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Hydrothermal versus microbial MEthane release From very shallow coastal systems: can differently sourced emISsions direcTly escape intO the atmosphere? (MEFISTO)

Deliverable 3. Pre-surveys in Southern Tyrrhenian Sea and Northern Adriatic Sea - *Final technical report* -

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Table of Content

1. SUMMARY

MEFISTO pre-surveys of hydrothermal vent area off the island of Panarea (Aeolian Archipelago, Southern Tyrrhenian Sea) and the most relevant bio-concretionned rocky buildups disseminated over the Northern Adriatic Sea (also known as “grébeni” “trezze” or “tegnue”) were carried out by OGS and INGV operational units in March 21-23, 2024 and in May 13, 2024, respectively. The surveys had the principal objective of detecting in each study area sites with different methane (CH_4) emission magnitudes, corresponding to the stations that would have been sampled during the successive field campaigns.

The survey, functional to the implementation of the experimental design, were both originally intended as visual investigations performed by mean of a remotely operated vehicle (ROV). However, while in the Northern Adriatic Sea the visual identification of the emission sites and the photogrammetric analyses of the seafloor was actually carried out using a ROV, in the Southern Tyrrhenian Sea GoPro cameras mounted on divers’ mask were preferred. This change to the original approved plan was due to logistical and scientific reasons. In fact, given the complexity of the rocky seabed in several of the areas investigated around the island and the generally shallow water depth (usually no more than 10 metres), the MEFISTO research team chose to simplify and speed up the activity by entrusting the mapping of the hydrothermal vent area and the identification of the emission sites to the divers of the INGV operational unit. It should also be noted that in the Aeolian Archipelago the leaking gases generally have an extremely high CO_2 content (95-98%), which means that the mere identification of bubble streams does not per se indicate CH_4 emissions. Therefore, the visual survey was implemented, in each investigated site, by the deployment along the water column of a multi-parametric probe equipped with a CH_4 sensor and by the measurement of the CH_4 efflux at the water-air interface through a floating accumulation chamber. Visualising all this data in real time allowed the target areas to be selected quickly and correctly, which in turn enabled the MEFISTO research team to carry out the sampling directly during the survey and thus complete the first campaign in the Southern Tyrrhenian Sea earlier than originally expected.

A total of 10 sites (8 in the Southern Tyrrhenian Sea and 2 in the Northern Adriatic Sea) were investigated during the two campaigns.

This report summarizes the results of the measurements with the accumulation chamber and the multiparametric probe in the hydrothermal vent area off the island of Panarea, as well as the most informative images taken by divers and ROV during the two campaigns, and includes the sampling plans developed for the two study areas.

2. MEFISTO RESEARCH PROGRAMME AND OBJECTIVES

2.1 General scientific background

Methane (CH_4), accounting for about 30% of the ongoing atmospheric warming, is today recognized as one of the most powerful greenhouse gases (GHGs), being an even stronger absorber of Earth's emitted thermal infrared radiation than carbon dioxide (Simson, 2021). Atmospheric CH_4 concentrations, which increased by only 700 ppb during the millennium before industrialization, are now more than 2.6 times above estimated pre-industrial equilibrium levels, reaching 1857 ppb in 2018 (Saunois et al., 2020). Such an increase is largely the result of anthropogenic emissions related to human activities, including agriculture, production and utilisation of fossil fuel and waste management practices (Ciais et al., 2014). Nevertheless, since the lifetime of CH_4 in the atmosphere barely exceeds 10 years (Prather et al., 2012), the concentrations and therefore the radiative forcing of this potent GHG are thought to be scaled down in a few decades by just stabilising or reducing the anthropogenic emissions (Shindell et al., 2012). Such an approach is considered an effective and realistic way to rapidly mitigate climate change, making it possible to limit the global temperature rise to 1.5-2.0 °C, as targeted by the Paris Agreement (Nisbet et al., 2019).
In order to verify future emission reductions, a precise quantification of the global CH_4 budget is actually needed but, according to the most recent modelling, important uncertainties still affect the calculations, since global emissions were estimated to range between 576 Tg CH_4 / yr and 737 Tg CH_4 / yr (Saunois et al., 2020). The most important source of uncertainty is attributable to natural emissions, accounting for 40% of the global CH4 budget, 1-13% of which is due to the oceans (Kirschke et al., 2013; Saunois et al., 2016). However, while the open ocean CH_4 emissions are relatively well constrained and are driven by variations that are steadily linked to the organic matter cycling, the global marine flux appears to be mostly influenced by shallow near-shore environments (0-50 mbsl), where CH_4 released from the seafloor can escape to the atmosphere before oxidation (Weber et al., 2019). Here, many forcings can severely affect the amount of CH_4 that reaches the air-sea interface, above all water depth, currents, tides, temperature, water column stratification and microbial methane oxidation (Boles and Clark, 2001; Jordan et al., 2022; Mc Ginnis et al., 2006), but due to limited and scarse data, the actual contribution of coastal areas to atmospheric CH_4 is still quite uncertain (Weber et al., 2019).

2.2 The MEFISTO project

The MEFISTO project aims to reduce the abovementioned uncertainties in the estimates of the natural CH_4 fluxes by providing new data on the emissions from shallow near-shore marine environments, where, rapidly bypassing the water column by bubble transport, this powerful GHG can be directly released into the atmosphere (Weber et al., 2019). The lack of data on CH_4 fluxes in coastal areas has significant implications for the accurate calculation of the atmospheric budget for this gas and the accuracy of this estimate is crucial for verifying potential emission reductions associated with the adoption of effective climate change mitigation strategies. The MEFISTO project, combining classical physical, chemical, and molecular methods with innovative hydroacoustic approaches, will help to fill this knowledge gap by focusing on the study of two Italian shallow coastal areas: a seepage zone recently identified in the Gulf of Trieste, centred on the Bardelli outcrop (Northern Adriatic Sea) and the hydrothermal vent area off the Panarea Island (Aeolian Archipelago, Southern Tyrrhenian Sea).

The project has three main objectives: 1) to ascertain possible differences in the water column degassing pathways and fates between microbially sourced and volcanic-related CH_4 emissions; 2) to assess the main physical and biological forcings (i.e., water depth, currents, tides, temperature, water column stratification and microbial community structure and composition) favouring or preventing the release of CH_4 to the atmosphere from the two investigated marine shallow areas; 3) to eventually develop local emission estimates that will contribute to the refinement of the global atmospheric CH_4 budget.

2.3 Volcanic-related CH_4 emissions: the hydrothermal vent system off the Panarea Island (Aeolian Archipelago, Southern Tyrrhenian Sea)

The Aeolian Archipelago is the expression of the volcanism migrating south-eastward from the Central and Southern Tyrrhenian Sea during the Lower Pleistocene. Submarine emissions of CO_2-rich gases and thermal water outflow is documented in this area since the Roman Age (De Astis et al., 2003). The archipelago includes the active volcanoes of Stromboli, Vulcano, Lipari and Salina. Panarea is the smallest among the Aeolian Islands and represents the subaerial portion of a 2000 m high and 20 km wide submarine stratovolcano, a dormant edifice with a known age range of ca. 150-200 ka (Anzidei et al., 2005; Calanchi et al., 1999; Dolfi et al., 2007).

As a matter of fact, Panarea volcano was generally considered extinct until November 3, 2002, when a low-energy submarine explosion, due to the injection of magmatic fluids in the deep geothermal body (Caracusi et al., 2005), caused an intense and long-lasting submarine gas eruption in the ~2.3 km^2 area rimmed by the islets of Lisca Bianca, Bottaro, Lisca Nera, Dattilo, and Panarelli, about 2.5 km east of Panarea, leading to the opening of a submarine crater 20x8 m wide and 9 m deep (Anzidei and Esposito, 2003; Anzidei et al. 2005; Esposito et al. 2006; Figure 2.1).

The explosion released a huge amount of CO_2 that provoked a drop of seawater pH to a value of 5.6-5.7 and the “degassing crisis” lasted several months (Caracusi et al., 2005; Romano et al., 2019).

Several studies conducted in the islet area indicated that, with about 98% CO_2, the composition of the persistent gaseous emissions is quite stable (Beaubien et al., 2014; Caliro et al., 2004; Espa et al., 2010; Marchini et al., 2021; Sani et al., 2024). Nevertheless, CH_4 concentrations, showed values up to 900 ppm, are generally not negligible (Romano et al., 2019), making the islet area of Panarea suitable for the MEFISTO project purposes.

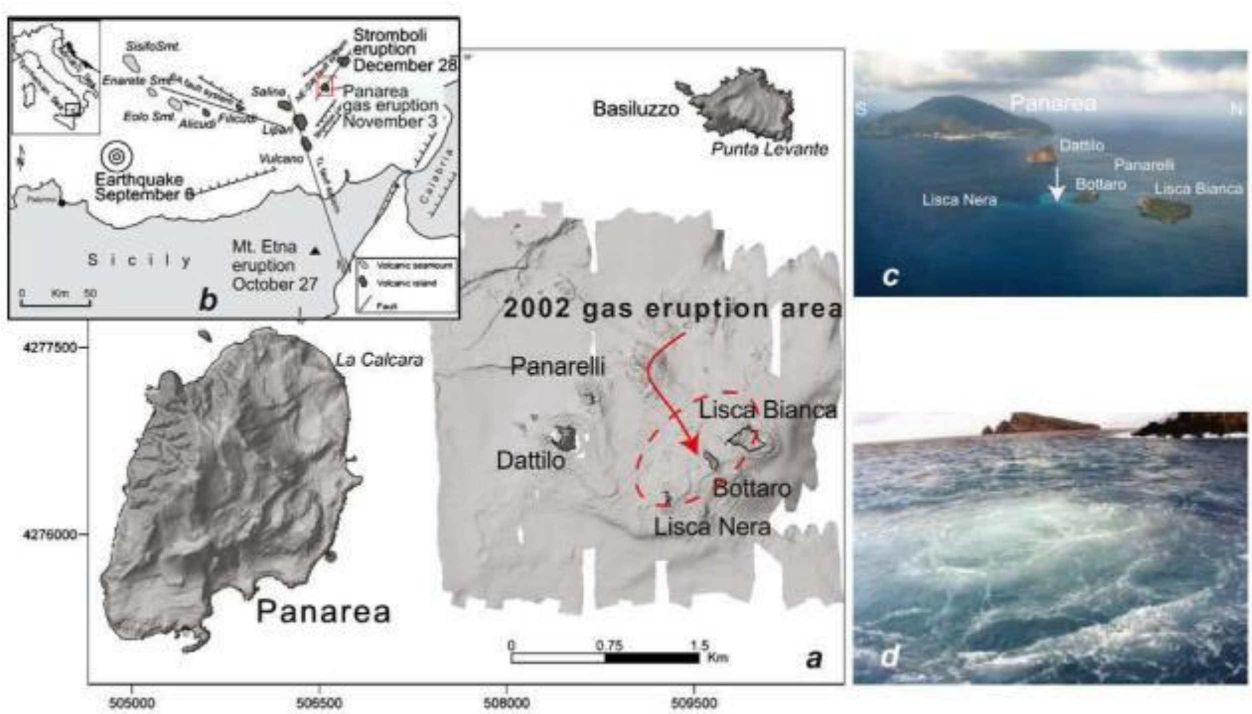

Figure 2.1. a) Location of the 2002 gas eruption. b) Structural sketch map of the Southern Tyrrhenian Sea and Aeolian Islands. c) Aerial view of Panarea Island and islet area with indication of major emission point. d) Gas bubbling in sea surface above the Bottaro crater. From Esposito et al. (2010).

