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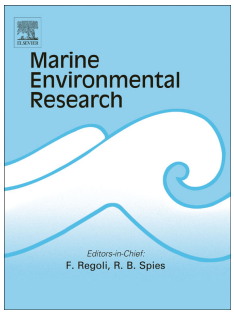

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COLONIZATION OF ORGANIC SUBSTRATES DEPLOYED IN DEEP-SEA REDUCING HABITATS BY SYMBIOTIC SPECIES AND ASSOCIATED FAUNA.

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ABSTRACT

In this study, our goal was to test whether typical vent/seep organisms harbouring symbionts or not, would be able to settle on organic substrates deployed in the vicinity of chemosynthetic ecosystems. Since 2006, a series of novel standardized colonization devices (**CHEMECOLI**: **CHEM**osynthetic **E**cosystem **CO**lonization by **L**arval **I**nvertebrates) filled with three types of substrates (wood, alfalfa and carbonate) have been deployed in different types of reducing habitats including cold seeps in the eastern Mediterranean, a mud volcano in the Norwegian Sea, and hydrothermal vents on the Mid-Atlantic Ridge for durations of 2 weeks to 1 year. For all deployments, highest species diversities were recovered from CHEMECOLIs filled with organic substrates. Larvae from species associated with thiotrophic symbionts such as thyasirid, vesicomyid and mytilid bivalves, were recovered in the eastern Mediterranean and at the Mid-Atlantic Ridge. At the Haakon Mosby Mud Volcano, larvae of symbiotic siboglinids settled on both organic and carbonate substrates. Overall, novel colonization devices (CHEMECOLI) filled with organic substrates attracted both fauna relying on chemosynthesis-derived carbon as well as fauna relying on heterotrophy the latter being opportunistic and tolerant to sulphide.

Key words: symbiosis, settlement, colonization, larvae of invertebrates, *Xylophaga* spp., *Idas* sp., *Thyasira* sp., *Sclerolinum contortum*.

1 INTRODUCTION

Deep-sea reducing ecosystems such as methane seeps and hydrothermal vents are sustained by chemosynthetic prokaryotes which use reduced compounds such as sulphide and methane, and produce organic matter from inorganic sources (Van Dover, 2000). These patchy and ephemeral marine habitats are colonized by symbiotic metazoans species (i.e. metazoans living in association with chemosynthetic bacteria) and associated fauna, depending primarily on metazoan dispersal capabilities during their early life stages (Tyler and Young, 2003), and secondly on food availability, settlement cues and predation/competition (Micheli et al., 2002). Some taxa are shared among the different deep-sea reducing habitats (hydrothermal vents, cold seeps and organic falls) both among symbiotic groups (siboglinid tubeworms, vesicomyid clams, bathymodiolid mussel) as well as among non-symbiotic groups such as dorvilleid, polynoid and spionid polychaetes.

In situ colonization experiments have been carried out over two decades at hydrothermal vents, using polycarbonate plates, basalt rocks, sponges or titanium rings (Van Dover et al., 1988; Shank et al., 1998; Taylor et al., 1999; Mullineaux et al., 1998, 2003; Pradillon et al., 2005, 2009; Kelly et al., 2007; Kelly and Metaxas, 2008), at methane seeps using sediment trays with or without agar layers releasing sulphide (Levin et al., 2006), at the deep-sea floor using natural or exotic sunken wood (Pailleret et al., 2007; Tyler et al., 2007; Voigt et al., 2007) and finally, using natural or artificially sunken whale carcases (Smith and Baco, 2003; Fujiwara et al., 2007; Lorion et al., 2009). But none of these colonization experiments involved deployment of organic substrates deployed directly on methane seeps or hydrothermal vents.

Because communities of metazoan are mostly sessile and dependant on the local chemosynthetic food web, colonization of these fragmented habitats has been assumed to be driven by larval dispersal (Mullineaux and France, 1985). Fertilization and development experiments at a certain pressure and temperature (see review Tyler and Young, 2003) have

recently allowed an estimate of the distance some marine larvae can disperse in oceanic currents (up to 1000 km), but only a few deep-sea species were investigated. Mullineaux et al. (1991, 2005) and Metaxas (2004) studied larval dispersal by sampling the seawater column using tows near deep-sea reducing habitats. However, very few of the recovered larvae could be identified to the species level. Recent techniques such as *in situ* hybridization (ISH) using group- or species-specific oligonucleotide probes (targeting 18S ribosomal RNA) have recently been developed which can circumvent this difficulty (Pradillon et al., 2007; Jones et al., 2008). At the time of settlement for these larvae from deep-sea species that may have dispersed for weeks to months, the geochemical factors triggering settlement into these reducing habitats are not yet known.

To tackle this issue, we conceived a novel colonization device allowing the settlement of early-life stages of organisms that colonize deep-sea reducing habitats excluding large-size predators. We designed a standardized colonization module named **CHEMECOLI** (**CHEM**osynthetic **E**cosystem **CO**lonization by **L**arval **I**nvertebrates) and filled it with organic and inorganic substrates (wood, plant material and carbonates) in order to simulate reducing habitats. Indeed, organic substrates (wood and plant) may produce sulphide through microbial degradation, while carbonate substrates (naturally present in seeps habitats) can be used as a chemically inert hard substrate for settlement. Devices were deployed in several reducing deep-sea habitats, though always away from direct influence of reducing fluids. The localized distribution of hydrothermal vents enables the direct influence of the fluid to be avoided, which is not possible at cold seeps where fluid emissions are widespread. However, sulphide enrichment in these latter habitats is considered to be limited to the sediment where it is produced by sulphate-reducing bacteria (SRB) associated with the anaerobic degradation of methane (Niemann et al., 2006). In all cases, methane enrichment in the water above the seafloor has been described (Charlou et al., 2002, 2003) and its influence on the colonization device cannot be ruled out. The questions to be addressed in this paper are: 1) Are larvae from symbiotic metazoan

93 species able to settle on natural organic substrates deployed in methane seep or hot vent areas? 2)
94 Are vent/seep fauna larvae able to settle on natural organic substrates and hence potentially use
95 them as stepping-stones for dispersal?
96
97 **2 MATERIAL AND METHODS**
98 **2.1 Colonization devices**
99 Standardized colonization devices (CHEMECOLIs, Fig. 1a) were made of a hollow PVC
100 cylinder (14 cm diameter x10 cm high, with a total volume of 1.539 dm^3). The cylinder was
101 drilled with lateral holes to permit circulation of fluids. Devices were filled either with dried
102 alfalfa grass, natural Douglas fir wood cubes (2*2*2 cm), or carbonate cubes (2*2*2 cm)
103 (Fig.1b, c & d). One device could harbour roughly 100 cubes. Substrates were retained within the
104 PVC cylinder by a plastic net of 2 mm mesh. Devices were weighted with stainless steel chain
105 and tagged using syntactic foam. Forty eight hours prior to deployment, wood-loaded devices
106 were soaked in cold filtered seawater in order to overcome the natural buoyancy of dry wood. At
107 each experiment site, a set of 3 CHEMECOLIs, each filled with a different substrate, was
108 deployed. The CHEMECOLIs were deployed and recovered *in situ* by ROV (Remotely Operated
109 Vehicle) or manned submersible. For deployment and recovery, a hermetic box was used with
110 separate compartments for each device, to avoid washing and mixing. Video and photos were
111 recorded during these *in situ* manipulations (Fig.2a & b).
112
113 **2.2 Study sites**
114 CHEMECOLIs were deployed during year 2006 in European waters exploring three
115 distinct reducing habitats of various depth and locations (Fig.3). Two sets of CHEMECOLIs
116 were deployed at the cold seep site ‘Central Zone 2A’ in Pockmark area in the Nile Deep-Sea
117 Fan in eastern Mediterranean (Table 1; Fig.3) (Dupré et al., 2007; Foucher et al., 2009). The first

