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The vesicomyid bivalve habitat at cold seeps supports heterogeneous and dynamic macrofaunal assemblages

Guillon Erwan [1], Menot Lénaïck [1], Decker Carole [1], Krylova Elena [2], Olu Karine [1]

[1] IFREMER, Centre de Bretagne, REM/EEP, Laboratoire Environnement Profond, F-29280 Plouzané, France
[2] P.P. Shirshov Institute of Oceanology, Russian Academy of Sciences, Nakhimovsky Prospekt, 36, Moscow, 117997, Russia

Abstract :

The high biodiversity found at cold seeps, despite elevated concentrations of methane and hydrogen sulfide, is attributed to multiple sources of habitat heterogeneity. In addition to geological and geochemical processes, biogenic habitats formed by large symbiont-bearing taxa, such as bivalves and siboglinid tubeworms, or by microbial mats drive the biodiversity of small-sized fauna. However, because these habitat-forming species also depend on geochemical gradients, the respective influence of abiotic and biotic factors in structuring associated macrofaunal communities is often unresolved. The giant pockmark Regab located at 3200 m depth on the Congo margin is characterized by different fluid-flow regimes, providing a mosaic of the most common biogenic habitats encountered at seeps: microbial mats, mussel beds, and vesicomyid clam beds; the latter being distributed along a gradient of environmental conditions from the center to the periphery of the pockmark. Here, we examined the structure of macrofaunal communities in biogenic habitats formed in soft sediment to (1) determine the influence of the habitats on the associated macrofaunal communities (inter-habitat comparison), (2) describe how macrofaunal communities vary among vesicomyid clam beds (intra-habitat comparison) and (3) assess the inter-annual variation in vesicomyid beds based on repeated sampling at a three-year interval. The highest densities were found in the microbial mat communities in intermediate fluid-flow areas, but they had low diversity — also observed in the sediment close to mussel beds. In contrast, vesicomyid beds harbored the highest diversity. The vesicomyid beds did not show a homogeneous macrofaunal community across sampled areas; instead, density and composition of macrofauna varied according to the location of the beds inside the pockmark. The clam bed sampled in the most active, central part of the pockmark resembled bacterial mat communities by the presence of highly sulfide-tolerant species living at the sediment surface, along with vesicomyid juveniles. This similarity suggests a gradual change in community composition from mats to clam beds. Inter-annual comparisons of the different clam beds highlighted that the most active central site had a more variable community than its peripheral counterparts. Finally, a rapid shift in community structure, particularly in polychaete families, in experimentally reduced oxygen concentrations in the central part of Regab, suggests that high beta-diversity communities can withstand intense variation in geochemical conditions. These community dynamics are likely related to the diversity and to the plasticity of the vesicomyids themselves, because they can cope with high spatial and temporal environmental variability at a very local scale.

Highlights

► The high plasticity and diversity of vesicomyids create habitat heterogeneity at seeps. ► The “vesicomyid habitat” sustains a heterogeneous macrofaunal community. ► High diversity of vesicomyid associated macrofaunal community allow rapid changes ► Microbial mats with juvenile clams is proposed as an initial stage for vesicomyid bed

Keywords : Cold-seep, macrofauna, diversity, dynamics, vesicomyid clams, Gulf of Guinea

1. Introduction

Local chemosynthetic primary production at cold seeps generally sustains very high biomass, dominated by large symbiont-bearing invertebrates such as Vesicomyidae clams, Bathymodiolinae mussels and Siboglinidae polychaete tubeworms (Levin, 2005; Sibuet and Olu, 1998), all of which have been described as ecosystem engineers (Levin, 2005). Along with microbial mats, these taxa generate a mosaic of habitats that enhance beta-diversity (Cordes et al., 2010). Geochemistry is thought to be the key to the foundation of cold-seep habitats: fluid flow, methane, hydrogen sulfide and oxygen concentrations, as well as the type of substratum, are the structuring factors behind the distribution of microbial mats, mussel beds, siboglinid tubeworm bushes and clam beds (Aharon, 1994; Levin et al., 2003; MacDonald et al., 2003; Mau et al., 2006; Olu-Le Roy et al., 2007a; Olu et al., 1997; Sahling et al., 2002; Sassen et al., 1994). This mosaic of habitats appears to reflect an interaction between the long-term spatio-temporal dynamics of fluid flow and the succession of key engineering species, from bacterial mats on newly formed sites where methane fluid flow is at its

maximum, to mussels and tubeworms settling on precipitated carbonate concretions, with tubeworm dominance increasing with decreasing methane fluid activity (Bergquist et al., 2003; Cordes et al., 2005b). However, the serial position of vesicomyid clam beds in this succession model remains unclear. Vesicomyids may be precursors of the mytilid/siboglinid settlement (Bowden et al., 2013; Sahling et al., 2008) as well as colonizers of transient fluid-flow areas (Marcon et al., 2014a). Vesicomyids are able to uptake hydrogen sulfide from the sediment and are usually associated with low methane fluxes, compared with bathymodiolin mussels or sponges associated with methanotrophic symbionts (Aharon, 1994; Mau et al., 2006; Olu-Le Roy et al., 2007a; Olu et al., 1997). Vesicomyid beds are also associated with lower sulfide fluxes than those experienced by microbial mats (Levin et al., 2003; Pop Ristova et al., 2012; Sahling et al., 2002). The ability to exploit low and transient fluxes (Marcon et al., 2014a), but also to cope with high sulfide levels within the sediment (Barry et al., 1997) likely explains the widespread distribution of vesicomyids at cold seeps along with their very high diversity at the genus and species level (Krylova and Sahling, 2010). Associated with either focused or more diffuse fluxes, they show high variability in their densities (less than 10 ind.m^{-2} to up to 1000 ind.m^{-2}) (Sibuet and Olu-Le Roy, 2002) and have variable growth rates (Barry et al., 2007). Inter-specific variability in sulfide-binding capacity (Goffredi & Barry 2002) or hypoxia tolerance (Decker et al., 2014) can explain the ability of this family to colonize spatially variable environments. Vesicomyids can also move, using their strong vascularized foot, to reach suitable environments (Arp et al., 1984). They are functionally important ecosystem engineers in cold seeps that may create habitat and modify their environment by bioturbation or bio-irrigation, both processes that may favor influxes of seawater sulfate within the sediment, stimulating anaerobic oxidation of methane (AOM) and sulfide production (Fischer et al., 2012; Wallmann et al., 1997). However, it is not known if, as shown for siboglinid tubeworms releasing sulfate through their roots (Dattagupta et al., 2008), vesicomyids may maintain the conditions of sulfide production without methane input and thus survive for a certain period of time after fluid emission stops. In any case, a certain period of time after sulfide production ceases the clam aggregation dies, or moves to find sulfide-rich sediments (Marcon et al., 2014a).

Cold-seep communities in the giant pockmark Regab located 3160 m deep along the Congo margin are likely sustained and structured by two different fluid regimes (Marcon et al., 2014b): along a central line on the pockmark, focus and intense flow through a fracture matches the patchy distribution of diverse assemblages including dense bushes of siboglinid tubeworms, assemblages of bathymodiolin mussels on hard carbonate crusts and more rarely on soft sediment, as well as aggregations of vesicomyid clams and microbial mats on soft sediments; at the periphery of the central line, lower diffuse flow through a porous substratum harbors only patches of vesicomyid clams (Olu-Le Roy et al., 2007a; Ondréas et al., 2005). The two vesicomyid species colonizing the pockmark (*Christineconcha regab* and *Laubiericoncha chuni*) differ in their distribution along this gradient (Cosel and Olu, 2009) according to differences in morphological (Krylova and Cosel, 2011) and physiological (Teixeira et al., 2013) adaptations. The Regab pockmark is assumed to have been relatively stable in terms of fluid emission over the last 10 years, as suggested by very little change in the distribution of most biogenic habitats, with the exception of some vesicomyid patches (Marcon et al., 2014a). Temporal variation in the distribution of the clam beds suggests that they are sustained by more transient fluxes than other habitats at an inter-annual scale. The Regab pockmark may thus provide an opportunity to study the spatio-temporal dynamics of vesicomyid assemblages and test the hypothesis that vesicomyids occupy a wide range of fluid-flow regimes.

At cold seeps, such as in the Regab pockmark, each habitat dominated by an engineering species is characterized by a unique meiofaunal and macrofaunal assemblage (Menot et al., 2010; Van Gaever et al., 2009). In chemosynthetic ecosystems, macrofaunal community structure and composition provide consistent indicators of fluid-flow patterns (Portail et al., 2015). In this study, the macrofauna associated with a mussel bed, a microbial mat and three clam beds were sampled to assess spatial variation in community structure and composition along a gradient of fluid flow. In addition, clam beds were sampled twice at a three-year interval, to assess the dynamics of the associated macrofauna. Throughout our analyses, we focused on testing three hypotheses: (1) habitat heterogeneity, a function of engineer species and environmental factors, is reflected in the beta-diversity of macrofaunal communities (2) clam beds, due to their wide distribution across the pockmark, provide a suitable environment for a wider range of macrofaunal species than any other habitat; (3) the observed

variability of associated macrofauna is related to the faster turnover rate in clam beds through the action of both seepage and clam activity.

