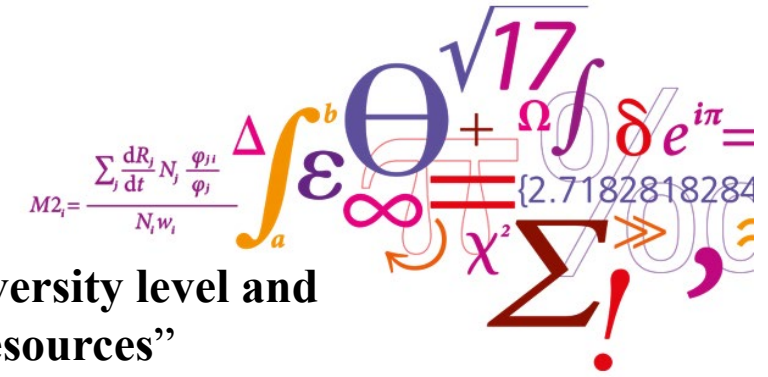


# *How do we coordinate QA of age reading practices across laboratories, when the common goal is to provide age data for stock assessment purposes?*

2023 TESA best practices in ageing workshop  
January 31<sup>st</sup> to February 2<sup>nd</sup>

# Overview

- A little about myself
  - DTU Aqua's otolith laboratory – otolith collection, lab set up, facilities/tools, methods applied
  - My role at DTU
  - Involvement in ICES
- ICES community
  - WKNARC
  - WGBIOP
    - knowledge sharing, standards, guidelines, workshops, exchanges, validation (WKVALPEL)
    - WebGR to SmartDots (more on this tomorrow)
    - Linking to assessment (WKAMEMSA)
- An example of how we implement QA procedures - WKARP2 online



What do we do? “**DTU Aqua** conducts research, provides advice, educates at university level and contributes to innovation in sustainable exploitation and management of aquatic resources”

**Provide scientific advice to Danish and international authorities** on the development of fish and shellfish stocks, fisheries management, effects of fisheries on ecosystems and stocks, aquatic restoration and aquaculture production

- Danish ministry for food, agriculture and fisheries
- International Council for the Exploration of the Sea (ICES)
- Contracted under the EU Data Collection Framework

**Section for Monitoring and Data:** fisheries dependent and independent sampling

- 2 locations, 3 vessels
- Lab technicians, age readers, biologists, programmers, statisticians, at-sea observers, port sampler and vessel crew



# DTU Aqua - Section for Monitoring and Data

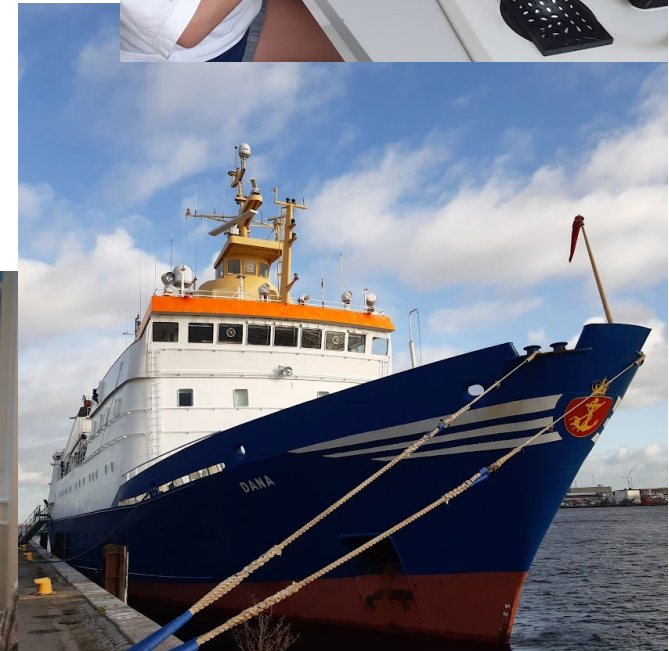
## National Otolith Laboratory

**DTU Aqua's otolith laboratory is equipped with:** state-of-the-art Leica stereomicroscopes and microscopes, dual viewing setups, Leica cameras, image capture software and image processing software.

**Three separate processing labs equipped with:** twin Struers grinding and polishing tables, a Struers ACCUTOM-100 sectioning machine, dedicated facilities for preparation and mounting of otoliths for sectioning.

**Expertise within the lab is centered on:** processing techniques, age determination, age validation, otolith microstructure examination for stock identification, shape analysis and data quality assurance

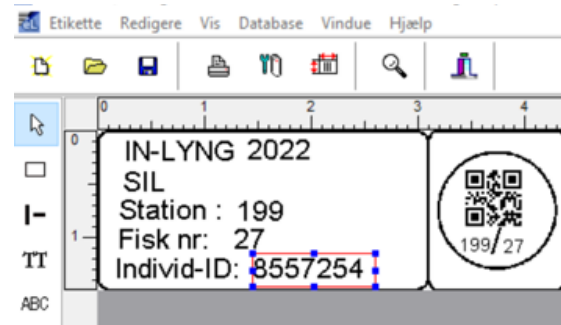
11 age readers, 33 species





- **33 different species** (range of taxonomic groups), e.g. cod (*Gadus morhua*), herring (*Clupea harengus*) and plaice (*Pleuronectes platessa*)
  - **approx. 2,000,000 otoliths covering 1982 to present.**
- A **digital archive of otoliths images** >15,000 for cod (back to 1996) and >45,000 for herring (back to 2010).
- Mostly from **scientific surveys** and **harbour collections** (targeting commercially important species).
- Provide the biological knowledge underpinning scientific advice on stock status. **Primarily used by scientists from ICES member states for stock assessment related studies.** Primarily covers the **Baltic Sea and North Sea.**
- Stored at DTU-Aqua in a water and fire proof archive.





