Universidade de Aveiro Departamento de Biologia
2010

Fábio Emanuel Lopes de Matos

COLONIZAÇÃO DE SUBSTRATOS ARTIFICIAIS EM ECOSSISTEMAS QUIMIOSSINTÉTICOS

COLONISATION OF ARTIFICIAL SUBSTRATES IN CHEMOSYNTHETIC ECOSYSTEMS

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, Biodiversidade e Gestão de Ecossistemas, realizada sob a orientação científica da Doutora Marina Ribeiro da Cunha, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro

O aluno foi suportado por uma Bolsa de Investigação no âmbito do Projecto CHEMECO (ESF/FCT, EURODEEP/0001/2007).

o júri

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palavras-chave

Colonização, quimiossíntese, fontes frias, vulcões de lama, macrofauna, substratos artificiais

resumo

A região do Golfo de Cádiz é caracterizada pela presença de vários vulcões de lama com diversas características geológicas. Estas estruturas têm sido alvo de investigação nos últimos anos nas áreas de geologia e ecologia. O presente trabalho visa o estudo dos processos de colonização em quatro desses vulcões recorrendo ao uso de dispositivos de colonização (CHEMECOLI) preenchidos com substratos orgânicos (madeira e alfalfa) e inorgânicos (carbonatos). Conjuntos de três CHEMECOLI, cada um com um tipo de substrato, foram depositados ao longo de um gradiente de profundidade: Mercator (354m), Meknès (698m), Darwin (1100m) e Carlos Ribeiro (2197m). No Mercator, três *sets* foram colocados com o objectivo de estudar a sucessão ecológica da comunidade de macrofauna. Dois desses conjuntos já foram recolhidos e analisados assim como cada um dos *sets* colocados no Meknès e no Darwin. O tempo de imersão variou entre 10 meses e dois anos. A biodiversidade de metazoários recrutados foi estudada com particular interesse pelas espécies de bivalves e poliquetas quimiotróficas. Em todos os dispositivos de colonização houve recrutamento independentemente da duração da experiência. Diferenças significativas foram observadas entre as comunidades dos diferentes substratos. Os substratos orgânicos foram mais densamente colonizados que os carbonatos. Na alfalfa os grupos de invertebrados mais abundantes foram os anfípodes e os poliquetas enquanto as amostras de madeira foram densamente colonizada por bivalves perfuradores de madeira. Espécies quimiotróficas, na sua maioria bivalves das famílias Solemyidae e Mytilidae, foram recrutadas com sucesso quase exclusivamente nos substratos orgânicos. O recrutamento de espécies características do ambiente circundante foi reduzido.

keywords Colonisation, chemosynthesis, cold seeps, mud volcanoes, macrofauna, artificial substrates

abstract The Gulf of Cadiz encompasses around forty mud volcanoes, with diverse geological settings, which have been the focus of geological and ecological surveys in the last years. The present study includes a combination of site surveys and replicate colonization experiments (CHEMECOLI) using organic (wood and alfalfa grass) and inorganic (carbonate) substrata in some of this chemosynthetic ecosystems. Sets of CHEMECOLI, each with one of the three substrates, were deployed in four mud volcanoes along a depth gradient: Mercator (354m), Meknès (698m), Darwin (1100m) and Carlos Ribeiro (2197m). In Mercator three sets were deployed in order to illustrate temporal variations of the assemblages. Two sets from Mercator and the ones from Meknès and Darwin were already recovered and analysed. The immersion times range from 10 months to two years. The biodiversity of the recruited metazoan, with particular attention to bivalve and polychaetes symbiotic species, are being analysed. The recruitment of metazoans was successfully recorded in all the CHEMECOLI independently of the duration of the experiments. Significant differences were observed between the substrate. The organic substrates were more densely colonised than the carbonates. In alfalfa grass, the amphipods and polychaetes are the most abundant groups while the wood experiments are densely colonised by wood-boring bivalves. Chemotrophic species, mostly Solemyid and Mytilid bivalves, were recovered mainly in organic substrates and the recruitment of background taxa was generally low.

Hobbes: *Do you have an idea for your story yet?*
Calvin: *No, I'm waiting for inspiration. You can't just turn on creativity like a faucet. You have to be in the right mood.*
Hobbes: What mood is that?
Calvin: Last-minute panic.

in: **Calvin & Hobbes: The 10th Anniversary Book, Bill Watterson**

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I. Introduction

The deep-sea starts approximately at 200 m depths below the euphotic zone, and can be classified as an extreme environment owing to the high pressures, low temperatures and reduced food inputs (Tyler 2003). In this environment the darkness is continuous and photosynthesis cannot be accomplished, therefore, in some locations life evolved exploiting other available energy sources based on chemical compounds (Etter and Mullineaux 2001).

1. Reducing habitats

The first contact with marine chemosynthetic environments occurred in 1977 with the discovery of deep-sea hydrothermal vents in the Galapagos Rift, and since then in other areas around the world oceans (Van Dover et al. 2002; Van Dover and Lutz 2004). Besides hydrothermal vents, several examples of chemosynthesis-based habitats are now known, including cold seeps and large organic falls. They are associated with fluid emissions rich in hydrogen sulphide originated by inorganic process in the seabed or by microbial-mediated sulphate reduction. Some times, methane produced by organic matter reduction via biogenic or thermogenic processes is also available. These ecosystems based in microbial chemoautotrophic production attracted the biologists' attention, particularly because of their exuberant metazoans communities and the establishment of unusual symbioses (Tunnicliffe et al. 2003; Tyler et al. 2003).

In 1984, the first deep-sea cold seeps were found in the Gulf of Mexico. They were characterised by hypersaline fluid emissions with high concentration of sulphides and methane. The cold seeps can result from a variety of processes including tectonic forces that induced the escape of high-pressure fluids or hydrocarbons through the sea floor, differential compaction of organic-rich sediments, gas hydrate dissociation and subsurface salt migration (Sibuet and Olu 1998; Vanreusel et al. 2009; Cordes et al. 2010). They are present both in passive and active margin around the world sea-floor throughout a wide bathymetric range (from 300 to 6000 m). While hydrothermal vents are dominated by basalt substrata, the cold seeps are predominantly sedimentary environments (Van Dover 2000). Nevertheless, hard substrates are present in cold seeps under various forms such as

carbonate concretions (Génio et al. 2008; Cordes et al. 2010), associated cold-water coral reefs (Cordes et al. 2010) or sunken wood that can travel long distances from the origin source (Gage 2003). Nonetheless, the importance of these substrates to seep-community composition and diversity is still poorly known (Tunnicliffe et al. 2003).

Among the different types of cold seeps discovered in the last decades, the mud volcanoes (MV) have attracted much of the attention of natural science researchers (Mazzini 2009). The mud volcanoes are geological structures present in many places (on land and offshore) where argillaceous material and fluids are expelled, by flow or eruption, through long narrow openings or fissures in the sea-floor (Dimitrov 2002; Olu-Le Roy et al. 2004). They can have a variety of shapes and sizes and their formation can be forced by geologic, tectonic, geochemical and/or hydro-geological factors (Milkov 2000). As a result of the escape of hydrocarbon-rich fluids, the environmental settings on mud volcanoes support a broad diversity of chemosynthetic organisms, including tubeworms and bivalves with symbiotic bacteria (Olu-Le Roy et al. 2004; Hilário et al. 2010; Rodrigues et al. 2010; Vanreusel et al. 2009). The chemosynthetic biota developed several adaptations to this extreme habitat including the tolerance to low oxygen levels to the high concentrations of chemical compounds that they use as energy source (Sibuet and Olu 1998).

Other examples of reducing habitats in the deep-sea are large organic falls from different origins that can be found in the sea-floor attracting a variety of organisms (e.g. Tyler 2003; Wahl 2009). Within those, wood and other plant remains are frequent in many areas of the sea-floor and widely dispersed throughout the deep (Pailleret et al. 2007). These materials can occur abundantly in some places with a remarkable variety of associated macroinvertebrates (e.g. Samadi et al. 2007; Young 2009). Several species of bivalves, gastropods, annelids, crustaceans and echinoderms exploit the plant remains as food sources (Wolff 1979). The most abundant and diverse group are the molluscs represented mainly by wood boring teredinid bivalves, mytilids and limpets (Hoyoux et al. 2009). This substratum is also commonly used as shelter by polychaetes. The activity of these organisms, together with bacteria and fungus, converts the wood in faecal pellets, a food source for the detritus feeders and larvae (Turner 1977). The wood remains may be colonised by a great diversity of crustaceans, which are the second-largest zoological group in this kind of organic remains. The knowledge on this fauna is essentially

taxonomic (Hoyoux et al. 2009) and more comprehensive studies about their role in these ecosystems are lacking. Besides wood and plant remains, organic falls include also the sulphide-rich whale skeletons (Baco and Smith 2003). When the whale carcass reaches the deep-sea floor it is rapidly processed by scavenging invertebrates and fishes, then the lipids, especially in the vertebrae, are decomposed by bacteria supporting chemosynthetic communities, often for several decades (Tyler et al. 2003).

The high biomasses and biodiversity found in hydrothermal vents and cold seeps show that is not the temperature or high-pressure that limits the deep-sea organism's activity. However, the similarities at high taxonomic levels between the biological assemblages associated to these two habitats (see Lutz and Kennish 1996 for a list with several examples) are not shared at the species level where significant differences concerning the composition, diversity and abundance were known (Sibuet and Olu 1998). Since 1977, more than 400 morphological species from hydrothermal vents and 200 more from seeps were documented (McArthur and Tunnicliffe 1998; Sibuet and Olu 1998). Although there are more vent species presently known, a single cold seep habitat shows often higher species richness than one hydrothermal vent (Sibuet and Olu 1998). In their review of deep-sea vent and seep communities, Sibuet and Olu (1998) show that only 13 species are present in both environments, while 6 are present in cold seeps, as well as, in whale remains. Table 1 and Figure 1 show comparisons of the number of species in these three chemosynthetic environments, vents, cold seeps and whale falls. Despite the relevance of these and other comparisons between reduced habitats, we must take in consideration that the available information is still scarce and usually limited to a single geographic region (Sibuet and Olu 1998).

Table 1. Comparison of global diversity among hydrothermal vents, cold seeps and whale skeletons. Adapted from Baco and Smith 2003.

Habitat	Substrate type	Number of macrofaunal species	Number of polychaetes species	Source
Whale skeletons	Hard*	>> 407	+ 201	Baco and Smith (2003), Dell (1987), Gibbs (1987), Marshall (1987), Waren (1989, 1991, 1993, 1996), Bennett et al. (1994), Marshall (1994), Dell (1995), Baco-Taylor (2002), Baco & Smith (unpubl. data)
Hydrothermal vents	Hard/soft	469	100	Tunnicliffe et al. (1998), Hashimoto et al. (2001)
Cold seeps	Soft/hard	229	25	Sibuet & Olu (1998), Poehls et al. (unpubl. data)

*Soft-sediment infauna were not included in these estimates

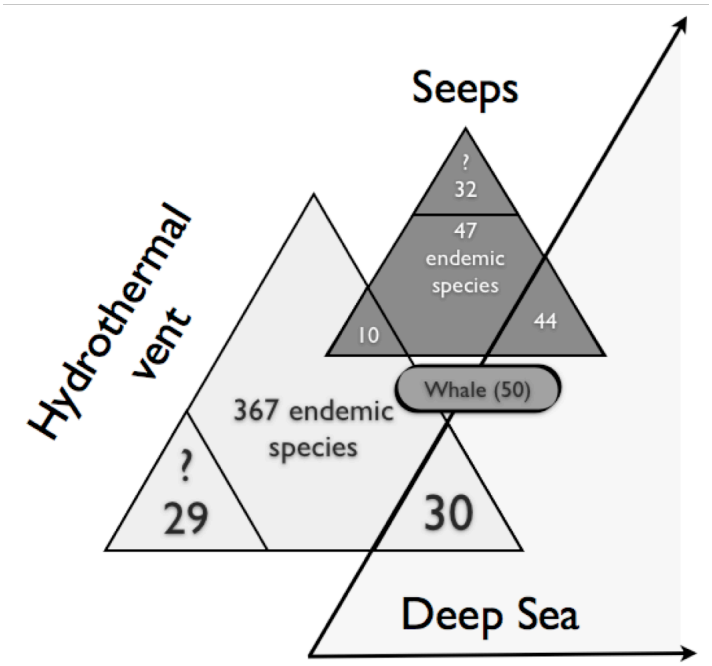


Figure 1. Shared and endemic species known in 1998 from vents, seeps, and the non-vent deep sea habitats. The whale division represents species found associated with bones on the seafloor. The "?" illustrate the species of uncertain habitat affinity. Until that year, two species were found at vents, seeps, and bones; four were identified in vents and bones; one species was found simultaneous in seep and bones; and another one at vents, non-vent deep sea and whale remains. The deep-sea triangle is open-sided for the reason that the species total number is impossible to determine but is certainly extremely large. From Tunnicliffe et al. 1998.

The hydrothermal vents and cold seeps share both physical and chemical characteristics and there is evidence of a common evolutionary history. Furthermore,

Distel et al. (2000) suggested that wood falls may function equally as stepping stones that may have, in the past, introduced mytilid taxa in vents and seeps therefore working as an important phylogenetic vector. For that reason, integrative approaches are important in the study of deep-sea reducing habitats (Van Dover et al. 2002).

Chemosynthesis and symbiosis

The methane oxidation and other reduced substrates used by microorganisms in chemosynthetic deep-sea environments produce energy to synthesise organic compounds from CO_2 dissolved in seawater. This autotrophic reaction can be summarized for hydrogen sulphide as:

$$CO_2 + H_2S + O_2 + H_2O \rightarrow Carbohydrate + H_2SO_4$$

Only microorganisms can perform this reaction in order to obtain biochemical energy (Miller 2004). These bacterial communities are grazed or filtered by other organisms and constitute the base of the food web in chemosynthetic habitats (Lalli and Parsons 1997). Beyond the adaptations to high pressure or environmental natural toxicity, one of the most remarkable characteristic in vent and seep fauna is the quantity and diversity of symbiotic relationships (exo- and endosymbiosis) identified between bacteria and metazoans (e.g. Distel and Roberts 1997). These symbiotic relationships allow the utilization of an energy source otherwise unavailable for the metazoan hosts. In their turn, the metazoans supply the bacteria with the necessary chemical substrate to maintain their metabolisms (Arp et al. 1987; Lutz and Kennish 1996) and provide them a suitable habitat (Figure 2). From the 211 species inventoried by Sibuet and Olu (1998) in cold seeps, 64 are known to establish symbiotic relationships with microorganisms; the majority is seep-endemic and recorded at a single seep location.

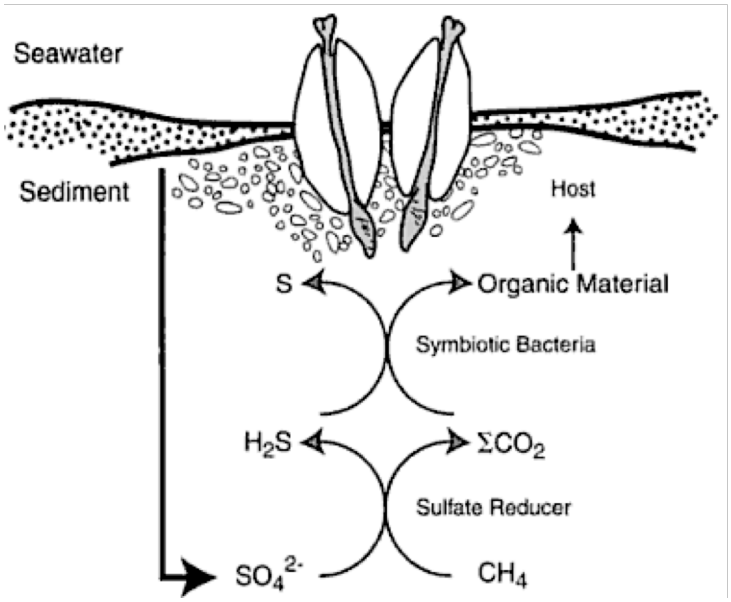


Figure 2. Biogeochemical cycle in cold seeps. Methane is used as a carbon substrate by sulphate reducing bacteria. Sulphide produced by microorganisms is taken up by invertebrates and assimilated by endosymbiotic chemoautotrophic bacteria. The symbiotic bacteria oxidise the sulphide, fix the inorganic carbon, and translocate the organic material produced to their host. Adapted from Masuzawa et al. 1992.

The way in which bacterial symbionts are acquired varies with the host species. The microorganisms can be acquired by the transmission from one generation to another (*vertical* transmission) or from the external environment, (*horizontal* acquisition) (Van Dover 2000).

Non-symbiotic species are also diverse and very abundant in cold seeps. These non-symbiotic organisms are attracted by the organic enriched chemosynthetic environments. Several species graze on bacteria, other are carnivores feeding mostly on the typical seep molluscs and polychaetes, for example, while other are detritivores (Sibuet and Olu 1998). Despite the importance of chemosynthetic communities in this environment, most of the deep sea floor and benthic boundary layer (portion of water and sediment immediately near the bottom) is nevertheless dominated by heterotrophic organisms (Gage 2003).

