Regular paper

Automatic method to transform routine otolith images for a standardized otolith database using R

by

Nicolas ANDRIALOVANIRINA* (1, 2), Alizée HACHE (1), Kélig MAHÉ (1), Sébastien COUETTE (3) & Émilie POISSON CAILLAULT (2)

Submitted: 18 Mar. 2022
Accepted: 14 Oct. 2022
Editors: E. Dufour, O. Otero

Key words
Otoliths
Image processing
Standardization
Rotation
Shape analysis
Binarization

Abstract. – Fisheries management is generally based on age structure models. Thus, fish ageing data are collected by experts who analyze and interpret calcified structures (scales, vertebrae, fin rays, otoliths, etc.) according to a visual process. The otolith, in the inner ear of the fish, is the most commonly used calcified structure because it is metabolically inert and historically one of the first proxies developed. It contains information throughout the whole life of the fish and provides age structure data for stock assessments of all commercial species. The traditional human reading method to determine age is very time-consuming. Automated image analysis can be a low-cost alternative method, however, the first step is the transformation of routinely taken otolith images into standardized images within a database to apply machine learning techniques on the ageing data. Otolith shape, resulting from the synthesis of genetic heritage and environmental effects, is a useful tool to identify stock units, therefore a database of standardized images could be used for this aim. Using the routinely measured otolith data of plaice (*Pleuronectes platessa* Linnaeus, 1758) and striped red mullet (*Mullus surmuletus* Linnaeus, 1758) in the eastern English Channel and north-east Arctic cod (*Gadus morhua* Linnaeus, 1758), a greyscale images matrix was generated from the raw images in different formats. Contour detection was then applied to identify broken otoliths, the orientation of each otolith, and the number of otoliths per image. To finalize this standardization process, all images were resized and binarized. Several mathematical morphology tools were developed from these new images to align and to orient the images, placing the otoliths in the same layout for each image. For this study, we used three databases from two different laboratories using three species (cod, plaice and striped red mullet). This method was approved to these three species and could be applied for others species for age determination and stock identification.

Résumé. – Méthode automatique de transformation des images d'otolithes acquises en routine pour une base de données d'otolithes standardisés en utilisant R.

La gestion des pêches est généralement basée sur des modèles structurés en âge. De ce fait, les données sur l'âge des poissons sont collectées par des experts qui analysent et interprètent des pièces calcifiées (écailles, vertèbres, rayons de nageoires, otolithes, *etc*.). L'otolithe, située dans l'oreille interne du poisson, est la principale pièce calcifiée utilisée, car elle est la seule métaboliquement inerte et est historiquement l'une des premiers proxys de données développées. L'otolithe contient également toutes les informations de l'histoire de vie du poisson et fournit des données d'âge pour toutes les évaluations de stocks des espèces commerciales. Cette méthode traditionnelle d'estimation de l'âge par un processus d'interprétation réalisée par un scientifique expert est donc très chronophage. L'analyse d'images peut être une méthode alternative peu coûteuse. Cependant, la première étape consiste à transformer les images d'otolithes prises en routine en images standardisées au sein d'une base de données afin d'appliquer des techniques d'apprentissage automatique sur les images. La forme des otolithes, résultant de la synthèse du patrimoine génétique et des effets de l'environnement, est un outil utile pour identifier les populations, une base de données d'images standardisées pourrait donc être également utilisée pour cela. À partir des données d'otolithes de plie (*Pleuronectes platessa* Linnaeus, 1758) et de rouget barbet de roche (*Mullus surmuletus* Linnaeus, 1758) en Manche Orientale ainsi que de la morue du nord-est de l'Arctique (*Gadus morhua* Linnaeus, 1758), un protocole de standardisation des données a été proposé. Toutes les étapes méthodologiques ont été développées sous un environnement R. Une matrice d'images en niveaux de gris a été générée à partir des images brutes dans différents formats. La détection des contours a été appliquée pour identifier les otolithes cassés, l'orientation de chaque otolithe et le nombre d'otolithes par image. Pour finaliser ce processus de standardisation, toutes les images ont été redimensionnées et binéarisées. Plusieurs outils mathématiques de morphologie ont été appliqués pour aligner et orienter les images, en plaçant les otolithes dans la même disposition pour chaque image. Pour cette étude, nous avons utilisé trois bases de données de deux laboratoires différents sur trois espèces (la morue, la plie et le rouget barbet de roche). Cette méthode a été approuvée sur ces trois espèces différentes et pourra être utilisée sur de multiples espèces pour la détermination de l'âge et l'identification des stocks.

