Fauna associated with shallow-water methane seeps in the Laptev Sea

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Abstract

Background. Methane seeps create unique benthic ecosystems in the deep-sea dependent on chemosynthetic (methane derived) organic matter. In the present study we focused on the recently described shallow-depth methane discharge area ~~on~~at the northern Laptev Sea shelf. The aim of this work was to ~~understand~~describe the ~~effect of seeping~~shallow-water methane ~~on benthic macrofauna at depths 60-70 m~~seep fauna and to understand whether there are differences in community structure between the methane seep and background areas.
Methods. Samples of macrofauna were taken during three expeditions of RV *Akademik Mstislav Keldysh* in 2015, 2017 and 2018 using 0.1 m^2 grabs and the Sigsbee trawl. In total, 21 grabs and two trawls were taken at two methane seep sites named *Oden* and *C15*~~.~~, located at depths of 60-70 m. For control, ~~six~~three 0.1 m^2 grabs were taken in an ~~areas~~area without methane seepage.
Results. The abundance of macrofauna was higher at methane seep stations, ~~also at *Oden* the biomass and diversity were higher~~ compared to ~~other areas.~~non-seep. Cluster analysis revealed five station groups corresponding to the control area, *Oden* site and three at the *C15* site. The taxa responsible for differences between the station groups were mostly ~~common and~~ widespread Arctic species, that were more abundant in samples from methane seep sites. However, large densities of symbiotrophic siboglinids *Oligobrachia* sp. were found exclusively at ~~all~~ methane seep stations. In addition, several species presumably new to science were found ~~only~~ at several methane seep stations, including the gastropod *Frigidalvania* sp. and the polychaete *Ophryotrocha* sp. The fauna at control stations was represented ~~exclusively~~only by well-known and widespread Arctic taxa. The number of station groups revealed from *C15* stations and high species richness in *C15* trawl samples compared to *Oden* indicated higher diversity of micro-niches within the *C15* site. The development of specific methane seep communities at such ~~a~~ shallow ~~depth apparently is~~depths can be related to pronounced oligotrophic environment on the northern Siberian shelf.

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Introduction

Methane gas seeping from the seafloor ~~provides environment and habitats~~can provide environmental conditions for unique fauna largely independent of photosynthetic primary production as occurs at hydrothermal vents (Van Dover, 2000). Distinct faunal response at methane seeps (also known as “cold seeps”) ~~expressed in sharp~~is associated with an increase in their total abundance and biomass, and presence of unique taxa absent in background areas. This pattern has been described from many areas of the Ocean (Baker & German, 2004; Levin, 2005). These taxa either develop symbiotic relationships with methanotrophic or sulphide-oxidizing bacteria or feed directly on benthic or suspended bacterial matter. At a higher trophic level, predators feeding exclusively on such taxa may be present (Gebruk, 2002; Dando, 2010).

In the Arctic Ocean, ~~only few~~several methane seep ecosystems have been discovered and investigated. The most studied include the Håkon Mosby mud volcano in the Norwegian Sea (Gebruk et al., 2003) and several sites around Svalbard and at Vestnesa Ridge (Åström et al., 2016~~). These areas~~; Åström et al., 2018). Other described cold seeps include the Lofoten-Vesterålen continental margin area (Sen et al., 2019a) and mud volcanoes in the Beaufort Sea (Paull et al., 2015). The cold seeps inhabited by specific benthic macrofauna ~~different from that in the surrounding ecosystems~~ are mostly located below the photic zone ~~(>~~ (depth >200 m ~~in the~~both around Svalbard ~~area~~ and ~~~1200 m~~ at Håkon Mosby) (Gebruk et al., 2003; Åström et al., 2016). At the same time, ~~in~~ areas with extensive methane ~~discharges~~discharge located at shallow depths ~~in coastal zones (for example~~(e.g. in the Norwegian and White Seas at depths <100 m) have been reported to have little or no ~~reaction~~response of macrofauna ~~is observed~~ (Savvichev et al., 2004; Levin, 2005). In general, ~~it was shown that there is~~ a ~~global trend of the~~ depth boundary is observed between shallow-water~~ vents and~~ cold seeps and their “deep-sea” counterparts at approximately 200 m (Tarasov et al., 2005; Dando, 2010). One of possible reasons for this boundary is the origin of organic matter: at depths <200 m photosynthetic organic matter is more available for benthic consumers due to stronger bentho-pelagic coupling. However, at greater depth the amount of photosynthetic organic matter decreases and chemosynthesis starts to play a significant role for local organic matter production. Therefore, despite the presence of methane and sulfides (unfavorable for most organisms), unique and diverse ecosystems develop most noticeably at deep-sea cold seeps (summarized by Dando, 2010).

Fauna associated with cold seeps in the Arctic includes symbiotrophic siboglinid polychaetes and thyasirid bivalves, but mainly consists of species not unique to~~to~~ methane seeps ~~but aggregating~~. Widespread Arctic species tend to aggregate in ~~such~~ habitats around methane seepage sites (Gebruk et al., 2003; Åström et al., 2016; Åström, Oliver & Carroll, 2017).

