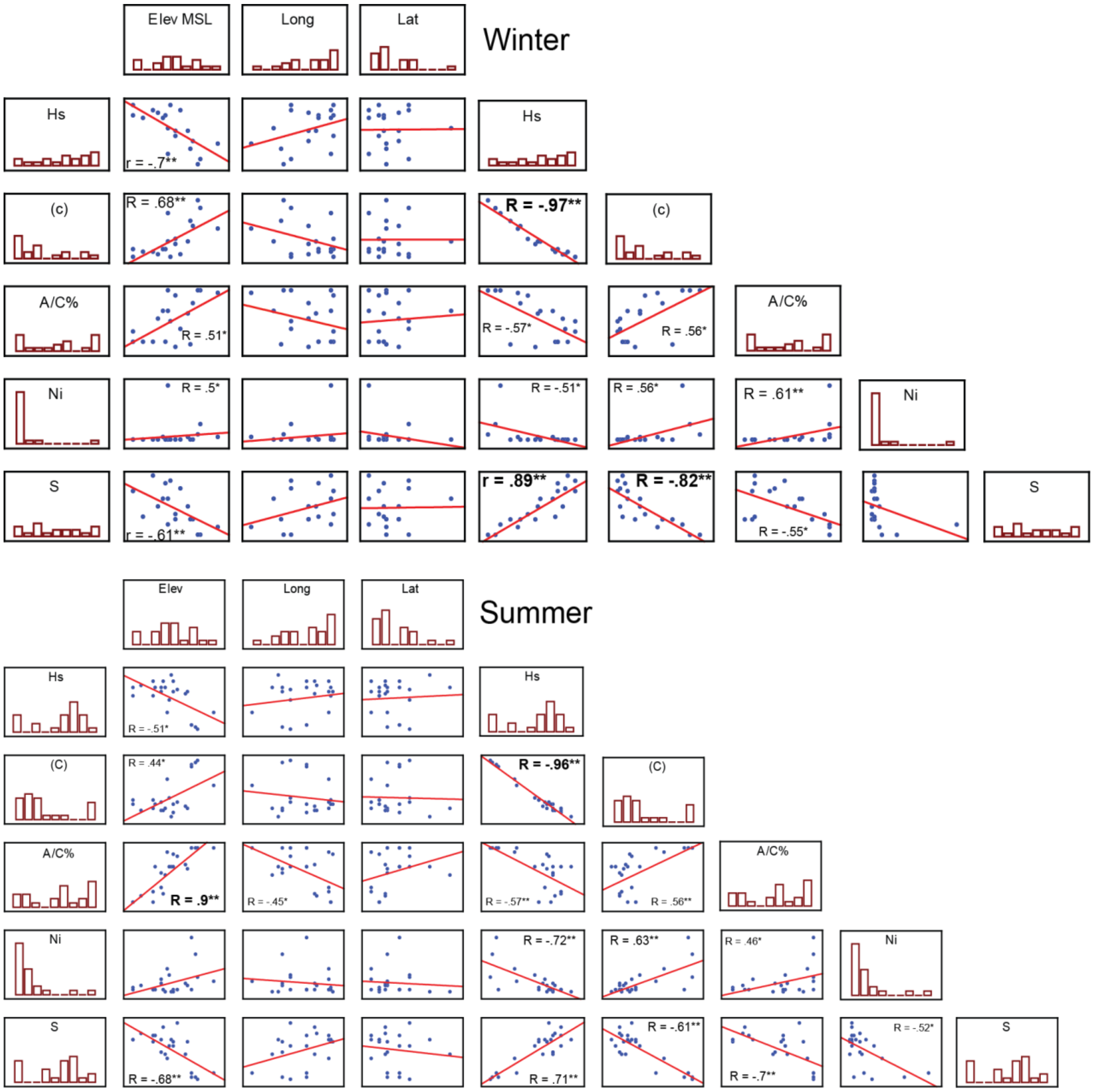

Figure 5.8. Multi-panel pairwise scatterplot of the spatial parameters Elevation (MSL), Latitude and Longitude with the ecological indices calculated for the living foraminifera in both seasons: diversity (Hs), dominance (c), A/C% ratio, faunistic density (Ni) and number of species (S). Spearman's Rank Correlation (R) and Pearson Correlation Coefficient (r) are indicated for each pairwise scatterplot only when significant (** for $p < 0.01$ and * for $p < 0.05$) and its font size is proportional to its strength. Multi-panel produced in STATISTICA 7 software.

For both seasons the applied metric showed that the diversity measures (Hs and S), which were strongly and positively correlated, were inversely correlated with the dominance index (c), (which was also an expected relation due to dominance's dependence on S), A/C % and Ni.

In turn, (c), A/C% and Ni were positively correlated and also correlated positively with Elev. Of those, the relationship between A/C% and Elev is noteworthy, being much stronger in summer (R = 0.9**) than in winter (R = 0.51*). Hs and S correlated negatively with Elev.

No significant relationships were found between the geographical variables latitude and longitude and the ecological variables, except for A/C% in summer, which had a moderate negative correlation with Long (R = -0.45*).

5. 3. 9. Sample similarity based on biological content

Similarities between samples based on benthic foraminifera content were evaluated using two complementary techniques: cluster analysis and Non-metric Multidimentional Scaling (NMDS) ordination. Cluster analysis individualizes the main groups, separated from each other by large differences in similarity, and NMDS highlights the natural sequence of changes in community across the main clusters (more information in Chapter 2).

Winter: The Q-mode dendrogram represented in Fig. 5.9A, performed by PRIMER E, distinguishes at a similarity level of 30%, two main groups of samples, represented by cluster I and cluster II. Figures 5.9B and C show the 3-dimentional solution of NMDS (stress = 0.069), as the stress level was relatively high when only two dimensions were used (stress = 0.12). In both projections the first axis separates the two main groups identified by cluster analysis. NMDS graphs were performed by WInKyst and CANOCO. Group I includes all samples collected in the upper elevations and 3 samples of the middle elevations from very confined locations. Group II includes all the lower elevations samples and middle elevations samples located in more exposed areas. From axis 2 no obvious information can be extracted, but axis 3 seems to show a north–south gradient, separating the northernmost samples (comprised by the shadow ellipse in the plot) from the southernmost samples in the lower part of the axis.

Summer: The Q-mode dendrogram represented in Figure 5.10A distinguishes three main groups of samples, represented by cluster I, II and III. Figure 5.10B shows the 2-dimentional solution of the NMDS with a level of stress = 0.09, which gives a good representation of sample divisions. When stress is lower than 0.1, higher dimension solutions will not add any additional information about the overall structure (Clarke and Warwick, 1994). In NMDS projection, the samples are grouped according to the superimposed clusters derived from the dendrogram.

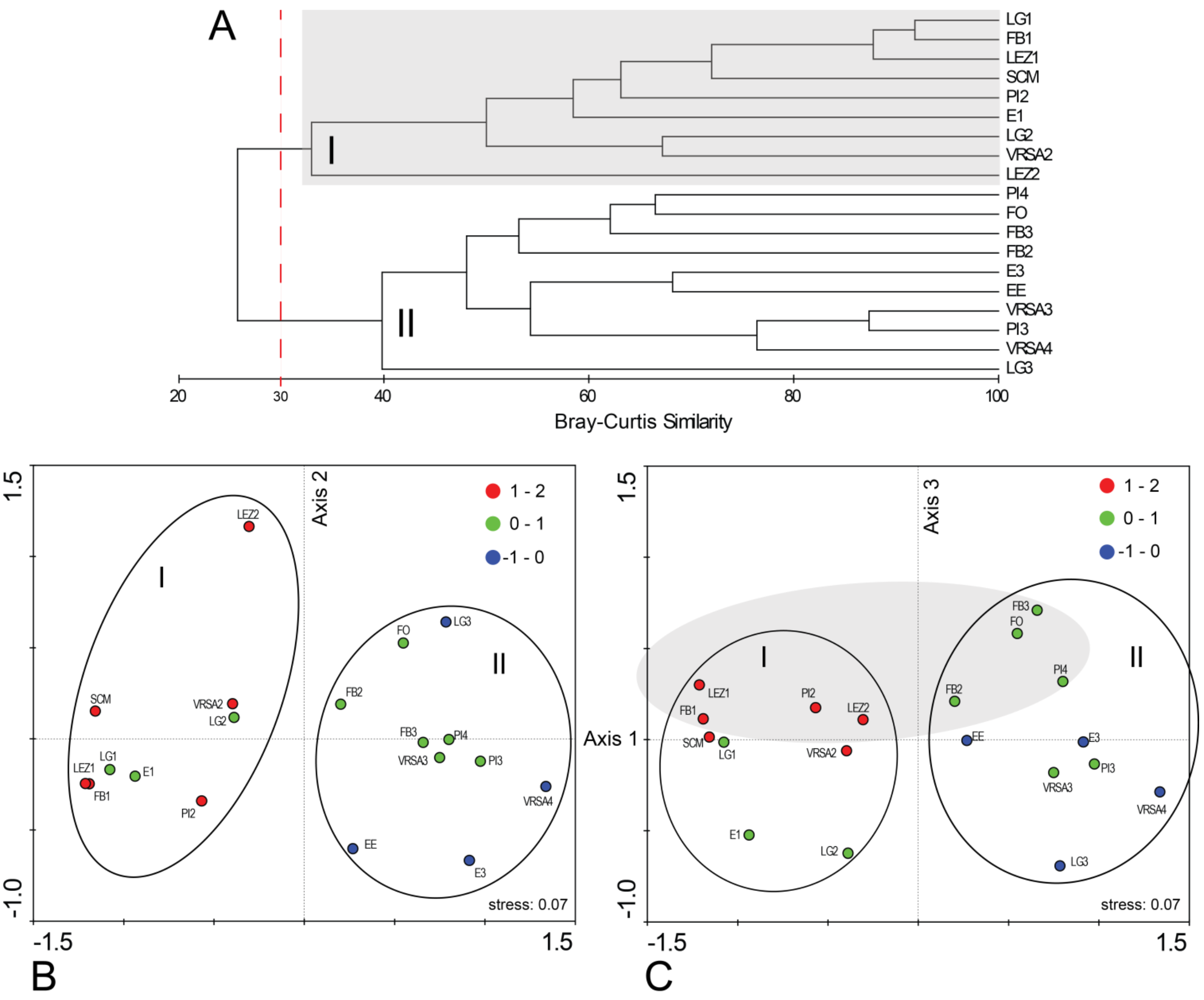

Figure 5.9. Representation of similarity among samples based on the content of living benthic foraminifera during winter. A – Q-mode dendrogram for hierarchical clustering of the 19 statistical valid samples, using group-average linking of Bray-Curtis similarities calculated on √-transformed relative abundance data of >5% species. Two main groups (I - II) are distinguished at a similarity level of 30%. B – NMDS plot of dimension 1 *versus* dimension 2 and C – NMDS plot of dimension 1 *versus* dimension 3. The samples are classify according to the elevation level at which they were collected: in red, samples collected between 1 and 2 meters above MSL; in green, between 0 and 1 meters; and in blue, between -1 and 0 meters.

The first axis clearly separates groups I and II from group III, indicating a division of samples according to elevation: groups I and II comprise samples collected in the lower and middle elevations (except LEZ2, which is an upper elevations sample) and group III comprises samples of the upper elevations and two samples of the middle elevations (E1 and LG1), which are both subject to high degrees of confinement. The second axis seems to indicate a subtle trend that follows the north–south gradient, with the northernmost samples at the positive extreme of the axis and the southernmost samples at the negative extreme.

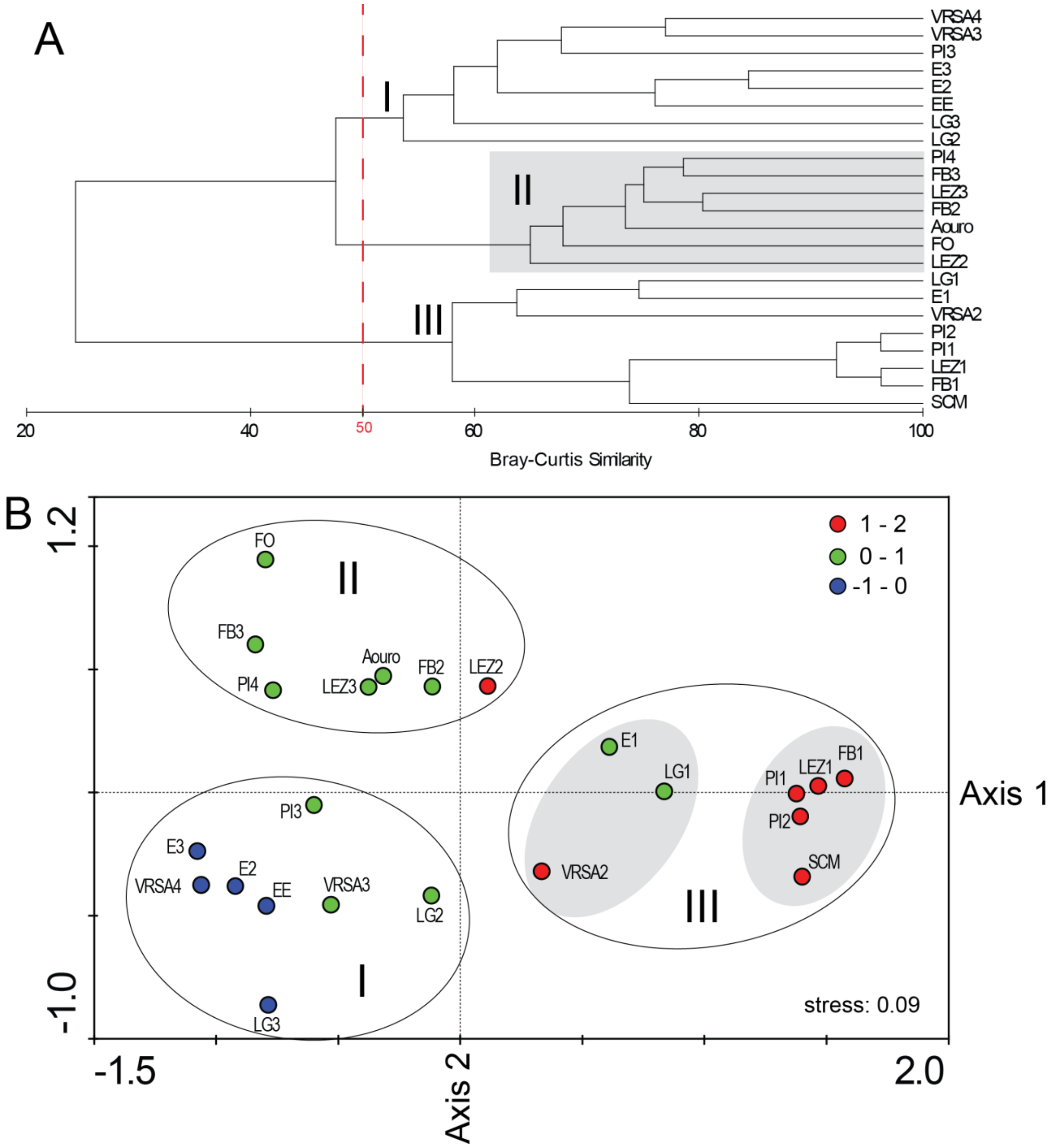

Figure 5.10. Representation of similarity among samples based on the content of living benthic foraminifera during summer. A – Q-mode dendrogram for hierarchical clustering of the 22 statistical valid samples, using group-average linking of Bray-Curtis similarities calculated on √-transformed relative abundance data of >5% or constant species. Three main groups (I, II and III) are distinguished at an arbitrary similarity level of 50%. B – 2-dimensional NMDS plot. Samples are classified according to the elevation level at which they were collected: in red samples collected between 1 and 2 meters above MSL, in green between 0 and 1 meters and in blue between -1 and 0 meters.

5. 3. 10. Environmentally driven patterns in species distribution

5. 3. 10. 1. Species data heterogeneity estimation (gradient length): The gradient length was measured by Detrended Correspondence Analysis (DCA), detrending by segments, in species square-root transformed data using CANOCO.

In winter, the largest gradient length was 2.847 (<3SD), suggesting that linear ordination methods are valid for the analysis of variability of benthic living foraminifera communities based on environmental differences (Leps and Smilauer, 2003).

In summer, the largest gradient length was 3.042 (falling in the grey zone), suggesting that either linear or unimodal ordination methods are suitable for the analysis of variability of benthic living foraminifera communities based on environmental differences (Leps and Smilauer, 2003). Having the liberty to choose, and in the interests of comparability, the same methodology adopted for winter was applied to summer data.

5. 3. 10. 2. Relationships among environmental variables: To infer the relationship between environmental variables based on species composition, a Redundancy analysis (RDA) was performed though CANOCO, using all the explanatory variables described in the previous chapter plus Longitude (Long).

Winter: Figure 5.11 is a simple ordination plot which brings out the correlation among all the environmental variables.

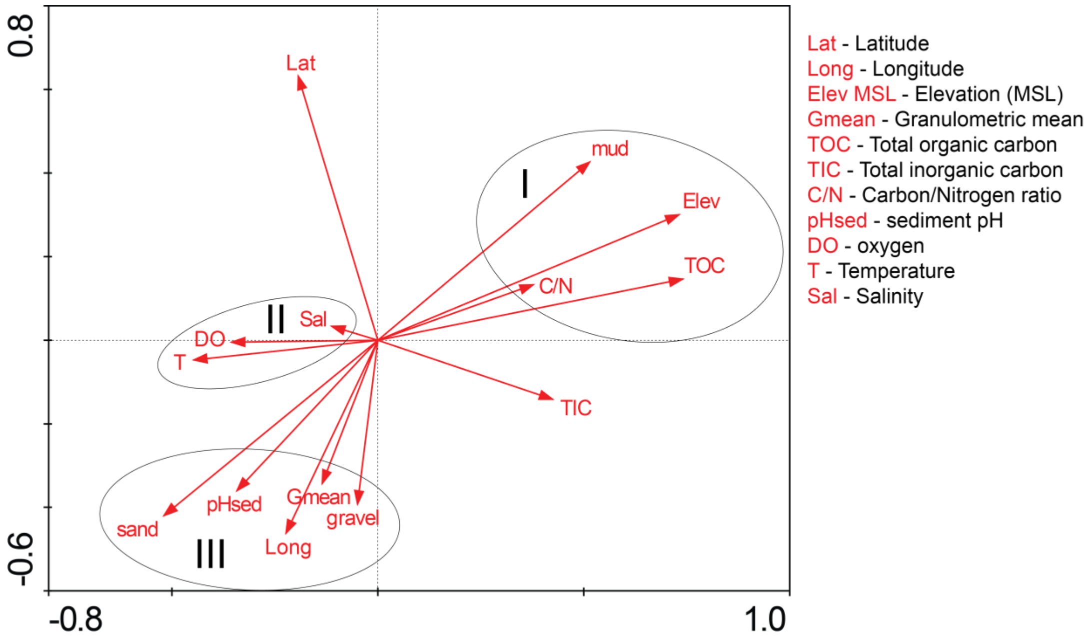

Figure 5.11. RDA simple plot of correlation and strength of environmental variables measured in winter.

The graph shows that there were strong correlations among variables and three groups of inter-correlated variables can be pointed out: Group I, positively correlated with the first axis comprised mud, Elev, TOC and C/N; Group II, negatively correlated with the first axis and comprised Sal, DO and T; and Group III, negatively correlated with the second axis and comprised sand, pH_{sed}, Gmean, gravel and Long. Lat appears independent and positively correlated with the second axis, whilst TIC appears independent although closer to the first group of variables.

The major environmental drivers in benthic foraminifera distribution were Lat, Elev, TOC, mud and sand. Mud and sand, as expected, had an almost perfect negative correlation.

Analyzing the RDA log file in CANOCO, high variation inflation factors (> 20) were observed among the environmental variables, revealing colinearity among them and consequently, redundancy (ter Braak and Smilauer, 2002).

Summer: Figure 5.12 brings out the correlation between all the environmental variables in summer and shows that there were strong correlations among variables, with three groups of inter-correlated variables: Group I, positively correlated with the first axis, comprising Elev, mud and TOC; Group II, negatively correlated with the first axis, comprised DO, T and C/N; and Group III, negatively correlated with the first and second axes, comprised pH_{sed}, sand, Sal, Gmean, gravel and Long. Lat appears independent and correlated positively with the second axis, and TIC also appears independent although closer to the third group of variables.

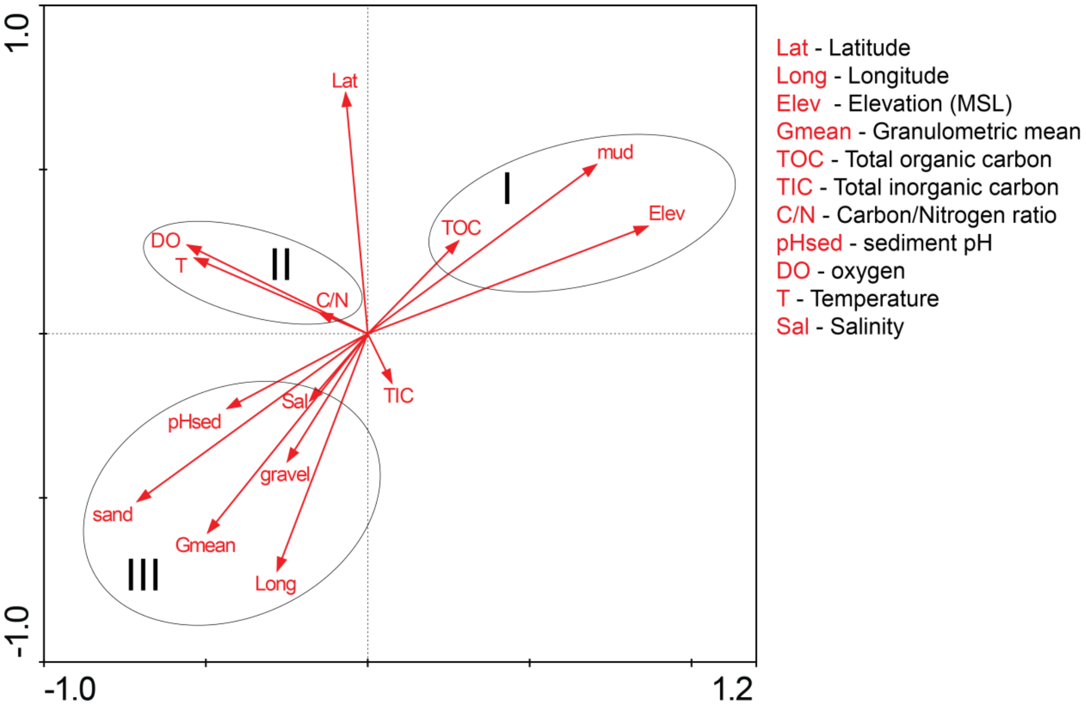

Figure 5.12. RDA simple plot of correlation and strength of environmental variables measured in summer.

The most relevant environmental variables in the distribution of benthic foraminifera fauna during summer were Lat, Elev, mud, Long, Gmean and sand. Mud and sand, as expected, have an almost perfect negative correlation.

As in winter, high variation inflation factors (> 20) were observed among the environmental variables, revealing colinearity among them.

5. 3. 10. 3. Selection of the most important environmental variables: To eliminate redundancy among environmental variables, several steps were performed. The environmental variables were divided into three different groups: spatial variables, which includes Lat, Long and Elev; physical variables, which includes T, Gmean, gravel, sand and mud; and chemical variables, which includes the TOC, TIC, C/N, Sal, DO, and pH_{sed}. An RDA was performed to each group of variables to select the most important in driving the biological distribution for each season.

Winter: Through manual selection using a Monte Carlo Permutation Test, it was possible to identify only Lat and Elev as significant ($p < 0.05$) among the spatial variables; only Gmean and sand were significant among the physical variables; and only TOC is significant among the chemical variables. A new RDA with the selected variables was performed (Fig. 5.13).

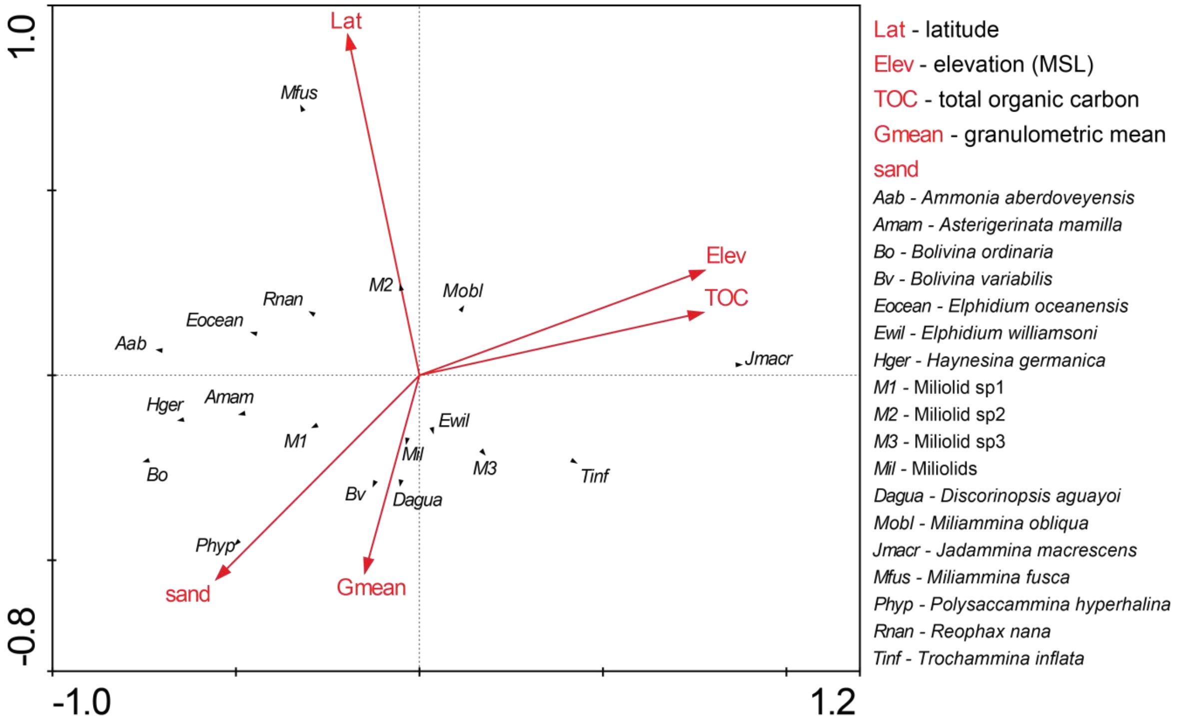

Figure 5.13. RDA biplot summarizing the effects of the five selected environmental variables upon benthic living foraminifera winter communities. In red the five environmental variables selected by RDA with automatic selection and in black the code names of common-dominant species.

The first two axes presented a good correlation between species distribution and environmental parameters, r = 0.91 and 0.89, respectively. The first gradient was by far the longest one, explaining about 37% of the total species variability. The selected environmental variables explained 53.1% of the total variance of which 70.5% was explained in the first axis. Both the tests of significance on the first canonical axis and on all canonical axes using Monte-Carlo Permutation Test, performing 499 permutations in a reduced model, were highly significant ($p = 0.002$ for both).

Summer: Through manual selection using Monte Carlo Permutation Test, it was possible to identify that, among the spatial variables, only Long and Elev were significant ($p < 0.05$); among the physical variables only T and sand were significant; and only DO was significant among the chemical variables. The new RDA is shown in Fig. 5.14.

The first two axes presented a good correlation between species distribution and environmental parameters, r = 0.90 and 0.82, respectively. The first gradient was by far the longest one, explaining about 44.5% of the total species variability. The selected environmental variables explained 63% of the total variance of which 70.7% was explained in the first axis. Both the tests of significance on the first canonical axis and on all canonical axes using a Monte-Carlo Permutation Test were highly significant ($p = 0.002$ for both).

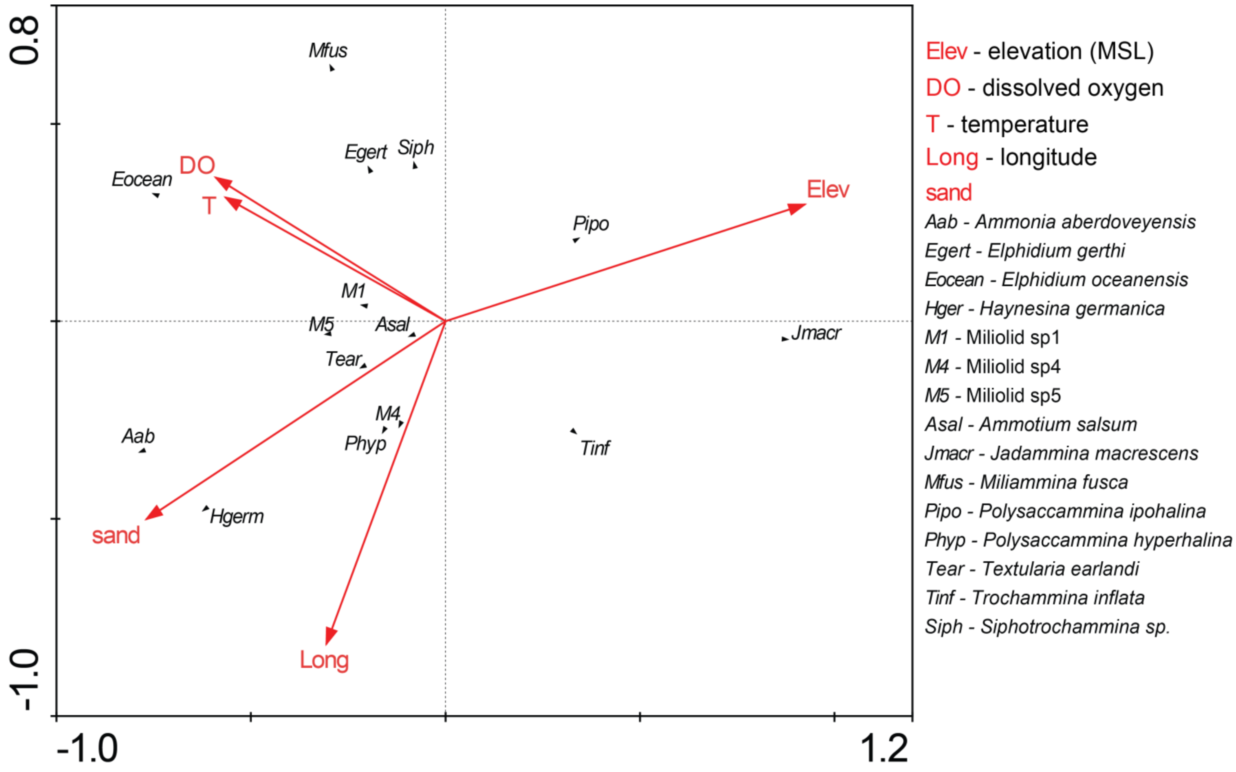

Figure 5.14. RDA biplot summarizing the effects of the five selected environmental variables upon benthic living foraminifera in summer communities. In red the five environmental variables selected by RDA with automatic selection and in black the code names of common-dominant species.

5. 3. 10. 4. Variance partitioning: To determine which of the groups of environmental variables explain most of the variability in species composition, variance partitioning was performed. First, a partial RDA was run, with the spatial variables (Lat and Elev) as environmental variables, and the chemical and physical variables (TOC, sand and Gmean) as covariables. By doing this it is possible to determine how much of the variability is explained by the spatial variables that cannot be explained by the chemical and physical variables. Secondly, another partial RDA was run with the chemical variables as environmental variables, and the spatial and physical variables as covariables. This gives the percentage of the species variability explained by chemical variables that cannot be explained by the spatial and physical variables. Finally, a third partial RDA was run with the physical variables as environmental variables and the spatial and chemical variables as covariables, which gives the percentage of species variability that can be explained by physical variables and cannot be explained by the spatial and physical variables.

Winter: Of the 53.1% of variation explained by the model, 18% is explained by the spatial variables, 19% by the chemical variables and 18% by the physical variables. The remaining 45% is explained by the combined effects of the three groups of variables (Fig. 5.15). The pure effect of each group of variables explains approximately the same variation, which suggests that each group *per se* is suitable to explain the variance in species composition.

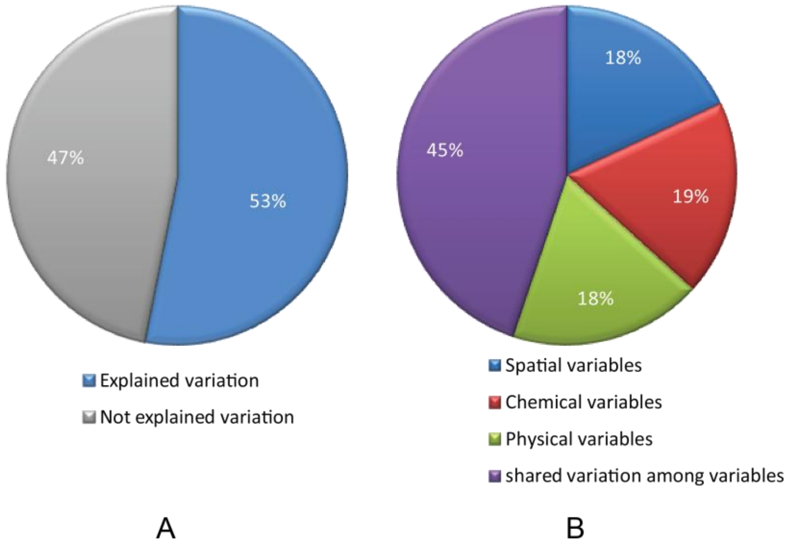

Figure 5. 15. Variance partitioning of species composition: A – total variance explained by the 3 groups of variables and B – partitioning of the total variance explained by each group of variables.

Despite the significance of the model (p = 0.002) and the low colinearity among environmental variables (variance inflation factors <15), the relatively high values of correlation between Elev and TOC, and between sand and Gmean (Fig. 5.13), as well as the high percentage of shared variance (Fig. 5.15), indicate some redundancy. To verify this, a new RDA was performed using the automatic selection which gives the marginal (the independent effect of each environmental variable) and the conditional (the effect that each variable brings in addition to all the variables already selected) effects of each variable (Table IV). According to these values, only Lat and Elev are significant among the five selected variables when a 0.05 probability threshold level for entry of a variable is adopted (values in red in Table IV).

Table III - Marginal and conditional effects obtained from the summary of forward selection.

Variable	**Marginal effects (λ1)**	**Variable**	**Conditional effects (λA)**	***p***
Elev	0.24	Elev	0.24	0.004
TOC	0.23	Lat	0.12	0.006
sand	0.15	TOC	0.07	0.064
Lat	0.09	Gmean	0.03	0.067
Gmean	0.05	sand	0.02	0.788

In Table III, the marginal effects show that Elev and TOC were the most important factors in determining species composition, followed by sand. Elev and TOC were closely correlated, as expected from their causal relationships seen in the previous chapter. Consequently it was not surprising that, the importance of TOC decrease dramatically (from 0.23 to 0.07) when Elev variable was selected. Moreover, when Elev was included in the model, Lat variable slightly increased its importance, becoming the second most important variable. Both Gmean and sand variables decreased their importance when Elev and Lat were included in the model due to their inverse relationship, especially with Elev.

Summer: Of the 63% of variation explained by the model, 34% was explained by the spatial variables, 2% by the chemical variables and 10% by the physical variables (Fig. 5.16). The remaining 54% was explained by the combined effects of the three groups of variables. The pure effect of the spatial variables solely explained much more variation than the other two groups, suggesting that it is the best group in explaining the variance of species composition.

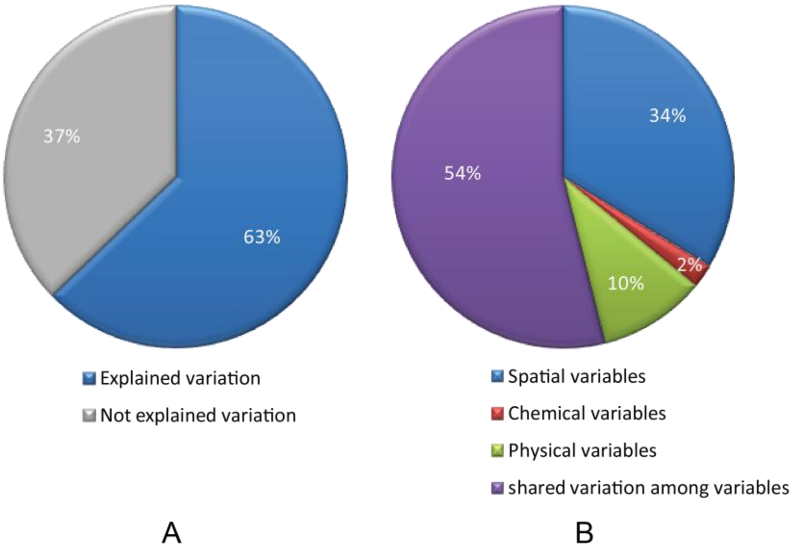

Figure 5.16. Variance partitioning of species composition: A – total variance explained by the 3 groups of variables and B – partitioning of the total variance explained per each group of variables.

Although the significance of the model (*p* = 0.002), T and DO had high variance inflation factors (> 20) indicating high shared variance with the other variables. Also, in Fig. 5.14 it is possible to see that T and DO are highly correlated, indicating redundancy. Applying the technique adopted for the winter data, Table IV shows that, for the summer data, only Elev and Long were significant among the five selected variables, when 0.05 probability threshold level for entry of a variable is adopted (values in red in the table).

Table IV - Marginal and conditional effects obtained from the summary of forward selection.

Variable	**Marginal effects (λ1)**	**Variable**	**Conditional effects (λA)**	***p***
Elev	0.39	Elev	0.39	0.002
sand	0.3	Long	0.15	0.002
DO	0.18	sand	0.05	0.068
T	0.16	DO	0.02	0.528
Long	0.14	T	0.02	0.56

Elev and sand were the most important factors influencing species composition, with a marginal effect of 0.39 and 0.3, respectively. If the Elev variable was selected, the effect of sand decreased dramatically (from 0. 3 to 0.05). When Elev was included in the model, the Long variable slightly increased its importance, becoming the second most important variable. Both DO and T variables decreased their importance when Elev was included in the model, due to their inverse relationship.

