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Influence of seep emission on the non-symbiont-bearing fauna and vagrant species at an active giant pockmark in the Gulf of Guinea (Congo–Angola margin)

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Abstract:

Detailed surveying with an ROV found that a dense and diverse cold-seep community colonises a giant pockmark located at 3200 m depth, 8 km north from the deep Congo channel. Several types of assemblages, either dominated by Mytilidae and Vesicomyidae bivalves or Siboglinidae polychaetes, are distributed on the 800-m diameter active area. The site is characterised by a most active central zone in a depression with abundant carbonate concretions and high methane fluxes where high-density clusters of mussels and siboglinids dominate. In contrast, the peripheral zones display large fields of dead and live vesicomyids on soft sediment, with a lower mean density and lower methane concentration in seawater. The associated megafauna includes Alvinocarididae shrimps, echinoids, holothurians of the family Synaptidae, several species of gastropods, two species of galatheids, and Zoarcidae and Ophidiidae fishes. Multivariate analyses of video transect data show that the distribution of these major megafauna species at the pockmark scale is influenced by the habitat heterogeneity due to fluid or gas emission, occurrence of hydrates, substratum variability and by the presence of large symbiont-bearing species. Several assemblages dominated either by mytilids, vesicomyids, or siboglinids have been sampled for megafauna densities and biomass estimations and stable isotope measurements (δ^{13}C and δ^{15}N) of dominant species and food sources. The highest estimates of megafauna densities have been obtained in mytilid beds. According to their stable isotopes values, non-symbiont-bearing species mainly rely on chemosynthesis-originated carbon, either as primary consumers of chemoautotrophic microorganisms, or at higher trophic level recycling organic matter, or relying on bivalve and tubeworm production. Most of them likely feed on different sources like shrimps, but differences according to habitat have been evidenced. Carbon and nitrogen isotope ratios of galatheids and benthic or benthopelagic fishes captured by trawls at increasing distances from the pockmark provide evidence of the high variability in the proportion of chemosynthesis-originated carbon in their diet, from 15% to 38%, according to the species captured as far as 4 km to the site.

Keywords: Cold seep; Megafauna; Isotopic signature; Vagrant species; Atlantic Equatorial African margin; Congo–Angola margin

1. Introduction

In deep chemosynthetic environments driven by fluids enriched in methane and sulphide, i.e. hydrothermal vents and cold seeps, extreme habitat heterogeneity and variability suggest that communities are mainly structured by abiotic forces (e.g., Barry et al., 1997; Bergquist et al., 2005; Henry et al., 1992; Levin et al., 2003; MacDonald et al., 2003; Olu et al., 1997; Sahling et al., 2002; Sarrazin et al., 1999; Van Dover, 1995). Nevertheless, the high biomass which characterizes these environments suggests that biotic interactions should also be important community structuring factors at seeps like at vents (Levesque et al., 2003; Micheli et al., 2002; Sarrazin and Juniper, 1999; Tunnicliffe, 1991).

Megafauna, or large-size epifauna at cold seeps, which are associated with biomass-dominant symbiont-bearing species, include high diversity of taxa and almost all the marine phyla (Levin, 2005; Sibuet and Olu, 1998). Diverse communities are probably favoured by substratum heterogeneity that includes both soft bottoms and carbonate concretions, and as well as environmental conditions that are moderate compared to hydrothermal vents. Symbiont-bearing megafauna are also considered as a source of habitat heterogeneity, because they generate extensive habitat complexity (Levin 2005). Megafaunal community structure and diversity are highly variable among seep sites, and are thought to be influenced by factors such as depth, substratum, pelagic or terrestrial inputs (Levin et al., 2000; Levin and Michener, 2002; Sahling et al., 2003; Sibuet and Olu-Le Roy, 2002; Sibuet and Olu, 1998), patch size or age of symbiont-bearing species (Cordes et al., 2005; MacAvoy et al., 2005).

Dense chemosynthetic communities were discovered on a large part of a 800-m-diameter pockmark discovered along the Congo-Angola margin a few kilometres from the Congo deep

channel (Olu-Le Roy et al., 2007a; Ondréas et al., 2005). These first studies described assemblages visually dominated by symbiont-bearing taxa, Vesicomyidae and Mytilidae bivalves and Siboglinidae polychaetes whose distributions seemed to be controlled by methane fluxes and substratum variability. This giant pockmark is, in fact, a complex (a pockmark 'cluster') of several individual pockmarks of about 100 m in diameter whose variable activities may contribute to the spatial heterogeneity observed on the seafloor (Ondreas et al. 2005). The distribution of other megafaunal species is probably controlled by habitat heterogeneity occurring at the pockmark scale, which is created both by fluid emission-related patterns and by the symbiont-bearing species, serving as 'ecosystem engineers' according to Levin (2005).

Following Carney (1994), associated fauna may be classified as endemic, colonist, and vagrant, depending on their abundance at seeps compared to background areas. Stable isotopes, which were first used to demonstrate chemosynthesis processes in seep community, and were mainly applied to symbiont-bearing species (Kennicutt II et al., 1992; Paull et al., 1984; Paull et al., 1985; Rau and Hedges, 1979), can be used to estimate trophic dependence of these 'associate' or 'heterotrophic' species on chemosynthetic production (Levin et al., 2000; Levin and Michener, 2002; MacAvoy et al., 2002). Carbon and nitrogen stable isotopes also have been used to decipher nutritional associations among fauna at vents (Colaço et al., 2002; Fisher et al., 1994; Levesque et al., 2006; Polz et al., 1998; Van Dover, 2002; Van Dover and Fry, 1989; Vereshchaka et al., 2000) and, more recently, at seeps (MacAvoy et al., 2005; Van Dover et al., 2003). The fauna closely associated with tube worm aggregations at cold seeps in the Gulf of Mexico obtain the bulk of its nutrition from local sources of primary production (MacAvoy et al. 2005) but the relative importance of chemosynthetic pathways have been suggested to vary regionally with depth and among microhabitats defined by dominant symbiont-bearing species (Levin and Michener, 2002).

The objective of the present study is to assess the influence of seep emissions on the non-symbiont-bearing megafauna at a giant pockmark recently discovered, and therefore the dependence of these species on the seep energy, by analysing the following: (i) species distribution relative to the distribution of active seeps at the pockmark scale, (ii) densities and biomass at more or less active local seeps, and (iii) isotopic signature of their tissues relative to chemoautotrophic or external sources of carbon and nitrogen. Export of local chemosynthetic biomass by large mobile predators captured in the background of the deep seep site is also estimated from stable isotope measurements.

2. Materials and methods

2.1. Video survey and image analysis for megafauna distribution

The giant pockmark named 'REGAB' (Ondréas et al. 2005) was explored by the ROV Victor 6000 in 2001 during the Ifremer-TOTAL collaborative programmes ZAIANGO and BIOZAIRE (Sibuet and Vangriesheim, 2009. This active cold-seep site is located at 3170 m depth on the Gabon continental margin close to the deep Congo channel (5°47,50′S; 9°42′40″E) (Figure 1). In this paper we will use the term 'pockmark' for the whole pockmark area, not to describe individual pockmarks. Regularly spaced video transects were first performed on the whole structure (Figure 2). Different types of faunal assemblages forming clusters were subsequently defined and mapped (Olu-le Roy et al. 2007a; Figure 2) within the pockmark; they were defined as 'chemosynthetic assemblages ' and either dominated by large bushes of the siboglinid polychaete *Escarpia southwardae* Andersen et al. 2005, or by two species of vesicomyid bivalves undistinguishable on images but identified from samples

as *Laubiericoncha chuni* (Thiele and Jaeckel, 1931), see Cosel and Olu (2008) and *Caplytogena regab* Cosel and Olu (2009), or by the Mytilidae *Bathymodiolus* sp. aff. *boomerang* (Olu-Le Roy et al., 2007b). The results of a second phase of video analysis, subsequent to the symbiont-bearing species cluster mapping, are presented in this paper. Video surveys of seven dives were analysed in order to map the distribution of megafaunal associate species along the dive tracks, at the pockmark scale. The video sequences from a down-looking camera, which was vertically mounted on the ROV, during 3-m-altitude surveys were analysed for the distribution of visible taxa of at least a 2-cm size, including the largest gastropods, some crustaceans, echinoderms and fishes. Maps of distribution along the dive tracks were compiled for the dominant associated species and compared with the previously acquired distribution maps of symbiont-bearing species, using the ADELIE extension for ArcGIS 9.0 developed at Ifremer. Each taxon record was associated with visual observations of habitat including the following: (i) substratum category (soft sediment, carbonate concretions, hydrate outcrops) and (ii) dominant symbiont-bearing species (living or dead vesciomyids, mytilids, siboglinids) or bacterial mats. Multidimentional scaling (MDS) of the species/biotope matrix of distance using the Bray Curtis distance (Primer software) was performed to identify the relationships between megafauna distribution and habitat characteristics.

In order to compare density and biomass of the associated megafauna in the clusters dominated by different symbiont-bearing species, 11 assemblages or ‘sites’ were selected at different locations on the pockmark, either in the central, more active part of the pockmark for methane emission (Charlou et al., 2004), or in peripheral areas (Table 1). There were three assemblages of different sizes dominated by *Bathymodiolus* aff. *boomerang*, five vesicomyid clusters with different proportion of living and dead individuals, and located in the different zones of the pockmark, and two escarpiid *E. southwardae* one, presumed to be

adults and the other one juveniles. Density, biomass of dominant symbiont-bearing species and also chemical characteristics at each site are described in Olu Le Roy et al. (2007a). Megafaunal species densities, when making density estimates on surfaces, were either estimated from close-up views using four laser points spaced 23 cm apart or from the scale provided by the ROV's sampling tools. When possible, density was averaged from five to 10 sequential, but non-overlapping images or from complete mosaics of the same site, produced from short video sequences using the ADELIE software. Individual wet weights were estimated from formalin-preserved specimens collected by the ROV grab or suction sampler, and dry weights of same specimens after 48 h at 60°C. Species biomass in the assemblages was estimated from three to five specimens of different sizes for each species.

