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Report of the Workshop on Age Reading of Turbot (WKART)

24–27 June 2008 Oostende, Belgium



International Council for the Exploration of the Sea Conseil International pour l'Exploration de la Mer

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Executive summary

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) meeting in Valetta, Malta in March 2007, identified turbot (*Psetta maxima*) as a species requiring an ageing workshop to evaluate and improve the age interpretation based on stained slides of the otoliths. The Workshop can build on the results of the otolith exchange organized in 2004 and will be the first ageing workshop for turbot.

Two otolith sets were included in the workshop: a North Sea turbot set (N=110), and a Baltic turbot set (N=96). Thirteen readers out of six countries attended the workshop namely Belgium (3), France (2), Germany (2), The Netherlands (2), UK (1), Sweden (2) and Latvia (1). Unfortunately, the Baltic otolith set has been lost after the exchange and only the photographs remained as the basis for further investigation.

For turbot, one of the main difficulties is the interpretation of the first annual ring, causing uncertainty among the readers during the exchange. Because validated otoliths or agreed reference collections do not exist at present, the final debate of whether or not the first ring is indeed the first annual ring is still ongoing. The workshop therefore dedicated its effort to conclude to a common interpretation of this particular ring and thus improve the agreement among readers.

A manual on the preparation of turbot otoliths has been compiled, documented with a reference set of annotated images. These documents can be used as a guideline and can form the template for discussion when refining the interpretation of the growth pattern and for identifying gaps and opportunities concerning the current knowledge of the age estimation of turbot.

The two regions, North Sea and Baltic Sea, are treated separately as the particularities between the datasets from the regions are too different. Also, these areas comprise different stocks so that the combination is clearly to be avoided.

The overall agreement rate of the North Sea sample was 82.8%. The range of agreement with the modal age was 70.5–91.1%. The overall agreement rate of the Baltic sample was 71.6%. The range of agreement with the modal age was 55.8–87.4%. The lower score for the Baltic area originates mostly from the poor quality of the image set. Furthermore, the Workshop participants were formed out of two clearly separated Expert Groups i.e. the North Sea and the Baltic Sea. Using results from age readers who are not familiar with the selected area, clouds the agreement within the area.

The overall results for this first turbot workshop are positive. For the North Sea area, expert readers for that area can reach an agreement of more than 90%. This indicates that the age estimation of turbot can be highly precise when the agreed interpretation is used. For the Baltic area, the results are more in the range of 70–80% but this is probably caused by the poor quality of the images and the reduced quality of the dataset especially the lack of younger ages.

The WK used ORAcle for analysing the results which is an improved version of the Eltinck spreadsheet and has been evaluated by the PGCCDBS 2008.

1 Results

The results of the re-reading at the Workshop revealed that the overall agreement rate for the two sets of otoliths combined was 77.8%. However, it was agreed that this figure was less meaningful than viewing the two sets of results separately because of the differences between the two samples.

The Swedish sample was unavailable at the Workshop and therefore the re-reading had to be done from images. These images were not of the best clarity, and could have led to some difficulties in interpretation. The Dutch otoliths were available and some readers chose to read them under the microscope while some readers viewed the images, which were of better quality than the Swedish ones. The age-structures of the samples were also significantly different. Therefore, analysis of the two sets was done separately.

The overall agreement rate of the North Sea sample was 82.8%. The range of agreement with the modal age was 70.5–91.1%. The overall agreement rate of the Baltic sample was 71.6%. The range of agreement with the modal age was 55.8–87.4%. The lower score for the Baltic area originates mostly from the poor quality of the image set. Furthermore, the Workshop participants came from two clearly separated Experts Groups i.e. the North Sea and the Baltic Sea. Using results from age readers who are not familiar with the selected area, clouds the agreement within the area.

The overall results for this first turbot Workshop are positive. For the North Sea area, expert readers for that area can reach an agreement of more than 90% for age classes where sufficient samples of good quality were present. This indicates that the age estimation of turbot can be highly precise when the agreed interpretation is used. For the Baltic area, the results are more in the range of 70–80% but this is probably caused by the poor quality of the images and the reduced quality of the dataset especially the lack of younger ages.