(1) Ifremer, Laboratoire Ressources Halieutiques, 150 quai Gambetta, BP 699, 62321 Boulogne-sur-Mer, France. nicolas.andrialovanirina@ifremer.fr, a.hch@laposte.net, kelig.mahe@ifremer.fr

(2) LISIC, Université Littoral Côte d'Opale (ULCO), 62228 Calais, France. emilie.caillault@univ-littoral.fr

(3) Ecole Pratique des Hautes Etudes, PSL, Paris & UMR Biogéosciences, université de Bourgogne, 21000 Dijon, France. sebastien.couette@ephe.psl.eu

* Corresponding author

INTRODUCTION

Fisheries management is generally based on an age structure model (Hilborn and Walters, 2013; Cadrin and Dickey-Collas, 2015). Fish ageing is therefore an essential biological tool, providing data for sustainable management of fish resources. In routine analysis, the age data is determined by several experienced readers who observe the surface of calcified structures (scales, vertebrae, fin rays, otoliths, etc.) showing seasonal zones (alternating opaque and translucent rings) in fishes (Panfili *et al*., 2002). Among these calcified structures, the otoliths, located in the vestibular system of the inner ear, are the most commonly used calcified structure for age estimation in fish (Fossum *et al*., 2000). They are metabolically inert (*i.e*. they can neither be altered nor generally resorbed, and they grow throughout the life of the fish; Casselman, 1987). Otoliths are also historically one of the first proxies developed; being easy to sample, store and process, age estimation using otoliths has been carried out since Reibisch (1899). These are all of the reasons why otoliths are the most commonly used calcified structures for ageing (Casselman, 1987; Campana and Thorrold, 2001). Campana and Thorrold (2001) estimated that nearly 800,000 otoliths were used worldwide each year to determine the age structures of commercial fish species, representing a cost of around 8 million Canadian dollars. In Europe, fisheries management relies on data collected, managed, and supplied by countries under the Data Collection Framework, which was first put into place in 2000. Under this framework, the member states are required to annually collect a number of calcified structures, and in 2010 22 countries analyzed 759,403 calcified structures (ICES, 2011). Each year, nearly one million otoliths are collected worldwide, including 35,000 in France, to provide age structure data for stock assessments of commercial species.

The traditional and most common use of otoliths is for age and growth determination, the internal structure of otoliths showing different growth increments with periodicity from a day to a year (*i.e*. daily, seasonal, and annual growth cycles) depending on the observed life stage (Panfili *et al*., 2002). There are also other uses of an otolith, in particular for microchemistry (Hüssy *et al*., 2021) and external shape analyses (Cadrin *et al*., 2013; Mahé, 2019). The morphology of otoliths is used to identify species in zooarchaeological and food-web studies. Otolith outline shape depends on the fish genotype, the influence of environmental factors (biotic and abiotic) during the life of the fish, and on the stage of development. Consequently, many studies have used otolith shape as a tool for stock identification (91 papers published from 1993 to 2017 exclusively on this topic) and this continues to develop substantially (Mahé, 2019; ICES, 2020). Since 2005, however, (according to Web of Science and the Stock Identification Methods Working Group [SIMWG] set up by the International Council for the Exploration of the Sea, ICES, see supplementary figure), there has been an increase in the number of otolith-based publications. This rise is due to two further research topics: otolith shape as a tool for stock identification, and otolith microchemistry to understand the ecology, habitat, and movement of individuals and to characterize the connectivity between different geographical areas for a studied species (*e.g*. Smith, 1992; Cardinale *et al*., 2004; Lombarte *et al*., 2006; Vignon *et al*., 2008; Cadrin *et al*., 2013; Chung *et al*., 2019; Randon *et al*., 2020; Hüssy *et al*., 2021).

