UAlg FCT
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FACULDADE DE CIÊNCIAS E TECNOLOGIA

**Universidade do Algarve**

Faculdade de Ciências e Tecnologia

# Bacteriobenthos diversity in the surface of different sediment environments in Ria Formosa

Ana Flor Torres Vidal

Mestrado em Biologia Marinha

**Trabalho efetuado sob orientação de:**

**Prof. Doutor Duarte Duarte**
**Prof.ª Doutora Margarida P. Reis**

Faro 2015

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Ana Flor Torres Vidal
(Licenciada em Biologia Marinha)


Tese de Mestrado em Biologia Marinha

Orientada por:

Prof. Doutor Duarte Duarte

Prof.ª Doutora Margarida P. Reis

CIMA – Laboratório de Ecologia Molecular e Microbiana

(No bench fees required)

Faro 2015

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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.

*Ana Flor Torres Vidal*

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*Ana Flor Torres Vidal*

### *Acknowledgements*

This part of the work can be the most challenging to write, not because it's hard to acknowledge others, but because it's hard to do so adequately.

It has no doubt been a journey up until now, one of many and nonetheless a long lasting one, filled with many challenges and undertakings.

I have more than many regards to present to all of you, though I don't seem to have enough pages to write them on, and so in a global sense… Thank You!

I have a wonderful bunch of friends who provide unwavering support for my writing endeavors, and whatever craziness would cross my mind at the moment. A magnificent family always ready to maintain my livelihood and spirit. Some great colleagues who where always eager to aid in any momentary lack of memory, clouding my judgment. And without a doubt not one but two amazing supervisors, who endured many non inspired moments and even then manage to encourage me a while more without any kind of pressure, which I'm not actually sure I totally deserved. And thought they were tireless in their teachings and managed to mitigate some of my ignorance, I asserted some personal character to a certain extent, so please know that any errors are of my own doing.

Working in the Microbial and Molecular Ecology Lab., under the supervision of Professor Margarida Reis has been very gratifying, and help me gain all sorts of knowledge, and develop my techniques.

I would like to thank everyone who has been through this lab, and in one-way or another has help me go forward with all the work. Special thanks to Sandra Mesquita, Filipa Esteves, Diana António, Cristina Costa, Sandra Caetano, Carla Freitas, Telma Valente and Catarina Diniz, for all their assistance and shared knowledge.

The fieldwork trips, with Professor Duarte Duarte, were some of the most enjoyable moments during this project, and besides providing with all the material for the analysis,

were also a way to catch some fresh air and exercise in the amazing scenario, that is the Ria Formosa.

A particular thanks is due to Maria Rodrigues, who was kind enough to assist us in fieldwork, when both Duarte and me were affected by injuries, and for all her assistance in the lab.

The conclusion of this work wouldn’t be possible without the indispensible help of Rodrigo Costa, Gianmaria Califano and Elham Karimi, for all their assistance, and for allowing me to use their equipment in the Microbial Ecology and Evolution Lab., when our systems failed.

Greetings are due, to Rita Andrade, Paulo Santana and Sónia Oliveira, for all their assistance with the sediment analysis, without which I would have struggled to find the correct way to treat and analyze my samples.

To all those who I assisted during this period, and helped me remember all the basic concepts, one sometimes tend to discard, Gisela Serrão, David Catita, Billy Hill, Nuno Magalhães, Fábio Anastácio, Nuno Soares, André Soares, Gonçalo Figueira…

Very special thanks to all my friends, and in particular to Ana Raposo, Alexandra Alves, Ricardo Alves, Pétala Almeida, Diana Rocha, Maria José Guedes, Patricia Silva and Micaela Justo, who endured most of my bad mood.

Never forgetting my family, and in particular my parents who were always supportive of all my choices, a unique acknowledgement is due.

Finally, to Cima and UAlg, for providing me with the infrastructures and opportunity to develop such project, and to all companies with whom I worked and who were essential for my ability to endure all this long period: Aquaexam, SeaExpert, FormosaMar, Lands.

Overall, thank you!

# Abstract

The number and variety of species present in an area and their spatial distribution is the common definition for diversity. In order to reveal eventual bacteriobenthos diversity patterns associated to the different origins of the surface layers from sediments of several environments, different sets of environments within the inlet boundaries of a coastal lagoon (Ria Formosa) were studied, taking in major account the hydrodynamics and temporal variation and how it might affect the different sediment environments, and it's biology. The general idea was to analyse the surface layer of given sets of sediments, and extract bacterial DNA that was aggregated to them, in the distinct locations. By doing so, the aim was to describe bacterial diversity in these environments, and find out how it could be related to different sediment origins. Are the bacterial populations the same in the different environments, or are there distinct pattern in their diversities? To ascertain the diversity of bacterial groups of different sediment environments, in the Ria Formosa Coastal Lagoon, a Nested PCR-DGGE (denaturing gradient gel electrophoresis) approach was used. Specific 16S cDNA primers were chosen according to the literature. After a first PCR (polymerase chain reaction) amplification, a second PCR was run in a more specific range within the first PCR product to produce a second PCR product, which was scattered by Denaturant Gradient Gel Electrophoresis (DGGE), creating specific group-patterns for further analysis. Statistical comparison between replicates and between different environments provided enough information to recognize specific bacteriobenthos diversity patterns.

Keyword(s): Diversity, Bacteriobenthos, Sediment Environment, DGGE, Ria Formosa

# Resumo

A diversidade pode ser definida como o número e variedade de espécies e a sua relação espacial, numa determinada área. De forma a observar eventuais padrões de diversidade bacteriobentónica associados com as diferentes origens das camadas superficiais de sedimentos de diferentes ambientes, pretendeu-se estudar diferentes conjuntos de ambientes sedimentares localizados nas imediações das barras do sistema lagunar da Ria Formosa, tendo em consideração o hidrodinamismo e a variação temporal e como poderão afectar os diferentes ambientes sedimentares e a sua biologia. Num contexto geral, pretendeu-se analisar a camada superficial de sedimentos, e proceder à extração de DNA das bactérias aderidas, nos diferentes locais. Pretendeu-se assim, avaliar a diversidade bacteriana nestes ambientes, e a forma como poderá estar relacionada com os diferentes ambientes sedimentares. Serão as populações bacterianas as mesmas nos diferentes ambientes, ou existirá um padrão distinto na sua diversidade? De forma a obter a diversidade dos grupos bacterianos de diferentes ambientes sedimentares, na Ria Formosa, procedeu-se à aplicação da técnica de "Nested PCR-DGGE" (electroforese desnaturante de amplicões obtidos em sucessivas reacções de PCR – reacção em cadeia da DNA polimerase). De acordo com a literatura, foram escolhidos fragmentos iniciadores («primer») específicos do 16S cDNA. Após a primeira amplificação, foi efetuada uma segunda com os produtos da anterior, resultando em amplicões mais específicos que foram subsequentemente separados por electroforese em gel desnaturante - DGGE, da qual se obtiveram perfis característicos. A comparação estatística entre os replicados e entre as amostras dos diferentes ambientes sedimentares, produziu informação suficiente para identificar padrões específicos de diversidade bacteriobentónica.

Palavra(s)-Chave: Diversidade, Bacteriobenthos, Ambientes Sedimentares, DGGE, Ria Formosa.

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*"As the oceans cover 70% of the earth's surface, marine sediments constitute the second largest habitat on earth, after the ocean water column, and yet we still know more about the dark side of the moon than about the biota of this vast habitat."*

*Gray & Elliott.*

# Introduction

## Aims

The aim of this project was to establish a correlation between the type of surface sediment intertidal environments of two natural inlets surrounding in Ria Formosa, and the general prokaryotes encountered, by the application of molecular methods for its detection and identification, and the evaluation of the effect of barrier islands dynamics in the microbial composition of the surface sediment environments, in a spatial-temporal context.

## Importance

This project was performed by the collection of data, from intertidal environments in Ria Formosa Coastal Lagoon, sampling its inlet surroundings. It contributes to the increase of knowledge about this vast system, through the molecular detection of prokaryotes present on specific surface sediment environments and by the

evaluation of the effect of the barrier dynamics on the microbial composition of such environments, according to its spatial distribution.

Through the integration of different field methods, a correlation between the type of sediments and organisms encountered in the environments was obtained, by applying molecular methods as analytical tools for its detection.

## Theoretical Framework

As stated by Gray & Elliot (2009) marine sediments constitute the second largest habitat on earth. Within coastal **sedimentary environments**, coastal lagoons represent more than 10% of the world's shoreline (Falcão & Vale, 1990), being defined by Kjerfve (1994) as, "shallow coastal water bodies separated from the ocean by a barrier, connected at least intermittently to the ocean by one or more restricted inlets, and usually oriented shore-parallel".

The formation and maintenance of **coastal lagoons** is due to sediment transport processes. Being carried by **rivers, waves, currents, winds, and tides** (Nichols & Boon, in Anthony et al., 2009). Coastal lagoons trap inorganic sediment and organic matter, serving as physical sinks or filters (Kjerfve, 1994). The sediment accumulates in river and tidal deltas, on marshes and flats where submerged aquatic vegetation slows the currents, and allows deposition (Anthony et al., 2009). The process of sedimentation can eventually fill lagoons (Nichols & Boon, in Anthony et al., 2009). Lagoons from humid temperate zones are refreshed by rainfall and run off from their catchments, typically having estuarine regimes (Bird, 1994). They often exhibit very high primary and secondary production rates and are valuable for fisheries and aquaculture, and sometimes for salt extraction (Kjerfve, 1994).

Accordind to Kjerve (1994), the hydrological characteristics of a coastal lagoon are determined partly by its configuration and the nature and dimensions of the connections to the sea, and partly by the bal**ance between freshwater and saltwater inflow**. This balance depends on one hand on evaporation, precipitation and freshwater input from **rivers**, **effluents** and **ground water aquifers** and on the other hand on seawater inflow and its relation to tidal range and tidal ventilation of the lagoon. **Sea-level** history, shore-face dynamics, and tidal range are three main factors that control the origin and maintenance of sandy barriers, and therefore coastal lagoons (Martin & Dominguez, 1994). Although seldom considered the wind also plays an important role in these environments.

Marine sediments can be classified by their source, size (Wentworth, 1922), shape, texture or composition and distribution. Sediment composition, in each environment, is mainly dependent on sediment source; sediment transport or deposition processes; and chemical processes occurring in the sediment environment and surrounding water column (Perry & Taylor, 2007). According to their source or origin, sediments can be lithogenous, biogenous, hydrogenous and cosmogenous (Goldberg, 1963). **Lithogenous (terrigenous) sediments** are rock-derived sediments formed by weathering processes, and transported from continent's soil and volcanoes, by wind, rivers, and currents. Their particles are deposited when the currents are too slow to keep them suspended. Wave action sorts the particles by size, moving the smaller ones further offshore, usually forming bands of sediments parallel to the shore. **Biogenous (biogenic) sediments** are of biological origin, as a result of organism's calcareous, siliceous, or phosphate-rich skeletal debris. Being scattered across a wide range of marine environments, biogenic sediments are mainly derived from planktonic organisms, when perished, settle throughout the water column till the

bottom, accumulating into layers. Others are a product of large organism's shells, coral reefs or plant-derived material, being the later mostly common in salt marshes and other coastal surroundings. **Hydrogenous (authigenic)** sediments are formed by the inorganic precipitation of seawater components as a consequence of chemical reactions favored by differences in geochemical and physical conditions, like the increase in pressure or temperature, proximity to hydrothermal vents, presence of chemical active substances, water properties, element saturation, oxidation state variation, among others. Because of authigenic slow accumulation rates they are more prompted to form within zones with little terrigenous and biogenic gathering, representing only a small portion of the total ocean sediments, although spread in various environments. **Cosmogenous (cosmogenic**) sediments, being of outer space origin are less abundant and the least significant part of ocean sediment. Resulting either from microscopic cosmic dust, pulled into Earth atmosphere or in less frequent cases from macroscopic meteor debris, most of which dissolves before reaching the ocean bottom. The composition of the sediment substrate is influenced by its location and respective environmental conditions; hence in deep sea sediments the predominance is of carbonate oozes, covering a large part of the ocean floor, whilst in costal and intertidal settings it is common to find biogenic production flooded by terrestrial inputs. Despite its origin, sediments are mostly described according to their shape and size. Ranging from coarse gravels in areas exposed to considerable wave and current action, to mud characteristic from low-energy estuarine areas or fine silts and clays in deep-sea sediments (Gray & Elliott, 2009).

Grain size is an important parameter both from a descriptive perspective and in relation to understanding sediment transport and deposition. For larger particles, measurements of three orthogonal axes are typically made and are used to calculate a

mean diameter. For smaller particles, grain size is typically determined by grading the samples through a set of sieves (see McManus 1988). A number of schemes have been devised to describe and measure grain size, but one of the most widely used is the Udden–Wentworth scheme (Fig. 1.4a).

Descriptions of sediment shape are somewhat more complex and may be taken to comprise elements of a particle's form, roundness and texture. Roundness is usually described on the basis of comparisons with visual identification charts. Form is also usually quantified by describing grains in terms of one of four standard classes: oblate, equant, bladed or prolate, which reflect the relationship between the short, intermediate and long axes of grains (Fig. 1.4b). Other useful schemes combine elements of both roundness and sphericity in visual comparison charts (e.g. Powers 1982). Particle sorting describes the range of grain sizes that occur within a sedimentary deposit (Fig. 1.4c) and can be calculated by measuring the dispersion of grain size around the mean. This is again a useful parameter as it can be used, along with grain size data, to infer information about the environments of sediment deposition and the history of sediment reworking (e.g. McManus 1988).

A **wide range of sediment environments** is usually found within coastal lagoons, among which fauna and flora have been widely researched. Yet, there is still a lack of knowledge on the complex **interactions between the microbiota** and its environment, be it the fauna, the flora or the sediment compartment (Gamito, 2006; Mirto *et al.,* 2004; Gomes & Mendonça-Hagler, 2004; Bertics & Ziebis, 2009; Pringault *et al.,* 2009; Palínska *et al.*, 2012).

**Prokaryotes** within coastal and shelf sediments play an important role in global biogeochemical cycles, and are the ultimate sink of most terrestrially derived compounds and a high proportion of marine particle flux (Hewson et al., 2007).

Studying microbial diversity associated with different types of sediments, especially bacteriobenthic diversity, should enlighten the contribution of different sediment environments to ecosystem dynamics. Nevertheless, despite their high abundances and reports of high diversity (Hewson et al., 2003), little is known about characteristic association of bacterial populations to different sediments. In fact, the marine sediment microbiome has been object of several studies; nevertheless no studies about **bacteriobenthic diversity** have been found for Ria Formosa coastal lagoon. Thus, the present study aims to be a first contribution to understand how bacteriobenthic diversity correlates to sediment characteristics and origin.

## Ria Formosa

The Ria Formosa, (Figure 1) is a **large shallow mesotidal coastal lagoon,** with a multi-inlet barrier system, extending along approximately 55km of the eastern part of Portugal's southern coast, being permanently connected to the sea through 6 narrow inlets, it has a high renewal rate, little freshwater or coarse sediment input, and therefore high values in salinity can be observed throughout the year. It is comprised of five barrier islands enclosed by two peninsulas, in the midst a system of sand flats and marshlands composed by tidal mud flats, salt marshes and partially dredged channels, of which only 14% are permanently flooded (Andrade *et al.*, 2004).

Washed by a semi-diurnal tidal period, with an average tidal range of 2,8 meters in spring tides and 1,3 meters in neap tides, it can reach a maximum range of 3,7 meters. Wave dominance is on 68% of the time from W-SW and 29% from E-SE, with a medium period of 4,7s.

Figure 1- Ria Formosa coastal lagoon 36º56'N 8º02'W to 37º03'N 7º32'W (Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community).

## Sediment environments

Like most global systems, the Ria Formosa **multi-barrier islands system**, is actively migrating towards the mainland, probably as a response to the current rise in mean sea level (Ceia, 2009). Due to littoral drift and wave dynamics, the ocean inlets can occasionally be sealed off by sediment deposition. In the Ria Formosa coastal lagoon, the **natural inlets are continuously migrating** towards east, until they reach a limit position in which they begin to be silted (São Luís, Armona, Fuseta and Lacém inlets). This is concomitant to the opening new **inlets** around the initial site, obligating this dynamic to restart west, where it originally begun (Weinholtz in Ceia, 2009) or where some inlets were fixed by rocky groins (Barra Faro-Olhão and Tavira inlets).

The **beaches** along the barrier islands are typically narrow and their behavior varies between being reflective through the western end (Martins et al. in Ceia, 2009) and intermediate to dissipative at the eastern end (Matias et al. in Ceia, 2009).

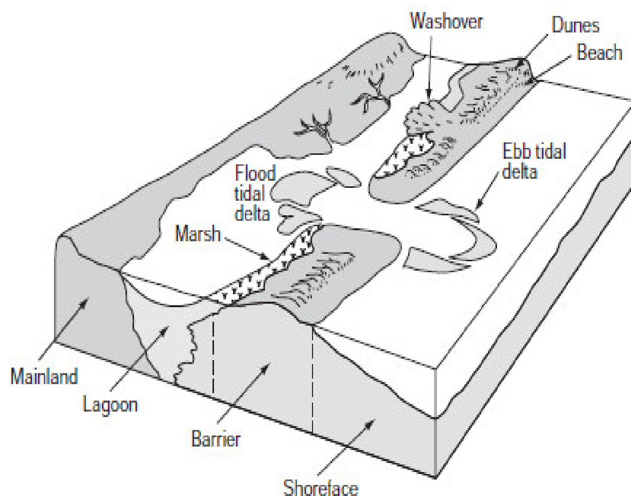



Figure 2 - (In Environmental Sedimentology, Temperate coastal environments by Andrew Cooper, 2007).

It is a large multi-inlet barrier island system, including mainland, **back-barrier lagoons**, **inlet deltas**, **barrier islands**, **barriers platforms** and **shore-face** (Pilkey et al., 1989 in Gamito, 2006). Due to the intense morphodynamics regime, climate and tidal currents, the growth /erosion rates of the barrier islands are very intense, making the system very unstable and vulnerable, under continuous change (Dias, 1988 in Ceia, 2009).

The type of **vegetation** and hydrodynamics of the unit constrict the several sediment environments. Numerous levels of intertidal platforms can be distinguished in Ria Formosa system. The **high marshes** are well established and secured by the vegetation, receiving sandy inputs coming from wash-over fans. Mediterranean saltmarsh scrubs dominate this environment. Sediment outputs are derived mainly

from the dismantling of high marshes by micro-depressions and minor tidal channels. The upper-middle marsh is covered by stands of the shrub sea-purslane (*Halimione portulcaoides*) and woody glasswort (*Sarcocornia fruticosa*), while the lower-middle marsh supports either flat- leaved *Spartina* swards or creeping glasswort (*Sarcocornia perennis*). Populations of glasswort (*Sarcocornia perennis*) and cord grass (*Spartina maritima*) colonize the lower levels (low marshes). With stronger dynamics, are the watts and tidal channels found (Rodrigues *et al*., 2005).

The **tidal channel**, **ebb-delta and saltmarsh** constitute the main depositional sub-environments of the barrier platform (Arnaud-Fassetta et al., 2006). The high-energy hydraulic conditions favor the strong transport capacity of the channel, limiting the deposition of fine-grained sediments in the adjacent fringe marshes. Thus, Ria Formosa inlet dynamics continuously changes sediment deposits of exposed channels. In a neap tide slack water phase the first millimeters of the surface layer sampled, are newly deposited.

An ongoing study of the morphodynamics of the floods tidal deltas of S. Luís and Fuseta inlets in Ria Formosa, focusing on the mass transport of water, sediments and nutrients in coastal systems (Alves, 2013) defines the following sediment environment units: marshland environments, tidal channels, tidal flats and lagoon beaches.

## Benthic Prokaryotes

The term benthos has its origin in the ancient Greek word ***βάθος*** (vathos), meaning depth. It refers to organisms living associated to the bottom of aquatic environments, be it oceans, lakes or rivers. In oceans, the benthic environment, or

benthal, extends from the shoreline (intertidal or eulittoral zone), descending along the surface of the continental shelf to the deepest oceanic trenches and submarine ridges, characterizing different ecosystems according to substrate composition and depth zonation. It ranges from organisms that live in and on sediments, those that inhabit rocky substrata, up to those residing on coral reefs, as well as on any other benthic organism (Steele, 2009). The composition, spatial distribution, and temporal variability of the benthos are profoundly affected by the nature of the substratum, being the result of a complex range of bio-physical-chemical interactions. In consequence, biological diversity may vary enormously between and within different types of sediments (Danovaro, 2010).

Sediments do not only represent shelter for benthic organism but are also feeding grounds for them, and due to the vast diversity of microbial populations (Kirk et al. 2004), the most complex and perplexing interactions can be uncovered. Distinctive types of macro- and microorganisms cohabit and interact in these complex and variable environments, where the role of plants within the substratum interactions should not be forgotten.

Marine sediments are extremely dynamic and interact continuous with the subjacent water column, being an important biological matrix, with high relevance in marine ecology. Organic matter deposition and degradation strongly influence sediment chemical composition. Prokaryotes take part in the oxidation, degradation and remineralisation of organic matter and consequent nutrient regenerations. They are capable of using the available organic matter in its dissolved form, which is essentially inaccessible to other heterotrophic groups. These organic compounds are incorporated into the bacterial biomass and reintroduced into food chains when bacterial cells serve as food for superior organism. Due to high quantities of DOM

(dissolved organic matter) and POM (particulate organic matter) in the estuaries water column and sediments, the bacterial processes have a primordial importance in these systems ecology. When these constituents are of high amount, the bacterial respiration causes oxygen depletion, resulting in hypoxic conditions, which may bring consequences into the ecosystem.

In general, in shallow coastal lagoons the water column does not present a lack of oxygen, due to its great interface with the atmosphere, and due to the high productivity from photosynthetic organisms. The turbulence triggered by the wind and high current dynamics increases the gas exchange and oxygen renewal, which is promoted by the overall shallow depth of the system. However, in sediments anaerobic conditions can be presented, as a result of the difficult renewal of interstitial water. On top sediments, organic matter is used by prokaryotes for respiration and biomass production (Danovaro et al. 2008), leading to oxygen depletion in subsurface layers. Prokaryotes are able to oxidize organic matter under these conditions, by processes of fermentation and sulfate reduction, from which many estuaries get the characteristic smell and color as a result of the production of sulfidric gas.

## Diversity

The two taxonomic domains of Prokaryotes, Bacteria and Archaea, can be found in marine sediments, thus impairing the general use of the word bacteriobenthos. In fact, benthic prokaryotes include benthic archaea as well as benthic bacteria. In this context, the concept of bacteriobenthos has also included archaea, especially since they are undistinguishable under light microscopy or general

cultivation methods. Actually, Archaea have been treated as other bacteria, until the development of molecular techniques, which revealed significant genetic distance between Archaea and Bacteria. Bacteriobenthos studies such as Roh *et al.* 2010, which did not use molecular methods providing taxonomic information, have in fact included all Archaea cell counts and activities in their bacteriobenthos results.

The Bacteria domain is sub divided in phyla: Acidobacteria, Actinobacteria, Aquificae, Armatimonadetes, Bacteroidetes, Caldiserica, Chlamydiae, Chlorobi, Chloroflexi, Chrysiogenetes, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Dictyoglomi, Elusimicrobia, Fibrobacteres, Firmicutes, Fusobacteria, Gemmatimonadetes, Lentisphaerae, Nitrospinae, Nitrospirae, Planctomycetes, Proteobacteria, Spirochaetes, Synergistetes, Tenericutes, Thermodesulfobacteria, Thermomicrobia, Thermotogae, Verrucomicrobia. (Benson, 2008)

As for Archaea, which comprise phyla: Crenarchaeota, Euryarchaeota, Korarchaeota, Diapherotrites, Geoarchaeota, Nanoarchaeota, Nanohaloarchaeota, Parvarchaeota, Thaumarchaeota, as well as the not yet classified archaea groups (Klenk, 2010).

A wide scope of archaea are found in extreme environments, forming sub categories established by the type of environment in which they are found. Extremophylic archaea include thermophiles, halophiles, acidophiles and alkilinophiles.

Nevertheless Archaea are not constrained to extreme settings, as they are growingly recognized as rather abundant within sea plankton, and can also be found as part of many animals microbiota. Extremely important in sediments are methanogens, which bear a type of metabolism exclusive to a group of Archaea, that

are strictly anaerobic and chemolithotrophs, generating methane as an outcome of $CO_2$ reduction.

As shown, in Table I, amongst the phyla included in the Bacteria domain the *Choloflexi* and *Gammaproteobacteria* are common in sediments, while recognition of Archaea in sediments only encompasses the phyla Crenarchaeota, Euryarchaeota (Schaechter, 2009).

Table I – Phyla encountered in sediment environments according to Bai (2012).

| **Bacteria in sediments** |
|---|
| Actinobacteria |
| Acidobacteria |
| Bacteroidetes |
| Chloroflexi |
| Firmicutes |
| Planctomycetes |
| Alphaproteobacteria |
| Betaproteobacteria |
| Deltaproteobacteria |
| Epsilonproteobacteria |
| Gammaproteobacteria |
| Uncultured or unclassified Bacteria |

To date, most studies examining prokaryote population's diversity within sediments have focused upon functional groups of bacteria, on single locations or environment types, as well as on effects of biological and chemical factors upon microbial assemblage's composition (Hewson et al., 2007). Microbes as

macroorganisms in an ecosystem are structured by temporal and spatial variability of physicochemical and biotic parameters (Hewson et al., 2007). It is well known that dense aggregations of bacteria colonize clean surfaces of hard or soft substrates fairly and promptly, through a succession that leads to a biofilm of adsorbed organic molecules and particles, thus being pioneers in the biofouling process (Thiyagarajan et al., 2010). In fact, each sediment particle provides clean surfaces for colonization and biofilm development, which might be limited by changing environmental factors such as abrasion, hydrodynamics, as well as nutrient and oxygen flow.

## Functional groups and their sediment interactions

Diverse functional groups of prokaryotes are known to live in sediments, including aerobic heterotrophs, chemolithotrophs (like hydrogen oxidizing bacteria, sulfur-oxidizing bacteria, iron-oxidizing bacteria, nitrifying bacteria, methanogens and methanotrophs), fermentative bacteria (acetogens), anaerobic heterotrophs (nitrate-respiring bacteria, sulfur- and sulfate- reducing bacteria) and syntrophic bacteria (Zhang *et al.* in Thiyagarajan et al., 2010).

Surface sediment prokaryotes play an important role, due to their high abundance relatively to the overlying water column, they are a major component of microbial food webs, biogeochemical cycles and energy flow (Hewson et al., 2007). Even though the understanding of sediment habitats global biogeochemistry is increasing, much is to learn of the prokaryote populations that inhabit them or the factors that might influence their distribution (Hewson et al., 2007).

Bearing in mind that microbial distribution is strongly related to the sediment properties (Danovaro et al. 1999), combined with seasonal and environmental

changes, bacterial abundance may be a signal of variations in nutrient flux. The carbon cycle is perhaps the most affected by microorganisms (Rex et al. 2006), together with the known impacts on nitrogen phosphorus and sulfur cycles. Organic mater is decomposed, releasing several compounds of nitrogen, phosphate, sulfate and sulfite, and generating complex marine humus. (Parson et al.)

## Bacteriobenthos study limitations

Like in most fields of microbiology, species identifications in sediments can be determined either with culture-dependent or culture independent methods. However, culture-dependent methods can be misrepresentative of environmental samples, since many species will not grow *in vitro* and low occurring species might be outrun by more abundant and opportunistic ones. Furthermore these techniques are usually time-consuming, since they rely on the isolation and culturing of microorganisms, followed by their identification which is based upon several characteristics. Whereas culture-independent methods rely on the direct analysis of nucleic acids, directly extracted from the sample, allowing the analysis of the whole diversity in fewer or even a single step, being therefore more reliable methods for analyzing complex microbial populations. Nevertheless some biases can arise, within each metadological step.

Difficulties in studying bacteriobenthos, as simple as limitations on how to enumerate the existing bacteria have hindered the use of this ecosystem compartment in ecosystem studies. The emergence of new molecular techniques has made the study of bacteriobenthos not only possible, but highly productive.

Nevertheless, in the extraction of DNA or RNA from whole environmental samples, the recovery yields can be low or insufficient, and even with high yields PCR (Polymerase Chain Reaction) amplification can loose sensibility with the presence of inhibitors, or by unequally targeting of all potential organisms, promoting the recovery of highly abundant species over others less occurring ones. Furthermore PCR might be affected by annealing competition of PCR products over primer availability; low hybridization rates due to divergent denaturation of templates; primer mismatch; and the formation of chimeric amplicons, by the re-annealing of templates, resulting in the misinterpretation of the actual microbial diversity.

Aside from all identification methods, limitations come to pass in the early stages of bacteriobenthos studies. The merely planning of sample collections is it self dependent on so many factors, that one may think twice before taking on this endeavor. Be it on deep-sea or highly dynamic coastal ecosystems sediment collections, a wide range of variables have to be considered.

In coastal ecosystems, bacterial diversity detection and their spatial-temporal variation in surface sediments are of great practical and scientific relevance. Despite their importance, our knowledge of the bacteria that inhabit surface sediments is very limited, especially in the heterogeneous marine ecosystems (Thiyagarajan *et al.*, 2010). The type of habitat constricts the easiness of its study. When talking about a sediment environment subjected to tidal patterns, these patterns must be taken into account when sampling these environments as well as the climate, and environment characteristics.

In another front, after sample collections are done with, special attention has to be paid to the transport, storage and manipulation of samples, in order to prevent

post sampling microbial and sample properties modification, which can lead to its degradation and contamination.

## Type of analysis and procedures

The estimation of bacterial abundances as well as their genetic diversity under *in situ* conditions a fundamental objective of aquatic microbial ecology (Thiyagarajan *et al.*, 2010). In the past, quantitative and qualitative analyses of microbial communities have been hindered by the inability to cultivate most of the bacteria originally isolated from natural samples. In the mean time, molecular finger printing techniques, have provided new insights into our understanding of variation in bacterial diversity in natural samples, and their response to environmental heterogeneity. Thus these modern tools have provided a complement to culture-dependent techniques (Brito *et al., 2009)*.

Bacterial diversity in aquatic habitats has been investigated using a number of approaches, which range in their sensitivity, including culture-based methods and sequencing of cloned randomly amplified 16S rDNA fragments (Hewson *et al.*, 2003).

Over the past two decades molecular tools have been developed that allow the estimation of over 95% of bacterial taxa, based on fingerprinting techniques, such as 16S rDNA terminal restriction fragment length polymorphism (TRFLP) and automated rDNA intergenic spacer analysis (ARISA), allow the acquirement of whole-community estimates of bacterial diversity, with far greater results than culture estimation approaches (Hewson *et al.,* 2003). Among these methods, polymerase

chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) has provided satisfactory results in the evaluation of microbial diversity of a variety of complex environments (Muyzer, 1999).

The direct extraction of nucleic acids (DNA or RNA) is the baseline for a large range of research protocols that have been made regular, in the major research areas. These procedures are currently made using commercial extraction kits that have evolved from the genetic material precipitation basis. Most commonly the step performed after isolating the genetic material, is the amplification of the extraction product, generally done through the use of PCR (Polymerase Chain Reaction), which enables the production of millions of copies of a specific DNA sequence in approximately two hours. This automated process bypasses the need to use bacteria for amplifying DNA, through cloning techniques.

In order to perform Denaturing Gradient Gel-Electrophoresis (DGGE), a sample of the previous extracted product is taken and amplified by an optimized NESTED-PCR protocol version (Lane, 1991; Huer & Smalla, 1997, *in* António, 2011) for bacterioplanktonic assemblages. The NESTED-PCR technique consists in using two pairs of PCR primers in a single locus. The first pair is a 16S gene universal bacterial primer, the second pair consisting in a more specific range will bind within the first PCR product and produce a second PCR product that will be shorter than the first one, thus diminishing the probability of wrong locus being amplified by mistake.

Denaturing Gradient Gel-Electrophoresis, introduced by Muyzer in 1993 for the study of the microbial community structure, is one of the most sensitive electrophoretic techniques. It is a molecular fingerprinting method that separates DNA products generated by polymerase chain reaction (PCR) (Ercolini, 2002).

Overcoming the limitation left by agarose gel electrophoresis results, in which products of similar size gather in a single DNA band that is largely non-descriptive, it allows to separate amplicons by sequence differences, according to the differential denaturing characteristics of the DNA amplicons, held together by guanidine and cytosine enriched clamps attached to one of the primers (Muyzer, 1999). While they migrate through the polyacrylamide gel, DNA fragments pass through increasing concentrations of chemical denaturants, as they begin to denature the migration will drastically slow down, as a result different denaturant concentrations will relate to unique patterns of bands, which theoretically represent a different DNA sequence (Wilson & Walker, 2005).

The separation of double-stranded DNA fragments identical in length, although different in sequence, is best accomplished by DGGE analyses. In the PCR DNA fragment products it seeks the difference in stability of G-C pairing (3 hydrogen bonds per pairing) as opposed to A-T pairing (2 hydrogen bonds). The acrylamide gel containing a gradient of increasing DNA denaturants is the base for this process. It allows double-stranded DNA fragments to migrate better, while denatured DNA molecules become effectively larger and slow down or stop in the gel. Thus DNA fragments richer in GC will be more stable and remain double-stranded until reaching higher denaturant concentrations. Sequence differing DNA fragments may be separated in an acrylamide gel (Wilson & Walker, 2005). The helpfulness of DGGE in the analysis of microbial populations rests on the fact that amplicons with different C-G proportions will migrate to different positions in DGGE gels, allowing to differentiate single nucleotide variation (Muyzer *et al*., 1993).

As DGGE, Temperature Gradient Gel Electrophoresis (TGGE) (Muyzer *et al*., 1993), allow the separation of DNA fragments of the same length but with different

base-pair sequences. Temporal Temperature Gradient Gel Electrophoresis (TTGE), was initially introduced by Yoshino *et al*. (1991), as a modification of TGGE, the temperature of a gel plate, with a constant concentration of denaturing chemicals, increases gradually and uniformly with time, which makes it easier to modulate the temperature over time. Based on the melting temperature (Tm) of the analyzed sequences a temperature ramp can be produced, allowing

The term "estimated spatial equivalent gradient resolution" (ESEGR) of TTGE was introduced in order to compare the temporal temperature-denaturing gradient directly with the spatial chemical denaturing gradient. To calculate the ESEGR for TTGE, the temporal temperature increase (ramping rate) was converted into a hypothetical chemical denaturant increase by using a conversion factor of 0-3 $^{o}$C temperature increase per 1% chemical denaturant (Abrams and Stanton 1992) and related to the amplicon migration rate in the gel; the amplicon migration rate was determined in a TTGE gel below denaturation conditions for the fragment. The TTGE analysis, performed according to Bùrresen-Dale et al. (1997), showed an ESEGR of 2-3% $cm^{-1}$ chemical denaturant increase.

TGGE allows the removing of certain disadvantages of DGGE, like the necessary casting of a chemical gradient (Bùrresen-Dale et al. 1997),

TTGE separates PCR products into distinct bands, which in theory will represent different strains from a microbial population sampled, providing a genetic profiling over the diversity *in situ* (Hastings *et al.*, 2006).

Furthermore, the bands in the gels can be excised and sequenced, allowing thus a phylogenic analysis of the microbial population present in a given environment.

## Organic matter analysis

In sediments, organic matter can be found in the form of macro and micro molecules, originating from natural or anthropic sources, as a result of plankton, bacterial, plant and animal debris (Fabiano & Danovaro 1994, Fabiano *et al.* 1995, Danovaro *et al.*1999).

Bacteria quickly eat the less resistant molecules, such as the nucleic acids and many of the proteins. Sediment organic matter from plant and animal detritus, bacteria or plankton formed in situ, or derived from natural and anthropogenic sources in catchments. Sewage and effluent from food-processing plants, pulp and paper mills and fish-farms are examples of organic-rich wastes of human origin (Meyer-Reil 1994, Meyer-Reil & Köster 2000).

Total Organic Carbon (TOC) refers to the amount of organic matter preserved within sediment. Sediment nutrients are assessed as Total Nitrogen (TN) and Total Phosphorus (TP) concentrations, and have inorganic as well as organic sources. The amount of organic matter found in sediment is a function of the amount of various sources reaching the sediment surface and the rates at which different types of organic matter are degraded by microbial processes during burial (Deming & Baross, 1993).

Organic matter influences many of the physical, chemical and biological properties of soils. Some of the properties influenced by organic matter include soil structure, soil compressibility and shear strength. In addition, it also affects the water holding capacity, nutrient contributions, biological activity, and water and air infiltration rates  (Meyer-Reil & Köster, 2000).

## Statistical analysis

The combination of molecular and statistical methods can be very useful to differentiate microbial communities (Boon, 2002). The use of statistics provides a significant advantage for the non-ambiguous interpretation of the spatial and temporal functioning of microbial communities (Fromin, 2002).

Depending on the data and results obtained, the community structure and diversity of the bacterial groups from the different samples are to be further analyzed by adequate statistical strategies for analyzing the gel patterns, from single band examination to the analysis of whole fingerprinting profiles.

# Procedures

## Fieldwork

Two natural inlets of Ria Formosa Coastal Lagoon (Figures 3 and 4) were adopted for sampling, namely Barra de São Luís (BSL) and Fuzeta (FUZ).

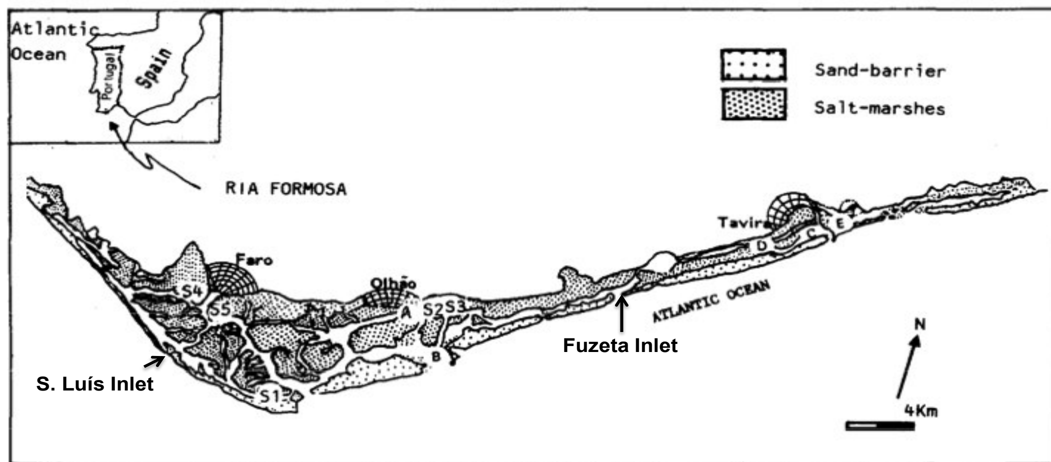



Figure 3: Map of Ria Formosa coastal lagoon, indicating the two inlets from which boundaries samples were collected (modified from Falcão e Vale, 1990).

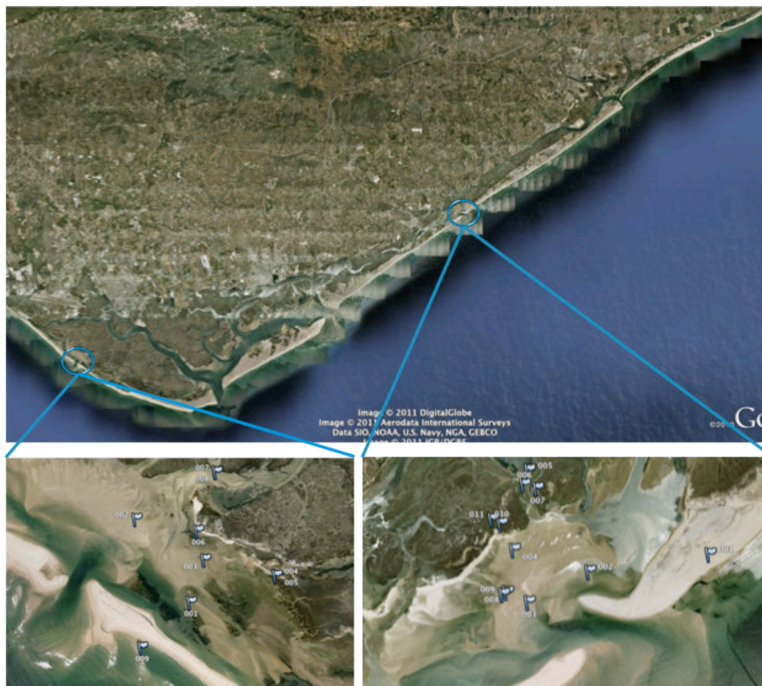


Figure 4: Satelite image of Ria Formosa, with a close-up view of the two inlets and the different sampling sites (Google Earth 2011).

In each of these inlets, nine different sediment environments (Figure 5) were chosen to be sampled, namely Intertidal flooding dunes (A), intertidal sand flats (B), low marsh/Spartina maritina (C), internal dune shore face (D), Zostera nolti prairie (E), intertidal sand flats with ebb dunes (F), intertidal sand channel (G), intertidal mud flats (H) and external dune shore face (I).

Figure 5: Sampling environments: A-intertidal flooding dunes (DEDE); B-intertidal sand flats (PIRE); C-low marsh/Spartina maritina (BSSM); D-internal dune shore face (FPIN); E-Zostera nolti prairie (PZNO); F-intertidal sand flats with ebb dunes (PIDV); G-intertidal sand channel (CIAP); H-intertidal mud flats (PILO); I-external dune shore face (FPEX).

## Sampling

Accounting for spatial and temporal variation, sampling strategy contemplated three sampling campaigns for BSL and two for FUZ, in Autumn and Winter 2010 and 2011, as detailed in table Table II.

Table II – Sampling schedule (Tidal info/seasonal distribution)

| | | Environment | | |
|---|---|---|---|---|
| | Coordinates | Nov/10 | Jan/11 | Nov/11 |
| BSL | 1 | DEDE | DEDE | DVDE |
| | 2 | PIPA | PIRE | DVDP |
| | 3 | BSSM | BSSM | BSSM |
| | 4 | FPIN | FPIN | FPIN |
| | 5 | PZNO | PZNO | PZNS |
| | 6 | PIDV | PIDE | PIRV |
| | 7 | MCIA | MCIA | MCIA |
| | 8 | PILO | PILO | PILO |
| | 9 | FPEX | FPEX | FPEX |
| FUZ | 10 | - | FPEX | - |
| | 11 | - | DEDE | - |
| | 12 | - | PIDE | MCMA |
| | 13 | - | PIRE | PIRV |
| | 14 | - | PILO | PILA |
| | 15 | - | MCIA | PIMC |
| | 16 | - | BSSM | BSSM |
| | 17 | - | PZNO | PIPC |
| | 18 | - | FPIN | PIPC |
| | 19 | - | CIAP | PIDI |
| | 20 | - | - | CIAP |
| Tide | | 7h45 0.8 m | 7h42 0.8 m | 6h41 1.0 m |
| | | - | 8h25 0.7 m | 7h15 0.9 m |

At both inlets sampling was performed during ebb in spring tides, according to the schedule and tidal hights as presented in Table II.

