# GUADIANA RIVER ESTUARY
## Investigating the past, present and future

**Edited by**
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# 3. Habitats of the Guadiana River estuary

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The Guadiana River estuary is the most important estuarine system in southern Portugal. The estuary's variety of habitats supports a great diversity of species. Some of these species have only recently been discovered and are found nowhere else on Earth. This chapter details the current state of knowledge of the estuary's habitats, with a particular focus on the unseen microscopic flora and fauna that support the entire food chain – and many economic activities – in the estuary.

## 3.1. Ecological zones of the Guadiana estuary and their environment

The Guadiana River estuary has two major environmental gradients, in which several important ecological zones occur. One such gradient is defined along the main axis of the estuary, as a result of the distance to the river mouth (a balance between tidal inflow and river outflow). The other gradient is set along an axis perpendicular to the estuarine channel, and relates to changes in elevation and their interaction with the tidal range.

The gradient defined along the main axis of the estuary divides it into three zones (Figure 3.1): (i) upper estuary (with tidal influence and salinity close to zero); (ii) the middle estuary (where salinity varies between 0.5–25 g/kg); and (iii) lower estuary (where the salinity is higher to 25 g/kg). The salinity of the Bay of Cadiz is, on average, 36 g/kg (see also Garel, chapter 1 in this book).

The gradient set along an axis perpendicular to the estuarine channel is classified into three zones (Figure 3.2): (i) subtidal (below the mean low tide level - always covered by the water); (ii) intertidal (between the mean low and high tide levels - flooded twice a day) and (iii) supratidal areas (above the mean high tide level, only flooded during spring or storm tides). Each of these zones has characteristic landforms and vegetation. In the intertidal area, unvegetated sand and mudflats are widespread, dependent on the local hydrodynamics (Figure 3.2). Saltmarshes may also occur in this zone – these areas differ by the presence of halophyte vegetation and a shorter duration of tidal inundation. Increased exposure to the atmosphere increases evaporation and makes the soil water of saltmarshes saltier than the surrounding soils. The fine, muddy soils of saltmarshes trap the organic matter from the halophyte vegetation and algae. The organic matter decomposes rapidly due to greater exposure to the atmospheric oxygen, making the soil water more acidic. In areas where saltmarshes remain inundated for longer, organic matter may be trapped in the soils for long periods of time, contributing to what is called 'blue carbon'. Disturbance of these blue carbon deposits through dredging or drainage can cause the carbon to be released as a greenhouse gas. Sulphuric acid may be released into the waterway at the same time, leading to problems for aquatic species. It is therefore important to avoid disturbing saltmarsh soils to keep our atmosphere, waterways and ecosystems healthy.
Salt water is heavier than fresh water, so the water in estuaries like the Guadiana may become 'stratified'

into layers, with salty water occurring near the bottom of the channel and fresh water sitting at the surface. During periods of low river flow, the water column is (i) well mixed during spring tides and (ii) partially stratified in neap tides. When the river flow is high, the water column is highly stratified. A feature called a 'salt wedge' appears in the estuary during these periods. The wedge is thickest at the estuary mouth and gradually becomes thinner further upstream (see also Garel, chapter 1 in this book).
It is this complexity of the estuary that creates the basis for the existence of a great diversity of habitats. As well as ecological diversity, along the estuarine margins there are: (i) urban areas (Vila Real de Santo António, Ayamonte, Castro Marim, Costa Esuri, Foz de Odeleite, Guerreiros do Rio, Laranjeiras, Alcoutim, Sanlúcar de Guadiana, Puerto de la Laja, Pomarão and Mértola), (ii) salt and aquaculture pans, (iii) agricultural areas and (iv) pinewoods (Figure 3.1) (see Box 3.1.).

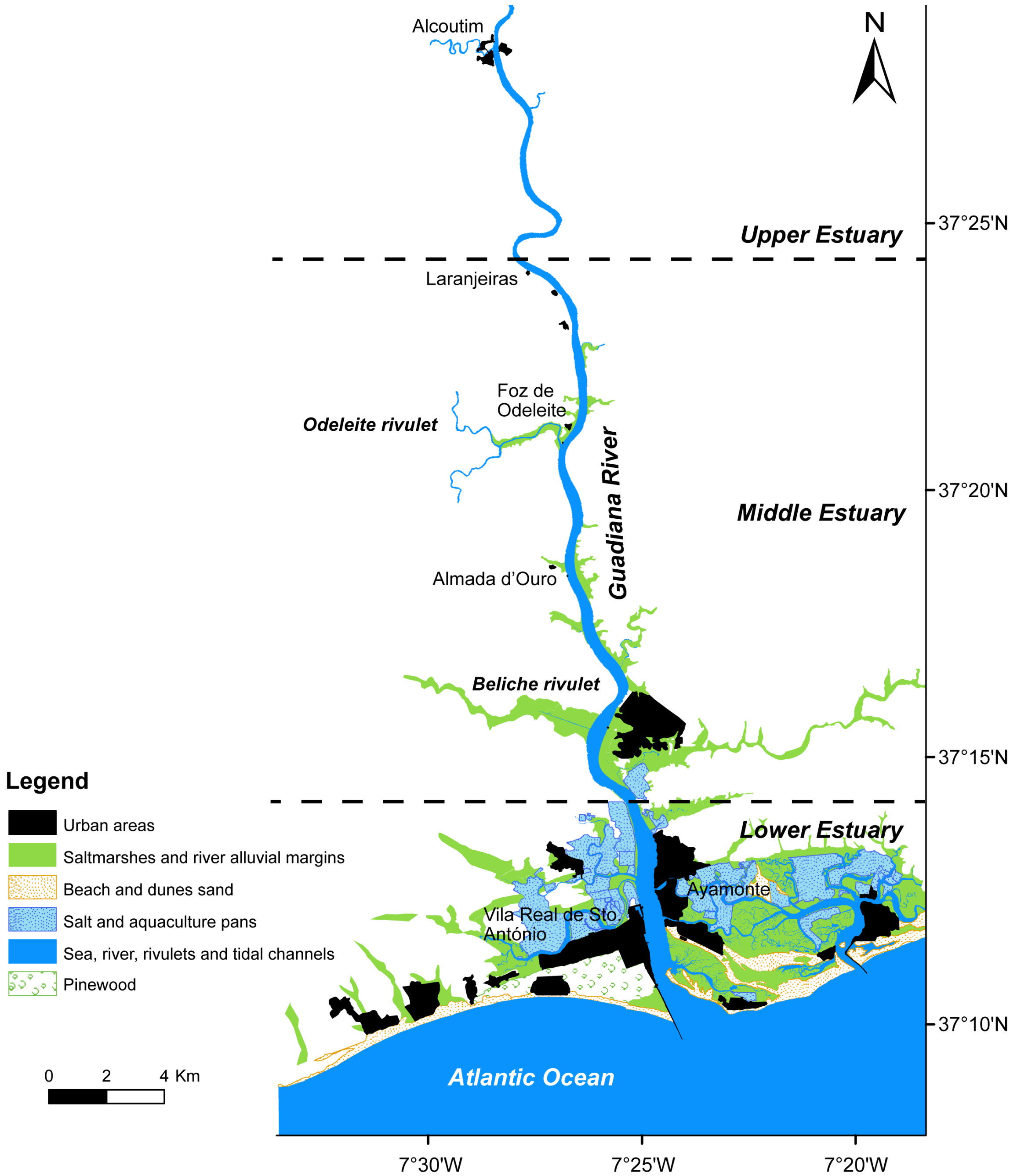


**Figure 3.1.**
Guadiana River estuary, showing the three main zones that are the result of distance to the river mouth (see also Garel, chapter 1 in this book).