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~~Common features of all known~~ Arctic ~~high latitude methane~~cold seep assemblages are characterized by the dominance of frenulate siboglinid worms ~~and lack of~~, while large chemosymbiotrophic methane seep taxa~~, such as~~ (vestimentiferan worms, bathymodioline and vesicomyid bivalves) are absent (Sen et al. 2018). A c~~Among common effects~~ommon effect of methane seeps on marine benthic communities ~~are~~ is an increased abundance~~,~~ and biomass ~~and diversity~~ of regular allochthonous taxa ~~comparing~~compared to the background (Gebruk, 2002; Levin, 2005). Species richness at cold seeps is not higher than in the background, though recent results obtained from the southwestern Barents Sea showed increased taxonomic richness within the seepage sites (Sen et al., 2019b).

In the Siberian Arctic, areas of intense ~~bubble~~ methane discharge (methane seeps) were discovered on the outer shelf of the Laptev Sea in 2008 (Yusupov et al. 2010). Further research revealed numerous gas flares ~~on~~in the northern Laptev Sea shelf (Lobkovsky et al., 2015; Shakhova 2015). Within this area specific microbial communities based on methane oxidation were discovered (Savvichev et al. 2018). Baranov et al. (2019) suggested that methane seeps occur through the fault system belonging to Laptev Sea Rift system and Khatanga-Lomonosov Fracture Zone located between the Eurasian and North American Tectonic Plates. The faults may conduit the gas from reservoirs deep in the sediment below the caprock formed by permafrost and gas hydrates (Baranov et al., 2019). Within the seep area, multiple bacterial mats and occasional methane bubbles and carbonate crusts were observed (Baranov et al., 2019). Notably, the methane associated fauna was recorded on the Laptev Sea shelf and slope much earlier: during expeditions of RV *Polarstern* in 1993 and 1995 five species of siboglinids were found in this area in the depth range 50-2000 m (Sirenko et al., 2004), which is more ??species?? than anywhere else in the high Arctic.

~~The Laptev Sea outer shelf was recently investigated in frames of the P.P. Shirshov Institute of Oceanology, Russian Academy of Sciences program "Ecosystems of the Siberian Arctic Seas" in three expeditions of the RV *Akademik Mstislav Keldysh* in 2015, 2017 and 2018 (Flint et al. 2018; 2019).~~

We examined benthic communities associated with methane seeps in the Laptev Sea at two ~~fields~~sites: *C15*, centred around 76°47.4′N and 125°49.5′E with depths 70-73 m and *Oden*, centred around 76.894°N and 127.798°E, with depths 63-67 m. A preliminary ~~results on bottom~~description of benthic fauna ~~with the focus~~observed on ~~megafaunal~~ video ~~data are described~~was published by Baranov et al. (2019). The aim of this study is to describe further the biological peculiarities of the methane seep fauna and to reveal the differences in either integral community characteristics or distribution of certain species between the methane seep and

Commented [MC5]: What measures ? species richness? Better to be specific.

Commented [MC6]: Is implies present authors, was would imply other authors, please clarify and if others cite who background areas. We hypothesized that the seep sites are different from the non-seep in terms of general community characteristics and certain species distribution.

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Materials & Methods

Samples of macrofauna were taken during three expeditions of RV *Akademik Mstislav Keldysh* in 2015 (AMK-63), 2017 (AMK-69) and 2018 (AMK-72) on the northern Laptev Sea shelf, in an area of active methane discharge. ~~Material was obtained using~~The gears used for sampling were the *Okean* (in 2015) and *Van Veen* (in 2017-2018) grabs (0.1 m^2 sampling area) and the *Sigsbee* trawl (2 m frame width) (Eleftheriou & McIntyre, 2005). There were 21 grab and 2 trawl stations at three sites: on two methane seep fields (12 grabs and 1 trawl at *C15*; and 6 grabs and 1 trawl at *Oden*) and at the control site with no methane seeping ~~methane~~ (3 grabs~~).~~) (Fig. 1). A single trawl was taken at each seep site to minimize the possible ecosystem damage from this gear. In 2015 ~~all the~~three seep stations were selected above the present gas flares visible on echo-sounder. Three more grabs were taken ~200 m away from the nearest gas flare to catch background community. In 2017 and 2018 station selection was based largely on the previously mapped methane flares. All the 2017 and 2018 grabs were taken above the gas flares (Fig. 1). Station data with coordinates and ~~depth~~depths are shown in Table 1. ~~The study area and location of stations are given in Fig. 1.~~ For additional information on methane seep fields see Flint et al. (2018) and Baranov et al. (2019).

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Table 1. Data on stations used in the present study. For trawl stations coordinates and depth of start and end are given.

Fig. 1. Study area.
Enlarged maps show sampling sites and corresponding stations. Detailed bathymetry is only available for *C15* and *Oden* sites; white circles indicate previously recorded gas flares (Baranov et al., 2019). Dotted line at *Oden* site enclosed map shows the approximate perimeter of seeping area.

