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Comparison of three different otolith-based methods for age determination of turbot (*Scophthalmus maximus*)

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Abstract

Three different methods to prepare the otoliths for age determination in fish are currently used. These are burning, grinding or using the untreated ethanol-washed otoliths. The optimal preparation method will depend on the fish species. Direct comparison of these three methods has, however, never been performed for the turbot (*Scophthalmus maximus*) before. This was the aim of the present study. Age determination were performed in one sample of turbot (n = 49) before and after burning the otoliths, and in another sample (n = 29) before and after grinding the otoliths. All samples were from the Western Baltic (ICES sub-division 24). None of the methods were significantly superior to the other two. It is therefore concluded that the use of untreated ethanol-washed otoliths for the age determination of turbot is as valid as the more time consuming use of burned or ground otoliths.

Keywords: otoliths, age determination, turbot (*Scophthalmus maximus*).

Introduction

Knowledge of the age of the fish in a given population greatly facilitates stock assessment. Age can be determined from different 'hard structures' such as scales, vertebrae and otoliths.

Otoliths are often used for marine species in the northern and temperate waters. Theoretically, a pair of annual rings is formed every year, an opaque summer ring and a hyaline winter ring. The opaque ring is formed during the growth season and is broader than the hyaline ring, which is formed during the winter period, where the growth slow down. The age of the fish is usually determined by counting the hyaline rings.

In the published works on methods to prepare turbot (*Scophthalmus maximus*) otoliths for age determination, there is only little agreement on which method is the best (Kändler, 1949; Mengi, 1963; Jones, 1970; Weber, 1979; van Leeuwen & Rijnsdorp, 1986; and Lucio, 1986). However the authors do agree that, it is difficult to determine the age of turbots.

In this paper three methods of preparing turbot otoliths for the use in age determination are described, the determined ages compared and the results discussed.

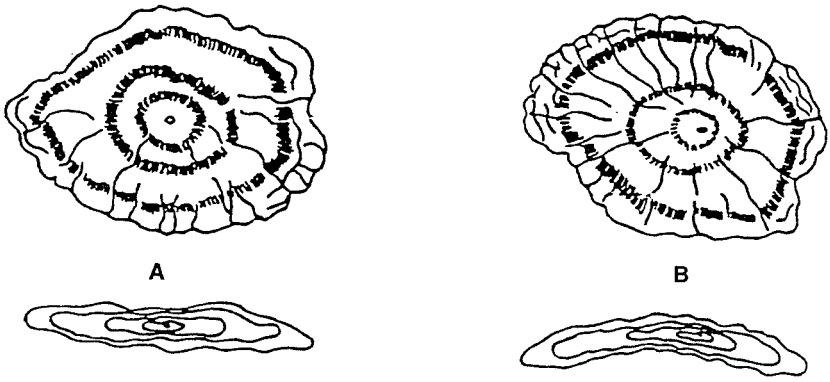

Fig. 1. Turbot (*Scophthalmus maximus*) otoliths. A: the dorsal otolith, and B: the ventral otolith.

Materials and methods

In turbot the dorsal (left) and the ventral (right) otoliths differ in shape (Fig. 1). The dorsal otolith has a symmetrical appearance, whereas the ventral otolith has a more asymmetrical shape. However, in spite of the differences in shape the number of annual rings formed on each otolith is the same and it is advisable to collect both otoliths in case one of them has a crystallized structure and is difficult to interpret.

