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## Tracing the microbial biosphere into the Messinian Salinity Crisis

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The Messinian salinity crisis (MSC), one of the largest environmental crises in Earth history, occurred in the Mediterranean Basin about 6 Ma ago. The isolation of the Mediterranean from the Atlantic Ocean caused the transformation of the Mediterranean sea into a giant salina. The establishment of harsh conditions (hypersalinity and anoxia) in the water mass had a strong impact on the aquatic biosphere, resulting in the apparent disappearance of many marine biota. This aspect is however controversial, mostly because of the finding of fossils of biota that actually survived the onset of the MSC. To trace the response of life to this catastrophic event, we studied the microbial biosphere (both body fossils and molecular fossils) archived in the sediments straddling the MSC onset (shales, carbonates and sulphates) from marginal subbasins (Piedmont Basin, northern Italy, and Nijar Basin, southern Spain). Despite the significant reduction of calcareous plankton, the progressive rise of other microorganisms (prokaryotes and eukaryotes) is documented in the studied sediments at the MSC onset. These microorganisms include remains of euryhaline and stenohaline diatoms and filamentous microfossils interpreted as vacuolated sulphide-oxidizing bacteria. This fossil assemblage, which typifies both marginal (gypsum) and more distal (carbonates and shale) deposits, indicates conditions of high primary productivity in the surface waters, favoured by increased nutrient influx in the course of high riverine runoff.

Molecular fossils allow tracing of the microbial biosphere into the geological past. The rise of algal compounds (e.g. dinosterol) in the basal MSC deposits (gypsum, carbonate and shales), accompanied by the simultaneous increase of terrigenous organic material (n-alkanes), agree with the eutrophication of the basin. In addition, the MSC deposits show an instant and significant increase of archaeal biomarkers, including the archaeal membrane lipids archaeol and extended archaeol, mostly produced by halophilic archaea, documenting a change toward harsh conditions. The co-occurrence of crenarchaeol, a compound mostly sourced by marine planktic Thaumarchaeota, above the MSC onset indicates that normal marine conditions were still present, probably in the upper water column, which was apparently underlain by a lower hypersaline layer. The local presence of tetrahymanol, a biomarker of ciliates or anoxygenic phototrophic bacteria, confirms water mass stratification, since this compound is predominantly found in stratified water bodies.

In conclusion, the comprehensive study of body fossils and molecular fossils from different peripheral Mediterranean subbasins reveals that the onset of the MSC was typified by an expansion of microorganisms. The microbial biosphere archived in the basal MSC sediments agrees with a stratified basin characterized by locally persistent marine conditions.