Sediment from ~~most~~ grab samples was washed by hand through the 0.5 mm mesh size sieve, and ~~then transferred to neutralized~~fixed with buffered 4% formalin~~.~~ solution afterwards. Two grab samples from the expedition in 2018 (Sts. 5947-3 at *C15* and 5953-2 at *Oden* site) were fixed with 96% ethanol. A 10-litre subsample of sediment taken from each trawl catch was washed through the 1 mm mesh size sieve and then fixed with neutralized 4% formalin. The material obtained was analyzed in the laboratory; all macrofaunal organisms were identified to the lowest possible taxonomical level with the help of taxonomic experts (see Acknowledgements) and counted. Species from grab samples were ~~weighted~~weighed (wet weight, all specimens of each species at a time). Molluscs were ~~weighted together~~weighed with shells, polychaetes with calcareous (spirorbids) or mucous tubes (*Spiochaetopterus typicus* and siboglinids) were ~~weighted together~~weighed with tubes. Density and biomass were calculated per square metre in case of grabs. Dominant species were distinguished by biomass. For trawl samples we calculated the contribution (in %) of each species to abundance. Biomass was not measured for trawl samples due to poor state of preservation. For ethanol fixed samples from Sts. 5947-3 and 5053-2, the biomass loss was corrected using taxa-specific coefficients after Brotskaya & Zenkevich (1939).

~~Total~~For grab samples total abundance, biomass, species richness (species number~~,~~), Pielou evenness, Hurlbert rarefaction index and Shannon-Wiener diversity index (H' ln) were calculated to get integral community characteristics.~~ For trawl samples, the species rank distributions were plotted. Differences between trawl catches were estimated by similarity percentage routine (SIMPER).~~ Abundance and biomass data from grab samples were square root transformed to increase the role of rare taxa. The similarity between grab samples and species was estimated using the Bray-Curtis similarity coefficient. Clusters were built based on similarity matrices using the unconstrained tree routine (UNCTREE); and SIMPROF used to distinguish station groups with significant differences in species composition. The results from cluster analysis were verified by non-metric multidimensional scaling (n-MDS). Clusters revealed by these methods were defined as separate station groups in terms of taxonomical similarity. Shade plots were built to visualize the species abundance and biomass differences between the stations and species in clusters. The Kruskal-Wallis test was used to verify differences in certain taxa occurrences between station groups. Results were corrected using the Tukey's pairwise post-hoc test. Species-individuals accumulation curves were plotted for each station group (McCune, Grace & Urban, 2002; Clarke & Gorley, 2015).

For all species present in any station group, an algorithm estimating the likelihood of accidental catch was applied. If a uniform distribution of species between two sampling efforts A and B is assumed, the probability of species absence at each station of B-sampling would be $(1-P_A)^{N(B)}$, where N(B) is the number of stations in B-sampling and P_A is the species occurrence (the proportion of stations where the species was present) in A-sampling. Using this equation, the likelihood of accidental absence of any species in either station group can be estimated. The number of grabs required for species catch in B-sampling can be calculated by the equation: $n = \lg(\alpha)\ \lg(1-P_A)^{-1}$, where α is the likelihood of species finding in B-sampling taken as 0.99 (Azovsky, 2018; Vedenin et al., 2019).

For trawl samples, the species rank distributions were plotted. Species richness, Pielou evenness, Hurlbert rarefaction index and Shannon-Wiener diversity index were calculated using

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the taxa-percentage values. Differences between trawl catches were estimated by similarity percentage routine (SIMPER).

Statistical analyses were performed in Primer V6, V7 and Past 3.0 software (Clarke & Warwick, 2001; Hammer, 2013; Clarke & Gorley, 2015).

Results

A total of 289 taxa of benthic macrofauna were identified in grab and trawl samples. In grab samples, density varied in the wide range from 580 ind. m^{-2} (St. 5624-3, Control site) to 9880 ind. m^{-2} (St. seep-3, *C15* site). Biomass ranged from 16.28 g ww m^{-2} (St. seep-1, *C15* site) to 405.79 g ww m^{-2} (St. 5623-3, *Oden* site). The list of all identified taxa from trawl and grab samples, with values of abundance and biomass is given in the Supplementary 1.

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Fig. 2. UNCTREE analysis with SIMPROF results (A) and non-metric multidimensional scaling plot (B) of grab stations using the Bray-Curtis similarity index ~~(square root transformed biomass data).~~. Square root transformed biomass data are used. Dashed lines connect statistically unreliable groupings (*p* > 0.05). Green lines indicate SIMPROF groups.

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Grab samples

Unconstrained tree with SIMPROF analysis revealed five significantly distinct groups of samples at the similarity level of 50 (Fig. 2). The UNCTREE parameters are shown in Supplementary 2. The groups partly corresponded with the station locations and presence/absence of methane seeps (*Control*, *C15* and *Oden* sites). To avoid a mix-up between the station groups and seeping sites hereinafter the corresponding names are used with either –station group or –site ending.

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Fig. 3. The species-individuals accumulation curves for the station groups. Colors are the same as in Figure 2.

