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► **To cite this version:**

S.M. Gaudron, F. Pradillon, M. Pailleret, Sébastien Duperron, N. Le Bris, et al.. Colonization Of Organic Substrates Deployed In Deep-Sea Reducing Habitats By Symbiotic Species And Associated Fauna. *Marine Environmental Research*, 2010, 70 (1), pp.1. 10.1016/j.marenvres.2010.02.002 . hal-00598197

HAL Id: hal-00598197

<https://hal.science/hal-00598197>

Submitted on 5 Jun 2011

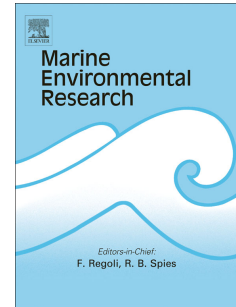
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Accepted Manuscript

Title: Colonization Of Organic Substrates Deployed In Deep-Sea Reducing Habitats By Symbiotic Species And Associated Fauna

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PII: S0141-1136(10)00036-X

DOI: [10.1016/j.marenvres.2010.02.002](https://doi.org/10.1016/j.marenvres.2010.02.002)

Reference: MERE 3423

To appear in: *Marine Environmental Research*

Received Date: 7 July 2009

Revised Date: 9 February 2010

Accepted Date: 13 February 2010

Please cite this article as: Gaudron, SM, Pradillon, F, Pailleret, M, Duperron, S, Le Bris, N, Gaill, F. Colonization Of Organic Substrates Deployed In Deep-Sea Reducing Habitats By Symbiotic Species And Associated Fauna, *Marine Environmental Research* (2010), doi: 10.1016/j.marenvres.2010.02.002

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

3

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24 **ABSTRACT**

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

39

40

41 **Key words:** symbiosis, settlement, colonization, larvae of invertebrates, *Xylophaga* spp., *Idas*42 sp., *Thyasira* sp., *Sclerolinum contortum*.

43 1 INTRODUCTION

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

55 *In situ* colonization experiments have been carried out over two decades at
56 hydrothermal vents, using polycarbonate plates, basalt rocks, sponges or titanium rings (Van
57 Dover et al., 1988; Shank et al., 1998; Taylor et al., 1999; Mullineaux et al., 1998, 2003;
58 Pradillon et al., 2005, 2009; Kelly et al., 2007; Kelly and Metaxas, 2008), at methane seeps using
59 sediment trays with or without agar layers releasing sulphide (Levin et al., 2006), at the deep-sea
60 floor using natural or exotic sunken wood (Pailleret et al., 2007; Tyler et al., 2007; Voigt et al.,
61 2007) and finally, using natural or artificially sunken whale carcasses (Smith and Baco, 2003;
62 Fujiwara et al., 2007; Lorion et al., 2009). But none of these colonization experiments involved
63 deployment of organic substrates deployed directly on methane seeps or hydrothermal vents.

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

68 recently allowed an estimate of the distance some marine larvae can disperse in oceanic currents
69 (up to 1000 km), but only a few deep-sea species were investigated. Mullineaux et al. (1991,
70 2005) and Metaxas (2004) studied larval dispersal by sampling the seawater column using tows
71 near deep-sea reducing habitats. However, very few of the recovered larvae could be identified to
72 the species level. Recent techniques such as *in situ* hybridization (ISH) using group- or species-
73 specific oligonucleotide probes (targeting 18S ribosomal RNA) have recently been developed
74 which can circumvent this difficulty (Pradillon et al., 2007; Jones et al., 2008). At the time of
75 settlement for these larvae from deep-sea species that may have dispersed for weeks to months,
76 the geochemical factors triggering settlement into these reducing habitats are not yet known.