- Various **storage methods** by sample type and in chronological order:
  - A4 plastic laminate sheets in folders, small paper envelopes, otolith trays, mounted on glass slides
- All samples are **individually labelled**:
  - a unique **IndivID** on an adhesive printed label (in recent years)
  - on an adhesive handwritten labels (back in time)
- **IndivID** – is **unique to each fish** in our **national database**
  - **Otolith image naming** e.g 8504116\_ALW\_RLX\_BX
  - **Tissue samples** for genetic analysis (stomach samples in the future)
  - Link to **AquaDots**



Image name: 8504116\_ALW\_RLX\_BX

[ICES Reference Codes - RECO](#)

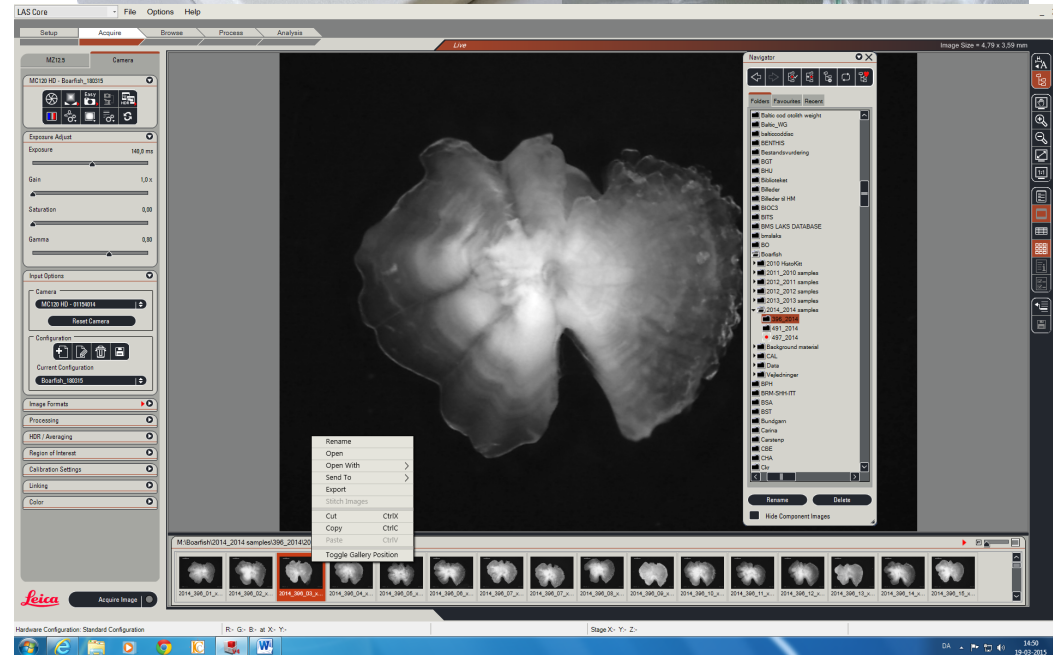
Aquadots management													
Aquadots administration													
Events													
+ Add event													
	Id	Name	Event type	Year	Species	Area			Start date	End date	Created by	Comments	
	21	2022 TOR 4A,4B,4C,20	Års aflæsning	2022	TOR	20, 4A, 4B, 4C	1	13	30-11-2022	30-03-2023	sel	Får aflæsninger fra	
	20	Microstomus kitt (Lemon Sole)	Sammenlæsning	2022	RTG	20	2	49	24-11-2022		gho		

# DTU Aqua - Section for Monitoring and Data

## National Otolith Laboratory

As the national otolith laboratory manager, I am responsible for:

- **routine otolith procedures**
  - **Nationally** across DTU Aqua's laboratories
  - **Internationally** (chair of ICES WGBIOP and WGSMART)
- adhering to internationally agreed standards and procedures to ensure **high quality service**
- **facilitating the link between data collectors and end users** (stock assessors, Regional Coordination Groups).



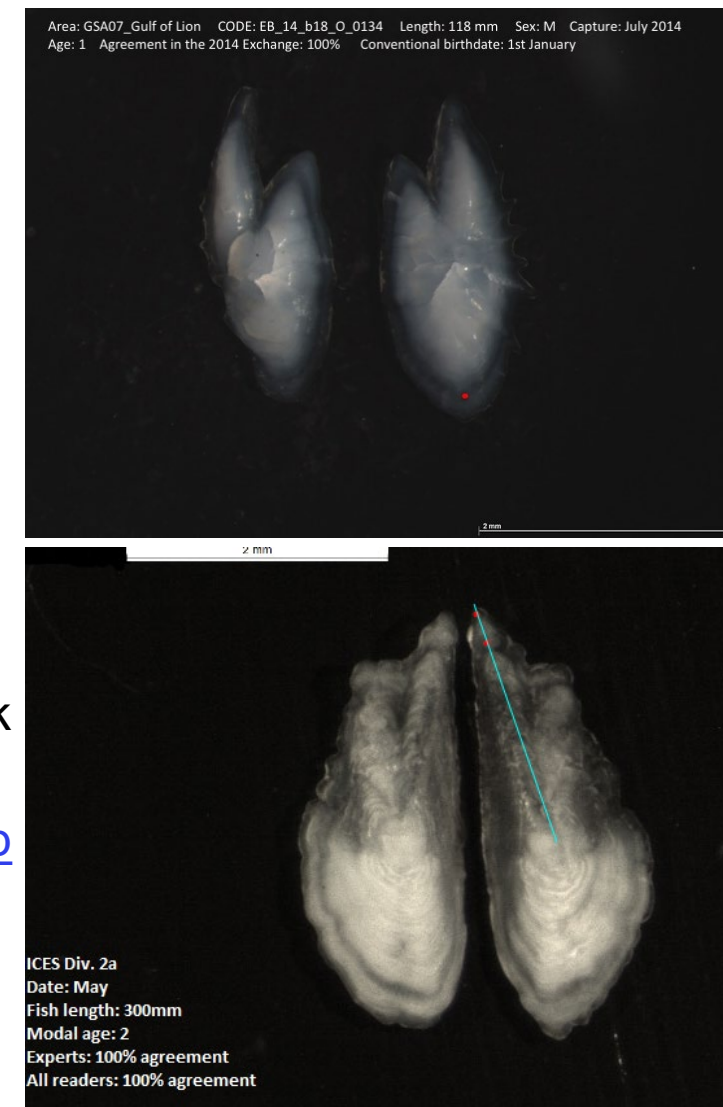
# Streamlining otolith procedures across DTU Aqua's laboratories and internationally