2. Materials and methods

2.1. Site characteristics

The 800 m wide in diameter, 15 to 20 m deep pockmark Regab is located over the oceanic crust (Ondréas et al., 2005), 8 km north of the Congo canyon at 3160 m depth. Although the term "pockmark" is used to name the whole depression, the Regab site is actually composed of several individual pockmarks <100 m in diameter and thus is considered as a "pockmark cluster" (Fig. 1) (Gay et al., 2006; Ondréas et al., 2005). Intensity and patterns of fluid flows have been further clarified based on a more accurate and comprehensive dataset acquired during the WACS cruise (Marcon et al. 2014b), splitting the pockmark in two main areas differing by fluid flow (Fig. 1). The central and eastern zone is an area of intense and focused fluid flow characterized by large and deep depressions, carbonate precipitation, gas emissions visible as bubble streams above the seafloor and gas hydrates outcrops. The second zone, corresponding to the periphery and western part of the pockmark is an area of diffuse and homogeneous fluid flow with mainly soft sediment, numerous shallow depressions and without visible gas emission nor hydrate outcrops (see Fig. 1 and Marcon et al. 2014b).

Chemosynthetic habitats are characterized by dense communities dominated by large engineer species including the vesicomyid bivalves *Christineconcha regab* and *Laubiericoncha chuni* (Cosel and Olu, 2008; Cosel and Olu, 2009; Krylova and Cosel, 2011), the mytilid *Bathymodiolus* aff. *boomerang* (Olu-Le Roy et al., 2007b) and the siboglinid polychaete *Escarpia southawardae* (Andersen et al., 2004), which are all associated with chemoautotrophic bacteria (Cambon-Bonavita et al., 2009; Decker et al., 2013; Duperron et al., 2005). Thiotrophic bacterial mats, although quite rare, also occur at Regab (Cambon-Bonavita et al., 2009; Pop Ristova et al., 2012). Most of the chemosynthetic habitats have been observed in the area of intense fluid flow, but a large vesicomyid field occur at the

south-western end of the pockmark, as well as many small clusters of mainly dead vesicomyids (not represented) in the small depressions spread over the whole area of diffuse flow (Fig. 1) .

Habitat chemistry was first described in 2001 by measuring concentrations in water within bivalve beds or siboglinid bushes, highlighting that the highest methane concentrations are associated with mussel beds (Olu-Le Roy et al., 2007a). Later, in 2008, benthic chamber deployments measured fluxes at the sediment interface within clam and mussel beds (Decker et al., 2012a; Pop Ristova et al., 2012). This last study also measured chemical gradients within the sediment, as well as microbial activities such as anaerobic methane oxidation (AOM) and sulfate reduction rates, demonstrating decreasing fluxes and bacterial activity in microbial mats and clam beds compared with those found in mussel beds.

Figure 1: Map of the Regab pockmark featuring the sites sampled in this study. Blade core samples were taken in 2008 (08) or 2011 (11), in vesicomyid beds (V), adjacent sediment (S), close to a mytilid bed (M) or in a microbial mat (B). Bathymetric map and the limit of the fluid activity zones are from Marcon et al. (2014b). The central and eastern zone is characterized by intense and focused fluid flow, the other part by diffuse and homogeneous emissions. Assemblage distribution is from Olu-Le Roy et al. (2007a). Vesicomyid fields are wide areas of both living and dead vesicomyid beds while vesicomyid clusters contains mostly living clams.

2.2. Sampling

Sampling was conducted in July-August 2008 during the GUINECO cruise, from the RV *Meteor* with the ROV *Quest 4000* (chief scientist, A. Boetius), and again in January 2011 during the WACS cruise, from RV *Pourquoi pas*? with the ROV *Victor 6000* (chief scientist, K. Olu) (Olu et al., 2011). Three habitats were included in this study: vesicomyid beds (Fig. 2a,b,c), sediment in close vicinity of a mussel bed (Fig. 2d) and a microbial mat (Fig. 2e) (Table 1). In 2008, sampling was focused on three vesicomyid sites V1, V2 and V3 as well as in sediment in the immediate periphery of V1 and V2 (S1, S2). In 2011, V2 and V3 were revisited in addition to sediment in the periphery of V3 (S3), sediment in the periphery of a mussel bed (M) and a bacterial mat (B). The vesicomyid sampling sites were chosen along a fluid-flow gradient with V2 in the center of the intense fluid flow area, V1 at its

northern border, and V3 in the diffuse flow area at the southwestern part of the pockmark (Fig. 1). V1, V2 and V3 respectively correspond to "Clam_N", "Clam_S" and "Clam_SW" and M to "Mussel_S_Env" in Pop Ristova et al. (2012); V2 and V3 are respectively "Site 1" and "Site 2" in Decker et al. (2012a). The bacterial mat B was sampled for the first time during the WACS cruise. These sites were physically marked during the Guineco cruise (Markers M3;5;7;10) or the WACS cruise (W03 to 06) (Table 1).

Sediment samples were collected with a large blade corer able to sample 360 cm^2 (20x18x25 cm). Duplicate or triplicate cores were usually sampled at each site, however where triplicates were available, the third core was taken after a benthic chamber (Calmar) was deployed to measure oxygen uptake and methane fluxes through the sediment interface (Caprais et al., 2010). The sediment was therefore isolated from 3 (in 2011) to 6 h (in 2008) for planned experiments and up to 44 h in the case of unfortunately shortened ROV dives leaving the chamber at the bottom until the next dive (Table 1). Complete oxygen consumption after only 2 h of deployment in 2008 (Decker et al., 2012a) led us to reduce the experiment duration to 3 h in 2011. These experiments may have disrupted the macrofauna enclosed under the chamber, at least for the longest experiments. Only a few vesicomyids were found in cores taken after chamber deployments done in 2008, despite initially similar density in beds with or without Calmar deployment according to counts of vesicomyids on seafloor images, suggesting that clams had escaped to avoid anoxic conditions although no clear explanation about how they had escaped was found (Decker et al., 2012a). Other details on Calmar deployments, oxygen consumption in vesicomyid beds and other gas fluxes at the sediment-water interface are available, respectively for the Guineco (2008) and Wacs (2011) cruises in Decker et al (2012a) and Khripounoff et al. (2015).

Table 1. List and characteristics of cores used to sample macrofauna in different habitats of the Regab pockmark in 2008 and 2011. The deployment of the benthic chamber (Calmar) was unusually long in 2008 due to technical issues, involving reduction of the number of vesicomyids retrieved in cores taken after the longest chamber deployments. Cr: *Christineconcha regab* and Lc: *Laubiericoncha chuni*. *Guineco cruise markers: Mx; WACS cruise markers: Wx.

Cruise	*Site*	*Habitat*	*Location (Marker)**	*Core*	*Vesicomyids in cores*	*Calmar (hours of deployment)*
GUINECO (2008)	08V1	Clam bed	North (M3)	08V1a	18 Cr	-
				08V1b	16 Cr	-
				08V1K	3 Cr	16 h
	08S1	Sediment near 08V1 bed	North (M5)	08S1a	1 Cr	-
				08S1b	-	-
	08V2	Clam bed	Center (M7/W05)	08V2a	28 Cr	-
				08V2b	32Cr	-
				08V2K	4 Cr	44 h
	08S2	Sediment near 08V2	Center (M7/W05)	08S2	2Cr	-
	08V3	Clam bed	Southwest (M10/W03)	08V3a	40 Cr+3 Lc	-
				08V3b	44 Cr+2 Lc	-
				08V3K	28 Cr+1 Lc	6 h
WACS (2011)	11V2	Clam bed	Center (M7/W05)	11V2a	20 Cr	-
				11V2b	26 Cr	-
				11V2K	20 Cr	3 h
	11V3	Clam bed	Southwest (M10/W03)	11V3a	21 Cr	-
				11V3b	20 Cr +1 Lc	-
				11V3K	13 Cr	3 h
	11S3	Sediment near 11V3	Southwest (M10/W03)	11S3	-	-
	11M	Mussel bed	Center (W06)	11Ma	-	-
				11Mb	-	-
	11B	Bacterial mat	Southwest (W04)	11Ba	-	-
				11Bb	1 Cr	-
				11BK	-	3 h

Figure 2: Images of the sampled habitats; a, b & c:vesicomyid beds from sites V1, V2 and V3, respectively, showing *Laubiericoncha chuni* and *Christineconcha regab* at V3; d: mytilid bed in soft sediment; e: microbial mat; f: blade core sampling of macrofauna in a vesicomyid bed.

