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Habitability and Potential Biosignatures of Psychrophilic Microorganisms on Europa and Enceladus

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ABSTRACT

Jupiter's and Saturn's satellites, Europa and Enceladus, respectively, are known for their ice crusts and subsurface oceans, which, as some scientists hypothesise, may harbour life, due to the thermal conditions in which some of the terrestrial organisms are able to thrive. These microorganisms are called psychrophiles, and they can live in temperatures from -20 to 10°C. The goal of this scientific paper was to test this hypothesis by comparing psychrophiles on Europa and Enceladus using 6 characteristics: temperature, habitats, oxygen, pH, energy sources, and metabolism. The results show that, though terrestrial psychrophiles might not be able to tolerate some of the pH levels of their oceans, all the other characteristics substantially overlap, which implies that Europa and Enceladus have a possibility to be habitable. This paper also discusses how methods such as mass spectrometry, fluorescence/reflectance spectroscopy, and gas chromatography can be used for detecting the biosignatures of psychrophiles, including breakdown products of biological molecules, microfossils, gases excreted by organisms, pigments, and complex organics. All of the above-mentioned suggest that extraterrestrial psychrophiles may indeed have a non-zero chance to exist on these moons due to their habitability, indicating that future astrobiological research should be directed in this direction.

INTRODUCTION

Cosmic missions, such as Cassini and Galileo, have revealed that icy moons like Europa and Enceladus, Jupiter's and Saturn's satellites, respectively, may harbour life in their subsurface oceans, ice crusts, and seafloors. Observations suggest that the presence of subsurface oceans, which might sustain psychrophilic life, along with hydrothermal activity and tidal heating,[1] may provide ideal environments for cold-loving organisms, called psychrophiles. Psychrophiles are microorganisms adapted to grow and reproduce at low temperatures.[2] Adding these moons' relatively close locations to our planet (within our Solar System), these factors make them one of the most prominent targets for future astrobiological research.

[1] Richard J. Greenberg, *Europa – the Ocean Moon* (Springer, 2005), 85–89.
[2] Khawar S. Siddiqui et al., "Psychrophiles," *Annual Review of Earth and Planetary Sciences* 41, no. 1 (2013): 87.

Europa and Enceladus were chosen for this investigation due to the similarity of their surface, the presence of a subterranean ocean underneath the icy crust, geological activity, warm interiors, and conditions that may support life.[3] Although we know that they might be habitable, only a few papers have thoroughly discussed the organisms that potentially exist or have existed there. However, both moons are going to be more thoroughly investigated in the future, with the Europa Clipper spacecraft already launched and the Enceladus Orbilander mission being in the planning stage.

These missions will likely look for biosignatures, signs that can provide evidence of prior or currently existing life. The examples of these are molecules like chlorophyll or carotenoids that strongly affect reflectance spectra, organics or unusual chemistry in surface ice, water, or gas plumes (like Enceladus' water plumes containing organics and possible H_2), or microscopic cellular remains preserved in ice or rock (microfossils). The methods for detecting them include the use of remote sensing, such as reflectance spectroscopy,[4] or *in situ* life detection methods, like penetrators.[5]

Psychrophiles are a specific branch of extremophiles (microorganisms that can survive in extreme conditions) that can withstand cold temperatures in terrestrial environments from -20 to 10°C.[6] Over millions of years of evolution, psychrophiles have developed various adaptations to withstand freezing environments. Their survival mechanisms feature numerous adaptations: cell membranes resistant to stiffening, polyunsaturated fatty acids, cold-adapted enzymes, and antifreeze proteins.[7, 8] These extremophilic organisms include archaea, bacteria, eukaryotes, and viruses. The cold biosphere consists of diverse types of environments—vast tracts of the deep sea, geographically dispersed alpine regions, geologically specific subterranean caverns, climatically challenged regions of permafrost, and biogeochemically diverse polar reaches.[9]

This research's main focus is to evaluate the habitability of two moons by comparing them with psychrophiles and answer the question: Is there a possibility of extraterrestrial psychrophilic microorganisms inhabiting Europa and Enceladus, and what are the methods for identifying their presence? Answering this question will help us understand how and where Europa Clipper and, prospectively, Enceladus Orbilander should search for life, what potential biosignatures to look out for (e.g., pigments, results of biological processes, etc.) on these moons, and determine whether these investigations are worthwhile in all the efforts. However, the paper only focuses on psychrophiles, leaving other types of extremophiles, like hyperthermophiles, alkaphiles, acidophiles, etc., unreviewed.