2.4 Grébeni/Trezze/Tegnúe: methane-derived deposits in the Northern Adriatic Sea

The Northern Adriatic Sea is generally characterized by a rather monotonous seabed, consisting mostly of mobile silty-sandy sediments. Nevertheless, numerous submarine rocky substrates of biogenic concretions, called “grébeni” or “trezze” in the Gulf of Trieste and “tegnúe” off the coast of Venice, are irregularly scattered over the soft bottom of this Adriatic Sea sub-basin (Casellato and Stefanon, 2008; Ingrosso et al., 2018; Lipej et al., 2016). Their size ranges from a single small block of 1 m^2 up to a few 1000 m^2, and their height rarely exceeds 4 m. These rocky substrates are suitable for the settlement and development of specific floristic and faunistic assemblages, that are favoured by the accumulation of calcareous incrusting algae, creating complex biostructures commonly referred to as “coralligenous” and giving the colourful underwater landscape a typical appearance (Lipej et al., 2016; Turicchia et al., 2022).

For this reason, since 2015, a limited number of biogenic outcrops in the Gulf of Trieste have been legally protected under the European Habitats Directive (92/43/EEC) and included in the European Natura 2000 network as Sites of Community Importance (Decision EU 2015/69 of December 3, 2014) (Bettoso et al., 2023). These sites are generally called “IT3330009 - Trezze San Pietro e Bardelli” (Figure 2.2).

Up to 4000 outcrops are currently recorded in the Northern Adriatic Sea (Figure 2.1), mostly off the Venetian coast, while in the Gulf of Trieste about 250 have been mapped so far, most of them off the lagoon of Marano and Grado, at a distance of 3 to 10 nautical miles (nm) from the coast and at a depth ranging between 13 and 25 m (Caressa et al., 2001).

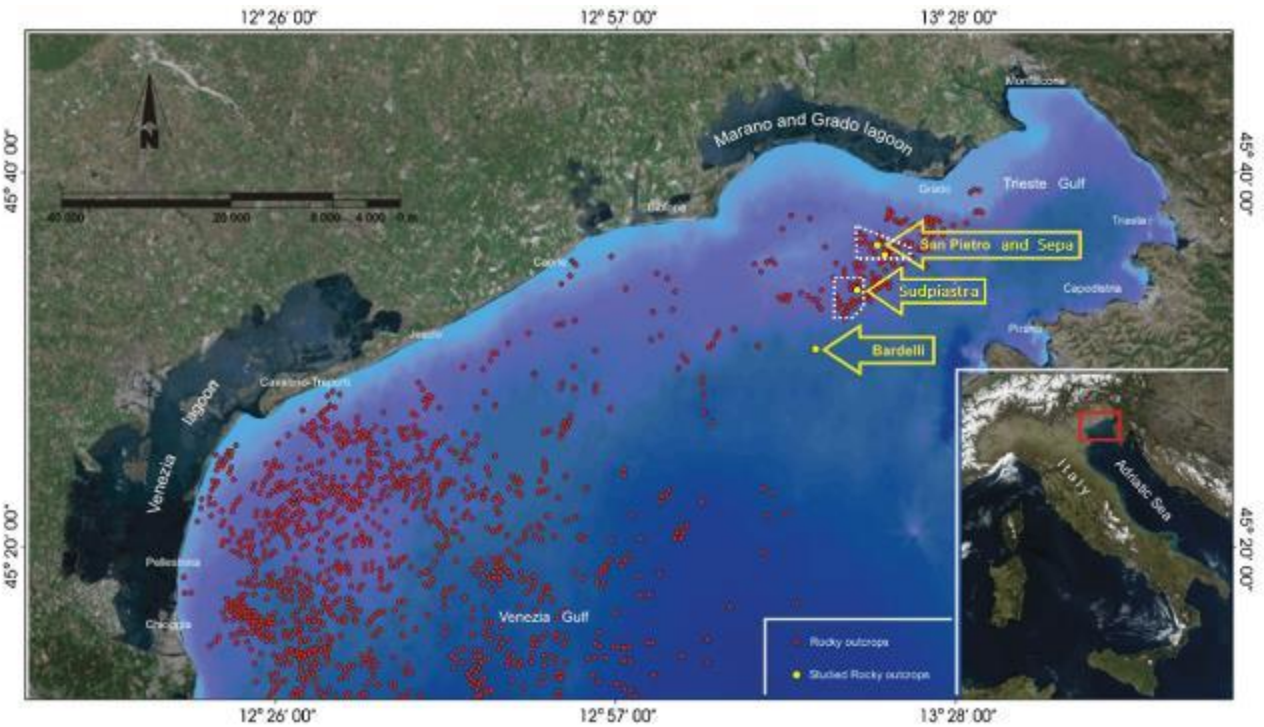

Figure 2.2. Location map of the northern Adriatic Sea rock outcrops (red dots) where the position of trezze San Pietro, Sepa, Sudpiastra and Bardelli is highlighted. Sites of Community Importance in the Gulf of Trieste are are indicated by white dashed polygons (mod. from Gordini et al., 2012).

These coralligenous buildups are often associated with gas seeps, thus leading to interpret such deposits as methane-derived carbonates. In fact, several gas fields were discovered and exploited during the 1960s in the Northern Adriatic Sea, where, particularly in the averagely 22 m deep Gulf of Trieste, gas seeps can produce up to 20 m-high gas flares and intermittent bubbling within the water column (Donda et al., 2019; Gordini et al., 2012). These seep gases, mainly composed of CH_4 and occurring both in deep and shallow Plio-Quaternary successions, are microbial in origin and mostly originate from laterally persistent Late Pleistocene peat layers, which are widely distributed throughout the Northern Adriatic Sea and represent the main source of organic matter feeding the gases (Donda et al., 2019).

2.5 Field research programs to accomplish MEFISTO objectives

The field research program in the hydrothermal vent area off the island of Panarea originally included the visual detection of bubble streams generated by leaking gases by mean of a remotely operated vehicle (ROV). However, the MEFISTO research team decided to carry out the survey using GoPro cameras mounted on divers' mask. This change to the original approved plan was due to logistical and scientific reasons. In fact, given the complexity of the rocky seabed in several of the areas investigated around the island and the generally shallow water depth (usually no more than 10 metres), the activity was simplified and accelerated up by entrusting the mapping of the hydrothermal vent area and the identification of the emission sites to the divers of the INGV operational unit. It should also be noted that, as previously mentioned, the gases leaking in this area have an extremely high CO_2 content (Beaubien et al., 2014; Caliro et al., 2004; Espa et al., 2010; Marchini et al., 2021; Sani et al., 2024), which means that the mere identification of bubble streams does not per se indicate CH_4 emissions.

Therefore, in each investigated site, the visual survey was conducted by the deployment along the water column of a multi-parametric probe equipped with a CH_4 sensor and by the measurement of the CH_4 efflux at the water-air interface through a floating accumulation chamber.

The evaluation of these preliminary data in real time allowed a quick and correct selection of the target areas, which in turn enabled the MEFISTO research team to carry out the sampling of each CH_4 emission site directly after its identification.

The new work plan included in each investigated site:

- gaseous emission visual detection and video recording and measurement of the CH_4 efflux at the water-air interface through a floating accumulation chamber (INGV scuba divers; Figure 2.3C);
- casting of the multi-parametric probe consisting of a RBRmaestro³ multi-channel logger (for conductivity, temperature, pressure, dissolved oxygen and pH detection), a CONTROS HydroC CO_2 sensor and a CONTROS HydroC CH_4 sensor (OGS personnel; Figure 2.3B);
- collection of hydrothermal fluid, pore-water and biofilm/sediment samples (INGV scuba divers; Figure 2.3A);
- deployment of Niskin bottles along the water column (OGS personnel);
- closure of Niskin bottles at discrete water depths above the emission site (INGV scuba divers; Figure 2.3A);
- recovery of Niskin bottles and collection of water samples for chemical and microbiological analyses (OGS and INGV personnel);
- deployment and recovery of hydrophones for passive hydroacoustic measurements (INGV scuba divers; Figure 2.3D).

A total of 8 stations were investigated during the campaign (Figures 2.4 and 2.5).

Two stations (hereafter named "Black Point Smoke" and "Black Point Bubbling") were sampled near the INGV meda-observatory system, in correspondence of a black-colored, sulfide-rich hydrothermal vent detected at 23 m depth in 2002 (Tassi et al., 2009).

Two stations (hereafter named “Hot/Cold1” and “Hot/Cold2”) were investigated in an area located NE of Panarea Island at 10–12 m depth and characterized by spots with very different temperatures at the distance of few meters from each other.

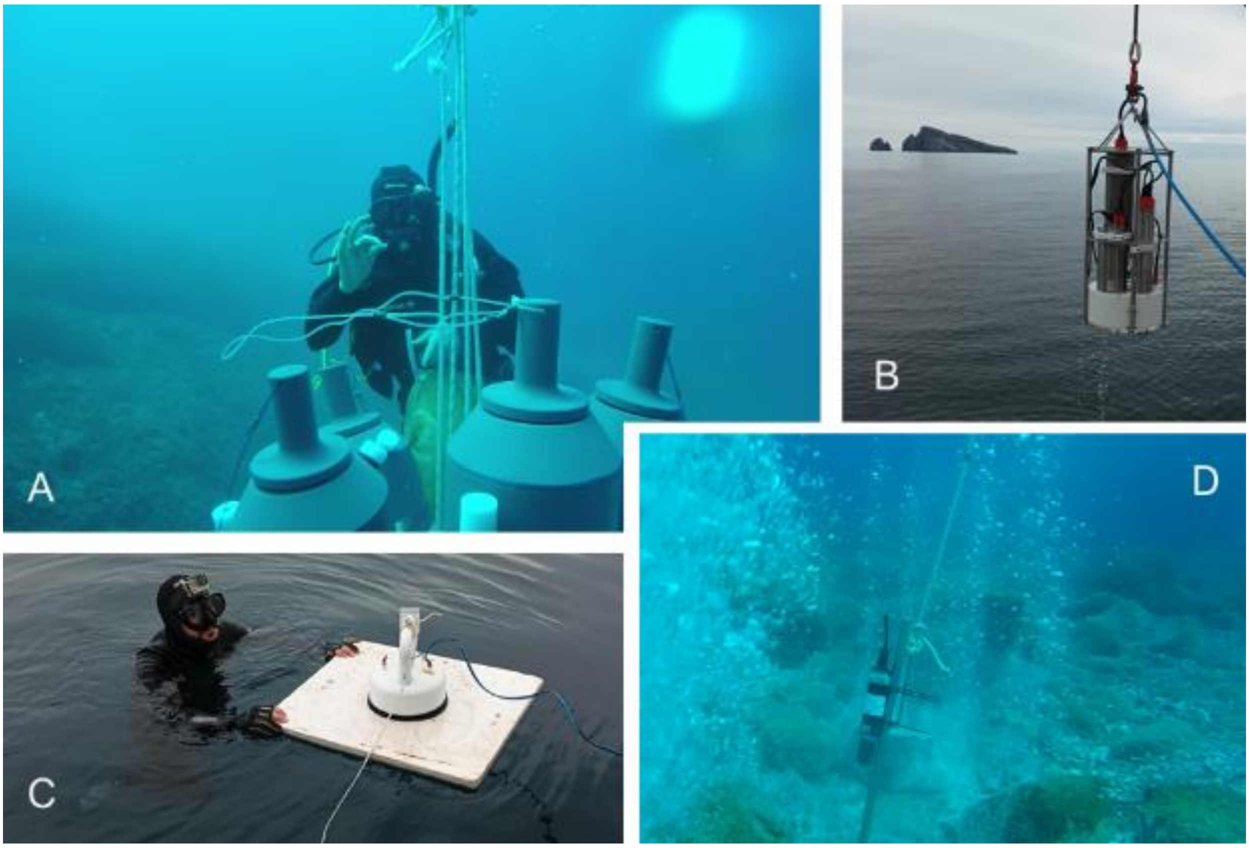

Figure 2.3. A) Collection of hydrothermal fluid, pore-water and biofilm/sediment samples (see scuba diver’s net mesh bag); B) Deployment of the multi-parametric probe; C) Gaseous emission visual detection and video recording (see GoPro camera mounted on diver mask) and measurement of the CH_4 efflux at the water-air interface through a floating accumulation chamber; D) Hydrophone for passive hydroacoustic measurements.

