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Crystal Ball

Hydrostatic pressure is the universal key driver of microbial evolution in the deep ocean and beyond

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Oceans cover approximately 70% of the Earth's surface, and microbes comprise ~90% of the ocean biomass and are regarded as an important 'hidden' driver of essential elemental cycling, such as carbon cycling, in the oceans (Karl, 2007; Salazar and Sunagawa, 2017). Although the general public – even many scientists – think of the oceans as unified, stable water systems, they contain varied environments, including extreme environments such as oxygen-deficient zones, oligotrophic open ocean, polar water regions, deep ocean, hydrothermal vents, cold seeps, and so on, where specific microbial communities have evolved (Fig. 1). Hydrostatic pressure influences the physiology of organisms living at depth in the oceans, which is the largest habitat of the biosphere in terms of volume (1.3×10^{18} m^3) (Whitman *et al*., 1998). While traditional biological oceanographic research focuses more on the photic zone of the ocean, more attention is now being paid to the dark deep ocean due to the advancement of deep-ocean engineering technologies and the rapid progress of molecular biology, particularly next-generation DNA sequencing technologies.

The deep ocean refers to those ocean waters with a depth greater than 1000 m, corresponding to a hydrostatic pressure of higher than 10 MPa. Knowledge of the composition and distribution of the microbiome in the deep ocean and subsurface environments is accumulating; for instance, mostly thermophilic, sulfur-metabolizing microbes, such as *Thermoccoccus*, *Pyrococcus*, and *Episilonproteobacteria*, are found to be the major functional groups in the hot fluid venting hydrothermal vent chimneys, and the microbial community has been discovered to shift to a community metabolizing iron–sulfur minerals after the venting ceased (Hou *et al*., 2020), indicating energy source is the driving force behind the microbiome shift. Microbes in the subsurface are shown to have metabolic flexibility, which helps them survive in energy-deficient environments (Li *et al*., 2020). Various environmental factors, such as temperature, salinity, nutrients, and chemical reactants (electron donors and electron acceptors), have been investigated to understand their roles on deep sea microbial growth, and some factors shown to be critical in shaping community structure and even the evolution of the microbiome.

Although high hydrostatic pressure (HHP) is the most common environmental factor in the ocean and subsurface (Fig. 1), its independent and joint effects with other environmental factors on microbiomes (structure, function, and evolution) have never been systematically investigated and understood. Therefore, here we call for the scientific community to give special attention to this largely present but mostly ignored environmental parameter, i.e., HHP, and its roles in shaping microbial physiology, community structure, and evolution.

HHP has profound effects on cellular physiology and cytological behaviour, including transcription, translation, membrane compositions, multimeric protein assemblages, protein structure, and cellular motility, which have been studied in piezosensitive model organisms like *Escherichia coli* and *Saccharomyces cerevisiae* as well as in several deep-sea piezophiles, mainly under pressures lower than 60 MPa (Bartlett, 2002). According to the growth capability under different HHP, microorganisms could be classified as piezosensitive (with slower growth at high pressure than at atmospheric pressure), piezotolerant (with similar growth at atmospheric pressure and at high pressure), piezophilic (with faster growth

