

Visualization of small lesions in skin of fish using fluorescein dye

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Assessing the effects of anthropogenic activities on the health of fish is important to safeguard the marine ecosystem. The impact of fisheries is mostly evaluated by monitoring the fish's reflexes or skin lesions. The latter is done with the naked eye. However, in this way, possibly present non-visible, microscopic lesions may be missed. Although these may seem small, they still may have a non-negligible impact on the health of fish. Indeed, they may be colonized by pathogenic bacteria such as *Vibrio tapetis* and *Aeromonas salmonicida* leading to the development of skin ulcerations. To allow the implementation of this risk factor in the impact-assessment of anthropogenic activities, these lesions need to be visualized.

The present study aimed at pinpointing a protocol to map microscopic skin lesions in salt water fish by means of fluorescein sodium salt. Fluorescein sodium salt is a yellow, nontoxic, water-soluble dye that is commonly used in ophthalmic medicine. Due to its high degree of ionization this dye is not able to penetrate or bind to intact epidermis. When the epithelial barrier is damaged, the fluorescein can penetrate the tissue and fill up intracellular spaces. Although fluorescein already proved its use in detecting skin lesions in fresh water fish it is not widely used in salt water species. In first instance, both macroscopic and microscopic skin lesions were inflicted on freshly euthanized marine fish by means of a scalpel, needle or rubbing with a net. Various immersion times (2min-4min-6min) in a fluorescein supplemented bath (0.2 mg/ml) and consequent rinsing protocols (2x 1min or 3x 1min) were tested. Fish were subsequently photographed under long wave-length ultraviolet (UV) light to visualize the lesions.

All induced lesions were clearly visible under ultraviolet light even if they were not discernible with the naked eye as they were stained with the fluorescein dye.

Generally, the shorter the immersion time, the weaker the staining. Therefore, the best staining was found when fish were placed in the fluorescein supplemented bath for a minimum of 6 minutes. With regard to the rinsing protocol, it was noted that three rinsing steps with clean sea water for one minute, were necessary to eliminate nonspecific staining of the fish which hampers a clear evaluation of the lesions.

To conclude, using the fluorescein dye allows to plot skin lesions in marine fish that are not macroscopically discernible. This rapid and cost-efficient method may prove valuable in various applications (e.g. fish disease monitoring; impact of fisheries, aquaculture), where an assessment of the impact on the fish's skin is needed, both macroscopic and microscopic.

Keywords: skin lesions; visualization; fluorescein dye; marine fish