2.3. Processing samples and identifying fauna

Once recovered, the cores were sliced into sections of various sizes. Whenever possible, the top 5 cm were sliced in three sections (0-1 cm, 1-3 cm, 3-5 cm). In cores containing vesicomyids, it was generally only possible to slice one to two sections in this top layer. Below 5 cm, the cores were sliced into 5 cm sections down to 25 cm. Very few individuals were found below 15 cm, which was the deepest layer common to all cores. Macrofaunal compositions were therefore compared among sites from 0 to 15 cm. Sediment were washed under filtered seawater and sieved on 250µm, 300µm, 500µm and 1mm mesh sizes. The macrofauna *sensu stricto* (i.e. excluding meiofaunal taxa as described by

Dinet et al. (1985)) was sorted under a dissecting microscope and specimens were identified to the phylum or order level except for Polychaeta and Gastropoda, which were identified down to the family level. It was not possible to identify all bivalve post-larvae in the macrofaunal samples under 500 µ because of the very similar round shape of vesicomyid and thyasirid at this size. Therefore only dominant bivalve family is obtained, not quantitative data. Mean macrofaunal densities and standard errors were estimated for each sampling site, without considering the samples taken after benthic chamber experiments. Large size (>1 cm) vesicomyids sampled in cores were not considered as part of the macrofauna communities but were used to characterize the biogenic part of the habitats. For that, all adult vesicomyids sampled with different sampling tools manipulated by the ROV (cores, nets) were identified, measured and weighed. Ash-free dry weights (AFDW) were obtained after combustion of soft tissues for 12 h at 550°C. Mean bivalve densities were also estimated from counts on images within 50 cm diameter circles and in blade core samples. Counts within cores and on images were both used for each site, but counts on images underestimate the density because it does not take into account buried specimens. However, core counts are less representative because they only take into account a small surface of sediment (Decker et al., 2012a).

2.4. Data analyses

Vesicomyid shell length histograms were done with a size-class interval of 2 mm. They were smoothed using a weighted moving average to rule out spurious peaks (Frontier and Pichod-Viale, 1991). Differences in shell length and individual AFDW among sites were tested using Kruskal-Wallis (n>2) and Wilcoxon-Mann Whitney (n=2) non-parametric tests.

Macrofaunal taxonomic composition at each habitat was depicted according to sediment depth. Diversity patterns across habitats and sampling periods were assessed using individual-based rarefaction curves (Gotelli and Colwell, 2001) computed on polychaete families. Both analyses were performed excluding samples taken after Calmar deployment. Variations in community composition across habitats, across sites and across sampling periods within vesicomyid clusters were analyzed using a between-groups hypergeometric principal component analysis. The Hypergeometric Principal

Component Analysis (H-PCA) uses the chord-normalized expected species shared (CNESS) distance instead of the Euclidean distance (Trueblood et al.,1994). CNESS is a metric distance based on the probability of sampling each taxa in each sample with a random draw of *m* individuals. The computation of probabilities is similar to that used for calculating the expected number of species ES(n) (Hurlbert, 1971). The standardization thus limits biases due to variations in sample size. Values of *m* may vary from 1 to the abundance of the least abundant sample, the former giving high weight to dominant taxa and the latter giving high weight to rare taxa. A comparison of standardized CNESS matrices for all possible values of *m* provides the basis for choosing the value of *m* that provides the best trade-off between these two extremes (Trueblood et al., 1994). The between-groups bH-PCA means that replicate samples have been grouped by sampling sites and these groups have been used as an explanatory variable in a constrained H-PCA to maximize the variance between sites (Doledec and Chessel, 1987). A co-inertia analysis (CoIA; Dolédec and Chessel, 1994) was used to assess relationships between spatio-temporal patterns in macrofaunal community composition and vesicomyid population characteristics (proportion of *C. regab*, density, length, weight). CoIA was computed to maximize covariance between a PCA of normalized vesicomyid population characteristics and a H-PCA of CNESS-transformed macrofaunal abundance in vesicomyid clusters. The validity of the CoIA was assessed by means of a Monte Carlo test.

All analyses were run using R packages Vegan and ade4.

3. Results

3.1. Vesicomyid population characteristics

Table 2 displays the characteristics of the vesicomyid populations at each sampled clam bed. The density of vesicomyids varied among sites, but the values are difficult to compare considering the high standard deviations due to differences in counting methods. Despite this bias, *C. regab* appeared to dominate across all sites and both sampling periods. *L. chuni* occurred at 11V3 (9.4%), 08V3 (5.6%) and 11V2 (1.5%) but no *L. chuni* individuals were sampled at V2 in 2008.

In terms of individual shell lengths, there were no significant differences among sites in 2008 (Kruskal-Wallis test: $X^2=2.59$; $p=0.27$). In contrast, a significant difference was observed between sites V2 and V3 in 2011 with larger individuals at V3 (Kruskal-Wallis test: $X^2=27.1$; $p<0.001$). For a given site, individual shell length increased from 2008 to 2011 (Fig. 3). This increase in mean shell length over time resulted from both a consistent shift of the main cohort of 20 mm and the absence of the smallest size classes in 2011 at V2 and V3 (Fig. 3).

Moreover, individual weights showed two different patterns, with significant differences among sites in 2008 (Kruskal-Wallis test: $X^2=14.6$; $p<0.001$), but not in 2011 (Wilcoxon-Man Whitney test: $W=218$, $p=0.09$). This difference suggests that individuals sampled in 2008 at V2, with higher individual weights and similar lengths, were in better condition than at V3 and V1 sampled the same year.

Table 2: Vesicomyid population characteristics. Vesicomyid densities were estimated using both images and blade cores. Mean individual weights (AFDW without shells) for the WACS cruise (11V2 and 11V3) were estimated after sub-sampling (25 individuals) the measured individuals. Standard errors for the density, length and weight are shown in parentheses.

	% of *Christineconcha regab*	Mean density (ind.m^{-2}) in beds	Individual length (mm)	Individual weight (g)	Nb of individuals	Reference
08V1	100	542 (124)	70.1 (9.1)	1.44 (0.57)	124	This paper
08V2	100	681 (297)	72.1 (8.6)	1.93 (0.6)	80	Decker et al. 2012a
08V3	94.4	1056 (218)	70.9 (12.3)	1.48 (0.4)	151	Decker et al. 2012a
11V2	98.5	625 (87)	84.3 (10.2)	2.48 (0.69)	137	This paper
11V3	90.6	474 (190)	90.2 (6.7))	2.17 (0.28)	128	This paper

Figure 3: Frequency histogram of vesicomyid clam shell length at V2 (a) and V3 (b) in 2008 (red) and 2011 (blue).

3.2. Densities and composition

Total macrofaunal densities varied considerably among and across habitats but also, to a lesser extent, within a same habitat type, and between replicate cores (Table 3; Table S1). The mean densities among sites are significantly different (Kruskal Wallis test, χ^2= 17.13, p = 0.016) despite variability among replicates within sites. The lowest densities were found in the sediment close to V1: 08S1 (1639 ±1611 ind.m^{-2}) and the highest in the bacterial mat (63597 ± 6776 ind.m^{-2}). Among clam habitats, macrofaunal densities ranged from 2208 (±845) ind.m^{-2} at 08V1 to 13667 (±2357) ind.m^{-2} at 08V2. At V2, however, densities dropped by half from 2008 to 2011, while at V3 densities were similar each year.

Polychaetes dominated in most habitats (Table 3; Fig. 4), their contribution ranging from 50% to over 90% of macrofaunal densities, with the exception of 11S3. In these sediments close to V3, the crustacean assemblage (mostly isopods and tanaids) accounted for 60% of the macrofaunal community. Crustaceans were also common at 08V1. Dense populations of hyalogyrinid gastropods were found in the bacterial mat, accounting for about 38% of the total macrofauna. Bivalves, represented mainly by juvenile vesicomyids, were also abundant in the bacterial mat, at the vesicomyid cluster V2 and adjacent sediment.

The taxonomic composition of polychaete assemblages greatly varied among and across habitats (Table 3; Fig. 4). Vesicomyid clusters and sediments nearby were mainly dominated by cossurids. However, at V2 there was a shift in dominance patterns between the two sampling periods. Ampharetids were highly dominant in 2008 while they were not found in 2011 and replaced by cossurids. Ampharetids were also found in abundance in the bacterial mats, together with hesionids. Polychaete assemblages showed an unusual composition at 11S3, where dorvilleids and nereidids were co-dominant. Two other families, capitellids and spioniids dominated in the sediment adjacent to mytilids (site M). Nautiliniellids were commensals in some vesicomyid bivalves, but also free-living in sediment cores of the vesicomyid beds.

Table 3: Macrofaunal community composition expressed in mean density (ind.m^{-2}) with standard error (in parentheses) at the different sites sampled in the Regab pockmark, without considering the core sampled after

benthic chamber deployment. See Supplementary Table (S1) for the densities detailed for each core, including cores associated with the benthic chamber. *Bivalvia were mostly represented by vesicomyid juveniles.