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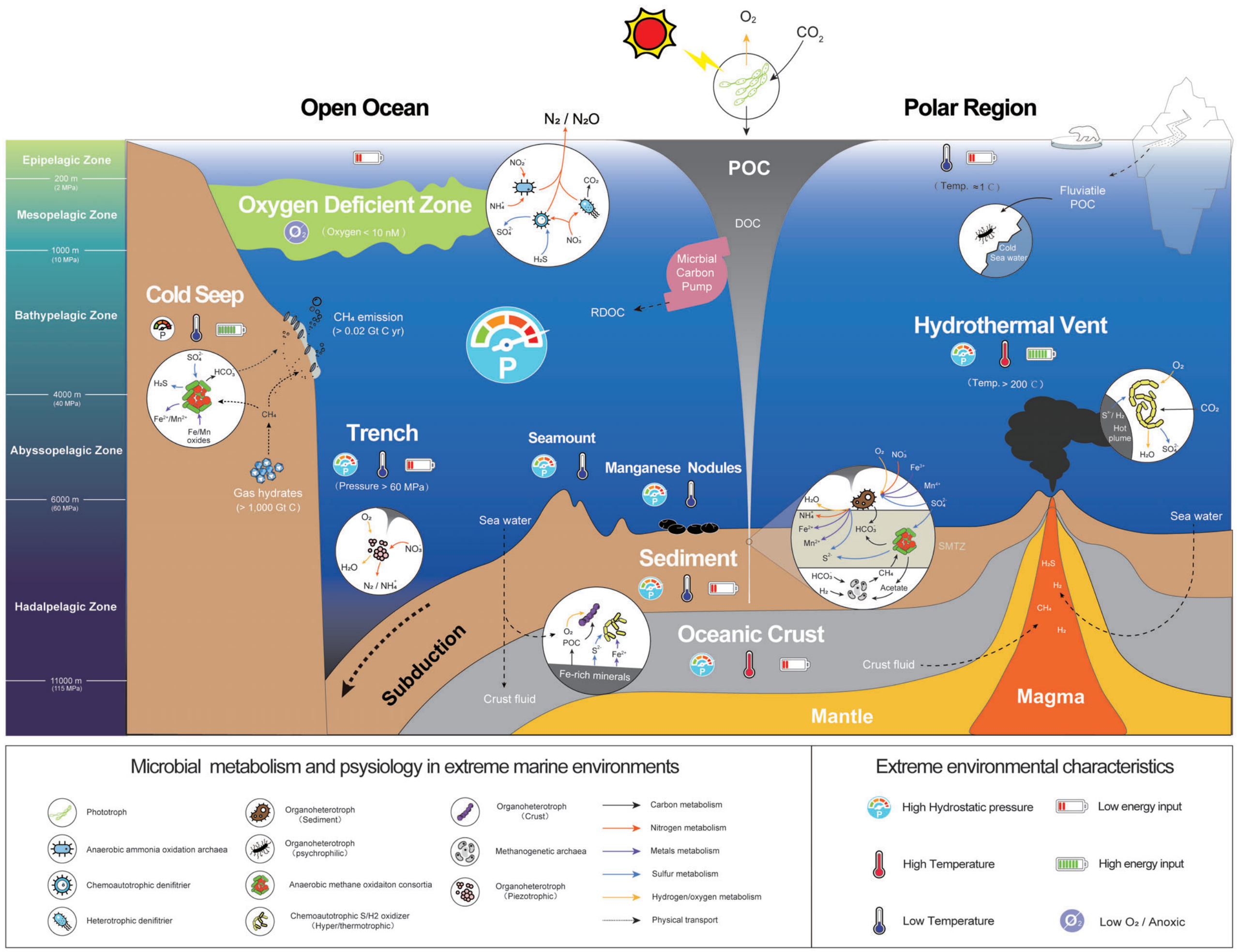

Fig 1. Cartoon shows representative extreme environments in the ocean: oxygen-deficient zone, oligotrophic open ocean, polar region, cold seep, marine trench, hydrothermal vent, deep-sea sediment, and oceanic crust. The major microbial functional groups and their mediated geochemical reactions in C, N, S elemental cycling in these specific environments are illustrated in the corresponding circles. Besides the canonical biological pump, the microbial carbon pump that has been proposed plays important roles in ocean carbon cycling (Jiao *et al*., 2010). Note that high hydrostatic pressure is the most universal physical parameter with which the majority of life on Earth must cope.

at high pressure than at atmospheric pressure), obligate piezophilic (with growth only at high pressure) (Fig. 2A). The adaptation mechanisms of microbes toward HHP are varied and include membrane adjustment, transcription regulation, and osmolyte production, as is the case when cells cope with other stresses, such as nutrient limitation and extreme pH/Tm/salt conditions (Oger and Jebbar, 2010). A common adaptation strategy triggered by excessive reactive oxygen species (ROS) is generally a response to oxidation imbalance under extreme conditions (Fig. 2B). This strategy was proposed to allow microorganisms to simultaneously adapt to HHP along with multiple other stresses (Zhang *et al*., 2015). The antioxidant defence mechanism has been shown to be the key mechanism for cells to cope with HHP, as illustrated with the deep-sea bacterium *Shewanella piezotolerans* WP3 as a model organism (Xie *et al*., 2018). Recently, the hypothesis that piezophiles have developed a common adaptation strategy to cope with multiple types of stresses, including HHP, was further underpinned by demonstrating that piezophilic characteristics are not restricted to strains from high pressure environments (Wang *et al*., 2020).

