finanziato dall'Unione Europea - NextGenerationEU a valere sul Piano Nazionale di Ripresa e Resilienza (PNRR) – Missione 4 Istruzione e ricerca – Componente 2 Dalla ricerca all'impresa - Investimento 1.1, Avviso PRIN 2022 PNRR indetto con DD N. 1409 del 14/09/2022, dal titolo "ENvironmental DRIvers affecting fattening and calcification process of wild and farmed MUSsels in the Adriatic Sea (ENDRIMUS)", codice progetto MUR P2022TEFRY - CUP J53D23013870001.

Titolo assegno di ricerca: Environmental drivers affecting calcification process of wild and farmed mussels in the Adriatic Sea

Progetto assegno

Mussels, belonging to the species *Mytilus galloprovincialis*, represent the most important species for the Italian shellfish production. The production comes mostly from aquaculture in lagoons and open-sea and to a smaller extent, from wild populations in the central and northern Adriatic Sea. In the last few years, both farmed and wild mussels have shown issues in terms of meat reduction and increased shell fragility, which negatively affect their quality, and hence their market value. Since farming technics have not been substantially modified with time, and changes also affect the wild populations, these impacts are inevitably caused by environmental phenomena that have occurred in recent years. In this contest, the project wants to pose attention on this phenomenon happening in the Adriatic Sea, identifying which environmental drivers can mainly affect the growth of mussels in this area. It has been established in literature that river discharges are the main source of nutrients and other inorganic elements, which are at the base of the trophic web in the Adriatic Sea. The project will evaluate which environmental drivers mainly affect the growth and quality of farmed and wild mussel populations along a latitudinal gradient from North to South of the Italian Adriatic coast. For this purpose, physical and biochemical features, as well as biological variables of the water column will be investigated, along with phytoplankton community abundance and composition, which represent an important source of food for these filter feeding animals. Biological analysis on mussel content and an in-depth study on the shell features at the macro, micro and nanoscale level will be carried out on samples from farmed and wild mussels collected at different sites, to evaluate how this bivalve species is sensitive to variations in environmental conditions. The project points out the importance of considering multiple environmental parameters to investigate bivalve growth and allow for a proper management of bivalve aquaculture. Indeed, given the great socio-economic relevance of *Mytilus galloprovincialis* along the Italian Adriatic coasts, projects like this one are crucial to guarantee a knowledge-based management of this important resource. The results produced will be disseminated to international, national, and regional stakeholders and the scientific community, in order to provide useful information for future development in mussel farming.

Piano di attività assegno.

Sampling of farmed and wild mussels. Wild mussels will be harvested by scuba diving from the artificial modules (from jacket of the offshore platform and from concrete modules of the artificial reefs), while farmed mussels will be directly taken from the culture ropes. All size classes will be sampled.

Mussel skeletal properties and calcification. Shell biometry (width, height, and thickness) will be measured with a caliper. Skeletal apparent porosity (percentage of the pore volume connected to the external surface), micro-density (mass per unit volume of the material which composes the shell, excluding the volume of pores) and bulk density (shell mass/volume ratio, including the volume of pores) will be measured by buoyant weight analysis. Net calcification rate (mass of $CaCO_3$ deposited per year per unit area) will be obtained as the product of shell bulk density and linear extension rate. Age will be measured in shells of different size in each site using annual internal growth bands and shell oxygen isotope ($\delta^{18}O$). By counting the total number of internal rings in each shell and the lighter $\delta^{18}O$ (summer) and heavier $\delta^{18}O$ (winter) peaks the age-length keys will be obtained and fitted with the von Bertalanffy growth function (VBGF). Variations in biometry, skeletal properties, and calcification rates will be compared among sites in relation to different environmental conditions and multivariate statistical approaches performed for data analysis.

Shell composition and microstructure. Shell characteristics (crystallography, polymorphism, pore structure, mechanical properties, organic matrix) will be determined using X-ray diffractometry (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric analysis (TGA), Scanning Electron Microscopy (SEM) and compression tests. Diffractometric measurements will determine the mineral phase that constitutes shells. The spectroscopic analyses (FTIR) will allow the characterization of the mineral phase, the organic matrix associated with the mineral phase and their interactions. Thermogravimetric analysis (TGA) will be used to estimate the % of organic matrix (OM) and the structurally associated intra-skeletal water content. The application of microscopic spectroscopic techniques (FTIR and Raman microscopies) will allow for mapping the distribution of the mineral and the organic matrices in the shells to verify if, during the skeleton growth, the relative amount and composition of the organic matrix along the shell may vary. The microscopic analyses, mainly scanning electron microscopy and scanning probe microscopy, will allow for observation of the shape and the architectural assembly of crystalline units that make the building blocks. To test for shell mechanical properties, compression tests will be conducted. The Young's modulus, and the required force to fracture will be recorded. It is well known that the architectural assembly of crystalline domains strongly influences the mechanical properties of materials. Thus, by combining the information obtained from X-ray diffraction and microscopic analyses, a complete view of the structural organization of the mineral phase of the mussel shell from the nano to the macro scale will be gained. Variations in the shell mineral composition and organic matrix content will be compared among sites in relation to different environmental conditions and multivariate statistical approaches performed for data analysis.