The three methods used in the present study for preparing the otoliths were:

1. Untreated otoliths ($Age_{untreated}$). The cleaned but otherwise untreated otoliths were first soaked in 95% ethanol, dried and then replaced in the ethanol, before they were placed under a stereo-microscope and the hyaline rings counted. (If the otoliths are allowed to dry after the first 'ethanol bath' the hyaline rings are often easier to see.)
2. Burning the otoliths (Age_{burned}). The burning of otoliths is a method where the otoliths are placed in the flames of a Bunsenburner (Møller Christensen, 1964). The otoliths will progressively change colour from white to brown, ash gray and finally black. When the otolith has obtained the colour ash gray, it was removed from the flame and broken by gentle pressure on the concave site in the centre of the otolith. The otolith fragments were then fixed on a microscope slide, the fractured surfaces facing upward. Before placing the slides under the microscope, the surfaces were gently brushed with 95% ethanol. With this method the hyaline rings appear as brown rings on a yellow background.
3. Grinding the otoliths (Age_{ground}). The otolith was coarsely ground transversely to the nucleus and the gently polished. The hyaline rings appear as dark rings on a clear background. For the burning experiment otoliths from a sample (n = 54) from November 1987 were used and for the grinding experiment otoliths from a sample (n = 47) from April 1987 were used. Both samples originates from the same area in the Baltic Sea (ICES sub-division 24). The samples were obtained from the commercial landings, which means that only fish measuring approximately 32.5 cm (the official minimum landing size for turbot caught in the Baltic

Sea at that time) and above were represented. Only otoliths from female turbots were used. Otoliths with a crystallized structure or otoliths damaged during the preparations for the various methods were discarded. All together 7 pairs of otoliths were discarded from the first sample resulting in 47 pairs for the burning experiment, and 18 from the second sample resulting in 29 pairs for the grinding experiment.

To compare the three methods, the age of each fish was determined first by using the untreated otoliths, and thereafter determined from either the burned or ground otoliths. In each case the age determination was done twice by the same reader, and without the reader knowing anything about the fish length or the previous age determination. This mean that for each fish there are four corresponding age determinations.

Results

To test the reproducibility of the methods, the double age determinations from each method were compared (Fig. 2). The following results were obtained: the age determinations were the same ±1 year in 83, 88 and 78% of the cases for the untreated, burned and ground otoliths, respectively.

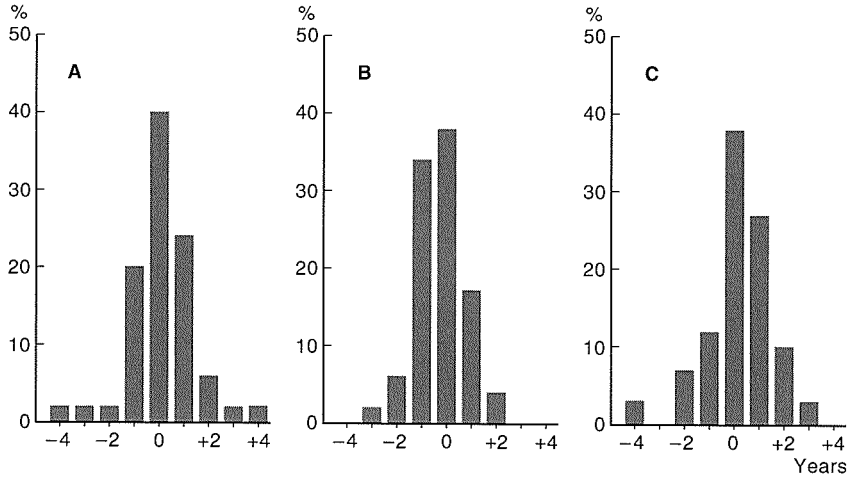

Fig. 2. Reproducibility of the three methods. The distribution of the difference (years) between the first and the second age determination are shown for the untreated (A), the burned (B), and the ground (C) otoliths.

In the following calculations, age represents the mean of the double age determinations. To test if there were any differences between the age determination when done on untreated versus burned and untreated versus ground otoliths, the results were tested with a Wilcoxon test for paired data. The test showed no significant differences in the age determinations between untreated/burned otoliths ($n = 47$, $T_{n=29} = 73.5$, n.s.) or between untreated/ground otoliths ($n = 29$, $T_{n=19} = 83$, n.s.).

