

Winogradsky Institute of Microbiology Russian Academy of Sciences (INMI)  
A.P.Karpinsky Russian Geological Research Institute (VSEGEI)

Cruise Report (30.06-03.07.2009)

Research vessel: “Ladoga”, 90 t, 1 winch, crew/scientist – 2/6 (Fig.1).

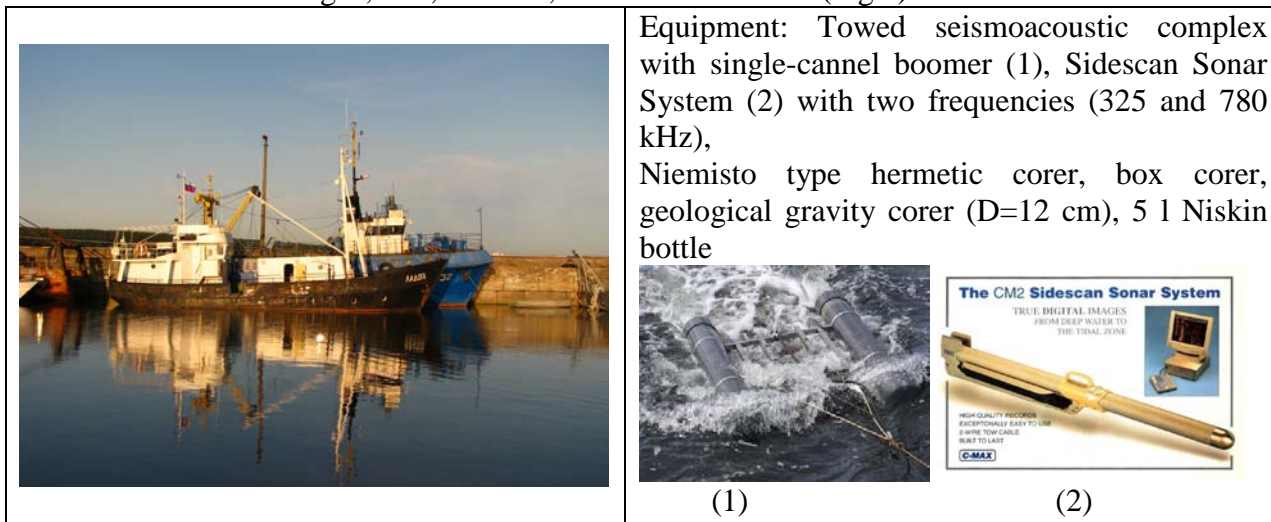


Fig. R/V “Ladoga”

**Main tasks of the July 2009 cruise.**

- Water and sediment sampling in the “pockmark” area (unusual geomorphological structure resembling pockmarks);
- Search for similar structures in this area using the seismoacoustic complex;
- Search for gas-saturated deposits in the Vyborg Bay using a Sidescan Sonar System;
- Near-bottom water and sediment sampling at the stations with pockmarks and gas-saturated surface sediments for microbiological and biogeochemical investigations.

**Scientific staff of the cruise:**

1. Dr. N.V.Pimenov (INMI), cruise leader – microbial biogeochemistry, isotopic geochemistry.
2. Dr. A.G. Grigorjev (VSEGEI), geochemist – collection of geological samples, description of the bottom sediments.
3. Dr. V.A. Gaimoyda (VSEGEI), geologist – collection of geological samples, description of the bottom sediments.
4. Dr. Yu.P. Kropachev (VSEGEI), geophysicist – operator of the geophysical equipment.
5. B.V.Stepanov (VSEGEI), electronics engineer, maintenance of the geophysical equipment.
6. T.A.Kanapatsky (INMI) – PhD student, microbial biogeochemistry

**Preliminary results.**

The map of the polygon, including the route and sampling sites, is presented on Fig. 2; the sampling sites' coordinates are presented in Table 1.

The complex of geophysical investigations included continuous seismoacoustic profiling (CSP) and side-looking hydrolocation (SLH). The range of geophysical investigations was 8 and 9 km of profiling by SLH and CSP, respectively.

Within the framework of geologo-geochemical research, 2 stations at pockmarks (crater-like structures), 2 stations for gas-saturated sediments, and 1 background station were investigated. At each station, surface sediments were sampled with a clamshell scoop; undisturbed surface

sediments (0–60 cm) and near-bottom water were sampled with a Niemisto type hermetic corer; geological gravity corer was used to sample the bottom sediments along the geological section up to 3 m long.

