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An efficient protocol and data set for automated otolith image analysis

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Abstract

Information on fish age constitutes one of the most important biological variables for a fish stock, and an accurate estimation of the age structure of the fish populations is essential for the reliable management of these natural resources. The age of individual cod (*Gadus morhua*) is determined by manually examining the layered structure of otoliths, a calcium carbonate structure of the inner ear. Image-based methods to age otoliths have been investigated for over 4 decades with varying results, but recent developments in automatic image analysis techniques are promising. The objective of this paper is to describe a method to efficiently image a manually broken otolith (avoiding the time-consuming embedding and cross-sectioning process) and to describe the organization and acquisition of imaged broken otolith images with associated metadata for a collection of north-east Arctic cod otoliths. A single-lens reflex camera was used for capturing photographs of the broken otoliths. A total of six images were acquired for each subject, consisting of three images in the first position with three different light exposures and three images in the second position with three different light exposures. This results in a simple and efficient procedure for capturing clear, satisfactory, and reproducible images of broken fish otoliths, and a more straightforward and less labour-intensive alternative to the commonly used methods that involve embedding and cross-sectioning of the otolith.

KEYWORDS

big data, deep learning, fish ageing, *Gadus morhua*, north-east arctic cod, Otolith

Dataset

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1 | INTRODUCTION

Information on fish age constitutes one of the most important biological variables, which is used for studying the life history (e.g. growth, sexual maturation) and population dynamics (Campana, 2001) of fish stocks. The age structure of fish populations is essential in any fisheries management system that is dependent on age-based analytical assessment models (Hilborn and Walters, 2013), and this includes a large proportion of commercially important fish stocks around the world (Hart and Reynolds, 2002). In most cases, this is obtained by physical sampling of individual fish, either from fish catches or from scientific surveys. Varieties of hard structures in fish, including opercula, vertebrae, spines or fin rays, are used for ageing purposes, but scales and otoliths are the most frequently used structures (Casselman, 1987; Campana and Thorrold, 2001).

Otoliths are calcified ear stones used by fish for balance and hearing (Panfili *et al.*, 2002). They are metabolically inert and grow throughout the life of the fish (Campana and Neilson, 1985). Changes in the structure of the otolith are visible in the seasonally dominated habitats (Godiksen *et al.*, 2010), where they form annual increments composed by two bands characterized by different opacity, that is the opaque and translucent zones (Katayama, 2018). These periodicities in the structure are widely used for ageing purposes (Høie and Folkvord, 2006), whereas seasonal zones can be visible in both whole (untreated) otoliths and/or after some form of preparation, such as cutting, breaking, burning or slicing Panfili *et al.*, 2002; Vitale *et al.*, 2019).

The north-east Arctic cod (*Gadus morhua*) is the world's largest cod stock and supports a large fishery in addition to being an important component of the Barents Sea ecosystem (Nakken, 2008; Yaragina *et al.*, 2011). The fish is distributed across the Barents Sea and migrates to the Norwegian coast for spawning (Bogstad *et al.*, 2016). Fishing occurs mainly in the Barents Sea and at the spawning grounds in the traditional Lofoten fishery (Opdal, 2010). Several scientific surveys in addition to catch sampling programmes are used to monitor the stock, and these monitoring programmes support age-based assessment models that are used for setting the total allowable catches (Olsen *et al.*, 2010; ICES, 2019).

The age of individual cod is routinely determined by a trained expert examining the otoliths. This involves removal of the otolith, cleaning, breaking it in two pieces and reading it under a stereomicroscope. The average time to become a certified age reader at the Institute of Marine Research (IMR) is approximately 4 years. Decisions to scale up sampling may be compromised by significant resource demands and training lead times. Alternatively, some otolith laboratories use thin slices for the age determination, which require labour-intensive embedding of the otolith in epoxy resin and cutting with precise cut-off machines (ICES, 2013). This is a rather time-consuming process and is not always feasible for large sets of otoliths (Panfili *et al.*, 2002), but offer clear surfaces suitable for traditional imaging techniques.

Imaging techniques are useful since they facilitate the development of online repositories of otolith pictures (Lombarte *et al.*, 2006) and efficient organisation of inter-institutional exchange workshops (Appelberg *et al.*, 2005). Different otolith imaging techniques have been developed, including 2-dimensional (2D) and 3-dimensional (3D) imaging (Schulz-Mirbach *et al.*, 2013). However, 3D images are rarely used for age estimation purposes (Fisher and Hunter, 2018).