## ICES WKNARC (2012/2013) – Workshop for National Age Reader Coordinators

- To develop tools and protocols for intercalibration between laboratories:
  - EFAN 2000; **Eltink**, Guidelines for validation studies, QA & reader comparisons
  - European Age Readers Forum; message board, reports & manuals, reference collections, Contacts
  - WebGR
- 3 point grading system for QC
- Identified gaps in **validation** and growth studies
- Birth of the state-of-the-art ICES Cooperative Research Report (CRR) Handbook of fish age estimation protocols and validation methods

[https://iceslibrary.figshare.com/articles/report/Handbook\\_of\\_fish\\_age\\_estimation\\_protocols\\_and\\_validation\\_methods/18624035](https://iceslibrary.figshare.com/articles/report/Handbook_of_fish_age_estimation_protocols_and_validation_methods/18624035)

Proposed **ICES WGBIOP** (2015 to present)





**WGBIOP** facilitate a strengthening of the link between the end users and the national laboratories while *supporting stock-based and ecosystem advice in terms of biological parameters.*

- Deals **mostly with QA of age and maturity data**, now broadening to include ichthyoplankton data
- Approx. 20 countries participating from year to year
- ToR's evolving with **stock assessment data needs**
- Maintains a **cycle of workshops and exchanges**:
  - in-line with the **ICES Benchmark cycle** (evaluation of the current assessment methodology and data)
  - **Fast track** on request from the assessment groups (EG's)
- Develops **calibration tools** – **SmartDots**
- Develops **guidelines** for:
  - exchanges and workshops
  - QA of age reading (and maturity staging) at institutes

Working Group on Biological Parameters



Under the umbrella of ICES EOSG (Ecosystem Observation Steering Group) - Groups meeting immediate data demands and contributing to the running and further development of effectively coordinated, integrated, quality assured and cost-effective monitoring in the ICES region and beyond.

## Need to be able to respond to requests from ICES EG's

- Stock based calibration events
- Communication is essential - Exchanges set up with input from the assessors
- SmartDots summary report specially tailored for EG's
  - Results presented at the EG's meetings
  - AEM (age error matrices)
- ICES Benchmark rolling issues

<http://stockdatabase.ices.dk/Manage/rollingissues.aspx>



Species name	English name	Stock code	Area	Area description	Stock description ICES	Assessment WG	Stock category	Age Based Assessment Y/N?	Age: % agreement from age readers, reading for assessment from most recent EX/WK	Age: CV% from most recent EX/WK	Age: APE% from most recent EX/WK	Age Validated Y/N	Age Validated based on table	Method	Reference	Links to validation reports/doi	2021	2020	
<i>Pleuronectes platessa</i>	Plaice	ple.27.420	27.4	Skagerrak and North Sea	Plaice ( <i>Pleuronectes platessa</i> ) in Subarea 4 (North Sea) and Subdivision 20	WGNSSK	1	Y	74%	32%	12%	N	Y	Released marked fish, Micromilling	Etherton (2015), Geffen (2012)	<a href="https://doi.org/10.1016/j.fishres.2015.05.009">https://doi.org/10.1016/j.fishres.2015.05.009</a> , <a href="https://doi.org/10.1007/s10641-012-0033-2">https://doi.org/10.1007/s10641-012-0033-2</a>	<a href="#">WKARP2 Workshop on Age reading of North Sea Plaice</a>	Otolith exchange of Plaice in Skagerrak and the North Sea. Coordinators:	Plaice maturity staging exchange to include immature fish. Coordinators:
<i>Pleuronectes platessa</i>	Plaice	ple.27.89a	27.8 and 27.9	Bay of Biscay, Portuguese waters	Plaice ( <i>Pleuronectes platessa</i> ) in	WGBIE	5,2	N				N							

### Exchanges (checklist)

1. Identifying participants
2. Selecting a representative set of otoliths images (covering all age groups, areas, quarters, preparation methods)
3. Give clear written instructions to the participants
4. Use the SmartDots tool <http://ices.dk/marine-data/tools/Pages/smartdots.aspx>
5. Analyzing the exchange results via SmartDots
6. Report results to the stock assessment EG

### Workshops (checklist)

1. Should always be preceded by an exchange
2. Use the SmartDots tool
3. Generic ToR's – review past ex's and wk's, ageing protocols, resolve interpretation issues, focus on standardization of methods, validation, compile reference collections
4. Make recommendations on how to improve the age reading quality

#### DATA QUALITY ASSURANCE REPOSITORY



##### Contents:

- Guidelines
- Methodological Workshop Reports
- Age Calibration Workshop and Exchange Reports
  - Herring Exchange
- Ageing manuals
- Maturity Staging Workshop Reports
- Maturity Manuals
- ICES Expert Groups Reports
- PGMED reports
- Others

##### Guidelines

- WGBIOP 2020 Guidelines for Exchanges And Workshops on Age Reading

**UPDATING**

Print it Send to f t in Share it



## Implementation of AQ scores <https://vocab.ices.dk/?ref=1682>

- Used in SmartDots
- Available in RBDES <https://github.com/ices-tools-dev/RDBES>
- Aiming for a generic score to be used for other biological variables(maturity staging, egg and larva identification...)