5. 3. 11. Winter ecological model

In developing a winter ecological model, a final RDA was performed with only the two most important environmental variables, Elev and Lat (Fig. 5.17). Alone, Elev and Lat were responsible for 36% of the total explained variance, of which 80% was explained on the first axis. Both axes presented a good correlation between species distribution and environmental parameters, r = 0.82 for the first axis and 0.84 for the second axis. Both the significance test on the first, and all axes, using Monte-Carlo permutation test, were highly significant *(p* = 0.002).

In Figure 5.17, sample scores were based on species composition (linear combinations of species in the samples; Samp scores in the CANOCO solution file) and the scaling was focused on inter-species correlations. Elev was correlated positively with the first axis (r = 0.72) and was responsible for the major division of samples into two groups: I and II, reflecting the results already shown by cluster and NMDS metrics. Lat was positively correlated with the second axis (r = 0.81) and was responsible for a secondary division of group II into: samples of lower elevations located in southern positions (Group IIa); and samples of the lower elevations located in northern positions (Group IIb).

Figure 5.17 indicates that, with the increase of both environmental variables, there was a decrease in diversity, with the majority of species concentrated in the lower elevations of the lower estuary. Also, with the increase of both variables, there was a decrease in tolerance by calcareous species, as the most elevated and northerly environments were dominated by agglutinated species.

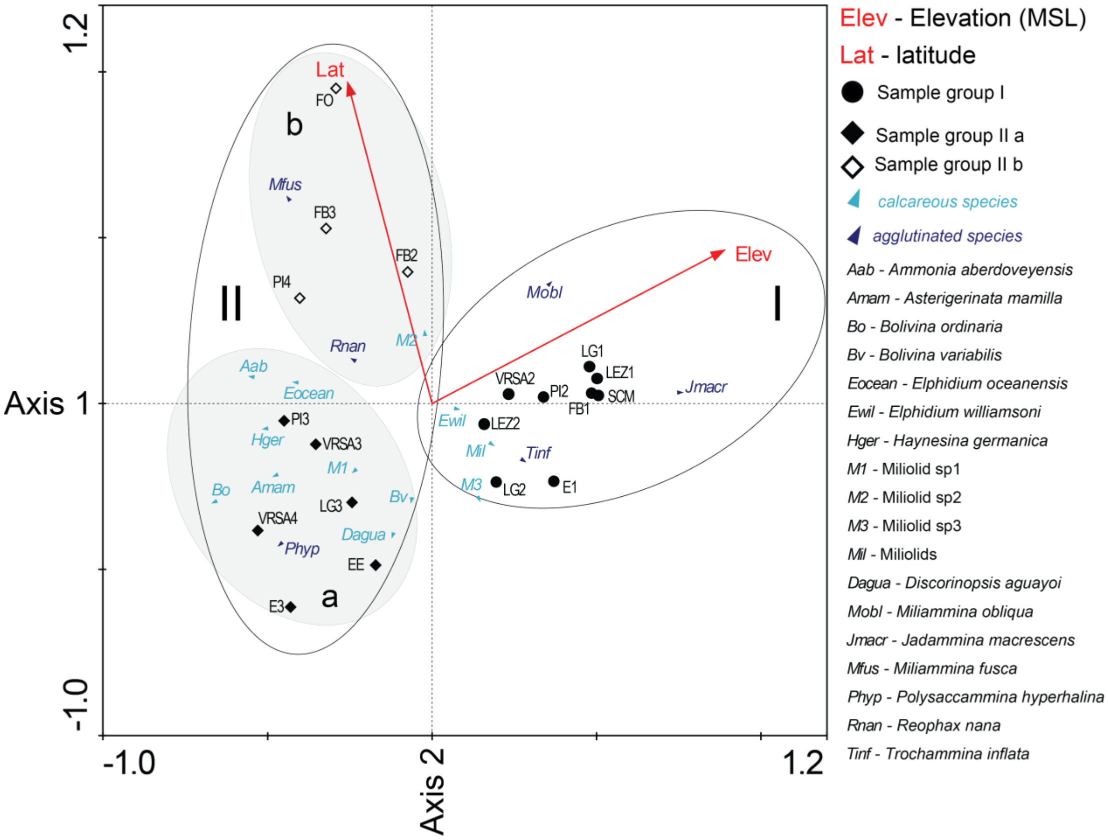

Figure 5.17. RDA triplot summarizing the effects of the main driving environmental variables (Elev and Lat) in benthic living foraminifera distribution in winter.

This model for interpretation of species distribution and assemblage trends can be enhanced with a shade matrix (Fig. 5.18), wherein samples and species are ordered according to their position in the RDA and the original abundances have been categorized and represented by symbols of increasing size and density.

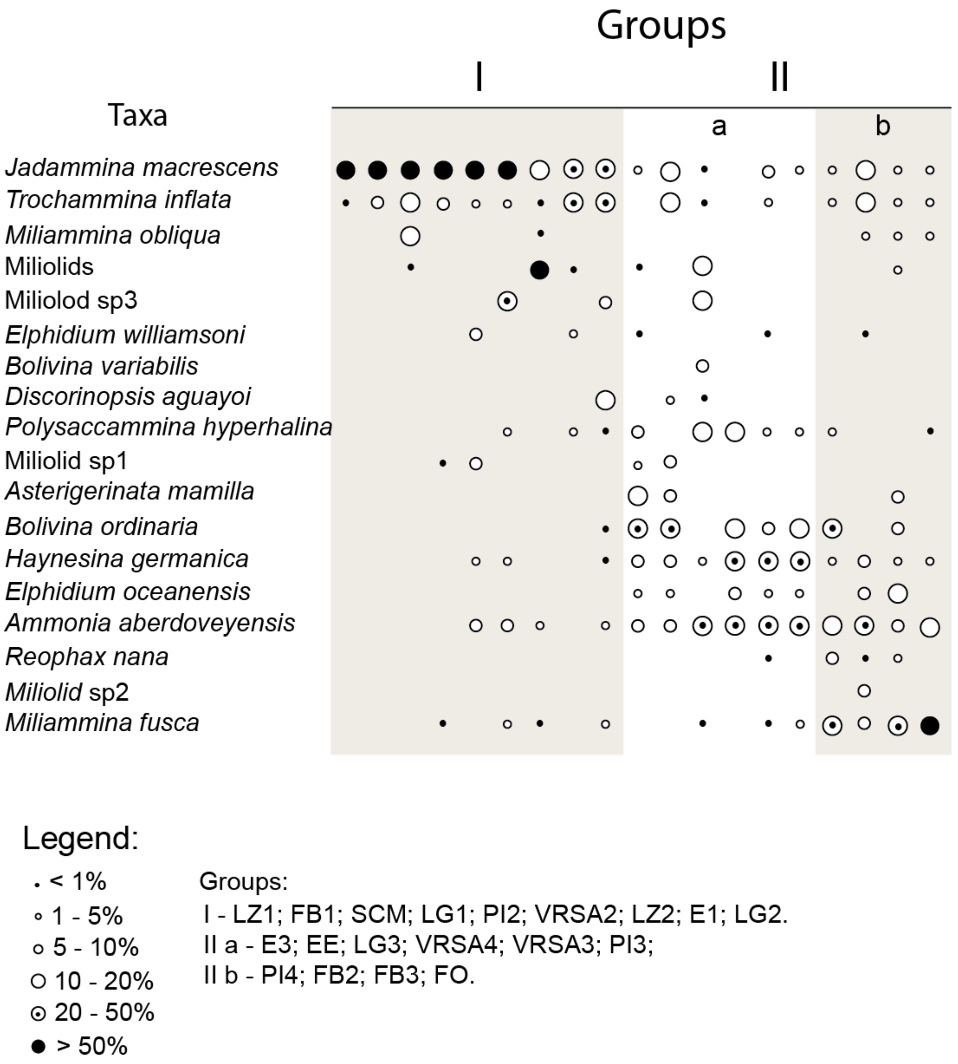

Figure 5.18. Shade matrix for the 18 living species (abundance > 5%) and 19 samples along Guadiana Estuary during the winter sampling season. The original abundances have been categorized and represented by symbols of increasing size and density and the rows and columns of the array re-ordered on the basis of RDA positions.

In winter, the two most ubiquitous species were *J. macrescens* and *A. aberdoveyensis*. Their presence in almost all samples indicates their high degree of resilience to daily changes in environmental factors, typical of estuarine intertidal milieu. *J. macrescens* has its optimum in the upper elevations of the lower estuary, where it dominated almost exclusively the assemblage. *J. macrescens* is closely related to *T. inflata*. This species was present in almost all the samples in which *J. macrescens* also occurs, although it reached its highest abundances in the middle elevations where *J. macrescens* abundance decreased slightly and diversity increased. *A. aberdoveyensis* has its optimum in the lower to middle elevations of lower and middle estuary and was associated with *H. germanica*, which was present in almost all the same samples as *A. aberdoveyensis*. In samples located far from sea influence, *A. aberdoveyensis* and *H. germanica* were associated with *M. fusca* and *E. oceanensis*, and in the samples located near the river mouth they were associated with *B. ordinaria*, *A. mamilla* and *P. hyperhalina*. It is also worth pointing out the stenotypic distribution of some species, which occurred only in specific environments, sometimes in significant numbers. This was the case with *D. aguayoi* and *B. variabilis,* which had a high incidence in lagoonal environments, and also with the porcelaneous taxa, which did not exhibit gradients in their distribution, occupying all the elevations and north–south levels, as long as there was vegetation. This indicates that the two most important environmental variables that explain the species general distribution, do not assume the same importance in the distribution of the porcelaneous taxa in particular.

5. 3. 12. Summer ecological model

For the summer model, the two most important environmental variables, Elev and Long, were used in a final RDA (Fig. 5.19). These two variables, alone, were responsible for 54.5% of the total explained variance, of which 80.6% was explained by the first axis. Both axes presented a good correlation between species distribution and environmental parameters, r = 0.89 for the first axis and 0.77 for the second axis. The significance tests on the first axis and on all axes using Monte-Carlo permutation test, were highly significant (p = 0.002). In Figure 5.19 the same rules of data projection applied for the winter data, were again used. Elev is correlated positively with the first axis (r = 0.92) and is responsible for the major division of samples: groups I and II were negatively correlated with Elev, whilst group III was positively correlated, which is in agreement with the results of cluster and NMDS analyses. Long was negatively correlated with the second axis (r = - 0.95) and was responsible for a secondary division of the samples: group I and almost all the samples of group III were positively correlated with Long, while group II and few samples of group III were negatively correlated. This last division of the samples could also be interpreted as a north–south gradient since Lat is inversely correlated with Long. Along the Guadiana sampling profile (except for SCM and LEZ, which occupy the westernmost position and were located in the lower estuary), the easternmost samples correspond to the southernmost samples and the westernmost samples correspond to the northernmost samples.

Fig. 5.19 shows that there were few species tolerant to increasing elevation and that these were all agglutinated. The majority of the species were concentrated in the lower elevations of the southeastern estuary, where both calcareous and agglutinated forms coexist. In samples from the northwestern middle elevations (group II) the diversity was also low and both forms, calcareous and agglutinated, co-exist.

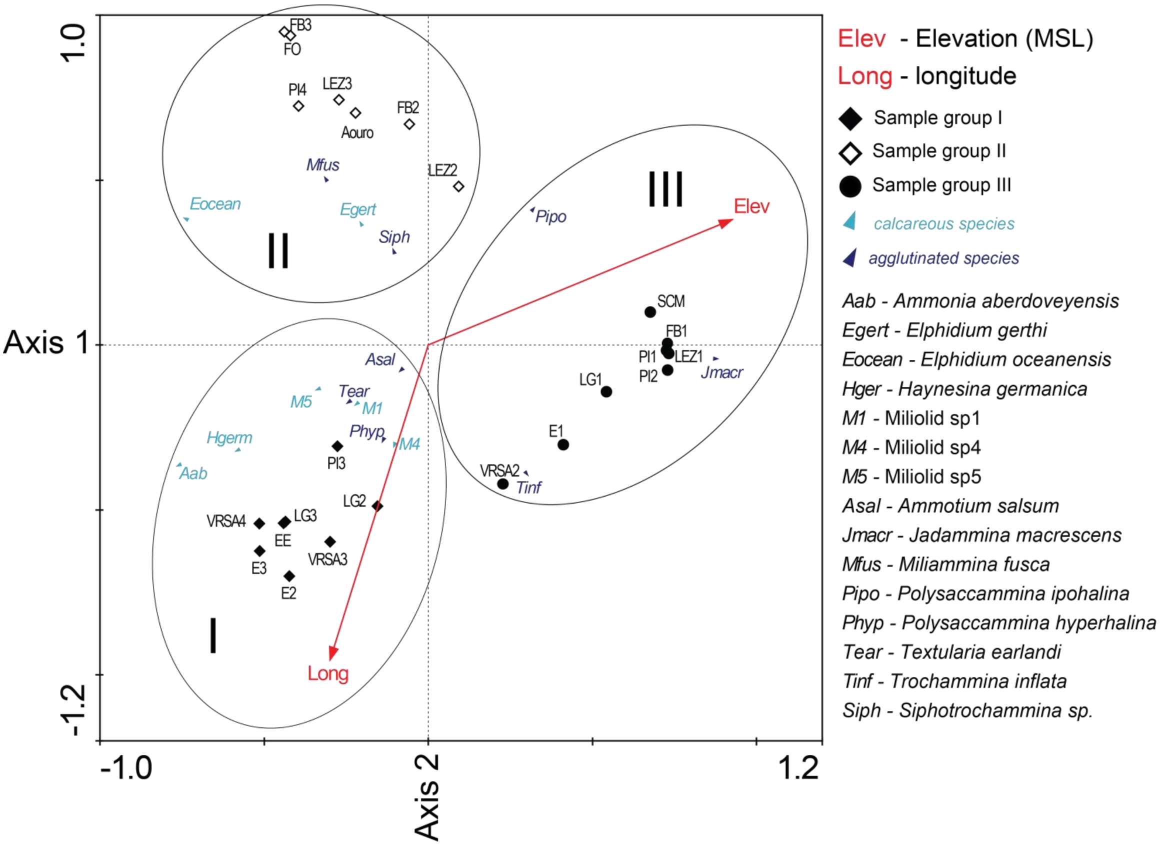

Figure 5.19. RDA triplot summarizing the effects of the main driving environmental variables (Elev and Long) in benthic foraminifera distribution in summer.

The shade matrix (Fig. 5.20) shows that in summer, like in winter, the two most ubiquitous species were *J. macrescens* and *A. aberdoveyensis*. The major difference between the two seasons is that *A. aberdoveyensis* had higher dominance in summer, especially in the samples of group I where it was associated with *H. germanica* and *P. hyperhalina*. In the samples of the middle elevations, with a north-westernmost position (group II), *A. aberdoveyensis* was still very prominent, being present in all samples, but was subdominant to *E. oceanensis* and *M. fusca*, which have their optimum there. *J. macrescens* had its optimum in the upper elevations of the lower estuary, where it dominated almost exclusively. *T. inflata* was subsidiary to *J. macrescens*, being present in almost all the same samples as the latter, but having a weaker expression when compared to its winter abundance.

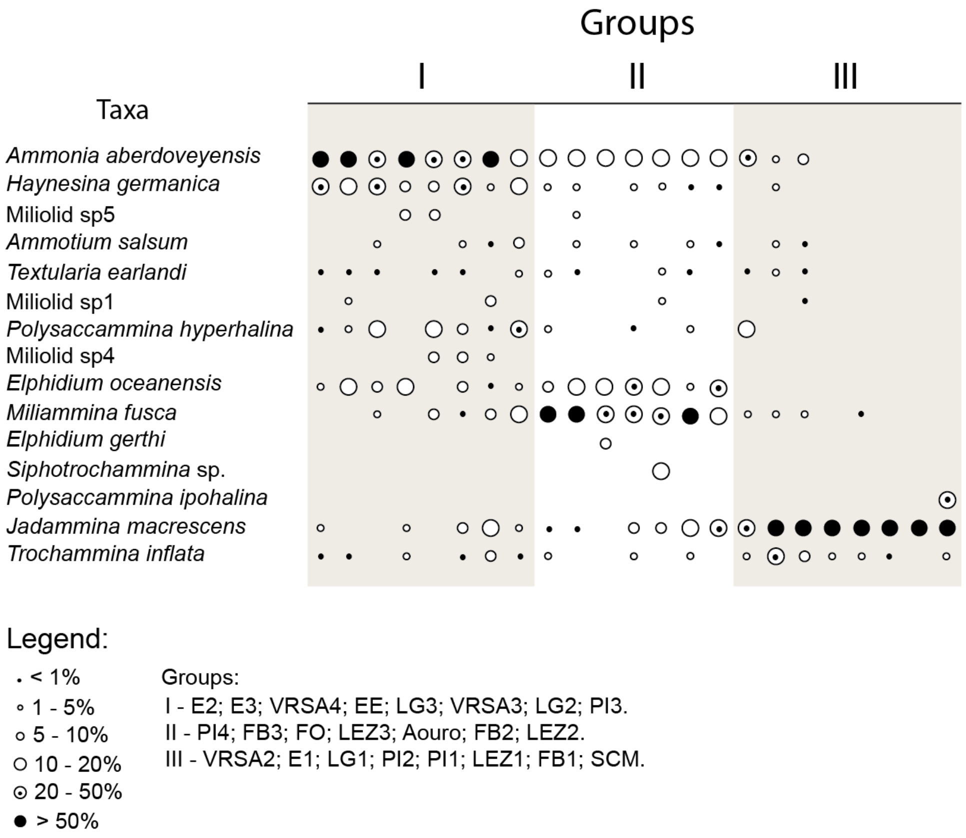

Figure 5.20. Shade matrix for the 15 living species (abundance > 5% or constant) and 23 samples along Guadiana Estuary during summer. The original abundances have been categorized and represented by symbols of increasing size and density and the rows and columns of the array re-ordered on the basis of RDA positions.

It is also worth noting the occurrence of some isolated species, such as *Polysaccammina ipohalina* at SCM and *Siphotrochammina sp.* at Aouro. Both species were specific to these sites, occurring only in summer with significant abundance values, 20.8 and 12%, respectively.

The porcelaneous forms, here represented by Miliolid sp1, sp4 and sp5, were not constant species and were restricted to the lower to middle elevations samples.

5. 4. Discussion

5. 4. 1. Framework

Despite the growing use of salt-marsh and estuarine foraminifera in the assessment of anthropogenic environmental problems, the most common application of this proxy remains in the reconstruction of paleoenvironments and former sea levels. To achieve such reconstructions, modern analogues are collected – either dead assemblages or total assemblages (live plus dead) – and compared to the paleoassemblages. Dead or total assemblages are preferable to live assemblages because they integrate seasonal compositional changes in the fauna, while living assemblages represent only a snapshot in time, pointing out the behavior of foraminifera in response to rapidly changing local conditions (Duchemin et al., 2005). Living assemblages thus cannot be used as independent and representative environmental indicators in paleoreconstruction studies (Scott et al., 2001). This problem may be overcome if monthly, or preferably fortnightly, samples are collected, ensuring a complete record of biologic and ecologic variations in foraminiferal life cycles (Debenay et al., 2006; Murray, 2003). Unfortunately, such studies are rare due to the time consuming tasks of foraminifera counting and identification. On the other hand, the study of living assemblages provides baseline knowledge on ecological trends and distribution patterns that is essential for interpreting paleoassemblages. Furthermore, the study of the living assemblages is fundamental in the understanding and identification of the post-mortem changes that could affect preservation (especially dissolution of calcareous tests and hydraulic transport of exotic taxa) of both, dead and total assemblages (Horton and Murray, 2007). In the present chapter, the presence, abundance and distribution of foraminifera are used as indicators of environmental factors variation and only the living assemblages are analyzed. Although samples of 20 cc of sediment are used (rather than the 50 cc recommended by Schönfeld et al., 2012), the majority of them are relatively rich in foraminifera, yielding more than 100 live (stained) individuals and thus providing a statistically reliable analysis.

Time-series studies, from month to month and year to year, provide an opportunity to visualize dynamic changes in species distribution and abundance under changing ecological and environmental conditions. Such studies are rare, being mostly based on single sampling periods (Horton and Murray, 2007). Given that monthly sampling was beyond the scope of this thesis, the two most extreme seasonal periods, winter and summer, were sampled to gauge the major environmental differences. In terms of environmental conditions, this goal was achieved, recording a very wet winter and a normal, hot summer.

In the present work, three to four samples were collected along several elevational transects in zones where major halophytic landscape differences occur. This relatively low sampling frequency is compensated by the broader spatial scale of the study, encompassing several transects and single points along a length of approximately 34 km of estuary. This sampling strategy was adopted to register a maximum of sub-environments in both latitudinal and elevation gradients.

Some foraminifera have been reported as having an infaunal behavior, living as deep as 30 cm in the sediment (Goldstein et al., 1995; Goldstein and Watkins, 1998) raising the question if the assemblage living at the first centimeter is representative of the local environmental conditions. A preliminary counting of a few 10 cm depth samples selected randomly from the winter set of samples show that, when existing, the living individuals were a negligent percentage of the total assemblage, thus discouraging to deeper sub-sampling, time consuming, task. Only the first centimeter (0 to 1 cm depth) was sampled as it contains the majority of the living population (Scott et al., 2001) and is recommended by Schönfeld et al. (2012) as providing a comparable dataset for the majority of bio-monitoring studies.

In order to neutralize the patchiness effect, the natural trend of seafloor organisms of spread heterogenic, three replicates should be taken per site (Schönfeld et al., 2012). Due to time limitations this methodological task was beyond the scope of this thesis and only one sample was taken per site. Nevertheless, in order to minimize the potential bias caused by patchiness a pseudoreplication procedure was used (Armynot du Châtelet et al., 2009; Debenay et al., 2006; Hulbert, 1984).

5. 4. 2. Spatial and temporal ecological trends

A series of biocenotic indices, such as diversity, number of species, dominance, faunistic density and agglutinated/calcareous ratio, allow for detection of changes in the assemblages and it is generally accepted that they can be used as preliminary descriptors (Debenay et al., 2000). In the present work, such indices are used to characterize the main ecological trends in space (elevation, latitudinal and longitudinal gradients) and time (winter and summer).

In the sub-environments of the Guadiana Estuary, foraminifera density is generally very high, particularly in the lower estuary where it surpassed 75 000 individuals/20 cc of sediment. High densities are observed frequently in the upper elevations of the lower estuary, caused by mainly oligospecific and sometimes monospecific numbers of agglutinated forms. In both seasons there is a trend of decreasing density with increasing latitude and decreasing elevation. In general, at all latitude and elevation levels, higher densities are observed in winter (Fig. 5.21A).

Both measures of species diversity, Shannon diversity (Hs) and the number of species (S), vary between values typical of estuarine and marsh environments (Murray, 2003), with Hs varying from 0.043 to 2.34 and S varying from 1 to 21 species. Diversity in the Guadiana Estuary is higher than in the Caminha Estuary, NW Portugal, where Hs never surpassed 1.70 and S never surpassed 11 species (Fatela et al., 2009). Nevertheless, the Guadiana Estuary have lower diversity than the adjacent continental shelf, where Mendes (2010) found Hs varying between 1.1 and 3.2 and S varying between 13 and 68 species. In the estuary, both indices show a similar trend of decreasing with increasing elevation but no significant relationship is found with latitude. Although always positively related due to the dependence of Hs on S, the relationship between these variables can be more or less strong depending on evenness variation, which is accounted for when calculating Hs. The relationship between both diversity measures is stronger in winter (R = 0.89) than in summer (R = 0.71), suggesting higher numbers of rare species and higher species dominance in summer.

Diversity is inversely correlated with foraminifera density, which is in accordance with Boltovskoy and Totah (1985), who stated that species number and dominance are often inversely correlated. In the upper marsh elevations, only few species, especially agglutinated, are able to survive the extreme and variable environmental conditions (Figs. 5.2, 5.4 and 5.5), i.e. the high content in organic matter trapped in the cohesive fine sediments promotes low pH and low oxygen concentrations; salinity (temperature) may be extremely high due to evaporation (insolation), especially during summer, or extremely low due to rainfall (frost), especially during winter; and the sediment may be very dry due to desiccation. Such extreme conditions promote high levels of physiological stress in the organisms, diminishing inter-species competition and favoring only the better adapted species.

High diversities require a considerable period of environmental stability (Buzas and Gibson, 1969) and thus, changes in species diversity are potentially a measure of stressful events (Murray, 2003). Both Hs and S were slightly higher during winter (Fig. 5.21B and C), suggesting higher environmental stability during this season. In fact, the Dominance index (c), which reflects the degree of environmental stress (Odum, 1997), exhibit an inverse trend to diversity indices in both seasons (Fig. 5.21E), suggesting generally harsher environmental conditions in summer and in the areas subject to longer periods of subaerial exposure between tidal cycles.

Greiner (1974, in Buzas, 1989) presents evidence that $CaCO_3$ availability controls the distribution of shell types, suggesting a gradient in estuaries in which environments with low carbonate availability would be dominated by agglutinated foraminifera, intermediate availability by hyaline foraminifera and high availability by porcelaneous foraminifera (miliolids). As $CaCO_3$ availability increases with the increase of salinity (Buzas, 1989) the same inferences could be made between the latter parameter and the distribution of the three types of tests. Thus, agglutinated foraminifera should be abundant in hyposaline conditions typical of marginal marine environments, the miliolids should be confined to hypersaline conditions (tropical and shallow waters), and hyaline forms should be abundant in all environments of brackish to marine waters (Douglas, 1979). The agglutinated/calcareous ratio (A/C%), which measures the proportion of agglutinated and calcareous tests in the assemblage, could be assumed as an indirect measure of salinity (fresh/marine water gradient) and an indirect measure of $CaCO_3$ availability (fresh/marine water and continent/water gradients). In the present work, the A/C% ratio gradually increased from the lower to the upper elevations in both seasons, although the trend is less marked in winter, which suggests greater homogeneity in the distribution of agglutinated species during this season. Lower temperatures, higher rainfall and riverine dominance during winter favor the dispersal of typically upper elevation agglutinated species to the lower levels of the intertidal margin. In fact, an overall dominance of agglutinated forms was observed during winter, where 81% of the individuals are agglutinated and only 19% are calcareous.

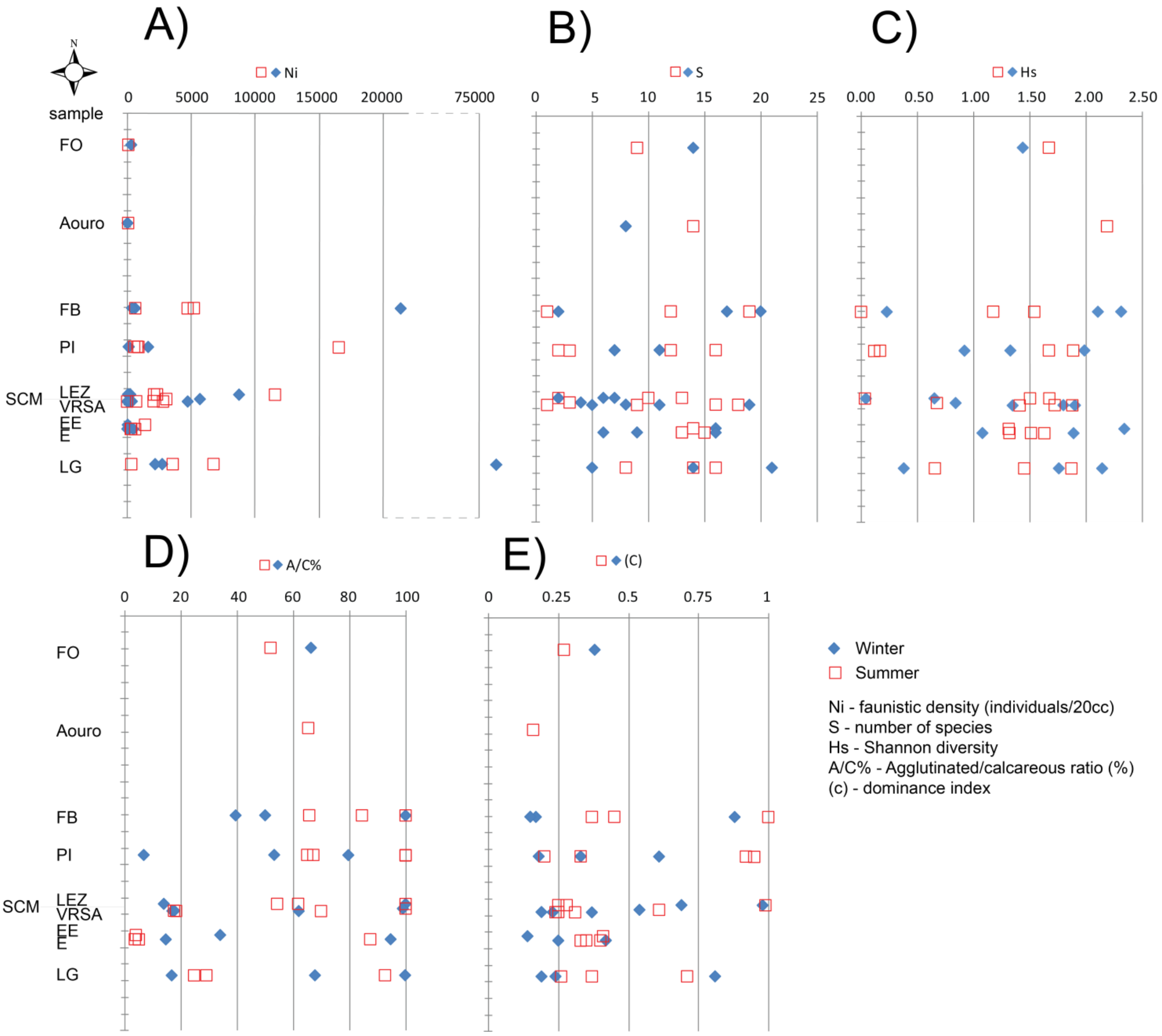

Figure 5.21. Comparison between biocenotic indices (A – faunistic density (Ni), B – number of species (S), C – Shannon diversity (Hs), D – Agglutinated/calcareous ratio (A/C%), and E – dominance index (c)) measured in winter and summer. The vertical axis represents latitude (i.e. distance from the river mouth).

In summer, the relationship between elevation and A/C% is much better defined (R = 0.9) and the relationship with the other spatial variables is also stronger, with higher values of A/C% in the western and northern samples. This environmental definition promotes disequilibrium in the proportions of agglutinated and calcareous in the elevation end-members, thus promoting very high or very lower values of A/C% during summer (Fig. 5.21D). Despite higher A/C% values in summer, an increase in the percentage of calcareous forms (41.6%) suggests an improvement in environmental living conditions for calcareous species. This opposing seasonal preference, with more agglutinated forms in winter and more calcareous forms in summer, was also observed in Chezzetcook Inlet, Nova Scotia by Scott and Medioli (1980b). No obvious gradient in A/C% is found from north to south as expected, due to the general trend observed in estuaries with a gradual change from agglutinated-dominated to calcareous-dominated assemblages (Debenay et al., 2000). The low number of samples collected in the middle and upper estuary, along with the inclusion of the lower estuary’s upper elevations samples (rich in marine agglutinated species such as *J. macrescens* and *T. inflata*) weaken the correlation between A/C% and latitude. Nevertheless, in the species distribution model for each season provided by redundancy analysis, there is a clear separation of the most diverse calcareous assemblages located at the lower elevations of the lower estuary from the less diverse agglutinated assemblages located at the upper elevations of the lower estuary and middle elevations of middle and upper estuary.

Contrary to expectations, in winter there is greater proportion (6.7%, compared to 2.7% in summer) and a greater diversity (9 species vs. 5 in summer) of porcelaneous tests. Miliolids are usually reported as preferring warm, shallow (Haynes, 1981), hypersaline waters (Douglas, 1979) and according to the literature (Colom, 1974; Ribes et al., 2000; Ribes and Gracia, 1991; Sen Gupta, 1999c), a high representation of miliolids could be characteristic of *Posidonia* meadows, being attached to the plant’s rhizomes. Their presence is also related to shelly substrates, which have higher availability of calcium carbonate (Phleger and Lankford, 1957 and Levy, 1970 in Debenay and Guillou, 2002). Laboratory experiments testing the resistance of hyaline, porcelaneous and agglutinated tests to different preservation environments indicate that porcelaneous tests are, after *Discorinopsis aguayoi* tests, the most susceptible to dissolution (Camacho, unpublished data). According to these results, higher densities and diversity of porcelaneous species were expected in the lower elevations during summer, where lower TOC and higher pH values are reported and both temperatures and salinities are higher, promoting higher $CaCO_3$ availability (Buzas, 1989). Instead, higher densities and diversities are reported in samples located in the upper elevations during winter. Comparable results, with large numbers of miliolids, have been reported in relatively stable, slightly hypersaline, restricted environments (Debenay et al., 1998b; Debenay and Guillou, 2002). Horton and Murray (2007), found among typically high marsh assemblages, a notable number of *Quinqueloculina* spp. and Armynot du Châtelet et al. (2009) found their most common miliolid species present at several locations along their salt-marsh transect, at different heights. The latter observations and those achieved in the present work, suggest that miliolids are somehow independent to elevation and organic matter presence, probably possessing any physiological strategy which enables them to live in such reductant environments.

5. 4. 3. Spatial and temporal environmental trends

The success of foraminifera in occupying a great diversity of niches is attributed to their ability to tolerate a broad range of environmental conditions (Murray, 2001). Among the many parameters studied in marginal environments, the elevation (time of aerial exposure between tidal cycles) is widely regarded as the principal factor controlling foraminifera distributions (Gehrels, 2000; González et al., 2000; Horton et al., 1999; Horton and Edwards, 2000; Horton and Murray, 2007; Jennings et al., 1995; Nydick et al., 1995; Scott and Leckie, 1990; Scott and Medioli, 1978; 1980; 1986; Thomas and Varekamp, 1991; Varekamp et al., 1992). Other parameters, such as shade from vegetation or leaf litter (presence or absence of vegetation), effects of drying (desiccation), and porewater salinity and pH have also been identified as important ecological controls on marsh foraminifera (Murray, 2006). In the great marshes of Massachusetts, De Rijk (1995) and De Rijk and Troelstra (1997) found that foraminifera distribution was not related to elevation, but with salinity variations, the result of a balance between infiltration, precipitation and flood. Under these conditions, those authors suggest that the foraminifera distribution is controlled mainly by spatial and temporal changes in a number of environmental variables, particularly salinity, concluding that there is no unique model which relates foraminifera and elevation applicable all over the world. Likewise, Goldstein and Watkins (1998), in a study of the salt-marsh of St. Catherines Island, Georgia, found significant differences in the foraminifera distribution patterns from those found by Scott and Medioli (1978; 1980; 1986), promoted by differences in geographical settings, including differences in salt-marsh physiography. In the mangrove swamps of French Guiana, elevation was found to have only minor influence on the distribution of foraminifera assemblages (Debenay et al., 2004).

Explaining distribution patterns require consideration of a broad range of environmental factors (Murray, 2001). Species will be able to survive and maybe prosper as long as conditions remain within the tolerance limits for the niche of an individual. Once conditions move outside the tolerance limits for any one limiting factor, the species is likely to die out (Murray, 2003). This is true not only for global scales but also for regional and local scales. Portugal, for example, is a relatively small country with a great diversity of environmental conditions in estuarine systems located in the north and in the south. In the northern systems, the fluvial-dominated signature promotes such extreme conditions (low pH values for instance), that most of the sites are barren of foraminifera and the existing assemblages are mainly agglutinated, with few calcareous occurrences (Moreno et al., 2005; Fatela et al., 2009). In the south, the estuarine systems have a higher marine contribution, promoting much more diversified and calcareous assemblages (Camacho, 2004).

In the present study, redundancy analysis was used to correlate several environmental variables and select the most important in controlling the distribution of foraminifera in winter and summer. The results accord Murray's (2001) model based on the niche concept, which states that local distributions are explained by a range of different factors (reaching critical thresholds singly or in combination) at different times and in different places. Indeed, the present data suggest that foraminifera distribution mirrors the seasonal variation of environmental factors and that the importance of each factor depends on the proximity of the species' ecological thresholds. When considering each environmental variable singly, elevation is the most important environmental parameter in explaining foraminifera distribution in both seasons, although it is more important in summer. In winter, TOC is also selected as a reliable environmental variable in explaining species distribution. Sediment type is important in both seasons. Although foraminifera do not feed directly on resistant and refractory organic matter (OM) of vegetal origin, which comprises almost all the OM present in the high marsh zone, its presence could promote the appearance of other microorganisms, such as bacteria, which are known to belong to foraminifera menu (Loubere and Fariduddin, 1999; Topping et al., 2006; Ward et al., 2003). Thus, foraminifera that live in high OM content environments could be indirectly dependent on it. The same approach could be done to sediment type, i.e. the species distribute in a granulometry gradient according to their demands in test construction. For instance *T. macrescens,* which is an upper marsh species, uses much more fine sediment (silts and clays) to build its test than *M. fusca*, which is a middle-lower marsh species, exhibiting a much coarser test, made essentially on quartz material. However, in the Guadiana Estuary, there are cases (e.g. E1 sample in the Spanish margin) located at relatively high positions (0.6 m above MSL) with an untypical physico-chemical composition (sand mean value: 72% and TOC mean value: 0.5%) which are colonized almost exclusively by trochamminiids. This seems to show that upper marsh species are independent on sediment type and TOC content, and are primarily controlled by elevation. Thus, in our opinion, both TOC and sediment type are selected by the model as secondary important variables due to their co-variability with elevation.

Surprisingly, neither salinity nor any other chemical water parameter appeared to be major drivers in species distribution and composition in the Guadiana Estuary's wetlands. The high daily, spatial and seasonal variability of salinity suggest that marsh and estuarine foraminifera species in general are osmoresistant, being present in both hypo- and hyper-saline environments (Debenay et al*.*, 2000).