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[3] Christopher D. Parkinson et al., "Habitability of Enceladus: Planetary Conditions for Life," *Origins of Life and Evolution of Biospheres* 38, no. 4 (2008): 361.
[4] Olivier Poch et al., "Remote Sensing of Potential Biosignatures From Rocky, Liquid, or Icy (Exo)Planetary Surfaces," *Astrobiology* 17, no. 3 (2017): 231.
[5] Julian Chela-Flores, "From the Moon to the Moons: Enceladus and Europa. The Search for Life and Reliable Biomarkers." *Journal of Cosmology* **5**, no.24 (2010): 973.
[6] Andrew Martin and Andrew McMinn, "Sea Ice, Extremophiles and Life on Extra-terrestrial Ocean Worlds," *International Journal of Astrobiology* 17, no. 1 (2017): 1.
[7] Martin and McMinn, "Sea Ice, Extremophiles and Life on Extra-Terrestrial Ocean Worlds," 3.
[8] Alberto Alcázar, Laura Garcia-Descalzo, and Cristina Cid, "Microbial Evolution and Adaptation in Icy Worlds." *ResearchGate*, (2009): 1.
[9] Siddiqui et al., "Psychrophiles," 88.

MOONS AND PSYCHROPHILES

Researchers emphasise that solar system satellites, such as Europa and Enceladus, are priority targets for the search for extraterrestrial life, as conditions there may support the existence of liquid water and a potential biosphere beneath the icy crust.[10] As of now, one of the most important missions of space science is the search for and discovery of a second genesis - a hypothetical independent origin of life beyond Earth. To determine if this hypothesis is true, it is proposed to use penetrators capable of reaching subsurface oceans and detecting reliable biosignatures that distinguish biological processes from abiotic ones.

The potential habitability of such icy moons as Europa and Enceladus has been widely discussed in many astrobiological research papers. Sea-ice microbes might be capable of occupying ice-associated biological niches on Europa and Enceladus.[11] The authors outline three main potential biospheres in which they can thrive: the ice-ocean interface, the subsurface ocean, and the seafloor. The paper also proposes that a specific branch of psychrophiles, called eutectophiles, could potentially inhabit the ice fissures and the ice-water interfaces. Studying the metabolism of these ice-associated bacteria demonstrates that the possible chemical processes important for life can occur on Europa and Enceladus. Additionally, their research collects information about the physical and chemical tolerances of psychrophiles, expected survival conditions, potential metabolic pathways, and biosignatures on both moons. However, this review does not specifically address the possibility of all types of psychrophiles thriving on Europa or Enceladus; it focuses primarily on eutectophiles, while briefly mentioning information about other kinds of extremophiles.

Research by Alcazar et al.[12] claims that the range of environmental conditions known to support microbial life has expanded with the recent discovery of cold-tolerant microorganisms in glaciated and permanently frozen environments. Now, there is a lot of research being done on terrestrial models of alien icy worlds. Among these, bacterial communities found in high mountains, subglacial lakes, and Arctic and Antarctic permafrost are considered typical of these settings, where psychrophilic bacteria are the predominant inhabitants. Their paper focuses on the mechanisms of adaptation and evolution that psychrophiles use to survive in extreme freezing environments. These features are uncommon in other prokaryotes and could serve as powerful biosignatures if similar molecules are found in icy samples from extraterrestrial bodies.

Research by Richard B. Hoover and Elena V. Pikuta[13] shows how terrestrial microbes living in extreme cold can help us understand the possibility of life existing in similar conditions on other celestial bodies. They can serve as analogues of possible extraterrestrial life in icy conditions on Mars, Europa,

[10] Chela-Flores, "From the Moon to the Moons: Enceladus and Europa. The Search for Life and Reliable Biomarkers," 973.
[11] Martin and McMinn, "Sea Ice, Extremophiles and Life on Extra-Terrestrial Ocean Worlds," 8.
[12] Alcázar, Garcia-Descalzo, and Cid, "Microbial Evolution and Adaptation in Icy Worlds," 1.
[13] Richard B. Hoover and Elena V. Pikuta, "Psychrophilic and Psychrotolerant Microbial Extremophiles in Polar Environments." *NASA Technical Reports Server*, (2010): 3.

Ganymede, Callisto, asteroids, comets, and other potentially habitable frozen bodies of the Solar System. Expeditions on Lake Glubokoye, Anuchin Glacier, Lake Untersee, Fox Tunnel, Lake Vostok (psychrophiles have been found more than two miles under the surface ice of Antarctica at Vostok Station[14]), and other polar regions of Earth, reviewed in this article, have shown that extraterrestrial life might not only be found in sea-ice, glaciers, ice sheets, and permafrost, but also in the cryptoendolithic, cryoconite, and especially ice-bubble environments. On Earth, cryoconite pools and ice bubble systems in Antarctica show that solar heating of dark rocks embedded in ice can cause localised melting, creating micro-environments with trapped gases that can support microbial life. Authors suggest that microorganisms should be capable of episodic growth within liquid water envelopes surrounding dark rocks in cometary ices and the permafrost and polar caps of Mars.