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Characteristics of station groups

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The *Control* station group ~~“Control”~~ included three stations located between methane seep sites~~.~~ ~~At all three stations, the~~ (Fig. 1). The bivalve *Portlandia arctica* was ~~the~~ dominant ~~species~~ by biomass~~, with~~ at all three stations; the starfish *Ctenodiscus crispatus* and the bivalve *Macoma calcarea* ~~also playing an important~~played a secondary role. Due to the low number of samples, the species-individuals accumulation curve did not reach an asymptote (Fig. 3). Compared to other groups, the values of density and species ~~number~~richness were the lowest at Control, whereas the evenness was the highest (Fig. 4).

The *C15-seep a* station group included five stations, all within the *C15* ~~methane~~ seep site. In this group, biomass and diversity values were relatively low. Dominant species in this group were the bivalve *Nuculana pernula*, the siboglinid *Oligobrachia* sp. and the polychaete *Cistenides hyperborea*. Species-individuals accumulation curve in this group reached saturation due to the largest number of samples (Fig. 3).

The *C15-seep b* station group consisted of only two stations~~, was characterized by~~ from *C15* site. This group demonstrated the highest abundance values and the lowest biomass values owing to high ~~density~~abundance of small polychaetes *Cossura longocirrata*, *Micronephthys minuta* and *Ophryotrocha* sp. at some stations (Fig. 4, Supplementary 1).

The *Oden* station group included six stations, all located within the *Oden* ~~methane~~ seep site. Values of biomass, species ~~number~~richness and diversity indices in this group were the highest among all station groups (Fig. 4). The most dominant species were the siboglinid *Oligobrachia* sp. and the other? polychaetes *Myriochele heeri* and *Nephtys ciliata*.

Fig. 4. Univariative characteristics of identified clusters.
Mean values of total density, biomass, species ~~number~~richness, Pielou evenness, Hurlbert rarefaction index and Shannon-Wiener index with standard deviation are shown. Exact values of these characteristics are shown in Supplementary 3.

~~In the~~The last group ~~*"Seep Background"* there were~~ *C15 background* contained five stations taken within the *C15* site~~ away from methane discharges~~. Three of these stations (Sts. background-1, 2 and 3) were taken several hundred metres away from active methane seeps. Two stations (5947-1 and 5947-3) were planned as active seep stations~~ but accidentally.~~ Accidentally, they were taken in the seep background area according to taxa composition and ~~cluster~~following analysis. Taxonomical composition at these stations was similar to that in the *Control* group, with the bivalve *Portlandia arctica* being the dominant species. Bivalves *Yoldiella lenticula* and *Y. solidula* were subdominant. In this station group, the biomass values were the lowest, other general community characteristics were intermediate (Fig. 4). As with the ~~"~~*Control*~~"~~ group, ~~"Seep Background"~~ *C15 background* did not reach the saturation point at species-individuals accumulation plot (Fig. 3).

Comparison of seep and non-seep ~~stations~~station groups

General community characteristics in the station groups appeared different in abundance, biomass and diversity (Fig. 4, Supplementary 3). The abundance of several taxa was significantly different in four station groups (Fig. 5). The Kruskal-Wallis test showed that differences in abundance of at least ten species are statistically reliable (Table 2). ~~Thus, the~~

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~~methane~~The seep sites were characterized by higher density of the polychaetes *Tharyx* sp. and *Cistenides hyperborea*, the bivalve *Macoma calcarea* and the ophiuroid *Ophiocten sericeum*. On the contrary, the bivalve *Portlandia arctica* was markedly more abundant in ~~the~~ *Control* and ~~*Seep*~~, to a lesser extent, in *C15* *background* ~~areas~~station groups (Fig. 5). Notable ~~are~~were extreme densities of small polychaetes at some seep stations, including *Cossura longocirrata* and *Ophtyotrocha* sp. (Fig. 5A~~).~~) at *C15 seep b*.

Fig. 5. Shade plot of species square root transformed abundance (A) and biomass (B) at stations arranged by clusters.
The species list is reduced to 20 most important taxa. Order of stations and colors the same as in Figure 2. Taxa grouped in clusters using UPGMA algorithm based on index of association.

Certain species present at some methane seep sites were completely absent at the non-seep sites (Fig. 5). Among them, at least four species (the polychaete *Spiochaetopterus typicus*, the siboglinid *Oligobrachia* sp., the bivalve *Axinopsida orbiculata* and the amphipod *Pleusymtes pulchellus*) were ~~found~~present only at *C15* and *Oden* sites. At least one species, the undescribed gastropod *Frigidalvania* sp., was present only ~~at~~in *Oden* station group and absent ~~at~~ ~~*C15*~~elsewhere (Table 3). The estimated number of grabs required to catch the latter species was slightly lower than the number of grabs taken.

Table 2. Results of the Kruskal-Wallis and Tukey's post-hoc tests for taxa with different abundance values in five station groups. Mean abundance in each station group is shown. Taxa are arranged according to *p*-value. ~~Taxa with *p* values lower than 0.05 are marked with plus.~~ Pairs in post-hoc column indicate significant comparisons (Tukey's $p < 0.05$).
1 – *Control* group; 2 – *C15 background* group; 3 – *Oden* group; 4 – *C15-seep a* group; 5 – *C15-seep-b* group.