Image-based methods for ageing fish have been tried with varying results (Fisher and Hunter, 2018), but automatic image analysis technique is a rapidly developing field, and great strides have been made recently, using a technique known as 'deep learning'. Deep learning employs deep neural networks which are convolutional neural networks (CNN) that are organised in many layers (hence 'deep') and work directly on the image pixels with increased abstraction (LeCun *et al.*, 2015). The method has been applied on images of Greenland halibut otoliths (Moen, 2018). The advantage of the Greenland halibut otoliths is that they can be imaged for ageing purposes without breaking them since annual increments are apparent on the whole otolith, whereas the cod otoliths need to be sectioned to make the increments visible. The rugged structure of the broken surface makes the imaging more challenging.

The objective of this paper is to describe a method to efficiently image a manually broken otolith (to avoid the embedding and cross-sectioning process) and to describe the organization and acquisition of broken otolith images with associated metadata for a subset of the historical north-east Arctic cod otolith material stored at the IMR. We have published the images and associative metadata as a reference internet archive for developing automated image-based methods.

1.1 | The surveys

Otoliths were taken from the two main fishery-independent surveys for north-east arctic cod: the joint Norwegian-Russian winter survey (Jakobsen *et al.*, 1997) and the Norwegian Spawning Cod acoustic-trawl survey (Korsbrekke, 1997). The winter survey covers the Barents Sea and has been conducted since 1981. The Norwegian Spawning Cod acoustic-trawl survey covers the Lofoten area and has been performed annually in March–April since 1982. The total number of trawl stations for the different surveys is given in Table 1.

On each of the surveys, the otoliths are sampled using a random-stratified sampling based on fish length for each trawl station. On the Norwegian-Russian Winter Survey, otoliths from individual fish are randomly sampled. Once there is one fish per 5-cm-length class, no further otoliths of that fish length are collected. This corresponds to a length-based random-stratified sampling scheme for each trawl station. On the Norwegian

Survey	Number of stations imaged	Total number of stations	Number of otoliths imaged
Winter Survey 2017	21	318	177
Winter Survey 2018	25	460	260
Spawning Survey 2017	17	38	294
Spawning Survey 2018	16	40	276

TABLE 1 The number of imaged otolith and sampling effort across stations, years and survey

Spawning Cod acoustic-trawl survey, otoliths from five fish are sampled for each five-centimetre size class. On rare occasions (approximately five times per survey), the catches can be very large, and the otoliths from ten fish will be sampled for each size class. Therefore, the amount of otolith samples collected from a station is dependent on the specific survey and on the size distribution of the fish in each catch.

We subsampled the complete material by randomly picking trawl stations within each survey and imaged all otoliths sampled for one station. We aimed to balance the data set between survey and year, and the number of stations imaged is given in Table 1. The number of otoliths per station depends on whether the respective length groups were present in the catches or not. For some stations, the catch may be split into two subsamples. In those cases, we imaged all sampled otoliths for both subsamples.

2 | MATERIALS AND METHODS

2.1 | Sample selection method

At the surveys, otolith samples were organized into boxes containing sets of envelopes which were separated by rubber bands (Figure 1). The boxes were labelled with the survey name, the year and the trawl station numbers. Each set of envelopes represented one station (trawl haul), labelled by a trawl sample serial number. Each set included a different number of envelopes depending on the available number of cod for the respective length groups at that station at the time of collection. From each fish, one pair of sagittae cod otoliths (the largest of the three pairs of otoliths) were collected and stored in the envelopes. Each envelope was labelled with individual fish data such as fish ID, length, weight, sex and age.

One of the otoliths was broken in half as a result of the preceding age reading process. Sometimes both otoliths were broken depending on how easy the first broken otolith was to read for the age reader. Each envelope was labelled with a number; determined age; and weight, length and sex of the fish.

A sampling frame was created by placing serial numbers in random order separated by survey and year, resulting in simple random sampling design within a year and survey. During the image capturing process, stations were chosen in the order of the randomized sampling frame.