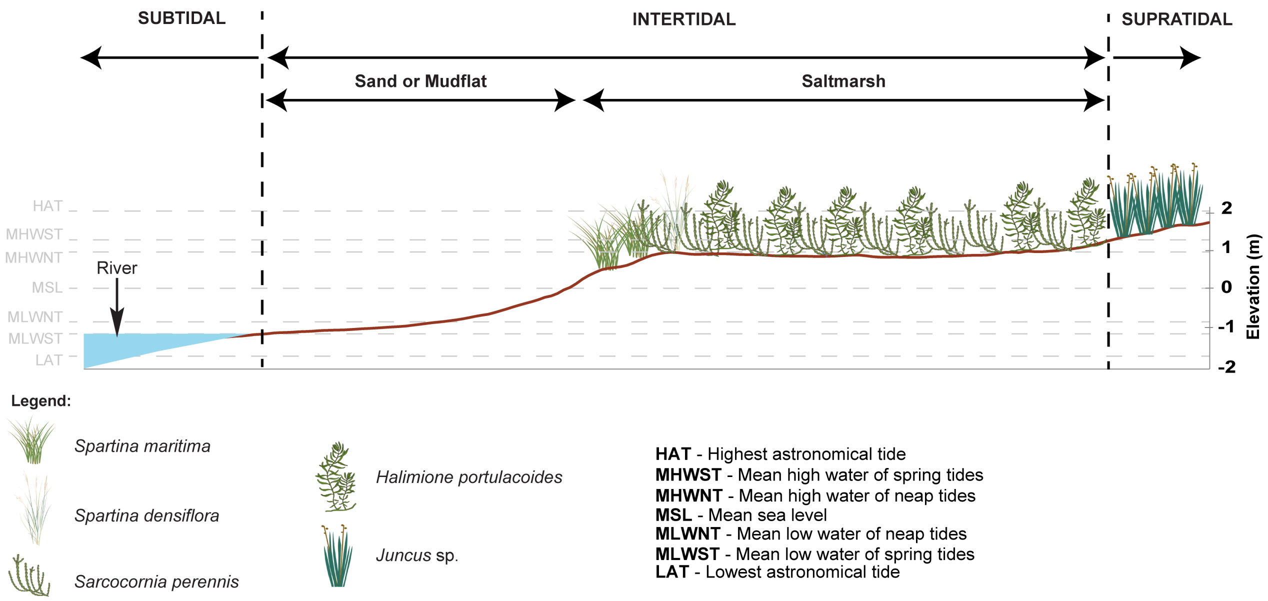


**Figure 3.2.**
Guadiana River estuary zonation as a result of tidal range. Note how each of the plant species inhabits a distinct zone along the gradient.

## 3.2. Biological communities

The various ecological zones in the Guadiana estuary are constantly changing as they respond to changes in the tides, river flows, climate and other aspects of the environment. Over the longer term, Guadiana estuary's ecosystems have also responded to large changes in sea level. These constant changes create highly diverse habitats hosting several groups of macro and microorganisms. In this section we will present some of the organisms that have been studied in the Guadiana estuary. We have chosen to focus on organisms with high scientific, ecological and economic value (see Box 3.2.).

### Box 3.1. Do you know that...?

...the saltmarsh area located at the Portuguese margin of the Guadiana River estuary is part of the first Natural Reserve created in the Portuguese mainland (additional information available at http://www.icnf.pt/portal/icnf/organica/apc-alg). The natural importance of this area is related to various ecological, botanical, ornithological, ichthyological, archaeological and economic aspects (e.g. fishing, salt production and tourism). For several birds (e.g. flamingo, *Phoenicopterus roseus*), fish (e.g. anchovy, *Engraulis encrasicolus*) and crustaceans (e.g. shrimp, *Crangon crangon*), this area assumes a special role during the nesting period and during subsequent migrations.

### Box 3.2. What are macro and microorganism?

Macroorganisms are all the organisms whose size is equal or higher than 0.5 mm, that is, the ones that are visible to the naked eye. On the other hand, microorganisms are ones that we can only see using a microscope (Figure 3.3).

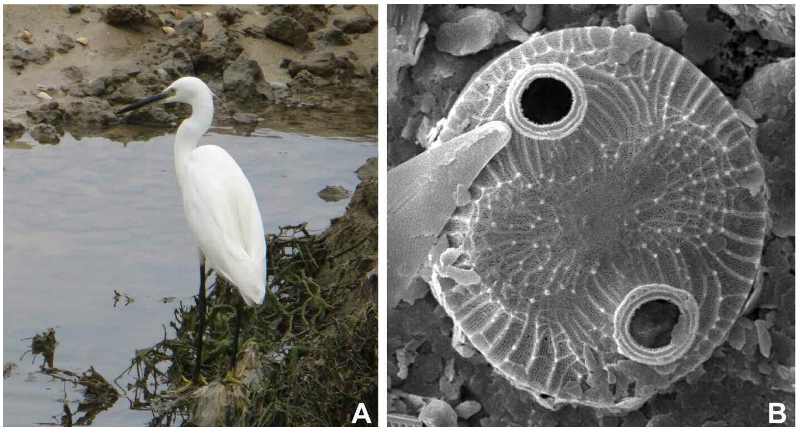


**Figure 3.3.**
Examples of a macroorganism (A – an egret, *Egretta garzetta*) and a microorganism (B – a diatom, *Auliscus sculptus*). Photographs by Ana Gomes (2012/2015).

## 3.2.1. Flora

In this section we will present some of the Guadiana macro and microflora. We understand as flora all the vascular plants, bryophytes, fungi, algae and lichens that grow up in this area.

### Macroflora

In the area of the Guadiana River estuary there are at least 462 registered species of plants. Between them it is important to highlight *Picris algarbiensis and Picris willkommii* (species endemic to the Iberian Peninsula), as well as *Limonium diffusum*, *Beta macrocarpa and Melilotus fallax,* for their and their habitats' state of conservation. All these species are considered vulnerable or threatened (species images and additional information available at http://flora-on.pt/ and http://www.icnf.pt/portal/ap/r-nat/rnscmvrsa/flora).
In the saltmarshes, we found a diversity of halophyte species such as *Spartina maritima*, *Sarcocornia perennis*, *Halimione portulacoides*, *Arthrocnemum macrostachyum, Suaeda vera*, *Frankenia laevis* and *Spergularia salin*a (Figure 3.4 and http://flora-on.pt/). All of these species have remarkable adaptations to living in an environment of high salinity, intense sunlight and changing water levels. Upward in the estuary, it is also possible to find *Spartina densiflora, Elymus* cf. *repens, Juncus* cf. *subulatus* and some riparian vegetation typical of freshwater habitats, such as *Scirpus maritimus* and *Phragmites australis* (Figure 3.4 and http://flora-on.pt/). In the surrounding forest areas, *Quercus suber* (cork oak) or mixed stands of *Quercus suber* and *Pinus pinaster or Pinus pinea* (pine trees) predominate, with an undeveloped undergrowth composed *Genista hirsuta, Ulex parviflorus, Lavandula luisieri, Cistus crispus* and *Cistus monspeliensis* (species images and additional information available at http://flora-on.pt/ and http://www.icnf.pt/portal/ap/r-nat/rnscmvrsa/flora). These habitats are typical of the Barrocal and Serra landscapes that characterize the Algarve region. Landscapes nearer the coast have been highly modified by economic development, including towns, saltpans, wharves, bridges, tourist facilities and other infrastructure.