Otolith shape is species-specific, but can also show intra-specific geographic differences (L'Abée-Lund, 1988; Lombarte and Lleonart, 1993; Vignon, 2012). Indeed, the otolith outline integrates both genetic determinism and the variation of environmental conditions during the life of the fish (Mahé, 2019). Species discrimination using the otolith shape is mostly done for species identification from the stomach contents of predators to reconstruct their diet and the food web. At the species level, otolith shape is used to identify the boundaries of stocks, which information is necessary for effective stock assessment to thus implement an efficient fishery management. In the majority of stock discrimination case studies, approaches based on otolith shape and/or morphometry represent the cheapest methodology, and appear as more efficient than several others (*e.g*. genetics, parasites, isotope and micro-chemical discrimination techniques) (Neves *et al*., 2011). Many studies have combined different markers to increase the power of stock discrimination (Randon *et al*., 2020). This explains partly the growing number of papers dealing with this subject, along with the development of methods (*i.e*. Morphometric variables, Shape indices, Elliptical Fourier Descriptors, Geometric methods from landmarks, Wavelet transform, etc.) and tools (packages in R dedicated to shape analysis (Libungan and Pálsson, 2015) or programs such as Shape (Iwata and Ukai, 2002)), to analyze otolith shape. Finally, several works have recently been published on otolith morphogenesis, and particularly on the symmetry between left and right otoliths (*e.g*. Mille, 2015; Palmer, 2016; Mahé *et al*., 2021). For several flatfishes, left and right otoliths are asymmetric so shape analysis is used to compare the degree of asymmetry between otoliths (Mille, 2015; Delerue-Ricard *et al*., 2019). For such shape comparisons, one otolith must be translated horizontally to compare to the second otolith of the same individual without bias. According to Palmer (2016) and Mahé *et al*. (2021), otolith asymmetry may be due to random (or stochastic) effects, genetic determinism, or environmental stress.

Since the 2000s, otolith imaging has been greatly enhanced by the development of web-based tools (*i.e*. SmartDots, https://www.ices.dk/data/tools/Pages/smartdots.aspx) to evaluate the precision of the age data during calibration exercises between experts or using the web-database to recognise species from otolith shape (Lombarte *et al.*, 2006). At the same time, solutions dedicated to otolith images processing have been developed in freeware such as ImageJ, or into dedicated software such as TNPC, which is specifically designed to estimate fish age (Mahé *et al.*, 2011). Consequently, a very large number of images acquired for routine fish ageing could be used for otolith shape studies, reducing the human cost of image acquisition and potentially increasing the number of images available for this type of study. For several species, age is obtained directly from the whole otolith, without prior preparation. This represents around 30 percent of the species studied in France (Mahé *et al.*, 2009), including a large number of flatfish, or roundfish (Vitale *et al.*, 2019). Quality and standardization levels required for ageing studies versus shape analyses are, however, not the same. An overview of two-dimensional imaging systems showed that there is also a great diversity in the methods and materials for routine acquisition, which also influences the quality of the raw images (Fisher and Hunter, 2018). For ageing data, the information is in the growth bands within the otolith, for which a full external shape is not necessary. Consequently, the objective of this paper is to describe an efficient method to transform a routinely acquired ageing database into a standardized otolith shape database to easily interpret ageing and shape analyses. We highlight available data, all steps contained in the methodological approach and, finally, we applied this protocol to a case study using the French database of plaice (*Pleuronectes platessa* Linnaeus, 1758; *n* = 11,274) in the Eastern English Channel and the southern North Sea from 2010 to 2019 and striped red mullet (*Mullus surmuletus* Linnaeus, 1758; *n* = 1,149) in the Eastern English Channel. Norwegian database of cod (*Gadus morhua* Linnaeus, 1758; *n* = 1,055) was also used to include northeast Arctic cod survey (Norwegian-Russian winter survey and Spawning Cod acoustic-trawl survey) (Myers *et al.*, 2020).