Unvegetated hot spots, characterized by CO2 emissions, temperatures up to 60 °C, and pH values ranging from 7 to 5.6, alternates here to cold spots featuring meadows of Posidonia oceanica seagrass, reduced venting activity, temperatures of about 26 °C and pH of about 7.9 (Di Bella et al., 2022).

One site (hereafter named “Zimmari”) located in a bay S of Panarea Island, where generally no emissions has been observed, was chosen as reference station.

Eventually, three stations (hereafter named “Bottaro Twins”, “Bottaro Single” and “Bottaro Downstream”) were sampled in the crater produced after the explosion of November 2002 and positioned a few tens of meters NW of Bottaro islet, where three particularly large vents occur (Anzidei et al., 2005; Capaccioni et al., 2005; Caracusi et al., 2005; Esposito et al., 2006).

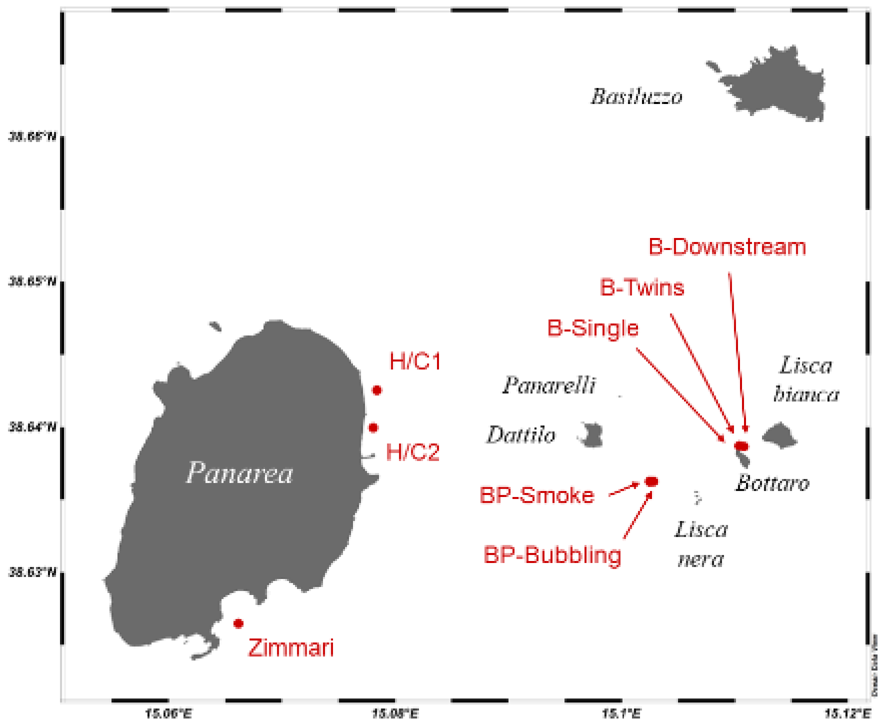

Figure 2.4. Location map of the study area displaying the investigated stations: Hot/Cold1 (H/C1), Hot/Cold2 (H/C2), Zimmari, Black point Smoke (BP-Smoke), Black Point Bubbling (BP-Bubbling) Bottaro Twins (B-Twins), Bottaro Single (B-Single) and Bottaro Downstream (B-Downstream).

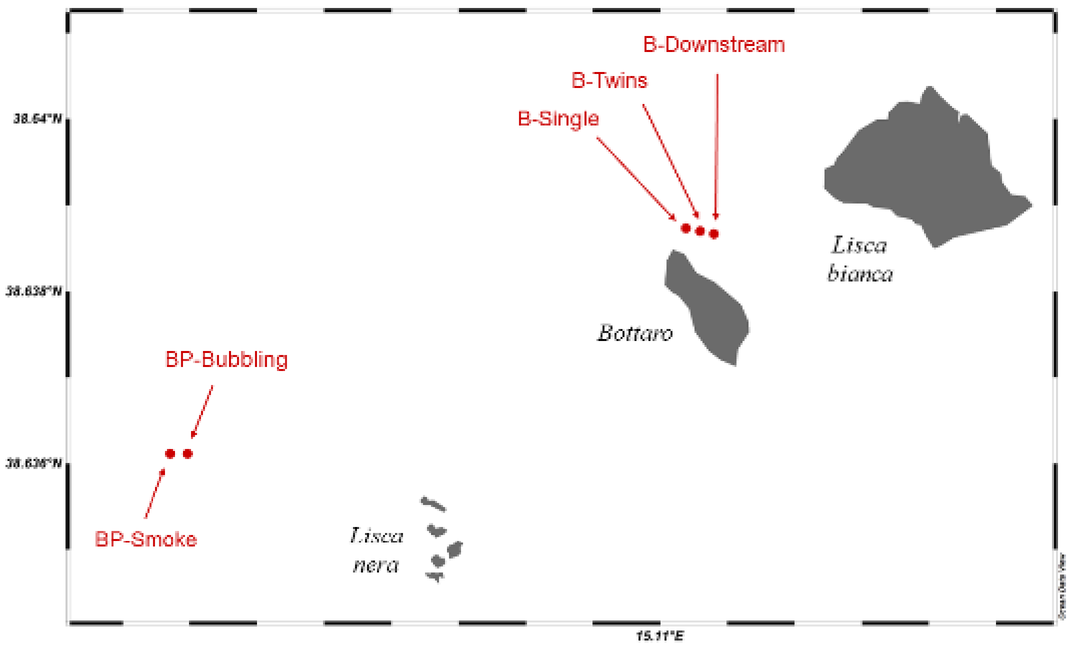

Figure 2.5. Detailed view of the stations in the Panarea islet area, showing the locations of Black point Smoke (BP-Smoke), Black Point Bubbling (BP-Bubbling) Bottaro Twins (B-Twins), Bottaro Single (B-Single) and Bottaro Downstream (B-Downstream).

The field research program of the trezze of the Northern Adriatic Sea included the visual detection of bubble streams generated by leaking gases by mean of a remotely operated vehicle (ROV). The survey was carried out by OGS operational unit in May 13, 2024 using a Blue Robotics BlueROV2 equipped with a 1080p low light camera and multiple 1500 lumen lights (Figure 2.6).

Figure 2.6. BlueROV2 by Blue Robotics.

The identification of the targeted outcrops (whose geographic position is well known) was supported by the employment of the vessel side-scan sonar and echosounder, which in turn gave also the opportunity to detect (before the ROV deployment) punctual gaseous emissions during the navigation above the rocky buildups.

Efforts focused on close visual inspection of the selected rock outcrops, particularly their edges, where gas emissions are most likely to be observed.

Two outcrops were investigated during the campaign, one off the Venetian coast, commonly referred to as “Bardelli”, and another (belonging to the so-called “Trezze San Pietro e Bardelli” site) located in the Gulf of Trieste and called “Sudpiastra” (Figure 2.2).

The Bardelli outcrop, covering an area of 250x90m, consists of two main groups separated by a flat sandy seafloor. The larger group, formed by smaller rounded shaped blocks separated by sandy to gravelly deposits, is 95x80m wide, while the second, composed of closely spaced rock slabs split by vertical fractures, is 55x38m wide (Gordini et al., 2012). At this site, geological and geophysical data at different scales of resolution indicated gas occurrences within the sedimentary succession, at the sea floor and in the water column (Donda et al., 2015; Donda et al., 2019; Gordini et al., 2012; Gordini et al., 2023), while geochemical analyses showed that the consisted mainly of methane (81-84%; Gordini et al., 2012; Donda et al., 2019).

“Sudpiastra” is a locally well-known but less studied outcrop (Bettoso et al., 2023), so that almost no data are available in terms of size, volume geometry and methane emissions. However, the vessel echosounder revealed potential gaseous emissions in correspondence of this study site, making it suitable for the MEFISTO project purposes.

3. RESULTS

Results of the CH_4 and CO_2 concentrations measured along the water column and at the air-sea interface in the hydrothermal vent area off the island of Panarea, as well as the most informative images taken by divers and ROV during the investigations of the10 investigated sites (8 in the Southern Tyrrhenian Sea and 2 in the Northern Adriatic Sea) are presented below.

3.1 Black Point sites

In “Black Point Smoke” station CH_4 concentrations showed a negative trend with depth along the water column, with higher values at the surface (4.60±0.09 ppm) and lower at the bottom (3.75±0.54 ppm). An opposite trend was observed for CO_2, with lower values at the surface (380.84±1.55 ppm) and higher at the bottom (428.75±0.54 ppm). Similar concentrations and trends of CH_4 (3.61±0.08 ppm in surface and 2.88±0.32 ppm at the bottom) and CO_2 (386.89±8.66 ppm in surface and 486.71±7.04 ppm at the bottom) were detected in “Black Point Bubbling” station.
Gas flux measurements at the air-sea interface were carried out at the “Black Point Bubbling” station. The CH_4 flux reached a value of 0.194 g m^{-2} d^{-1}, whereas a maximum CO_2 flux of 15.04 g m^{-2} d^{-1} was measured.

Figure 3.1. Biofilm collection in “Black Point Smoke” station. The black vent fumarole can be seen pouring out of the hole between the rocks.

Figure 3.2. Hydrophone recording the noise radiated by the gas bubble oscillating walls in “Black Point Bubbling” station.

3.2 Hot/Cold sites

In “Hot/Cold1” station both CH_4 (7.25±0.12 ppm) and CO_2 (502.83±1.12 ppm) showed quite homogeneous distributions along the water column. Similarly, CH_4 concentrations (4.34±0.12 ppm) poorly varied with depth in “Hot/Cold2” site, while CO_2 values, ranging between 426.24 ppm measured at the surface and 613.55 ppm detected at the bottom, showed a strong positive trend along the water column.