### Why is the study and conservation of the macroflora so important?

The study and conservation of the macroflora is essential, because it provides many things that are vital to sustaining life – these are called 'ecosystem services' and include the primary production of energy from sunlight, nutrient cycling, production of food and medical resources, climate regulation, Green Tourism, and


**Figure 3.4.**
Macroflora from the Guadiana River estuary: (A) *Spartina maritima* (closer to the river water, top right) and *Spartina densiflora*; (B) close up of *Spartina maritima*; (C) *Sarcocornia perennis*; (D) *Halimione portulacoides*; (E) *Elymus* cf. *repens* (see arrow); (F) *Phragmites australis*. Photographs by Ana Gomes (2010/2011).

environmental education. All of these ecosystem services are vital for the region's economic activities. Furthermore, the large macroflora diversity existent in and around the Guadiana River estuary must be preserved and valued, since this natural heritage is essential as habitat for other species. Many of these species may also give us early-warning signals to detect environmental changes caused by human pressures, climate change and sea-level rise (see Box 3.3.).

## Box 3.3. Did you know that...?

...*Sarcocornia perennis* produce succulent shoots suitable for human consumption which are used in gourmet cuisine, due to their salty taste and nutritional properties. *Arthrocnemum macrostachyum* is also edible and is used in the pharmaceutical industry and in the functional foods industry, because of its high concentration of polyunsaturated fatty acids and antioxidants.

### *Microflora*

Guadiana River estuary microflora is very diverse. However, this book will only focus on diatoms.

### What are diatoms?

Diatoms are single-celled algae that have cell walls of silica (called frustules). Each frustule is made up of two valves (Figure 3.5). As algae, diatom cells contain pigments (carotenoids) that give them a brownish/golden colour (Figure 3.6). They can vary in size between 2 – 500 µm and belong to the Chromista Kingdom, which includes all algae (additional information available at http://westerndiatoms.colorado.edu) (see Box 3.4.).

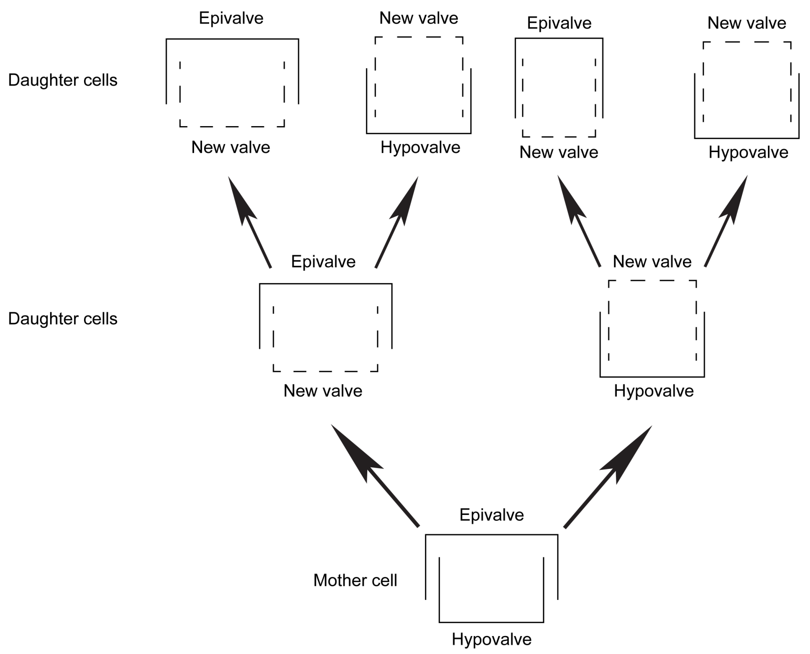


**Figure 3.5.**
Schematic representation of asexual reproduction of diatoms. Mother and daughter cells are composed of two siliceous valves (epi- and hypovalve).

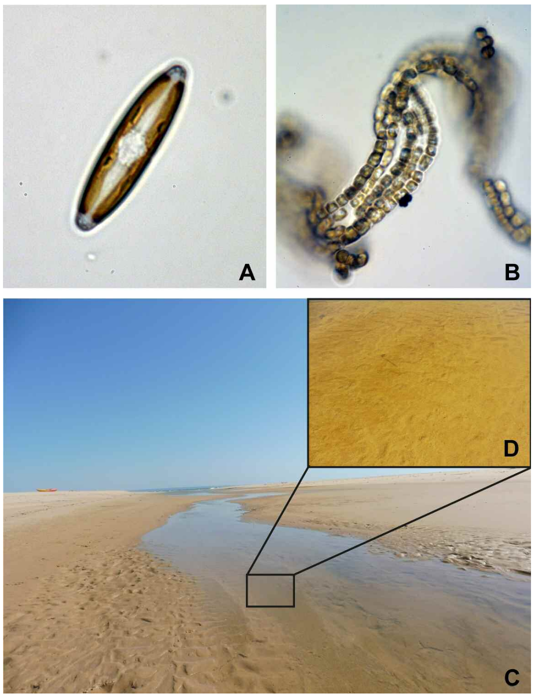


**Figure 3.6**
Diatom cells brownish/golden colour given by the carotenoids: (A) solitary diatom under a light microscope (*Navicula* sp.); (B) diatom colony under a light microscope (*Nanofrustulum* sp.); (C) tidal channel covered by diatom cells; (D) close up of the bottom of the tidal channel. Photographs by Ana Gomes (2012/2016).

**Box 3.4. Did you know that...?**

...diatoms were first described by an English gentleman in 1703. Due to their small size, it was only possible to visualize them after the invention of the microscope. Diatoms are so finely patterned that they were later used by German manufacturers to calibrate microscope optics.

### How can we identify different diatom species?

Diatom identification, to the species level, is based on the analysis of its valve shape, size and ornamentation. Diatoms are traditionally divided in two groups based on shape: (i) the centric diatoms (which have a circular, bi- or multipolar shape and a radial perforation pattern) and (ii) the pennate diatoms (which are bipolar and have a sternum or a raphe - Figure 3.7).