The quality of the preparation method and the related images are key stone aspects for age reading. They form the basis for a solid standard age interpretation. In combination with a well designed dataset, age reading of turbot proved to be highly precise for the North Sea. The images of the Baltic set did not allow for an adequate evaluation and because the actual otoliths were not present during the meeting, this could not be solved. Nevertheless, the WKART is of the opinion that the stocks in the Baltic area are not more difficult to age. Sliced and stained otoliths have proved to be a good and trustworthy preparation method. Nevertheless, multiple preparation methods currently exist. Therefore the recommendations by WKART are as follows:

- 1) Compare the different preparation methods that currently exist to determine a standard international procedure.
- 2) The Dutch reference set is compiled very well and could be used as an international approved set. Images of the otoliths could even further be improved by Imares, using their improved camera.
- 3) The Baltic reference set should be completed with younger ages. Images should be improved extensively.
- 4) Build collection of the edge growth. For the North Sea with emphasis on the period May–August, for the Baltic with emphasis on the period June– August.

5) For the Baltic, a new exchange should be organized. For the North Sea, only a small exchange with emphasis on the edge growth could be envisaged.

6) Certified ototliths should be compiled to determine the accurate status of the "first ring".

2 Manual for age reading of turbot

Methods

Transverse sections of otholiths are used for age reading. Thin cross sections, 0.5 mm, are cut through the centre of the otoliths with a diamond cut-off wheel. The cross sections are etched and thereafter stained with a solution of neutral red.

Interpretation of otolith annulli

Age is estimated from the cross sections of the otoliths using a binocular microscope with transmitted and reflected light. Annual rings in the cross sections can be visualized as narrow distinctly darker coloured bands. Age estimation is based on the red protein bands.

Definition of annuli

Sagittae otoliths of turbot deposit annular growth increments, so called annuli. One annulus consists of one opaque and one translucent zone. The opaque zone represents the seasonal period of fast growth (summer ring) and the translucent zone represents the period of slower growth (winter ring). In Baltic turbot a complete annuli is visible in summer or late summer when the opaque zone of the new year has started to form outside the translucent zone of the previous year. This transition will be visible earlier in otoliths from turbots that have been caught in the southern part of the Baltic compared with turbots that have been caught in the northern part of the Baltic. In general, this appears in the period June–August.

Details of the various areas of a turbot otolith and the best positions to read the age

The most consistent place for ageing turbot is usually from the nucleus towards the dorsal or ventral ends and the area out to the inside edge either side of the sulcus.

Reading should be done in all four directions but if that is not possible it is recommended to count the rings in the most visible (contrast) area.

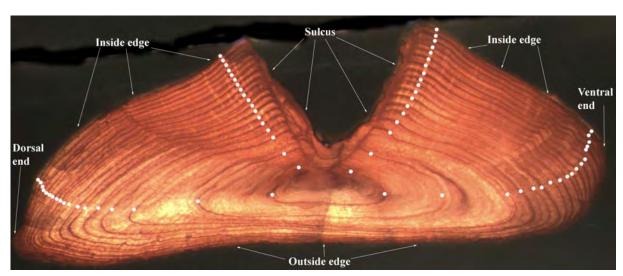


Figure x. Baltic turbot male 306 mm caught in June 2007 SD 28 age 18 years.

Individual differences in the size of the first year

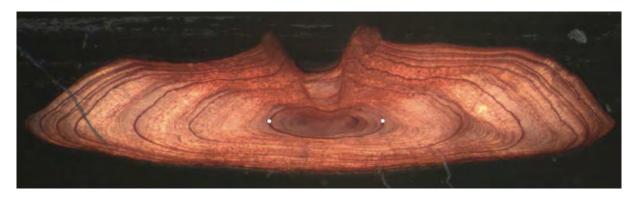


Figure x. HV 2007 no 6 June small first year.

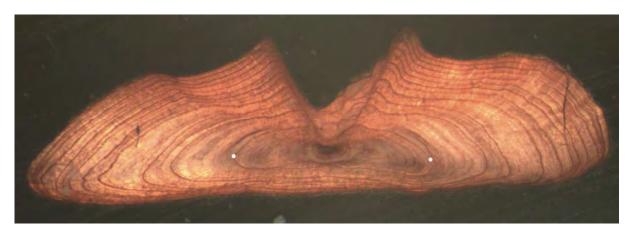


Figure x. HV 2007 no 100 June large first year.

Formation of the new annulus

In SD 27, 28 the new annulus is visible June–August. In young fish the growth starts earlier.

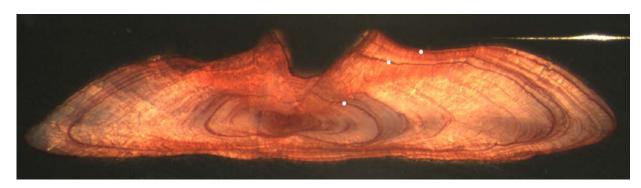


Figure HV 2007 no 116 June.