2.2. Stable isotope measurements

Carbon and nitrogen isotopic ratios were analysed for three symbiont-bearing species and ten associated species, sampled by ROV in the chemoautotrophic assemblages. Between two and five individuals were analysed for each species. With this sample set, it was possible to correlate isotopic signature to habitat for associated species collected in multiple assemblages that were dominated by different symbiont-bearing species. Specimens used for isotopic measurements were dissected on board; muscle samples were removed and stored in liquid nitrogen. In the laboratory, samples were dried under vacuum and analysed in triplicate for their carbon and nitrogen isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) calculated by a DELTA Plus (thermo Finnigan) isotopic mass spectrometer (LPTC, Bordeaux) and for a few samples by a FINNIGAN DELTA S IRMS (Station Biologique de Roscoff) as follows:

$$\delta^{x}E = \left[\frac{\left({}^{x}E/{}^{y}E \right)_{sample}}{\left({}^{x}E/{}^{y}E \right)_{standard}} - 1 \right] \times 1000$$

where *E* is the element analysed (C or N), *x* is the atomic weight of the heavier isotope, and *y* is the atomic weight of the lighter isotope (*x* = 13, 15 and *y* = 12, 14 for C and N, respectively). The standard materials to which the samples were compared were PDB (Pee Dee Belemnite) for carbon and air N2 for nitrogen. Reproducibility of all measurements was about 0.3‰. The standard compounds used to correct samples' values from deviation due to the spectrometer were usg24 (16.1‰) for the carbon and N1 (0.4‰) for the nitrogen. Inter-comparison of measurements was performed to test the reproducibility of the samples analysed by the two mass spectrometers.

In addition to the fauna, sediments from ROV push cores collected adjacent to the different assemblages were analysed after vertical subsampling of 2-cm slices from the interface (0 cm) to a depth of 6 cm. Isotopic measurements were performed after acidification to remove carbonates. Particles from sediment traps deployed for a year on the REGAB site, at 400 m above the seafloor, were also analysed for carbon and nitrogen isotopic ratios.

Methane $\delta^{13}C$ was measured on sediment from tube-cores (first 5 cm) collected in crimping boxes and on water in sealed vials (20 ml) and analysed by Head-Space/Gas Chromatography/Isotope Ratio Mass Spectrometry (HS/GC/IRMS) in Total Laboratory.

The REGAB and background megafauna were also sampled by beam trawls. Trawl samples were taken at increasing distances from the centre of the pockmark as follow: at 400 (peripheral edge), 580, 1560, 3680 and 5800 m, southward (CP19 to CP15), and at 610, 680, 1660 and 3600 m northward (CP20 to CP23) (Figure 1b). Benthic fishes and galatheids were analysed for their isotopic composition, with particular attention being paid to species collected in the cold-seep site and in the background. Pieces of tail muscles of 38 specimens belonging to 12 species of fishes and abdomen muscles of 9 specimens of galatheids were dissected on board. The proportion of chemosynthetic carbon in the diet of these mobile

predators or scavengers was estimated with the following isotopic mixing equation proposed by MacAvoy et al. (2002):

$\delta xEpredator - F = (\delta xEseep \times \mathrm{fseep}) + (\delta xEocean \times (1 - fseep))$ (1)

where *f*seep is the fraction of diet from chemosynthetic sources and δ*xE*seep and δ*xE*ocean are the mean isotopic signatures of the chemosynthetic material and background ocean, respectively. The parameter *F* corrects for trophic enrichment and is dependent on the isotope used. When using $\delta^{13}C$ and $\delta^{15}N$, F was given a value of 1 and 3.5‰, respectively, which is the trophic enrichment typically associated with these isotopes (Minegawa and Wada, 1984; Rau et al., 1983) The values used for the mean isotopic signatures of the chemosynthetic prey, δ*xE*seep ($\delta^{13}C$seep and $\delta^{15}N$seep), were calculated by averaging values for selected resident fauna exclusive of vagrant species, and for sediment collected at different places in the active site. The phytodetritus-based food sources of the background, δ*xE*ocean, were similarly estimated from values of megafauna species collected in the same beam trawl samples as fishes, except for the one sampling part of the cold-seep site (CP20). This trawl haul (CP20) was performed in the north part of the pockmark and collected some species of the chemoautotrophic assemblages characteristics of the cold-seep site. Some of these species were analysed for their isotopic signature, in order to increase the number of specimens, but were separated in the results, as they cannot be referred to a particular habitat (e.g., mussel bed, etc.) as for ROV samples.

3. Results

3.1. Distribution of megafauna species all over the pockmark

On the giant pockmark, 10 invertebrate taxa were identified on videos as associate species of chemosynthetic assemblages; their occurrence is reported together with substratum and symbiont-bearing species, to characterise the habitat. A total of 4352 individuals were

counted along video tracks, with half being galatheids ascribed to *Munidopsis* sp.. The species were not recognizable on the images, but *Munidopsis geyeri* was more abundant in the samples than was *Munidopsis hirtella* (Macpherson and Segonzac, 2005). Shrimps described from samples as *Alvinocaris muricola* (Komai and Segonzac, 2005), holothurians of the genus *Chiridota*, a species close to *Chiridota heveva* Pawson and Vance, 2004 (M. Sibuet, pers. com.) and large gastropods of family Turridae, species *Phymorhynchus coseli* Warén and Bouchet (2009) were also well represented. Other taxa are irregular echinoids and several families of fishes, including the families Zoarcidae, Macrouridae and Ophidiidae, which were the most abundant and easily recognisable. The distribution maps (Figure 3) of the four most abundant taxa (*Munidopsis* sp., *Alvinocaris muricola*, *Chiridota* sp. and echinids) show that, galatheids were observed along the dive tracks throughout the pockmark, while the other species seem to be closely associated with fluid emission, as co-occur with symbiont-bearing species. Shrimps were mainly observed in the central part of the pockmark associated with mytilid and escarpiid assemblages; echinids were only observed in soft sediment areas, in the close vicinity of vesicomyid beds, and holothurians were observed in the entire active area. Multivariate analyses were performed for the entire data set to avoid misinterpretation due to denser sampling coverage in the active region. The result of the multidimensional scaling analysis, using the Bray Curtis distance between species and analysing first the similarity between biotopes (Figure 4a), identified the symbiont-bearing species assemblages (mytilids, vesicomyids and escarpiids), as the exception of dead escarpiid bushes, as a primary habitat for associated megafauna, the bare substratum (soft, reduced sediment, carbonate) as a secondary habitat and hydrate outcrops, dense gas bubbles and bacterial mats as a final one (stress = 0.03). Analysis of the similarity between species (Figure 4b) grouped together Alvinocarid shrimps, *Chiridota* sp. holothurians, species associated with living symbiont-bearing species assemblages and, to a lesser degree,

galatheids (stress = 0.02). Whether or not *Phymorrhynchus* belongs to this group depends on the 'classification' or grouping method employed. Its relationship with symbiont-bearing species is much less strong than for other species. The zoarcid fish *Lycodes* sp. and echinoids are grouped together; they were generally both observed in the close vicinity of symbiont-bearing bivalve assemblages. Other fishes and swimming holothurians defined another group, whose distribution is probably independent of that of chemosynthetic fauna.

3.2. Megafaunal community structure in chemosynthetic assemblages

The densities of megafaunal species were estimated from images at 10 sites, which were either characterised as vesicomyids, mytilid clusters, or escarpiid bushes (Tables 2a and 2b). The symbiont-bearing species generally show the highest densities in clusters with 31 to 86% of the total individuals, and largely dominate the megafaunal biomass with 71-76% in escarpiid bushes and 84-98% in bivalve clusters. The associated species observed in the symbiont-bearing species clusters are dominated by the shrimps *Alvinocaris muricola*, independent of the symbiont-bearing species, except in the vesicomyid clusters V1 and Vext. Their very high density, up to 744 ind.m^{-2} in mytilid beds, 246 ind.m^{-2} in vesicomyid clusters and 302 ind.m^{-2} in escarpiid bushes, represents between up to 12% of the total biomass in dry weight in bivalve clusters and up to 26% in escarpiid bushes. The gastropods include the Provannidae *Provanna reticulata* Warén and Bouchet (2009) and *Provanna chevalieri* Warén and Bouchet (2009) (not distinguishable on images) and the limpet *Paralepetopsis sasakii* Warén and Bouchet (2009). Both genera have high numerical densities in mytilid beds and adult tube-worm bushes. Nevertheless, owing to their small size, they make a small contribution to the total biomass. In the vesicomyid clusters, their densities are more variable,

only the Provannidae are present, in association with high bivalve density (>500 ind./m²). The Turridae *Phymorrhynchus coseli* is sometimes associated with vesicomyids in soft sediment areas in the SW part of the pockmark in clusters with numerous empty shells (V1,VB). Actiniaria colonise concretions and among mussels. The holothurid *Chiridota* sp. can be locally abundant, particularly among bivalves, and has higher densities in vesicomyid clusters than in mytilid beds. *Munidopsis geyeri* is only observed in the clusters located in the periphery areas. The zoarcid fish *Lycodes* sp. is commonly observed among bivalves, but not with high densities (no more than one or two ind/cluster).

The densities and biomass of associated species were highest in the three mytilid beds (Table 2), whereas among vesicomyid clusters and escarpiid bushes they have lower densities/biomass. These differences are only significant for density and biomass between mytilid and vesicomyids assemblages (Kruskall Wallis test; p value<0.05). The densities and biomass are highly variable in vesicomyid clusters, and higher in the adult escarpiid bush than in the juvenile one. Among the vesicomyids, densities of associated species mainly depend on the bivalve density, but also on their location in the pockmark. The clusters located in the central part of the REGAB site (V3 and VC) have higher densities, particularly of *Alvinocharis muricola* and *Provanna* sp. than V1 and VB that are located in the peripheral fields and Vext, which is an isolated cluster away from the active part of the pockmark (Figure 2b). Considering the total density of the symbiont-bearing and non-symbiont-bearing species, the density and biomass of mytilid clusters are significantly higher than in the other two cluster types (mean = 1882 ind.m^{-2}, 691 ind.m^{-2} and 629 ind.m^{-2}, respectively, for vesicomyids and escapiid assemblages).

3.3. Isotopic measurements

3.3.1. Sources: methane, particles from traps and sediment

Methane carbon isotopic ratio measured from sediment samples varies from −68.5 to −96.2 ‰ (mean = −80.23 ‰, sd = 9.58, n=7) (Table 3). The values were more homogeneous in the four water samples analysed with a mean $\delta^{13}C$ of −68.57‰ (sd = 1.34). Methane was the main component of the hydrocarbon gas extracted from sediment (98.8 %) and water (99.97 %).