77 To tackle this issue, we conceived a novel colonization device allowing the settlement of
78 early-life stages of organisms that colonize deep-sea reducing habitats excluding large-size
79 predators. We designed a standardized colonization module named **CHEMECOLI**
80 (**CHEM**osynthetic **E**cosystem **CO**lonization by **L**arval **I**nvertebrates) and filled it with organic
81 and inorganic substrates (wood, plant material and carbonates) in order to simulate reducing
82 habitats. Indeed, organic substrates (wood and plant) may produce sulphide through microbial
83 degradation, while carbonate substrates (naturally present in seeps habitats) can be used as a
84 chemically inert hard substrate for settlement. Devices were deployed in several reducing deep-
85 sea habitats, though always away from direct influence of reducing fluids. The localized
86 distribution of hydrothermal vents enables the direct influence of the fluid to be avoided, which
87 is not possible at cold seeps where fluid emissions are widespread. However, sulphide
88 enrichment in these latter habitats is considered to be limited to the sediment where it is produced
89 by sulphate-reducing bacteria (SRB) associated with the anaerobic degradation of methane
90 (Niemann et al., 2006). In all cases, methane enrichment in the water above the seafloor has
91 been described (Charlou et al., 2002, 2003) and its influence on the colonization device cannot be
92 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 dm³). 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

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

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

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

146 Finally, one set of CHEMECOLIs was deployed at the Haakon Mosby Mud volcano site
147 (HMMV) in the Norwegian Sea (Fig.3; Table 1). There, methane is abundant at the surface of the
148 sediment but no sulphide was usually detected in seawater above the area of deployment,
149 characterized by the presence of siboglinid tubeworms (Niemann et al., 2006; Lösekann et al.,
150 2008). Bacteria *Beggiatoa* spp. and siboglinid Annelid (*Sclerolinum contortum* (Smirnov,
151 2000) and *Oligobrachia haakonmosbiensis* (Smirnov, 2000)) constitute the dominant micro-
152 organisms and macro-fauna respectively. Both use hydrogen sulphide for chemoautotrophy, but
153 siboglinids achieve chemosynthesis through the help of symbionts located within the trophosome
154 (Lösekann et al., 2008). Colonization devices were deployed by ROV *Victor 6000* (*Ifremer,*
155 *France*) and recovered by the ROV *Quest 4000* (*MARUM, Germany*) after 12 months (Table 1)
156 in the south Hummocky periphery of the mud volcano where there was generally a high density
157 of siboglinids (>50%) and lower density of *Beggiatoa* spp. (<20%) (Jerosh et al., 2007).

158

159 **2.3 Sample processing methods**

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

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

182

183 **2.4 COI and 18S rRNA genes sequencing**

184 DNA was extracted from soft-tissues of some specimens recovered from CHEMECOLIs
185 deployed at the Nile Deep-Sea Fan (Table 2) and of polychaetes recovered from CHEMECOLIs
186 deployed at HMMV, using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's
187 protocol. Fragments of the mitochondrial cytochrome oxidase I-encoding gene (COI_{mt} DNA)
188 were amplified using primers LCO 1490 and HCO 2198 (Folmer et al., 1994). Polymerase Chain
189 Reaction (PCR) was performed as follows: an initial denaturing step of 5 min at 94°C, followed
190 by 35 cycles at 94°C for 30s, 48°C for 40s for hybridization, then 1 min at 72°C, and a final
191 extension for 10 min at 72°C. PCR products were sequenced by Genoscope (France).
192 Alternatively, a ~1800 bp fragment of the 18S rRNA gene was amplified with primers 1f and

193 2023r (Pradillon et al., 2007) using Ex Taq PCR kit (TaKaRa, Kyoto, Japan) and the following
194 PCR protocol: initial denaturation at 96°C for 5 min, 30 cycles of (96°C for 1 min, 51°C for 1
195 min, and 72°C for 3 min) and a final extension at 72°C for 10 min. Amplification products were
196 purified using gel extraction following the manufacturer's protocol (Wizard kit Promega).
197 Sequencing reactions using the PCR products as template were performed with BigDye
198 Terminator Cycling Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA,
199 USA), in both directions, using additional internal primers (see Pradillon et al., 2007). DNA
200 sequencing was conducted with an ABI-PRISM 3130x Genetic Analyser (Applied Biosystems
201 Japan Ltd., Tokyo, Japan) following the manufacturer's protocols. Sequences were inspected by
202 eye and assembled with AutoAssembler 2.1 (Applied Biosystems). All sequences were deposited
203 in the EMBL database under given accession numbers.