## Questionnaire based with a long term goal to agree on QA methods at a regional level

Q.'s:

- ✓ Do you implement AQ scores?
- ✓ Individual or 2 readers per species?
- ✓ How are QC checks carried out – no. of samples, frequency, image based or not, what analysis is used?
- ✓ Availability of manuals?
- ✓ Accreditation or Quality Management Plan?
- ✓ Information requested on validation or method comparisons

EDIT ANNOTATION BOF\_2022\_Q2\_7J\_WESPAS\_H13\_015.jpg - daviesj - 17-08-2022 11:24:19

PARAM	OWR	
AQ-code	AQ2	
Approved	Code	Description
	AQ1	Rings can be counted with certainty
	AQ2	Rings can be counted with difficulty and some doubt
Comment	AQ3	Rings cannot be counted, the calcified structure is considered unreadable (...)
	AQ3_QA	Unreadable or very difficult to age with acceptable precision



**WGBIOP proposed the** workshop in response to repeated recommendations for validation

- Collate info on existing ageing protocols, past workshops and exchanges, provide guidelines on how to overcome the cycle of repeated low agreement
- Share knowledge

## Ageing is a step by step process

1. Based on scientific information
2. Aim to improve precision
3. Perform studies to support the ageing method (direct or indirect validation)
4. True validation is often too complex or expensive
5. Very often not available

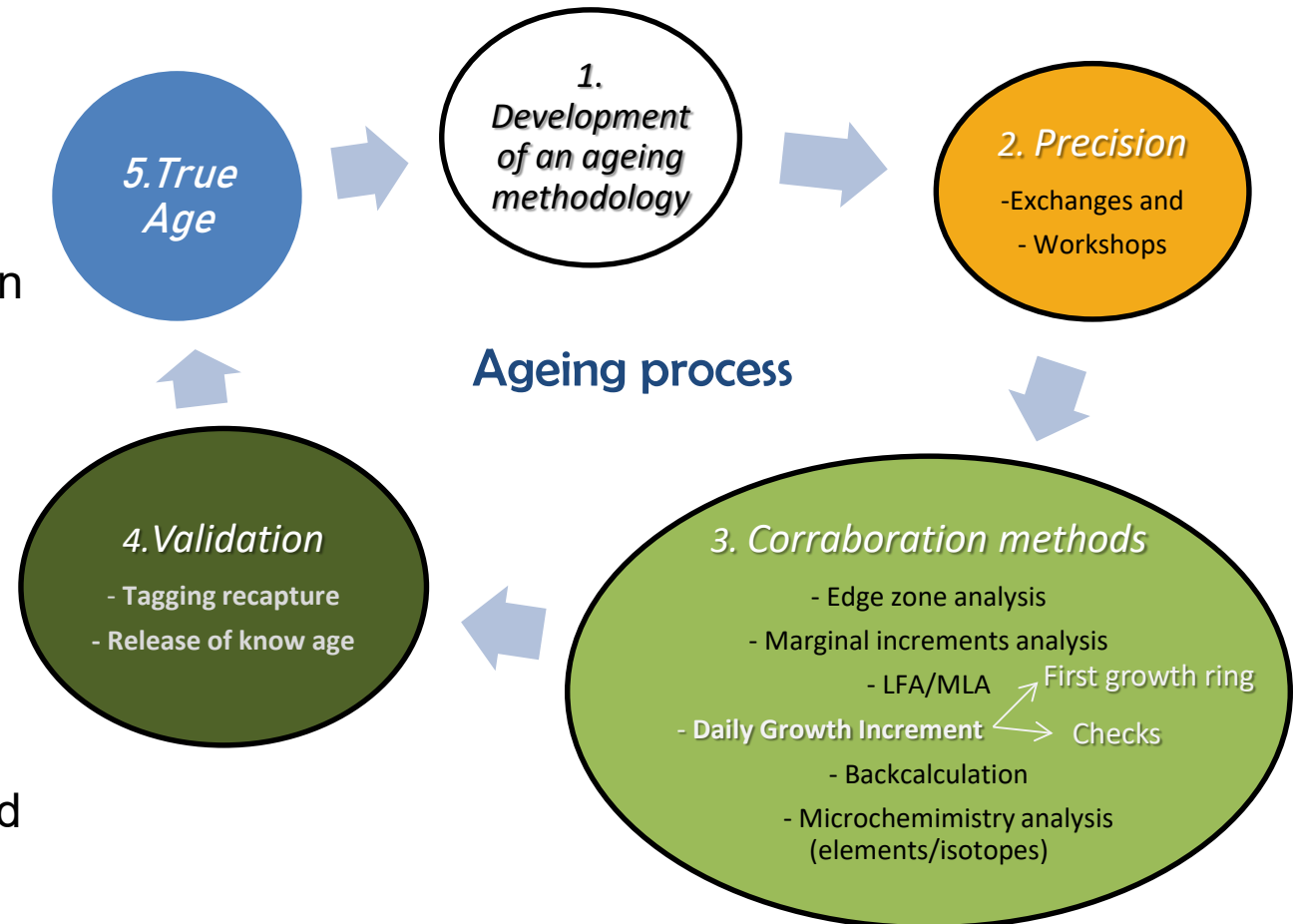


Figure 26: An ageing process for small pelagic species. (WKVALPEL, 2019)

