



# Mediterranean loggerhead sea turtle as potential sentinel species for anthropogenic threats: biomarker identification and protocol development to evaluate effects on reproduction and embryo development

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## Introduction:

In the Mediterranean Sea, anthropogenic threats are now widely spread and impact on the ecosystems and marine biota. To this, the Loggerhead Sea turtle, the most common sea turtle species in the Mediterranean Sea, was elected as sentinel species for bioaccumulation of contaminants and pathogens and as the official bioindicator for the descriptor 10 "Marine litter" in the Marine Strategy Framework Directive. Indeed, from embryos to adults, Loggerheads are affected by the presence of pollutants (microplastics MPs, heavy metals HMs, POPs, phthalates, etc.). The bioaccumulation of these pollutants could influence some physiological processes and the health status of these specimens. Particularly, even in embryonic stages, was highlighted the presence of several compounds suggesting a maternal transfer.

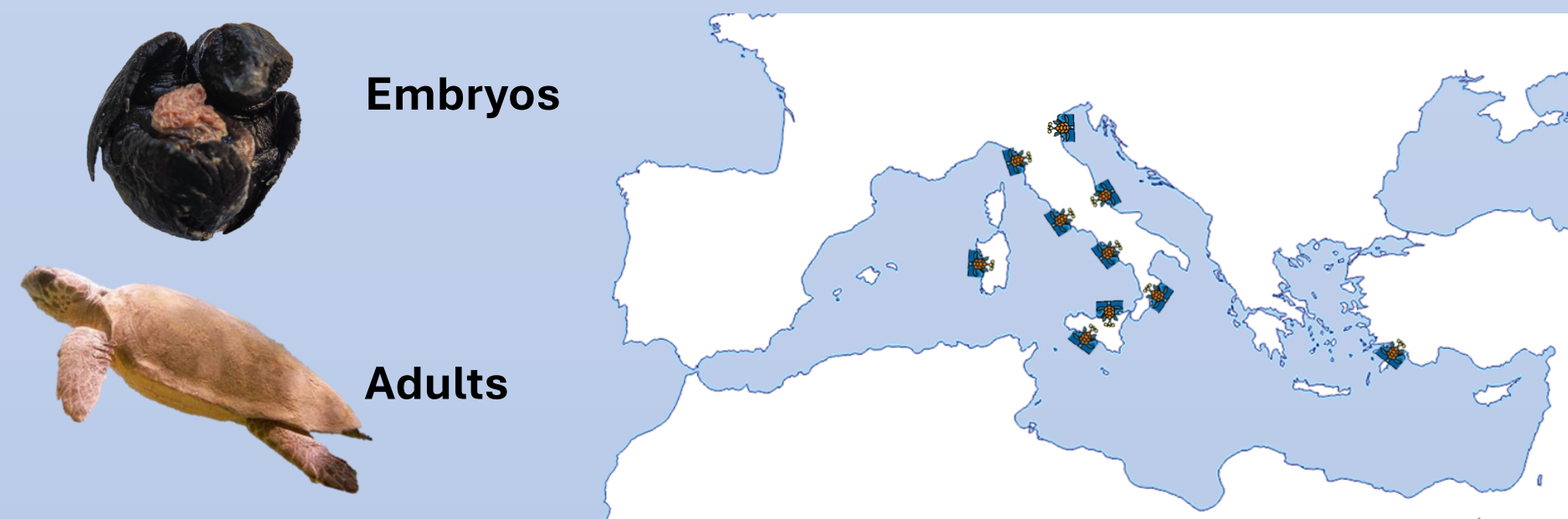
## Aim and Methods:

This study aims to identify suitable biomarkers to investigate the effects of anthropogenic threats on the development, health and reproductive status of Loggerheads, at different life stages in different Mediterranean areas and at different temporal scales. The final purpose will be the set up of sampling and analytical protocols to develop further conservation monitoring programs. Hence, any aspects of the development, health and reproductive status will be investigated through the achievement of specific objectives:

- Center Network and Sampling survey organization:** Building of an international network with scientific centers and development of shared standardized sampling protocol
- Pollutants assessment:** quantification and characterization of several compounds through Raman Spectroscopy, Atomic absorption spectrophotometer and High-Performance Liquid Chromatography) analyses
- Stress assessment:** stress status evaluation through Histology, Histochemistry, Proteomic and Transcriptomic analyses
- Metabolism assessment:** molecular characterization of yolk and liver in embryonic stages and adults with Histology, Proteomic, Metabolomic and Fourier Transform InfraRed spectroscopy analyses
- Sexual determination in embryos and reproductive status assessment in adults** through Histology, Histochemistry, Proteomic, Fourier Transform InfraRed spectroscopy analyses
- Validation of biomarkers:** Data analysis and statistical biomarker signature with the purpose of develop scientific standardized protocols for further conservation strategies

