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CRUISE REPORT

CAGE Worm-cruise to outer Kvalsund collecting tubeworms and sediment for eDNA and aDNA

on R/V Helmer Hanssen, October 28th, 2020

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Photo: A. Sen

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1. Summary

From the morning of October 28th to the evening of October 28th 2020, fieldwork was performed comprising retrieval of live siboglinid worms for genetic and environmental studies hereunder pore water content of sulphate, sulphide and methane and sediment TOC, TC and isotopes. The working area was a basin located in outer Kvalsund, Troms, northern Norway. A total of 2 box core stations and 2 gravity core stations were obtained and two CTD casts. Echosounding and chirp data were recorded on stations and on transits.

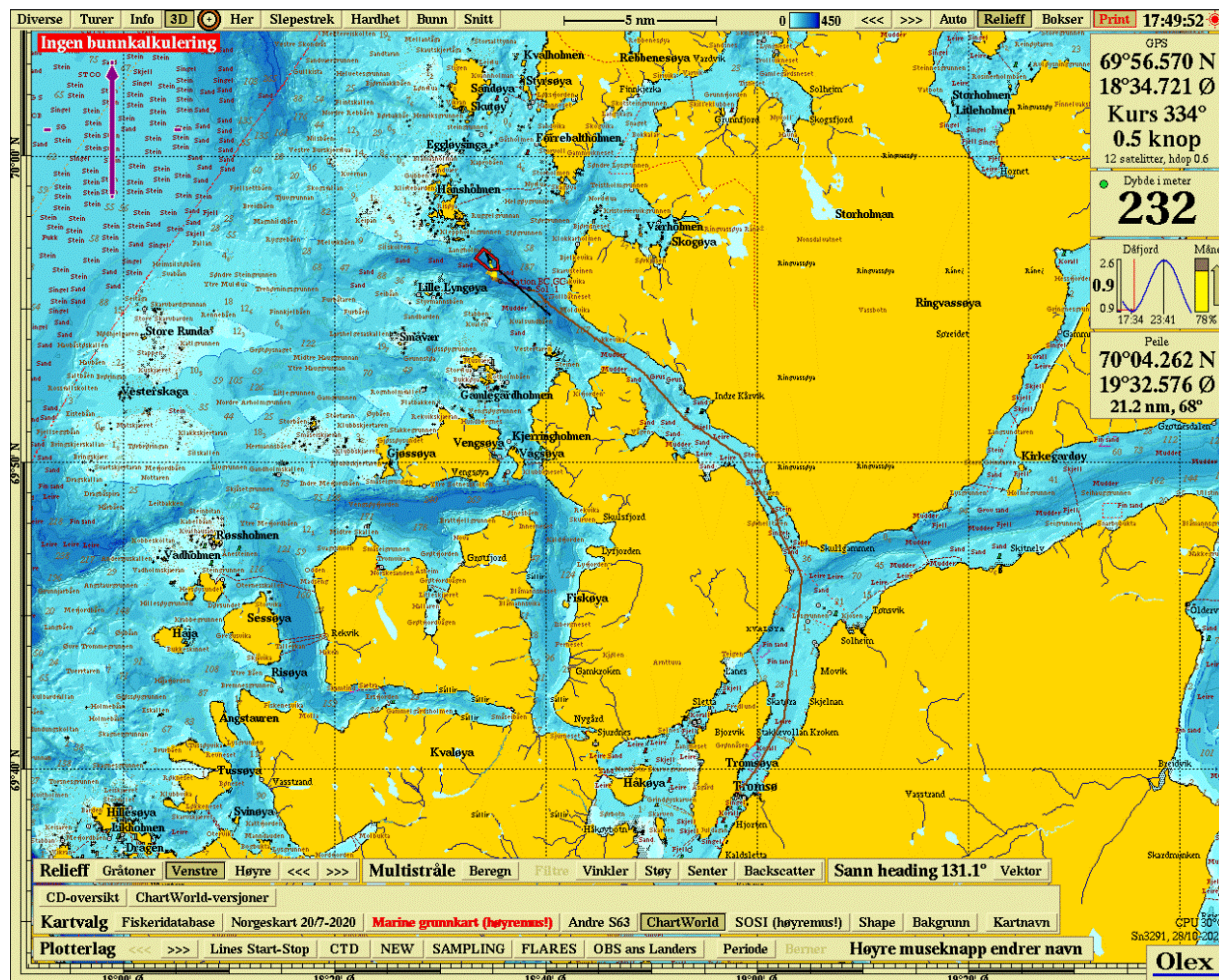
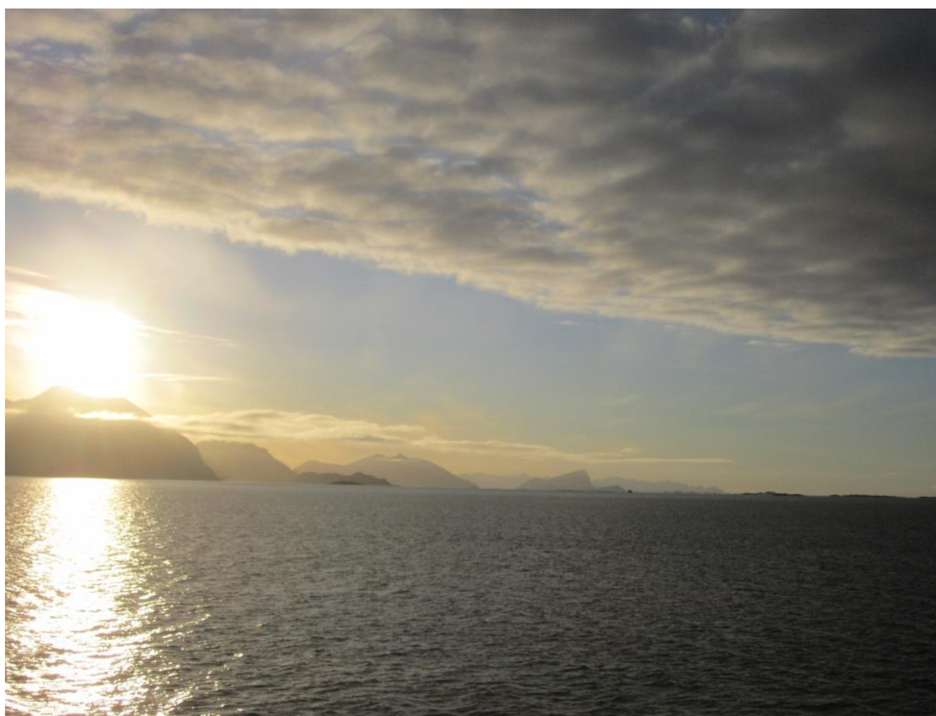