Table 3. Likelihood of not finding a species calculated for species present only at methane seep sites and only at the *Oden* site.

Trawl samples

The overall Bray-Curtis similarity between the two trawls was 66%. Species ranking graphs showed high level of dominance by abundance for both trawl stations (Fig. 6). The ~~most abundant~~dominant species in both trawls was the ophiuroid *Ophiocten sericeum*: 37% of the total abundance at *C15* and 46% at *Oden*. The second most abundant species at *C15* was the gastropod *Frigidalvania* sp. (12%) and at *Oden* the bivalve *Yoldiella solidula* (11%). Ten most abundant species accounted for >70% of the total abundance ~~un~~in both trawls (Fig. 6).

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Fig. 6. Species ranking for *C15* and *Oden* trawl samples.
The most numerous species are indicated. X-axis is logarithmic.

Species ~~number~~richness, Pielou evenness, Hurlbert rarefaction for 100 individuals and Shannon-Wiener index are shown in Table 4. Diversity ~~appeared~~was higher in the *Oden*-trawl than in the *C15*-trawl, similarly as in the grab samples. However, the species ~~number~~richness (as well as the total amount of individuals) in the *C15*-trawl was higher than in the *Oden*-trawl (Table 4, Supplementary 1).

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Species responsible for taxonomical difference between the two trawl samples are shown in Table 5. Most notable was a high amount of the gastropod *Frigidalvania* sp. at *C15*. At *Oden* *Frigidalvania* sp. was also present, but in much smaller densities (only 2.3 % of the total abundance). In addition, *C15*-sample differs from *Oden* by high amount of various filter-feeders including 6 species of sponges (with *Craniella polyura* being most numerous), at least 6 species of cnidarians, 17 species of bryozoans and 3 species of tunicates (Supplementary 1).

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At *C15* trawl sample, a large piece of carbonate crust was found. Cavities of its pores were inhabited by numerous polychaetes, typical also for the soft sediments around the seepage area (e.g. members of families Nephthyidae, Nereididae, Oweniidae and Terebellidae, see Supplementary 1), and by several filter-feeders (Hydrozoa).

Table 4. Species ~~number~~richness, Pielou evenness, Hurlbert rarefaction for 100 individuals and Shannon-Wiener index calculated for trawl samples.

Table 5. Similarity percentage routine for trawl samples.
Species with contribution >0.5% are shown. Species more abundant at *C15* are marked with bold.

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Comparison of ~~grab~~C15 and ~~trawl data~~Oden sites

All gears showed significant differences between the *C15* and *Oden* sites expressed in different taxonomical composition and quantitative characteristics. The Bray-Curtis similarity between the sites according to the grab samples and trawl samples was 26.2 and 65.6, respectively. The main differences ~~were~~in species composition included the high abundance of the sponge *Craniella polyura* and the gastropod *Frigidalvania* sp. at *C15* site and higher numbers of the ophiuroid *Ophiocten sericeum* at *Oden* site.

The grab data ~~indicate~~indicated a high level of heterogeneity of benthic fauna on the scale of several meters at both seep-sites. Some species formed patches, for example *Oligobrachia* sp., *Cossura longocirrata* and *Ophryotrocha* sp., being extremely numerous at several grab stations. There were also species with rather uniform distribution based on combined data, for example

Ophiocten sericeum. According to the cluster analysis, the *C15* site is more heterogenic forming at least three different species complexes within its area (Fig. 2). Dissimilarity within the *C15* and *Oden* sites was 64.7 and 26, respectively (Supplementary 2).

Dominant species were different in grab and trawl samples. The ~~main~~ dominant species in trawls at both methane seep sites was the ophiuroid *Ophiocten sericeum*. Whereas based on grab data, the ~~main~~ dominants at seep sites were the siboglinid *Oligobrachia* sp., the bivalve *Nuculana pernula* and the polychaete *Myriochele heeri*. ~~In non-seep areas, the main dominant was the bivalve *Portlandia arctica*, based on the only available grab data.~~

Discussion

Integral community parameters: methane seep vs. non-seep ~~***stations***~~

The abundance of macrofauna was higher at the ~~methane~~ seep stations compared to the background~~, also~~. In addition, at the *Oden* site the biomass ~~and diversity were~~was higher compared to non-seep sites. Increased values of abundance and biomass have been reported from both hydrothermal vents and cold seeps all over the world. In the Arctic, a twofold increase of biomass compared to control sites was observed at cold seeps south off Svalbard (mean values of 20.7 vs. 9.8 g ww m^{-2}), the abundance increase was less prominent (770 vs. 590 ind. m^{-2}) (Åström, 2016). For the Håkon Mosby mud volcano, the comparison of abundance and biomass with the background is not available. In our study, the abundance at the methane seep sites *C15* and *Oden* was more than four times higher than at the control. However, differences in biomass although pronounced were not statistically reliable. ~~Among important controls of increased~~Increased biomass in seep habitats is commonly ~~are discussed an~~explained by enhanced organic matter content~~,~~ and habitat heterogeneity ~~and the occurrence of hard substrates~~ (Gebruk et al. 2003; Sen et al. 2018).