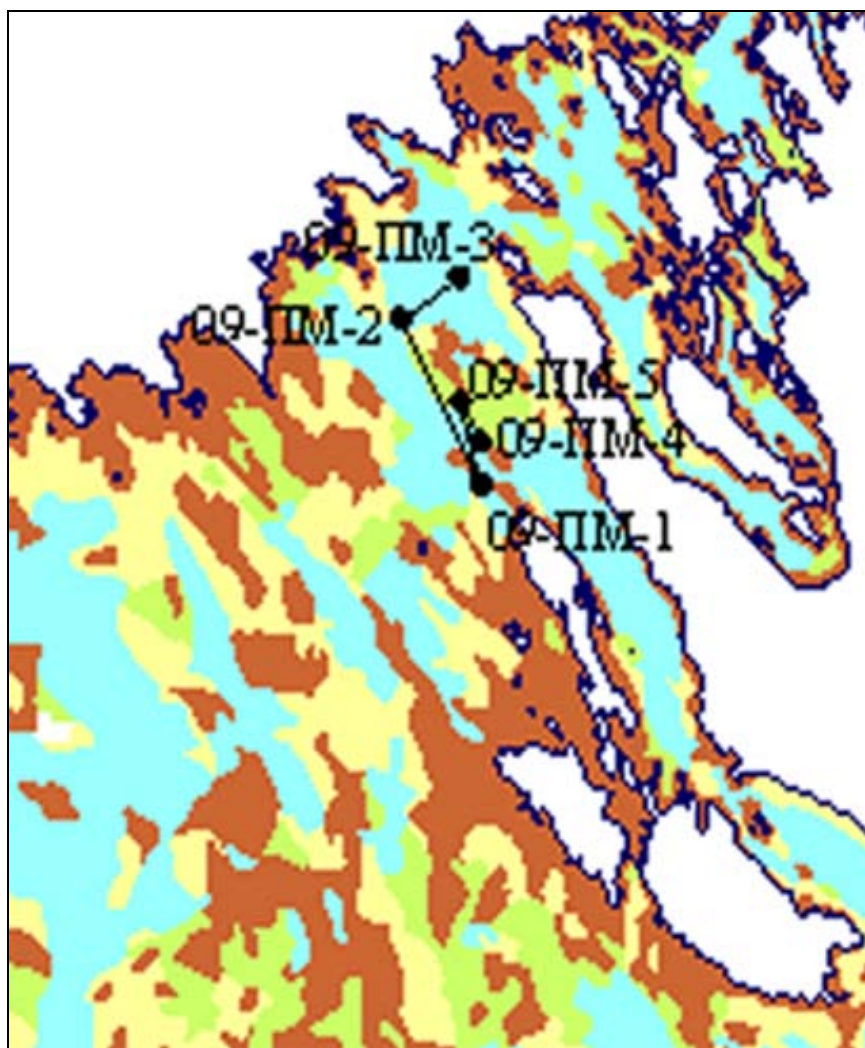


Fig. 2 . Map of the polygon, including the route and sampling sites

Table 1.

Station no.	Sampling	Longitude, degrees	Latitude, degrees
09-PM-1	Crater-like structures/Pockmarks?	28,4168	60,48343
09-PM-2	Gas-saturated sediments	28,35024	60,55024
09-PM-3	Gas-saturated sediments	28,40014	60,56694
09-PM-4	Crater-like structures/Pockmarks?	28,41672	60,50026
09-PM-5	Background	28,40015	60,51693

The results of geophysical research are presented on Figs. 3–8.