The first trip made to BSL, in November 2010, was used to test collection procedures and for technical optimizations on experimental samples. A second trip was then made, as planed, in January, and sampling was done both in BSL and FUZ. The third trip was not possible till November 2011(Table II).

Two more sampling campaigns were made in order to collect samples for the construction of a reference molecular marker, used in the **DGGE/TTGE** for comparative analysis of DGGE patterns (Table III).

Table III – Molecular reference marker sampling campaingns.

| | Sampling site | Environment |
|---|---|---|
| SL Jul/11 | 1A | DE |
| | 2B | PIA |
| | 3C | FIDE |
| SL Jan/12 | 4D | PIA |
| | 5E | PZN |
| | 6F | CIA |
| ide | 10h04 0.8 m<br>10h48 0.7m | |

Sampling locations were reached by kayaking. Once arriving to the given spot, the georeferencial location was determined using DGPS (Differential GPS), seen in Figure 6-A, which was comprised by the Trimble 5800 RTK controler, the DGPS antena and a celphone to establish the conection with the Geographic Portuguese Institute (IGeo), according to the Datum 73 Hayford Gauss IPCC coordinates system.

Samples were taken with sterile seringes, previously cut with asseptic technique. Triplicate sediment cores were taken (Figure 6-B), for each point, and syringes were sealed with Parafilm®, stuck to the bottom side of the sediment core. At each site a fourth sediment sample was collected for further grain-size analysis (Figure 6-C). Sealed plastic bags provided the necessary containment for samples, that were immediately cooled in a thermal container. The samples were transported in a cooled thermic container to the laboratory, where they were immediately stored at -20ºC, till DNA extraction.

Figure 6: Sampling procedure:

A– Sampling moment;

B– Syringes;

C– Sediment container.

## Laboratory procedures

The samples collected were prepared, for all respectively laboratory analyses. Each point of triplicates (A, B, C) was used for molecular analysis, while the sediment sample (D), was divided into two parts: for organic matter percentage (%M.O.) determination, and granulometric analysis (Figure 7).

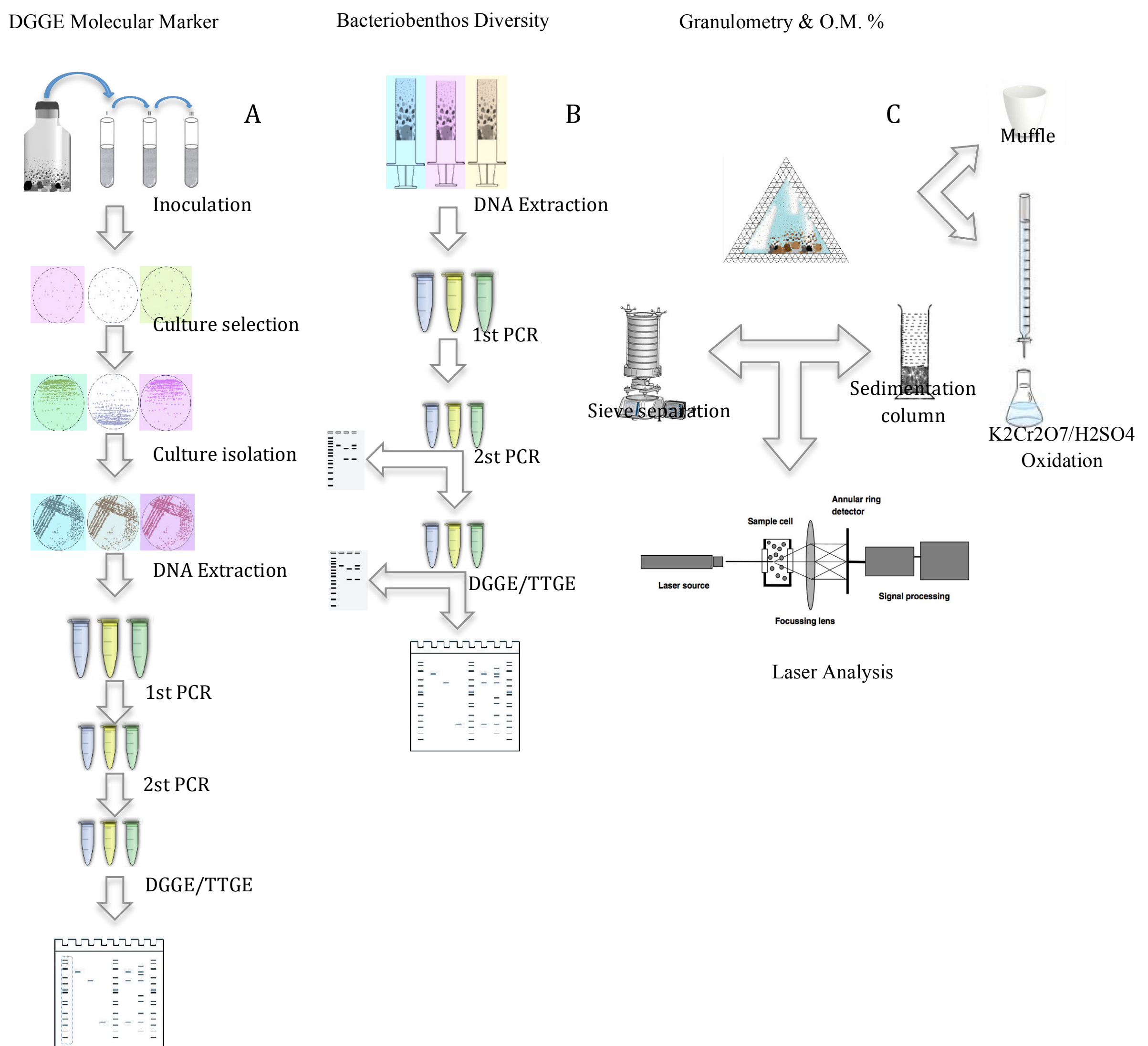



Figure 7: Procedures Schematics, with the several steps taken in each part of the laboratory analysis. A: DGGE molecular marker construction; B: Bacteriobenhos diversity analysis; C: Granulometric and organic matter analysis (Schematic diagram of a laser diffraction instrument based on the Malvern Instruments apparatus - Methods for the study of Marine Benthos, 2005).

## Geological analysis

Granulometric analysis is the assessment of particulates dimensions for a given sediment sample, conjugated by its corresponding statistical analysis.

Several methods for grain size analysis can be chosen, depending on the type of sample being studied. Most detrital sediment samples present mixed grain sizes, and therefore require the conjugation of several techniques for evaluating the full range sizes existing.

For evaluating grain size distribution, a part of the fourth sediment sample, for each site, was used. Being primarily separated into 3 groups: Fine, Coarse and Mixed sediments.

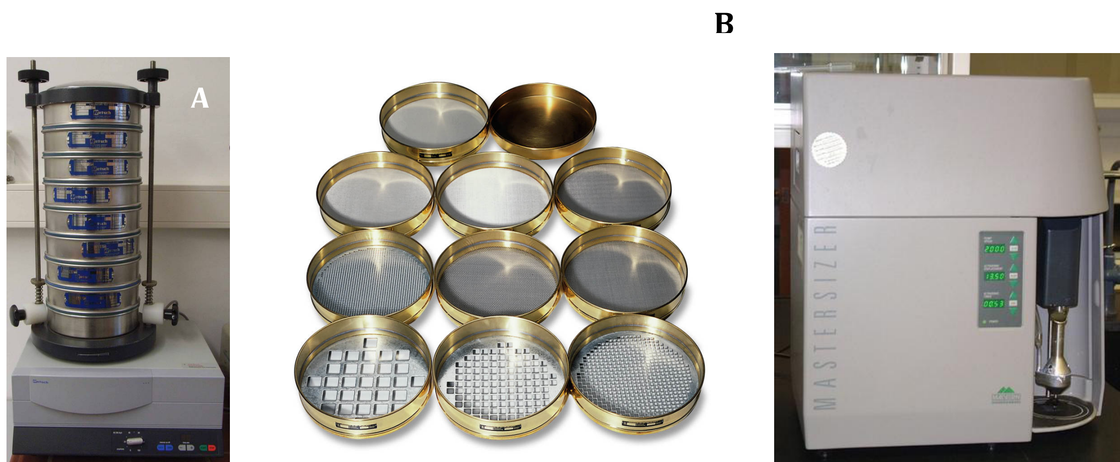



Figure 8: Granolumetric analysis equipment. A-Automatic sieving column; B-Sieves; C-Malvern Mastersizer.

## Fine Sediments: Laser diffraction particle sizing

Fine sediments, were laser analyzed in a "Malvern Mastersizer" 2000 laser sizer (Herrenberg, Germany). A sub-sample of each sand/silt samples was diluted with water and dispersant, to allow the separation of all grains. These results allowed the calculation of the mean particle size, later on referred as grain size.

Laser diffraction was used for particle sizing; this technique was developed over the past decades by several manufacters, being based on the measurement by low angle light scattering (LALS), generated by a laser beam passing through a suspended particle sample (Eleftheriou & McIntyre, 2005). Because the suspension of heavy sand or coarse samples is very difficult to obtain, the samples chosen for this technique were mainly composed of silt and clay, with some low amounts of sand in between, to avoid bias in the distribution. When required a pre-screening with a 63µm sieve was preformed, in which the larger particles were then sieved and separated according to the meshes sizes.

For the measurement of the samples a previous background measurement had to be taken to differentiate the total light scaterring generated by the sediment sample from the one from the one caused by the surrounding environment.

Measured values were exported into an Excel spreadsheet, and later on treated with Gradistat 8.0, by Simon Blott (2010).

## Coarse Sediments

Coarse sediments were completely dried at 40-50ºC, weighted and afterwards separated by automatic sieving (Figure 7-A). The series of sieves selected had meshes of 4,00mm, 2,80mm, 1,40mm, 1,00mm, 0,710mm, 0,50mm, 0,350mm, 0,250mm, 0,180mm, 0,125mm, 0,090mm and 0,063mm. Sieves were put together in a stack, with increasing mesh size, on the collecting pan; the weighted sample was laid on the uppermost sieve (biggest mesh size); the complete sieve stack filled with the sample material was placed on the sieve shaker and fasten, finally the timer was set for a period of 10-15 minutes with an amplitude of 40 ppm (per minute/per million). After

sieving, each remaining fraction of sediment, on each sieve and collecting pan, was weighted and the values were analyzed on a Microsoft Excel macro "Gradistat" (Blott & Pye, 2001), from which various textural parameters were obtained (Folk & Ward, 1957), through the logarithm method of Blott, 2001.

## Mixed Sediments

Mixed sediments were dried together with coarse samples and sieved in the same way, howeverthe fractions smaller than 0,063mm were submitted to a sedimentation test, the pipette method. In this, remaining samples wereplaced into a 1L water column, mixed with a dispersant (sodium hexametaphosphate or Calgon®), and stirred vigorously for 10 min to disperse all material. The column was put to rest to evaluate if no flocculation occurred, given so the column was mixed from the base up, until all particulates were scattered uniformly within the cylinder, ending with prolonged and even stroke vertically from the bottom of the column to the water surface (Folk, 1974).

A procedure data sheet by Dias (2004) was followed (Table IV); in which pre-determined intervals for each withdraw were schedule, hence each extract was made at the depth of 10 cm at 24ºC, by inserting a pipette and taking 20 ml of solution to a weighted petri dish. The pipette was then rinsed into the same petri dish, with distilled water to recover all sediment residues. This procedure was followed for each withdraw without remixing the columns, after which the petri dishes were placed at 40-50ºC, to evaporate all water, and thus weighted to at room temperature to determine the proportion of sediments residue remaining suspended at each given time.

Table IV –Withdraw intervals data sheet, for fine sediments granulometry depth test, in a 5-column essay (adapted from Dias, 2004).

| h | m | s | Column 1 | Column 2 | Column 3 | Column 4 | Column 5 |
|---|---|---|---|---|---|---|---|
| 0 | -02 | 00 | Agitation | | | | |
| 0 | 00 | 00 | 4 Φ | | | | |
| 0 | 01 | 45 | 5 Φ | | | | |
| 0 | 07 | 00 | 6 Φ | | | | |
| - | - | - | - | Agitation | | | |
| 0 | 11 | 00 | - | 4 Φ | | | |
| 0 | 12 | 45 | - | 5 Φ | | | |
| 0 | 18 | 00 | - | 6 Φ | | | |
| - | - | - | - | - | Agitation | | |
| 0 | 22 | 00 | - | - | 4 Φ | | |
| 0 | 23 | 45 | - | - | 5 Φ | | |
| 0 | 29 | 00 | - | - | 6 Φ | | |
| 0 | 28 | 00 | 7 Φ | - | - | | |
| - | - | - | - | - | - | Agitation | |
| 0 | 37 | 00 | - | - | - | 4 Φ | |
| 0 | 38 | 45 | - | - | - | 5 Φ | |
| 0 | 39 | 00 | - | 7 Φ | - | - | |
| 0 | 44 | 00 | - | - | - | 6 Φ | |
| 0 | 50 | 00 | - | - | 7 Φ | - | |
| 1 | 05 | 00 | - | - | - | 7 Φ | |
| - | - | - | - | - | - | - | Agitation |
| 0 | 56 | 00 | - | - | - | - | 4 Φ |
| 0 | 57 | 45 | - | - | - | - | 5 Φ |
| 1 | 03 | 00 | - | - | - | - | 6 Φ |
| - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - |
| 1 | 24 | 00 | - | - | - | - | 7 Φ |
| - | - | - | - | - | - | - | - |
| 1 | 50 | 00 | 8 Φ | - | - | - | - |
| 2 | 01 | 00 | - | 8 Φ | - | - | - |
| 2 | 12 | 00 | - | - | 8 Φ | - | - |
| 2 | 27 | 00 | - | - | - | 8 Φ | - |
| 2 | 46 | 00 | - | - | - | - | 8 Φ |

* Collection depth – 10 cm at 24ºC.

## Gradistat

Granulometric data was input Gradistat 8.0 (December 2010 version), from which statistical results were obtained, through the method of Folk, 1957. This methods allowed the determination of certain granulometric parameters as the Aritmetric and Geometric Mean, Calibration, Assimetric quoeficient, curtose quoeficient, textural group, amoung others (*Annex I – Gradistat data, Blott 2001).*

Results were treated according to the Udden/Wentworth scale (Table V) and ploted into Folk diagrams, by their textural composition fractions.

Table V: Modified grain-size scale from Udden (1914) & Wentworth (1922) for siliciclastic sediment, adopted by Gradistat.

| Grain Size | | |
|---|---|---|
| **ϕ phi** | **mm** | **μm** |
| **-10** | 1024 | |
| **-9** | 512 | |
| **-8** | 256 | |
| **-7** | 128 | |
| **-6** | 64 | |
| **-5** | 32 | |
| **-4** | 16 | |
| **-3** | 8 | |
| **-2** | 4 | |
| **-1** | 2 | |
| **0** | 1 | |
| **1** | | 500 |
| **2** | | 250 |
| **3** | | 125 |
| **4** | | 63 |
| **5** | | 31 |
| **6** | | 16 |
| **7** | | 8 |
| **8** | | 4 |
| **9** | | 2 |

| Decriptive term | |
|---|---|
| Very large | Boulder |
| Large | Boulder |
| Medium | Boulder |
| Small | Boulder |
| Very small | Boulder |
| Very Coarse | Gravel |
| Coarse | Gravel |
| Medium | Gravel |
| Fine | Gravel |
| Very Fine | Gravel |
| Very Coarse | Sand |
| Coarse | Sand |
| Medium | Sand |
| Fine | Sand |
| Very Fine | Sand |
| Very Coarse | Silt |
| Coarse | Silt |
| Medium | Silt |
| Fine | Silt |
| Very Fine | Silt |
| Clay | |

## Organic Matter Determination

After granulometric analysis, the rest of the fourth sediment sample was used for determining organic matter percentage (OM %) in each case, by the use of two different techniques, $K_2Cr_2O_7/H_2SO_4$ Oxidation and muffle furnace methods Procedures were followed according to *Annex II – Guerrero & Portela Determinação de Matéria Orgânica – Laboratório de Solos e Resíduos (Plataforma do Golfe)*.

Pearson's coefficient of correlation was made between components of the organic matter of surface sediments and granulometric variables.

## DNA extraction from sediments

For the extraction of total bacterial DNA, the sediment samples replicates were unfrozen 12h prior to the procedure, on a vertical position to drain off interstitial water. To avoid contamination, samples were prepared in a laminar flux chamber, using sterile bistoury, spatula and small petri plates, the top layer of the sediment sample was taken from the syringe, by inverting the plunger and pressing out the sediment. After scraping the surface, 2.0 - 5.0 mm of top layer sediment was cut and weighted, about 0.5 - 1g of collected sample was used for extraction, whereas the rest was reweighed and stored at -20$^{O}$C.

The extraction was made using MoBio Laboratories, Inc. UltraClean® Soil DNA Isolation Kit (*figure 10*), with the Alternative protocol - for maximum yields. The cell disruption (step 6 of protocol) was preformed in the Bertin Technologies PRECELLYS®24 tissue homogenizer/grinder automated equipment. A program of two cycles of 30 seconds at 6700rpm was chosen. The remaining steps (Figure 10)

were performed according to the original procedure (*Annex III – MoBio Aternative protocol (Maximum yields)*). The amount of DNA in the extracted samples was measured using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer. The extraction products were stored at -20$^{O}$C till subsequent PCR amplification.

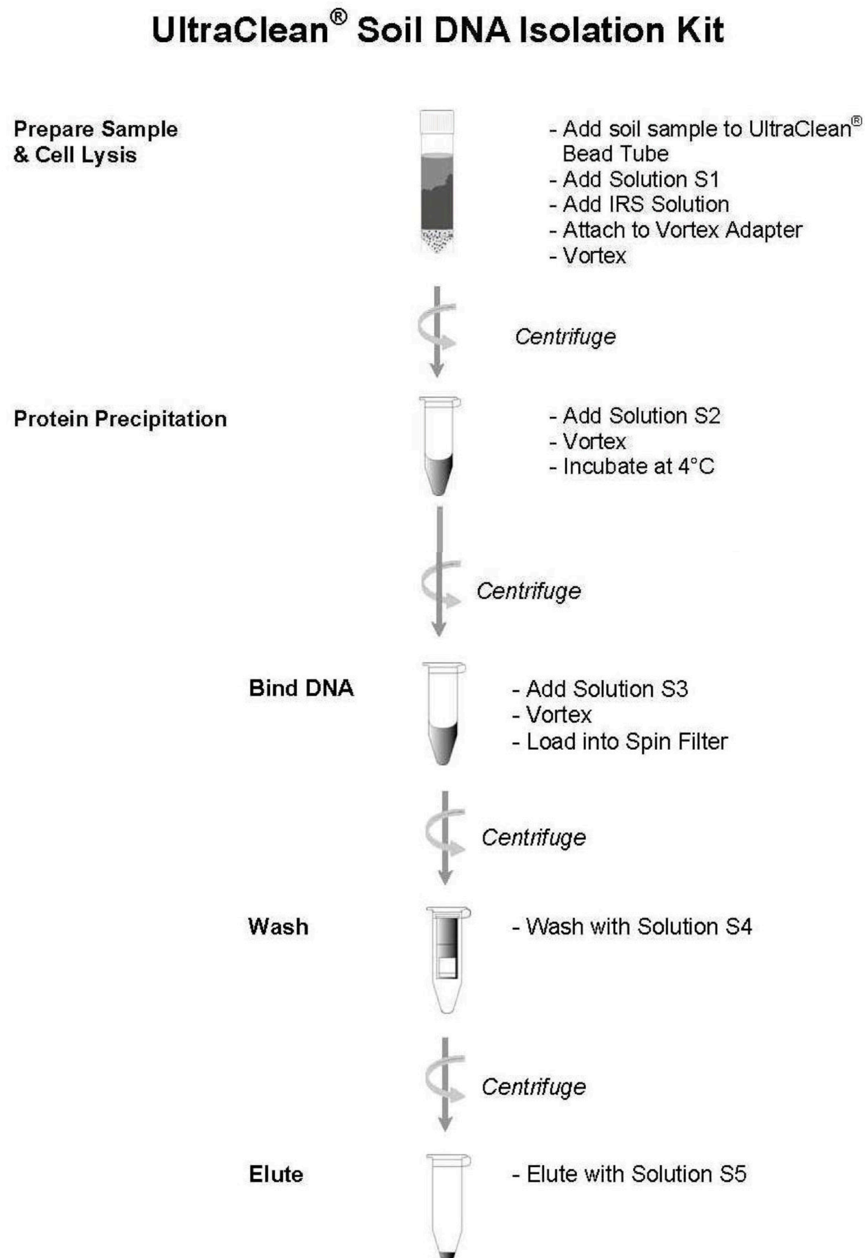



Figure 10: DNA extraction schematics (adapted from MoBio UltraClean® Soil DNA Isolation Kit protocol).

## Quantitative analysis of nucleic acids

Quantitative analysis using the NanoDrop® ND-1000 device, was performed according to the manufacturers manual, by pipetting 1.0 µl of the nucleic acid solution to the measurement pedestal. Concentrations were measured with UV-Vis at λ = 260 nm. Additionally, ratios of 260/230 nm and 280/260 nm were evaluated, to assess the purity of the sample.

## Primers

Table VI – Primers used in bacteriobenthos molecular identification.

| Primer | Sequence (5' - 3') | Specificity | Region | Tm (°C) | Reference |
|---|---|---|---|---|---|
| **1492 R** | 5’ TACGGYTACCTTGTTACGACTT 3’ | *Bacteria* | V9 (C8) | 54 | (Lane *et al*. 1991) |
| **27 F** | 5’ AGAGTTTGATCMTGGCTCAG 3’ | *Bacteria* | V1 (C1) | 54 | (Lane *et al*. 1991)) |
| **1378R** | 5’ CGGTGTGTACAAGGCCCGGGAACG 3’ | *Bacteria* | V8-V9 (C7) | 55 | (Heuer&Smalla, 1997) |
| **F968 GC** | GC Clamp - 5’ AACGCGAAGAACCTTAC 3’ | *Bacteria* | V5-V6 (C5) | 55 | (Heuer&Smalla, 1997) |
| **GC Clamp** | 5’ CGCCCGGGGCGCGCCCCGGGCGGGGCGG GGGCACGGGGGG 3’ | - | - | 90 | (Nübel *et al*., 1996) |
| **334fc**[a] | 5´ ACGGGGYGCASSAGKCGVGA 3’ | *Archaea* | V2-V3 (C2) | 54 | (Modified by Pires, 2010) |
| **Arch958R**[b] | 5` YCCGGCGTTGAVTCCAATT 3` | *Archaea* | V5-V6 (C5) | 54 | (Modified by Pires, 2010) |
| **Arch Bac 524-533F** | 5` GCCGCGGTAA 3` | *Archaea* | V3-V4 (C3) | 55 | (Isenbarger *et al*. 2008) |
| **Arch958R –GC**[b] | GC Clamp - 5' CCGGCGTTGAVTCCAATT 3` | *Archaea* | V5-V6 (C5) | 55 | (Modified by Pires, 2010) |

[a] Modified from ARC344f Bano et al. 2004.
[b] Modified from ARCH958R DeLong, 1992.

The adopted amplification procedure was a Nested-PCR technique. The first primer pair was a 16S gene universal bacterial primer, 27F (Table VI) and 1492R, producing a fragment with around 1400 bp of the rDNA 16S gene. The second pair targeted to a more specific range, F968 and 1378R that binds within the first PCR product and produce a second shorter PCR product of about 400bp, matching the

variable region V6-V8. This procedure diminishes the probability of the wrong locus being amplified. The forward primer for the second amplification was adapted for DGGE use, by adding a GC clamp introduced by Nübel *et al*. (1996).

## PCR Amplification protocols

The Nested-PCR amplification was based on an optimized NESTED-PCR protocol version (Lane, 1991; Huer&Smalla, 1997, in António, 2011) for bacterioplanktonic assemblages. All the primers used in this study are described in Table VI.

After several optimizations, the 1st PCR reaction mixtures (Table VII) were composed by 10 pmol of each primer (27F and 1492R), 200 µM of dNTPs, 1 x PCR buffer (Invitrogen™), 3.75 mM $MgCl_2$, 1 U Platinum®Taq DNA Polymerase (Invitrogen™), and 1% DMSO, combined with $H_2O$ (Sigma-Aldrich, Inc.) to 25µl in a 0.2 ml microfuge tube. After addition of 10 ng of template DNA, the mixtures were incubated in a VWR Collection DOPPIO® thermal cycler (Gene Technologies, Ltd.) programmed (Table VIII) with initial denaturation of double-stranded DNA for 5 min at $94^{O}C$; folllowed by 30 cycles consisting of 30 sec at $95^{O}C$, 30 sec at $55^{O}C$, and 30 sec at $72^{O}C$ and extension for 5 min at $72^{O}C$.

The 2nd PCR reaction mixtures were composed by 10 pmol of each primer (1378R and F968 GC), 200 µM of dNTPs, 1 x PCR buffer (Invitrogen™), 3.75 mM $MgCl_2$, 1 U Platinum®Taq DNA Polymerase (Invitrogen™), and 1% Acetamide adjusted with $H_2O$ (Sigma-Aldrich, Inc.) to 25µl in a 0.2 ml microfuge tube. After addition of DNA template diluted 1:5 from the 1st amplification products, the mixtures were incubated with initial denaturation of double-stranded DNA for 5 min at $94^{O}C$;

following 30 cycles of 30 sec at 95$^{O}$C, 30 sec at 55$^{O}$C, and 30 sec at 72$^{O}$C and extension for 5 min at 72$^{O}$C.

Table VII – PCR recipes

| Primer Set | 16S | 16S – DGGE | Primer Set | Arch | Arch - DGGE |
|---|---|---|---|---|---|
| **BufferGoTaq** | 1X | 1X | **BufferGoTaq** | 1X | 1X |
| **$MgCl_2$** | 3,75 mM | 3,75 mM | **$MgCl_2$** | 2,75 mM | 2,5mM |
| **DMSO/Acetamida** | 2% | 4% | **Formamida/Acetamida** | 1% | 2% |
| **dNTP's Mix** | 0,2 mM | 0,2 mM | **dNTP's Mix** | 2,5mM | 2,5mM |
| **PrimerFoward** | 0,2 µM | 0,2 µM | **PrimerFoward** | 0,1 µM | 0,1 µM |
| **Primer Reverse** | 0,2 µM | 0,2 µM | **Primer Reverse** | 0,1 µM | 0,1 µM |
| **GoTaq polymerase** | 0,625 U | 0,625 U | **GoTaq polymerase** | 0,5 U | 0,5 U |
| **DNA template** | 2-10 ng | 2-10 ng | **DNA template** | 2-10 ng | 2-10 ng |

Table VIII – PCR Protocol

| PCR step 16S | T (°C) | Time | Number of cycles | PCR step Archaea | T (°C) | Time | Number of cycles |
|---|---|---|---|---|---|---|---|
| **Initial denaturation** | 94 | 5 min. | 1 | **Initial denaturation** | 94 | 5 min. | 1 |
| **Denaturation** | 94 | 1 min | | **Denaturation** | 94 | 1 min | |
| **Annealing** | 56 | 1 min | 25 | **Annealing** | 58 | 1 min | 30 |
| **Elongation** | 72 | 1 min 30 sec | | **Elongation** | 72 | 1 min | |
| **Final elongation** | 72 | 10 min | 1 | **Final elongation** | 72 | 7 min | 1 |
| **PCR step 16S-DGGE** | **T (°C)** | **Time** | **Number of cycles** | **PCR step Archaea - DGGE** | **T (°C)** | **Time** | **Number of cycles** |
| **Initial denaturation** | 94 | 2 min. | 1 | **Initial denaturation** | 94 | 5 min. | 1 |
| **Denaturation** | 95 | 30 sec | | **Denaturation** | 94 | 1 min | |
| **Annealing** | 53 | 45 sec | 25 | **Annealing** | 50 | 1 min | 36 |
| **Elongation** | 72 | 30 sec | | **Elongation** | 72 | 1 min | |
| **Final elongation** | 72 | 10 min | 1 | **Final elongation** | 72 | 7 min | 1 |

## Electrophoresis

To confirm the success of the PCR, both steps of amplification were analyzed by electrophoresis on a 1% (m/v) agarose gel (SeaKem® LE Agarose - Lonza Rockland, Inc.) in 1X Tris–acetate–EDTA (TAE), using the ready-to-use ZipRuller™Express DNA Ladder Set (Fermentas, Life Sciences), broad range from 100bp to 20000bp (*Annex IV - Zip Ruler Fermentas*), allowing the comparison in size an intensity of the bands obtained. Gels wer precasted with GelStar™ Nucleic Acid Gel Staining (Lonza Rockland, Inc.) or GreenSafe Premium (NzyTech, Lda.) or post-stained, after electrophoresis, in GelRed® Nucleic Acid Gel Stain (Biotium, Inc.) for 30 min and washed in TAE before being viewed and photographed under ultraviolet (UV) illumination (312 nm).

## Purification

Some amplification products were purified, for better screening, using the NZYTech NZYGelpure® purification kit (Annex V – nzytech for PCR clean up protocol).

## DGGE and TTGE of 16S rDNA amplicons

DGGE and TTGE were performed using the Temporal Temperature Gel Electrophoresis – TTGE-2401/240v Model (*C.B.S. Scientific, Company Inc.*). The apparatus was prepared according to the instructions (*Annex VI– Intructions for apparatus preparation, glass plate's mouting and gradient making)*.

Two different solutions were prepared to obtain the DGGE polyacrylamide gel (6 to 9% acrylamide/bisacrylamide [Rotiphorese® Gel 30 (37,5:1), Carl Roth GmbH + Co. KG] in 1x TAE buffer; using a denaturant gradient ranging from 32 to 60% [where 100% denaturant was 7 M urea (Merck Millipore) and 40% deionized formamide (Eurobio Laboratoires)]. A gradient of both solutions was then pumped into the assembled gel plate sandwich, using the Linear Gradient Maker GM-40 (C.B.S. Scientific). Polymerization was obtained by adding 0,05% - 0,06% (wt/vol) ammonium persulfate (APS) and 0,1% - 0,14% N,N,N′,N′-tetra-methylethylenediamine (TEMED), added immediately before gel casting.

The Nested-PCR products were loaded onto the wells in approximately equal amounts of DNA, with loading dye (saccharose 50%, bromophenol blue 0.1%), and electrophoresis was carried out in 1x TAE buffer at 160 V for 16 h at 60°C. A marker composed by 8 bands, a mixture of the 16S rDNA of bacterial fragments, was included in each gel. Additionally, to help ensure the correct run of the DGGE, an extra lane with the ready-to-use ZipRuller™ Express DNA Ladder 2 (Fermentas, Life Sciences), was loaded in each gel. (*Annex IV – zip ruller bands)*

TTGE gels were initially made with an 45% urea/formamide denaturing for 7,5% Acrylamide, however even though the running temperature was adjusted to denaturant in the gel, to much denaturation was observed in the gels, hence gels were subsequently made without chemical denaturing gradient.

The new TTGE were adapted from Roudière *et al.* (2009), being composed of 8% acrylamide/bisacrylamide [Rotiphorese® Gel 30 (37,5:1), Carl Roth GmbH + Co. KG] and 7 M urea (Merck Millipore), in 1X TAE. For polymerization 0.1% (wt/vol) TEMED and APS was added to the mix immediately before casting of the gels. Gels were run at 46V with a temperature ramp from 63 °C to 70 °C during 16 h (0.4 °C/h.)

in 1X Tris–acetate–EDTA buffer at pH 8.4. A pre-migration step was introduced, comprising of a 15min run at 20V and 63ºC.

After electrophoresis, the gels were stained using an adapted a silver staining method (*Annex VII – Silver nitrate staining protocol)*, and scanned after drying. Later on, to try to obtain better resolution, the gels were stained in several fluorescence dyes, the latest employed was SYBR® Gold Nucleic Acid Gel Stain (invitogen$^{TM}$) at a 1:10,000 dilution, for 15 to 30 min and washed in 1X TAE, prior to visualization.

## Preparation of a Reference Marker from Bacterial strains

To allow comparative analysis of DGGE patterns, a marker containing standard collections and bacterial isolates PCR products, amplified with 16S primers, was added to every gel. This marker was composed by 16S amplicons of DNA extracted from NCTC (Health Protection Agency) *Escherichia coli 9001, Enterobacteraerogenes 10006, Enterococcus faecalis 775, Pseudomonas aeruginosa 10662, Staphilococcusaureus 6571, Closrtidium pertringeus 13170;* and isolates form cultures, maintained in Laboratório de Ecologia Molecular e Microbiana (CIMA-UALG), of *Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Bacillus licheniformis, Bacillus subtilis* and a marine isolate designated as H4. In addition, to create the molecular marker these standard bacterial strains were mixed with amplified DNA, obtained from the sediment samples bacterial isolates.

The sample sediment bacteria were obtained by suspending sediments with sterile aged seawater (SASW), and diluted three times. The different dilutions were then plated into “ZoBell” marine agar plates. Isolated colonies were purified, by repeated streaking on the same type of plates, in order to obtain pure clones.

From each isolate a bacterial suspension was centrifuged for 5 min at 4500 G to pellet the bacteria, a colony was suspended in 50µL lysis buffer (0.05% SDS; 0.03 M NaOH), incubating at 95ºC for 15 min., after which 450 µL of distilled water was added and centrifuged for 5 min. at 15000 G (13000xrpm), 1 µL of supernatant was hence used in a 16S PCR amplification. The marker combination was obtained by analyzing the migration behavior of each amplicon; hereafter the ones with different migration distances, on the DGGE gel, were combined (1x each) into a reference lane.

In similarity to Roudière et al. 2009, additional runs were made in which two different loads were made into DGGE wells, one of the loads consisted barely of the amplification product, and another containing a mix of the product with known standard isolates from the reference marker.

Marker samples were stored at −20°C and thawed in ice before each use.

## Genetic Diversity Data Analysis

The analysis of the DGGE gel was done using Gelcompar 4.0 program (Applied Math, Ghent, Belgium) software package by Smalla *et al.* (*2001*).

A data matrix was loaded into the program together with the gel image, lanes and bands were identified and selected, for a final comparison, where several similarity coeficients where tested.

## Other Techniques…

Several optimization steps were performed; including procedures form several different techniques. Described are solely the final optimizations acquired through a long lasting sequence of studies.

## Results and Discussion

To help define the several environments sampled, a list of nomenclature and abbreviations was adopted, as shown in table IX; the correspondence of each sampling point with its respective environment and assigned code is also shown bellow, on table X.

Table IX – Sediment environment adopted nomenclature.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **PILO** | Intertidal mud flats | **PZNS** | Buried *Zostera nolti* prairie | **FPIN** | Internal dune shore face | **PILA** | Intertidal mud flats with green algae |
| **MCIA** | Intertidal sand channel | **PIRV** | Intertidal sand flats with ebb ripples | **CIAP** | Intertidal protected sand channel | **PIMC** | Intertidal sand flats channel with macroalgae |
| **BSSM** | Low marsh (*Spartina maritina*) | **PIDV** | Intertidal sand flats with ebb dunes | **DVDE** | Intertidal ebb dunes | **PIPC** | Intertidal sand flats with shells |
| **PZNO** | *Zostera nolti* prairie | **MCMA** | Intertidal sand channel margin | **DVPD** | Intertidal ebb dunes in flooding delta periphery | **PIDI** | Intertidal sand flat at the base of the internal dune shore face |

Table X – Respective sampling point's codes and environments.

| Sampling Point | Code | Environment | Sampling Point | Code | Environment |
|---|---|---|---|---|---|
| **O301101** | **BJ01** | DEDE | **O4011101** | **FJ01** | FPEX |
| **O301102** | **BJ02** | PIRE | **O4011102** | **FJ02** | DEDE |
| **O301103** | **BJ03** | BSSM | **O4011103** | **FJ03** | PIDE |
| **O301104** | **BJ04** | FPIN | **O4011104** | **FJ04** | PIRE |
| **O301105** | **BJ05** | PZNO | **O4011105** | **FJ05** | PILO |
| **O301106** | **BJ06** | PIDE | **O4011106** | **FJ06** | MCIA |
| **O301107** | **BJ07** | MCIA | **O4011107** | **FJ07** | BSSM |
| **O301108** | **BJ08** | PILO | **O4011108** | **FJ08** | PZNO |
| **O301109** | **BJ09** | FPEX | **O4011109** | **FJ10** | FPIN |
| | | | **O4011110** | **FJ11** | CIAP |
| **O8111103** | **NF03** | MCMA | **O9111101** | **NB01** | DVDE |
| **O8111104** | **NF04** | PIRV | **O9111102** | **NB02** | DVDP |
| **O8111105** | **NF05** | PILA | **O9111103** | **NB03** | BSSM |
| **O8111106** | **NF06** | PIMC | **O9111104** | **NB04** | FPIN |
| **O8111107** | **NF07** | BSSM | **O9111105** | **NB05** | PZNS |
| **O8111108** | **NF08** | PIPC | **O9111106** | **NB06** | PIRV |
| **O8111109** | **NF09** | PIPC | **O9111107** | **NB07** | MCIA |
| **O8111110** | **NF10** | PIDI | **O9111108** | **NB08** | PILO |
| **O8111111** | **NF11** | CIAP | **O9111109** | **NB09** | FPEX |

## Grain size analysis

The Folk diagram (1954) bellow shows the ratio between sand and mud (silt+clay) in all sample locations. The use of the Udden scale (1914) and Wentworth scale (1922), projected and treated by GRADISTAT® software, allowed the adoption of the appropriate nomenclature for each sediment sample.

For all analyzed sediments, the grain size sediment textural parameters (gravel %, sand % and mud %) were projected in Figures 11-14. Thus through the percentual representation of the textural class distribution in the sampled sediments, these diagrams will characterize them based on the 3 triangular vertices, as 3 main classes present in typical marine sediments, these being Gravel, Sand and Mud.

Most of the samples analyzed were classified as sands, ranging from muddy sand to sandy gravel, as shown in the diagrams below. The average percentage of each textural class, was therefore higher for sands, showing a value of 76.3%, whereas only 15.6% of mud and 8.1% of gravel were observed.

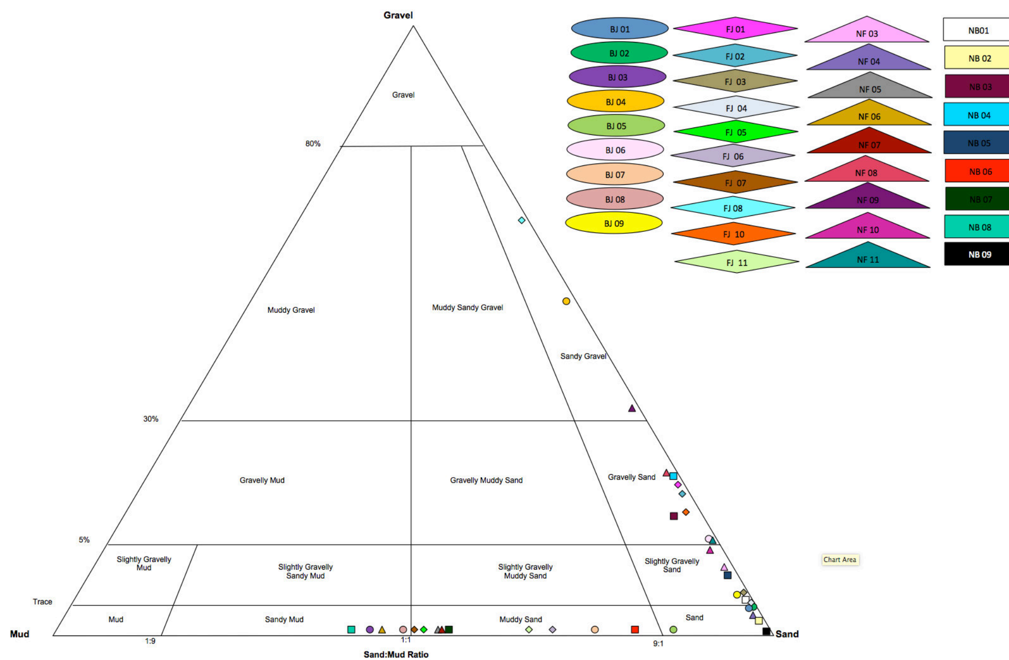



Figure 11 – Folk Gravel, Sand and Mud diagram, obtained by sediment samples grain size analyses in GRADISTAT.

While most samples presented an amount of sand above 80.0%, only a few samples could be set apart from the others, either: (i) by a higher content of small particles, thus belonging to the muds, or (ii) by being composed by coarser particles, and therefore acquiring the classification of gravel sediments. Samples BJ03, BJ08, NB08 and NF06 were included in the first case, with mud contents of 56.8%, 51.8%, 59.9 and 54.6%, respectively and thus, named as sandy mud sediments. Three samples had relevant higher gravel percentages, respectively of 56.8%, 70.2% and 35.7%, namely samples BJ04, FJ08 and NF09, which were therefore considered as sandy gravel sediments.

Separated diagrams within the different sediment types allowed for a better comparison between samples (Figures 12-14).