~~*Taxa*~~Pielou's evenness was distinctly higher at the *Control* and *C15 background* station groups, which reflects the increased dominance of certain species at seep stations compared to non-seep. Many authors reported high abundance and biomass values of one to few dominant species at various cold seeps (Gebruk et al. 2003; Åström, 2016; Åström et al., 2018). This can be caused by conditions less favorable for some background species, but more favorable for symbiotrophs or grazers (summarized in Dando, 2010).

The cold seeps usually demonstrate lower diversity values compared to the background areas (Levin, 2005). However, combined species list from grab and trawl samples showed a high

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Commented [MC26]: Exactly, so one already knows which are dominant and Pielous adds no additional information and is thus redundant; similarly Shannon index is a hybrid of richness and dominance and tells us nothing (despite its popularity)

Commented [MC27]: Clarify which of the many measures is meant diversity although only two trawls were sampled. Our studies on the Siberian shelf using the same gear under the same conditions obtained less than 150 species per trawl (Galkin & Vedenin, 2015; Vedenin, Galkin & Kozlovsky, 2015), while a total of 203 species were found in a single *C15* sample. The unusually high diversity may reflect a higher amount of microniches within the *C15* site. This is indirectly confirmed by lower similarity values observed between all *C15* grab samples. Higher habitat heterogeneity at seep sites can increase the overall diversity of benthic fauna (Gebruk et al. 2003; Levin, 2005). The scale of heterogeneity is hard to assess, but based on stations coordinates and the fact that stations 5947-1 and 5947-2 from *C15*-site were grouped in *C15 background*, while station 5947-2 was grouped as *C15-seep a* we can assume that the scale is less than 5 m (distance between these stations) (Fig. 1; Table 1).

In addition, the diversity values at the *Oden* station group were significantly higher than at other sites. The reasons for this are unknown so far, since no environmental parameters measured directly at benthic stations are available. Interestingly, the peculiarly higher values of diversity within the cold seeps are known only for the seep areas in the Arctic, e.g. for the Vestnesa Ridge (Åström et al., 2018) and for the South-Western Barents Sea (Sen et al., 2019b).

Common shelf taxa responsible for differences in station groups

The station groups revealed by UNKTREE and n-MDS analysis largely corresponded to the geographical position of the *C15*, *Oden* and control sites. A number of common species widely distributed across the Siberian shelf (see Supplementary 1, Sirenko, 2001) were largely responsible for increased integral community parameters in our study. Most of these taxa are listed in Table 2. Among such species (based on grab samples) were the polychaetes *Spiochaetopterus typicus*, *Cossura longocirrata* and *Tharyx* sp., the bivalve *Macoma calcarea*, the amphipod *Pleusymtes pulchella* and the ophiuroid *Ophiocten sericeum*. In addition, based on trawl data, the sponge *Craniella polyura* was present in high densities at the *C15* site~~.~~, together with other filter-feeders including cnidarians and bryozoans. Apparently the same species aggregations were visible on the video reported by Baranov et al. (2019). All these species were previously reported from a wide range of areas of the Laptev Sea and adjacent regions (Sirenko et al., 2004).

The increased density of common taxa at deep-sea hydrothermal vents and cold seeps is a well-known phenomenon usually explained by increased availability of organic matter in these habitats (Hessler & Kaharl, 1995; Levin, 2005). In the Arctic, the increased biomass and abundance of common allochthonous species was reported for the Håkon Mosby mud volcano (Rybakova et al., 2013), Svalbard (Åström et al., 2016) and Vestnesa Ridge cold seeps (Åström

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Commented [MC31]: Please be specific, this could be anything from 1 to 100 or more et al., 2018). Also, a significant increase of abundance of filter feeders (especially sponges) was shown for the Aurora Seamount on the Gakkel Ridge, the only investigated hydrothermal vent in the Central Arctic Ocean (Boetius, 2015).

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Fig. 7. Taxa found only at seep stations.
A – *Oligobrachia* sp. (left – tube with several fragments enlarged; center – complete specimen extracted from tube; right – anterior and posterior fragments of the specimen); B – *Frigidalvania* sp.; C – *Ophryotrocha* sp. (upper left – several specimens, total view; upper right – anterior fragment; lower – enlarged parapodia); D – *Axinopsida orbiculata*. Photos by A. Vedenin and V. Kokarev.

