Testing a mass marking technique using alizarin complexone on reared European flounders (*platichthys flesus*) to quantify restocking efficacy

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Introduction

Since 1993, more than 1.6 million reared juvenile European flounders (*Platichthys flesus*) have been released in the western part of the Limfjorden to support stock enhancement.[1] Despite this effort, little is known about their survival, growth and contribution to the local and regional fishery. Tagging hatchery-reared fishes prior to release enables differentiation from wild stocks and assessment of restocking success. Alizarin complexone (ALC) is a fluorescent dye and a well-known mass-marking technique that leaves a lasting mark in otoliths without harming the fish when applied with an appropriate dose. However, the optimal dose varies by species and life stage.[2] While ALC tagging has been used in related species such as Japanese flounder (*Paralichthys olivaceus*) and turbot (*Scophthalmus maximus*), no established protocol exists for European flounder.[2] The aim of the study is to find a cost-benefit balance between the amount of ALC applied in the annual restocking programme and obtaining optimum marks, i.e., obtaining marks that remain viable for a long period, while keeping the doses as low as possible.

Methods

Reared *P. flesus* (*n* = 136) originating from adults caught in the western Limfjorden were randomly selected from tanks at a hatchery to test different doses of ALC on juvenile European flounder (see Fig. 1 & Fig. 2).

The experiment comprised five treatment groups in various ALC concentrations (30, 40, 50, 75 and 100 mg/L) and a control group in pure seawater, all immerged for 24 hours. Salinity, temperature, pH and dissolved oxygen were measured from the tanks prior to the experiment and every hour during the 24-hours experiment to test for variations. Total length and weight were obtained on all individuals to test for different growth pattern within the six groups. Another group of individuals was double-tagged with ALC, 21 days apart, to evaluate the potential of separating close cohorts.

Sagittal otoliths were extracted from each of the groups after 1, 6 and 12 months of the ALC immersion to assess the strength of marking retention and durability. Light exposure was limited as much as possible. The otoliths were mounted on glass slides with clear thermoplastic resin, lightly polished and viewed using a Leica DM4B microscope, illuminated with UV light and photographed with a Leica MC190 HD camera.

