

## Experimental infection of the sea urchin *Paracentrotus lividus* by three *Vibrio splendidus*-related strains

Carella Francesca<sup>1</sup>, Nil Gonzalez Fibla<sup>2,3</sup>, Anna Callau<sup>2</sup>, Edgar Bertomeu<sup>2</sup>, Ana Roque<sup>2</sup>

1. Department of Biology, University of Naples Federico II, Napoli, Italy.
2. Institute of Agrifood Research and Technology (IRTA)-La Ràpita, Tarragona, Spain.
- 3 Institute of Professional Studies in Aquaculture and Environment in Catalonia (IEPAAC) La Ràpita, Tarragona, Spain.

The purple Sea urchin *Paracentrotus lividus* is an important commercial species for the Mediterranean countries. Mortality of *P. lividus* are reported during Spring at low incidences in the Bay of Naples, Campania (Italy) since 2020. Sick individuals typically display body wall lesions where spines, podia, pedicellaria and epidermis are lost, reported as Bald Sea Urchin Disease or BSUD syndrome. Previous bacteriological analysis from affected animals revealed the presence of 3 *Vibrio* species (*V. cicytrophicus*, *V. crassostreae* and *V. tasmaniensis*) belonging to the *Splendidus* clade. The 3 isolates were tested for pathogenic potential. Thus, *in vivo* infection assays were performed in three separated tanks with the 3 *Vibrio sp.* over 1 month on adults of *P. lividus* under controlled conditions. Prior to the experimental challenge, sea urchins were acclimatized in a water tank facilitated with a flow through system, with UV treated, and filtered seawater adjusted to a low water flow rate and oxygen saturation > 100%. The tank temperature was controlled and kept at ± 23-25 °C to mimic the local seawater temperature. The bacteria were injected (~10<sup>8</sup> CFU/ml) at level of peristomal membrane surrounding the mouth. Animals were evaluated for presence for disease symptoms (loss of appendages, test ulceration) over the experimentation. Animals were then collected for bacteriological, microscopical (histopathology, TEM) and molecular analysis.