The organic matter obtained in particle traps partly contained detritical matter from a terrestrial origin (Treignier et al. 2006). Its $\delta^{13}C$ value (−23.22‰) is consistent with this origin and with the $\delta^{13}C$ value of sediments sampled off the cold-seep site (Table 4) while the $\delta^{15}N$ (4.43‰) is lower due to less recycling. The $\delta^{13}C$ of the sediment sampled next to the different types of assemblages in the active site is depleted compared with these values and on average is more depleted close to mytilid than vesicomyid clusters (Table 4). The microorganism community, which probably contributes significantly to the carbon pool in the sediment, may have a different composition depending on the site and environment (Cambon-Bonavita et al., 2009) and thus a different signature. $\delta^{15}N$ values show a strong contrast between mytilid and vesicomyid surrounding sediments. The values close to zero are probably due to a higher contribution of the microbial primary producers compartment to the sediment close to the mytilids, where the methane fluxes are higher than close to vesicomyids (Olu-Le Roy et al. 2007a), while the less depleted $\delta^{15}N$ values close to less active areas colonised by vesicomyids may indicate a higher content of detrital/recycled material.

3.3.2. Chemoautotrophic symbiont-containing species

Bathymodiolus aff. *boomerang* tissues have quite homogeneous isotopic signatures and demonstrated the most depleted values (Table 5), consistent with dominant nutrition via

methanotrophic symbionts. Mussels from the M2 site are significantly more depleted in $\delta^{13}C$, particularly the gills (−67.05‰), than those from M1 and M3. Only at M2, have gills significantly different $\delta^{13}C$ than from the mantle.

The relative importance of methanotrophy vs. sulphide oxidation in the nutrition of the mussels can be estimated by a two-end-member mixing equation (Fry and Sherr, 1984) where:

$\delta^{13}C\ consumer = f \times \delta^{13}Csource\ 1 + (1-f) \times \delta^{13}Csource\ 2$ (2) where f is the proportion of carbon originated from *source 1* in the nutrition of the consumer.

or by Equation (1) : $\delta xEpredator - F = (\delta xEseep \times f) + (\delta xEocean \times (1-f))$ (1) already cited, taking into account the parameter F that corrects for trophic enrichment. We used F = 1 between carbon source and mussels, as isotopic fractionation is negligible during methanotrophy, from carbon source to the symbionts (Conway et al., 1994), and the two possible carbon sources are the CH_4-derived carbon ($\delta^{13}C$ = 67–70 ‰) and DIC ($\delta^{13}C$ = 23 ‰). The estimated contribution of methanotrophy in the mussel nutrition is, following the Fry and Sherr equation (2), from 84 to 100% and following MacAvoy equation (1) from 85 to 88%. Equation (2) was used by Van Dover et al. (2003) to estimate the relative importance of methanotrophy (source 1) *vs.* sulphide oxidation (source 2) in the nutrition of mussels, assuming that photosynthetically derived material is negligible. Considering the methane signature as carbon source (1) and the vesicomyid signature for thiotrophically generated biomass as source (2), REGAB mytilids has a contribution of methanotrophy from 76 to 94%. Vesicomyids showed typical values for sulphur-oxidizing symbionts, with no significant differences between sites (V1/V3) or tissues. Escarpiids were characterised by more heterogeneous signatures, with significantly different values between sites with the less depleted at M1, dominated by mytilids, and the more for the adult bush EA. Juveniles

sampled at EB had an intermediate isotopic signature, between those of adults from EA and M1.

3.3.3. Associate megafauna in chemosynthetic assemblages

Results of the 55 individuals of 10 species of presumably non-symbiont-bearing invertebrates analysed for carbon and nitrogen stable isotopes are summarised in Table 6 and plotted in Figure 5. The tissue $\delta^{13}C$ values of the species collected either during ROV dives in chemoautotrophic assemblages or by the trawl haul realised in the north part of the pockmark (CP20) ranged from −35.12 to −60.09‰ and revealed that the major part of their diet is from a chemoautotrophic origin. Indeed, POM from particle traps has $\delta^{13}C$ value of −23.22‰ (Table 4). The sole exception is the galatheid *Munidopsis hirtella* ($\delta^{13}C$ = −22.4‰) only collected in the trawl, that is probably an occasional or vagrant species in the cold-seep site. The $\delta^{15}N$ values, ranging from 1.9 to 9.37‰, suggest that the majority of individuals derive their nutrition heterotrophically rather than from symbionts.

The isotopic signature of individuals collected by trawls in the north part of the pockmark (*A. muricola* and *Chiridota* sp.) compared with those sampled by ROV in the central part of the pockmark showed a lesser contribution of chemosynthesis-based production in their diet (−20‰ shift in $\delta^{13}C$ for the holothurid). The commensal polychaete *Branchipolynoe* sp. collected in *Bathymodiolus* mussels has a $\delta^{13}C$ signature very close to its host. The grazers including the gastropods *Provanna* and *Paralepetopsis*, the shrimp *A. muricola* possessed depleted values of −35.5 ‰ for *Provanna*, −48.8‰ for the shrimps, and up to −55.0‰ for the limpet specimens analysed, which suggests variable but a large contribution of bacteria in their diet, or different microbial communities with distinct signatures. $\delta^{15}N$ of 3.28 (*Alvinocaris*) to 3.89‰ (*Provanna*) are consistent with a trophic regimen of primary consumers, probably mainly based on microbial communities, considering a shift of 3.5 to

4‰ between prey and predator and a signature of archaeal and bacterial populations between −1‰ (mytilid methanotroph symbionts) and 1‰ (vesicomyid sulphur-oxydising symbionts). The light signatures in carbon and nitrogen of *Paralepetopsis* collected at *Bathymodiolus* beds suggest a main consumption of methanotrophic Archaes. The turrid gastropod *Phymorrhynchus* has the same $\delta^{13}C$ signature as the vesicomyids, suggesting that bivalve tissues could partially be included in its diet. *Phymorrhynchus* has been observed in abundance among vesicomyid clusters. Nevertheless, its $\delta^{15}N$ values suggest another prey with a lighter signature. The echinoderms observed in the chemosynthetic assemblages such as *Chiridota* sp. or those close to them like the irregular echinoid, also have light $\delta^{13}C$ values (respectively −57.1 and −41.3‰). Their $\delta^{15}N$ values however differ, suggesting that echinids are primary consumers, probably grazing bacteria, while the holothurid has more enriched and more variable $\delta^{15}N$ values suggestive of more recycling material in its diet. This species also could partially feed on bivalve tissues from which they have an enrichment of about 4.0-4.5‰ in $\delta^{15}N$. The detritivore/scavenger galatheids has nevertheless the most enriched $\delta^{15}N$ values (up to 9.7‰), corresponding to a higher trophic level. Finally, the zoarcid fish *Lycodes* sp. shows a quite variable signature in carbon (−25.4 to −68.0‰) and nitrogen (−1.23 to 9.4‰), probably revealing a fairly variable diet; some individuals were observed lying on bacterial mats while others are probably predators or scavengers of invertebrates.

Within some other species, especially *A. muricola* and *Chiridota* sp., $\delta^{13}C$ and $\delta^{15}N$ values vary according to the sampling site. Signatures of shrimps collected on mussel beds show lighter values than those collected in vesicomyid clusters (Figure 6a). These shrimps which have been observed grazing on bivalve mantles (Figure 6c) may have a nutritional link with bivalves, characterised by different isotopic signatures. The more variable $\delta^{13}C$ signature of the shrimps at mytilid beds than at vesicomyid beds may also correspond to a higher variability of microbe populations linked to methane- or sulphur-related processes. Similarly,

a difference between habitats has been observed for the holothurid, with a correspondence between $\delta^{13}C$ values of the holothurid and of the dominant bivalves (mytild/vesicomyid) (Figure 6b). This species, which has been observed among bivalves (Figure 6c), could be a potential scavenger of bivalve tissues. Moreover, the very depleted value of its digestive wall ($\delta^{13}C$ = −66.71‰) compared with the tegument or tentacles (respectively −53.9 and −55.1‰) may suggest the presence of bacteria in its digestive tract, although this was not seen by electronic microscope observations.

3.3.4. Mobile megafauna collected by trawls

Twelve benthic or benthopelagic fish species were sampled in the trawls located from the REGAB site 14 km southward and northward. Some were collected only once or twice, others such as the *Acantaurus armatus* (Ophidiidae) were present in most of the trawl samples. Among the 11 species sampled close to the pockmark, 5 *(Coryphaenoides striatus, Stomias boa boa, Stylephorus chordatus, Bassozetus robustus and Porogadus milles)* were also collected at 4000-m sites and south of the Congo channel at 3000 m depth. The Ophidiidae (*A. armatus* and *B. robustus)* were the most abundant in trawls in the close vicinity of the seep area. *Lycodes* sp. (Zoarcidae) is the sole fish sampled only at the REGAB site, where it was observed by ROV among bivalve assemblages. Despite a strong heterogeneity in the isotopic signatures of the three specimens analysed, this species likely has a high percentage of chemosynthesis-originated carbon in its diet (35–100%) (Fig. 5, Table 7).

Among the other fishes sampled in the trawl hauls closest to the active area (CP19-20-21), the Ophidiidae *B. robustus, A. armatus* and *Holomycteronus squamosus* partially take advantage of this local production with a mean contribution of chemosynthesis-originated carbon from 15% for *A. armatus* to 38% for *H. squamosus* (Table 7). For some benthopelagic

fishes, *Scopeloberyx robustus* (Melamphaidae) and *Stylephorus chordatus* (Stylephoridae), the estimated percentage of chemosynthetic material is about 20%. Specimens of *Porogadus milles* showed variable signatures and the other species seem to be independent of the chemosynthetic production. A decrease in the chemosynthetic contribution to the fish diet with increasing distance from the pockmark was not observed, probably due to the large distribution perimeter of these individuals.

The two species of galatheids greatly differ in the isotopic signatures of their tissues and, therefore, by their dependence on the chemosynthetic production. Their distribution also appears to be different according to their distribution in trawls and as only *M. geyeri* was sampled during dives on REGAB.

4. Discussion

4.1. Level of dependence on chemosynthesis production

The distribution of the megafauna species along the dive tracks at the pockmark scale shows that the non-symbiont-bearing species, which are abundant within chemosynthetic assemblages (*A. muricola*, *Chiridota* sp.), are closely associated with symbiont-bearing species and as they are not, or rarely, observed outside the assemblages. *Phymorrhynchus* and the galatheids are mainly located in the periphery of the chemosynthetic assemblages but also do not have a regular distribution all over the pockmark, like fishes for example. Although few species can be included in such analysis based on video processing, there is evidence of the three levels of relationship, and possibly dependence, of the megafauna species relative to the seeps and chemoautotrophic processes, as defined by Carney (1994) as: endemic, colonists and vagrant species. *A. muricola* and *Chiridota* sp. may be more

dependent on the chemosynthetic production and may be endemic, whereas *Phymorrhynchus* and the galatheids may be colonists of the seep ecosystem. Carbon isotopic signatures of these two species are, moreover, less depleted than other heterotrophs sampled in the assemblages; this seems to confirm a lesser degree of chemosynthetic dependence than for *A. muricola* or *Chiridota* sp.. The small gastropods *Provanna* and *Paralepetopsis*, whose distribution of the chemosynthetic assemblages could not be estimated using video transects, are also probably strongly dependent on seep processes, judging from to their $\delta^{13}C$ values. These two species were also abundant in chemosynthesis assemblages in the central active part of the pockmark (mytilid and vesicomyid beds, adult escarpiid bushes), but absent in the vesicomyid clusters located at the periphery of the pockmark, which was less active in terms of fluid emission.