## Preliminary results:

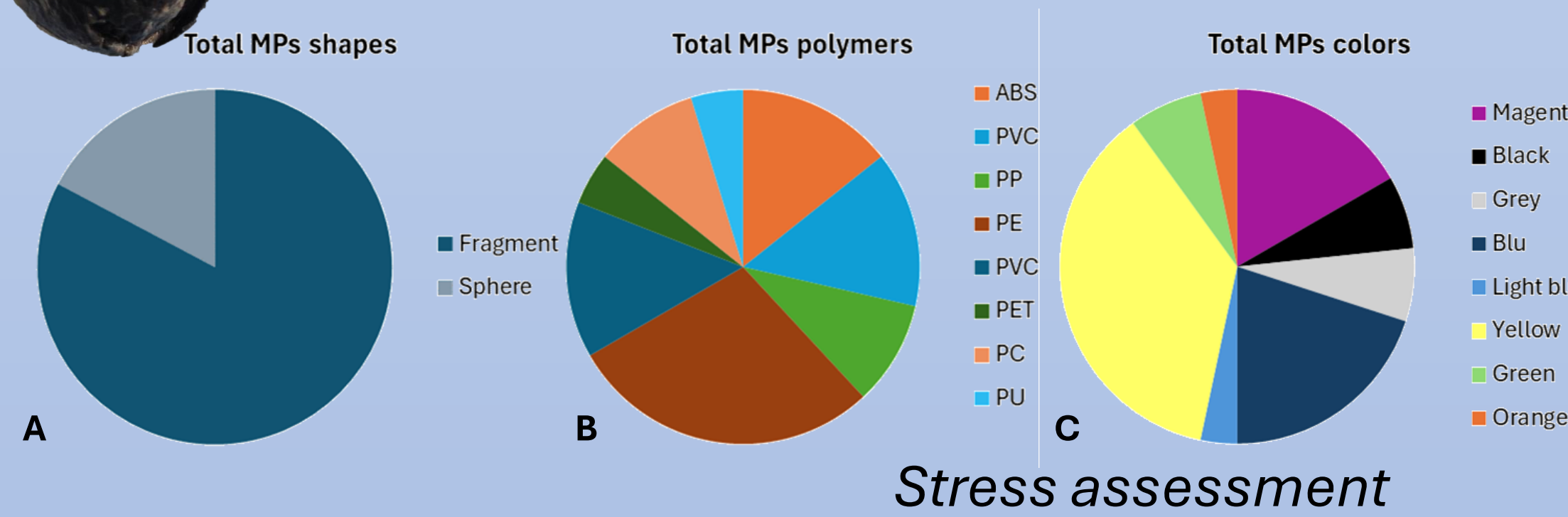
### Center network and sampling survey organization



**Figure 1.** Creation of a network with national and international scientific centers for sharing protocol and samples for analysis.