Temperature and oxygen seem to play some role in summer, probably due to the proximity to threshold levels, extremely high temperatures (29º C) and extremely low oxygen saturation (35.2%). When running winter and summer models with the most important variables, considering the effects that each variable brings in addition to elevation, the majority of the variables lose importance in explaining foraminifera distribution (see conditional effects in Table III and IV). Elevation is the primary driver of foraminifera assemblages as it combines the effects of a series of other environmental variables (TOC, C/N and mud content increase with increasing elevation; and $\mathrm{pH_{sed}}$, sand, gravel and temperature increase with decreasing elevation). The other geographic variables assume second place on a scale of importance in species distribution. Latitude is the second most important variable in species distribution during winter and longitude during summer. As both latitude and longitude are not true ecological variables, their importance is here interpreted as the integration of a series of conditions related to the prevalent hydrodynamics that have a riverine predominance in winter and marine predominance in summer. Latitude includes the effects of salinity gradients, with which it is strongly negatively correlated (winter R = -0.74** and summer R = -0.85**, Table IV, in Chapter 4). In turn, longitude presents an opposing trend to latitude due to the physiography of the estuary, meaning that the northernmost samples are also the westernmost, and the southernmost samples are as well the easternmost (Fig. 3.4, Chapter 3).

5. 4. 5. Dominant species distribution

Of the 52 species of living foraminifera found in the Guadiana Estuary during winter and summer, 11 are dominant (more than 20% in relative abundance in at least one sample) (Fig. 5.22). Of those, only *J. macrescens, T. inflata, M. fusca, A. aberdoveyensis* and *H. germanica* are dominant in both seasons. *B. ordinaria*, Miliolid sp3 and the miliolids group are only dominant in winter and *P. hyperhalina, P. ipohalina* and *E. oceanensis* are only dominant in summer. Only *P. hyperhalina* and the species that are dominant in both seasons are constants (present in more than 50% of the samples) in at least one season. Among the non-constant species, *P. ipohalina* have the narrowest distribution, occurring in only one sample in summer. Almost all the dominant species are cosmopolitan, occurring in other estuaries all over the world (Sen Gupta, 1999c). In the Guadiana in particular, several of these species were previously recorded by Ruiz et al. (1995; 2005) and González et al. (2000), although direct comparison between their assemblages and the ones studied presently is complicated, mainly due to differences in methodology: they use the total assemblage (living + dead individuals), which increases the number of different assemblages they found by including several species here considered exotic (with no living representatives in the estuary - see chapter 7 for further details), and the criteria used to choose key species and nomenclature are quite different. A major difference is the absence of *M. fusca* and *P. hyperhalina* in the previous works, which seems inexplicable considering the fact that those species are very abundant and constant along the estuary presently. On the other hand, the species *Nonion incrassatum* (González et al., 2000) and *Astrononion stelligerum* (González et al., 2000; Ruiz et al., 2005) are not recognizable among the species encountered in our samples and thus no synonymy is possible. Also nomenclature incongruities for the elphidiids hampered attempts to find synonymy between the species of this group.

Jadammina macrescens is probably the species with the greatest scientific consensus in terms of its life position and indicative meaning. The first applications of marsh foraminifera in reconstruction of episodes of mean sea-level change were based in monospecific assemblages of *J. macrescens* (cf. *Trochammina macrescens*, according to Scott and Medioli, 1980) collected near the continental limit (high marsh) in Nova Scotia marshes (Scott and Medioli, 1978; 1980). Being the most tolerant species to subaerial exposure, its relative abundance is very useful in sea-level change studies because it reflects the vertical distance of the depositional environment to the highest high-water level, where it may represent 100% of foraminifera species, decreasing towards the low marsh, where it is almost absent (Scott and Medioli, 1978; 1980). Therefore, its relative abundance is inversely related to tide-flood frequency and ‘other species’ relative abundance (diversity). It has thus been used as an indicator of flood frequency (Thomas and Varekamp, 1991; Varekamp et al., 1992). *J. macrescens* is the most widespread species in the present work, occurring in almost all winter samples (94.7%) and summer (82.6%) samples. In the most elevated positions, the assemblages are almost exclusively (and in some cases, exclusively) composed of *J.*

macrescens. This species exhibits uncommonly high densities in some places, surpassing 68 000 individuals/20 cc in sample LG1 in winter.

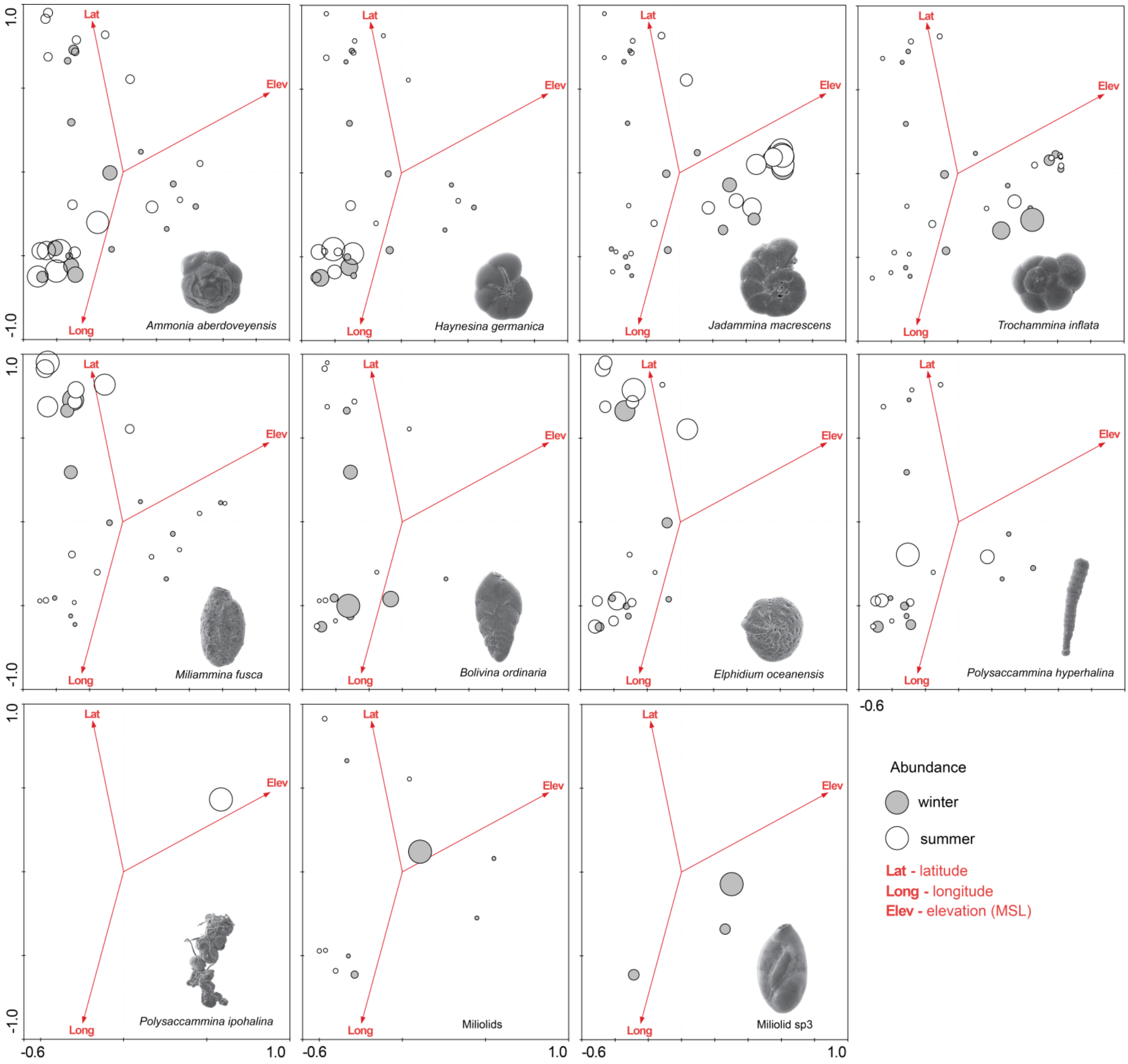

Figure 5.22. RDA biplot representing the distribution and abundance of the dominant species in Guadiana Estuary according to the three most important environmental variables.

In a time-series study performed at Tees Estuary, UK, Horton and Murray (2007) monitored *J. macrescens* standing crops fortnightly over 12 months and recorded peaks in abundance during the year. The maximum standing crop was 864/10 cc in October, being far less than the 68 266 individuals/20 cc (= 34 133 individuals/10 cc) in Guadiana sample LG1. Furthermore, Horton and Murray (2007) observed a progressive decline of this species from May/June, where it represents more than 80%, to March (5th day), when it temporarily disappeared. Our winter sampling, with high densities of *J. macrescens*, coincided exactly with the absence period verified by Horton and Murray (2007). These samples have a strong component of juveniles (generally more than 50% of the total; data not presented herein), extremely small and fully stained, which were 'hidden' in small mucous sediment aggregates, and were only visible when these were disaggregated with a probe. The counting of these 'hidden' specimens may explain the large differences between the densities found in this work and others in which *J. macrescens* was also dominant. In summer, the juvenile component is always present and, although generally less than 50%, there are some samples in which it is higher. These observations lead us to conclude that this species have the capacity of reproducing in both seasons, although winter seems more favorable, at least in the Guadiana Estuary. While juveniles are always present in samples containing adult *J. macrescens*, there are considerable differences between the adult/juvenile proportions from sample to sample, especially in summer. This seems to suggest that, within the same geographical area, the species does not reproduce at the same time, or at least at the same scale, which points out to the influence of other abiotic and/or biotic factors acting locally and in parallel with seasonality, triggering reproduction.

High proportions of juveniles hamper the distinction between *Jadammina macrescens* f. *macrescens* and *Jadammina macrescen*s f. *polystoma*, the latter having supplementary apertures in the last chamber and being indicative of high salinity marshes. The former has no supplementary apertures and is restricted to areas where salinity is lower than 20‰ (Scott and Medioli, 1980). In the specimens collected in Guadiana Estuary the supplementary apertures are clearly visible in SEM pictures, but not so clear under the light microscope, especially in small individuals. Considering that this morphological characteristic is environmentally controlled (Boltovskoy, 1958 in Scott and Medioli, 1980), the two forms are unlikely to occur together, leading us to believe that the form present in the Guadiana is *J. macrescens* f. *polystoma*. This agrees with the species optimum in the upper-middle elevations of the lower estuary, where salinities are high.

Like *J. macrescens, T. inflata* also have higher abundances during winter. *T. inflata* is closely related to *J. macrescens*, occurring together in almost all the samples, although it have higher abundances in middle elevations (0.2 to 0.8 m above MSL at samples E1, LG2 and FB2) where *J. macrescens* abundance starts to decrease and species diversity starts to increase. This relationship was reported previously for the Guadiana (González et al., 2000; Ruiz et al., 2005), and at other places world widely (some of the Nova Scotia marshes - Scott and Medioli, 1980; Fraser River Delta - Patterson, 1990). In other estuarine systems all over Iberian Peninsula (Morbihan, Basque, Minho-Lima and Sado – Leorri et al., 2010), both *J. macrescens* and *T. inflata* are dominant components at the most elevated zones but the species association/succession is not exactly the same as in Guadiana due to, probably, local environmental differences.

No other species is intrinsically related to *J. macrescens* and *T. inflata*, but several species with ephemeral occurrences seem to share similar habitat preferences. In the lower estuary in winter, the Miliolids group and the Miliolid sp3 have peaky occurrences, each in a distinct sample of the upper elevations: LEZ2 (82.3%) and VRSA2 (28.8%), respectively. As previously debated, these occurrences are surprising due to the expected dependence of these taxa on warmer waters, with higher $CaCO_3$ availability and higher pH. Ambiguity related to their identification somewhat clouds the interpretation of these occurrences.

In summer it is worth noting the ephemeral occurrence of *P. ipohalina* at SCM, where it is subdominant to *J. macrescens* (20.8 and 75%, respectively). *P. ipohalina* is a high marsh species (upper mangrove, Barbosa et al., 2005), usually representing a minor component in estuarine systems (Scott, 1976b; Scott and Medioli, 1980; Scott et al., 1990). It was firstly described by Scott (1976b) in San Dieguito Marsh, California, associated with a few other species, especially with *J. macrescens polystoma* and *T. inflata*. Although the latter species are common to almost every marsh, from brackish to hypersaline, associations with *P. ipohalina* should indicate a brackish marsh environment (Scott, 1976b). In the present study, salinity was measured in both seasons at site SCM, a sample located at the margin of a salt-works pond. Here, salinity is relatively high in both seasons (winter – 14.3 and summer – 26.1) when compared with those measured by Scott (1976b) (2–10‰). A common characteristic of SCM and San Dieguito marsh is the lack of tidal activity, which raises the possibility that this species is conditioned primarily by confinement and temperature.

In winter, at SCM site, *P. ipohalina* is replaced by *T. inflata* and *M. obliqua* (Fig. 5.4). The latter, is here reported for the first time in Guadiana Estuary, and, to our knowledge, in Portugal. Hayward et al. (1999) have characterized *M. obliqua* as largely restricted to Southern Hemisphere shores. Based on the present experience, we believe that the strong morphological similarities between *M. obliqua* and *M. fusca* could be preventing accurate identification, not only in Portuguese estuarine systems, but also worldwide, since the former taxon is rarely mentioned in the literature. In the Guadiana, *M. obliqua* is common, with higher incidence in winter, at middle elevations of the northernmost positions and at two samples of the upper elevations with a more confined position. The occurrence of the association between *M. obliqua*, *J. macrescens* and *T. inflata* was also found at New Zealand marsh environments, at the highest heights (Hayward et al., 1999).

Discorinopsis aguayoi, like *M. obliqua*, is a species of narrow distribution, occurring with *J. macrescens* and *T. inflata* in significant abundances (11.9%) during winter. In the living assemblage, its occurrence seems limited to confined sites of the lower estuary (LG and E1), at lower to middle elevations where low marsh vegetation occurs. *D. aguayoi* is regarded as an indicator of landlocked marine/brackish ponds, mangrove marshes (Javaux and Scott, 2003) and high marsh in warm temperate environments (Scott, 1976; Scott et al., 1990). Despite being very well documented by the latter authors, *D. aguayoi* was never mentioned as an epiphytic species. In the Guadiana it is found highly attached to vegetal structures, each specimen having to be carefully detached using a needle probe.

The *Ammonia* genus includes species that are among the most common benthic foraminifera in the shallow waters of marginal marine environments (Debenay and Guilou, 2002; Langer and Leppig, 2000). In the Guadiana Estuary, *A. aberdoveyensis* is the most ubiquitous calcareous species, being absent only from the most elevated samples (PI1, FB1, SCM, LEZ1). Its optimum occurrence is in the lower to middle elevations of the lower and middle estuary. Its peak of occurrence is observed in summer, especially in the lower elevations where it is always the dominant species. It is noteworthy that, in summer, *A. aberdoveyensis* is able to migrate farther up the marsh, occupying higher elevation stands, probably due to the absence of inter-species competition. *A. aberdoveyensis* is a dominant element in two of the main assemblages found for Guadiana: in the middle elevations north assemblage, where it is subdominant to *Miliammina fusca*, and in the lower elevations of the lower estuary assemblage, where is co-dominant with *B. ordinaria* in winter.

H. germanica is closely associated with *A. aberdoveyensis*, occurring as subsidiary species in almost all samples where *A. aberdoveyensis* is present. The dominance of these two hyaline forms (*Ammonia tepida* or *A. beccarii* instead of *A. aberdoveyensis*) has been documented for the estuarine systems of the whole of Europe (see Redois and Debenay, 1996). Both species are resistant to strong physico-chemical changes such as anoxia crises (common in the Mediterranean Sea and stratified lagoons), which require rapid recolonization (Debenay et al., 1998b). In the temperate paralic environments of the Atlantic coast of France, *H. germanica* is the most euryhaline and opportunistic hyaline species, marking the transition between agglutinated forms from upper paralic environments (where is dominant) to calcareous forms from the higher marine influence environments, being progressively replaced by *A. tepida* (Debenay et al. 2000; Debenay and Guilou, 2002; Redois and Debenay, 1996). Horton and Murray (2007) found *H. germanica* among the dominant calcareous species from the tidal flats to middle marsh environments, and *Ammonia* spp. as a not dominant, ephemeral occurrence species. Martins et al. (2010), found *H. germanica* as the most abundant species, followed by *A. tepida*, in the Ria de Aveiro, NW Portugal. In the Guadiana Estuary, *H. germanica* is not as prominent as *A. aberdoveyensis* but is very constant and is even dominant in few samples of the middle and lower elevations. However, our data does not permit to see the zonation observed by Redois and Debenay (1996), Debenay et al. (2000) and Debenay and Guilou (2002). Instead, it seems that *A. aberdoveyensis* is the most well established calcareous species in the estuary, and together with *E. oceanensis*, are the most able to migrate farther up the marsh and farther upstream. A similar distribution is found in Venice Lagoon (Serandrei-Barbero *et al*., 1999) and at Acheloos and Evinos Estuaries (Greece) (Scott *et al*., 1979), where in both areas the lower stands are dominated by *Ammonia* with significant components of *Elphidium* and *Haynesina*. These and the Guadiana Estuary distributions seems to indicate that *Ammonia* is the dominant component in the low stands of the estuarine wetlands in south Europe, Mediterranean warmer environments, whilst in north Europe, colder oceanic environments, *Haynesina germanica* has higher prominence as lower stands species.

According to Hayward et al. (1997), *E. gunter*i (here *E. oceanensis*) has a middle to low latitude distribution, with preference for warmer waters. It is also associated with brackish waters (Debenay et al., 2003; Fatela and Silva, 1990). In the Guadiana *E. oceanensis* have its optimum during summer, being a dominant species at middle and lower elevations (-0.7 to 1.1 m in relation to MSL). In terms of distribution range it is almost as eurytopic as *A. aberdoveyensis*, occupying the entire study range of elevation and latitude.

Bolivina ordinaria has a controversial taxonomic history in the literature, as various names have been used, by different authors, to describe similar specimens' worldwide (see Mendes, 2010 for review). The direct comparison of our specimens with the specimens of Mendes (2010), led us to conclude that both belong to the same species. Thus, the same identification criteria as that author were adopted. *B. ordinaria* distribution within the Guadiana Estuary was surprising, as it is primarily described as an abundant species living at great depths of the Portuguese continental shelf (Martins et al. 2006; 2007; Mendes et al., 2004; Mendes, 2010). Mendes (2010) considered *B. ordinaria* as a ubiquitous, very abundant species, highly adaptable and tolerant to a wide range of different factors or combination of factors, living at two different continental shelf depth ranges (25-100 m in the southeast and 20-60 m in the northwest Gulf of Cadiz), and strongly associated with the direct influence of river discharges. The latter author also found a peak of abundance of *B. ordinaria* at 50 m depth and related it to a high rate of productivity at that depth. Mendes et al. (2004) previously described *B. ordinaria* as an important component of the deep water assemblage, relating it to low hydrodynamic levels, low-oxygen fine-grained sediments and to cold-water incursion derived from upwelling events. Debenay and Redois (1997a,b), in a survey regarding foraminiferal distribution on the continental shelf in North Senegal, related the presence of *Bolivina* spp. to the fertility of upwelling waters and Senegal River discharges. Likewise, Levy et al. (1993) related two species of *Bolivina* with upwelling events along the southern coast of Portugal.

In the Guadiana Estuary, *B. ordinaria* occurs as a dominant species in winter. In summer it is less frequent and less abundant. Depth and low hydrodynamics are not regarded as conditioning factors, as the species occurs in environments above MSL (< 0.61 m) and during winter, when the estuary experiences higher hydrodynamics. Passive upriver transportation by tides could be a hypothesis, but then it would be expected to happen mainly in summer when the residence times are higher due to low river flows (Oliveira et al., 2006). From the overall environmental drivers discussed here, the productivity, allied to low temperatures seem to be the common factors in controlling the occurrence of *B. ordinaria* in both, the estuary and deep shelf environments. In Guadiana Estuary, during the rainy seasons (winter and spring), the inputs of river fresh water are higher, enhancing the bioavailability of nutrients, especially Si, promoting the bloom of diatoms, which are the preferred food source for healthy foodwebs (Dias et al., 2001; Rocha et al., 2002). The low temperatures indirectly and the productivity directly could be triggering *B. ordinaria* reproduction and proliferation inside the estuary.

Miliammina fusca is a very common species in worldwide paralic environments, dominating the continental end-member, and associated with direct freshwater influence (see Debenay and Guilou, 2002 for review). It is considered as an indicator species of the low-middle marsh zone (Varekamp et al., 1992) and the most ubiquitous agglutinated shallow-water species (Murray and Alve, 1999). In the Guadiana estuary, *M. fusca* is the most ubiquitous agglutinated shallow- water species, dominating assemblages either in the upper reaches of the lower estuary and upstream, at middle elevation zones. Along with lower-middle elevation indigenous calcareous species (*A. aberdoveyensis* and *E. oceanensis*), *M. fusca* is most abundant during summer, when it often represents more than 50% of the total assemblage (Fig. 5.4). The species also seems able to migrate farther up the marsh in summer (e.g. LEZ and FB transects), reaching 16.3% at 1.06 m above MSL (LEZ2 sample).

Polysaccammina hyperhalina is a common and nearly constant agglutinated species in the Guadiana Estuary. In winter, its distribution seems restricted to the lower elevation zones, whilst in summer it reaches higher abundances at both the middle and lower elevations. *P. hyperhalina* is now reported in the Guadiana Estuary for the first time and, as far it was possible to ascertain, in Portugal. It is a species of hypersaline environments and was first reported and described from Venice Lagoon (Petrucci et al., 1983). In the Guadiana Estuary, the species has its optimum at the lower elevations of the lower estuary (Figs. 5.3 and 5.4), where marine processes are more pronounced. In the northwest of Portugal paralic systems, nor Martins et al. (2010) at the Canals of Ria de Aveiro, nor Moreno et al. (2005) and Fatela et al. (2009), at Minho and Coura estuaries, found *P. hyperhalina*. The presence/absence of this species may relate not only to salinity but also to temperature, as *P. hyperhalina* seems to prefer warmer climates, as noted by Petrucci et al., (1983) when he compared faunas from Venice Lagoon, Greece and Southern California.

5. 4. 5. Seasonal zonation model of benthic foraminifera in the Guadiana Estuary

Seasonal variations in living foraminifera populations of the Guadiana Estuary did not significantly alter the composition of the dominant elements. These elements are also dominant in paralic environments worldwide, allowing comparison between various environments in different geographical areas (Debenay and Guilou, 2002). Based on those dominant elements and on the seasonal variations exhibited by their interrelationships, it was possible to define four main assemblages:

- *J. macrescens* assemblage: Corresponds to the most elevated highly vegetated marsh environments (between 1 and 2 m above MSL, or lower, in sheltered environments) of the lower estuary, where the sediments are finer (> 90% mud), pH is the lowest and TOC is the highest. *J. macrescens* is the dominant species, usually with abundance values usually higher than 70%, with *T. inflata* as a subsidiary species. Occasionally, significant occurrences of *M. obliqua*, *P. ipohalina* and miliolids are recorded.
- *J. macrescens* and *T. inflata* assemblage: corresponds to the middle elevation zones of the lower estuary, between 0.1 and 0.6 m above MSL, at lagoonal vegetated sites with high marine influence. The physico-chemical parameters may be highly variable. Both *T. inflata* (6–49%) and *J. macrescens* (13-54%) are dominant and, at the lower elevations, *D. aguayoi* occasionally occur in winter and *A. aberdoveyensis* in summer.
- *M. fusca* assemblage: is observed in the middle elevation zones (0 to 0.75 m above MSL) of the upper reaches of the lower estuary up until the sampling northern limit, usually in non vegetated areas. *M. fusca* is the dominant species, associated with *A. aberdoveyensis* and *E. oceanensis*.

- *A. aberdoveyensis* assemblage: this is observed in the lower elevation zones of the lower estuary, between -7 to 0.3 m in relation to MSL. It corresponds to the estuarine zone of higher marine influence, where the sediment is composed of more than 50% sand, with the occasional occurrence of significant bioclastic content. *A. aberdoveyensis* is the dominant species, associated with *H. germanica*, *P. hyperhalina* and *E. oceanensis*. In winter, *B. ordinaria* is co-dominant with *A. aberdoveyensis*, and, to a lesser extent, *A. mamilla* may be an important component.

Debenay et al. (2000) and Debenay and Guilou (2002), based on *ca* 1500 samples collected worldwide by several authors, in various types of paralic environments, were able to synthesize the main foraminiferal distribution trends for each geographical and climatic zone. Despite several similarities between the Guadiana Estuary distribution model and the generalist model proposed by the previously cited authors for temperate North Atlantic paralic environments, greater similarities were found with the distribution trends observed in Mediterranean paralic environments. In Mediterranean paralic environments, *J. macrescens* and *T. inflata* are prominent species in the upper marsh zone, occasionally related to *D. aguayoi*. The middle marsh is characterized by the presence of *M. fusca*, occasionally associated with *Ammonia* (*tepida* or *beccarii*), *P. hyperhalina*, *T. inflata*, and *Quinqueloculina seminulum*. Mudflats and low marshes are characterized by the presence of *Ammonia*, sometimes associated with *H. germanica*.

Although the results achieved in the present study are novel and interesting, we believe that a higher sampling frequency could improve the refinement of the distribution boundaries, especially in the northern portion of the estuary, where a series of interesting species occur (*P. ipohalina*, *M. obliqua*, *Haplophragmoides* spp., *Siphotrochammina* sp., etc.) but where distribution trends could not be defined due to a lack of sampling resolution.

5. 4. 6. Application to sea level and paleo-reconstruction studies

The results in this chapter suggest that foraminiferal adaptations to multiple and inter-related parameters lead to distinct life positions of the different species. In a paleoenvironmental record, each of these life positions is more or less difficult to interpret depending on the strength of the indicative meaning of the existing species or assemblages. Each species has its own unique niche influenced by a large number of abiotic and biotic factors. For a species to survive, the numerical values of all those factors must lie within the upper and lower critical threshold tolerance limits peculiar to that species (Pielou, 1974, in Murray, 2001). The species with high range of tolerance for a high number of factors are those with higher probability of having a broad distribution, occupying a high diversity of habitats. These are called eurytopic species. Conversely, species with narrow ranges of tolerance to environmental factors have narrow distributions and are called stenotopic species. The latter have higher indicative meaning as they are better bioindicators due to their environmental exclusivity (Odum, 1997). However, when environmental exclusivity is associated to ephemerality, as is the case of *D. aguayoi*, *Siphotrochammina* sp. and *P. ipohalina*, which occur at high densities but only once in time and space, is difficult to understand which of the environmental parameters is conditioning the species distribution. Among the widely distributed species in the Guadiana Estuary, *J. macrescens, M. fusca* and, to a lesser extent, *P. hyperhalina* and *T. inflata* offer the highest ecological indicator value as they are strongly related to at least one of the main environmental factors. Figure 5.23, an RDA triplot comprising both seasons' environmental, species and sample data, shows that *J. macrescens* is indicative of upper elevations in the lower estuary, *M. fusca* is indicative of middle elevations in the middle to upper estuary, *P. hyperhalina* is indicative of lower elevations in the lower estuary and *T. inflata* is indicative of middle elevations in confined environments lower estuary. Although the environmental signal provided by the eurytopic species alone is weak, the great quantity of data that those species usually provide can be very useful when integrated with other species data. Numeric relationships between species, populations and entire communities frequently provide more accurate environmental indications than species alone, as the whole community better reflects the integrated environmental conditions than its individual parts (Odum, 1997).

Certain indicator species, which are common in the modern record, are absent from the paleo-record due to higher susceptibility to taphonomic effects. This is the case of *M. fusca* and *P. hyperhalina* (Camacho, 2004). In these cases, only the signal given by the remaining paleo-assemblage can provide some answers and, together with other environmental proxies, can even detect the probable 'presence' of the absent species. Thus, species like *A. aberdoveyensis*, which is the most ubiquitous species in the present estuary, are indicative of a relatively high range of habitats and thus offer limited interpretative power if not considered alongside the total assemblage.

Figure 5.23 also shows that, for sea-level studies where elevation is the variable of interest, the middle elevation environments are the most difficult to interpret due to their more variable nature. Middle elevation samples are the most variable in terms of species composition, assuming quite different positions in the RDA projection in winter and summer (e.g. FB2, LG2 and PI4). Their transitional position can favor species migration from the elevational extremes, promoting seasonal variability in species composition. The upper elevation group of samples (group A in Fig. 5.23) provides the most accurate information for sea-level reconstruction as they have a very constant species composition through time. Scott and Medioli (1980) also found this trend. High resolution sampling along their Chezzetcook transects showed that the most elevated faunal zone yielded higher accuracy as it has the lowest vertical range and because the top of this zone is distinguished by a sharp decrease in foraminiferal numbers that accurately locates the highest high water (HHW) datum.

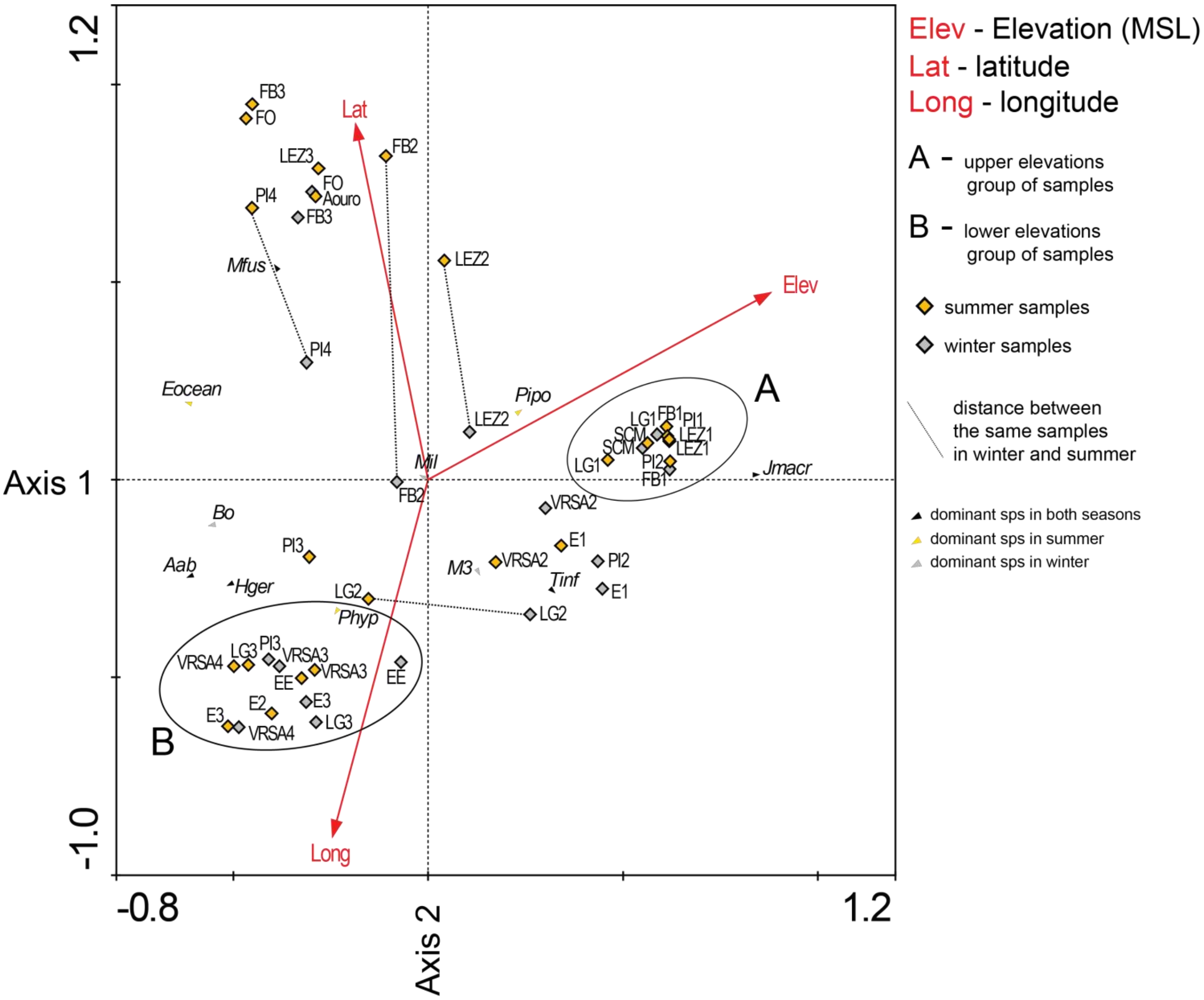

Figure 5.23. RDA triplot summarizing the effects of the main driving environmental variables (Elev, Lat and Long) in the distribution of the dominant species of benthic foraminifera in both seasons. Samples scores (grey – winter and yellow – summer) are based on species composition.

While not as cohesive as the upper elevation group, the lower elevation group of samples (group B in Fig. 5.23) also maintains some coherence in species composition through time, potentially providing reliable information.

The information in this chapter is of paramount importance in providing ecological baselines in the interpretation of paleoenvironments and past sea-levels. Nevertheless, the taphonomic processes acting on benthic foraminifera after death will be recorded, and sometimes aggravated, in the paleo-record. The effects of taphonomic processes are only accounted for when considering the total assemblage of living and dead individuals, including the empty tests of living indigenous species, ephemeral occurrences and transported, and sometimes reworked, exotic species. In Chapter 6, the biocenosis and taphonomic effects information will be brought together and used as base lines in the interpretation of the Guadiana Estuary's paleoenvironments

5. 5. Conclusions

This chapter identifies the main trends in the benthic foraminifera distribution and biodiversity patterns along a spatial and seasonal gradient in the Guadiana Estuary, Southern Portugal. The most important conclusions are summarized as follows:

In both winter and summer there is a trend of decreasing faunistic density with increasing latitude and decreasing elevation.

Species density can be abnormally elevated if the usually discarded juvenile component (< 63µm) is considered. In some agglutinated species the juvenile component can reach 80% of the total abundance and can provide valuable information about species reproduction cycles.

Along the elevation gradient there is a negative correlation between diversity and dominance. In the upper elevation zones, where the environmental conditions are generally harsher, only a few well adapted agglutinated species are able to survive, occurring at elevated densities. At the lower elevation zones, where the time of aerial exposure is lower and the environmental conditions are steadier, there is more diverse faunas, mainly composed of calcareous species.

In winter, when the fluvial processes prevail, agglutinated species proliferate, especially those living at the uppermost zones of the marshlands. In summer, when marine conditions prevail, calcareous species become more competitive, increasing their numbers and moving further up the marsh and river.

Lower-elevation agglutinated species such *Miliammina fusca* and *Polysaccammina hyperhalina* exhibit the same trends as the other lower-elevation calcareous species, having their optimum during summer. This suggests that there are environmental factors other than salinity and $CaCO_3$ availability that drive the growth of the lower elevation assemblages as a whole.

Foraminifera distribution in the Guadiana Estuary mirrors the seasonal variation of environmental factors and the relative importance of these factors varies according to the proximity of species thresholds. When considering each environmental variable individually, elevation is the most important environmental parameter in explaining foraminifera distribution as it integrates the effects of a series of other environmental variables.

No significant relationship is found between species distribution and salinity along the elevational gradient, which is in accordance with the belief that marsh foraminifera are highly osmoresistant, tolerating extreme salinity variations promoted by daily and seasonal environmental changes.

Jadammina macrescens, among the agglutinated species, and *A. aberdoveyensis*, among the calcareous species, are the most ubiquitous and dominant species in the Guadiana Estuary in the winter and summer, during the year of study.

Based on dominant species and seasonal variations in their relationships, it is possible to define the foraminifera zonation ecological model for Guadiana Estuary, which lays on the definition of four main assemblages:

> ***J. macrescens* assemblage:** this corresponds to the most elevated, highly vegetated marsh environments (between 1 and 2 m above MSL, or lower, in sheltered environments) of the lower estuary, where the sediments are finer (> 90% mud), with low pH and high TOC. *J. macrescens* is the dominant species, usually with abundance values higher than 70%, associated with *T. inflata* as a subsidiary species. Occasionally, significant occurrences of *M. obliqua*, *P. ipohalina* and miliolids are recorded.
>
> ***J. macrescens* and *T. inflata* assemblage:** this corresponds to the middle elevation zones of the lower estuary, between 0.1 and 0.6 m above MSL, at lagoonal vegetated sites with high marine influence. Physico-chemical parameters can be highly variable. Both *T. inflata* (6–49%) and *J. macrescens* (13-54%) are dominant and, at lower elevations, *D. aguayoi* occasionally occurs in winter and *A. aberdoveyensis* in summer.
>
> ***M. fusca* assemblage:** this was observed in the middle elevation zones (0 to 0.75 m above MSL) of the upper reaches of the lower estuary until the sampling northern limit, usually in unvegetated areas. *M. fusca* is the dominant species, associated with *A. aberdoveyensis* and *E. oceanensis*.
>
> ***A. aberdoveyensis* assemblage:** this is observed in the lower elevation zones of the lower estuary, between -0.7 to 0.3 m in relation to MSL. It corresponds to the estuarine

zone of highest marine influence, where the sediment is composed of more than 50% sand, occasionally with a significant bioclastic component. *A. aberdoveyensis* is the dominant species, associated with *H. germanica*, *P. hyperhalina* and *E. oceanensis*. In winter, *B. ordinaria* is co-dominant with *A. aberdoveyensis*, and *A. mamilla* may also be an important component.

The species distribution model developed for the Guadiana Estuary is more similar to models for the Mediterranean climatic zone than those for the temperate North Atlantic climatic zone.

The upper (and to a lesser extent the lower) elevation zones provides the most accurate information for sea-level reconstruction as they have the most constant species composition through time.