4.2. Trophic guilds and contribution to the biomass

Biomass of megafauna in chemosynthetic assemblages of the REGAB site is dominated by symbiont-bearing species. With the exception of *E. southwardae* which presents variability already observed for the Siboglinidae polychaetes (Conway et al., 1994; MacAvoy et al., 2005); all the other symbiont hosts have a homogeneous isotopic signature. The variability of the carbon isotopic signature according to the different sites, could be related to the use of different DIC sources (which might reflect different carbon signatures), to methane concentration and to the age of the tubeworm. However, at the well-studied Gulf of Mexico seeps, no differences between old and young tube-worm aggregations were observed for *Lamellibrachia luymesi* (−20‰) and *Seepiophyla jonesi* (−22‰) (MacAvoy et al., 2005). The mytilid *Bathymodiolus* sp. aff. *boomerang* possesses both methanotrophic and sulphur-oxidising symbionts (Duperron et al., 2005), but at the sites sampled for this study the carbon

isotopic signature of their tissues is very close to that of methane and mainly derives its carbon from methanotrophy according to the mixing equations applied to our stable isotope measurements. The most depleted $\delta^{13}C$ values, suggesting a higher contribution of methanotrophic symbionts, originated from the M2 site mytilids where the highest methane concentrations were recorded. This situation is closer to that of mussels in the Gulf of Mexico seeps that have only methanotrophic symbionts (Brooks et al., 1987) than that of the Blake ridge mussels *Bathymodiolus heckerae*, which, like REGAB mytilids, harbour two types of symbionts (Van Dover et al., 2003). The vesicomyids and *E. southwardae* harbour only sulphur-oxidising bacteria (Nadalig et al., in press). After symbiont-bearing species, the second contributor to the biomass is the shrimp *A. muricola*, representing up to 10% of the total dry weight of mytilid and vesicomyid clusters (M1, VC), and 25 % in the tube-worm aggregations. Densities reach 450ind.m^{-2} in mussel beds. In contrast, they show low densities in some vesicomyid clusters of peripheral areas (V1, Vext). According to its nitrogen isotopic signature, the shrimp seems to be mainly a primary consumer. Similarly, in the Gulf of Mexico seeps, *Alvinocaris stactophila* was one of the most abundant species associated with mussel beds, accounting for up to 17% of the individuals collected, apart from mussels, and this species being related to the higher methane concentrations (Bergquist et al., 2005), which is consistent with our observations. Other primary consumers mainly relying on microbial biomass (*Provanna*., *Paralepetopsis*) may have very high densities, equivalent to those of shrimps in most of the bivalves clusters, and sometimes higher (at M1); however the biomass is much less owing to their small size. Quantitative sampling could, nevertheless, give slightly different results, with image analysis biased toward the larger species. Small gastropods are also the most abundant species in Gulf of Mexico mussel beds, with *Provanna sculpta* accounting for up to 14% of the individuals (Bergquist et al. 2005). Echinids are not included in density/biomass estimation as they are mainly located in the vicinity of the

chemosynthetic assemblages. Nevertheless, they are abundant in soft sediment areas close to vesicomyid beds and are another species that seem to be mainly primary consumers of microbial chemoautotrophic communities.

Higher trophic levels including the holothurid, galatheids and zoarcid fish are not very abundant in the sampled assemblages, except locally for *Chiridota* sp. in bivalve beds (93 ind.m^{-2} and 5% of the biomass in V3), which could be a scavenger of both vesicomyids and mussels. The density and biomass of these higher trophic levels (detritivorous, scavengers, predators) increase with the proportion of empty shells in vesicomyid clusters, *M. geyeri* and *Phymorrhynchus* in V1, *Chiridota* (11 ind/m^2), *Phymorrhynchus* and *M. geyeri* in VB. At the pockmark scale, distribution based on transects show that holothurids and galatheids are quite abundant in the area colonised by chemosynthesis-based communities (Fig. 3). Galatheids belonging to the genus *Munidopsis* have been reported to be dependent on chemosynthetic production at deep seeps (Levin and Michener, 2002) and to feed on vesicomyid clams (Sahling et al., 2003). At the REGAB site their $\delta^{15}N$ signature suggests a diet mainly based on recycled material. Low predation pressure in living chemosynthetic assemblages has been suggested at deep cold-seep sites compared with shallower sites (Sahling et al. 2003). Nevertheless, *Phymorrhynchus* observed in abundance in some vesicomyid clusters, particularly in the periphery areas, may not only be a scavenger but also an active predator (A. Warén, pers. com.).

The primary “consumers”, via endosymbioses with chemoautotrophic bacteria, or mainly grazing on free-living microbial communities, therefore dominate the chemosynthetic assemblages in the active centre of the pockmark. There are some possible predators/scavengers, but their densities and biomass are low, at least in the assemblages sampled for this study. In the periphery, the low-density vesicomyid clusters with a high proportion of empty shells provide detritical material for detritivores and scavengers, and

higher trophic levels dominate the heterotrophic fauna. Such a community structure, with a clear dominance of primary consumers, symbiont hosts and bacterivores, has been observed from quantitative sampling in the hydrothermal vent community dominated by the siboglinid *Ridgeia piscesae* (Bergquist et al., 2007). The commensal Polynoidae *Branchipolynoe sp.* has been found in most of the mussels collected on REGAB but the sampling effort is not sufficient to estimate its abundance, and it could represent a non-negligible part of the biomass. As observed in other hydrothermal vent and cold-seep sites, its isotopic signature suggests a strong nutritional relationship with its host (Colaço et al., 2002; Fisher et al., 1994; Suzuki et al., 1989; Van Dover et al., 2003). According to Desbruyères et al. (1985) from gut content analyses, commensal polynoids nutrition is based on mussel mucus-rich pseudo-faeces and gills. More generally, the polychaete community that is not included in this study, likely play an important role in the community structure and could contribute to the biomass of predators, as has been shown in hydrothermal vent communities for polynoids in particular (Bergquist et al. 2007). At seeps, the Amphinomid polychaete has been suggested to obtain significant dietary carbon directly from the symbiont-containing *Bathymodiolus childressi* (MacAvoy et al. 2005).

Finally, biomass of associated species is highest in mytilid beds and more variable in vesicomyid clusters, mostly due to the presence or absence of only one species *Chiridota* sp. Nevertheless, the infauna that are not included in this study probably contribute more to the biomass than in the case of mytilid and siboglinids.

4.3. Transfer of seep organic matter to the surrounding ecosystem

The mobile benthic predators/scavengers that use chemosynthetic biomass may contribute to the export of seep organic production to the surrounding ecosystem. Isotopic measurements suggest that the contribution of chemosynthetic material in fish diet is variable among the

species but not negligible (more than 15% and up to 38%) for 5 of the species captured off the cold-seep site among the 12 analysed. These species have been sampled at least in the northern part of the cold-seep site (CP20) or in a 100-m radius around the pockmark and some (Ophidiidae) have been observed during ROV dives. Only the Zoarcidae *Lycodes* sp. is resident in the seeps, and may be endemic, according to its high degree of dependence from chemosynthetic production and their absence in trawl samples outside the seep site. The two species of galatheids differ greatly in the isotopic signatures of their tissues and, therefore, by their dependence on the chemosynthetic production. Their distribution also appears to be different according to their distribution in trawls and as only *M. geyeri* was sampled during dives on REGAB.

In comparison, a similar study in the shallower sites of the Gulf of Mexico (MacAvoy et al. 2002) showed several species of fish and invertebrates that obtained between 50 and 100% of their nutrition from seep production, indicating that they are resident to the seeps. There is also a greater abundance of vagrants at bathyal depths than at 3200 m depth. MacAvoy et al. indicated that several vagrant predators/scavengers sampled on seep sites, including fishes (*Nezumia* sp., *Oligopus* sp., and the eels *Synaphobranchus* sp., *Ophicthus cruentifer* and *Dysomma rugosa*) and three invertebrates (*Bathynomus giganteus, Buccinum canetae, Scleracterias tanneri*) have a significant contribution of chemosynthetic material in their diet. Predation by large and mobile species of seep resident species is probably much higher at these shallow sites, because the surrounding benthos is probably more depauperate, as suggested by Sahling et al. (2003) in the Sea of Okhotsk. However, all these species were captured on the Louisiana slope cold-seep sites whereas those collected off-site by traps (2 km from the location of the seep communities) used from 0 to 40% of chemosynthetic material, which is not different from our study.

4.4. Comparison with other cold-seep communities in the Atlantic

The chemosynthetic assemblages of the REGAB site share many genera and even species with other cold-seep sites in the Atlantic. The alvinocarid shrimp *Alvinocaris muricola* has been collected in the Gulf of Mexico (Carney, 1994; Cordes et al., 2007), Blake Ridge (Van Dover et al. 2003) and Barbados seep aggregations (Olu et al., 1996), and the chirodotid holothurian *Chiridota heveva*, which seems to be very close to the REGAB species, was first described from the Blake Ridge mussel beds (Pawson and Vance, 2004). The gastropod *Provanna sculpta* is abundant within Gulf of Mexico mussel beds (Bergquist et al., 2005) and tube-worm aggregations (Bergquist et al., 2003). Actinians have also been reported in Blake Ridge mussel beds and from the Florida escarpment (Cordes et al., 2007; Van Dover et al., 2003). Galatheid crabs *Munidopsis* sp. are generally associated. The commensal polynoid *Branchipolynoe seepensis* may also be a common species to West Atlantic and East Atlantic cold-seep mussels (Desbruyères and Hourdez, unpubl.) The community inhabiting the REGAB pockmark is very similar to the group of communities described for the deep seeps of the Gulf of Mexico (2200–3300 m), the intermediate-depth sites of the Barbados prism (1700–2000) and the 2150 m Blake Ridge sites, which have been included in the same group, clustered by depth instead of geography by Cordes et al. (2007). Indeed this 'deep' seep community from the western Atlantic includes *E. laminata* and *B. heckerae* or *B. boomerang* as structuring species, and *A. muricola, Munidopsis* sp., *B. seepensis, Chiridota* sp., *Phymorrhynchus* sp. a Nautillinellid polychaete and the ophiurid *Ophioctenella acies*. Except for these last two species, all the other species or genera have been sampled on REGAB. Most intriguing is the co-occurrence of similar species from both sides of the Atlantic, that has been discussed for *Bathymodiolus boomerang* (Olu-Le Roy et al. 2007b) as example of two amphi-atlantic Bathymodiolinae species complexes, Alvinocharid shrimps (Komai and

Segonzac 2005), and more generally by Cordes et al. (2007) comparing associated fauna from box core sampling on the Nigerian margin and cold-seep communities from the Eastern Atlantic.