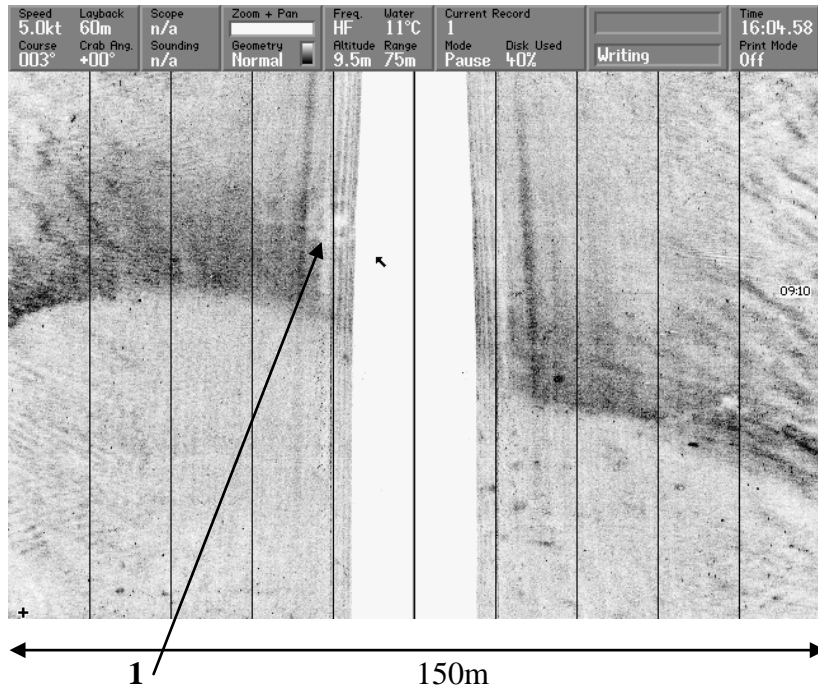


Fig. 3. A pockmark (1), 12–14 m in diameter, located at the lower edge of the slope. Coordinates: 60°30.925N 28°25.202E, Gulf of Finland. Side-looking hydrolocation. Range HF75; frequency, 325 kHz. Sampling station 09-PM-4.

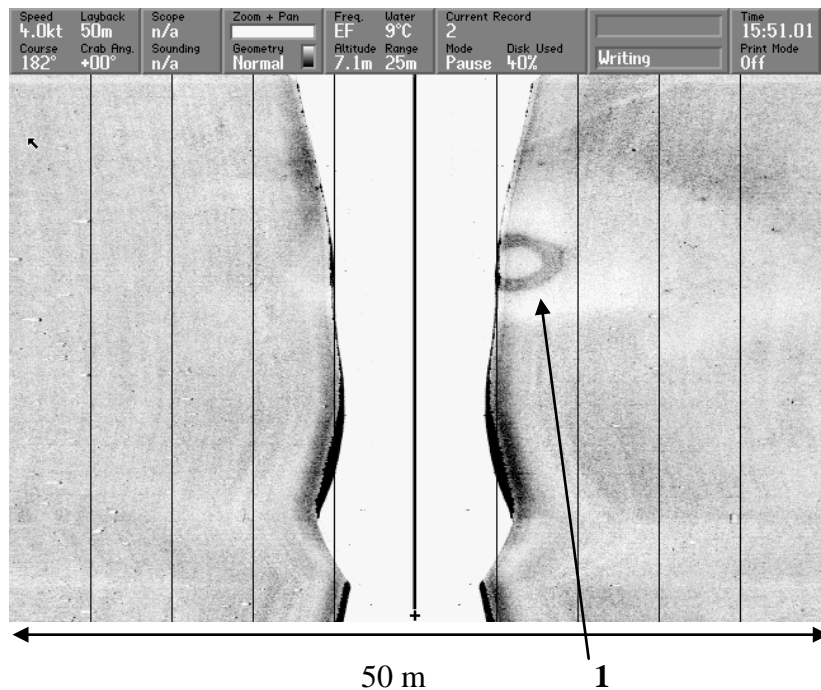


Fig. 4. A pockmark (1), 5–7 m in diameter, located at the lower edge of the slope. Coordinates: 60°29.335N 28°25.470E, Gulf of Finland. Side-looking hydrolocation. Range EF25; frequency, 780 kHz. Sampling station 09-PM-1.