Figure 3.3. INGV diver collecting biofilm and sediment samples in “Hot/Cold1”.

Gas flux measurements were carried out both in “Hot/Cold1” and “Hot/Cold2” stations, but ideal gas concentrations inside the accumulation chamber were obtained only for CH_4 in “Hot/Cold1” station, where a maximum CH_4 flux of 0.103 g m^{-2} d^{-1} was measured.

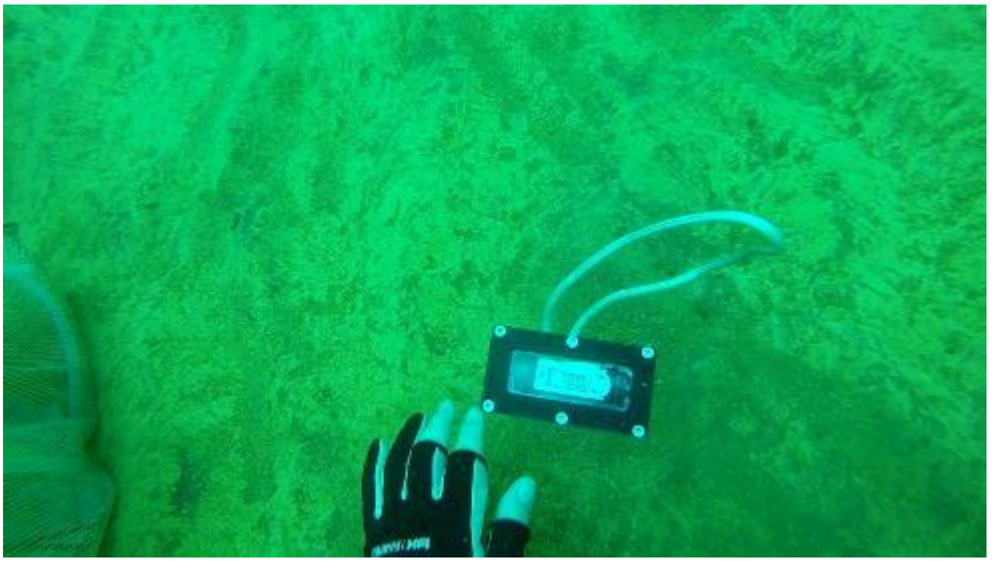

Figure 3.4. INGV diver measuring surface sediment temperature in a hot patch detected in “Hot/Cold1”. The digital thermometer display indicated 70.0 °C.

Figure 3.5. INGV diver carrying operating in “Hot/Cold1” station.

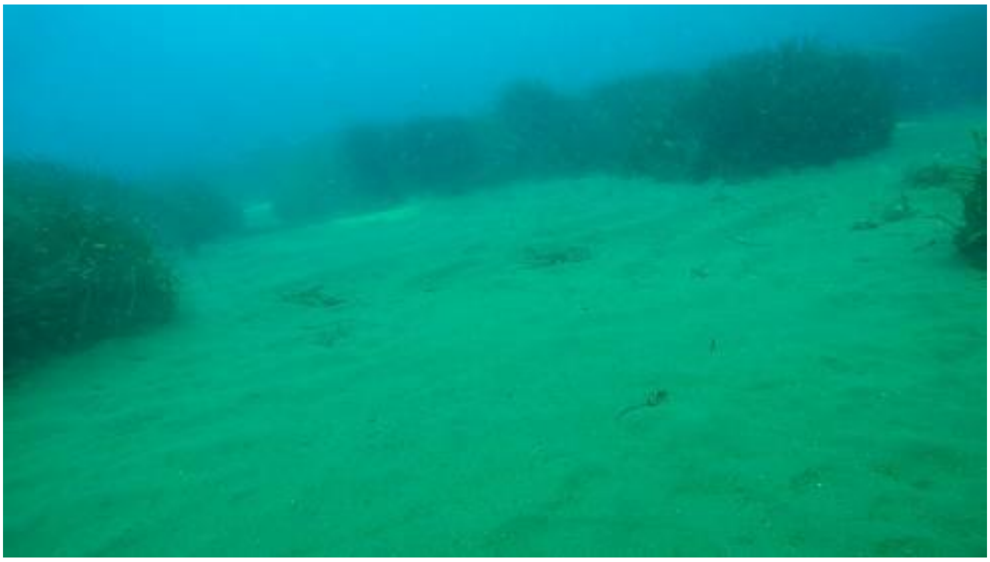

Figure 3.6. “Hot/Cold2” site.

3.3 Zimmari

A quite homogeneous distribution of CH_4 along the water column was observed in “Zimmari” station, in which an average value of 4.42±0.06 ppm was measured. CO_2 concentrations, ranging between 449.82 ppm and 463.52 ppm, showed a positive trend with depth along the water column, with lower values at the surface and higher at the bottom
Gas flux measurements at the air-sea interface were carried out also in this site but neither CH_4 or CO_2 reached ideal concentration values inside the accumulation chamber.

3.4 Bottaro sites

CH_4 concentrations showed a quite homogeneous distribution along the water column of “Bottaro Twins” station, with a mean value of 2.45±0.09 ppm. The comparison between bottom and surface waters indicated that CH_4 concentrations detected at 7 m b.s.l (2.29±0.63 ppm) were on average slightly lower than those observed at 1 m b.s.l. (2.65±0.22 ppm). CO_2 concentrations, ranging between 5675.53 ppm and 6122.94 ppm, had a negative trend with depth along the water column, with higher values at the surface and lower at the bottom.

Figure 3.7. Hydrophone recording the noise radiated by the gas bubble oscillating walls in "Bottaro Twins" station.

A quite homogeneous distribution of CH_4 along the water column was also observed in "Bottaro Single" and "Bottaro Downstream" stations (2.14±0.10 ppm and 2.04±0.12 ppm, respectively), while CO_2 concentrations, ranging between 617.89 ppm and 642.96 ppm in "Bottaro Single" and between 440.56 ppm and 442.61 ppm in "Bottaro Downstream", were one order of magnitude lower than those measured in "Bottaro Twins" but showed the same negative trend with depth.

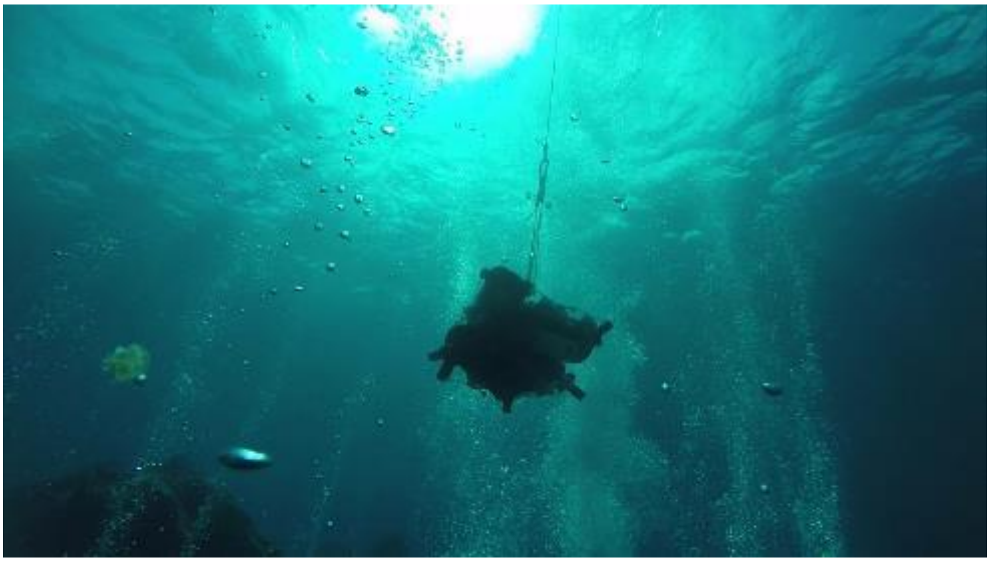

Figure 3.8. Deployment of Niskin bottles in "Bottaro Twins".

Gas flux measurements at the air-sea interface were carried out between “Bottaro Twins” and “Bottaro Single” emission sites. The CH_4 flux reached a value of 0.030 g m^{-2} d^{-1}, whereas a maximum CO_2 flux of 11910 g m^{-2} d^{-1} was detected. A similar CH_4 value of 0.034 g m^{-2} d^{-1} was observed in “Bottaro Downstream” station, in which ideal gas concentrations inside the accumulation chamber were not obtained for CO_2.

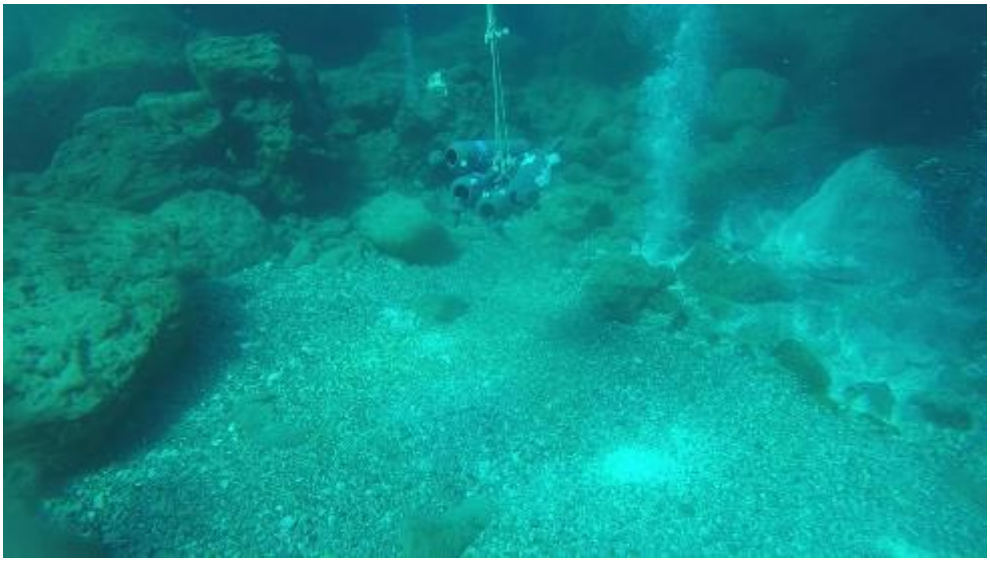

Figure 3.9. Deployment of Niskin bottles in “Bottaro Single”.