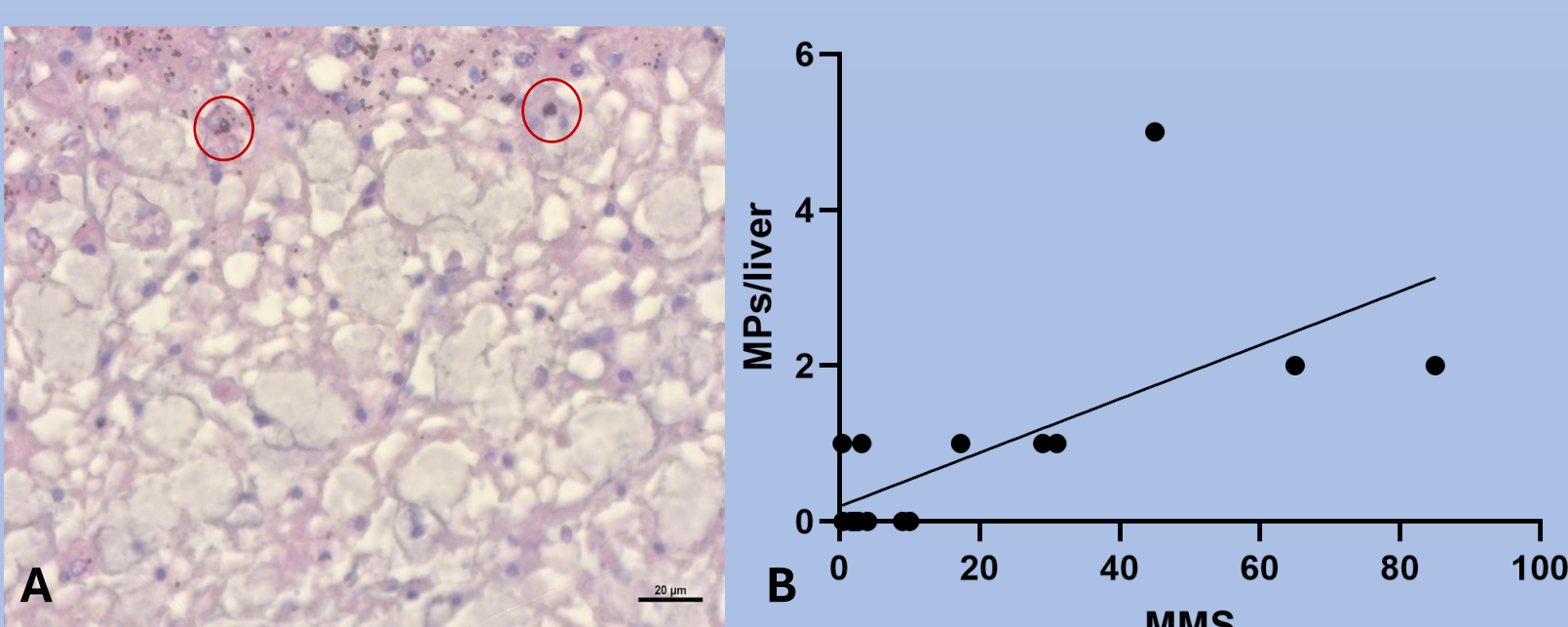
### Embryos

### Pollutant assessment

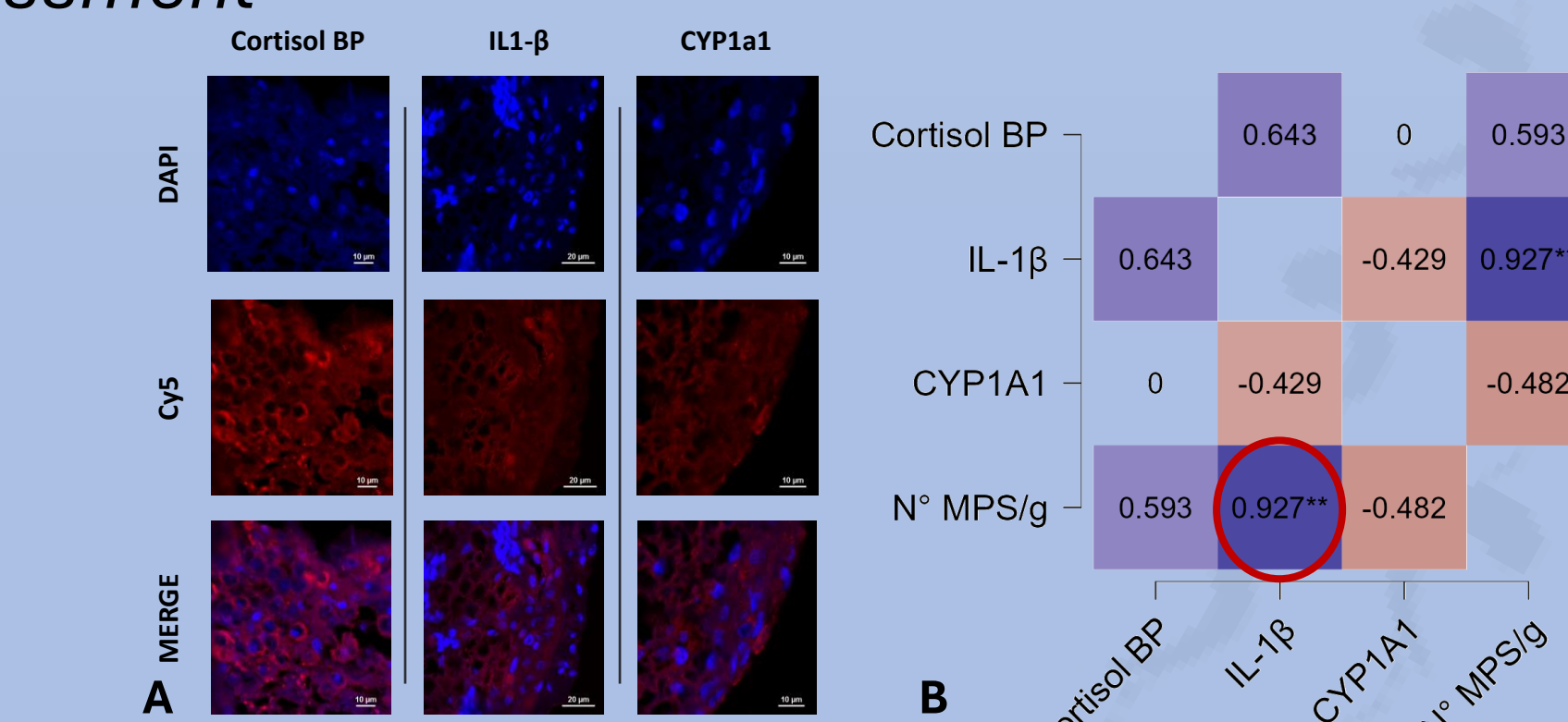


**Figure 2.** From liver samples, MPs were extracted and characterized. The most represented shape (A), polymers (B) and colors were the fragment (83%), Polyethylene (PE), Acrylbutadien styrene (ABS) and Polivini chloride (PVC) and yellow (36%) and blue (20%). All MPs identified were smaller than 5µm.

### Stress assessment

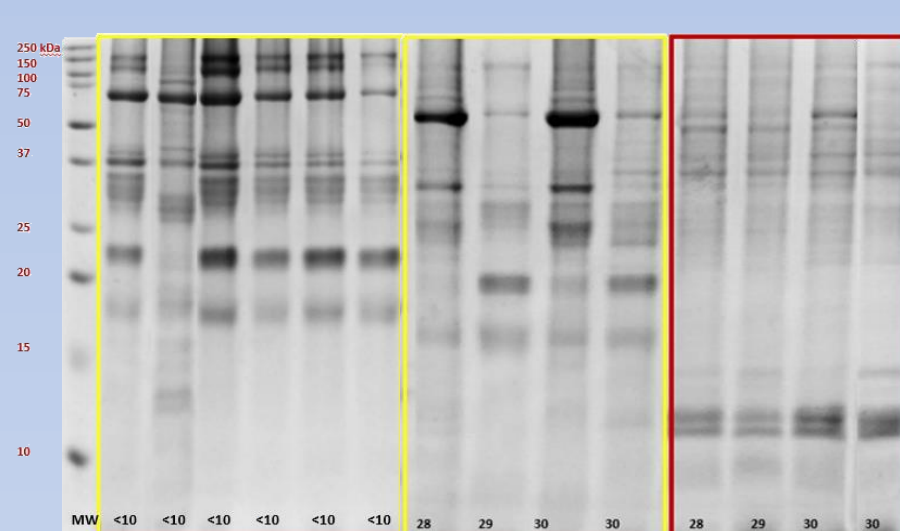


**Figure 3.** Histological images (A) of liver samples highlighting (red circle) the presence of melanomacrophages (MMs) biomarkers of stress condition. The following statistical analysis performed (B) showed a significant positive correlation between the number of MMs and the number of MPs (p value: 0,041; Spearman's rho: 0,501).



**Figure 4.** Assessment of immunofluorescence expression levels (A) of Cortisol Binding Protein, IL-1β and CYP1a1 biomarkers for stress condition, inflammatory process and contaminant exposure respectively. The heatmap (B) describing the Spearman correlation performed shows a positive strong correlation (red circle) between the expression level of IL-1β and the number of MPs (p value: 0,003; Spearman's rho: 0,927).

