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Bivalve gut microbiome responses to sediment characteristics and anthropogenic activities in *Cerastoderma edule* (Linnaeus, 1758)

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Abstract:

Estuaries are highly dynamic ecosystems that are influenced by fluctuating environmental conditions and sediment-associated contamination. These factors can significantly affect microbiomes associated with their hosts. In this study, we investigated the gut microbiome of the bivalve *Cerastoderma edule* by integrating bacteria (16S rRNA) and fungal (ITS) diversity data with sediment physicochemical parameters across three estuarine sites subjected to varying degrees of anthropogenic pressure. Sampling was conducted in the Sado Estuary (Portugal), a warm-temperate, mesotidal system. The selected areas, Tróia (TR), Gâmbia (GAM), and Navigator (NAV), represent contrasting environmental settings, from low-contaminated (TR) to more impacted zones influenced by industrial and aquaculture activities (GAM and NAV). Microbial diversity and composition were assessed on a seasonal basis using next-generation sequencing and multivariate analyses. Alpha diversity metrics revealed differences in bacterial communities between sites and seasons, whereas fungal diversity remained relatively stable across locations. This suggests that gut-associated fungi are more resilient to environmental pressures, although specific compositional shifts were observed in the impacted areas, with some taxa showing correlations with trace metal concentrations. Principal Component Analysis (PCA) indicated strong associations between gut microbial composition and sediment parameters, including organic matter, salinity, and trace metals. In particular, the bacterial communities exhibited strong responses to trace metals, such as Hg, Zn, and Pb. Functional inference (FAPROTAX) highlighted the microbial involvement in nitrogen, sulfur, and carbon cycling, indicating potential adaptive responses to contamination. Together, these findings support the utility of the *C. edule* gut microbiome as a sensitive indicator of estuarine environmental status and host–environment interactions. Moreover, they underscored the importance of including both bacterial and fungal components to obtain a more holistic understanding of microbiome-mediated ecosystem functions.

Keywords: Environmental disturbance; 16S rRNA; ITS sequencing; Estuarine ecotoxicology; Host–microbe interactions; Functional prediction

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