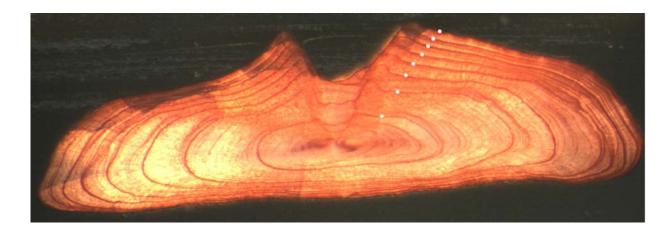


Figure HV 2007 no 146 June.

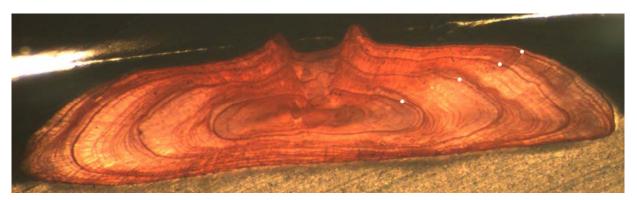


Figure HV 2007 no 41 July.

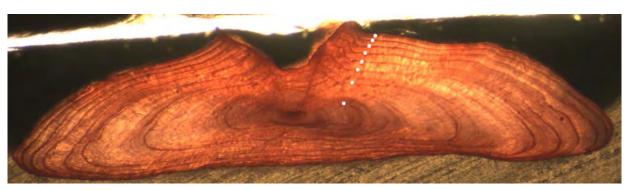


Figure HV 2007 no 2 July.



Figure HV 2007 no 126 August.

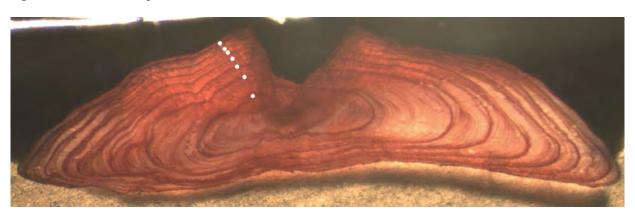
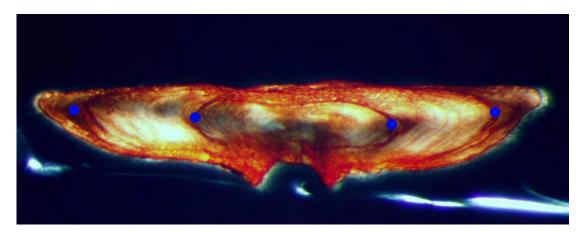


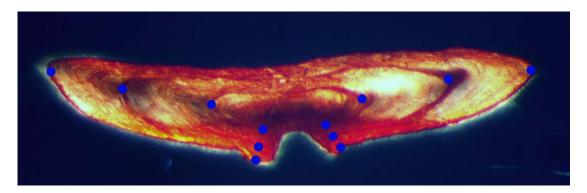
Figure HV 2007 no 128 August.

3 Reference images of the Turbot

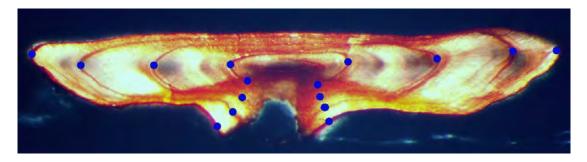
3.1 The North Sea



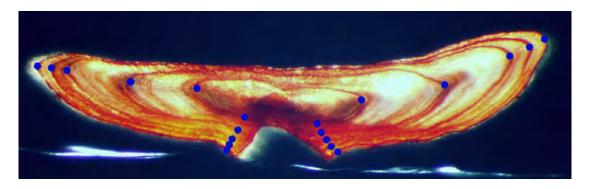
2 years old, fish length: 36 cm, Sex: M, sampling: month 9.



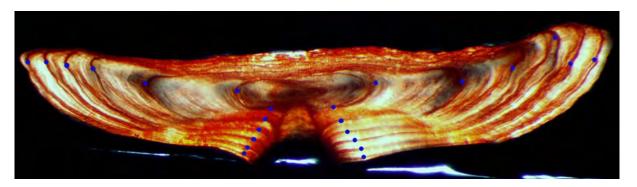
3 years old, fish length: 34.7 cm, Sex: M, sampling: month 5.