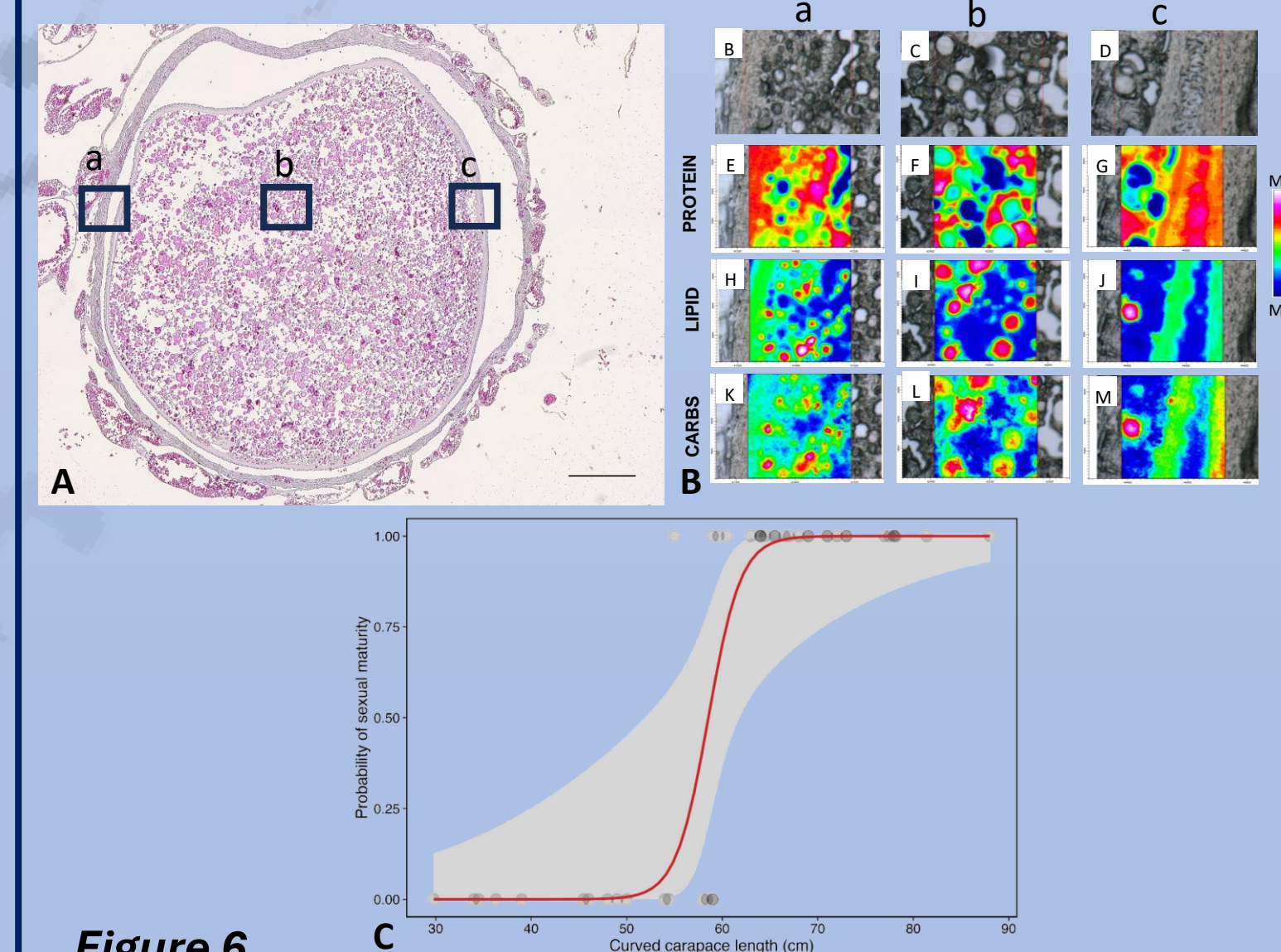
### Metabolism assessment



**Figure 5.** SDS page images characterizing the proteic pattern of yolk (yellow rectangle) at early and late embryonic developmental stages and liver (red rectangle) at late embryonic developmental stages (from <10 to 30 developmental stages).

### Adults

### Reproductive status assessment



**Figure 6.** Histological (A) and FTIR-imaging (B) images characterizing the female gonads. These analysis were used to characterize oogenesis and in particular vitellogenesis processes and to identify the size at first maturity for loggerhead females in the Adriatic Sea by the L50 analysis (C).

## Conclusion:

The presence of MPs and other pollutants even in the embryonic stages highlight a maternal transfer of these compounds that could induce effects on the health status of these embryos, such as stress conditions and inflammatory process as described in these results. The validation of further specific biomarkers could represent an optimal tool for investigating the health status of the sea turtle population in future conservational strategies.