Taxa specific for methane seep sites

The most distinctive species of the methane seeps in our study was the siboglinid *Oligobrachia* sp. (Fig. 7a). This species was present at every seep station and absent at every background and control station. This species is morphologically very close to *Oligobrachia haakonmosbiensis* originally described from the Håkon Mosby mud volcano from the depth of ~1200 m (Smirnov, 2000). Colonies of *O. haakonmosbiensis* with the biomass reaching 350 g ww m^{-2} were reported from this area (Gebruk et al., 2003). Recent phylogenetical analyses showed that the species from the Laptev Sea belongs to a separate, undescribed species of *Oligobrachia* (Sen et al., 2018). In the Laptev Sea, *Oligobrachia* sp. is known from different localities, seep and noon-seep, occurring in a wide depth range 100-2166 m (Buzhinskaja, 2010). Our record at 63 m is the shallowest for this species, with high population density and biomass: >1000 ind. m^{-2} and 45 g ww m^{-2} at Sts. 2623-1 and 5953-2~~.~~ (*Oden* site). Several specimens from 2015-samples (erroneously identified as *O. haakonmosbiensis*) were investigated using transmission electron microscopy (Savvichev et al., 2018). Usually the endosymbionts of siboglinids are represented by sulphide-oxidizing bacteria (Rodrigues et al., 2011; Lee e al., 2019), but here methanotrophic bacteria were found inside its trophosome.

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Some samples from the seep sites besides the siboglinids also were marked by several species of molluscs. The gastropod *Frigidalvania* sp. (Rissoidae) occurred in high density at *C15* site: up to 2340 ind. m^{-2} and 25 g ww m^{-2} at St. 5625-3 (Fig. 7b). According to trawl samples, this species occurs at the *Oden* site, but was low in number. This species is new to science. Large numbers of unknown rissoid gastropods were previously reported from the Håkon Mosby, referred to as *Alvania* sp. in Gebruk et al. (2003). Later, the stable isotope analysis has shown that the rissoids at Håkon Mosby are grazing on bacterial mats (Decker & Olu, 2012). Another rissoid gastropod, *Pseudosetia griegi*, was observed grazing on bacterial mats at the hot vent Loki Castle on the Mohn's Ridge (Sweetman et al., 2013). At the recently investigated Lofoten canyon seep site dense aggregations of unidentified rissoids were observed from ROV (Sen et

Commented [MC33]: Please be more quantitative, this could be 2 or 100 depending on the context al., 2019a). Based on the details available from the published photo, we suggest that the gastropods are very likely to belong to genus *Frigidalvania*, based on the shell shape and rusty-brownish periostracum (Sen et al., 2019a, see Fig. 4b). Unfortunately, in our study we were not able to identify the behavior or lifestyle of *Frigidalvania* sp. This species remained unnoticed in the video data ~~(Baranov et al., 2019).~~due to its small size (Baranov et al., 2019). However, multiple bacterial mats observed from video-transects and caught by box corer provide an opportunity for such species to graze on them (Savvichev et al., 2018; Baranov et al., 2019). Another species common at seep sites and lacking in the background was the thyasirid bivalve *Axinopsida orbiculata*~~.~~ (Fig. 7d). Some species of thyasirids are known as symbiotrophic. However, the information on symbiotic bacteria in the gills of *A. orbiculata* is controversial: Zhukova, Kharlamenko & Gebruk (1991) have demonstrated the presence of bacteria in bivalve specimens from the Kraternaya Bight, the Kuril Islands, whereas according to Dufour (2005) this species lacks bacterial symbionts. It is possible that *A. orbiculata* is attracted by increased food availability at seep sites, as may another bivalve, *Macoma calcarea*, which is also common in seep background areas (Fig. 5). Overall, no bivalves restricted to cold seeps are known so far in the Arctic with the exception of two large thyasirids recently described by a few empty shells (Åström, Oliver & Carroll, 2017) and Pleistocene subfossils (e.g. *Archivesica* spp., Sirenko et al., 2004; Hansen et al., 2017). The subfossils suggest that previously the Arctic cold seeps (and possibly hydrothermal vents) were inhabited by richer fauna that became extinct after Quaternary glaciation.

There was a notably high density (>3600 ind. m^{-2}) of the dorvilleid polychaete *Ophryotrocha* sp. ~~at~~in one grab ~~station~~sample at *C15-seep*, b station group (Supplementary 1 (Fig. 7c). At least 15 species of *Ophtyotrocha* have been described from reducing habitats (Taboada et al., 2013; Salvo et al., 2014; Ravara et al., 2015), including two species considered as obligate for cold seeps in the Kagoshima Bay, Japan (Miura, 1997). On the other hand, many species of this genus are common in regular marine ecosystems including Arctic seas (Sirenko, 2001).

Another taxon common in reducing habitats is Tanaidacea. In our material three species were present (Supplementary 1), all widely distributed in the Arctic (Sirenko, 2001). The density of tanaids in our samples was low, although this taxon was reported in high ~~density~~densities from the Håkon Mosby (Gebruk et al., 2003) and the Vestnesa Ridge (Åström et al., 2018) with several species (described as new) restricted to the methane seep habitats (Błażewicz-Paszkowycz and Bamber, 2011). It seems likely that many species of tanaids remain unidentified and diversity in this taxon remains underestimated owing to difficulties of identification of these small crustaceans (summarized by Błażewicz-Paszkowycz & Bamber, 2011). The low number of

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Commented [MC36]: Similarly how can it be known that a fossil was living ina cold seep?