Figure 1: Overview map of the cruise.

Figure 2: Detailed map showing the investigated areas during the cruise.



2. Background and objectives

The cruise was arranged as a follow-up of previous attempts to collect siboglinid worms of the species *Oligobrachia webbi*, to investigate if this worm is linked to seep environments or not. This species has been described based only on the morphology of dead individuals, fifty years ago, before DNA sequencing and genetic analyses became widespread. It is morphologically practically identical to *Oligobrachia haakonmosbiensis* and *Oligobrachia* sp. CPL-clade, which are found at different cold seep sites in the Arctic (Sen et al., 2018, Sen et al., 2020). Due to the similarities in physical appearance, there is some debate as to whether *O. webbi* and the seep *Oligobrachia* species truly are distinct species. DNA analysis would easily resolve the issue however, the stored *O. webbi* samples were processed in a way that makes DNA analysis difficult, but doable with some limitations (preserved specimen obtained from Tromsø Museum for aDNA analysis). The goal of this cruise is to hopefully collect fresh samples of *O. webbi* in order to examine DNA sequences and compare them with those of *O. haakonmosbiensis* and the *Oligobrachia* sp. CPL-clade, thereby settling the question of species identities. Furthermore, this work would answer the question of whether Arctic cold seeps are inhabited by seep specific siboglinid worms or more generalist species that are capable of occupying habitats spanning a wide range of local environmental conditions. The site was first sampled (unsuccessfully) during a teaching cruise in November 2-5, 2017 (Rasmussen et al., Cruise Report 2017). A new cruise was arranged as a collaboration between former WP3 and WP6 within CAGE at the Department of Geoscience, the Arctic University of Norway in 2018. As a follow-up of the results of this cruise new sampling was done in 2020 by box coring and gravity coring in the same small depression at the mouth of Kvalsund c. 2 hours sailing from Tromsø. Subcores from the boxcores were taken for e- and aDNA, gravity cores were taken with the same purpose. Additional subcores and gravity cores were taken for pore water sulphide, sulphate and methane and TC and TOC sediment analyses.
The obtained worms will be dissected and the symbiont containing organ (trophosome) removed for bacterial DNA analysis.

