The Bald disease in a natural population of the purple sea urchin *Paracentrotus lividus* from the Bay of Naples: From spines to tissues

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In the Mediterranean Sea, the spread of marine diseases represents a growing concern for biodiversity, especially when key ecological species are involved, including the purple sea urchin *Paracentrotus lividus*, currently affected by the Bald Sea Urchin Disease (BSUD). The BSUD has been described in several echinoids' species worldwide and in *P. lividus* populations from different coastal areas. However, the related studies are fragmented, poorly standardized and often limited to local observations.

This study characterized the BSUD in a natural population of *P. lividus* from the Bay of Naples in 2021, using an integrated approach, including gross examination, histopathology, scanning electron microscopy (SEM), immunohistochemistry, using two different cellular stress markers (Hsp70 and Hsp90), and metagenome. Nine individuals were examined and classified, according to the severity of their external lesions (Stage 0-3), in asymptomatic and symptomatic.

Symptomatic individuals showed the typical clinical signs of this disease, with spine loss, biofilm presence on the bare test and, in the most severe cases, ulcers. SEM observations revealed bacterial microcolonies, penetrating the lesions, often associated with filamentous algae and the presence coelomocytes migrating to the damaged areas. Histopathology highlighted the presence of lesions only in the most advanced disease stages (2-3), with inflammatory and regressive lesions, and lipofuscin-like pigment accumulation. Moreover, ciliated protozoa were observed in all the symptomatic individuals, often associated with Gram (+/-) bacteria.

Immunohistochemistry showed a basal expression of Hsp70 chaperones in the digestive epithelia and coelomocytes, with stronger immunopositivity in symptomatic individuals, suggesting the activation of the cellular stress response, associated with inflammation. Hsp90 was detected only in severe cases, in phagocytic or apoptotic coelomocytes. Finally, metagenome data highlighted an increased viral and bacterial presence in symptomatic individuals. In this context, this integrated approach provided a broader view of the BSUD in the population of the Mediterranean Sea.