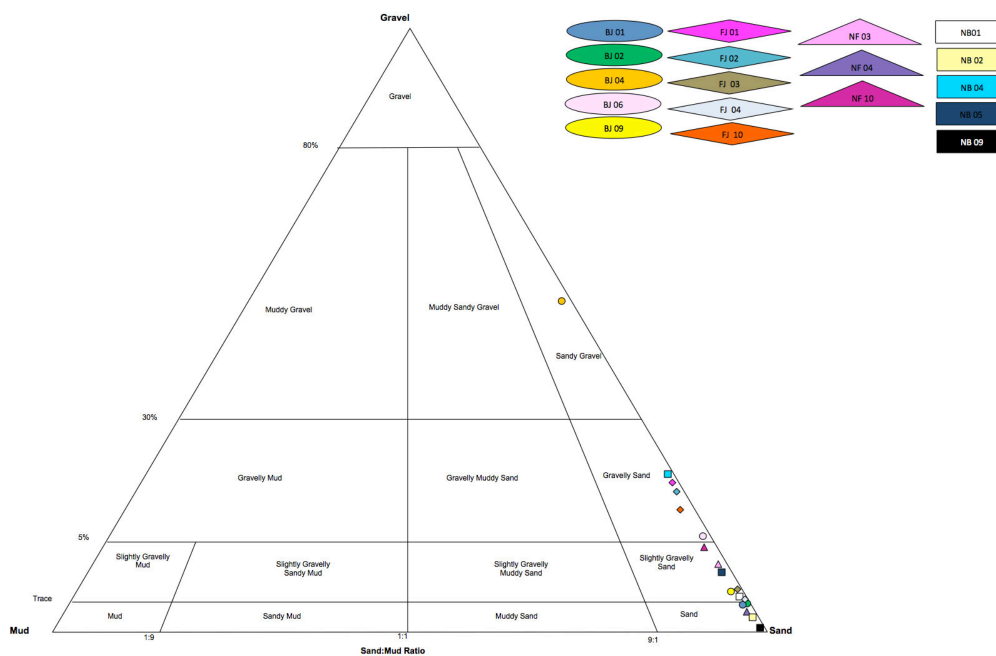



Figure 12 – Folk Gravel, Sand and Mud diagram, obtained for coarse sediment size analyses with GRADISTAT.

Coarse sediment samples, separated by the sieve method, were found to be mainly composed by gravely sand and sand (Figure 12), after data treatment with GRADISTAT. One sample stood out from all others, BJ04, by having 56.8% gravel, mentioned before as being a sandy gravel sample. As for the remaining coarse

sediment samples, five were considered as gravely sand, being these NB04 (21.2%), FJ01 (18.8%), FJ02 (16.7%), FJ10 (14.0%) and BJ06 (7.2%), and apart from these all others samples presented values above 95.0% of sand, being NB09 (99.9%) the one with the highest percentage within the coarse sediment samples and in general.

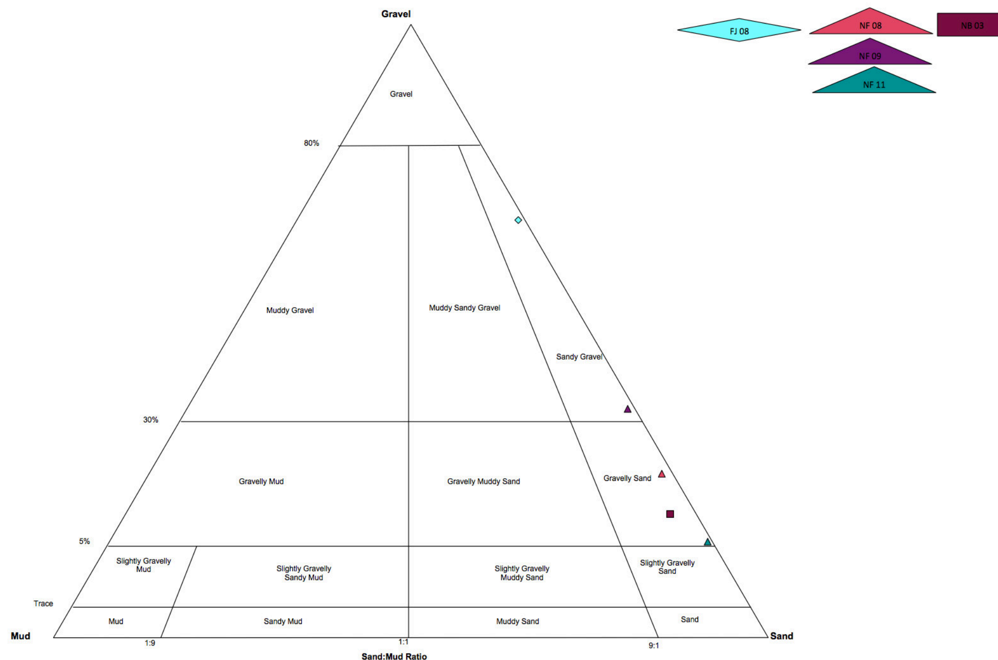



Figure 13 – Folk Gravel, Sand and Mud diagram, obtained for mixed sediment size analyses with GRADISTAT.

After analyzing data for mixed sediment sorting (Figure 13), one sample was found to be coarser than the ones previously analyzed as coarse sediments. The sample FJ06, with 70.2% gravel, is the coarser sample of them all, followed, within the mixed samples, by NF09 with 35.7% and thus forming sandy gravel in this group of mixed sediment samples. In these sediments, besides sandy gravel the presence of gravely sand with percentages of gravel of 21.4% (NF08), 15.3% (NB03) and 7.9% (NF11) was also observed, contrasting with the fine sediments samples which were predominantly constituted by sandy mud and muddy sand (Figure 14).

In Figure 14, a single sample was predominantly composed by sand, though in lesser amounts than most samples in the other groups. The green circle representing BJ05, stood as sand sample, composed of 90.6% of sand.

All other samples in this group had less than 90.0% of sand, with special emphasis for sandy mud samples, mentioned before as having higher amounts of mud, which presented less than 50.0% of sand.

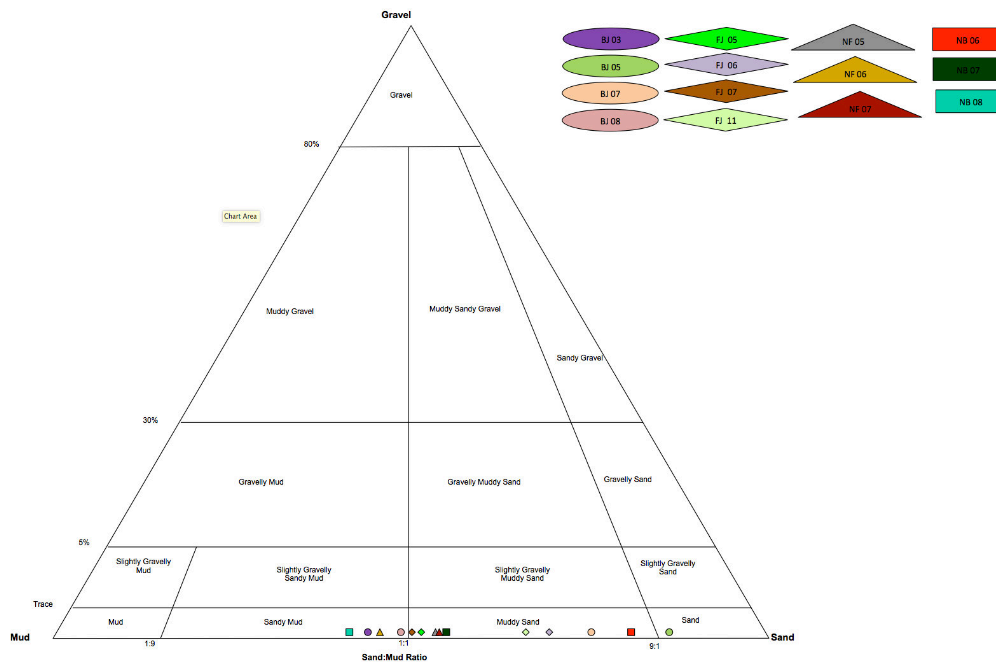


Figure 14 – Folk Gravel, Sand and Mud diagram, obtained for fine sediment size analyses with GRADISTAT.

From the same GRADISTAT data analysis a different diagram was acquired, this time showing the ratio between silt and clay, as shown bellow in Figures 15-18. These figures confirmed the results from the previous Gravel, Sand and Mud diagram, by showing that most samples were manly composed by sand. Like the previous diagrams, these are a percentual representation of the textural class distribution in the sampled sediments, in 3 main classes, yet in this case in Clay, Silt and Sand.

These new diagrams allowed a better understanding of the composition of the fine sediments, as these were, as seen before, the only ones with an amount of mud worth considering.

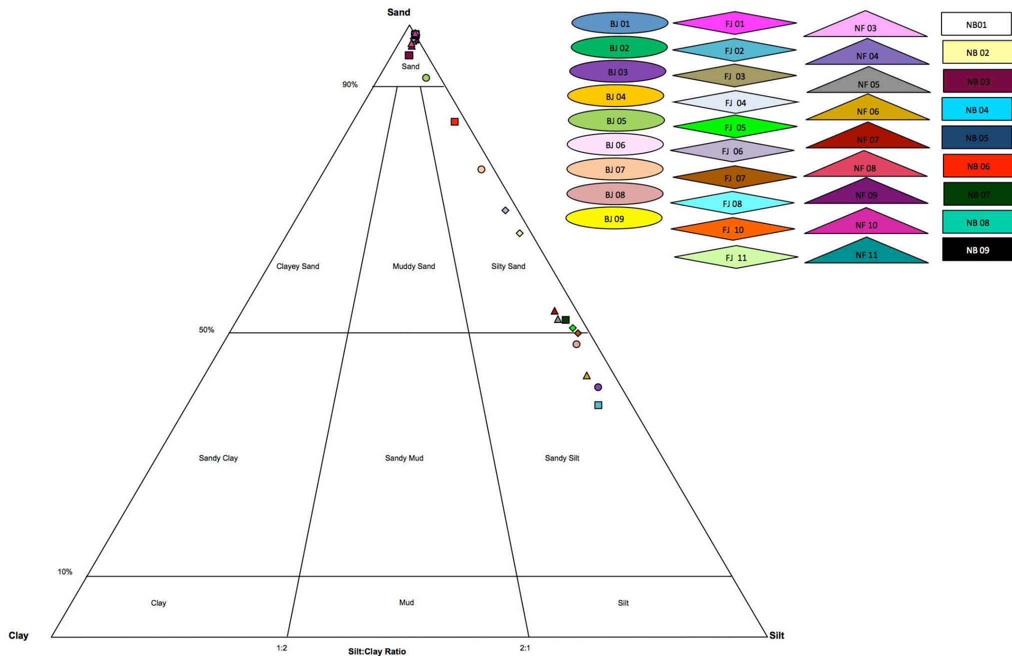


Figure 15 – Folk Sand, Silt and Clay diagram, obtained for sediment size analyses with GRADISTAT.

Coarse and mixed sediments revealed to be entirely composed by sands (Figures 16 and 17), a minimum value on the amount of sand was observed for the sample FJ08, in the mixed sediment group (Figure 16), with a value of 82.4% sand, but also composed by 15.4% gravel, and therefore having less than 10.0% of fine particles.

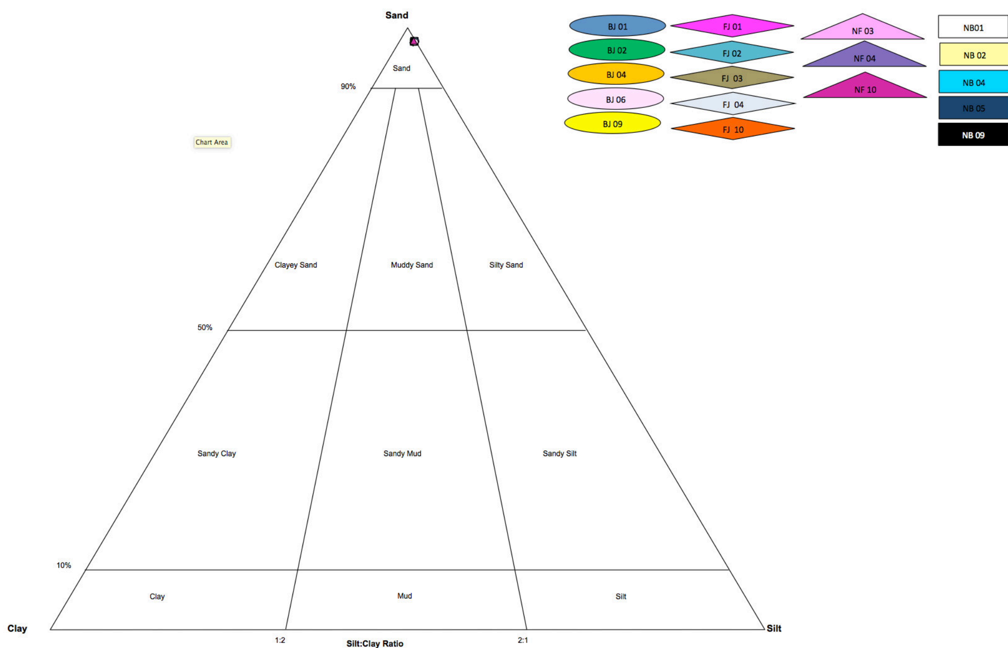


Figure 16 – Folk Sand, Silt and Clay diagram, obtained for coarse sediment size analyses with GRADISTAT.

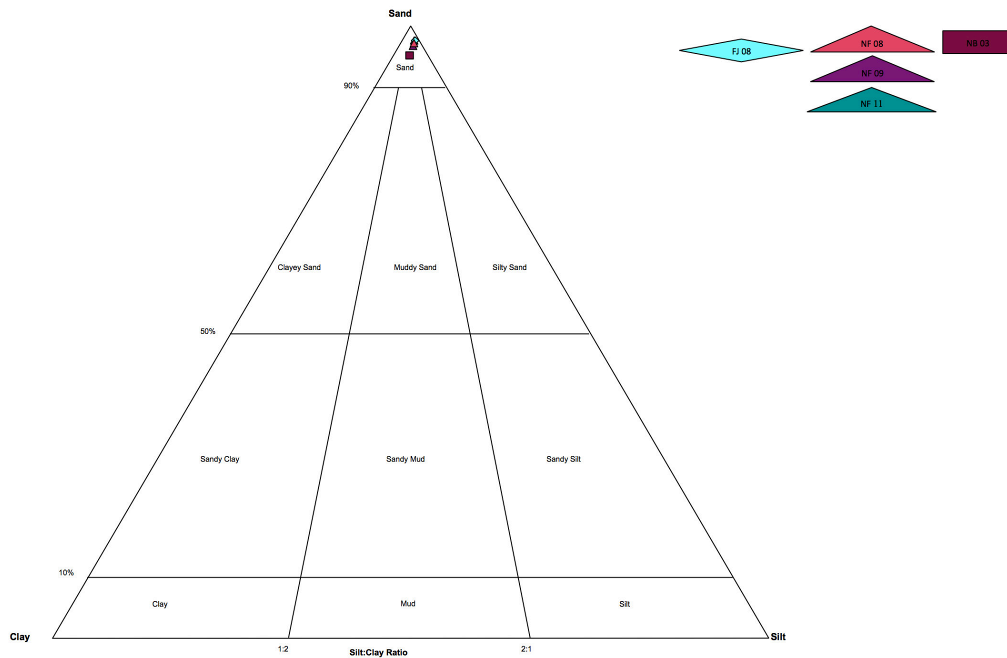



Figure 17 – Folk Sand, Silt and Clay diagram, obtained for mixed sediment size analyses with GRADISTAT.

As observed before, in the Gravel, Sand and Mud diagrams, BJ05 stood once more as sand, while the muddy sand and sandy mud samples were now classified as silty sand and sandy silt, respectively. This allowed the inference that the muddy sediments observed before, were mainly composed of silt, with very low amounts of clay.

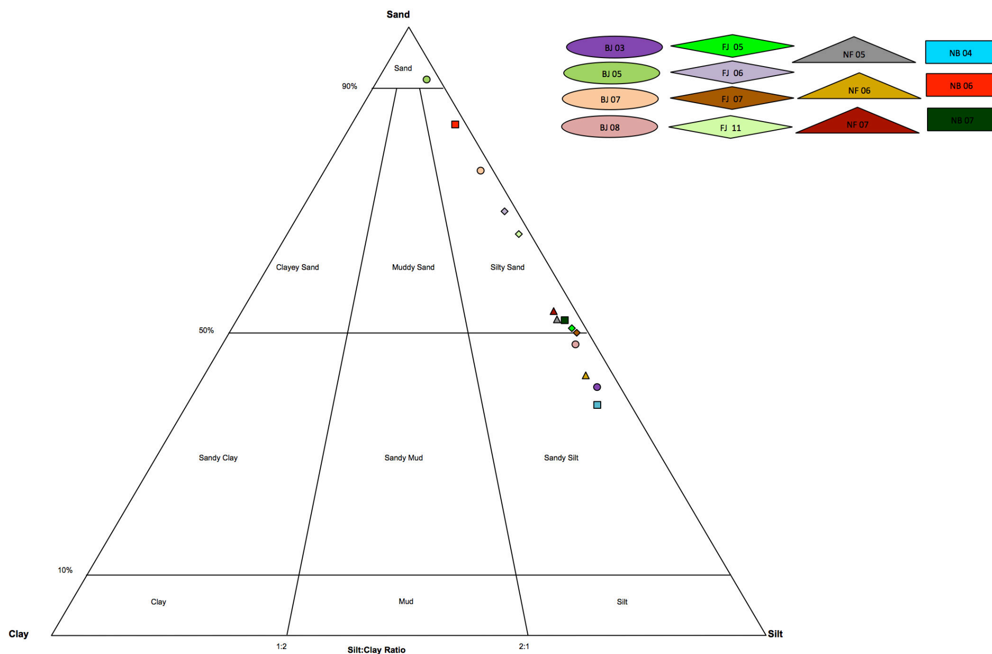



Figure 18 – Folk Sand, Silt and Clay diagram, obtained for fine sediment size analyses with GRADISTAT.

The grain size was also analyzed against the environments types in each sampling site, results can be observed in Figure 19. With the exception of the *Zostera noltii* prairie, sampled at Fuzeta in January, external more hydrodynamic sites (FPIN, PIDE, FPEX, DEDE, PIPC, DVDE) were caracterized by a higher amount of gravel, while internal and more sheltered ones present larger contents of mud (BSSM, PZNO, MCIA, PILO, CIAP, PILA, PIMC), however the environment PIRV (BSL Nov) had a very high amount of mud, even though it was a site of high hydrodynamics, possible due to the natural shift of the inlet, which gradually causes a shift in the adjacent environments. The ones where very high amounts of gravel have been noticed, are related to the presence of big size bioclasts in these sediments. High amounts of sand are a predominant caracteristic amoung all samples, ranging from values of 39 % to 100 % sand.

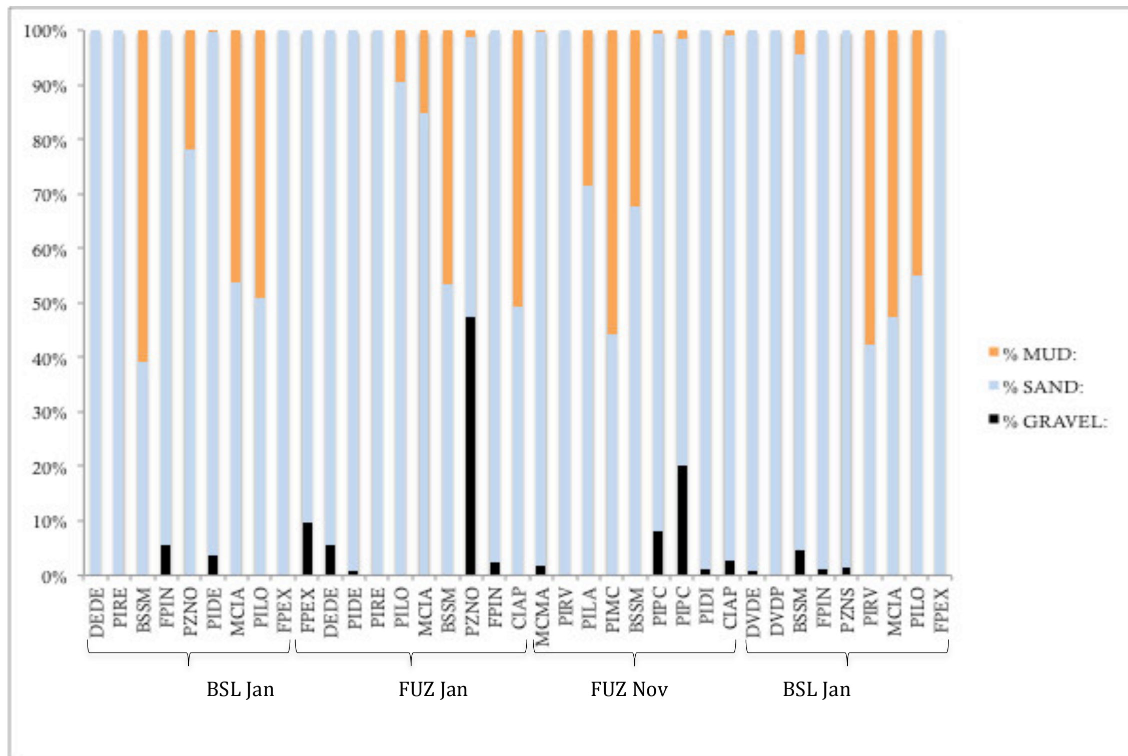



Figure 19 – Percentage of grain size type sediment in each environment.

## Organic matter determination

Largely, organic matter values obtained by weight-loss-on-ignition (designated in figures as % M.O. Muffle) were higher than the ones obtained by the organic carbon oxidation technique (designated in figures as % M.O. Average), as shown in Figure 20.

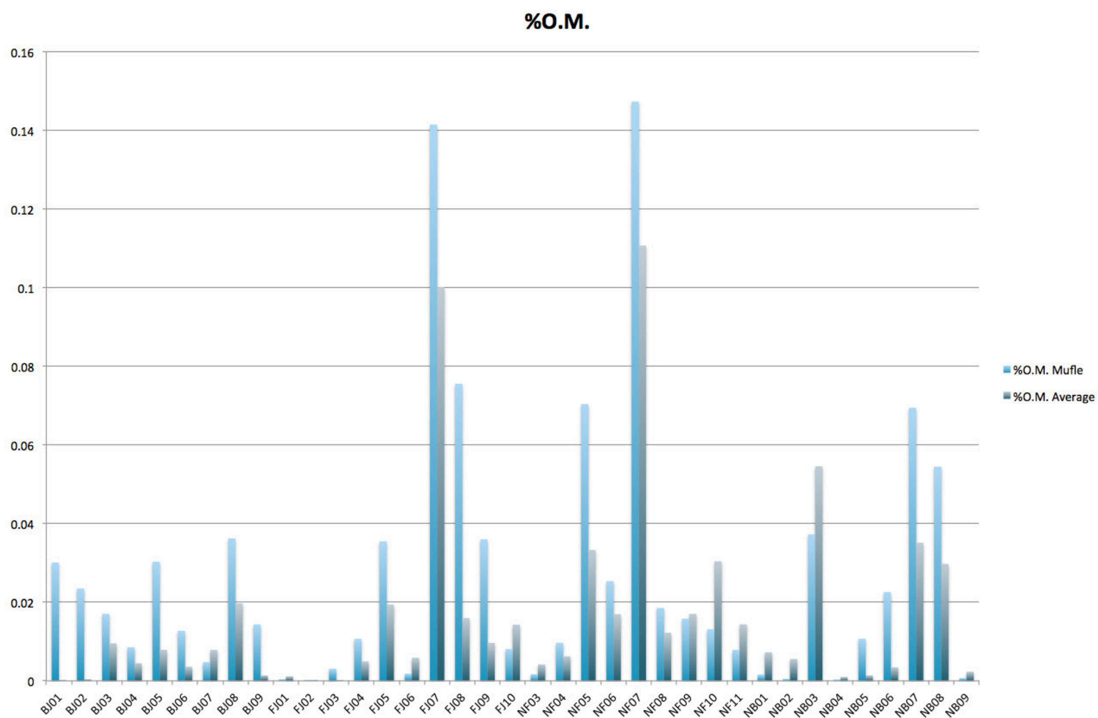



Figure 20 – Organic matter percentage at the different sampling points – comparison of values from different techniques.

No significant correlation was observed between organic matter contents and sampling period (Figure 21), but one was found with the type of environment sampled. The grain size for each sediment environment presented a negative correlation with the percentage of organic matter in a given sample, of which smaller grain sizes corresponded to higher percentages of organic matter (Figure 21).

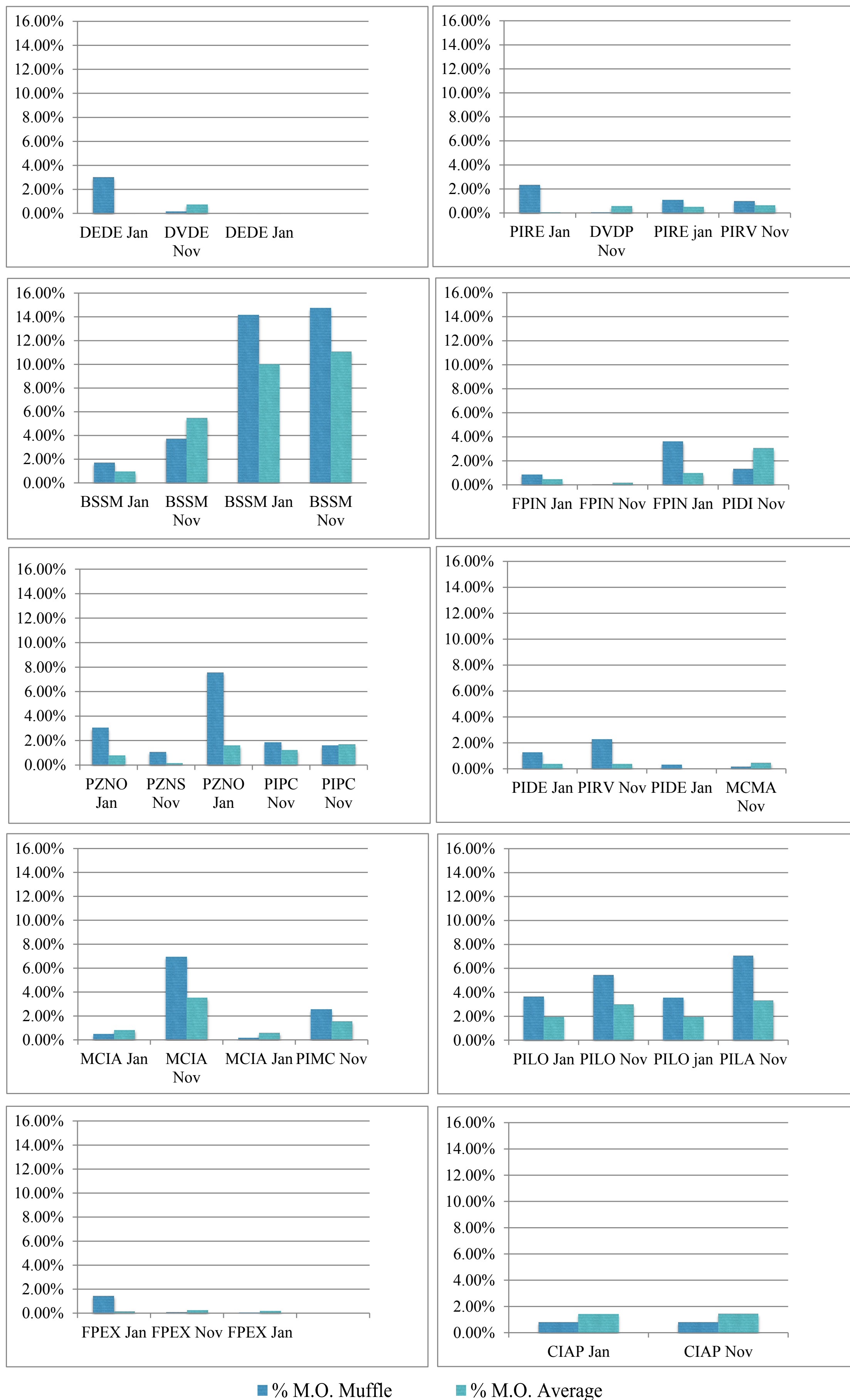



Figure 21 – Organic matter comparison between similar environments in different periods.

In Figure 21 % of sand and % of mud was plotted against % O.M. for each of the sampled sediment environments. In graph **A** of figure 22 the percentage of organic matter (% M.O.) was correlated with the percentage of sand (% Sand) in the sediments collected at the São Luís inlet sampling site, while in graph **B** the percentage of organic matter (% M.O.) was correlated with the percentage of mud (% Mud = % Silt + % Clay) for the same sampling site. Graphs C and D present the same type of data for Fuzeta inlet sampling site, respectively for sand and mud as well.

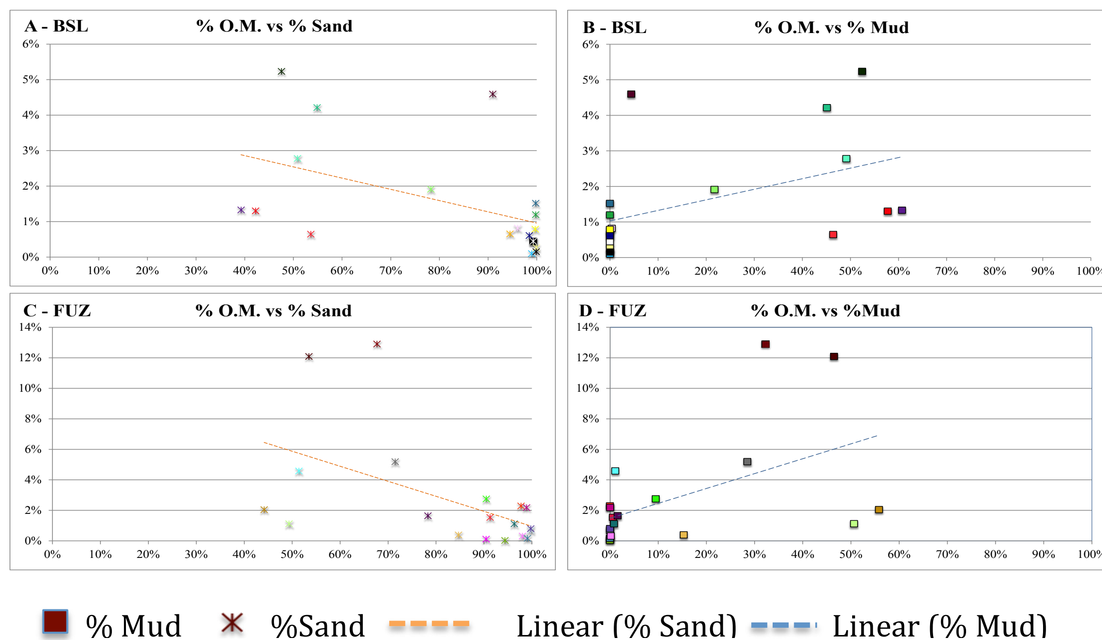



Figure 22 – Correlation between grain size of all sediment environments samples and the % O.M. In graph **A** the percentage of organic matter (% M.O.), was correlated with the percentage of sand (% Sand) in the sediments, while in graph B the percentage of organic matter (% M.O.), was correlated with mud percentage (% Mud = % Silt + % Clay). In graphs C and D the same type of data is given for the Fuzeta inlet sampling site. The colors for each data point indicate the different environments, according to the color codes given in table X.

From the analysis of graphs A and C, in figure 22, it was deduced that the percentage of organic matter tends to decrease for the sandier sediments in both inlets, while in graphs B and D the muddier sediments had higher percentages of organic matter. Samples BJ01, BJ02, BJ04, BJ06, BJ09, FJ01, FJ02, FJ03, FJ04, FJ05, FJ10, NF03, NF04, NF08, NF10, NF11, NB01, NB02, NB03, NB04, NB05 and NB09, with sand contents higher than 90%, and organic matter contents lower then 2%, corresponded to

environments of elevated hydrodinamism, which justifies their poor particulate organic matter content, not only by poorer settling of organic particulate matter, but also through greater dilution of incoming matter, and poorer microbial biofilm growth (Cravo et al., 2015) and probably also by their own coastal origin. To these samples correspond the sandy environments: DEDE, PIRE, FPIN, PIDE, FPEX, PIRE, Intertidal Mud Flat (PILO), MCMA, PIRV, PIPC, PIDI, CIAP, DVDE, DVDP, BSSM and also *Zostera noltii* prarie (PZNO).

Another group of sediments occurred for samples with lower than 90 % sand, corresponding to the Intertidal Mud Flat (PILO), to the Internal Sand Channel (MCIA), Low Saltmarsh (BSSM), *Zostera noltii* prarie (PZNO) and Intertidal Sand Flat with flooding dunes (PIDE), of samples BJ03, BJ05, BJ07, BJ08, FJ06, FJ07, FJ08, FJ11, NF05, NF06, NF07, NF09, NB06, NB07, NB08. These environments are characterized by slow currents which are responsible for the settlement of suspended fine particles of sand, mud and organic matter (*Curran et al., 2007*). Thus with the decrease of sand, hence increase of mud contents, a higher percentage of oragnic matter was observed for these envirnoments, with special increase in the November samples.

Graphs B and D, O.M. % vs Mud, show a opposing variation tendencies since the same samples showing sand content higher than 90 %, show now a close to zero value in terms of mud, and therefore low values of organic matter. These evironments are caracterized by high hydrodynamism, resulting in a low rate sedimentation of fine particles, and hence are not being trapped here (*Mayer, 1993*). The second group of sediment samples, with a symetric polting to the previous graphs A and C, present higher mud contents, and so also more organic matter.

From the temporal analysis, higher organic matter values were observed in November samples, compared to the January campain. The wide-ranging decrease of organic

matter contents from November to January, can be a behaviour resulting from the increase in the hydrodynamic (higher amplitude tides in equinocium), and decrease in microbial activity, related to the low temperatures, organica matter leaching and lowering on the antropogenic activity (*Cravo et al., 2015*).

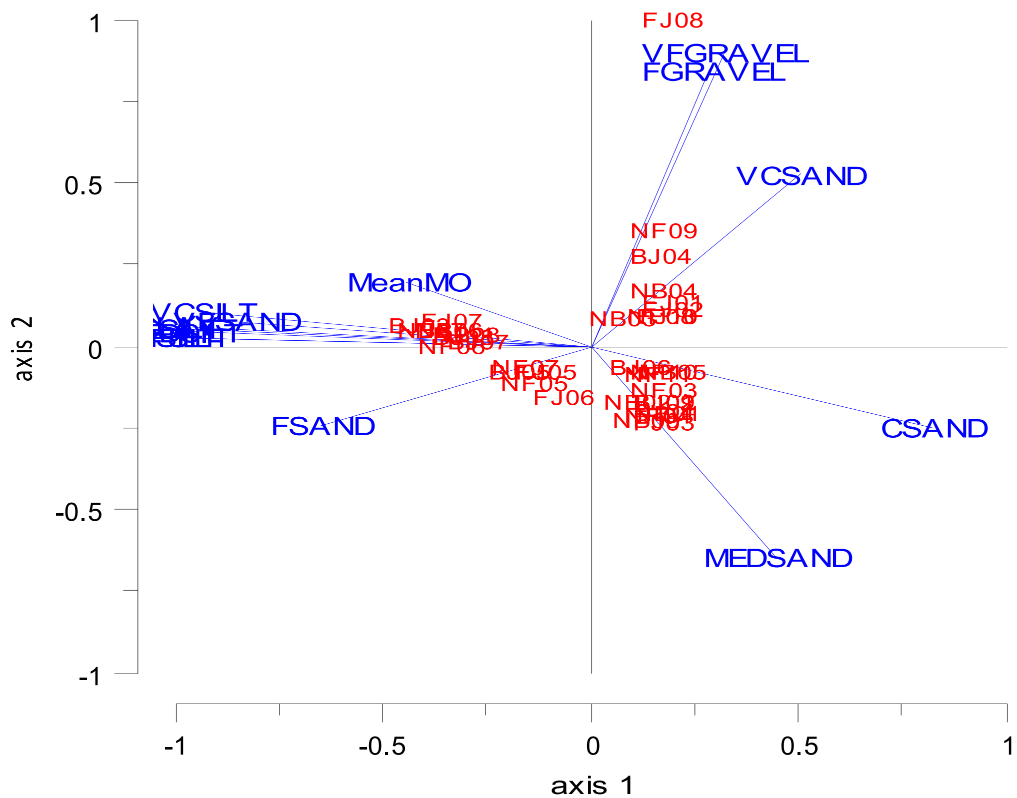
There is a lack of information in the Fuzeta inlet, from one sampling period to the other, as a result of an artificial shift in the actual inlet. A new inlet was open in early 2011, about a mile west from the natural one and consequentely this last one was drastically closed. By the time the second sampled was possible, in Fuzeta, the natural inlet was completely closed, the artificial one was now the main inlet, and a new channel was dredged crossing on top of the previous flooding delta was located, site where FJ02 was sampled, and cutting access to the external dune face, sampling site for FJ01. From this modification a complete difference behaviour in the environmentn was displayed, and most of the previous sampled sites were now very different environments, compared to January. A slight difference between environments was at S. Luís inlet, however, as the shifts in this this inlet occurred naturally, the impact wasn't as noticible as the Fuzeta intel, and the main affected environments where the ones near the delta, and due to the natural occuring east migration of this inlet.

## Principal component analysis

The analysis of Figure 23 led to identification of a strong gradient within the different grain size fractions, defined by sequential size variation. This gradient can be justified by the 84% sediment grain size classes variability, in the 4 main axis, for the 37 samples analyzed. The Figure shows that Clay and Silt rich sediments, are lacking of coarser fractions, meaning Gravel, and are very poor in coarse and very coarse sands. Contrasting gravel rich sediments, have no Silt or Clay, and low amounts of fine or

very fine sand. Sandy sediments, can either present some coarse fractions (Gravel) or fine (Clay and Silt), this tendency will thus reflect into the environments. From the ploting of organic matter into the prcincipal component analysis, it is clear that a higher affinity is found with mud rich sediments, being in ccordance with was previously discussed.

PCA on sediment textural analysis and M.O.



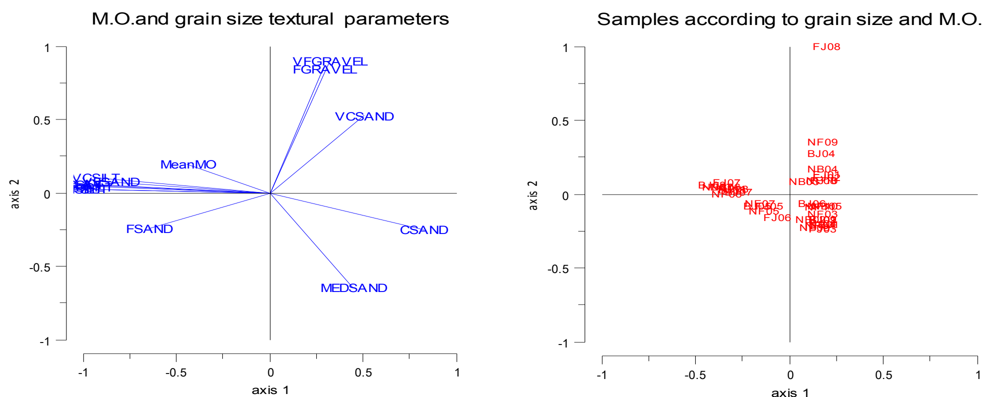




Figure 23 – Principal Component Analysis (PCA) applied on grain size textural analysis data and organic matter mean content for each of the 37 samples. Samples represented in red by their codes (table X). Explanatory variables represented in blue by the following ciphers: VFGRAVEL - very fine gravel %; FGRAVEL – fine gravel %; VCSAND – very coarse sand %; CSAND – coarse sand %; MEDSAND – Medium sand %; FSAND – fine sand %; VCSILT – very coarse silt %; CSILT – coarse silt %; MEDSILT – Medium silt %; FSILT- fine silt %; VFSILT – very fine silt %; CLAY –clay %; MeanMO – average organic matter %.

## DNA extracted from sediments

It was possible to extract DNA from colected sediment cores by the methodology described above in the Procedures section. The successful DNA extraction can be visualized on figure 24, presenting the agarose gel photography. Some samples had a higher recovery yield than others, nevertheless all showed traces of extracted DNA.

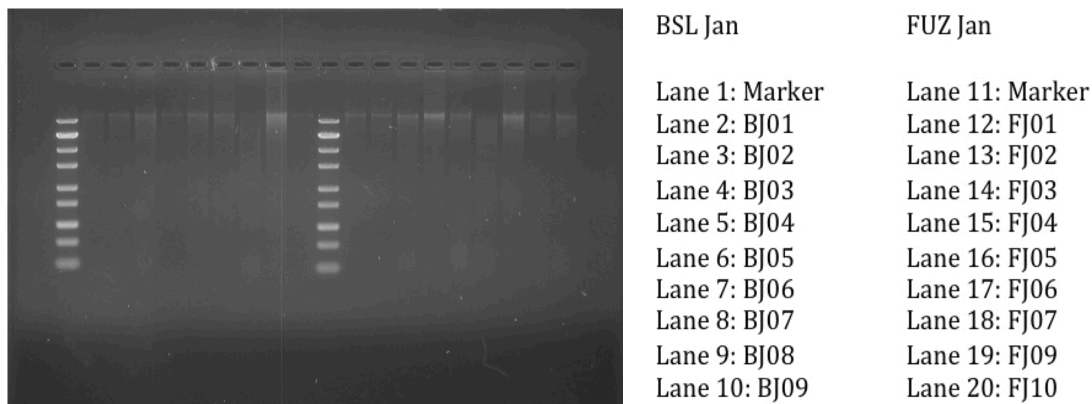


Figure 24 – 1% agarose gel run with DNA extraction products from samples of both sampling sites.

## Nested-PCR Amplification

DNA amplification with 16S cDNA primers was possible for most of the DNA samples, although in some instances results were only visible in the first or in the second part of the Nested-PCR. Figure 25 shows obtained amplicons for DNA samples of sediment environments of both inlets with first and second primer sets for 16S rDNA specific for Prokaryotes, while Figure 26, shows the results of first amplification with archaea specific primers. All bands had the characteristic size, namely about 1500 bp for the first PCR and about 400 bp for the second PCR. Some lanes presented less visible results, probably due to losses in the gel loading or in some cases less optimal conditions for the amplification. Negative controls ensured the non contamination of the sample.