set was left on the bottom for two weeks during November 2006, and was then replaced with a second set that was deployed at the exact same location for one year by ROV *Quest 4000 (MARUM, Bremen, Germany)* (November 2006 to November 2007; see Table 1). Recovery after one year was done using the ROV *Victor 6000 (Ifremer, France)*. Devices were deployed on outcropping authigenic carbonate crusts which are generally considered to limit the inflow of methane and sulphide from underlying sediments. However, small siboglinid tubeworms *Lamellibrachia* sp. nov., were observed within a crack close to the devices, meaning that direct influence of seepage, at least for the fraction of methane that is not oxidized in the sediment, could not be completely ruled out. Surrounding sediment epifauna included lucinid clams *Lucinoma* aff. *kazani* (Salas & Woodside, 2002) and *Myrtea* sp., mussels *Idas* sp., thyasirid and vesicomyid bivalves, and echinoids. Site 2A is dominated by emission of methane and heavier hydrocarbons, and there is co-occurrence of soft sedimentary and hard carbonate substrates (Dupré et al., 2007; Foucher et al., 2009).

A set of CHEMECOLIs was deployed for 11 months by ROV *Victor 6000 (Ifremer, France)* at the hydrothermal vent field Rainbow close to the Azores Triple Junction on the Mid-Atlantic Ridge (MAR), (Fig.3; Table 1), about 10 m away from an edifice composed of several venting chimneys. No active diffuse flow was detected in the immediate surrounding of the devices, and temperature around devices equalled the seawater baseline temperature of 3.7°C. High temperature fluids at Rainbow are highly enriched in hydrogen, methane, ferrous iron and relatively depleted in sulphide in comparison to other MAR vent sites such as Lucky Strike (Charlou et al., 2002). The high iron content in fluids leads to further depletion in bioavailable sulphide (Schmidt et al., 2007) with a negligible fraction of it in the form of free sulphide (Le Bris and Duperron, in press). Macrofaunal communities at Rainbow are typically dominated by the two shrimps *Rimicaris exoculata* (Williams & Rona, 1986) and *Mirocaris fortunata* (Martin & Christiansen, 1995) that form swarms around walls of large edifices, and by the mussel

Bathymodiolus azoricus (Cosel & Comtet in Cosel, Comtet & Krylova, 1999) which occurs in beds on chimneys and around diffuse vent flows. Recovery of CHEMECOLIs was done by the manned submersible Nautile (*Ifremer, France).*

Finally, one set of CHEMECOLIs was deployed at the Haakon Mosby Mud volcano site (HMMV) in the Norwegian Sea (Fig.3; Table 1). There, methane is abundant at the surface of the sediment but no sulphide was usually detected in seawater above the area of deployment, characterized by the presence of siboglinid tubeworms (Niemann et al., 2006; Lösekann et al., 2008). Bacteria *Beggiatoa* spp. and siboglinid Annelid (*Sclerolinum contortum* (Smirnov, 2000) and *Oligobrachia haakonmosbiensis* (Smirnov, 2000)) constitute the dominant micro-organisms and macro-fauna respectively. Both use hydrogen sulphide for chemoautotrophy, but siboglinids achieve chemosynthesis through the help of symbionts located within the trophosome (Lösekann et al., 2008). Colonization devices were deployed by ROV *Victor 6000 (Ifremer, France)* and recovered by the ROV Quest 4000 *(MARUM, Germany)* after 12 months (Table 1) in the south Hummocky periphery of the mud volcano where there was generally a high density of siboglinids (>50%) and lower density of *Beggiatoa* spp. (<20%) (Jerosh et al., 2007).

2.3 Sample processing methods

Within a few hours of recovery, CHEMECOLIs were sorted on board in a cold room. For each CHEMECOLI, external aspect was recorded and then the top mesh was opened to collect the colonized substrate into a bucket. One fifth of content (or 20 cubes) randomly selected from the bottom, middle and top of the device, was fixed in 4% buffered formaldehyde in twice-filtered seawater (TFSW), 2/5 (or 40 cubes) were fixed in 95% ethanol, 1/5 (or 20 cubes) was fixed for Fluorescence *in situ* hybridization (FISH) analyses (4% buffered formaldehyde in TFSW for few hours at 4°C, rinsed three times in TFSW then transferred into 50/50 Ethanol/TFSW and stored in 95% ethanol), the rest was shared between frozen in 10% glycerol

or/and fixed in buffered 2.5% Glutaraldehyde. Once sorting was completed, the medium in which cubes were sorted was filtered through a 64-µm mesh to recover small fauna that was not attached to substrates, and fixed either in formaldehyde, ethanol, FISH, frozen or Glutaraldehyde. If organisms had settled outside the device, they were fixed but not included in further analyses. All species fixed in buffered formaldehyde were morphologically identified using a dissecting microscope. Wood cubes were dissected in thin slices using wood dissecting scissors in order to collect endofauna. For alfalfa, each piece of grass was examined including the inside of the grass itself. For carbonate cubes, only external observation was carried out. Specimens were counted, identified to the lowest taxonomic level possible, and pictures were taken. Some specimens from the dominant fauna such as polychaetes and molluscs were sent to taxonomists for species identification. Some species recovered from substrates fixed in formaldehyde from CHEMECOLIs deployed at the Nile Deep-Sea Fan and HMMV, were also recovered from substrates fixed in ethanol and used for molecular-based identification using marker genes such as COI and 18S rRNA (see below).

2.4 COI and 18S rRNA genes sequencing

DNA was extracted from soft-tissues of some specimens recovered from CHEMECOLIs deployed at the Nile Deep-Sea Fan (Table 2) and of polychaetes recovered from CHEMECOLIs deployed at HMMV, using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's protocol. Fragments of the mitochondrial cytochrome oxidase I-encoding gene (COImt DNA) were amplified using primers LCO 1490 and HCO 2198 (Folmer et al., 1994). Polymerase Chain Reaction (PCR) was performed as follows: an initial denaturing step of 5 min at 94°C, followed by 35 cycles at 94°C for 30s, 48°C for 40s for hybridization, then 1 min at 72°C, and a final extension for 10 min at 72°C. PCR products were sequenced by Genoscope (France). Alternatively, a ~1800 bp fragment of the18S rRNA gene was amplified with primers 1f and

2023r (Pradillon et al., 2007) using Ex Taq PCR kit (TaKaRa, Kyoto, Japan) and the following PCR protocol: initial denaturation at 96ºC for 5 min, 30 cycles of (96ºC for 1 min, 51ºC for 1 min, and 72ºC for 3 min) and a final extension at 72ºC for 10 min. Amplification products were purified using gel extraction following the manufacturer's protocol (Wizard kit Promega). Sequencing reactions using the PCR products as template were performed with BigDye Terminator Cycling Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA, USA), in both directions, using additional internal primers (see Pradillon et al., 2007). DNA sequencing was conducted with an ABI-PRISM 3130x Genetic Analyser (Applied Biosystems Japan Ltd., Tokyo, Japan) following the manufacturer's protocols. Sequences were inspected by eye and assembled with AutoAssembler 2.1 (Applied Biosystems). All sequences were deposited in the EMBL database under given accession numbers.