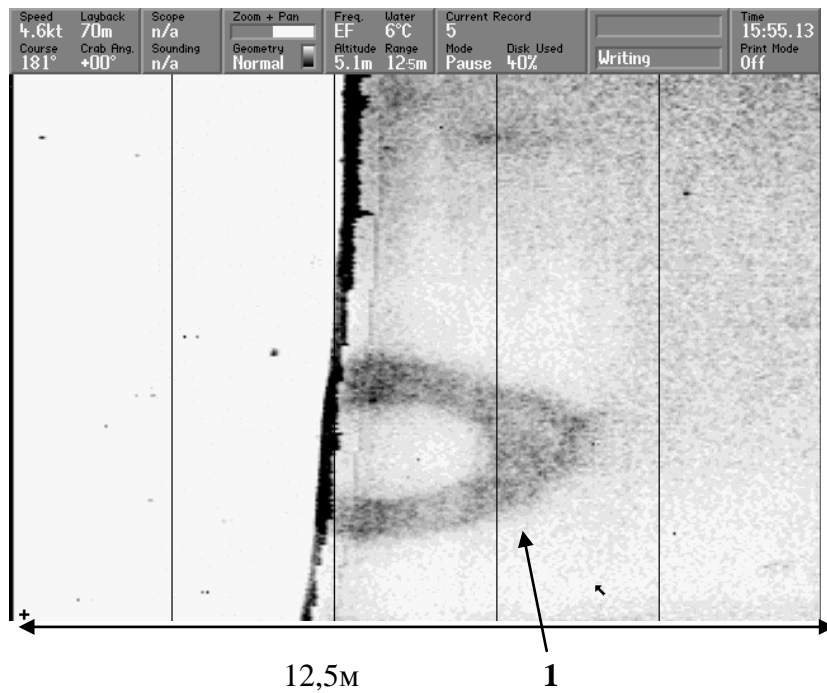


Fig. 5. Detailed image of a pockmark (1), 5–7 m in diameter, located at the lower edge of the slope. Coordinates: 60°29.335N 28°25.470E, Gulf of Finland. Side-looking hydrolocation. Range EF12.5; frequency, 780 kHz. Sampling station 09-PM-1.

