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# The challenge of proving the existence of metazoan life in permanently anoxic deep-sea sediments

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Please see related Commentary article: Animals, anoxic environments, and reasons to go deep, <http://dx.doi.org/10.1186/s12915-016-0266-1>

## Abstract

The demonstration of the existence of metazoan life in absence of free oxygen is one of the most fascinating and difficult challenges in biology. Danovaro et al. (2010) discovered three new species of the Phylum Loricifera, living in the anoxic sediments of the L'Atalante, a deep-hypersaline anoxic basin of the Mediterranean Sea. Multiple and independent analyses based on staining, incorporation of radiolabeled substrates, CellTracker Green incorporation experiments and ultra-structure analyses, allowed Danovaro et al. (2010) to conclude that these animals were able to spend their entire life cycle under anoxic conditions. Bernhard et al. (2015) investigated the same basin. Due to technical difficulties in sampling operations, they could not collect samples from the permanently anoxic sediment, and sampled only the redoxcline portion of the L'Atalante basin. They found ten individuals of Loricifera and provided alternative interpretations of the results of Danovaro et al. (2010). Here we analyze these interpretations, and present additional evidence indicating that the Loricifera encountered in the anoxic basin L'Atalante were actually alive at the time of sampling. We also discuss the reliability of different methodologies and approaches in providing evidence of metazoans living in anoxic conditions, paving the way for future investigations.

This paper is a response to Bernhard JM, Morrison CR, Pape E, Beaudoin DJ, Todaro MA, Pachiadaki MG, Kormas KA, Edgcomb VG. 2015. Metazoans of redoxcline sediments in Mediterranean deep-sea hypersaline anoxic basins. *BMC Biology* 2015 13:105.

See research article at <http://bmcbiol.biomedcentral.com/articles/10.1186/s12915-015-0213-6>

## Background

The Deep-sea Hypersaline Anoxic Basins (DHABs) of the Mediterranean Sea are one of the most extreme oceanic realms known on Earth. The bottom sediments of these regions are completely anoxic and covered by a thick and dense brine (from tens to hundreds of meters), which hampers oxygen exchange. In particular, in the L'Atalante basin, the anoxic conditions are present since more than 50.000 years [1]. These conditions have been assumed for a long time to be excessively harsh to allow

the life of multicellular eukaryotes, at least until the recent discovery of three new species belonging to Loricifera, a group of microscopic invertebrates (Metazoa). These three species were apparently able to live and complete their entire life cycle without access to free oxygen [2]. Using different and independent analyses based on incubations with radioactive tracers and specific fluorogenic probes (e.g. CellTracker Green), quantitative micro X-ray and infrared spectroscopy, and accurate analyses of different components of life cycles, Danovaro et al. [2] concluded that the loriciferans inhabiting the L'Atalante basin are metabolically active and show specific adaptations to the anoxic conditions. Furthermore, SEM and TEM analyses provided evidence that the cellular tissues were not degenerated.

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