2.5 Chemical analyses

For the Nile Deep-Sea Fan and Rainbow experiments, the presence of free sulphide was examined using potentiometric electrodes *in situ* before the recovery of CHEMECOLIs. The potentiometric probe used is equipped with a conventional Ag/Ag_2S electrode (Le Bris et al., 2008; Laurent et al., 2009), which was prepared from a silver wire and calibrated in the laboratory. Above a threshold of c.a. 20 µM the electrode has a logarithmic response that allows quantitative determination of sulphide providing that the pH is known. In agreement with the theoretical response of the Ag/Ag_2S electrode, the slope of the electrode is about 30 mV/ per decade of HS^-, after correction of pH variation. Below this threshold, however, the slope tends to increase and can reach much higher values. This increase in sensitivity is combined with a lower reproducibility which does not allow fully quantitative determination of $[HS^-]$ in the lower part of this range. For this reason, only a maximum concentration could be defined when sulphide was detectable but still lower than 20-30 µM. Using the manipulator arm of the submersible, the tip

of the electrodes (<5 mm total) was placed on different locations, on the top, side and base of the CHEMECOLI devices. In addition to this *in situ* approach, additional measurements were done on board, while the CHEMECOLIs were still in the collection box filled with bottom seawater, the electrodes were inserted among cubes or into alfalfa grass.

2.6 Data analysis

Density of species colonizing wood cubes within a given CHEMECOLI was calculated taking into account that twenty cubes represent a volume of 160 cm^3. To compare densities among the different substrates this volume was also used for the carbonate substrate even if the substrate could not be explored in 3 dimensions. Alfalfa was colonized by organisms inside stems and therefore the 3D was explored. Species recovered from CHEMECOLIs from the Nile Deep-Sea Fan were compared with what was known from Sibuet et al. (1998) and Olu-Le Roy et al. (2004); species recovered from CHEMECOLIs from Rainbow were compared with what was known from Desbruyères et al. (2001) and Desbruyères et al. (2006); species recovered from CHEMECOLIs from HMMV were compared with what was known from Gebruk et al. (2003) and Paxton and Morineaux (2009). For each CHEMECOLI, we counted both the number of new taxon (N) compared to the surrounding habitat and the number of taxon (X) that was already recorded in the surrounding habitat. Biodiversity was estimated based on three different univariate indices using PRIMER v.6 (PRIMER-E): species richness (*S*), Shannon-Wiener index of diversity (*H'*, log (e) such as Kelly and Metaxas (2008)), and Pielou's evenness index (*J'*).

3 RESULTS

3.1 Species recovered within CHEMECOLIs

For each experiment we tried to assign organisms to described species (Sibuet et al., 1998; Desbruyères et al., 2001; Gebruk et al., 2003; Olu-Leroy et al., 2004; Desbruyères et al.,

243 2006 and Paxton and Morineaux, 2009). DNA barcode sequences (COI mitochondrial gene)
244 were obtained from specimens of the two mollusc gastropods *Coccopigya* sp. and *Clelandella*
245 *myriamae* (Gofas, 2005), for *Idas* sp., and for the Annelid *Prionospio* sp., all from modules
246 deployed in Nile Deep-Sea Fan (Table 2). COImt DNA sequences were compared with the NCBI
247 nucleotide database. The sequence from *Idas* sp. matched 100% against Genbank accession no
248 **EF210072-1b** obtained from adult *Idas* sp. specimens from the Central Zone in the eastern
249 Mediterranean during the 2003 Nautinil cruise (Duperron et al., 2008). Other sequences did not
250 match 100% with any published sequence. The 18S rRNA sequence obtained from one
251 polychaete species, *Paramphinome* sp. (J. Blake) recovered within both organic substrates from
252 CHEMECOLIs deployed in HMMV, was compared with the Genbank database and was
253 identified as identical with a sequence from *Paramphinome jeffreysi* (McIntosh, 1868).
254 Overall, a total of 33 taxa were recovered from all CHEMECOLIs deployed in the three
255 study areas hosting reducing habitats. Main groups recovered were Mollusca (bivalves and
256 gastropods) and Annelida (polychaetes), followed by Arthropod Crustacean, Nemertea,
257 Sipuncula, Foraminifera, Actinopodia and Echinoderma (Table 3). Species recovered varied
258 among study sites, but also among the different CHEMECOLI substrates deployed within a
259 given study site (Table 3). For both the Rainbow and the Nile Deep-sea Fan 1-year experiments,
260 more than 50% of the species encountered within both organic and carbonate substrates were not
261 previously reported in the surrounding chemosynthetic communities. In the HMMV experiment,
262 only 30% of the fauna recovered from CHEMECOLIs was not documented in the surrounding
263 habitat. Overall densities of specimens (Table 3) were the highest for organic substrates and
264 especially for the wood substrate, reaching 14,987.5 specimens per dm^3 in the CHEMECOLI
265 deployed at HMMV due to the high number of wood-borers that colonized a cube (~117
266 specimens).

Mollusc dominated as colonists within CHEMECOLIs deployed for 2-weeks and 1-year experiments at the Nile Deep-Sea Fan (Fig. 4a). High densities of bivalves were counted within the wood substrates due to the abundance of wood-boring bivalves (*Xylophaga dorsalis* (Turton, 1819)) (~287.5 specimens per dm^3 for the short-term 2-week experiment and 525 specimens per dm^3 for the long-term 1-year experiment), while gastropods were more abundant on the alfalfa substrate (~300 specimens per dm^3), along with polychaetes (~ 200 specimens per dm^3). In the long-term experiment, polychaetes as well densely colonized the wood substrate (~200 specimens per dm^3). Lower densities of crustaceans (max ~60 specimens per dm^3) and Sipuncula were also present on organic substrates in the long-term experiment. Carbonate substrates deployed for one year were colonized only by low densities of gastropods *Clelandella myriamae*, hesionid polychaetes and sipuncle (Table 3). Young settler-stages (juveniles) of symbiotic bivalve species harbouring sulphur-oxidizing bacteria were successfully recovered. These included *Idas* sp., *Vesicomyid* sp. and *Thyasira* sp. (Fig. 5a). These three species were all recovered within the wood substrate after the two-weeks and after the one-year experiments. *Idas* sp. was also recovered in the alfalfa substrate after the one-year experiment (Fig. 5a).

CHEMECOLIs recovered after one year at the Rainbow site, were poorly colonized compared to CHEMECOLIs deployed into cold seep habitats for one year. Wood-boring bivalves (*Xylophaga atlantica* (H.G. Richards, 1942)) dominated in the wood-containing device, and juveniles of symbiotic bivalves were also recovered (~200 specimens per dm^3, Table 3, Fig. 5b) similar morphologically to vesicomyid juveniles, however a molecular identification is necessary to ascertain this observation such as ISH (see introduction). Some polychaetes (*Prionospio* sp. nov. 3, ~40 specimens per dm^3) were recovered within both wood and alfalfa substrates (Fig. 4b).

CHEMECOLIs deployed for one year at the HMMV were densely colonized, especially the wood and alfalfa substrates (Fig. 4c). Wood-boring bivalves (*Xyloredo ingolfia* (Turner, 1972)) were dominant in the wood substrate reaching the highest density documented in our

292 study with 14,412.5 specimens per dm^3. The gastropod *Alvania* aff. *griegi* occurred on the alfalfa
293 substrate reaching ~220 specimens per dm^3. Polychaetes occurred on all substrates, more
294 abundant on the wood (400 specimen per dm^3) but were as well present in high densities in
295 alfalfa and carbonate substrates (>100 specimens per dm^3). Juveniles of the chemosymbiotic
296 species *Sclerolinum contortum* (Fig. 5c) were recovered inside all three CHEMECOLIs,
297 displaying tube lengths ranging from few mms to few cms (Table 3). Adult siboglinid tubeworms
298 were attached to the outside mesh of each colonization device that was in contact with the soft
299 sediment (but not counted as not *within* the mesh). Among crustaceans (Fig. 4c), amphipods and
300 copepods occurred on wood and alfalfa substrates, while *Metacaprella* sp. occurred on all three
301 substrates (Table 3).