**Resources are often the issue so there is a need for prioritisation of species (stocks) to be validated**

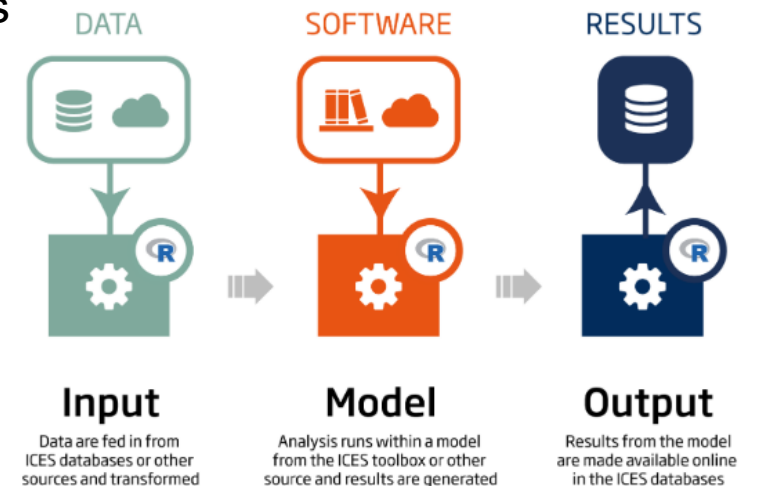
## Numerous EG's requesting age error data (AEM's or raw data)

**Aim:** develop and promote new approaches to incorporate uncertainty in age/maturity in stock assessment and advice

**Members:** age and maturity coordinators, model developers, stock assessors and statisticians

**Format:**

- Literature summary on age reading error integration (...maturity)
- Discussion on guidelines for exchanges and workshops
- Discussion on the SmartDots report
- Presentations of models: Stock Synthesis (SS), State-space Assessment Model (SAM) and Gadget 3
- Presentation of TAF (Transparent Assessment Framework)



**TAF** The open framework enables anyone to easily find, reference, download, and run the assessment from any stage in the process leading to the published ICES advice

### Suggested improvements to the exchange set-up:

- More **communication** needed between calibration exercise coordinators and stock assessors
- Information to obtain **BEFORE** an exchange/workshop
  - what data is used in the stock assessment (SA) model: areas samples, seasons (especially if survey data is used), what is the age+ group, months used for building maturity ogive, only mature-immature is important
- Report back to SA **AFTER** exchange/workshop
  - SmartDots summary report
  - Join the SA EG's and present the results

**Smartdots report is suitable but having easy access to the raw data is necessary**

- **Recommended approach:**
  - SS3, SAM and gadget 3 are able (or nearly able) to use raw data from exchange events to model an AEM and MSEM (use within the model)
- **Alternative approach:**
  - the AEM can be modelled externally and provided to the assessment model
  - the AEM can be calculated empirically (assuming modal age as true age), and be used in the models instead of estimating them

### Important points:

- Stratification of samples
- Participation of advanced readers/stagers
- Seasonal variation in age reading errors (v.imp. if survey indexes are used)
- AQ scores - can this information be used to model the errors?
- Need for regular calibration exercises to check if an AEM is not changing
- Importance of reference collections to support this



# WKARP2 - Workshop 2 on age reading of North Sea plaice (*Pleuronectes platessa*)

Held online 6–10 December 2021

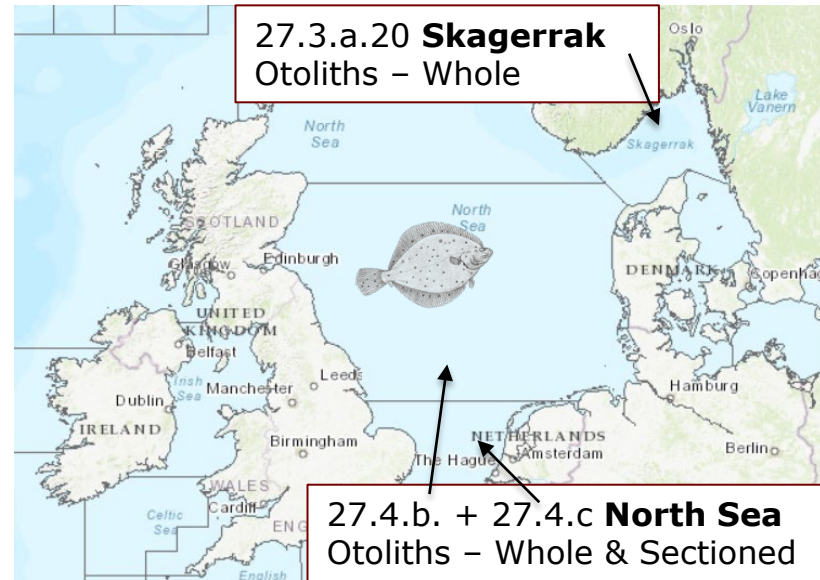
17 readers (9 advanced) from 8 countries

## Review

- 2020 North Sea Skagerrak plaice ex. (SmartDots ID 281)
- 2010 – WKARP (2002, 2003)

## Main issues

- Evaluate level of ageing agreement for the stock (*ple.27.420*)
- Compare and standardize laboratory procedures and age reading methods



## Goals

- Provide **guidelines** for reliable age interpretation - including 1st winter ring
- Provide **age error data** to the stock assessment working group
- Create a **reference collection** of otoliths with agreed age



Both whole & sectioned otoliths from the same fish



# WKARP2 - Workshop 2 on age reading of North Sea plaice (*Pleuronectes platessa*)

Providing **guidelines** for reliable age interpretation - including 1st winter ring