Despite accumulating knowledge on cells' adaptation mechanisms to HHP, its impact on the geochemical functions of cells *per se*, microbial ecology, and evolution remains poorly understood. Most marine microorganisms live in HHP environments below 1000 m (higher than 10 MPa), whereas less than 100 piezophilic strains have been isolated and reported since the first obligate piezophilic bacterium was isolated at 1981 (Yayanos *et al*., 1981). HHP is not taken into account as a critical factor when considering the origin and evolution of life on early Earth, when the extent of HHP ecosystems was

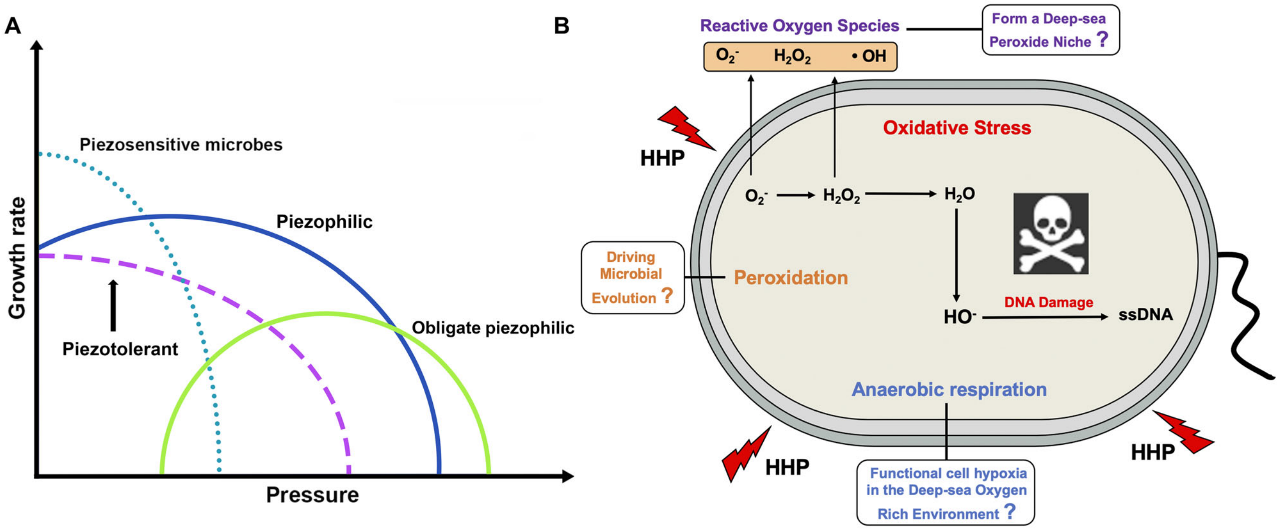

Fig 2. Cartoon shows the effects of HHP on microbial growth (modified from Fang *et al*., 2010) (A) and the potential influences of intracellular peroxidation triggered by HHP (B).A. The current terminology defines pressure-adapted microorganisms as piezotolerant (with similar growth rates at atmospheric pressure and at high pressure), piezophilic (with faster growth at high pressure than at atmospheric pressure), and obligate piezophilic (with growth only at high pressure).B. Graphic display of how high pressure-induced peroxidation influences metabolism, ecology and evolution of life in the deep ocean. Functional cell hypoxia: due to the intracellular peroxidation induced by high pressure, even under aerobic conditions, cells may tend to have anaerobic respiration instead of aerobic respiration to avoid further peroxidation damage. Form a deep-sea peroxide niche: HHP induces an increase of intracellular ROS concentration (Oger and Jebbar, 2010), their release (such as cell lysis by virus attacking or cell death) to the surrounding area may form a super-oxidation zone. Driving Microbial evolution: ROS scavenging is assumed to have originated early in the evolution history of life (Khademian and Imlay, 2020), and to be an essential driving force of life evolution. Nevertheless, it is unclear if and how HHP induced peroxidation would have a role in driving the evolution of deep life.

even larger than now (Picard and Daniel, 2013). Whether HHP is one criterion to define the habitable zone is also debatable as the ‘killing-pressure’ (~200 MPa) revealed in laboratory conditions (Abe, 2007), has never been verified in a natural habitat.