MATERIALS

Sampling

Age-structured models need year-round monitoring, thus samples are taken during all four annual quarters, either at sea or on land. At sea, the calcified structures are taken either on board a professional fishing vessel or during scientific surveys. On land, they are sampled in the fish market or in the scientific laboratory.

When a fish is sampled to estimate its age, both biological (species, individual size and weight, sex, sexual maturity staging, etc.) and sampling parameters (catch date and location, sampling area, gear, etc.) are identified. Accuracy of this information is directly dependent on the sampling method; for example, samples in the fishing market have many uncertainties, especially the geographical positions, but all the necessary data are acquired with a very high degree of precision during scientific surveys. Consequently, the quality of the metadata associated with the otolith may limit its use for shape analysis, depending on the scientific question to be answered. In addition, the sampling location also has a direct influence on sample quality, as it relates to the percentage of damaged and/or dirty otoliths (Stevenson and Campana, 1992).

The final step before image acquisition is the transport and storage from the sampling location to the laboratory. Paper envelopes and micro-tubes are two main means of storage. Paper envelopes, recommended by Williams and Bedford (1974), are very practical during sampling and filing to keep calcified structures, but their use increases the percentage of broken otoliths, which can be used for ageing but are unusable for shape analysis. A last means of transport and storage, which is specifically used for small otoliths from pelagic species during scientific surveys, is a black plastic strip with cavities where the otoliths are embedded in resin (Vitale *et al.*, 2019). This last method simplifies aging acquisition on board but it reduces the quality of images due to the resin (*i.e.* poor contrast, presence of impurities or air bubbles, etc.) that is present above the otolith.

Image acquisition for ageing database

Each sagittal otolith is prepared and analyzed for age determination according to the international ageing protocol (Vitale *et al.*, 2019). Depending on the study species, whole, broken and/or sectioned otoliths are used for ageing (Vitale *et al.*, 2019), however sectioned and broken otoliths cannot be used for shape analysis.

To prepare the otoliths for analysis, they are first cleaned of organic matter using sponge, paper towels, a soft brush, or an ultrasonic cleaner. For some species (*e.g.* striped red mullet, *Mullus surmuletus* Linnaeus, 1758; Mahé *et al.*, 2013), otoliths can be burned to increase the visibility of growth increments as defined by Christensen (1964).

Depending on sampling, otolith images are taken using only one otolith or both left and right otoliths, leading to these two different types of otolith images in routine otolith image databases. As shown in figure 1, some of the acquisitions are non-standardized, and sometimes otoliths are broken or there is an overlap between left and right otoliths. Moreover, for the same species, the size of the otoliths can be very different and, in several cases, various magnifications are used. Three different tools are used to take the otolith images: a scanner (low resolution), a binocular dissecting microscope (medium resolution) and a high resolution microscope, which enables visualization of internal (growth increments) and outline structures (shape). With both types of microscope, cameras with different technical characteristics (*i.e.* lens, magnification, sensor, resolution) can be