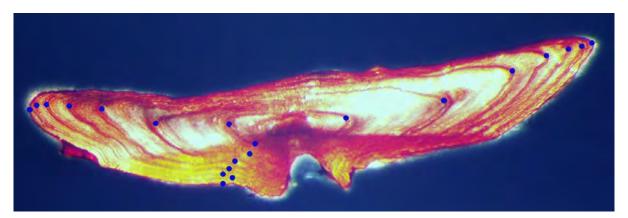
4 years old, fish length: 48.1 cm, Sex: F, sampling: month 5.



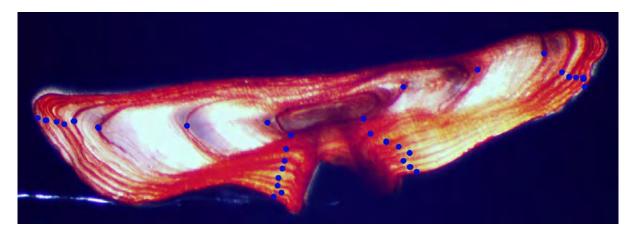
5 years old, fish length: 55 cm, Sex: F, sampling: month 5.



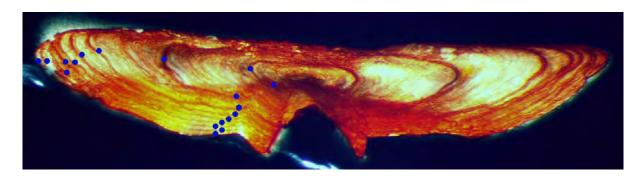
6 years old, fish length: 64.6cm, Sex: F, sampling: month 8.



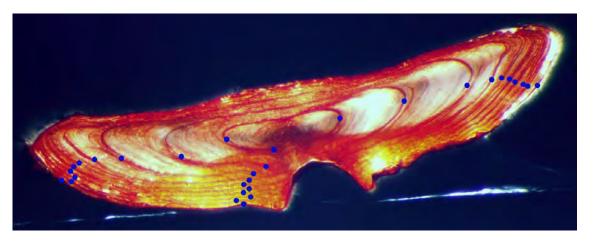
7 years old, fish length: 45.7 cm, Sex: M, sampling: month 1.



8 years old, fish length: 49.9 cm, Sex: M, sampling: month 6.

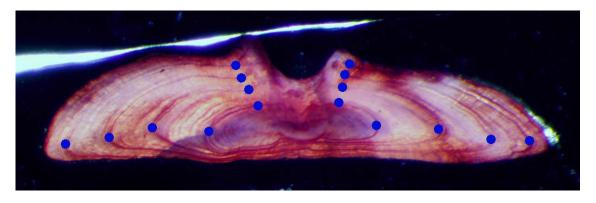


9 years old, fish length: 48.5 cm, Sex: M, sampling: month 4.

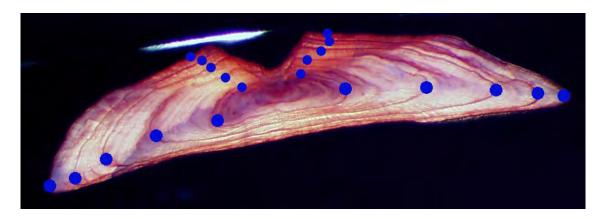


10 years old, fish length: 48.1 cm, Sex: M, sampling: month 2.

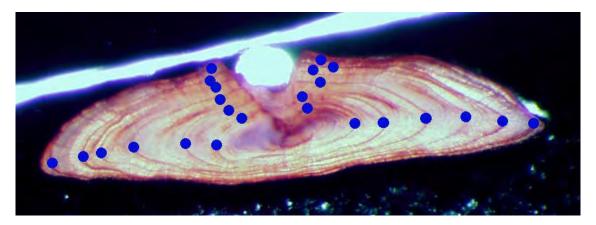
3.2 The Baltic Sea



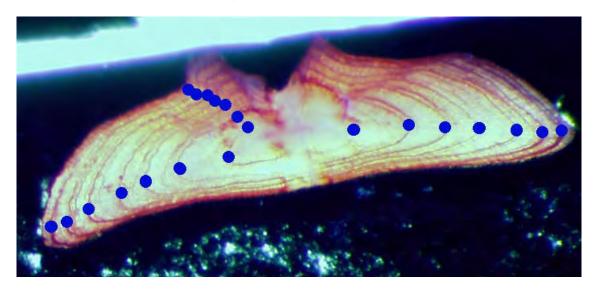
4 years old, fish length: 34 cm, Sex: F, sampling: Quarter 3.