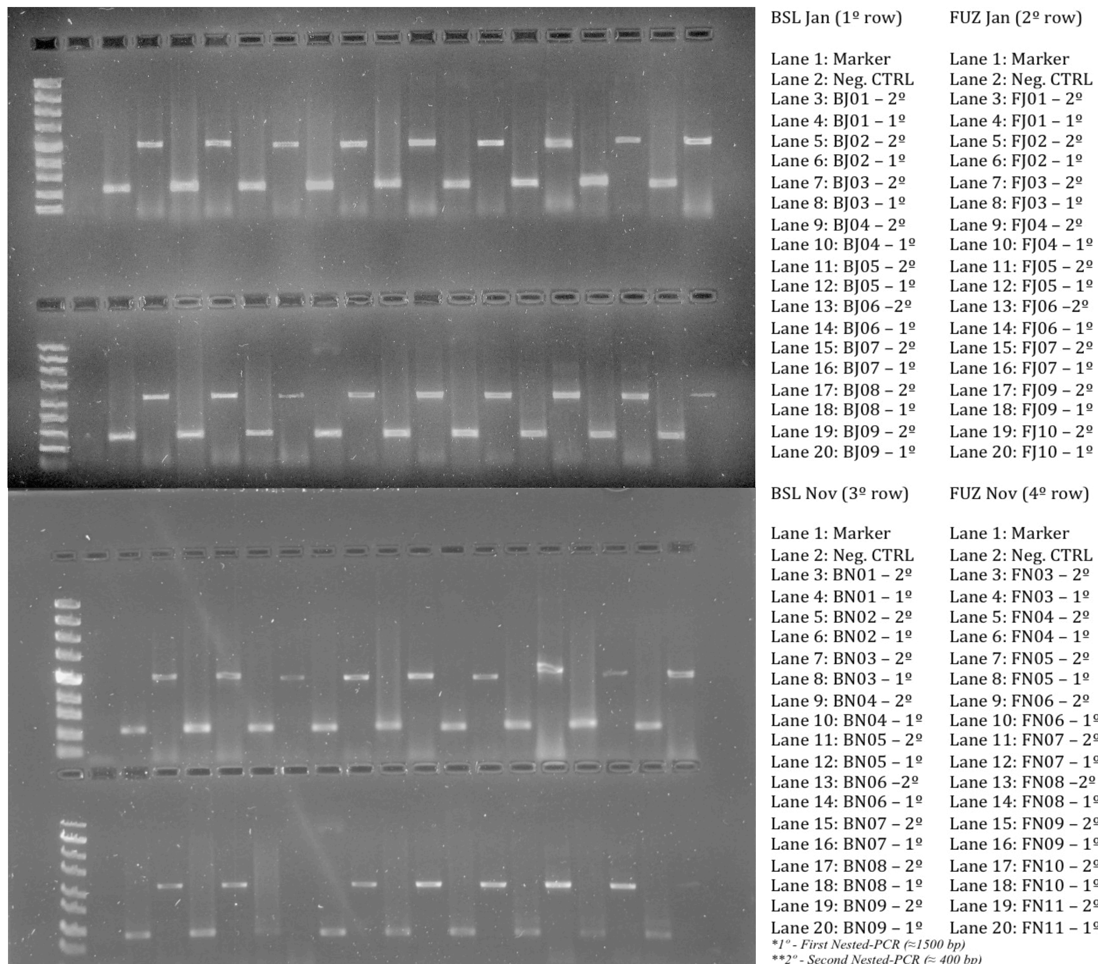



Figure 25 – 1% agarose gel with amplicons both Nested-PCR amplifications products from samples of both sampling sites.

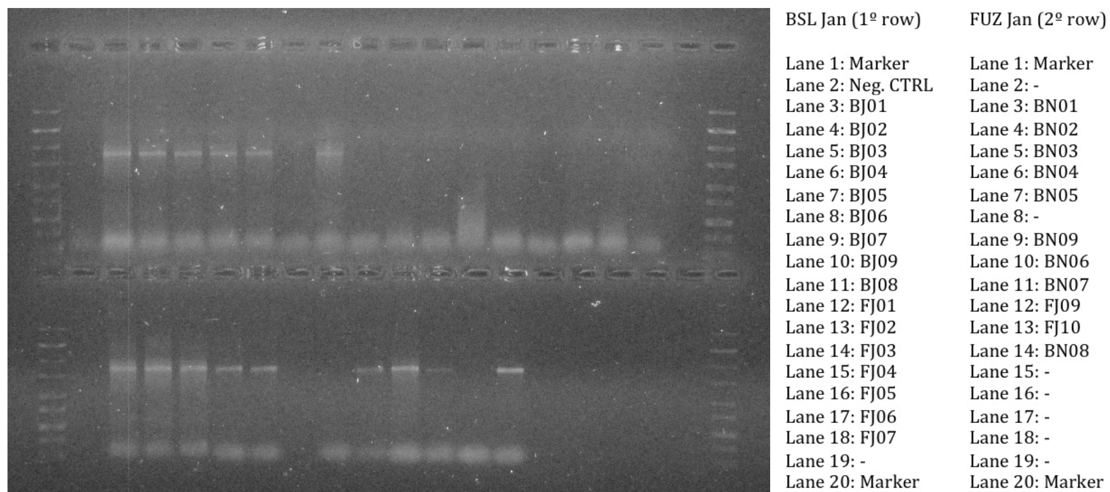



Figure 26 – 1% agarose gel ran with 1º Nested-PCR amplifications products from Archaea Primers.

## Reference Marker from Bacterial strains

Isolated colonies obtained from ZoBell agar medium were cultivated and purified. DNA from thes cultured bacterial strains was extracted and amplified with 16S rDNA

primers, in order to construct a reference marker, to serve as reference in the DGGE runs. Figure 26 shows products of DNA isolation, and amplification with first and second primer set for some of amplified strains. Again, some have shown better yield within the several steps.

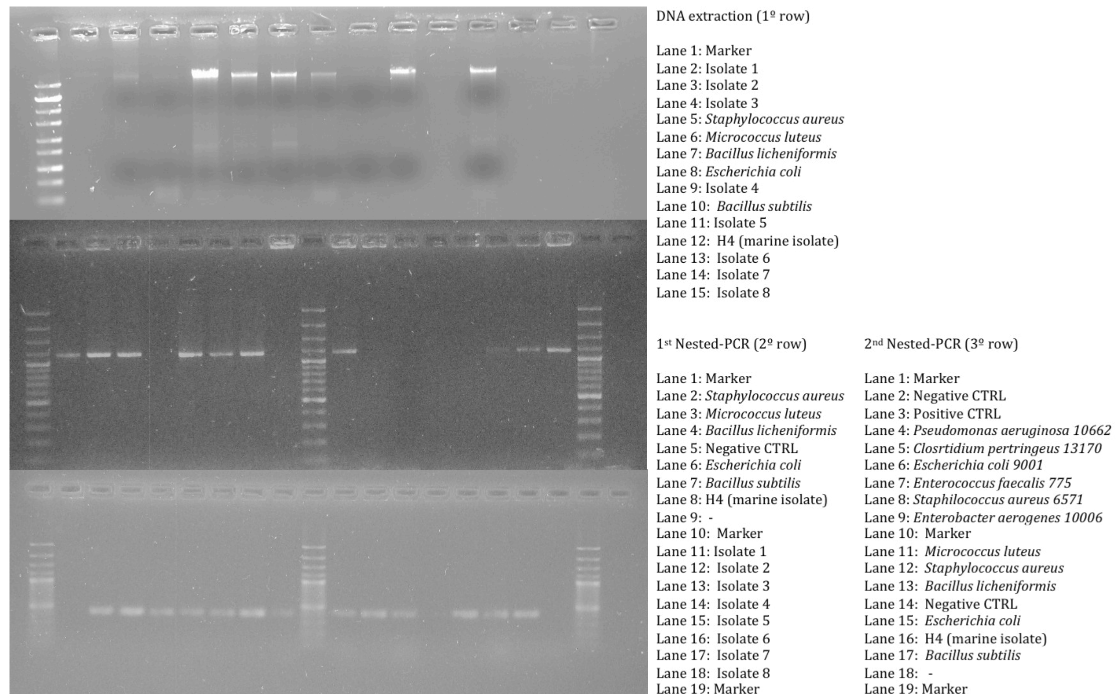



Figure 27 – 1% agarose gel ran with 2º Nested-PCR amplifications products from bacterial standards used on reference maker.

Figure 28 was the result of one of the reference markers designed, showing a lane taken form the DGGE gel, wich displays the patern of bands corresponding to the different strains used in this specific marker: *Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Bacillus licheniformis, Bacillus subtilis, the* marine isolate designated as H4, as well as the bacterial isolates from the sediment samples.

More strains were included in the marker, than the number of bands that are visible, this can be due to the proximity between the isolated strains, and the known ones, showing in this case a stronger band, instead of two different ones.

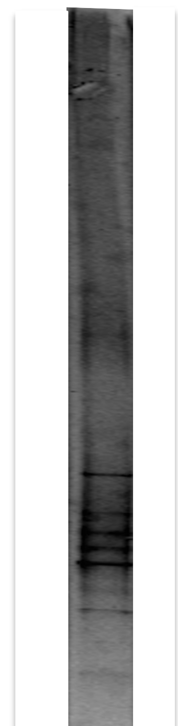


Figure 28 – Reference marker obtained in DGGE run.

## DGGE Analysis

DGGE allows the separation of same size amplicons, based on their sequence, in a GC content dependent manner. The obtained bands, are single stranded DNA fragments with different melting domains, their position on the gel give it an unique signature, the more they migrated, the higher the amount of denaturant the fragments were subject to, later they denatured into single stranded, hence higher was its GC content.

Figure 29 shows DGGE gel, after treatment with GelCompar® II, in which lanes were aligned, and bands were obtained, in comparison to standard reference markers.

From a first analysis, it was possible to observe, some band repetition across samples, and some similarities could be seen, in close sampling locations, from which some seem to be present when comparing same sampling locations, in different periods.

Some correspondence could be found between the bands in the marker and the ones the sample lanes, representing proximity of these organisms, or even that the same organism in the marker was present in the respective sample. Band 1 of the reference marker could be found on samples FJ01, NF03 and NB06; band 2 on samples BJ0, FJ04, NB05, NB06 and NB08; band 3 on BJ02, FJ07, FJ10, NB06, NF04 and NF05; band 4 on FJ04, FJ05, FJ06, NB04, NB07, NB09 and NF06; band 5 on FJ02, FJ03, FJ04, FJ07, FJ10, NB05, NB07, NF03 and NF08; band 6 on NF03, NF04 and NF07; band 7 on BJ05, FJ02, FJ05, NB01, NB02, NB08 and NF07; band 8 on BJ09, FJ04, FJ05, FJ06, NB01, NB06 and NF05; and band 9 on NF09.

However, some variation might be due to sample disposition within the gel, since band richness (species richness) in the middle lanes appeared to be higher when

compared to the ones in the outside lanes, resulting in a different diversity according to the position of the lanes on the gel. This presents a serious concern in terms of gel interpretability and reproducibility, which could be solved by comparing gels done separately for each environment with all the collected replicates.

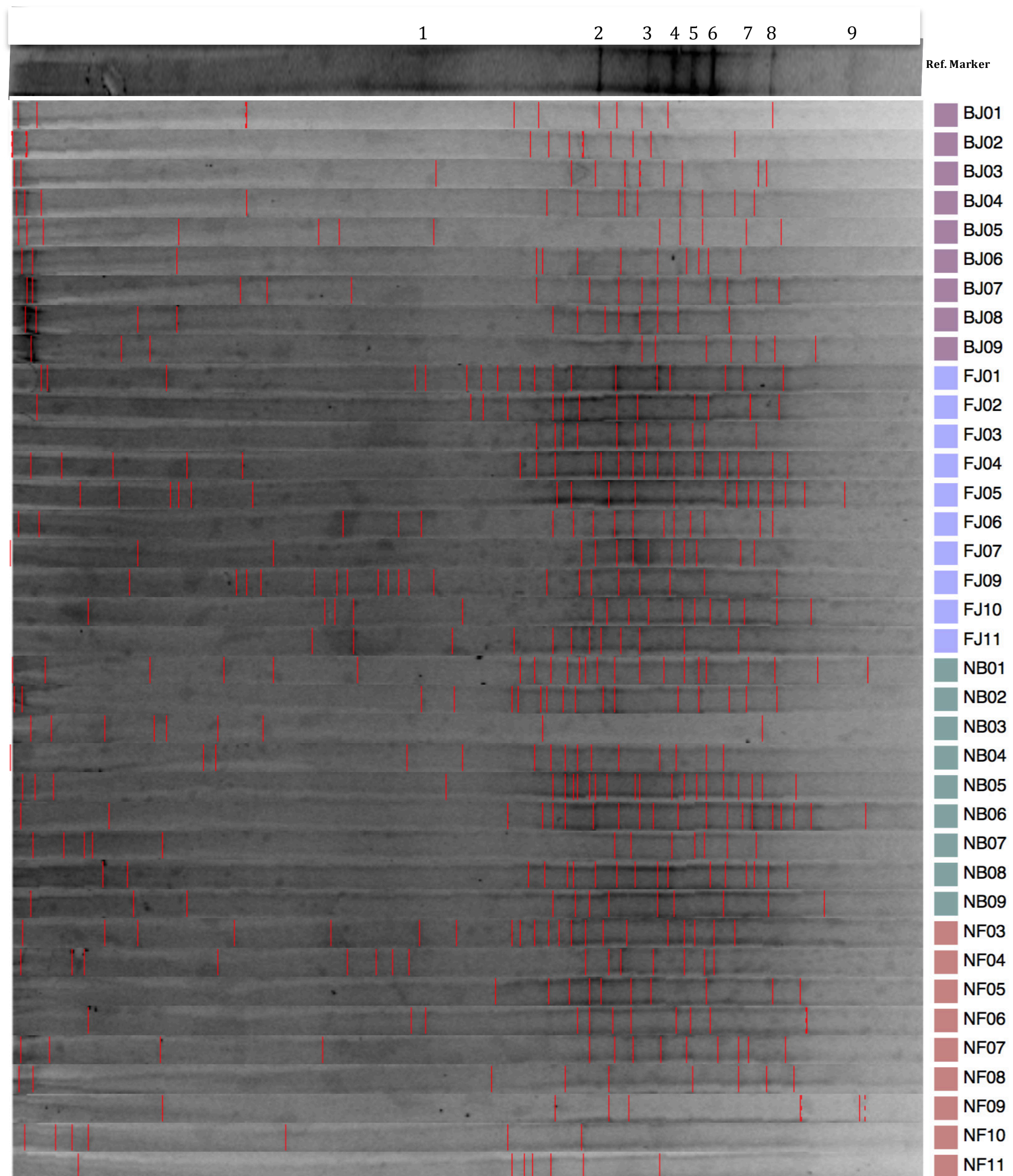



Figure 29 – Acrylamide denaturing gel with 2º Nested-PCR amplifications products from BSL and FUZ, in both sampling periods. Gel stained with SybrGold®, the to lane represents the marker ran in this DGGE (samples ran from left to right).

Nevertheless, a relatively high diversity was encountered throughout all samples, this assuming every band represent a different organism, while representing

an amplicon with different sequence, which was inferred from its position in the gel, and therefore as a general rule a different strain. Hence there can be some situations where a single organism could be represented by more than one band in the DGGE Gel, but we opted to adopt the analysis based on each band representing one single organism.

## Statistical Analysis

A range of similarity coefficients was used for cluster analysis, namely: Dice, Ochiai and Jaccard, to produce different similarity matrices, from which data was subsequently tentatively clustered with algorithms such as Neighbor Joining, UPGMA and single-linkage. For final data analysis the chosen clustering method used the Ochiai similarity coefficient, with neighbor joining. The reason for this choice was the better interpretability of the results, together with similarity found between this method and the one using the Dice coefficient with neighbor joining. In fact, results were very similar using Ochiai and Dice indices (*Annex VIII*), but the Jaccard coefficient produced very different results.

Figure 30 shows the alignment of the DGGE gel, according to the Ochiai Similarity coefficient and Neighbor Joining clustering dendogram, with the grouping of the different samples according to their similarity. For better understanding, Figure 31 can be found on the next foldable page, this Figure is wider version of Figure 30, containing the same information and few more data.

From the clustered analysis shown in Figures 30 and 31, we can see the grouping by diversity similarity, of the different samples. The macroorganisms found in the different sampling points seem to be influential in the type of diversity in the different samples; there was also found some correlation with the sediment type and

the diversity similarity, possibly meaning the similar surfaces are colonized by similar organims; and also a high level of similarity in sampling locations in specific months, which could denote the location different environments to be close enough for the same organims to colonize them, and how different temperatures and previous climatic regimes could influence the type of organism at a certain moment. The colors in this figure represent the two inlets in the different sampling trips, and allow to better understanding how the different samples group, and how closely related they can be.

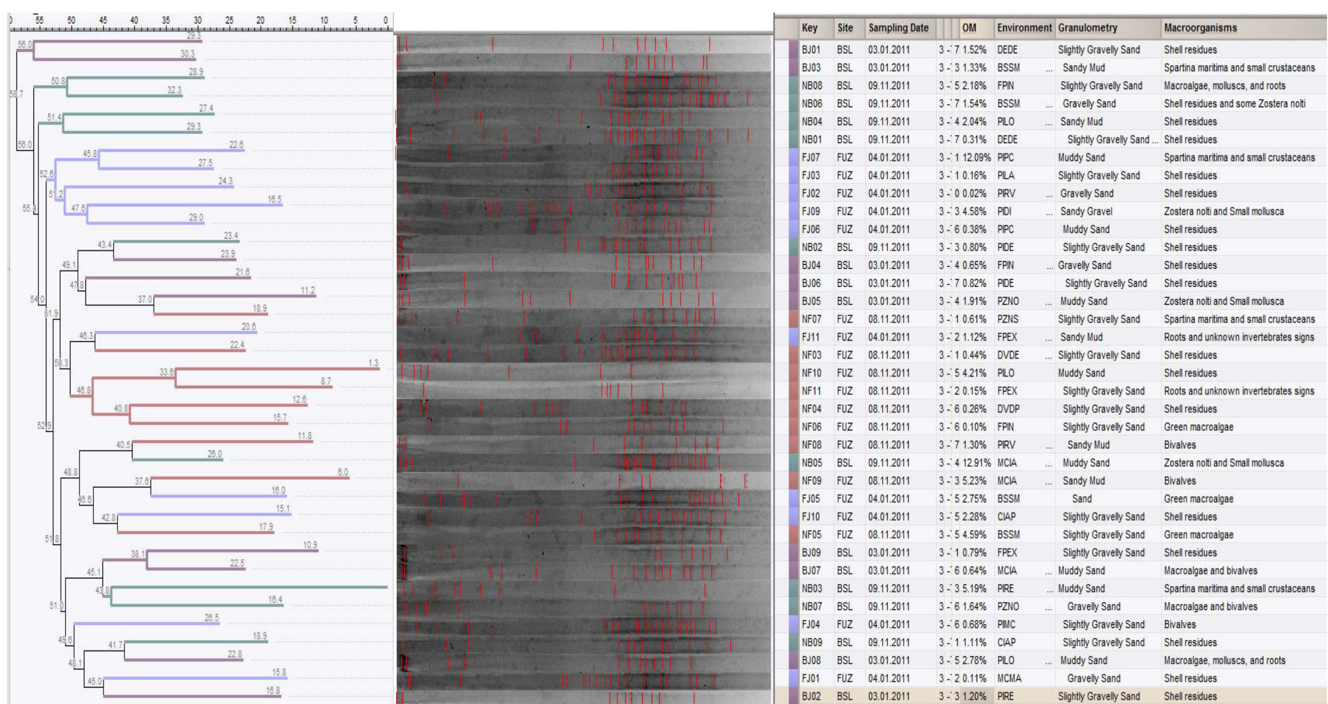
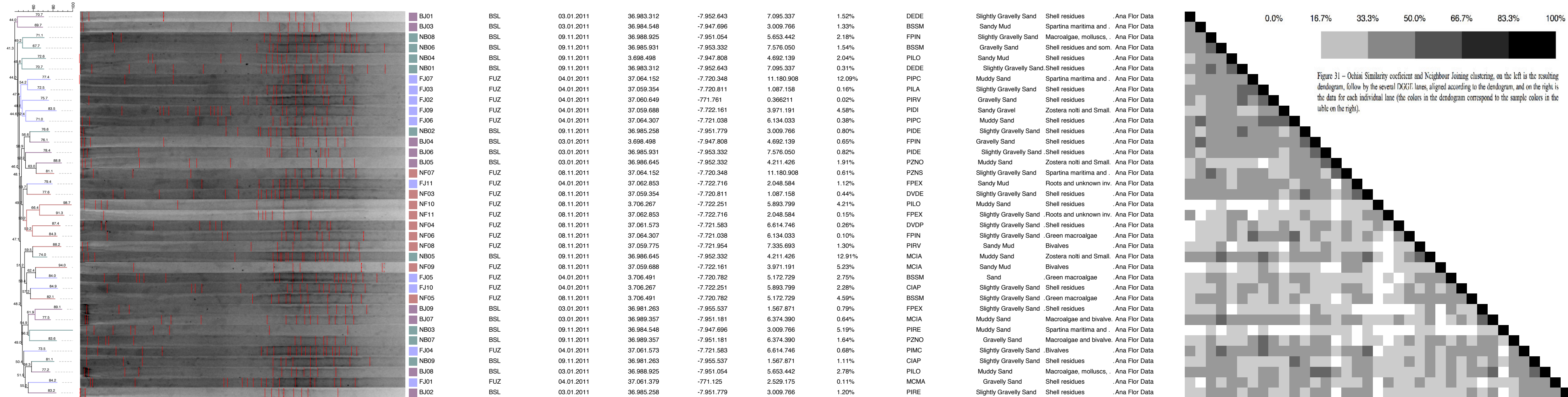


Figure 30 – Ochiai Similarity coeficient and Neighbour Joining clustering, on the left is the resulting dendogram, follow by the several DGGE lanes, aligned according to the dendogram, and on the right is the data for each individual lane (the colors in the dendogram correspond to the sample colors in the table on the right).

This clustering method, brings together samples FJ01 and BJ02, which are from different environments and inlets, but have a closely related sediment type, the same type of observed macroorganisms, and were sampled in the same period of the year; BJ05 and NF07, which where sampled in different inlets and periods of the year but the same type of environment; NB02 and BJ04, of different environments and periods of the year, but same inlet and resembling sediment type; NB04 and NB01, from the same inlet and sampling trip, but with different environments and sediment

type, among others. This data may suggest, the diversity is dependent on different factors, in some cases the sediment type is the dominant one, in other the type of seeding organisms present in the location, or even the season and location proximity.

The parameters for the clustering were later adjusted with an optimization factor of 1% and also a 1% error factor for the alignment divergences, from which a new band-matching matrix was obtained (*Annex IX*), which diminished the number of band variability and brought together close bands (position-wise), this increased the number of common bands between the lanes, hence the similarity between samples.



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BJ01 | BSL | 03.01.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues .Ana Flor Data |
| BJ03 | BSL | 03.01.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 1.33% | BSSM | Sandy Mud | Spartina maritima and . .Ana Flor Data |
| NB08 | BSL | 09.11.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, . .Ana Flor Data |
| NB06 | BSL | 09.11.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 1.54% | BSSM | Gravelly Sand | Shell residues and som. .Ana Flor Data |
| NB04 | BSL | 09.11.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 2.04% | PILO | Sandy Mud | Shell residues .Ana Flor Data |
| NB01 | BSL | 09.11.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 0.31% | DEDE | Slightly Gravelly Sand. | Shell residues .Ana Flor Data |
| FJ07 | FUZ | 04.01.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 12.09% | PIPC | Muddy Sand | Spartina maritima and . .Ana Flor Data |
| FJ03 | FUZ | 04.01.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.16% | PILA | Slightly Gravelly Sand | Shell residues .Ana Flor Data |
| FJ02 | FUZ | 04.01.2011 | 37.060.649 | -771.761 | 0.366211 | 0.02% | PIRV | Gravelly Sand | Shell residues .Ana Flor Data |
| FJ09 | FUZ | 04.01.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 4.58% | PIDI | Sandy Gravel | Zostera nolti and Small. .Ana Flor Data |
| FJ06 | FUZ | 04.01.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.38% | PIPC | Muddy Sand | Shell residues .Ana Flor Data |
| NB02 | BSL | 09.11.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues .Ana Flor Data |
| BJ04 | BSL | 03.01.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 0.65% | FPIN | Gravelly Sand | Shell residues .Ana Flor Data |
| BJ06 | BSL | 03.01.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 0.82% | PIDE | Slightly Gravelly Sand | .Shell residues .Ana Flor Data |
| BJ05 | BSL | 03.01.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 1.91% | PZNO | Muddy Sand | Zostera nolti and Small. .Ana Flor Data |
| NF07 | FUZ | 08.11.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and . .Ana Flor Data |
| FJ11 | FUZ | 04.01.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 1.12% | FPEX | Sandy Mud | Roots and unknown inv. .Ana Flor Data |
| NF03 | FUZ | 08.11.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues .Ana Flor Data |
| NF10 | FUZ | 08.11.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 4.21% | PILO | Muddy Sand | Shell residues .Ana Flor Data |
| NF11 | FUZ | 08.11.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 0.15% | FPEX | Slightly Gravelly Sand | .Roots and unknown inv. .Ana Flor Data |
| NF04 | FUZ | 08.11.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.26% | DVDP | Slightly Gravelly Sand | .Shell residues .Ana Flor Data |
| NF06 | FUZ | 08.11.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.10% | FPIN | Slightly Gravelly Sand | .Green macroalgae .Ana Flor Data |
| NF08 | FUZ | 08.11.2011 | 37.059.775 | -7.721.954 | 7.335.693 | 1.30% | PIRV | Sandy Mud | Bivalves .Ana Flor Data |
| NB05 | BSL | 09.11.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 12.91% | MCIA | Muddy Sand | Zostera nolti and Small. .Ana Flor Data |
| NF09 | FUZ | 08.11.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 5.23% | MCIA | Sandy Mud | Bivalves .Ana Flor Data |
| FJ05 | FUZ | 04.01.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 2.75% | BSSM | Sand | .Green macroalgae .Ana Flor Data |
| FJ10 | FUZ | 04.01.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues .Ana Flor Data |
| NF05 | FUZ | 08.11.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 4.59% | BSSM | Slightly Gravelly Sand | .Green macroalgae .Ana Flor Data |
| BJ09 | BSL | 03.01.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 0.79% | FPEX | Slightly Gravelly Sand | .Shell residues .Ana Flor Data |
| BJ07 | BSL | 03.01.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 0.64% | MCIA | Muddy Sand | Macroalgae and bivalve. .Ana Flor Data |
| NB03 | BSL | 09.11.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 5.19% | PIRE | Muddy Sand | Spartina maritima and . .Ana Flor Data |
| NB07 | BSL | 09.11.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalve. .Ana Flor Data |
| FJ04 | FUZ | 04.01.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.68% | PIMC | Slightly Gravelly Sand | .Bivalves .Ana Flor Data |
| NB09 | BSL | 09.11.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues .Ana Flor Data |
| BJ08 | BSL | 03.01.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.78% | PILO | Muddy Sand | Macroalgae, molluscs, . .Ana Flor Data |
| FJ01 | FUZ | 04.01.2011 | 37.061.379 | -771.125 | 2.529.175 | 0.11% | MCMA | Gravelly Sand | Shell residues .Ana Flor Data |
| BJ02 | BSL | 03.01.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues .Ana Flor Data |



Figure 31 – Ochiai Similarity coeficient and Neighbour Joining clustering, on the left is the resulting dendogram, follow by the several DGGE lanes, aligned according to the dendogram, and on the right is the data for each individual lane (the colors in the dendogram correspond to the sample colors in the table on the right).

From this new alignment, new values were obtained, assuming that each band on the gel lanes represent a single species, Richness (r) was acquired from the optimized Ochiai Similarity coefficient and Neighbor Joining clustering, shown in Table XI, as the number of species in each lane (r). The diversity was also obtained for the same clustering optimization, through the Shannon-Wiener index, values can also be observed on table XI, under the H' column.

The Shannon-Wiener index takes into consideration the abundance (evenness) of each band/specie, assuming the individuals are randomly sampled from an infinite population and that all species are represented in it (Magurran, 1988).

Table XI- Values of Richness and Shannon -Wiener Diversity Index of clustered samples (with 1% optimization and error of clustering).

| **BSL** | | | | | | **FUZ** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | **H'** | | r | **H'** | | r | **H'** | | r | **H'** |
| **BJ01** | 10.00 | **0.99** | **NB01** | 23.00 | **1.36** | **FJ01** | 18.00 | **1.20** | **NF03** | 21.00 | **1.32** |
| **BJ02** | 11.00 | **1.04** | **NB02** | 17.00 | **1.23** | **FJ02** | 13.00 | **1.11** | **NF04** | 15.00 | **1.18** |
| **BJ03** | 11.00 | **0.95** | **NB03** | 9.00 | **0.95** | **FJ03** | 11.00 | **1.04** | **NF05** | 10.00 | **1.00** |
| **BJ04** | 13.00 | **1.11** | **NB04** | 15.00 | **1.18** | **FJ04** | 22.00 | **1.34** | **NF06** | 11.00 | **1.00** |
| **BJ05** | 12.00 | **1.08** | **NB05** | 20.00 | **1.30** | **FJ05** | 19.00 | **1.28** | **NF07** | 13.00 | **1.08** |
| **BJ06** | 12.00 | **1.00** | **NB06** | 20.00 | **1.30** | **FJ06** | 16.00 | **1.20** | **NF08** | 9.00 | **0.90** |
| **BJ07** | 15.00 | **1.14** | **NB07** | 12.00 | **1.08** | **FJ07** | 13.00 | **1.08** | **NF09** | 6.00 | **0.77** |
| **BJ08** | 12.00 | **1.00** | **NB08** | 17.00 | **1.23** | **FJ09** | 20.00 | **1.30** | **NF10** | 7.00 | **0.84** |
| **BJ09** | 10.00 | **0.99** | **NB09** | 11.00 | **1.04** | **FJ10** | 16.00 | **1.20** | **NF11** | 7.00 | **0.84** |
| | | | | | | **FJ11** | 12.00 | **1.08** | | | |

## Principal Component analysis

The analysis of Figure 32 shows higher variability (H4log10) associated with sandy sediments, high O.M.% associated with muddy sediments, and also a very high variability in very fine sandy sediments associated with the observed macroorganisms (Observ). The inlet in which the sample occurred seems to be influential mainly for samples from FUZ. It led to the identification of a gradient justifying nearly 62% of the

detected variance, for the 37 samples analyzed, along 2 main axis, Axis 1 - mainly defined by sand and mud content, and Axis 2 defined by Prokaryotic diversity versus inlet sampled.

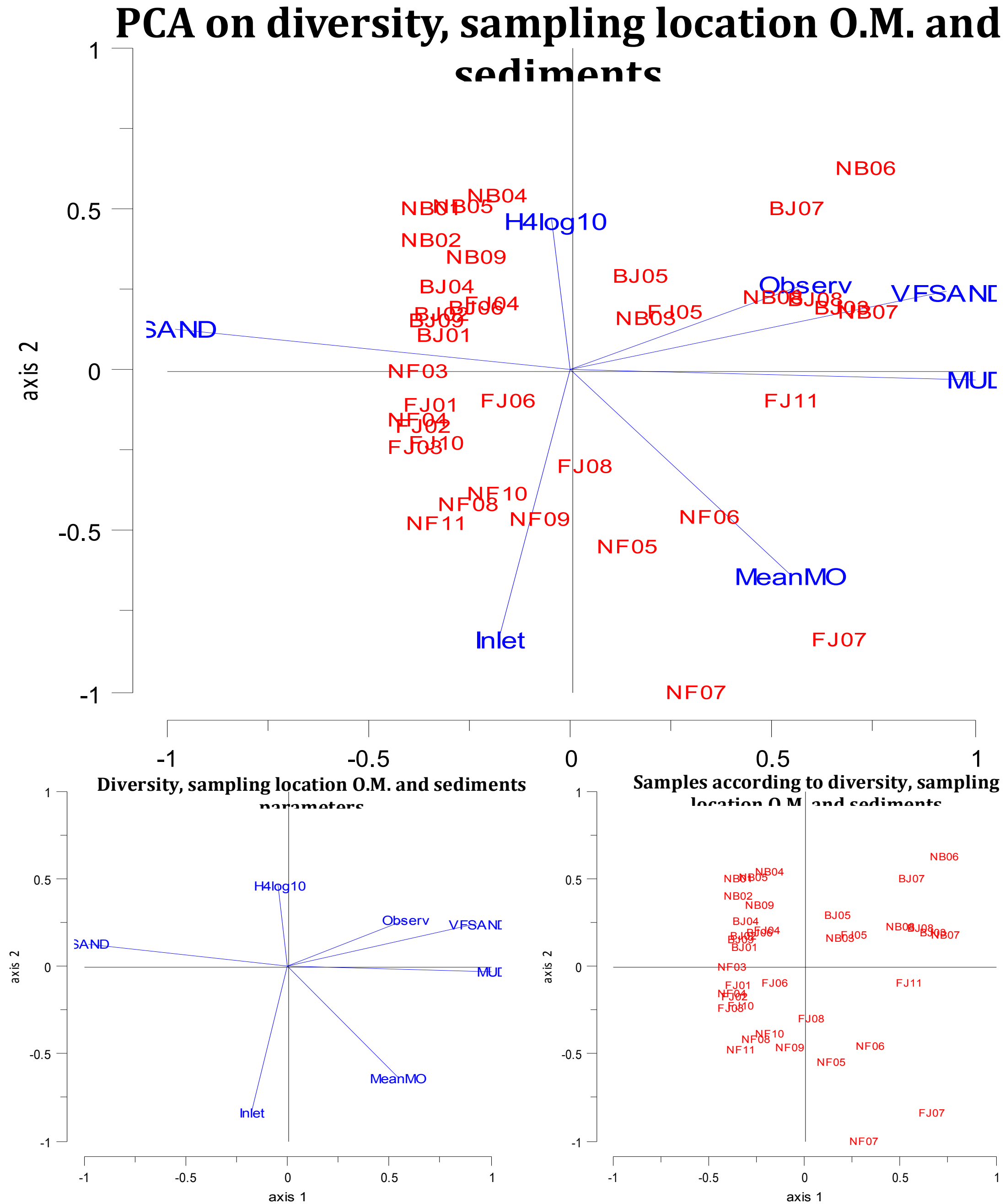



Figure 32 – Principal Component Analysis (PCA) applied on diversity, grain size textural analysis data, organic matter mean content and environment type for each of the 37 samples. Samples represented in red by their codes (table X). Explanatory variables represented in blue by the following ciphers: SAND – sand %; VFSAND – Very fine sand %; MUD –Mud %; MeanMO – average organic matter %; Inlet – Inlet sampled (BSL or FUZ); Observ – Observed macroorganisms; H4log10 – Shannon-Wiener index.

## Modifications and Incomplete analysis…

Several staining techniques were applied to stain DGGE, although silver staining protocols seem to be widely use, we found it might be better to elude all the artistic variables it depends on, and opted for a not so permanent stain with fluorescent dyes, in particular SybrGold and GelStar.

Optimization was carried out in the TTGE technique, unfortunately, due to some unforeseen equipment problems, no results were possible to be shown, and this part of the work was put on stand-by. The same applies to the Archaea diversity, from which some successful amplifications were obtained, as we could see in Figure 26, nevertheless some optimizations is still to be made.

## Data comparison - Diversity/sediments relation

Tables XII and XII display a final gathering of all data acquired with this project, showing the samples collected and analyzed, the organims observed upon sampling, the type of environment selected, the sediment size type of each sample, the organic matter percentage found in the sediment, and the species richness in the samples, as well as the diversity index, according to Shannon-Wiener.

The Archaea diversity was not calculated, for the reasons stated previously, hence the value N/A in the table.

Table XII- Relation Sediment environment – Microbial Diversity, in BSL inlet.

| Sample | CODE | Environment | Observed Macroorganisms | Grain Size Type | O.M. % | 16S rDNA Richness/Diversity | Archea Diversity | Sample | CODE | Environment | Observed Macroorganisms | Grain Size Type | O.M. % | 16S rDNA Richness/Diversity | Archea Diversity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101<br>102<br>103 | BJ01 | DEDE | Shell residues | Slightly Gravelly Sand | 0.02 | 10.0/0.99 | N/D | 301<br>302<br>303 | NB01 | DVDE | Shell residues | Slightly Gravelly Sand | 0.45 | 23.0/1.36 | N/D |
| 104<br>105<br>106 | BJ02 | PIRE | Shell residues | Slightly Gravelly Sand | 0.04 | 11.0/1.04 | N/D | 304<br>305<br>306 | NB02 | DVDP | Shell residues | Slightly Gravelly Sand | 0.73% | 17.0/1.23 | N/D |
| 107<br>108<br>109 | BJ03 | BSSM | *Spartina maritima* and small crustaceans | Sandy Mud | 0.95 | 11.0/0.95 | N/D | 307<br>308<br>309 | NB03 | BSSM | *Spartina maritima* and small crustaceans | Muddy Sand | 0.47 | 9.0/0.95 | N/D |
| 110<br>111<br>112 | BJ04 | FPIN | Shell residues | Sandy Gravel | 0.45 | 13.0/1.11 | N/D | 310<br>311<br>312 | NB04 | FPIN | Shell residues | Sandy Mud | 5.46 | 15.0/1.18 | N/D |
| 113<br>114<br>115 | BJ05 | PZNO | *Zostera nolti* and Small mollusca | Gravel | 0.79 | 12.0/1.08 | N/D | 313<br>314<br>315 | NB05 | PZNS | *Zostera nolti* and Small mollusca | Muddy Sand | 0.16 | 20.0/1.30 | N/D |
| 116<br>117<br>118 | BJ06 | PIDE | Shell residues | Sand | 0.36% | 12.0/1.00 | N/D | 316<br>317<br>318 | NB06 | PIRV | Shell residues and some *Zostera nolti* | Gravelly Sand | 0.14 | 20.0/1.30 | N/D |
| 119<br>120<br>121 | BJ07 | MCIA | Macroalgae and bivalves | Muddy Sand | 0.81% | 15.0/1.14 | N/D | 319<br>320<br>321 | NB07 | MCIA | Macroalgae and bivalves | Sandy Gravel | 0.35 | 12.0/1.08 | N/D |
| 122<br>123<br>124 | BJ08 | PILO | Macroalgae, molluscs, and roots | Sandy Mud | 1.93% | 12.0/1.00 | N/D | 322<br>323<br>324 | NB08 | PILO | Macroalgae, molluscs, and roots | Gravelly Sand | 2.52 | 17.0/1.23 | N/D |
| 125<br>126<br>127 | BJ09 | FPEX | Shell residues | Slightly Gravelly Sand | 0.14% | 10.0/0.99 | N/D | 325<br>326<br>327 | NB09 | FPEX | Shell residues | Gravelly Sand | 2.97 | 11.0/1.04 | N/D |
| 128<br>129<br>130 | | - | - | - | - | - | - | 328<br>329<br>330 | | - | - | - | - | - | - |

Table XIII- Relation Sediment environment – Microbial Diversity, in FUZ inlet.

| Sample | CODE | Environment | Observed Macroorganisms | Grain Size Type | O.M. % | 16S rDNA Richness/Diversity | Archea Diversity | Sample | CODE | Environment | Observed Macroorganisms | Grain Size Type | O.M. % | 16S rDNA Richness/Diversity | Archea Diversity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 201<br>202<br>203 | FJ01 | FPEX | Shell residues | Gravelly Sand | 0.17% | 18.0/1.20 | N/D | 401<br>402<br>403 | NF03 | MCMA | Shell residues | Slightly Gravelly Sand | 0.45 | 21.0/1.32 | N/D |
| 204<br>205<br>206 | FJ02 | DEDE | Shell residues | Gravelly Sand | o,o2 | 13.0/1.11 | N/D | 404<br>405<br>406 | NF04 | PIRV | Shell residues | Slightly Gravelly Sand | 0.62 | 15.0/1.18 | N/D |
| 207<br>208<br>209 | FJ03 | PIDE | Shell residues | Slightly Gravelly Sand | 0.01 | 11.0/1.04 | N/D | 407<br>408<br>409 | NF05 | PILA | Green macroalgae | Gravelly Sand | 3.33 | 10.0/1.00 | N/D |
| 210<br>211<br>212 | FJ04 | PIRE | Bivalves | Slightly Gravelly Sand | 0.28 | 22.0/1.34 | N/D | 410<br>411<br>412 | NF06 | PIMC | Green macroalgae | Gravelly Sand | 1.54 | 11.0/1.00 | N/D |
| 213<br>214<br>215 | FJ05 | PILO | Green macroalgae | Muddy Sand | 1.94 | 19.0/1.28 | N/D | 413<br>414<br>415 | NF07 | BSSM | *Spartina maritima* and small crustaceans | Slightly Gravelly Sand | 11.08 | 13.0/1.08 | N/D |
| 216<br>217<br>218 | FJ06 | MCIA | Shell residues | Muddy Sand | 0.59 | 16.0/1.20 | N/D | 416<br>417<br>418 | NF08 | PIPC | Bivalves | Muddy Sand | 1.22 | 9.0/0.90 | N/D |
| 219<br>220<br>221 | FJ07 | BSSM | *Spartina maritima* and small crustaceans | Muddy Sand | 10.02 | 13.0/1.08 | N/D | 419<br>420<br>421 | NF09 | PIPC | Bivalves | Muddy Sand | 1.7 | 6.0/0.77 | N/D |
| 222<br>223<br>224 | FJ08 | PZNO | *Zostera nolti* and Small mollusca | Sandy Gravel | 1.6 | 20.0/1.30 | N/D | 422<br>423<br>424 | NF10 | PIDI | Shell residues | Sandy Mud | 3.04 | 7.0/0.84 | N/D |
| 225<br>226<br>227 | FJ10 | FPIN | Shell residues | Gravelly Sand | 0.96 | 16.0/1.20 | N/D | 425<br>426<br>427 | NF11 | CIAP | Roots and unknown invertebrates signs | Slightly Gravelly Sand | 1.44 | 7.0/0.84 | N/D |
| 228 | FJ11 | CIAP | Roots and unknown invertebrates signs | Muddy Sand | 1.43 | 12.0/1.08 | N/D | 428<br>429<br>430 | | - | - | - | - | - | - |
| 229<br>230 | | - | - | - | - | - | - | | | | | | | | |

## Conclusion

Much can be assumed from how the sediment environments relate to the type of organisms living in them and how the types of sediments and their dynamics will influence the biota. Prokaryotes are probably the widest group of organisms to be considered, but how to relate their diversity with a wide range of variables was a difficult task to undertake.

In this work we tried to correlate bacterial and archaeal diversity to the type of sediment environment where they were found and furthermore considered as part of.

A very important step in this type of study was the collection of the environmental samples, and pre-selection of the type of samples to be collected.

Different types of techniques were preformed, for which the samples were divided. A geological approach was undertaken to analyze the type of sediments found in the sampling environment. As a result we were able to ascertain the relative particle sizes for each site, and later on correlate them with the amount of organic matter found in each sample, and possibly the diversity of prokaryotes inhabiting or to be found in such environments, taking in consideration the type of flora and fauna observed at the collection site.