5. Conclusion

The REGAB pockmark cold-seep community is the first to be described in the Equatorial East Atlantic and the second in the Eastern Atlantic after the Haakon Mosby Mud Volcano located in high latitudes (Gebruk et al. 2003). REGAB's striking feature is the abundance and diversity of large symbiont-bearing species, mytilid and vesicomyid bivalves and siboglinids, forming puzzling habitats hosting chemosynthetic assemblages, whose highest biomasses are found in the mussel beds likely located in the highest fluid flow areas. Associated megafauna is very similar among assemblage types, with few same dominant taxa but whose individuals may be closely associated with local aggregates as suggested by the variation of isotopic signatures according to the types of aggregates or habitat. These associate species, which are likely endemics of the seep community, mainly use chemosynthesis-derived carbon but with variable contribution of methanotrophy or thiotrophy. Biomass in the sampled assemblages was dominated by primary "consumers", either symbiont-bearing species or feeders of free living microbes, such as shrimps or gastropods. Scavengers (galatheids, probably holothurids) and possible predators (*Phymorhynchus* and zoarcid fishes) representing the higher trophic levels are less dense distributed but occur all over the area colonised by chemosynthetic assemblages. Export of local production by colonists or vagrant species is reduced compared to shallower depth seeps but has been shown for 7 species of fish with more than 10% contribution of chemosynthesis-based carbon and for two galatheids. The REGAB community bears a high resemblance to those associated with seeps in the western Atlantic (Barbados prism, Gulf of Mexico, Blake Ridge). Further sampling efforts using

quantitative tools are necessary to describe better the communities associated with each of the three chemosynthetic assemblages, and for comparison with studies on similar habitats in the Gulf of Mexico seeps. The REGAB pockmark is an unusual cold-seep site by the high spatial heterogeneity of habitats in a relatively restricted area, which makes comparisons easier between habitats and chemosynthetic assemblages. Thus it is possible to identify the factors controlling the community structure without the influence of factors that may play a role in a broader scale, such as bathymetry, geology of the structures, or biogeography.

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Table and figure caption

Table 1: Characteristics of the sampling sites based on dominant symbiont-bearing species. Abbreviations: Mx: site dominated by Mytilidae; Vx: by Vesicomyidae, E: by Escarpiidae. For the location, see Fig.2b.

Table 2: Densities (ind.m^{-2}) (a) and biomass (g dry weight.m^{-2}) (b) of megafauna species from image analysis and sampling in the clusters defined by symbiont bearing species (M: clusters dominated by mytilids; V: by vesicomyids, E: by escarpiids). n.e.: non estimated

Table 3: Isotopic carbon signature ($\delta^{13}C$) of methane extracted from sediment or expelled fluid on the REGAB pockmark. Percentage of methane in the total gas fraction is calculated from gas chromatography peak integration.

Table 4: Isotopic signatures of sediment and particles sampled by cores or traps in and off the REGAB pockmark.

Table 5: Isotopic signatures of symbiont-bearing species from different sampling sites in the REGAB pockmark.

Table 6: Mean isotopic signatures of associated megafauna sampled by ROV in the clusters dominated either by mytilids (M), vesicomyids (V) or escarppids (E) or by trawl (CP20) in the REGAB cold-seep site.

Table 7: Isotopic signature ($\delta^{13}C$) of fishes sampled by trawls at increasing distance from the REGAB site and estimated percentages of chemosynthetic material in their diet. See fig. 1 for the localization of trawls.

Figure 1: Location of the REGAB pockmark along the Congo-Angola margin and of the benthic trawls

Figure 2:a. Location of the dive tracks in the REGAB site, analyzed for megafauna distribution, and of sampling sites. Two dive tracks are represented by the black and grey lines; the box indicates the area surveyed by five more dives. b. Distribution of chemosynthetic assemblages along the dive tracks, classified by the dominance of symbiotic species (from Olu-Le Roy et al. 2007a).

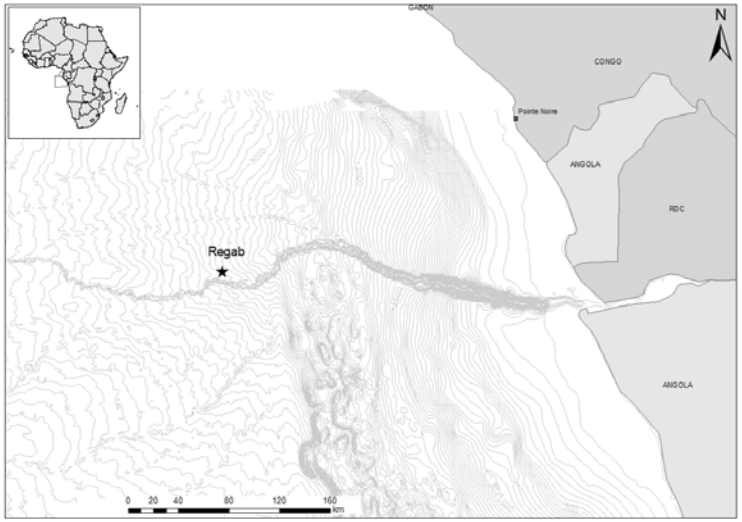
Figure 3: Distribution maps of major associated megafauna species along the dive tracks. a. *Chiridota* sp., b. *Munidopsis* sp., c. *Alvinocaris muricola*, d. Irregular echinid.

Figure 4: Multidimentional scaling (MDS) plots of the species/biotope matrix of distance using the Bray Curtis distance (Primer software). **a.** Similarity between biotopes, **b.** Similarity between species. H=hydrate outcrop, B=gas bubbles, BM=bacterial mat, Conc=carbonate concretion, S= soft sediment, RS=reduced sediment, Ed=dead escarpiids, El=live escarpiids, M=mytilids, Vd=dead vesicomyids, Vl=live vesicomyids, Vm=mixed (live and dead) vesicomyids.

Figure 5: Mean $\delta^{13}C$ vs $\delta^{15}N$ values of each species, sediment samples or particles analysed from the REGAB pockmark. The signatures of the three Zoarcidae fish are represented to show the high variability between specimens. Methane $\delta^{13}C$ values are also plotted. CI to CIII correspond to the different levels of consumers (from primary to tertiary)

Figure 6: $\delta^{13}C$ vs $\delta^{15}N$ values of shrimps *Alvinocaris muricola* (a) and holothurids *Chiridota* sp. (b) from different sites dominated either by mytilids (M) or vesicomyids (V) with signature of the bivalves at the different sampling sites and sediment for holothurids.(c) from left to right: *A. muricola* among mussels, *Chiridota* sp. in mussel and vesicomyid aggregates.

Fig. 1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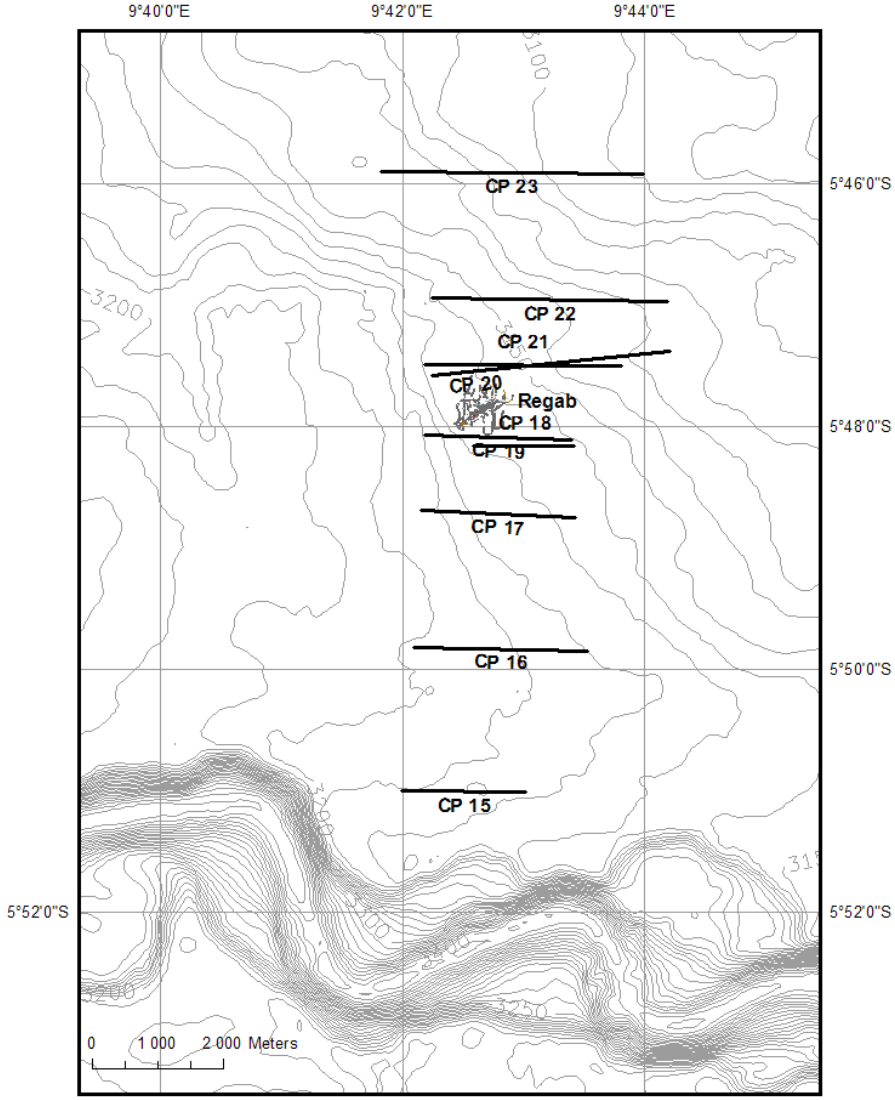
CONGO
Pointe Noire
ANGOLA
RDC
Regab
ANGOLA
N
0 20 40 80 120 160 km