302 Species richness (*S*), Shannon-Wiener diversity (*H'*) and Pielou's evenness (*J'*) indices
303 differed among substrates at all deployment sites (Table 4). No statistical support can be
304 summoned due to the lack of replicates, however for each study site, *S* and *H'* were the highest
305 for wood and alfalfa substrates compared to carbonate substrates, except for the wood substrate
306 in HMMV where the species richness is the highest but Shannon-Wiener diversity index is very
307 low due to the dominance of the species *Xyloredo ingolfia* that gives an equitability of 0.1 (*J'*).
308

309 **3.2 Chemical data**

310 *In situ* punctual sulphide measurements on colonization devices after one year
311 deployment at the Nile Deep-Sea Fan did not reveal any sulphide enrichment in their immediate
312 surrounding before recovery (data not shown). Yet very low sulphide levels, micromolar to
313 submicromolar in alfalfa and wood respectively, were detected inside the devices after recovery
314 suggesting that sulphide had been produced (Table 5). Measurement in the immediate vicinity
315 (<1 m from colonization device) conversely revealed that local free sulphide enrichment could be

316 detected in the cracks of the underlying carbonate pavement (data not shown). Sulphide was
317 however not detected in the water above these cracks.
318 At the Rainbow hydrothermal vent, no free sulphide was detected *in situ* at the top, side
319 or basis of the devices. On board, neither the alfalfa pieces, nor the wood cube devices displayed
320 significant sulphide enrichment when the electrodes were inserted deeper inside. Compared to
321 Nile deep-sea fan, the situation is however quite different in Rainbow since free sulphide is also
322 extremely low even in the habitat of *Bathymodiolus* mussels or shrimps in the immediate vicinity
323 of the venting chimneys.
324

325 **4 DISCUSSION**

326 The goal of this study was to test whether organic substrates within CHEMECOLIs
327 supported the colonization of marine invertebrates' endemic from geologically-driven reducing
328 habitats such as hydrothermal vents and cold seeps, and to test whether larvae of symbiont-
329 bearing metazoans were able to settle on these substrates hence potentially use them as stepping-
330 stones for dispersal. For both questions we can respond positively.
331

332 **4.1 Density of species and species richness recovered within CHEMECOLIs**

333 The most abundant taxa recovered from CHEMECOLIs were molluscs (bivalves and
334 gastropods) and annelid polychaetes. This observation fits with the results of previous
335 colonization experiments in deep-sea reducing habitats (Van Dover et al., 1988; Shank et al.,
336 1998; Mullineaux et al., 1998; Smith and Baco, 2003; Levin et al., 2006; Fujiwara et al., 2007;
337 Pailleret et al., 2007; Kelly and Metaxas, 2008; Pradillon et al., 2009). In the Nile Deep-Sea Fan
338 (short-term and long-term), HMMV and MAR experiments, organic substrates (wood and
339 alfalfa) displayed higher metazoan abundances compared to carbonate substrates. The high
340 bivalve abundances reported for wood-filled CHEMECOLIs are explained by the high densities

of wood-boring bivalves represented by a single species per site. Overall, thirty to sixty percent of species which have colonized wood substrates deployed in the eastern Mediterranean, in the Mid-Atlantic Ridge and in the Norwegian Sea, have not been described previously from the surrounding seep or vent communities (Table 3).

Several physical and chemical factors may influence the settlement of larvae of species endemic to deep-sea reducing habitats. Kelly et al. (2007) suggested that settlement of colonists on basaltic plates deployed at hydrothermal vents was correlated with temperature and hydrogen sulphide. Similarly, temperature was presented as a cue for colonists to locate preferred habitat conditions in a gradient of hydrothermal vent flow (Bates et al., 2005). Both these parameters are tracers of the impact of vent fluids on the environment. It is therefore impossible to discriminate a direct influence of sulphide from other factors correlated to the vent fluid ratio. CHEMECOLIs at the vent site Rainbow were deployed ten meters away from chimneys and species abundances were very low compared to the two other experiments carried out in cold seep habitats. A low supply of larvae in the particular period 2006-2007 at Rainbow may explain this result as shown in Pacific hydrothermal vent colonization experiments for particular years (Kelly et al., 2007). The distance from active venting results in no significant signature of fluid emission in the surrounding water, as evidenced by the low background temperature measured (3.5°C). The lack of any attractive signature of fluid venting could be an alternative explanation for the low rates of colonization observed at Rainbow. Kelly et al. (2007) nevertheless demonstrated that vent species were recovered at a similar distance from vents, even though settlement and post-settlement survival rates were lower compared to basaltic plates deployed closer to the vent edifice. The difference in fluid geochemistry (low free H_2S and high rate of precipitation close to vent sources at Rainbow) might also contribute to the difference between those experiments.

Species richness calculated from our three *in situ* experiments (Table 4) were in the order of magnitude reported by Kelly & Metaxas (2008) from colonization experiments at hydrothermal vents, where *S* ranged from 11 on sponge substrates to 6 on basaltic plates. Species richness from our experimental study were however, lower than those obtained from colonization experiments carried at methane seeps by Levin et al. (2006) where *S* values were between 16-19 species and where sulphide added to tray experiments increased the species richness. Kelly & Metaxas (2008) hypothesised that the physical structure may have influenced the species richness in their experiments, with complex physical structure of the sponge favouring a more diverse faunal assemblage by creating interstitial spaces that could provide protection against predation. In our experiments, degradation of wood by wood-boring bivalves increased the area available for colonization by other species such as spionid polychaetes at the Nile Deep-Sea Fan or at Rainbow (pers. observ.). Similarly, available surfaces in alfalfa-filled colonizers were large. In both organic substrates, micro-niches offered a variety of chemical micro-environments similar to sponges in Kelly & Metaxas (2008) allowing more species to colonize and therefore increasing the species richness. These probably differentiate compared to carbonate substrates, where species richness was more similar to that recovered on basaltic plates in Kelly & Metaxas (2008).

4.2 Occurrence of species harbouring sulphur-oxidizing bacteria in CHEMECOLIs

Results from both the two-weeks and one-year experiments at the Nile Deep-Sea Fan suggested a sulfophilic stage, which is a particular stage in the faunal succession, documented from whale carcasses, during which chemosynthetic fauna harbouring thiotrophic symbionts establishes (Smith and Baco, 2003), as indicated by the presence of *Vesicomyid* sp., *Thyasira* sp. and *Idas* sp. These taxa, also reported from whale falls and methane seeps, are typical from reducing ecosystems (Kiel and Godaert, 2006). Indeed, all known members of families

Vesicomyidae and Thyasiridae harbour sulphur-oxidizing bacterial symbionts (Peek et al., 1998; Taylor et al., 2007; Dubilier et al., 2008). Regarding *Idas* sp., barcoding demonstrated that it was the same species previously documented to harbour 6 distinct bacterial symbionts, including sulphur- and methane-oxidizing bacteria and found at cold seeps in the eastern Mediterranean (Duperron et al., 2008). Phylogenetically, *Idas* sp. clusters within the large group which includes all large mussels (*Bathymodiolus*) associated with deep-sea seeps and vents as well as many smaller species associated with organic falls worldwide (Duperron et al., 2008, Lorion et al., 2009). In our *in situ* experiment, *Idas* sp. was found associated with the two organic substrates, wood and alfalfa whereas *Thyasira* sp. and *Vesicomyid* sp. juveniles settled on wood cubes but were not recovered in the alfalfa.