2.2 | Camera settings

The camera used for capturing photographs of the broken otoliths was a Canon model EOS 5D Mark II. It is a digital single-lens reflex camera with a full-frame complementary metal oxide semiconductor sensor of approximately 36 mm × 24 mm and with approximately 21.10 effective megapixels. The camera was attached to an LMscope (Micro Tech Lab) mount and connected to a computer (Figure 1) using the 'EOS Utility 2' (Canon U.S.A., 2019) application allowing communication between the camera and the computer. This application includes

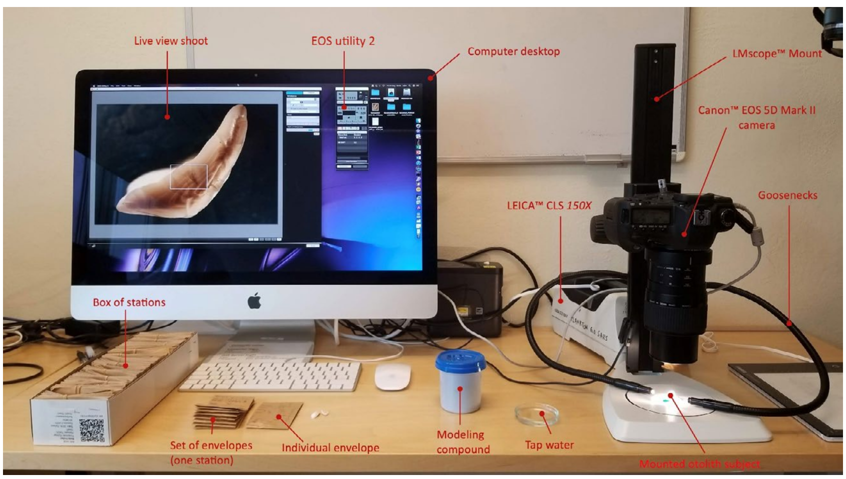

FIGURE 1 Schematic displaying the procedure set-up for capturing images of broken cod otoliths. The red oval arrows are placed on the respective object description

functions like downloading and displaying images, remote shooting and camera setting control.

The camera and the computer were connected to one another after making sure they were both turned on. The EOS Utility 2 application on the computer was manually initiated if it did not automatically start-up after connection. The 'Camera settings/Remote shooting' option was selected from the menu that appeared on the computer. The correct camera settings were applied to the camera (Table 2). The auto exposure bracketing (AEB) setting was used to get three images of the same subject at three different light exposures. When the capture button is clicked three times successively, each picture will have a different light exposure setting (in order of: standard exposure, decreased exposure, increased exposure) (Figure 2) according to the exposure compensation amount and the AEB amount that was manually set by the user (Table 2).

2.3 | Light settings

For illumination, a LEICA CLS 150× photonic cold light source (Leica Microsystem) was used. The top and bottom dial were set to 4 and 6, respectively. Two photonic goosenecks were attached to the illumination system to allow for free configuration of light to the specimen. The goosenecks were adjusted to illuminate each side of the otolith from below the mount (Figure 1). This allows the light to beam up into the otolith from the bottom making the growth lines visible. The goosenecks may be moved slightly after each otolith was placed under the camera to adapt the lighting to fit each otolith, but the general configuration stayed constant. This was done to make sure parts of the otolith fracture surface are not too overexposed or too underexposed in order to get a good picture of all the growth lines as clearly as possible.

TABLE 2 Manual settings used on the Canon EOS 5D Mark II. These can either be activated on the camera itself or through the EOS Utility 2 application on the computer. Mode and magnification must be activated on the camera itself

Camera settings		
Mode	'Av' (Aperture-Priority AE)	
Magnification	3×	
Aperture	F16	
ISO Speed[a]	320	
Image resolution	Large (3,744 × 5,616 pixels)	
jpg compression	Fine	
AEB[b]	Exposure Compensation Amount	−2
	AEB Amount	−3, −2, −1

[a]ISO Speed = image sensor's sensitivity to light.

[b]AEB = auto exposure bracketing; this setting must be activated manually.

2.4 | Selection of otolith

The otolith half that seemed to have the clearest otolith growth lines were chosen from each envelope. In order to do this, a criterion was followed: the subjects chosen were ones where the fracture surface was flat enough so the image could be focused at all parts of the subject area as much as possible. If the break was rugged and it was difficult to see the growth lines clearly, otoliths that presented a clear core or nucleus were selected as this ensured that the right number of age zones were visible. A clean break at the core is beneficial when reliably determining the age.

2.5 | Preparation of otolith for imaging

In order to hold the otolith in place when capturing the images, a 'mount' was formed with a dark blue modelling compound, like Play-Doh™ (Figure 3). This modelling compound is the same as the age readers use. The otolith was placed onto the mount with the fracture surface facing towards the camera lens as horizontal as possible. The mounted subject was placed centrally in the camera frame, and the camera height was adjusted to gain a clear image without adjusting the magnification setting. After placement under the camera, a thin layer of moisture was added onto the surface of the otolith fraction to make the growth lines smoother and more visible.