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Sites	08V1	08V2	08V3	11V2	11V3	08S1	08S2	11S3	11M	11B
Cnidaria	0	0	0	0	0	0	0	0	0	0
Nemertea	0	0	0	55.5 (78)	0	55.5 (39)	278	0	166.5 (235)	55.5 (78)
Chaetognatha	0	0	28 (40)	0	0	14 (20)	0	0	0	0
Mollusca										
Bivalvia*	180.5 (138)	1375 (530)	125 (177)	111.5 (78)	97.5 (59)	181 (177)	2889	278	458.5 (216)	2916.5 (275)
Gastropoda										
Patellacea	0	14 (20)	0	0	0	0	0	0	0	0
Hyalogyrinidae	56 (0)	319.5 (138)	0	0	0	0	278	0	0	23847 (4813)
Neolepetopsidae	0	0	0	28 (0)	0	0	0	0	0	0
Provannidae	0	569 (177)	0	472.5 (472)	55.5 (78)	0	0	0	194.5 (275)	0
Chelicerata	0	0	0	28 (40)	0	0	0	0	69.5 (98)	28 (40)
Crustacea										
Amphiphoda	0	0	0	41.5 (59)	0	0	0	28	0	0
Cumacea	0	0	0	0	0	0	0	0	0	0
Caridea	139 (0)	181 (177)	0	0	0	14 (20)	306	0	0	0
Isopoda	430.5 (412)	55.5 (39)	14 (20)	0	41.5 (59)	14 (20)	167	1083	0	0
Tanaidacea	194.5 275)	0	14 (20)	0	0	0	56	444	0	0
Annelida										
Polychaeta										
Ampharetidae	69.5 (59)	7792 (3516)	28 (0)	42 (20)	0	55.5 (78)	15694	0	42 (20)	20264 (17187)
Amphinominae	0	0	0	0	0	0	0	0	0	0
Capitellidae	28 (40)	152.5 (98)	0	250 (79)	0	347.5 (295)	1056	0	2722 (40)	125 (177)
Chrysopetalidae	0	0	0	0	0	0	0	0	0	0
Cirratulidae	0	0	41.5 (59)	194.5 (158)	14 (20)	14 (20)	56	0	0	28 (40)
Cossuridae	472 (0)	2153 (1277)	2444 (1414)	5305.5 (1846)	2555.5 (2082)	750 (824)	2389	56	2889 (707)	69.5 (98)
Dorvilleidae	0	0	0	0	55.5 (78)	0	0	278	28 (0)	2444.5 (589)
Hesionidae	264 (294)	708 (177)	166.5 (39)	194.5 (235)	28 (40)	42 (20)	694	28	180.5 (138)	13555.5 (4989)
Lacydoniidae	0	0	0	0	0	0	28	0	0	0
Lumbrineridae	0	0	0	0	14 (20)	0	0	0	0	0
Maldanidae	28 (40)	0	0	0	0	0	0	0	0	0
Nautiliniellidae	69.5 (19)	139 (40)	42 (20)	514 (570)	375 (98)	28 (40)	28	0	0	14 (20)
Nephtyidae	0	0	0	0	0	0	28	0	0	0
Nereididae	14 (20)	0	0	42 (20)	0	0	0	194	0	0
Opheliidae	0	0	0	0	0	0	0	0	0	0
Paraonidae	0	0	0	0	0	0	0	111	0	0
Phyllodocidae	0	0	0	0	0	14 (20)	0	0	0	0
Pilargiidae	0	55.5 (39)	111.5 (78)	14 (20)	14 (20)	28 (40)	56	83	0	14 (20)
Pisionidae	0	0	0	0	0	0	0	0	0	0
Polynoïdae	0	14 (20)	69.5 (98)	0	0	0	0	0	0	14 (20)

Sphaerodoridae	42 (20)	14 (20)	14 (20)	0	0	28 (40)	139	0	125 (98)	0
Spionidae	69.5 (98)	69.5 (19)	41.5 (59)	125 (98)	41.5 (59)	0	0	0	1513.5 (1316)	69.5 (59)
Syllidae	139 (157)	42 (20)	139 (40)	28 (40)	0	42 (20)	0	0	0	153 (177)
Trichobranchidae	14 (20)	0	0	0	0	0	0	0	0	0
Unknown	0	14 (20)	0	14 (20)	0	14 (20)	0	0	0	0
Total	**2208** (845)	**13666** (2357)	**3278** (1925)	**7459**	**3292** (2298)	**1639** (1611)	**24139**	**2583**	**8389** (2161)	**63597** (6776)

Figure 4: Vertical distribution of the different macrobenthic taxa in the different sampled habitats. The size of the upper section was increased to 5 cm in vesicomyid beds due to bivalve abundance. Cores taken after Calmar benthic chamber deployments were not included.

3.3. Taxonomic diversity

Polychaete taxonomic richness per habitat ranged from 7 to 11 families. Rarefaction curves (Fig. 5) indicate an insufficient sampling effort for all sites apart from the microbial mat and the mussel bed. The lowest diversity and richness were found in the sediment near the mussel bed 11M. Although rarefaction curves for vesicomyid beds and nearby sediments did not reach an asymptote, all curves exceed those of bacterial mats and mussel beds, suggesting higher diversity. Moreover, the slope of the curves revealed that evenness was the highest at 08V1 and 08S1 and lowest at both V2 and S2 in 2008. In 2011, evenness increased at V2, but decreased at V3.

Figure 5: Rarefaction curves based on polychaete families sampled at the different habitats of Regab. Cores taken after Calmar benthic chamber deployments were not included. The inset graphs features the rarefaction curves for the site 11S3 (<30 individuals).

3.4. Vertical distribution

Vertical distribution varied according to habitat and year (Fig. 4). At the bacterial mat, more than 65% of the sampled macrobenthos was within the 0-1 cm section (not shown) and 92% was sampled within the 0-5 cm section (Fig. 4). The central clam bed sampled in 2008 (08V2) exhibited a similar pattern with ~90% of the macrobenthos sampled within the 0-5 cm section. At the northern and southwestern clam beds, both sampled in 2008 (08V1 and 08V3), 72% to 75% of the macrobenthos was sampled within the 0-5 cm layer. In addition, in the adjacent sediments (08S1, 11S2 and 11S3), most of the macrofauna was found within the 0-5 cm layer (70 to 85%). Finally, in the sediment near the mussel bed, the macrofaunal community dwelled deeper in the sediment, with 23% and 18% of the macrobenthos within the 5-10 cm and 10-15 cm layers, respectively. This pattern was mainly driven by capitellid polychaetes, whose relative density increased with depth (12% in the 5-10 cm layer and 17% in the 10-15 cm layer).

In 2011, the macrobenthos sampled at the southwestern clam bed (11V3) was more evenly distributed than in 2008 when less than 40% was sampled in the top 5 cm of sediment and 50% was sampled between 5 and 10 cm. The cossurids were more abundant below 5 cm depth than above 5 cm depth. In the central clam bed V2, the vertical distribution of the macrofaunal community was also less skewed toward the top layer of sediments in 2011: 60% of the macrobenthos was sampled between 0 and 5 cm of sediment (~90% in 2008), and 25% of it was sampled in the 5-10 cm layer (6% in 2008). This pattern coincided with a shift in dominance from ampharetids in 2008 to the deeper dwelling cossurids in 2011.

3.5. Community dissimilarities

The dissimilarities of the macrofaunal communities between the different habitats are shown in the two first axes of the bH-PCA with a random draw of m = 8 individuals (Fig. 6). The first axis, explaining 39% of the variance, discriminated two groups of sites: (1) the bacterial mat 11B as well as the central vesicomyid bed and its periphery in 2008 (08V2 and 08S2) and (2) the other vesicomyid sites 08V3, 11V2, 11V3 as well as 11M. The former group was characterized by a dominance of

ampharetids, hesionids and hyalogyrinids in high density. The latter group was dominated by cossurids. The second axis, explaining 15% of variance, isolated 11S3 and two samples of 08V1, mostly due to the high density of crustaceans. Outliers within sites were observed for 08V1 and 08V2 corresponding to samples taken under benthic chamber after measurements that due to shortened dives, lasted for 16 h and 44 h, respectively. The macrobenthos at 08V1 was characterized by a high dominance of cossurids. At 08V2, the outlying replicate was characterized by a more abundant assemblage of crustaceans and lower densities of ampharetids. No outlier was found after benthic chamber measurements lasting 3 to 6 h (11B, 11V2, 11V3 and 08V3).

Figure 6: Between-groups Hypergeometric Principal Component Analysis (bH-PCA) (scale type=3) on macrofauna (>250µm) on CNESS-transformed densities (PC1=39.4%; PC2=14.7%). All cores are included. Larger dots correspond to samples taken after benthic chamber deployment.

The CoIA and Monte-Carlo test showed a significant co-structure ($p < 0.05$) between the characteristics of vesicomyid populations and the taxonomic composition of the macrofauna in vesicomyid beds (Fig. 7). The first axis (75% of total variance) illustrated temporal changes. The vesicomyid beds sampled in 2011 (11V2 and 11V3) were characterized by larger vesicomyids with higher individual mean biomass and mean length compared to vesicomyid beds sampled in 2008. Nautilinillids were more abundant in the beds of larger vesicomyids. The second axis (17% of total variance) illustrated spatial variations. Sites located in the central area of Regab (V1 and V2) had a higher proportion of *C. regab*, higher biomass (especially at V2), and higher relative abundance of ampharetids, whereas at V3, located at the periphery of Regab, vesicomyid densities were higher, *L. chuni* more abundant, but with lower biomass, and the syllid, polynoid, pilargiid and cossurid polychaetes occurred in higher relative abundance.