NASA's Cassini mission, launched toward the Saturn system in 1997, has discovered several signs showing that Enceladus might be suitable for life.[15] In particular, it has found evidence of a global subsurface ocean, hydrothermal activity, and, more importantly, carbon dioxide (CO_2), methane (CH_4), ammonia (NH_3), and other organic particles — potential biosignatures that make Enceladus one of the key candidates for life-detection missions like Enceladus Orbilander and Tianwen-4. Similarly, a Europa Clipper spacecraft was launched to Europa in October 2024, meaning that we are going to find out about these moons' habitability in just a few years. Additionally, the Galileo mission (1995–2003) confirmed the existence of a global saltwater ocean beneath Europa's ice crust.

The goal of this paper is to evaluate the possibility that terrestrial-like psychrophiles might be able to inhabit the icy ocean worlds within our solar system. While previous studies have examined the habitability and environments of icy moons, terrestrial psychrophiles, and potential biosignatures separately or in broader contexts, this study provides a structured comparison of known psychrophilic requirements with the estimated conditions of both Ocean Worlds across six factors, alongside with implications for targeted biosignature searches. To achieve this, it gathers and compares information about optimal temperature, pH level tolerance, habitat preference, etc. The process and results are interpreted, displayed, and then discussed in the following sections. Potential habitable zones, biosignatures, and their detection methods are reviewed in the discussion sections.

METHODS

This study uses a comparative habitability assessment to evaluate the potential for terrestrial-like psychrophilic life to inhabit Europa and Enceladus. It consists of analysing the existing literature and comparing average characteristics of psychrophiles to conditions on each moon using 6 key factors: temperature, habitats, oxygen, pH, energy sources, and metabolism. These factors were selected for further reasons:

[14] J. Cameron Thrash, "Microbes That Like It Cold: Psychrophiles – One World One Ocean," May 8, 2012.
[15] Rocco L. Mancinelli, Chris P. Mckay, and Alfonso Davilla, "Enceladus: Biosignatures." *NASA Technical Reports Server*, (2014): 1.

- Temperature — directly determines whether known psychrophiles can grow and reproduce.
- Habitats — establishes whether suitable physical conditions analogous to terrestrial psychrophiles occur.
- Oxygen — distinguishes aerobic, anaerobic, and potentially microaerobic metabolic pathways required to survive on Europa and Enceladus.
- pH levels — determines compatibility with known physiological ranges.
- Energy sources — show whether available physical/chemical energy could support metabolism.
- Metabolism — evaluates metabolic pathways appropriate for available environmental resources.
- Comparable information could be identified for both psychrophiles and moons.
- Other variables, including salinity, pressure, gravity, light absence/abundance, and cosmic radiation, were mentioned, but excluded from the core comparison due to the lack of sufficient comparable information across all three columns.

The data are then presented in a table for comparison and visual demonstration. Matching results would suggest that Europa and Enceladus may be habitable for some forms of life, leaving a slight possibility that extraterrestrial psychrophiles may exist, have existed, or may someday exist there, making these moons one of the most promising targets for upcoming astrobiological research and space missions.

Several types of data were collected for the comparison, allowing the results to be more precise. Since the information about psychrophiles in this research is primarily averaged, it does not compare any particular species of microorganism; rather, it is derived from books and research papers about extremophiles, and more specifically, psychrophiles.[16, 17] The data related to living conditions on the moons under review were gathered from NASA reports, books, and studies on the moons' habitability.[18, 19, 20] Average suitable temperatures, suitable/present environments, energy sources, metabolisms, and oxygen requirements/presences were analysed for both psychrophiles and moons, as they are one of the most important aspects for creating conditions favourable for life.[21]

Step-by-step procedures:

1. Data collection. The required data were gathered from the sources mentioned above.
2. Comparison. The parameters of psychrophiles and the living conditions of Ocean Worlds were combined into a single table for visual demonstration, with various levels of compatibility assessed.

[16] Craig L. Moyer, R. Eric Collins, and Richard Y. Morita, "Psychrophiles and Psychrotrophs," *Elsevier eBooks*, (2016): 1.
[17] Saleem Farooq Rather, "Psychrophiles: Their Habitat and Applications," *ResearchGate*, (2019): 28.
[18] Parkinson et al., "Habitability of Enceladus: Planetary Conditions for Life," 361.
[19] T. M. Becker et al., "Exploring the Composition of Europa with the Upcoming Europa Clipper Mission." *Space Science Reviews* 220, no.49 (2024): 49.
[20] Robert T. Pappalardo et al., *Europa* (University of Arizona Press, 2009), 353.
[21] Siddiqui et al., "Psychrophiles," 98.

3. Analysis. The acquired results were summarised, discussed, and conclusions were made.

These procedures were chosen because they can simply and distinctly demonstrate the similarities and disparities between psychrophiles and the moons, without relying on more precise experimental data, which, unfortunately, was not available during this research. This study does not imply, however, that further empirical evidence is unnecessary.

Current estimates of icy moons' habitability are limited by the fact that the data and calculations in this research are based on indirect interpretations of other researchers' studies and speculative modelling, and are not yet supplemented by any empirical evidence. This limitation was taken into consideration during the comparative habitability assessment, and the evidentiary status of each value was presented in the table. Hence, it is imperative to explore these environments in more detail with both remote and in situ detection methods.