From what we gather, a negative correlation was found between the particle size and the amount of organic matter encountered, which surprisingly did not mean a wider diversity in this case, as expectable since the colonization surface was wider for smaller particles and higher values of organic matter should favor bacteria.

In the molecular approach, two lines were followed, one to ascertain the diversity of the environments, and another to help identify such diversity. In a first instance DNA was extracted from the collected samples, and tested in the different type of procedures to be applied. Secondly some samples were recollected, the sediment was then suspended and microbial cultures were made, and further on purified. These purified colonies were amplified and together with some known bacterial strains were used to form a molecular reference marker, for comparison with the environmental samples.

A conjugation of 16S bacterial and archaeal primers were used to amplify the extracted DNA, in addition a G-C clamp was introduced in the second set of primers to prevent total denaturation in the DGGE run. Successful results were obtained with DGGE analysis, but only one of the three collected replicates was processed (due to equipment failure) and thus no sufficient data was gathered to allow definite conclusions, although the existence of differences within the several sampled environments was noticeable.

Nested PCR-DGGE/TTGE was successful in the identification of environmental samples in several studies and although there remain some reservations in reliability of the technique, it is still widely used to detect untargeted and uncultured organisms, where culture based techniques may be insufficient or impracticable (Bong-Soo, 2004), creating diversity profiles of the encountered organisms.

These techniques need a high level of optimization, before they can be used widely and promptly. Small variations in the voltage, denaturing conditions, gel concentration, temperature and time can be of significant impact in the subsequent results.

Some difficulties may arise in terms of low sensitivity and limited detection of bands in complex profiles, but which can be improved by using an automated standardized reading process.

Nonetheless, another setback resides on the existence of some preferential amplifications within the 16S gene, which also explains certain biases arisen on some analyses. Also the fact that although some bacteria may contain a single copy of 16S rDNA, other may reach up to 15 copies, will also increase the difficulties in reading the profiles, in terms of diversity. But in a broad sense, we can say the amount of DNA should reflect the number of expected bands in the profile. When more precise identification is needed, further steps can be used, in particular DNA sequencing.

## Practical applications

The main benefit of the DGGE/TTGE techniques was the possibility of further analysis of the obtained fingerprints in order to detect sequences specifically associated to some sediment environments, as well as almost ubiquitous sequences. Once technical problems with the TTGE equipment are surpassed, this technique should be used to compare multiple gels obtained for all replicates.

# References

Abreu, A., & Machado, M. (2000). *Sedimentos e comunidades da Ria Formosa estado atual dos conhecimentos* (Final Report). Universidade do Algarve.

Adam, S., Ibrahim, E., Foster, R. M., Monbaliu, J., Sabbe, K., Toorman, E., & Van der Wall, D. (2009). Assessment of sediment erodibility of intertidal mudflats using hyperspectral remote sensing. *In Proceedings 6th EARSeL Special Interest Group Imaging Spectroscopy workshop, Israel*.

Andrade, C. F. (1990). *O ambiente de Barreira da Ria Formosa: Algarve - Portugal* (Doctoral dissertation, Universidade de Lisboa, Gaia).

Anthony, A., Atwood, J., August, P., Byron, C., Cobb, S., Foster, C., Fry, C., Gold, A., Hagos, K., Heffner, L., Kellogg, D.Q., Lellis-Dibble, K., Opaluch, J.J., Oviatt, C., Pfeiffer-Herbert, A., Rohr, N., Smith, L., Smythe, T., Swift, J. & Vinhateiro, N. (0). Coastal Lagoons and Climate Change: Ecological and Social Ramifications in U.S. Atlantic and Gulf Coast Ecosystems. *Ecology and Society*, *1*(4), 8.

António, D. S. (2011). *Fito- e bacterioplâncton em albufeiras dominadas por cianobactérias no Algarve* (Master's thesis, Universidade do Algarve, Faro).

Arnaud-Fassetta, G., Bertrand, F., Costa, S., & Davidson, R. (2006). The western lagoon marshes of the Ria Formosa (Southern Portugal): Sediment-vegetation dynamics, long-term to short-term changes and perspective. *Continental Shelf Research*, *26*(3), 363–384.

Bettencourt, P. (1994). *Les Environnements Sedimentaires de la Côte Sotavento (Algarve, Sud Portugal) et leur Évolution Holocéne et Actuelle* (Doctoral dissertation, University Bordeaux I, Bordeaux).

Bird, E. C. (1994). Physical setting and geomorphology of coastal lagoons. In B. Kjerfve (Ed.), *Coastal lagoon processes* (60th ed., pp. 9-39). Amsterdam: Elsevier Oceanography Series.

Boon, N., Windt, W. D., Verstraete, W., & Top, E. M. (2002). Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiology Ecology*, *39*(2), 101–112.

Brito, A., Newton, A., Tett, P., & Fernandes, T. F. (2009). Temporal and spatial variability of microphytobenthos in a shallow lagoon: Ria Formosa (Portugal). *Estuarine Coastal and Shelf Science*, *83*(1), 67–76.

Ceia, F. R. (2009). Vulnerabilidade das Ilhas-Barreira e Dinâmica da Ria Formosa na. *Gestão Costeira Integrada*, *9*(1), 57-77.

*Coastal lagoon processes* (60th ed.). (1994). B. Kjerfve (Ed.). Amsterdam: Elsevier Oceanography Series.

Costa, M., Silva, R., & Vitorino, J. (2001). *Contribuição para o estudo do clima de agitação marítima na costa Portuguesa*. 2as Jornadas Portuguesas de Engenharia Costeira e Portuária in CD-ROM.

Cravo, A., Fernandes, D., Damião, T., Pereira, C., & Reis, M. (2015). Determining the footprint of sewage discharges in a coastal lagoon in South-Western Europe. *Marine Pollution Bulletin*, *96*(1-2), 197–209.

Curran, K. J., Hill, P. S., Milligan, T. G., Mikkelsen, O. A., Law, B. A., Madron, X. D., & Bourrin, F. (2007). Settling velocity, effective density, and mass composition of suspended sediment in a coastal bottom boundary layer, Gulf of Lions, France. *Continental Shelf Research*, *27*(10-11), 1408–1421.

Cury, J. C. (2006). *Diversidade de Bacteria e Archaea em solos de mangue e marisma* (Doctoral dissertation, Universidade de São Paulo Escola Superior de Agricultura " Luiz de Queiroz ", Piracicaba, Brazil).

Danovaro, R. (2009). *Methods for the study of deep-sea sediments, their functioning and biodiversity* (1st ed.). Boca Raton: CRC Press.

Davis, R. A. (1978). *Coastal sedimentary environments* (1st ed.). New York: Springer-Verlag.

Ercolini, D., Blaiotta, G., Moschetti, G., & Coppola, S. (2002). Evaluation of PCR-DGGE analysis for molecular typing of cheeses. *Annals of Microbiology*, *52*, 81–87.

Falcao, M., & Vale, C. (1990). Study of the Ria Formosa ecosystem: benthic nutrient remineralization and tidal variability of nutrients in the water. *Hydrobiologia*, *207*(1), 137–146.

FitzGerald, D. M., Kraus, N. C., & Hands, E. B. (2000). Natural Mechanisms of Sediment Bypassing at Tidal Inlets. *Erdc/Chl Chetn*, *4*(30), 1-10.

Fontolan, G., Pillon, S., Quadri, F. D., & Bezzi, A. (2007). Sediment storage at tidal inlets in northern Adriatic lagoons: Ebb-tidal delta morphodynamics, conservation and sand use strategies. *Estuarine Coastal and Shelf Science*, *75*(1-2), 261–277.

Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Gillet, F., Aragno, M., & Rossi, P. (2002). Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. *Environ Microbiol*, *4*(11), 634-43.

Garrity, G. M., Brenner, D. J., Krieg, N. R., & Staley, J. T. (Eds.). (2005). *Bergey's Manual of Systematic Bacteriology: Volume 2: the Proteobacteria: Part B: the Gammaproteobacteria* (2nd ed.). New York: Springer.

Garrity, G. M., Brenner, D. J., Krieg, N. R., & Staley, J. T. (Eds.). (2005). *Bergey's manual of systematic bacteriology. Volume 2. The proteobacteria. Part A. Introductory essays* (2nd ed.).

Garrity, G. M., Brenner, D. J., Krieg, N. R., & Staley, J. T. (2005). *Bergey's manual of systematic bacteriology. Volume 2. The proteobacteria. Part C. The alpha-, beta-, delta-, and epsilonproteobacteria* (2nd ed.). New York, NY: Springer.

Gray, J. S., & Elliott, M. (2009). *Ecology of marine sediments: From science to management* (2nd ed.). Oxford: Oxford University Press.

Haff, L. A. (1994). Improved quantitative PCR using nested primers. *Genome Research*, *3*(6), 332–337.

Halos, L., Mavris, M., Vourc'h, G., Maillard, R., Barnouin, J., Boulouis, H., & Vayssier-Taussat, M. (2006). Broad-range PCR-TTGE for the first-line detection of bacterial pathogen DNA in ticks. *Veterinary Research*, *37*(2), 245–53.

Hewson, I., Jacobson-Meyers, M. E., & Fuhrman, J. A. (2007). Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. *Environmental Microbiology*, *9*(4), 923–933.

Hewson, I., Vargo, G. A., & Fuhrman, J. A. (2003). Bacterial Diversity in Shallow Oligotrophic Marine Benthos and Overlying Waters: Effects of Virus Infection, Containment, and Nutrient Enrichment. *Microbial Ecology*, *46*(3), 322–336.

Hulscher, S. J., & Dohmen-Janssen, C. M. (2005). Introduction to special section on marine sand wave and river dune dynamics. *Journal of Geophysical Research*, *110*(4), 1-6.

Kim, B. K., Kang, H., Oh, H. M., Chun, J., & Park, S. S. (2004). Remarkable bacterial diversity in the tidal flat sediment as revealed by 16S rDNA analysis. *Journal of Microbiology*, *14*(1), 205–211.

Kim, B. K., Oh, H. M., Kang, H., & Chun, J. (2005). Archaeal diversity in tidal flat sediment as revealed by 16S rDNA analysis. *Journal of Microbiology*, *43*(2), 144–151.

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., & Sass, H. (2005). Microbial Diversity in Coastal Subsurface Sediments: a Cultivation Approach Using Various Electron Acceptors and Substrate Gradients. *Applied and Environmental Microbiology*, *71*(12), 7819–7830.

Madeira, F. (2012). *Caracterização das variações espaciais das distribuições granulométricas dos sedimentos superficiais, intermareais, dos bancos de areia do Parchal de São Francisco, da Ilha dos Tesos, da Vaza da Galé, do Banco do Berbigão e do delta de enchente da Barra Faro-Olhão*. Universidade do Algarve.

Magurran, A. E. (1988). *Ecological diversity and its measurement* (1st ed.). Princeton, NJ: Princeton University Press.

Magurran, A. E. (2004). *Measuring biological diversity* (1st ed.). Malden, Ma: Blackwell Pub.

Mancini, L., Rosemann, S., Marcheggiani, S., & Aulicino, F. A. (2008). Microbiological indicators and sediment management. *Annali dell'Istituto Superiore di Sanita*, *44*(3), 268–272.

Martin, L., & Dominguez, J. M. (1994). Geological history of coastal lagoons. In B. Kjerfve (Ed.), *Coastal lagoon processes* (1st ed., p. 163–167). Amsterdam: Elsevier.

Mayer, L. M. (1994). Relationships between mineral surfaces and organic carbon concentrations in soils and sediments. *Chemical Geology*, *114*, 347–363.

Mendonça, A. (2001). *Simulação da hidrodinâmica de maré na Ria Formosa com um modelo de elementos finitos*. Instituto Superior Técnico.

Moss, J. A., Nocker, A., Lepo, J. E., & Snyder, R. A. (2006). Stability and Change in Estuarine Biofilm Bacterial Community Diversity. *Applied and Environmental Microbiology*, *72*(9), 5679–5688.

Muyzer, G. (1999). DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology*, *2*, 317–322.

Muyzer, G., Uitterlinden, A. G., & De Waal, E. C. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Apply Environmental Microbiology*, *59*(3), 695–700.

Newton, A., & Mudge, S. M. (2003). Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal. *Estuarine Coastal and Shelf Science*, *57*(1-2), 73–85.

Pacheco, A., Carrasco, A. R., Vila-Concejo, A., Ferreira, Ó., & Dias, J. A. (2006). Recent Evolution of Faro Channel and its Association to Dredging Operations (Algarve, Portugal). *Journal of Coastal Research*, *39*(39), 572–577.

Pacheco, A., Carrasco, A. R., Vila-Concejo, A., Ferreira, Ó., & Dias, J. A. (2007). A coastal management program for channels located in backbarrier systems. *Ocean & Coastal Management*, *50*(1-2), 119–143.

Pacheco, A., Ferreira, Ó., Williams, J. J., Garel, E., Vila-Concejo, A., & Dias, J. A. (2010). Hydrodynamics and equilibrium of a multiple-inlet system. *Marine Geology*, *274*(1-4), 32–42.

Pacheco, A., Vila-Concejo, A., Ferreira, Ó., & Dias, J. A. (2003). Recent bathymetric evolution of the Faro Channel (Algarve, Portugal). *4º Symposium on the Iberian Atlantic Margin*, 1986–1987.

Pacheco, A., Vila-Concejo, A., Ferreira, Ó., & Dias, J. A. (2008). Assessment of tidal inlet evolution and stability using sediment budget computations and hydraulic parameter analysis. *Marine Geology*, *247*(1-2), 104–127.

Pansu, M., & Gautheyrou, J. (2006). *Handbook of soil analysis: Mineralogical, organic and inorganic methods* (1st ed.). Berlin: Springer.

Pastor, J. (2012). *Caracterização granulométricas de sedimentos do delta de enchente da Barra de São Luís/Ancão do sistema lagunar da Ria Formosa*. Universidade do Algarve.

Peart, M. R. (1993). Using sediment properties as natural tracers for sediment source: two case studies from Hong Kong. *Tracers In Hydrology. Proc. International Symposium*, (215), 313-318.

Pilkey Jr., O. H., Neal, W. J., Monteiro, J. H., & Dias, J. M. (1989). Algarve Barrier Islands: A Noncoastal-Plain System in Portugal. *Journal of Coastal Research*, *5*(2), 239-261.

Pravdic, V. (1970). Surface charge characterization of sea sediments. *Limnology and Oceanography*, *15*(2), 230–233.

Reineck, H. E., & Singh, I. B. (1978). Depositional Sedimentary Environments. With Reference to Terrigenous Clastics. In H. Caspers (Ed.), *International Review of Hidrobiology* (60th ed., p. 130–131). Berlin: Springer-Verlag.

Rodrigues, A., Carvalho, A., Reia, J., Azevedo, B., Martins, C., Duarte, P., . . . Falcão, M. (2005). A Geographical Information System for Ria Formosa (South Coast of Portugal).

Rosati, J. D. (2009). *Barrier island migration over a consolidated substrate* (Unpublished doctoral dissertation). Louisiana State University and Agriculture and Mechanical College, Baton Rouge.

Rosati, J. D., Dean, R. G., & Stone, G. W. (2010). A cross-shore model of barrier island migration over a compressible substrate. *Marine Geology*, *271*(1-2), 1–16.

Saintilan, N. (2009). *Australian saltmarsh ecology* (1st ed.). Collingwood, Vic: CSIRO Pub.

Salles, P. (2001). *Hydrodynamic Controls On Multiple Tidal Inlet Persistence* (Doctoral dissertation, Massachusetts Institute of Technology and the Woods Hole Oceanographic Institution).

Salles, P., Voulgaris, G., & Aubrey, D. G. (2005). Contribution of nonlinear mechanisms in the persistence of multiple tidal inlet systems. *Estuarine Coastal and Shelf Science*, *65*(3), 475–491.

Schaechter, M. (2009). *Encyclopedia of microbiology* (3rd ed.). Amsterdam: Elsevier/Academic Press.

Schönborn, W. (1982). J. S. Gray, The Ecology of Marine Sediments — An Introduction to the Structures and Functions of Benthic Communities (Cambridge Studies in Modern Biology 2). XI + 185 S., 61 Abb., 21 Tab. Cambridge 1981. *Journal of Basic Microbiology*, *22*(6), 427.

Sheffield, V. C. (1989). Attachment of a 40-Base-Pair G+C-Rich Sequence (GC-Clamp) to Genomic DNA Fragments by the Polymerase Chain Reaction Results in Improved Detection of Single-Base Changes. *Proceedings of The National Academy of Sciences*, *86*(1), 232–236. doi:10.1073/pnas.86.1.232

Silva, T. (2012). *Caracterização das variações espaciais das distribuições granulométricas dos sedimentos superficiais intermareais, do delta de enchente da Barra da Fuseta e ambientes adjacentes*. Universidade do Algarve.

Soares, J. (2011). *Caracterização das variações espaciais das distribuições granulométricas dos sedimentos superficiais intermareais, do delta de enchente da barra da Armona e ambientes adjacentes*. Universidade do Algarve.

Stauble, D. K. (1998). *Techniques for measuring and analyzing inlet ebb-shoal evolution* (CETN-IV-13). Coastal Engineering Technical Note.

Steele, J. H., Thorpe, S. A., & Turekian, K. K. (2009). *Marine Biology* (2nd ed.). Burlington: Academic Press, Elsevier.

Sánchez-García, L., Andrés, J. R., Martín-Rubí, J. A., & Louchouarn, P. (2009). Diagenetic state and source characterization of marine sediments from the inner continental shelf of the Gulf of Cádiz (SW Spain), constrained by terrigenous biomarkers. *Organic Geochemistry*, *40*(2), 184–194.

Sørlie, T., Johnsen, H., Vu, P., Lind, G. E., Lothe, R., & Børresen-Dale, A. L. (2005). Mutation Screening of the TP53 Gene by Temporal Temperature Gradient Gel Electrophoresis. *Methods in Molecular Biology, Molecular Toxicology Protocols*, *291*, 207–216.

Thiyagarajan, V., Lau, S., Tsoi, M., Zhang, P., & Qian, P. Y. (2010). Monitoring Bacterial Biodiversity in Surface Sediment Using Terminal Restriction Fragment Length Polymorphism Analysis ( T-RFLP ): Application to Coastal Environment. *Coastal Environmental and Ecosystem Issues of the East China Sea*, 151-163.

U.S. Army Corps of Engineers. (1995). *Engineering and design: Coastal geology*. Washington, D.C.: Author.

Van Rijn, L. C. (1993). *Principles of sediment transport in rivers, estuaries, and coastal seas* (1st ed.). Amsterdam, the Netherlands: Aqua Publications.

Van Rijn, L. C., Walstra, D. R., & Ormondt, M. V. (2007). Unified View of Sediment Transport by Currents and Waves. IV: Application of Morphodynamic Model. *Journal of Hydraulic Engineering-asce*, *133*(7), 776–793.

Vasquez, A., Ahrne, S., Pettersson, B., & Molin, G. (2001). Temporal temperature gradient gel electrophoresis (TTGE) as a tool for identification of Lactobacillus casei, Lactobacillus paracasei, Lactobacillus zeae and Lactobacillus rhamnosus. *Letters in Applied Microbiology*, *32*(4), 215-9.

Vila-Concejo, A., Dias, J. M., Ferreira, O., & Matias, A. (1999). *Proceedings Coastal Sediments'99 Conference*. New York: ASCE.

Vila-Concejo, A., Matias, A., Ferreira, O., Duarte, C., & Dias, J. A. (2002). Recent Evolution of Faro Channel and its Association to Dredging Operations (Algarve, Portugal). *Journal of Coastal Research*, (36), 741-752.

Vila-Concejo, A., Matias, A., Pacheco, A., Ferreira, Ó., & Dias, J. A. (2006). Quantification of inlet-related hazards in barrier island systems. An example from the Ria Formosa (Portugal). *Continental Shelf Research*, *26*(9), 1045–1060.

Whitman, W. B., De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., & Schleifer, K. H. (Eds.). (2009). *Bergey's Manual of Systematic Bacteriology Volume 3: The Firmicutes* (2nd ed.). New York: Springer.

Whitman, W. B., Staley, J. T., Brown, D. R., Hedlung, B. P., Paster, B. J., Ward, N. L., & Ludwig, W. (Eds.). (2010). *Bergey's Manual of Systematic Bacteriology Volume 4: The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes* (2nd ed.). New York: Springer.

Wilson, K., & Walker, J. M. (2005). *Principles and techniques of biochemistry and molecular biology* (6th ed.). Cambridge: Cambridge University Press.

# Annex I

| | O3011101 | O3011102 | O3011103 | O3011104 | O3011105 | O3011105 | O3011107 | O3011108 | O3011109 |
|---|---|---|---|---|---|---|---|---|---|
| ANALYST AND DATE: | | | | | | | | | |
| SIEVING ERROR: | 0,3% | 0,9% | | -0,1% | 9,0% | | | | 0,0% |
| SAMPLE TYPE: | Unimodal, Moderately Well Sorted | Unimodal, Moderately Well Sorted | Bimodal, Very Poorly Sorted | Unimodal, Very Well Sorted | Unimodal, Very Well Sorted | Unimodal, Poorly Sorted | Unimodal, Poorly Sorted | Bimodal, Very Poorly Sorted | Unimodal, Moderately Sorted |
| TEXTURAL GROUP: | Slightly Gravelly Sand | Slightly Gravelly Sand | Sandy Mud | Sandy Gravel | Gravel | Sand | Muddy Sand | Sandy Mud | Slightly Gravelly Sand |
| SEDIMENT NAME: | Slightly Very Fine Gravelly Coarse Sand | Slightly Very Fine Gravelly Coarse Sand | Fine Sandy Very Coarse Silt | Sandy Very Fine Gravel | Very Coarse Gravel | Poorly Sorted Fine Sand | Fine Silty Fine Sand | Fine Sandy Medium Silt | Slightly Very Fine Gravelly Coarse Sand |
| METHOD OF MOMENTS Arithmetic (µm): MEAN | 828,2 | 815,2 | 77,42 | 2139,1 | 168987,6 | 154,2 | 142,0 | 86,37 | 843,4 |
| SORTING | 367,8 | 389,8 | 79,23 | 474,9 | 70859,9 | 81,52 | 94,09 | 88,01 | 449,4 |
| SKEWNESS | 2,329 | 1,714 | 1,078 | 0,544 | -0,448 | -0,391 | -0,244 | 0,790 | 1,505 |
| KURTOSIS | 19,21 | 9,656 | 3,014 | 3,646 | 2,970 | 2,365 | 1,758 | 2,273 | 6,477 |
| METHOD OF MOMENTS Geometric (µm): MEAN | 747,4 | 725,2 | 36,62 | 2056,1 | 81868,2 | 96,19 | 70,47 | 34,74 | 733,1 |
| SORTING | 1,527 | 1,572 | 4,294 | 1,252 | 17,95 | 4,365 | 5,456 | 5,233 | 1,642 |
| SKEWNESS | -0,206 | -0,016 | -0,707 | -0,319 | -3,607 | -2,173 | -1,383 | -0,592 | 0,167 |
| KURTOSIS | 3,524 | 3,063 | 2,992 | 4,378 | 14,24 | 6,572 | 3,556 | 2,355 | 2,600 |
| METHOD OF MOMENTS Logarithmic (φ): MEAN | 0,420 | 0,464 | 4,574 | -1,040 | -6,957 | 2,833 | 3,224 | 4,574 | 0,448 |
| SORTING | 0,611 | 0,653 | 2,073 | 0,324 | 1,830 | 1,573 | 2,058 | 2,356 | 0,716 |
| SKEWNESS | 0,206 | 0,016 | 0,471 | 0,319 | 3,263 | 1,888 | 1,237 | 0,445 | -0,167 |
| KURTOSIS | 3,524 | 3,063 | 3,064 | 4,378 | 12,66 | 8,153 | 3,978 | 2,489 | 2,600 |
| FOLK AND WARD METHOD (µm): MEAN | 756,3 | 726,4 | 43,91 | 2050,3 | 177487,2 | 162,0 | 97,78 | 43,36 | 726,7 |
| SORTING | 1,516 | 1,583 | 4,008 | 1,262 | 1,261 | 2,194 | 3,684 | 4,782 | 1,668 |
| SKEWNESS | -0,112 | -0,007 | -0,135 | -0,099 | -2,669 | -0,401 | -0,662 | -0,265 | 0,106 |
| KURTOSIS | 0,993 | 0,977 | 0,844 | 0,783 | 0,163 | 2,292 | 2,122 | 0,731 | 0,890 |
| FOLK AND WARD METHOD (φ): MEAN | 0,403 | 0,461 | 4,509 | -1,036 | -7,472 | 2,626 | 3,354 | 4,528 | 0,461 |
| SORTING | 0,600 | 0,663 | 2,003 | 0,336 | 0,335 | 1,134 | 1,881 | 2,258 | 0,738 |
| SKEWNESS | 0,112 | 0,007 | 0,135 | 0,099 | 2,669 | 0,401 | 0,662 | 0,265 | -0,106 |
| KURTOSIS | 0,993 | 0,977 | 0,844 | 0,783 | 0,163 | 2,292 | 2,122 | 0,731 | 0,890 |
| FOLK AND WARD METHOD (Description): MEAN: | Coarse Sand | Coarse Sand | Very Coarse Silt | Very Fine Gravel | Very Coarse Gravel | Fine Sand | Very Fine Sand | Very Coarse Silt | Coarse Sand |
| SORTING: | Moderately Well Sorted | Moderately Well Sorted | Very Poorly Sorted | Very Well Sorted | Very Well Sorted | Poorly Sorted | Poorly Sorted | Very Poorly Sorted | Moderately Sorted |
| SKEWNESS: | Fine Skewed | Symmetrical | Fine Skewed | Symmetrical | Very Fine Skewed | Very Fine Skewed | Very Fine Skewed | Fine Skewed | Coarse Skewed |
| KURTOSIS: | Mesokurtic | Mesokurtic | Platykurtic | Platykurtic | Very Platykurtic | Very Leptokurtic | Very Leptokurtic | Platykurtic | Platykurtic |
| MODE 1 (µm): | 855,0 | 855,0 | 206,5 | 2400,0 | 206536,5 | 206,5 | 206,5 | 206,5 | 605,0 |
| MODE 2 (µm): | | | 38,50 | | | | | 11,35 | |
| MODE 3 (µm): | | | | | | | | | |
| MODE 1 (φ): | 0,247 | 0,247 | 2,280 | -1,243 | -7,686 | 2,280 | 2,280 | 2,280 | 0,747 |
| MODE 2 (φ): | | | 4,703 | | | | | 6,466 | |
| MODE 3 (φ): | | | | | | | | | |
| $D_{10}$ (µm): | 413,8 | 395,7 | 6,291 | 1495,9 | 104781,1 | 69,53 | 8,640 | 4,544 | 387,3 |
| $D_{50}$ (µm): | 774,8 | 732,9 | 46,84 | 2091,6 | 181181,0 | 171,4 | 172,0 | 55,38 | 703,9 |
| $D_{90}$ (µm): | 1268,9 | 1307,8 | 221,7 | 2718,4 | 284984,6 | 280,7 | 284,7 | 243,4 | 1414,4 |
| $(D_{90} / D_{10})$ (µm): | 3,067 | 3,305 | 35,23 | 1,817 | 2,720 | 4,037 | 32,95 | 53,56 | 3,652 |
| $(D_{90} - D_{10})$ (µm): | 855,1 | 912,0 | 215,4 | 1222,5 | 180203,5 | 211,2 | 276,1 | 238,8 | 1027,1 |
| $(D_{75} / D_{25})$ (µm): | 1,746 | 1,855 | 8,254 | 1,444 | 1,741 | 1,898 | 2,356 | 14,13 | 2,101 |
| $(D_{75} - D_{25})$ (µm): | 420,4 | 452,4 | 118,4 | 758,0 | 100408,7 | 108,3 | 134,5 | 155,6 | 559,5 |
| $D_{10}$ (φ): | -0,344 | -0,387 | 2,174 | -1,443 | -8,155 | 1,833 | 1,812 | 2,039 | -0,500 |
| $D_{50}$ (φ): | 0,368 | 0,448 | 4,416 | -1,065 | -7,501 | 2,544 | 2,539 | 4,174 | 0,507 |
| $D_{90}$ (φ): | 1,273 | 1,337 | 7,312 | -0,581 | -6,711 | 3,846 | 6,855 | 7,782 | 1,369 |
| $(D_{90} / D_{10})$ (φ): | -3,706 | -3,455 | 3,364 | 0,403 | 0,823 | 2,099 | 3,782 | 3,817 | -2,737 |
| $(D_{90} - D_{10})$ (φ): | 1,617 | 1,725 | 5,139 | 0,862 | 1,444 | 2,013 | 5,042 | 5,743 | 1,869 |
| $(D_{75} / D_{25})$ (φ): | 35,97 | 33,92 | 2,053 | 0,592 | 0,898 | 1,435 | 1,589 | 2,482 | -10,355 |
| $(D_{75} - D_{25})$ (φ): | 0,804 | 0,892 | 3,045 | 0,530 | 0,800 | 0,924 | 1,236 | 3,820 | 1,071 |
| % GRAVEL: | 0,7% | 0,9% | 0,0% | 56,8% | 100,0% | 0,0% | 0,0% | 0,0% | 1,7% |
| % SAND: | 99,3% | 99,1% | 43,2% | 43,2% | 0,0% | 90,6% | 78,3% | 48,2% | 98,3% |
| % MUD: | 0,0% | 0,0% | 56,8% | 0,0% | 0,0% | 9,4% | 21,7% | 51,8% | 0,0% |
| % V COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 100,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % MEDIUM GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % FINE GRAVEL: | 0,1% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % V FINE GRAVEL: | 0,6% | 0,9% | 0,0% | 56,8% | 0,0% | 0,0% | 0,0% | 0,0% | 1,7% |
| % V COARSE SAND: | 22,6% | 22,5% | 0,0% | 42,9% | 0,0% | 0,0% | 0,0% | 0,0% | 26,9% |
| % COARSE SAND: | 59,9% | 55,9% | 0,0% | 0,3% | 0,0% | 0,0% | 0,0% | 0,0% | 47,7% |
| % MEDIUM SAND: | 16,0% | 20,0% | 6,5% | 0,0% | 0,0% | 17,9% | 19,4% | 8,9% | 23,2% |
| % FINE SAND: | 0,8% | 0,7% | 20,5% | 0,0% | 0,0% | 54,9% | 49,0% | 25,7% | 0,5% |
| % V FINE SAND: | 0,0% | 0,0% | 16,2% | 0,0% | 0,0% | 17,8% | 9,9% | 13,5% | 0,0% |
| % V COARSE SILT: | 0,0% | 0,0% | 16,9% | 0,0% | 0,0% | 0,6% | 2,9% | 10,6% | 0,0% |
| % COARSE SILT: | 0,0% | 0,0% | 15,8% | 0,0% | 0,0% | 2,2% | 4,7% | 11,4% | 0,0% |
| % MEDIUM SILT: | 0,0% | 0,0% | 11,5% | 0,0% | 0,0% | 2,1% | 4,9% | 12,1% | 0,0% |
| % FINE SILT: | 0,0% | 0,0% | 6,8% | 0,0% | 0,0% | 2,2% | 5,0% | 9,3% | 0,0% |
| % V FINE SILT: | 0,0% | 0,0% | 3,0% | 0,0% | 0,0% | 1,5% | 3,1% | 4,5% | 0,0% |
| % CLAY: | 0,0% | 0,0% | 2,8% | 0,0% | 0,0% | 0,8% | 1,2% | 3,9% | 0,0% |

| | 4011101 | 4011102 | 4011103 | 4011104 | O4011105 | O4011106 | O4011107 | 4011109 | 4011110 |
|---|---|---|---|---|---|---|---|---|---|
| ANALYST AND DATE: | | | | | | | | | |
| SIEVING ERROR: | 0,1% | 0,2% | -0,6% | 0,1% | | | | 1,1% | -0,1% |
| SAMPLE TYPE: | Bimodal, Moderately Sorted | Unimodal, Moderately Sorted | Unimodal, Well Sorted | Unimodal, Moderately Well Sorted | Bimodal, Very Poorly Sorted | Unimodal, Very Poorly Sorted | Bimodal, Poorly Sorted | Bimodal, Poorly Sorted | Unimodal, Well Sorted |
| TEXTURAL GROUP: | Gravelly Sand | Gravelly Sand | Slightly Gravelly Sand | Slightly Gravelly Sand | Muddy Sand | Muddy Sand | Muddy Sand | Sandy Gravel | Gravelly Sand |
| SEDIMENT NAME: | Very Fine Gravelly Coarse Sand | Very Fine Gravelly Very Coarse Sand | Slightly Very Fine Gravelly Coarse Sand | Slightly Very Fine Gravelly Coarse Sand | Very Coarse Silty Fine Sand | Medium Silty Medium Sand | Very Coarse Silty Fine Sand | Sandy Very Fine Gravel | Very Fine Gravelly Very Coarse Sand |
| METHOD OF MOMENTS Arithmetic (µm): MEAN | 1488,4 | 1525,7 | 697,5 | 764,6 | 94,20 | 110,2 | 88,12 | 3069,1 | 1556,3 |
| SORTING | 1312,0 | 1011,1 | 363,3 | 391,5 | 89,23 | 117,6 | 82,39 | 1852,2 | 509,5 |
| SKEWNESS | 2,470 | 2,945 | 4,512 | 2,285 | 0,697 | 0,382 | 0,867 | 0,555 | 1,238 |
| KURTOSIS | 9,687 | 14,95 | 31,14 | 12,67 | 2,115 | 1,343 | 2,562 | 2,587 | 5,213 |
| METHOD OF MOMENTS Geometric (µm): MEAN | 1126,1 | 1288,3 | 635,8 | 679,7 | 42,39 | 22,83 | 43,74 | 2239,2 | 1460,2 |
| SORTING | 1,989 | 1,712 | 1,434 | 1,553 | 4,861 | 10,21 | 4,313 | 3,024 | 1,363 |
| SKEWNESS | 0,561 | 0,271 | 1,163 | 0,387 | -0,831 | -0,254 | -0,926 | -4,674 | 0,135 |
| KURTOSIS | 2,912 | 3,633 | 7,085 | 3,390 | 2,907 | 1,384 | 3,338 | 43,71 | 3,553 |
| METHOD OF MOMENTS Logarithmic (φ): MEAN | -0,171 | -0,366 | 0,653 | 0,557 | 4,231 | 2,800 | 4,263 | -1,163 | -0,546 |
| SORTING | 0,992 | 0,776 | 0,520 | 0,635 | 2,195 | 2,588 | 2,036 | 1,597 | 0,447 |
| SKEWNESS | -0,561 | -0,271 | -1,163 | -0,387 | 0,597 | 0,803 | 0,640 | 4,674 | -0,135 |
| KURTOSIS | 2,912 | 3,633 | 7,085 | 3,390 | 3,016 | 2,566 | 3,375 | 43,71 | 3,553 |
| FOLK AND WARD METHOD (µm): MEAN | 1096,0 | 1257,9 | 630,3 | 666,4 | 54,49 | 117,7 | 54,15 | 2520,3 | 1447,3 |
| SORTING | 1,994 | 1,676 | 1,402 | 1,551 | 4,168 | 5,343 | 3,815 | 2,218 | 1,369 |
| SKEWNESS | 0,137 | -0,076 | 0,138 | 0,103 | -0,284 | -0,574 | -0,238 | -0,246 | 0,062 |
| KURTOSIS | 0,929 | 1,022 | 1,315 | 1,018 | 0,808 | 0,962 | 0,882 | 1,331 | 0,998 |
| FOLK AND WARD METHOD (φ): MEAN | -0,132 | -0,331 | 0,666 | 0,586 | 4,198 | 3,087 | 4,207 | -1,334 | -0,533 |
| SORTING | 0,995 | 0,745 | 0,488 | 0,633 | 2,059 | 2,418 | 1,932 | 1,149 | 0,453 |
| SKEWNESS | -0,137 | 0,076 | -0,138 | -0,103 | 0,284 | 0,574 | 0,238 | 0,246 | -0,062 |
| KURTOSIS | 0,929 | 1,022 | 1,315 | 1,018 | 0,808 | 0,962 | 0,882 | 1,331 | 0,998 |
| FOLK AND WARD METHOD (Description): MEAN: | Very Coarse Sand | Very Coarse Sand | Coarse Sand | Coarse Sand | Very Coarse Silt | Very Fine Sand | Very Coarse Silt | Very Fine Gravel | Very Coarse Sand |
| SORTING: | Moderately Sorted | Moderately Sorted | Well Sorted | Moderately Well Sorted | Very Poorly Sorted | Very Poorly Sorted | Poorly Sorted | Poorly Sorted | Well Sorted |
| SKEWNESS: | Coarse Skewed | Symmetrical | Coarse Skewed | Coarse Skewed | Fine Skewed | Very Fine Skewed | Fine Skewed | Fine Skewed | Symmetrical |
| KURTOSIS: | Mesokurtic | Mesokurtic | Leptokurtic | Mesokurtic | Platykurtic | Mesokurtic | Platykurtic | Leptokurtic | Mesokurtic |
| MODE 1 (µm): | 605,0 | 1700,0 | 605,0 | 605,0 | 206,5 | 280,3 | 206,5 | 2400,0 | 1200,0 |
| MODE 2 (µm): | 1700,0 | | | | 38,50 | | 60,87 | 605,0 | |
| MODE 3 (µm): | | | | | | | | | |
| MODE 1 (φ): | 0,747 | -0,743 | 0,747 | 0,747 | 2,280 | 1,839 | 2,280 | -1,243 | -0,243 |
| MODE 2 (φ): | -0,743 | | | | 4,703 | | 4,042 | 0,747 | |
| MODE 3 (φ): | | | | | | | | | |
| $D_{10}$ (µm): | 512,0 | 626,9 | 411,0 | 390,2 | 6,535 | 7,914 | 7,534 | 703,4 | 1021,3 |
| $D_{50}$ (µm): | 1058,7 | 1306,2 | 613,8 | 657,6 | 68,09 | 242,0 | 63,47 | 2697,2 | 1437,1 |
| $D_{90}$ (µm): | 2754,3 | 2452,0 | 970,7 | 1247,0 | 253,2 | 829,3 | 237,6 | 5816,0 | 2241,7 |
| $(D_{90} / D_{10})$ (µm): | 5,380 | 3,912 | 2,362 | 3,196 | 38,74 | 104,8 | 31,53 | 8,268 | 2,195 |
| $(D_{90} - D_{10})$ (µm): | 2242,3 | 1825,1 | 559,7 | 856,8 | 246,7 | 821,4 | 230,0 | 5112,6 | 1220,4 |
| $(D_{75} / D_{25})$ (µm): | 2,741 | 2,011 | 1,448 | 1,792 | 9,440 | 9,117 | 7,159 | 2,416 | 1,558 |
| $(D_{75} - D_{25})$ (µm): | 1121,0 | 905,5 | 232,4 | 398,4 | 162,0 | 288,9 | 134,7 | 2414,2 | 647,6 |
| $D_{10}$ (φ): | -1,462 | -1,294 | 0,043 | -0,318 | 1,982 | 0,270 | 2,074 | -2,540 | -1,165 |
| $D_{50}$ (φ): | -0,082 | -0,385 | 0,704 | 0,605 | 3,876 | 2,047 | 3,978 | -1,431 | -0,523 |
| $D_{90}$ (φ): | 0,966 | 0,674 | 1,283 | 1,358 | 7,257 | 6,981 | 7,052 | 0,507 | -0,030 |
| $(D_{90} / D_{10})$ (φ): | -0,661 | -0,521 | 29,89 | -4,264 | 3,662 | 25,85 | 3,401 | -0,200 | 0,026 |
| $(D_{90} - D_{10})$ (φ): | 2,428 | 1,968 | 1,240 | 1,676 | 5,276 | 6,711 | 4,979 | 3,048 | 1,134 |
| $(D_{75} / D_{25})$ (φ): | -0,775 | -0,187 | 2,295 | 6,639 | 2,314 | 2,964 | 2,062 | 0,377 | 0,251 |
| $(D_{75} - D_{25})$ (φ): | 1,455 | 1,008 | 0,534 | 0,841 | 3,239 | 3,189 | 2,840 | 1,273 | 0,640 |
| % GRAVEL: | 18,8% | 16,7% | 1,3% | 1,2% | 0,0% | 0,0% | 0,0% | 70,2% | 14,0% |
| % SAND: | 81,2% | 83,3% | 98,7% | 98,8% | 51,6% | 71,5% | 50,4% | 29,4% | 86,0% |
| % MUD: | 0,0% | 0,0% | 0,0% | 0,0% | 48,4% | 28,5% | 49,6% | 0,4% | 0,0% |
| % V COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % MEDIUM GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % FINE GRAVEL: | 5,3% | 2,6% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 26,3% | 0,0% |
| % V FINE GRAVEL: | 13,6% | 14,1% | 1,3% | 1,2% | 0,0% | 0,0% | 0,0% | 43,9% | 14,0% |
| % V COARSE SAND: | 33,9% | 52,9% | 7,0% | 16,1% | 0,0% | 0,0% | 0,0% | 16,8% | 78,5% |
| % COARSE SAND: | 38,8% | 27,0% | 72,3% | 58,3% | 0,0% | 0,0% | 0,0% | 6,6% | 7,3% |
| % MEDIUM SAND: | 8,4% | 3,4% | 19,4% | 23,9% | 10,6% | 47,7% | 8,2% | 2,8% | 0,2% |
| % FINE SAND: | 0,1% | 0,0% | 0,1% | 0,4% | 27,2% | 22,7% | 23,8% | 1,7% | 0,0% |
| % V FINE SAND: | 0,0% | 0,0% | 0,0% | 0,0% | 13,8% | 1,2% | 18,4% | 1,6% | 0,0% |
| % V COARSE SILT: | 0,0% | 0,0% | 0,0% | 0,0% | 14,2% | 4,7% | 17,4% | 0,0% | 0,0% |
| % COARSE SILT: | 0,0% | 0,0% | 0,0% | 0,0% | 12,6% | 6,5% | 12,8% | 0,0% | 0,0% |
| % MEDIUM SILT: | 0,0% | 0,0% | 0,0% | 0,0% | 9,6% | 7,4% | 9,0% | 0,0% | 0,0% |
| % FINE SILT: | 0,0% | 0,0% | 0,0% | 0,0% | 6,2% | 5,7% | 5,5% | 0,0% | 0,0% |
| % V FINE SILT: | 0,0% | 0,0% | 0,0% | 0,0% | 2,9% | 2,9% | 2,5% | 0,0% | 0,0% |
| % CLAY: | 0,0% | 0,0% | 0,0% | 0,0% | 2,8% | 1,2% | 2,4% | 0,4% | 0,0% |