Figure 7: Co-inertia analysis on macrofauna (>250 µm) and the characteristics of vesicomyids at each site (Table 2) on cores from sites 08V1, 08V2, 08V3, 11V2 and 11V3 (Axis 1=75%; Axis 2=17%). Only taxa with a contribution more than 5% are represented.

4. Discussion

4.1. Influence of fluid flow and engineering species on macrofaunal community composition?

The variability of fluid flow in both space and time within the Regab pockmark cluster creates a mosaic of ecological niches exemplified by the patchy distribution of bacterial mats and symbiont-bearing macrofaunal species such as siboglinid tubeworms, bathymodiolin mussels and vesicomyid clams (Ondréas et al., 2005; Olu-Le Roy et al., 2007a). These assemblages are differently distributed in the two areas defined by (Marcon et al., 2014b)(Fig. 1). In the central area, the intense and focused fluid, along with numerous carbonate concretions, favors the settlement of mussels and siboglinid tubeworms, whereas the surrounding area – fueled by diffuse fluid-flow– and weaker methane fluxes through sediments hosts only clam beds, which are patchily distributed all over this area at the exception of one large vesicomyid field in the South West part.

The gradient of methane flux from the center to the periphery and associated sulfide flux are assumed to shape the distribution of macrofaunal assemblages and bacterial mats. Methane concentrations and fluxes decreased both in 2001 and 2008 from mussel to clam beds (Olu-Le Roy et al., 2007a; Pop Ristova et al., 2012); microbial anaerobic methane oxidation (AOM) and sulfate reduction rates measured in sub-surface sediments follow this gradient, leading to the highest sulfide fluxes at the mussel site, intermediate fluxes in the microbial mats and lowest fluxes in the vesicomyid clam beds (Pop Ristova et al., 2012). In clam beds, sulfide produced by AOM does not reach the upper sediment layers (the sulfide peak was at found at 2.5 cm depth in microbial mats versus up to 8.5 cm in vesicomyids) (Pop Ristova et al., 2012)..

Similar to symbiont-bearing species relying directly on chemoautotrophy, the density, diversity and taxonomic composition of macrofaunal communities follow this gradient of chemical fluxes and

microbial activity. Macrofaunal density showed a parabolic relationship with methane and sulfide flux. The densities were the lowest where flux intensities were the highest, i.e. near the mussel bed, and where flux intensity was the lowest, i.e. most vesicomyid beds and their surroundings. The highest macrofaunal densities in 2008 were recorded at the central clam bed and its surrounding sediment as well as in the bacterial mat, where both microbial activity and sulfide fluxes exhibited intermediate values (Pop Ristova et al., 2012).

Diversity decreased along the gradient of increasing fluid flux. The bacterial mat and mussel bed exhibited the least diverse assemblage of polychaetes; the northern vesicomyid bed, its surrounding sediment and the southwestern clam bed sampled in 2008 showed the highest polychaete diversity. Similar to sedimented vents and seeps in the Pacific (Bernardino et al., 2012; Portail et al., 2015), macrofaunal density and diversity patterns in the Regab pockmark thus show a typical response to organic enrichment and hypoxia (Diaz and Rosenberg, 1995; Pearson and Rosenberg, 1978). At cold seeps, sulfide concentrations and fluxes and associated hypoxia likely drive patterns of endofaunal macrobenthic community structure (Decker et al., 2012b; Levin et al., 2003; Sahling et al., 2002), although methane and oxygen may structure epifauna associated with mussel beds (Bergquist et al., 2005). Sulfide concentrations, together with low oxygen concentrations are sources of physiological stress that only a few taxa can cope with (Diaz and Rosenberg, 1995). These opportunistic species benefit from local primary production by methanotrophic and thiotrophic microorganisms to flourish until their own tolerances are exceeded and populations collapse.

Organisms known to tolerate such conditions are mainly polychaetes, such as ampharetids (Levin, 2005), hesionids and dorvilleids (Levin et al., 2003; Menot et al., 2010; Sahling et al., 2002) or capitellids (Decker et al., 2012b; Gamenick et al., 1998), as well as the hyalogyrinids and provannids, two gastropod families including species commonly found in deep-sea reducing environments (Warén and Bouchet, 2009). These taxa were dominant at sites with the most intense fluxes and highest concentrations of methane and hydrogen sulfide. In the bacterial mat, the macrofaunal community, was dominated by ampharetids, hesionids, dorvilleids and hyalogyrinids, all possibly microbial feeders (Jumars et al., 2015; Levin et al., 2013; Thurber et al., 2012; Thurber et al., 2013; Warén and Bouchet, 2009). The macrofauna was located mainly in the top layers of sediment whereas at sites exposed to

lower fluxes and concentrations, such as the peripheral clam bed, the macrobenthos was dominated by cossurids and was more evenly distributed in the sediment layers.

Sulfide fluxes within the sediment increase with methane fluxes in Regab pockmark habitats (Pop Ristova et al., 2012). However, sulfide and oxygen concentrations in the sediments also depend on the interplay between methane fluxes and the rates of bio-irrigation by large symbiont-bearing species such as vesicomyids (Fischer et al., 2012) and siboglinids (Dattagupta et al., 2008). Determining the roles of these abiotic and biotic factors on macrobenthic community patterns is thus difficult. According to our results, macrofauna, along with engineer species, follow the inter-habitat chemical gradient observed at the pockmark scale. However, it is also possible that the action of engineer species influences the taxonomic composition of the associated macrobenthic communities. As shown by the Co-Inertia Analysis, larger vesicomyids favor the presence of nautiliniellids which is likely related to the commensal or parasitic relationship of the latter with the clams (Dreyer et al., 2004; Miura and Laubier, 1989), but could also be related to the numerous empty clam shells (dead vesicomyids) at the site they were sampled. Also, dense vesicomyids favor higher densities of syllids, polynoids, pilargiids and cossurids. Vesicomyids, with their long, strong foot, pump sulfate deep into the sediment, creating bio-irrigation (Arp et al., 1984; Fischer et al., 2012; Wallmann et al., 1997). Vesicomyid activity may thus deepen the sulfide production zone, cutting off food sources for surface deposit-feeders, such as ampharetid polychaetes or hyalogirinid gastropods, possibly relying on free-living sulfur-oxidizing bacteria (Warén and Bouchet 2009; Bowden *et al*. 2013), while enhancing oxygen availability and penetration in the top sediment layer (Fischer et al., 2012) and thereby favoring sub-surface deposit feeders such as cossurids and capitellids (Levin et al., 2003). The peculiar vertical distribution of the macrobenthos in the northern clam bed, featuring high relative abundance of hesionids in the two deeper layers of sediment, is a good example of clam bio-irrigation of the sediment. Small predators with strong digging abilities and tolerance to anoxia, such as hesionids, may be forced to bury themselves deeper in the sediment to prey on sulfur-bacteria which live near the sulfide-production zone. Finally, the possible action of mussels on the sediment (at the rare places where they live in soft sediment) and the resulting impact on the composition of the associated macrobenthos is difficult to assess here as large mussel size made it impossible to sample the

macrofauna directly under the mussel bed. However, when living in soft sediment, mussels are likely to move horizontally which may also exert physical action on the sediment, e.g. bioturbation. In the central and most active area of Regab, oxygen penetration depth is higher in the sediment near the mussel bed (2.08 mm) than in vesicomyids (1.15 mm) or in the microbial mat (0.45 mm) (Caprais, unpubl.). This may explain the high density of spionids, capitellids and cossurids deep inside the sediment. Finally, engineering species, including the bacteria in microbial mats, may also favor some associated taxa through trophic interactions (e.g. grazing of mats by hyalogrinid gastropods).

4.2. Variability of vesicomyid habitat macrofaunal community through time and space

Macrofaunal community structure and taxonomic composition showed large variation among vesicomyid clam beds. In 2008, the central clam bed had the most abundant, but least diverse macrobenthos, which was dominated by ampharetids and hyalogyrinids, both characteristic of high-sulfide conditions. The macrobenthos in the southwestern clam bed was less abundant, but more diverse and dominated by cossurids along with syllids and pilargiids. Finally, the clam bed located north of Regab harbored the least abundant but most diverse macrobenthic community, composed of a large assemblage of crustaceans (mostly isopods) and a polychaete assemblage dominated by cossurids, hesionids and syllids. These discrepancies may be the result of the wide distribution of vesicomyids along the chemical gradients.