At Rainbow, juveniles bivalve morphologically similar to vesicomyid specimens were recovered within the wood substrate. Interestingly, vesicomyid clams are not, to date, reported from the Northern Mid-Atlantic Ridge (Desbruyères et al., 2001) and only further studies using DNA sequencing (Comtet et al., 2000) may ascertain their identification.

Sclerolinum contortum (Monolifera siboglinid) was recovered from all 3 substrates at HMMV. According to Sahling et al. (2005), the genus *Sclerolinum* occurs at hydrothermal vents, methane seeps, decaying wood and other organic falls. *S. contortum* occurs buried 15 cm deep in the sediment at HMMV, where sulphide concentrations are around 0.15 mM, and harbours sulphide-oxidizing autotrophic symbionts within its trophosome (Lösekann et al., 2008). We herein confirm that *S. contortum* can settle and grow in decaying plant debris, in wood and mineral substrates. Specimens recovered within the wood substrate were very tiny (only few mms) compared to those from alfalfa and carbonate substrates (few cms). The high densities of wood-boring bivalves per wood cube may have limited the development of *S. contortum* in the latter CHEMECOLI. Concentrations of sulphide are certainly different within these three substrates and may also have affected the growth, survival rate and development of this

symbiotic tubeworm. Adults of *S. contortum* were recovered at the bottom of the three CHEMECOLIs deployed at HMMV. Larvae must have been released in the three devices by mature reproductive adults from below. In soft sediments, abundance of siboglinid frenulates is documented to be inversely correlated with the abundance of other benthic fauna (Dando et al., 2008). Besides, frenulates usually 'mine" insoluble sulphide into an anoxic zone within the sediment by their root that is deeply buried in this area (Dando et al., 2008).

4.3 New species, opportunistic species and tolerance to sulphide

The biodiversity of Nile Deep-Sea Fan and HMMV cold seeps is still under investigation (Vanreusel et al., 2009), and some of the species recovered within CHEMECOLIs may not yet be documented from the background fauna. Gebruk et al. (2003) have listed 19 species so far from HMMV and Olu-Le Roy et al. (2004) have numerated 27 species from 4 mud volcanoes located in the eastern Mediterranean. At least some recovered species specialized in the exploitation of organic substrates are probably not present as adults in the surrounding habitat in our experiment. Wood-boring bivalves from the family Xylophagainae were for example recovered from all wood samples, albeit with different species (T. Haga). *Xylophaga* spp. are opportunistic species in the deep-sea which settle exclusively on sunken wood (Turner et al., 1977; Tyler et al., 2007). Small cocculiniform gastropods identified as *Coccopigya* sp. (maybe 2 new species according to A. Warén) and rissoid gastropods identified as *Alvania* aff. *griegi* (A. Warén) were recovered within the alfalfa-filled CHEMECOLIs from the Nile Deep-Sea Fan and HMMV, respectively. Juveniles of a new *Alvania* species (sp.1) were recovered at the HMMV site in dense aggregations by Gebruk et al. (2003) and could be the same species as retrieved from the alfalfa-filled colonizer. The genus *Alvania* is commonly found around hot vents, on sulphidic rocks or at the base of black smokers (Desbruyères et al., 2006) and the species *A.* aff. *griegi* was described

130 years ago from a piece of sunken driftwood (Friiele, 1879, A. Warén personal communication).

Three new species of Spionidae polychaetes (within genus *Prionospio*) were identified within devices containing organic substrates: two from CHEMECOLIs deployed at the Nile Deep-Sea Fan, and a third from devices deployed at Rainbow (J. Blake). According to Sigvaldadottir & Desbruyères (2003), only five polychaetes species occurred at Rainbow including two species of Spionidae *P. unilamellata* (Sigvaldadottir & Desbruyères, 2003) and *Laonice asaccata* (Sigvaldadottir & Desbruyères, 2003). Spionid polychaetes are considered as opportunistic species in the deep-sea fauna (Van Dover et al., 1988) and very tolerant to sulphide exposure (Desbruyères et al., 2001; Levin et al., 2006). A new species of Glyceridae, *Glycera* sp. nov. (M. Böggemann) was recovered from biogenic substrates deployed in Nile Deep-Sea Fan. Glyceridae polychaete such as *G. dibranchiate* (Ehlers, 1868) from mudflats has been shown to be highly tolerant to sulphide exposure (Hance et al., 2008) and this may explain why a new species of *Glycera* was recovered from both biogenic substrates in CHEMECOLIs deployed at the Nile Deep-Sea Fan.

Other opportunistic species were encountered within CHEMECOLIs such as the Amphinomidae polychaete *Paramphinome jeffreysi* identified to the species level as a result of 18S rRNA sequencing (A. Nunes Jorge). A great number of Amphinomidae have been reported associated with the siboglinid *Sclerolinum contortum* at HMMV (Gebruk et al., 2003) but they were not identified to the species level. This opportunistic species is generally associated with other polychaete species such as *Prionospio steenstrupi* (Malmgren, 1867), *Capitella capitata* (Fabricius, 1780) and *Heteromastus filliformis* (Claparède, 1864) in soft marine sediment enriched in organic matter and associated with the symbiotic thyasirid bivalve *Thyasira sarsii* (Philippi, 1845) (Kutti et al., 2007). At HMMV, a nematode *Halomonhystera disjuncta* (Bastian,

1865) that is usually described in intertidal organically enriched mudflats was also found in high abundance in the microbial mats area (Vanreusel et al., 2009).

Another opportunistic polychaete species, *Ophryotrocha* sp., was found within wood substrate deployed at HMMV and the genus *Ophryotrocha* is common in organics- enriched habitats (Rouse and Pleijel, 2001). This species is morphologically similar to the one newly described by Paxton & Morineaux (2009), *O.* cf. *spatula* (Fournier & Conlan, 1994) that lives in the white microbial mats and nearby sediments at HMMV. Levin et al. (2006) also recovered one species of dorvilleid polychaete within tray with sulphide deployed within the seep: *O. platykephale* (Blake, 1985) that lives as well on bacterial mats in the methane seeps at the northern California margin. This species can tolerate up to 1mM concentration of sulphide (Levin et al., 2006).

Overall non-symbiotic fauna associated with symbiotic fauna recovered within CHEMECOLIs filled with wood and alfalfa substrates, were opportunistic and tolerant to sulphide confirming that CHEMECOLIs actually mimic reducing habitats.

4.4 Sulphide production from organic substrates and potential role in the colonization process

Sulphide enrichment in sunken woods has been reported in a number of studies related to archaeological shipwreck and sulphide concentration exceeding hundreds of micromolar have been recently documented at the surface in naturally sunken wood in a mangrove swamp (Laurent et al. 2009). Sulphide production from wood decomposition is considered to result of the activity of anaerobic sulphate-reducing bacteria (SRB). Palacio et al. (2006) have measured cellulolytic activities in Fir wood after 2 months in laboratory experiments using Mediterranean seawater at 14°C (same temperature recorded at the Nile Deep-Sea Fan). The dynamics of

487 sulphide enrichment from this process is still poorly understood. Especially, nothing is known for
488 the deep environments considered in this study (> 1500 m).

