



EXTENDED SYNOPSIS OF THE X-PRESS PROJECT

Title of the Project: EXtreme environments: High PRESSure Sampling and experimentation system for deep sea hydrocarbon releases

The deep sea, defined as water and sediments beneath 1000 m depth, is the Earth's largest ecosystem. Defined by permanent darkness, low temperature, limited organic carbon concentration, and hydrostatic pressure increasing by 1 MPa per 100 m of depth, the deep ocean can be largely considered an extreme environment for life. Yet, distinctive and previously unknown communities of remarkably diverse microbes are thriving in this habitat, performing key ecological processes and providing essential goods and services for human well-being, such as genetic resources and climate regulation. Nevertheless, the diversity and functioning of microbial life in deep-sea ecosystems remain poorly understood. Our limited understanding largely stems from the combined challenges of the remoteness and inaccessibility of this habitat, along with the technical difficulty in retrieving intact samples for study and experimentation in the lab while maintaining the extreme pressure that defines this environment. The latter is crucial in deep-sea research, as the pressure-adapted microorganisms of the deep sea are highly sensitive to depressurization. Previous work by our team demonstrated a substantial loss of microbial diversity with decompression, thereby affecting measurements of biogeochemical cycling. These findings brought to life the X-PRESS project.

The X-PRESS project—*EXtreme environments: High PRESSure Sampling and experimentation system for deep-sea hydrocarbon releases*—set out to close a crucial gap in our ability to study the deep ocean. Led by the *Institute of Geoenergy of the Foundation for Research and Technology – Hellas (FORTH/IG)*, the X-PRESS project addressed this challenge by developing a **new, fully operational system for high-pressure sampling and experimentation**—technology that now places Greece at the forefront of deep-sea environmental research. The X-PRESS pressure-retaining sampler can collect seawater from depths up to 6,000 m which is then transferred into a linked high-pressure laboratory incubation system without decompression at any stage of the process—an essential requirement for credible biological observations. Within these vessels, seawater can be manipulated as needed, depending on the scientific objective. Together, these instruments form a complete operational platform for high-pressure microbiology allowing scientists to study microbial processes exactly as they occur *in situ*, under the extreme pressures of the deep ocean. The system was engineered by the project's collaborator, Top Industrie (France), and subsequently modified and tested—both in the laboratory and at sea—by FORTH/IG scientists, with the support of Greece's two seagoing public research institutions: the Hellenic Centre for Marine Research (HCMR) and the Fisheries Research Institute of ELGO-DIMITRA (FRI). A major achievement of the project was its redesign for dual compatibility: it now fits both HCMR's and FRI's multi-water samplers, greatly expanding the national research fleet's ability to use the system.



Once fully functional, the X-PRESS infrastructure supported a series of experiments that explored the ecology of oil-eating microbes in deep waters of the Eastern Mediterranean Sea. These microorganisms are fascinating not only because they can break down oil, but also because of the unique biochemical and ecological traits they have evolved to perform this function under extreme conditions. They excrete biosurfactants—surface-active molecules that help emulsify and degrade hydrocarbons—and are a source of novel enzymes and antimicrobial or bioactive compounds with potential applications across industries and in bioremediation. Moreover, their metabolic versatility and resilience make them promising candidates for the biodegradation of plastic waste, providing sustainable solutions to one of today’s most urgent environmental challenges.

In the X-PRESS project, we examined the production of biosurfactants by a deep-water hydrocarbon-degrading microbial community. We specifically isolated and characterised the structure and properties of excreted rhamnolipids. We found that our deep-sea consortium produced sufficient rhamnolipids to stabilize micro-scale oil droplets with an efficiency comparable to commercial dispersants under subsea conditions, while avoiding the elevated toxicity associated with the latter.

We subsequently examined the oil biodegradation capacity of the same consortium -originating from a depth of 1000m- at different pressure levels (0, 10, 20, 30 MPa) to test its adaptability to changing hydrostatic pressure. Our results indicated that the consortium was primarily composed of *piezotolerant* microorganisms, meaning those capable of growing equally well at atmospheric pressure and at pressures up to approximately 10MPa, which corresponds to their depth of origin. We also made an interesting observation: although elevated pressures of 20 and 30 MPa inhibited overall cell growth, the pressure-adapted microorganisms that persisted exhibited higher biodegradation efficiency on a per-cell basis. This finding is consistent with recent studies and supports an emerging concept in deep-sea microbiology—that a small fraction of the microbial community accounts for a disproportionately large share of total metabolic activity in the deep ocean.

A third experiment expanded the project’s scientific scope by establishing a new method for high-pressure experimentation with methane, a major greenhouse gas. This technique allowed the team to enrich aerobic methanotrophic microorganisms from samples collected at 2,000 m in the Southeast Aegean. Methanotrophs possess a unique capability—they can thrive solely on methane as their carbon and energy source thereby forming a crucial biological barrier that mitigates methane fluxes to the atmosphere. This process plays a central role in regulating emissions from both natural sources (e.g., dissociating gas hydrates, mud volcanoes) and anthropogenic disturbances (e.g., subsea well blowouts). The development of this experimental protocol demonstrates that the X-PRESS system provides a valuable platform for investigating broader environmental processes, including deep-sea methane cycling and the microbial mechanisms contributing to greenhouse gas mitigation.

Throughout the project, dissemination was a priority. Results were presented at more than fifteen national and international events, including scientific symposia, oil-and-gas industry meetings, and workshops with local authorities. Project progress and visuals from field expeditions were shared through FORTH/IG’s official social media accounts, while two journal manuscripts are currently in preparation and further data analysis is ongoing.



Overall, X-PRESS aimed to equip Greece with the tools and scientific knowledge needed to respond effectively to potential deep-sea hydrocarbon releases, particularly in the context of ongoing natural gas exploration within the Hellenic Exclusive Economic Zone (EEZ) at depths exceeding 2,500 m. However, its capabilities extend far beyond oil spill response. The X-PRESS system now represents a national research asset, enabling the exploration of microbial life in extreme environments. Studying these extremophiles can uncover novel enzymes and biochemical pathways for applications in biotechnology, pharmaceuticals, and green chemistry, while pressure-adapted deep-subsurface microbes may also inform energy transition strategies, including CO₂ and H₂ storage and the formation of natural hydrogen reservoirs. Finally, insights into pressure adaptation shed light on the limits of life on Earth and potentially other planets, linking extremophile microbiology to astrobiology. Through these broader research opportunities, X-PRESS has helped position FORTH/IG at the forefront for the study of extreme ecosystems and blue biotechnology in the Eastern Mediterranean.