Vesicomyid clams et Regab appear to encompass a large gradient of chemical fluxes and concentrations, following the general gradient over the pockmark with higher fluxes in central clam beds compared with those in the periphery with consistency among years (2008 vs 2011) and benthic chamber types (Decker et al., 2012a; Pop Ristova et al., 2012). AOM rates are consequently higher in vesicomyid beds from the center where highest peaks of AOM were observed closer to the surface (5cm) than at peripheral sites (at 8.5 cm, Pop Ristova et al., 2012). The co-structure between the composition of macrofaunal assemblages and the population characteristics of vesicomyids further suggests an interaction between abiotic and biotic factors in shaping macrobenthic communities. The three sites differ in their vesicomyid population, although *C. regab* was dominant all over the

pockmark, *L. chuni* was present at the peripheral site but absent or rare at central sites. These relative proportions of vesicomyid species may favor some polychaete families, by differing in their bio-irrigation or bioturbation activity. *L. chuni* is assumed to burrow deeper due to its longer siphons (Krylova and Cosel, 2011) and higher ability to cope with hypoxia than *C. regab*. The oxygen carrier (hemoglobin vs. myoglobin) and affinity differ in the two vesicomyid species (Decker et al., 2014). The two clam species may therefore differently affect the endofauna by modifying the geochemistry of the sediment more or less deeply. This may explain the more homogeneous vertical distribution of macrofauna in the peripheral clam bed and the occurrence of deep-dwellers such as cossurids; in this bed, bio-irrigation may be deeper than in its central counterpart, where only *C. regab* occurs. However, at the central site, the shell of *C. regab,* which has short siphons, may better protect the species from high sulfide concentrations (Krylova and Cosel, 2011) and the highly sulfide-tolerant polychaete families co-occur with the vesicomyids close to the surface. Therefore, the variability at the species level of biogenic habitat forming taxa may increase beta-diversity. Such intra-habitat variability has also been suggested for siboglinid fields dominated by different species and for different type of microbial mats of the Haakon Mosby mud volcano (Decker et al., 2012b). For vesicomyids, niche separation according to hydrogen sulfide and methane concentrations had been already observed (Barry et al., 1997) and may involve variability in associated macrofauna in other cold seep areas.

The proportion of *C. regab* within the vesicomyid bed sampled at Regab also slightly decreased from 2008 to 2011, which may have played a role in temporal changes of the macrobenthos. The most striking evolution however is the increase in mean biomass and mean shell length of vesicomyids at both the peripheral and central sites. The dominant cohort of both populations grew at an estimated rate of 7 mm.year^{-1} over the three-year period. Moreover, the smaller size classes were consistently absent in 2011. These patterns are consistent with spatial segregation between large and small vesicomyids reported at cold seeps of the Makran continental margin (Fischer et al., 2012). There, bio-irrigation triggered by large vesicomyid clams may deepen the sulfide production zone in the sediment and thus block the sulfide source for smaller clams as well as surface deposit-feeders that may rely on sulfur-oxidizing bacteria (Fischer et al., 2012). Interestingly, at Regab, vesicomyid juveniles are

observed in microbial mats where they seem to tolerate high sulfide concentrations. They were also abundant in the central vesicomyid bed V2 and nearby sediment in 2008, but their density decreased greatly three years later. More generally, the macrobenthos associated with the central clam bed shifted between 2008 and 2011 from a community resembling that of the bacterial mat to a community resembling that of the southwestern clam bed. The large assemblage of ampharetids and the high abundance of hesionids and hyalogyrinids sampled in 2008 were replaced by deeper-dwelling organisms such as cossurids which accounted for the vast majority of the macrobenthos. This change in the macrobenthic community may also be linked to fluid flow activity at this site which may have decreased over the years. However, in 2011, the presence of capitellids and provannids — also sampled in the mussel bed — suggests that the chemical fluxes were still relatively high at this site. Compared with the southwestern clam bed, chemical fluxes were higher still at the center (Khripounoff et al., 2015).

Variations in macrofaunal community composition after benthic chamber measurements suggest that the macrofauna are able to quickly respond to changes in local conditions. As explained above, some cores were sampled after unusually long benthic chamber measurements. It was expected that these long deployments would disturb the local macrofauna and thus bias the macrofaunal results for these samples, especially for the longest experiments, because oxygen was completely consumed after 3 h (Decker et al., 2012a). In 2008, in the central bed, the macrobenthos in the core after chamber experiment exhibited relatively lower densities of ampharetids and cossurids and relatively higher density of isopods than other sampled cores when the incubation lasted for 44 consecutive hours. Isopods may have been attracted by the sediment re-suspended when chamber was removed just before sampling. The same year, in the V1 clam bed, where the measurements lasted 16 h, cossurids attained densities that were one order of magnitude higher than the two other cores. Cossurids dwell deep in organic-rich, oxygen-poor sediments and are likely to actively burrow to feed on particles (Jumars et al., 2015). In Regab, these polychaetes occurred down to the deepest 10-15 cm sediment layer (the benthic chamber was inserted into the sediments down to 10 cm). Cossurids may thus have positively responded to the disturbance created by the 16 h incubation at the northern (V1) site and negatively responded to the more stringent 44 h incubation at the central (V2) site.

Our results highlight the high adaptability of macrobenthic species reflected by a rapid response of these organisms to a change in environmental conditions. Rapid changes in macrofauna community structure after oxygen decreases may be favored by the high beta-diversity of the vesicomyid habitat, observed in response to the spatial heterogeneity, and may be due to the temporal dynamics of this habitat.

4.3. Dynamics of cold-seep habitats

The dynamics of cold-seep habitats is thought to involve a succession of foundation taxa, from the bacterial mats on sediments of the youngest and most active flow seepages to large bushes of siboglinid tubeworms on carbonate concretions (Bowden et al., 2013). This succession is driven by the interaction between the rate of fluid advection and the rate of bio-irrigation. Fluid advection controls the upward flow of methane whereas bio-irrigation has a strong influence on the downward flow of sulfates: methane and sulfate are the two reduced and oxidized chemicals that fuel AOM and thus the production of sulfides (Fischer et al., 2012). On the Hikurangi margin, ampharetid tubeworms are likely the first bio-irrigators to colonize bacterial mats, setting the stage for vesicomyid recruitment by enhancing sulfide availability (Bowden et al., 2013). In Regab, ampharetids were dominant in the bacterial mat, attaining similarly high densities as on the Hikurangi margin (over 20,000 ind.m^{-2}). The co-occurrence of a large number of vesicomyid juveniles further suggests the initiation of a new stage of succession that will eventually lead to a vesicomyid-dominated community. On the Makran convergent continental margin, Fisher *et al.* (2012) observed a spatial succession of (i) bacterial mats and ampharetids; (ii) ampharetids and small vesicomyids; and (iii) large vesicomyids. The authors attributed the spatial distribution of these habitats to temporal dynamics. As vesicomyids grow, their irrigating activity profoundly modifies sediment biogeochemistry, thereby oxygenating surface sediments, deepening the sulfate-methane transition zone and thus sulfide production to a point where small clams and surface deposit-feeders (such as ampharetids) no longer have access to their primary food source. The growth of the dominant vesicomyid cohort from 2008 to 2011 along with the absence of small vesicomyids and a shift in macrofaunal community composition from an ampharetid-

dominated to a cossurid-dominated assemblage in 2011 support the prominent role of vesicomyid bioengineering on the dynamics of cold-seep communities at Regab. Because bio-irrigation also accelerates the formation a carbonate crust by enhancing AOM (Luff et al., 2004), vesicomyids may eventually favor the next stage of succession leading to a dominance of bathymodiolin mussels and siboglinid tubeworms, which both need hard substrata for settlement (Bowden et al., 2013). Fossilized bivalve shells, that may be either vesicomyids or mytilids associated with carbonate concretions, have been found at Regab (Pierre and Fouquet, 2007). Marcon *et al.* (2014a) mapped some dead vesicomyid clams on carbonate concretions in Regab, providing further support for a successional transition from vesicomyid beds to carbonate crusts. However, the authors also reported large fields of dead clams in soft sediment, suggesting that seep succession may end after the vesicomyid-dominated phase in areas where fluid seepage is too low or transient to give rise to carbonate environments. From 2001 to 2011, changes in the distribution of living vesicomyids were also more frequent than for mussels and siboglinid tubeworms, suggesting that large vesicomyids are able to move from unsuitable to suitable areas according to local-scale variation in fluid seepage. Vesicomyids thus are key actors in the dynamics of cold-seep habitats as well as versatile colonists of a wide range of fluid-flow settings.

The ability of clams to settle in various places and to move according to changes in geochemical gradients and the capacity for their associated endofauna to respond rapidly to environmental changes make clam beds a highly variable habitat which may actively contribute to the macrofaunal beta-diversity in the Regab pockmark and in seeps in general.

5. Conclusions

We visited twice, at a three-year interval, the Regab pockmark first described in 2001 to study macrofaunal community structure in various chemosynthetic habitats, defined visually by several engineer species and microbial mats.

Macrofaunal community patterns were explained by both habitat heterogeneity and by the general pattern of fluid flow at the pockmark scale, which also drives the distribution of the biogenic habitats.