2. Some aspects of the deep-sea benthic ecology

The macrofauna in the deep sea is dominated by the Polychaeta (bristle worms), representing between half to three-quarters of the total abundance of organisms, followed by the peracarid crustaceans. Almost identical in number, the phylum Mollusca is

represented by Gastropoda, Bivalvia (including mussels and clams), and Scaphopoda. Less common are a variety of other worm-like phyla such as the Nemertea, Sipuncula, Echiura, and others (Gage and Tyler 1996). The benthic macrofauna, is arbitrarily determined by the size fraction of animals retained in sieves with meshes of 0.25-0.5 mm and have their upper size limit determined subjectively by their visibility in photographs of sea bottom (Gage 1996).

A fundamental ecological question in the study of deep-sea communities is how the macrofaunal species maintain their populations in patchy and ephemeral habitat such as cold seeps and hydrothermal vents. The insular nature of chemosynthetic habitats in the deep-sea and their patchy distribution raises fundamental issues concerning the life history (reproduction, dispersal and recruitment) of their inhabitants.

Larval Dispersal, Colonisation and Recruitment

In order to understand marine populations' dynamics it is necessary to investigate what influences propagule supplies and their dispersal (Marshall et al. 2009). The larvae production depends of the fecundity (the production of gametes) and fertilisation (production of zygotes) success. Many aspects affect the fecundity and fertilisation of organisms. The condition of progenitors at first and then the local concentration of sperm in the case of external fertilisation or the population density are some of those aspects that limit the recruitment and colonisation success. If the zygotes are released in the water column, its also necessary to consider the mortality in the plankton. This factor is important because it affects not only the number of larvae that can recruit into a population, but also how far larvae can disperse. This mortality can result from predation, starvation in the case of planktotrophic larvae, incapability to reach an adequate place to metamorphosis or bad larval condition (Marshall et al. 2009).

The most common reproductive strategy in benthic marine invertebrates involves the production of planktonic larvae. Depending on the larval type, they may be dispersed for long distances in the water column until settling and metamorphosis. Planktotroph larvae are able to remain for longer periods (from weeks to months) in the water column but require feeding. On the other hand, the pelagic lecithotrophic larvae can only persist in the water column for a short period (from minutes to days), after which they settle and

metamorphose. The lecithotrophic larvae show a much more limited dispersal capacity in comparison to the planktotrophic larvae (Todd 1998).

Studies on 30 hydrothermal mollusc species showed that only three species have planktotrophic development; the other 27 were inferred as non-planktotrophic. Examples like this led to conclude that very few vent species have planktotrophic larval phases with high dispersal capability (Van Dover and Lutz 2004). Many species (e.g. peracarid crustaceans) have also direct development, a reproductive strategy that results in a very limited dispersal potential (Van Dover 2000). Taking into consideration estimations of gene flow it is possible to measure rates and modes of dispersal. High gene flow rates across thousands of kilometres were documented for species with planktonic larva (e.g. vent molluscs and polychaetes) in the East Pacific Rise (Vrijenhoek 1997). The opposite was also observed, namely in the amphipod, *Ventiella sulfuris*. Lacking a larval phase in its development, amphipods carry their broods in a marsupium. In this case, the gene flow is limited to the species capability to cross the habitat barriers (France et al. 1992). Environmental factors such as the role of oceanographic currents in larval transportation are also of utmost importance since that they can carry the species beyond their range limits (Kotta and Witman 2009). The near-bottom flow is also fundamental for the transport of propagules of many sessile organisms to the settlement sites (Tyler 2003).

During the time spent in the water column the larvae reach maturity but they need to find a suitable substrate founding order to metamorphose. They can select actively the settling habitat (e.g. Bourget and Harvey 1998), being able to accept or reject the substrate and, if necessary, they may release to re-enter in the water column. These characteristics ensure a higher survival chance and better growth and reproductive rates (Jenkins et al. 2009).

The settlement on a substrate depends of several factors. The surface proprieties such as rugosity, or biogenic signals released by early colonisers are some of those variables (e.g. Crisp and Barnes 1954; Wahl and Hoppe 2002). The settlement and colonisation process in the deep-sea can occur virtually in any solid surface, living or not. This process can be resumed in four principal phases (Figure 3): i) thermodynamic adsorption of

macromolecules; ii) attachment of bacterial communities; iii) colonisation by unicellular organisms; iv) metazoan settlement.

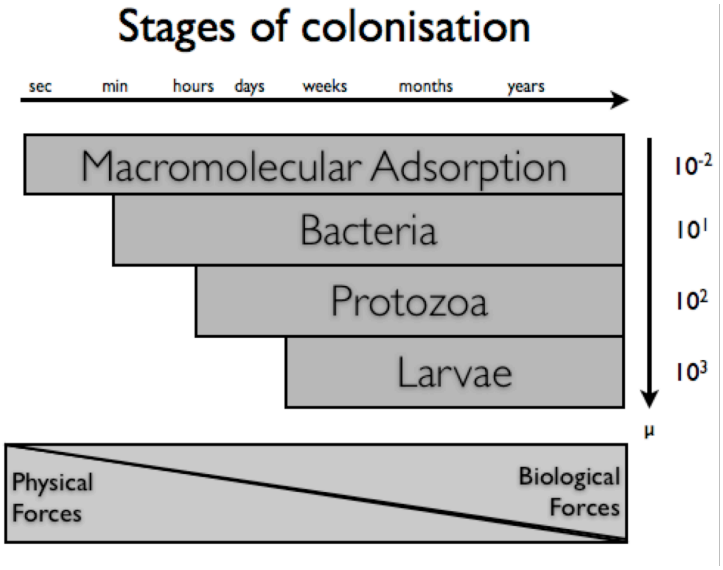


Figure 3. Different stages of hard substrate colonisation in aquatic environments. The biological forces gain more importance along the time. The top bar corresponds to time and the right bar to the size of macromolecules or organisms. From Wahl 2009b.

Although these colonisation steps take place sequentially, the succession process depends as well on the presence of other colonisers previously settled and on the reaction time of organisms. The protist and fungal densities on the water column, for example, are conditioned by seasonal cycles in some regions while autotrophic unicellular organisms have lower colonising capacity during the winter. Inevitably, changes in the substrata by biological activity of the first colonisers affect all the following organisms, inhibiting or facilitating their settlement (Wahl 2009b). Metazoan organisms may also modify the three-dimensionality of the substrate creating different levels of rugosity that can be explored by other organisms (e.g. Wahl and Hoppe 2002; Wahl 2009a).

The metazoan community development is closely related to the nature and location of the substrate (Young 2009). Studies in intertidal regions showed that colonisation process is very unpredictable and is normally characterised by variations in spatial and temporal scale. Additionally, the development of the community depends greatly of the initial settlement process (see e.g., Sutherland and Karlson 1977). The success of substrate colonisation depends of the number of competent larvae present in the water column and

the rate at which larvae meet a given substrate. Much of knowledge about larval responses to surfaces was produced from laboratory experiments or using man-made substrata deployed in the field during a defined period (Davis 2009). Studies developed in the deep-sea with wood panels by Turner (1973) showed that woodborer species start to appear within a few months after the experimental substrate exposure. Within one year of immersion, at 2000 m depth, the same author identified in some panels 41 species of different groups. Turner (1977) suggested that the beginning of colonisation occurs within one or two months after immersion and the number of taxa present increases gradually with time. Other experiments with organic-rich materials showed that they are rapidly colonised too (e.g. Turner 1973; Bertram and Cowen 1999). In the Norwegian Sea, the colonisation of phytodetritus by benthic foraminifera has been documented within eight days after deployment (Bertram and Cowen 1999). Recently, standardized experiments using artificial substrates have also been deployed in several deep-sea chemosynthetic habitats of the European margins (Gaudron et al. 2010). The conclusions drawn from these approaches have limitations inherent to the simulations of natural process and the incapability to reproduce all the variables that rule them. However, they were essential to understand the complexity of colonisation processes.

Almost all marine populations are demographically open and their continuity depends on the supply of larvae present in the plankton. The process of addition of new individuals within populations is designated as recruitment. A population will persist if the recruitment is continued (Caley et al. 1996). The recruitment is, in its turn, affected by multiple factors at spatial and temporal scales. At a local scale, the substrate availability, the micro-hydrodynamics and the larval behaviour are the most important factors. In a global scale, the communities depend essentially of the larval pool size and physical transport of larvae (Cornell and Lawton 1992; Marshall et al. 2009).

Ecological Succession

The ecosystem and the development biology of organisms are intimately related. Odum (1963) defined three parameters for ecological succession: i) the community development follows an orderly process that turns it predictable; ii) the succession is conditioned by the presence of early colonisers and the modification made by them in the

physical environment; the community control the succession process even though the environment determines the pattern and change speed, imposing development limits of community; iii) eventually the ecosystem reaches a stability in which the maximum biomass and symbiotic relation between organisms were stabilised (a climax state).

Normally, the species-richness tends to increase during the early stages of ecologic succession and no dominant species or groups of species normally occur. The increase of organisms' size and complexity of their life histories, as well as, the interspecific relationships may lead ultimately to species exclusion. As a result, the number of species that a given area can support is significantly reduced (Odum 1969). The common pattern during a bloom stage of ecology succession (Table 2) is the dominance of organisms with small sizes with high reproduction rates and simple life histories (species with r development strategy). Organisms with such characteristics have selective advantages in a mineral nutrient-rich ecosystem. Otherwise, with the habitat development the selection pressure tends to favour organisms with more complex life histories. These new colonisers (of the same early species but larger, other larger species with more storage capacities, or both) show a K-strategy. They are more adapted to exploiting seasonal or periodic resources. Summarising, the r-selection species predominates during the first stages of colonisation while the K-selection increase their influence towards the mature stages. The continuing increase of species richness in an ecosystem will depend on if the niche potential exceeds the counter-effects of the increasing competition (Odum 1969).

Table 2. Ecological succession: trends to be expected in the community structure development, life histories of organisms and selection pressure. Adapted from Odum 1969.

Ecosystem attributes	Developmental stages	Mature stages
Community structure		
Total organic matter	Small	Large
Species diversity: variety component	Low	High
Species diversity: equitability component	Low	High
Stratification and spatial heterogeneity (pattern diversity)	Poorly organized	Well-organized
Life history		
Niche breadth	Broad	Narrow
Size of organism	Small	Large
Life cycles	Short, simple	Long, complex
Selection pressure		
Growth form	For rapid growth (r-selection)	For feedback control (K-selection)
Production	Quantity	Quality

The fauna of chemosynthetic-based ecosystems exploits a limited energy source both in space and in time. Despite the high environmental variation in these habitats, the colonisation processes are quite rapid. The fluid discharge rate is the best-understood variable and it is the most important at local scale. Several species do not support low levels of seep fluid emissions but the opposite may also occur including, possibly, negative effects of the high levels of methane emission on organisms. The instability of substrates is another variable that can influence the colonisation in cold seeps. Extremely rapid mud expulsions can exclude all fauna (Tunnicliffe et al. 2003).

Cold seeps are at first a sedimentary habitat, within which carbonate concretions may develop secondarily. The sulphide appears also as secondary product, resulting mainly from the anaerobic bacterial oxidation of methane. The species that are dependent of the existence of hard substrata or sulphide only would appear later in the community seep succession. At early stages of colonisation in cold seeps, the process is dominated by symbiont-containing species. After their establishment, the community development would support non-symbiont organisms enabling new trophic links and the presence of extremely high densities of meiofauna, suspension and deposit feeders, and carnivores (Sibuet and Olu 1998; Tunnicliffe et al. 2003).

Biodiversity of benthic metazoan assemblages

The study and documentation of the biodiversity of deep-sea and their pattern is essential to understand the evolution and ecology of the ocean (Baco and Smith 2003). There are regional and local scale processes that control the local diversity (Figure 4). The biodiversity is usually measured as: α-diversity, the diversity within a habitat; β-diversity, the degree of variability that communities show between different sites or locations; and, finally, the γ-diversity that encompasses all diversity in a whole region (Whittaker 1975). In the analysis of species diversity the α-diversity can be estimated as species richness, i.e., the number of species found at a particular place and in terms of their relative frequencies or abundance — evenness or equitability (Terlizzi and Schiel 2009).

Concerning the species richness of the benthic metazoan communities, several factors are important, however, the food supply probably plays the most significant role, limiting the number of species that a given ecosystem can support. Nonetheless, the importance of interactions between other environmental and biological regulators must be considered (Figure 5).

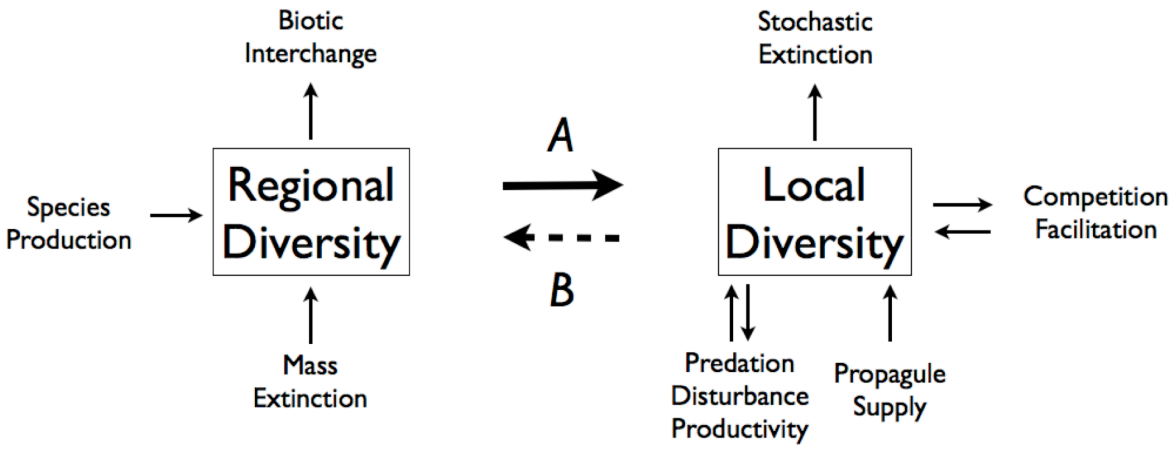


Figure 4. Regional and local scale processes that control the local diversity. The A arrow represents dispersal or habitat selection from the regional pool, the B dashed arrow the beta diversity or between-habitat diversity. The increase of beta diversity in local-scale variability increases consequently the regional diversity. From Kotta and Witman 2009, modified from Ricklefs and Schluter 1993.

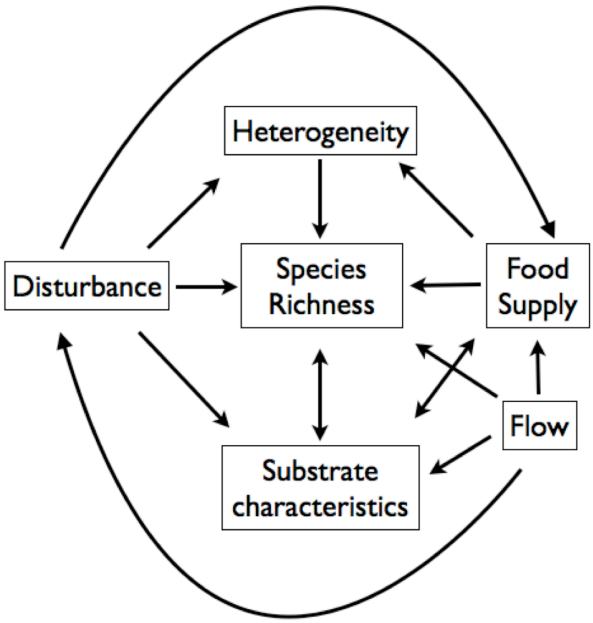


Figure 5. Relation of direct and indirect environmental factors that affect the species richness at local scale. From Levin et al. 2001

The biodiversity observed in a particular place depends of the species number present in the regional species pool, the metacommunity. In its turn, the diversity in metacommunity is controlled by several processes such as those represented in Figure 4. The species richness in a given area results from the differences observed in the birth, death and migration rates of the different species. On the other hand, these rates are affected by the biotic and abiotic components of ecosystem at local and regional scales (Gaston 2000). The deep-sea communities are not free of seasonal changes. The carbon and sediment content, for example, varies seasonally and the organisms respond to these oscillations (Gili and Petraitis 2009). The study and documentation of the biodiversity of deep-sea and their pattern is essential to understand the evolution and ecology of the ocean (Baco and Smith 2003).