a.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5°46'0"S
5°48'0"S
5°50'0"S
5°52'0"S
5°52'0"S
CP 23
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CP 20
Regab
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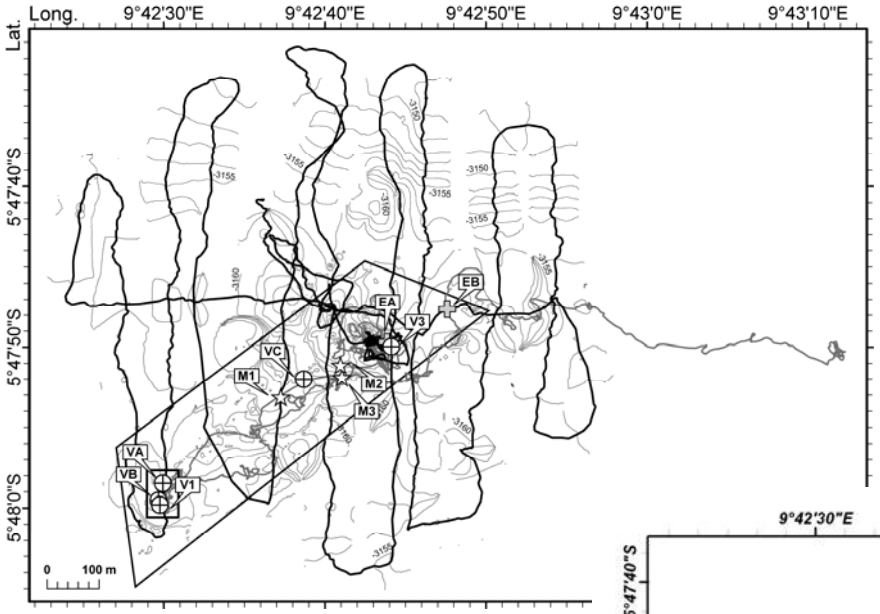
CP 16
CP 15
3200
0 1 000 2 000 Meters

b.

Fig 2


Long.
9°42'30"E
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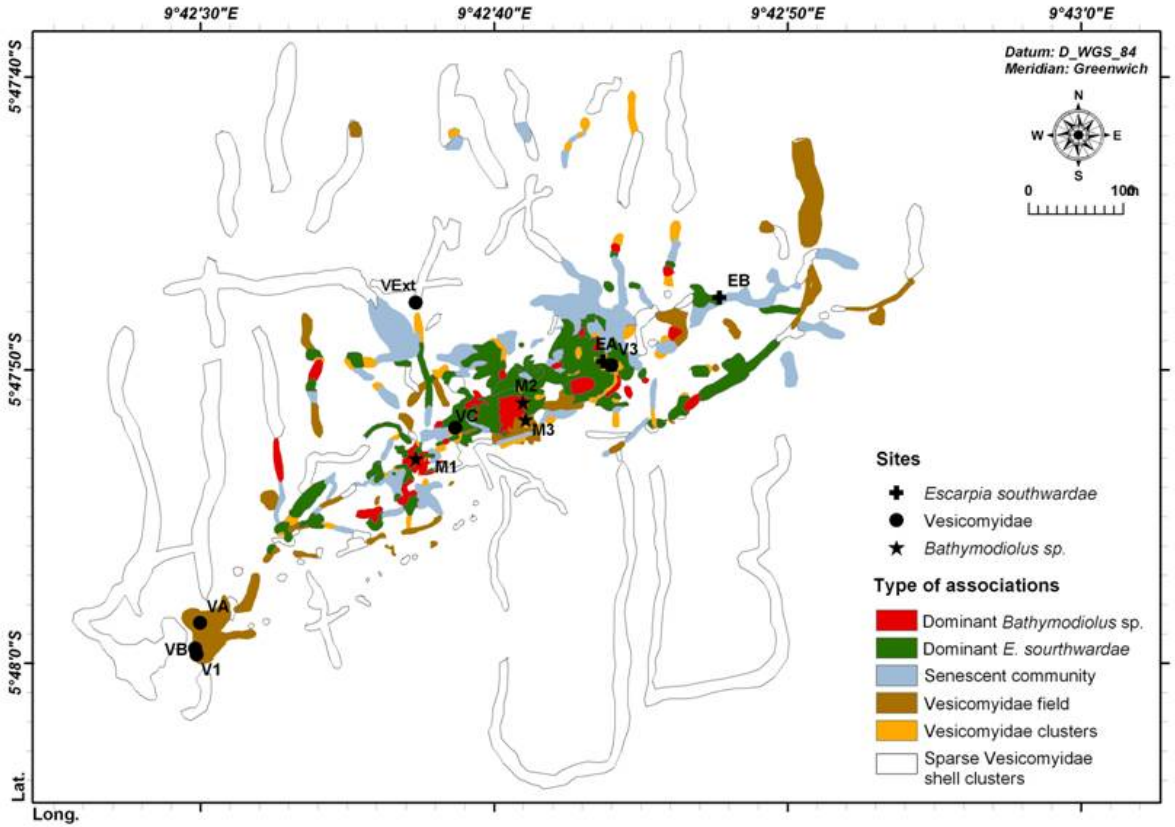
9°43'10"E
Lat.
5°47'40"S
5°47'50"S
5°48'0"S
EA
EB
V3
VC
M1
M2
M3
VA
VB
V1
0
100 m

a.


9°42'30"E
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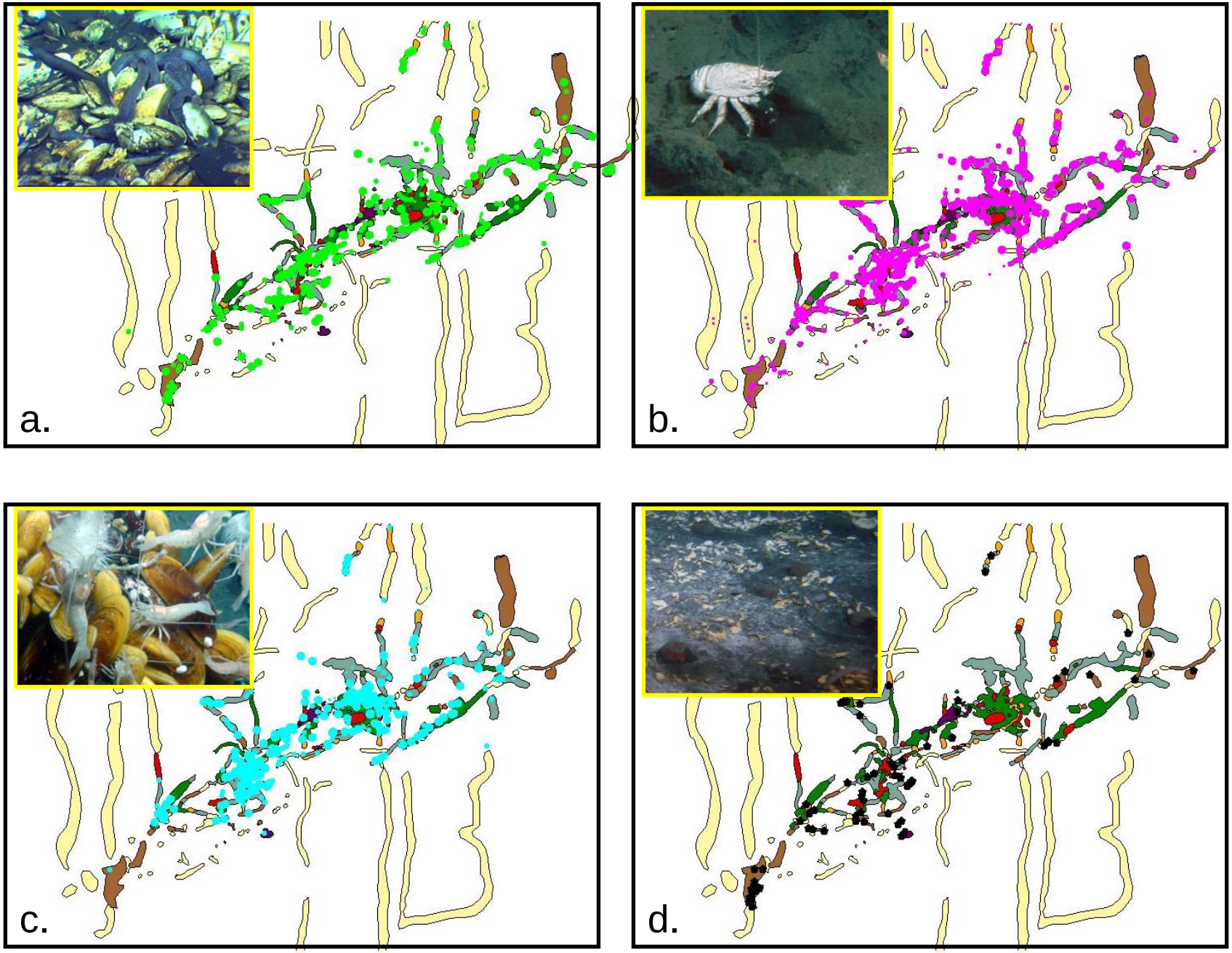
5°47'40"S
5°47'50"S
5°48'0"S
Lat.
Long.
Datum: D_WGS_84
Meridian: Greenwich
N
W
E
S
0
100m
VExt
EB
EA
V3
M2
VC
M3
M1
VA
VB
V1
Sites
Escarpia southwardae
Vesicomyidae
Bathymodiolus sp.
Type of associations
Dominant Bathymodiolus sp.
Dominant E. sourthwardae
Senescent community
Vesicomyidae field
Vesicomyidae clusters
Sparse Vesicomyidae shell clusters

b.


a.
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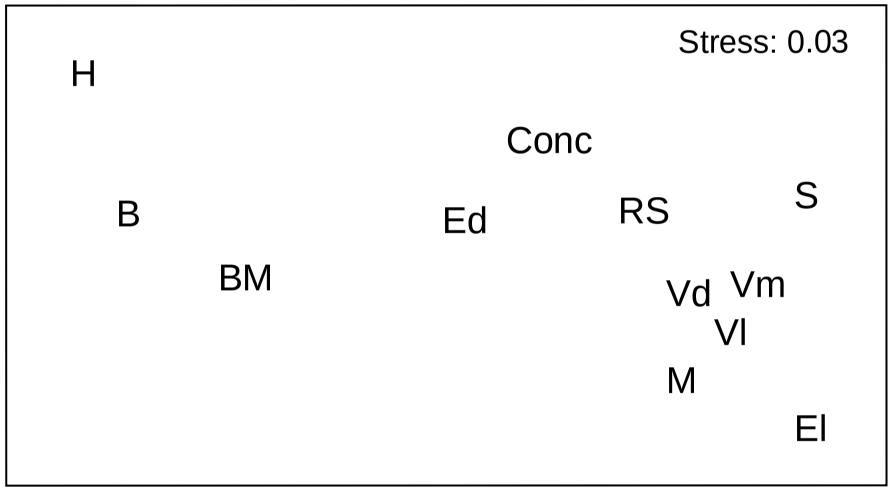
c.
d.

