Age reading quality assurance manual 2021

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Aim

The aim is to ensure a high quality of the age data from the commercial and survey sampling programs, for commercially important species, which is used in stock assessment.

Background

DTU Aqua has a state-of-the-art otolith laboratory equipped with Leica stereomicroscopes and microscopes, dual viewing setups, Leica cameras, image capture software and image processing software. Across two locations there are three processing labs equipped with twin Struers grinding and polishing tables, a Struers ACCUTOM-100 sectioning machine and dedicated facilities with fume hood and consumables for preparation, mounting, fine scale polishing, grinding and sectioning of otoliths.
The otolith collection comprises 35 different species, primarily from the Baltic Sea and North Sea; the most extensive collections are cod (*Gadus morhua*), herring (*Clupea harengus*) and plaice (*Pleuronectes platessa*) which combined total approximately 2,000,000 otoliths covering the years 1982 to present. In addition, a digital archive of otolith images for cod (>15,000 dating back to 1996) and herring (>45,000 dating back to 2010).
Areas of expertise include; age reading expertise (daily and seasonal growth increments), otolith microstructure expertise, technical expertise in sample preparation (sectioning and polishing), image analysis expertise (macro/micro structure, UV-fluorescent tag detection), expert support for the ICES SmartDots platform, scientific expertise in growth studies, life history analysis, age validation methods and shape analysis for stock identification. Tasks are carried out under the supervision of a national laboratory manager who is responsible for the routine otolith procedures both across DTU Aqua's laboratories and internationally (chair of ICES WGSMART and former chair of WGBIOP).
The majority of DTU Aqua's otolith collection originates from scientific surveys and harbour collections funded through EUs Data Collection Framework. The primary objective is to provide the biological knowledge necessary to give scientific advice on stock status and exploitation patterns. Both the DTU Aqua data group and the otolith laboratory fall under the Section for Monitoring and Data with close cooperation ensuring the delivery of high quality data, adhering to internationally agreed standards and procedures plus facilitating the link between data collectors and end users (stock assessors, managers and RCG's).
The DTU Aqua otolith laboratory manager is part of the SmartDots development team, which in 2018 launched the platform as a tool for quality assurance of biological parameters as input for stock assessment. Contributions from DTU Aqua include the development of report outputs through r-scripts, development and review of user handbooks, testing and review of the platform functionality and chairing the ICES Working Group on SmartDots Governance (WGSMART) who oversee all improvements and ensure all developments are in line with the ICES quality assurance framework (QAF). Since 2018, the laboratory manager has been responsible for coordinating 28 age reading calibration and training exchanges and workshops with over 10 international laboratories participating.

Quality assurance of age reading at DTU Aqua

The aim is to ensure a high quality of the age data from the commercial and survey sampling programs. This is achieved by the following:

1. Two age readers per stock

Since 2019, each stock has two designated age readers responsible for the delivery of age data, one primary and one secondary. The primary age reader being the more experienced and providing the majority of the age data for stock assessment purposes and the secondary reader at varying levels of training. Training of secondary readers can be by a number of training methods depending on the material and available.

2. Age reader training and reader comparison

An annually updated plan gives an overview of what reader comparisons are carried out for each stock on an annual basis. Reader comparisons are carried out between the primary and secondary readers, this can be either an internal DTU calibration (national), as described in a) and b) below or in cooperation with other institute(s) who also read the stock (international), as described in c), d) or e) below. If the readers take part in an international age reading exchange or workshop via SmartDots then a national calibration event is not deemed necessary.

For all reader comparisons, the sample selection should to include fish covering the entire age range used in the stock assessment plus all quarters of the year where both the routine commercial and survey sampling takes place.

a) Both readers annotate a set of *images* in the SmartDots software, reader results (ages and annotations) are compared, the results and all otolith images where there is disagreement are discussed between the readers. A small report is made available and stored on the otolith laboratory server in chronological order.

b) Both readers read a set of *physical otoliths* and record their ages in an excel workbook which produces a set of results. Results and all otolith images where there is disagreement are discussed between the readers. This can be on a dual view or standard stereomicroscope if both readers are in the same laboratory or on a video call where the camera view of the stereomicroscope is shared onscreen. The workbook with results is stored on the otolith laboratory server in chronological order. This method can also be used to carry out self-checks from time to time and especially after extended periods where no age reading has been carried out. A reader can take a set of otoliths, which have previously been read, re-read them and compare their results.

c) In cooperation with another institute who are reading the same stock, an exchange of physical otoliths takes place. This requires an exchange of samples and corresponding data. Reader results (ages) are compared using an excel workbook. The results and all otoliths where there is disagreement are discussed between the readers, either via an online meeting or images are taken and shared with follow-up email correspondence.