3. Framework and objectives

CHEMECO project - Monitoring colonisation processes in chemosynthetic ecosystems

This dissertation is integrated in the multidisciplinary European project CHEMECO (Figure 6), an international Consortium of research teams formed in the framework of the European Science Foundation EUROCORES programme, EuroDEEP call. This project is specifically interested in the study of colonisation process in different reducing environments (cold seeps and hydrothermal vents) of European deep-waters in the Atlantic Ocean, Mediterranean and Nordic seas (Figure 7) (Gaudron et al. 2010). In the Atlantic Ocean, the colonisation study was carried out in the Mid-Atlantic Ridge hydrothermal vents (MoMAR sites Rainbow and Lucky Strike), and in the Gulf of Cadiz cold seeps' area. In the Norwegian Sea, the experiments were deployed in the Håkon Mosby mud volcano while in the Mediterranean the Nile deep-sea fan was selected as the study area. The CHEMECO project is mainly focused on the pioneer microbial communities, the recruitment of metazoan larvae, the development of symbioses and their importance in the biodiversity and trophic structure of newly established communities. Other aspect in consideration is the impact of metazoan colonisation on chemical exchanges and biogeochemical process. The research plan included a group of site surveys, a set of replicate colonisation experiments, comparison between natural and experimental biological assemblages, *in situ* monitorizations of environmental parameters, and reactive transport modelling. For colonisation experiments, similar colonisation devices were used, hosting the same type of inorganic and organic substrates. With this approach and the long-term replicate multi-site experiments, it is expected to gain a better understand of the factors that govern the establishment of metazoan communities in different habitats. Additionally, these proposed experiments may contribute to the implementation of *in situ* experimentation platforms in the context of long-term deep-sea observatory sites. Together with Portugal and France teams from Germany and Belgium are also involved as Associated Partners in the Consortium. The present MSc dissertation is strongly embedded in the CHEMECO project and focus in the biodiversity of metazoan, namely, the macrofauna communities of mud volcanoes in Gulf of Cadiz.

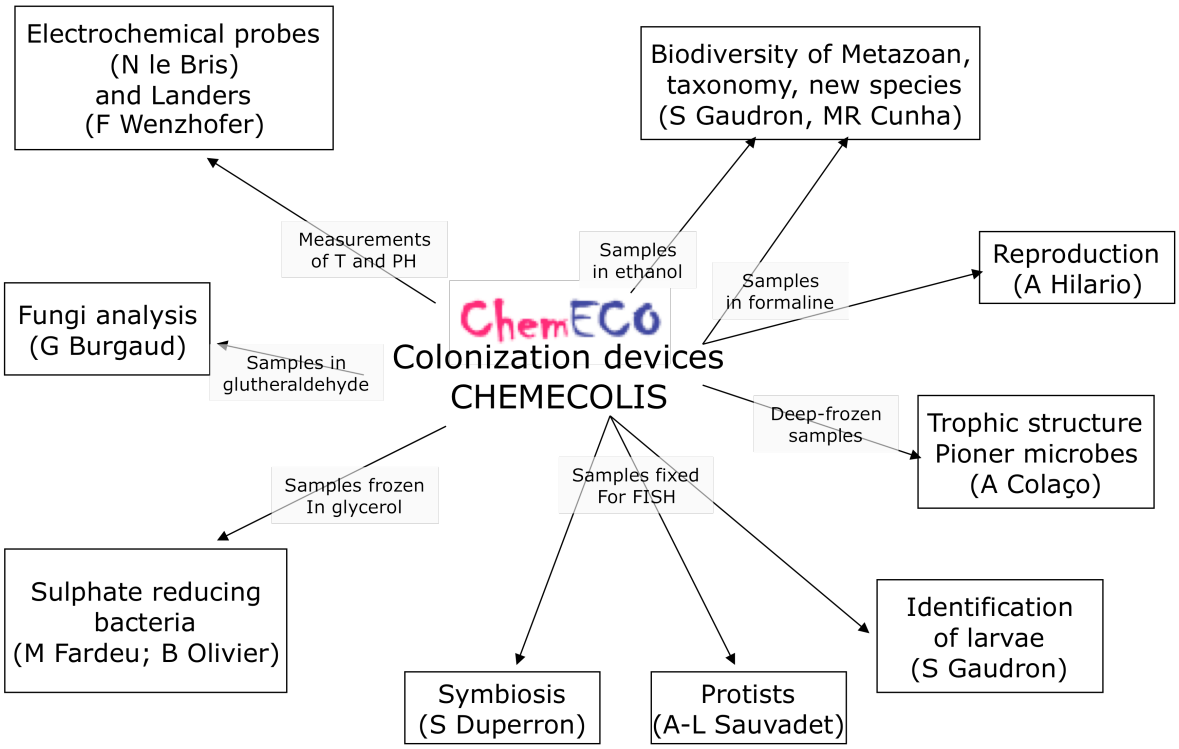


Figure 6. Multidisciplinary approach of the CHEMECO project and the researcher's responsible for each component study.

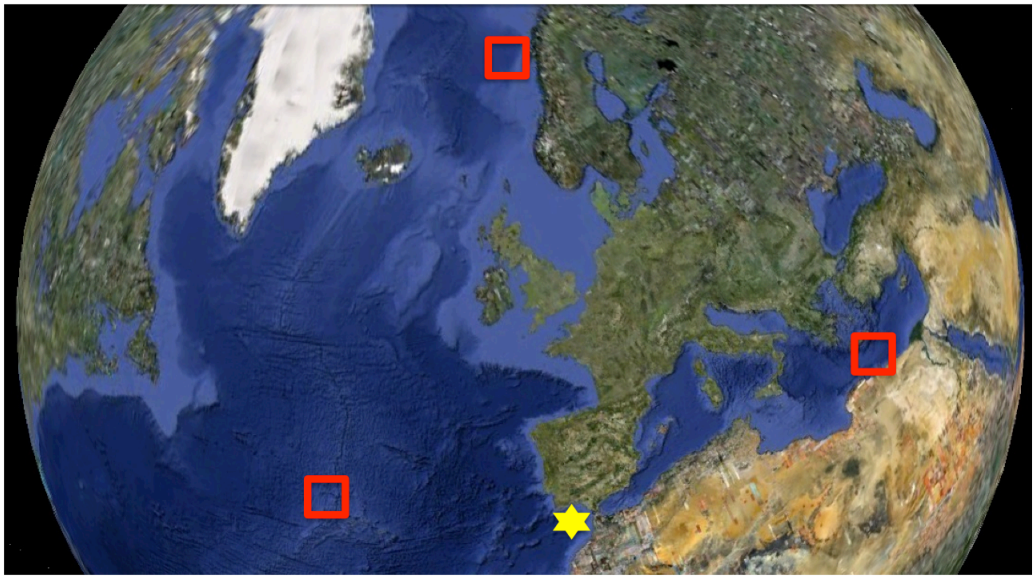


Figure 7. Study sites in the CHEMECO project. Mid-Atlantic Ridge; Håkon Mosby mud volcano; Nile Fan; and Gulf of Cadiz (yellow star). Map from Google Earth.

Objectives

The present dissertation is focused on the results of the CHEMECO project obtained for the Gulf of Cadiz area and aims to contribute to a better understanding of colonisation processes and biodiversity in the deep-sea. The specific objectives of this study are:

a) to characterize the composition and community structure of macrofauna in organic (wood and alfalfa) and inorganic (carbonate) substrates deployed in three mud volcanoes (Mercator, Meknès and Darwin);

b) to investigate the presence of species endemic from reducing habitats, specially the settlement of symbiont-bearing metazoans;

c) to test the effect of contrasted environmental variables such depth and substrate type on the recruited macrofauna assemblages;

d) to compare the results with the background fauna data of the studied mud volcanoes.

II. Material and Methods

1. Study area

The Gulf of Cadiz is the region connecting the Atlantic Ocean to the Mediterranean Sea. The bathymetry increases progressively from 200m in the shelf edge to over 4000m in the deepest region (Horseshoe and Seine abyssal plains) (Zitellini et al. 2009). The local circulation features, therefore, are strongly influenced by the Atlantic and Mediterranean waters, particularly by the undercurrents of the Mediterranean Outflow (Peliz et al. 2006). When the warm and salty water of Mediterranean Sea flows through the strait of Gulf of Cadiz a special type of eddies, the Mediterranean water eddies (Meddies) are formed enhancing the recirculation of the water masses within the Gulf of Cadiz. The Mediterranean water, more dense that the Atlantic flowing out beneath, falls down through the continental slope to a depth of around 1000 meters reaching its natural buoyancy (Richardson 1996). The Gulf of Cadiz geology is intimately related to the tectonic forces interacting in the region between the Southern Euroasia and North Africa plates. In the Gulf of Cadiz, active mud volcanism is a widespread phenomenon (Figure 8) present from the Iberian-Moroccan shelf to the deepest areas inside the gulf (Foucher et al. 2009, Zitellini et al. 2009), encompassing over forty mud volcanoes (Kenyon *et al*. 2000, 2001, 2002, 2003, 2006; Pinheiro *et al*. 2003; Akhmetzhanov *et al*. 2007, 2008).

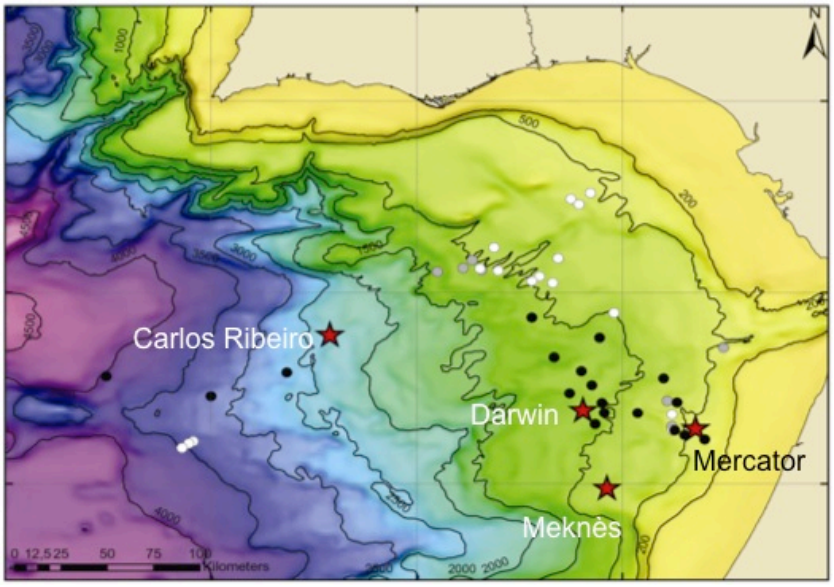


Figure 8. Bathymetric map of Gulf of Cadiz and location of the mud volcanoes where the CHEMECO experiments were deployed.

In 2000, during the 10th Training Through Research cruise (TTR10), the study of the biological assemblages associated to mud volcanoes in Gulf of Cadiz was initiated. Since then, this area has been the object of frequent biologic investigations and provided the possibility to learn more about ecological process related to these chemosynthetic communities. The ecosystems of Gulf of Cadiz are discontinuous environments controlled by several factors of different nature (chemical, physical, topographic and geological) that contribute to the exuberant biodiversity present in this region (Cunha et al. 2006; Vanreusel et al. 2009).

In the shallow Moroccan margin, the El Arraiche MV field encompasses the Renard (including Pen Duick Escarpment) and Vernadsky Ridges, as well as several mud volcanoes (e.g. Mercator) at depths that range from 230 to approximately 600m depth. The proximity to the euphotic zone and to the African coast adds to the great productivity observed in the area. Mercator (Figure 9), one of the shallowest mud volcanoes, differs significantly from the other mud volcanoes by the high chloride enrichment of its pore water (Van Rensbergen et al. 2005). The top of Mercator shows patches of disturbed sediments from which gas venting is occasionally observed. Solitary corals (*Caryophyllia* sp.), accompanied by Cidaridae echinoids and Onuphidae polychaetes (*Hyalinoecia tubicola*) are the most conspicuous organisms seen during video surveys of its crater (M.R.

Cunha pers. comm.). The macrofauna samples collected from Mercator mud volcano (Figure 14) yielded a total of 271 species (data from Rodrigues 2009). The most diverse groups of invertebrates are the Arthropoda and the Polychaeta, with 112 and 88 species respectively. Rarefaction biodiversity ($ES_{(100)}$) for this volcano was estimated in 54.1 species (M. R. Cunha pers. comm., estimation based on 5 box corers and 1 TV-grab).

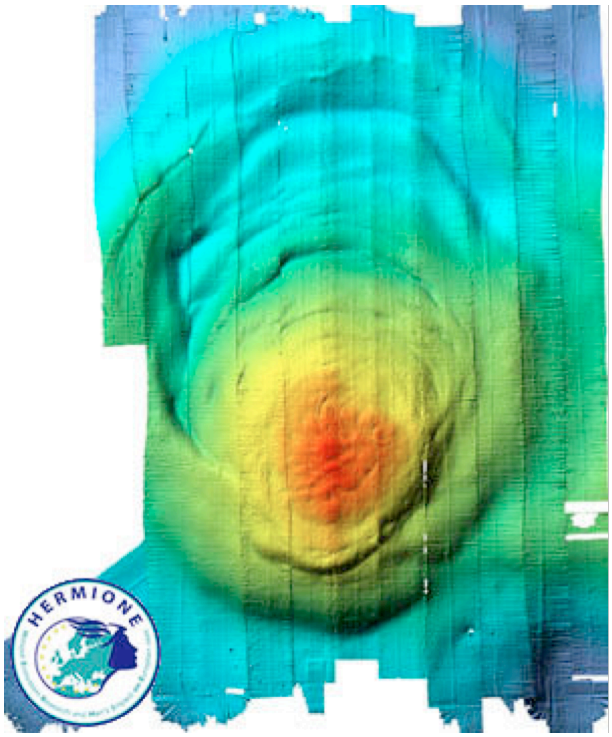


Figure 9. Multibeam bathymetric map of the surface of the Mercator mud volcano (courtesy of NOCS).

Extensive authigenic carbonate provinces occur at intermediate depths (700-1200m) along the margins of Morocco and Spain. In the Moroccan margin the carbonate provinces are accompanied by the frequent occurrence of mounds, thickets and debris of mostly dead cold-water scleractinean corals coral (e.g. around and at the flanks of the Meknès; Figure 10). Meknès is the southernmost Moroccan mud volcano rising isolated among an extensive field of small coral mounds. The crater is formed by stiff, sometimes heavily disturbed, green mud breccia with scattered clasts and a striking large number of empty shells of the gastropod *Neptunea contraria*. Except for a few *Paromola cuvieri* individuals, living megafauna is rarely sighted in the crater (M.R. Cunha, pers. comm.). In Meknès, a

total of 161 species were recorded, the Arthropoda contributed with 77 species and the Polychaeta with 48 species (data from Rodrigues 2009; Figure 14). Rarefaction biodiversity ($ES_{(100)}$) was estimated in 41.3 species (M.R. Cunha pers. comm., estimation based on 7 samples, 3 TV-grab and 4 box corers). The carbonate province further includes other mud volcanoes from the western Moroccan field among which the Darwin (Figure 11). The widespread presence of authigenic carbonates and also extensive *Neptunea* and *Bathymodiolus* graveyards (usually within the crater of the mud volcanoes) suggest that this was a very active seepage area in the past. Darwin mud volcano differs from the other mud volcanoes in this area because its crater is completely covered by large carbonate slabs and crusts (Figure 12); the fissures among slabs and depressions with scattered crust are filled with abundant shell ash and occasionally small clumps of living *Bathymodiolus mauritanicus* (Figure 13). In Darwin the sampling effort was lower, with only one Tv-grab collected and a total of 74 species recorded (data from Rodrigues 2009; Figure 14). The most diverse group was the Polychaeta with 30 species followed by the Arthropoda with 24. There are no rarefaction biodiversity values estimated yet for this volcano.

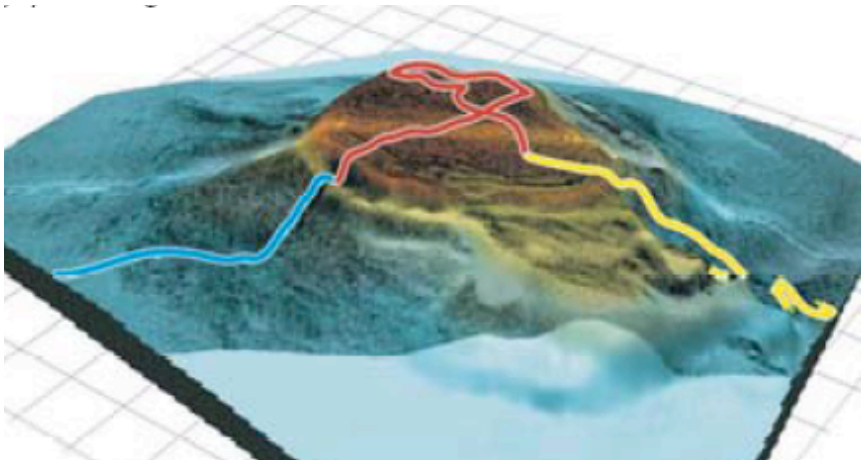


Figure 10. Topography of the Meknès mud volcano located in the Moroccan margin. Courtesy of MSU, Floating University.