The data analyzed in this chapter bring new insights into foraminifera ecology and biology that are of paramount importance in providing the ecological baselines for studies of reconstruction of the paleoenvironments and paleo-sea-levels on the southern Iberian Peninsula and in related climatic zones. Future studies should concentrate on higher sampling resolution to more accurately define the distribution boundaries of foraminifera species, especially those living in the northernmost portion of the estuary.

Chapter 6

Holocene paleoenvironmental reconstruction of the Guadiana Estuary based on modern assemblages of benthic foraminifera

Chapter 6

6 - Holocene paleoenvironmental reconstruction of the Guadiana Estuary based on modern assemblages of benthic foraminifera

6. 1. Chapter objectives

In the present chapter, the Holocene evolution of the Guadiana Estuary is assessed through the interpretation of the paleoenvironments recorded in fossil sedimentary sequences. Two drilled boreholes (CM3 and CM5), previously studied by Boski et al. (2002; 2008), are revisited and interpreted at the light of a modern analogue approach. The total assemblage of foraminifera (living + dead individuals) is used as a baseline in the interpretation of the paleoassemblages as it combines seasonal and taphonomic effects relevant to the paleo-record. Along with sedimentary features and ^{14}C dates, the dominant components, diversity and agglutinated/calcareous ratio (A/C%) are expected to provide a temporal and spatial framework for the different transgressive/regressive events recorded in the sediments accumulated in the Guadiana Estuary during the Holocene. The paleosequences will be discussed and compared with other data, particularly from the Gulf of Cadiz, generally, from Iberian Peninsula zone.

6. 2. Benthic foraminifera as paleoenvironmental indicators

Most estuaries were established by the flooding of river-eroded or glacially-scoured valleys during the Holocene rise in sea level (Wolanski, 2007). The Holocene is the most recent Epoch of the earth’s history, comprising the last 10 000 radiocarbon years or 11 700 calibrated years (Smith et al., 2011). It is characterized by a climate much milder than in the preceding glacial period, during which huge ice caps covered not only Antarctica and Greenland (as today), but also part of North America and Northern Europe, as well as several other high latitude and high altitude areas (Pirazzoli, 1991). Because estuaries progressively fill with sediments, bioclasts and organic matter resulting from land and sea exchange, the sediments can tell the story of the estuaries’ evolution. The presence of *in situ* fossil assemblages can be used as proxies for paleoenvironmental reconstruction with higher accuracy than sedimentological evidence alone. This type of paleoenvironmental reconstruction is based on the principle of uniformitarism, which states that "the present is the key to the past" and assumes that the natural processes that operate in the present are the same as the natural processes that operated in the past (Lowe and Walker, 1997).

Furthermore, historical and paleoecological perspectives on the natural processes acting in the geodynamic evolution of an estuary are essential to provide guidelines for more efficient and sustainable management of modern estuaries.

Traditionally, Holocene sea-level studies have applied saltmarsh bioindicators in a qualitative way. The zones in which the bioindicators are found on the contemporary surface of the marsh are surveyed and this information is applied to the fossil sequences (Gehrels et al., 2000). Among the various biological proxies used in paleoenvironmental reconstructions, benthic foraminifera are one of the most reliable, providing both a modern and an historical context. Other biological proxies, like thecamoebians, tintinnids, diatoms and radiolarians, have also proven to be important (Gehrels et al., 2001; Salgado-Labouriau, 2007; Scott et al., 2001). It has been suggested that combined training sets of these bioindicators may improve the precision and accuracy of sea-level reconstructions. This is especially important when modern analogues of one or more of the bioindicator groups are not available or when they cannot be considered representative of paleoenvironments in stratigraphical sequences (Gehrels, et al., 2001).

An important recent advance in sea-level investigations on centennial time-scales has been the construction of 'marsh-paleoenvironmental curves' or 'marsh paleosurface elevation diagrams' (Gehrels and van de Plassche, 1999). These diagrams are based on the biozonation concept proposed by Scott and Medioli (1978; 1980), which allows elevation changes in the past to be inferred, based on the ecological significance of each biozone. Microfauna, and benthic foraminifera in particular, present a distinct distribution in most marshes of the world (De Rijk, 1995; Scott and Medioli, 1980; Scott et al., 2001), since their tolerance to tidal exposure performs an important role in competition for space (Scott and Medioli, 1980). The different levels of tidal exposure tolerated by foraminifera, or, in other words, immersion frequency, produce a marked vertical zonation in assemblages (Gehrels, 2000; Scott and Medioli, 1980). The marked vertical zonation in foraminifera populations constitutes an important tool in the reconstruction of sea-level change episodes when living assemblages on the marsh surface relate to specific elevation ranges and analogous paleoassemblages are collected from the sediments (Gehrels, 2000).

The first applications of marsh foraminifera in reconstruction of mean sea-level change episodes were based on monospecific assemblages of *Jadammina macrescens* collected near the continental limit (high marsh) of Nova Scotia marshlands (Scott and Medioli, 1978; 1980). The relative abundance of this species reflects the vertical distance of the deposition environments to the highest high water (HHW). In other words, 100% *J. macrescens* occurs near this zone, declining to almost absent in the low marsh zone (Scott and Medioli, 1980). The application of any model based on modern distributions to the fossil record has also to consider taphonomical processes (Duchemin et al., 2005). Taphonomic effects can limit the resolution and reliability of nearshore foraminiferal proxy data in both space and time (Schafer, 2000). In general, the most significant postmortem processes are test transport (loss and/or gain) and destruction, especially dissolution of calcareous tests but also differential disintegration of agglutinated taxa (Murray and Alve, 1999).

An old, but heated, taphonomic debate surrounds the choice of the best modern assemblage to represent the paleoassemblages, stemming from the uncertainty of knowing precisely the proportion of living foraminifera incorporated into the fossil record (Gehrels et al., 2001). Two major options are debated between paleoenvironmentalists. The first is the option adopted by Murray (1973), who believes that a distinction must be made between ecological and paleoecological studies; ecological research should rely on living assemblages, ignoring transport and taphonomic problems, while paleoecology should rely on the dead assemblage, which represents the addition of material from a succession of previous living assemblages time-averaged over several to many years and modified to a lesser or greater extent by taphonomic processes (Murray, 1982; Murray, 2000; Murray and Alve, 1999). The second approach, advocated by Scott et al. (1980b) and other authors (e.g. Patterson et al., 1999; Tobin et al., 2005), relies on the use of total assemblage (living + dead individuals) in paleoenvironmental interpretations, as it integrates most small-scale seasonal and spatial variations into a definable assemblage and appears to reliably reflect long term conditions.

6. 3. Previous studies

The present chapter follows on from the work of Boski et al. (2002), who studied postglacial sea-level rise and its sedimentary signal in the Guadiana Estuary. Based on sedimentary features, ^{14}C dates and preliminary faunal analysis of the first 4 boreholes drilled in the estuary (Fig. 6.1) (CM1, CM2, CM3 and CM4), they proposed the first chronological approach to post-glacial marine transgression in the Guadiana Estuary. The interpretation of these multi-proxy data led to the recognition of two distinct phases of sea-level rise: an accelerated phase of infilling by clay sediments containing the salt marsh foraminifer *Trochammina*, beginning ca 9800 ^{14}C yr BP, and a second phase of slower sea-level rise since ca 6500 ^{14}C yr BP, with predominantly sandy sedimentation within an estuary enclosed by sand spits. The rate of sea-level rise inferred for the first phase is 0.85 cm per year, compared to 0.3 cm per year during the second phase. The second phase lasted until *ca* 5000 yr BP, when the sea attained its present level.

Later, a fifth borehole (CM5) was drilled upstream of the previous study sites (Fig. 6.1). The CM5 borehole accumulated in a sheltered environment and reached 51 m depth, containing an almost continuous record of sea-level variation since *ca* 13 000 cal yr BP.

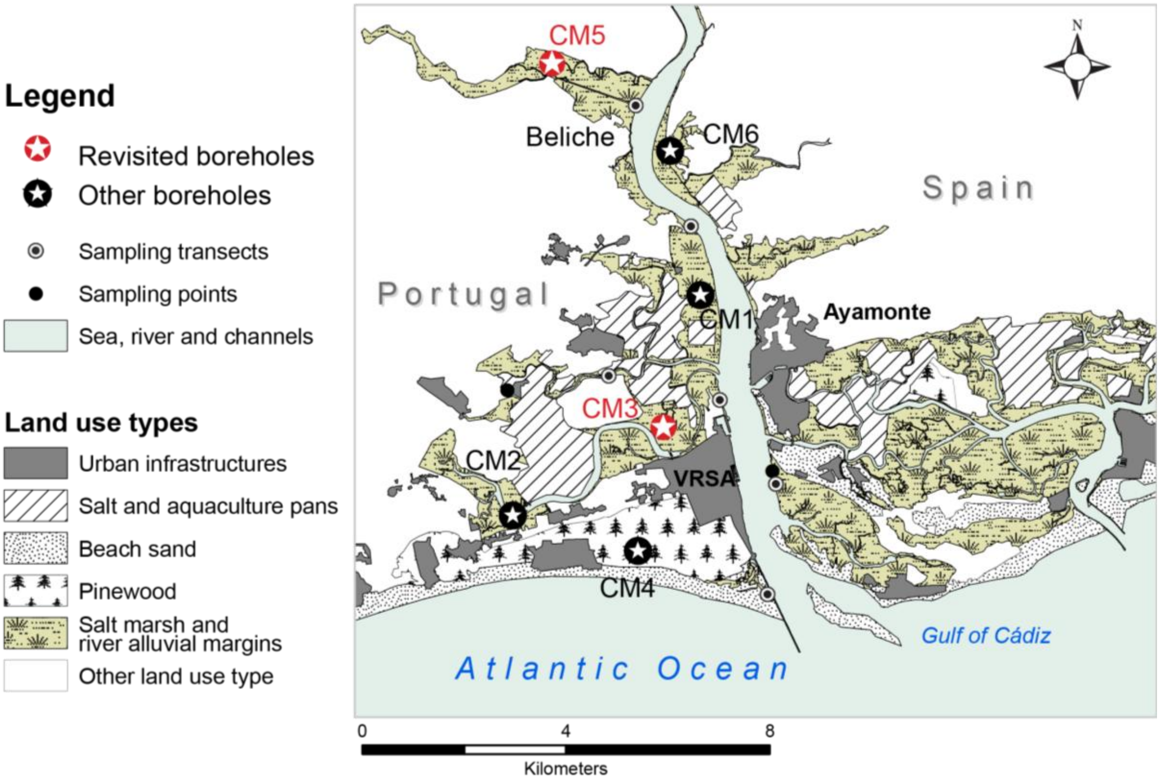

Figure 6.1. Map of the Guadiana lower estuary, the focus of borehole sampling.

Together with three other boreholes in the Gilão-Almargem estuarine system, CM5 was subject of research by Boski et al. (2008), who studied the chronology of sedimentary processes during postglacial sea-level rise at both sites. Foraminifera played a prominent role, contributing to better understanding of the nature and organization of the sedimentary facies in both systems. CM5 was the subject of two additional studies: González-Vila et al. (2003), who studied the vertical distribution of terrestrial and phytoplankton biomarker compounds in the CM5 sedimentary sequence, and Fletcher et al. (2007), who studied the palynological evidence in CM5 and related it to environmental and climatic changes.

Very recently, the 6th and last borehole drilled in Guadiana Estuary (CM6) was studied by Delgado et al. (2012). These authors propose a curve of sea-level rise for the SW Iberian Atlantic margin based on the integration of sedimentological, geochemical and paleontological proxies provided by 63 m of accumulated sediments in the CM6 borehole.

6. 4. Methodology of paleoenvironmental reconstruction

6. 4. 1. Field methodology

With the aim of studying the postglacial Guadiana Estuary sedimentary infill, a total of six rotary boreholes were drilled from 1997 onwards. The first four boreholes (CM1, CM2, CM3 and CM4) were drilled along the estuary according to the sedimentary diversity potential and considering logistical limitations (Fig. 6.1). CM1 borehole was located in the tidally active salt-marsh of Castro Marim, and was the closest borehole to the present main channel of the Guadiana River. CM2 was the most landward borehole, located in a confined sector of the estuary close to a subsidiary tidal channel of the Guadiana. CM3 was located within the Vila Real de Santo António saltmarsh, about 3 km south of CM1 (see geographical coordinates in Table I, Appendix 10). Its present position, close to the main estuarine channel, is affected sporadically by spring tides. CM4 is the southernmost borehole, drilled closer to the coastline than the others, and located in the Monte Gordo dunes. In 1999, a fifth borehole (CM5) of 51 meters was drilled. Located in the present Beira marsh, near the Beliche Rivulet, CM5 was the northernmost borehole, being 11 km from the present coastline (see geographical coordinates in Table I, Appendix 10). More recently, a sixth (CM6) borehole was drilled, the only one located in the Spanish side of the river between CM5 and CM1 (Fig. 6.1). CM6 was the deepest borehole, reaching 63 meters.

The rotary boreholes were collected by a WIRTH Bo rig operated by the Portuguese Geological Survey (IGM). Recovery and handling processes are described in detail in Boski et al. (2002) for the cores CM1 to CM4, in Boski et al. (2008) for core CM5 and in Delgado et al. (2012) for core CM6.

Prior to sample extraction, the cores were split longitudinally, photographed and macroscopically described in terms of lithology, presence of sedimentary structures and macrofossil content (see Boski et al., 2002; 2008; Delgado et al., 2012 for more details).

6. 4. 2. Laboratory methodology

Of the six boreholes drilled in the Guadiana Estuary, only CM3 and CM5 were revisited and interpreted at the light of the modern foraminifera assemblages in the present study. The data from both boreholes were very congruent, with a satisfactory number of samples and relatively well preserved foraminifera assemblages. Thus, the following methodologies are relative to those two boreholes only.

In the CM3 borehole a sample was collected approximately at each meter of the sedimentary sequence resulting in a total of 20 samples for microfauna analysis. CM5 was sampled at higher resolution, with a mean of 2 samples per meter depth, resulting in a total of 82 samples for microfauna analysis. From each sampled depth, 10 cc of sediment were taken for micro-faunal analysis. Sample preparation procedures were the same as for the living assemblages, except that samples were not stained (see Chapter 2).

6. 4. 3. Data analysis of paleoassemblages

Several biocenotic indices were calculated; namely, abundance, faunistic density (Ni extrapolated for 20 cc of sediment), specific dominance, agglutinated/calcareous ratio (A/C%) and Shannon diversity (Hs), all described in Chapter 2. Although tests were generally well preserved, several samples were barren of foraminifera. Of the 20 samples collected in CM3, 10 had less than 300 individuals and 1 had 0 individuals. Of the 82 samples collected in CM5, 2 had less than 300 individuals and 26 had 0 individuals. Samples with at least 100 individuals were considered as statistically valid (Fatela and Taborda, 2002). In some monospecific samples, or samples with only two or three species (e.g. *Trochammina* sp., *J. macrescens* and *T. inflata*), only the first aliquot of the plankton splitter was counted as further counting would not alter the assemblage structure. In some of these cases, the minimum threshold of 100 individuals was not reached, although the samples were still considered as statistically valid.

The identification of several fossil foraminifera presented some uncertainties and these were grouped in artificial groups: indeterminate forms, consisting of very small and/or damaged tests; planktic tests; and the miliolids group, consisting of all porcelaneous forms. For the Shannon diversity calculation, the miliolids group was separated into *incertae sedis* species (see Tables VII and VIII, Appendix 4).

For stratigraphical data organization and presentation, Bennett’s (2005) program, Psimpoll (version 4.25) was used. Both CM3 and CM5 diagrams were edited and improved with Adobe illustrator (Ai) CS5. The sediment description presented is based on the Troels-Smith (1955) system provided by Psimpoll (see Birks and Birks, 1980). The Foraminiferal Zones were differentiated through the Zonation menu of Psimpoll, using a binary splitting by sums-of-squares approach, which splits the data set into successively smaller groups by splitting existing zones (Bennett, 2005).

6. 4. 4. Geochronology

Bulk sediment, peat and wood for radiocarbon dating were all kept at -20ºC and freeze dried before being sent to commercial laboratories (see Table I, Appendix 10). Organic matter samples were taken from the centre of the cores in order to avoid edge contamination. Age determinations from the inorganic, sandy layers were made on hand-picked mollusc shells. All ^{14}C data are expressed as calibrated years Before Present (cal yr BP).

6. 5. Results

6. 5. 1. Modern analogues

The total assemblage (living + dead individuals) was chosen to be used has reference in paleoenvironmental reconstructions as it integrates the seasonal compositional changes derived from the living assemblages but also some taphonomical effects, only accounted when total or dead assemblages are studied. Nevertheless, the living components were studied to better understand the differences between modern and paleoassemblages.

6. 5. 1. 1. Living versus total assemblage: Living population percentage (LPP) was calculated for all samples in both winter and summer seasons (Table III and IV, Appendix 4). LPP was generally higher in summer, when it varied between 0.63 and 80%, at VRSA1 and FB3, respectively (mean 40.5%). In summer, the highest LPP values were observed in the lower elevation samples (Fig. 6.2a), where the calcareous species dominate. In winter, the LPP was substantially lower, varying from 2 to 59.2% (mean 25.6%). The highest values were observed in upper elevations and confined locations, where the agglutinated species dominate. Only in those samples is LPP similar to or higher than the values observed in summer (Fig. 6.2a).

The total assemblages represent approximately 3 times more species than the living assemblage (Fig. 6.2b). Seasonal variation in the number of species (S) (represented by the whiskers in Fig. 6.2b) is generally higher in the total assemblages, especially in the lower elevation samples where the hydrodynamics is also higher.

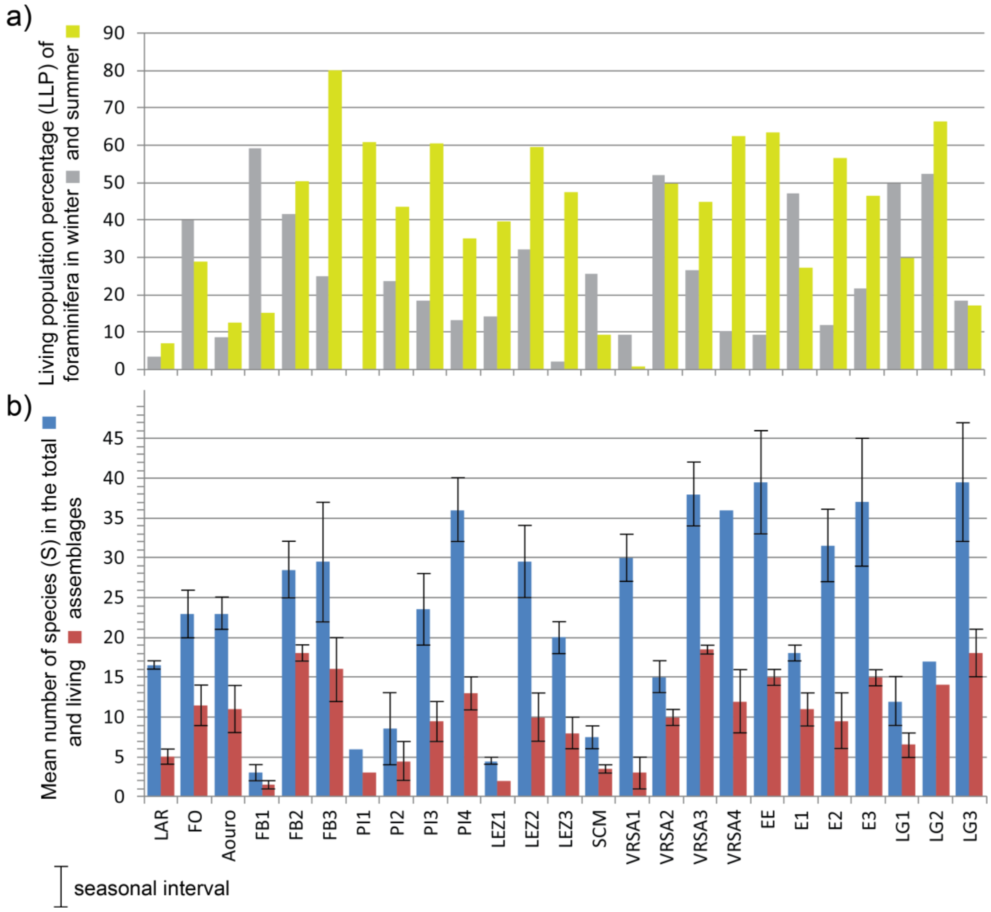

Figure 6.2. Living assemblage data: a) variation of the living population percentage (LPP) in all winter and summer samples; b) variation of the mean number of species (S) in the total and living assemblages, with whiskers representing the seasonal interval.

Table I includes all taxa found in the living, total and fossil assemblages (thanatocenosis). It shows in which assemblages each taxon is present and its status in terms of abundance. It also highlights those taxa which were dominant in at least one of the three assemblages and classifies each taxon in terms of origin, either indigenous (I) or exotic (E) (further discussed).

According to Table I, the taxa which were dominant in the living assemblage generally kept their abundance status in the total assemblage. The species which were dominant in the living assemblage in both studied seasons (Chapter 5), namely *J. macrescens*, *M. fusca*, *T. inflata*, *A. aberdoveyensis*, *H. germanica* and the miliolids group, were also dominant in the total assemblage. Of the species in the living assemblage which were dominant in only one season, only *P. hyperhalina* remained dominant in the total assemblage. The species *P. ipohalina*, *B. ordinaria*, *E. oceanensis* and Miliolid sp3, which were dominant in at least one season, lose importance when the total assemblage is considered.

Table I – Presence, abundance status (R – rare, A – accessory, C – common and D – dominant) and origin (I – indigenous or E – exotic) of the taxa found in the living, total and fossil (thanatocenosis) assemblages. The blue highlights the taxa which were dominant in at least one of the three assemblages.

	Assemblage abundance status			
Taxa	living	total	fossil	origin
Agglutinated taxa				
Ammobaculites cf. *exigus*	R	R		I
Ammobaculites sp.	R	A	D	I
Ammobaculites sp2		R		I
Ammotium cf. *cassis*	R	R		I
Ammotium salsum	A	A		I
Ammotium sp.		R		I
Ammovertellina sp.	R	R		I
Arenoparrella mexicana	R	R		I
Cribrostomoides crassimargo		R		I
Deuterammina eddystonensis	R	A		I
Eggerelloides scaber	R	A		I
Haplophragmoides maniliensis		D		I
Haplophragmoides wilberti		R		?
Hormosina pilulifera		R		?
Jadammina macrescens	D	D	D	I
Lepidodeuterammina ochracea	R	A		I
Lepidodeuterammina plymouthensis	R	A		E
Leptohalysis scottii	R	R		E
Miliammina fusca	D	D	R	I
Miliammina obliqua	C	D		I
Polysaccammina hyperhalina	D	D		I
Polysaccammina ipohalina	D	R		I
Portatrochammina sp.	R	R		I
Remmaneica helgolandica		R		E
Reophax nana	A	A		I
Septotrochammina gonzalezi		R		E
Septotrochammina plicata		R		E
Siphotrochammina sp.	C	D		I
Textularia earlandi	R	A		I
Textularia sp.			R	E
Tiphotrocha comprimata	R	R		I
Trochammina inflata	D	D	D	I
Trochamminita irregularis		R		I
Trochammina sp. (linings)			D	I
Calcareous taxa				
Ammonia aberdoveyensis	D	D	D	I
Ammonia beccarii		A	R	E
Amphicoryna sp.		R		E
Asterigerinata mamilla	C	C	D	I
Bolivina cf. *variabilis*	A	R		E
Bolivina ordinaria	D	C	A	I
Bolivina pseudoplicata		R	R	E
Bolivina sp.		R		E
Bolivina striatula		R	R	E
Bulimina sp.		R	R	E
Buliminella elegantissima	R	R	R	E
Cassidulina sp.		R		E
Cornuspira involvens	R	R	R	I
Elphidium poeyamn	R	R	A	I
Discorbinella sp.			R	E
Discorbis cf. *williamsoni*		R		E
Discorbis spp.	R	R	R	E
Discorinopsis aguayoi	C	A		I
E. incertum			R	E
Elphidium (reworked)		A		E
Elphidium advenum	R	R	R	E
Elphidium advenum var. *margaritaceum*		R	C	E
Elphidium cf. *discoidale*		C	A	E
Elphidium complanatum		R	R	E
Elphidium crispum		A	R	E
Elphidium excavatum	R	R	A	E
Elphidium excavatum williamsoni	A	R	R	I
Elphidium gerthi	A	A		I
Elphidium macelum var. *aculcatum*		R		E
Elphidium oceanensis	D	C	R	I
Elphidium spp.		R	A	E
Epistominella sp.		R		E
Eponides cf. *lateralis*		R	R	E
Fissurina sp.	R	R	R	E
Glabratella sp.		R	R	E
Globulina sp.		R	R	E
Haynesina depressula	R	R	R	I
Haynesina germanica	D	D	D	I
Helenina anderseni	R	R		I
Hopkinsina sp.		R		E
Lagena gracilima			R	E
Lamarckina haliotidea	R			E
Lobatula lobatula		C	R	E
Miliolid sp1	A	A		I
Miliolid sp11		R		?
Miliolid sp12		R		?
Miliolid sp2	A	A		I
Miliolid sp3	D	C		I
Miliolid sp4	A	R		I
Miliolid sp5	A	A		I
Miliolid sp6	R	A		E
Miliolid sp7		R		E
Miliolid sp8	R	R		E
Miliolid sp9	R	R		E
Miliolid sp10	R	R		E
Miliolids	D	D	C	I
Neoconorbina sp.		R		E
Nodosaria sp.		R		E
Nonion boueanum			R	E
Nonion fabum		R		E
Nonion sp.		R	R	E
Nonionella sp.		R	R	E
Oolina sp.		R		E
Parafissurina cf. *inaequalabiata*		R		E
Pararotalia cf. *Spinigera*			C	E
Planorbulina mediterranensis		R	A	E
Poroeponides sp.		R	R	E
Porosononion granosum		R		E
Pullenia bulloides		R		E
Rosalina sp.	R	R	R	E
Rotalia sp.		R		E
Stainforthia sp.		R		E
Trifarina sp.		R		E
Uvigerina sp.		R	R	E
Valvulineria sp.		R		E
Planktic		R	R	E

On the other hand, a few agglutinated species which were absent or less common in the living assemblages, gain importance in the total assemblages: *Miliammina obliqua* and *Siphotrochammina* sp., which were classified as common in the living assemblage, and *Haplophragmoides manilaensis*, which was absent from the living assemblage. These are all classified as dominant species in the total assemblage.

6. 5. 1. 2. Indigenous versus exotic species: All the species found living in the estuary were considered indigenous, except those with occasional occurrences with abundances lower than 1% and those with higher abundances than 1%, but occurring only in the southernmost site (LG), which were considered exotic species. The species which were found exclusively dead were also considered exotic, except those with patchy occurrences in the northernmost samples (LAR, Aouro and FO). Table I shows all species classified as indigenous or exotic, except those with an inconsistent low abundance distribution; these were left unclassified (question mark in Table I).

6. 5. 2. Paleoassemblages

Biocenotic information on CM3 and CM5 borehole samples, i.e. number of species (S), faunistic density (Ni) and taxa absolute and relative frequencies, is provided in Tables VII-X, Appendix 4.

6. 5. 2. 1. Diagenetic settings: A number of very prominent species in the living assemblages were not found at all in the paleorecord. Except *D. aguayoi*, which is believed to have very low preservation potential, all the prominent calcareous species in the living assemblage (i.e. *A. aberdoveyensis, A. mamilla, B. ordinaria, E. oceanensis, H. germanica* and the Miliolids) were present in the paleoassemblages. Among the prominent agglutinated species of the living assemblages, only *J. macrescens* and *T. inflata* were present in the paleoassemblages. The species *M. obliqua*, *P. hyperhalina*, *P. ipohalina* and *Siphotrochammina* sp. were absent from the paleoassemblages and only one specimen of *M. fusca* was recorded in one sample of the CM5 borehole (CM5-890).

In both boreholes, especially in the older samples in CM5, the inner-linings and the badly preserved tests of Trochamminiid forms were very abundant. Although bearing a strong resemblance to *J. macrescens* (see Atlas, p. 33), the poor preservation necessitated the creation of the group *Trochammina* spp., into which all the undifferentiated Trochamminiids were placed.

6. 5. 2. 2. Indigenous versus exotic species: Of the taxa found in the paleoassemblages, 15 were indigenous and 31 were exotic (these are minimum values, as the Miliolids group includes several unidentified species and both indigenous and exotic species can occur). The majority of the exotic species are rare to accessory species (< 5% in abundance) (see Table VIII and X, Appendix 4) and if only the 5% abundance threshold is adopted, approximately 67% of species information would be lost. On the other hand, using a threshold below 5% would increase the entropy in the data set, especially because several of the exotic taxa are not represented in modern assemblages. In order to use the exotic species signal, the taxa were organized in new Tables (Table XI and XII, Appendix 4). According to this organization, each species representing ≥ 10% in at least one sample corresponds to a single entry in the database; the exotic agglutinated species representing < 10% were combined in group; the exotic calcareous taxa representing < 10% were likewise combined; as were the indigenous calcareous representing < 10% (see list of species in each group in Table XIII, Appendix 4).

Although the majority of the exotic species in the paleoassemblages have occurrences lower than 5%, there are three species in the CM3 borehole worthy of note, as they are not present or are of little importance in the modern assemblages: *Elphidium advenum* var. *margaritaceum* is present in 4 of the 20 samples from the CM3 borehole and its abundance varies from 0.72 to 9%; *Planorbulina mediterranensis* is present in 5 samples of the CM3 and varies from 0.62 to 9%; and *Pararotalia* cf. *spinigera* is present in only one sample of the CM3 borehole, with 10% representation.

6. 5. 3. Distribution model based on modern analogue total assemblages

Prior to the identification of the main environmental groups based on the modern analogue total assemblage, both winter and summer data sets were reorganized to harmonize the taxa groups defined for the paleoassemblages (Table V and VI, Appendix 4). According to this new organization, the winter total assemblage matrix comprises 24 samples and 25 taxa (19 species and 6 artificial groups: Miliolids, planktic, Σ exotic agglutinated < 10%, Σ exotic calcareous < 10%, Σ indigenous agglutinated < 10% and Σ indigenous calcareous < 10%; see list of taxa in Table XIII, Appendix 4). The summer total assemblage matrix comprises 25 samples and 27 taxa (21 species and the same 6 artificial groups as in winter; see list of taxa in Table XIII, Appendix 4). Data from the winter and summer reorganized matrices were collated, resulting in an original data matrix of 49 samples and 28 taxa. Hierarchical cluster analysis of this dataset produced four groups of samples, each characterized by different species composition (Fig. 6.3).

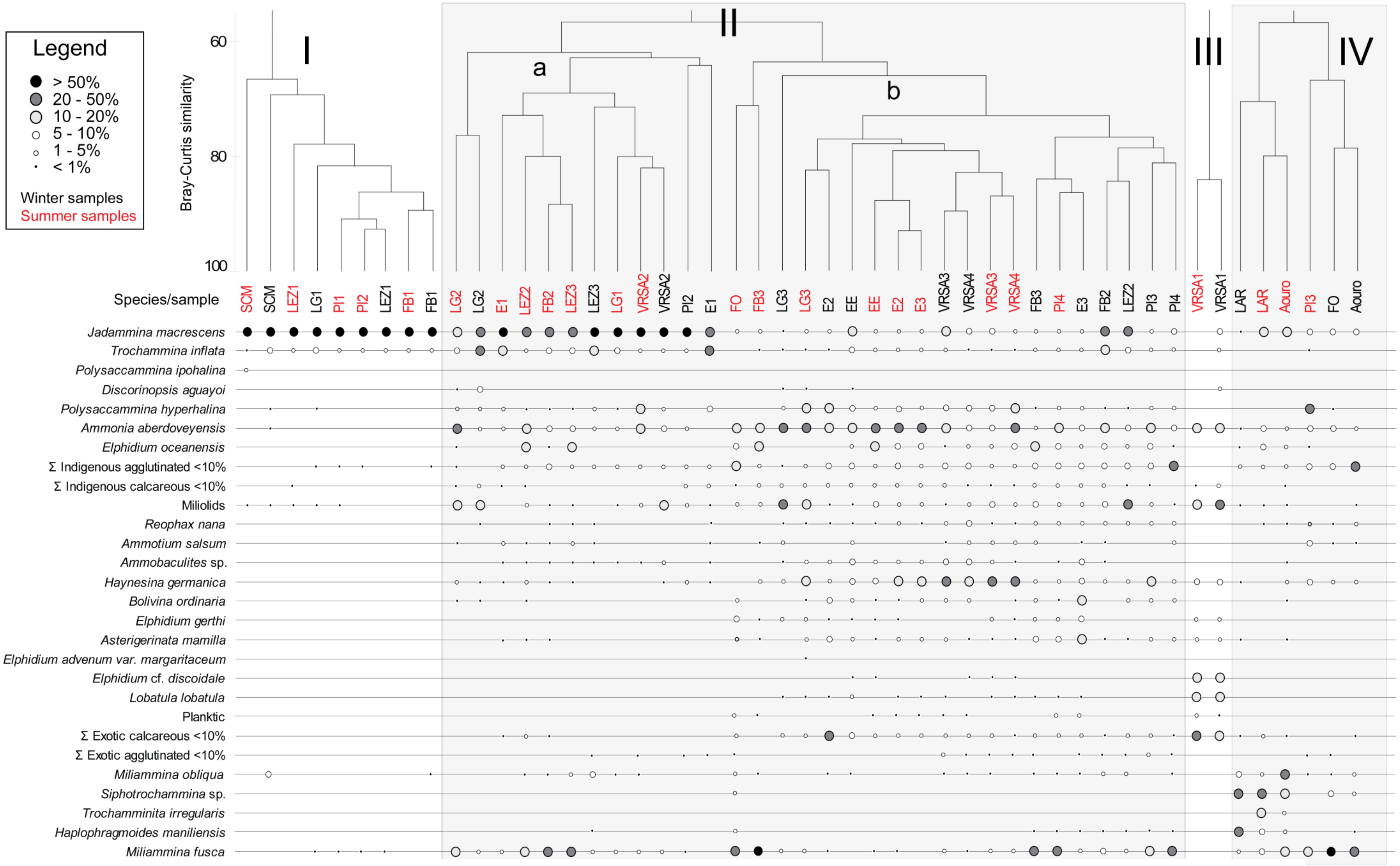

Figure 6.3. Q-mode cluster analysis, using Bray-Curtis similarity coefficient, applied to the data matrix containing data from foraminifera total assemblages in winter and summer: 49 samples x 28 taxa. Clusters cut at approximately 55% similarity separate 4 environmental groups: I, II, III and IV. Group II is divided into sub-groups *a* and *b*. Associated with the dendrogram is the shade matrix of the main taxa abundance variation along the different clusters. The original abundances have been categorized and represented by circles of increasing density and shade.

Each of these groups represents the different sub-environments of the Guadiana Estuary, serving as basis for comparison with the paleoassemblages. The main criteria are the ecology of the dominant species, diversity, test type dominance and exotic species contribution.

Cluster I – groups 9 samples from both seasons, located above the Mean High Water (MHW), or below it, at sites protected from direct sea influence (e.g. LG). These samples are predominantly vegetated by *Sarcocornia perennis* ssp *alpini*, *Suaeda vera* and *Salicornia fruticosa* at the most elevated sites and by *Halimione portucaloides*, *Salicornia ramosissima*, *Arthrocnemum macrostachyum* and *Spartina densiflora* at the less elevated sites (Fig. 6.4). Foraminifera species diversity is very low, usually ranging between 0.12 and 0.41. The population is dominated by a few, highly abundant agglutinated species (10 500 to 49 000 individuals/20 cc), which causes very high A/C% values (99 to 100%) (Fig. 6.5). These environments are almost exclusively dominated by *J. macrescens* (83 to 97.3%, mean 94%), associated with *T. inflata* (0.95 to 9.6%, mean 4%) as secondary species (Fig. 6.3).

Cluster IIa – groups 12 samples from both seasons, the majority of them located between the MSL and the MHW. These sites are predominantly vegetated with *S. densiflora*, and to a lesser extent, *S. perennis* spp *perennis* and *H. portucaloides*. The lower elevation sites may also be unvegetated (Fig. 6.4). Foraminifera diversity is relatively low, ranging from 0.7 to 2.1 (Fig. 6.5). *J. macrescens* is still the dominant species (17.4 to 83.2%, mean 49.4%), but loses some space to *T. inflata*, a secondary species that reaches its optimum in this zone (1.5 to 34%, mean 12%). Other species, both agglutinated and calcareous, start to appear, and both the A/C% ratio (40 to 97.5%) and the Ni (360 to 9160 individuals/20 cc) decrease. Among secondary taxa, *A. aberdoveyensis* (0 to 45.4%, mean 8.5%) and *M. fusca* (0 to 36.8%, mean 9%) are the most constant species. The Miliolids (0 to 18%, mean 4.5%), *P. hyperhalina* (0 to 13.4%, mean 3%) and *E. oceanensis* (0 to 19.3%, mean 3%) are occasionally important (Fig. 6.3).

Cluster IIb – includes 20 samples from both seasons, the majority located between the MHW and the Mean Low Water (MLW). From the most to the least elevated sites, the sediments are usually vegetated with *S. densiflora*, followed by *Spartina maritima* to unvegetated mud-flats (sometimes with *Ruppia maritima*) (Fig. 6.4). This cluster has the highest diversity (1.6 to 3) and the lowest Ni (126 to 6528 individuals/20 cc), comprised essentially of calcareous tests (A/C% ranging from 10 to 81%) (Fig. 6.5). The dominant species are *A. aberdoveyensis* (5 to 45%, mean 20%), associated with *H. germanica* (0 to 27%, mean 10%) in the southern samples and *M. fusca* (0 to 55.5% mean 11%) in the northern samples. A series of other species are occasionally important: *J. macrescens* (0.6 to 30.4%, mean 7%), *P. hyperhalina* (0 to 16%, mean 6%), Miliolids (0.2 to 42%, mean 6%) and *E. oceanensis* (0 to 15%, mean 5%). Although not particularly abundant (0 to 20.6%, mean 3.2%), the group of exotic calcareous species is a very constant, present in 19 of the 20 samples. It is also noteworthy that the planktic group, although occurring in very low abundances (always lower than 2%) is a very constant in this cluster, despite being totally absent from Clusters I and IIa (Fig. 6.3).