d) WGBIOP Exchange via SmartDots
Both readers take part in an international age reading exchange via the SmartDots platform where readers from all national laboratories reading a certain stock take part. Reader results (ages and annotations) are compared, analysed and reporting on using a standardised, SmartDots report output. Reports are publically available from the SmartDots List of events

e) WGBIOP Workshop via SmartDots
Both readers take part in an international age reading workshop via the SmartDots platform where readers from all national laboratories reading a certain stock participate in a workshop setting (physically or online) to discuss the outcomes of an age reading exchange. Reader results (ages and annotations) are compared, analysed and reported on using a standardised SmartDots report output. Reports are publically available from the SmartDots List of events.
A workshop will also focus on updating age reading guidelines and criteria, review of validation studies and results from previous calibration events, compilation of reference collections and determination of best age reading methods (if applicable).

4. International calibration (WGBIOP exchanges and workshops) events

High priority is given to age reader participation in international calibration events (exchanges and workshops) which take place using images on the SmartDots platform. The ICES Working Group on Biological Parameters (WGBIOP) coordinates the cycle of international age reading exchanges and workshops and produce guidelines for coordinating these events. Annually updated guidelines can be found here. All national laboratories age reading a stock are recommended to participate in these events. Results are published using the standardised report output and annotated images and the report become publically available on the platform.

5. Data Quality Checks

To ensure the quality of the age data provided to the stock assessors is of a high standard data checks are carried out per quarter for commercial sampling programs or after a survey for the survey sampling programs. Checks include some standardised graphs to check for outliers in the data, an example is shown in Figure 1. Figures 2 and 3 show examples of a plots showing distribution of length by age, these are produced by sample type, area and quarter.