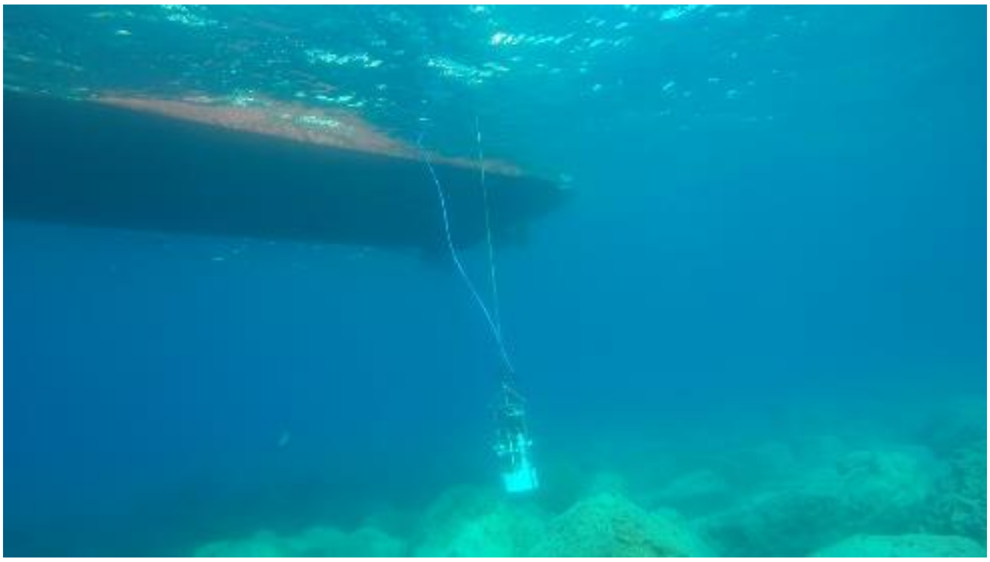

Figure 3.10. Multi-parametric probe cast in “Bottaro Downstream”.

Figure 3.11. Niskin closure and sediment retrieval from “Bottaro Downstream” rocky seabed.

3.5 Bardelli outcrop

The site was extensively explored during the ROV survey. The strong water turbidity prevented the photos and videos taken by the underwater vehicle from being of good quality, so that the outcrop was barely recognisable above 21.5 m b.s.l. (Figure 3.12).

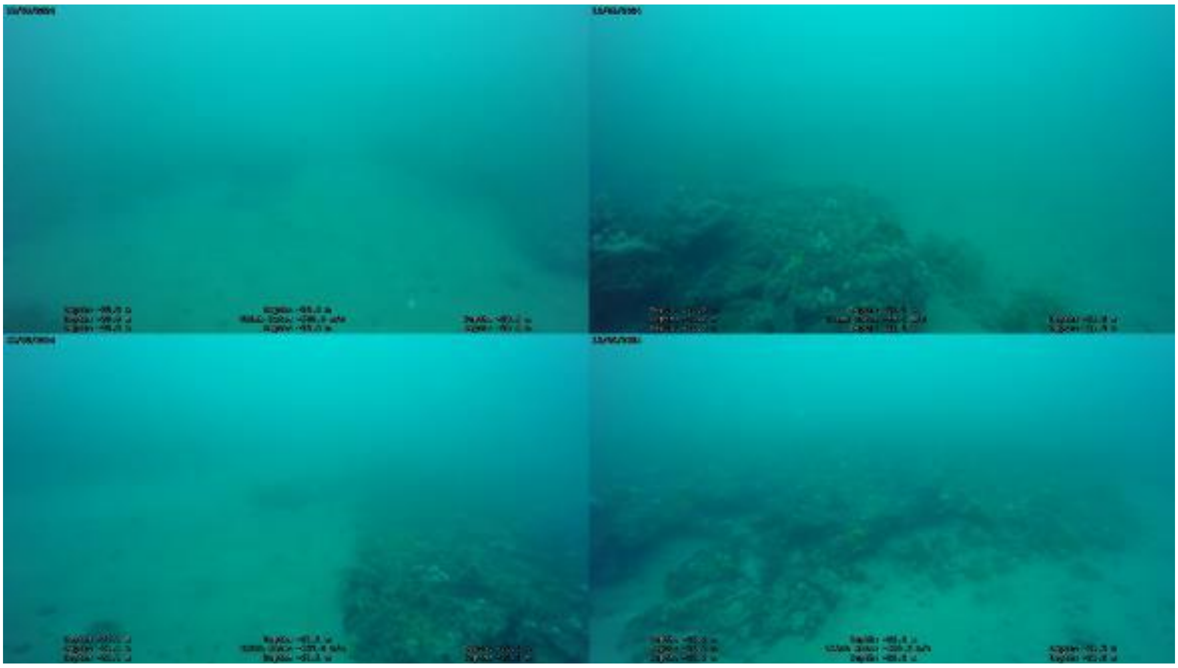

Figure 3.12. Bardelli outcrop pictures taken by the ROV at about 21 m b.s.l. The rocky buildup is hardly distinguishable due to the strong water turbidity.

Slightly better resolution was obtained at greater depths, even though no bubble stream released from the seafloor was detected during the visual inspection of this trezza (Figures 3.13, 3.14 and 3.15). However, isolated microbial mats were observed on the sandy seafloor separating the blocks forming the outcrop, thus confirming that intermittent gas emissions occur in the area (Figure 3.16).

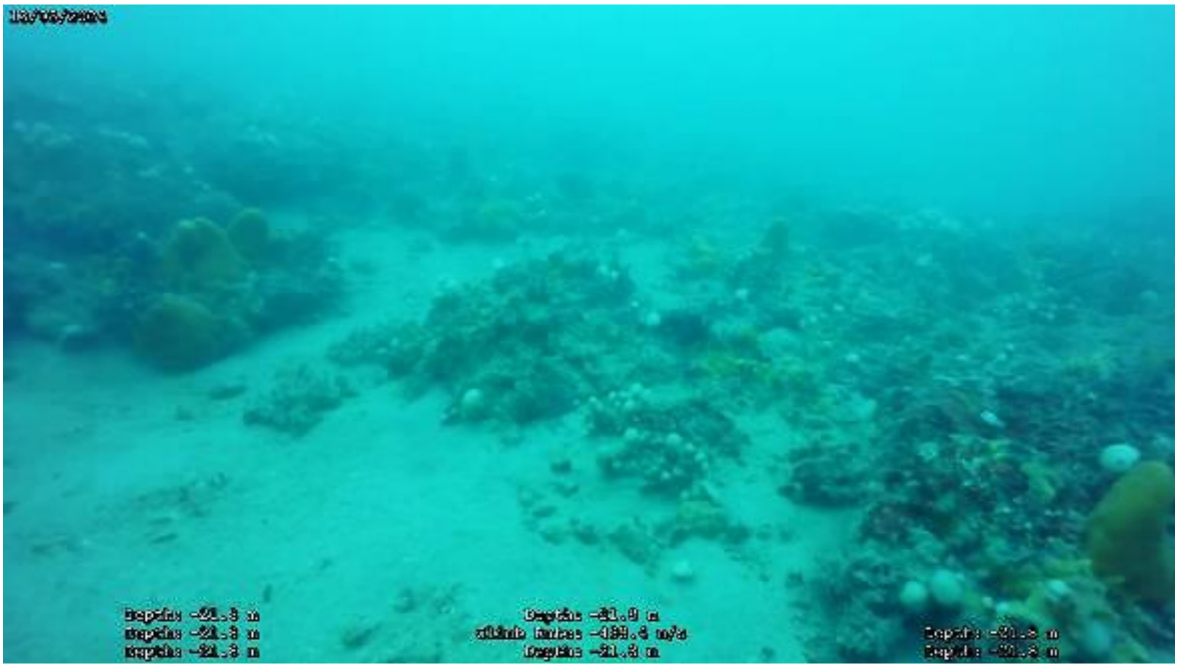

Figure 3.13. Seafloor picture taken at 21.8 m b.s.l. showing a slight better resolution despite the water turbidity.

Figure 3.14. Seafloor picture taken at 21.8 m b.s.l. showing a slight better resolution despite the water turbidity.

Figure 3.15. Detail of the coralligenous biostructures (photo taken by the ROV at 22 m b.s.l.).

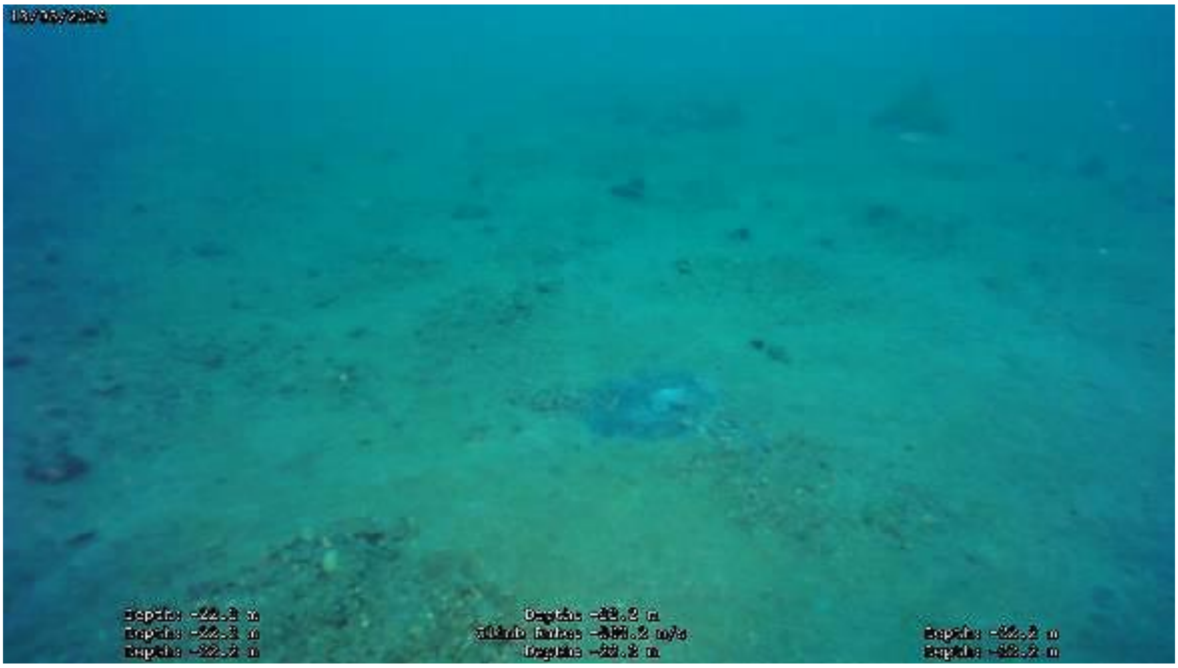

Figure 3.16. Isolated microbial mat on the sandy seafloor separating the blocks forming the "Bardelli" outcrop.

3.6 Sudpiastra outcrop

At this site, active gas seepage was observed during the survey, with several bubble streams frequently (even though intermittently) released from the seafloor, in particular from the gravel-to-sandy patches between the rocky deposits (Figures from 3.17 to 3.23). Seven emission points were observed during the investigation of the area.