HHP may induce significant metabolic alterations within cells, as it alters the dissolved gas concentration and the intracellular oxidation status (Xie *et al*., 2018; Yang *et al*., 2020). The former impact is straightforward in that more gas is dissolved under elevated hydrostatic pressure. Large populations of living organisms on Earth exchange gas with their environments, and many core metabolic processes are related to gas. The effects of the oxidative stress caused by high pressure on cells are totally unknown, and we wish to draw special attention to this topic in future research (Fig. 2B). First, do cells prefer anaerobic respiration to avoid more oxygen damage caused by HHP (Xie *et al*., 2018), even in oxygen-rich deep-sea environment? Is functional cell hypoxia common in the deep ocean? Second, are the reactive oxygen species induced by HHP released into the extracellular space to form a periphery oxidized niche? Third, has HHP-induced peroxidation played an important role in life evolution? Another question is whether interactions between microorganisms in microbial communities may improve resistance to HHP? By exchanging compatible solutes, stress tolerance may be improved at the community level.

Moreover, virus-induced lateral gene transfer and antioxidant responses may further help the community gain genomic advantages for living in pressurized conditions. Thus, genomic diversity is expected to be largely expanded in the deep biosphere. The impact of HHP should be considered on a planetary scale over geological time. HHP may change the element stable isotope fractionation ratio, which should be considered for data interpretation; however, this aspect is being ignored due to scant information and a lack of available data. In short, little is known regarding the effects of HHP on microbial structure, function, and evolution. We should not remain blind to this aspect when planning the next phase of deep ocean microbiome exploration.

What has been learned from the piezophilic life on Earth has inspired thoughts about extraterrestrial life. Martian life – if it existed or exists – would continuously shrink its surface footprint and ultimately retreat to the subsurface environments, where pressure increases (Cabrol, 2017). The icy moons of Jupiter or Saturn, and even the outer solar system, present the possibility of potential abodes for life in subsurface liquid waters (Sephton *et al*., 2018). The pressures at the water–rock interfaces of Europa, Titan, and Enceladus are estimated