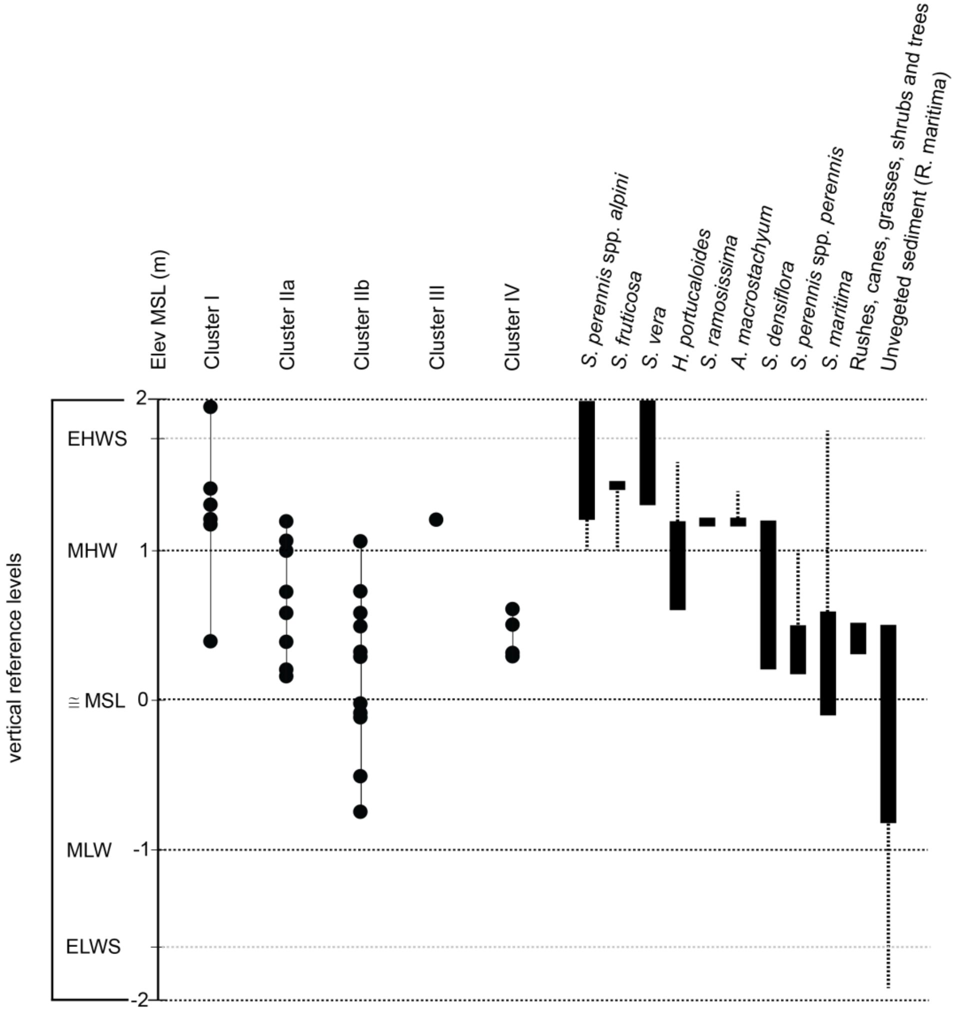

Figure 6.4. Vertical range of the identified clusters (on the left side of the graph); EHWS – extreme high water spring; MHW – mean high water; MSL – mean sea level; MLW – mean low water; ELWS – extreme low water spring. Halophyte vertical distributional range (on the right side of the graph); black columns – identified range; dashed line – range considered in the literature (ALFA, 2004).

Cluster III – includes only two samples of the site VRSA1 in winter and summer. Despite its elevational location (1.2 m above MSL) and halophyte vegetation (*S. perennis* ssp. *alpini*, *S. vera*, among others typical of high marsh), this site has a very unusual sedimentology and foraminifera population. VRSA1 is characterized by more than 92% sand, 5-7% mud and a negligible percentage of gravel (see Chapter 4) and contains a relatively high diversity (mean 2.8), low density (mean 235 individuals/20 cc) foraminifera population, almost exclusively dead (mean LPP: 5%) and agglutinated individuals (A/C% mean 4.4%).The dominant species are the miliolids (mean 17%), *A. aberdoveyensis* (mean 15%) and a series of exotic species: *Lobatula lobatula* (12.2%) and *Elphidium discoidale* (mean 15%), as well as the group of exotic calcareous species with less than 10% representation (mean 18%). The specimens are usually large, reworked and dull. The general characteristics of this small group of samples points to the presence of a relict fauna, probably reworked from older marine deposits, with some mixture of modern specimens brought to the site by spring tides.

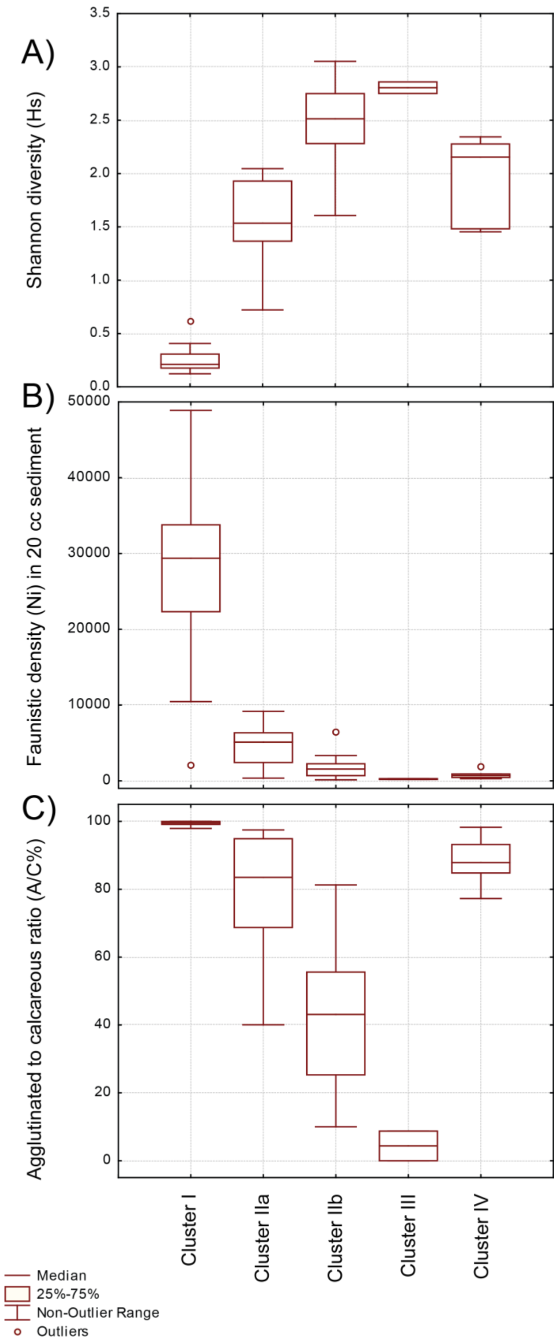

Figure 6.5. Box-plots representing: A) Shannon diversity (Hs); B) faunistic density (Ni/20 cc sediment); and C) Agglutinated/calcareous ratio (A/C%) of the modern total foraminifera assemblage. Median value is represented by the horizontal line within the box, 25% and 75% quartiles are defined in the ends of the box, the whiskers represent the non-outlier range and the circles represent the outliers. Extreme values not shown.

Cluster IV – includes 5 samples from both seasons, the majority located in the middle to upper estuary (Fig. 6.3), in a very narrow range of elevation (between 0.3 and 0.6 above MSL). Due to its northernmost position, the sampling area is predominantly colonized by fresh-water vegetation, such as rushes, canes, grasses, shrubs and trees. Only PI3 (summer) had a typical low marsh vegetation, composed exclusively of *S. maritima* (Fig. 6.4). Foraminifera diversity is relatively high, ranging from 1.5 to 2.3, consisting almost exclusively of agglutinated forms (with A/C% ranging from 77.3 to 98.3%). Ni is relatively low (ranging from 279 to 912 individuals/20 cc) compared to Cluster I and II, where agglutinated fauna are also strongly represented (Fig. 6.5). *M. fusca* is the dominant species (9 to 67%, mean 24.4%) and associated with *Siphotrochammina* sp. (0 to 45%, mean 16%), *J. macrescens* (0.7 to 18%, mean 9.3%), *Haplophragmoides manilaensis* (0 to 31%, mean 7%) and *M. obliqua* (1.1 to 24%, mean 6%)

6. 5. 4. CM3 borehole environmental description

The first meter of sediment was discarded for microfauna analysis due to probable anthropogenic disturbance. The Paleozoic substratum was reached at a depth of 36 m. The 20 samples collected in this sequence contain microfauna in a relatively good state of preservation, with only 8 samples lacking foraminifera. In general, the upper half faunas were better preserved. Based on an analysis of sedimentological facies, Boski *et al*., (2002) distinguished 4 lithological units. Eight Foraminiferal Zones were defined using the binary splitting algorithm in Psimpoll (Fig. 6.6):

F Zone 1 (-36 to -31.7 m) – The first zone from CM3 corresponds to lithological Unit I, which consists of a gravelly layer comprised of 80% quartz and quartzite pebbles and cobbles, intermixed with 20% of greywacke and shale fragments. One sample for foraminifera was analyzed, at -32.70 m depth, and no tests were found.

F Zone 2 (-31.7 to -27.5 m) – Starting at the end of Unit I, the second zone is mostly included in the lowermost level of Unit II, which consists of dark grey clay, rich in organic matter and containing plant detritus and bioclasts. Only 2 samples were analyzed in this interval, both containing only *Trochammina* spp. inner linings, with relatively high faunal densities (mean value of 3880 individuals/20 cc of sediment). The environment was interpreted as typical of middle-high marsh, resembling the Cluster I in the modern analogue model. It marks the early stages of the Holocene marine transgression recorded at around -30.6 m depth, prior to 10 738 cal yr BP (the nearest available radiocarbon date at -26.9 m).

F Zone 3 (-27.5 to -22.7 m) – Included in the middle part of Unit II, this zone was barren of foraminifera, having only a few *Trochammina* spp. inner linings (16-80).

F Zone 4 (-22.7 to -19.6 m) – The fourth zone includes only 2 samples, which appear in the middle-uppermost part of Unit II. It is very similar to F Zone 2 in terms of microfauna content, with both samples containing only *Trochammina* spp. inner linings, and relatively high faunal densities (mean value of 3832 individuals/20 cc of sediment). Together with F Zone 2 and 3, F Zone 4 seems to indicate a pre-invasion phase where restricted environments were constant along *ca* 10 m of sedimentary sequence, suggesting that, during this period, accretion kept pace with sea-level rise.

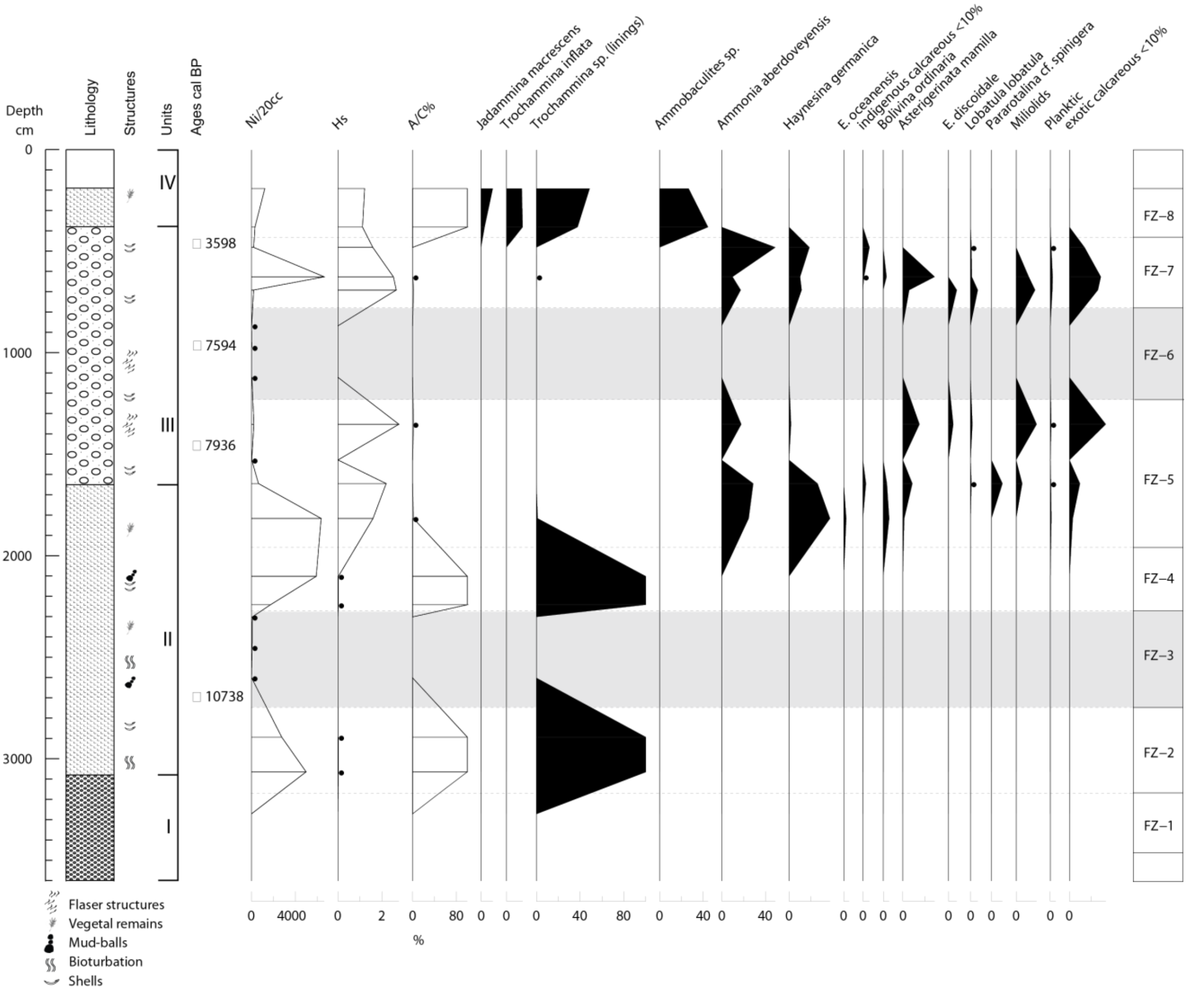

Figure 6.6. Stratigraphical representation of CM3 borehole; from left to right: depth (cm), lithology (after Troels-Smith, 1955), sedimentary structures, ^{14}C ages (cal yr BP), lithological units (Boski et al., 2002), Ni, Hs and A/C% (white areas), taxa relative abundances (black areas) and foraminiferal zones (grey: barren of foraminifera).

F Zone 5 (-19.6 to -12.3 m) – The fifth zone includes 4 samples from the upper part of Unit II and the first 4 meters of Unit III. Unit III sediments range from dark medium sand to fine sand with flaser structures and occasional gravel layers of a few centimeters thickness. Compared with the previous F zones, this sub-sequence has a very strong oceanic signal, characterized by relatively high diversity (mean 2.2), relatively low Ni (mean 1817 individuals/20 cc) and very low A/C% values (mean 1.8%). The first sample of this sequence (-18.1 m) resembles the most oceanic environment found in the modern assemblages (Cluster IIb), where *A. aberdoveyensis* and *H. germanica* dominate in association with other calcareous species, such as *E. oceanensis*, *B. ordinaria* and a low abundance of exotic calcareous species. The second and fourth samples (-16.4 to -13.5 m, separated by a sample barren of foraminifera) have a more oceanic nature, conferred by a decrease in the *A. aberdoveyensis/H. germanica* association, and an increase in the diversity of exotic species. Among these, it is important to note the presence of *Pararotalia* cf. *spinigera*, with 10% representation. This species is characteristic of coastal warm environments (Arieli et al., 2010) and is absent from the samples of Guadiana modern environments as well from other fossil samples.

F Zone 6 (-12.3 to -7.8 m) – This zone includes 3 samples barren of foraminifera in the middle part of Unit III. The few individuals (16 to 46/20 cc) belong to several calcareous species, including exotic species, resembling the Cluster III in the modern analogue model.

F Zone 7 (-7.8 to -4.3 m) – The seventh zone includes 3 samples from the upper levels of Unit III. Assemblage composition is very similar to F Zone 5, but with the opposite sequence. The first 2 samples have a more oceanic nature, similar to the last 2 samples from F Zone 5, and the last sample is richer in *A. aberdoveyensis* and *H. germanica*, with an increase in the indigenous calcareous species group.

F Zone 8 (-4.3 to -0.8 m) – The eighth zone includes the 2 most recent samples of the undisturbed part of Unit IV in the CM3 borehole, which takes in the top 3.8 m of black clay with plant remains. The assemblages are represented by only a few species (Hs 1.2) of agglutinated foraminifers (A/C% is 100%) at low densities (mean Ni 774 individuals/20 cc). The dominant taxa are *Trochammina* spp. (inner linings) (mean 43%) and the *Ammobaculites* sp. (mean 35.4%), associated with *T. inflata* (mean 14.4%) and *J. macrescens* (mean 7%). Changes in both lithology and microfauna in relation to the previous F Zone indicate a declining marine influence at the CM3 site, which, according to the radiocarbon chronology, occurred around 3500 cal yr BP.

6. 5. 5. CM5 borehole environmental description

This borehole was deeper than CM3 and more complete in terms of sampling frequency, with a mean of two samples per meter, providing an excellent record of the environmental evolution of the site in question since *ca* 13 000 cal yr BP (Fig. 6.7). Like CM3, the faunistic material was relatively well preserved, with only 30% of the samples having no statistical meaning. The upper half is best preserved, having high faunal densities (208 to 37 392 individuals/20 cc). The lower half also has relatively high densities (44 to 44 800 individuals/20 cc), although almost exclusively composed of inner linings of Trochamminiids. Boski et al. (2008) distinguished five lithological units in the sedimentary column overlying the Paleozoic substratum at 50.8 m depth. Based on the binary splitting performed by Psimpoll, 10 Foraminiferal Zones were distinguished (Fig. 6.7):

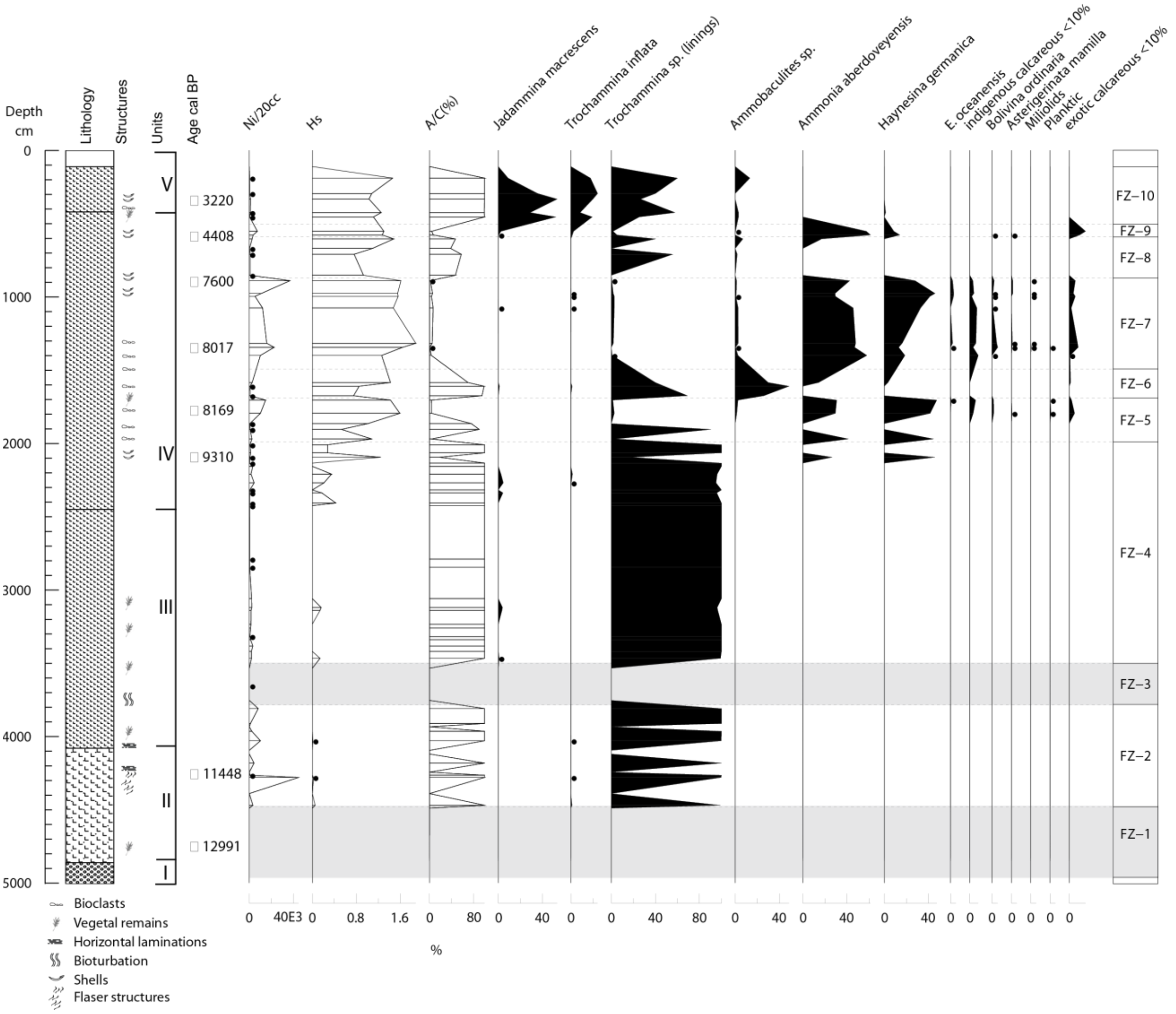

Figure 6.7. Stratigraphical representation of CM5 borehole; from the left to the right: depth (cm), lithology (after Troels-Smith, 1955), sedimentary structures, ^{14}C ages (cal yr BP), lithological units (Boski et al., 2008), Ni, Hs and A/C% (white areas), taxa relative abundances (black areas) and foraminiferal zones (grey: barren of foraminifera).

F Zone 1 (-48.5 to -44.8 m) – This includes 12 samples with no evidence of foraminifera in the first half of Unit II, which is a layer of silt with several intercalations of clay and sand. The unit below, Unit I, comprises two different layers: a layer of shale, greywacke and quartz gravel; and an upper layer of medium to coarse sand, containing abundant mica and sparse shale. The lithology, lack of foraminifera tests or other bioclasts, plus the available dating, seem to suggest a pre-Holocene fluvial environment.

F Zone 2 (-44.8 to -37.8 m) – The second zone includes 14 samples, 6 of which were barren of foraminifera and were intercalated with 8 samples dominated by *Trochammina* spp. inner linings, which in some cases had negligible percentages of *T. inflata* (Cluster I in modern analogue model). This zone occurs in the upper part of lithological Unit II and the beginning of Unit III. The upper part of Unit II contained 5 sandy layers with hydromuscovite, frequent flaser and bioturbation structures. At the beginning of Unit III the sediment is composed of fine, compact, dark-grey silt, containing layers enriched with plant remains. Foraminifera assemblages point to the presence of a middle to high-marsh environment – the first indication of marine influence at the CM5 site at -44.7 m depth, prior to 11 448 cal yr BP (the nearest available dating, at -42.7 m depth).

F Zone 3 (-37.8 to -35 m) – This zone, still included in Unit II, comprises 6 samples barren of foraminifers.

F Zone 4 (-35 to -19.9 m) - The fourth zone is the longest of CM5 borehole, including 23 monotonous samples composed by *Trochammina* spp. inner linings, in some cases associated with *T. inflata* and *J. macrescens* (both < 5%) (Cluster I). However, near the top of this zone, at -20.9 m, *Trochammina* spp. (inner linings) is still prominent (18%), but the dominant species are *A. aberdoveyensis* (26%) and *H. germanica* (46%) (Cluster IIb). This shift in the foraminifera assemblage is accompanied by the first appearance of bioclasts. This F Zone comprises the middle and upper part of Unit III and the beginning of Unit IV. The beginning of Unit IV is very similar to Unit III, with dark, fine and very compact silt with sporadic plant remains. The persistence of a strongly confined environment throughout such a long interval of time suggests a more stable phase of sea-level rise, keeping pace with accretion rates at the site.

F Zone 5 (-19.9 to -16.9 m) – The fifth zone includes 5 samples with different foraminifera assemblages and spans a layer of Unit IV rich in bioclasts. The zone starts (-19.7 m) with an assemblage rich in *A. aberdoveyensis* (41.3%), associated with *H. germanica* (44.7%) and a negligible percentage of *Trochammina* spp. (inner linings) (Cluster IIb). In the following sample (-19 m), *Trochammina* spp. (inner linings) are again dominant (90%) and the remaining specimens are corroded calcareous tests, which were impossible to identify (Cluster IIa). The sample at -18.6 m had no statistical significance, with only a few *Trochammina* spp., *Ammobaculites* sp. and corroded calcareous tests. In the two last samples of this zone, foraminifera composition changes again, this time to an assemblage dominated by *A. aberdoveyensis* (mean 30%) and *H. germanica* (mean 45%), associated with a series of other calcareous and agglutinated species (Cluster IIb). The alternating foraminifera assemblage suggests a transitional phase, from what could be a stable high-middle marsh environment to a low intertidal environment subject to higher marine influence. Based on the available chronological data, it seems that this phase started around 9300 cal yr BP and lasted at least 1140 years.

F Zone 6 (-16.9 to -14.9 m) – This zone falls within Unit IV and includes 3 samples of low diversity (mean Hs 1) and relatively low density (mean Ni 1416 individuals/20 cc). The assemblages are dominated by a few agglutinated species (mean A/C% 88.2%), of which *Trochammina* spp. (40 to 69.2%, mean 52.4%) and *Ammobaculites* sp. (26.2 to 49%, mean 35%) are dominant. *A. aberdoveyensis* (14%) and *H. germanica* (3.3%) are also present in the last sample of this zone (Cluster IIa).

F Zone 7 (-14.9 to -8.7 m) – This zone comes from the upper half of Unit IV and includes 7 samples that together represent the most oceanic period registered in the CM5 borehole. Diversity reaches its highest values in this interval (mean Hs 1.6) and the species are predominantly calcareous (mean A/C% 3.7%). Well preserved tests and high faunistic densities (mean Ni 16318 individuals/20 cc) suggest fast sedimentation rates for this period. The assemblages are dominated by *A. aberdoveyensis* (29.3 to 58.2%, mean 43%) and *H. germanica* (3.3 to 46%, mean 28%), associated with a series of secondary species.

F Zone 8 (-8.7 to -5.9 m) – The eighth zone encompasses the upper half of Unit IV and includes 4 samples, 2 of which were barren of foraminifera. Ni (mean 1190 individuals/20 cc) and Hs (mean 1.1) decrease in relation to the previous zone, and A/C% increases to median values (mean 47.6%). The assemblages are composed by *Trochammina* spp. (mean 48%) and *Ammobaculites* sp. (mean 4.4%). *A. aberdoveyensis* is also present in the last sample (-6 m), with an abundance of 17% (Cluster IIa). All samples in this interval contained a high percentage of partially dissolved calcareous tests (data in Table X, Appendix 4), suggesting extended exposure to subaerial conditions and/or low sedimentation rates. Indeed, this relative short sedimentary sequence (2.8 m) endures approximately 3200 years, based on the available dates.

F Zone 9 (-5.9 to -5 m) – The ninth zone, with 2 samples, appears in the upper meters of Unit IV. Again, a shift in the composition of the assemblages occurs, with a renewed increase in diversity (mean 1.3). *A. aberdoveyensis* becomes once more the dominant species (mean 60%), associated with *H. germanica* (11.5%) (Cluster IIb). The group of exotic calcareous species is well represented (mean 12%) and A/C% is low (mean 5.5%). This zone seems to suggest a short episode of renewed marine influence around 4400 cal yr BP.

F Zone 10 (-5 to -1.1 m) – This zone, although starting at the end of Unit IV, spans the entirety of Unit V, which is very similar to the previous one in terms of lithology, except for the reappearance of vegetal remains. Five samples are included in this zone, with only the uppermost sample being barren of foraminifera (-1.9 m). This last sample came from a layer with a mottled appearance due to redox reactions (Boski *et al*., 2008). The tenth zone indicates a very confined environment, through the dominance of the *Trochammina* spp. (25.4 to 60%, mean 42%) and association with other agglutinated species: *J. macrescens* (9 to 53.4%, mean 36%), *T. inflata* (8.4 to 24.35, mean 18%) and *Ammobaculites* sp. (0 to 13.3%, mean 4%) (Cluster I). The presence of calcareous species is negligible (mean A/C% 99.9%).

6. 6. Discussion

6. 6. 1. Taphonomic problems

Studies which involve the use of microfossils to interpret fossil sequences run the risk of failing to account for post-depositional changes. Reliable interpretations are generally made by using either modern dead or total (living + dead) assemblages for comparison (Horton and Murray, 2007). Although Murray (2000) advises the use of modern dead assemblages in the interpretation of paleoenvironments, he also accepts that the total and dead populations are often statistically identical when the living/total ratio is low (Murray, 1982). The use of the dead assemblage seems essential in cases where the study environment does not provide good preservation conditions for calcareous tests, such as brackish estuaries, where pH is usually so low that the foraminifera tests start to dissolve immediately after death (Alve and Murray, 1997; Hayward et al., 1999; Murray and Alve, 1999a; Scott, 1976b). In the Guadiana Estuary, where the total assemblage is approximately 3 times larger than the living and thus, the dead and total assemblages are very similar. In such cases, the total assemblage approach seems more attractive as it is less time consuming than analyzing the living and dead components separately (Scott et al., 1980b). Regardless of the analogue assemblage chosen to interpret the paleoassemblages, dead or total, there will be always *postmortem* changes that will affect preservation to varying degrees (Horton and Murray, 2007). Hence it is always essential to study the living assemblages in order to determine the pathways to fossilization (Murray and Alve, 1999b).

In the present work, all three assemblages (living, total and fossil) were compared in order to see whether the signal of the dominant species, which are the most important characteristic in the interpretation of any assemblage, kept their value in the three assemblages. The species *J. macrescens*, *M. fusca*, *T. inflata*, *A. aberdoveyensis*, *H. germanica* and the miliolids group, all of which were dominant in the living assemblage in both studied seasons, were also dominant in the total assemblage. Of the remaining species, only one rare species was recorded in the living dataset that was not found in the total assemblage, indicating that the total assemblage is a good representation of the indigenous fauna. The total assemblage also records some agglutinated dominant species that were not found alive, though their location and abundance prove them to be indigenous to the estuary. Their absence from the living fraction may be related to the seasons in which the sampling occurred, probably not being the ideal period for their reproduction.

One of the most important taphonomic processes affecting the transition of biocenosis to taphocenosis in superficial sediments is selective preservation, which especially affects calcareous species in low pH saltmarsh environments (Alve and Murray, 1997; Fatela et al., 2009; Moreno et al., 2005; Murray and Alve, 1999a). In environments where carbonate dissolution naturally occurs on a large scale, calcareous-dominated living assemblages become preserved as agglutinated dead assemblages (Murray and Alve, 1999a; Murray and Alve, 1999b), which could lead to erroneous interpretations of fossil material. The Guadiana boreholes, although showing some signs of severe dissolution in certain samples, especially in the northernmost borehole (CM5), were generally in a good state of preservation up to 20 m depth. All the prominent calcareous species in the living and total assemblages were present in the paleoassemblages, except *D. aguayoi*, which is believed to have a very low preservation potential (Camacho, unpublished data). The presence of large numbers of dead calcareous specimens suggests that these forms may be preserved in subsurface sediments of the Guadiana Estuary, in contrast to most other marsh deposits (Scott, 1976b). More than calcareous dissolution, differential disintegration of agglutinated tests seems to greatly affect the analogy between modern and fossil assemblages. A series of important agglutinated taxa which were found to be dominant in modern total assemblages of the Guadiana Estuary, such *H. manilaensis*, *M. obliqua*, *P. hyperhalina*, *P. ipohalina* and *Siphotrochammina* sp., were totally absent from the paleo-record. Additionally, *M. fusca* was only found as one specimen in the CM5-890 sample. On the other hand, taphonomic processes enhance the relative abundance of certain taxa within the paleoassemblages that are more resilient to these processes. This was the case of *Ammobaculites* sp., *J. macrescens* and *T. inflata*, the only three agglutinated species present in paleoassemblages that are also present in the modern assemblages as prominent species. None of the 20 remaining agglutinated species found in the modern assemblages were present in the paleorecord.

Goldstein et al. (1995) and Goldstein and Watkins (1998; 1999) studied the taphonomic processes acting in the salt marsh foraminifera from St. Catherine's Island, Georgia, USA, and found that although *M. fusca* was the most abundant marsh foraminifera in surface and shallow subsurface sediments, it was one of the most susceptible to *post-mortem* degradative processes. The tests of *M. fusca* have non-mineralized, organic cement and they appear to be degraded via one of two distinct processes: one leading to fragmentation (along sutures) and the other producing 'half-tests', both increasing dramatically down core (Goldstein and Watkins, 1999). The same authors also found that *J. macrescens* and *T. inflata* had taphonomically resistant tests and persist in subsurface and Holocene relict marsh-sediment. Indeed, in the Guadiana Estuary boreholes, Trochamminiids are the earliest foraminifera forms found at 44.7 m depth, approximately 11 500 years ago. However, especially in the older half of the sedimentary sequences, acid-resistant inner linings, sometimes with vestiges of the agglutinated original test, are the commonest forms, casting some doubts on the identification of the original species. Along with Trochamminiids, *Ammobaculites* sp. was also preserved in the Holocene sediments of the Guadiana Estuary, although only present in the more recent part of the sedimentary column. The absence of the uncoiled portion of tests of *Ammobaculites* genus meant that identification of the taxon could not be defined with greater taxonomic precision. The mixed test organization of *Ammobaculites*, with a planispiral and a tubular portion, are suited to an infaunal mode of life and a passive detrital or bacterial scavenging feeding habit (Jones and Charnock, 1985 in Wightman, 1990). According to Wightman (1990), *Ammobaculites* are opportunistic, flourishing in the absence of competition in environments that are subject to extreme daily or seasonal fluctuations in ecological factors, such as those normally observed in estuarine environments (e.g. salinity and temperature). When it occurs with *Trochammina*, *Ammobaculites* are indicative of marsh environments (Wightman, 1990). Other authors have related the *Ammobaculites* genus to the lower marsh zone, usually associated with *Ammotium salsum* (e.g. Debenay, 2000; Debenay et al., 2000; Patterson, 1990; Scott and Medioli, 1980). Goldstein and Watkins (1998) also found a species of *Ammobaculites* in low to high marsh settings and this seemed mainly associated with moist sites, regardless of elevation. However, these authors found *Ammobaculites* to be less likely to be preserved in older sediments. In the Guadiana's modern total assemblages, *Ammobaculites* sp. has its highest expression together with calcareous species in lower elevation samples exposed to the highest marine influence. Here the sediment is slightly sandy and usually unvegetated. Its presence along with *J. macrescens, T. inflata* and *Trochammina* spp. (inner linings) at the paleoassemblages of both boreholes could indicate one of two things: a different species from the one occurring in modern sediments, or; calcareous species were originally more abundant, but were erased from the paleo-record due to taphonomic processes.

The reworking and mixture of estuarine sediments is one of the most significant problems in microfossil-based palaeoecological studies (Salgado-Labouriau, 2007). In particular, the distinction between indigenous species that live, reproduce and remain in the original estuarine habitat (such as *A. aberdoveyensis* and *H. germanica*) and exotic species that live outside this habitat and are brought into the estuary due to hydrodynamic transport (such as planktonic forms) is of major importance (Cearreta et al. 2007; Wang et al., 1985). The higher the tidal range, the higher the transport and the sorting effect experienced by tests, causing thanatocenosis distinguished by small test size, high species diversity and low dominance (Wang et al., 1985).

In some microfossil-based palaeoecological studies, the exclusion of allochthonous taxa from modern assemblages has been tried, invariably increasing the number of fossil taxa without a modern analogue (Szkornik, 2007). This is also true for the Guadiana Estuary, suggesting that the allochthonous taxa should be considered in interpretation. Furthermore, if the allochthonous species are removed from the tanathocoenosis dataset, only a few species would be available for comparison (15 of 47, Table I). Instead of removing the allochthonous species, one can exploit their indicative meaning. As they are transported into the estuary by ocean currents, and thus are indicative of marine influence, their percentage in the fossil assemblage can be used as an indicator of oceanic influence of former sea-levels (Cearreta et al., 2007; Scott and Medioli, 1980b). In the Guadiana total assemblage, several taxa were considered allochthonous, and their presence, alone (*E. margaritaceum*, *E.* cf. *discoidale* and *L. lobatula*) or along with other taxa (planktic, exotic calcareous and exotic agglutinated), was interpreted as an indication of marine influence. The number of allochthonous taxa was higher in the thanatocenosis than its live counterpart because it represented many generations added over a long period of time, with some of those resulting from events of higher sea-level stands, presumably higher than today.