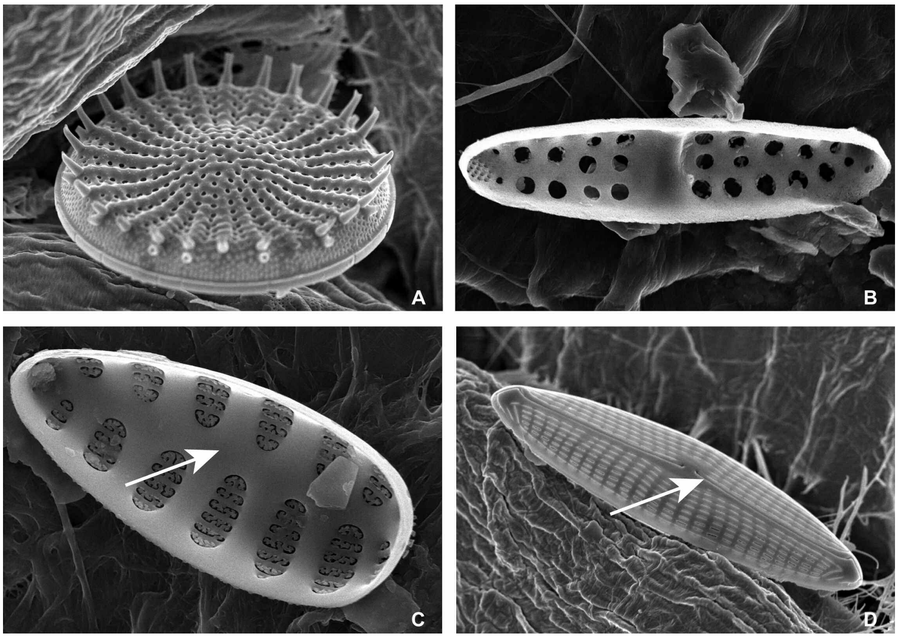


**Figure 3.7**
Examples of diatoms of different morphological groups: (A) centric diatom with a circular shape (*Stephanodiscus alpinus*); (B) centric diatom with a bipolar shape (*Plagiogrammopsis minima*); (C) pennate diatom with a sternum (longitudinal siliceous structure indicated by the arrow; *Opephora guenter-grassi*); (D) pennate diatom with a raphe (fissures positioned through the valve face - see arrow; *Navicula erifuga*). Photographs by Ana Gomes (2011).

### When did diatoms appear in the history of the Planet Earth?

Centric diatoms were the first to appear in the geological time scale. That happened during the Jurassic Period, ca. 190 million years ago, which is a period better known for its dinosaurs than for its diatoms (see the geological scale at http://www.stratigraphy.org/index.php/ics-chart-timescale). The appearance of the bi- or multipolar centric diatoms occurred during the Cretaceous, ca. 100 million years ago, a time when flowering plants were overtaking conifers in the world's land vegetation. The pennate diatoms with a sternum were detected in the fossil record 65 million years ago and the pennate with a raphe appeared ca. 30 million years ago. The appearance of the raphe in diatoms allow them to greatly expand their ecological niches and probably had a major impact in their diversification (see Box 3.5.).

## Box 3.5. How do diatoms reproduce?

Diatoms reproduce mainly by asexual reproduction (Figure 3.5), which are infrequently interrupted by sexual events. During the asexual reproduction, diatom cells continuously decrease size, because each daughter cell has a valve that belong to the mother cell and forms a new valve inferior to this (Figure 3.5). Thus, only sexual reproduction allows diatoms to reestablish the maximum dimensions of the cells. Diatoms have very short generation times which enables the rapid development of algal blooms, increasing the number of cells in many orders of magnitude in only a few days. In a gram of saltmarsh soil from the Guadiana estuary, there can be many thousands of diatoms.

### How many diatom species exist in the world?

Diatom species may number in the tens of thousands globally. This number arises, most probably, from the variety of environments that diatoms can colonize. In the Guadiana River estuary there are hundreds of diatom species. Among those are a new diatom genus (*Syvertsenia*) and two new species recently described: *Syvertsenia iberica* and *Simonsenia aveniformis* (Figure 3.8). Additionally, there are still some diatom species in the Guadiana River estuary that are yet unknown to science.

### Where can diatoms live?

Diatoms can live in salt, brackish and freshwater environments and they have different life-forms. They can be planktonic (they float in the water column), tycoplanktonic (they spend part of their lives associated with some kind of substrate and the other part floating in the water column) or they can be benthic (they live attached to a substrate). Benthic diatoms can colonise several substrates, including: plants or other algae, rocks, sand, mud and animals. Diatoms may also live solitarily or form colonies (Figure 3.6).

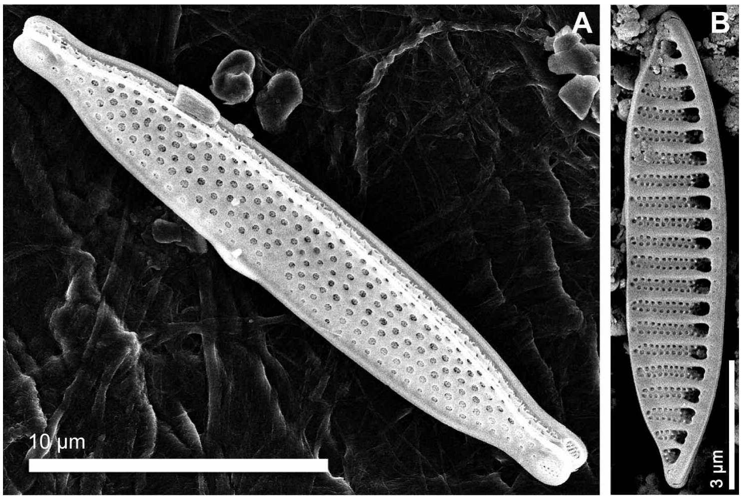


**Figure 3.8.**
Images from the diatom species found and recently described in the Guadiana River estuary: (A) *Syvertsenia iberica*; (B) *Simonsenia aveniformis* (figure adapted from Gomes et al., 2013 and Witkowski et al., 2015).

### Why are they so important? What can they be used for?

Diatoms are responsible for 1/5 of the Earth's photosynthesis and they are the key players in the carbon, nitrogen, phosphorous, silica and iron cycles. Additionally, diatoms are an important component of the phytoplankton and microphytobenthos, which are the basis of the food chain in aquatic systems. Moreover, they are highly sensitive to changes in environmental variables such as salinity, sediment type, duration of tidal inundation, nutrient levels and pH. These characteristics, along with their short generation time, allow diatoms to quickly respond to environmental changes. Thus, since the begin of the 20$^{th}$ century they have been often used to study the evolution of estuaries, like the Guadiana River estuary, in relation to sea level and climate changes. Particularly in the Guadiana estuary, diatoms were a determinant indicator to identify climatic events of short time duration and reconstruct in detail the estuary's evolution after the Last Glacial Maximum. Recently, diatoms have also started to be used as indicators for environmental quality in freshwater and coastal systems . Diatoms have proven to be very sensitive to changes caused by increasing human pressure on natural systems and are therefore useful in pollution monitoring. On the other hand, some diatoms or the toxins they produce represent a threat to fisheries, aquaculture and public health. The ingestion, for example, of shellfish during periods of these harmful diatom blooms may cause diarrhea, amnesia or even death. Better knowledge and understanding of diatoms can allow us to value and preserve our natural heritage, ecosystem services and our health.