| | O4011111 | 8111103 | 8111104 | O8111105 | O8111106 | O8111107 | 8111108 | 8111109 | 8111110 | 8111111 |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYST AND DATE: | | | | | | | | | | |
| SIEVING ERROR: | | 0,3% | -1,3% | | | | 3,4% | -0,3% | 0,6% | -0,5% |
| SAMPLE TYPE: | Unimodal, Very Poorly Sorted | Unimodal, Moderately Sorted | Unimodal, Moderately Well Sorted | Bimodal, Very Poorly Sorted | Bimodal, Very Poorly Sorted | Bimodal, Poorly Sorted | Unimodal, Poorly Sorted | Unimodal, Poorly Sorted | Unimodal, Moderately Sorted | Bimodal, Moderately Sorted |
| TEXTURAL GROUP: | Muddy Sand | Slightly Gravelly Sand | Slightly Gravelly Sand | Muddy Sand | Sandy Mud | Muddy Sand | Gravelly Sand | Sandy Gravel | Gravelly Sand | Gravelly Sand |
| SEDIMENT NAME: | Coarse Silty Medium Sand | Slightly Very Fine Gravelly Coarse Sand | Slightly Very Fine Gravelly Coarse Sand | Very Coarse Silty Fine Sand | Fine Sandy Coarse Silt | Very Coarse Silty Fine Sand | Very Fine Gravelly Very Coarse Sand | Sandy Very Fine Gravel | Very Fine Gravelly Coarse Sand | Very Fine Gravelly Coarse Sand |
| METHOD OF MOMENTS Arithmetic (µm): MEAN | 84,59 | 873,6 | 679,3 | 99,11 | 89,01 | 95,26 | 1431,5 | 1919,5 | 1063,9 | 1017,2 |
| SORTING | 113,1 | 776,9 | 385,0 | 91,00 | 98,90 | 84,60 | 1028,4 | 1499,5 | 582,8 | 789,0 |
| SKEWNESS | 0,880 | 4,779 | 7,637 | 0,600 | 0,795 | 0,723 | 1,833 | 1,429 | 1,655 | 2,939 |
| KURTOSIS | 1,948 | 33,84 | 113,3 | 1,965 | 2,006 | 2,291 | 8,215 | 4,946 | 8,220 | 16,70 |
| METHOD OF MOMENTS Geometric (µm): MEAN | 13,57 | 707,0 | 609,3 | 44,60 | 31,02 | 48,32 | 1056,7 | 1301,2 | 914,9 | 771,0 |
| SORTING | 10,10 | 1,770 | 1,516 | 4,992 | 5,777 | 4,337 | 2,874 | 3,334 | 1,693 | 2,700 |
| SKEWNESS | 0,097 | 0,789 | 0,327 | -0,893 | -0,443 | -1,040 | -5,427 | -4,584 | -0,089 | -6,079 |
| KURTOSIS | 1,377 | 4,903 | 4,938 | 2,954 | 2,215 | 3,548 | 55,37 | 41,61 | 2,950 | 66,42 |
| METHOD OF MOMENTS Logarithmic (φ): MEAN | 2,646 | 0,500 | 0,715 | 4,120 | 4,424 | 4,081 | -0,080 | -0,380 | 0,128 | 0,375 |
| SORTING | 2,675 | 0,824 | 0,600 | 2,210 | 2,468 | 2,010 | 1,523 | 1,737 | 0,759 | 1,433 |
| SKEWNESS | 0,710 | -0,789 | -0,327 | 0,667 | 0,235 | 0,722 | 5,427 | 4,584 | 0,089 | 6,079 |
| KURTOSIS | 2,291 | 4,903 | 4,938 | 3,099 | 2,373 | 3,557 | 55,37 | 41,61 | 2,950 | 66,42 |
| FOLK AND WARD METHOD (µm): MEAN | 134,9 | 693,2 | 605,4 | 59,02 | 44,01 | 61,36 | 1139,8 | 1426,8 | 908,9 | 802,2 |
| SORTING | 5,318 | 1,708 | 1,523 | 4,160 | 4,905 | 3,670 | 2,031 | 2,328 | 1,718 | 1,865 |
| SKEWNESS | -0,551 | 0,234 | 0,001 | -0,354 | -0,038 | -0,289 | -0,050 | -0,103 | -0,048 | 0,102 |
| KURTOSIS | 0,800 | 0,977 | 1,033 | 0,828 | 0,708 | 0,901 | 0,974 | 1,027 | 0,939 | 0,930 |
| FOLK AND WARD METHOD (φ): MEAN | 2,890 | 0,529 | 0,724 | 4,083 | 4,506 | 4,027 | -0,189 | -0,513 | 0,138 | 0,318 |
| SORTING | 2,411 | 0,773 | 0,607 | 2,057 | 2,294 | 1,876 | 1,022 | 1,219 | 0,781 | 0,899 |
| SKEWNESS | 0,551 | -0,234 | -0,001 | 0,354 | 0,038 | 0,289 | 0,050 | 0,103 | 0,048 | -0,102 |
| KURTOSIS | 0,800 | 0,977 | 1,033 | 0,828 | 0,708 | 0,901 | 0,974 | 1,027 | 0,939 | 0,930 |
| FOLK AND WARD METHOD (Description): MEAN: | Fine Sand | Coarse Sand | Coarse Sand | Very Coarse Silt | Very Coarse Silt | Very Coarse Silt | Very Coarse Sand | Very Coarse Sand | Coarse Sand | Coarse Sand |
| SORTING: | Very Poorly Sorted | Moderately Sorted | Moderately Well Sorted | Very Poorly Sorted | Very Poorly Sorted | Poorly Sorted | Poorly Sorted | Poorly Sorted | Moderately Sorted | Moderately Sorted |
| SKEWNESS: | Very Fine Skewed | Coarse Skewed | Symmetrical | Very Fine Skewed | Symmetrical | Fine Skewed | Symmetrical | Fine Skewed | Symmetrical | Coarse Skewed |
| KURTOSIS: | Platykurtic | Mesokurtic | Mesokurtic | Platykurtic | Platykurtic | Mesokurtic | Mesokurtic | Mesokurtic | Mesokurtic | Mesokurtic |
| MODE 1 (µm): | 280,3 | 605,0 | 605,0 | 206,5 | 280,3 | 206,5 | 1700,0 | 1700,0 | 855,0 | 605,0 |
| MODE 2 (µm): | | | | 44,85 | 20,90 | 60,87 | | | | 1200,0 |
| MODE 3 (µm): | | | | | | | | | | |
| MODE 1 (φ): | 1,839 | 0,747 | 0,747 | 2,280 | 1,839 | 2,280 | -0,743 | -0,743 | 0,247 | 0,747 |
| MODE 2 (φ): | | | | 4,483 | 5,585 | 4,042 | | | | -0,243 |
| MODE 3 (φ): | | | | | | | | | | |
| $D_{10}$ (µm): | 9,482 | 380,0 | 363,6 | 6,670 | 5,304 | 8,541 | 472,0 | 467,0 | 436,8 | 383,2 |
| $D_{50}$ (µm): | 264,1 | 645,0 | 606,3 | 79,34 | 42,35 | 74,76 | 1149,7 | 1491,2 | 928,3 | 776,8 |
| $D_{90}$ (µm): | 1711,4 | 1518,7 | 998,0 | 258,8 | 279,8 | 246,3 | 2668,3 | 3931,1 | 1814,5 | 1867,4 |
| $(D_{90} / D_{10})$ (µm): | 180,5 | 3,997 | 2,745 | 38,80 | 52,75 | 28,84 | 5,653 | 8,417 | 4,154 | 4,873 |
| $(D_{90} - D_{10})$ (µm): | 1701,9 | 1138,7 | 634,4 | 252,1 | 274,5 | 237,8 | 2196,3 | 3464,0 | 1377,6 | 1484,2 |
| $(D_{75} / D_{25})$ (µm): | 13,54 | 2,063 | 1,750 | 9,023 | 16,32 | 6,599 | 2,699 | 3,087 | 2,111 | 2,431 |
| $(D_{75} - D_{25})$ (µm): | 400,1 | 504,7 | 345,1 | 169,9 | 196,8 | 145,3 | 1173,6 | 1702,7 | 698,2 | 734,9 |
| $D_{10}$ (φ): | -0,775 | -0,603 | 0,003 | 1,950 | 1,838 | 2,021 | -1,416 | -1,975 | -0,860 | -0,901 |
| $D_{50}$ (φ): | 1,921 | 0,633 | 0,722 | 3,656 | 4,561 | 3,742 | -0,201 | -0,576 | 0,107 | 0,364 |
| $D_{90}$ (φ): | 6,721 | 1,396 | 1,460 | 7,228 | 7,559 | 6,871 | 1,083 | 1,098 | 1,195 | 1,384 |
| $(D_{90} / D_{10})$ (φ): | -8,670 | -2,316 | 497,2 | 3,706 | 4,113 | 3,399 | -0,765 | -0,556 | -1,390 | -1,536 |
| $(D_{90} - D_{10})$ (φ): | 7,496 | 1,999 | 1,457 | 5,278 | 5,721 | 4,850 | 2,499 | 3,073 | 2,054 | 2,285 |
| $(D_{75} / D_{25})$ (φ): | 4,104 | 35,91 | 3,585 | 2,329 | 2,788 | 2,069 | -0,593 | -0,220 | -1,642 | -3,005 |
| $(D_{75} - D_{25})$ (φ): | 3,759 | 1,045 | 0,807 | 3,174 | 4,029 | 2,722 | 1,432 | 1,626 | 1,078 | 1,282 |
| % GRAVEL: | 0,0% | 4,3% | 0,4% | 0,0% | 0,0% | 0,0% | 21,4% | 35,7% | 5,8% | 7,9% |
| % SAND: | 67,9% | 95,7% | 99,6% | 54,2% | 44,6% | 54,6% | 78,0% | 63,6% | 94,2% | 91,5% |
| % MUD: | 32,1% | 0,0% | 0,0% | 45,8% | 55,4% | 45,4% | 0,6% | 0,7% | 0,0% | 0,6% |
| % V COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % MEDIUM GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % FINE GRAVEL: | 0,0% | 1,1% | 0,2% | 0,0% | 0,0% | 0,0% | 2,4% | 9,5% | 0,3% | 1,2% |
| % V FINE GRAVEL: | 0,0% | 3,2% | 0,3% | 0,0% | 0,0% | 0,0% | 19,0% | 26,2% | 5,5% | 6,7% |
| % V COARSE SAND: | 0,0% | 19,7% | 9,4% | 0,0% | 0,0% | 0,0% | 35,8% | 32,0% | 38,8% | 28,9% |
| % COARSE SAND: | 0,0% | 47,6% | 59,9% | 0,0% | 0,0% | 0,0% | 32,0% | 21,2% | 40,5% | 39,9% |
| % MEDIUM SAND: | 53,8% | 27,6% | 29,2% | 11,7% | 16,1% | 9,4% | 7,3% | 6,8% | 14,7% | 21,5% |
| % FINE SAND: | 13,3% | 0,6% | 1,1% | 29,0% | 23,6% | 26,6% | 2,1% | 1,6% | 0,3% | 0,8% |
| % V FINE SAND: | 0,8% | 0,3% | 0,0% | 13,5% | 4,9% | 18,6% | 0,9% | 2,1% | 0,1% | 0,4% |
| % V COARSE SILT: | 7,4% | 0,0% | 0,0% | 13,8% | 11,4% | 16,7% | 0,0% | 0,0% | 0,0% | 0,0% |
| % COARSE SILT: | 8,9% | 0,0% | 0,0% | 11,6% | 14,9% | 11,7% | 0,0% | 0,0% | 0,0% | 0,0% |
| % MEDIUM SILT: | 7,7% | 0,0% | 0,0% | 8,7% | 13,6% | 7,8% | 0,0% | 0,0% | 0,0% | 0,0% |
| % FINE SILT: | 4,7% | 0,0% | 0,0% | 5,9% | 8,5% | 4,8% | 0,0% | 0,0% | 0,0% | 0,0% |
| % V FINE SILT: | 2,2% | 0,0% | 0,0% | 2,9% | 3,6% | 2,2% | 0,0% | 0,0% | 0,0% | 0,0% |
| % CLAY: | 1,2% | 0,0% | 0,0% | 2,9% | 3,4% | 2,1% | 0,5% | 0,6% | 0,0% | 0,5% |

| | 9111101 | 9111102 | 9111103 | 9111104 | 9111105 | O9111106 | O9111107 | O9111108 | 9111109 |
|---|---|---|---|---|---|---|---|---|---|
| ANALYST AND DATE: | | | | | | | | | |
| SIEVING ERROR: | -0,8% | 1,1% | -2,7% | 0,0% | 0,1% | | | | 0,0% |
| SAMPLE TYPE: | Unimodal, Moderately Well Sorted | Unimodal, Moderately Well Sorted | Bimodal, Poorly Sorted | Unimodal, Well Sorted | Unimodal, Moderately Well Sorted | Unimodal, Poorly Sorted | Unimodal, Poorly Sorted | Bimodal, Very Poorly Sorted | Unimodal, Moderately Well Sorted |
| TEXTURAL GROUP: | Slightly Gravelly Sand | Slightly Gravelly Sand | Gravelly Sand | Gravelly Sand | Slightly Gravelly Sand | Muddy Sand | Muddy Sand | Sandy Mud | Slightly Gravelly Sand |
| SEDIMENT NAME: | Slightly Very Fine Gravelly Coarse Sand | Slightly Very Fine Gravelly Coarse Sand | Very Fine Gravelly Very Coarse Sand | Very Fine Gravelly Very Coarse Sand | Slightly Very Fine Gravelly Coarse Sand | Medium Silty Fine Sand | Very Coarse Silty Fine Sand | Fine Sandy Coarse Silt | Slightly Very Fine Gravelly Medium Sand |
| METHOD OF MOMENTS Arithmetic (µm): MEAN | 777,3 | 701,3 | 1150,7 | 1775,2 | 1070,5 | 157,0 | 97,06 | 69,41 | 552,5 |
| SORTING | 368,1 | 311,9 | 902,0 | 485,7 | 718,6 | 105,4 | 86,32 | 74,70 | 278,5 |
| SKEWNESS | 3,320 | 1,716 | 0,949 | 3,112 | 5,384 | -0,454 | 0,639 | 1,181 | 2,098 |
| KURTOSIS | 21,17 | 9,175 | 3,987 | 28,24 | 41,66 | 1,652 | 2,146 | 3,381 | 15,14 |
| METHOD OF MOMENTS Geometric (µm): MEAN | 708,4 | 632,8 | 620,8 | 1695,7 | 938,0 | 62,51 | 46,72 | 30,64 | 488,6 |
| SORTING | 1,456 | 1,521 | 5,862 | 1,274 | 1,569 | 7,657 | 4,659 | 4,509 | 1,586 |
| SKEWNESS | 0,665 | -0,071 | -3,647 | 0,393 | 0,614 | -1,204 | -0,981 | -0,554 | 0,068 |
| KURTOSIS | 4,826 | 3,677 | 21,26 | 5,657 | 5,991 | 2,780 | 3,214 | 2,602 | 3,344 |
| METHOD OF MOMENTS Logarithmic (φ): MEAN | 0,497 | 0,660 | 0,688 | -0,762 | 0,092 | 2,585 | 4,125 | 4,901 | 1,033 |
| SORTING | 0,542 | 0,605 | 2,551 | 0,349 | 0,650 | 1,960 | 2,124 | 2,172 | 0,666 |
| SKEWNESS | -0,665 | 0,071 | 3,647 | -0,393 | -0,614 | 1,380 | 0,746 | 0,422 | -0,068 |
| KURTOSIS | 4,826 | 3,677 | 21,26 | 5,657 | 5,991 | 4,873 | 3,316 | 2,711 | 3,344 |
| FOLK AND WARD METHOD (µm): MEAN | 700,6 | 630,7 | 681,3 | 1703,8 | 916,4 | 192,9 | 59,44 | 35,00 | 487,5 |
| SORTING | 1,425 | 1,530 | 3,151 | 1,290 | 1,520 | 2,580 | 3,978 | 4,397 | 1,597 |
| SKEWNESS | 0,126 | 0,020 | -0,447 | 0,026 | -0,104 | -0,479 | -0,371 | -0,137 | 0,077 |
| KURTOSIS | 1,043 | 1,097 | 0,933 | 1,231 | 1,096 | 3,310 | 0,872 | 0,820 | 1,036 |
| FOLK AND WARD METHOD (φ): MEAN | 0,513 | 0,665 | 0,554 | -0,769 | 0,126 | 2,374 | 4,072 | 4,836 | 1,036 |
| SORTING | 0,511 | 0,613 | 1,656 | 0,367 | 0,604 | 1,367 | 1,992 | 2,137 | 0,676 |
| SKEWNESS | -0,126 | -0,020 | 0,447 | -0,026 | 0,104 | 0,479 | 0,371 | 0,137 | -0,077 |
| KURTOSIS | 1,043 | 1,097 | 0,933 | 1,231 | 1,096 | 3,310 | 0,872 | 0,820 | 1,036 |
| FOLK AND WARD METHOD (Description): MEAN: | Coarse Sand | Coarse Sand | Coarse Sand | Very Coarse Sand | Coarse Sand | Fine Sand | Very Coarse Silt | Very Coarse Silt | Medium Sand |
| SORTING: | Moderately Well Sorted | Moderately Well Sorted | Poorly Sorted | Well Sorted | Moderately Well Sorted | Poorly Sorted | Poorly Sorted | Very Poorly Sorted | Moderately Well Sorted |
| SKEWNESS: | Coarse Skewed | Symmetrical | Very Fine Skewed | Symmetrical | Fine Skewed | Very Fine Skewed | Very Fine Skewed | Fine Skewed | Symmetrical |
| KURTOSIS: | Mesokurtic | Mesokurtic | Mesokurtic | Leptokurtic | Mesokurtic | Extremely Leptokurtic | Platykurtic | Platykurtic | Mesokurtic |
| MODE 1 (µm): | 605,0 | 605,0 | 1700,0 | 1700,0 | 1200,0 | 280,3 | 206,5 | 152,2 | 427,5 |
| MODE 2 (µm): | | | 107,5 | | | | | 20,90 | |
| MODE 3 (µm): | | | | | | | | | |
| MODE 1 (φ): | 0,747 | 0,747 | -0,743 | -0,743 | -0,243 | 1,839 | 2,280 | 2,720 | 1,247 |
| MODE 2 (φ): | | | 3,237 | | | | | 5,585 | |
| MODE 3 (φ): | | | | | | | | | |
| $D_{10}$ (µm): | 450,5 | 373,8 | 116,8 | 1195,4 | 530,6 | 16,29 | 7,055 | 4,501 | 271,6 |
| $D_{50}$ (µm): | 679,0 | 626,4 | 1021,7 | 1688,4 | 951,0 | 222,8 | 80,84 | 38,01 | 474,8 |
| $D_{90}$ (µm): | 1169,7 | 1106,1 | 2360,3 | 2410,0 | 1518,0 | 365,7 | 247,8 | 198,4 | 912,4 |
| $(D_{90} / D_{10})$ (µm): | 2,596 | 2,959 | 20,21 | 2,016 | 2,861 | 22,44 | 35,13 | 44,07 | 3,359 |
| $(D_{90} - D_{10})$ (µm): | 719,2 | 732,4 | 2243,6 | 1214,6 | 987,4 | 349,4 | 240,8 | 193,9 | 640,8 |
| $(D_{75} / D_{25})$ (µm): | 1,626 | 1,708 | 4,454 | 1,341 | 1,710 | 1,765 | 7,733 | 10,06 | 1,834 |
| $(D_{75} - D_{25})$ (µm): | 344,3 | 348,6 | 1294,0 | 497,6 | 510,3 | 119,7 | 153,2 | 105,4 | 303,2 |
| $D_{10}$ (φ): | -0,226 | -0,146 | -1,239 | -1,269 | -0,602 | 1,451 | 2,013 | 2,334 | 0,132 |
| $D_{50}$ (φ): | 0,559 | 0,675 | -0,031 | -0,756 | 0,073 | 2,166 | 3,629 | 4,718 | 1,075 |
| $D_{90}$ (φ): | 1,150 | 1,420 | 3,098 | -0,258 | 0,914 | 5,940 | 7,147 | 7,796 | 1,880 |
| $(D_{90} / D_{10})$ (φ): | -5,085 | -9,756 | -2,501 | 0,203 | -1,518 | 4,093 | 3,551 | 3,340 | 14,22 |
| $(D_{90} - D_{10})$ (φ): | 1,377 | 1,565 | 4,337 | 1,012 | 1,517 | 4,488 | 5,134 | 5,462 | 1,748 |
| $(D_{75} / D_{25})$ (φ): | 5,346 | 4,092 | -1,917 | 0,562 | -1,604 | 1,442 | 2,177 | 2,076 | 2,497 |
| $(D_{75} - D_{25})$ (φ): | 0,701 | 0,772 | 2,155 | 0,424 | 0,774 | 0,820 | 2,951 | 3,331 | 0,875 |
| % GRAVEL: | 1,1% | 0,4% | 15,3% | 21,2% | 3,1% | 0,0% | 0,0% | 0,0% | 0,1% |
| % SAND: | 98,9% | 99,6% | 82,4% | 78,8% | 96,9% | 84,7% | 55,7% | 40,1% | 99,9% |
| % MUD: | 0,0% | 0,0% | 2,3% | 0,0% | 0,0% | 15,3% | 44,3% | 59,9% | 0,0% |
| % V COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % COARSE GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % MEDIUM GRAVEL: | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% | 0,0% |
| % FINE GRAVEL: | 0,0% | 0,0% | 0,6% | 0,5% | 1,1% | 0,0% | 0,0% | 0,0% | 0,0% |
| % V FINE GRAVEL: | 1,1% | 0,4% | 14,7% | 20,7% | 2,0% | 0,0% | 0,0% | 0,0% | 0,1% |
| % V COARSE SAND: | 14,3% | 12,8% | 35,8% | 78,0% | 42,4% | 0,0% | 0,0% | 0,0% | 6,0% |
| % COARSE SAND: | 70,8% | 61,0% | 20,2% | 0,8% | 47,3% | 0,0% | 0,0% | 0,0% | 39,0% |
| % MEDIUM SAND: | 13,5% | 24,3% | 6,7% | 0,0% | 7,0% | 36,9% | 9,6% | 4,5% | 48,8% |
| % FINE SAND: | 0,2% | 1,5% | 10,6% | 0,0% | 0,1% | 45,2% | 28,5% | 18,7% | 5,8% |
| % V FINE SAND: | 0,0% | 0,1% | 9,1% | 0,0% | 0,0% | 2,6% | 17,6% | 16,9% | 0,3% |
| % V COARSE SILT: | 0,0% | 0,0% | 0,1% | 0,0% | 0,0% | 1,9% | 13,9% | 13,9% | 0,0% |
| % COARSE SILT: | 0,0% | 0,0% | 0,1% | 0,0% | 0,0% | 3,7% | 11,0% | 14,9% | 0,0% |
| % MEDIUM SILT: | 0,0% | 0,0% | 0,1% | 0,0% | 0,0% | 3,8% | 8,4% | 13,5% | 0,0% |
| % FINE SILT: | 0,0% | 0,0% | 0,1% | 0,0% | 0,0% | 3,6% | 5,6% | 9,1% | 0,0% |
| % V FINE SILT: | 0,0% | 0,0% | 0,1% | 0,0% | 0,0% | 1,8% | 2,7% | 4,5% | 0,0% |
| % CLAY: | 0,5% | 0,0% | 2,0% | 0,0% | 0,0% | 0,5% | 2,7% | 4,1% | 0,0% |

# Annex II

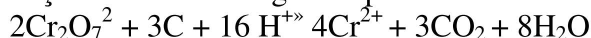
UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologia

Laboratório de Solos e Resíduos
Plataforma do Golfe

**Determinação da Matéria Orgânica**

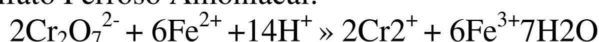
## 1. Fundamento do método

A matéria Orgânica é determinada pelo método proposto por Walkley e Black (1934) que consiste na oxidação do carbono orgânico do solo com dicromato de potássio (em excesso) e ácido sulfúrico normal, determinando o excesso de dicromato através de posterior titulação com Sulfato Ferroso Amoniacal (sal de Mohr) 0,5 N em presença de Acido Fosfórico., usando Difenilamina como indicador.
As reacções químicas são:
Oxidação do carbono orgânico pelo dicromato de potássio:



$$2Cr_2O_7^{2} + 3C + 16\ H^{+} » 4Cr^{2+} + 3CO_2 + 8H_2O$$

Titulação do excesso de dicromato (que não foi gasto na oxidação do C) com Sulfato Ferroso Amoniacal:



$$2Cr_2O_7^{2-} + 6Fe^{2+} + 14H^{+} » 2Cr2^{+} + 6Fe^{3+}7H2O$$

Para que a viragem do indicador seja brusca e o erro do indicador de titulação desprezável, faz-se a titulação em presença de $H_3PO_4$ que fixa o ião $Fe^{3+}$ num complexo estável ( Fe $(PO_4)_2)^{3-}$.

$$Fe^{3+} + PO_4^{3-} » (Fe(PO_4)_2)^{3-}$$

## 2.2. Reagentes

Dicromato de Potássio ($K_2Cr_2O_7$)
Difenilamina ($NH(C_6H_5)_2$)
Ácido Sulfúrico Concentrado ( $H_2SO_4$)
Sulfato Ferroso Amoniacal ou sal de Mohr ($Fe(NH_4)_2(SO_4)_2$
Acido Fosfórico 85% ($H_3PO_4$)

## 2.3 Soluções

Solução de Dicromato de Potássio 1 N

Pesar 60g de Dicromato de Potássio. Secar na estufa a 105 ºC durante 2 horas. A temperatura ambiente pesar 49,04g e dissolver num litro de água destilada.

Solução de Difenilamina

Deitar 20 ml de H2O num balão Erlenmeyer de 250 ml. Juntar lentamente 100 ml de H2SO4 e deixar arrefecer á temperatura ambiente. Adicionar 0,5g de Difenilamina e dissolver completamente o indicador.

Sal de Mohr

Pesar 196,1g de Sulfato Ferroso Amoniacal $(SO_4)_2$ Fe $(NH_4)_2.6H_2O$ dissolver em 80 ml de $H_2O$ com 20 ml de H2SO4 concentrado e diluir em H2O até perfazer um litro.

Universidade do Algarve, *Campus* de Gambelas, Edifício 8, Faro, Laboratório 1.36
Contactos: Responsável Prof. Carlos Guerrero cguerre@ualg.pt
Técnico: Camilo Portela cportela@ualg.pt
Tel.: 289 800 100 Ext: 7309

**2.4 Método**

Faça passar a amostra que colheu por um crivo de 2 mm.

Pese 0,5g (no caso de solos com menos de 5% de M.O.) e transfira para balões Erlenmeyer de 500 ml.

Adicionar 10 ml de Dicromato de Potássio 1 N.

Juntar lentamente 20 ml de H2SO4 directamente na solução (este procedimento deverá ser efectuado na Hote).

Agitar imediatamente durante 1 minuto deixando seguidamente o balão a repousar durante 30 minutos sobre uma placa de amianto.

Preparar um ensaio em branco medindo 10 ml de solução de Dicromato de Potássio e 20 ml de H2SO4 para um balão Erlenmeyer de 500 ml deixando seguidamente o balão a repousar durante 30 minutos sobre uma placa de amianto.

Ao fim de 30 minutos adicionar ao balão que contém a amostra e ao do ensaio em branco: 200 ml de $H_2O$, 10 ml de $H_3PO_4$ e 10 gotas do indicador Difenilamina.

Titular imediatamente com uma solução de Sulfato Ferroso Amoniacal 0,5 N (é conveniente titular o ensaio em branco antes da amostra).

A solução ficará azul e virará para verde brilhante quando atingir o ponto de equivalência.

**2.5 Cálculos**

O método utilizado um método indirecto uma vez que a percentagem de M.O. no solo é determinada com base na oxidação do C orgânico pelo Dicromato.

1. Como o Sulfato Ferroso Amoniacal em solução é muito instável, a sua concentração ou título (Cs) deve ser calculada diariamente através do volume (Vs) gasto na titulação do ensaio em branco com Dicromato de Potássio ($V_D$) cuja concentração é representada por $C_D$:

$$Cs = \frac{V_D x C_D}{Vs}$$

2. Pela diferença entre os ml de Sulfato Ferroso Amoniacal gastos na titulação do ensaio em branco (B) e da amostra (A) e considerando a concentração ( Normal ou meq/L desta solução (Cs) determinam-se os meq de Fe gastos na redução do Dicromato em excesso.

$$meq\ Fe = (B-A) \times (Cs)$$

3. A conversão dos meq de Fe em g de C faz-se sabendo que a 1 meq de Fe corresponde ¼ meq de C (12g):

$$g^C\ (oxidado) = meq\ Fe \times 0{,}003$$

4. Como através deste método apenas 75% do C orgânico é oxidado, o C total existente é dado por:

$$gC(Total) = gC\ (\ oxidado) \times 1{,}333$$

5. A quantidade de M.O existente no solo calcula-se admitindo que a M.O contem em média 58% de carbono:

$$G\ M.O = gC \times 1{,}724$$

Universidade do Algarve, *Campus* de Gambelas, Edifício 8, Faro, Laboratório 1.36
Contactos: Responsável Prof. Carlos Guerrero cguerre@ualg.pt
Técnico: Camilo Portela cportela@ualg.pt
Tel.: 289 800 100 Ext: 7309

6.A percentagem de matéria orgânica é calculada com base na quantidade de solo pesado (p).

$$\% \text{ M.O.} = \text{g M.O.} \times \frac{100}{P}$$

Os cálculos podem ser resumidos na seguinte equação:

$$\% \text{ M.O} = \frac{(B-A) \times Cs \times 0{,}003 \times 1{,}724 \times 1{,}333 \times 100}{\text{Peso da amostra (g)}}$$

**Classificação dos solos quanto ao teor de matéria orgânica:**

| Percentagem de Matéria Orgânica | | Classificação |
|---|---|---|
| Solos ligeiros | Solos médios e pesados | |
| < 0,5 | < 1,0 | Muito baixo |
| 0,6 – 1,5 | 1,1 – 2,0 | Baixo |
| 1,6 – 5,0 | 2,1 – 7,0 | Médio |
| 5,1 – 10,0 | 7,1 – 15,0 | Alto |
| > 10,0 | > 15,0 | Muito alto |

(Santos, 1995)

# Annex III

## Alternative Protocol (For Maximum Yields)

**Please wear gloves at all times**

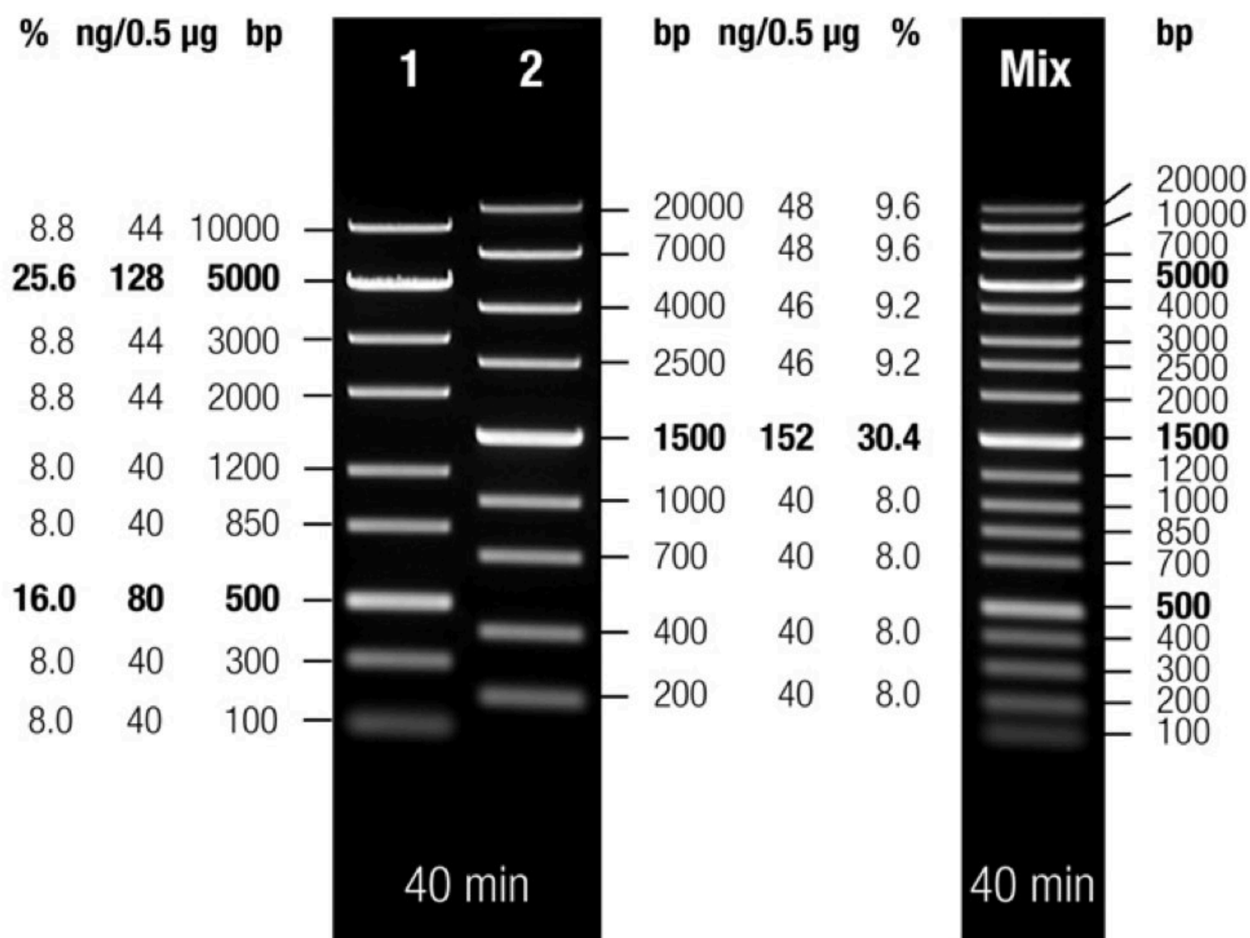
1. To the 2 ml **Bead Solution Tubes** provided, add 0.25 – 1 gram of soil sample. (For larger sample sizes up to 10 grams, we offer the UltraClean® Mega Soil DNA Isolation Kit, Catalog# 12900-10).
2. Gently vortex to mix.
3. **Check Solution S1.** If **Solution S1** is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60 µl of **Solution S1** and invert several times or vortex briefly.
5. Add 200 µl of **IRS Solution** (Inhibitor Removal Solution). This is only required if the DNA is to be used for PCR.
6. Secure **Bead Solution Tubes** horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing).
   **Note:** If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.
7. Make sure the 2 ml **Bead Solution Tubes** rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).
   **Note**: With 0.25 grams of soil and depending upon soil type, expect between 400 to 450 µl of supernatant. Supernatant may still contain some soil particles.
9. Add 250 µl of **Solution S2** and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
10. Centrifuge the tubes for 1 minute at 10,000 x *g.*
11. Avoiding the pellet, transfer entire volume of supernatant to a clean **2 ml Collection Tube** (provided).
12. Shake to mix Solution S3 before use. Add 1.3 ml of **Solution S3** to the supernatant and vortex for 5 seconds. **Note:** High volume of solution will touch the rim of the tube. Take care when handling tube.
13. Load approximately 700 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute.
14. Discard the flow through, add the remaining supernatant to the **Spin Filter**, and centrifuge at 10,000 x *g* for 1 minute. Repeat until all supernatant has passed through the **Spin Filter**.
    **Note**: A total of three loads for each sample processed are required.
15. Add 300 µl of **Solution S4** and centrifuge for 30 seconds at 10,000 x *g*.
16. Discard the flow through.
17. Centrifuge again at 10,000 x *g* for 1 minute.
18. Carefully place **Spin Filter** in a new clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution S4** onto the **Spin Filter**.
19. Add 50 µl of **Solution S5** to the center of the white filter membrane.
20. Centrifuge at 10,000 x *g* for 30 seconds.
21. Discard the **Spin Filter**. DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C to -80°C). **Solution S5** contains no EDTA.

**Thank you for choosing the UltraClean® Soil DNA Isolation Kit.**

# Annex IV

# ZipRuler™ Express DNA Ladder Set, ready-to-use




1% TopVision™ Agarose (#R0491)
5 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, 40 min

# Annex V

nzytech
genes & enzymes

# NZYGelpure

**Catalogue numbers:** MB01101 (50 preps)

MB01102 (200 preps)

**Description:** NZYGelpure kit is designed for the purification of DNA from TAE/TBE agarose gels and for the direct purification of PCR products. The kit can be used to purify DNA fragments from 100 bp to 10 kb. NZYGelpure purification kit utilizes a silica-gel based membrane which selectively adsorbs up to 10 µg of DNA fragments in the presence of specialized binding buffers. Soluble agarose, nucleotides, oligos (<30mer), enzymes, mineral oil and other impurities do not bind to the membrane and are washed away. DNA fragments are then eluted off the column and can be used for downstream protocols without further processing. Binding Buffer contains a pH indicator, allowing evaluation of optimal pH for DNA binding. The pH indicator does not interfere with DNA binding.

**Storage:** All kit components can be stored at room temperature (20-25 °C) and are stable for up to one year. For longer storage, keep all contents at 4 °C. Add 32 ml (MB01101) or 100 ml (MB01102) of ethanol to each bottle of Wash buffer.

**System Components**

| | 50 preps (MB01101) | 200 preps (MB01102) |
|---|---|---|
| Binding Buffer [1] | 2 × 30 ml | 2 × 120 ml |
| Wash Buffer (concentrated) | 8 ml | 25 ml |
| Elution Buffer [2] | 15 ml | 60 ml |
| NZYTech spin columns | 50 | 200 |
| Collecting columns (2 ml) | 50 | 200 |

[1] Contains guanidine thiocyanate. Wear gloves when using this kit.
[2] Elution Buffer is a 10 mM Tris-HCl, pH 8.5, solution.



nzytech
genes & enzymes

**Protocol for DNA purification from Agarose Gels**

All purification steps should be carried out at **room temperature**.

All centrifugations should be carried out at room temperature in a table-top microcentrifuge at **>12000 x g** (10000-15000 rpm depending on the rotor type).

2. Excise the DNA fragment from the gel with a clean, sharp scalpel. Weight the gel slice and transfer to a 1.5 ml microcentrifuge tube.
3. Add 300 µl of Binding Buffer for each 100 mg of gel weight (example – a gel slice weighing 125 mg would require 375 µl of Binding Buffer). For high concentration gels (2.0-3.0%), 500 µl of Binding Buffer per 100 mg of agarose gel should be added. The maximum amount of gel slice per NZYTech spin column is 400 mg. For gel slices >400 mg use more than one column.
4. Incubate at 55-60 °C for 10 minutes and shake occasionally until agarose is **completely** dissolved.
5. Check that the colour of the mixture is yellow (similar to the colour of the Binding Buffer). If the colour of the mixture is orange or violet, add 10 µl of 3 M sodium acetate pH 5.0, and mix well.
6. **(Optional)** For DNA fragments <500 bp or >10 kb long add 1 gel volume of isopropanol to the sample and mix well by pipetting several times (example – a gel slice weighing 125 mg would require 125 µl of isopropanol).
7. Load the above mixture into the NZYTech spin column placed into a Collection tube (2 ml). Centrifuge for 1 minute and discard the flow-through in the collection tube. The maximum volume of the column reservoir is 700 µl. For sample volumes of more than 700 µl, simply load and spin again.
8. **(Optional)** Add 500 µl of Binding Buffer and centrifuge for one minute. Discard the flow-through in the collection tube. This step is only important if DNA is intended to be used for direct sequencing, *in vitro* transcription or microinjection.
9. Add 600 µl of Wash Buffer and centrifuge for 1 minute. Discard the flow-through in the collection tube.

10. Centrifuge for 1 minute to dry NZYTech spin membrane of residual ethanol.
11. Place the NZYTech spin column into a clean 1.5 ml microcentrifuge tube. Add 50 µl of Elution Buffer to the centre of the column and incubate at room temperature for 1 minute. Centrifuge for 1 minute to elute DNA. Ultrapure water may be used in place of elution buffer. However, DNA recovery with acidic waters may be significantly reduced.

    Note: It is extremely important to add the Elution Buffer to the centre of the column. Incubating the column at higher temperatures (37 to 50 °C) may slightly increase the yield. Pre-warming the Elution Buffer at 55 to 80 °C may also slightly increase elution efficiency.

    Note: If a higher DNA concentration is desirable, 30 µl (or less) of Elution Buffer can be used to elute the DNA. It is critical that the Elution Buffer is applied directly in the centre of the column. (To recover maximum amount of DNA it is recommended to repeat the elution step.)
12. Store the purified DNA at -20 °C.

**Protocol for PCR clean-up**

1. Transfer the volume of the reaction mixture into a 1.5 ml microcentrifuge tube and add five volumes of Binding Buffer. Mix by inverting the tube a few times. Centrifuge briefly to collect the sample.
2. Add the above mixture to the NZYTech spin column and let stand for 2 minutes. The maximum loading volume of the column is 700 µl. For sample volumes greater than 700 µl simply load again. Centrifuge for 1 minute and discard the flow-through in the tube.
3. Add 600 µl of Wash and centrifuge for 1 minute. Discard the flow-through in the collection tube.
4. Centrifuge for 1 minute to dry NZYTech spin membrane of residual ethanol.





1. Place the NZYTech spin column into a clean 1.5 ml microcentrifuge tube. Add 50 µl of Elution Buffer to the centre of the column and incubate at room temperature for 1 minute. Centrifuge for 1 minute to elute DNA. Ultrapure water may be used in place of elution buffer. However, DNA recovery with acidic waters may be significantly reduced.

    Note: It is extremely important to add the Elution Buffer to the centre of the column. Incubating the column with the Elution Buffer at higher temperatures may slightly increase the yield especially of fragments larger than 10,000 bp. Pre-warming the Elution Buffer at 55 to 80 °C may also slightly increase elution efficiency. If a higher DNA concentration is desirable, 30 µl (or less) of elution buffer may be used. It is recommended that the elution step be repeated to recover the maximum amount of DNA.

5. Centrifuge for 1 minute to elute the DNA.
6. Store the purified DNA at -20 °C.

**Protocol for DNA purification from enzymatic reactions**

1. Transfer the volume of the reaction mixture into a 1.5 ml microcentrifuge tube and add five volumes of Binding Buffer. Mix by inverting the tube a few times. Centrifuge briefly to collect the sample.
2. Continue with step 2 of the protocol for PCR clean-up.