2.6 | Image capture

In the EOS Utility application, the 'Live View Shoot' option was selected with the 'Depth-of-Field Preview' on. This allowed the subject to be viewed in real time on the computer screen (Figure 1). After establishing this setting, the target shutter speed, before image capturing, should be 1/80 seconds. If it was not, then the goosenecks were slightly adjusted by pushing them close together or pulling them farther apart. Then, the Live View Shoot setting was reinitiated until the target shutter speed was reached.

A total of six images were acquired for each broken otolith subject, consisting of three images with different exposures for two different orientations of the otolith (Figure 2). For the first position setting, the subject was positioned so the ventral side of the otolith was near the bottom of the camera frame in an overall vertical arrangement. The spot metering box which is shown in the Live View Shoot window was positioned on the otolith as much as possible rather than on the

FIGURE 2 Resulting images of AEB image capturing. The layered structure that is formed during growth is visible in the images. Dimensions of each image are 11.9 × 8.0 mm with an image resolution of 470.7 pixels/mm

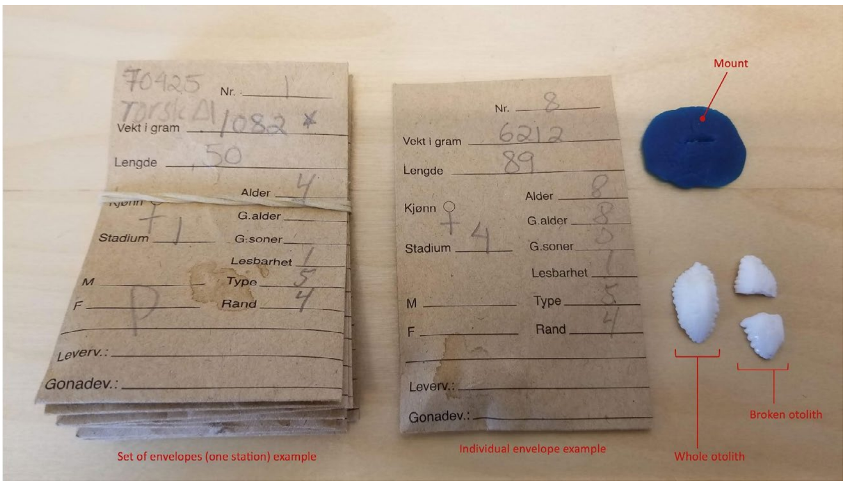

FIGURE 3 This picture displays the mount made of modelling compound and the contents of an individual envelope taken from a set of envelopes, or station

background. This ensured that the changing light exposures were respective to the otolith subject rather than the dark background. The three shots were taken by clicking the capture button three times successively. The three results were the same image but at three different light exposures (Figure 2). Then the second position was arranged by turning the subject 180° so that the ventral side of the otolith was near the top of the camera frame, again, in an overall vertical arrangement. Three successive shots were taken again, and the spot metering box was positioned again. At the end of the image capturing process, a collection of six images should result (Figure 2). If the otolith was very big and could not fit in the camera frame in the vertical direction, it was positioned to make it fit in the frame for the first position and then turned 180° for the second position.

Capturing images of the same otolith in different positions will provide slightly different images of the same subject. This can provide additional data (like data augmentation), and also a useful control for automatic image analysis applications.

2.7 | Example images

The images taken during this study consisted of cod otoliths that were aged between 1 and 16 years old. Figure 4 shows the range of variability in different ages with respect to size