Diatoms may also be used in forensic and archaeological research. Diatoms are useful, for example, to determine where a homicide/suicide took place (e.g. if a death by drowning occurred in a swimming pool, in a river or in the sea) or to determine if a person was at the crime scene (e.g. comparing diatoms of a garden pond with the diatoms attached to the wet jeans of a suspect who could have gained access to a house through the garden, after the suspect stated that the jeans were wet because he had been in the sea). In archaeological research, among other applications, diatoms can tell us about clay sources for pottery and provenance of finished pottery.
Finally, other economic gains associated with diatoms are: (i) the exploitation of diatomite (rock formed by are large concentrations of diatom frustules) and (ii) oil and gas exploration. Diatomite is used, for example, to make bricks (good insulator), dynamite, polishers and filters. Diatoms are useful tools in oil and gas exploration for age dating and for correlating rocks that form in different aquatic environments. Such information is important to understand the geological history of the basin and determine the prospects of the basin for hydrocarbon production.

### *3.2.2. Fauna*

In this section we will present a group of organisms that belong to the Guadiana microfauna.

#### *Microfauna*

This category includes all animal, protist and chromist organisms with size less than 0.2 millimeters that can only be observed with the help of a microscope. In estuarine sediments, the microfauna is mainly constituted by single-celled eukaryotes, which are autonomous living beings.

#### Can such microorganisms be important in science, especially in the study of an estuary?

Indeed, all eukaryotic estuarine species play a role in the base stages of the food chain and participate actively in the carbon cycle. They have short lifecycles and react quickly to changes, which makes them very sensitive to environmental impacts and, thus, excellent bioindicators in the assessment of the ecological quality of a given place. In these environmental studies, eukaryotes that possess a hard test (testate eukaryotes) that preserves in the sediment have some advantages compared to those called naked eukaryotes, which do not preserve well (Figure 3.9). By leaving a permanent record in the sediment, the testate eukaryotes enable the reconstruction of the environmental history of a site in the absence of the original physico-chemical baseline data. So the better we know them and their relations with the surrounding environment in the present, the better we can interpret their occurrences in the past and make reliable predictions for the future (see also Mendes and Rosa, chapter 4 in this book).
Despite the great diversity of single-celled eukaryotes we may find in the sediments, in this section we will focus on one special group of testate chromists, the foraminifera, and how their study has been contributing to the understanding of Guadiana estuary, both present and past.

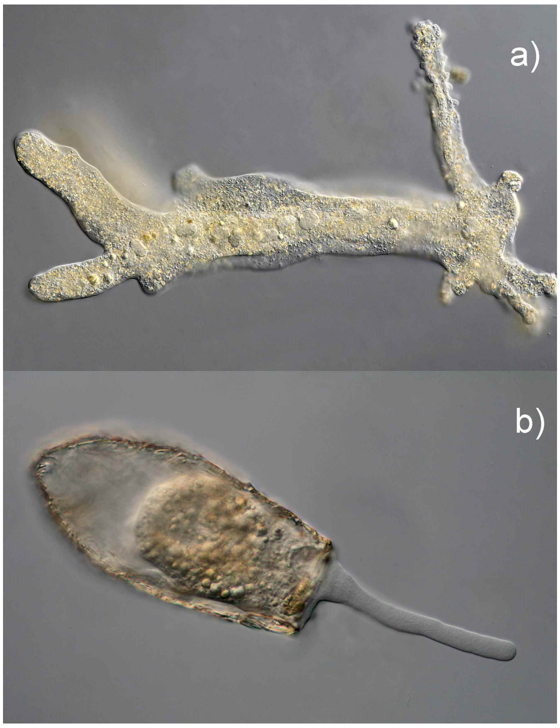


**Figure 3.9.**
Difference between a (a) naked amoeba (Genus *Chaos*) and a (b) testate amoeba (Genus *Difflugia*) (courtesy of Prof. Ferry Siemensma – http://www.arcella.nl/).

### What are foraminifera?

Foraminifera are testate chromists that occupy a great diversity of habitats, from the deepest oceanic environments to the upper limits of the tidal zones in coastal wetlands. Most foraminifera possess a hard shell which, after death, remains in the sediment where it may eventually fossilize (Figure 3.10) (see Box 3.6.). Whereas metazoans evolved organs and other specialized features through the multicellularity, the foraminifera, as other eukaryotes, specialized by diversifying subcellular components or organelles to perform their vital functions.

**Box 3.6. Did you know that...?**

The foraminifera were first described and illustrated in the sixteenth century, but were not studied systematically until the latter part of the nineteenth century following the remarkable voyage of HMS Challenger which began in 1872 (Figure 3.11). The discovery of living foraminifera in deep-sea waters occurred during that expedition, alongside fossil remains in sediments that were dredged from the sea floor.

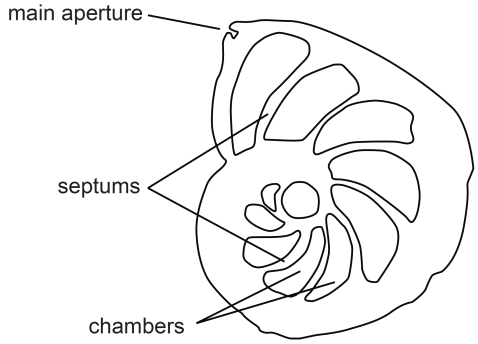


**Figure 3.10.**
Schematic thin section of a foraminifer test. The foraminifer shell is often distinctively coiled resembling that of a gastropod or a cephalopod. Illustration by Sarita Camacho (2017).

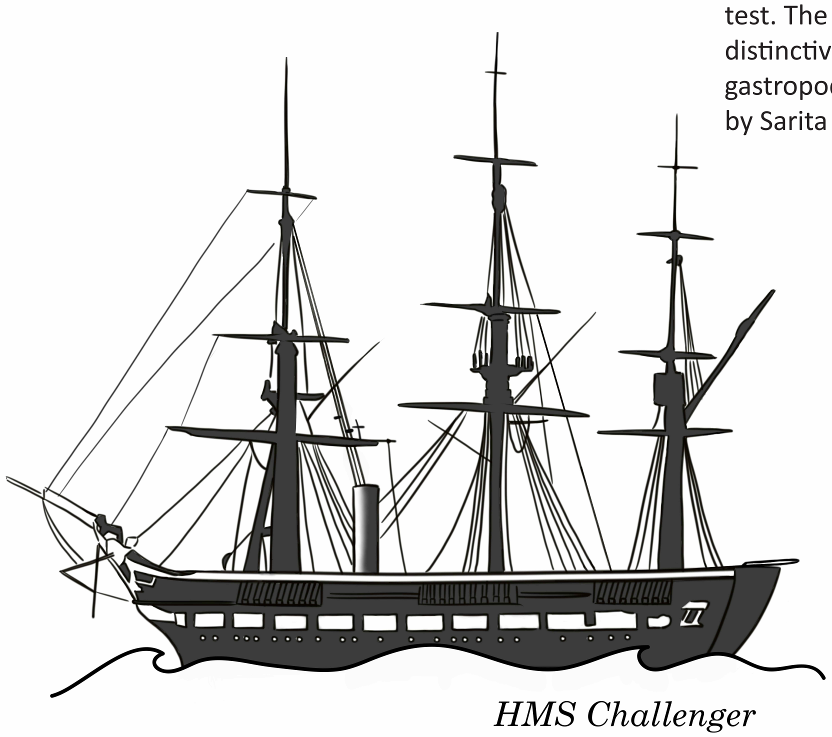


**Figure 3.11.**
HMS Challenger was a steam-assisted corvette built for the British Royal Navy in 1858. In 1872 she undertook the first global marine research expedition. The Challenger Expedition was a grand tour of the world, covering 68,000 nautical miles (125,936 km) organized by the Royal Society in collaboration with the University of Edinburgh. Illustration by Sara Encarnação (2017).