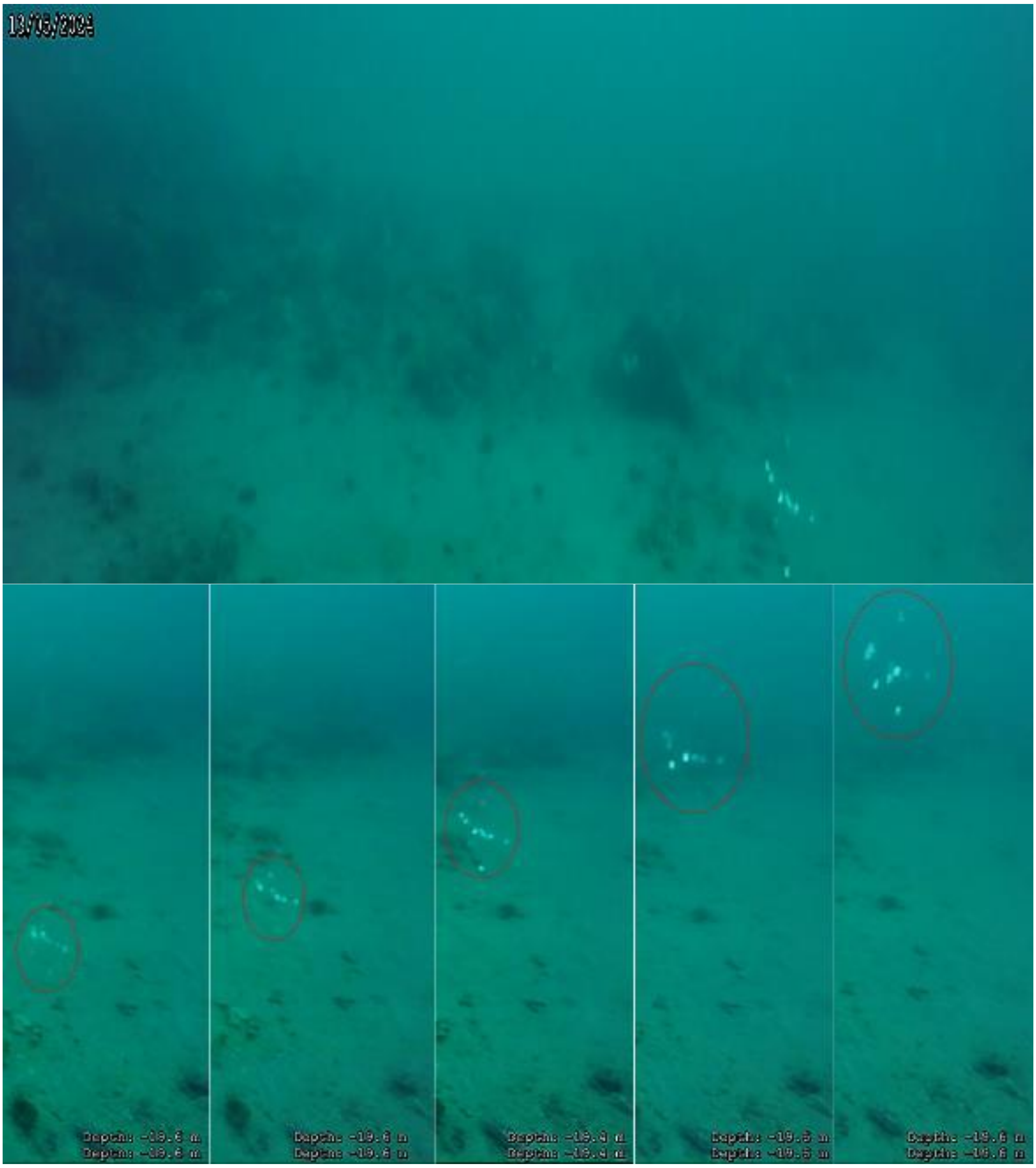

Figure 3.17. a) Bubbles in water column at emission point 1; b) Emission sequence frame by frame.

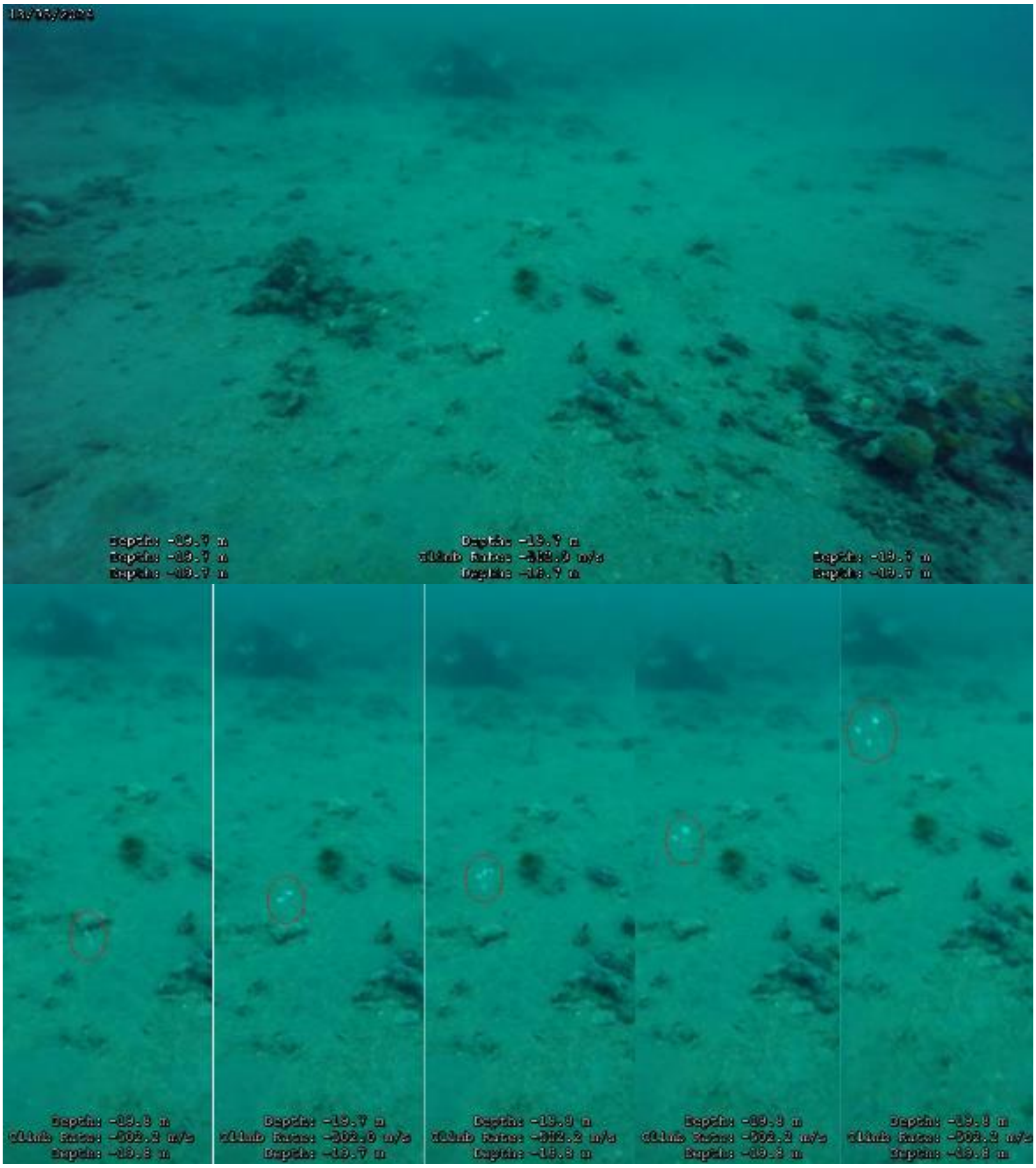

Figure 3.18. a) Bubbles in water column at emission point 2; b) Emission sequence frame by frame.

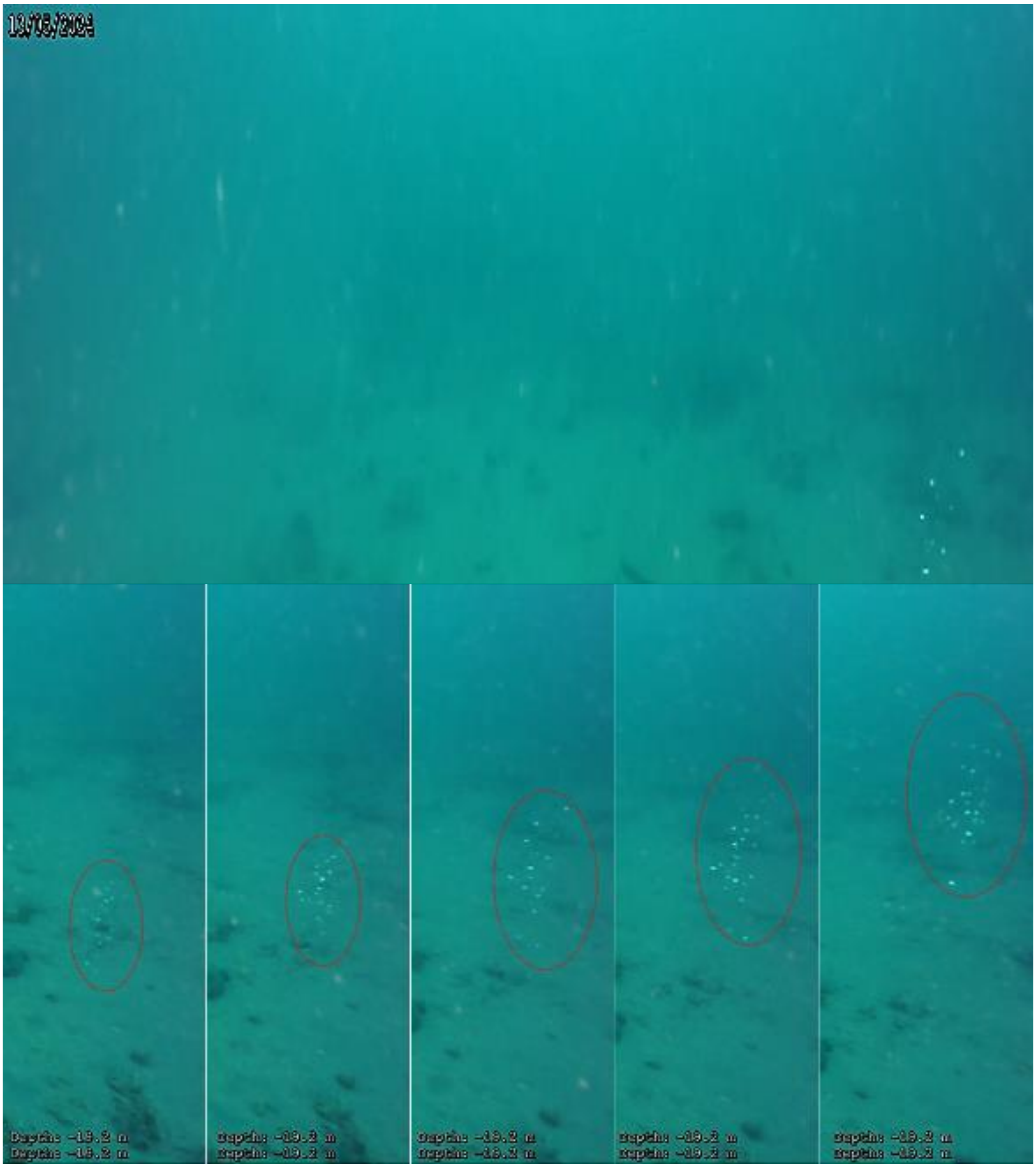

Figure 3.19. a) Bubbles in water column at emission point 3; b) Emission sequence frame by frame.