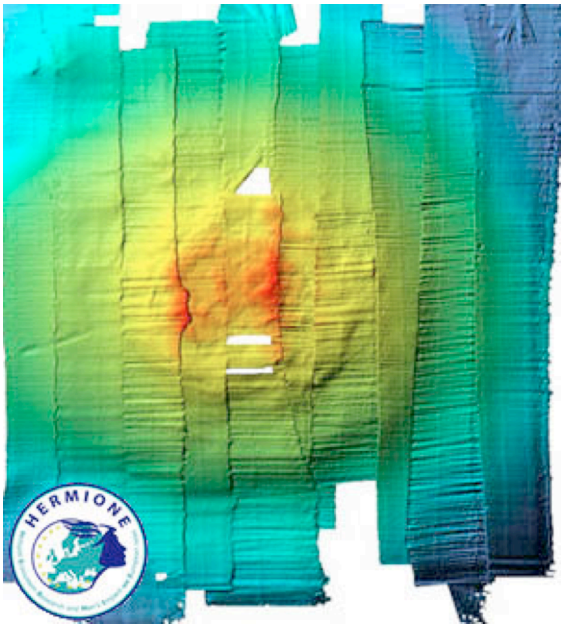


Figure 11. Multibeam bathymetric map of Darwin mud volcano. Courtesy of NOCS.

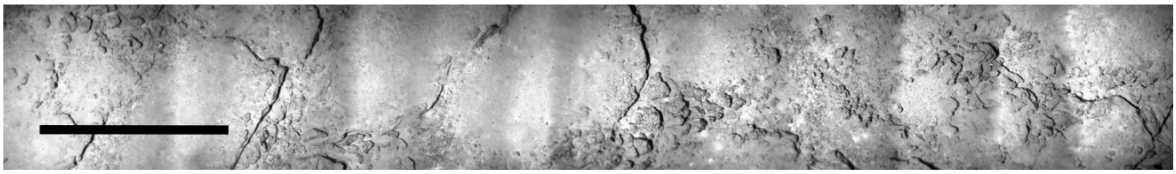


Figure 12. Carbonate slabs that cover the crater in Darwin mud volcano. The scale bar corresponds to 5 meters. Courtesy of NOCS (JC10 – ROV ISIS).

Figure 13. *Bathymodiolus* graveyards, typically occurring within the crater of Darwin mud volcano, suggest that this was a very active seepage area in the past. Courtesy of NOCS (JC10 – ROV ISIS).

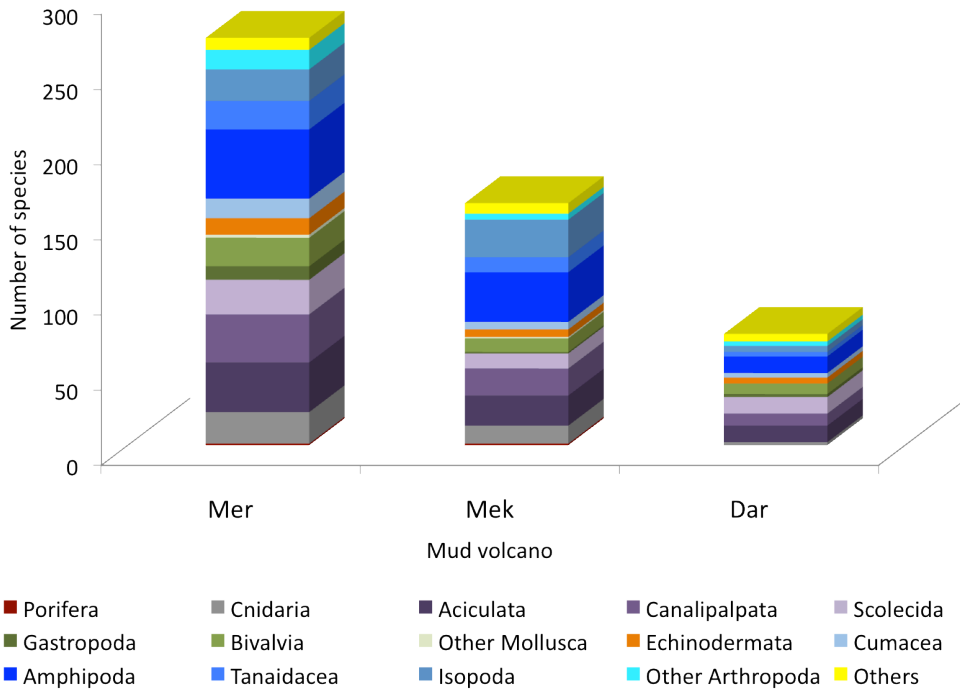


Figure 14. Background faunal assemblages from Mercator (Mer), Meknès (Mek) and Darwin (Dar) mud volcanoes. Data from Rodrigues, 2009.

The deep-water field (1300-4000m), mostly within the Portuguese margin includes several mud volcanoes (e.g. Carlos Ribeiro) that are aligned along major crustal strike–slip faults associated with the African-Eurasian plate boundary (Duarte 2005). Gas hydrates were recovered from these mud volcanoes and the methane concentrations yield the highest records from the Gulf of Cadiz (Kenyon et al. 2000, 2001, 2002, 2003, 2006; Akhmetzhanov et al. 2007, 2008). Video surveys of Carlos Ribeiro mud volcano (Figure 15) often show exuberant sponge and gorgonian patches at the crater rim and upper flank (M.R. Cunha pers. comm.).

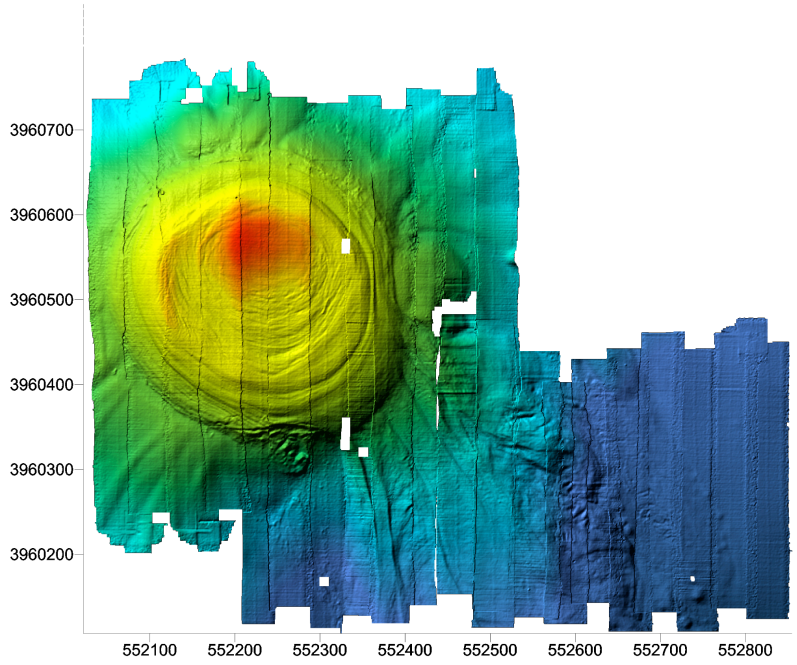


Figure 15. Bathymetric map of Carlos Ribeiro mud volcano showing the central "mud pie". Courtesy of NOCS.

2. Experimental design and colonisation devices - CHEMECOLI

This study includes a combination of site surveys and replicate colonization experiments (CHEMECOLI - CHEMosynthetic Ecosystem COlonization by Larval Invertebrates) using organic and inorganic substrata (Gaudron et al. 2010). Sets of CHEMECOLI, each with one of the three substrates, were deployed in four mud volcanoes

along a depth gradient (Table 3): Mercator at 350m in the shallow Al Arraiche MV field, Meknès at 700m and Darwin at 1100m, both in the carbonate province, and Carlos Ribeiro at 2200m in the deep MV field. Three types of substrate were used: dried alfalfa grass, wood cubes and carbonate cubes (2 x 2 x 2 cm). The organic substrate is not intended to simulate the background environment of mud volcanoes, but it is supposed to create the chemical conditions to attract and support chemotrophic species by the degradation and subsequent production of sulphide compounds. The colonisation devices were built with a PVC cylinder (14 cm diameter x 10 cm high) drilled with lateral holes. The artificial substrate was included inside used a Nylon net of 2 mm mesh that will allow the colonization of the substrates by metazoan larvae or juveniles but not by adults of most species. The CHEMECOLI were maintained in place at the sea-floor by means of a stainless steel chain fixed to the PVC cylinder.

Table 3. Deployment and recovery data of the colonisation experiments and their location in mud volcanoes. DD: Deployment duration.

Site coordinates					Deployment		Recovery		DD
Site	Depth	Latitude	Longitude		Cruise/Dive	Date	Cruise/Dive	Date	(days)
Mercator	354m	35º17.916'N	06º38.709'W	Mer01	JC10-D28	2007.05.19	64PE284-D8/9	2008.03.02-3	290
				Mer02	JC10-D28	2007.05.19	B09/14b-D01	2009.05.19	631
				Mer03	JC10-D28	2007.05.19			
Meknès	698m	35º59.091'N	07º04.424'W	Mek01	64PE284-D07	2008.03.01	B09/14-D03	2009.05.20	446
Darwin	1100m	35º23.523'N	07º11.513'W	Dar01	JC10-D33	2007.05.21	B09/14-D02	2009.05.19	629
Carlos Ribeiro	2197m	35º47.244'N	08º25.282'W	CR01	JC10-D36	2007.05.27			

The deployment and recovery of the *in situ* colonisation experiments were made using a ROV (Remotely Operated Vehicle) during the JC10 cruise onboard the RRS James Cook (ROV Isis, National Oceanographic Center Southampton), the 64PE284 cruise onboard the RV Pelagia (ROV Cherokee, MARUM, Bremen) and the B09/14 cruise onboard the RV Belgica (ROV Genesis, Renard Centre for Marine Geology, Gent). In Mercator three sets of CHEMECOLI were deployed in order to illustrate temporal variations of the assemblages, while in the other three mud volcanoes only on set was used. The immersion time ranged from 290 to 631 days (Table 3, Figures 16-17).

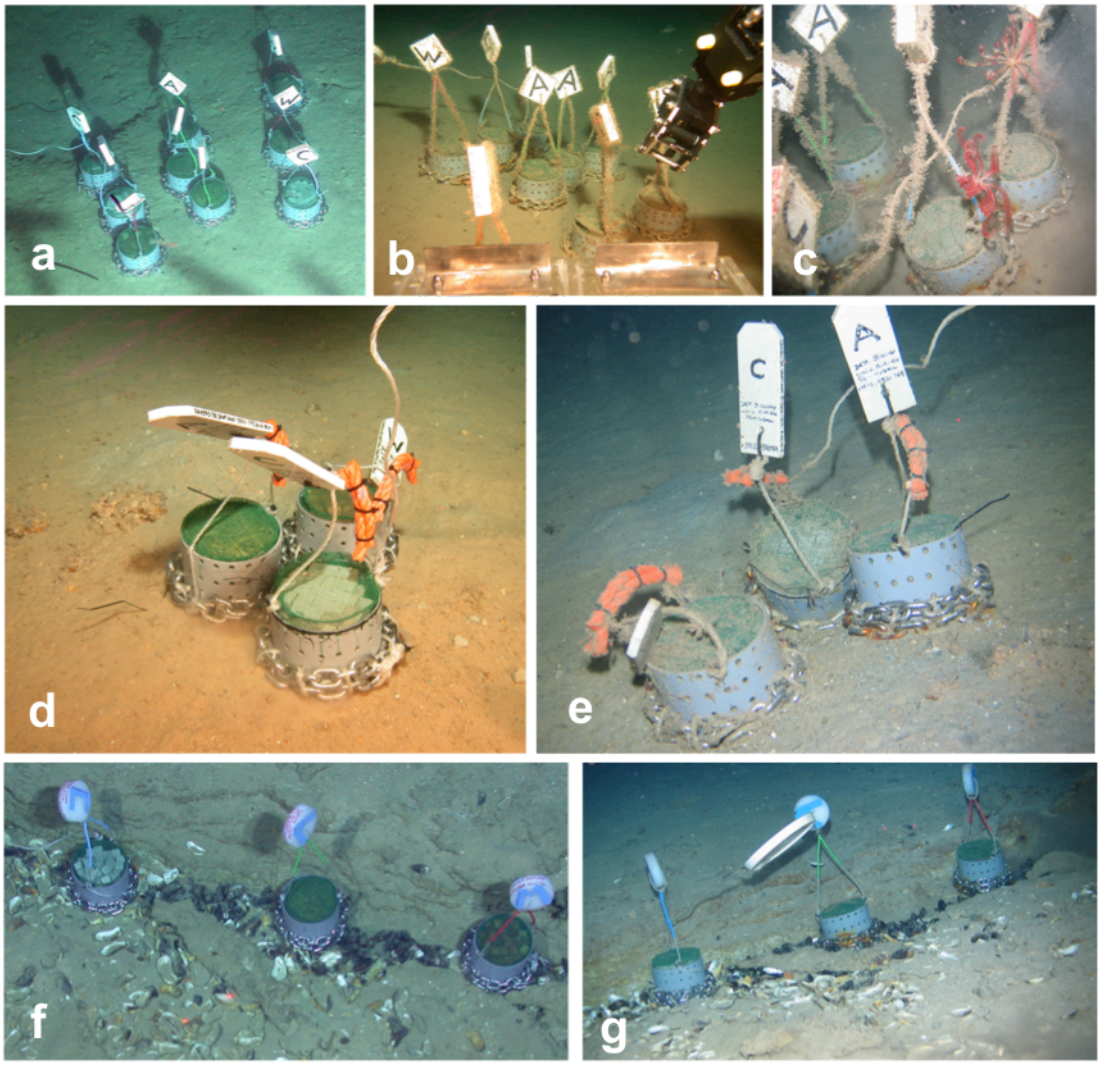


Figure 16. a) Deployment of three sets of colonisation experiments in Mercator MV at 354m. b) Recovery of the first set in Mercator MV after 290 days of immersion. c) Recovery of the second set in Mercator MV after 631 days of immersion. d) Deployment of one set in Meknès MV at 698m e) recovery after 446 days of immersion in Meknès MV. f) Deployment of one set in Darwin MV at 1100m. g) Recovery of the Darwin MV set after 629 days of immersion. Photos from NOCS (JC10, ROV ISIS), MARUM (64PE284, ROV Cherokee) and RCMG (B09/14, ROV Genesis).

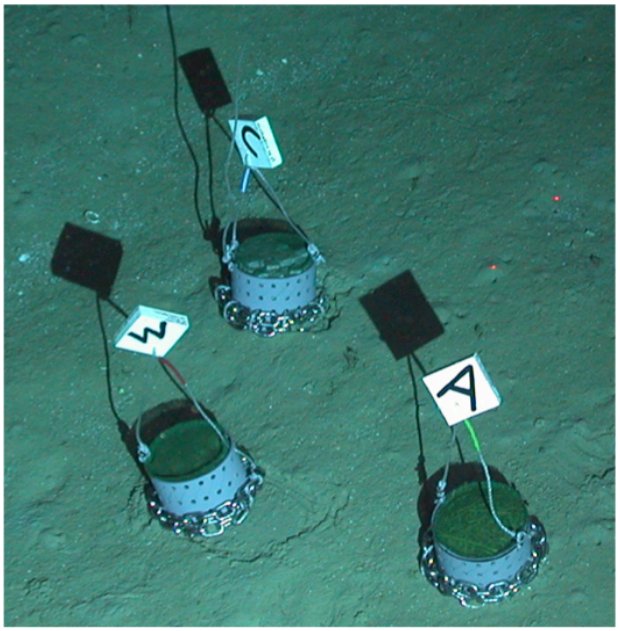


Figure 17. Deployment of one set of colonisation experiments in Carlos Ribeiro MV at 2197m.

3. Processing of samples

After recovery two thirds of the different substrates were sub-sampled for macrofaunal studies. The substrates were photographed (Figure 18) and fixed in 95% ethanol (one third) and formalin (one third) The animals were sorted later in the laboratory and separated into major taxonomic groups. After the taxonomic identification, the organisms will be curated and deposited in the Biological Research Collection of the University of Aveiro, in the Department of Biology and will be available for further studies.

