

Interactive comment on “Geophysical and geochemical controls on the megafaunal community of a high Arctic cold seep” by A. Sen et al.

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General Comments

This is a well-written manuscript describing the geochemistry, physical habitat and fauna at a series of methane-seeps in the Arctic. The authors relate changes in faunal distribution, from photographic images, to the local chemical and physical environment and discuss the micro-habitats available at such seeps. The manuscript would be improved if it were possible to make comparisons with the background fauna away from seep influence. There are a number of problems with the analytical procedures, presentation and interpretation of results as described below.

Specific Comments

Methane

Since the study is based on methane seepage it is essential to have some reliable measurements of methane concentrations available to the biota. Unfortunately the method described for methane analysis does not measure “dissolved pore water methane”, as stated, but a mixture of free methane plus methane sorbed to the sediment and released by the sodium hydroxide addition (Ertefai et al. 2010). Since authigenic carbonate is present, the concentration of the sorbed methane can be up to two orders of magnitude higher than the dissolved methane (Ijir et al. 2009). It is unclear why pore water obtained from the rhizons was not used for on-board methane analysis. There is no information available, to my knowledge, on the extent to which sorbed methane is available to the biota. Thus comparisons between sites based on methane availability are thus not valid.

Sulphide

Dissolved reduced sulphur species are utilised by chemoautotrophic free-living and symbiotic bacteria as an energy source. The concentration of dissolved “sulphide” ($H_2S + HS^- + S^{=}$) and thiosulphate is thus an important measurement. The authors state that sulphide was below the detection limit in the bottom water and in the upper “few” cm of the sediment of all the cores. However, sulphide must have been present to support the bacterial mats visible on the surface. The detection limit is not given but since the lowest standard used in the assay was 40 μM (Hong et al., 2017) the method described may not have been able to detect concentrations of a few μM . Many thiotrophic symbiotic associations exist in sediments with dissolved sulphide concentrations of $< 1 \mu M$. It is very difficult to prevent oxidation of low concentrations of sulphide in pore water and since the samples were not analysed immediately it is probable that oxidation occurred during preservation and storage.

H_2S would have been carried into the upper sediment and the water column in the methane bubbles as well as in the associated water plume (Reeburgh 2009, Dando et al. 1994a). In addition, the drawdown of seawater induced by the rising methane bubbles (O’Hara et al. 1995, Zimmermann et al. 1997) would have locally generated reduced sulphur species from iron sulphides within the sediment (Dando et al. 1994b) as well as producing a halo of less reducing areas surrounding the bubble outlets.

Identification of biota

The statements “Visible fauna (at least a few centimeters across) were identified” (p5) and the statement in the following paragraph that “Numerous individuals of siboglinid worms were seen”, appear contradictory. *Oligobrachia haakonmosbiensis* is large for a frenulate, with a tube diameter of 0.6-0.9 mm (Smirnov, 2014). It would be useful to have a high resolution image, perhaps as a

supplementary file, to show how these individual siboglinids were visible in the photographs, since a lot of the Discussion is based on their presence or absence.

Most of the identifications relied on interpretations of images taken by a towed camera with a resolution of 16 million pixels and with stereo cameras mounted on a ROV with a resolution of 1.4 million pixels. We are not told the respective field of views photographed by these cameras so it is not possible to estimate the respective resolutions. It would help interpretation if the authors would calculate the sizes that the respective pixels represent. Rough calculations, from the dimensions given in Figure 2, suggest that the pixel size in images from the stereo cameras may have been inadequate to resolve smaller organisms, such as *Oligobrachia* tubes, 0.6-0.9 mm in diameter (Smirnov, 2014) unless they occurred in clumps. The core samples at GHP 5 were taken around the periphery of the pingo (Figure 1) so that it is not possible to deduce from these that *Oligobrachia* was absent from pingo 5.

Another problem in comparing tow cam and ROV pictures is that the ROV imaging was always from fairly discrete areas on pingo 5 while the tow cams were transects covering from the outside into the centre of the pingo (Figure 1). This might explain why *Nothria*, for example, was identified in all the tow cams but not in the ROV pictures. The tow cam epifaunal data, presented in Table 1, should therefore be divided into “on pingo” and “off pingo” sections. The reason that TC25 GHP3 clusters with the GHP5 ROV camera tracks is probably because tow cam 25 has the greatest proportion of off-pingo track of any of the tow-cams.