489 Only micromolar to submicromolar levels of sulphide were recorded within both organic
490 substrates from CHEMECOLI deployed in eastern Mediterranean recovered on board, much less
491 than measured on natural sunken wood in a tropical shallow water mangrove (Laurent et al.,
492 2009). The fact that sulphide was undetectable *in situ* in contact with the CHEMECOLI indicates
493 that the influence of substrate degradation on the micro-environment was insignificant with
494 respect to this parameter at the time of recovery (i.e. after one year). The presence of species
495 known to harbour sulphide-oxidizing symbionts and of numerous sulphide-tolerant species
496 characteristic of sulphidic habitats suggests that the presence of these species is not necessarily
497 related to high sulphide content. Two hypotheses can be proposed to explain this result: 1) the
498 microenvironment has been enriched to a larger extent over a limited period of time preceding
499 the recovery and colonizers still reflect these earlier sulphide-enriched stages; 2) sulphide
500 production by SRB is just starting and thiotrophic species settlement is triggered by indirect clues
501 correlated to sulphide production, such as compounds produced by cellulose-degrading bacteria.
502 This stresses the need for a better characterisation of the variability of physico-chemical
503 conditions over time in future sunken wood deep-sea experiments. The fact that taxa such as
504 thyasirid, vesicomyid and mytilid bivalves were already recovered after 2 weeks in the Nile
505 Deep-Sea Fan wood experiment, whereas these species were absent from the carbonate
506 colonization device, supports the latter hypothesis. However, the idea that the presence of
507 organic matter is the only factor triggering the settlement of these species should be taken with
508 caution since methane, and even sulphide enrichment in the local environment can not be
509 excluded and may have influenced the settlement of these bivalves. A 2-week record on the
510 carbonate at the base of one colonization device indeed revealed pulsated sulphide enrichment,
511 likely originating from a crack located beneath it (Le Bris et al., 2008).

5 CONCLUSION

This simple, standardized, colonization device, CHEMECOLI, was designed to recover young stages of endemic fauna from reducing habitats and to study colonization processes in the absence of large predators. Overall, colonization devices deployed at the Nile Deep-Sea Fan, HMMV, and Rainbow were mostly colonized by molluscs (gastropods or bivalves) and annelid (polychaetes), and juvenile stages were successfully recovered. Organic substrates were more densely colonized than the carbonate substrate. Wood-boring bivalves were found on every wood substrate at all sites, although with different species. Organic substrates yielded several species presumably harbouring chemosynthetic bacterial symbionts, as well as associated heterotrophic fauna common at hot vents, methane seeps and organic falls and tolerant to sulphide. Species that were not previously reported in these reducing habitats, but that were common in other type of reducing habitats, were also present.

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772 **Table 1.** Sampling details of deployment and recovery of CHEMECOLIs including dates, cruises, latitude & longitude, depth and substrates.

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Substrates	Deployment date and dive	Cruises	Sites	Details of sites	Recovery date & dive	Cruises
Alfalfa Wood Carbonate	06/06/2006 Dive 277-7	VICKING	Haakon Musby Mud Vulcano (HMMV)	72°00’14’’N, 14°43’22’’E, 1257m	29/06/2007 Dive 166-3	ARK XXII/1b
Alfalfa Wood Carbonate	21/08/2006, Dive 292-9	MOMARETO	Rainbow/ MAR	36°13’76’’N, 33°54’19’’W, 2300m	14,15/07/2007 Dives 1676&1677	MOMARDREAM
Wood Carbonate	4/11/2006 Dive 120	BIONIL	Nile Deep-Sea Fan/eastern Mediterranean	32° 31’97’’N, 30°21’18’’E, 1693m	18/11/2006 Dive 126	BIONIL
Alfalfa Wood Carbonate	18/11/2006 Dive 126	BIONIL	Nile Deep-Sea Fan/ eastern Mediterranean	32°31’97’’N, 30°21’18’’E, 1693m	10/11/2007 Dive 338-17	MEDECO

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780 **Table 2.** Details of the specimens from the MEDECO cruise sampled in eastern Mediterranean
781 at the Nile Deep-Sea Fan, which mitochondrial partial COI gene for cytochrome oxidase subunit
782 I have been sequenced and recorded into the EMBL database with GenBank accession number.

Species	Specimens	Substrates	Site details	COI accession number
Clelandella myriamae	1	Alfalfa	32°31’97’’N,	**FM212782**
Clelandella myriamae	2	Alfalfa	30°21’17’’E,	**FM212783**
Clelandella myriamae	3	Alfalfa	1693m	**FM212784**
Coccopigya sp.	1	Alfalfa		**FM212785**
Coccopigya sp.	2	Alfalfa		**FM212786**
Idas sp.	1	Alfalfa		**FM212787**
Prionospio sp.	1	Wood		**FM212788**
Prionospio sp.	2	Alfalfa		**FM212789**
Prionospio sp.	3	Alfalfa		**FM212790**
Prionospio sp.	4	Alfalfa		**FM212791**
Prionospio sp.	5	Alfalfa		**FM212792**

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796 **Table 3.** List of taxa identified within CHEMECOLIs and their estimated densities per dm^3
797 within the device. N: is for taxon that is a new record for the site and X is for taxon that has been
798 recorded already in the surrounding habitat. 1) short-term experiment at the Nile Deep-Sea Fan:
799 MW1 (wood substrate) and MC1 (carbonate substrate); 2) long-term experiment at the Nile
800 Deep-Sea Fan: MW2 (wood substrate), MA2 (alfalfa substrate) and MC2 (carbonate substrate);
801 3) long-term experiment at Rainbow: RW (wood substrate), RA (alfalfa substrate) and RC
802 (carbonate substrate; 4) long-term experiment at HMMV: HW (wood substrate), HA (alfalfa
803 substrate) and HC (carbonate substrate).
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Species list	**EASTERN MEDITERRANEAN NILE DEEP-SEA FAN**					**ATLANTIC OCEAN RAINBOW**			**NORWEGIAN SEA HMMV**		
Densities per dm^3	**2 weeks**		**1 year**			**1 year**			**1 year**		
	MW1	**MC1**	**MW2**	**MA2**	**MC2**	**RW**	**RA**	**RC**	**HW**	**HA**	**HC**
MOLLUSCA											
Bivalvia											
Xylophaga dorsalis (including juvenile)	N287.5	-	N525	N18.75	-	-	-	-	-	-	-
Xylophaga atlantica	-	-	-	-	-	N 150	-	-	-	-	-
Xyloredo ingolfia	-	-	-	-	-	-	-	-	N14412.5	-	-
Idas sp. Med (including juvenile)	X12.5	-	X31.3	X12.5	-	-	-	-	-	-	-
Thyasira sp. (juvenile)	X25.0	-	X6.3	-	-	-	-	-	-	-	-
Vesicomyid sp. (juvenile)	X37.5	-	X12.5	-	-	-	-	-	-	-	-
Unidentified bivalves juveniles	-	-	-	-	-	X43.8	-	-	-	-	-
Gastropoda											
Clelandella myriamae (juvenile)	X6.3	X18.75	-	X168.8	X18.8	-	-	-	-	-	-
Coccopigya sp. (juvenile)	-	-	N25	N112.5							
Alvania aff. *griegi* (juvenile)	-	-	-	-	-	-	-	-	-	N225	-
Caenogastropod sp.	-	-	-	N6.3	-	-	-	-	-	-	-
ANNELIDA											
Polychaeta											
Prionospio sp. nov.1	-	-	N75	-	-	-	-	-	-	-	-
Prionospio sp. nov.2	-	-	-	N143.8	-	-	-	-	-	-	-
Prionospio sp. nov.3	-	-	-	-	-	N18.8	N12.5	-	-	-	-
Glycera sp. nov.	-	-	N43.8	N25	-	-	-	-	-	-	-
Eupolymnia sp.	-	-	N6.3	-	-	-	-	-	-	-	-
Nicolea sp.	-	-	-	N12.5	-	-	-	-	-	-	-
Sclerolinum contortum (juvenile)	-	-	-	-	-	-	-	-	X68.8	X18.8	X93.8