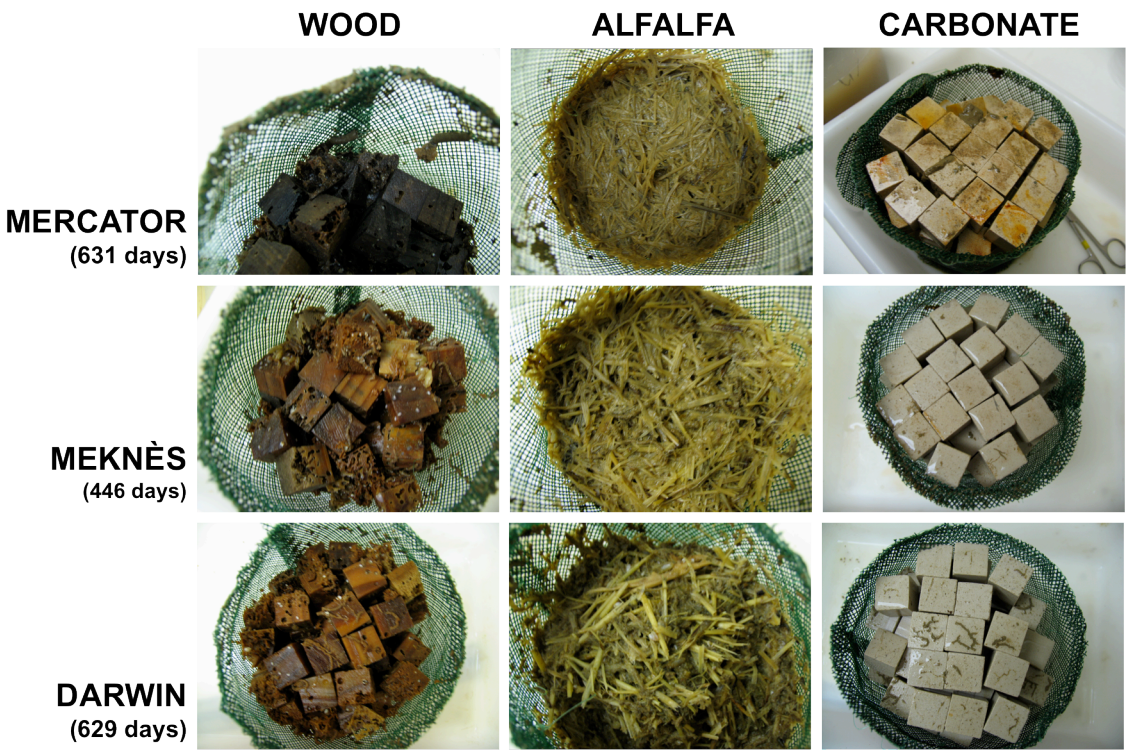


Figure 18. Detail of samples immediately after recovery from the second set of CHEMECOLI's deployed in Mercator and of the Meknès and Darwin sets.

4. Data Analysis

Data analyses were performed using the statistical package PRIMER 6 (Clarke and Gorley 2006). The main attributes of the CHEMECOLI metazoan assemblages were described by univariate descriptors: i) abundance, expressed as number of individuals in the subsample (2/3 of the substrate for alfalfa and carbonate and 1/3 for wood); ii) taxa richness (S), expressed as the number of taxa, whenever possible identified to species level; iii) diversity, expressed as the Shannon-Wiener index (H´); iv) equitability, expressed as the Pielou's (1969) evenness index (J') and Hulbert (1971) expected species richness ($ES_{(n)}$). Distributional analysis of the CHEMECOLI metazoan assemblages was carried out using k-dominance curves. K-dominance curves consist of plotting the cumulative ranked abundances against species that are ordered by decreasing abundances, in a logarithmic scale (Lambshead et al. 1983).

For the multivariate analysis the abundance data were first organised into a sample *vs*. species matrix. Non-metric multidimensional scaling (MDS) ordination was performed

using the Bray-Curtis similarity measure after fourth root transformation of the data (Field et al. 1982). Analyses of similarities by randomisation/permutation tests (ANOSIM) were performed on the MDS results (Clarke 1993). One-way ANOSIM tests were directed to assess the significance of differences between i) mud volcanoes; ii) substrate type. SIMPER analysis (Similarity Percentages – species contributions) was performed to indicate the percentage contribution of the species to the similarity within and dissimilarity between groups of samples.

III. RESULTS

The results correspond to 2/3 of the CHEMECOLI sample for alfalfa and carbonate substrates, and only 1/3 for the wood substrate. A total of 5644 specimens were ascribed to 99 taxa in this study (Table 4). From these, 10 species were cnidarians, 43 polychaetes, 13 amphipods, 13 other crustaceans (isopods, cumaceans and decapods and nebalids), five bivalves, 11 gastropods and four taxa from other metazoan groups. The variations in the number of species from the major taxonomic groups in the different samples is summarised in Figure 19. The polychaetes are typically the most common taxonomic group followed by amphipods and gastropods.

Table 4. Univariate measures of the metazoan assemblage by sample, pooled by mud volcano, pooled by substrate and total (all samples pooled). Mer: Mercator; Dar: Darwin; Mek: Meknès. W: wood, A: alfalfa grass; and C: carbonate. H'(log_e): Shannon-Wiener diversity; J': Pielou's evenness; and $ES_{(100)}$: Hulbert's expected species number in a sample of 100 individuals. Abundance: total number of individuals in the sub-sample. * Number of specimens recovered lower than 100.

	S	Abundance	H'(log_e)	J'	$ES_{(100)}$
Mer01 W	31	362	2.014	0.586	16.3
Mer02 W	21	864	0.470	0.155	6.3
Mek01 W	22	666	1.712	0.554	10.3
Dar01 W	20	570	2.130	0.711	13.2
Mer01 A	21	900	1.101	0.362	7.4
Mer02 A	18	34	2.589	0.879	18.0*
Mek01 A	21	455	1.770	0.581	12.1
Dar01 A	30	1640	2.125	0.625	14.0
Mer01 C	6	7	1.748	0.976	6.0*
Mer02 C	25	116	2.044	0.635	22.9
Mek01 C	4	5	1.332	0.961	4.0*
Dar01 C	12	25	2.203	0.887	12.0*
Mer	75	2283	---	---	16.5
Mek	32	1126	---	---	12.5
Dar	42	2235	---	---	15.1
C	36	153	---	---	29.0
W	55	2462	---	---	15.4
A	62	3029	---	---	18.0
Total	99	5644	---	---	20.6

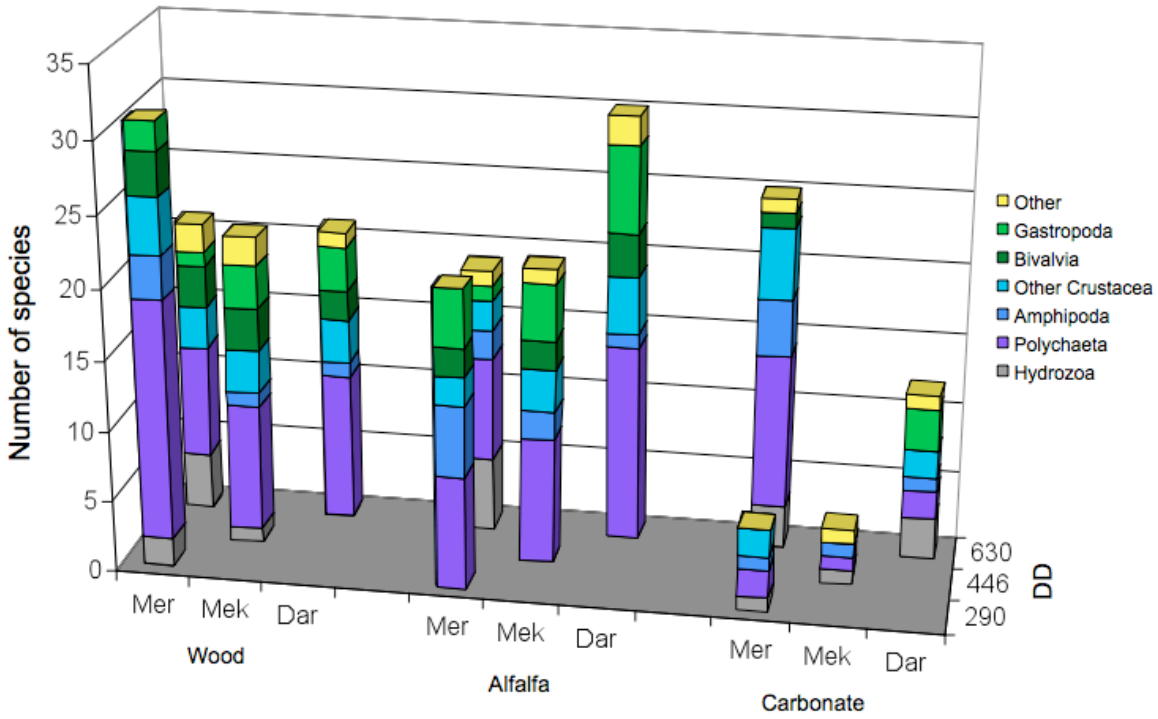


Figure 19. Species richness for each mud volcano in the different substrate and the time of immersion. DD: Deployment duration.

The macrofauna assemblages show important variations in the community structure according to the substrate type, location, and immersion time (Figure 20). The wood experiments were densely colonised by wood-boring bivalves and gastropods. The alfalfa grass was highly colonised by amphipods and polychaetes in Mercator, and mostly by gastropods and amphipods in Meknès and Darwin. Settlement on the carbonate substrata was generally scarce, but the sediment deposited among the cubes yielded some invertebrates, mostly crustaceans (amphipods, isopods and tanaids) and gastropods.

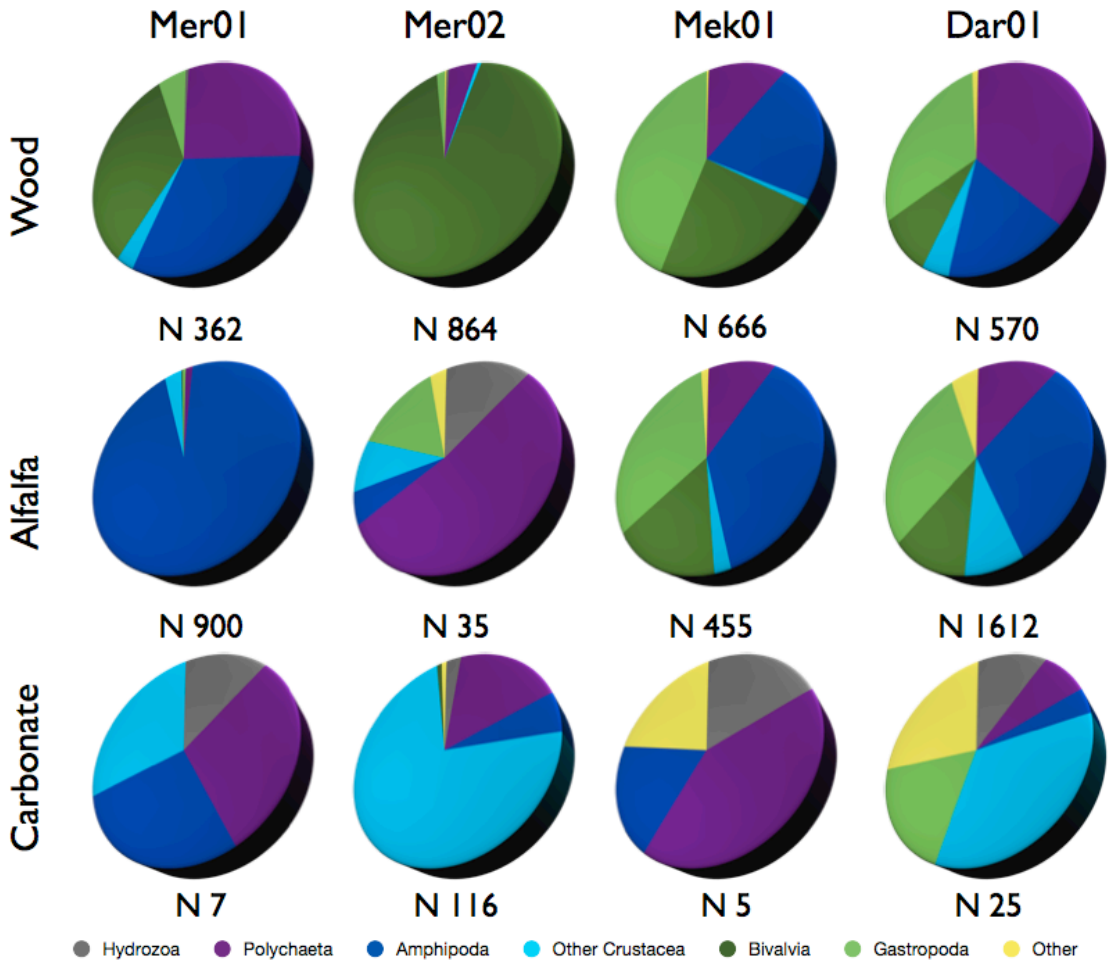


Figure 20. Community structure of the assemblages settled in the studied samples. Two thirds of the sample were analysed for all CHEMECOLI except for the wood samples (only one third analysed).

1. Chemotrophic species

The presence of chemosynthetic bivalves (Mytilidae and Solemyidae) was recorded in the three mud volcanoes almost exclusively in organic substrates (Figure 21). The Mercator MV was scarcely colonised by chemotrophic species independently of the substrate. Mytilid and solemyid bivalves occurred in both organic substrates a single individual of solemyid bivalve was also found in carbonate. In all cases chemotrophic species was less than 1% of total abundance in the Mercator samples. In Meknès and Darwin the abundance of chemotrophic individuals recovered was considerable higher. From the bivalves group only the family Mytilidae were represented in these two volcanoes. In Meknès, 116 (~17% of total abundance) and 78 individuals (~12%) were found in the wood and alfalfa samples, respectively; in Darwin, 54 (~8%) and 203 (40%)

specimens were found in wood and in alfalfa, respectively. In all volcanoes the mytilid bivalves *Idas* sp. were recruited with success in wood but these mytilids were never observed in the natural substrates of the mud volcanoes. The recruitment of *Bathymodiolus mauritanicus* in the samples from Darwin could not be confirmed as the juveniles of the two mytilid species are morphologically very similar and molecular methods are needed to differentiate between the two species. Solemyid bivalves that are known to occur in all studied mud volcanoes (*Petrasma* sp. in Mercator and *Acharax* sp. in Meknès and Darwin) only recruited in Mercator in the organic substrates. The siboglinid polychaetes are represented by several species in all studied sites but only one specimen was found in the alfalfa sample from Meknès.

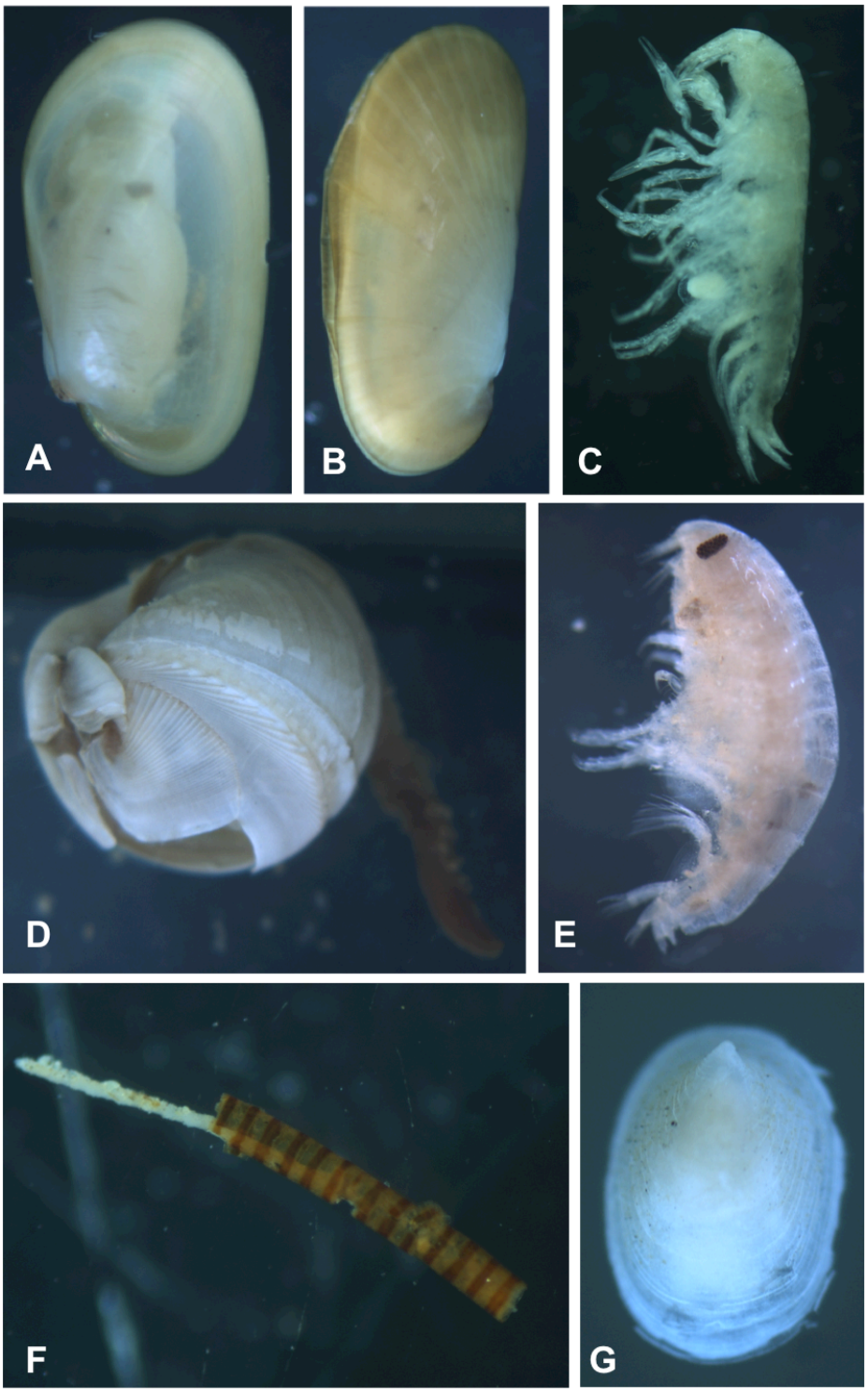


Figure 21. Chemotrophic species (A, B anf F) and some of the common species recovered in the CHEMECOLI. A) Mytilidae (cf. *Idas* sp.), B) Solemyidae (*Petrasma* sp.), C) *Seba aloe*, D) *Xylophaga* sp., E) *Orchomene grimaldii*, F) Siboglinidae und. and G) Gastropod sp. A.