204

205 2.5 Chemical analyses

206 For the Nile Deep-Sea Fan and Rainbow experiments, the presence of free sulphide was
207 examined using potentiometric electrodes *in situ* before the recovery of CHEMECOLIs. The
208 potentiometric probe used is equipped with a conventional Ag/Ag₂S electrode (Le Bris et al.,
209 2008; Laurent et al., 2009), which was prepared from a silver wire and calibrated in the
210 laboratory. Above a threshold of c.a. 20 µM the electrode has a logarithmic response that allows
211 quantitative determination of sulphide providing that the pH is known. In agreement with the
212 theoretical response of the Ag/Ag₂S electrode, the slope of the electrode is about 30 mV/ per
213 decade of HS⁻, after correction of pH variation. Below this threshold, however, the slope tends to
214 increase and can reach much higher values. This increase in sensitivity is combined with a lower
215 reproducibility which does not allow fully quantitative determination of [HS⁻] in the lower part of
216 this range. For this reason, only a maximum concentration could be defined when sulphide was
217 detectable but still lower than 20-30 µM. Using the manipulator arm of the submersible, the tip

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

222

223 **2.6 Data analysis**

224 Density of species colonizing wood cubes within a given CHEMECOLI was calculated
225 taking into account that twenty cubes represent a volume of 160 cm³. To compare densities
226 among the different substrates this volume was also used for the carbonate substrate even if the
227 substrate could not be explored in 3 dimensions. Alfalfa was colonized by organisms inside
228 stems and therefore the 3D was explored. Species recovered from CHEMECOLIs from the Nile
229 Deep-Sea Fan were compared with what was known from Sibuet et al. (1998) and Olu-Le Roy et
230 al. (2004); species recovered from CHEMECOLIs from Rainbow were compared with what was
231 known from Desbruyères et al. (2001) and Desbruyères et al. (2006); species recovered from
232 CHEMECOLIs from HMMV were compared with what was known from Gebruk et al. (2003)
233 and Paxton and Morineaux (2009). For each CHEMECOLI, we counted both the number of new
234 taxon (N) compared to the surrounding habitat and the number of taxon (X) that was already
235 recorded in the surrounding habitat. Biodiversity was estimated based on three different
236 univariate indices using PRIMER v.6 (PRIMER-E): species richness (*S*), Shannon-Wiener index
237 of diversity (H' , log (e) such as Kelly and Metaxas (2008)), and Pielou's evenness index (J').

238

239 **3 RESULTS**

240 **3.1 Species recovered within CHEMECOLIs**

241 For each experiment we tried to assign organisms to described species (Sibuet et al.,
242 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 *Coccolpigya* 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). COI mt 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 dm³ in the CHEMECOLI
265 deployed at HMMV due to the high number of wood-borers that colonized a cube (~117
266 specimens).

267 Mollusc dominated as colonists within CHEMECOLIs deployed for 2-weeks and 1-year
268 experiments at the Nile Deep-Sea Fan (Fig. 4a). High densities of bivalves were counted within
269 the wood substrates due to the abundance of wood-boring bivalves (*Xylophaga dorsalis* (Turton,
270 1819)) (~287.5 specimens per dm³ for the short-term 2-week experiment and 525 specimens per
271 dm³ for the long-term 1-year experiment), while gastropods were more abundant on the alfalfa
272 substrate (~300 specimens per dm³), along with polychaetes (~ 200 specimens per dm³). In the
273 long-term experiment, polychaetes as well densely colonized the wood substrate (~200
274 specimens per dm³). Lower densities of crustaceans (max ~60 specimens per dm³) and Sipuncula
275 were also present on organic substrates in the long-term experiment. Carbonate substrates
276 deployed for one year were colonized only by low densities of gastropods *Clelandella myriamae*,
277 hesionid polychaetes and sipuncle (Table 3). Young settler-stages (juveniles) of symbiotic
278 bivalve species harbouring sulphur-oxidizing bacteria were successfully recovered. These
279 included *Idas* sp., *Vesicomysid* sp. and *Thyasira* sp. (Fig. 5a). These three species were all
280 recovered within the wood substrate after the two-weeks and after the one-year experiments. *Idas*
281 sp. was also recovered in the alfalfa substrate after the one-year experiment (Fig. 5a).