Unidentified polychaete juvenile	-	-	-	-	-	-	-	-	X68.8	X43.8	-
Unidentified polychaete larvae	25	-	-	-	-	-	-	-	-	-	-
Paramphinome jeffreysi	-	-	-	-	-	-	-	-	X225	X43.8	-
Ophryotrocha cf. *spatula*	-	-	-	-	-	-	-	-	X25	-	-
Flabelligerid sp.	-	-	-	-	-	-	-	-	-	N6.3	N12.5
Hesionid sp.	-	-	-	N6.3	N6.3	-	-	-	-	-	-
Unknown Polychaete	-	-	-	N6.3	-	-	-	-	-	-	-
ARTHROPODA											
Crustacea											
Amphipod spp.	62.5	-	18.8	12.5	-	-	-	-	162.5	93.8	-
Metacaprella sp.	-	-	-	-	-	-	-	-	X6.3	X6.3	X12.5
Copepod spp	12.5	-	-	-	-	-	-	-	6.3	6.3	-
Chelicerata											
Pycnogonid sp.	-	-	-	-	-	-	-	X6.3	-	-	-
NEMERTEAN											
Nemertean spp.	-	-	-	-	-	X6.3	X43.8	-	N6.3	N43.8	-
SIPUNCULA											
Sipuncle sp.	-	-	N25	N12.5	N6.3	-	-	-	-	-	-
FORAMINIFERA											
Foraminifera sp.	-	-	-	-	-	N68.8	-	N9.7	-	-	-
ACTINOPODIA											
Actinopod sp.	-	-	-	-	-	-	N6.3	-	-	-	-
ECHINODERMA larvae	-	-	-	-	-	-	-	-	N6.3	-	-
Total density	468.8	18.8	768.8	537.5	31.2	287.5	62.5	16.0	14987.5	487.5	118.8
Ratio of N: species from surrounding habitat	0.13	-	0.6	0.75	0.66	0.6	0.66	0.5	0.3	0.33	0.33
Ratio of X : species from surrounding habitat	0.13	1	0.3	0.17	0.33	0.4	0.33	0.5	0.5	0.44	0.66

Table 4. Univariate indices of colonist assemblage structure from the different substrates within CHEMECOLIs deployed: at the Nile Deep-Sea Fan: short-term experiment, MW1 (wood substrate), MC1 (carbonate substrate), and long-term experiment, MW2 (wood substrate), MA2 (alfalfa substrate) and MC2 (carbonate substrate); at Rainbow: RW (wood substrate), RA (alfalfa substrate) and RC (carbonate substrate); at Haakon Mosby Mud Volcano (HMMV): HW (wood substrate), HA (alfalfa substrate) and HC (carbonate substrate). *S*: species richness, N: the number of individuals, *H'*: Shannon-Wiener diversity and *J'*: Pielou's evenness.

	S	N	*J'*	*H'*
Nile Deep-sea Fan				
2 weeks				
MW1	8	75	0.64	1.33
MC1	1	3	-	-
1 year				
MW2	10	123	0.54	1.24
MA2	12	86	0.73	1.80
MC2	3	5	0.86	0.95
Rainbow				
1 year				
RW	5	46	0.76	1.23
RA	3	10	0.73	0.80
RC	2	4	0.81	0.56
HMMV				
1 year				
HW	10	2398	0.01	0.22
HA	9	78	0.74	1.62
HC	3	19	0.6	0.66

Table 5. Detection of H_2S at the periphery or centre of the CHEMECOLI devices filled with organic substrates deployed at the Nile Deep-Sea fan for one year after their recovery on board during the MEDECO cruise (IFREMER) in 2007: MW2 (wood substrate) and MA2 (alfalfa substrate).

MW2	
Periphery	undetectable
Periphery	undetectable
Center - 1	< 10 µM
Center - 2	< 1 µM
Center - 3	< 10 µM
MA2	
Periphery	undetectable
Center - 1	< 20 µM
Center - 2	< 10 µM

Figures caption

Fig. 1. Description of a CHEMECOLI: a: colonization device filled with cubes of Douglas fir (scale bar equalled to 3.3 cm); b: carbonate substrate; c: wood substrate; d: alfalfa dried substrate.

Fig.2. CHEMECOLIs deployed *in situ* in 2006 at Haakon Mosby Mud Volcano during the VICKING cruise (IFREMER) (a), and CHEMECOLIs recovered in eastern Mediterranean at the Nile Deep-Sea Fan during the MEDECO cruise (IFREMER) (b).

Fig.3. Map (after Interridges website) of study sites where CHEMECOLIs were deployed: at Haakon Mosby Mud Volcano in the Norwegian Sea; at Rainbow on the Mid-Atlantic Ridge and at the Nile Deep-Sea Fan in eastern Mediterranean.

Fig.4. Densities of the 4 dominant groups of invertebrates that have colonized the CHEMECOLI within the 3 different substrates. a- short-term experiment at the Nile Deep-Sea Fan: MW1 (wood substrate) and MC1 (carbonate substrate); long-term experiment at the Nile Deep-Sea Fan: MW2 (wood substrate), MA2 (alfalfa substrate) and MC2 (carbonate substrate); b-at Rainbow: RW (wood substrate), RA (alfalfa substrate) and RC (carbonate substrate; c-at Haakon Mosby Mud Volcano: HW (wood substrate), HA (alfalfa substrate) and HC (carbonate substrate).

Fig.5. Densities of thiotrophic species versus non thiotrophic species that have colonized the CHEMECOLI within the 3 different substrates. a- short-term experiment at the Nile Deep-Sea Fan: MW1 (wood substrate) and MC1 (carbonate substrate); long-term experiment at the Nile Deep-sea Fan: MW2 (wood substrate), MA2 (alfalfa substrate) and MC2 (carbonate substrate);