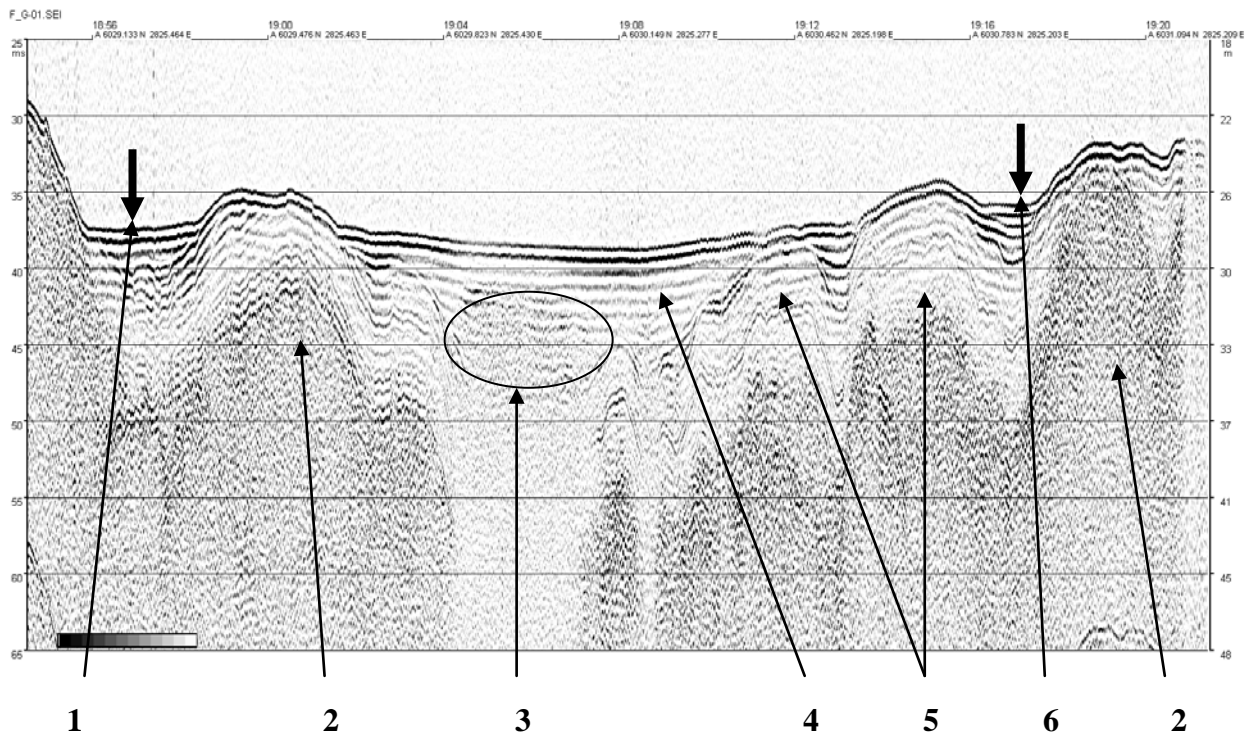


Fig. 6. A seismoacoustic profile demonstrating locations of pockmarks and gas-saturated sediments. Gulf of Finland.  
**1**, pockmark location (60°29.335N 28°25.470E), sampling station 09-PM-1; **2**, glacial complex; **3**, zone of gas-saturated precipitates (sediments); **4**, modern sediments; **5**, complex of over-ribbon and ribbon clays; **6**, pockmark location (60°30.925N 28°25.202E), sampling station 09-PM-4.

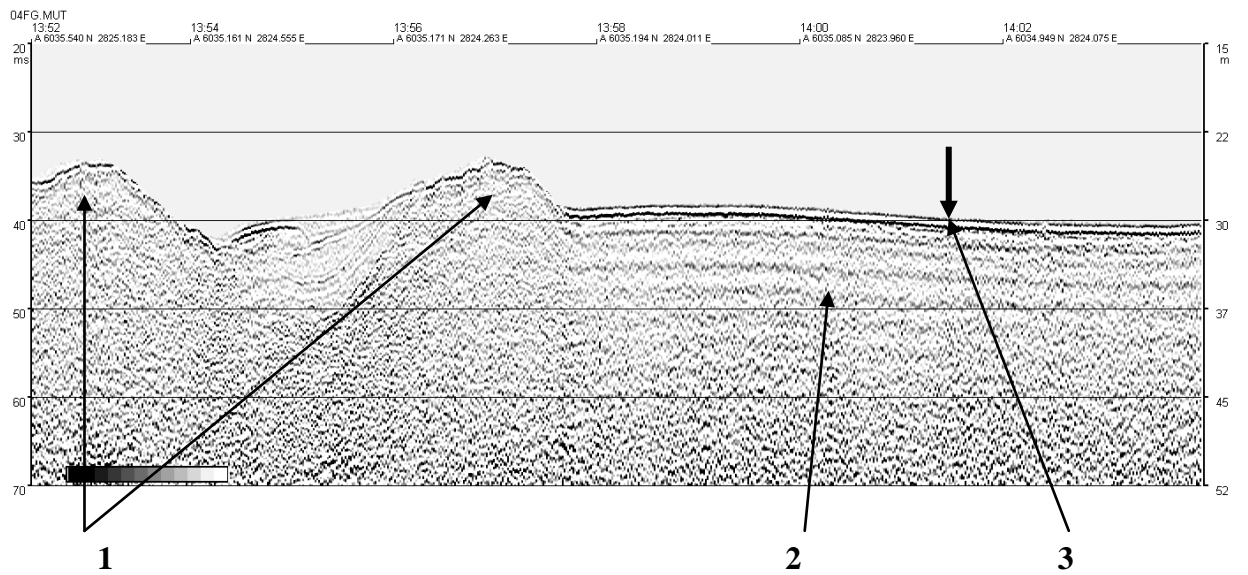


Fig. 7. A seismoacoustic profile of a zone of gas-saturated sediments. Gulf of Finland. **1**, glacial complex; **2**, zone of gas-saturated precipitates (sediments); **3**, location of the 09-PM-3 sampling station (60°34.970N 28°24.050E).