282 CHEMECOLIs recovered after one year at the Rainbow site, were poorly colonized
283 compared to CHEMECOLIs deployed into cold seep habitats for one year. Wood-boring bivalves
284 (*Xylophaga atlantica* (H.G. Richards, 1942)) dominated in the wood-containing device, and
285 juveniles of symbiotic bivalves were also recovered (~200 specimens per dm³, Table 3, Fig. 5b)
286 similar morphologically to vesicomysid juveniles, however a molecular identification is necessary
287 to ascertain this observation such as ISH (see introduction). Some polychaetes (*Prionospio* sp.
288 nov. 3, ~40 specimens per dm³) were recovered within both wood and alfalfa substrates (Fig. 4b).

289 CHEMECOLIs deployed for one year at the HMMV were densely colonized, especially
290 the wood and alfalfa substrates (Fig. 4c). Wood-boring bivalves (*Xyloredo ingolfia* (Turner,
291 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

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

345 Several physical and chemical factors may influence the settlement of larvae of species
346 endemic to deep-sea reducing habitats. Kelly et al. (2007) suggested that settlement of colonists
347 on basaltic plates deployed at hydrothermal vents was correlated with temperature and hydrogen
348 sulphide. Similarly, temperature was presented as a cue for colonists to locate preferred habitat
349 conditions in a gradient of hydrothermal vent flow (Bates et al., 2005). Both these parameters are
350 tracers of the impact of vent fluids on the environment. It is therefore impossible to discriminate
351 a direct influence of sulphide from other factors correlated to the vent fluid ratio. CHEMECOLIs
352 at the vent site Rainbow were deployed ten meters away from chimneys and species abundances
353 were very low compared to the two other experiments carried out in cold seep habitats. A low
354 supply of larvae in the particular period 2006-2007 at Rainbow may explain this result as shown
355 in Pacific hydrothermal vent colonization experiments for particular years (Kelly et al., 2007).
356 The distance from active venting results in no significant signature of fluid emission in the
357 surrounding water, as evidenced by the low background temperature measured (3.5°C). The lack
358 of any attractive signature of fluid venting could be an alternative explanation for the low rates of
359 colonization observed at Rainbow. Kelly et al. (2007) nevertheless demonstrated that vent
360 species were recovered at a similar distance from vents, even though settlement and post-
361 settlement survival rates were lower compared to basaltic plates deployed closer to the vent
362 edifice. The difference in fluid geochemistry (low free H₂S and high rate of precipitation close to
363 vent sources at Rainbow) might also contribute to the difference between those experiments.
364

365 Species richness calculated from our three *in situ* experiments (Table 4) were in the order
366 of magnitude reported by Kelly & Metaxas (2008) from colonization experiments at
367 hydrothermal vents, where S ranged from 11 on sponge substrates to 6 on basaltic plates. Species
368 richness from our experimental study were however, lower than those obtained from colonization
369 experiments carried at methane seeps by Levin et al. (2006) where S values were between 16-19
370 species and where sulphide added to tray experiments increased the species richness. Kelly &
371 Metaxas (2008) hypothesised that the physical structure may have influenced the species richness
372 in their experiments, with complex physical structure of the sponge favouring a more diverse
373 faunal assemblage by creating interstitial spaces that could provide protection against predation.
374 In our experiments, degradation of wood by wood-boring bivalves increased the area available
375 for colonization by other species such as spionid polychaetes at the Nile Deep-Sea Fan or at
376 Rainbow (pers. observ.). Similarly, available surfaces in alfalfa-filled colonizers were large. In
377 both organic substrates, micro-niches offered a variety of chemical micro-environments similar
378 to sponges in Kelly & Metaxas (2008) allowing more species to colonize and therefore
379 increasing the species richness. These probably differentiate compared to carbonate substrates,
380 where species richness was more similar to that recovered on basaltic plates in Kelly & Metaxas
381 (2008).