2. Community structure and species composition

Differences between mud volcanoes

The highest number of species was recorded in Mercator with 75 taxa, followed by Darwin with 42 and Meknès with 32 (Table 4, Figure 19). The number of colonisers in each volcano was very similar in Mercator and Darwin, 2283 and 2235 respectively, while in Meknès the abundance was only about one half of these values (Table 4). The pooled $ES_{(100)}$ for Mercator MV was of 16.5 (Table 4) but there was a high variability within the individual samples that were characterised by the presence of highly dominant species in the organic substrates (xylophaginid bivalves in wood and Lysianassid amphipods in alfalfa, Figure 20). In Darwin and Meknès the pooled $ES_{(100)}$ was slightly higher than the values of their individual samples but Meknès recorded the lowest $ES_{(100)}$ value (12.5). The Shannon-Wiener index reached both the lowest and the highest record in Mercator MV (0.470 in wood to 2.589 in alfalfa, both after 631days of immersion). This index fluctuated more among samples from Mercator than in the samples from the other two mud volcanoes (1.330-1.772 in Meknès; 2.125-2.203 in Darwin). In Mercator MV the range of Pielou's evenness is equally high, varying from 0.155 in wood, due to the dominance of *Xylophaga* sp., to 0.976 in carbonates that recruited an assemblage with low abundance and no dominant species. In Darwin and especially in Meknès, J' showed lower variations and much higher minimum values (0.554-0.961 in Meknès; 0.625-0.887 in Darwin).

Differences between substrata

The organic substrates recorded both higher abundance and number of species than the carbonates (Table 4). The alfalfa and wood (Table 4; note that for wood only one third of the sample was sorted) were densely colonised, in contrast with the assemblage from the carbonates that showed a number of individuals one to two orders of magnitude lower. Although the number of species in individual samples of wood and alfalfa was similar, the pooled number was higher for the latter (A: 62; W:55, Table 4). The samples with the lowest evenness were recorded in organic substrates (Table 4 and Figure 22) due the high dominance of the wood-boring bivalve *Xylophaga* sp. (more than 90% in the wood sample Mer02) and the amphipod *O. grimaldii* (approximately 70% in the alfalfa sample Mer01). The highest evenness was consistently recorded in carbonates.

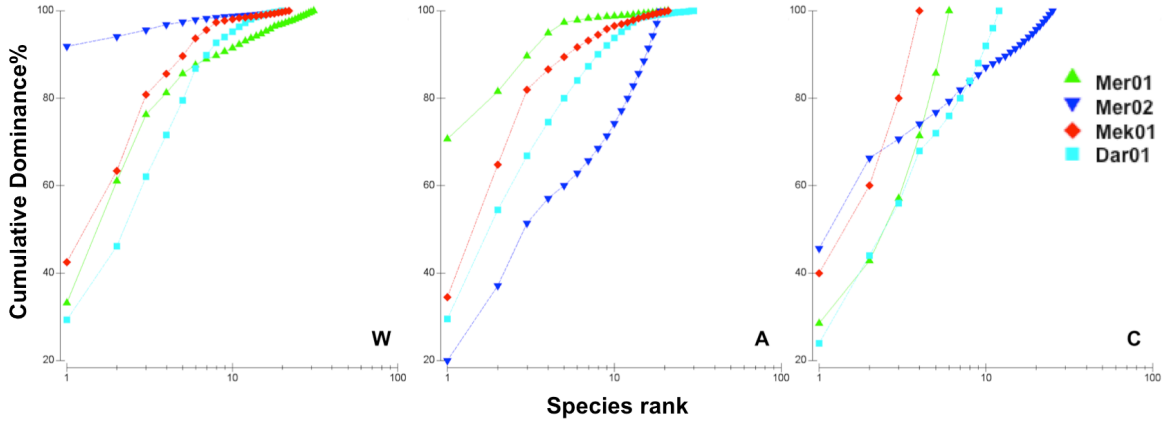


Figure 22. K-dominance curves for the three substrates. Mer: Mercator; Dar: Darwin; Mek: Meknès. W: wood; A: alfalfa grass; and C: carbonate. All samples show low dominance except for W-Mer02 (left graph) and A-Mer01 (center graph).

The Shannon-Wiener index showed no clear pattern in relation to the substrate type. However the Hulbert rarefaction curves (Figure 23) clearly illustrated the high biodiversity of the carbonate samples (indicated by the steepness of the curves) also confirmed by the pooled $ES_{(100)}$ value (29.0). Except for the organic samples in Darwin the rarefaction curves show levels far from the community saturation.

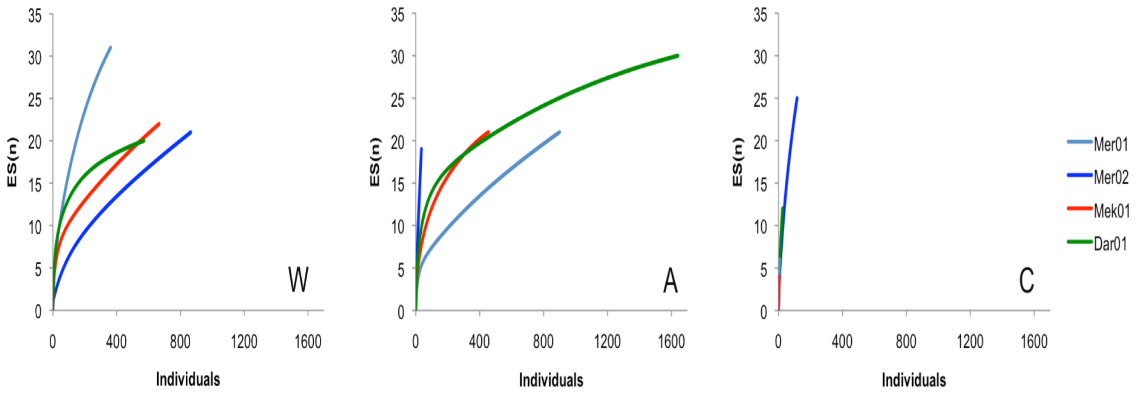


Figure 23. Hulbert's rarefaction curves for the assemblages found in the different substrates. Mer: Mercator; Dar: Darwin; Mek: Meknès. W: wood; A: alfalfa grass; and C: carbonate. $ES_{(n)}$: Expected number of species for a given number of individuals (n).

Time series in Mercator MV

The time series experiment in the Mercator MV allows following the ecological succession in two different moments in the three substrate types. The species turnover between the two periods of time analysed, varies from 21 in the carbonates and 31-32, in

the organic substrates (Table 5). In both organic samples the number of species decreased with the increasing immersion time. The opposite pattern was observed in the carbonate samples, where the number of taxa increased with the immersion time. The abundance increased with time in wood and carbonate and decreased in alfalfa. The higher loss of species and the lower number of species gained was observed in wood. In this substrate, the decrease in the number of species together with the increase in abundance and especially in the dominance of *Xylophaga* sp. resulted in a considerable reduction of diversity (H' decreased from 2.014 to 0.470, Table 4). In alfalfa the number of species lost and gained was very similar, but the great reduction in abundance and increased evenness of the assemblage due to the loss of amphipod dominance lead to an increase in diversity (Table 4). The carbonate was the only substrate where both the abundance and the number of species showed a great increase with the immersion time; the number of species gained was the highest (20) and only one species was removed. This resulted in the increase of the diversity with the immersion time (Table 4).

Table 5. Species turnover in Mercator MV. Numbers were estimated based on the samples recovered after 290 and 631 days of immersion. DD: deployment days.

	DD		Number of taxa excluded	Number of taxa gained	Species Turnover
	290	631			
Wood	31	21	21	11	32
Alfalfa	20	18	16	15	31
Carbonate	6	25	1	20	21

Multivariate Analysis

The MDS plot (Figure 24) shows a clear segregation between the samples from the shallow Mercator MV and the two mud volcanoes from the deeper carbonate province. The organic substrates also appear segregated from the carbonates. Overall there is little dispersion between the organic samples from the mud volcanoes in the carbonate province (Darwin and Meknès).

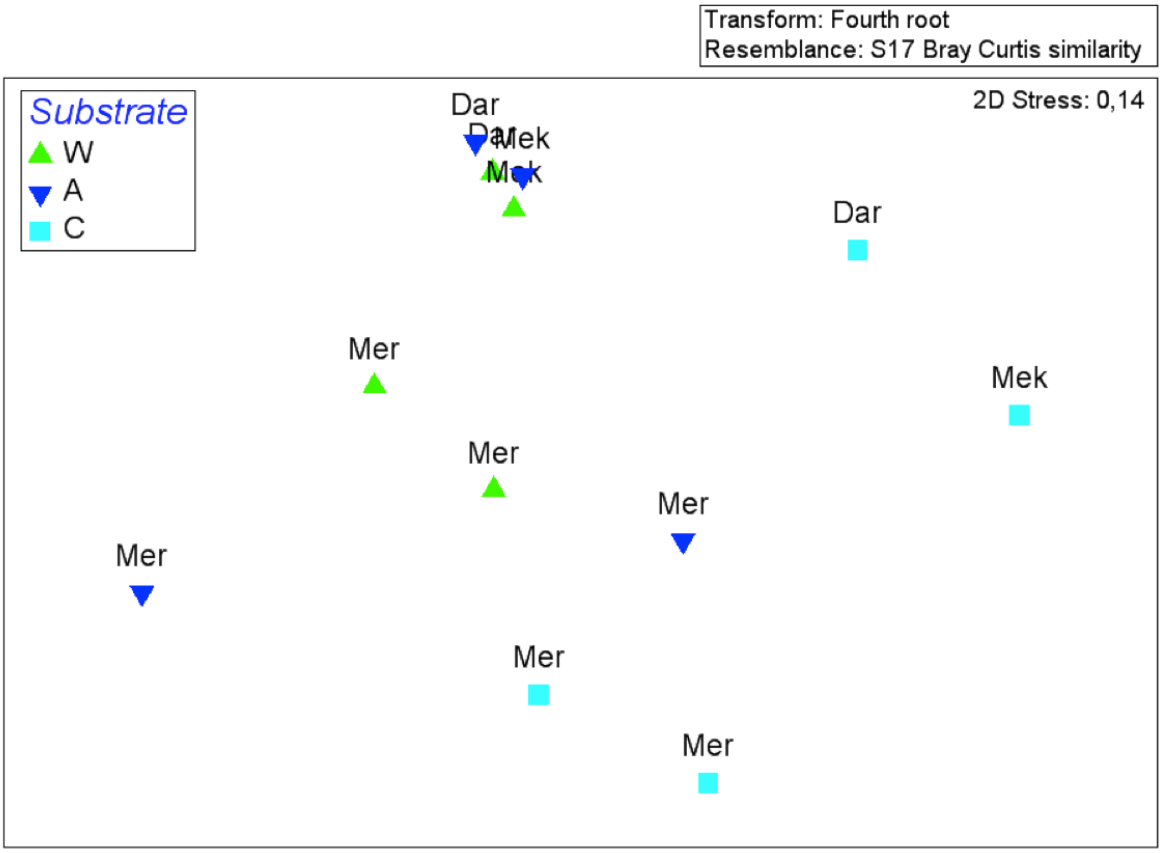


Figure 24. MDS plot of the studied samples based on the species abundance after fourth root transformation. Mer: Mercator; Dar: Darwin; Mek: Meknès; A: Alfalfa; C: Carbonate; W: Wood.

The one-way ANOSIM tests for the differences among mud volcanoes showed no significant results (Global R=0.225; significance level=9%), but these were based in only three samples from Darwin and Meknès and six from Mercator and the low number of samples influences the power of test. However, the one-way ANOSIM tests for the difference among substrate type (Table 6) show significant global and pairwise results. Despite the low number of samples (and therefore of possible permutations), the pairwise tests show the significant difference between wood and carbonate samples (R=0.651, significance level =2.9) contrasting with the low distance between the assemblages of organic substrata (R=0.000; significance level=62.9%).

Table 6. Results of the ANOSIM global and pairwise tests; one-way analysis for substrate type. (a): all permutation possible; *: significant values (p<0.05).

	Sample statistic	Permutations used	Significant Statistics	Significance level (%)
Global test	0.292	5775[(a)]	23	2.4*
Pairwise tests				
Wood, Alfalfa	0.000	35[(a)]	22	62.9
Wood, Carbonate	0.651	35[(a)]	1	2.9*
Alfalfa, Carbonate	0.245	35[(a)]	7	20

The SIMPER results (Table 7) for substrates show that the average dissimilarity is lower between the two organic substrates (70%) than between the carbonates and the organic substrates (~90%). The low values of average similarity (15-36%) for the substrate type groups are indicative of an important heterogeneity of the samples of different mud volcanoes. The higher contributions for the similarity in carbonate samples are from Hydrozoa und. and Ophiuroidea und. (both with more than 20 % contribution); in wood samples are from *Xylophaga* sp. and *Ophryotrocha* sp. (both with ~15% contribution); and in alfalfa samples are from the polychaetes *Amage* sp. and *Melinnopsis* sp. (both with only ~9% contribution) and several other species with slightly lower contributions. The SIMPER results further indicate that the differences between substrate types are explained mainly by abundance variations in the most dominant species (especially the ones from organic substrates): the amphipods *S. aloe* and the *O. grimaldii*, the molluscs gastropod sp. A, *Xylophaga* sp. and Mytilidae (cf. *Idas* sp.) and the polychaete *Ophryotrocha* sp. Although the inorganic substrate was dominated by isopods (*Gnathia* sp. and *Munna* sp.) these species were not important contributors for the average dissimilarity between substrate types because of their very low abundances.

Table 7. Breakdown of percentual contributions from SIMPER analysis for comparisons between substrate type (all samples of each substrate combined). The taxa listed contribute at least 1%. Numbers in bold mark the six dominant species in each substrate. W: wood; A: Alfalfa; C: carbonate; AS: average similarity; AD: Average dissimilarity; und.: undetermined species; ●: contributions lower than 1%.

	Abundance				% Contribution				% Contribution		
	W	**A**	**C**		**W**	**A**	**C**		**W/C**	**A/C**	**W/A**
				AS:	36.55	20.19	15.09	AD:	90.31	89.18	70.31
Cnidaria											
Clytia linearis	0.5	0.1	0.3		●	●	●		1.21	1.25	1.10
Clytia sp.	0.5	0.0	0.1		●	---	6.40		1.22	●	1.10
Hydrozoa und.	0.0	0.0	0.4		---	---	22.18		1.43	1.75	---
Zygophylax biarmata	0.0	0.1	0.1		---	●	●		●	1.07	●
Mollusca											
Gastropoda sp. A	**116.5**	**68.5**	0.0		9.04	6.14	●		5.60	3.90	4.34
Gastropoda sp. B	1.8	**12.0**	0.1		1.48	7.08	●		1.59	2.62	2.02
Gastropoda sp. D	3.5	1.3	0.0		1.39	8.58	---		1.93	2.60	1.71
Gastropoda sp. F	0.0	0.6	0.4		---	●	●		●	1.41	0.96
Bivalvia und.	0.8	0.3	0.0		1.39	●	---		1.43	●	1.25
Mytilidae (cf. *Idas* sp.)	**42.8**	**35.1**	0.0		5.65	5.32	---		4.07	3.33	3.41
Solemyidae (cf. *Petrasma* sp.)	1.0	0.1	0.1		●	●	●		1.38	●	1.25
Xylophaga sp.	**236.5**	0.4	0.0		15.54	●	---		7.69	1.09	6.04
Sipuncula											
Sipuncula und.	0.5	0.0	0.0		1.39	---	---		1.30	---	1.16
Annelida											
Amage sp.	**30.8**	3.1	0.3		2.83	8.98	●		3.20	2.66	2.95
cf. *Amphiduros* sp.	2.0	0.4	0.0		3.97	●	---		2.18	1.13	1.54
Aphelochaeta sp.	9.3	6.8	0.0		5.57	3.18	---		3.26	2.12	2.39
Capitellidae sp. 1	0.5	0.4	0.1		●	●	●		1.14	1.16	1.08
Capitellidae sp. 2	4.5	0.0	0.0		●	---	---		1.07	---	1.01
Capitellidae sp. 3	3.3	0.0	0.0		3.53	---	---		2.20	---	2.02
Capitellidae sp. 5	0.0	0.1	**0.5**		---	●	●		1.06	1.38	●
Exogoninae und.	1.3	2.1	0.0		1.35	●	---		1.39	●	1.50
Harmothoe evei	1.8	0.9	**0.8**		1.49	●	6.05		1.67	1.87	1.54
Hesionidae und.	0.8	0.3	0.0		●	●	---		1.21	1.02	1.17
Leocrates atlanticus	1.0	0.1	0.4		●	●	12.35		1.37	1.46	1.22
Melinnopsis sp.	19.5	11.1	0.0		5.24	8.94	---		3.46	3.33	2.52
cf. *Nereimyra* sp.	3.3	2.5	0.0		4.34	2.13	---		2.38	1.55	1.83
Ophryotrocha sp.	**30.3**	5.9	0.0		14.73	3.40	---		5.32	2.13	3.21
Polycirrus norvegicus	0.8	0.1	0.4		●	●	●		1.14	1.18	1.04
Subadyte pellucida	0.3	0.6	0.1		●	6.89	●		●	1.80	1.33
Arthropoda											
Amphipoda sp. A	0.0	9.1	0.4		---	●	●		●	2.12	1.37
Amphipoda sp. B	0.0	12.4	0.0		---	3.06	---		---	2.85	2.03
Ensayara c.f. *carpinei*	0.8	5.9	0.0		●	●	---		●	1.64	1.54
Ischyroceridae und.	0.0	0.0	0.4		---	---	11.11		1.16	1.46	---
Orchomene grimaldii	25.3	**79.8**	0.0		●	2.94	---		1.65	3.88	3.42
Seba aloe	**57.5**	**80.1**	0.0		3.84	6.34	---		3.86	4.04	3.84
Gnathia sp.	0.5	0.6	**6.8**		●	●	6.05		1.72	2.15	0.99
Munna sp.	1.5	0.0	**3.1**		7.54	---	6.05		1.90	1.69	2.36
Pseudotanaidae und.	4.3	**16.6**	0.4		●	2.91	●		1.52	2.47	2.23
Pseudotanaidae sp.A	0.3	0.0	**0.6**		●	---	●		1.04	●	●
TAN SP 019	0.5	0.3	0.0		●	---	---		1.22	1.04	1.10
Tanaidae und..	1.8	0.0	0.0		●	---	---		0.95	---	●
Nebalia sp.	1.8	3.3	0.0		●	7.21	---		●	2.56	1.93
Monodaeus couchi	1.0	0.1	0.1		●	●	●		1.16	1.04	1.10
Echinodermata											
Ophiuroidea und.	1.8	7.5	**1.0**		●	7.91	23.77		1.63	2.07	1.90