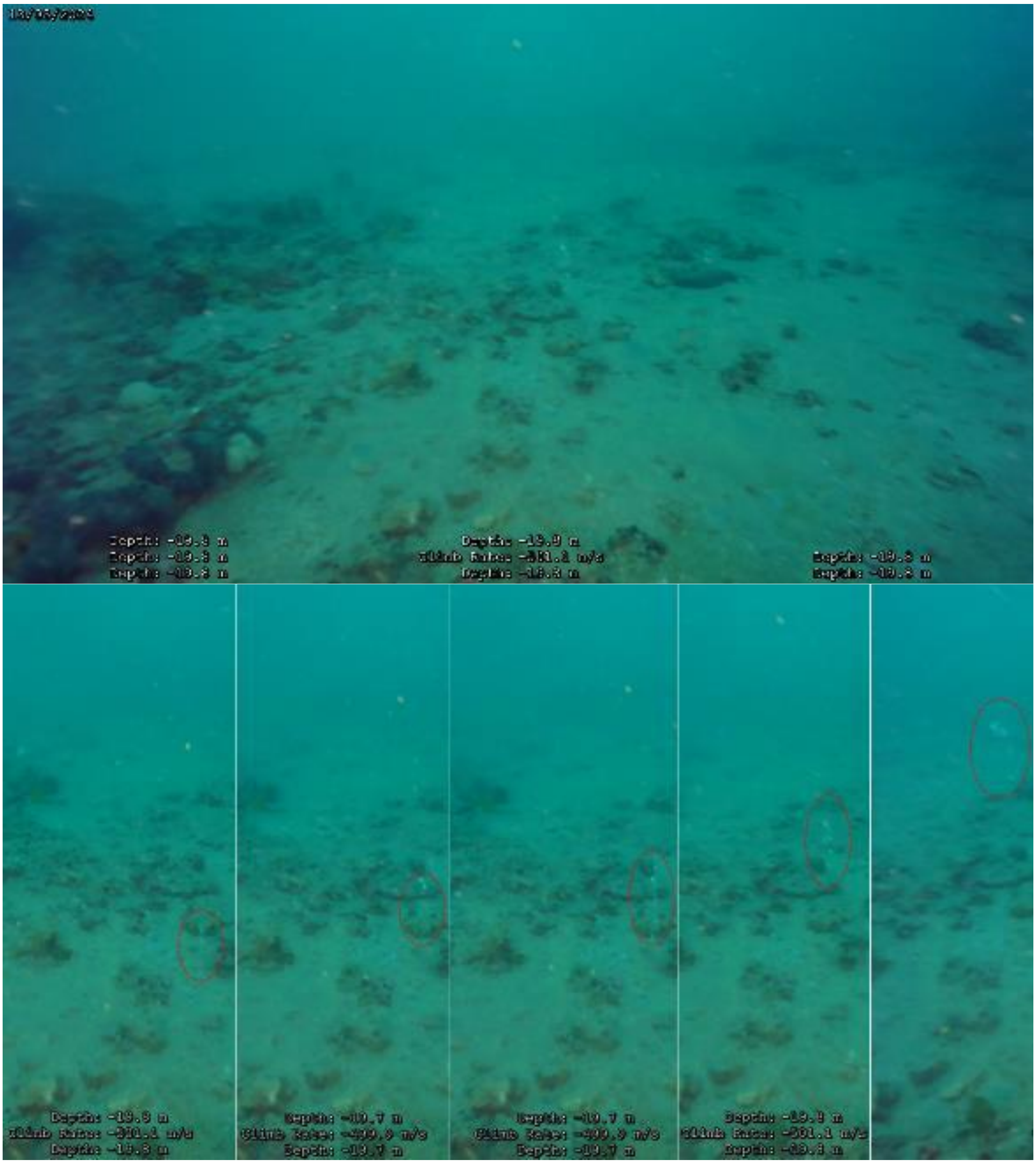

Figure 3.20. a) Bubbles in water column at emission point 4; b) Emission sequence frame by frame.

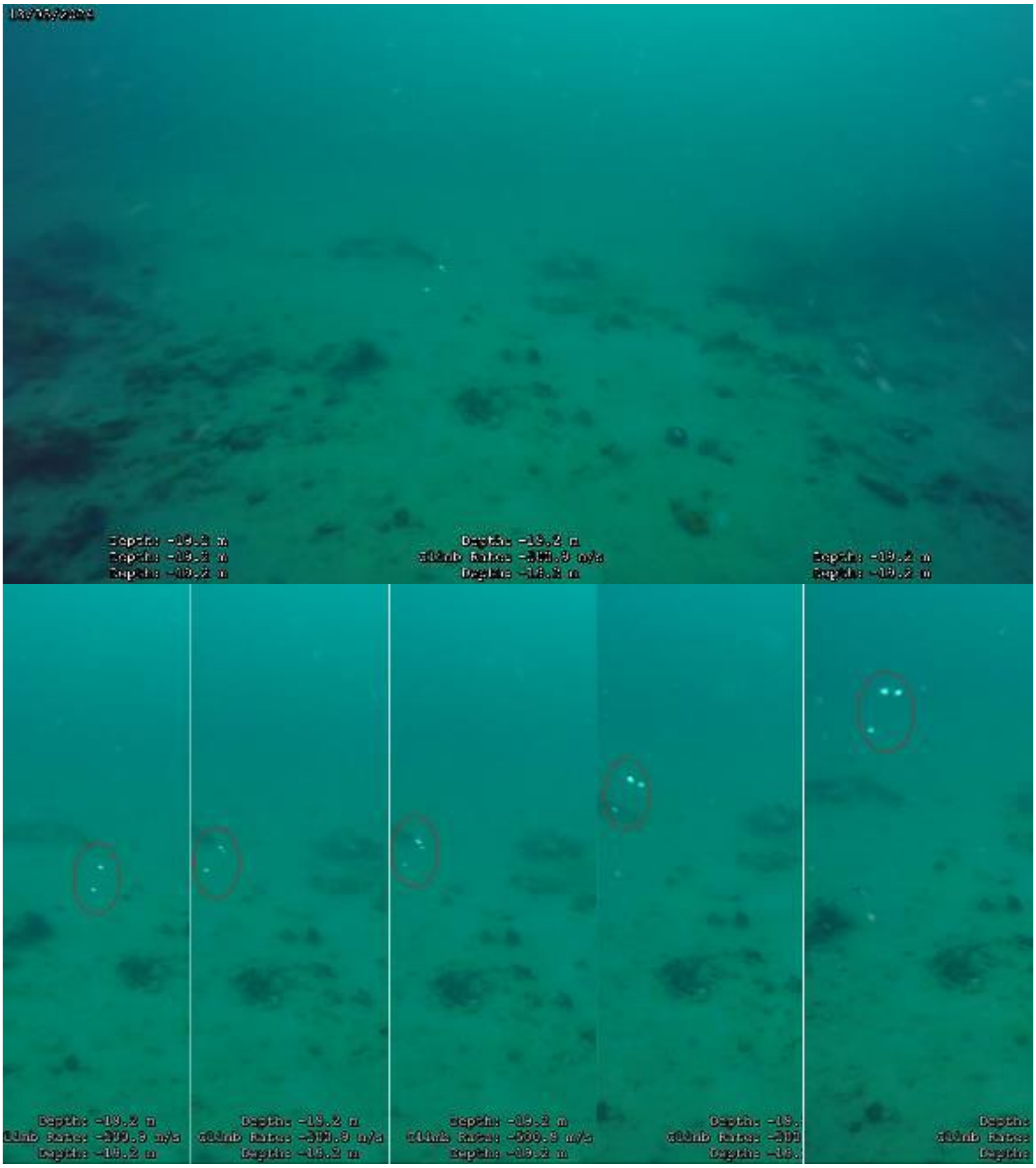

Figure 3.21. a) Bubbles in water column at emission point 5; b) Emission sequence frame by frame.

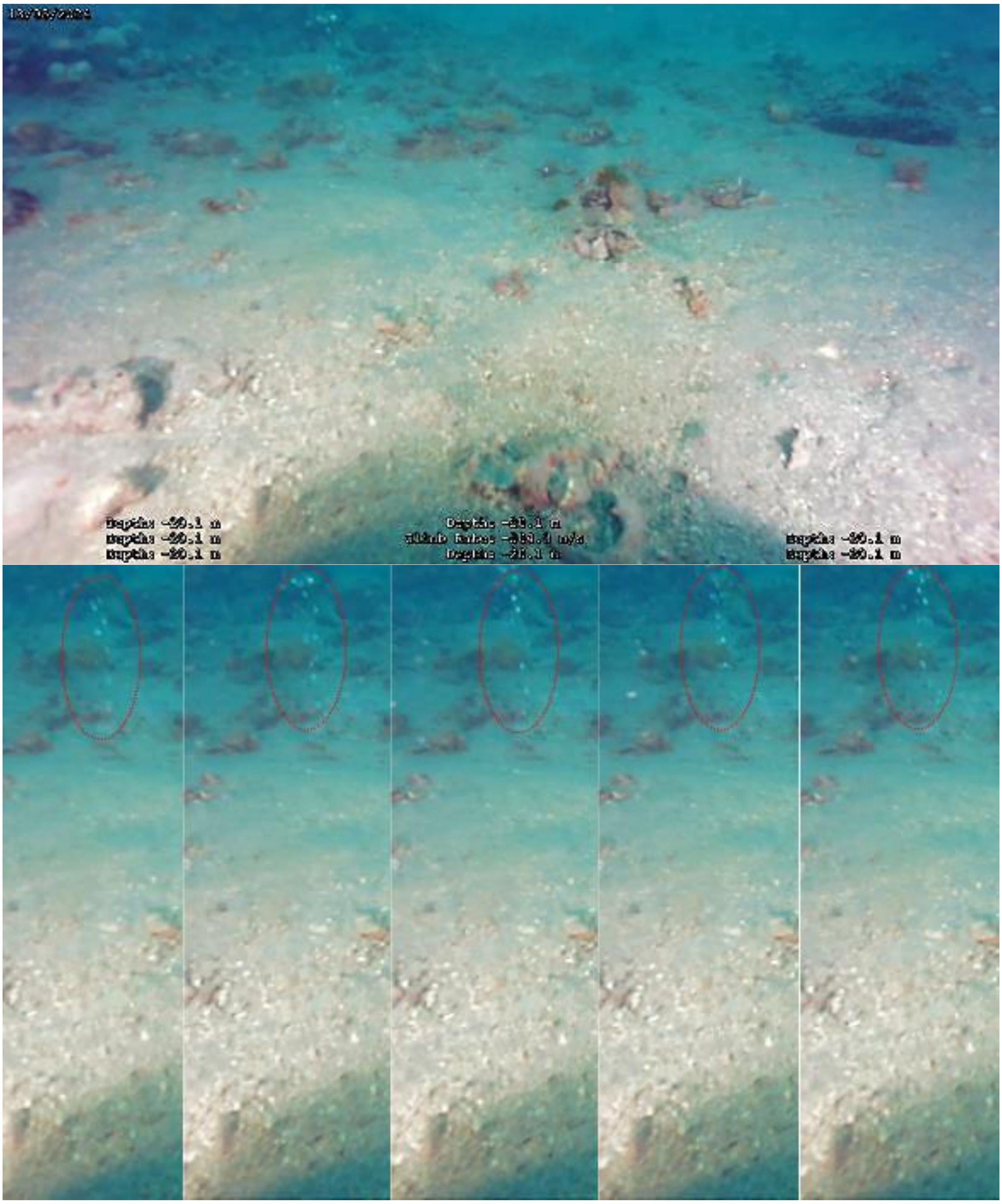

Figure 3.22. a) Bubbles in water column at emission point 6; b) Emission sequence frame by frame.