382

383 **4.2 Occurrence of species harbouring sulphur-oxidizing bacteria in CHEMECOLIs**

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

390 Vesicomidae and Thyasiridae harbour sulphur-oxidizing bacterial symbionts (Peek et al., 1998;
391 Taylor et al., 2007; Dubilier et al., 2008). Regarding *Idas* sp., barcoding demonstrated that it was
392 the same species previously documented to harbour 6 distinct bacterial symbionts, including
393 sulphur- and methane-oxidizing bacteria and found at cold seeps in the eastern Mediterranean
394 (Duperron et al., 2008). Phylogenetically, *Idas* sp. clusters within the large group which includes
395 all large mussels (*Bathymodiolus*) associated with deep-sea seeps and vents as well as many
396 smaller species associated with organic falls worldwide (Duperron et al., 2008, Lorion et al.,
397 2009). In our *in situ* experiment, *Idas* sp. was found associated with the two organic substrates,
398 wood and alfalfa whereas *Thyasira* sp. and *Vesicomid* sp. juveniles settled on wood cubes but
399 were not recovered in the alfalfa.

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

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

415 symbiotic tubeworm. Adults of *S. contortum* were recovered at the bottom of the three
416 CHEMECOLIs deployed at HMMV. Larvae must have been released in the three devices by
417 mature reproductive adults from below. In soft sediments, abundance of siboglinid frenulates is
418 documented to be inversely correlated with the abundance of other benthic fauna (Dando et al.,
419 2008). Besides, frenulates usually ‘mine’ insoluble sulphide into an anoxic zone within the
420 sediment by their root that is deeply buried in this area (Dando et al., 2008).

421

422 **4.3 New species, opportunistic species and tolerance to sulphide**

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

439 130 years ago from a piece of sunken driftwood (Friiele, 1879, A. Warén personal
440 communication).

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

454 Other opportunistic species were encountered within CHEMECOLIs such as the
455 Amphinomidae polychaete *Paramphinome jeffreysi* identified to the species level as a result of
456 18S rRNA sequencing (A. Nunes Jorge). A great number of Amphinomidae have been reported
457 associated with the siboglinid *Sclerolinum contortum* at HMMV (Gebruk et al., 2003) but they
458 were not identified to the species level. This opportunistic species is generally associated with
459 other polychaete species such as *Prionospio steenstrupi* (Malmgren, 1867), *Capitella capitata*
460 (Fabricius, 1780) and *Heteromastus filliformis* (Claparède, 1864) in soft marine sediment
461 enriched in organic matter and associated with the symbiotic thyasirid bivalve *Thyasira sarsii*
462 (Philippi, 1845) (Kutti et al., 2007). At HMMV, a nematode *Halomonhystera disjuncta* (Bastian,

463 1865) that is usually described in intertidal organically enriched mudflats was also found in high
464 abundance in the microbial mats area (Vanreusel et al., 2009).

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

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

477

478 **4.4 Sulphide production from organic substrates and potential role in the colonization** 479 **process**

480 Sulphide enrichment in sunken woods has been reported in a number of studies related to
481 archaeological shipwreck and sulphide concentration exceeding hundreds of micromolar have
482 been recently documented at the surface in naturally sunken wood in a mangrove swamp
483 (Laurent et al. 2009). Sulphide production from wood decomposition is considered to result of
484 the activity of anaerobic sulphate-reducing bacteria (SRB). Palacio et al. (2006) have measured
485 cellulolytic activities in Fir wood after 2 months in laboratory experiments using Mediterranean
486 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, vesicomid 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 pulsed sulphide enrichment,
511 likely originating from a crack located beneath it (Le Bris et al., 2008).

512

513 **5 CONCLUSION**

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

525

526

527

528 **6 ACKNOWLEDGEMENTS**

529 Authors would like to thank the people who have deployed and recovered some of the
530 CHEMECOLIs: Olivier Gros, Mélina Laurent, Mélanie Bergmann, Catherine Pierre and
531 Delphine Cottin. We are grateful to chief scientists, captains and crews of RVs *Pourquoi pas?*,
532 *Meteor*, *L'Atalante*, *Polarstern*, and teams operating ROVs *Victor 6000* (Ifremer, France), *Quest*
533 *4000* (MARUM, Bremen, Germany), and submersible *Nautilie* (Ifremer, France). Sample
534 collection was funded by CHEMECO (European Sciences Foundation
535 (ESF)/Eurocores/EURODEEP), HERMES (EC) and DIWOOD (EC) programs, and by
536 IFREMER, CNRS, and MPI institutes. Analysis and interpretation of data was supported by
537 CHEMECO (ESF/Eurocores/EURODEEP) and UPMC. Marie Pailleret and Florence Pradillon
538 were supported respectively by a Ph D grant from ANR Deepoases and by a Post-doctoral
539 fellowship from HERMES (EC). We are indebted to taxonomists for their precious expertises: J.
540 Blake, M. Böggemann, and P. Hutchings for the polychaetes, A. Warén for the gastropods, T.
541 Haga for the wood-boring bivalves. Genoscope, J. Lambourdière, M-C. Boisselier and S. Samadi
542 are acknowledged for their technical expertise in COI sequencing. We are also grateful to A.
543 Nunes Jorge and the JAMSTEC (Japan) that did the 18S rRNA sequencing.