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tanaids in our samples could be a result of a too large sieve mesh size used onboard (see Materials & Methods). Tanaids commonly are < 0.5 mm in size and require a corresponding mesh size to be found (Pavithran et al., 2009).

Overall, considering grab and trawl data combined, all the seep-specific taxa were the same at both seep sites. The only exception is the polychaete *Ophryotrocha* sp., which could be missed from the *Oden* trawl sample due to the large sieve mesh size (Supplementary 1).

Presence of specific benthic communities at C15 and Oden

Up to now no distinct macrofaunal changes in response ~~of macrofauna~~ to methane seeps ~~was~~were reported ~~in~~from the Arctic Ocean at depths < 80 m. In general, at depths <200 m both hydrothermal vents and cold-seeps are usually ~~are~~ colonized by a subset of the local fauna (Tarasov et al., 2005; Dando, 2010). Some species notable at shallow-water methane seeps belong to opportunistic taxa common in various reducing habitats. These include siboglinid polychaetes and thyasirid bivalves reported from Skagerrak, Kattegat, coastal areas of Florida, Japan, New Zealand, New Guinea etc. (Southward & Culter, 1986; Schmaljohann & Flügel, 1987; Schmaljohann et al., 1990; Malakhov, Obzhirov & Tarasov, 1992; Gebruk, 2002). The isotope data suggest that food sources of macrofauna at shallow-water methane seeps are largely photosynthesis-based (Levin, 2005). It was suggested that the faunistic depth boundary between the deep-sea and shallow-water vents and seeps at approximately 200 m is controlled by the amount of ~~POC~~organic matter input from the photosynthetic production (decreasing below the photic zone) and the greater number of predators at shallow depths. Definite seep-obligate species were not reported from depths <200 m (Tarasov et al., 2005; Dando, 2010).

At the same time, methane seep habitats even at shallow depths increase a number of microniches owing to increased organic matter availability, variety of substrates and repeated disturbance (Dando, 2010). Shallow cold-seeps ~~thus~~ may therefore support greater species diversity compared to the background ~~or attract species specialists to reducing habitats.~~. In our study at both methane seep sites, *C15* and *Oden*, community characteristics were significantly different from those in non-seep areas, ~~largely~~among other things owing to presence of obligate species to ~~to~~reducing habitats. In addition, the communities found at *C15* site formed several ~~clusters~~station groups and were more scattered at the n-MDS plot (Fig. 2A, B) which could indicate a larger diversity of microniches within this site. Large numbers of filter-feeders (Hydrozoa and Bryozoa) found in *C15*-trawl indicated the presence of hard substrata (including carbonate crusts). The larger amount of microniches is partly supported by the video-data, where

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the landscape within the active seepages was more complex than in non-seep areas (Flint et al., 2018; Baranov et al., 2019).

~~We~~Unfortunately, no environmental data except for the echo-sounding showing certain gas flares and CTD-measurements obtained from the area of the seeps from two points away from the benthic samples were available (Flint et al., 2018; Baranov et al., 2019). Nevertheless, we suggest that the observed ~~reaction~~response of macrofauna to methane seeps at the shallow depths of 60-70 m ~~is~~can be related to very low primary productivity on the outer shelf of the Laptev Sea, dropping from ~~~15mgC~~720mg C m$^{-\cancel{3}2}$ per day at ~~400 km from the~~ Lena river delta to ~~3.53~~<100 mg C m$^{-\cancel{3}2}$ per day at 600 km (~~Fahl & Stein, 1997; Sukhanova et al., 2017; Flint et al. 2018).~~Sorokin & Sorokin, 1996) during September. Outside the short Arctic summer months, these values tend to zero. In these extremely oligotrophic conditions, methane is a source of energy for the methane-oxidizing bacteria and stimulates the development of local patchy benthic communities at these depths. As a comparison, the specific communities with siboglinids around Svalbard located at similar latitude are developed only at depths >200 m (Åström et al., 2016). Unlike the Laptev Sea shelf, the primary production south and west off Svalbard reaches much higher values up to 1800 mg C m^{-2} per day during May blooms (Wassman et al., 2006). Furthermore, the Barents Sea remains uncovered with ice during most of the year, while the Laptev Sea shelf is ice-free during one to two months annually.

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Conclusions

Our study is the first description of shallow-water (< 100 m) methane seep communities in the Siberian Arctic. On the northern Laptev Sea shelf, significant differences were found between two methane seep sites (*C15* and *Oden*, located at depths of 63-73 m) and the background areas. The differences ~~include~~included integral community parameters and presence at seep sites of species ~~specialists to~~typical for reducing habitats, such as siboglinids *Oligobrachia* sp. and thyasirid bivalves. Several species at methane seeps are presumably new to science, including the gastropod *Frigidalvania* sp. and the polychaete *Ophryotrocha* sp~~.~~., found in large quantities at *C15* site. We suggest that the ~~reaction~~response of macrofauna to methane seeps at shallow depths is related to very low primary productivity on the outer shelf of the Laptev Sea.

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