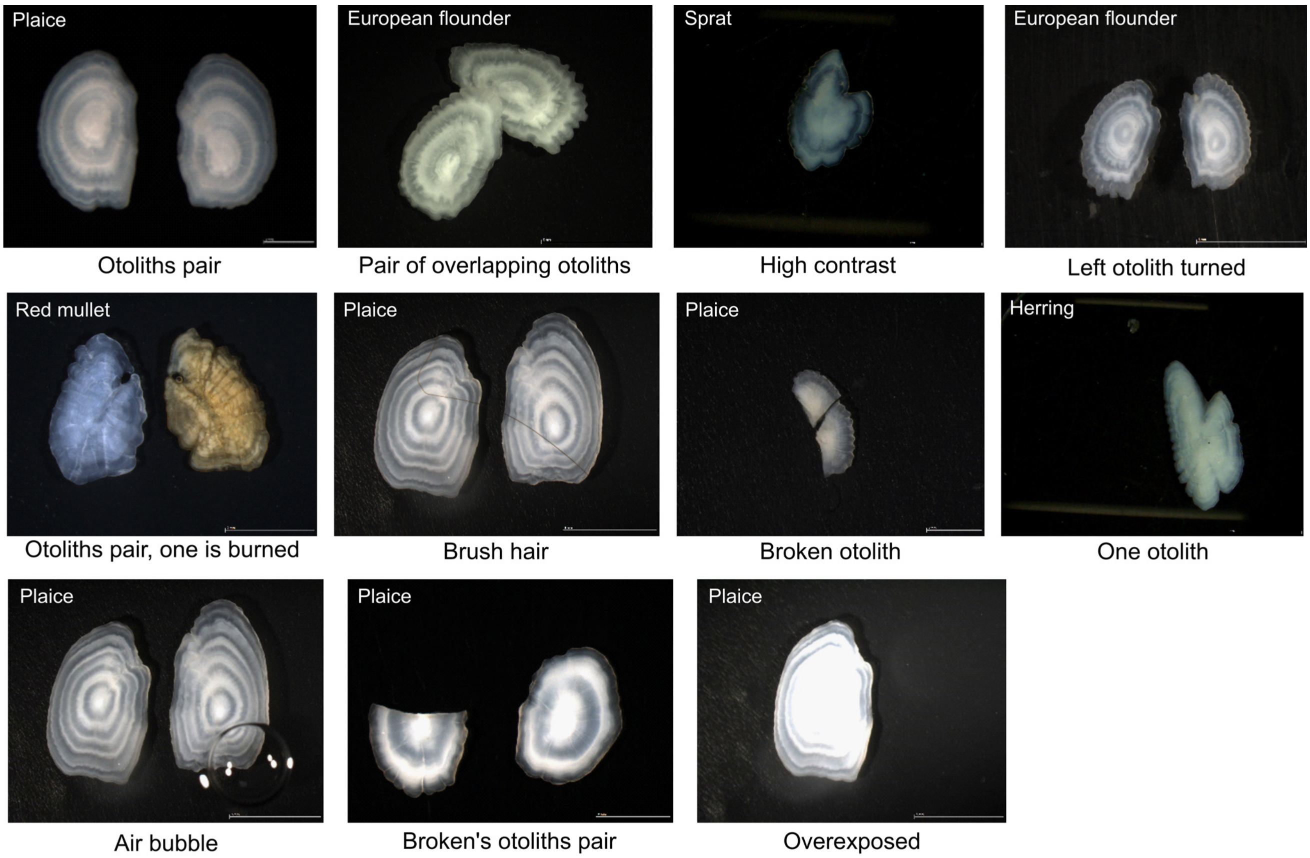


Figure 1. – Different types of otolith images showing common issues. Scale is different between images depending on the sample, some broken otoliths, various exposures and colors, some particles may be present (hair, bubbles).

used, resulting in different pixel sizes. Images are generally acquired made using an RGB (RedValue + GreenValue + BlueValue) channel, but can sometimes, depending on the reader, be taken in greyscale. Image light intensity is also adjusted by the reader. All of these possibilities for image acquisition in terms of resolution, colour, magnification, light intensity or number of otoliths per image (or broken / decalcified otoliths) lead to a lack of standardization in the routine images (Fig. 1).

For all acquisition methods, readers can choose reflected or transmitted light to illuminate the otoliths. It is assumed that one annulus (*i.e.* one annual growth increment) consists of one opaque and one translucent zone (Panfili *et al.*, 2002). Under reflected light, the opaque zones of the otoliths are white and the translucent zones are dark (on a black background). In transmitted light (with a white background), the opposite is true: the opaque zones of the otoliths are dark and the translucent zones are white (Fig. 2).

Finally, a database is created with biological parameters, images and ageing data for each fish. Table I summarises the various sources of potential differences with their respective solutions, which are described later in this paper.