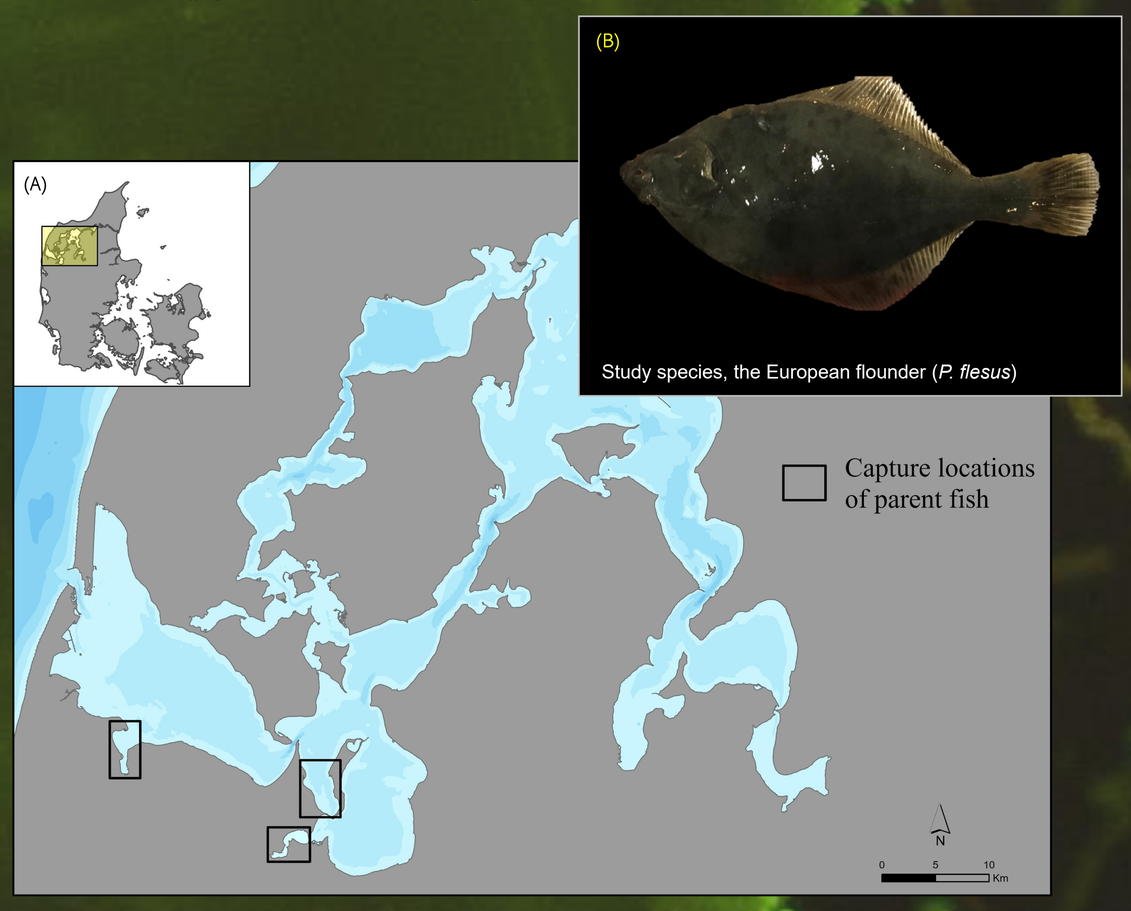

Fig. 1: (A) Map of the western part of the Limfjorden with capture sites of the wild caught adults around (1) Kilen, (2) Lemvig and (3) Venø Sound. (B) Dorsal view of the European flounder (*Platichthys flesus*).

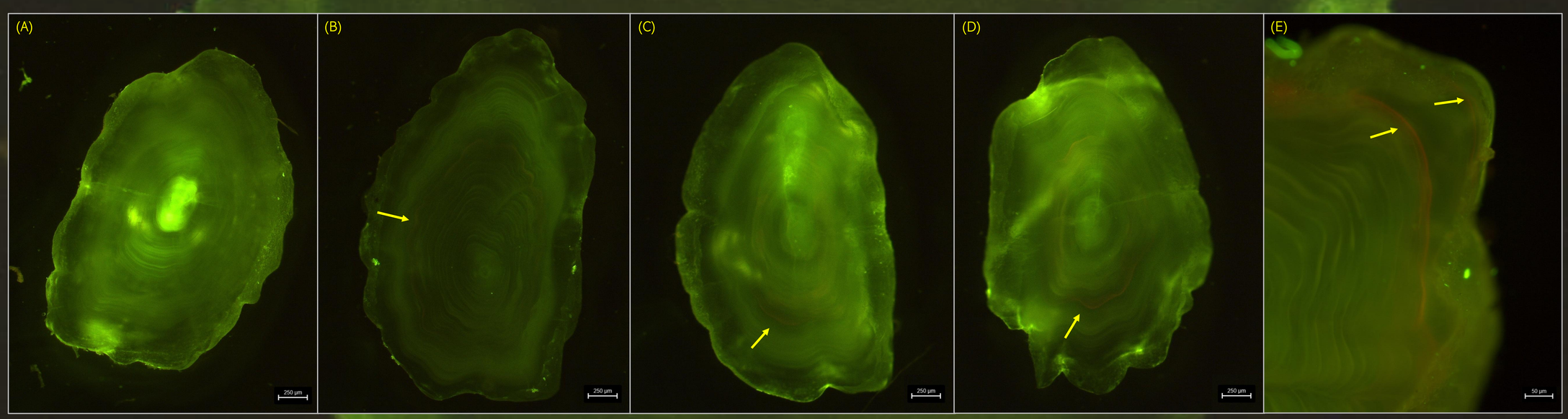

Fig. 2: Varying degrees of detectable marks in the otoliths. (A) No ALC marks (0 mg/L); (B) low ALC marks (40 mg/L); (C) moderate ALC marks (75 mg/L); (D) strong ALC marks (100 mg/L). (E) Strong and visible double ALC rings (100 mg/L per immersion).

Preliminary results and discussion

No mortalities occurred during the experiment. The flounders ranged between 31-110 mm in total length and weighed between 0.6-18.3 grams prior to the ALC immersion, resembling the release size of the annually reared juvenile flounders (3-10 cm). A preliminary visual assessment indicates that concentrations of 50 mg/L or higher produce varying, but clear, detectable marks under fluorescence microscopy, and that double tagging can be used to separate close cohorts. Further spectral and statistical analyses are, however, needed to define the cost-benefit balance between the ALC used in the restocking programme while creating optimum marks. This is to our knowledge the first study to apply ALC immersion tagging to European flounder (*P. flesus*), offering a promising and practical tool for long-term monitoring of restocking efforts in the Limfjorden.

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