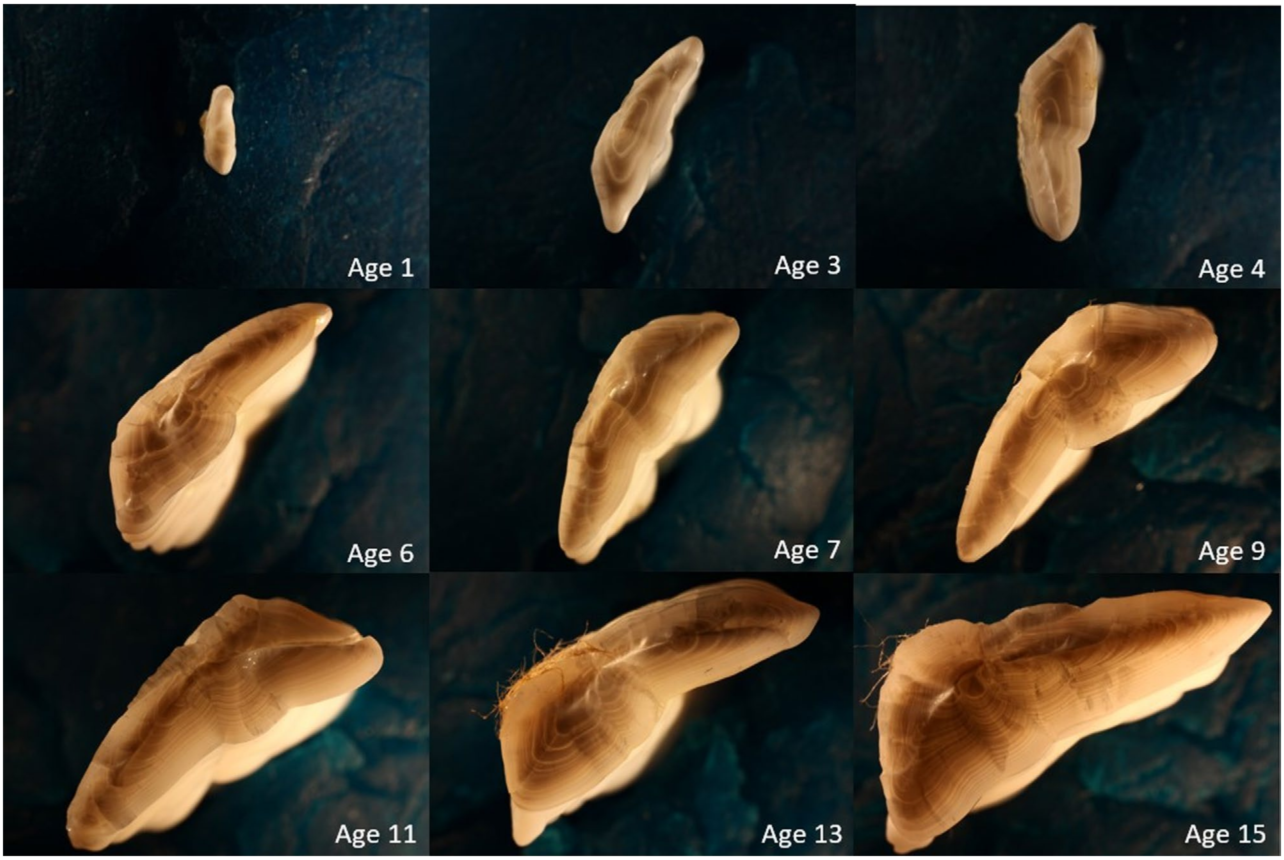

FIGURE 4 Example images of broken cod otoliths of different ages. Images were captured using the procedure created in this study. Dimensions of each image are 11.9 × 8.0 mm with an image resolution of 470.7 pixels/mm

of the otolith and quality of the image. For instance, an age 1 otolith does not have any growth lines yet, while the age 15 otolith has many growth lines, and the density can make them harder to distinguish.

3 | DATA ORGANIZATION

The data can be downloaded from the Norwegian Marine Data Centre and the data are licenced under the Creative Commons Attribution 4.0 International License.

3.1 | Images

The six images were automatically stored by the Canon software using a default name. They were then placed in folders organized as follows:

■Year	(example: 2018)
■Serial Number_Del#	(example: 70024 or 70024_Del1)
■Nr#_age#	(example: Nr03_age06)
This folder contains six image files with consecutive numbers	

Some sets of envelopes have the same serial number but were split into two sets with one labelled as 'Del 1' and the other labelled as 'Del 2'. This was done at the time of the survey when two samples came from one catch. This occurs either when (1) both juveniles and adults are present in the catch and they are separated because the juveniles are measured in millimetres (mm) and the adults are measured in centimetres (cm) or (2) when a large amount of one size is present (for example, if there are many medium-sized fish and very few large-sized fish in one catch). These two groups of different sized fish will be handled as two subsamples (parts 1 and 2) from one catch in order to get a better size distribution.