**Aim:** to provide some measurement guidelines to apply when age reading

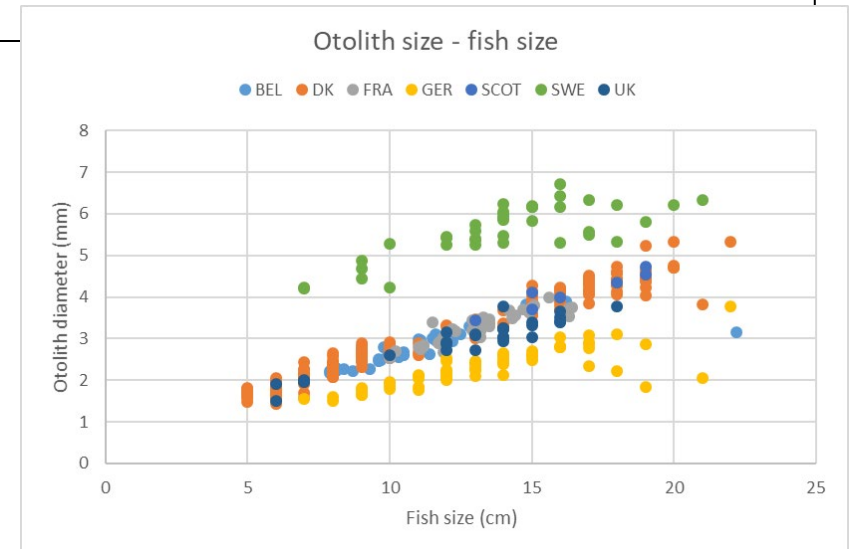
- Measure
- Examine edge type

UK, SCOT, FRA, GER, BEL, SWE and DK contributed with images of age 0 (2020) and age 1 (2021)

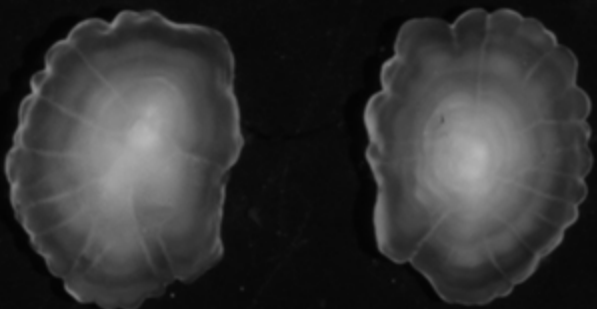
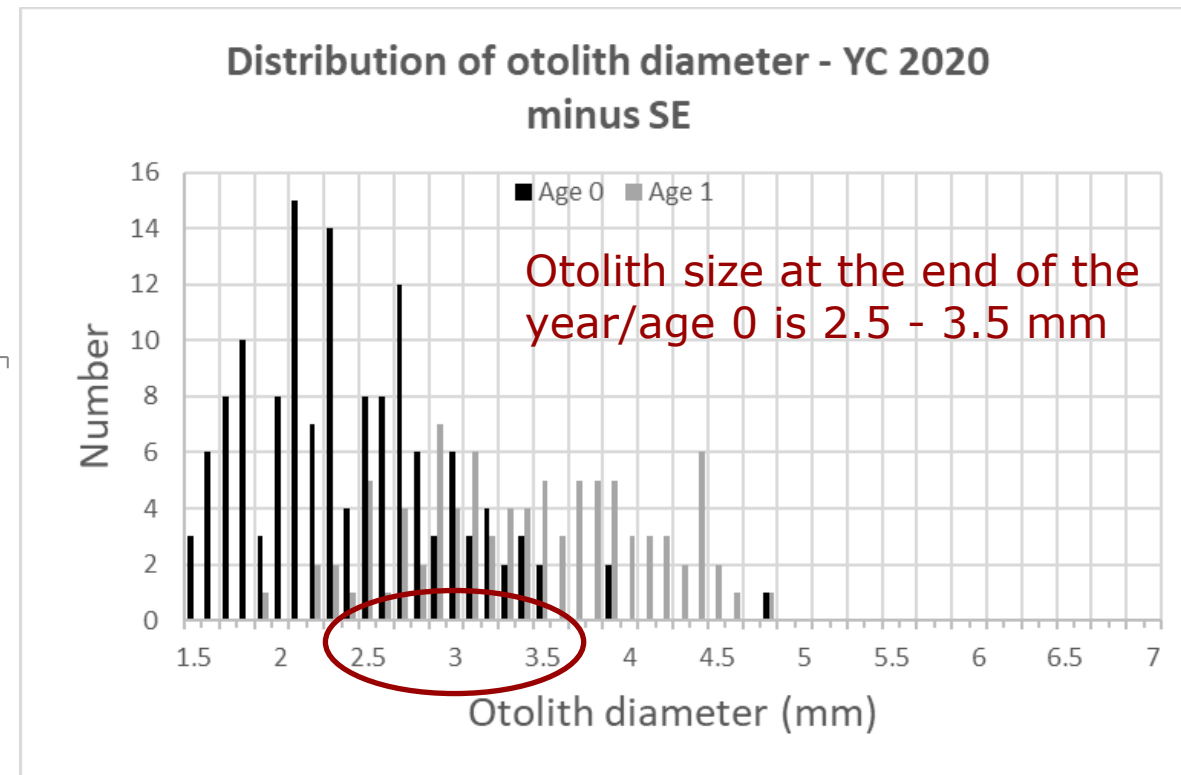
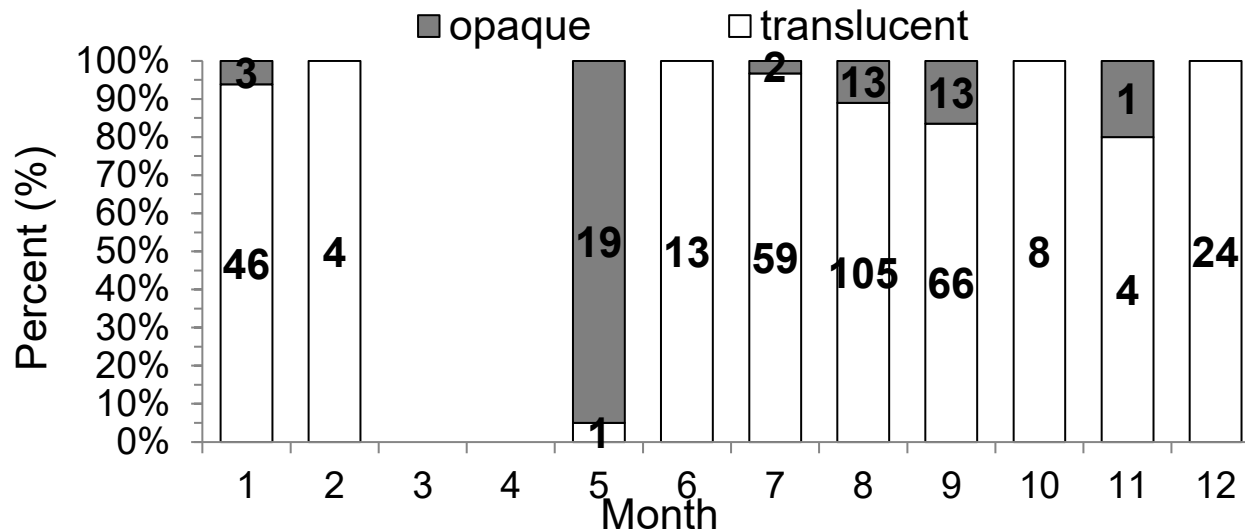
- Whole otoliths, reflected light, different magnifications and image quality

