

## Optimization of the preparation technique for the age reading of vertebrae of thornback ray (*Raja clavata*)

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Age composition of a fish stock is essential to determine the status of a fish stock and formulate advice for ICES and the European Commission. To be able to perform qualitative stock assessments, it is imperative to develop reliable age determination methods. The use of vertebrae to study age of rays and skates has proved to be a valuable method in the past. However, each species requires adapted cleaning, staining and drying methods. Therefore, during the SUMARIS project, an optimized protocol for age reading of thornback ray was developed.

Before observing and interpreting the growth rings on the vertebrae of rays, some preparatory steps are necessary such as vertebrae selection, cleaning, possibly embedding followed by sectioning of the vertebrae and band pattern enhancement by staining or burning. A segment of 10 to 20 vertebrae of the vertebral column were removed starting from behind the eyes up to the beginning of the tail. Cleaning is an essential step to observe the growth bands, but care needs to be taken not to denature the structure of the centra. Different cleaning methods were tested, from using hot tap water and ethanol to enzymatic cleaning using pepsin. Alizarin red, chrystal violet and silver nitrate illuminated with an UV-light were tested as staining methods for band pattern enhancement. The staining was tested on whole as well as on sectioned vertebrae.

As a result of the tests, an optimized protocol for age reading of thornback ray was developed. The used vertebral segment always needs to be sampled at the same place, because the number of growth marks may vary depending on the location of the vertebra on the skeletal axis of the same animal. Therefore, the first 10 vertebrae will be used because they are the largest. For cleaning the vertebrae, the enzymatic pepsin digestion was selected as best and for the band pattern enhancement chrystal violet was selected on whole vertebrae.

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