6. 6. 2. Baselines for paleoenvironmental interpretation

The application of hierarchical cluster analysis to the winter and summer total modern assemblages identified five different sub-environments (clusters) in the Guadiana Estuary, each characterized by specific species composition. Each sub-environment is related to the relationship of sediment to water levels and distance to the sea at the time of deposition. The indicator value of these assemblages hinges on the ecology of the dominant species, but other factors, such as diversity, test type dominance and exotic species contribution, are also important. Cluster I, IIa and IIb represent the main faunistic zonation promoted by daily tidal activity in the lower estuary. Cluster I is characterized by an almost monospecific assemblage of *J. macrescens* and represents the highest zone of the marsh, which is subject to significant periods of subaerial exposure. Cluster IIa is characterized by the presence of *J. macrescens* still as a dominant species, but associated with *T. inflata* and, to a lesser extent, to a few calcareous species. Here, the tidal inundation time is slightly higher than cluster I. Cluster IIb is characterized by the dominance of *A. aberdoveyensis*, which is associated with *H. germanica* in the lower reaches of the lower estuary and with *M. fusca* in the upper reaches of the lower estuary. Both associations are typical of lower to middle elevation samples and are subject to higher tidal inundation times. A similar sequence was also found by Haslett et al. (2001) in the Severn Estuary, UK, with three foraminiferal zones of decreasing marsh heights distinguished, related to decreasing subaerial exposure times. According to these authors, the alternation between these zones in a sedimentary sequence tells us how the marsh is evolving in relation to sea-level rise: accreting in quasi-equilibrium if a *J. macrescens–T. inflata* zone is found, submerging if an *Ammonia beccarii–H. germanica* zone is found, and emerging if a zone barren of foraminifera is found after a *J. macrescens–T. inflata* zone.

Cluster III had a more ambiguous character as it is mainly composed of miliolids and the indigenous species, *A. aberdoveyensis,* and associated with a series of exotic species: *L. lobatula*, *E. discoidale* and the group of the exotic calcareous species. The specimens are usually large, dull and sometimes broken, suggesting reworking, possibly from pre-existing older marine deposits. The mixture with some modern specimens may also suggest a taphocoenosis resulting from hydrodynamic sorting, which is a common process in tidal channels (Wang and Chappell, 2001), estuarine deltas (Salgado-Labouriau, 2007) and gravel banks on shelf areas (Scott et al., 1980b). In either case, the relict fauna seems to be strongly related to high energy environments with low sedimentation rates, which are often common in the lower elevation environments of estuaries.

Cluster IV is characterized by the dominance of *M. fusca* and associated with a series of other agglutinated fresh/brackish water preferred species: *Siphotrochammina* sp., *H. manilaensis* and *M. obliqua* (although *J. macrescens* is also a constant species). These indicate an environment with higher fluvial influence. Nevertheless, no direct analogies were found between this assemblage and any of the borehole paleoassemblages. As seen above, with the exception of *J. macrescens*, none of the agglutinated species occurring in Cluster IV were recorded in the paleo-record. The lack of fresh-/brackish-water assemblage in the paleo-record could also be related to the fact that both boreholes are located in the lower estuary and thus never experienced truly fluvial conditions.

6. 6. 3. Guadiana Estuary - paleoenvironmental interpretation

The recognition of several distinct foraminiferal assemblages in saltmarsh sediments and their relationship with sequential saltmarsh surfaces and sea level provides the foundation for the present paleoenvironmental interpretation.

To a certain extent, the different dating and sampling frequency of the CM3 and CM5 boreholes hampers direct comparison between the two sequences. The higher resolution of CM5 showed, not only the major faunal shifts also recognized in CM3, but also other minor shifts, including important stages suggested mainly by detailed pollen time-series (Issar, 2003). These stages were not hitherto identified in studies performed in Gulf of Cadiz area. Borehole CM5 exhibited a low diversity fauna with fewer exotic species, which is in agreement with its more sheltered, northward location. On the other hand, CM3 exhibited a much more marine nature, having a greater percentage of calcareous exotic species, including *Pararotalia* cf. *spinigera*, which occurs in significant numbers at 16.5 m depth (*ca* 8000 cal BP), but are absent from the present day estuary. Despite the differences, it seems that the observed faunal shifts for both boreholes occur synchronously when the calibrated dates are considered (Fig. 6.8).

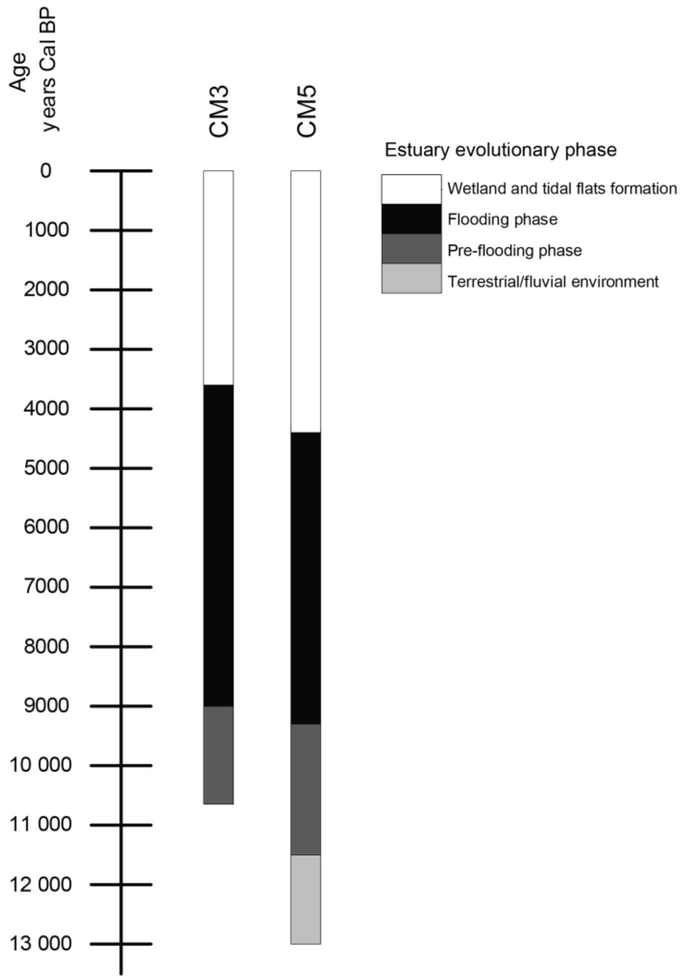

Figure 6.8. Simplified diagram of environmental evolution along the CM3 and CM5 boreholes according to calibrated ages.

6. 6. 3. 1. Pre-Holocene stage:

Over the Paleozoic substratum, reached at -36 m depth in CM3 and at -50.8 m depth in CM5, is a unit of gravel (Unit I in both boreholes) composed by shale, greywacke and quartz pebbles, remnants of a fluvial regime prevailing during past low-stands (Boski et al., 2002; 2008). Prior to the first evidence of Holocene marine transgression in the Guadiana Estuary, the deepest zones of the river valley, in which CM5 borehole is located, started silting up with intercalated layers of silt, clay and sand, probably resulting from the alternation between different hydrodynamic periods driven by seasonality. This siltation phase starts before 13 000 cal yr BP and included the brief cold phase of the Younger Dryas (*ca* 12 900 – 11 650 cal yr BP; Smith et al., 2011). The phase ends prior to 11 500 cal yr BP, when the first evidence of Holocene transgression is recorded. According to Dias et al. (2000), the sea level during this period was 40 to 60 m lower than at present. The global cooling, lack of rain (Pirazzoli, 1991) and sea retreat promoted by the Younger Dryas (Dias et al. 2000), along with the lack of foraminifera or any other bioclasts in the sedimentary column, suggests that continental processes were strengthened and these deposits have a fluvial/terrestrial origin.

6. 6. 3. 2. Beginning of estuary formation – pre-flooding phase:

The first signs of marine influence are recorded at -44.7 m depth, with the presence of Trochamminiids inner linings and *T. inflata* species. In CM3 there is no record of a pre-transgression silting phase and the paleovalley starts to be infilled with transgressional sediments, although somewhat later (*ca* 11 000 cal yr BP) and shallower (*ca* 30.6 m depth) than at CM5 due to its location in a less pronounced valley. According to Dabrio et al. (2000), who presented a regional synthesis of postglacial infilling in the Gulf of Cadiz based on material from the Tinto-Odiel and Guadalete estuaries, the first marine evidence of transgressive origin occurred at -30 m depth below the present mean sea-level in the Guadalete and -22 and -30 m in the Odiel and Tinto, respectively. These differences reflect essentially topographic irregularities along the rivers' thalwegs (Dabrio et al., 2000).

The beginning of marine sedimentation is somehow unstable, with intercalating phases of marsh accretion in quasi-equilibrium and marsh emergence (Haslett et al., 2001) – the first occurring when the marsh formation kept pace with sea-level rise and the marsh was continuously occupied by *Trochammina* spp. (inner linings) and *T. inflata*; the second occurring when accretion and, consequently, elevation, outpaced sea-level rise, promoting the progressive decrease of tidal inundation and consequent collapse of the resistant, but tidally dependent, agglutinated foraminifera forms. This unstable sequence continues during *ca* 0.66 m and ends in a zone barren of foraminifera of *ca* 0.22 m (6 continuous samples). This could be a longer period of marsh emergence, probably promoted by the occurrence of a colder phase. No dates are available at or near the barren zone, which makes it difficult to link this phase to any previously reported cold period.

After the barren zone, the marsh resumes its rapid growth in quasi-equilibrium with sea-level rise, proven by the constant presence of *Trochammina* spp., sometimes associated with *J. macrescens* and *T. inflata,* through 14.6 m of sediment until a little later than 9300 cal yr BP (*ca* 20 m depth), when another faunal shift takes place. This agrees with Dias et al. (2000), who determined a sea level of -20 m for this period. The pre-submergence phase is recorded in three foraminiferal zones (2, 3 and 4) and is included in the first phase of estuary formation proposed by Boski et al. (2008). This phase is characterized by accelerated sea-level rise, while the estuary was infilled with clay sediments at a rate of 7 mm yr^{-1} in the period between 13 000 to 7500 cal yr BP (approximately 11 000 to 6500 ^{14}C yr BP). CM3 borehole also recorded the foraminiferal zones 2, 3 and 4, although with a temporal and spatial lag in relation to CM5.

6. 6. 3. 3. Holocene transgression:

Lower transgression – maximum flooding stage: Approximately at the same depth in CM5 and CM3 (21 m and 18 m depth, respectively), the foraminifera assemblages indicate a new phase, where the replacement of marsh species by estuarine species is observed. The changing composition of the species association of *A. aberdoveyensis* and *H. germanica* indicates fluctuations in marine influence. This faunal shift suggests that sea-level rise outpaced the accretion rate (submergence phase: Haslett et al., 2001), creating the most oceanic conditions in the Guadiana Estuary for the whole Holocene. The first signs of faunal shift are observed in the end of F Zone 4, at approximately 9300 cal yr BP (*ca* 8200 ^{14}C yr BP) and are expressed by the presence of *A. aberdoveyensis* and *H. germanica*, associated with *Trochammina* spp. (inner linings) and abundant calcareous tests in dissolution. The presence of other bioclasts is also noted for the first time. This shift is coincident with the 8200 BP cooling event recorded in the Northern Europe during the Early-Mid Holocene (Davis and Stevenson, 2007 and references therein). Records from the Greenland ice cores indicate that a cold event occurred around 8.2 cal ka BP, constituting the most significant climate excursion of the past 10 000 years (Clarke et al., 2004). In Southern Europe, this event has not been widely observed; instead, arid conditions have been reported (Davis and Stevenson, 2007; Fletcher et al., 2007; Reed et al., 2001). Aridity best explains the faunistic shift observed in the Guadiana Estuary at this time, which suggests the beginning of a submergence phase. The water cooling resulting from the major discharge of Lake Agassiz (Clarke et al., 2004; Smith et al., 2011), probably reduced evaporation from the ocean and therefore decreased orographic rainfall in Iberia. Aridity could also explain the occurrence of dissolved calcareous tests, by reducing runoff and preventing the arrival of sediment at the estuary, thus promoting low sedimentation rates favorable to poor test preservation (Bertram and Cowen, 1998).

From 9300 to *ca* 8000 cal yr BP (-21 to -14 m depth) the estuary surface was still rising fast, between shorter submergence and accretion phases, suggesting brief oscillations of sea-level rise and retreat that could be related to the short-lived aridity events reported by Fletcher (2007).

Based on the data from the most complete sequence, the CM5 borehole, the maximum Holocene flooding of the Guadiana Estuary took place between *ca* 8000 to 7600 cal yr BP (*ca* 7600 to *ca* 6700 ^{14}C yr BP). During this interval, an open estuarine environment prevails, characterized by the dominance of *A. aberdoveyensis* and *H. germanica* estuarine species, associated not only with indigenous species (as occurs in the modern marsh at CM5 location), but also with exotic calcareous species. Dabrio et al., (2000), Boski et al., (2002) and Lario et al., (2002) suggest a date of 6500 ^{14}C yr BP for maximum flooding in the Guadiana Estuary during the Holocene.

In the CM3 borehole, the few samples available do not record the same oscillating phase recorded in CM5 before maximum flooding and its estuarine fauna has much more marine influence, including not only calcareous indigenous species like *A. aberdoveyensis*, *H. germanica*, *E. oceanensis*, *A. mamilla* and *B. ordinaria*, but also a significant number of exotic species in significant abundances. The group of exotic species are noteworthy (reaching > 33%), along with the species *P.* cf. *spinigera* (reaching 10%) and the Planktic group that, despite low abundance (<2%), is constantly present during the time of maximum flooding. None of these values are observed in the modern estuary; *P.* cf. *spinigera* is not even reported from the estuary. *Pararotalia* is a marine genus (Hayward et al., 2012), indicative of marine influence and water circulation in estuaries (Debenay et al., 2000; 2001b) and is associated with warm waters (Arieli et al., 2010). During a marine transgression, the newly created habitats are quickly occupied by species characteristic of the new environmental setting. When a high sea-level is followed by a regression, the marine species vacate the space, which is then colonized by their terrestrial coevals (Buzas and Culver, 1994).

During the period of maximum flooding in CM5, in CM3 there was only a repetitive record of relict fauna, with only a few reworked calcareous tests. The sediment is medium to fine sand, with flaser structures and occasional centimeter-scale gravel layers, which, combined with the relict fauna, suggests a subtidal environment with enhanced hydrodynamics. In open bays, the transport of foraminifera tests in bedload and suspension is more frequent or continuous and occurs at a larger scale than in the intertidal zone (Diz, 2009). Cearreta et al. (2007), in a study of Melides Lagoon, SW Portugal, found evidence for a similar high energy environment (Subzone 4a) in their most seaward borehole (MB). Although no dates are available for this Subzone, the similarity between depths (extended at MB probably due to the higher proximity to sea) and the same environmental trend between MB and CM3 boreholes suggests the same cause, although with different consequences. According to Cearreta et al. (2007), a coarse clastic aggrading barrier developed, isolating the lagoon, which was then inundated ephemerally. The strong submergence observed at the flooding maximum at the CM3 location, the presence of *P.* cf. *spinigera*, and the increase in the number of exotic species, even at the more protected, upstream location of CM5, suggests that the estuary was much more marine during the Holocene highstand than it is today.

Upper transgression – the chronozone of ca 4 ky BP?:

Around 7600 cal yr BP (*ca* 6700 ^{14}C yr BP), a new faunal shift is recorded. Boski et al. (2002; 2008), Dabrio et al. (2000) and Lario et al. (2002) suggest 6500 ^{14}C yr BP as the time when the rate of sea-level rise diminished considerably and the rate of sedimentation in the estuaries surpassed the rate of sea-level rise, initiating a phase of sand-spit development and predominantly sandy sedimentation within the estuary. This second phase of sea-level rise corresponds to F Zone 8 of the CM5 borehole (Fig. 6.7), which is interpreted as a transitional environment, where open estuary microfauna are replaced by confined marsh communities. This change is accompanied by high proportions of dissolved carbonate tests and inner linings. The corroded aspect of the tests could be attributed to the lower sedimentation rates verified for this period, promoting the degradation and abrasion of the calcareous tests due to longer exposure to the elements.

At 4400 cal yr BP (*ca* 4300 ^{14}C yr BP), a short but well defined episode of renewed marine influence is recorded in the CM5 borehole at 5 meters depth, which could be related to the 4 ky BP warmer period (Issar, 2003). The estuarine assemblage composed by *A. aberdoveyensis* and *H. germanica*, associated with the exotic calcareous group reappears, suggesting a new phase of submergence, when sea-level rises faster than marsh accretion. Issar (2003), who made a remarkable review on climate changes during the Holocene in Europe and, in particular, the Iberian Peninsula, concluded, supported in various pollen time-series studies, that there was indeed a severe change to a warmer climate, resulting in dryness, at 4 ky BP, at least in the southeastern part of the Iberian Peninsula. This change was global, as it coincided with a high sea level (Issar, 2003). According to the author, evidence for a key horizon at 4 ky BP are so strong and so generalized in both Iberian Peninsula and Levant regions that he suggests its recognition as a chronozone in the Holocene.

Immediately after maximum flooding, there is a shallowing at the CM3 site, evolving from a subtidal environment, where only relict fauna was found, to a low elevation intertidal environment with the dominance of *A. aberdoveyensis*, *H. germanica* and *A. mamilla*, associated with the Miliolids and exotic calcareous foraminifera. The site's environmental evolution suggests a decrease in eustatic sea-level rise. However, there was always some degree of inundation, which probably blurred the boundary between the lower and upper transgressions recorded in CM5. The strong marine influence felt at the CM3 location since the beginning of the transgressive phase is only reduced when the last faunal shift occurs, around 3500 cal yr BP.

6. 6. 3. 4. Wetland and tidal flat formation:

Based on the CM3 and CM5 records, the last major paleoecological shift occurred around 3500 cal yr BP, when a estuarine low-marsh fauna is replaced by a middle-high marsh fauna, colonized exclusively by the agglutinated forms *J. macrescens*, *T. inflata*, *Ammobaculites* sp. and *Trochammina* spp. inner linings. The continuous indication of middle-high marsh environments until the surface of the boreholes suggests that the phase of accretion in quasi-equilibrium with sea-level remained constant since *ca* 3500 cal yr BP. During this phase, non eustatic factors drove the evolution of nearshore morphology (Dias et al., 2000), with lateral progradation dominating in relation to vertical accretion, causing accelerated expansion of tidal flats and rapid growth of sandy barriers (Dabrio et al., 2000; Kirby and Plater, 2006). The coastline reached a position close to present one approximately 200 years ago (Morales, 1997).

6. 7. Conclusions

The present work follows on from the work of Boski et al. (2002; 2008), who proposed the first chronological approach to post-glacial marine transgression in the Guadiana Estuary. This was based on sedimentary features, ^{14}C dates and faunal analysis (mollusc and fossil foraminifera) of 5 boreholes drilled in the estuary. Of those, the CM3 and CM5 boreholes were revisited and interpreted at the light of a modern analogue approach. The total foraminifera assemblage (living + dead individuals) was chosen as baseline in the interpretation of the paleoassemblages as it combines seasonal and taphonomic effects, both relevant to the interpretation of the paleo-record. A comparison between living, total and fossil foraminifera assemblages proved that the total assemblage is a good representation of the indigenous fauna of the Guadiana Estuary and that the inclusion of exotic species in the study provides important supplementary information in the interpretation of paleoassemblages.

Carbonate dissolution is recognized as an important taphonomic process acting during the transition from biocenosis to taphocenosis in estuarine sediments. The dissolution of calcareous tests was observed in the paleoassemblages, especially when low sedimentation rates were associated with the moment of deposition. However, in the Guadiana’s Holocene sediments, differential disintegration of agglutinated tests seems to be a major problem when drawing analogies between modern and fossil assemblages. Important dominant agglutinated species in the modern Guadiana Estuary, such *H. manilaensis*, *M. fusca*, *M. obliqua*, *P. hyperhalina*, *P. ipohalina* and *Siphotrochammina* sp., were totally absent from the paleo-record. Trochamminiids and *Ammobaculites* sp. proved to be the most resilient species to taphonomic degradation. *Trochammina inflata*, along with the acid-resistant inner linings of *Trochammina* spp., were the earliest foraminifera forms found in the Holocene sediments accumulated in the estuary.

Based on the modern total assemblage analysis, five different sub-environments were identified, each characterized by specific species composition. Each of the 5 groups indicate the relationship of sediment to water level and distance to the sea at the time of deposition: 1) *J. macrescens* and 2) *J. macrescens* and *T. inflata*, both representing the most elevated stands of the saltmarsh at lower estuary, indicating an emergent environment, where accretion follows sea-level rise; 3) *A. aberdoveyensis* and *H. germanica*, representing the lower elevation stands of the lower estuary (saltmarsh and tidal flats) and indicating a submergent environment where sea-level rise surpasses accretion rates; 4) Miliolids, *A. aberdoveyensis* and exotic calcareous species, representing high energy environments, with strong marine influence; and 5) *M. fusca* and other agglutinated species representing fresh/brackish environments. This last environment was not recorded in the paleoenvironments of the Guadiana and this may be related to the low preservation potential of these species, or to the fact that both boreholes are located in the lower estuary and thus never experienced exclusively fluvial conditions.

Some major differences were observed between the two boreholes: some related to sampling effort, with CM5 having higher sampling frequency and a more ^{14}C dates available, and others related to the physiography of borehole locations, with CM5 having a sheltered, northward position in a much deeper zone of the river thalweg than CM3. Despite the differences, it seems that the major faunal shifts for both boreholes occur synchronously, although a time lag is observed, such that all the shifts are first recorded at CM5.

The analysis of foraminifera assemblage shifts along the CM3 and CM5 sedimentary sequences, complemented by sedimentological and chronological data, enabled the identification of four distinct phases in the evolution of the Guadiana Estuary since *ca* 13 000 cal yr BP:

Pre-Holocene phase: This phase starts prior to 13 000 cal yr BP and comprises the cold climate event of the Younger Dryas, and is characterized by the silting up of the estuary promoted mainly by dominant continental processes, with no foraminifera or other traces of marine life;

Pre-flooding phase: The first signs of marine influence are recorded prior to 11 500 cal yr BP, at -44.7 m depth, marked by the presence of Trochamminiids inner linings and *T. inflata* species. The pre-flooding sequence has an unstable evolution, characterized by alternating phases of marsh accretion, in equilibrium with rapid sea-level rise (*Trochammina* spp., *T. inflata* and *J. macrescens*), and marsh emergence, where marsh accretion surpasses sea-level rise (Trochamminiids to barren zones).

Holocene transgression: Based on the foraminiferal faunal shifts, two transgressional phases are distinguished: the *lower transgression*, which occurs between *ca* 9300 and 7600 cal yr BP (*ca* 8200 to *ca* 6700 ^{14}C yr BP), and the *upper transgression*, which occurs around 4400 cal yr BP (*ca* 4300 ^{14}C yr BP). The *lower transgression* corresponds to the most oceanic conditions in the Guadiana Estuary for the whole of the Holocene, when sea-level rise outpaces accretion rates, indicated by the replacement of middle-high marsh species by estuarine species. The estuary's maximum flooding occurs between *ca* 8000 and 7600 cal yr BP (*ca* 7600 to *ca* 6700 ^{14}C yr BP). The most oceanic conditions are recorded in the southern borehole, where, along with *A. aberdoveyensis, H. germanica, A. mamilla* and miliolids, a great abundance of exotic species (> 33%) and the constant presence of planktic forms are recorded. During this phase, optimal conditions were created for the proliferation of the marine warm-water species, *Pararotalia* cf. *spinigera*, which occurred in significant abundance (10%). At the peak of transgression, the environment was subtidal, subject to strong hydrodynamic conditions, as suggested by the presence of reworked foraminifera. Evidence from the Holocene highstand suggests that the estuary was subject to higher marine influence and warmer conditions than it is today.

The *upper transgression* corresponds to a short but well defined episode of renewed marine influence at approximately 4400 cal yr BP (*ca* 4300 ^{14}C yr BP), after a phase of sea-level deceleration when sand-spit accretion and sandy sedimentation occurred in the estuary. *A. aberdoveyensis, H. germanica* and exotic calcareous species replace the marsh accretion assemblage (*Trochammina* spp., *Ammobaculites* sp. and some calcareous tests in dissolution), suggesting a new phase of submergence, with sea-level rising faster than marsh accretion. This stage, apparently not reported in other studies from the Gulf of Cadiz zone, could be related to the warm climatic period reported for the southeastern part of the Iberian Peninsula around 4 ky BP (Issar, 2003).

Wetland and tidal flat formation: From 3500 cal yr BP onwards, only a monotonous sequence of middle-high marsh fauna (*J. macrescens*, *T. inflata*, *Ammobaculites* sp. and *Trochammina* spp. inner linings) is recorded, suggesting that the environment kept accreting in quasi-equilibrium with sea-level rise. This conclusion is in accordance with the accelerated expansion of tidal flats and rapid growth of sandy barriers suggested by other authors (e.g. Dabrio et al., 2000; Dias et al., 2000).

Chapter 7

Thecamoebians and their seasonal and spatial patterns in the intertidal margins of Guadiana Estuary

Chapter 7

7 – Thecamoebians and their seasonal and spatial patterns on the intertidal margins of the Guadiana Estuary

7. 1. Chapter objectives

In Portugal, the group of organisms known as thecamoebians is almost unknown to science. To date, as far as it was possible to ascertain, no studies have been conducted, neither in freshwater nor brackish systems. Hence, the present chapter gathers pioneering data on their taxonomy, biocenotic and distributional trends in an estuarine system in Portugal.

The present chapter is a study of the seasonal and spatial distribution patterns of thecamoebians and two agglutinated tintinnid species in relation to hydrodynamics, in order to evaluate their potential as indicators of elevation, marine influence (distance to sea) and flooding events in the mesotidal Guadiana Estuary. To achieve this, a series of biocenotic indices and the living proportion of both groups were calculated and compared and related to major spatial environmental variables. Thecamoebian and tintinnid distributions were also related to major chemical and physical variables (see Chapter 4) and to foraminifera total assemblages (see Chapter 6) by means of multivariate analysis. The distribution of the three groups of organisms was compared in order to understand the deposition associations likely to be found in the paleorecord and how they can be interpreted in terms of ecology. Prior to the presentation and discussion of the results, the importance of thecamoebians as indicators of environmental change is introduced, along with a brief introduction to tintinnids, highlighting their importance in estuarine food-webs and how they can be important in paleoenvironmental reconstruction studies. Further details about thecamoebian and tintinnid biology and ecology can be found in the Chapter 9, the Atlas of the present thesis.

The aims of this chapter are therefore to present thecamoebians to the Portuguese scientific community, explore their potential as indicators of spatial and seasonal hydrodynamics and evaluate their usefulness as a tool, individually or complementarily, in paleoenvironmental reconstructions.

7. 2. Thecamoebians as indicators of environmental change

Thecamoebians, also known as arcellaceans, are testate amoebae (Protozoa) closely related to foraminifera and, like them, possess a hard test that, after death, can fossilize in the sediment (Scott et al., 2001). They are present in a wide range of moist and freshwater habitats, including moss, soil, peat, standing water and even sewage-treatment works. They are most commonly found in any moist situation where there are mosses, even occurring above ground level on the bark of trees and the roofs of buildings (Ogden and Hedley, 1980). In an unusual situation, thecamoebians proliferated in tap water in Faro when chlorine levels in the public network dropped significantly (Camacho, unpublished data). Thecamoebians also occur in low salinity areas of estuaries, either as transported or indigenous occurrences (Scott et al., 2001).

The main reason for studying the ecology of modern thecamoebian species assemblages is to build contemporary databases with which past environmental changes can be interpreted. Their distribution trends have been studied in peatlands (*Sphagnum* moss) (Bobrov et al., 1999), lakes (Asioli et al., 1996; Patterson et al., 1996; Scott and Medioli, 1983), streams (Holcová, 2007) and estuaries (Charman et al., 2002; 2010; Duleba and Debenay, 2003; Gehrels et al. 2001; Scott et al., 1991; 1995). Thecamoebian assemblages preserved in sediments have enabled the detection of flooding events (Holcová, 2007), eutrophication (Asioli et al., 1996; Medioli et al., 1987; Patterson et al., 1996; Scott and Medioli, 1983), heavy metal pollution (Asioli et al., 1996; Patterson et al., 1996) and past sea-levels (Charman et al., 2002; Gehrels et al. 2001). As they are almost invariably among the first colonizers of periglacial lakes formed immediately after glacial retreat, they are usually present at the bottom of lacustrine sequences in northern latitudes, where they record pristine conditions and, as such, provide an ideal baseline for the detection of subsequent environmental changes (Scott et al., 2001). The potential of thecamoebians as soil indicators has been useful in other unlikely areas, such as forensic investigation. Recently, in a re-opened murder case in Northern Ireland, testate amoebae were recovered from dried sediment residues on the clothes of the victim and helped to determine where exactly he was killed (Swindles and Ruffel, 2009).

Despite the great quantity of information obtained since the first studies (Cash *et al.*, 1915, 1919; Cash and Hopkinson, 1905, 1909; Leidy, 1879; Penard, 1902; all available in Siemensma, 2012), the thecamoebians are still a largely unknown group, with much-dispersed information and enormous taxonomic disagreement. The early studies described the habitat preferences of individual species qualitatively and only recently have quantitative values for each environmental parameter of importance been considered (Bobrov et al., 1999). Studies have shown that hydrological conditions exercise the strongest controls on the distribution of testate amoebae in peatlands (Bobrov et al., 1999), although water chemistry (especially pH levels), substrate (Patterson and Kumar, 2002) and nutrient status may also be important (Charman, 2001).

In freshwater sediments, where foraminiferal tests are not present, thecamoebian tests take their place. In transitional areas (e.g. estuaries), the two types of tests are found mixed together in varying proportions that have paleoecological significance. Studies of the combination of these two types of organisms allow the characterization and monitoring of all aquatic environments typically found in marginal marine settings (Scott et al., 2001). If the use of thecamoebians as environmental indicators in peat and lake sediments is relatively well established, and still being extensively developed and refined, in saltmarsh sediments (and in their application in reconstructing sea-level change) relatively little is known (Charman, 2001; Gehrels et al. 2001). Gehrels et al. (2001) studied the vertical distribution of foraminifera, testate amoebae and diatoms in UK saltmarshes to assess the use of multiproxy indicators in sea-level reconstructions. Although the vertical range of testate amoebae in saltmarshes is small – from the supratidal to the uppermost intertidal zone sediments – Gehrels et al. (2001) found that, statistically speaking, their precision in mean sea-level prediction is unrivalled.

However, there are still some problems of crucial importance to be solved. Fossil testate amoebae are present in a variety of sediments, but their numbers are much lower than in modern analogues assemblages. The effects of fossilization on tests are largely unknown and differential preservation is difficult to assess. Further studies on taphonomy are required and further work is needed to better concentrate the fossil tests for analysis, providing a workable technique for sea-level studies (Charman, 2001).

Like thecamoebians, studies of tintinnids in the Guadiana Estuary are lacking. Tintinnids are ciliate chromists, which possess a shell (or ‘lorica’) and constitute a ubiquitous component of the microzooplankton (Dolan, 2010; Gavriola and Dolan, 2003). Among microzooplakton, tintinnids are the most thoroughly investigated component, due to their role as grazers, in regenerating nutrients and as food for higher trophic levels (Verity, 1987 and references therein). The majority (98%) of tintinnid species are marine (Dolan et al., 2012) and cosmopolitan in the world oceans, but some have a more restricted distribution, with some species occurring in shallow, brackish-water environments like estuaries (Pierce and Turner, 1990). A few species are known from freshwater plankton, but no tintinnids are benthic (Dolan et al., 2012). The estuarine agglutinated forms, although living in the water column, show some dependency on the sediment bottom. During their life cycles they have to interact several times with the bottom sediment, in the collection and incorporation of mineral flakes into the structure of their loricae, in the release of resting cysts which sink into the sediment and excyst only when the conditions are favorable (Pierce and Turner, 1990) due to seasonal feeding strategies (e.g. aggregating near the bottom when the nanoplankton is less abundant) (Verity, 1987) and when they die and their empty loricae settle into the sediment. Among the living species, only these agglutinated forms seem capable of fossilization. The tintinnids with gelatinous or membranous loricae, which are the majority, do not seem to preserve in the sediment (Tappan and Loeblich, 1973). The forms that do fossilize constitute one of the most extensive and continuous fossil records for zooplanktonic organisms, dating back to the Ordovician period (Dolan et al., 2012), with their greatest diversity attained in the Late Jurassic and Early Cretaceous of the Tethyan region (Tappan and Loeblich, 1973). Foraminifera, thecamoebians and diatoms have been the preferred micro-proxies in estuarine Holocene paleoenvironmental studies and only rarely have a few tintinnids species been described alongside the other micro-proxies (Echols and Fowler, 1973; Scott et al., 1995; Scott et al., 2005).

7. 3. Results

Thecamoebians were obtained from the same superficial sediment samples as the foraminifera, covering exactly the same spatial gradients in the study area, but with the inclusion of the AL (Alcoutim) sample, which comes from the northernmost site and was collected only in summer. Although lacking foraminifera, this site was very rich in thecamoebians.

For the statistical analysis, all samples were used, except for Constancy, which only samples with more than 50 individuals (living + dead individuals) were used. Of the 24 samples collected in winter, 9 had less than 50 individuals: FB1, LEZ1, VRSA1, VRSA2, E1, E2, LG1, LG3 and LG3. Of the 26 samples collected in summer, 10 had less than 50 individuals: FB1, PI1, LEZ1, SCM, VRSA1, E1, E3, LG1, LG2 and LG3 (columns in grey in Tables XIV and XV, Appendix 4).

7. 3. 1. Faunistic density (Ni)

Thecamoebian and tintinnid faunistic density (Ni, individuals/20 cc sediment) was analyzed separately for all samples in winter and summer (Table XIV and XV, Appendix 4) Fig. 7.1. Ni variation was analyzed along a longitudinal profile, where only low elevation samples (-0.74 to

0.75 m in relation to MSL) from the river margin were considered (Fig. 7.2) and according to a sample grouping based on the visible environmental characteristics such as elevation, latitude and vegetation (Fig. 7.3). Four groups of samples were distinguished:

- Fluvial environments – comprising the samples collected in the middle and upper estuary, between 0 and 0.5 m above MSL, usually unvegetated or colonized by fluvial grasses, canes and rushes: AL (only at summer), LAR, FO and Aouro;
- Semi-enclosed ponds and high marsh – comprising the samples located in the lower estuary, between -0.1 and 1.96 m, usually densely colonized by diverse halophytic vegetation at the highest stands: FB1, PI1 (only at summer), LEZ1, SCM, VRSA1, E1, LG1, LG2 and LG3;
- Middle marsh – comprising the samples located at the middle levels of the marsh in the lower estuary, between 0.75 and 1.2 m, vegetated exclusively by *Spartina densiflora*: FB2, PI2, LEZ2 and VRSA2;
- Low marsh – comprising the samples located at the lowest levels of the marsh in the lower estuary, between -0.74 and 0.6 m, unvegetated or colonized by *Ruppia maritima* or *Spartina maritima*: FB3, PI3, PI4, LEZ3, VRSA3, VRSA4, EE, E2 and E3.

In winter, thecamoebian Ni (TeNi) varied from 0, at LEZ1 and VRSA1, to 10592 individuals/20 cc, at LAR. In summer, there were generally less thecamoebians (Fig. 7.1A), varying from 0, at SCM, VRSA1 and LG1, to 7272 individuals/20 cc, at AL. The opposite trend was verified for the tintinnids Ni (TiNi). In winter, there were less tintinnids, varying from 0 at LEZ1, VRSA1 and LG1, to 2245 at VRSA3, while in summer (Fig. 7.1B) TiNi varied from 0, at SCM and VRSA1, to 4893 individuals/20 cc at PI4.

In both seasons, but especially in winter, there was a clear decreasing trend in TeNi with decreasing latitude (i.e. toward the river mouth; Fig. 7.2A and B), corroborated by a relatively strong correlation between TeNi and latitude (r = 0.75 in winter and R = 0.73 in summer) (Table I). The opposite trend was observed for TiNi, which increases with decreasing latitude in both seasons, although these relationships were not significant (Table I), probably due to the decrease of TiNi in the 3 southern samples (Fig. 7.2C).

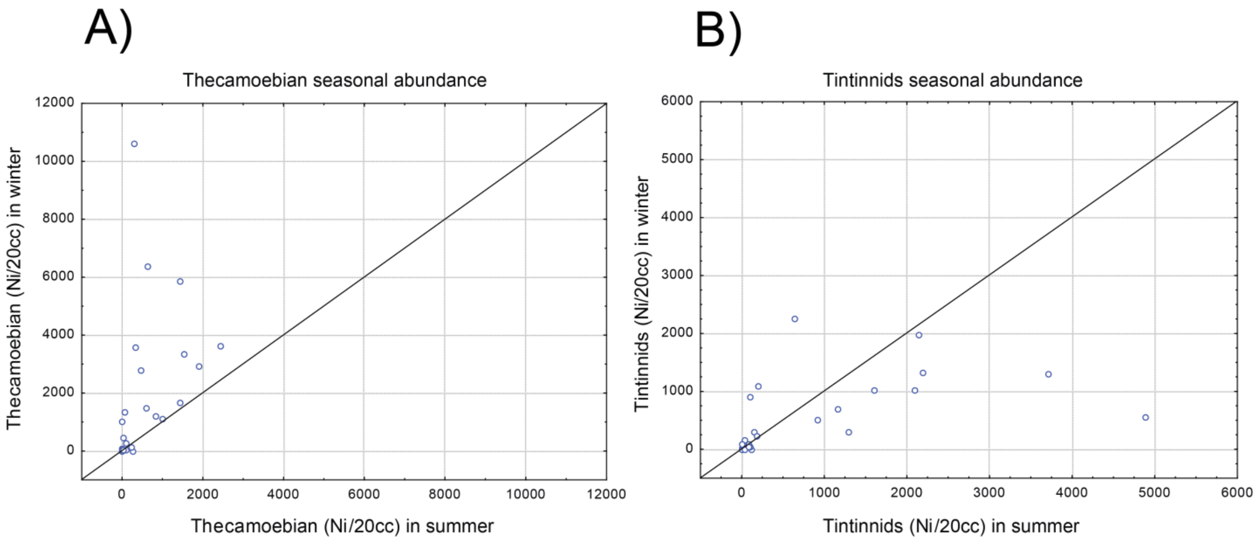

Figure 7.1. XY plots expressing the seasonal differences in thecamoebians/20 cc (A) and tintinnids/20 cc (B). Above the line, there are more individuals in winter; below the line there are more individuals in summer.