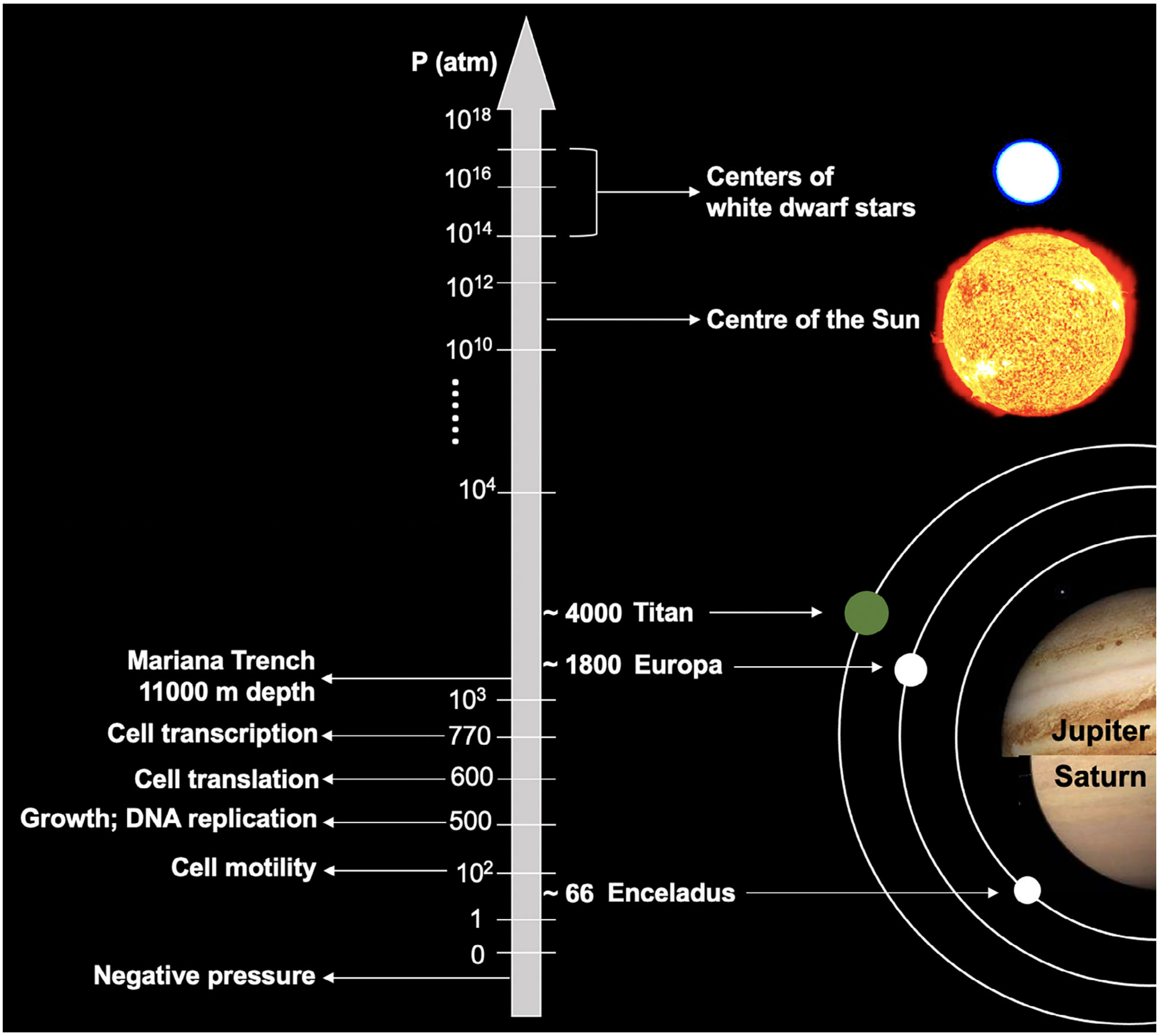

Fig 3. Cartoon shows pressure range in solar system (right panel) and the effects of HHP on cellular process (left panel). The pressure range of Titan, Europa, and Enceladus were calculated by sea water density, acceleration of gravity, ice thickness, water depth. Cell motility, Growth and DNA replication, Translation, Transcription are influenced by HHP at 10, 50, 60, 77 MPa respectively. The pressures at the water-rock interfaces of Enceladus, Europa, and Titan are estimated to be ca. 6.6, 180, 400 MPa respectively.

to be ca. 180, 400, and 6.6 MPa, respectively (Fig. 3). Given that there are synergistic and antagonistic effects between high pressure and other conditions (e.g., temperature and ions), some of the stresses may act in a compensatory manner: e.g. increases in pressure and decreases in temperature reduce membrane fluidity – a rise in pressure of 1000 atm is equivalent to a decrease in temperature of approximately 13–21°C (Somero, 1992; Zhao *et al*., 2020) – further enabling cells to inhabit even deeper, hotter environments. We observed that supplementation of $MgCl_2$ increased the highest growth pressure of a microbial community enriched from Mariana Trench sediment from 152 to 182 MPa (unpublished data). Therefore, when the co-adaptation of multiple environmental factors is considered, the habitable zone for life may be much larger than we previously expected.

Most life on the planet lives in a HHP condition, and even life probably originated in a HHP environment, yet it is astonishing how little we know about the effects of HHP on cell physiology, community structure, function, and evolution. With the testable hypotheses proposed above, and exploiting novel technologies especially biosensors for measuring cellular physiological conditions, biochemical reactions under HHP both under *in situ* and laboratory conditions, we anticipate our understanding on

HHP's roles in shaping microbial structure, functions, and evolution will be elucidated in the near future.

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