3.2 | Metadata file

The information about the trawl stations and individual samples are taken from the IMR's central data repository. The raw biotic data are given as XML files adhering to IMR's biotic ver 3 format.* The files are placed in the 'biotic' folder

■biotic

and are named based on the surveys, that is:

*https://www.imr.no/formats/nmdbiotic/v3/nmdbioticv3_en.html

biotic_cruiseNumber_2017102_G+O+Sars.xml
biotic_cruiseNumber_2017625_Kristine+Bonnevie.xml
biotic_cruiseNumber_2017849_Helmer+Hanssen.xml
biotic_cruiseNumber_2018836_Helmer+Hanssen.xml
biotic_cruiseNumber_2018203_Johan+Hjort.xml
biotic_cruiseNumber_2018202_Johan + Hjort.xml

A table for the individual data is extracted from the XML files and presented in the 'individualdata.csv' file. The metadata contains the age and other parameters recorded for the individual otolith, c.f. Table 3 for an explanation of the fields.

TABLE 3 Fish collection metadata field names and descriptions

Field name	Description
Serialno	A trawl station is uniquely identified within a year by its serial number.
Platform	Platform code
Species	Species of interest
Noname	Norwegian name
Aphia	Code for taxonomic classification in the World Register of Marine Species (WORMS) database
Samplenumber	Number to identify the sample given species and station
No	Fish number in a catch
Weight	Fish weight
Length	Fish length
Age	Fish age
Sex	Fish sex
Stage	
Liverweight	Weight of liver
Gonadweight	Weight of the gonad
Stomachweight	Weight of stomach
Lengthunit	cm (3) or mm (1)
Weightmethod	kg (1)
Spawningage	Age of specimen at first spawning
Spawningzones	Count of annual zones in the otolith after first spawning
Readability	The reader's description of how difficult the otolith is to age and determine spawning zones. 1. Spawning zones can be read and age determined 2. Spawning zones can be counted 3. Not readable 4. Can read age but no spawning zones can be determined 5. Lower age bound (of two consecutive) 6. Higher age bound (of two consecutive)
Otolithtype	North-east Arctic cod or Coastal cod 1. Coastal cod 2. Possible coastal cod 3. Svalbard cod stock 4. Possibly NEA Cod 5. NEA cod 6. Farmed cod 7. Possibly farmed cod
Otolithedge	What kind of growth (hyaline or opaque) is at the edge of the otolith 1. Narrow opaque zone 2. Wide opaque zone 3. Narrow hyaline zone 4. Wide hyaline zone
Otolithcentre	What kind of growth (hyaline or opaque) is at the centre of the otolith 1. Hyaline 2. Opaque
Calibration	The number of divisions in the eyepiece graticule on 2 mm. Calibration is done for the lens and magnification used when reading the growth zones

4 | CONCLUDING REMARKS

A variety of methods of fish otolith ageing have been developed for different species. Whole, cut, broken, broken and burnt or sectioned otoliths are used, depending on the characteristic of the otolith, like the overall size, thickness, clarity of the annual increments (Panfili *et al.*, 2002). The procedure presented in this paper has the potential to simplify and ameliorate otolith age reading and is limited to a few straightforward steps, which can be done within a relatively short time (i.e. within minutes). For example, it can mitigate the need for the embedding in epoxy resin and cross-sectioning of cod otoliths (ICES, 2013), and even, through machine learning application, automate otolith age reading techniques (see e.g. Moen, 2018).

The AEB setting applied in the presented study is a manual technique for high dynamic range (HDR) imaging, which is the method of gaining one image automatically by taking multiple images at different light exposures. For this study, we chose to individually store the three images for subsequent merging. Although several camera systems have automatic HDR abilities, storing the individual images allow for reprocessing the merge, or, alternatively, allow for the three images to be added as separate channels in a CNN.

Additionally, for future applications, the step of weighing the imaged otolith could conceivably be beneficial (see e.g. Dub *et al.*, 2013). It has been proven that there is a highly significant relationship between otolith weight and fish age for different fish species, including cod (Cardinale *et al.*, 2000; Araya *et al.*, 2001; Lou *et al.*, 2005). After imaging the otolith, an extra step of placing the otolith on a scale to record a weight could add a supplemental parameter to the results.

We have developed a simple procedure for capturing clear, satisfactory and reproducible images of broken fish otoliths. This is a more straightforward and less labour-intensive alternative to the widely and currently used imaging method involving embedding and cross-sectioning of the otolith (Panfili *et al.*, 2002; ICES, 2013). Due to its comparative simplicity, the new procedure has the potential to be implemented on standard surveys. At the same time, we provide a reference data set for developing new automated reading algorithms. In combination, this may lead to a more efficient method for ageing fish in operational surveys.

OPEN PRACTICES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.21335/NMDC-1826273218. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

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