The information for the 'psychrophiles' column (Table 1) was collected from research papers by Siddiqui et al.,[22] Maayer et al.,[23] and Moyer et al.[24] Books like "Habitability of Enceladus: Planetary Conditions for Life"[25] and "Europa"[26] provide valuable data about the moons' structure and environments. Research articles by Hand et al.,[27] Szalay et al.,[28] and Glein et al.[29] convey information about the presence of chemical elements, crucial for sustaining life, and the pH of Enceladus' ocean. Similar data can also be found in the review article by Martin and McMinn.[30]

COMPARATIVE HABITABILITY ASSESSMENT

Many research papers and scientific reports have confirmed the presence of hydrothermal activity on Enceladus. The composition and the limited size range (2 to 8 nanometres in radius) of stream particles indicate ongoing high-temperature (>90 °C) hydrothermal reactions associated with global-scale geothermal activity that quickly transports hydrothermal products from the ocean floor at a depth of at least 40 kilometres up to the plume of Enceladus.[31] In October 2015, the Cassini spacecraft flew directly

[22] Siddiqui et al., "Psychrophiles."
[23] Pieter De Maayer et al., "Some Like It Cold: Understanding the Survival Strategies of Psychrophiles," *EMBO Reports* 15, no. 5 (2014): 508.
[24] Moyer, Collins, and Morita, "Psychrophiles and Psychrotrophs," 1–2.
[25] Parkinson et al., "Habitability of Enceladus: Planetary Conditions for Life," 361.
[26] Robert T. Pappalardo et al., *Europa*, 431.
[27] Kevin P. Hand, Robert W. Carlson, and Christopher F. Chyba, "Energy, Chemical Disequilibrium, and Geological Constraints on Europa," *Astrobiology* 7, no. 6 (2007): 1009.
[28] J. R. Szalay et al., "Oxygen Production From Dissociation of Europa's Water-ice Surface," *Nature Astronomy* 8, no. 5 (2024): 572.
[29] Christopher R. Glein, John A. Baross, and J. Hunter Waite, "The pH of Enceladus' Ocean," *Geochimica Et Cosmochimica Acta* 162 (2015): 2.
[30] Martin and McMinn, "Sea Ice, Extremophiles and Life on Extra-Terrestrial Ocean Worlds," 8–9.
[31] Hsiang-Wen Hsu et al., "Ongoing Hydrothermal Activities Within Enceladus," *Nature* 519, no. 7542 (2015): 209.

through the plume of escaping material and sampled its chemical composition. Waite et al.[32] found that the plume contains molecular hydrogen, H_2, a sign that the water in Enceladus' ocean is reacting with rocks through hydrothermal processes.

Temperature comparisons in Table 1 reveal that Europa and Enceladus satisfy the psychrophiles' requirement of a comfortable temperature. Terrestrial cold-loving microorganisms best thrive from –20 to 10°C, while average thermal conditions of Europa and Enceladus' subsurface oceans are predicted to be −4 to 0°C and −23 to 0°C, respectively [33]. Their ice shells and subsurface oceans also provide environments with conditions that mostly overlap with those tolerated by terrestrial psychrophiles, since their ordinary habitats are ice, permafrost, and cold oceans.[34] Psychrophiles on Earth are generally anaerobic, meaning that they do not require oxygen for life. Biospheres which they could potentially inhabit on Europa and Enceladus are unlikely to have plenty of oxygen, too. Although terrestrial psychrophiles naturally reside in neutral-alkaline habitats (6–9), pH levels in at least some places on these moons are either highly acidic or basic (2–11 and 8.5–12), suggesting that they may not be suitable for them. Many terrestrial psychrophiles acquire energy through chemosynthesis of H_2, H_2S, Fe^{2+}, NH_3, or organics, a process where microorganisms convert chemical energy into food energy (carbohydrates), rather than using sunlight as in photosynthesis. The Cassini mission has found hydrothermal H_2, CO_2, CH_4, and NH_3 in Enceladus' plumes, while Europa is assumed to possess radiolytic oxidants and seafloor H_2. A lot of psychrophilic microorganisms are methanogens, organisms that use the process of methanogenesis for metabolism. This anaerobic respiration process involves the reduction of a simple carbon compound, such as carbon dioxide (CO_2) or acetate, to methane (CH_4), both of which were, to some extent, found on Enceladus. Also, life on Europa would likely be chemolithoautotrophic, meaning that it obtains energy by oxidising inorganic chemical compounds (like H_2, H_2S, Fe^{2+}, NH_3, etc.)

Table 1

Comparative Habitability Assessment of Psychrophiles and the Moons

Criteria	**Description**	**Psychrophiles**	**Europa**	**Enceladus**	**Compatibility**	**Evidence Status**
Temperature	The average temperature at which psychrophiles can thrive	-20 to 10°C	−4 to 0°C	−23 to 0°C	Strong	Modeled/estimated

[32] J. Hunter Waite et al., "Cassini Finds Molecular Hydrogen in the Enceladus Plume: Evidence for Hydrothermal Processes," *Science* 356, no. 6334 (2017): 2.
[33] Martin and McMinn, "Sea Ice, Extremophiles and Life on Extra-Terrestrial Ocean Worlds," 8–9.
[34] Siddiqui et al., "Psychrophiles," 88.