876 b-at Rainbow: RW (wood substrate), RA (alfalfa substrate) and RC (carbonate substrate; c-at
877 Haakon Mosby Mud Volcano: HW (wood substrate), HA (alfalfa substrate) and HC (carbonate
878 substrate).
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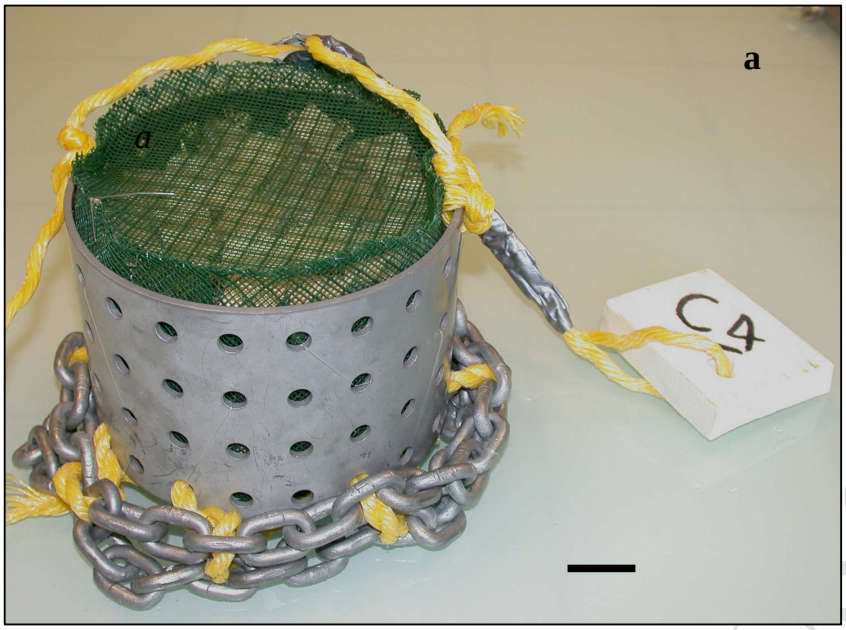
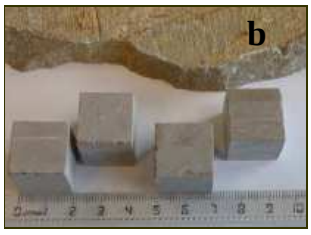
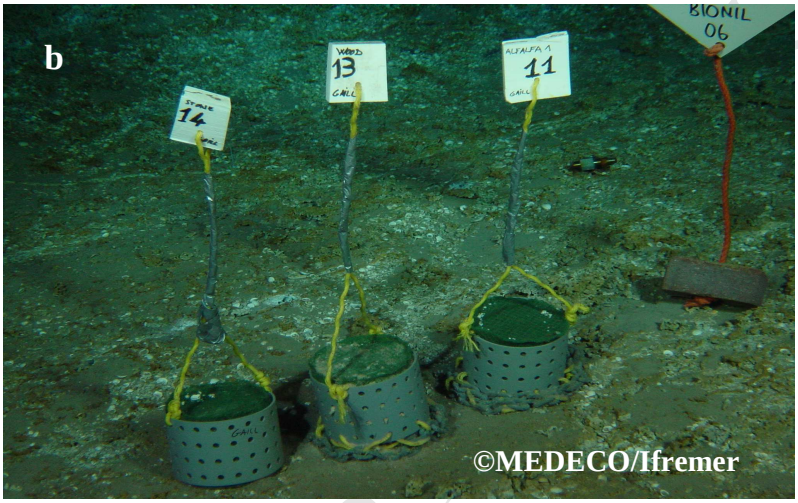
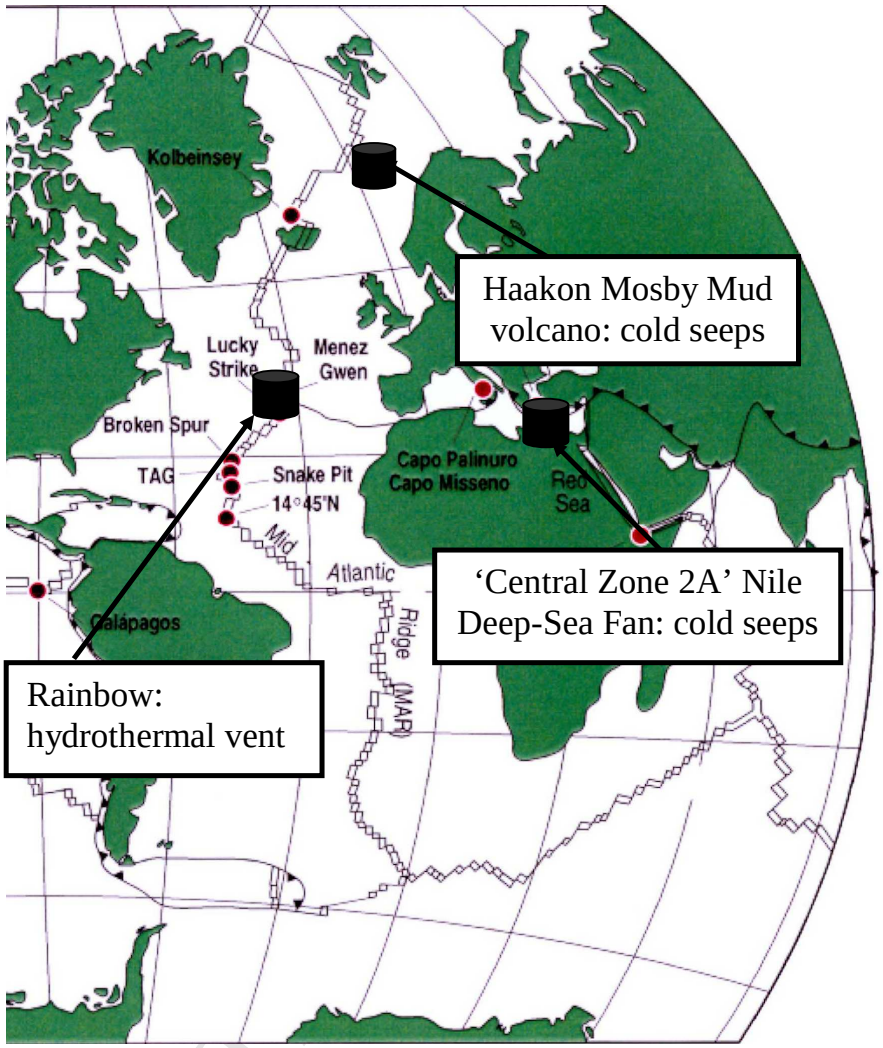
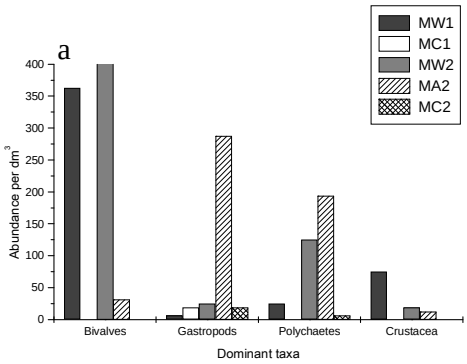
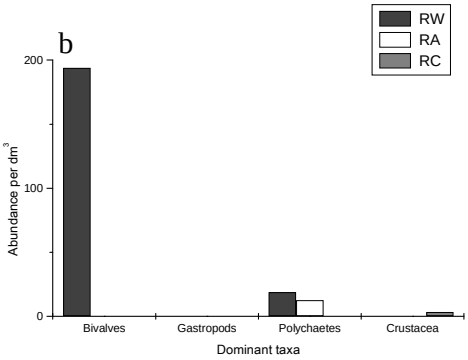


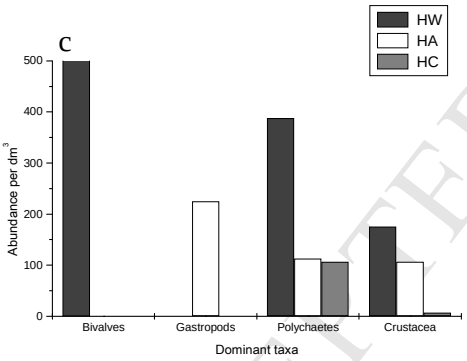





Kolbeinsey
Haakon Mosby Mud volcano: cold seeps
Lucky Strike
Menez Gwen
Broken Spur
TAG
Snake Pit
14°45'N
Capo Palinuro
Capo Misseno
Red Sea
Mid
Atlantic
Ridge (MAR)
Galápagos
'Central Zone 2A' Nile Deep-Sea Fan: cold seeps
Rainbow: hydrothermal vent







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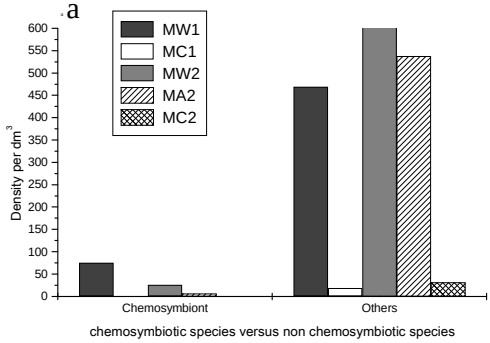

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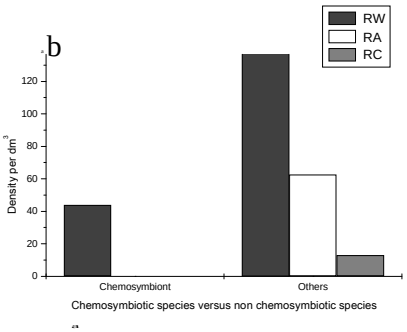

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