a.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Conc
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RS
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Vd
Vm
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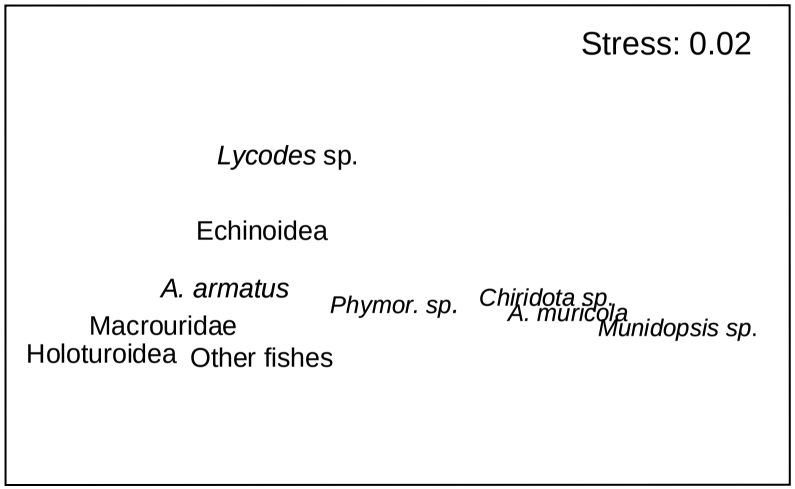
M
El

b.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Lycodes sp.
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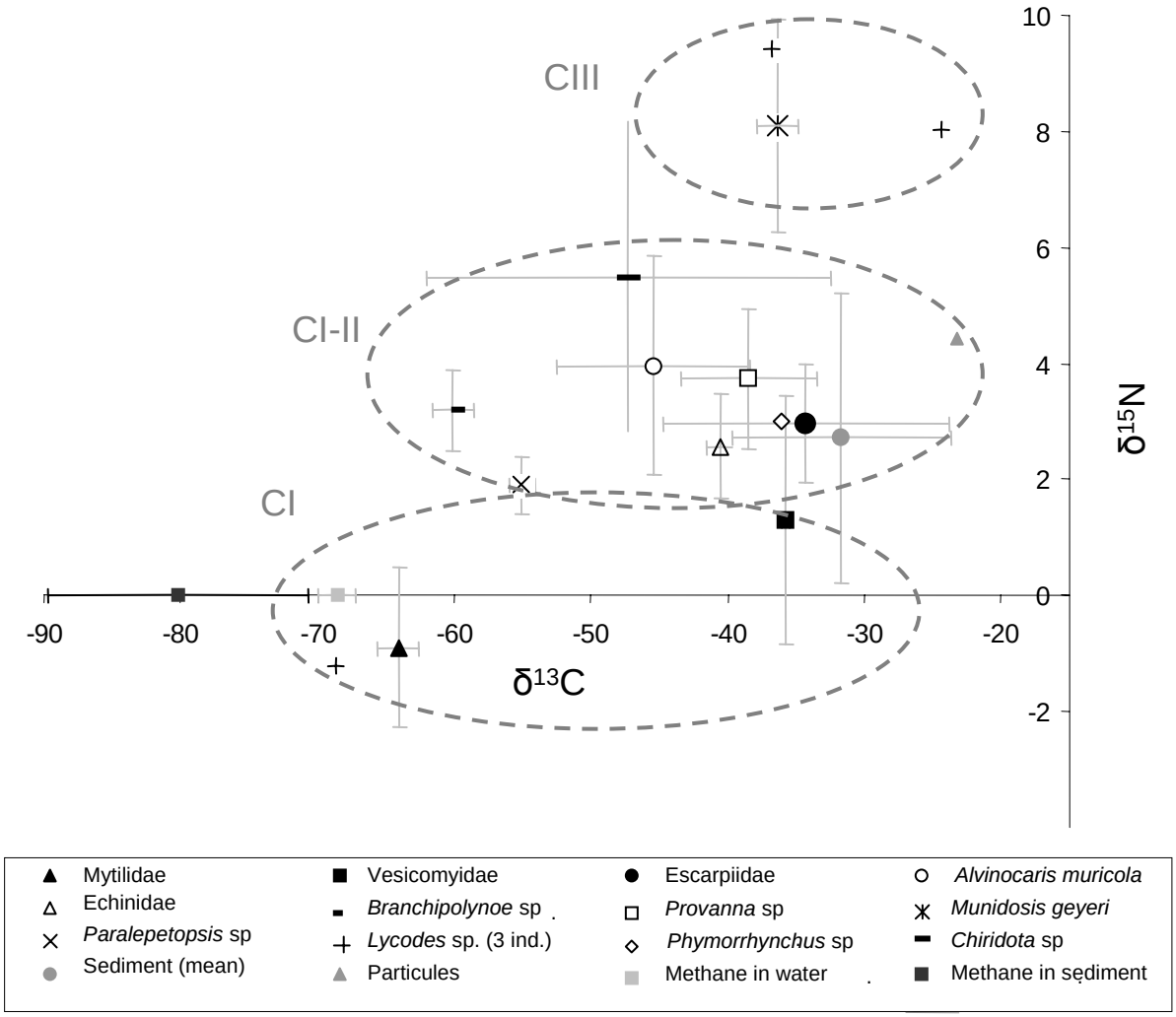
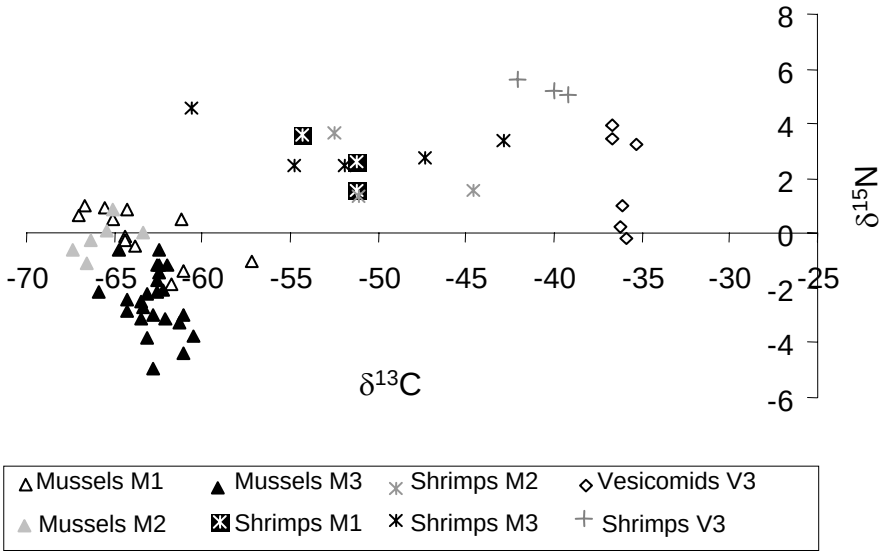
A. armatus
Phymor. sp.
Chiridota sp.
A. muricola
Munidopsis sp.
Macrouridae
Holoturoidea
Other fishes


CIII
CI-II
CI
10
8
6
4
2
0
-2
-90
-80
-70
-60
-50
-40
-30
-20
δ13C
δ15N
Mytilidae
Echinidae
Paralepetopsis sp
Sediment (mean)
Vesicomyidae
Branchipolynoe sp
Lycodes sp. (3 ind.)
Particules
Escarpiidae
Provanna sp
Phymorrhynchus sp
Methane in water
Alvinocaris muricola
Munidosis geyeri
Chiridota sp
Methane in sediment

a.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4
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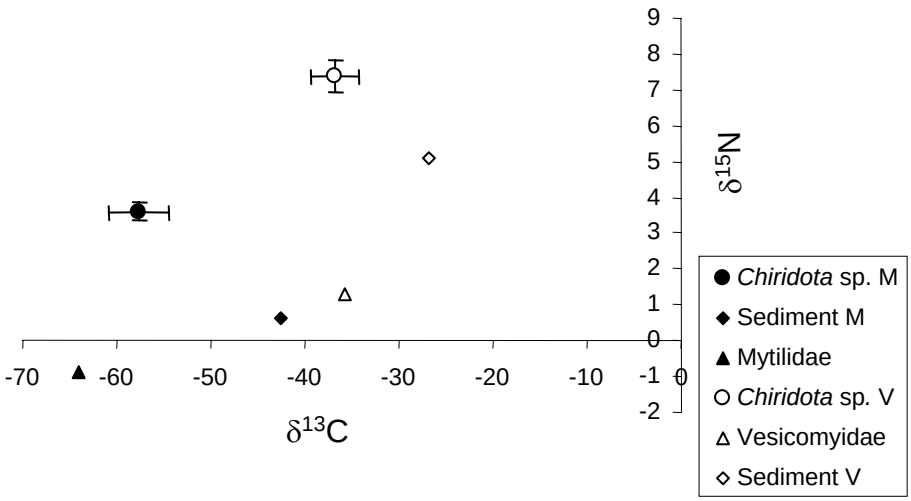
0
-2
-4
-6
-70
-65
-60
-55
-50
-45
-40
-35
-30
-25
δ15N
δ13C
Mussels M1
Mussels M3
Shrimps M2
Vesicomids V3
Mussels M2
Shrimps M1
Shrimps M3
Shrimps V3

b.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-60
-50
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-30
-20
-10
0
δ15N
δ13C
Chiridota sp. M
Sediment M
Mytilidae
Chiridota sp. V
Vesicomyidae
Sediment V

c.

Table 1.

Characteristics of the sampling sites based on dominant symbiont-bearing species.

Site	Dominant species	Percentage of living individuals	Cluster size (m^2)	Other comments
M1	Mytilidae *Bathymodiolus* aff. *boomerang*[a]	100	400	
M2	*B.* aff. *boomerang*	100	1	
M3	*B.* aff. *boomerang*[a]	100	4	
V1	Vesicomyidae: *Laubiericoncha chuni*	>80	4	External, main large field
VB	Vesicomyidae	0	Unknown	External, main large field
Vext	Vesicomyidae	c. 50	0.45	External, isolated cluster
V3	Vesicomyidae: *Calyptogena regab*	>80	0.35	Central zone
VC	Vesicomyidae	>80	0.75	Central zone
EA	Siboglinidae: *Escarpia southwardae*	c. 100	<0.5	Adults (>1.5 m long)
EB	*E. southwardae*	c. 100	<0.25	Young bush (<0.5 m long)

Table 2.