Environments	Usual/comfortable habitats	Ice, permafrost, cold oceans	Ice shell, subsurface ocean	Ice shell, subsurface ocean, hydrothermal vents	Strong	Inferred
Oxygen	Aerobic/anaerobic respiration	Often low-O_2, many anaerobes	O_2 from the surface, but limited delivery	Anoxic ocean; O_2 absent	Partial	Inferred
pH level	Acidity and alkalinity of the environment	Neutral–alkaline (6–9)	Acidic–alkaline (~2–11)	Alkaline (~8.5–12)	Partial	Modeled/estimated
Energy source	What they use to acquire energy	Chemosynthesis (H_2, H_2S, Fe^{2+}, NH_3, organics)	Radiolytic oxidants + seafloor H_2	Hydrothermal H_2 (serpentinization); CH_4, CO_2, NH_3	Strong	Observed/inferred
Metabolism	Reactions that provide energy	Aerobic & anaerobic; includes methanogenesis	Likely chemolithoautotrophy; O_2	Likely anaerobic methanogenesis	Partial	Hypothesized

Notes: The table compares terrestrial psychrophiles, Europa, and Enceladus across six criteria: temperature, environment, oxygen, pH levels, energy sources, and metabolism. The descriptions of the criteria are also provided in a particular column.

The next section begins by discussing the potential habitability of Europa and Enceladus for psychrophilic microorganisms, considering environmental conditions that could sustain life. It then examines the possible adaptations and survival strategies that such microorganisms may employ in these icy ocean worlds. Third, it explores the types of potential biosignatures that could indicate the presence of psychrophilic life and how they might be detected in future missions. Lastly, it suggests other characteristics that are not reviewed in this paper or are not thoroughly reviewed from the perspective of psychrophiles.

ENVIRONMENTAL CONSIDERATIONS FOR THE HABITABILITY OF PSYCHROPHILES

Psychrophiles are typically found in environments as cold as ice, permafrost, and cold oceans. Enceladus's subterranean ocean was found by NASA's Cassini spacecraft through two main types of evidence: analysis of its "tiger stripe" geysers and gravity measurements from its Doppler-shifting orbital path. Europa's subterranean ocean was discovered by analysing magnetic field data from NASA's Galileo spacecraft, which detected an induced magnetic field consistent with a salty, conductive layer beneath the moon's icy crust. Other key evidence includes surface geological features like fractures and ridges that suggest movement of subsurface material. Thus, Jupiter's and Saturn's satellites can indeed provide environments suitable for alien analogues of terrestrial psychrophilic life.

Facultatively anaerobic psychrophilic bacteria are widely distributed in nature and can easily survive in oxygen-free habitats, such as ice shells, the subsurface ocean, and deep-sea hydrothermal vents. Szalay et al.[35] found that $12 \pm 6\ \mathrm{kg\ s^{-1}}$ ($2.2 \pm 1.2 \times 10^{26}\ \mathrm{s^{-1}}$) O_2 is produced within Europa's surface, less than previously thought, with a narrower range to support habitability in Europa's ocean. However, charged particles breaking molecular bonds in surface ice, thus dissociating the water to ultimately produce H_2 and O_2, might provide a potential oxygenation mechanism for Europa's subsurface ocean. The same idea might also be applied to Enceladus. But the current scientific consensus is that Enceladus's ocean is largely anoxic (lacking free oxygen).

Estimates for the Enceladus subsurface ocean pH vary significantly; Hsu et al.[36] derived pH values of 8.5–10.5 from plume nanoparticle analysis, while a thermodynamic model of carbonate speciation produced by Glein et al.[37] alludes to a 'soda ocean' (pH ~ 11–12). Research by Weber et al.[38] alludes to a range that would include potential for both acidic and basic ocean conditions for Europa: ~2–11 and ~4–6. Generally, most of the cold-adapted microorganisms are known to yield maximum enzyme activity in the neutral to alkaline pH range.[39] However, if extraterrestrial psychrophilic life hypothetically exists on these moons, it does not mean that it's identical to our terrestrial life; it would presumably develop adaptations to these harsh conditions.