**REVISED 09/11**

NZYTech, Lda. Estrada do Paco do Lumiar, Campus do Lumiar, Edifício E – R/C

# Annex VI

### 3.4 Preparation/Cleaning of Glass Plate for Gel Casting

Hand wash both plates with a high quality lab detergent followed by a complete rinsing with $dH_2O$. Air dry or use a lint-free tissue. Spray/wipe the chosen inner surfaces of the plate set with 95% ethanol and dry with lint-free tissue.

### 3.5 Gel Casting Techniques.

A. Gel WrapTM Gasket Casting Method. B. Vertical gradient gel casting using GM-40 gradient maker and gravity flow. C. Vertical gradient gel casting using GM-40 gradient maker and a Mini-pump. D. Vertical gradient gel casting using GM-40 gradient maker, Mini-pump and

Multi-gel Caster.

**A. Gel Casting using Gel Wrap™ Gasket Casting method** For Vertical DGGE, use the set of spacers which do not have the small hole or channel milled into the lower end, these are for casting perpendicular gels.



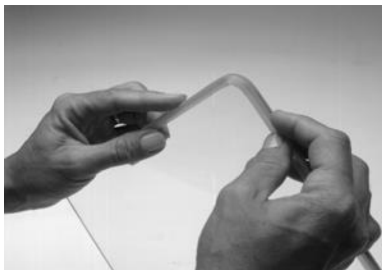
1. Start by holding the rectangular back plate with the rounded bottom corners and start applying the gasket around one side of the glass plate. Note: one side of the “U” shaped gasket is flat,

and the other side has tubing that will act as a seal around the spacers.

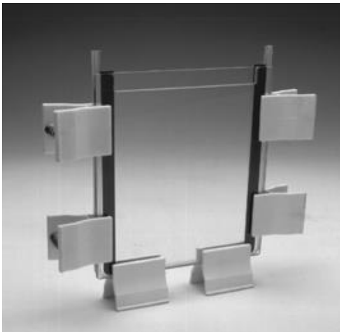
2. When applying the gasket over the rounded corners of the back glass plate, make sure the notches on the gasket align with the rounded corners of the glass plate. Once the gasket is pushed over the bottom edge and corners, work it down the remaining side.

3. Place the gasketed plate on the lab bench with the tubing side up, and extend the bottom of the plate over the edge of the bench, approximately 3⁄4 of an inch. Place

the spacers along side the inside edges of the gasket. Be sure the rounded corner end of each spacer is facing the outside bottom of the plate, following the radius of the glass.



4. Place the notched plate on top of the bottom assembly, starting from the bottom edge and gently easing the plate down. Verify the gasket is smooth around the edges and then clamp along the bottom.

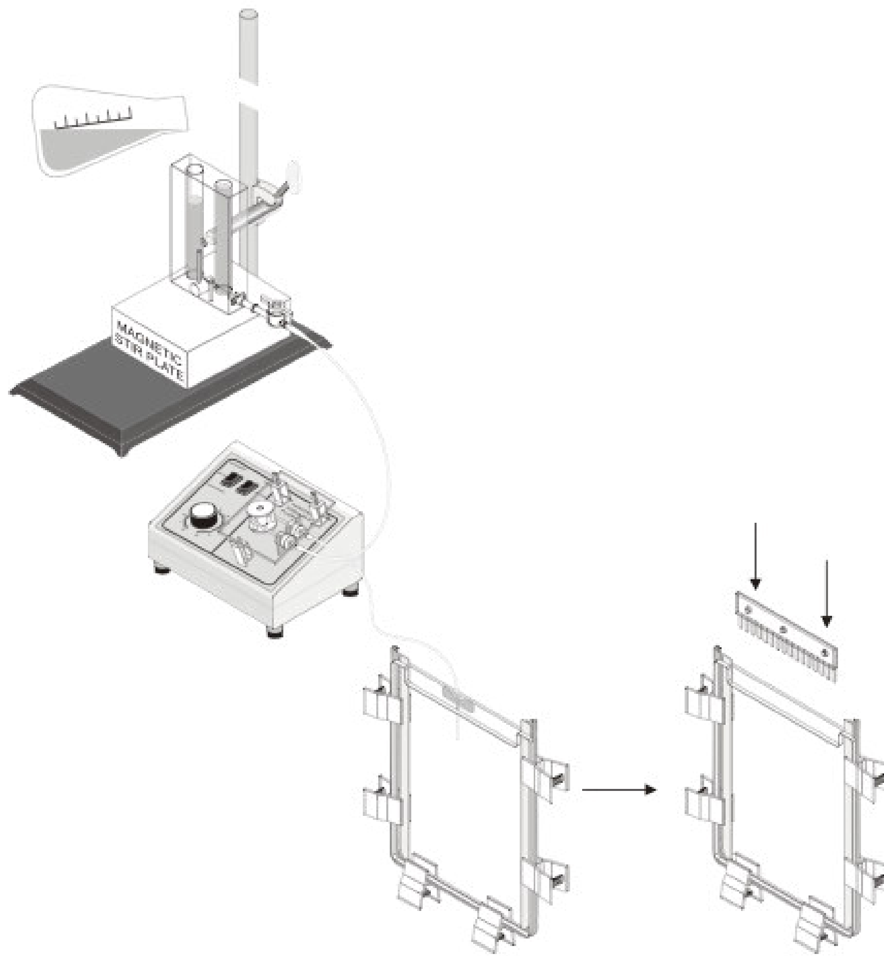


5. Lift the assembly and stand it on the base of the clamps. For leveling, push glass plate assembly down until it stops against clamp body. Clamp the sides of the assembly with additional casting clamps on either side. As each clamp is attached, be sure the gasket is aligned between the plates forming a seal.

**5 Gel Casting Techniques- continued**

**C. Vertical gradient gel casting using gradient maker (GM-40) and a mini-pump.**

1. Alternatively, you may choose to use a “mini-pump ”or other peristaltic pump to cast gels as shown in (Fig 3-5). If so, secure the gradient maker to a ring stand and connect the outlet tubing to the mini-pump tubing adapter. Connect tubing from mini-pump to a 20ga. needle for affixing between glass plates.


MAGNETIC
STIR PLATE

# Annex VII

## Acrylamide Silver Staining Protocol

## (Adapted from António, 2010 & Esteves, 2013)

1. Fixation solution - 10% Glacial Acetic Acid – 20 min.

2. Wash twice in distilled water – 3 min.

3. Staining solution - Silver nitrate (1g/L), 1% Glacial Acetic Acid and 10% Absolute Ethanol.

4. Wash three times in distilled water – 1 min.

5. 2$^{nd}$ Fixation solution – 3% Sodium Hydroxide with 1mL Formaldehyde 37%.

6. Revelation solution – 3% Sodium Carbonate, 1mL Formaldehyde 37% and Sodium Thiosulfate (2 mg/mL).

7. Stopping solution - 10% Glacial Acetic Acid – 5 min.

8. Conservation solution – 10% Glycerol and 25% Ethanol – 30 min.

9. Cover with cellophane and let it dry.

# Annex VIII

Page 1 - Clustering with Dice coefficient and Neighbor Joining

Page 2 - Clustering with Dice coefficient and Single Linkage

Page 3 - Clustering with Dice coefficient and UPGMA

Page 4 - Clustering with Jaccard coefficient and Neighbor Joining

Page 6 - Clustering with Jaccard coefficient and Single Linkage

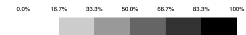
Page 7 - Clustering with Jaccard coefficient and UPGMA

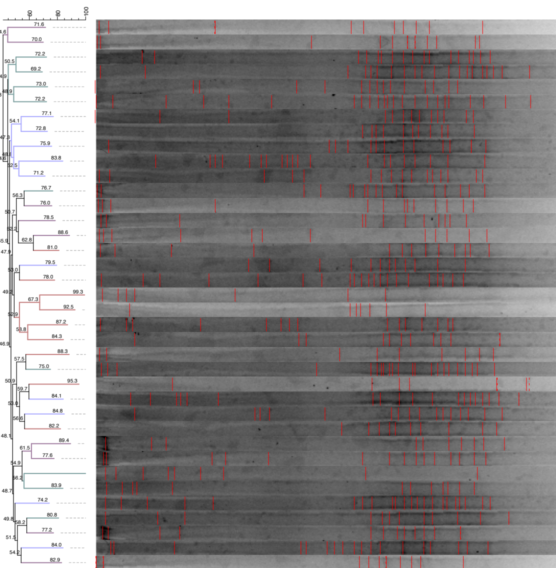
Page 8 - Clustering with Ochiai coefficient and Single Linkage

Page 9 - Clustering with Ochiai coefficient and UPGMA

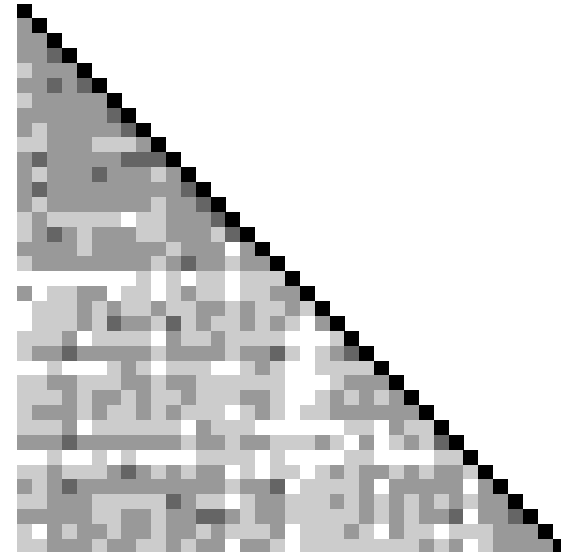
Ana_Bacteria

Ana_Bacteria



| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| BJ01 | BSL | 03.01.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues . | Ana Flor Data |
| BJ03 | BSL | 03.01.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 1.33% | BSSM | Sandy Mud | Spartina maritima and . | Ana Flor Data |
| NB08 | BSL | 09.11.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, . | Ana Flor Data |
| NB06 | BSL | 09.11.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 1.54% | BSSM | Gravelly Sand | Shell residues and som. | Ana Flor Data |
| NB04 | BSL | 09.11.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 2.04% | PILO | Sandy Mud | Shell residues . | Ana Flor Data |
| NB01 | BSL | 09.11.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 0.31% | DEDE | Slightly Gravelly Sand. | Shell residues . | Ana Flor Data |
| FJ07 | FUZ | 04.01.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 12.09% | PIPC | Muddy Sand | Spartina maritima and . | Ana Flor Data |
| FJ03 | FUZ | 04.01.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.16% | PILA | Slightly Gravelly Sand | Shell residues . | Ana Flor Data |
| FJ02 | FUZ | 04.01.2011 | 37.060.649 | -771.761 | 0.366211 | 0.02% | PIRV | Gravelly Sand | Shell residues . | Ana Flor Data |
| FJ09 | FUZ | 04.01.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 4.58% | PIDI | Sandy Gravel | Zostera nolti and Small. | Ana Flor Data |
| FJ06 | FUZ | 04.01.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.38% | PIPC | Muddy Sand | Shell residues . | Ana Flor Data |
| NB02 | BSL | 09.11.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues . | Ana Flor Data |
| BJ04 | BSL | 03.01.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 0.65% | FPIN | Gravelly Sand | Shell residues . | Ana Flor Data |
| BJ06 | BSL | 03.01.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 0.82% | PIDE | Slightly Gravelly Sand . | Shell residues . | Ana Flor Data |
| BJ05 | BSL | 03.01.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 1.91% | PZNO | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF07 | FUZ | 08.11.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and . | Ana Flor Data |
| FJ11 | FUZ | 04.01.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 1.12% | FPEX | Sandy Mud | Roots and unknown inv. | Ana Flor Data |
| NF03 | FUZ | 08.11.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues . | Ana Flor Data |
| NF10 | FUZ | 08.11.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 4.21% | PILO | Muddy Sand | Shell residues . | Ana Flor Data |
| NF11 | FUZ | 08.11.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 0.15% | FPEX | Slightly Gravelly Sand . | Roots and unknown inv. | Ana Flor Data |
| NF04 | FUZ | 08.11.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.26% | DVDP | Slightly Gravelly Sand . | Shell residues . | Ana Flor Data |
| NF06 | FUZ | 08.11.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.10% | FPIN | Slightly Gravelly Sand . | Green macroalgae . | Ana Flor Data |
| NF08 | FUZ | 08.11.2011 | 37.059.775 | -7.721.954 | 7.335.693 | 1.30% | PIRV | Sandy Mud | Bivalves . | Ana Flor Data |
| NB05 | BSL | 09.11.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 12.91% | MCIA | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF09 | FUZ | 08.11.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 5.23% | MCIA | Sandy Mud | Bivalves . | Ana Flor Data |
| FJ05 | FUZ | 04.01.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 2.75% | BSSM | Sand . | Green macroalgae . | Ana Flor Data |
| FJ10 | FUZ | 04.01.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues . | Ana Flor Data |
| NF05 | FUZ | 08.11.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 4.59% | BSSM | Slightly Gravelly Sand . | Green macroalgae . | Ana Flor Data |
| BJ09 | BSL | 03.01.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 0.79% | FPEX | Slightly Gravelly Sand . | Shell residues . | Ana Flor Data |
| BJ07 | BSL | 03.01.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 0.64% | MCIA | Muddy Sand | Macroalgae and bivalve. | Ana Flor Data |
| NB03 | BSL | 09.11.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 5.19% | PIRE | Muddy Sand | Spartina maritima and . | Ana Flor Data |
| NB07 | BSL | 09.11.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalve. | Ana Flor Data |
| FJ04 | FUZ | 04.01.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.68% | PIMC | Slightly Gravelly Sand . | Bivalves . | Ana Flor Data |
| NB09 | BSL | 09.11.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues . | Ana Flor Data |
| BJ08 | BSL | 03.01.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.78% | PILO | Muddy Sand | Macroalgae, molluscs, . | Ana Flor Data |
| FJ01 | FUZ | 04.01.2011 | 37.061.379 | -771.125 | 2.529.175 | 0.11% | MCMA | Gravelly Sand | Shell residues . | Ana Flor Data |
| BJ02 | BSL | 03.01.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues . | Ana Flor Data |



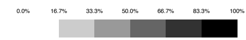
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| BJ02 | BSL | 03.01.2011 | 36.-7. 3.. | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues | . |
| NF05 | FUZ | 08.11.2011 | 3.. -7. 5.. | 4.59% | BSSM | Slightly Gravelly Sand | Green macroalgae | . |
| BJ05 | BSL | 03.01.2011 | 36.-7. 4.. | 1.91% | PZNO | . Muddy Sand | Zostera nolti and Small mollusca | . |
| NF07 | FUZ | 08.11.2011 | 37.-7. 11. | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and small crustaceans | . |
| NB06 | BSL | 09.11.2011 | 36.-7. 7.. | 1.54% | BSSM | . Gravelly Sand | Shell residues and some Zostera nolti | . |
| NB08 | BSL | 09.11.2011 | 36.-7. 5.. | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, and roots | . |
| FJ04 | FUZ | 04.01.2011 | 37.-7. 6.. | 0.68% | PIMC | Slightly Gravelly Sand | Bivalves | . |
| NB05 | BSL | 09.11.2011 | 36.-7. 4.. | 12.91% | MCIA | . Muddy Sand | Zostera nolti and Small mollusca | . |
| NF08 | FUZ | 08.11.2011 | 37.-7. 7.. | 1.30% | PIRV | . Sandy Mud | Bivalves | . |
| BJ07 | BSL | 03.01.2011 | 36.-7. 6.. | 0.64% | MCIA | . Muddy Sand | Macroalgae and bivalves | . |
| BJ09 | BSL | 03.01.2011 | 36.-7. 1.. | 0.79% | FPEX | Slightly Gravelly Sand | Shell residues | . |
| BJ04 | BSL | 03.01.2011 | 3.. -7. 4.. | 0.65% | FPIN | . Gravelly Sand | Shell residues | . |
| NB02 | BSL | 09.11.2011 | 36.-7. 3.. | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues | . |
| NF03 | FUZ | 08.11.2011 | 37.-7. 1.. | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues | . |
| BJ08 | BSL | 03.01.2011 | 36.-7. 5.. | 2.78% | PILO | . Muddy Sand | Macroalgae, molluscs, and roots | . |
| NB09 | BSL | 09.11.2011 | 36.-7. 1.. | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues | . |
| BJ06 | BSL | 03.01.2011 | 36.-7. 7.. | 0.82% | PIDE | Slightly Gravelly Sand | Shell residues | . |
| FJ03 | FUZ | 04.01.2011 | 37.-7. 1.. | 0.16% | PILA | Slightly Gravelly Sand | Shell residues | . |
| FJ06 | FUZ | 04.01.2011 | 37.-7. 6.. | 0.38% | PIPC | Muddy Sand | Shell residues | . |
| FJ07 | FUZ | 04.01.2011 | 37.-7. 11. | 12.09% | PIPC | Muddy Sand | Spartina maritima and small crustaceans | . |
| FJ02 | FUZ | 04.01.2011 | 37.-7. 0.. | 0.02% | PIRV | . Gravelly Sand | Shell residues | . |
| NB07 | BSL | 09.11.2011 | 36.-7. 6.. | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalves | . |
| BJ03 | BSL | 03.01.2011 | 36.-7. 3.. | 1.33% | BSSM | . Sandy Mud | Spartina maritima and small crustaceans | . |
| NF06 | FUZ | 08.11.2011 | 37.-7. 6.. | 0.10% | FPIN | Slightly Gravelly Sand | Green macroalgae | . |
| NB01 | BSL | 09.11.2011 | 36.-7. 7.. | 0.31% | DEDE | Slightly Gravelly Sand | Shell residues | . |
| NB04 | BSL | 09.11.2011 | 3.. -7. 4.. | 2.04% | PILO | Sandy Mud | Shell residues | . |
| FJ09 | FUZ | 04.01.2011 | 37.-7. 3.. | 4.58% | PIDI | . Sandy Gravel | Zostera nolti and Small mollusca | . |
| FJ11 | FUZ | 04.01.2011 | 37.-7. 2.. | 1.12% | FPEX | . Sandy Mud | Roots and unknown invertebrates signs | . |
| BJ01 | BSL | 03.01.2011 | 36.-7. 7.. | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues | . |
| FJ05 | FUZ | 04.01.2011 | 3.. -7. 5.. | 2.75% | BSSM | Sand | Green macroalgae | . |
| FJ10 | FUZ | 04.01.2011 | 3.. -7. 5.. | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues | . |
| NF04 | FUZ | 08.11.2011 | 37.-7. 6.. | 0.26% | DVDP | Slightly Gravelly Sand | Shell residues | . |
| FJ01 | FUZ | 04.01.2011 | 37.-7. 2.. | 0.11% | MCMA | Gravelly Sand | Shell residues | . |
| NF09 | FUZ | 08.11.2011 | 37.-7. 3.. | 5.23% | MCIA | . Sandy Mud | Bivalves | . |
| NF10 | FUZ | 08.11.2011 | 3.. -7. 5.. | 4.21% | PILO | Muddy Sand | Shell residues | . |
| NF11 | FUZ | 08.11.2011 | 37.-7. 2.. | 0.15% | FPEX | Slightly Gravelly Sand | Roots and unknown invertebrates signs | . |
| NB03 | BSL | 09.11.2011 | 36.-7. 3.. | 5.19% | PIRE | . Muddy Sand | Spartina maritima and small crustaceans | . |

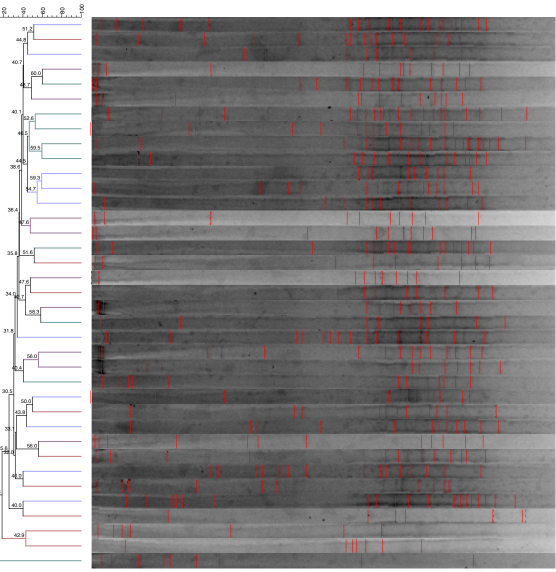
| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BJ02 | 100 | | | | | | | | | | | | | | | | | | |
| NF05 | 47.6 | 100 | | | | | | | | | | | | | | | | | |
| BJ05 | 9.1 | 9.1 | 100 | | | | | | | | | | | | | | | | |
| NF07 | 34.8 | 17.4 | 56.0 | 100 | | | | | | | | | | | | | | | |
| NB06 | 38.7 | 40.0 | 31.3 | 42.4 | 100 | | | | | | | | | | | | | | |
| NB08 | 35.7 | 37.0 | 27.6 | 53.3 | 59.5 | 100 | | | | | | | | | | | | | |
| FJ04 | 37.5 | 37.5 | 29.4 | 40.0 | 52.4 | 46.2 | 100 | | | | | | | | | | | | |
| NB05 | 31.3 | 37.5 | 29.4 | 45.7 | 52.4 | 46.2 | 45.5 | 100 | | | | | | | | | | | |
| NF08 | 30.0 | 42.1 | 19.1 | 27.3 | 34.5 | 30.8 | 25.8 | 51.6 | 100 | | | | | | | | | | |
| BJ07 | 33.3 | 32.0 | 29.6 | 35.7 | 51.4 | 43.8 | 48.7 | 43.2 | 16.7 | 100 | | | | | | | | | |
| BJ09 | 28.6 | 30.0 | 27.3 | 17.4 | 46.7 | 29.6 | 37.5 | 31.3 | 21.1 | 56.0 | 100 | | | | | | | | |
| BJ04 | 34.8 | 26.1 | 40.0 | 38.5 | 42.4 | 33.3 | 40.0 | 45.7 | 27.3 | 35.7 | 34.8 | 100 | | | | | | | |
| NB02 | 30.8 | 29.6 | 41.4 | 33.3 | 48.7 | 35.3 | 41.0 | 41.0 | 30.8 | 31.3 | 14.8 | 60.0 | 100 | | | | | | |
| NF03 | 32.3 | 32.3 | 18.2 | 35.3 | 43.9 | 36.8 | 51.2 | 51.2 | 26.7 | 22.2 | 12.9 | 35.3 | 57.9 | 100 | | | | | |
| BJ08 | 43.5 | 36.4 | 41.7 | 24.0 | 43.8 | 41.4 | 47.1 | 47.1 | 19.1 | 51.9 | 36.4 | 56.0 | 48.3 | 36.4 | 100 | | | | |
| NB09 | 45.5 | 45.5 | 16.7 | 24.0 | 37.5 | 41.4 | 47.1 | 47.1 | 28.6 | 37.0 | 27.3 | 24.0 | 34.5 | 36.4 | 58.3 | 100 | | | |
| BJ06 | 36.4 | 27.3 | 50.0 | 32.0 | 37.5 | 34.5 | 41.2 | 41.2 | 38.1 | 44.4 | 27.3 | 56.0 | 41.4 | 36.4 | 50.0 | 25.0 | 100 | | |
| FJ03 | 45.5 | 28.6 | 8.7 | 33.3 | 45.2 | 42.9 | 48.5 | 48.5 | 20.0 | 46.2 | 28.6 | 41.7 | 35.7 | 43.8 | 34.8 | 26.1 | 43.5 | 100 | |
| FJ06 | 37.0 | 46.2 | 35.7 | 41.4 | 44.4 | 48.5 | 42.1 | 47.4 | 40.0 | 38.7 | 23.1 | 48.3 | 42.4 | 37.8 | 42.9 | 50.0 | 35.7 | 59.3 | 100 |
| FJ07 | 36.4 | 26.1 | 32.0 | 46.2 | 42.4 | 33.3 | 40.0 | 45.7 | 27.3 | 42.9 | 17.4 | 46.2 | 33.3 | 35.3 | 32.0 | 32.0 | 40.0 | 58.3 | 41.4 |
| FJ02 | 24.0 | 33.3 | 23.1 | 29.6 | 47.1 | 38.7 | 38.9 | 44.4 | 26.1 | 34.5 | 25.0 | 37.0 | 45.2 | 34.3 | 38.5 | 30.8 | 38.5 | 56.0 | 53.3 |
| NB07 | 26.1 | 18.2 | 16.7 | 32.0 | 25.0 | 34.5 | 47.1 | 41.2 | 28.6 | 44.4 | 36.4 | 48.0 | 27.6 | 18.2 | 33.3 | 33.3 | 33.3 | 52.2 | 42.9 |
| BJ03 | 28.6 | 38.1 | 34.8 | 33.3 | 45.2 | 42.9 | 30.3 | 43.8 | 30.0 | 46.2 | 19.1 | 50.0 | 28.6 | 37.5 | 34.8 | 26.1 | 26.1 | 36.4 | 51.9 |
| NF06 | 27.3 | 40.0 | 17.4 | 33.3 | 25.8 | 28.6 | 30.3 | 36.4 | 20.0 | 30.8 | 9.5 | 33.3 | 28.6 | 37.5 | 26.1 | 34.8 | 26.1 | 36.4 | 51.9 |
| NB01 | 31.3 | 36.4 | 28.6 | 33.3 | 46.5 | 50.0 | 40.0 | 44.4 | 25.0 | 36.8 | 30.3 | 44.4 | 50.0 | 40.9 | 28.6 | 28.6 | 34.3 | 47.1 | 46.2 |
| NB04 | 38.5 | 32.0 | 22.2 | 28.6 | 45.7 | 43.8 | 43.2 | 37.8 | 8.3 | 46.7 | 16.0 | 35.7 | 37.5 | 44.4 | 44.4 | 44.4 | 37.0 | 46.2 | 45.2 |
| FJ09 | 19.4 | 26.7 | 31.3 | 18.2 | 35.0 | 37.8 | 33.3 | 28.6 | 0.0 | 45.7 | 20.0 | 36.4 | 27.0 | 24.4 | 31.3 | 25.0 | 25.0 | 32.3 | 50.0 |
| FJ11 | 43.5 | 36.4 | 16.7 | 40.0 | 43.8 | 34.5 | 41.2 | 47.1 | 28.6 | 37.0 | 9.1 | 40.0 | 41.4 | 48.5 | 41.7 | 33.3 | 33.3 | 34.8 | 28.6 |
| BJ01 | 20.0 | 20.0 | 27.3 | 17.4 | 40.0 | 37.0 | 43.8 | 31.3 | 31.6 | 33.3 | 30.0 | 36.4 | 44.4 | 32.3 | 36.4 | 27.3 | 36.4 | 38.1 | 46.2 |
| FJ05 | 26.7 | 41.4 | 19.4 | 31.3 | 46.2 | 44.4 | 43.9 | 43.9 | 35.7 | 29.4 | 34.5 | 31.3 | 38.9 | 30.0 | 38.7 | 45.2 | 19.4 | 33.3 | 34.3 |
| FJ10 | 29.6 | 46.2 | 28.6 | 41.4 | 44.4 | 24.2 | 42.1 | 36.8 | 24.0 | 38.7 | 30.8 | 20.7 | 36.4 | 32.4 | 21.4 | 28.6 | 28.6 | 22.2 | 31.3 |
| NF04 | 24.0 | 24.0 | 29.6 | 42.9 | 28.6 | 18.8 | 21.6 | 27.0 | 16.7 | 40.0 | 16.0 | 35.7 | 31.3 | 27.8 | 29.6 | 22.2 | 37.0 | 30.8 | 32.3 |
| FJ01 | 41.4 | 28.6 | 26.7 | 32.3 | 31.6 | 40.0 | 45.0 | 30.0 | 37.0 | 30.3 | 14.3 | 19.4 | 40.0 | 35.9 | 40.0 | 33.3 | 33.3 | 34.5 | 35.3 |
| NF09 | 23.5 | 37.5 | 0.0 | 31.6 | 16.0 | 17.4 | 14.3 | 21.4 | 26.7 | 0.0 | 0.0 | 21.1 | 17.4 | 22.2 | 22.2 | 22.2 | 11.1 | 35.3 | 27.3 |
| NF10 | 0.0 | 11.8 | 10.5 | 20.0 | 14.8 | 8.3 | 13.8 | 20.7 | 12.5 | 18.2 | 11.8 | 20.0 | 16.7 | 21.4 | 21.1 | 21.1 | 21.1 | 11.1 | 17.4 |
| NF11 | 23.5 | 23.5 | 10.5 | 20.0 | 22.2 | 25.0 | 27.6 | 13.8 | 0.0 | 27.3 | 11.8 | 20.0 | 33.3 | 35.7 | 31.6 | 31.6 | 31.6 | 22.2 | 17.4 |
| NB03 | 10.0 | 10.5 | 19.1 | 18.2 | 13.8 | 23.1 | 12.9 | 19.4 | 22.2 | 25.0 | 31.6 | 18.2 | 7.7 | 20.0 | 9.5 | 19.1 | 19.1 | 20.0 | 8.0 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FJ07 | 100 | | | | | | | | | | | | | | | | | |
| FJ02 | 37.0 | 100 | | | | | | | | | | | | | | | | |
| NB07 | 40.0 | 38.5 | 100 | | | | | | | | | | | | | | | |
| BJ03 | 41.7 | 32.0 | 17.4 | 100 | | | | | | | | | | | | | | |
| NF06 | 50.0 | 40.0 | 43.5 | 27.3 | 100 | | | | | | | | | | | | | |
| NB01 | 44.4 | 48.7 | 22.9 | 35.3 | 29.4 | 100 | | | | | | | | | | | | |
| NB04 | 35.7 | 34.5 | 29.6 | 46.2 | 38.5 | 52.6 | 100 | | | | | | | | | | | |
| FJ09 | 24.2 | 35.3 | 18.8 | 25.8 | 32.3 | 32.6 | 40.0 | 100 | | | | | | | | | | |
| FJ11 | 40.0 | 38.5 | 8.3 | 43.5 | 26.1 | 40.0 | 29.6 | 37.5 | 100 | | | | | | | | | |
| BJ01 | 26.1 | 33.3 | 27.3 | 47.6 | 9.5 | 42.4 | 32.0 | 27.6 | 36.4 | 100 | | | | | | | | |
| FJ05 | 25.0 | 36.4 | 32.3 | 26.7 | 27.6 | 23.8 | 23.5 | 20.5 | 25.8 | 20.7 | 100 | | | | | | | |
| FJ10 | 41.4 | 33.3 | 35.7 | 22.2 | 46.2 | 35.9 | 32.3 | 27.8 | 35.7 | 23.1 | 34.3 | 100 | | | | | | |
| NF04 | 35.7 | 20.7 | 29.6 | 23.1 | 46.2 | 31.6 | 40.0 | 40.0 | 29.6 | 16.0 | 11.8 | 32.3 | 100 | | | | | |
| FJ01 | 19.4 | 37.5 | 26.7 | 13.8 | 20.7 | 39.0 | 42.4 | 21.1 | 26.7 | 28.6 | 37.8 | 17.7 | 18.2 | 100 | | | | |
| NF09 | 21.1 | 30.0 | 33.3 | 11.8 | 26.7 | 13.8 | 9.5 | 7.7 | 33.3 | 12.5 | 40.0 | 18.2 | 19.1 | 16.7 | 100 | | | |
| NF10 | 10.0 | 19.1 | 31.6 | 11.1 | 22.2 | 6.7 | 9.1 | 7.4 | 21.1 | 11.8 | 7.7 | 8.7 | 36.4 | 8.0 | 0.0 | 100 | | |
| NF11 | 10.0 | 28.6 | 10.5 | 11.1 | 11.1 | 33.3 | 36.4 | 14.8 | 31.6 | 35.3 | 15.4 | 0.0 | 27.3 | 32.0 | 15.4 | 42.9 | 100 | |
| NB03 | 9.1 | 8.7 | 28.6 | 10.0 | 0.0 | 18.8 | 16.7 | 13.8 | 0.0 | 10.5 | 14.3 | 0.0 | 8.3 | 22.2 | 13.3 | 12.5 | 12.5 | 100 |

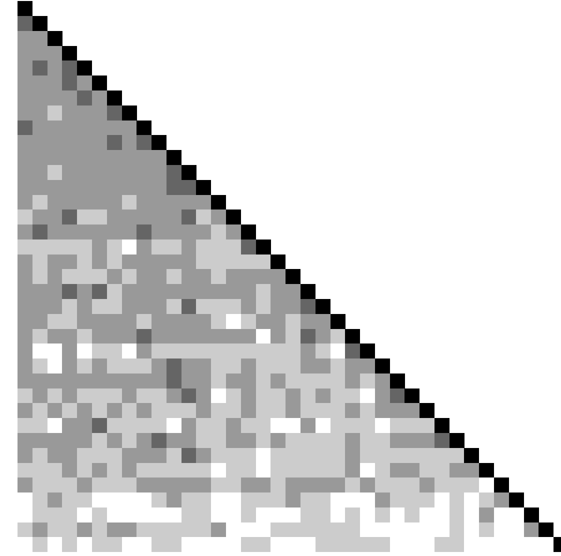
Ana_Bacteria

Ana_Bacteria





| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| FJ04 | FUZ | 04.01.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.68% | PIMC | Slightly Gravelly Sand . | Bivalves | . Ana Flor Data |
| NF03 | FUZ | 08.11.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ11 | FUZ | 04.01.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 1.12% | FPEX | Sandy Mud | Roots and unknown inv. | Ana Flor Data |
| BJ04 | BSL | 03.01.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 0.65% | FPIN | Gravelly Sand | Shell residues | . Ana Flor Data |
| NB02 | BSL | 09.11.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ06 | BSL | 03.01.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 0.82% | PIDE | Slightly Gravelly Sand . | Shell residues | . Ana Flor Data |
| NB01 | BSL | 09.11.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 0.31% | DEDE | Slightly Gravelly Sand. | Shell residues | . Ana Flor Data |
| NB04 | BSL | 09.11.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 2.04% | PILO | Sandy Mud | Shell residues | . Ana Flor Data |
| NB06 | BSL | 09.11.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 1.54% | BSSM | Gravelly Sand | Shell residues and som. | Ana Flor Data |
| NB08 | BSL | 09.11.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, . | Ana Flor Data |
| FJ03 | FUZ | 04.01.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.16% | PILA | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ06 | FUZ | 04.01.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.38% | PIPC | Muddy Sand | Shell residues | . Ana Flor Data |
| FJ02 | FUZ | 04.01.2011 | 37.060.649 | -771.761 | 0.366211 | 0.02% | PIRV | Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ01 | BSL | 03.01.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ03 | BSL | 03.01.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 1.33% | BSSM | Sandy Mud | Spartina maritima and . | Ana Flor Data |
| NB05 | BSL | 09.11.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 12.91% | MCIA | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF08 | FUZ | 08.11.2011 | 37.059.775 | -7.721.954 | 7.335.693 | 1.30% | PIRV | Sandy Mud | Bivalves | . Ana Flor Data |
| BJ02 | BSL | 03.01.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF05 | FUZ | 08.11.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 4.59% | BSSM | Slightly Gravelly Sand . | Green macroalgae | . Ana Flor Data |
| BJ08 | BSL | 03.01.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.78% | PILO | Muddy Sand | Macroalgae, molluscs, . | Ana Flor Data |
| NB09 | BSL | 09.11.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ01 | FUZ | 04.01.2011 | 37.061.379 | -771.125 | 2.529.175 | 0.11% | MCMA | Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ07 | BSL | 03.01.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 0.64% | MCIA | Muddy Sand | Macroalgae and bivalve. | Ana Flor Data |
| BJ09 | BSL | 03.01.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 0.79% | FPEX | Slightly Gravelly Sand . | Shell residues | . Ana Flor Data |
| NB07 | BSL | 09.11.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalve. | Ana Flor Data |
| FJ07 | FUZ | 04.01.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 12.09% | PIPC | Muddy Sand | Spartina maritima and . | Ana Flor Data |
| NF06 | FUZ | 08.11.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.10% | FPIN | Slightly Gravelly Sand . | Green macroalgae | . Ana Flor Data |
| FJ10 | FUZ | 04.01.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ05 | BSL | 03.01.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 1.91% | PZNO | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF07 | FUZ | 08.11.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and . | Ana Flor Data |
| FJ09 | FUZ | 04.01.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 4.58% | PIDI | Sandy Gravel | Zostera nolti and Small. | Ana Flor Data |
| NF04 | FUZ | 08.11.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.26% | DVDP | Slightly Gravelly Sand . | Shell residues | . Ana Flor Data |
| FJ05 | FUZ | 04.01.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 2.75% | BSSM | Sand . | Green macroalgae | . Ana Flor Data |
| NF09 | FUZ | 08.11.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 5.23% | MCIA | Sandy Mud | Bivalves | . Ana Flor Data |
| NF10 | FUZ | 08.11.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 4.21% | PILO | Muddy Sand | Shell residues | . Ana Flor Data |
| NF11 | FUZ | 08.11.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 0.15% | FPEX | Slightly Gravelly Sand . | Roots and unknown inv. | Ana Flor Data |
| NB03 | BSL | 09.11.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 5.19% | PIRE | Muddy Sand | Spartina maritima and . | Ana Flor Data |