Two broad physical features characterize the foraminifera:
They possess *granuloreticulopodia*, which are fine, thread-like, pseudopodia ('false feet') that anastomose and have a granular texture when viewed under a microscope (Figure 3.12).
Nearly all foraminifera possess a *test* or shell that contains the organism and separates it from the surrounding environment. The test may be organic (not mineralized), agglutinated (constructed of foreign particles cemented together by the foraminifer), composed of calcium carbonate or, in rare cases, silica.
There are considerable variations in the dimension, morphology, composition and microstructure of the foraminifera tests. Those features are of great taxonomic importance for being intimately related with physiological differences, habitat, ecological niche and various types of reproductive strategies, each with repercussions in their life cycles.

**Figure 3.12.**
Light microscope micrograph of a *Lacogromia cassipara* exhibiting its distinctive 'granuloreticulopodia' (courtesy of Prof. Ferry Siemensma - http://www.arcella.nl/). The granuloreticulopodia emerge from one or several orifices existent in the test (known as 'foramina', a term from which the name of the group is derived) existent in the test, and are used in the captures of prey, to hold onto surfaces, to remove waste, to travel and in test construction.

The agglutinated forms, which are very common in estuarine sediments, collect organic and mineral matter from the sea floor and bind it together with an organic, calcareous or ferric oxide cement. Like the better-known caddisfly larvae, agglutinated foraminifera carefully select the sediment grains for their size, texture and composition when building their homes (Figure 3.13). The type of cement and composition of bonded particles provide information about the type of sediment and environmental conditions at the time when the foraminifer was building its test. Some genera, however, are not selective and use, indiscriminately, every type of material available on the seafloor.
The calcareous forms come in two main types: porcelaneous and hyalines (also called glassy), according to

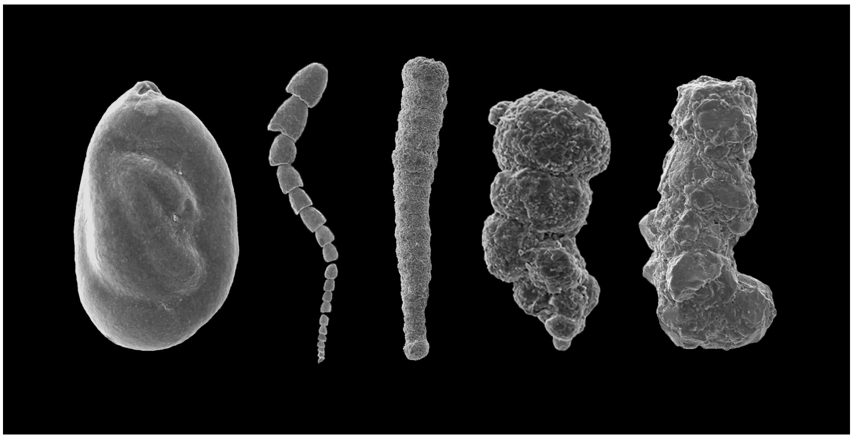

**Figure 3.13.**
Sequence of different species of agglutinated foraminifera, with increasing test roughness from left to the right. Micrographs by Sarita Camacho (2011).

their appearance under transmitted light. The porcelaneous types are opaque, reflecting all the light, while the glassy types, as the name suggests, are translucent (Figure 3.14).
Porcelaneous tests have a solid surface with a milky or amber aspect and are constructed by the secretion of tiny needles of high magnesium calcite within tiny vesicles in the cytoplasm, which are then exported

**Figure 3.14.**
Foraminifera stained with Rose Bengal, a vital stain for differentiating the living from the dead individuals (the fuchsia-stained specimens were alive at collection time). The porcelaneous ones (white circles) have a semi-opaque test, avoiding the observation of the vividly stained protoplasm as is visible in the translucent test of the glassy forms (black circles). Photography by Sarita Camacho (2011).

to the outer margin of the cell. In the hyaline (glassy) forms the test is built by a bio-mineralization process which takes place outside the protoplasmatic body. This type of wall has numerous perforations (pores) of very small diameter – foraminifers of this kind are known as "calcareous perforate" (Figure 3.15). Together with the aperture, the pores function as passages for the cytoplasm to carry food or waste products between the interior and the exterior of the test (see Box 3.7.).

## Box 3.7. What are the foraminifera feeding mechanisms?

Most benthic foraminifers are opportunistic omnivores. They have adopted a broad range of feeding mechanisms including herbivory, bacterivory, suspensivory, detritivory, carnivory and parasitism, allowing them to access a wide range of nutritional resources. Some large calcareous and planktic forms can carry naked photosybionts in their endoplasm, especially diatoms and dinoflagellates which aid in supplying energy to the foraminifera.

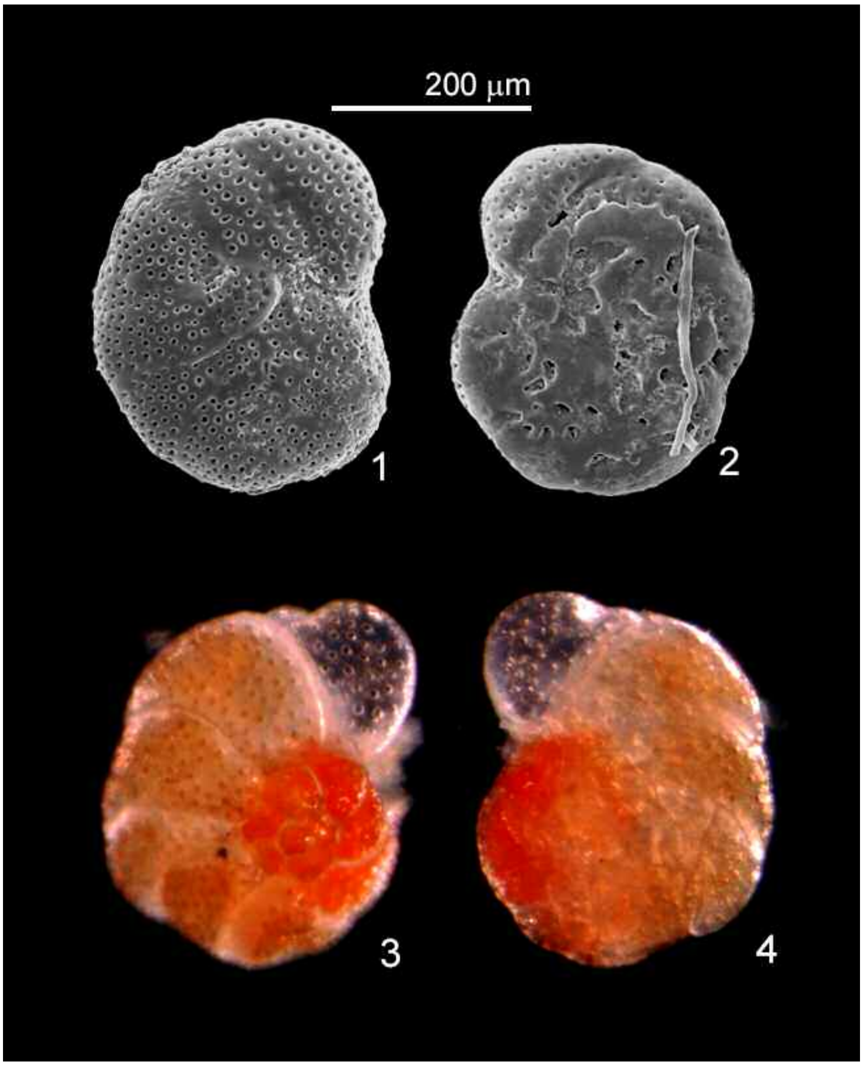


**Figure 3.15.**
The well visible pores of *Discorinopsis aguayoi,* a hyaline, epiphytic species: 1) dorsal view, and 2) ventral view, of a specimen photographed at scanning electronic microscope; 3) dorsal view and 4) ventral view, of a living specimen photographed at light microscope (natural colours). Stereoscope micrographs by Dr Maria Holzmann and scanning electron microscope (SEM) micrographs by Sarita Camacho (2011).