Although no significant differences were found between mud volcanoes the SIMPER analysis was also performed and is summarised in Table 8. The results show that the average dissimilarity is lower between the two organic mud volcanoes from the carbonate province (~65%) than between the mud volcanoes from different regions (~87%). The low values of average similarity (19-32%) for the mud volcano groups result from the significant differences between the samples of different substrate types. The higher contributions for the similarity in Darwin are from Ophiuroidea und. and Pseudotanaidae und., (both with more than 13 % contribution); in Meknès are from the polychaete *Leocrates atlanticus* and Ophiuroidae und. (both with more than 16% contribution); and in Mercator are from the polychaete *Harmothoe evei* (~17% contribution) and the isopod *Gnathia* sp. (9% contribution). Again, the differences between mud volcanoes are explained mainly by abundance variations in some of the most dominant species: the molluscs gastropod sp. A, *Xylophaga* sp. and Mytilidae (cf. *Idas* sp.), the polychaete *Amage* sp. the amphipod *S. aloe* and Pseudotanaidae und.

Table 8. Breakdown of percentual contributions from SIMPER analysis for comparisons between volcanoes (all samples of each volcano combined). The taxa listed contribute at least 1%. Numbers in bold mark the six dominant species in each volcano. Mer: Mercator; Mek: Meknès; D: Darwin; AS: average similarity; AD: Average dissimilarity; und.: undetermined species; ●: contributions lower than 1%.

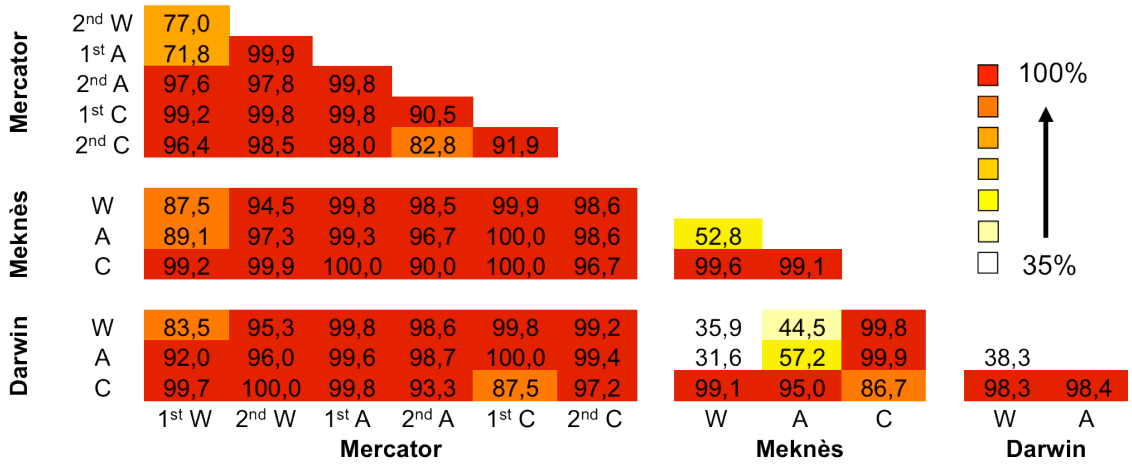
	Abundance				% Contribution				% Contribution		
	Mer	Mek	Dar		Mer	Mek	Dar		Mer/Mek	Mer/Dar	Mek/Dar
				AS:	18.89	26.70	31.89	AD:	86.98	87.50	64.94
Cnidaria											
Clytia linearis	0.5	0.2	0.0		6.53	●	---		1.62	1.26	1.58
Clytia sp.	0.4	0.0	0.0		2.92	---	---		1.08	●	---
Eudendrium sp.	0.0	0.0	0.2			---	●		---	1.01	1.62
Hydrozoa und.	0.2	0.0	0.2		1.93	---	●		1.28	●	1.62
Zygophylax biarmata	0.1	0.0	0.2		●	---	●		●	1.00	1.62
Mollusca											
Gastropoda. Sp. A	2.7	**117.3**	**124.0**		●	11.14	8.70		4.81	4.23	6.01
Gastropoda. Sp. B	0.1	1.7	16.7		●	4.60	10.12		1.77	3.05	3.43
Gastropoda. Sp. D	2.6	0.8	0.3		1.88	3.87	●		1.98	1.35	1.70
Gastropoda. Sp. E	0.0	0.0	0.7		---	---	●		---	1.17	1.66
Gastropoda. Sp. F	0.0	1.2	1.2		---	●	3.82		●	1.96	2.69
Gastropoda. Sp. H	0.0	0.2	0.2		---	●	●		●	1.01	1.62
Bivalvia und.	0.3	0.3	0.3		●	●	●		●	●	1.14
Mytilidae (cf.*Idas* sp.)	0.2	**51.7**	**51.8**		●	9.66	6.56		4.02	3.38	4.78
Solemyidae (cf. *Petrasma* sp.)	0.8	0.0	0.0		5.90	---	---		1.53	1.27	---
Xylophaga sp.	**152.3**	**9.2**	2.0		3.22	●	●		4.13	3.29	2.78
Annelida											
Amage sp.	0.7	**9.3**	**34.8**		1.64	3.87	4.13		2.40	2.90	4.14
Aphelochaeta sp.	3.5	1.7	12.7		●	●	4.84		1.80	2.36	3.36
Capitellidae sp. 1	0.3	0.3	0.3		2.82	●	●		1.18	0.96	1.14
Capitellidae sp. 3	0.2	3.7	0.3		●	●	●		1.34	●	1.89

Capitellidae sp. 5	0.4	0.0	0.0	5.92	---	---	1.87	1.30	---
cf. *Amphiduros* sp.	1.0	0.3	0.8	●	●	●	1.22	1.30	1.73
cf. *Nereimyra* sp.	0.5	1.0	5.7	●	3.87	4.07	1.57	1.95	2.69
Exogoninae und.	0.3	0.0	3.8	●	---	3.19	●	1.71	2.48
Harmothoe evei	2.3	0.0	0.0	16.96	---	---	3.19	2.39	---
Hesionidae und.	0.3	0.0	0.8	●	---	●	●	1.18	1.71
Leocrates atlanticus	0.5	0.8	0.2	●	16.12	●	2.90	1.16	1.82
Melinnopsis sp.	0.3	**14.2**	**26.2**	●	6.96	5.82	2.93	2.90	3.89
Nicolea cf. *venustula*	0.0	0.0	0.2	---	---	●	---	1.01	1.62
Ophryotrocha sp.	**11.0**	6.5	19.7	1.77	6.17	4.91	2.73	2.70	3.61
Polycirrus norvegicus	0.8	0.0	0.0	4.06	---	---	1.49	1.19	---
Protodrilus sp.	0.0	0.0	3.8	---	---	●	---	0.97	1.37
Subadyte pellucida	0.3	0.2	0.5	3.22	●	●	1.21	1.07	1.22
Oligochaeta und.	0.0	0.0	4.7	---	---	●	---	1.02	1.44
Arthropoda									
Amphipoda sp A	**6.3**	0.0	0.0	1.47	---	---	1.52	1.24	---
Amphipoda sp B	**8.3**	0.0	0.0	●	---	---	1.58	1.27	---
Ensayara c.f. *carpinei*	4.4	0.0	0.0	●	---	---	1.32	1.10	---
Ischyroceridae und.	0.2	0.0	0.2	●	---	●	1.08	1.02	1.62
Lysianassidae und.	0.0	0.2	0.0	---	●	---	1.50	---	1.58
Orchomene grimaldii	**69.8**	0.3	0.0	3.16	●	---	3.09	2.28	●
Seba aloe	0.0	**72.5**	**111.0**	---	11.51	7.48	4.51	4.06	5.51
Gnathia sp.	**5.3**	0.0	0.0	9.03	---	---	2.80	2.10	---
Janira maculata	0.0	0.5	0.0	---	●	---	1.29	---	1.56
Munna sp.	2.4	1.0	0.3	8.15	●	●	2.41	1.64	1.49
Pseudotanaidae und.	0.0	1.2	**27.2**	---	●	13.47	1.04	4.12	5.07
Pseudotanaidae sp.A	0.0	0.3	0.8	---	●	●	●	1.51	2.50
TAN SP 019	0.5	0.0	0.0	3.63	---	---	1.27	1.02	---
Tanaidae und.	0.0	0.0	2.3	---	---	●		1.04	1.49
Nebalia sp.	3.0	0.3	0.3	1.62	●	●	1.61	1.28	1.21
Monodaeus couchi	0.8	0.0	0.0	3.72	---	---	1.41	1.13	---
Echinodermata									
Ophiuroidea und.	0.2	1.5	11.8	1.38	17.73	13.97	2.32	3.12	2.08

3. Biodiversity from local to regional scale

The Bray-Curtis dissimilarity matrix (Table 9) can be used as an overview of the β-diversity in the studied samples. The Meknès and Darwin MV both located in the carbonate province show much lower dissimilarities between them than with Mercator (El Arraiche shallow MV field). High dissimilarities between samples of the same mud volcano occur especially between organic and inorganic substrates. In fact the lowest dissimilarities are between samples of the same substrate in Darwin and Meknès even with different times of immersion while in Mercator the samples of the same substrate but different immersion duration are highly dissimilar. In summary, the nature of the substrate appears to overcome the influence of location in the case of the two volcanoes (Meknès and Darwin) belonging to the same province. Nevertheless, β-diversity between mud volcanoes of different provinces (Mercator vs Meknès and Mercator vs Darwin) is always high regardless of the substrate type.

Table 9. β-diversity - Bray-Curtis dissimilarity matrix for samples recovered from the three mud volcanoes: Mercator, Darwin and Meknès; W: wood; A: alfalfa grass; C: carbonates. In Mercator 1st and 2nd correspond to the first and the second sets of CHEMECOLI recovered.



The high heterogeneity observed in the assemblages that recruited in different substrates together with the high β-diversity between mud volcanoes of different provinces resulted in an estimated $ES_{(100)}$ regional value (γ-diversity: 20.6) that is overall higher than α- diversity values estimated either for each single sample or for the samples pooled by MV of substrate type (Table 4). The only exceptions are the pooled value for carbonates (29.0) and the individual sample Mer02-carbonate (22.4). Although the carbonates were the less colonised substrate, both in abundance and number of species, the high evenness of the recruited assemblages resulted in these relatively high $ES_{(100)}$ values.

4. Background fauna

In the comparison between the background fauna and the assemblages recovered in the CHEMECOLIs it is important to consider that the species from several families such as the Capitellidae, Mytilidae (*Idas* sp. but in Darwin probably also *Bathymodiolus mauritanicus*), Solemyidae (probably *Petrasma* sp.) and the gastropods were not determined for the CHEMECOLI samples. Therefore, the low taxonomic resolution limits such comparison and the values below probably underestimate the overlap between the CHEMECOLI and the background fauna (Table 10).

Table 10. Number and list of recovered taxa in CHEMECOLI by substrate and the correspondent number of species recruited from background environment. T: number of taxa; TSL: taxa identified to species level; BR: background fauna recruited in CHEMECOLI; %: Percentage of the total species identified in CHEMECOLI.

		T	TSL	BR	%	List of species recruited from background
Mercator	W	42	14	7	16.7	*Aricidea suecica meridionalis, Eunereis longissima, Leocrates atlanticus, Subadyte pellucida, Monodaeus couchi,* Solemyidae (probably *Petrasma* sp.), *Munna* sp.
	A	36	15	8	22.2	*Campanulina panicula, Zygophylax biarmata, Eunereis longissima, Leocrates atlanticus, Subadyte pellucida, Dulichiopsis nordlandicus, Monodaeus couchi,* Solemyidae (probably *Petrasma* sp.)
	C	28	10	8	28.6	*Leitoscoloplos* cf. *mammosus, Paradoneis lyra, Subadyte pellucida, Eriopisa elongata, Melphidippella macra, Monodaeus couchi,* Solemyidae (probably *Petrasma* sp.), *Munna* sp.
Meknès	W	22	4	3	13.6	*Leocrates atlanticus, Prionospio* cf. *aluta*, *Munna* sp.
	A	21	5	1	4.8	*Leocrates atlanticus*
	C	4	2	1	25.0	*Leocrates atlanticus*
Darwin	W	20	2	3	15.0	*Seba aloe*, Mytilidae sp. (probably *Idas* sp. and *Bathymodiolus mauritanicus*), *Munna* sp.
	A	30	4	3	10.0	*Seba aloe, Austrofilius* sp.
	C	12	3	1	8.3	*Leocrates atlanticus*

The levels of colonisation by background species of the local environment were globally low. The highest levels in percentage were recorded in carbonate samples of Mercator and Meknès MV. In all volcanoes the recruitment of chemotrophic species from the surrounding environment was low (see results on chemotrophic species mentioned above). Concerning the non-chemotrophic background fauna, the polychaetes and the Crustacea were the most successful group to colonise the CHEMECOLI substrates. The amphipod *Seba aloe*, known as a background species in Darwin, was remarkable by attaining a dominant position in the recruited assemblages of organic substrates.

IV. Discussion

1. Recruitment success of experiments

One of the objectives of this study was to investigate the potential of using artificial substrates for obtaining initial life cycle stages of chemotrophic species. Although the Mercator was the volcano with less percentage of chemotrophic fauna recruited it was the site where the recruitment of several individuals of a resident species (*Petrasma* sp.) could be observed. It was not possible to identify the siboglinid specimen found in Meknès as one of the species known to occur in this mud volcano and the recruitment of *Bathymodiolus mauritanicus* in Darwin experiments needs molecular confirmation. The identification to species level based on the morphology of all these chemotrophic taxa is also very difficult because the individuals obtained are all in the initial stages of their life cycles. In Meknès and Darwin the percentage of chemotrophic fauna was considerably higher (up to 17% of the total abundance) but the species recruited *Idas* sp. is a cosmopolitan species commonly associated to wood falls that has never been recorded in the natural substrates of these mud volcanoes. In all mud volcanoes the chemotrophic species were almost exclusively found associated to organic substrates. The higher success in the recruitment of chemotrophic species in these substrates can be related to their degradation and subsequently to the amount of sulphide released.

Despite the importance of chemotrophic fauna for our study, the majority of taxa recruited by the CHEMECOLI were heterotrophic, being the polychaetes the most diverse taxonomic group found in the samples. The results of the multivariate analysis showed that most of the variability in the species composition and structure of the recruited assemblages could be explained by the substrate type. The deep-sea fauna composition has been related essentially to the depth, substrate type (hard-substrate or sediments with different particle sizes) and food availability (Levin et al. 2001; Miller 2004). Within their bathymetric range of occurrence, deep-sea invertebrate larvae can probably detect differences between surface textures that are important cues for recruitment (Mullineaux 1989). Concerning species richness the food availability will play the most significant role, determining the number of species that a given ecosystem can support. However, the explanation for the diversity and structure of the deep-sea assemblages is often complex

and several factors may play an important role (Levin et al. 2001; Snelgrove and Smith 2002). The high sulphide concentration, for example, is correlated with the structure of the community (Levin et al. 2003). The organic substrates in the CHEMECO experiments showed a more complex assemblage with a higher number of species than the carbonates and in some cases with a highly dominant species. These aspects are in agreement with the diverse assemblages associated to organic falls observed by other authors (e.g. Wolff 1979; Samadi et al. 2007; Young 2009) and may be explained by the high food availability and sulphide concentrations associated to the alfalfa and wood. The organic substrates are usually colonised by specialised fauna that rapidly explore the available food source and may become dominant. The carbonates, on the other hand, are a common substrate in mud volcanoes but their colonisation may occur slowly owing to the low food availability. Despite the low levels of colonisation, the carbonates exhibited highly diverse assemblages characterised by the absence of dominant species. If sufficient time is allowed, it is expected that slow growing, sessile species and other metazoan will establish in this substrate. According to Wahl (2009b) it may take up to several years to create adequate conditions for metazoan larvae recruitment in hard substrates.