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| FJ01 | FUZ | 04.01.2011 | 37.-7. 2.. | 0.11% | MCMA | Gravelly Sand | Shell residues |
| FJ04 | FUZ | 04.01.2011 | 37.-7. 6.. | 0.68% | PIMC | Slightly Gravelly Sand | Bivalves |
| NF10 | FUZ | 08.11.2011 | 3.. -7. 5.. | 4.21% | PILO | Muddy Sand | Shell residues |
| NF11 | FUZ | 08.11.2011 | 37.-7. 2.. | 0.15% | FPEX | Slightly Gravelly Sand | Roots and unknown invertebrates signs |
| FJ11 | FUZ | 04.01.2011 | 37.-7. 2.. | 1.12% | FPEX | . Sandy Mud | Roots and unknown invertebrates signs |
| NF03 | FUZ | 08.11.2011 | 37.-7. 1.. | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues |
| NF08 | FUZ | 08.11.2011 | 37.-7. 7.. | 1.30% | PIRV | . Sandy Mud | Bivalves |
| NB05 | BSL | 09.11.2011 | 36.-7. 4.. | 12.91% | MCIA | . Muddy Sand | Zostera nolti and Small mollusca |
| NF09 | FUZ | 08.11.2011 | 37.-7. 3.. | 5.23% | MCIA | . Sandy Mud | Bivalves |
| FJ05 | FUZ | 04.01.2011 | 3.. -7. 5.. | 2.75% | BSSM | Sand | Green macroalgae |
| NB09 | BSL | 09.11.2011 | 36.-7. 1.. | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues |
| BJ08 | BSL | 03.01.2011 | 36.-7. 5.. | 2.78% | PILO | . Muddy Sand | Macroalgae, molluscs, and roots |
| BJ02 | BSL | 03.01.2011 | 36.-7. 3.. | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues |
| NF05 | FUZ | 08.11.2011 | 3.. -7. 5.. | 4.59% | BSSM | Slightly Gravelly Sand | Green macroalgae |
| FJ02 | FUZ | 04.01.2011 | 37.-7. 0.. | 0.02% | PIRV | . Gravelly Sand | Shell residues |
| FJ07 | FUZ | 04.01.2011 | 37.-7. 11. | 12.09% | PIPC | Muddy Sand | Spartina maritima and small crustaceans |
| FJ03 | FUZ | 04.01.2011 | 37.-7. 1.. | 0.16% | PILA | Slightly Gravelly Sand | Shell residues |
| BJ01 | BSL | 03.01.2011 | 36.-7. 7.. | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues |
| BJ03 | BSL | 03.01.2011 | 36.-7. 3.. | 1.33% | BSSM | . Sandy Mud | Spartina maritima and small crustaceans |
| FJ09 | FUZ | 04.01.2011 | 37.-7. 3.. | 4.58% | PIDI | . Sandy Gravel | Zostera nolti and Small mollusca |
| FJ06 | FUZ | 04.01.2011 | 37.-7. 6.. | 0.38% | PIPC | Muddy Sand | Shell residues |
| NB08 | BSL | 09.11.2011 | 36.-7. 5.. | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, and roots |
| NB06 | BSL | 09.11.2011 | 36.-7. 7.. | 1.54% | BSSM | . Gravelly Sand | Shell residues and some Zostera nolti |
| NB04 | BSL | 09.11.2011 | 3.. -7. 4.. | 2.04% | PILO | Sandy Mud | Shell residues |
| NB01 | BSL | 09.11.2011 | 36.-7. 7.. | 0.31% | DEDE | Slightly Gravelly Sand | Shell residues |
| BJ05 | BSL | 03.01.2011 | 36.-7. 4.. | 1.91% | PZNO | . Muddy Sand | Zostera nolti and Small mollusca |
| NF07 | FUZ | 08.11.2011 | 37.-7. 11. | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and small crustaceans |
| BJ06 | BSL | 03.01.2011 | 36.-7. 7.. | 0.82% | PIDE | Slightly Gravelly Sand | Shell residues |
| NB02 | BSL | 09.11.2011 | 36.-7. 3.. | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues |
| BJ04 | BSL | 03.01.2011 | 3.. -7. 4.. | 0.65% | FPIN | . Gravelly Sand | Shell residues |
| FJ10 | FUZ | 04.01.2011 | 3.. -7. 5.. | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues |
| NF04 | FUZ | 08.11.2011 | 37.-7. 6.. | 0.26% | DVDP | Slightly Gravelly Sand | Shell residues |
| NF06 | FUZ | 08.11.2011 | 37.-7. 6.. | 0.10% | FPIN | Slightly Gravelly Sand | Green macroalgae |
| NB07 | BSL | 09.11.2011 | 36.-7. 6.. | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalves |
| NB03 | BSL | 09.11.2011 | 36.-7. 3.. | 5.19% | PIRE | . Muddy Sand | Spartina maritima and small crustaceans |
| BJ09 | BSL | 03.01.2011 | 36.-7. 1.. | 0.79% | FPEX | Slightly Gravelly Sand | Shell residues |
| BJ07 | BSL | 03.01.2011 | 36.-7. 6.. | 0.64% | MCIA | . Muddy Sand | Macroalgae and bivalves |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FJ01 | . | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| FJ04 | . | 71.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NF10 | . | 95.8 | 92.6 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NF11 | . | 80.9 | 84.0 | 72.7 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| FJ11 | . | 84.6 | 74.1 | 88.2 | 81.2 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NF03 | . | 78.1 | 65.6 | 88.0 | 78.3 | 68.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NF08 | . | 77.3 | 85.2 | 93.3 | 100.0 | 83.3 | 84.6 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NB05 | . | 82.3 | 70.6 | 88.5 | 92.6 | 69.2 | 65.6 | 65.2 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NF09 | . | 90.9 | 92.3 | 100.0 | 91.7 | 80.0 | 87.5 | 84.6 | 88.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| FJ05 | . | 76.7 | 71.9 | 96.0 | 91.7 | 85.2 | 82.3 | 78.3 | 71.9 | 75.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NB09 | . | 80.0 | 69.2 | 88.2 | 81.2 | 80.0 | 77.8 | 83.3 | 69.2 | 87.5 | 70.8 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BJ08 | . | 75.0 | 69.2 | 88.2 | 81.2 | 73.7 | 77.8 | 89.5 | 69.2 | 87.5 | 76.0 | 58.8 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | |
| BJ02 | . | 73.9 | 76.9 | 100.0 | 86.7 | 72.2 | 80.8 | 82.3 | 81.5 | 86.7 | 84.6 | 70.6 | 72.2 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| NF05 | . | 83.3 | 76.9 | 93.7 | 86.7 | 77.8 | 80.8 | 73.3 | 76.9 | 76.9 | 73.9 | 70.6 | 77.8 | 68.7 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | |
| FJ02 | . | 76.9 | 75.9 | 89.5 | 83.3 | 76.2 | 79.3 | 85.0 | 71.4 | 82.3 | 77.8 | 81.8 | 76.2 | 86.4 | 80.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | |
| FJ07 | . | 89.3 | 75.0 | 94.7 | 94.7 | 75.0 | 78.6 | 84.2 | 70.4 | 88.2 | 85.7 | 80.9 | 80.9 | 77.8 | 85.0 | 77.3 | 0.0 | | | | | | | | | | | | | | | | | | | | | |
| FJ03 | . | 79.2 | 68.0 | 94.1 | 87.5 | 78.9 | 72.0 | 88.9 | 68.0 | 78.6 | 80.0 | 85.0 | 78.9 | 70.6 | 83.3 | 61.1 | 58.8 | 0.0 | | | | | | | | | | | | | | | | | | | | |
| BJ01 | . | 83.3 | 72.0 | 93.7 | 78.6 | 77.8 | 80.8 | 81.2 | 81.5 | 93.3 | 88.5 | 84.2 | 77.8 | 88.9 | 88.9 | 80.0 | 85.0 | 76.5 | 0.0 | | | | | | | | | | | | | | | | | | | |
| BJ03 | . | 92.6 | 82.1 | 94.1 | 94.1 | 72.2 | 76.9 | 82.3 | 72.0 | 93.7 | 84.6 | 85.0 | 78.9 | 83.3 | 76.5 | 80.9 | 73.7 | 77.8 | 68.7 | 0.0 | | | | | | | | | | | | | | | | | | |
| FJ09 | . | 88.2 | 80.0 | 96.2 | 92.0 | 76.9 | 86.1 | 100.0 | 83.3 | 96.0 | 88.6 | 85.7 | 81.5 | 89.3 | 84.6 | 78.6 | 86.2 | 80.8 | 84.0 | 85.2 | 0.0 | | | | | | | | | | | | | | | | | |
| FJ06 | . | 78.6 | 73.3 | 90.5 | 90.5 | 83.3 | 76.7 | 75.0 | 69.0 | 84.2 | 79.3 | 66.7 | 72.7 | 77.3 | 70.0 | 63.6 | 73.9 | 57.9 | 70.0 | 65.0 | 66.7 | 0.0 | | | | | | | | | | | | | | | | |
| NB08 | . | 75.0 | 70.0 | 95.6 | 85.7 | 79.2 | 77.4 | 81.8 | 70.0 | 90.5 | 71.4 | 73.9 | 73.9 | 78.3 | 77.3 | 76.0 | 80.0 | 72.7 | 77.3 | 72.7 | 76.7 | 68.0 | 0.0 | | | | | | | | | | | | | | | |
| NB06 | . | 81.2 | 64.5 | 92.0 | 87.5 | 72.0 | 71.9 | 79.2 | 64.5 | 91.3 | 70.0 | 76.9 | 72.0 | 76.0 | 75.0 | 69.2 | 73.1 | 70.8 | 75.0 | 70.8 | 78.8 | 71.4 | 57.7 | 0.0 | | | | | | | | | | | | | | |
| NB04 | . | 73.1 | 72.4 | 95.2 | 77.8 | 82.6 | 71.4 | 95.6 | 76.7 | 95.0 | 86.7 | 71.4 | 71.4 | 76.2 | 80.9 | 79.2 | 78.3 | 70.0 | 80.9 | 70.0 | 75.0 | 70.8 | 72.0 | 70.4 | 0.0 | | | | | | | | | | | | | |
| NB01 | . | 75.8 | 75.0 | 96.5 | 80.0 | 75.0 | 74.3 | 85.7 | 71.4 | 92.6 | 86.5 | 83.3 | 83.3 | 81.5 | 77.8 | 67.9 | 71.4 | 69.2 | 73.1 | 78.6 | 80.6 | 70.0 | 66.7 | 69.7 | 64.3 | 0.0 | | | | | | | | | | | | |
| BJ05 | . | 84.6 | 82.8 | 94.4 | 94.4 | 90.9 | 90.0 | 89.5 | 82.8 | 100.0 | 89.3 | 90.9 | 73.7 | 95.2 | 95.2 | 87.0 | 80.9 | 95.5 | 84.2 | 78.9 | 81.5 | 78.3 | 84.0 | 81.5 | 87.5 | 83.3 | 0.0 | | | | | | | | | | | |
| NF07 | . | 80.8 | 75.0 | 88.9 | 88.9 | 75.0 | 78.6 | 84.2 | 70.4 | 81.2 | 81.5 | 86.4 | 86.4 | 78.9 | 90.5 | 82.6 | 70.0 | 80.0 | 90.5 | 80.0 | 90.0 | 73.9 | 63.6 | 73.1 | 83.3 | 80.0 | 61.1 | 0.0 | | | | | | | | | | |
| BJ06 | . | 80.0 | 74.1 | 88.2 | 81.2 | 80.0 | 77.8 | 76.5 | 74.1 | 94.1 | 89.3 | 85.7 | 66.7 | 77.8 | 84.2 | 76.2 | 75.0 | 72.2 | 77.8 | 85.0 | 85.7 | 78.3 | 79.2 | 76.9 | 77.3 | 79.3 | 66.7 | 80.9 | 0.0 | | | | | | | | | |
| NB02 | . | 75.0 | 74.2 | 90.9 | 80.0 | 73.9 | 59.3 | 81.8 | 74.2 | 90.5 | 75.9 | 79.2 | 68.2 | 81.8 | 82.6 | 70.8 | 80.0 | 78.3 | 71.4 | 83.3 | 84.4 | 73.1 | 78.6 | 67.9 | 76.9 | 66.7 | 73.9 | 80.0 | 73.9 | 0.0 | | | | | | | | |
| BJ04 | . | 89.3 | 75.0 | 88.9 | 88.9 | 75.0 | 78.6 | 84.2 | 70.4 | 88.2 | 81.5 | 86.4 | 61.1 | 78.9 | 85.0 | 77.3 | 70.0 | 73.7 | 77.8 | 66.7 | 77.8 | 68.2 | 80.0 | 73.1 | 78.3 | 71.4 | 75.0 | 76.2 | 61.1 | 57.1 | 0.0 | | | | | | | |
| FJ10 | . | 90.3 | 73.3 | 95.5 | 100.0 | 78.3 | 80.6 | 86.4 | 77.4 | 90.0 | 79.3 | 83.3 | 88.0 | 82.6 | 70.0 | 80.0 | 73.9 | 87.5 | 87.0 | 87.5 | 83.9 | 81.5 | 86.2 | 71.4 | 80.8 | 78.1 | 83.3 | 73.9 | 83.3 | 77.8 | 88.5 | 0.0 | | | | | | |
| NF04 | . | 90.0 | 87.9 | 77.8 | 84.2 | 82.6 | 83.9 | 90.9 | 84.4 | 89.5 | 93.7 | 87.5 | 82.6 | 86.4 | 86.4 | 88.5 | 78.3 | 81.8 | 91.3 | 87.0 | 75.0 | 80.8 | 89.7 | 83.3 | 75.0 | 81.2 | 82.6 | 72.7 | 77.3 | 81.5 | 78.3 | 80.8 | 0.0 | | | | | |
| NF06 | . | 88.5 | 82.1 | 87.5 | 94.1 | 85.0 | 76.9 | 88.9 | 77.8 | 84.6 | 84.0 | 78.9 | 85.0 | 84.2 | 75.0 | 75.0 | 66.7 | 77.8 | 95.0 | 84.2 | 80.8 | 65.0 | 83.3 | 85.2 | 76.2 | 82.8 | 90.5 | 80.0 | 85.0 | 83.3 | 80.0 | 70.0 | 70.0 | 0.0 | | | | |
| NB07 | . | 84.6 | 69.2 | 81.2 | 94.4 | 95.6 | 90.0 | 83.3 | 74.1 | 80.0 | 80.8 | 80.0 | 80.0 | 85.0 | 90.0 | 76.2 | 75.0 | 64.7 | 84.2 | 90.5 | 89.7 | 72.7 | 79.2 | 85.7 | 82.6 | 87.1 | 90.9 | 80.9 | 80.0 | 84.0 | 68.4 | 78.3 | 82.6 | 72.2 | 0.0 | | | |
| NB03 | . | 87.5 | 93.1 | 93.3 | 93.3 | 100.0 | 88.9 | 87.5 | 89.3 | 92.9 | 92.3 | 89.5 | 95.0 | 94.7 | 94.4 | 95.5 | 95.2 | 88.9 | 94.4 | 94.7 | 92.6 | 95.8 | 87.0 | 92.6 | 90.9 | 89.7 | 89.5 | 90.0 | 89.5 | 96.0 | 90.0 | 100.0 | 95.6 | 100.0 | 83.3 | 0.0 | | |
| BJ09 | . | 92.3 | 76.9 | 93.7 | 93.7 | 95.2 | 93.1 | 88.2 | 81.5 | 100.0 | 79.2 | 84.2 | 77.8 | 83.3 | 82.3 | 85.7 | 90.5 | 83.3 | 82.3 | 89.5 | 88.9 | 87.0 | 82.6 | 69.6 | 91.3 | 82.1 | 84.2 | 90.5 | 84.2 | 92.0 | 78.9 | 81.8 | 91.3 | 95.0 | 77.8 | 81.2 | 0.0 | |
| BJ07 | . | 82.1 | 67.9 | 90.0 | 84.2 | 77.3 | 87.5 | 90.9 | 72.4 | 100.0 | 82.8 | 77.3 | 65.0 | 80.0 | 80.9 | 79.2 | 72.7 | 70.0 | 80.0 | 70.0 | 70.4 | 76.0 | 72.0 | 65.4 | 69.6 | 77.4 | 82.6 | 78.3 | 71.4 | 81.5 | 78.3 | 76.0 | 75.0 | 81.8 | 71.4 | 85.7 | 61.1 | 0.0 |

Ana_Bacteria

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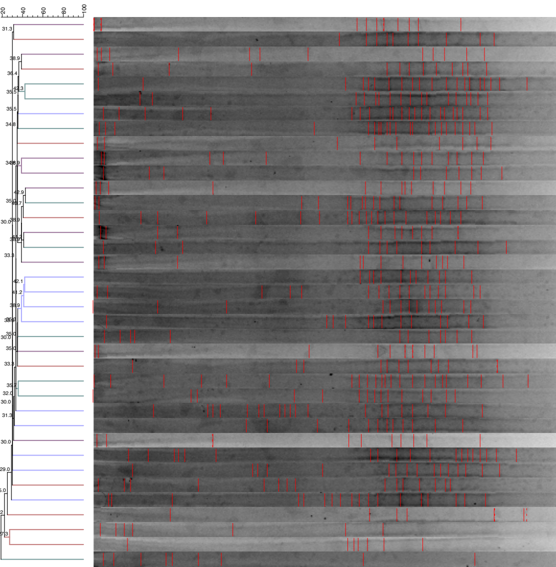

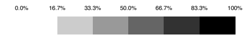


| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| BJ02 | BSL | 03.01.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF05 | FUZ | 08.11.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 4.59% | BSSM | Slightly Gravelly Sand . | Green macroalgae | . Ana Flor Data |
| BJ05 | BSL | 03.01.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 1.91% | PZNO | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF07 | FUZ | 08.11.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and . | Ana Flor Data |
| NB06 | BSL | 09.11.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 1.54% | BSSM | Gravelly Sand | Shell residues and som. | Ana Flor Data |
| NB08 | BSL | 09.11.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, . | Ana Flor Data |
| FJ04 | FUZ | 04.01.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.68% | PIMC | Slightly Gravelly Sand . | Bivalves | . Ana Flor Data |
| NB05 | BSL | 09.11.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 12.91% | MCIA | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF08 | FUZ | 08.11.2011 | 37.059.775 | -7.721.954 | 7.335.693 | 1.30% | PIRV | Sandy Mud | Bivalves | . Ana Flor Data |
| BJ07 | BSL | 03.01.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 0.64% | MCIA | Muddy Sand | Macroalgae and bivalve. | Ana Flor Data |
| BJ09 | BSL | 03.01.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 0.79% | FPEX | Slightly Gravelly Sand . | Shell residues | . Ana Flor Data |
| BJ04 | BSL | 03.01.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 0.65% | FPIN | Gravelly Sand | Shell residues | . Ana Flor Data |
| NB02 | BSL | 09.11.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF03 | FUZ | 08.11.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ08 | BSL | 03.01.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.78% | PILO | Muddy Sand | Macroalgae, molluscs, . | Ana Flor Data |
| NB09 | BSL | 09.11.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ06 | BSL | 03.01.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 0.82% | PIDE | Slightly Gravelly Sand . | Shell residues | . Ana Flor Data |
| FJ03 | FUZ | 04.01.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.16% | PILA | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ06 | FUZ | 04.01.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.38% | PIPC | Muddy Sand | Shell residues | . Ana Flor Data |
| FJ07 | FUZ | 04.01.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 12.09% | PIPC | Muddy Sand | Spartina maritima and . | Ana Flor Data |
| FJ02 | FUZ | 04.01.2011 | 37.060.649 | -771.761 | 0.366211 | 0.02% | PIRV | Gravelly Sand | Shell residues | . Ana Flor Data |
| NB07 | BSL | 09.11.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalve. | Ana Flor Data |
| BJ03 | BSL | 03.01.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 1.33% | BSSM | Sandy Mud | Spartina maritima and . | Ana Flor Data |
| NF06 | FUZ | 08.11.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.10% | FPIN | Slightly Gravelly Sand . | Green macroalgae | . Ana Flor Data |
| NB01 | BSL | 09.11.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 0.31% | DEDE | Slightly Gravelly Sand. | Shell residues | . Ana Flor Data |
| NB04 | BSL | 09.11.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 2.04% | PILO | Sandy Mud | Shell residues | . Ana Flor Data |
| FJ09 | FUZ | 04.01.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 4.58% | PIDI | Sandy Gravel | Zostera nolti and Small. | Ana Flor Data |
| FJ11 | FUZ | 04.01.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 1.12% | FPEX | Sandy Mud | Roots and unknown inv. | Ana Flor Data |
| BJ01 | BSL | 03.01.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ05 | FUZ | 04.01.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 2.75% | BSSM | Sand . | Green macroalgae | . Ana Flor Data |
| FJ10 | FUZ | 04.01.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF04 | FUZ | 08.11.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.26% | DVDP | Slightly Gravelly Sand . | Shell residues | . Ana Flor Data |
| FJ01 | FUZ | 04.01.2011 | 37.061.379 | -771.125 | 2.529.175 | 0.11% | MCMA | Gravelly Sand | Shell residues | . Ana Flor Data |
| NF09 | FUZ | 08.11.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 5.23% | MCIA | Sandy Mud | Bivalves | . Ana Flor Data |
| NF10 | FUZ | 08.11.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 4.21% | PILO | Muddy Sand | Shell residues | . Ana Flor Data |
| NF11 | FUZ | 08.11.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 0.15% | FPEX | Slightly Gravelly Sand . | Roots and unknown inv. | Ana Flor Data |
| NB03 | BSL | 09.11.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 5.19% | PIRE | Muddy Sand | Spartina maritima and . | Ana Flor Data |

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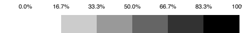


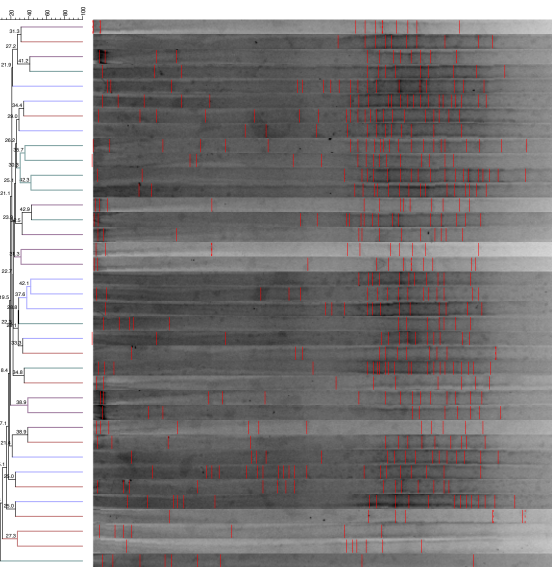

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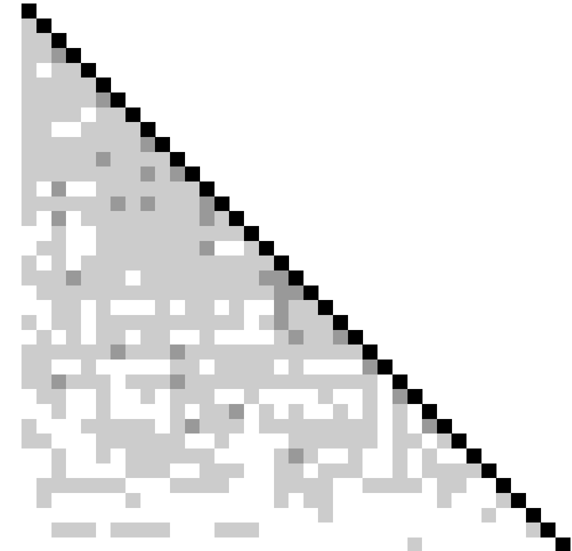
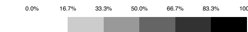
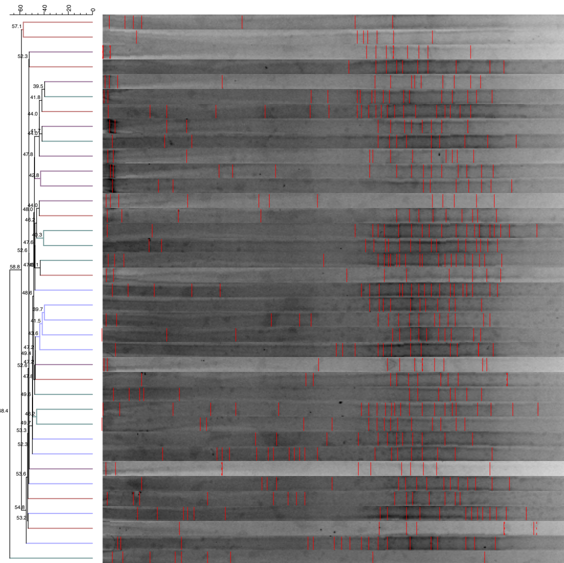
|---|---|---|---|---|---|---|---|---|---|---|
| BJ02 | BSL | 03.01.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF05 | FUZ | 08.11.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 4.59% | BSSM | Slightly Gravelly Sand | .Green macroalgae | .Ana Flor Data |
| BJ08 | BSL | 03.01.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.78% | PILO | Muddy Sand | Macroalgae, molluscs, . | Ana Flor Data |
| NB09 | BSL | 09.11.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ01 | FUZ | 04.01.2011 | 37.061.379 | -771.125 | 2.529.175 | 0.11% | MCMA | Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ04 | FUZ | 04.01.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.68% | PIMC | Slightly Gravelly Sand | .Bivalves | . Ana Flor Data |
| NF03 | FUZ | 08.11.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ11 | FUZ | 04.01.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 1.12% | FPEX | Sandy Mud | Roots and unknown inv. | Ana Flor Data |
| NB01 | BSL | 09.11.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 0.31% | DEDE | Slightly Gravelly Sand. | Shell residues | . Ana Flor Data |
| NB04 | BSL | 09.11.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 2.04% | PILO | Sandy Mud | Shell residues | . Ana Flor Data |
| NB06 | BSL | 09.11.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 1.54% | BSSM | Gravelly Sand | Shell residues and som. | Ana Flor Data |
| NB08 | BSL | 09.11.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, . | Ana Flor Data |
| BJ04 | BSL | 03.01.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 0.65% | FPIN | Gravelly Sand | Shell residues | . Ana Flor Data |
| NB02 | BSL | 09.11.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ06 | BSL | 03.01.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 0.82% | PIDE | Slightly Gravelly Sand | .Shell residues | . Ana Flor Data |
| BJ01 | BSL | 03.01.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ03 | BSL | 03.01.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 1.33% | BSSM | Sandy Mud | Spartina maritima and . | Ana Flor Data |
| FJ03 | FUZ | 04.01.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.16% | PILA | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ06 | FUZ | 04.01.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.38% | PIPC | Muddy Sand | Shell residues | . Ana Flor Data |
| FJ02 | FUZ | 04.01.2011 | 37.060.649 | -771.761 | 0.366211 | 0.02% | PIRV | Gravelly Sand | Shell residues | . Ana Flor Data |
| NB07 | BSL | 09.11.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalve. | Ana Flor Data |
| FJ07 | FUZ | 04.01.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 12.09% | PIPC | Muddy Sand | Spartina maritima and . | Ana Flor Data |
| NF06 | FUZ | 08.11.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.10% | FPIN | Slightly Gravelly Sand | .Green macroalgae | .Ana Flor Data |
| NB05 | BSL | 09.11.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 12.91% | MCIA | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF08 | FUZ | 08.11.2011 | 37.059.775 | -7.721.954 | 7.335.693 | 1.30% | PIRV | Sandy Mud | Bivalves | . Ana Flor Data |
| BJ07 | BSL | 03.01.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 0.64% | MCIA | Muddy Sand | Macroalgae and bivalve. | Ana Flor Data |
| BJ09 | BSL | 03.01.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 0.79% | FPEX | Slightly Gravelly Sand | .Shell residues | . Ana Flor Data |
| BJ05 | BSL | 03.01.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 1.91% | PZNO | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF07 | FUZ | 08.11.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and . | Ana Flor Data |
| FJ10 | FUZ | 04.01.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ09 | FUZ | 04.01.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 4.58% | PIDI | Sandy Gravel | Zostera nolti and Small. | Ana Flor Data |
| NF04 | FUZ | 08.11.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.26% | DVDP | Slightly Gravelly Sand | .Shell residues | . Ana Flor Data |
| FJ05 | FUZ | 04.01.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 2.75% | BSSM | Sand | .Green macroalgae | .Ana Flor Data |
| NF09 | FUZ | 08.11.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 5.23% | MCIA | Sandy Mud | Bivalves | . Ana Flor Data |
| NF10 | FUZ | 08.11.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 4.21% | PILO | Muddy Sand | Shell residues | . Ana Flor Data |
| NF11 | FUZ | 08.11.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 0.15% | FPEX | Slightly Gravelly Sand | .Roots and unknown inv. | Ana Flor Data |
| NB03 | BSL | 09.11.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 5.19% | PIRE | Muddy Sand | Spartina maritima and . | Ana Flor Data |



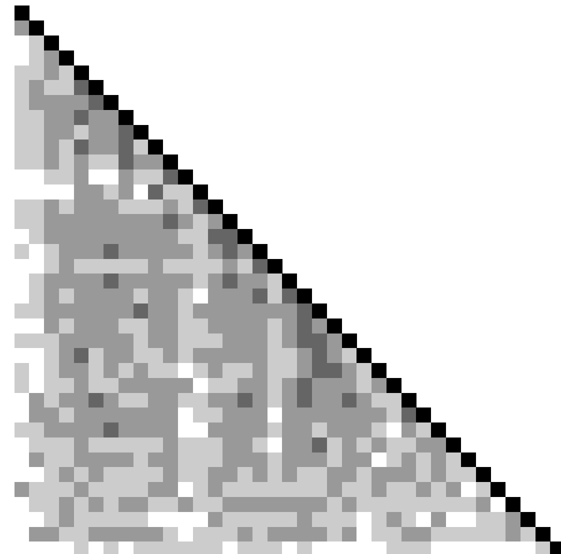
Ana_Bacteria

Ana_Bacteria



| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NF10 | FUZ | 08.11.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 4.21% | PILO | Muddy Sand | Shell residues | . Ana Flor Data |
| NF11 | FUZ | 08.11.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 0.15% | FPEX | Slightly Gravelly Sand | .Roots and unknown inv. | Ana Flor Data |
| BJ02 | BSL | 03.01.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 1.20% | PIRE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF05 | FUZ | 08.11.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 4.59% | BSSM | Slightly Gravelly Sand | .Green macroalgae | . Ana Flor Data |
| BJ04 | BSL | 03.01.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 0.65% | FPIN | Gravelly Sand | Shell residues | . Ana Flor Data |
| NB02 | BSL | 09.11.2011 | 36.985.258 | -7.951.779 | 3.009.766 | 0.80% | PIDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF03 | FUZ | 08.11.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.44% | DVDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ08 | BSL | 03.01.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.78% | PILO | Muddy Sand | Macroalgae, molluscs, . | Ana Flor Data |
| NB09 | BSL | 09.11.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 1.11% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ06 | BSL | 03.01.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 0.82% | PIDE | Slightly Gravelly Sand | .Shell residues | . Ana Flor Data |
| BJ07 | BSL | 03.01.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 0.64% | MCIA | Muddy Sand | Macroalgae and bivalve. | Ana Flor Data |
| BJ09 | BSL | 03.01.2011 | 36.981.263 | -7.955.537 | 1.567.871 | 0.79% | FPEX | Slightly Gravelly Sand | .Shell residues | . Ana Flor Data |
| BJ05 | BSL | 03.01.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 1.91% | PZNO | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF07 | FUZ | 08.11.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 0.61% | PZNS | Slightly Gravelly Sand | Spartina maritima and . | Ana Flor Data |
| NB06 | BSL | 09.11.2011 | 36.985.931 | -7.953.332 | 7.576.050 | 1.54% | BSSM | Gravelly Sand | Shell residues and som. | Ana Flor Data |
| NB08 | BSL | 09.11.2011 | 36.988.925 | -7.951.054 | 5.653.442 | 2.18% | FPIN | Slightly Gravelly Sand | Macroalgae, molluscs, . | Ana Flor Data |
| NB05 | BSL | 09.11.2011 | 36.986.645 | -7.952.332 | 4.211.426 | 12.91% | MCIA | Muddy Sand | Zostera nolti and Small. | Ana Flor Data |
| NF08 | FUZ | 08.11.2011 | 37.059.775 | -7.721.954 | 7.335.693 | 1.30% | PIRV | Sandy Mud | Bivalves | . Ana Flor Data |
| FJ04 | FUZ | 04.01.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.68% | PIMC | Slightly Gravelly Sand | .Bivalves | . Ana Flor Data |
| FJ03 | FUZ | 04.01.2011 | 37.059.354 | -7.720.811 | 1.087.158 | 0.16% | PILA | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ06 | FUZ | 04.01.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.38% | PIPC | Muddy Sand | Shell residues | . Ana Flor Data |
| FJ07 | FUZ | 04.01.2011 | 37.064.152 | -7.720.348 | 11.180.908 | 12.09% | PIPC | Muddy Sand | Spartina maritima and . | Ana Flor Data |
| FJ02 | FUZ | 04.01.2011 | 37.060.649 | -771.761 | 0.366211 | 0.02% | PIRV | Gravelly Sand | Shell residues | . Ana Flor Data |
| BJ03 | BSL | 03.01.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 1.33% | BSSM | Sandy Mud | Spartina maritima and . | Ana Flor Data |
| NF06 | FUZ | 08.11.2011 | 37.064.307 | -7.721.038 | 6.134.033 | 0.10% | FPIN | Slightly Gravelly Sand | .Green macroalgae | .Ana Flor Data |
| NB07 | BSL | 09.11.2011 | 36.989.357 | -7.951.181 | 6.374.390 | 1.64% | PZNO | Gravelly Sand | Macroalgae and bivalve. | Ana Flor Data |
| NB01 | BSL | 09.11.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 0.31% | DEDE | Slightly Gravelly Sand. | Shell residues | . Ana Flor Data |
| NB04 | BSL | 09.11.2011 | 3.698.498 | -7.947.808 | 4.692.139 | 2.04% | PILO | Sandy Mud | Shell residues | . Ana Flor Data |
| FJ11 | FUZ | 04.01.2011 | 37.062.853 | -7.722.716 | 2.048.584 | 1.12% | FPEX | Sandy Mud | Roots and unknown inv. | Ana Flor Data |
| FJ09 | FUZ | 04.01.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 4.58% | PIDI | Sandy Gravel | Zostera nolti and Small. | Ana Flor Data |
| BJ01 | BSL | 03.01.2011 | 36.983.312 | -7.952.643 | 7.095.337 | 1.52% | DEDE | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| FJ10 | FUZ | 04.01.2011 | 3.706.267 | -7.722.251 | 5.893.799 | 2.28% | CIAP | Slightly Gravelly Sand | Shell residues | . Ana Flor Data |
| NF04 | FUZ | 08.11.2011 | 37.061.573 | -7.721.583 | 6.614.746 | 0.26% | DVDP | Slightly Gravelly Sand | .Shell residues | . Ana Flor Data |
| FJ05 | FUZ | 04.01.2011 | 3.706.491 | -7.720.782 | 5.172.729 | 2.75% | BSSM | Sand | .Green macroalgae | .Ana Flor Data |
| NF09 | FUZ | 08.11.2011 | 37.059.688 | -7.722.161 | 3.971.191 | 5.23% | MCIA | Sandy Mud | Bivalves | . Ana Flor Data |
| FJ01 | FUZ | 04.01.2011 | 37.061.379 | -771.125 | 2.529.175 | 0.11% | MCMA | Gravelly Sand | Shell residues | . Ana Flor Data |
| NB03 | BSL | 09.11.2011 | 36.984.548 | -7.947.696 | 3.009.766 | 5.19% | PIRE | Muddy Sand | Spartina maritima and . | Ana Flor Data |



# Annex IX

| | 0,60% | 1,20% | 1,70% | 2,90% | 4,60% | 6,20% | 7,20% | 8,50% | 9,20% | 11,00% | 12,40% | 13,90% | 15,80% | 17,40% | 18,40% | 19,90% | 21,50% | | 25,20% | 26,10% | 26,60% | 28,10% | 29,10% | 30,50% | 34,30% | 36,30% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BJ01 | | 149 | | 124 | | | | | | | | | | | | | | ? | | 112 | | | | | | | | | |
| BJ03 | 159 | 101,1 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NB08 | | | | | | | | | | 212 | | 188 | | | | | | | | | | | | | | | | | |
| NB06 | | | 181 | | | | | | | 175 | | | | | | | | | | | | | | | | | | | |
| NB04 | 195 | | | | | | | | | | | | | | | | 168 | 168 | | | | | | | | | | | |
| NB01 | 187 | | | | 179 | | | | | | | | 169 | | | | | 173 | | | | | 166 | | | | 170 | | |
| FJ07 | 181 | | | | | | | | | | | 193 | | | | | | | | | | | | | | | | | |
| FJ03 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| FJ02 | | | | 180 | | | | | | | | | | | | | | | | | | | | | | | | | |
| FJ09 | | | | | | | | | | | | 187 | | | | | | | 184 | 181 | | 180 | | | 181 | 183 | 181 | 183 | 179 |
| FJ06 | | 179 | | 180 | | | | | | | | | | | | | | | | | | | | | | 195 | | | |
| NB02 | | | 201 | 198 | | | | | | | | | | | | | | | | | | | | | | | | | |
| BJ04 | | 168 | 86 | | 143 | | | | | | | | | | | | | | | 140 | | | | | | | | | |
| BJ06 | | | 155,4 | 105,1 | | | | | | | | | | | 150 | | | | | | | | | | | | | | |
| BJ05 | | 148 | 147 | | 142 | | | | | | | | | | 143 | | | | | | | | | | 140 | 139 | | | |
| NF07 | | | 175 | | 162 | | | | | | | | | 167 | | | | | | | | | | | 160 | | | | |
| FJ11 | | | | | | | | | | | | | | | | | | | | | | | | | 175 | | 200 | | |
| NF03 | | 175 | | | | | | | 179 | | | 179 | | | | | | | 172 | | | | | | 182 | | | | |
| NF10 | | | 133 | | 131 | | 138 | 141 | | | | | | | | | | | | | | | | 136 | | | | | |
| NF11 | | | | | | | | 123 | | | | | | | | | | | | | | | | | | | | | |
| NF04 | | | 188 | | | | 195 | 189 | | | | | | | | | | 172 | | | | | | | | | 177 | 177 | 174 |
| NF06 | | | | | | | | | 111,5 | | | | | | | | | | | | | | | | | | | | |
| NF08 | | | 90,3 | 157 | | | | | | | | | | | | | | | | | | | | | | | | | |
| NB05 | | | 181 | 176 | 176 | | | | | | | | | | | | | | | | | | | | | | | | |
| NF09 | | | | | | | | | | | | | | | 147 | | | | | | | | | | | | | | |
| FJ05 | | | | | | | | 184 | | | 188 | | | 182 | 182 | 185 | | | | | 182 | | | | | | | | |
| FJ10 | | | | | | | | 193 | | | | | | | | | | | | | | | | | 179 | 179 | 184 | | |
| NF05 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BJ09 | | | | 236 | | | | | | | 175 | | 171 | | | | | | | | | | | | | | | | |
| BJ07 | | | 110 | 165,3 | | | | | | | | | | | | | | | 164 | | | 165 | | | | | 158 | | |
| NB03 | | | 169 | | 170 | | | | 178 | | | | 169 | 168 | | | | 165 | | | | 164 | | | | | | | |
| NB07 | | | | 182 | | 184 | | 184 | 185 | | | | | 177 | | | | | | | | | | | | | | | |
| FJ04 | | | | 178 | | 177 | | | | 182 | | | | | | 186 | | | | 185 | | | | | | | | | |
| NB09 | | | | 185 | | | | | | | | 181 | | | | 177 | | | | | | | | | | | | | |
| BJ08 | | | 216,2 | 198,1 | | | | | | | | 173 | | | 184 | | | | | | | | | | | | | | |
| FJ01 | | | | 186 | 194 | | | | | | | | | 181 | | | | | | | | | | | | | | | |
| BJ02 | ? | 148 | 148 | 134 | | | | | | | | | | | | | | | | | | | | | | | | | |

| | 42,80% | 44,30% | 45,60% | 46,40% | 48,60% | 49,10% | 50,30% | 51,90% | 53,70% | 55,10% | 56,10% | 57,70% | 58,80% | 59,60% | 61,00% | 62,20% | 63,80% | 65,20% | 66,60% | 67,30% | 68,50% | 69,30% | 71,20% | 73,00% | 74,00% | 75,20% | 76,40% | 77,40% | 78,70% | 79,90% | 81,50% | 82,20% | 83,70% | 84,80% | 86,00% | 87,20% | 88,20% | 91,40% | 93,00% | 93,80% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BJ01 | | | | | | | | | | 103 | | 105 | | | | | | 107 | 104 | | | 102 | | 97 | | | | | | | | | 76 | | | | | | | |
| BJ03 | | | | 126 | | | | | | | | | | | 142,9 | | 131 | | 134 ? | | 129 ? | | 119 | 119 | | | | | | | 112 | 109 | | | | | | | | |
| NB08 | | | | | | | | | | | | 171 | 173 | | 174 | 177 | | 181 | 182 | | 185 | | 185 | 187 | | | | 172 | 169 | | 161 | 160 | 159 | | 147 | | | | | |
| NB06 | | | | | | | | | | 166 | | | 172 | 177 | 185 | | 200 | | 188 | | | 182 | 181 | 176 | | | 174 | | 162 | 163 | 183 | | 170 | 163 | 154 | | 155 | | | 134 |
| NB04 | | 163 | | | | | 158 | | | | | 170 | | 167 | 169 | 173 | 167 | | 169 | | | | 164 | 159 | | | 151 | | 149 | | | | | | | | | | | |
| NB01 | | | | | | | | | | | 163 | 165 | | 162 | 166 | 165 | 166 | 169 | 165 | | | 166 | 160 | | 159 | 164 | 163 | | | | 149 | | 151 | | | | 131 | | | 124 |
| FJ07 | | | | | | | | | | | | | | | | 176 | 182 | | 185 | | 196 | 157,4 | | 172 | 169 | 169 | | | | 158 | 157 | | | | | | | | | |
| FJ03 | | | | | | | | | | | | 180 | | 177 | 188 | 183 | | | 198 | | 182 | 181 | | 174 | | 165 | 165 | | | | | 156 | | | | | | | | |
| FJ02 | | | | | | | 171 | 171 | | 169 | | | | 186 | 191 | 194 | | | 194 | | 196 | | | | | 181 | 179 | | | | 167 | | | 164 | | | | | | |
| FJ09 | 175 | 174 | | 176 | | | | | | | | | 178 | | | 183 | 177 | | 185 | | | 182 | | 173 | | | 173 | | | | | | 152 | | | | | | | |
| FJ06 | 180 | | 180 | | | | | | | | | | | 166 | | 166 | 172 | | 179 | | 175 | | 160 | 163 | | 159 | 156 | | | | | 144 | 139 | | | | | | | |
| NB02 | | | 167 | | | 159 | | | | 173 | 172 | | 167 | 165 | 169 | 173 | | 185 | | 187 | | | | | 164 | 0 | 168 | | | 153 | 149 | | | 146 | | | | | | |
| BJ04 | | | | | | | | | | | | | 139 | | | 141 | | | 142 | 142 | 138 | | | | 136 | 0 | 128 | | | 124 | 122 | | | | | | | | | |
| BJ06 | | | | | | | | | | | | 143 | 145 | | | 158 | | | 152 | | | | 142 | | 142 | 138 | 137 | | | 131 | | | | | | | | | | |
| BJ05 | | | | 138 | | | | | | | | | | | | | | | | | | | 121 | 121 | | 117 | | | | 113 | | | | 107 | | | | | | |
| NF07 | | | | | | | | | | | | | | | | | 164 | | 162 | | 171 | | 155,6 | | 158 | | | 153 | | 148 | 146 | | | 138 | | | | | | |
| FJ11 | | | | | 176 | | | | | 176 | | | | 176 | | 185 | 185 | 184 | | 178 | | 182 | | | 176 | | | | | 167 | | | | | | | | | | |
| NF03 | | 183 | | | 169 | | | | 169 | 172 | | 176 | 172 | 174 | 188 | 184 | 180 | | | 179 | | | 180 | 176 | 172 | | 176 | | 175 | | | | | | | | | | | |
| NF10 | | | | | | | | | 131 | | | | | | | 146 | | | | | | | | | | | | | | | | | | | | | | | | |
| NF11 | | | | | | | | | | 114 | 117 | 117 | | 122 | | 113 | | | | | | | 112 | | | | | | | | | | | | | | | | | |
| NF04 | | 178 | | | | | | | | | | | | | | | 173 | 178 | | 193 | | | 175 | | 168 | | 170 | 168 | | | | | | | | | | | | |
| NF06 | | 163 | 167 | | | | | | | | | | | | | 166 | 177 | | 169 | | 167 | | | 167 | | 161 | 158 | | | | | | | | ? | | 134 | | | |
| NF08 | | | | | | | | | 150 | | | | | | | 149 | | | 157 | | | | | | | 140 | | | | 138 | | | 136 | | 126 | | | | | |
| NB05 | | | | | 162 | | | | | | | | | 179 | 192 | 188 | 180 | 178 | | | 181 | 181 | | 177 | 166 | 164 | 163 | | 160 | 157 | 154 | 153 | | | 136 | | | | | |
| NF09 | | | | | | | | | | | | | | | 141 | | | | 137 | | 129 | | | | | | | | | | | | | | | 106 ? | | | 95 ? | |
| FJ05 | | | | | | | | | | | | | | 191 | | 193 | | 204 | | | 193 | | | 181 | | | | | 172 | 174 | 166 | 161 | 160 | 155 | | 147 | | 134 | | |
| FJ10 | | | | | | 171 | | | | | | | | | | | 175 | 179 | | 178 | | 175 | | 175 | | 167 | 169 | | 167 | 168 | | | 159 | | | 145 | | | | |
| NF05 | | | | | | | | | 166 | | | | | 176 | | 181 | 191 | 189 | | | 181 | | 177 | | | | 168 | | | | | | 153 | | | 144 | | | | |
| BJ09 | | | | | | | | | | | | | | | | | | | | | | 161 | 158 | | | | 153 | | 148 | | 147 | | 139 | | | | 129 | | | |
| BJ07 | | | | | | | | | | | | 151 | | | | | 157 | | 161 | | | 156 | 154 | 149 | | | 153 | | 155 | | 149 | | 145 | | | | | | | |
| NB03 | | | | | | | | | | | | 152 | | | | | | | | | | | | | | | | | | | | 132 | | | | | | | | |
| NB07 | | | | | | | | | | | | | | | | | | | 173 | | 170 | | | 167 | | 164 | 157 | | 155 | | | 149 | | | | | | | | |
| FJ04 | | | | | | | | | | | 181 | 182 | | 188 | | | 191 | 191 | 192 | | 187 | 184 | 186 | 180 | | 177 | 178 | 169 | 172 | 166 | | | 157 | 152 | | | | | | |
| NB09 | | | | | | | | | | | | | | 170 | | 178 | 178 | 184 | | | | | 195 | 180 | | | | | 177 | | | | 165 | | | | | | | |
| BJ08 | | | | | | | | | | | | | | 163 | | 165 | | 164 | 168 | | | 169 | 160 | 161 | | | | | 151 ? | | | | | | | | | | | |
| FJ01 | | 169 | 168 | | | | 167 | 165 | 166 | | 168 | 174 | | 182 | | 189 | | | 207,9 | | | | 122,1 | 184 | | | | | 173 | 174 | | | | 164 | | | | | | |
| BJ02 | | | | | | | | | | | | 119 | | 121 | | 122 | 128 | | 118 | | | 117 | 120 | | | | | | | 96 | | | | | | | | | | |