Intermediate perturbations (methane, sulfide fluxes) sustain the highest macrofaunal densities in microbial mats, while densities decrease in higher fluid flow mytilid beds and in lower fluid flows vesicomyid habitats. In turn, vesicomyid habitats sustain higher alpha-diversity than any other habitat, also likely facilitated by vesicomyid bio-irrigation. Moreover, the high plasticity of vesicomyids, exemplified by the occurrence of two different species with different biological traits and by their ability to move horizontally and vertically, allows them to colonize a range of geochemical habitats, including microbial mats in their juvenile stages at Regab. The "vesicomyid habitat" is therefore heterogeneous and sustains a heterogeneous macrofaunal community. This high beta-diversity also allows rapid changes in the macrofaunal community in response to environmental variation, such as oxygen depletion observed during experiments of only a few hours, and even in the areas of the weakest fluid flow that supports less specialized fauna. These observations suggest high adaptability of the vesicomyid-associated macrofauna to spatial heterogeneity and temporal dynamics of fluid flow even at very small scales. Changes in associated macrofauna also respond to vesicomyid growth and species replacement. Vesicomyids thus play a prominent bioengineering role in the dynamics of cold-seep communities at Regab, from microbial mats to different vesicomyid beds that can be successional stages or spatial patterns driven by fluid flow heterogeneity. The resulting high variability of vesicomyid-associated macrofauna in apparently similar habitats must be considered in biodiversity assessments.

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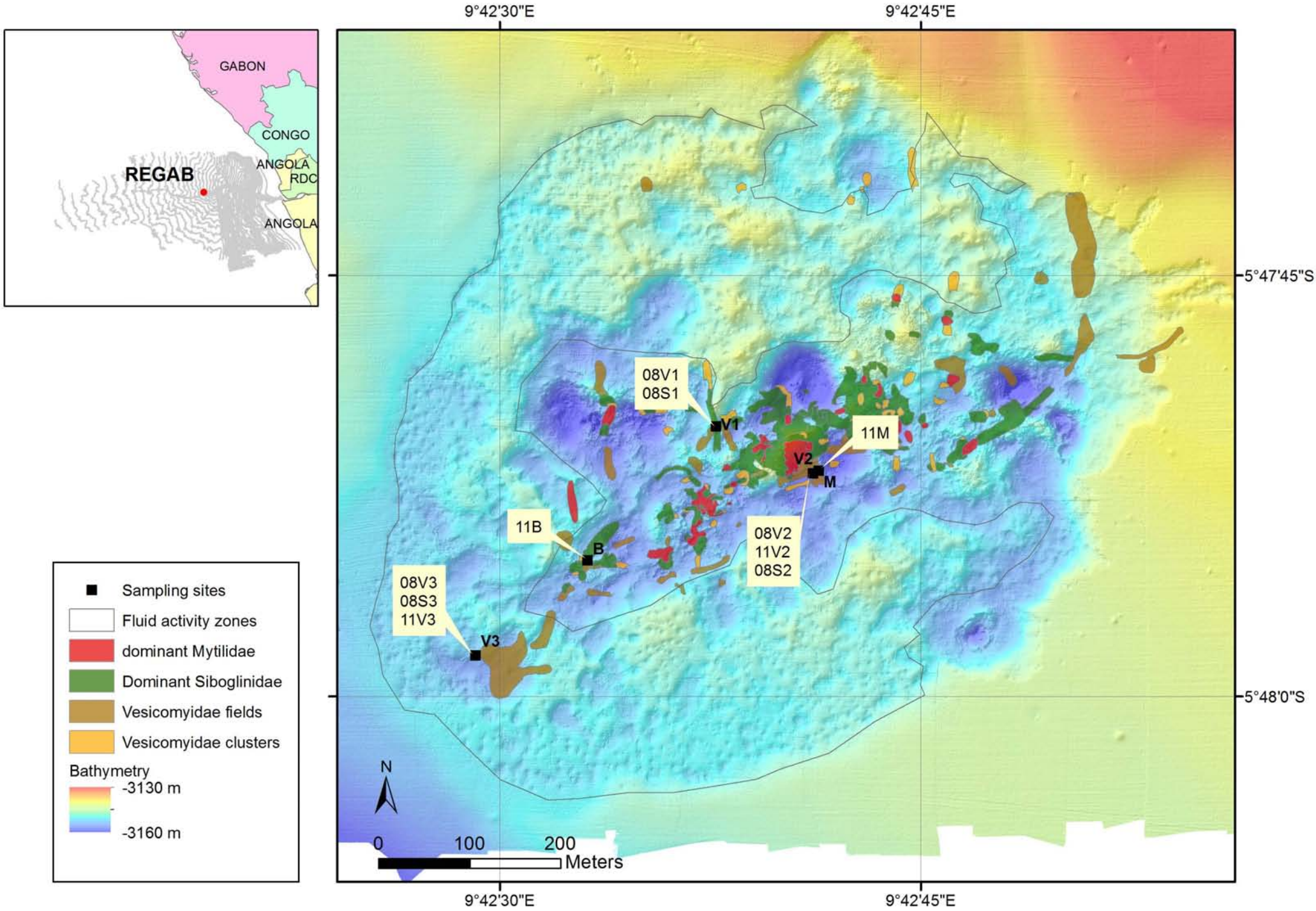
Highlights

- The high plasticity and diversity of vesicomyids create habitat heterogeneity at seeps.
- The “vesicomyid habitat” sustains a heterogeneous macrofaunal community.
- High diversity of vesicomyid associated macrofaunal community allow rapid changes
- Microbial mats with juvenile clams is proposed as an initial stage for vesicomyid bed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08V3
08S3
11V3
V3
Sampling sites
Fluid activity zones
dominant Mytilidae
Dominant Siboglinidae
Vesicomyidae fields
Vesicomyidae clusters
Bathymetry
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-3160 m
N
0
100
200
Meters

a.

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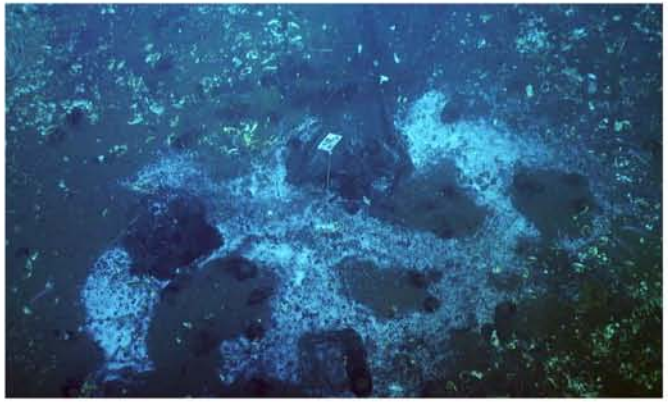

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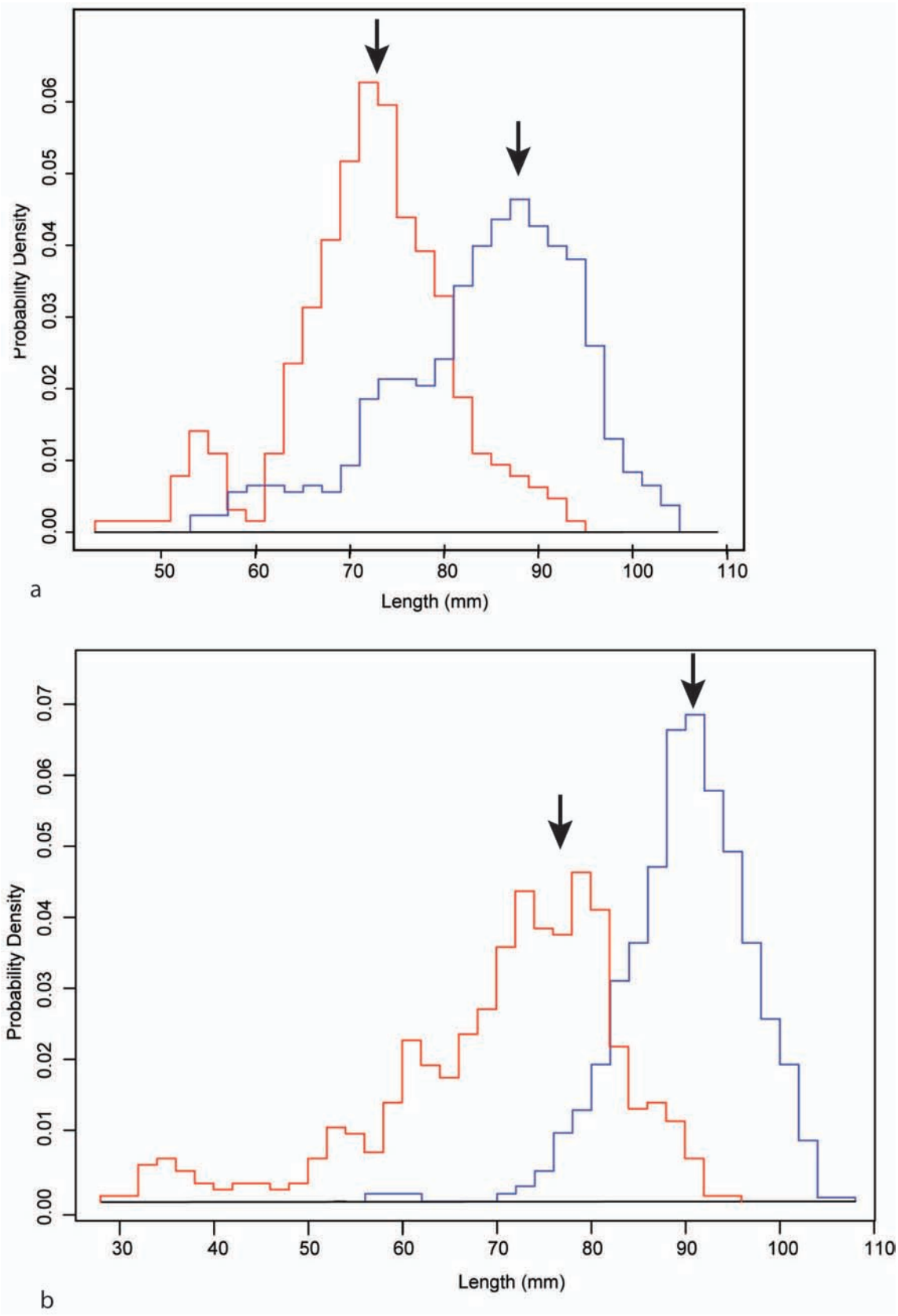