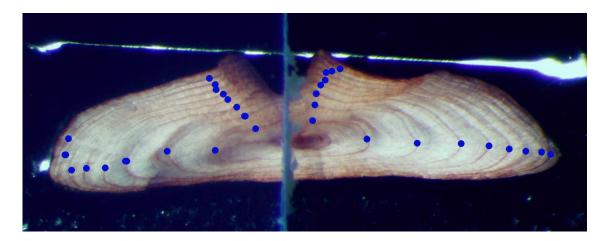
5 years old, fish length: 37.1 cm, sex: F, sampling: Quarter 2.



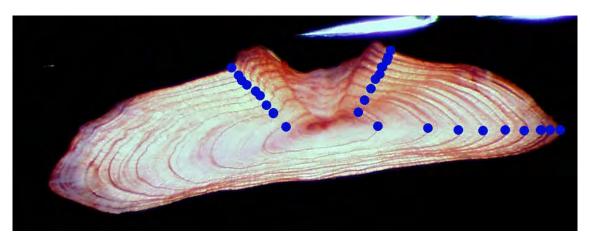
6 years old, fish length: 28 cm, Sex: F, sampling: Quarter 3.



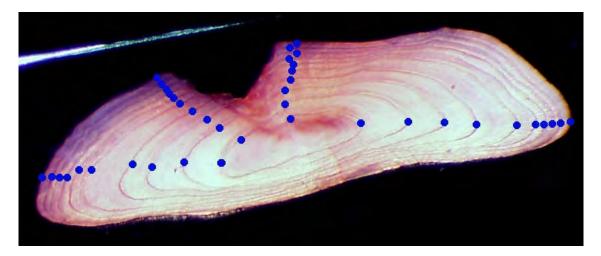
7 years old, fish length: 28 cm, Sex: F, sampling: Quarter 3.



8 years old, fish length: 31.5 cm, Sex: F, sampling: Quarter 3.



9 years old, fish length: 37.6 cm, Sex: F, sampling: Quarter 2.



10 years old, fish length: 36.1 cm, Sex: F, sampling: Quarter 2.

Annex 1 List of participants

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Annex 2 Agenda

ToRs of the Workshop on Age Reading of Turbot (WKART), Oostende, Belgium, 24–27 June, 2008

- a) decrease the relative/absolute bias and improve the precision (reduce CV) of age determinations between age readers of the different age reading laboratories;
- b) evaluate the results of the previous exchange;
- c) create a manual;
- d) collate agreed age reference collection;
- e) formulate follow-up actions
- f) answer to the relevant generic ToRs defined by PGCCDBS.

Agenda

Tuesday 24 June

09.00	Introduction, time schedule and assigning responsibilities.
10.30	Coffee break
11.00	Overview exchange age readings, ToR b
12.30	Lunch break
13.30	Report structure and intersession tasks
15.00	Coffee break
15.30	Age reading methodology, selected otoliths especially ring 1
18.00-18.30	End of day 1.

Wednesday 25 June

09.00	Finalise methodology, reread of exchange otoliths
10.30	Coffee break
11.00	Reread of exchange otoliths, ToR c-f
12.30	Lunch break
13.30	Reread of exchange otoliths, ToR c-f
15.00	Coffee break
15.30	Reread of exchange otoliths, ToR c-f
18.00-18.30	End of day 2.

Thursday 26 June

09.00	Overview reread otoliths, ToR a
10.30	Coffee break
11.00	ToR c-f
12.30	Lunch break

13.30 ToR c-f

15.00 Coffee break

15.30 ToR c-f

18.00–18.30 End of day 3.

Friday 27 June

09.00 Draft report

10.30 Coffee break

11.00 Plenary final report

Lunch break and end of day 4.

Annex 3 Recommendations

RECOMMENDATIONS OF THE WORKSHOP ON AGE READING OF TURBOT (WKART),	
Oostende, Belgium, 24–27 June, 2008	FOR FOLLOW UP BY:
1. Compare the different preparation methods that currently exist to determine a standard international procedure.	PGCCDBS
2. The Dutch reference set is compiled very well and could be used as an international approved set. Images of the otoliths could even further be improved by Imares, using their improved camera.	Approved set: WEBGR, Improved images: Imares
3. The Baltic reference set should be completed with younger ages. Images should be improved extensively.	Participating institutes/labs
4. Build collection of the edge growth. For the North Sea with emphasis on the period may-august, for the Baltic with emphasis on the period june-august.	Participating institutes/labs
5. For the Baltic, a new exchange should be organized. For the North Sea, only a small echange with emphasis on the edge growth could be envisaged.	PGCCDBS
6. Certified ototliths should be compiled to determine the status of the "first ring".	