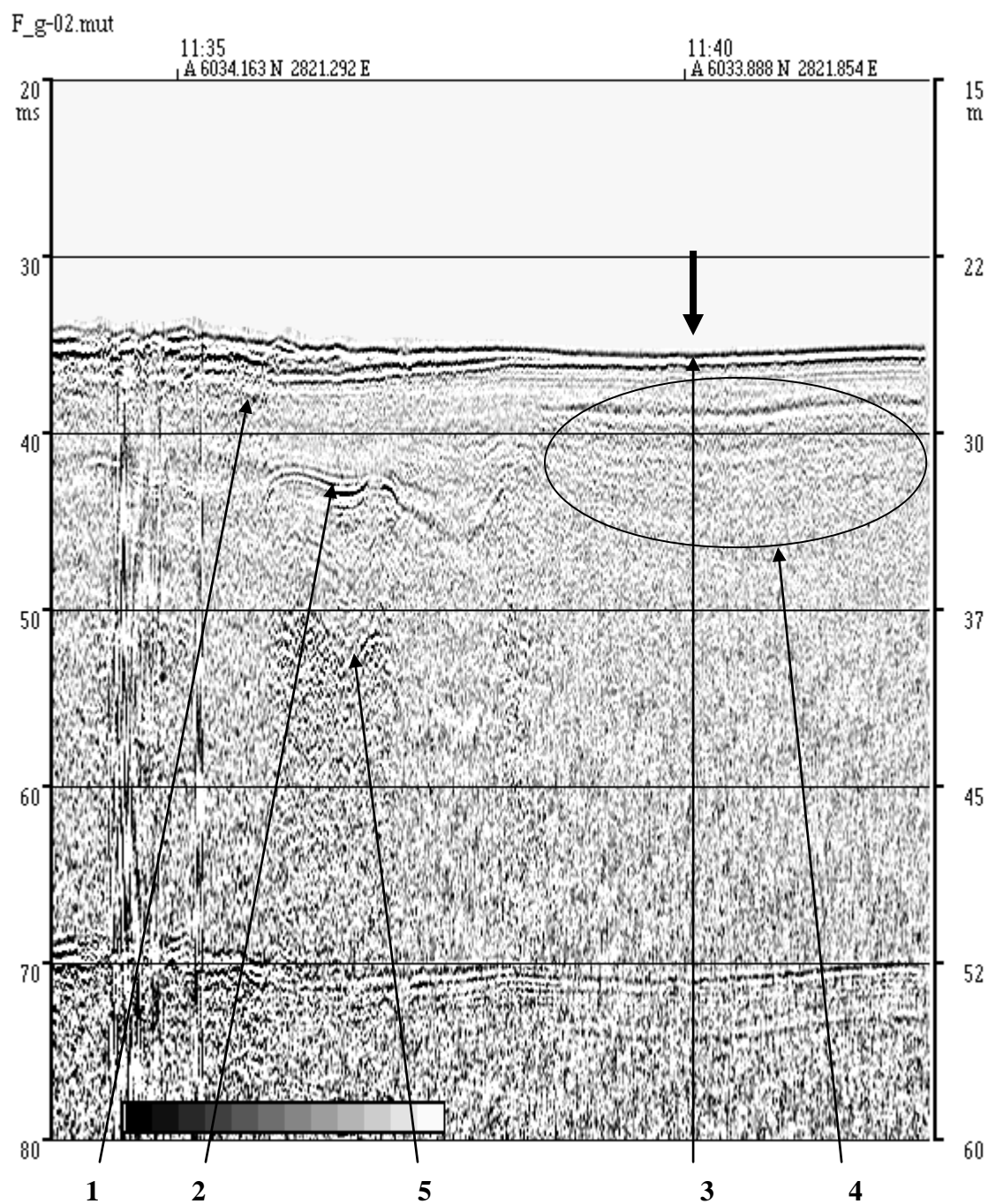


Fig. 8. A seismoacoustic profile of a zone of gas-saturated sediments. Gulf of Finland. **1**, modern sediments; **2**, top of the complex of over-ribbon and ribbon clays; **3**, location of the 09-PM-2 sampling station (60°33.880N 28°21.863E); **4**, zone of gas-saturated precipitates (sediments); **5**, top of the glacial complex.

Sampling of the bottom sediments and near-bottom water for microbiological, biogeochemical, and isotopic geochemical investigation was carried out at three stations, PM-1, PM-2, and PM-3.

Immediately on board the ship, samples were taken for the following analyses:

1. Determination of methane content;
2. Determination of alkalinity and sulfate content in pore water and near-bottom water;

3. Determination of the rates of microbial methanogenesis, methane oxidation, and sulfate reduction;
4. Determination of stable-isotopic ( $\delta^{13}\text{C}$ ) composition of methane, dissolved mineral carbon, and organic matter of the sediments;
5. Determination of total microbial numbers in the near-bottom water and in the upper sediment layer.