Regarding latitude, there was a positive relationship between TeNi and TiNi in both seasons, although it was stronger and only significant in summer (Table I).

Figure 7.2 also shows the Ni percentage that represents the living fraction for both thecamoebians and tintinnids, in winter and summer. In both seasons, TeNi% was very low, although higher and more constant in summer. In winter, living thecamoebians were found only in 3 samples (LAR, FB3 and PI4), never surpassing 2% of the total assemblage (Fig. 7.2A). In summer, living thecamoebians were found in all the marginal samples, varying between 0.56 to 9.5%, in FB3 and LAR, respectively. At LG3 no living individuals were found (Fig. 7.2B). In both seasons, the living assemblage exhibited different patterns from the total assemblage in north–south distribution, showing high peaks of abundance at specific sites, such as the mouth of Beliche rivulet (FB) in winter, and at the LAR, VRSA and E sites in summer.

In winter, there were no living tintinnids, contrasting with high TiNi% in summer, when they represent between 0 (LAR, Aouro, PI4, E3 and LG3) to 82% (FB2) of the total assemblage (Fig. 7.2C). It is worth noting that only the samples located near a freshwater source had living representatives (AL – 7%, FO – 16%, FB – 82%, EE – 50%), except VRSA, with 61% living tintinnids, with which no visible freshwater source is associated.

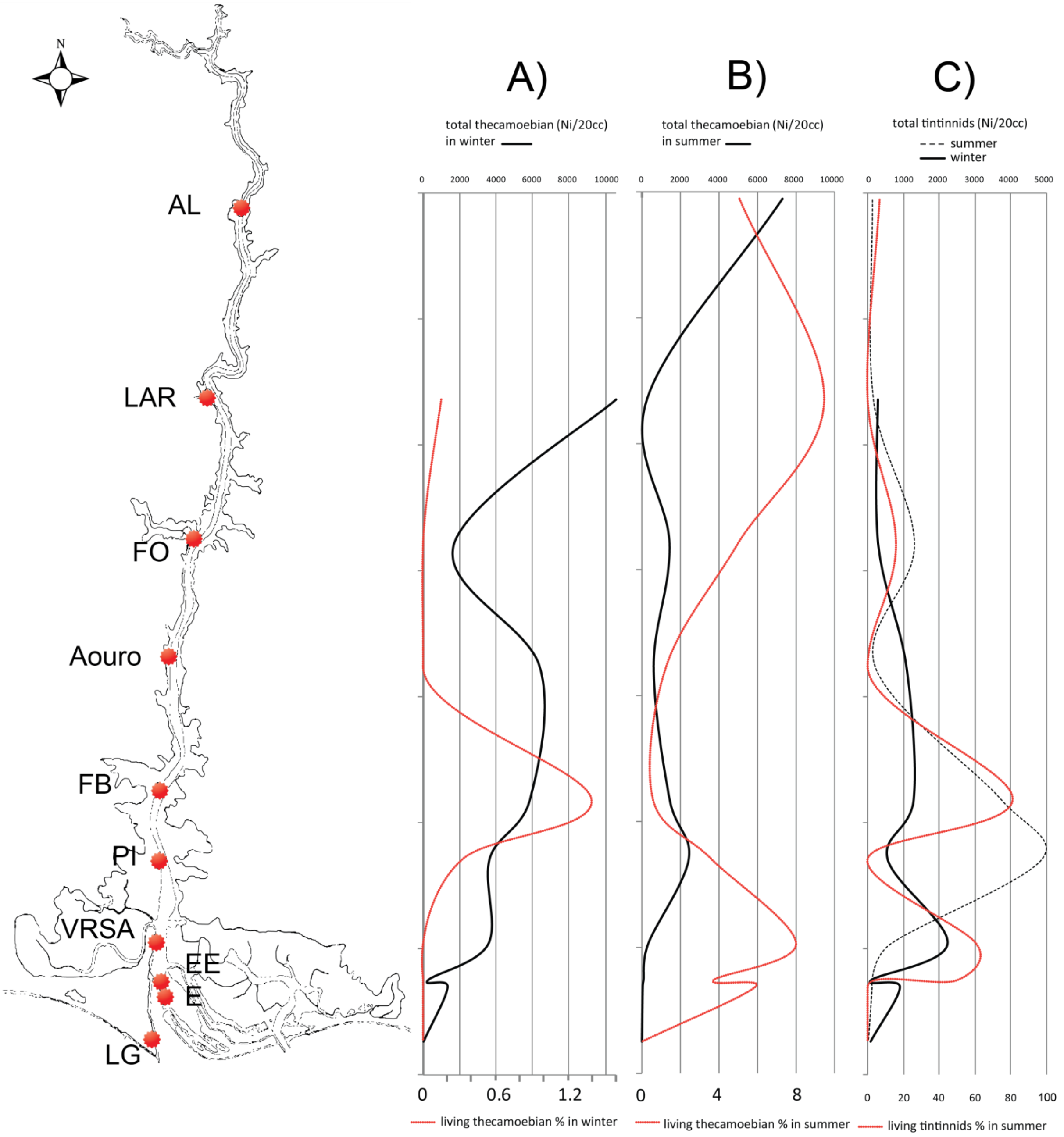

Figure 7.2. Variation of the number of individuals (living + dead) per 20 cc of sediment (Ni) along a north/south gradient, considering only the lowest elevation samples at the river margin; A) thecamoebians in winter; B) thecamoebians in summer; C) tintinnids in winter and summer. The curves in red represent the Ni percentage in living individuals.

Table I – Correlation between faunistic densities of thecamoebian (TeNi), tintinnids (TiNi) and latitude, considering only the river margin samples (-0.74 to 0.75 m in relation to MSL).

Correlation	**Spearman *R***	***P* - level**	**Pearson *r***	***p* - level**
TeNi winter *vs* latitude			**0.75**	**0.02**
TeNi summer *vs* latitude	**0.73**	**0.01**		
TiNi winter *vs* latitude			-0.16	0.67
TiNi summer *vs* latitude	0.4	0.24		
Thecas *vs* tintinnids winter			0.21	0.6
Thecas *vs* tintinnids summer	**0.71**	**0.02**		

According to the environmental grouping of samples (Fig. 7.3), it is possible to see two distribution patterns in the thecamoebians (Fig. 7.3A and B) in both seasons. The first is a latitudinal trend, previously observed, with more thecamoebians in the northern samples where the fluvial influence is higher. The second is an elevational trend observed in the 3 groups of samples of the lower estuary, with increasing abundance with decreasing elevation. It is also possible to observe two distribution patterns in the tintinnids (Fig. 7.3C and D) in both seasons, i.e. a latitudinal trend previously observed, with more tintinnids in the lower estuary than at the middle/upper estuary, and an elevational trend similar to the thecamoebians, with increasing tintinnid abundance from higher to lower elevations.

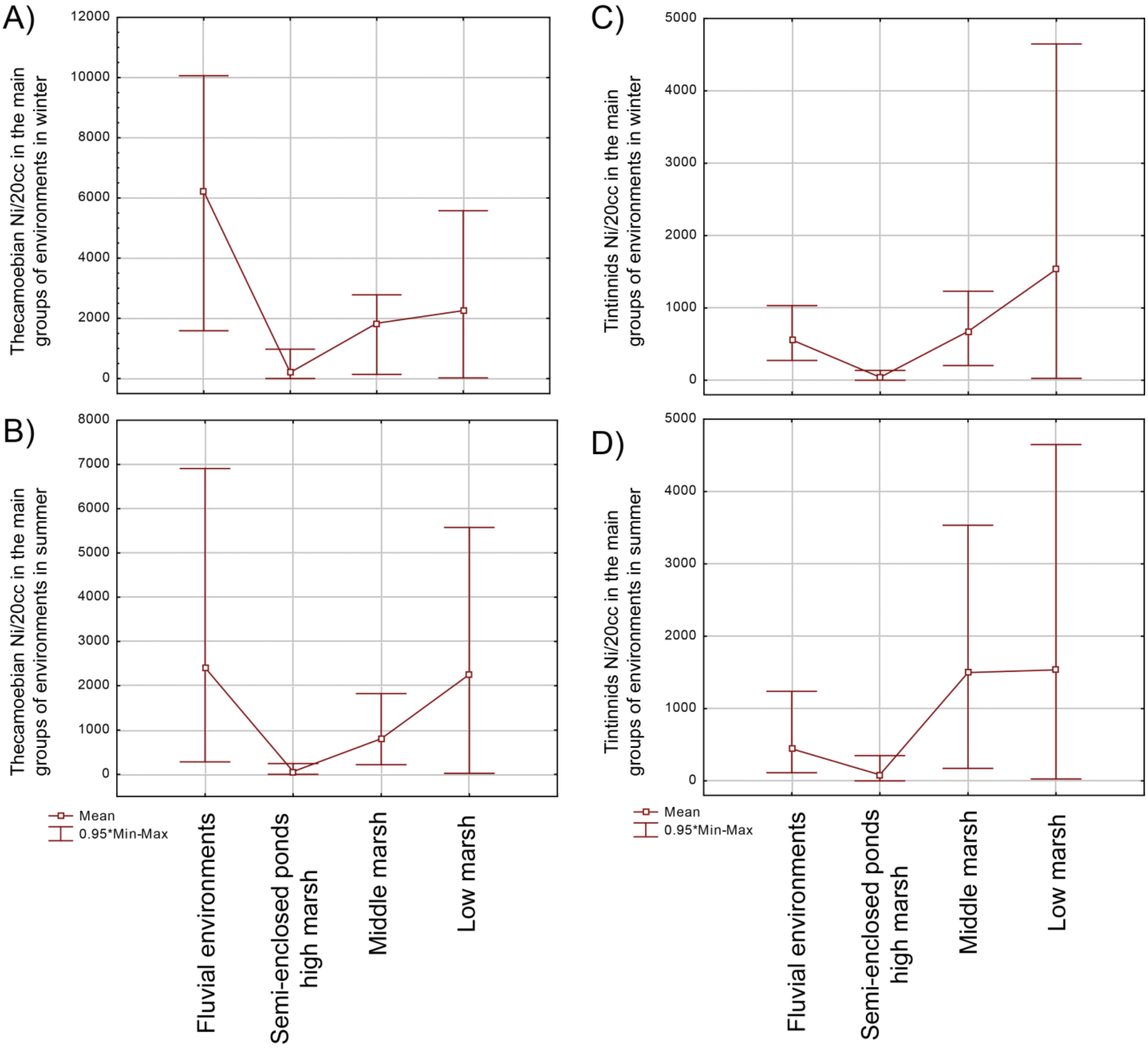

Figure 7.3. Total thecamoebians per 20 cc of sediment in winter (A) and summer (B) and total tintinnids per 20 cc of sediment in winter (C) and summer (D), in the four main environmental groups of samples – Fluvial environments: AL (only at summer), LAR, FO and Aouro; Semi-enclosed ponds/high marsh: FB1, PI1 (only at summer), LEZ1, SCM, VRSA1, E1, LG1, LG2 and LG3; Middle marsh: FB2, PI2, LEZ2 and VRSA2; Low marsh: FB3, PI3, PI4, LEZ3, VRSA3, VRSA4, EE, E2 and E3.

The elevational trend for both thecamoebians and tintinnids in both seasons was also confirmed by a negative correlation with elevation (Table II), although not significant, probably due to the inclusion of the samples of the northern stretch (Fluvial environments in Fig. 7.3), which exhibit a different trend from equivalent elevations in the lower estuary (middle marsh in Fig. 7.3).

When considering all samples, spanning both latitudinal and elevational gradients, TeNi and TiNi were strongly positively correlated (R = 0.78 in winter and R = 0.88 in summer, in Table II).

Table II - Correlation between faunistic densities of thecamoebians (TeNi), tintinnids (TiNi) and elevation (Elev MSL), considering all samples.

Correlation	**Spearman *R***	***P* - level**
TeNi winter *vs* Elev MSL	-0.07	0.75
TeNi summer *vs* Elev MSL	-0.15	0.45
TiNi winter *vs* Elev MSL	-0.22	0.30
TiNi summer *vs* Elev MSL	-0.32	0.10
Thecas *vs* tintinnids winter	**0.78**	**0.00**
Thecas *vs* tintinnids summer	**0.88**	**0.00**

7. 3. 2. Number of species (S)

Numbers of thecamoebian and tintinnid species (TeS and TiS, respectively) were analyzed in all winter and summer samples (Table XIV and XV, Appendix 4). TeS varied between 0 and 13, for both seasons and the TiS varied between 0 and 2, for both seasons. Generally, there was greater TeS in winter compared to summer (Fig. 7.4).

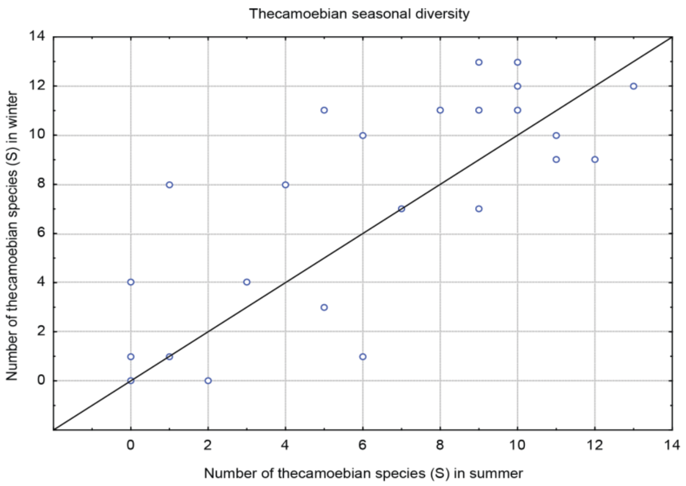

Figure 7.4. Scatter plot expressing the differences between the number of thecamoebian species in winter and summer at the same sampling points.

Figure 7.5. shows the summer and winter variation in TeS according to the grouping of samples with similar environmental characteristics. TeS shows a similar trend to TeNi, with higher values in fluvial and low-marsh environments, followed by middle marsh and semi-enclosed ponds/high-marsh environments in descending order. TeS varied from 9 and 13 species in fluvial environments, from 3 and 13 in low marsh, from 4 to 11 in middle marsh and from 0 to 8 in semi-enclosed ponds/high marsh.

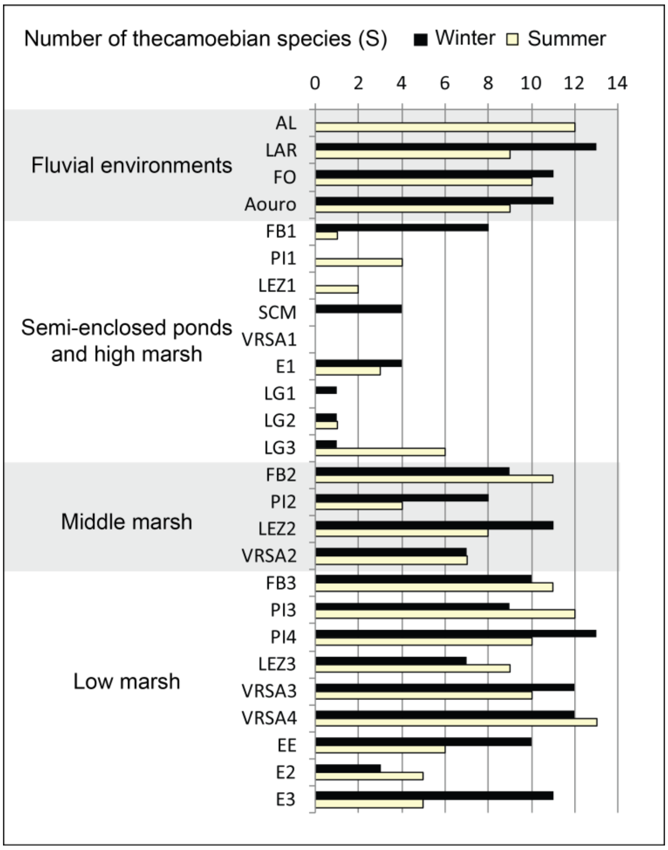

Figure 7.5. Variation of the number of thecamoebian species in winter and summer, in the four main environmental groups.

7. 3. 3. Species abundance, dominance and constancy

Relative abundances were calculated for all taxa in all samples of both seasons (see Table XIV and XV, Appendix 4).

In the Guadiana Estuary, a total of 17 species of thecamoebian were counted and identified. Although only species were used in the present work, an effort was made to appreciate the ecophenotypic variability within each species. We found that almost all species showed a large number of strains and, in some cases, more than 5 strains were recognized (Atlas, p. 78-81). The 17 identified thecamoebian species belong to at least 9 genera, 6 families, 2 sub-orders and 1 order (Table III). The sub-order Difflugiina was the best represented, comprising 82.4% of the total species identified. Of these, the Difflugidae family was the best represented in number of species (6 species), but the family Centropyxidae (excluding *Centropyxis orbicularis*, which was counted together with *Bullinularia* cf. *indica* due to identification issues) was the best represented in terms of faunistic density, comprising 5853 (71%) of the 8243 total individuals counted and identified.

Table III - Taxonomic context of the 17 thecamoebian and 2 tintinnids species occurring in the Guadiana Estuary (in **bold –** species only found in winter).

Group	Order	Suborder	Family	Genus	Species
Thecamoebians	Arcellinida	Arcellina	Arcellidae	*Arcella* *Pyxidicula*	*Arcella arenaria* *Arcella* sp. *Pyxidicula* sp.
		Difflugiina	Difflugidae	*Difflugia* *Pontigulasia*	*Difflugia bidens* *Difflugia corona* *Difflugia protaeiformis* *Difflugia oblonga* *Difflugia urceolata* *Pontigulasia compressa*
			Centropyxidae	*Centropyxis*	*Centropyxis aculeata* *Centropyxis arcula* *Centropyxis constricta* *Centropyxis orbicularis*
			Trignopyxidae	*Cyclopyxis*	***Cyclopyxis* sp.**
			Plagiopyxidae	*Bullinularia*	*Bullinularia* cf. *indica*
			Lesquereusiidae	*Lesquereusia*	***Lesquereusia* sp.**
			Nebelidae	*Nebela*	***Nebela* cf. *penardiana***
Tintinnids	Tintinnida	-	Codonellopsidae	*Stenosemella*	*Stenosemella ventricosa*
		-	Codonelidae	*Tintinnopsis*	*Tintinnopsis* cf. *lata*

Both the 2 identified species of tintinnids belong to the same order, Tintinnida (Table III). *Stenosemella ventricosa*, from the family Codonellopsidae, was the best represented, comprising 4472 (92.5%) of the total of 4834 individuals counted and identified.

Of the 17 thecamoebian and 2 tintinnid species identified in the Guadiana Estuary, only 4 were classified as dominants, representing more than 20% of the total individuals in at least one sample, namely, *Centropyxis aculeata*, *Centropyxis arcula* and *Stenosemella ventricosa* in winter and summer, plus *Centropyxis constricta* in summer. Three were classified as common species, representing 10–20% of the total individuals in at least one sample, namely, the group *C. orbicularis* + *B.* cf. *indica* in both seasons, *C. contricta* in winter and *Tintinnopsis* cf. *lata* in summer. *Arcella arenaria*, *Difflugia oblonga*, *Difflugia urceolata* and *Pyxidicula* sp. were accessory species (5-10%) in at least one sample in at least one season.

In winter, all species were constant (present in more than 50% of the samples), except *Arcella* sp., *Cyclopyxis* sp., *Difflugia bidens*, *Lesquereusia* sp., *Nebela* cf. *penardiana* and

Pontigulasia compressa. The most constant species were *C. aculeata*, *C. arcula*, *C. constricta* and *Tintinnopsis* sp., which were present in all the statistically valid samples. *A. arenaria* and *Difflugia corona* were the least constant species, being present in only 53% of the samples. In winter all species were constant except *Arcella* sp., *D. bidens*, *D. corona* and *P. compressa*. The most constant species were *C. orbicularis* + *B.* cf. *indica*, *C. arcula* and *Stenosemella* sp., present in all statistically valid samples. *Pyxidicula* sp. was the least constant species, being present in only 56% of the samples. Constancy values for the remaining species appear in Table XIV and XV, Appendix 4.

7. 3. 4. Environmental forcing and species associations

With the aim of inferring the main environmental drivers on thecamoebian and tintinnid distribution along the Guadiana Estuary, and how these species are related with foraminifera, species-environment relationships in the winter and summer datasets were analyzed by a constrained ordination method. The biological dataset used for foraminifera was the total assemblage (living + dead individuals) (Tables III and IV, Appendix 4). Several steps were taken to choose the most appropriate method:

7. 3. 4. 1. Species data heterogeneity estimation (gradient length): The gradient length was measured by Detrended Correspondence Analysis (DCA), detrending by segments, in square-root transformed data from all groups of species (total foraminifera, thecamoebians and tintinnids) for winter and summer. For the matrix including both seasons' data, the largest gradient was 2.9 SD, suggesting that the linear ordination method is the most appropriate for the analysis (Leps and Smilauer, 2003).

7. 3. 4. 2. Selection of environmental variables: In selecting environmental variables, two aspects were considered: removal of the superfluous variables and the addition of variables thought to be related to the most important determinants of the distribution of total assemblages (living + dead). The variables considered superfluous were those that showed colinearity with other most important variables (see Chapters 4 and 5). Thus, the variables considered for analysis were: Lat, Elev MSL, TOC, TIC, C/N, sand, pH_{sed}, Sal, DO and T.

7. 3. 4. 3. Redundancy analysis (RDA): The first two axes present a good correlation between species distribution and environmental parameters, r = 0.82 and 0.86, respectively. The first gradient was the longest one, explaining about 36% of the total species variability

(Table IV). The selected environmental variables explained 55% of the total variance of which 84% was explained on the first two axes. Variance inflation factors were low (< 6), indicating low shared variance among variables (Table IV). Both the test of significance on the first canonical axis and on all canonical axes using a Monte-Carlo Permutation Test were highly significant ($p = 0.002$ for both).

Table IV – CANOCO log-file results of the RDA with the selected environmental variables: Eigenvalues, correlation of axes 1 to 4 with environmental variables, correlation among environmental variables, variance inflation factors, marginal effects, conditional effects and significance of the environmental variables (*p*-value) with automatic selection. In red: the most significant results.

	Axis 1	Axis 2	Axis 3	Axis 4										
Eigenvalue	0.363	0.102	0.031	0.019										
species-environment correlation	0.819	0.859	0.639	0.604										
					Elev MSL	**Lat**	**TOC**	**TIC**	**C/N**	**sand**	**pHsed**	**Sal**	**DO**	**T**
Elev MSL	0.642	0.300	0.031	0.034	1									
Lat	-0.207	0.726	-0.120	-0.022	0.142	1								
TOC	0.538	-0.017	0.043	-0.284	0.393	-0.057	1							
TIC	0.382	-0.291	-0.227	-0.183	-0.065	-0.367	0.518	1						
C/N	0.127	0.276	-0.071	0.062	0.215	0.386	0.157	-0.176	1					
sand	-0.526	-0.392	-0.140	0.261	-0.766	-0.133	-0.536	-0.087	-0.103	1				
pHsed	-0.292	-0.038	-0.450	0.134	-0.269	0.067	-0.331	-0.030	0.031	0.415	1			
Sal	-0.100	-0.453	-0.108	-0.211	-0.152	-0.301	-0.054	-0.068	0.101	0.258	0.038	1		
DO	-0.357	0.272	-0.156	0.031	-0.393	0.330	-0.345	-0.307	0.246	0.378	0.340	0.263	1	
T	-0.408	0.009	-0.040	-0.104	-0.442	0.218	-0.237	-0.269	0.315	0.462	0.201	0.596	0.753	1
VIFs					3.313	2.092	2.434	2.013	1.515	3.470	1.401	2.730	3.043	5.660
Marginal effects (λ1)					0.240	0.100	0.160	0.100	0.020	0.180	0.060	0.040	0.080	0.100
Conditional effects (λA)					0.240	0.110	0.010	0.080	0.010	0.020	0.020	0.030	0.020	0.010
automatic selection *p* - value					0.002	0.002	0.592	0.006	0.382	0.088	0.146	0.084	0.236	0.232

When performing the RDA with automatic selection, Elev MSL, Lat, TOC and sand were the most important variables in explaining species distribution when considered alone (marginal effects in Table IV). When considered together (conditional effects), Elev MSL and Lat are still important in explaining species variance, but TOC and sand lose importance due to their causal relationship with other variables. Monte Carlo permutation test selects only Elev MSL, Lat and TIC as significant variables ($p < 0.05$) in explaining species variability (Table IV).

The ordination biplot (Fig. 7.6) shows that Elev MSL and Lat were the most important variables in explaining species distribution, with the first being positively correlated with the first axis and the second positively correlated with the second axis. Axis 1 separates the assemblages according to an elevational gradient, being positively correlated with Elev MSL and TOC, and to a lesser extent, to C/N and TIC contents. Associated with these environmental conditions was the typically high-marsh agglutinated species-assemblage, comprising *Jadammina macrescens, Trochammina inflata* and *Polysaccammina ipohalina*, and classified as assemblage I in Fig. 7.6.

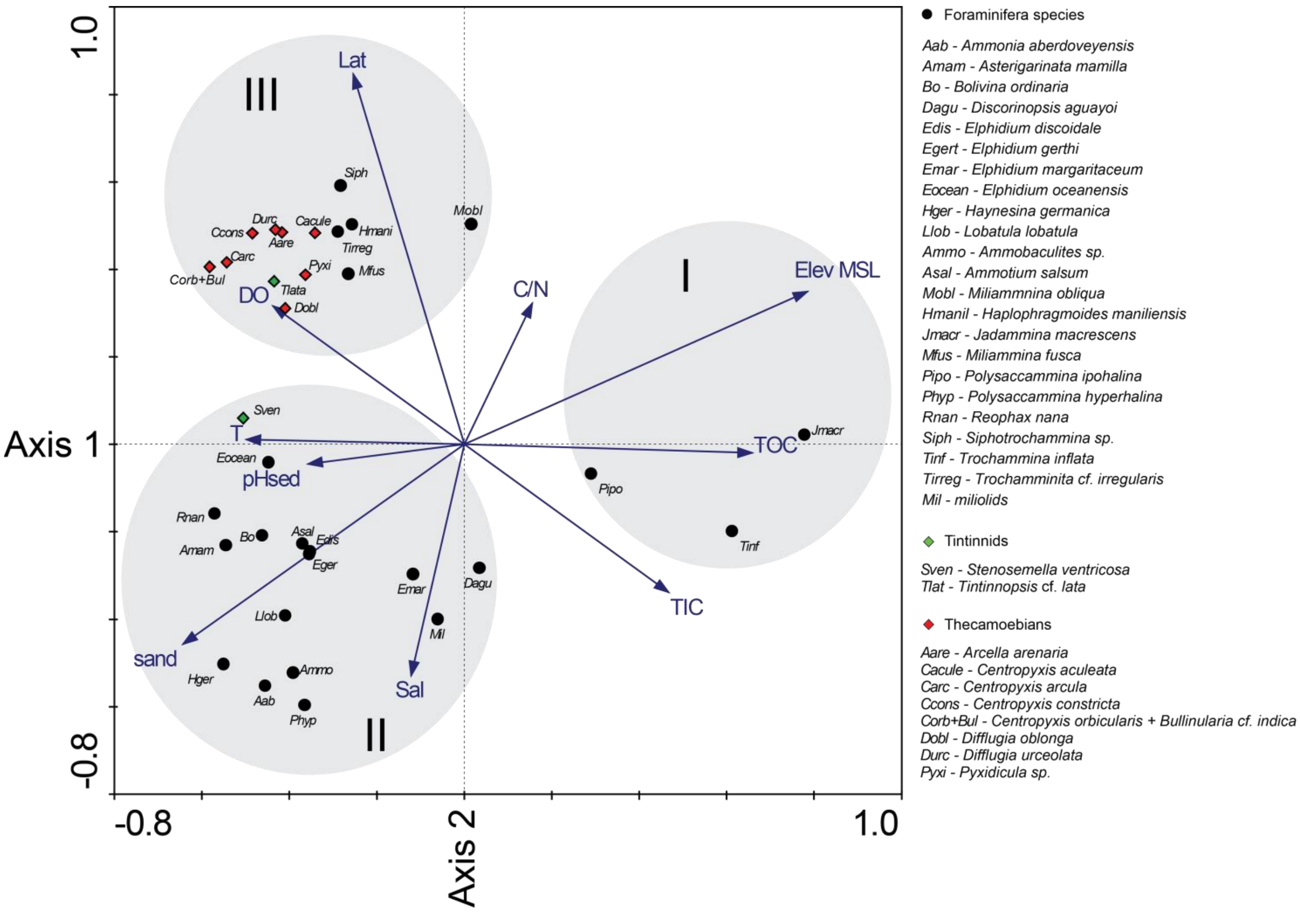

Figure 7.6. RDA biplot summarizing the effects of the main environmental variables in driving the distribution of thecamoebians (red diamonds), tintinnids (green diamonds) and foraminifera (black dots) in the Guadiana Estuary. All taxa are projected as symbols. Shaded areas represent the main distinguishable species assemblages: I – high marsh at lower estuary; II – lower marsh at lower estuary; and III – fresh/brackish environment assemblage.

Axis 1 is negatively correlated with sand and T, and, to lesser extent, to DO, Sal, pH_{sed} and Lat. Two assemblages were negatively correlated with axis 1: assemblage II, which was strongly correlated with Sal, sand, pH_{sed} and T and was comprised mainly of calcareous species (*Discorinopsis aguayoi*, Miliolids, *Elphidium margaritaceum*, *Ammonia aberdoveyensis*, *Haynesina germanica*, *Lobatula lobatula*, *Elphidium gerthi*, *Elphidium discoidale*, *Asterigerinata mamilla*, *Bolivina ordinaria* and *Elphidium oceanensis*), some coarse agglutinated species (*Ammobaculites* sp., *Polysaccammina hyperhalina*, *Ammotium salsum* and *Reophax nana*) and the tintinnid species *S. ventricosa*; and assemblage III, strongly correlated with Lat and DO and comprising all thecamoebian species, some agglutinated foraminifera (*Siphotrochammina* sp., *Haplophragmoides* sp., *Trochamminita* cf. *irregularis*, *Miliammina fusca* and *Miliammina obliqua*) and the tintinnid species, *Tintinnopsis* cf. *lata*. Axis 2 distributes the species according to a latitudinal gradient (distance to sea), thus separating the middle/upper estuarine assemblage III from assemblages I and II, both from the lower estuary and hence subject to higher marine influence.

7. 4. Discussion

7. 4. 1. Framework

If foraminifera had previously received little attention in the estuarine systems of Portugal, one can say that thecamoebians had been completely overlooked. As far as we know, this is the first time thecamoebians are described and analyzed in terms of taxonomy, abundance, diversity and distribution in an estuary. When the present study was initially conceived, thecamoebians were considered to be just a complementary proxy to foraminifera, since they live preferentially in freshwater sediments and on terrestrial macrophytes (Bonnet, 1974). Thus, the expectations of finding interesting data were low. The sampling effort targeted foraminiferal distribution and was thus limited to the intertidal zone along the Guadiana Estuary. Despite the fact that no samples were collected in the supratidal zone (where estuarine thecamoebians have been reported: Gehrels et al. 2001) and only a relatively coarse sediment fraction (63-500 µm) was analyzed (possibly missing smaller taxa: Charman, 2001; Roe et al., 2002), the data are very profitable, providing samples with more than 10 500 tests/20 cc of sediment. A total of 8243 individuals were identified, with a total of 17 species and at least 26 strains. Two species of tintinnids were misidentified as thecamoebians and are also included in the data set. Their great abundance (some samples with more than 4500 individuals/20 cc of sediment) and opposing seasonal and latitudinal trends to thecamoebians, led to their inclusion in this study. Both thecamoebians and tintinnids were sampled out of their preferential habitat (thecamoebians being freshwater/terrestrial organisms and tintinnids being planktonic) and their highest concentrations are observed in the lower elevation samples of the sampled intertidal zone. This suggests that the assemblages found could have an allochothonous origin. In the case of thecamoebians, transported by downstream discharges, being displaced from their natural habitats, both from the river and streams and from marginal terrestrial vegetation. In the case of tintinnids, they may have settled out of the water column, both from the brackish waters in the lower estuary or carried from the neritic zone by tides. As thecamoebians and tintinnids possess a hard shell, both were found in great quantities in the intertidal sediments of the estuary and because they have divergent origins, their taphocoenosis offers great potential as indicators of estuarine hydrodynamics in terms of elevation, marine influence and flooding events.

7. 4. 2. Spatial and temporal distribution

Thecamoebians live in all types of freshwater bodies and in a variety of moist environments. In estuaries, the majority of the tests are reworked from upstream environments and only a small fraction belong to species that are able to survive and thrive in slightly brackish water (Scott et al., 2001). In the Guadiana Estuary, a clear decreasing trend is observed in thecamoebian faunistic density with decreasing latitude (i.e., distance to sea) in both winter and summer. Regarding temporal variation, thecamoebian test densities are much higher in winter than in summer which could be related to the raised hydrometric level and increased current observed during winter (see Chapter 3). In winter, the communities from the river alluvial margins are more susceptible to being entrained by intense rainfall and flooding episodes and, along with species of freshwater lotic environments, are flushed downriver by the strong currents. Living thecamoebian percentages are very low in both seasons, which is in accordance with Scott et al. (2001), who state that only a few species are able to survive (especially through the encystment process), and only a smaller number of species are capable of reproducing in brackish environments. Nevertheless, there is a significant difference between, not only the living percentage in winter and summer, but also in the number of samples bearing living individuals. In winter, living individuals are found in only 3 samples with percentages never surpassing 2%. In summer, all marginal samples have living individuals, although never surpassing 9.5%. In both seasons, the living assemblage exhibit different patterns from the total assemblage in the north–south distribution, with high peaks of abundance at specific sites (i.e. the mouth of Beliche rivulet (FB) in winter, and at LAR, VRSA and E sites in summer). If, in the case of FB, AL and Lar sites, the higher concentration of living species could be a reflection of the freshwater influx of adjacent streams, in the case of VRSA and E, which are located near the river mouth, no major stream input can be associated. Nevertheless, in between the two sites is the outfall of the Ayamonte Sewage Treatment Plant (STP) (see Fig. 3.9 in Chapter 3 and Figs. 12-14, Appendix 1), with a permanent discharge of freshwater into the channel that could be positively influencing thecamoebian abundance. The present data seem to suggest that a permanent freshwater influx, combined with less hydrodynamic conditions during the summer, offer adequate conditions for a few species to live and maybe prosper, even in the lower reaches of the lower estuary.

Although some tintinnid species have been reported as exclusively from fresh to slightly brackish waters (e.g. *Tintinnopsis rioplatensis* in Scott et al., 2008; Wells and Daborn, 1997), the majority of tintinnids are pelagic, with few species being restricted to neritic-estuarine systems. This is the case with *Tintinnopsis* and *Stenosemella* (Pierce and Turner, 1990), the two genera occurring in the Guadiana Estuary. As primarily pelagic organisms, tintinnids should be dependent on salinity and the organisms' upriver intrusion would depend on the species' tolerance to freshwater. In the Guadiana, in what concerns the north–south gradient and seasonal abundance, tintinnids exhibit an opposing trend to thecamoebians, with increasing faunistic densities with decreasing distance to the sea, and a higher number of individuals/20 cc in summer. This trend is also observed for the 20 tintinnid species (and to ciliates in general) found in Nervión Estuary, Bay of Biscay, by Urrutxurtu et al. (2003). In contrast, Dollan and Gallegos (2001), found maximum tintinnid abundance and diversity in the mesohaline part of the Chesapeake Bay. Likewise, Sanders (1987), in Maine Estuary, USA, found that tintinnids' highest abundances were recorded in the most upriver samples of the studied transect where chlorophyll values were also higher, suggesting that tintinnid distribution was primarily controlled by food availability, rather than salinity. Sanders (1987) also found a strong correlation between tintinnid abundance and temperature, reporting the maximum occurrences in spring and summer. To the west of the Guadiana Estuary in the Ria Formosa, a mesotidal lagoonal system with a negligible freshwater contribution, Barbosa (2007) also found the highest tintinnid densities in the spring and late summer and a strong correlation with the presence of nanoplakton, especially the cryptophytes group. Hargraves (1981) and Verity (1987) also found similar seasonal patterns in tintinnid abundance in Narragansett Bay, Rhode Island. Here, tintinnid abundance was negatively correlated with blooms of the noxious flagellate, *Olisthodiscus luteus*.

In accordance with the highest tintinnid densities verified in summer, only in this season living individuals are recorded. In contrast to thecamoebians, tintinnid living percentages are relatively high (varying from 0 to 82% in the marginal samples along the estuary), with several specific occurrences, i.e. only the samples located near a freshwater source have living representatives (AL – 7%, FO – 16%, FB – 82%, EE – 50%), except VRSA with 61% of living tintinnids, with which no visible freshwater source is associated. AL, FO and FB are all located at, or near, the mouth of hydrologically important tributaries of the Guadiana, and site EE is located in the vicinity of Ayamonte STP. VRSA is located in between Beliche rivulet (FB) and the Ayamonte STP. In the Beliche rivulet, where the highest tintinnid abundances are recorded, a freshwater plume is clearly observed during summer (Fig. 39, Appendix 1). Tintinnids have been reported as tracers of suspended organic matter in the vicinity of aquaculture installations (Scott et al., 1995b) and in sewage outfalls (Scott et al., 2005), as well as indicators of freshwater plumes (Echols and Fowler, 1973). Studies on pelagic food chains have demonstrated the effect of resource availability, such that increasing nutrient concentrations bring about an increasing algal production and biomass, with a subsequent increase in bacteria, flagellate and ciliate abundance (Lansac-Tôha et al., 2004 and references therein). In the Guadiana Estuary, as far as it was possible to ascertain, there are no data on tintinnid distribution. However, there are abundant phytoplankton data (Rocha et al., 2002; Domingues et al., 2005; Domingues and Galvão, 2007; Barbosa et al., 2007; Domingues et al., 2012), indicating the same annual succession, with a diatom bloom in spring (usually March–April), followed by a mid-spring increase in the abundance of green algae (April–May), and a summer to early-autumn bloom of cyanobacteria. The highest tinnitid abundances, both total and living assemblages, in the Guadiana Estuary during summer could be associated with cyanobacteria blooms, as these organisms are on the varied menu of tintinnids (Sanders, 1987; Urrutxurtu, et al., 2003). However, cyanobacteria are not the preferred food source for large-sized tintinnids such as the two species found in the Guadiana, which are mainly phytophagous (Urrutxurtu, et al., 2003). Tintinnids feed essentially on nanoplankton, selecting different size categories of prey according to the oral diameter of their lorica (Pierce and Turner, 1992). Among the vast diet of tintinnids (see review in Pierce and Turner, 1992), microflagellates seem to be preferential prey (Hargraves, 1981; Verity, 1987; Barbosa, 2007, among others) and these are known to graze on cyanobacteria (Perez, et al., 1996). The great abundance of tintinnids observed in the Guadiana Estuary during summer could then be indirectly related to cyanobacteria blooms triggering nanoplankton productivity.