## Challenges:

- Not all sample data was provided & data needed to be standardised
- Issues with scale bars - missing or incorrectly calibrated, images from the same institute calibrated differently
- Images were not all in jpeg format (tiffs are very large)
- Image J (FIJI) used to measure.
- **Important** to have the correct calibration information



# WKARP2 - Workshop 2 on age reading of North Sea plaice (*Pleuronectes platessa*)

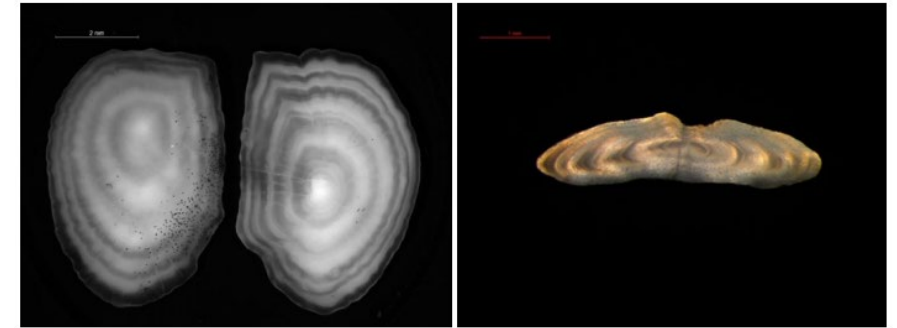


Catch Date: July 2020  
Length: 8 cm  
Area: 4b  
Age: 0  
Edge: Is difficult!

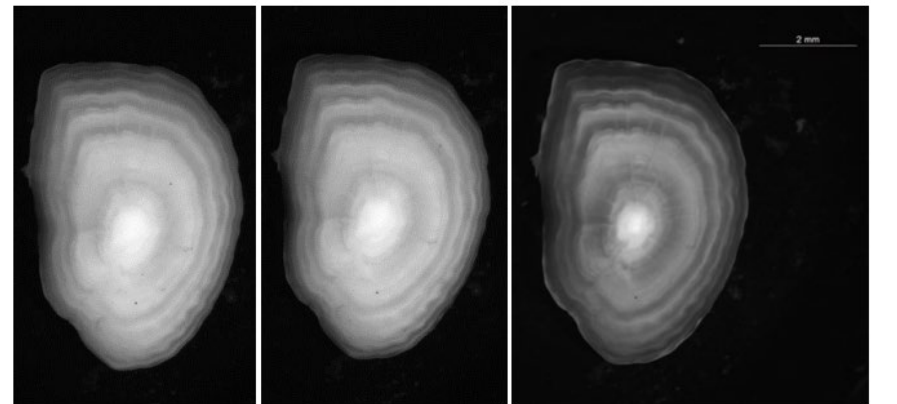
## WKARP2 - Workshop 2 on age reading of North Sea plaice (*Pleuronectes platessa*)

2020 Exchange	N samples	N readers	Modal age range	Comparison	PA (%)	CV (%)
Skagerrak 27.3.a.20, whole	90	14 (7)	0–14	All readers	66	43
				Adv. readers	69	55
North Sea 27.4.b & c, whole	106	14 (7)	0–11	All readers	75	50
				Adv. readers	76	46
North Sea 27.4.b & c, sectioned	106	7 (6)	0–16	All readers	75	38
				Adv. readers	75	38
Stock level (ple.27.420), whole	196	11 (9)	0–16	Adv. readers	72	32

2021 Workshop	N samples	N adv. readers	Modal age range	PA (%)	CV (%)
Skagerrak 27.3.a.20, whole	50	9	1–15	74	18
North Sea 27.4.b & c, whole	50	9	0–11	74	20
North Sea 27.4.b & c, sectioned	50	7	0–12	76	14
Stock level (ple.27.420), whole	100	9	0–15	74	20



Same otolith  
3 preparation methods



Same otolith from l - r, after 0 hours, 2 hours and 24 hours in water



## WKARP2 - Workshop 2 on age reading of North Sea plaice (*Pleuronectes platessa*)

### Conclusions:

- differences in the identification of the first winter ring
- differences in the estimated ages of older fish - unreliable (narrowing of the annuli close to the edge)

### To provide guidelines - Further work is required

**Reflected light** is optimal for whole and sectioned otoliths – whole is most common

No obvious benefit from sectioning plaice otoliths **under age 6** – sectioning may *increase* overestimation

Sectioning otoliths **above age 6** can help to identify outer age rings – sectioning may *prevent* underestimation

Reliability of the age reading depends on **image quality**.