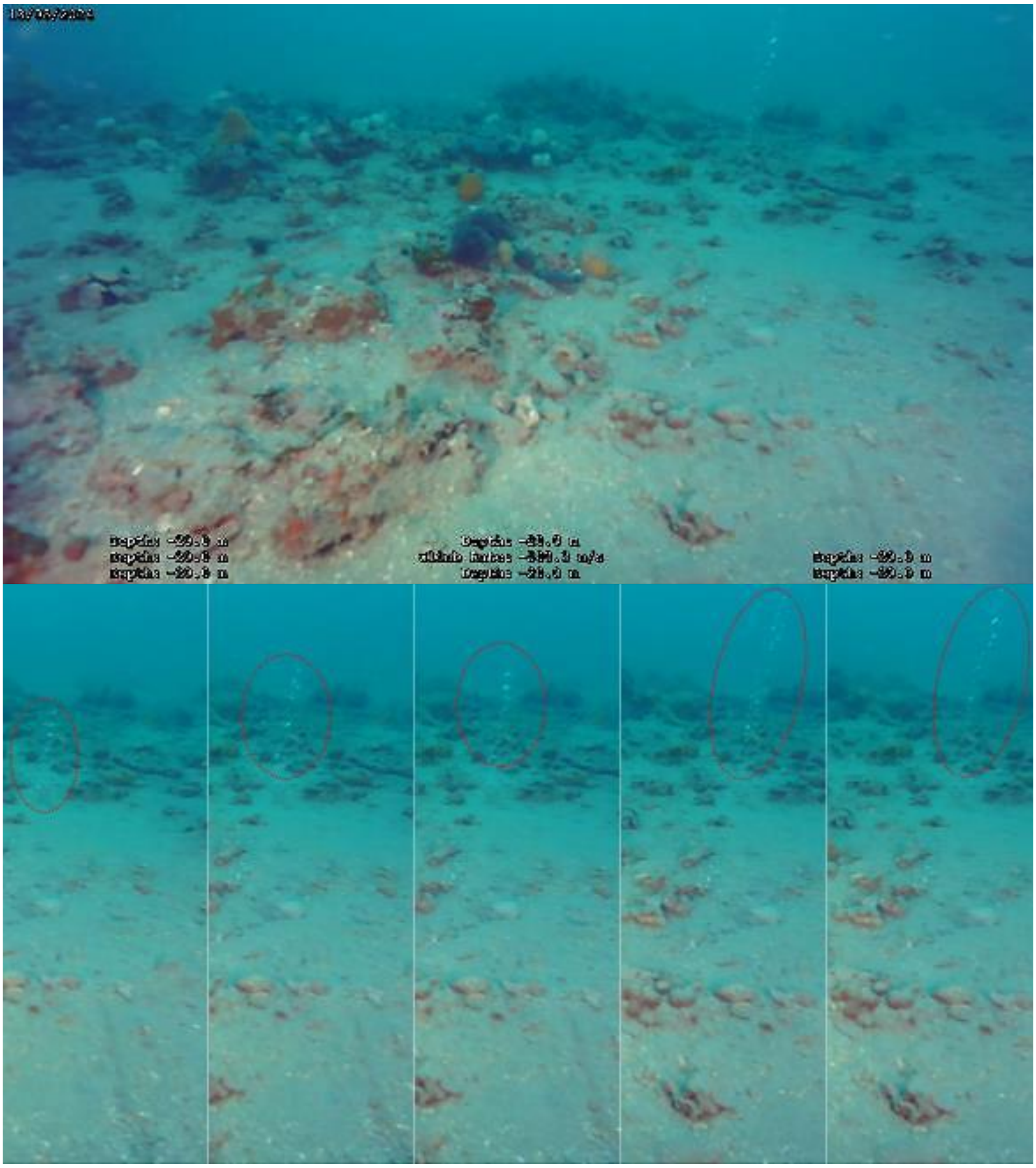

Figure 3.23. a) Bubbles in water column at emission point 7; b) Emission sequence frame by frame.

The seafloor was characterized by the presence of numerous pockmarks (Figure 3.24) and microbial mats (Figure 3.25 and 3.26).

Figure 3.24. Pockmarks at the seafloor of the trezza Sudpiastra.

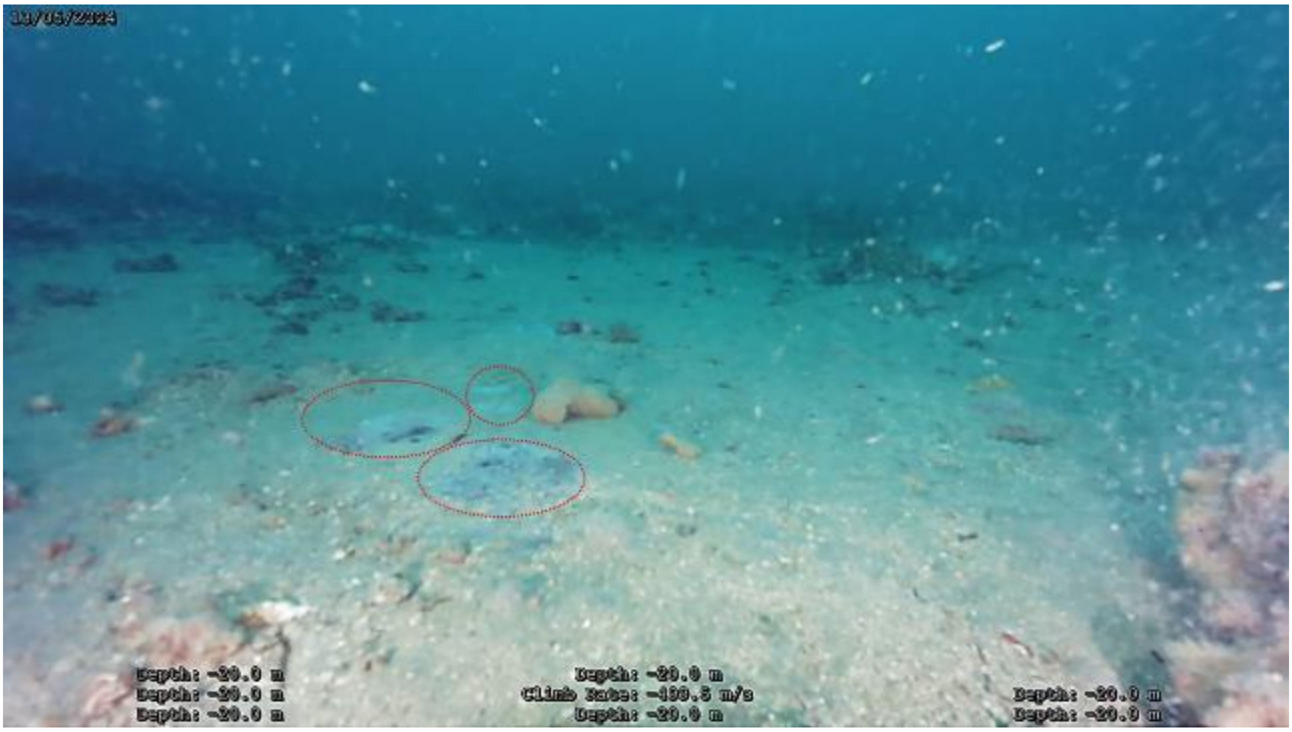

Figure 3.25. Isolated microbial mats on the sandy seafloor separating the blocks forming the Sudpiastra outcrop.

Figure 3.26. Isolated microbial mats on the sandy seafloor separating the blocks forming the Sudpiastra outcrop.

4. EXPERIMENTAL RESEARCH DESIGN

4.1 Hydrothermal vent area off Panarea island

The evaluation of the data collected in real time made it possible to quickly and correctly select the target locations in the study area. Considering the magnitude of the CH_4 emissions, each of the eight stations surveyed (Black Point Smoke, Black Point Bubbling, Bottaro Twins, Bottaro Single, Bottaro Downstream, Hot/Cold1, Hot/Cold2 and Zimmari) proved to be suitable for the purposes of the MEFISTO project. This in turn enabled the MEFISTO research team to carry out the sampling of each CH_4 emission site immediately after its identification, allowing the first campaign in the Southern Tyrrhenian Sea (March 21-23, 2024) to be completed earlier than originally expected. The second campaign in the area will be carried out in the same sites by the end of September 2024, in order to determine the effect of seasonality on CH_4 release and methanotrophic communities.
Site locations and station coordinates and depths are shown in Figures 2.4 and 2.5 and in Table 4.1, respectively.

Table 4.1 - Hydrothermal vent area selected sites (Panarea - Southern Tyrrhenian Sea)

Station	*Depth (m)*	*Longitude (Degree Minutes)*	*Latitude (Degree Minutes)*
Black Point Smoke	21.0	15°06.630' E	38°38.217' N
Black Point Bubbling	21.0	15°06.630' E	38°38.217' N
Bottaro Twins	6.25	15°06.623' E	38°38.324' N
Bottaro Single	6.25	15°06.623' E	38°38.324' N
Bottaro Downstream	4.0	15°06.648' E	38°38.320' N
Hot/Cold1	10.0	15°04.704' E	38°38.553' N
Hot/Cold2	11.3	15°04.653' E	38°38.280' N
Zimmari	8.5	15°03.969' E	38°37.589' N

4.2 Trezze of the Northern Adriatic Sea

The ROV visual inspection of the two study sites (Bardelli and Sudpiastra outcrops) clearly indicated the intermittent nature of the gas emissions in the Northern Adriatic Sea. In fact, although the presence of isolated but spatially diffused microbial mats on the seafloor of both areas suggested a constant seepage activity, bubble-driven ebullitive processes along the water column were only observed at the Sudpiastra site.
This prompted the MEFISTO research team to remove the Bardelli outcrop from the pool of sampling stations and to focus the efforts on investigating the rocky buildups belonging to the "Trezze San Pietro e Bardelli" Site of Community Importance, which also includes the Sudpiastra outcrop.
In fact, although gas flares detected at the Bardelli outcrop can reach considerable heights of 4 to about 20 m (Donda et al., 2019), this trezza is quite isolated and far from the coast (about 12 nautical miles). Practically speaking, the navigation to this rocky formation is time-consuming, does not allow

other sampling sites to be investigated in one day, and the result (i.e. the detection of gas) is not guaranteed.
Therefore, the selection of the sampling stations (San Pietro, Sepa and Sudpiastra) was limited to the nearest outcrops of the Gulf of Trieste which are known for their frequent gas emissions (Bettoso et al., 2023; Gordini, 2009; Gordini et al., 2012; Gordini et al., 2023).
Site locations and station coordinates and depths are shown in Figure 2.2 and in Table 4.2, respectively (a reference station will be identified during the first sampling campaign).
The first and second sampling campaigns will be carried out in the area in July 2024 and in March 2025.

Table 4.2 - Selected sampling sites (trezze - Northern Adriatic Sea)

Station	*Depth (m)*	*Longitude (Degree Minutes)*	*Latitude (Degree Minutes)*
San Pietro	14.0	13°20.276	45°36.191
Sepa	16.7	13°20.581	45°35.622
Sudpiastra	19.0	13°18.305	45°33.218

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