According to Dolan et al. (2012), nearshore tintinnids like *Tintinnopsis* and *Stenosemella* are restricted to relatively shallow and turbulent environments due to their dependence on small mineral particles used to form the lorica. These particles are gathered from suspension in the water column, rather than collected from the bottom. On the other hand, nearshore tintinnids have the capacity of encysting, in contrast to their oceanic counterparts (Pierce and Turner, 1993), which suggests that the bottom sediments exert some dependency on them. Furthermore, Verity (1987) observed that tintinnids aggregate near the bottom during periods of low nanoplankton chlorophyll *a* and may then utilize an alternative benthic food supply. In fact, on the surface of the intertidal margins of the Guadiana River, exposed during low tide, there were significant numbers of living tintinnids in summer. The question is whether they are entrapped involuntary in the sediment during the ebb or if they strategically remain within the estuary. Barbosa (2007) observed that *Tintinnopsis* cf. *rotundata*, the dominant species in the Ria Formosa, migrated to the bottom during night ebb, suggesting that this behavior could be a strategy for remaining within the system. This behavior was also observed for other ciliates elsewhere (Crawford and Purdie, 1992, in Barbosa, 2007). In terms of energy, it could be more efficient to remain within the estuary waiting for the new high tide than to be passively washed away by the ebb tide and to come back with the flood tide into the same place where food is potentially abundant.

In the lower reaches of the lower estuary (EE, E and LG), both total assemblages of thecamoebians and tintinnids decrease. In the southernmost site, LG, which is a small, shallow lagoon protected from direct sea/river influence by a jetty eastwards and by a sand spit southwards (Fig. 3.5, Chapter 3), the exchanges with the main channel waters are limited. This could limit the transport of thecamoebian tests and tintinnid loricae into the lagoon. At the Spanish sites (EE and E), the decrease in the abundance of both groups could be related to the proximity of the main channel mouth, where the hydrodynamics are stronger, with near-bottom currents reaching 90 cm/s during ebb spring tide (Lobo et al., 2004). Tintinnids are very small, ranging from 20 to 100 µm (Dolan, 2010) and possess an internal cavity. Thus, their bulk specific gravity is lower than that of a mineral grain of the same external diameter. They are similar hydrodynamically to silt-size detrital particles and therefore are readily retained in suspension (Echols and Fowler, 1973). The same characteristics seem to apply to thecamoebians. In the case of tintinnids, transportation of the loricae is further facilitated because they are exclusively planktonic, and the loricae are already in suspension when the organism dies (Echols and Fowler, 1973). Echols and Fowler (1973), in a study concerning the occurrence of agglutinated tintinnids on the continental shelves of Oregon and Washington, found that intense wave turbulence, typical of the nearshore zone, apparently prevents loricae from accumulating there. Instead, they are transported seaward to the central part of the shelf.

Presence of testate amoebae in fossil coastal sediments has previously been taken to indicate the transition between saline and freshwater conditions, but recent work suggests that the detail of the assemblage composition may yield more specific information than this (Charman, 2001). According to Gehrels (2001), within the upper marsh and supratidal areas of a marsh, the elevational zonation (i.e. relative to sea-level) is distinctive and strong. In the Guadiana Estuary, the distribution and abundance of thecamoebians and tintinnids is also evaluated in terms of elevation. In the lower estuary, where all 6 transects were made, there is a clear trend in decreasing abundance, both for thecamoebians and tintinnids, with increasing elevation in both seasons. The highest elevation samples have few or no tests or loricae. Also, although not always representing the highest elevation, all semi-enclosed ponds are impoverished in tests and loricae, suggesting that a kind of sorting in the deposition of these structures could occur due to intertidal confinement. These patterns also suggest that, for those points located farthest from river/sea influence, the contribution of terrestrial thecamoebians (supratidal environments, Gehrels, 2001) is negligible or absent. Gehrels et al. (2001) studied the vertical distribution of thecamoebians in UK saltmarshes to assess their use as indicators in sea-level reconstructions and found that they are present (< 20% living) from above the highest astronomical tide level down to a level where flooding duration is of 2% (i.e. a combined total of 7 days in a year). While the flooding duration approach was not attempted in this study, broadly speaking we can say that, from land to water, our transects started where Gehrels's ended, hence the thecamoebian distribution range found by Gehrels et al. (2001) did not cross with the one sampled in the present work. Also, Gehrels's most landward genera (*Trachleuglypha*, *Euglypha* and *Trinema*, all filose testate amoebae with siliceous shells) are not found among the Guadiana's assemblages. On the other hand, some of the most seaward occurring genera (e.g. *Centropyxis* and *Difflugia*) are recognized among the genera found in the Guadiana.

As previously documented, according to Echols and Fowler (1973), tintinnids loricae are similar hydrodynamically to silt-size detrital particles. Nevertheless, a closer look to the granulometric data (Chapter 4 and Tables III and IV, Appendix 6), shows that the percentage of silts has an opposing trend to that of thecamoebian and tintinnids in relation to elevation in the lower estuary (see also RDA biplot, Fig. 7.6). The highest percentages of silts are observed in the highest elevations, decreasing towards the lower elevations, where the abundances of thecamoebians and tintinnids are higher. This could mean two things: the processes controlling tintinnid and thecamoebian distribution are stochastic, and then they do not behave hydrodynamically like silts; or they could behave hydrodynamically like silts but then their distribution is not stochastic. The first hypothesis seems most applicable to thecamoebians, or at least to the majority of them, as few individuals are alive and the salinity is probably too high for their survival in the lower estuary, even during ebb tide. The second hypothesis seems the most probable for tintinnids, at least in summer, when the living percentages may be very high. Tintinnids could remain strategically at the lower levels of the marsh and tidal mud flats, where emersion is rather brief. In the intertidal flat of Schelde Estuary, Netherlands, Hamels et al. (2005) found a strong positive correlation of ciliate density and diversity with sandy sites rather than with silty sites. According to the authors, these occurrences are not related to food availability (higher in silty sites) nor to predator forcing (higher in sandy sites) but are rather related to the lack of large interstitial spaces in the silty sites, which can hamper ciliate movement and prevent the colonization of the sediment by ciliates.

7. 4. 3. Species abundance and community composition

In the Guadiana Estuary, a total of 17 species of thecamoebian are counted and identified, with 7 of these occurring at abundances < 5%. Although the following comparison is not completely fair – due to some disagreement over the degree of variability in morphology that should be allowed within taxa (for more details see Thecamoebian taxonomic remarks in the Atlas) and because different sieves have been used in the collection of specimens – in a general way there are less species in the Guadiana Estuary than in any peatland, lentic and lotic environment (between 18 to 89 taxa, based on data from: Asada and Warner, 2009; Asioli et al., 1996; Bobrov et al., 1999; Holcová, 2007; Lansac-Tôha et al., 2008; Mucio et al., 2010; Patterson and Kumar, 2000; Velho et al., 2000). However, the Guadiana's thecamoebian diversity is comparable to that of other estuarine systems (varying between 7 to 18 taxa, based on data from: Duleba and Debenay, 2003; Riveiros et al., 2007; Scott et al., 1991; Scott et al., 1995; Zucon and Loyola e Silva, 1993), except the case of Gehrels et al. (2001) and Roe et al., 2002, which extended the size range of the studied fractions and sampled beyond the high marsh, thus encountering more than 40 taxa. Thecamoebian species richness is usually lower in estuaries and coastal lagoons where salinity is a limiting factor (Lansac-Tôha et al., 2007). In a survey of species richness in the different habitats in Brazilian freshwater environments, Lansac-Tôha et al. (2007) recorded 346 infrageneric taxa. Of those, 282 taxa were recorded in freshwater plankton, 80 taxa in aquatic macrophytes, 81 taxa in sediment and 73 taxa in *Sphagnum*. Among the 17 species found in the Guadiana Estuary, it is possible to identify at least 26 different strains (see Atlas, Chapter 10), which suggests a great diversity of source sub-environments. We believe that, have the sieve smaller than 63 µm been used, the number of species and strains would have been higher (Charman et al., 2010; Mitchell et al., 2008; Roe et al., 2002).

Thecamoebian species numbers follows the same trend as density, reaching higher values in winter, when the river level is highest. This seasonal variation was also observed in flowing freshwater systems elsewhere (Costa et al., 2011; Holcová, 2007; Lansac-Tôha et al., 2009). Furthermore, the number of species varies the same way as faunistic density in the different environments, decreasing in the following order: fluvial, lower estuary low marsh, lower estuary middle marsh and lower estuary high marsh and semi-enclosed ponds, with mean number species of 11, 9, 8 and 2, respectively. According to Lansac-Tôha et al. (2009), periods of elevated hydrometrical levels favor faunal exchange among the environments and consequently, greater turnovers in local communities. Even so, in the Guadiana, the increase of both thecamoebian faunistic density and diversity in winter seems to be related to the same causal process, resulting from the accumulation of reworked tests during winter flows.

The number of tintinnid species is very low, with only 2 species identified. A third species *Tintinnopsis campanula*, is also seen, although its rare occurrence and premature identification in a phase when only foraminifera and thecamoebian were targeted, led to only its presence/absence being recorded. This very low diversity of tintinnid assemblages in the sediment samples is assumed to result from selective loss of species with small loricae during sieving, as few species have lorica lengths greater than 63 µm (Echols and Fowler, 1973). Echols and Fowler (1973), who used the same 63 µm mesh, only found three different agglutinated tintinnid species in the three estuarine systems they studied (two *Tintinnopsis* species and *Stenosemella ventricosa*, the latter being one of the two species found in Guadiana). In estuaries, when the appropriate sampling methods are used, is common to find more than 20 tintinnid species (Dolan and Gallegos, 2001; Hargraves, 1981; Sanders, 1987; Urrutxurtu et al., 2003; Verity, 1987). In the Ria Formosa Lagoon, the most abundant species was *Tintinnopsis* cf. *rotundata* (Barbosa, 2007) and in Óbidos Lagoon the species *Tintinnopsis minuta* was the most common (Silva, 1953), the latter having loricae smaller than 63 µm and the former varying between 45 and 90 µm in length (ICES, 1939-2001).

Both tintinnid genera found in the Guadiana Estuary, *Stenosemella* and *Tintinnopsis*, belong to the order Tintinnida and are described as neritic, or restricted to relatively shallow waters (Pierce and Turner, 1990). In estuaries, *Tintinnopsis* is usually the most abundant genus in terms of number of species (Hargraves, 1981; Sanders, 1987; Urrutxurtu et al., 2003; Verity, 1987), but *Stenosemella* is more generalist, being also present in neritic waters (Echols and Fowler, 1973). In the Guadiana Estuary, the identified and counted species were *Stenosemella ventricosa* and *Tintinnopsis* cf. *lata*. Both were recorded in Óbidos lagoon (Silva, 1953) and Barbosa (2007) recorded the presence of *Stenosemella* spp. and *Tintinnopsis* spp. in the Ria Formosa Lagoon. In the Guadiana Estuary, *S. ventricosa* is the most abundant species, representing 92.5% of the total tintinnids.

The most representative families of thecamoebians recorded in the present study are Centropyxidae, Difflugiidae and Arcellidae. Difflugiidae is the best represented in number of species (6), but the family Centropyxidae is the best represented in terms of faunistic density, comprising 71% of total individuals counted and identified. Both families have been reported in other estuaries (Duleba and Debenay, 2003; Gehrels et al., 2001; Scott et al., 1991; Scott et al., 1995; Scott *et al.*, 2001; Zucon and Loyola e Silva, 1993). In estuaries, the genus *Centropyxis* tends to dominate the thecamoebian assemblage (Scott et al., 2001). It is characterized as an opportunistic genus, adapted to a variety of unfavorable conditions, including low food supplies, low temperature, low salinities and contaminated water (Holcová, 2007).

At thecamoebians and tintinnids assemblages, only 4 species were dominant, namely *Centropyxis aculeata*, *Centropyxis arcula* and *S. ventricosa* in winter and summer, plus *Centropyxis constricta* in summer.

7. 4. 4. Environmental forcing in species distribution and paleoenvironmental application

Redundancy analysis (RDA) selects elevation, herein interpreted as tidal exposure time and latitude, interpreted as fluvial–marine gradient, as the two main environmental drivers in thecamoebian, tintinnid and foraminifera distribution along the Guadiana Estuary. These parameters had been considered among the main drivers in species distribution in paralic environments (Debenay et al., 2006; Duleba and Debenay, 2003; Mitchell et al., 2008; Scott and Medioli, 1980). Based on the gradients of these two environmental variables, RDA suggests a major distinction between three different environmental zones, each bearing a different faunal assemblage, i.e. the fresh/brackish water zone, the high marsh zone and the low marsh/mud flats zone. The fresh/brackish assemblage is composed by a high diversity of thecamoebians, including all the species found in the estuary, although dominated by the centropyxids, especially the species *C. aculeata*, *C. arcula* and *C. constricta*. Five agglutinated foraminifera species are strongly correlated with these environmental conditions, namely, *M. fusca*, *Siphotrochammina* sp., *H. manilaensis*, *T.* cf. *irregularis* and *M. obliqua*. The fresh/brackish assemblage is controlled mainly by latitude, as it is usually more common in the middle/upper estuary environments, where the input of freshwater is higher (salinity always < 1 during low tide, Chapter 4). Dissolved oxygen also shows a positive correlation with the fresh/brackish water assemblage, although it is most likely related to increased solubility in cold water during winter (see Chapter 4) than to a direct effect on the species assemblage. This is supported by the summer data, when the living percentages of thecamoebians are the highest and the oxygen content in water is the lowest (Chapter 4). *Miliammina fusca*, as seen previously, is a very common species in worldwide paralic environments, dominating the continental end-member, and associated with direct freshwater influence (see Debenay and Guilou, 2002 for review). In the foraminifera biocenosis survey (Chapter 5), it is one of the most ubiquitous agglutinated species, dominating environments in the upper reaches of the estuary. In other studies concerning thecamoebian and foraminifera distribution in estuaries, *M.*

fusca is usually a common component among the thecamoebians dominating transitional zones between fresh and marine environments. It may occur as the only foraminifera species or associated with a panoply of other freshwater taxa such as: *Haplophragmoides wilberti*, *H. manilaensis*, *Pseudothurammina limnetis*, *Jadammina macrescens*, *Balticammina pseudomacrescens*, etc. (Duleba and Debenay, 2003; Scott *et al*., 1991; Scott *et al*., 1996; Riveiros *et al*. 2007; Zucon and Loyola e Silva, 1993).

In the biocenosis, *M. obliqua* is a common species, with higher incidence in winter, at middle elevations of the northernmost positions and at some sites of the lower estuary, occupying higher elevations and more confined positions (Chapter 5). In the total assemblage, *M. obliqua* is a dominant species (Chapter 6). Likewise, *Siphotrochammina* sp., *H. manilaensis* and *T.* cf. *irregularis* are less prominent or even absent from the biocenosis, but dominant, or at least more prominent, in total assemblages. These three species are confined to the upper estuarine environments in both living and total assemblages, thus having a narrow distribution, not only spatially but also temporally. None of the agglutinated foraminifera species in the fresh/brackish assemblage are found preserved in Holocene sediments (Chapter 6), not even *M. fusca* with its more generalist distribution. This fact, allied to the poor preservation of thecamoebians in saltmarsh and coastal deposits (Charman, 2001; Roe, et al., 2002), constitutes a major challenge in the identification of fresh/brackish water paleoenvironments from multi-proxy assemblages.

According to Redundancy Analysis, elevation is negatively correlated with the fresh/brackish assemblage, which is mainly found at middle to lower stands of the intertidal margins. If this generalization is true for thecamoebians collected in the middle and lower estuary (where elevation is inferred through transects spanning -0.5 to 2 m above MSL), in the case of the upper reaches (LAR, FO and Aouro), this could not be the case, as samples are collected only once per site, ranging through a narrow elevation interval (0.3 to 0.53 m above MSL) due to the lack of marsh zonation at these sites. Based on data from Gehrels et al. (2001) and Riveiros et al. (2007), both of whom found the opposite elevational trend due to a different sampling strategies, and based on the fact that in upper estuary the land/fluvial processes are enhanced, one must consider the possibility of a turnover on thecamoebian elevational trend at the upper reaches of the estuary. However, according to Scott et al. (1980), thecamoebian distribution is more heavily influenced by the amount of freshwater influx than by elevation.

Tintinnopsis cf. *lata* was associated with thecamoebians and agglutinated freshwater-tolerant foraminifera species rather than with *S. ventricosa*, which in turn is associated with the lower-reaches, calcareous-dominated foraminifera assemblage. This relation seems to result, essentially, from the similar ubiquitous distribution pattern of *T.* cf. *lata* and some thecamoebians during winter. Like centropyxids, *T.* cf. *lata* had a constancy of 100% (present in all samples with statistical meaning). In winter, only empty lorica of *T.* cf. *lata* are found and, in contrast to *S. ventricosa*, there are more empty loricae in winter than in summer. This could be related to differences in hydrodynamic characteristics of loricae and life mode of the two species. Verity (1987) found persistent differences between the vertical distribution of *Stenosemella* spp. and *Tintinnopsis minuta*. *Stenosemella* spp. are most abundant in bottom samples and least abundant at the surface. *Tintinnopsis minuta* occur in the highest concentrations at the surface, with lower abundance at mid-depth and bottom. In the Guadiana, empty *S. ventricosa* loricae may sink directly into the sediment, in the location where the species is living, while *T.* cf. *lata* could have a more planktonic nature; when the organism dies the lorica, in suspension, takes more time to sink and may be more easily displaced by currents. In summer, both *T.* cf. *lata* and *S. ventricosa* have a similar distribution pattern, in both living and total assemblages. The high abundance of both species in the living component of the Beliche Rivulet mouth (FB site) is noteworthy.

The low marsh/mud flats assemblage is strongly associated with proximity to the ocean, where salinity is the highest and more constant, as well as lower times of tidal exposure and sandy sediments, impoverished in organic matter. The assemblage is mainly composed of foraminifera calcareous species, dominated by *A. aberdoveyensis* and *H. germanica*. A few coarse agglutinated species are also present, although only *P. hyperhalina* is dominant. In this assemblage, it is important to note the slight distancing of *D. aguayoi*, Miliolids and *E. margaritaceum*, which are strongly correlated with salinity and slightly higher elevations found in the protected small lagoon at the southernmost site (LG). On the other extreme of the assemblage is the association of *E. oceanensis* and the tintinnid *S. ventricosa*. Although temperature and pH_{sed} are not significant ($p < 0.05$) environmental variables in explaining species distribution overall, these variables are strongly correlated with *E. oceanensis* and *S. ventricosa*. Both species have their maximum abundances in summer, at the upper reaches of the lower estuary (slightly upstream of the other species of the main assemblage), especially near freshwater inflows. Temperature has been identified as one of the main environmental factors in triggering tintinnids growing rates in estuaries. However, this does not imply that temperature directly causes an increase in tintinnid abundance, as other factors also influence the intrinsic rate of tintinnid growth, such as daylight, food supply and predator abundance, and these also increase in summer (Hargraves, 1981; Pierce and Turner, 1992; Sanders, 1987; Verity, 1987). *Elphidium oceanensis*, as seen previously, is generally associated with warmer, brackish waters (Debenay et al., 2003; Fatela and Silva, 1990; Hayward et al., 1997), being, along with *A. aberdoveyensis*, the calcareous species most able to migrate farther up the marsh and farther upstream in estuary during summer (Chapter 5). If these assumptions are correct, it could be assumed that high abundances of *S. ventricosa* with calcareous foraminifera species, *E. oceanensis*, could be indicative of warmer climatic periods.

Although not clearly highlighted by the redundancy analysis, a high abundance of thecamoebians, mainly empty tests, may occur in the lower elevation stands of the lower estuary, associated with calcareous foraminifera species during winter. As this association is not expected in living assemblages due to the low salinity tolerance of thecamoebians, if found in the fossil record, it should be interpreted as evidence of high-water periods or flood events.

The high marsh assemblage is strongly controlled by elevation, preferring the highest elevations that are densely vegetated and subject to longer aerial exposition times due to less frequent tidal inundation. Here, the sediments are finer (> 90% mud), pH is the lowest and TOC is the highest. High marsh assemblages only occur at the lower estuary where tide effects are major and an intertidal marginal zonation exists. Farther up the Beliche Rivulet (FB) this zonation gradually starts to disappear. The assemblage is mainly composed of agglutinated, acid-resistant foraminifera and is dominated by the highly abundant *J. macrescens* species, associated with the subsidiary species *T. inflata* and *P. ipohalina*. Both thecamoebians and tintinnids are usually absent from this assemblage and the rare individuals are allochothonous occurrences, most probably brought by tides and river freshets.

7. 5. Conclusions

In general, there are still several limitations in the current knowledge of the taxonomy, ecology and biogeography of testate amoebae, especially in estuarine communities. In Portugal, particularly in the Guadiana Estuary, these limitations are considerable, not only for thecamoebians, but also for tintinnids. To improve the use of these organisms as reliable indicators in further studies of paleoenvironmental reconstruction, we need to know more about their ecology and distribution trends, especially in the environments where they are native. This must also be accompanied by a taxonomic census, attempting the lowest possible taxonomic divisions to maximize the ecological indicator value of the assemblages. The present, preliminary study of thecamoebians and tintinnids has given rise to a series of observations and assumptions, sometimes weakly supported or even contradictory, which need further work to be tested and, eventually, strengthened. Even without the appropriate sampling methods, 17 species of thecamoebians were identified. Despite the low diversity, the ecophenotypic variability observed within species is high, suggesting that there is a great potential to be explored in surrounding freshwater environments. The most representative families of thecamoebian recorded in the present study are Centropyxidae, Difflugiidae and Arcellidae. Difflugiidae is the best represented in number of species but the family Centropyxidae, mainly represented by *C. aculeata*, *C. arcula* and *C. constricta*, is the best represented in terms of faunistic density, comprising 71% of the total counted and identified individuals.

In winter, when the river level and current flow are the highest, there is an increase in thecamoebian species diversity and faunistic density doubled. Independent of the season, the highest thecamoebian diversity and density are observed in the middle and upper estuary, where salinity is negligible, associated with a few agglutinated foraminifera species, such as *M. fusca, H. manilaensis, M. obliqua* and *Siphotrochammina* sp. None of these agglutinated foraminifera species have good preservation potential in the Guadiana Estuary paleoenvironments which, allied to the low preservation potential of thecamoebians reported for other estuaries, constitutes a major challenge in the identification of fresh/brackish water environments in the paleorecord.

In the lower estuary, abundant empty thecamoebian tests can occur alongside calcareous foraminifera. These occurrences tend to decrease towards the sea and are observed mainly at the lower stands of the marsh, related to *post-mortem* transport after the winter heavy rain periods. In summer, a significant increase of living thecamoebians in the lower estuary, especially near freshwater outfalls, could indicate that their presence is not due to stochastic processes alone and that some species are able to live in the lower reaches of the estuary, as long as salinity is diminished.

In the sediments of the intertidal margins of the Guadiana Estuary, without using directed methods, two species of agglutinated tintinnids (> 63 µm), *Stenosomella ventricosa* and *Tintinnopsis* cf. *lata*, are found in significant numbers. Their higher abundances and living percentages, especially *S. ventricosa* which represented more than 92% of the tintinnid total assemblage, are observed in summer and are strongly correlated with inflows of freshwater and sewage, where the nutrient availability may be enhanced, contributing positively to the flourishing of nanoplankton, the main food source for tintinnids.

If fossils of *Tintinnopsis* cf. *lata*, and especially *Stenosemella ventricosa*, are discovered in abundance in Holocene sediments, they should be useful paleoenvironmental indicators, suggesting proximity to sites of freshwater discharge or, at least, brackish shallow waters. High abundances of *S. ventricosa* associated with the calcareous foraminifera species, *E. oceanensis*, may be indicative of warmer climatic periods.

The study of tintinnids in the Guadiana Estuary provides additional insights for future studies aimed to monitoring the climatic and paleoenvironmental record of estuaries and their inclusion as a proxy should be considered. However, further work is needed to better understand the processes behind tintinnid distribution within the estuary, as their loricae are easily transported and allochthonous deposition could lead to erroneous interpretations.

The obtained results emphasize the importance of improving the knowledge on thecamoebians and tintinnids and provide a critical baseline for planned future environmental and paleoenvironmental studies in this region.

Chapter 8

General conclusions

Chapter 8

8 – General conclusions

This thesis explores the potential of benthic foraminifera as environmental indicators in the Guadiana River Estuary according to an actual perspective, where we study the species distribution patterns and their responses to seasonal environmental changes and a paleoenvironmental perspective, in which the previous information in combination with the knowledge of diagenetic processes is applied in interpreting analogues environments that formed in the estuary during the Holocene.

The variation of environmental parameters in the estuary showed strong dependence on spatial and seasonal gradients, exhibiting prevailing river conditions in winter and marine conditions in summer. During the winter of 2010, heavy rains forced the continuous discharge of the Alqueva dam, promoting exceptional hydrodynamic conditions, which contributed to increased coarser sediment deposition in the estuary, particularly in the less elevated stands. However, in general, the sediments are poorly calibrated and with a fine skewness, which corresponds to the low energy conditions expected for deposition environments as are the case of marginal intertidal environments.

In winter, the salinity was always low (< 3) throughout the estuary, except in the most confined environments which are subject to higher evaporation. In summer, the estuary waters are better mixed and saltwater intrusion is significant until the International Bridge zone. The area of greatest salinity extends until approximately the latitude of Beliche Rivulet, from which, upstream, salinity values are similar to those observed in winter.

The confined soaked areas are subject to extreme seasonal variations in salinity and dissolved oxygen. In winter, when the contribution in river water and rainwater is higher there is low salinities and high values of oxygen concentration; in summer when insolation and evaporation are the most important factors, salinity increases and oxygen decreases.

The seasonal variation of the water surface temperature is very high and there is a difference of *ca* 14 º C between the maximum temperatures recorded in winter and summer

There is a dependency relationship between organic matter, pH and particle size with elevation. In the higher areas of the marsh, where the hydrodynamics is attenuated, occurs differential deposition of fine sediments. The cohesive characteristics of these sediments promote the entrapping of organic matter and its posterior degradation causes low pH.

The organic matter in the sediments of the Guadiana is characterized by a mixture of native aquatic and terrestrial origin. The organic material in the mid-upper estuary areas and higher stands of the saltmarsh at marine estuary incorporate a greater contribution from terrestrial sources, whereas the low-mid areas of the marine estuary experience a substantial contribution from indigenous aquatic sources.

The distribution of foraminifera in the Guadiana estuary mirrored the seasonal variation of environmental factors, whose relative importance depended on the proximity of species tolerance limits. The elevation proved to be the most important parameter in the distribution of foraminifera, since it combines the effect of a series of other variables, such as organic matter and fine sediment content, which tend to increase as elevation increases, and the pH of the sediment, coarse sediment content and temperature, which tend to increase with decreasing elevation. The salinity gradient, integrated in the fluvial-marine profile, has also proved to be important in the microfauna distribution. However, showed little significance in species distribution along the elevation gradient in the intertidal zone, probably due to the high osmoresistence reported for marsh species. Along the elevation gradient there was a negative correlation between diversity and species dominance. In the most elevated marsh areas, where environmental conditions are usually more severe, only some agglutinated species are able to survive. In the lower areas of the marsh, where time to air exposure is diminished and environmental conditions are generally more uniform, there is dominance of more diversified faunas, mainly composed by calcareous species.

During winter, when the fluvial processes are dominant, agglutinated species of the highest stands of saltmarsh proliferate, constituting more than 80% of the total individuals counted. In summer, when marine conditions prevail, calcareous species become more competitive, increasing their faunal densities and expanding into higher marsh areas and areas further upstream.

The species *Miliammina fusc*a and *Polysaccammina hyperhalina*, both agglutinated and typical of mid-low marsh stands, showed a similar behavior to calcareous species, showing optimum occurrences in summer. The *Jadammina macrescens*, among agglutinated species, and *Ammonia aberdoveyensis*, among calcareous species, were the most ubiquitous and dominant in the two studied seasons.

Based on the dominant species of living foraminifera and seasonal variations in their relations was possible to define a model of ecological zonation for the Guadiana estuary that is based on the recognition of four different assemblages:

- ***J. macrescens* assemblage:** dominates the higher marsh areas (between 1 and 2 meters above MSL) or lower zones which are subject to high degrees of confinement. These zones are densely vegetated and the sediments are predominantly fine, pH is low and organic matter content is high. The species *J. macrescens* is dominant, accounting for over 70% of individuals, and is associated with *Trochammina inflata*. Occasionally *Miliammina obliqua*, *Polysaccammina ipohalina* and the miliolids group may occur in significant abundance;
- ***J. macrescens* and *T. inflata* assemblage:** dominates in areas of intermediate elevation, between 0.1 and 0.6 m above MSL, especially in wet areas (intertidal pools), densely vegetated and near the sea. The physico-chemical parameters are very variable. Both species *J. macrescens* and *T. inflata* are dominant. In areas of lower elevation the species *Discorinopsis aguayoi* can occur in winter and the species *A. aberdoveyensis* in summer;
- ***M. fusca* assemblage:** it is common in areas of intermediate elevation, between 0 and 0.75 m above the MSL, particularly upstream of the low estuary, usually in unvegetated areas. *M. fusca* is the dominant species and is associated with the secondary species *A. aberdoveyensis* and *Elphidium oceanensis*.
- ***A. aberdoveyensis* assemblage:** is common in low elevation areas of the marine estuary, between -7 and 0.3 m in relation to MSL. The sediments consist of over 50% sand, occasionally with significant bioclasts contribution. *A. aberdoveyensis* is the dominant species, being associated with *Haynesina germanica*, *P. hyperhalina* and *E. oceanensis*. In winter, the *Bolivina ordinaria* is co-dominant with *A. aberdoveyensis* and *Asterigerinata mamilla* is also abundant.

The ecological zonation model of benthic foraminifera from the Guadiana estuary showed more similarities with the foraminifera distribution models described for the Mediterranean climate zone than with the North Atlantic area.

The comparison between the living, total (living plus dead) and fossil foraminifera assemblages, showed that the total assemblage, which combines the seasonal and taphonomic effects on modern microfaunas, represent reliably the indigenous microfauna of the Guadiana estuary and that the inclusion of exotic species in the study is an asset in the paleoassemblages interpretation. The taphonomic dissolution of the calcareous tests was observed in the paleoassemblages, especially when episodes of low sedimentation rates were associated with the time of tests deposition. However, the study of Guadiana Holocene sediments seems to indicate that the differential disintegration of agglutinated tests is the major problem in the finding of analogies between modern and fossil faunas. In the Guadiana biocenoses, important agglutinated species such as *Haplophragmoides manilaensis*, *M. fusca*, *M. obliqua*, *P. hyperhalina*, *P. ipohalina* and *Siphotrochammina* sp., are not represented in the fossil record. Moreover, the trochamminiids and the species *Ammobaculites* sp. proved to be the most resistant to taphonomic processes.

Through cluster analysis of total foraminifera assemblages it was possible to identify five associations, each with different faunal composition, indicative of its position in relation to intertidal level and distance to the sea, with which the paleoenvironments were interpreted: 1) monospecific assemblage of *J. macrescens* and 2) *J. macrescens* and *T. inflata* assemblage, both assemblages indicating emergent environments, in which the rates of accretion keep pace with MSL rise, 3) *A. aberdoveyensis* and *H. germanica* assemblage, indicative of submergent environment in which the rising in MSL exceeds the rates of accretion, 4) miliolids, *A. aberdoveyensis* and exotic species assemblage, indicating energetic environments subject to strong marine influence and 5) *M. fusca* and other agglutinated species assemblage, indicating environments subject to strong fluvial influence.

The analysis of the faunal changes that occurred along the sedimentary sequences CM3 and CM5, together with the sedimentological and chronological data, allowed the identification of four distinct phases of evolution of the Guadiana estuary since *ca* 13 000 cal yr BP:

- ***Pre-Holocene phase:*** started before 13 000 cal yr BP and covered the short cold climate event Younger Dryas, characterized by a period of silting up of the estuary, promoted by dominant continental processes, with no foraminifera or other traces of marine life;
- ***Pre-flooding phase:*** began with the first signs of trochamminiids inner and *T. inflata*, occurring before 11 500 cal yr BP, when sea level was *ca* 45 m depth. It is characterized by alternating phases of marsh accretion in equilibrium with MSL rise (*J. macrescens* and *T. inflata*) and phases of marsh emergence (presence of *Trochammina* spp. evolving into areas barren of foraminifera);
- ***Holocene Transgression:*** occurred between *ca* 9300 and 3500 cal yr BP and included two phases of marked marine influence:

  - *Lower transgression* – records the transgressive maximum during the Holocene, between *ca* 8000 and 7600 cal yr BP and is characterized by the presence of *A. aberdoveyensis, H. germanica, A. mamilla*, miliolids, high abundance in exotic species and constant presence of planktic forms. The presence of the warm water species *Pararotalia* cf. *spinigera* and evidences of subtidal environments occurrence in CM3 borehole suggest that the Guadiana estuary experienced periods of higher oceanity and, probably, warmer conditions than today;
  - *Upper transgression* - records at 4400 cal yr BP a short episode of marine renewal characterized by the presence of *A. aberdoveyensis*, *H. germanica* and exotic species, interrupting a longer deceleration phase of the MSL, characterized by *Trochammina* spp. and *Ammobaculites* sp. This short phase of marine influence, apparently not identified in other studies conducted in the Gulf of Cadiz, could be related to the warm climatic period reported to the Southeast of the Iberian Peninsula, based on detailed palynological time series.
- ***Wetland and tidal flat formation*:** from 3500 cal yr BP up to present, there was a monotonous sequence of mid-high marsh, characterized by the continuous presence of trochamminiids and *Ammobaculites* sp., suggesting an accreting environment in balance with the rise of MSL.

In a pioneering study on the distribution of thecamoebian and tintinnids in the Guadiana estuary, 17 species of thecamoebian (integrating over 25 strains) and 3 species of tintinnids were identified. The high ecophenotipic variability observed among thecamoebian reveals proximity and diversity of freshwater environments in the estuary and a high scientific potential still to explore.

Among thecamoebians, Centropyxidae family, represented mainly by species *Centropyxis aculeata*, *Centropyxis arcula* and *Centropyxis constricta,* was the best represented in terms of faunistic density, integrating more than 70% of total counted individuals.

In winter, when river water level and currents are higher there is an increase in thecamoebian diversity and the faunistic density doubles. Regardless of the season, the highest thecamoebian diversity and density are always recorded in the mid-upper estuary, where salinity is negligible. In these environments, the thecamoebian are associated with agglutinated foraminifera species such as *M. fusca*, *H. manilaensis*, *M. obliqua* and *Siphotrochammina* sp. In the lower estuary, along with the calcareous foraminifera, there is a significant number of empty tests of thecamoebian which tends to decrease in direction to sea. These occurrences are more frequent in the lower marsh and are related to *postmortem* transport which increases in periods of high rainfall during winter. In summer, a significant increase of living thecamoebian in the lower estuary, especially located in the vicinities of freshwater outflows, may indicate that the presence of these organisms in the estuary is not only due to stochastic processes, but that some species are able to live even in the most marine estuary as long as salinity is reduced.

Two species of tintinnids, *Tintinnopsis* cf. *lata* and *Stenosemella ventricosa*, were accidentally included in this study. Its morphological similarities with thecamoebian led them to be misidentified during the counting task. However, their study proved to be an asset, giving rise to new information that can be applied in future studies of paleo-climate and environmental monitoring in estuaries. When the tintinnids species presently studied are found in the fossil form in Holocene sediments it may suggest proximity to places of freshwater discharge or, more broadly, brackish shallow environments. The occurrence of high abundances of *S. ventricosa* associated to the *E. oceanensis* calcareous foraminifer may indicate warmer climatic periods. The present study demonstrated that the three indicators discussed here, when related with environmental conditions, offer great potential in environmental monitoring studies as well as in paleoenvironmental reconstruction. This potential is increased if the three groups of organisms are analyzed in an integrated perspective, thereby increasing the range of possible environmental scenarios to gauge in a paleoenvironmental interpretation.

The data analyzed in this thesis bring new insights into foraminifera ecology and biology that are of paramount importance in providing the ecological baselines for studies of reconstruction of the paleoenvironments and paleo-sea-levels on the southern Iberian Peninsula and in related climatic zones. Future studies should concentrate on higher sampling resolution to more accurately define the distribution boundaries of foraminifera species, especially those living in the northernmost portion of the estuary.

The majority of the agglutinated foraminifera species found in Guadiana Estuary exhibited low preservation potential and were not recorded in the paleoenvironments. This, allied to the low preservation potential reported for thecamoebians in other estuaries, constitutes a major challenge in the identification of fresh/brackish water environments in the paleorecord. Further studies on taphonomy are required for both foraminifera and thecamoebians to better understand the effects of fossilization and the paths of differential preservation.

Much remains to be known, especially with regard to testate amoebae and tintinnids, both little known to science in Portugal and whose potential as ecological indicators was proven here.