### How can we identify different foraminifera species?

The test is the best studied element of foraminifera as the shape of the test serves as the basis of its classification. More recently, the analysis of ribosomal ribonucleic acid (RNA ribosomal) sequences has been applied in the identification of the foraminiferal species, providing a new taxonomic criterion, independent of the morphologic characteristics of the test. Molecular analysis also enables an evaluation of the ecological significance of the different morphologic characteristics and helps to determine the subtle morphological differences within the species avoiding erroneous and superfluous taxonomic subdivisions.

### When did foraminifera appear in the history of the Planet Earth? How many foraminifera species exist in the world?

Stratigraphically, the foraminifers appear in the Early Cambrian (see the geological scale at http://www.stratigraphy.org/index.php/ics-chart-timescale) (the first unambiguous ones are from the lowermost Cambrian of West Africa), at about the same time that the metazoans with skeletons. Foraminifers began differentiating into countless species which adapted to all marine environments, from the surface to the deepest oceanic depths. At the moment, around 4000 species of foraminifera are estimated to exist in the world.

### Where can foraminifera live?

The benthic species live on the seafloor, where they can be free or sessile, epifaunal (attached to the substrate - sand, stones, rocks, animal shells, etc.), epifaunal epiphyte (attached to algae or sea-grass) or infaunal, living within the sediment. The planktic forms float passively in the water column, moved only by currents but capable of vertical migration (Figure 3.16).

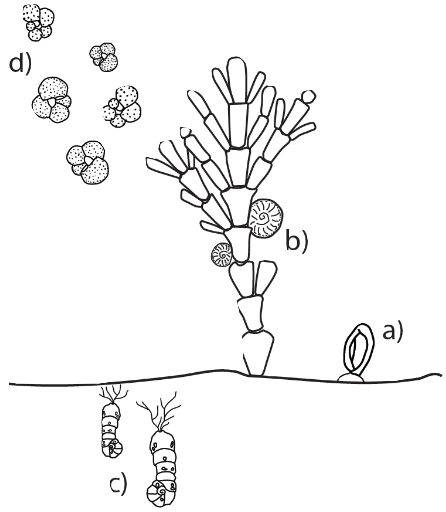


**Figure 3.16.**
The different life positions of foraminifera: a) epifaunal; b) epiphyte; c) infaunal; and d) planktic.
Illustration by Sarita Camacho (2017).

### How helpful can the foraminifera be in the study of an estuary?

Inferences about the evolution of estuaries are possible through the study of sediments that progressively accumulate, preserving ancient bioclasts and organic matter. The presence of fossil assemblages that represent the live communities in the moment of deposition can be used to make reconstructions of the past environment with greater accuracy than can be achieved from studying the sediment alone. This type of palaeoenvironmental 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. Foraminifera are particularly useful in finding out about changes in sea-level in saltmarsh environments. The zones in which the foraminifera are found on the present-day surface of the marsh are surveyed. They are so sensitive to subtle environmental changes that in an huge marsh, where the physical-chemical parameters are constantly changing, we may find many different assemblages. This modern analogues assemblages which ecological meaning is known serve then as a base for identification and interpretation to the fossil sequences.

Foraminifera have been used as reliable bioindicators as they provide information about both modern and historical contexts, their biological and ecological features are usually well known, they are easily identifiable and may be sampled in great quantities in small sediment volumes, providing ideally reliable statistical results. The better the foraminiferal modern assemblages and the ecological conditions which determine their spatial and temporal abundance are known, the better scientists' palaeoecological interpretations will be (see also Mendes and Rosa, chapter 4 in this book).

In Portugal, most ecological studies based on estuarine foraminifera have been performed in order to establish modern databases through which fossil foraminiferal assemblages can be compared and interpreted. But they are also successful used to identify the different ecological provinces, to detect environmental stress conditions and to monitor pollution. Before foraminiferal assemblages can be used as stress and pollution indicators in the marshlands which mark the transition between the continent and the sea, a precise understanding of their response to varying environmental conditions is necessary in order to distinguish between the stress promoted by human activities and natural environmental changes. This requirement is particularly critical in estuaries and coastal lagoons that are subject to a complex interaction of numerous physico-chemical parameters, each presenting spatial and temporal variability, and because these environments are often exposed to various human impacts, such as chemical pollution, industrial effluent and agricultural pesticides. Explaining foraminiferal distribution patterns thus requires consideration of a broad range of environmental factors. Species will be able to survive and potentially prosper as long as conditions remain within their tolerance limits. Once conditions move beyond the tolerance limits for any limiting factor, the species is likely to disappear (see Box 3.8.).

## Box 3.8. How to study the foraminiferal ecology of an estuary?

Observing foraminifera is much simpler than we think. A short walk through the marsh, a spatula, a small bag, a magnifying glass and a pinch of luck will be enough to observe hundreds, if not thousands of foraminifera. The complexity of the sampling plan will vary according to the objectives we want to achieve. But whatever study we want to achieve, for example, variation and distribution of species, diversity analysis, assessment of the health of the ecosystem, there are two trends in the spatial distribution of foraminifera that should be considered: 1) most foraminifera prefer to live in marine environments, and so in coastal areas species richness (biodiversity) is generally high, gradually decreasing as we move upstream where the waters are brackish, and eventually ending up with just one or two species, or disappearing in the areas where the salinity is close to zero; 2) foraminifera need moisture to survive and therefore their distribution is strongly dependent on the tides. Because of this, it is expected that greater diversity of species and evenness will occur in the lower zones of the marsh where the time of tidal inundation is greater. As we move upslope into the marshland towards land, diversity tends to decrease. These higher zones of the marsh, flooded only at high tide, are exposed to the atmosphere for longer periods and consequently more exposed to variations of the elements. Also in these higher stages of the marsh there is a greater accumulation of organic matter, which causes the pH to drop (that is, become more acidic), which is not compatible with the carbonate shells of the foraminifera. In these extreme conditions, only a few agglutinated species, with greater osmoregulation capacity, are able to survive. In the upper stages of the marsh, it is therefore common to find only one or two species  in assemblages of agglutinated foraminifera.