The thick ice shell and surface snow on the Ocean World planets cause high light attenuation. Midday

[35] Szalay et al., "Oxygen Production From Dissociation of Europa's Water-Ice Surface," 572.
[36] Hsu et al., "Ongoing Hydrothermal Activities Within Enceladus," 209.
[37] Glein, Baross, and Waite, "The pH of Enceladus' Ocean," 25.
[38] Jessica M. Weber et al., "A Review on Hypothesized Metabolic Pathways on Europa and Enceladus: Space-Flight Detection Considerations," *Life* 13, no. 8 (2023): 2.
[39] Junaid Furhan, "Adaptation, Production, and Biotechnological Potential of Cold-adapted Proteases From Psychrophiles and Psychrotrophs: Recent Overview," *Journal of Genetic Engineering and Biotechnology* 18, no. 1 (2020): 5.

summer irradiance beneath the ice can be as low as 1–2% of the incident surface irradiance.[40] Because of this, photoautotrophic life will likely not be present underneath the surface. It is also highly unlikely for them to exist on the surface due to the intense UV radiation, which can either directly damage DNA, alter biomolecules, inactivate biochemical activities (e.g., alteration of protein synthesis), or induce the formation of reactive oxygen species (ROS), which requires an energetically expensive antioxidant response. Thus, Europa and Enceladus' fauna would probably consist of chemotrophic organisms, which would also reside under the surface.

Metabolism on Europa and Enceladus can be both aerobic and anaerobic; however, the second option is more likely to occur due to the O_2 deficiency. If oxidants reach the ocean, aerobic/microaerobic chemotrophy could occur; otherwise, largely anaerobic chemolithoautotrophy using seafloor reductants. Hydrogenotrophic methanogenesis, powered by H_2 from hydrothermal activity, may be utilised by psychrophiles on Enceladus.[41, 42]

Hydrostatic pressure and salinity were not considered in the main comparison due to the lack of reliable information for the "Psychrophiles" column. However, some missing information was assumed, allowing an unreliable conclusion about these criteria compatibilities to be made. According to Byrne et al.[43], the pressure at the ocean floor of Enceladus is about 7 MPa, while the pressure at the ocean floor of Europa is considerably larger, at about 210 MPa. Similarly, Jansen et al.[44] claim that pressures on Enceladus and Europa are <50 bar (5 MPa) and >1000 bar (100 MPa), respectively. They also estimate the salinity to be around 5–30 g/kg on Enceladus and below 100 g/kg on Europa. If we only consider terrestrial marine psychrophiles, we can assume that their salinity value is close to normal seawater salinity, approximately 35 g/kg, and their pressure value would range from ~0.1 MPa to ~40 MPa. This puts them in a strong compatibility range for Enceladus, but an extremely low compatibility range for Europa. Though if we consider deep-sea psychropiezophiles, the compatibility level would likely rise.

All the values presented in the table almost entirely overlap with the range of environments suitable for terrestrial psychrophiles to live, although some, such as pH levels, may not be fully suitable for psychrophilic life. Although if independently evolved extraterrestrial life truly emerged there, it would not necessarily be identical to terrestrial psychrophiles, since, as shown in the results of this comparison, environmental conditions on Jupiter's and Saturn's moons do not entirely coincide with preferable tolerance ranges of terrestrial psychrophiles. As Jebbar et al.[45] claim, because of this, life on Europa and

[40] Martin and McMinn, "Sea Ice, Extremophiles and Life on Extra-Terrestrial Ocean Worlds," 6.
[41] Waite et al., "Cassini Finds Molecular Hydrogen in the Enceladus Plume: Evidence for Hydrothermal Processes," 2.
[42] Rory M McKeown et al., "Psychrophilic Methanogenic Community Development During Long-term Cultivation of Anaerobic Granular Biofilms," *The ISME Journal* 3, no. 11 (2009): 1231.
[43] Paul K. Byrne, "The Geology of the Rocky Bodies Inside Enceladus, Europa, Titan, and Ganymede," paper presented at the 49th Lunar and Planetary Science Conference, LPI Contribution No. 2085, Lunar and Planetary Institute, Houston, May 22, 2018.
[44] Malte F. Jansen et al., "Energetic Constraints on Ocean Circulations of Icy Ocean Worlds," *The Planetary Science Journal* 4, no. 6 (2023): 117
[45] Mohamed Jebbar et al., "Microbial Diversity and Biosignatures: An Icy Moons Perspective," *Space Science Reviews* 216, no. 1 (2020): 1.

Enceladus would most likely be polyextremophilic — able to survive and thrive in multiple extreme environmental conditions simultaneously — and also methanogenic, since they are among the proposed earliest independent lineages in the tree of life. Having numerous extremotolerances that make them suitable candidate organisms for ocean world biomes, understanding biosignatures of methanogenic life in the fossil record may be informative for their detection on ocean worlds.

Although both Europa and Enceladus may provide suitable conditions for psychrophiles, their environments are not entirely identical. Europa features a deep ocean, much deeper than that of Enceladus, which puts higher oceanic pressure on the organisms at the seafloor. Therefore, Europan microorganisms may also be piezophilic (thriving at high pressures), in addition to being psychrophilic. Europa is also presumably warmer (Table 1), meaning that its potential psychrophiles would have to adapt to less extreme temperatures than on Enceladus. On the other hand, Enceladus has evidence of hydrothermal activity and molecular hydrogen, making hydrogen-dependent metabolism, including methanogenesis, a potentially relevant pathway. Thus, the potential habitability of two moons should not be interpreted as implying identical biological and metabolic conditions.