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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 |
|-----------------------------|-----------|------------|--------------|----------------------------|
| <i>Clelandella myriamae</i> | 1 | Alfalfa | 32°31'97''N, | <u>FM212782</u> |
| <i>Clelandella myriamae</i> | 2 | Alfalfa | 30°21'17''E, | <u>FM212783</u> |
| <i>Clelandella myriamae</i> | 3 | Alfalfa | 1693m | <u>FM212784</u> |
| <i>Coccolpigya</i> sp. | 1 | Alfalfa | | <u>FM212785</u> |
| <i>Coccolpigya</i> sp. | 2 | Alfalfa | | <u>FM212786</u> |
| <i>Idas</i> sp. | 1 | Alfalfa | | <u>FM212787</u> |
| <i>Prionospio</i> sp. | 1 | Wood | | <u>FM212788</u> |
| <i>Prionospio</i> sp. | 2 | Alfalfa | | <u>FM212789</u> |
| <i>Prionospio</i> sp. | 3 | Alfalfa | | <u>FM212790</u> |
| <i>Prionospio</i> sp. | 4 | Alfalfa | | <u>FM212791</u> |
| <i>Prionospio</i> sp. | 5 | Alfalfa | | <u>FM212792</u> |

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796 **Table 3.** List of taxa identified within CHEMECOLIs and their estimated densities per dm³
 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 | | |
|---|--|--------|--------|------------|-----------|---------------------------|-------|----|-----------------------|-------|-------|
| | 2 weeks | | 1 year | | | 1 year | | | 1 year | | |
| Densities per dm ³ | MW1 | MC1 | MW2 | MA2 | MC2 | RW | RA | RC | HW | HA | HC |
| MOLLUSCA | | | | | | | | | | | |
| Bivalvia | | | | | | | | | | | |
| <i>Xylophaga dorsalis</i> (including juvenile) | N287.5 | - | N525 | 5 | - | - | - | - | - | - | - |
| <i>Xylophaga atlantica</i> | - | - | - | - | - | N 150 | - | - | - | - | - |
| <i>Xyloredo ingolfia</i> | - | - | - | - | - | - | - | - | N14412.5 | - | - |
| <i>Idas</i> sp. Med (including juvenile) | X12.5 | - | X31.3 | X12.5 | - | - | - | - | - | - | - |
| <i>Thyasira</i> sp. (juvenile) | X25.0 | - | X6.3 | - | - | - | - | - | - | - | - |
| <i>Vesicomysid</i> sp. (juvenile) | X37.5 | - | X12.5 | - | - | - | - | - | - | - | - |
| Unidentified bivalves juveniles | - | - | - | - | - | X43.8 | - | - | - | - | - |
| Gastropoda | | | | | | | | | | | |
| <i>Cleandella myriamae</i> (juvenile) | X6.3 | X18.75 | - | X168. 8 | X18. 8 | - | - | - | - | - | - |
| <i>Coccolpigya</i> sp. (juvenile) | - | - | N25 | 5 | N112. | - | - | - | - | - | - |
| <i>Alvania</i> aff. <i>griegi</i> (juvenile) | - | - | - | - | - | - | - | - | - | N225 | - |
| Caenogastropod sp. | - | - | - | N6.3 | - | - | - | - | - | - | - |
| ANNELIDA | | | | | | | | | | | |
| Polychaeta | | | | | | | | | | | |
| <i>Prionospio</i> sp. nov.1 | - | - | N75 | - | - | - | - | - | - | - | - |
| <i>Prionospio</i> sp. nov.2 | - | - | - | 8 | - | - | - | - | - | - | - |
| <i>Prionospio</i> sp. nov.3 | - | - | - | - | - | N18.8 | N12.5 | - | - | - | - |
| <i>Glycera</i> sp. nov. | - | - | N43.8 | N25 | - | - | - | - | - | - | - |
| <i>Eupolymnia</i> sp. | - | - | N6.3 | - | - | - | - | - | - | - | - |
| <i>Nicolea</i> sp. | - | - | - | N12.5 | - | - | - | - | - | - | - |
| <i>Sclerolinum contortum</i> (juvenile) | - | - | - | - | - | - | - | - | X68.8 | X18.8 | X93.8 |