Scientific objective of the cruise:

- Collection of siboglinid worms in Kvalsund was done with the purpose of improving the understanding of faunas related to environments of seepage of methane and resolving issues of species identities of physically similar species through molecular means.
- Collections of trophosomes for symbiotic bacterial identification by DNA studies
- Collection of sediment for characterizing the physical environment inhabited by the worms.
- Collections of cores for eDNA analysis to characterize sediment microbial communities (from which symbionts are drawn) and to obtain DNA from remains of *O. webbi.*

3. Equipment

Acoustic equipment
- 3.5 kHz Pinger *Geo Acoustics GeoPulse transducer*

Sediment sampling
- Box corer (BC) 25x25x50 cm barrel
- Gravity corer 6 m tubes

Water properties:
- CTD (Seabird 911 Plus)

4. Methods

Seismic profiling:
High-resolution seismic profiles (hull-mounted sub-bottom profiler), were collected along the ship tracks and during sampling. The equipment worked well and the data are generally of good quality.

Water properties

The water properties – temperature, salinity – were measured using a *Seabird 911 Plus* CTD. Data collection was performed during down- and up-casts at a speed of approx. 1.0 m/s

Sediment sampling

The boxcores were taken in two different stations at outer Kvalsund-Lyngøya and were sampled for tubeworms, porewater, sediment properties (organic carbon content, stable isotope signatures) and sediment microbial communities (eDNA). Three subcores from each boxcore were taken for DNA studies and pore water properties, one was drilled with small holes every 2 cm for pore water sampling with rhizons and one with 2.5 cm holes each 5 cm for methane and TOC samples.Holes were taped before pushing the cores into the sediment. Two gravity cores were taken, one for DNA and one for porewater and sediment analysis. The GC for DNA was frozen, while the core for methane, TOC and pore water was drilled with 2.5 cm holes each 10 cm and taped before coring. CTD casts were done at each station for water properties. After subcores were removed, sediment from the box cores were sieved over a 1 mm mesh sieve to retrieve live tubeworms.

One Van Veen grab was taken at the first station in outer Kvalsund. The entire grab sample was sieved to collect worm samples.

Photo (Naima El bani Altuna): drilling for rhizons

Photo (Naima El bani Altuna): sub core drilled for methane and TOC

Photo (Naima El bani Altuna): Subsampling box core

Sampling for methane:
3 ml of sediment was taken out with a cut 3 ml syringe and put in crimp vials prepped with 3 ml 5%NaOH. Thereafter vials were crimped and put in cooler at 4°C.

Sampling for TOC:
Sediment was taken out from each 2 cm hole with a 20 ml cut syringe and put in jars and in the cooler at 4°C.

Sampling for pore water:

Pore water samples for sulphate and sulphide were sampled by rhizons connecte to 10 ml syringes with wooden sticks for suction. The first 1 to 1.5 ml pore water was discarded. After syringes were full 6ml were put in 6 ml exetainers and put in cooler at 4°C. Thereafter 1 ml was put in Eppendorf vials pre-prepped with 1 ml Zinc acetate (19.6 mM). These were frozen at -20°C.