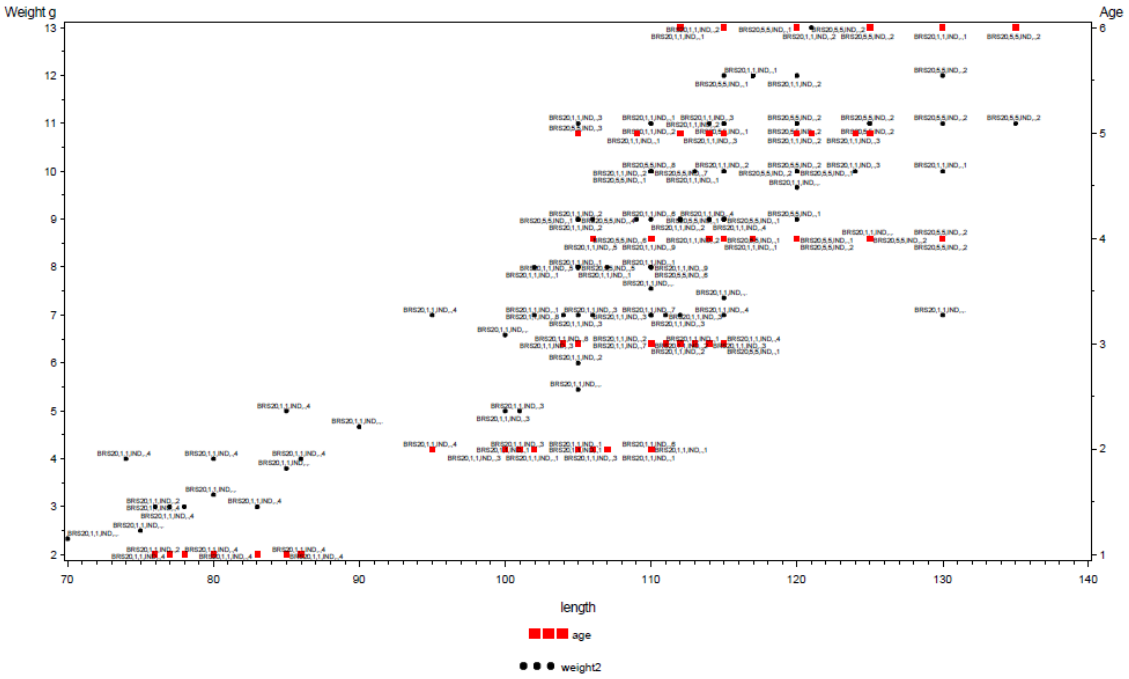

Figure 1. Length, weight age plot

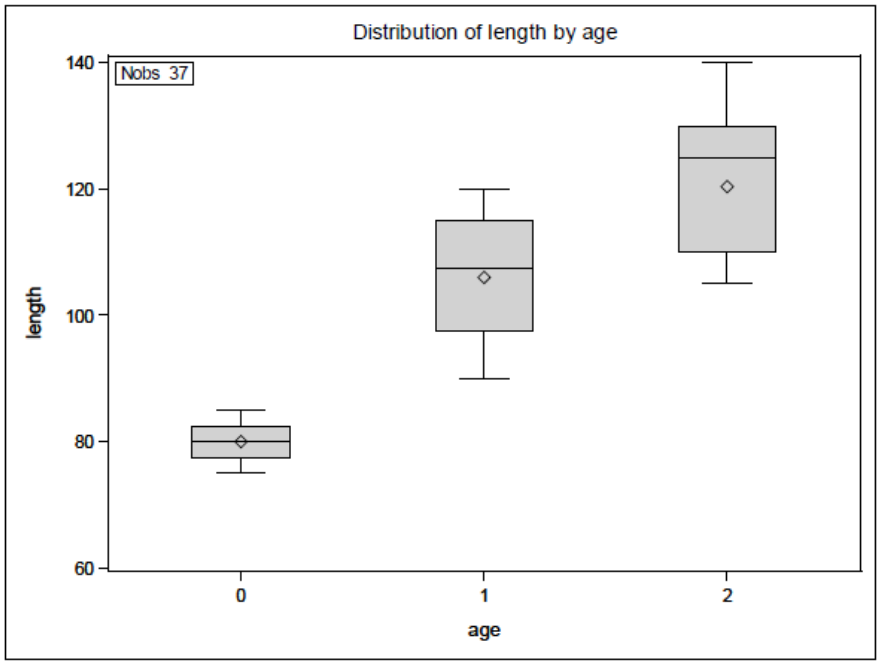

Figure 2. Box plot showing the distribution of length by age

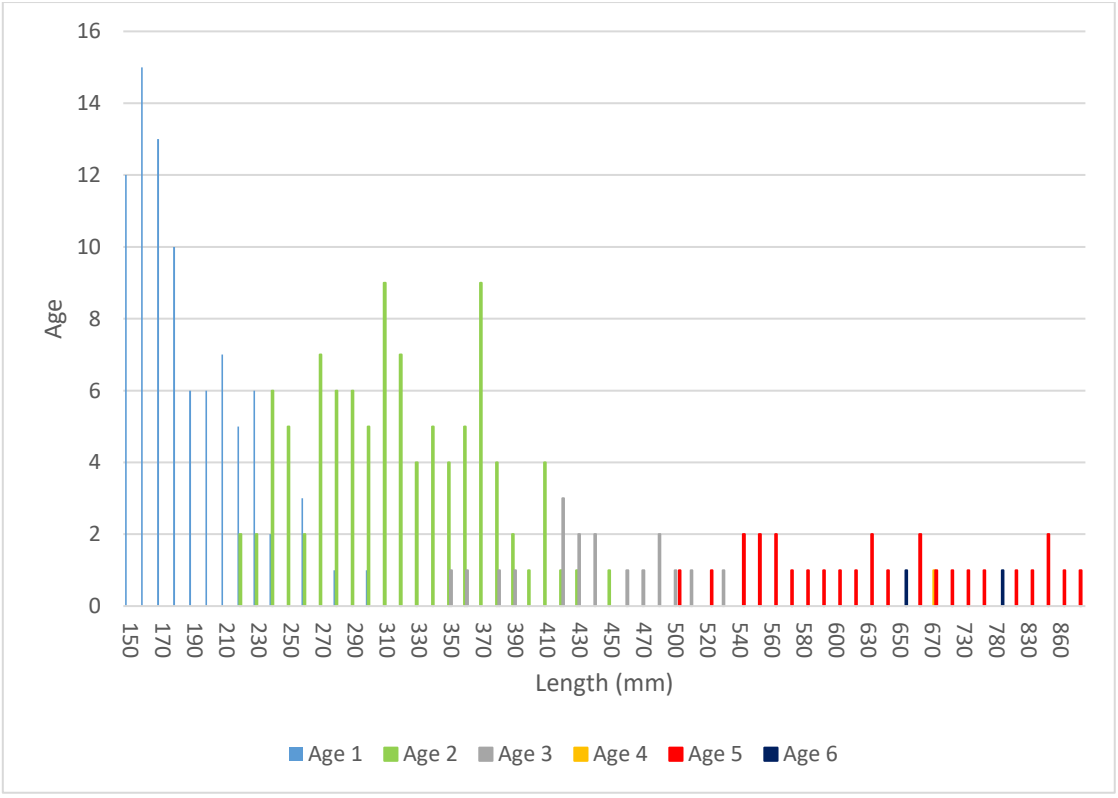

Figure 3. Bar plot showing the frequency of length by age

After the data checks have been carried out any data issues are discussed with those conducting the biological sampling, the age readers and those entering the data into Fishline, the aim being to identify where the error originated. Any necessary corrections are then made to the data directly in the database.