The frenulates observed were identified from specimens in the core samples and density estimates for them were calculated from the densities observed in the cores. It would have helped the interpretations if information had been provided on the depth they reached in the sediment. This may be site specific, since the penetration depth of a species has been shown to vary between cores (Dando et al. 2008). *Oligobrachia haakonmosbiensis* were reported penetrating the sediment to a depth of 55 cm at the Híkon Mosby mud volcano (Lösekann et al. 2007). It would also be helpful to know whether other macrofauna were recovered from the cores, since most faunal species with chemoautotrophic bacteria at shallower seeps are infaunal and would not show on surface photographs. An example is at a methane seep at 170 m depth in the N. Sea where 3 such species were found living within the sediment and shells of a fourth, the bivalve *Lucinoma borealis*, were recovered (Dando et al. 1991, Dando 2001): no epifauna with chemoautotrophic symbionts were observed. Many frenulates have tubes completely buried within the sediment; thus chemosynthesis is probably more common at the pingo sites than this study of mainly epifauna suggests.

Discussion

On discussing the distribution of the frenulate *Oligobrachia*, the authors wrote: “the image transects containing siboglinid frenulates were much less even in terms of species abundances compared to the transect and mosaics which did not contain any frenulates”. This would be expected since it has been shown that, on the Rockall slope, frenulate distribution did not cluster with most other taxa and there was an inverse relationship between frenulate density and the density of other benthos (Dando et al. 2008). This was explained because sediment disturbance by other organisms would increase sulphide oxidation and displace, or bury, the thin tubes of the frenulates. It should be noted that in the latter study most of the frenulates had a low abundance and none of the tubes projected from the sediment, if they did at all, as far as those of *Oligobrachia haakonmosbiensis* and thus would not provide an epifaunal habitat. In the one obligate, methane seep frenulate species that occurred in high densities, *Siboglinum poseidoni* (Dando et al. 1994c), no epifauna were noted between or above the projecting tubes.

The authors consider that chemoautotrophic primary production at the pingos might exceed the photosynthetic primary production reaching the sea floor. The examples they cite are from deeper water, where less photosynthetic production reaches the sea floor. This is unlikely to be true at 400 m where much more photosynthetic production will reach the seabed. As shown in a comparative study (Bernadino et al. 2012), the isotopic difference between background and seep fauna was much lower at the Eel River seeps (250-500m) than at deeper seeps at 770 m depth and deeper. Isotopic evidence of

food inputs is needed to support the authors' hypothesis. Since methane solubility increases with pressure there will, potentially, also be more methane available to the biota at deeper sites.

Regarding sulphide in the upper sediment, p.14 line 11: only 1 measurement at 5 cm depth is shown in Fig. 4 (for core 1045, off the edge of pingo 3). The exact value for this sample is not shown. However, a single measurement does not justify the statement that "sulfide was not detectable - - - even in the upper 5 cm of the sediment at the pingo site". Should this read "sites"?

As mentioned above, it is probable that sulphide was present at significant concentrations for the biota, including the bacterial mats at the surface, but was not detected using the stated analytical procedure. Serov et al. (2017 Fig. S2C) shows a picture from one of the pingos with a white bacteria mat, presumably of sulphur-oxidising bacteria, on top of "tubeworms" that project approximately 4 cm above the sediment, if the scale on the photograph is correct. If these are sulphur-oxidising bacteria then sulphide or thiosulphate must be present in the water column. The "tubeworms" are approximately 10 mm across, measured against the scale on the photograph, and thus cannot be *Oligobrachia*.

P14, line 21 and subsequent text: "this particular image transect did not contain frenulates." *Oligobrachia haakonmosbiensis* has large tubes for a frenulate, many species have tubes 100 μM or less across and would not be visible if they did project above the surface, although many species are completely buried within the sediment. More than one species frequently occur in the same core sample (Dando et al. 2008) so that it is not possible to state that frenulates were absent. To be correct you should write that "this transect did not show any 'visible' frenulates".