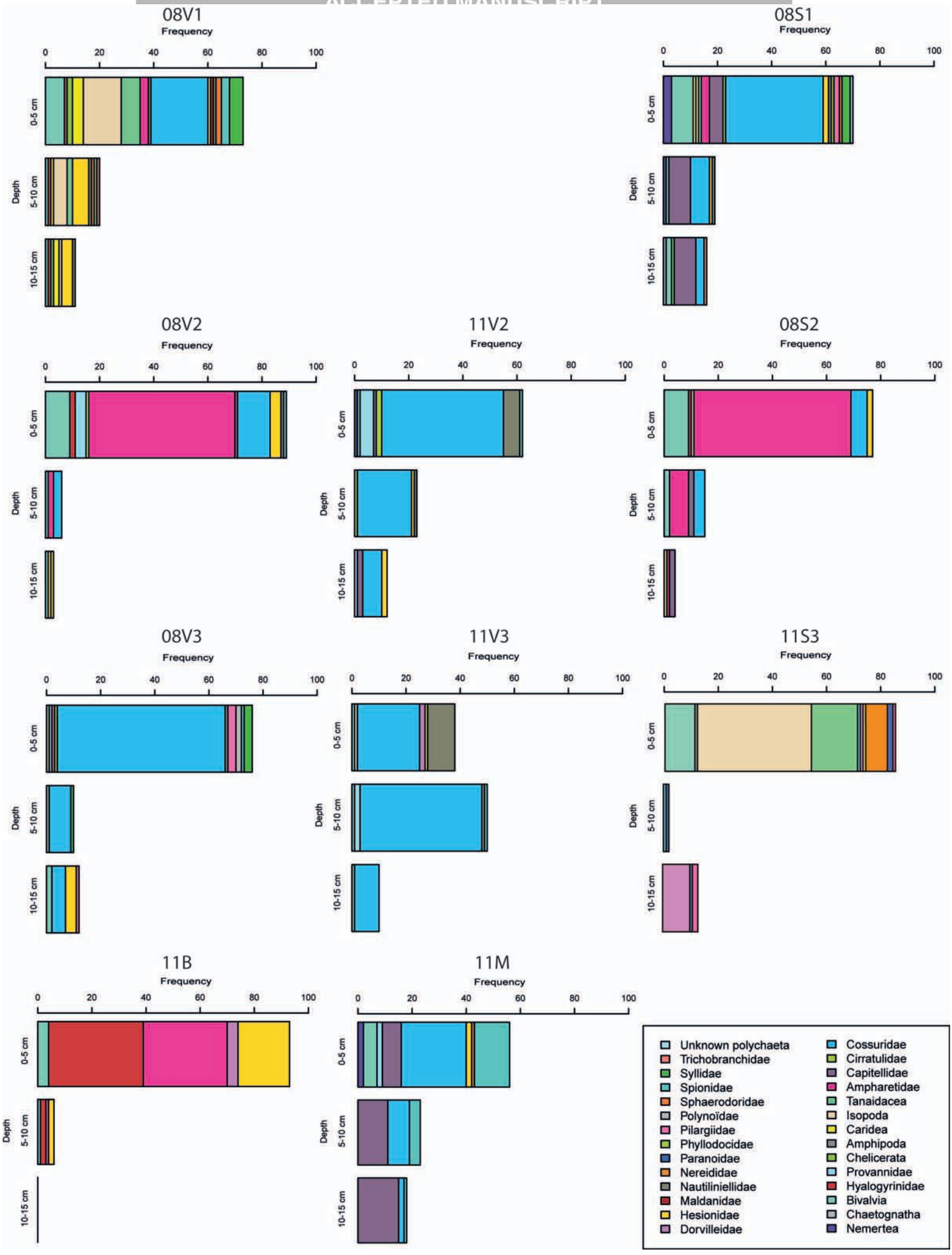

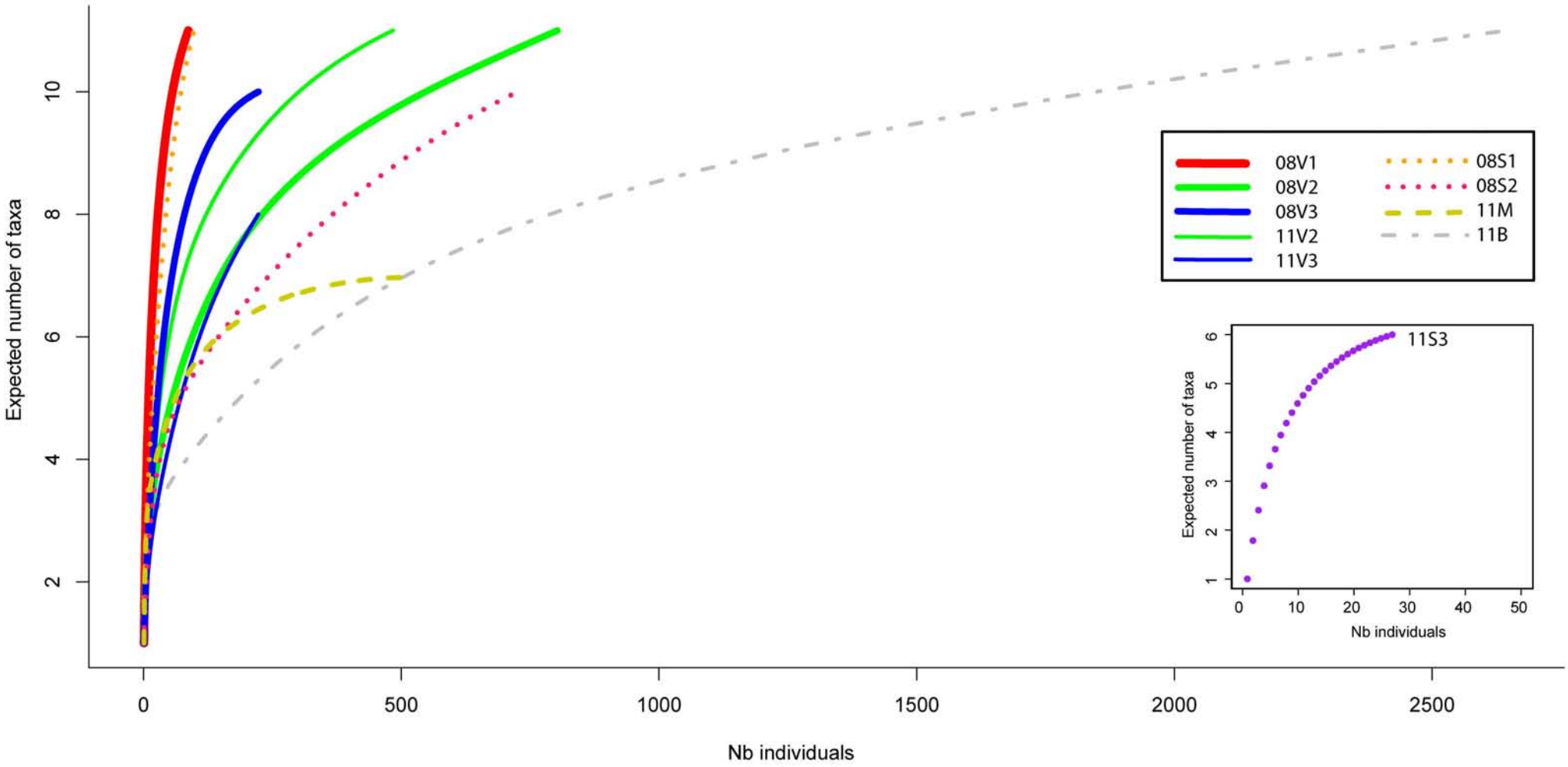
Expected number of taxa
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1500
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2500
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6
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11V3
08S1
08S2
11M
11B
11S3
Expected number of taxa
Nb individuals
1
2
3
4
5
6
0
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50