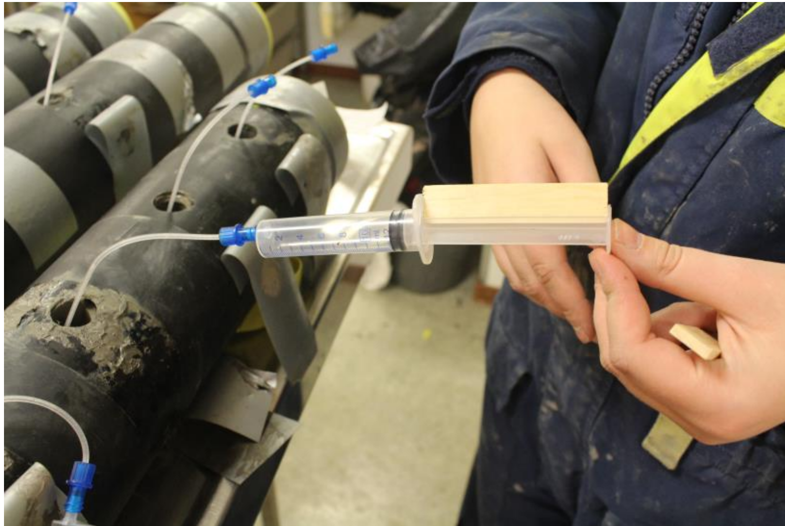


Photo (Anna Mamadzhanian, St. Petersborg University): rhizon for pore water sampling

Photo (Troels Laier, GEUS, Copenhagen). Vials for pore water (exetainer and Eppendorf tube), crimp vial for methane samples, and jar for TOC samples.

Worm sampling:
Worms that were visible in the box cores were manually pulled out with forceps, taking care not to break them. Since they are not always easily visible, the box core sediment was then sieved on a 1 mm sieve to remove all sediment to access the rest of the worms present in the cores. Retrieved worms were transferred immediately to chilled seawater and kept in the dark, until they were processed and fixed. This allows for the worms to be kept alive, which is necessary for processing.

Worms were removed from their tubes under a microscope, using forceps and a fine paintbrush. Extracted individuals were divided into worm only tissue (anterior parts) and the trophosome tissue (symbiont containing organ in the posterior of the worms). Opisthosomes (the very end of the worms) were not retrieved. Both types of samples were frozen at -20°C for DNA analyses (worm species and symbiont species for worm only tissue and symbiont tissue respectively). Tubes were also frozen, for stable isotope analyses. In addition to worms extracted from tubes, a number of whole worms, and including tubes were frozen as is, due to time constraints.

5. Participants

Participants:

Rasmussen, Tine L. (professor, chief scientist)	CAGE, UiT
Arunima Sen (post. doc.)	Nord Uni.
Silje Andreassen (master student)	IG, UiT
Lucie Alain (master student)	IG, UiT
Naima El bani Altuna (Ph.D. student)	CAGE, UiT
Christine Lockwood Ireland (Ph.D. student)	IG, CAGE, UiT
Steinar Iversen (engineer)	IG, UiT
Fabio Sarti (engineer)	CAGE, UiT

IG, UiT = Department of Geoscience, the Arctic University of Norway, Tromsø, Norway
CAGE = Centre for Arctic Gas Hydrate, Climate and Environment

6. Journal

Remark: UTC one hour difference.

Wednesday, October 28th, 2020

Weather: clear, 2 °C; winds 1 m/s

Summary

Embarkation of the vessel. Safety drill and departure for outer Kvalsund at 07.04 UTC to find more tubeworms. Heading for first station 1: 69°55.900N, 18°35.700E. On transit pass slowly 69°55.650 N, 18°38.420E with chirp (for eventual later return station 3). Planned box core with three subcores -1 for rhizons (small holes every 2 cm), 1 for methane large holes each 5 cm, and one for DNA to be frozen in upright position. Thereafter depending on success GC or GRAB. Two GC planned – one with large holes each 10 cm, one to be frozen for DNA.
Continued box coring at a deeper site within the basin off Lyngøy – no worms therefore return to first station for grab sampling.