Densities (ind m^{-2}) (a) and biomass (g dry weight m^{-2}) (b) of megafauna species from image analysis and sampling in the clusters defined by symbiont-bearing species (M: clusters dominated by mytilids; V: by vesicomyids, E: by escarpiids).

	M1	**M2**	**M3**	**V3**	**VC**	**V1**	**VB**	**Vext**	**EA**	**EB**
(a)										
Symbiont-bearing species										
Bivalvia										
B. aff. boomerang	591.7	1066.7	869.4							
Vesicomyidae				741.6	607.5	586.4	0 (sh.)	66.7		
Polychaeta										
E. southwardae	125.0		189.2						769.1	250.0
Associated species										
Actiniaria	52.8	13.5	3.03					4.2		
Gastropoda										
Paralepetopsis sp.	227.8	236.3	68.7						79.5	
Provanna sp.	508.3	87.8	129.3	210.4	38.8	67.8			105.8	

	M1	M2	M3	V3	VC	V1	VB	Vext	EA	EB
Phymorhynchus sp.					3.0	30.3	27.8		2.8	
Crustacea										
Alvinocaris muricola	450.0	344.3	743.7	245.6	53.4	0.7	55.6	0	302.4	58.5
Munidopsis geyeri						8.1	27.8			2.7
Holothuridea										
Chiridota sp.	2.8	6.8		93.3	4.9		111.1			31.9
Chordata										
Lycodes sp.						2.8		2.1		
Total	1958.3	1741.8	2003.2	1290.9	707.5	696.1	222.3	73.0	1256.7	343.1
Associated species	1313.9	675.1	1130.9	549.3	100.0	107.0	222.3	6.3	487.6	93.08
(b)										

	M1	M2	M3	V3	VC	V1	VB	Vext	EA	EB
Symbiont-bearing species										
Bivalvia										
B. aff. *boomerang*	1966.9	3546.0	2890.2							
Vesicomyidae				1964.5	1609.2	1553.2	0	0		
Polychaeta										
E. southwardae	91.4		138.4						562.2	90.0
Associated species										
Actiniaria	n. e.							n.e.		
Gastropoda										
Paralepetopsis sp.	1.8	1.8	0.53						0.64	
Provanna sp.	10.4	1.8	2.65	4.3	0.8	1.4			2.22	
Phymorhynchus sp.					3.0	29.9	27.5		2.8	
Crustacea										
Alvinocaris muricola	255.0	195.1	421.4	139.2	30.2	0.4	31.5	0	171.3	33.2

	M1	M2	M3	V3	VC	V1	VB	Vext	EA	EB
Munidopsis geyeri						23.0	78.6			7.5
Holothuridea										
Chiridota sp.	3.4	8.3		114.6	6.0		136.4			0.7
Chordata										
Lycodes sp.						n.e.		n.e.		
Total	>2329	3753.1	3453.2	2222.6	1649.1	>1608	222.3	n.e.	736.4	126.4
Associated species	270.6	207.1	401.3	258	39.9	54.8	222.3	n.e.	174.2	36.4

n.e.: non-estimated.

Table 3.

Isotopic carbon signature ($\delta^{13}C$) of methane extracted from sediment or expelled fluid on the REGAB pockmark.

Sampling site	**Sample type**	**% CH_4**	**$\delta^{13}C$ CH_4 (‰)**
Vesicomyid field	Sediment	100	−82
Regab centre	Sediment	99.8	−81
Regab centre	Gas from sediment core	99.8	−84.3
Hydrate outcrop	Gas from sediment core	100	−68.5
Next to M2	Sediment	99.94	−68.7
Next to M1	Sediment	99.87/99.92	−84.8/−96.2
Regab centre	Fluid	99.941	−70.4
Regab centre	Fluid	99.954	−68.69
Regab bubble site	Fluid	99.989	−67.3
Regab bubble site	Fluid	99.955	−67.9

Table 4.

Isotopic signatures of sediment and particles sampled by cores or traps in and off the REGAB pockmark.

Sample	**$\delta^{13}C$ (‰) mean**	**$\delta^{13}C$ (‰) sd**	**$\delta^{15}N$ (‰) mean**	**$\delta^{15}N$ (‰) sd**
Sediment M1	−30.9		0.14	

Sample	$\delta^{13}C$ (‰) mean	$\delta^{13}C$ (‰) sd	$\delta^{15}N$ (‰) mean	$\delta^{15}N$ (‰) sd
Sediment M2	−47.69	2.37	1.36	0.34
Sediment M3	−29.46		0.07	
Sediment V1	−25.58		5.72	
Sediment V2	−26.4		3.92	
Sediment V3	−28.04	0.72	4.44	0.37
Particulars from traps	−23.22		4.43	
Sediment 600 m/1700 m from Regab	−23.77	0.59	7.88	0.03

Table 5.

Isotopic signatures of symbiont-bearing species from different sampling sites in the REGAB pockmark.

Species/sites	$\delta^{13}C$ (‰) mantle	$\delta^{13}c$ (‰) gill	$\delta^{15}N$(‰) mantle	$\delta^{15}N$ (‰) gill	$\delta^{13}C$(‰) mean*	$\delta^{15}N$(‰) mean*
Bathymodiolus aff. *boomerang*						
M1	−63.3±3.5 (12)	−63.7±2.1	−0.5±0.8 (12)	−0.6±0.3		
M2	−64.5±0.8 (6)	−67.0±0.8	0.3±0.5 (6)	−0.7±0.4		
M3	−63.7±1.5 (24)	−62.4±0.9	−1.9±0.9 (24)	−3.1±1.0		
Vesicomidae sp.						

Species/sites	δ^{13}C (‰) mantle	δ^{13}c (‰) gill	δ^{15}N(‰) mantle	δ^{15}N (‰) gill	δ^{13}C(‰) mean*	δ^{15}N(‰) mean*
V1	−35.6±1.6 (5)	−35.7±1.1	2.9±2.0 (5)	−1.0±2.1		
V3	−36.0±0.8 (5)	−36.0±0.15	3.5±0.3 (5)	2.9±2.0		
Escarpia southwardae						
M1					−24.2±5.5 (5)	2.5±1.6 (5)
EA (adults)					−36.22±3.3 (5)	2.9±0.6 (5)
EB (youngs)					−29.1±3.4 (3)	–

Table 6.

Mean isotopic signatures of associated megafauna sampled by ROV in the clusters dominated either by mytilids (M), vesicomyids (V) or escarppids (E) or by trawl (CP20) in the REGAB cold-seep site.

Species	Site	δ^{13}C (‰) mean	δ^{13}C (‰) sd (*n*)	δ^{15}N (‰) mean	δ^{15}N (‰) sd (*n*)
Polychaeta					
Branchipolynoe sp.	M1	−60.09	–	2.46	–
Gastropoda					
*Provanna s*p.	VB,E2E3	−35.12	1.66 (6)	3.89	1.33 (6)
	CP20	−44.31	4.43 (5)	4.71	0.49 (5)

Species	Site	$\delta^{13}C$ (‰) mean	$\delta^{13}C$ (‰) sd (*n*)	$\delta^{15}N$ (‰) mean	$\delta^{15}N$ (‰) sd (*n*)
Paralepetopsis sp.	M1	−55.0	–	1.9	–
Phymorrhynchus sp.	E2	−36.13	1.46 (3)	No value	–
	CP20	−36.03	1.51 (5)	2.99	0.41 (5)
Crustacea					
Alvinocaris muricola	M1M2M3	−50.4	0.92 (11)	2.6	0.45 (11)
	V3	−40.4	1.47 (3)	5.3	0.26 (3)
	CP20	−38.5	2.03(3)	4.40	0.54 (3)
Munidosis geyeri	E2	−36.31	–	9.37	–
	CP20	−36.33	1.05 (3)	6.50	0.42 (3)
Munidopsis hirtella	CP20	−22.4	–	11.25	–
Echinodermata					
Chiridota sp.	M1M3	−57.06	5.59 (5)	3.93	1.96 (5)
	CP20	−36.78	1.84 (2)	7.37	0.49 (2)
Echinidae sp.	Near to M3	−41.29	2.53 (3)	1.95	1.25 (3)

Species	Site	δ^{13}C (‰) mean	δ^{13}C (‰) sd (*n*)	δ^{15}N (‰) mean	δ^{15}N (‰) sd (*n*)
Chordata					
Lycodes sp.	CP20	−43.23	22.83 (3)	5.41	5.79 (3)

Table 7.

Isotopic signature (δ^{13}C) of fishes sampled by trawls at increasing distance from the REGAB site and estimated percentages of chemosynthetic material in their diet.

	δ^{13}C (‰) mean (*n*)							Mean	% chemosynthetic material
Trawls	**CP17**	**CP18**	**CP19**	**CP20**	**CP21**	**CP22**	**CP23**		
Distance from REGAB	**1560 m**	**580 m**	**400 m**	**610 m**	**680 m**	**1660 m**	**3600 m**		
Acantaurus armatus	−20.1 (2)		−21.8 (3)	−22.7	−19.3	−22.4 (2)	−22.0	−21.4±1.6	4–27
Bassossetus robustus	−20.6 (2)				−25.0	−21.2 (4)	−23.2 (2)	−21.7±1.6	16–39
Coryphaenoides striaturus	−18.8						−18.8	−18.8±0.6	7
Porogadus milles	−21.5 (2)	−22.0		−18.6		−21.0		−21.0±1.5	6–23
Malacoteus niger		−18.7						−18.7±1.0	6

	δ^{13}C (‰) mean (*n*)							Mean	% chemosynthetic material
Trawls	**CP17**	**CP18**	**CP19**	**CP20**	**CP21**	**CP22**	**CP23**		
Distance from REGAB	**1560 m**	**580 m**	**400 m**	**610 m**	**680 m**	**1660 m**	**3600 m**		
Scopeloberyx robustus		−18.5			−212			−19.9±1.6	19
Holcomysteronus squamosus			−21.3	−28.3				−24.8±3.8	20–56
Stomias boa boa			−18.1 (2)			−15.9		−17.5±1.7	0–3
Stylephorus chordatus			−20.7		−22.6			−21.8±1.1	17–26
Histobranchius bathybius						−18.0		−18.0±0.1	0
Manducus maderensis				−21.1		−16.9		−19.0±2.6	6
Lycodes sp.				−45.2 (3)				−45.2±19.7	35–100
Munidopsis geyeri			−34.4 (2)	−36.3 (3)					86–100
Munidopsis hirtella			−23.6 (2)	−22.0		−21.4			19–31