#### **Determination of methane content**

Methane content in the samples of water and sediments was determined by the headspace analysis method (Kvenvolden and Redden 1980). Water samples were distributed into 30-ml glass vials supplemented with KOH to suppress microbial processes. A constant water volume was then removed, and the vials were immediately sealed with gas-tight butyl rubber stoppers. Sediment samples were collected with a cut-off 2-ml plastic syringe, placed into penicillin vials, and filled with degassed water. After removal of the standard volume of water, the vials were sealed with butyl rubber stoppers.

Methane content in the gas phase was determined in the laboratory on a Kristall 2000 gas chromatograph (Russia) equipped with the flame ionization detector.

#### **Determination of alkalinity and sulfate content in near-bottom and pore water**

Pore water was obtained by centrifugation of the sediments at 8000 rpm for 10 min in a TsUM-1 centrifuge (Russia). Alkalinity was determined by standard titration. Sulfate content in pore water was determined on a «Staier» ion chromatograph (Russia).

#### **Determination of the rates of microbial processes**

The rates of methane oxidation, methanogenesis, and sulfate reduction in the sediments were determined by the radioisotope method (Table 1).

Table 1. Radioactive compounds used for determination of the rates of microbial processes

Process	Substrate	Amount of labeled compound per sample, $\mu\text{Ci}$	Fixative
CH <sub>4</sub> oxidation	<sup>14</sup> CH <sub>4</sub>	2	2N KOH
CH <sub>4</sub> formation	NaH <sup>14</sup> CO <sub>3</sub>	20	2N KOH
	<sup>14</sup> CH <sub>3</sub> COONa	10	2N KOH
Sulfate reduction	Na <sub>2</sub> <sup>35</sup> SO <sub>4</sub>	10	2N KOH

Bottom sediment samples were incubated in cut-off 5-ml plastic syringes. The sediment (3 ml) was placed into a syringe, sealed with gas-tight rubber stoppers, supplemented with the

relevant labeled substrate, and incubated at 4°C for 1–3 days. After incubation, the samples were fixed with 2N KOH and treated as described [Pimenov et al, Microbiologia 2008, 77(5), 651-659 in Russian]. Radioactivity was determined on a Rackbeta liquid scintillation counter.

### **Determination of the stable isotopic ( $\delta^{13}\text{C}$ ) composition of methane, dissolved mineral carbon, and organic matter of the sediments**

For mass spectrometric analysis, methane from the sediments was collected as follows. A 250-ml glass vial was half-filled with the sediment and the concentrated salt solution was added to 230 ml; the vial was then sealed hermetically with a rubber stopper and shaken vigorously. In the laboratory, the gas phase was collected with a syringe by methane substitution with the salt solution; the gas was stored above the salt solution. The  $\delta^{13}\text{C}$  content of methane was determined on a TRACE GC gas chromatograph (Germany) combined with a Delta plus mass spectrometer (Germany).

Mass spectral measurement of  $\delta^{13}\text{C}$  (‰), the value characterizing the isotopic composition of pore water mineral carbon and the carbon of organic matter in the sediments, was carried out with gaseous carbon dioxide ( $\text{CO}_2$ ) as the working gas. Organic matter was oxidized to  $\text{CO}_2$  by high-temperature incineration (560 °C) in the presence of copper oxide as a catalyst. Mineral carbon ( $\text{NaHCO}_3$ ) was converted to  $\text{BaCO}_3$ ;  $\text{CO}_2$  was then obtained by fusion of barium carbonate with tin salts at 560 °C. The  $\delta^{13}\text{C}$  value, a relative characteristics of carbon isotopic composition, was determined on a Delta Plus isotopic mass spectrometer (Thermo Electron Corporation, Germany) and calculated from the known equation:

$$\delta^{13}\text{C} = \left( \frac{[^{13}\text{C}]/[^{12}\text{C}]_{\text{sample}}}{[^{13}\text{C}]/[^{12}\text{C}]_{\text{st}}} - 1 \right) \cdot 1000\text{‰},$$

where  $([^{13}\text{C}]/[^{12}\text{C}]_{\text{sample}})$  and  $([^{13}\text{C}]/[^{12}\text{C}]_{\text{st}})$  are the ratios of  $^{12}\text{C}$  and  $^{13}\text{C}$  abundance in the sample and standard, respectively. The international PDB standard was used, with the  $[^{13}\text{C}]/[^{12}\text{C}]$  ratio of 0,001172. The error of  $\delta^{13}\text{C}$  determination did not exceed  $\pm 0.1$  ‰.