POTENTIAL HABITABLE ZONES FOR PSYCHROPHILES

According to both Martin & McMinn[46] and Pappalardo et al.,[47] the potential niches on Europa and Enceladus in which psychrophiles might live are the seafloor-ocean boundaries and ice-ocean boundaries. The tidal current and hydrothermal activity warm up the ocean, creating pleasant thermal conditions, especially at the bottom.

Regions within the icy shell may also be habitable: fissures caused by bending and thermal effects at the ice-water interface; a sub-ice mixing layer, in which materials are exchanged between the ice and ocean; a crushed-ice "keel," corresponding with isostatic support for surface ridges; migration of subsurface ice material to other regions, causing subsequent upwarping. In the thick-shell model, convection and the resulting diapirs and brine channels could provide habitable niches.[48]

BIOSIGNATURES AND POTENTIAL DETECTION METHODS

Biosignatures are the traces that indicate the presence of organic life. As shown in research by Schwieterman et al.,[49] biosignatures can be grouped into three broad categories: gaseous, surface, and temporal biosignatures. In this scheme, gaseous biosignatures are direct or indirect products of metabolism, surface biosignatures are spectral features imparted on radiation reflected or scattered by

[46] Martin and McMinn, "Sea Ice, Extremophiles and Life on Extra-Terrestrial Ocean Worlds," 8.
[47] Robert T. Pappalardo et al., *Europa*, 589.
[48] Robert T. Pappalardo et al., *Europa*, 702.
[49] Edward W. Schwieterman et al., "Exoplanet Biosignatures: A Review of Remotely Detectable Signs of Life," *Astrobiology* 18, no. 6 (2018): 666.

organisms, and temporal biosignatures are measurable time-dependent modulations that indicate the presence of a biosphere acting on a planetary environment. Detecting combinations of all these biosignatures (rather than one alone) is what would make the case compelling.

Gaseous signatures include O_2, O_3, nitrous oxide (N_2O), CH_4, methyl chloride (CH_3Cl), ethane (C_2H_6), NH_3, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and methanethiol (CH_3SH). Surface signatures include absorption and reflection of light by pigments in living organisms, scattering by the physical structures of organisms (including individual organisms and community architectures), degradation products of biological molecules, complex organics, fluorescence of pigments, and bioluminescence. Life on these moons will most likely possess peptides containing prebiotic amino acids. Mancinelli et al.[50] hypothesise that the combination of homochirality and the frequency distribution of amino acids makes them attractive as a biosignature for Enceladus and Europa. Additionally, the state of chemical disequilibrium (the long-term coexistence of chemically incompatible species, whose persistent presence suggests a continual resupply by biological sources) has been proposed as a biosignature and can be used to guide the search for life in exoplanet atmospheres as well.[51] However, many of these signatures can have an abiotic explanation, such as CH_4, which can be explained by hydrothermal/geochemical processes, or chemical disequilibrium, which can be caused by geological sources. To be certain of their biological origin, we must look for specific combinations of observations. For instance, observing CH_4, chemical disequilibrium, and surface signatures, like pigmentation, altogether in one area would make the biological interpretation more persuasive. Detecting molecularly complex organics, which could also be results of an abiotic synthesis, along with a fitting chemical context and additional biosignatures would make the case more compelling. Pigments also require substantial contextual evidence.

Space missions to other planets require portable instruments that use minimal power, require no sample preparation, and provide suitably diagnostic information to an Earth-based exploration team.[52] Certain specific techniques, which include mass spectrometry, fluorescence/reflectance spectroscopy, and gas chromatography, can offer strong insights into potential biosignatures.

Mass spectrometry, an analytical technique used to measure the mass-to-charge ratio (m/z) of ions to determine the mass, elemental composition, and structure of molecules, is one of the satisfactory methods for this purpose (e.g., Europa Clipper's SUDA, intended to analyse the fragments of Europa's surface constantly ejected into space by micrometeorites). This method is capable of detecting molecular and chemical biosignatures, like breakdown products of biological molecules, cold-adapted enzymes, proteins, polyunsaturated fatty acids, or cell membranes that may be found as fragments in ice grains ejected from plumes. The presence of these signatures would strongly indicate the presence of life and psychrophilic adaptation.

[50] Mancinelli, Mckay, and Davilla, "Enceladus: Biosignatures," 1.
[51] Schwieterman et al., "Exoplanet Biosignatures: A Review of Remotely Detectable Signs of Life," 692.
[52] Laura Garcia-Descalzo et al., "Mass Spectrometry for Direct Identification of Biosignatures and Microorganisms in Earth Analogs of Mars," *Planetary and Space Science* 72, no. 1 (2012): 1.