To further explore whether there were any size dependent influence on the three methods, and whether this influenced the three methods differently, the following correlations were calculated. Linear regression analysis was used:

1. ($Age_{untreated} - Age_{burned}$) against fish length ($y = 0.09 \cdot$ fish length $- 2.82$; $r^2 = 0.08$, $p < 0.05$) and
2. ($Age_{untreated} - Age_{ground}$) against fish length ($y = 0.19 \cdot$ fish length; $r^2 = 0.18$, $p < 0.05$).

These results are shown in Fig. 3A and B.

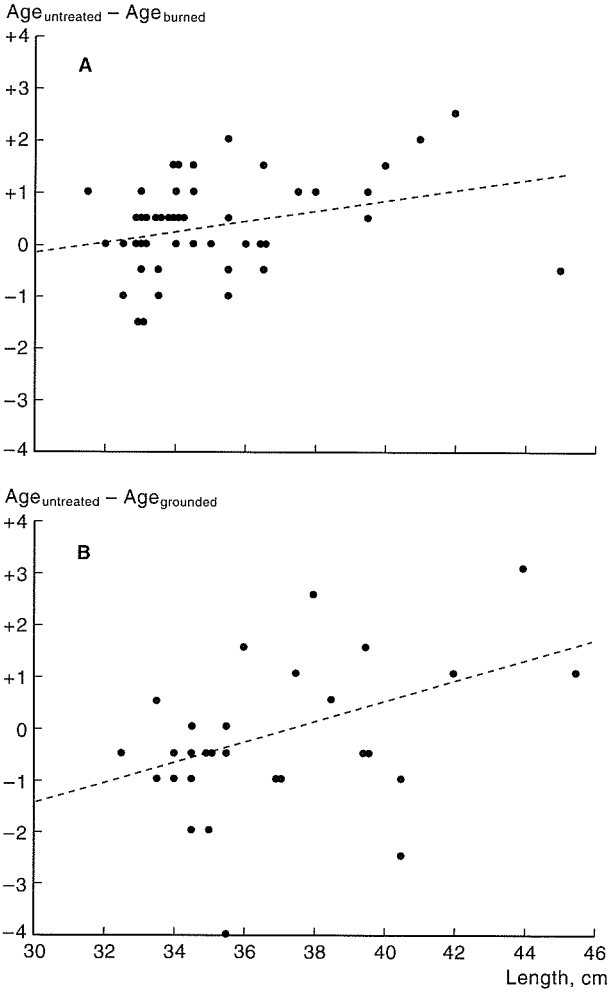

Fig. 3. A: the difference between age determinations done on untreated versus burned otoliths ($Age_{untreated} - Age_{burned}$) against fish length ($y = 0.09x - 2.82$; $r^2 = 0.08$). B: the difference between age determinations done on untreated versus ground otoliths ($Age_{untreated} - Age_{ground}$) against fish length ($y = 0.19x - 7.14$; $r^2 = 0.18$).

Discussion

The first published attempts to determine the age of turbots were described by Kändler in 1949, followed by Mengi (1963), Jones (1970), Weber (1979), van Leeuwen & Rijnsdorp (1986) and Lucio (1986). In the cited papers, different methods have been used for the preparing of turbot otoliths for age determination. But they have not been directly compared. There is only little agreement on which method is the best suited for the turbot otoliths.

In this study the reproducibility of the three methods were comparable and the determined ages were considered to be identical ($Age_{untreated} - Age_{burned}$ and $Age_{untreated} - Age_{ground}$). Further analysis showed a tendency towards a length-related difference between the three methods. In Fig. 3A a tendency toward slightly higher age determinations in 'bigger' fish can be seen, when untreated otoliths are used compared to the burned. In Fig. 3B the tendency is toward lower ages in 'smaller' fishes, when the untreated otoliths are used compared to the ground. These results indicate that there can be some minor differences between the three methods, which can not be detected in this study. Due to the low r^2 values and the shallow slopes, however, these differences are probably not of practical significance regarding the use in assessment work, within the age range (4-10 year) studied here.

In conclusion, none of the above results gave any indications to prefer one preparation method to the other. All three methods were found to have a certain degree of uncertainty which can only be reduced by larger samples.

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