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|---|-------|------|-------|-------|------|-------|-------|------|---------|-------|-------|
| Unidentified polychaete juvenile | - | - | - | - | - | - | - | - | X68.8 | X43.8 | - |
| Unidentified polychaete larvae | 25 | - | - | - | - | - | - | - | - | - | - |
| <i>Paramphinome jeffreysi</i> | - | - | - | - | - | - | - | - | X225 | X43.8 | - |
| <i>Ophryotrocha cf. spatula</i> | - | - | - | - | - | - | - | - | X25 | - | - |
| <i>Flabelligerid</i> sp. | - | - | - | - | - | - | - | - | - | N6.3 | N12.5 |
| <i>Hesionid</i> sp. | - | - | - | N6.3 | N6.3 | - | - | - | - | - | - |
| <i>Unknown Polychaete</i> | - | - | - | N6.3 | - | - | - | - | - | - | - |
| ARTHROPODA | | | | | | | | | | | |
| Crustacea | | | | | | | | | | | |
| <i>Amphipod</i> spp. | 62.5 | - | 18.8 | 12.5 | - | - | - | - | 162.5 | 93.8 | - |
| <i>Metacaprella</i> sp. | - | - | - | - | - | - | - | - | X6.3 | X6.3 | X12.5 |
| <i>Copepod</i> spp | 12.5 | - | - | - | - | - | - | - | 6.3 | 6.3 | - |
| Chelicerata | | | | | | | | | | | |
| <i>Pycnogonid</i> sp. | - | - | - | - | - | - | - | X6.3 | - | - | - |
| NEMERTEAN | | | | | | | | | | | |
| <i>Nemertean</i> spp. | - | - | - | - | - | X6.3 | X43.8 | - | N6.3 | N43.8 | - |
| SIPUNCULA | | | | | | | | | | | |
| <i>Sipuncle</i> sp. | - | - | N25 | N12.5 | N6.3 | - | - | - | - | - | - |
| FORAMINIFERA | | | | | | | | | | | |
| <i>Foraminifera</i> sp. | - | - | - | - | - | N68.8 | - | N9.7 | - | - | - |
| ACTINOPODIA | | | | | | | | | | | |
| <i>Actinopod</i> sp. | - | - | - | - | - | - | N6.3 | - | - | - | - |
| ECHINODERMA larvae | | | | | | | | | | | |
| 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 |

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

| | <i>S</i> | <i>N</i> | <i>J'</i> | <i>H'</i> |
|-------------------|----------|----------|-----------|-----------|
| 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 |

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830 **Table 5.** Detection of H₂S at the periphery or centre of the CHEMECOLI devices filled with
831 organic substrates deployed at the Nile Deep-Sea fan for one year after their recovery on board
832 during the MEDECO cruise (IFREMER) in 2007: MW2 (wood substrate) and MA2 (alfalfa
833 substrate).

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| MW2 | |
|------------|--------------|
| Periphery | undetectable |
| Periphery | undetectable |
| Center - 1 | < 10 μ M |
| Center - 2 | < 1 μ M |
| Center - 3 | < 10 μ M |
| MA2 | |
| Periphery | undetectable |
| Center - 1 | < 20 μ M |
| Center - 2 | < 10 μ M |

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852 Figures caption

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

855

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

859

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

863

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

871

872 **Fig.5.** Densities of thiotrophic species versus non thiotrophic species that have colonized the
873 CHEMECOLI within the 3 different substrates. a- short-term experiment at the Nile Deep-Sea
874 Fan: MW1 (wood substrate) and MC1 (carbonate substrate); long-term experiment at the Nile
875 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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