Fluorescence spectroscopy utilises a beam of light that excites the electrons in molecules of specific compounds, causing them to emit light. That light is directed towards a filter and onto a detector for measurement and identification of the molecule or changes in the molecule. Psychrophiles may be detected by the fluorescence of their pigments using fluorescence spectroscopy methods.[53, 54]

Similarly, reflectance spectroscopy is a non-destructive analytical technique that measures the amount of light reflected from a sample as a function of wavelength to determine its chemical, physical, and optical properties. This method can be utilised for the detection of pigmented microorganisms on diverse surfaces of potentially habitable worlds.[55, 56] Because these types of measurements can be made without contacting the sample, they become powerful techniques for the remote non-destructive detection of extraterrestrial life on the surfaces of bodies in the solar system.

One of the most commonly used techniques for organic detection is gas chromatography. It is based on an analytical technique that separates, identifies, and quantifies chemical compounds in a mixture by using a gas to transport the sample through a column containing a stationary phase. This method is also used for detecting complex organics, and finding them would very likely indicate the existence of extraterrestrial life.

Since rover missions to Mars require portable instruments that use minimal power and require no sample preparation,[57] these types of devices can be equipped and utilised for this purpose. For instance, organic-carbon-bearing mudstones, which can be identified as potential biosignatures, have been recently discovered by the Perseverance rover.[58] The discovery relied on *in situ* spectroscopy and imaging by Perseverance's instrument suite (SHERLOC, PIXL, SuperCam, WATSON, Mastcam-Z) — a great demonstration of how portable, non-destructive instruments can detect organics and minerals on another planet. Similarly, the Mars Curiosity rover carries plenty of different tools for various tasks, such as the Sample Analysis at Mars (SAM) Instrument Suite, which includes both a gas chromatograph and a mass spectrometer, and also a tunable laser spectrometer. Curiosity's instrumentation also includes several other spectrometers, like APXS, ChemCam, and CheMin.[59]

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[53] Poch et al., "Remote Sensing of Potential Biosignatures From Rocky, Liquid, or Icy (Exo)Planetary Surfaces," 231.
[54] Siddharth Hegde et al., "Surface Biosignatures of exo-Earths: Remote Detection of Extraterrestrial Life," *Proceedings of the National Academy of Sciences* 112, no. 13 (2015): 3886.
[55] Poch et al., "Remote Sensing of Potential Biosignatures From Rocky, Liquid, or Icy (Exo)Planetary Surfaces," 231.
[56] Hegde et al., "Surface Biosignatures of Exo-Earths: Remote Detection of Extraterrestrial Life," 3887.
[57] Garcia-Descalzo et al., "Mass Spectrometry for Direct Identification of Biosignatures and Microorganisms in Earth Analogs of Mars," 1.
[58] Joel A. Hurowitz et al., "Redox-driven Mineral and Organic Associations in Jezero Crater, Mars," *Nature* 645, no. 8080 (2025): 332.
[59] John P. Grotzinger et al., "Mars Science Laboratory Mission and Science Investigation," *Space Science Reviews* 170, no. 1–4 (2012): 6.

LIMITATIONS AND FURTHER DIRECTIONS FOR STUDY

There are still at least five characteristics by which the habitability of psychrophilic microorganisms on Europa and Enceladus has not yet been comprehensively reviewed in this article. It remains unknown whether they can survive in non-terrestrial conditions, including salinity, pressure, gravity, light absence/abundance, and cosmic radiation. However, some of those conditions are already discussed in the research by Martin & McMinn,[60] but it does not directly focus on psychrophiles. Moreover, this paper does not review other branches of extremophiles, such as hyperthermophiles, alkaliphiles, acidophiles, etc., which may also exist on the same moons. For instance, hyperthermophilic microorganisms may possibly live next to the high-temperature (>90 °C) hydrothermal vents of Enceladus.[61] This leaves a gap for further scientific research to close.

CONCLUSION

This research paper aimed to determine whether Europa and Enceladus can be habitable, whether psychrophilic microorganisms can inhabit them, and what biosignatures might indicate their presence. The comparative habitability assessment was used to evaluate the habitability of Europa and Enceladus-like environmental conditions. The comparison showed substantial overlap between the environmental conditions estimated for Europa and Enceladus and the known tolerance ranges of terrestrial psychrophiles, particularly temperature, oxygen, habitat, energy sources, and potential metabolic pathways, while pH suitability remains uncertain. Such results suggest a possibility that some environments on Europa and Enceladus may be potentially habitable for some forms of psychrophilic life, making these moons one of the most promising targets for upcoming astrobiological research and space missions. They are not, however, definitive evidence of life existing there. Furthermore, these calculations are based on indirect interpretations of other researchers' studies and are not yet supplemented by any empirical evidence. Hence, it is imperative to explore these environments in more detail with both remote and in situ detection methods.

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[60] Martin and McMinn, “Sea Ice, Extremophiles and Life on Extra-Terrestrial Ocean Worlds,” 5–8.
[61] David Deamer and Bruce Damer, “Can Life Begin on Enceladus? A Perspective From Hydrothermal Chemistry,” *Astrobiology* 17, no. 9 (2017): 6.

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