### Issues:

- High CV values for young fish (modal ages 0, 1 and 2) indicates problems to interpret 1<sup>st</sup> winter ring
- Correct identification of otolith edge type – highly dependent on image quality (also soaking time)

### Recommendations:

- Workshop to establish guidelines for image quality
- Functions in SmartDots for using **reference** (test) sets

### Future work:

- Compilation of AEM (age error matrices)
- Results to be presented at the *ple.27.420* benchmark data compilation workshop
- Expand the collection of otoliths to be included in the reference sets

## Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods

S. E. CAMPANA

*Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2*

*(Received 6 March 2001, Accepted 21 May 2001)*

*Why use reference collections?*

*Monitor consistency in age reading overtime (check for drift)*

*Monitor consistency between readers*

*Training new readers*

*Known or agreed age – covering age range, geographical (stock) and temporal range, multiple years...*

“Through use of quality control monitoring, ageing errors are readily detected and quantified; **reference collections** are the key to both quality control and reduction of costs”

**SmartDots Reference Set Module in development in cooperation between WGBIOP and WGSMA**

# Guidelines for image taking are on the way...



Image of whole otoliths under water of *Mullus surmuletus* (reflected light) with the right otolith burnt.

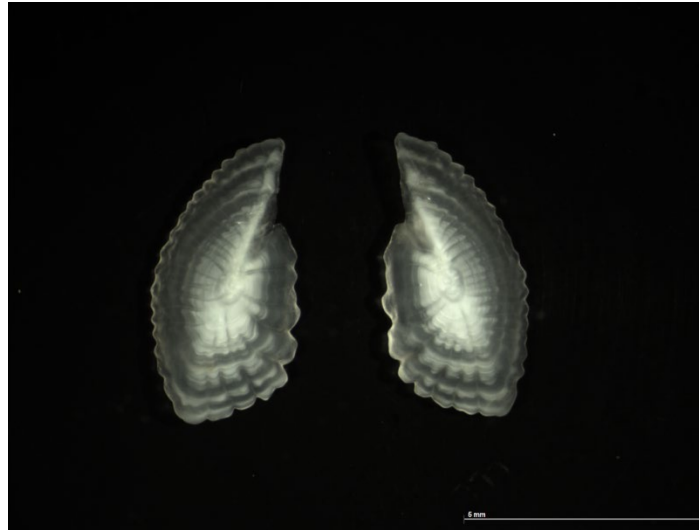


Image of whole otoliths under water of *Trachurus trachurus* (reflected light)

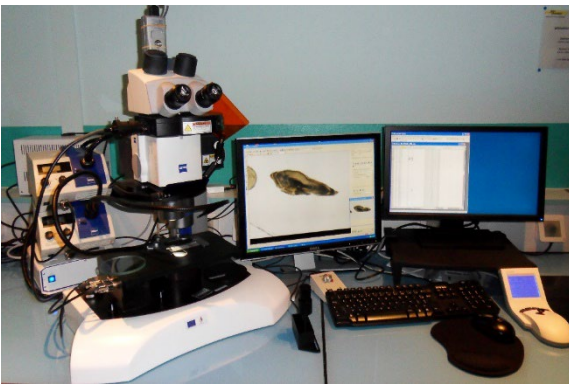
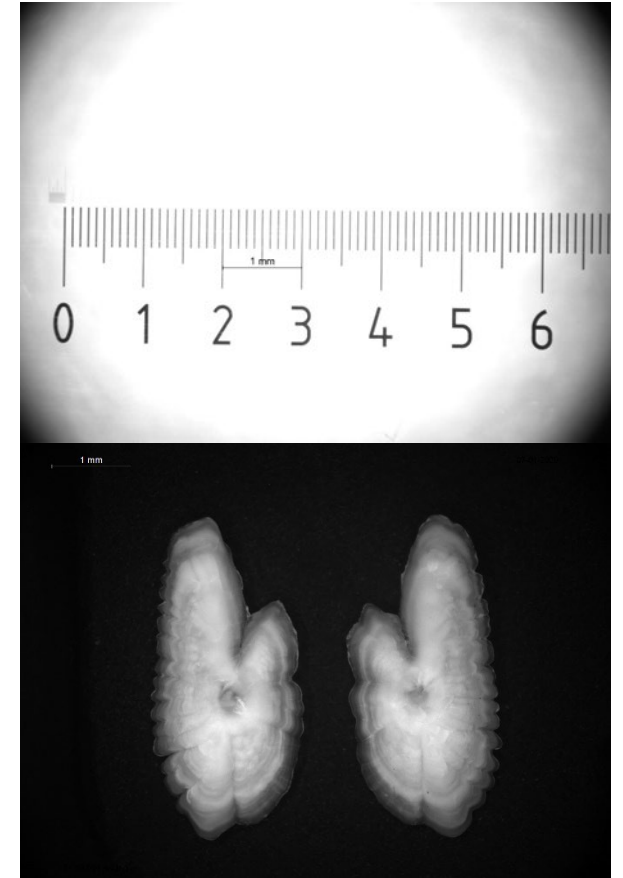


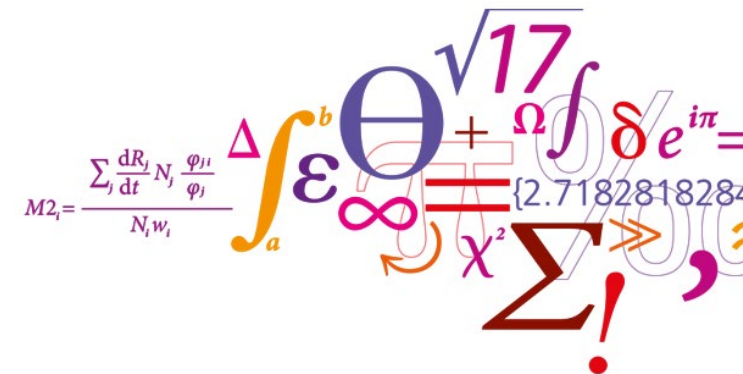
Image acquisition by binocular with a camera and an image acquisition and processing software.



Issues to avoid when preparing and imaging samples



Correct calibration ensures that readers can rely on the images magnification and that any measurements taken of features within the otolith are correct



Thanks for your attention!



DTU