### The study of the Guadiana's microfauna

Detailed scientific study of the Guadiana's foraminifera began in February 2010, conducted by Dr Sarita Camacho and a team of researchers at the University of the Algarve. Several field trips were carried to Guadiana estuary in order to analyse variations of the foraminifera communities in the two periods of the year with the greatest environmental contrast – summer and winter. The sampling area extended over 34 km of the river's length, from Alcoutim to the mouth of the Guadiana, thus covering the entire salinity gradient of the estuary. Several samples were collected along profiles at each sampling site, usually perpendicular to the course of the river. The exact location of the samples was related to the zonation of the halophyte (salt-adapted) vegetation, sampling the different stages of marsh, from the non-vegetated mudflats to the highest areas, which are covered with water only during periods of spring tides (Figure 3.17).

**Figure 3.17.**
Edited photograph of the river margin highlighting the different intertidal zones. Photography by Sarita Camacho (2010).

Samples for analysis of the foraminifera were collected at the sediment surface (~0-1 cm depth) where most of the living foraminifera are found (Figure 3.18 a). Simultaneously sediment samples were collected for analysis of organic matter and grain size. The salinity, temperature and oxygen dissolved in the water, as well as the pH of the sediment, were measured in each campaign (Figure 3.18 b and c).

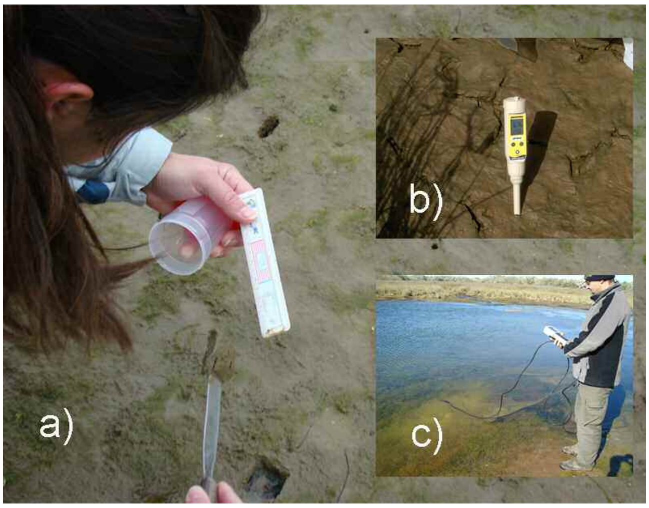


**Figure 3.18.**
Field sampling procedures: a) collection of the first centimeter of sediment; b) sediment pH measurement; and c) water parameters measurement. Photography by Sarita Camacho (2010).

Through this research, it was possible to identify 52 different species of foraminifera in the Guadiana estuary. Their distribution mirrored the seasonal variation of environmental factors, whose relative importance depended on environmental differences in the estuary. Elevation above sea level 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, which tend to increase as elevation increases, and the pH of the sediment, coarse sediment and temperature, which tend to decrease as elevation increases. The salinity gradient of the fluvial–marine transition has also proved to be important in 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.

In the most elevated marsh areas, where environmental conditions are usually more severe, only some agglutinated species were able to survive. In the lower areas of the marsh, where the duration of air exposure is diminished and environmental conditions are generally more uniform, there was dominance of more diversified faunas, mainly composed of calcareous species. During winter, when 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.

Based on the dominant species, on their interrelationships and their relation to the environmental parameters, it was possible to define three main foraminiferal assemblages in the Guadiana estuary:

i) The *Miliammina fusca* assemblage, was the most common in the mid-low elevation zones of the upper reaches of the lower estuary up until the sampling northern limit (Alcoutim), usually in unvegetated areas. *Miliammina fusca* was the dominant species, associated with *Ammonia aberdoveyensis* and *Elphidium oceanensis* (Figure 3.19 a);

ii) The *Jadammina macrescens* assemblage occurs in the most elevated and highly vegetated marsh stands (or lower, in sheltered environments) of the lower estuary, where the sediments are very fine, pH is low and organic matter is high. *Jadammina macrescens* was the dominant species but *Trochammina inflata* was also very common. Occasionally, significant occurrences of *Miliammina obliqua*, *Polysaccamminaipo halina* and miliolids were observed (Figure 3.19 b);

iii) The *Ammonia aberdoveyensis* assemblage was observed in the lower elevation zones of the lower estuary, where marine influence is high and the sediment is sandy. *Ammonia aberdoveyensis* was the dominant species, associated with *Haynesina germanica*, *Polysaccammina hyperhalina* and *Elphidium oceanensis*. In winter, *Bolivina ordinaria* is co-dominant with *A. aberdoveyensis* (Figure 3.19 c).

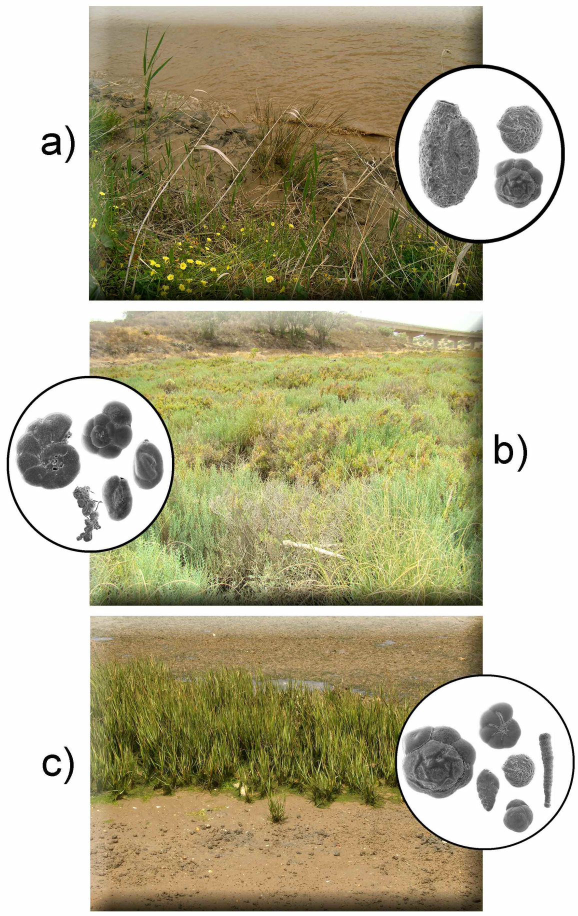


**Figure 3.19.** The three main foraminiferal assemblages in Guadiana estuary: a) *Miliammina fusca* assemblage, most often found in the upper reaches of the lower estuary; b) *Jadammina macrescens* assemblage, found mainly in the most elevated, strongly vegetated marsh stages; and C) *Ammonia aberdoveyensis* assemblage, found primarily in the lower elevation zones of the lower estuary. Infography by Sarita Camacho.

When compared to other estuarine systems in the north of Portugal, the foraminiferal distribution in the Guadiana estuary shows slight differences, pointing to a dominance of warmth-preferring species, resembling more the ecological distributions typical of the Mediterranean climatic zone than those of the North Atlantic climatic zone. This trend seems to make sense considering the Guadiana's geographical position which grant it both North Atlantic and Mediterranean climatic characteristics.

These results bring new insights into foraminiferal distribution and are expected to improve their value as bioindicators, providing a benchmark for future environmental quality assessments and to improve the ecological interpretation of palaeoenvironmental data on the southern Iberian Peninsula and related bioclimatic zones.

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