In terms of abundance, opportunistic species such as lysianassid amphipods, wood-boring Xylophagainae bivalves, cocculinid limpets (Gast. sp. A) and the polychaetes *Ophryotrocha* sp. and Capitellidae spp. accounted for most of the colonisation of organic substrates. The Xylophagainae bivalves and cocculinid limpets are specialised on organic falls (e.g. Young 2009). Turner (1973) hypothesised that the amount of woodborer bivalves that rapidly colonises wood falls suggests that there is a large pool of larvae present in the water column waiting to explore such opportunities of colonisation. Many other opportunistic amphipod and polychaete species are adapted to exploiting seasonal or periodic resources. Furthermore, the Capitellidae and *Ophryotrocha* are non-symbiont bearing polychaetes that show a high tolerance to the presence of sulphide (Levin et al. 2003).

2. Trends in succession

In Mercator MV two sets of experiments were recovered after approximately 10 and 24 months of immersion. Despite the lack of replication some aspects of the ecological succession in the different substrates may be noted. The species assemblage and ecological succession depends of many interacting biotic (competition for resources, predation and other interactions between colonisers) and abiotic factors (Sousa 1984; Lissner et al. 1991; Jenkins et al. 2009; Marshall et al. 2009). The modification of organic substrates, and therefore of the associated food availability, resulting from the organisms' activity since the beginning of colonisation is one of the most important changes that may determine the course of succession (Snelgrove et al. 1996). Although the assemblages in the three substrate types revealed different trends with the increasing immersion time these can be related with the alterations in the organic substrates.

In the alfalfa samples of Mercator the increasing immersion duration was accompanied by a decrease in the number of species, abundance and dominance of the colonising assemblage. After 10 months the alfalfa was densely colonised mainly by amphipods with the lysianassid *O. grimaldi* accounting for 70% of the total abundance. After 24 months of immersion a high turnover of species occurred; the amount of alfalfa remaining was very low and may explain the drastic decrease in abundance (from 900 to 35 individuals) and the absence of *O. grimaldi* that lead to a substantial increase in the evenness of the assemblage. The observed absence of chemotrophic bivalves after 24 months may be due to the low level of sulphide released by the degradation of the small amount of alfalfa. In their experiments using *Sargassum* as substrate, Snelgrove et al. (1996) also observed a decrease in faunal densities along the time and suggested that with time (and decreasing food availability) the initial opportunistic species are replaced by other background fauna.

In the wood experiments the amount of substrate remained high even after 24 months of immersion. Although there was also a high turnover there were more species removed than gained with a net decrease in species richness. Unlike alfalfa, wood is not easily degraded; specialised wood boring bivalves (e.g. *Xylophaga* spp.), together with bacteria and fungus, convert the wood in faecal pellets, a food source that can be then used by detritus feeders (Turner 1977). The wood-boring bivalve *Xylophaga* sp. reached a

dominance of more than 90% and was the major contributor to the great increase of the total abundance suggesting that the colonization of wood by this specialised taxon was still in an initial phase after 10 months of immersion and was probably near its peak after 24 months of immersion. The decrease in the number of species and in the abundance of opportunistic polychaetes from the Dorvilleidae and Capitellidae families was evident after 24 months and may be due to the interaction with the dominant species (competition by interference). A reduction in the abundance and dominance of *Xylophaga* sp. is expected for a longer immersion time as the wood will be degraded and no longer able to support a dense population of the bivalve.

The species turnover observed in organic substrates was higher than in carbonates. The assemblages from inorganic substrates are mostly ruled by their geologic characteristics (Cordes et al. 2010) and hard substrates also require more time to attain the necessary conditions for the establishment of metazoan communities. After 24 months of immersion the carbonate experiment in Mercator showed a considerable amount of fine sediments deposited among the cubes which may create more favourable conditions to species settlement and recruitment. With the increase in immersion time the carbonate assemblages showed an increase in species number, abundance and diversity which suggest that the succession is in a very early stage of succession.

3. Local scale patterns

The local diversity is affected by the small-scale habitat heterogeneity (Cordes et al. 2010) while the β-diversity is mainly affected by the species depth range limits that are a balance of several characteristics of the species and environmental variables (Rex and Etter 2010). The species pool together with the environmental characteristics (e.g. substrate type) in each location will determine the species that are recruited. In the present study the utilisation of artificial substrates is probably a constraint to the recruitment and may explain the low colonization rates by the local background fauna which consists mainly on species adapted to a sedimentary environment. Also the $ES_{(100)}$ values estimated for the experiments in each mud volcano (pooled samples from different substrates) are much lower than the ones estimated from the background assemblages (Mercator: 16.5 vs 54.1;

Meknès: 12.5 vs 41.3, M.R. Cunha, pers.comm.) confirming the incapability to reproduce all the environment variables and the great limitations to the simulation of natural processes (including the limited time for ecological succession) inherent to the artificial substrate approach.

Despite the lack of replication that did not allowed proving a significant difference between the assemblages of the different mud volcanoes, the importance of location was evident from the results of this study. The three studied mud volcanoes are located along a bathymetric gradient that ranges from 350 m (Mercator) to 1100m (Darwin). The rate of faunal replacement in benthic assemblages is closely related with the depth, being higher at lower depths than in the deepest locations (Rex and Etter 2010). Therefore changes in the composition and structure of the assemblage are expected to be greater between Mercator and Meknès (about 350m difference in depth) than between the two deeper mud volcanoes, Meknès and Darwin (about 310m difference in depth). Furthermore, Mercator is located in the Al Arraiche field and Meknès and Darwin in the carbonate province. The environmental conditions (both in geology and oceanography) of the two areas are very different and have been described above. In summary, Mercator is essentially a mud environment with a relative proximity to the surface and coastal waters and consequently under their direct influence (important organic input), while Meknès and Darwin show a high availability of natural hard substrates such as cold-water corals and carbonated structures and are located deeper where the influence of the euphotic layer of ocean is less significant. One important factor is the oceanography: the water masses that interact with the sea-floor are different in the two areas and are likely to affect the larval supplies and recruitment. This may explain the lower dissimilarity between the assemblages recruited in the same type of substrate in Meknès and Darwin (31-57% in organic substrates) when compared to the dissimilarity between Mercator and these two volcanoes (83-100% for organic substrates). The characteristics of the water masses may also determine the course of succession: this can be exemplified by the higher near-bottom temperatures in Mercator that favour a more rapid degradation of the organic substrates.

4. Regional patterns

The global $ES_{(100)}$ value (representing the γ-diversity) based on quantitative samples from mud volcanoes of the Gulf of Cadiz was estimated in 58.1 (M.R. Cunha, pers. comm.), matching the highest values reported by Snelgrove and Smith (2002) for different deep-sea regions. The global value obtained in this study (all pooled samples) was much lower (20.6) and can be explained by the limitations of the artificial substrate approach as already mentioned above. The biodiversity patterns in benthic assemblages are dependent on time and space scales (Rex and Etter 2010), both limited in the artificial substrate approach.

In a global scale the patterns of species diversity reflect the evolutionary and the ecological processes that vary between regions of the deep ocean (Rex and Etter 2010). Important differences in taxonomic structure of communities among chemosynthetic habitats are known (Metaxas and Kelly 2010). The substrate characteristics and chemical conditions may limit the recruitment allowing only the settlement of specialist species or organisms with physiological tolerance to high levels of sulphide (Metaxas and Kelly 2010). Other factors such the currents, depth, temperature or the distance between habitat patches joining with the larvae life span will establish the virtual boundaries between communities of different chemosynthetic habitats, resulting in distinct global diversity values found between regions. By using a standardised experimental design, the CHEMECO project allowed to compare the colonization patterns in different deep-sea areas. Despite the differences in the duration of experiments, the number of taxa record in Gulf of Cadiz was globally much higher than the documented by Gaudron et al. (2010) in the other three CHEMECO areas (the Nile deep sea fan, Rainbow hydrothermal vent and Håkon Mosby mud volcano (HMMV)). The highest number of species was recorded in the Nile deep-sea fan (12) while in the Rainbow hydrothermal vent and HMMV the number of species recovered varies between 2 and 10 in experiments with 1 year of immersion. The species pool present in each of the four regions of CHEMECO determines the abundance and species richness of the recruited assemblages and the high colonization rates observed in the Gulf of Cadiz highlight the strategic biogeographic location and the importance of this region as one of the most biodiverse in the European margins.

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ANNEX I

List of taxa found in the CHEMECOLIs deployed in Gulf of Cadiz. Taxonomic data according WoRMS – World Register of Marine Species (http://www.marinespecies.org)

Phyllum CNIDARIA

Class Hydrozoa

Hydrozoa und.

SubClass Hydroidolina

Order Anthoathecatae

SubOrder Filifera

Family Campanulinidae

Campanulinidae und.

Genus *Campanulina* van Beneden, 1847

Campanulina paniculata Sars, 1873

Family Haleciidae

Genus *Halecium* Oken, 1815

Halecium tenellum Hincks, 1861

Order Leptothecatae

Family Campanulariidae

Genus *Clytia* Lamouroux, 1812

***Clytia* sp.**

Clytia linearis Thornely, 1899

Family Lafoeidae

SubFamily Lafoeinae

Genus *Cryptolaria* Busk, 1857

Cryptolaria pectinata Allman, 1888

SubFamily Zygophylacinae

Genus *Zygophylax* Quelch, 1885

Zygophylax biarmata Billard, 1905

Genus *Filellum* Hincks, 1868

Filellum* cf. *serratum Clarke, 1879

Order Anthoathecatae

SubOrder Filifera

Family Eudendriidae

Genus *Eudendrium* Ehrenberg, 1834

Eudendrium sp.

Phyllum NEMERTEA

Nemertea und.

Phyllum SIPUNCULA

Sipuncula und.

Phyllum ANNELIDA

Class Oligochaeta

Oligochaeta und.

Class Polychaeta

Polychaeta sp. A

Polychaeta sp. B

Family Protodrilidae

Genus *Protodrilus* Czerniavsky, 1881

***Protodrilus* sp.**

Subclass Aciculata

Order Amphinomida

Family Amphinomidae

Genus *Linopherus* Quatrefages, 1865

Linopherus* cf. *hemuli Fauchald, 1972

Order Eunicida

Family Dorvilleidae

Dorvilleidae und.

Genus *Ophryotrocha* Claparède & Mecznikow, 1869

***Ophryotrocha* sp.**

Genus *Protodorvillea*

Protodorvillea kefersteini McIntosh, 1869

Genus *Iphitime* Marenzeller, 1902

***Iphitime* sp.**

Family Lumbrineridae

Lumbrineridae und.

Genus *Lumbrinerides* Orensanz, 1973

Lumbrineriopsis paradoxa Saint Joseph, 1888

Order Phyllodocida

Family Chrysopetalidae

Chrysopetalidae und.

Family Hesionidae

Hesionidae und.

Genus *Amphiduros*

cf. *Amphiduros* sp.

Genus *Leocatres* Kinberg 1866

Leocrates atlanticus McIntosh, 1885

Genus *Nereimyra* Blainville, 1828

Nereimyra sp.

Family Nereididae

Genus *Eunereis* Malmgren 1865

Eunereis longissima Johnston, 1840

Family Phyllodocidae

Phyllodocidae und.

Genus *Anaitides* Czerniavsky 1882

Anaitides madeirensis Langerhans 1880

Family Syllidae

Subfamily Exogoninae

Exogoninae und.

Subclass Canalipalpata

Order Sabellida

Family Siboglinidae

Siboglinidae und

Order Spionida

Family Cirratulidae

Genus *Aphelochaeta* Blake 1991

***Aphelochaeta* sp.**

Family Spionidae

Spionidae und.

Genus *Prionospio* Malmgren 1867

Prionospio* cf. *aluta Maciolek 1985

***Prionospio* sp.**

***Prionospio* sp. 1**

Order Terebellida

Family Ampharetidae

Genus *Melinnopsis* McIntosh, 1885

***Melinnopsis* sp.**

Genus *Amage* Malmgren, 1865

***Amage* sp.**

Family Polynoidae

Polynoidae und.

Genus *Harmothoe* Kinberg, 1856

Harmothoe evei Kirkegaard, 1980

Genus *Neoamphitrite* Hessle, 1917

Neoamphitrite affinis Malmgren, 1866

Genus *Subadyte* Pettibone, 1969

Subadyte pellucida Ehlers, 1864

Family Terebellidae

Terebellidae und.

Genus *Nicolea*

Nicolea* cf. *venustula Montagu, 1818

Genus *Polycirrus* Grube, 1850

Polycirrus norvegicus Wollebaek, 1912

Subclass Scolecida

Family Scalibregmatidae

Scalibregmatidae und.

Family Paraonidae

Genus *Aricidea* Webster, 1879

Aricidea suecica meridionalis Laubier & Ramos, 1974

Genus *Paradoneis*

Paradoneis lyra Southern, 1914

Order Capitellida

Family Capitellidae

Capitellidae sp. 1

Capitellidae sp. 2

Capitellidae sp. 3

Capitellidae sp. 4

Capitellidae sp. 5

Order Orbiniida

Family Orbiniidae

Genus *Leitoscoloplos* Day, 1977

Leitoscoloplos* cf. *mammosus Mackie, 1987

Phyllum ARTHROPODA

Class Malacostraca

Superorder Leptostraca

Order Nebaliacea

Family Nebaliidae

Genus *Nebalia* Leach, 1814

***Nebalia* sp.**

SuperOrder Eumalacostraca

Order Decapoda

Infraorder Brachyura

Family Xanthidae

Genus *Monodaeus* Guinot 1967

Monodaeus couchi Couch, 1851

Superorder Leptostraca

Order Nebaliacea

Family Nebaliidae

Genus *Nebalia* Leach, 1814

***Nebalia* sp.**

Superorder Peracarida

Order Amphipoda

Amphipoda sp. A

Amphipoda sp. B

Suborder Corophiidea

Family Dulichiidae

Genus *Dulichiopsis* Laubitz, 1977

Dulichiopsis nordlandicus Boeck, 1871

Family Ischyroceridae

Ischyroceridae und.

Suborder Gammaridea

Family Lysianassidae

Lysianassidae und.

Genus *Ensayara* J.L. Barnard, 1964

Ensayara* cf. *carpinei Bellan-Santini, 1974

Genus *Orchomene* Boeck, 1871

Orchomene grimaldii Chevreux, 1890

Genus *Tryphosella* Bonnier, 1893

Tryphosella simillima Ruffo, 1985

Family Melitidae

Melitidae und.

Genus *Eriopisa*

Eriopisa elongata

Family Melphidippidae

Genus *Melphidippella* Sars, 1894

Melphidippella macra Norman, 1869

Family *Phoxocephalidae*

Phoxocephalidae und.

Family Sebidae

Genus *Seba* Bate, 1862

Seba aloe Karaman, 1971

Order Isopoda

Isopoda und.

Suborder Asellota

Family Janiridae

Genus *Austrofilius* Hodgson 1910

***Austrofilius* sp.**

Genus Janira Leach, 1814

Janira maculosa Leach, 1814

Family Munnidae

Genus *Munna* Krøyer, 1839

***Munna* sp.**

Family Desmosomatidae

Genus *Chelator* Hessler, 1970

***Chelator* sp.**

Suborder Cymothoida

Family Gnathiidae

Genus *Gnathia* Leach, 1814

***Gnathia* sp.**

Order Tanaidacea

Tanaidacea sp. 004

Family Apseudotanaidae

Apseudotanaidae sp. 019

Family Pseudotanaidae

Pseudotanaidae und.

Pseudotanaidae sp. A

Family Tanaidae

Tanaidae und.

Phyllum MOLLUSCA

Class Gastropoda

Gastropoda sp. A

Gastropoda sp. B

Gastropoda sp. C

Gastropoda sp. D

Gastropoda sp. E

Gastropoda sp. F

Gastropoda sp. H

Gastropoda sp. I

Order Mesogastropoda

Family Rissoidae

Rissoidae und.

Erithoidae und

Class Bivalvia

Bivalvia und.

Order Euheterodonta *incertae sedis*

Family Xylophagidae

Genus *Xylophaga* Turton, 1822

***Xylophaga* sp.**

Order Mytiloida

Family Mytilidae

Genus *Idas* Jeffreys, 1876

***Idas* sp.**

Order Solemyoida

Family Solemyidae

***Petrasma* sp.**

Phyllum ECHINODERMATA

Class Stelleroidea

Subclass Ophiuroidea

Ophiuroidea und.