Protocol of October 28th (times in UTC)

~0645: Embarkation of the vessel at "*Kullkransvingen*" in Tromsø
~0704: Departure for outer Kvalsund
0924: On station for first CTD HH20-639CTD
0928: CTD HH20-639CTDstart, temp 0.5, sal. 33.5
0942: CTD HH20-639CTDstop
0957: Box core station HH20-640BC, 47 cm, three subcores, sieved for worms
1130: Moving to 69.56.040, 18.35.300 for 2 GC stations
1203: GC station HH20-641GC, 6 m! pore water and TOC, CH_4 core
1310: Transit back to core for DNA same position
1327: GC station HH20-642GC, to be frozen
1357: Moving to deeper site at c. 280 m slightly further northwest for BC
1445: Boxcore station HH20-643BC (40 cm, disappointing – no worms, normal sediment thyasirids though)
1513: CTD HH20-644CTDstart, temp 0.5, sal. 33.5
1525: CTD station HH20-644CTDstop
1707: transit back to first station for grab sampling
1732: grab station HH20-646GRABstart, full
1732: grab station HH20-646GRABstop, full
1815: begin transit to Tromsø
2010: arrival

7. Preliminary results

The acoustic data, as well as the data collected with the box corer and gravity corer, will be analysed at a later stage on land. Onboard species determination showed the tubeworms to belong to a different species (*Siboglinum fjordicum* most likely) – no *O. webbi* were collected although a fragmented worm had multiple tentacles (VVG646#5) and could be *O. webbi*. It is believed that it may have disappeared and been replaced by the *Siboglinum* species since its first recording in 1965 by Brattegård (1966). Genetic studies of collected worms, their trophosome, sediment samples and pore water analyses for methane and sulphate will be performed at the Department of Bioscience, Aarhus University Denmark, TOC and isotope analyses will be performed at IFREMER, France, sulphide analyses at Stockholm University, foraminiferal faunas and their stable isotopes at UiT.

Table Collected worm samples

HH sample	sample # and name	purpose	preservation
HH2020-640	BC1#1 tube SI	isotopes	frozen
HH2020-640	BC1#2 post troph	DNA	frozen
HH2020-640	BC1#2 worm only	DNA	frozen
HH2020-640	BC1#2 troph DNA	DNA	frozen
HH2020-640	BC1#2 troph FISH	FISH	FISH protocol
HH2020-640	BC1#3 pot. post troph	DNA	frozen
HH2020-640	BC1#3 tentacle	DNA	frozen
HH2020-640	BC1#3 tube	DNA/isotopes	frozen
HH2020-640	BC1#3 eggs	morphology	4% formaldehyde
HH2020-640	BC1#4 worm and tube	DNA/isotopes	frozen
HH2020-640	BC1#5 tube with meat	isotopes	frozen
HH2020-640	BC1#5 troph DNA	DNA	frozen
HH2020-640	BC1#5 troph FISH	FISH	FISH protocol
HH2020-640	BC1#5 troph SI	isotopes	frozen
HH2020-640	BC1#5 worm only DNA	DNA/isotopes	frozen
HH2020-640	BC1#5 tube	DNA/isotopes	frozen
HH2020-640	BC1#6 worm SI	isotopes	frozen
HH2020-640	BC1#7 worm SI	isotopes	frozen
HH2020-643	BC2#1 tentacle and tube	DNA/isotopes	frozen
HH2020-643	BC2#1 worm only DNA	DNA	frozen
HH2020-643	BC2#1 poss. troph DNA	DNA	frozen
HH2020-643	BC2#1 tube	DNA/isotopes	frozen
HH2020-643	BC2#2	DNA/isotopes	frozen
HH2020-643	BC2#3	DNA/isotopes	frozen
HH2020-643	BC2#4	DNA/isotopes	frozen
HH2020-643	BC2#5	DNA	frozen
HH2020-646	VVG(grab)#1	DNA/isotopes	frozen
HH2020-646	VVG(grab)#2	DNA/isotopes	frozen
HH2020-646	VVG(grab)#3	DNA/isotopes	frozen
HH2020-646	VVG(grab)#4	DNA/isotopes	frozen
HH2020-646	VVG(grab)#5	DNA/isotopes	frozen
HH2020-646	VVG(grab)#6	DNA/isotopes	frozen
HH2020-646	VVG(grab)#7	DNA/isotopes	frozen

HH2020-646	VVG(grab)#8	DNA/isotopes	frozen
HH2020-646	VVG(grab)#9	DNA/isotopes	frozen

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