GHP5 gas release: in many submarine seeps gas is only released at low tide when slight differences in bottom pressure cause the sub-surface gas volume to increase. At other frequently visited methane seep sites, such as the Scanner pockmark, continuous gas release may, or may not, be present during any given cruise. In the absence of data on the tidal conditions when observations were made over GHP5 it is not possible to state that gas was not released from this pingo. The enhanced reflectors below GHP5 indicate subsurface gas and, on enlargement of Figure 6, it is possible to see a small gas "chimney" towards the edge of the pingo (see Fig 6 section), although this is considerably smaller than the chimneys below the other pingos. Core 920 on the edge of GHP 5 contains methane of thermogenic origin (Serov 2017, Table S1), implying a deep source for the gas. Active release of methane from the sediment will channel the methane into the higher porosity release channels. The sediment at the sides of these channels will have a low methane concentration, due to the down-flow of seawater from the sediment surface (Dando et al. 1994a, O'Hara et al. 1995). Thus it is not correct to argue that methane release will stimulate overall sulphate reduction and methanotrophy in a seeping pingo when compared to a non-seeping pingo with a high sediment methane concentration (p17 first paragraph). Microbes may also be removed from the system by the rising fluids.

Sulphate reduction (p16): "In most cores, the ratio of inorganic carbon to sulfate consumption was found to be close to 1:1 regardless of depth (both GHP5 and GHP3). The one exception was core 1048 from GHP5, for which, almost all values were closer to the 2:1 ratio." Core 1048 is shown in Figure 1 to be the furthest away from any pingo, i.e. it is in background sediment. Thus it should be no surprise that in this core sulphate reduction is not dependent upon the presence of methane.

"The dual need for inorganic and organic carbon sources (plus thiotrophic chemoautotrophy) likely results in frenulates **in general**, and, *O. haakonmosbiensis* specifically, relying heavily on a highly active sediment methanotrophic microbial community" (p16 line 31). This is not true as a general statement. Dando et al. (2011), in a study of the relationship between 10 species of frenulates and the chemistry of their habitat, found that, with the exception of 1 obligate methane seep species, all occupied sediments where the dissolved methane concentration was $< 1 \mu\text{M}$.

"On the other hand, at GHP5, seepage of methane is low due to the lack of a sub-surface gas chimney. Methane is still present in the sediment, but in lower concentrations and as a result, methanotrophic microbes are likely less abundant and methanotrophic activity is considerably lower, as evidenced by lower AOM linked sulfate flux rates" p17. As mentioned earlier the authors do not know the concentration of available methane in the sediment and hence cannot make such comparisons

regarding different methane concentrations at different sites. The values in Figure 4 may just equate to the amount of authigenic carbonate in the samples. A small gas chimney appears to be visible below GHP5 in Figure 6.

The discussion regarding hydrothermal vents is not very appropriate for this paper with respect to *O. haakonmosbiensis*. This is a cold-water species that penetrates approximately 0.5 m into the sediment. At vent sites the temperature within the sediment would, almost certainly, be lethal for the species.

Although *O. haakonmosbiensis* was the only metazoan with chemoautotrophic symbionts found, it does not mean that it was the only one present, since the infauna, where, for example, other frenulates and thyasirid and lucinid bivalves might be expected, was not studied. It is therefore also not correct to state that “the community at the pingos does not contain specialized seep endemics” (p22 line 13) since the infauna were not studied and *O. haakonmosbiensis*, if distinct from *O. webbi*, is probably a seep obligate species. “Endemic” is incorrect in this context since it refers to geographic regions, not habitats.

Figure 1

It would aid interpretation if the positions of the observed gas flares were pinpointed in figures b-e.

Figure 2, 8 & 9

These would benefit from scales in the camera pictures, since the laser spots, when present, are difficult to see.

Figure 3

Figure 3b has TC21 and TC18GHP3 plotted on top of each other, including the labels, so it is not clear what this point represents.

Figure 4

The lines after the final points appear to be extrapolated randomly. If this is because the graphs are part of larger ones and have been cut off at 60 cm then it would be sensible to give the depth and values of the next points in parenthesis at the end of the lines. The coloured open circles are not well differentiated at the scale of the Figure and should be replaced by coloured filled circles to differentiate the cores.

Figure 6

I think the vertical scale is m depth below the sea surface and not sediment depth. Fig. 6b is too small to be useful without enlargement.

Discussion

The term “megafaunal taxa” is used in the Discussion. Megafauna are large animals such as cetaceans and large fish. The correct term for the taxa observed is “macrofauna”

P14 lines 20 & 21, Fig. 2 should read Fig. 3

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Figure 6 section