6. Development of best methods

Age reading methods can vary from stock to stock and different institutes apply different age reading methods when age reading. Investigations into the best age reading methods are ongoing at DTU Aqua to ensure that the methods applied are those that give the most accurate results. International collaboration aims to standardise the age reading methods per stock because a) comparison and calibration is difficult when a number of different age reading methods are used at national laboratories and b) advancements in laboratory techniques can support the research required to investigate the most suitable age reading methods for each stock.

One of the generic Terms of Reference for WGBIOP age reading workshops is to create or update an ageing manual, this requires that most accurate age reading method for a stock is determined and agreed upon. An outcome would then be a recommendation that national laboratories apply these methods. DTU Aqua aim to apply the recommended age reading method or each stock. An overview of methods currently applied can be found in Table 1.

Table 1. Overview of age reading methods applied per stock at DTU Aqua

Species	Stock	Age Reading Method https://vocab.ices.dk/?ref=1511
Gadus morhua	cod.27.22-24	Se
Gadus morhua	cod.27.21	Br
Gadus morhua	cod.27.47d20	Br
Sprattus sprattus	spr.27.22-32	ALEt
Sprattus sprattus	spr.27.3a4	ALEt
Clupea harengus	her.27.3a47d	ALEt
Clupea harengus	her.27.25-2932	ALEt
Clupea harengus	her.27.6a7bc	ALEt
Clupea harengus	her.27.1-24a514a	ALEt
Ammodytes spp.	san.sa.1r	ALEt
Ammodytes spp.	san.sa.2r	ALEt
Ammodytes spp.	san.sa.3r	ALEt
Ammodytes spp.	san.sa.4	ALEt
Ammodytes spp.	san.sa.5r	ALEt
Ammodytes spp.	san.sa.6	ALEt
Ammodytes spp.	san.sa.6a	ALEt
Ammodytes spp.	san.sa.7r	ALEt
Scomber scombrus	mac.27.nea	ALEt
Micromesistius poutassou	whb.27.1-91214	ALS
Merlangius merlangus	whg.27.3a	Br
Merlangius merlangus	whg.27.47d	Br
Trisopterus esmarkii	nop.27.3a4	Br
Pollachius virens	pok.27.3a46	Br
Melanogrammus aeglefinus	had.27.46a20	Br
Pleuronectes platessa	ple.27.420	ALS

Pleuronectes platessa	ple.27.21-23	ALS
Pleuronectes platessa	ple.27.24-32	ALS
Microstomus kitt	lem.27.3a47d	ALS
Limanda limanda	dab.27.3a4	ALS
Limanda limanda	dab.27.22-32	ALS
Solea solea	sol.27.20-24	SS
Solea solea	sol.27.4	SS
Platichthys flesus	fle.27.2223	SS
Platichthys flesus	fle.27.3a4	SS

7. Age validation studies

One of the main sources of error in age determination is the uncertainty around whether structures seen within the otolith are the true annuli used to determine the age of the fish or other false rings or checks. These false rings or checks should not be included in the count of age and occur due to a range of environmental and/or physiological conditions leading to adverse fish growth. Repeated low levels of agreement between readers indicates poor accuracy and precision in the age readings. To ensure the age reading criteria followed by the readers for training and calibration purposes are correct, age validation studies (direct or indirect) are carried out. Validation techniques applied at DTU Aqua include marginal increment analysis, microstructure analysis and micro chemical analysis.

8. Otolith Archive

At DTU Aqua in Lyngby, there is a storage facility that is water and fireproof and where all otoliths are archived by sample type and in chronological order. The same archiving procedures are followed at the storage facilitates at DTU Aqua in Hirtshals. Otoliths are stored individually using a number of methods (A4 plastic laminate sheets in folders, small paper envelopes, otolith trays, mounted on glass slides) and stored collectively in labelled cardboard boxes by sample type (surveys, landings and at sea sampling of catches) in chronological order and on easily accessible shelves. Samples are individually labelled, in more recent years using adhesive printed labels but back in time using adhesive handwritten labels. There is a cataloguing system for all in house samples. A new system is currently being developed which will enable labels to be printed directly from a database output. All otoliths will be labelled with an individual ID corresponding to our national database (Fishline). Labels will also have a QR or Barcode that will be used in the future for cataloguing. This will improve the internal quality assurance protocols and make the otolith collection more easily accessible for QA purposes.