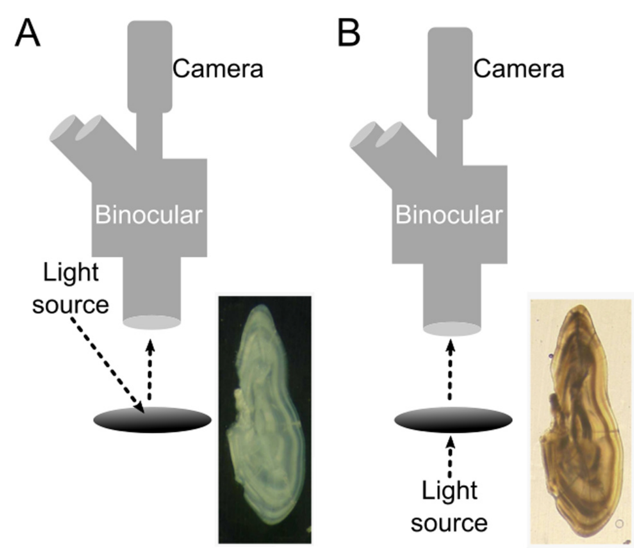


Figure 2. – Otolith images from a binocular dissecting microscope under different types of illumination: A. Reflected light and B. Transmitted light.

Data organization

The database for routine otolith processing has two com-

Table I. –Sources of differences between otolith images with proposed solutions.

Source of bias	Potential differences	Methodological steps to standardize
Otolith presentation	Whole / Broken / overlapped otoliths / pairs of otoliths / left or right otoliths	Sorting (with several groups with different characteristics)
Preparation method	Otolith in water / burnt otolith / otolith embedded in resin	Sorting (with several groups with different characteristics)
Light type	Transmitted light / Reflected light	Sorting (with several groups with different characteristics)
Acquisition system	Scanner / Binocular microscope / High-resolution microscope	1. Greyscale image / 2. Count and cut otolith image: resizing / 3. Oriented image: binarized image
Light intensity	Various exposures	1. Greyscale image / 3. Oriented image: binarized image
Contrast of image	Depends on readers and camera	1. Greyscale image / 3. Oriented image: binarized image
Colour channel	RGB / Greyscale	1. Greyscale image / 3. Oriented image: binarized image
Number of otoliths	Left otolith / Right otolith / Both otoliths	2. Count and cut otolith image: cutting and mirror effect
Magnification	From 1x to 100x	2. Count and cut otolith image: resizing
Ratio of otolith to image size	Percentage from 10 to 95 %	2. Count and cut otolith image: resizing
Image resolution	Between 658 and 1432 pixel/cm	2. Count and cut otolith image: resizing
Orientation of longest axis	Orientation from 0 to 360 degrees	3. Oriented image: main axis alignment
Format of image	jpg / png / raw / tiff	Save in single format

ponents: firstly, the images database with a name which is composed of the sampling quarter and year, the name of the reader, and the location (*i.e.* survey name or fishing port); secondly, the metadata file which includes the name of the otolith image and the metadata on the fish catch (survey/commercial sampling, date, location, vessel and gear) and species (sex, sexual maturity stage, length and weight).

METHODS

To standardize otolith images, the algorithm in R used three successive steps (Fig. 3). Before the image processing, sorting of the images was done to identify those that were unusable for the standardization process, such as the broken or overlapping otoliths. The algorithm we developed selects otoliths by greyscale and then by size. For an image with a single broken otolith our algorithm cannot detect that it is a broken otolith (therefore excluded). On the other hand, on an image with a pair of otoliths with a whole otolith and a broken one, the algorithm will be able to select only the whole otolith and not take into account the broken one.

After this pre-treatment, the standardization steps were implemented. Firstly, the otolith image was transformed into greyscale (Fig. 3; Step 1) using the magick package (Ooms, 2021). The transformation was done by converting the three-channel image (*i.e.* RGB) to a single channel using the formula in equation 1 (Ooms, 2021):

$$\text{Greyscale} = (\text{RedValue} + \text{GreenValue} + \text{BlueValue}) / 3 \quad (1)$$

Step 2 of the algorithm comprises several functions to count and segment individual otoliths present in captured imagery. A basic R function “contourLines” with 8 levels was used to detect the otolith outlines (a greyscale or binarized image can be used). To detect whether objects are otoliths or not, a minimum area is defined. The area of each outline within the image is then calculated. This step also allows the exclusion of broken otoliths. Contours with a high area that are not included in another shape are kept and counted. Thus, the number of otoliths is deduced with the perimeter of an image. If there are two otoliths in the same image, the algorithm identifies the minimum and maximum point on the y axis (y and ymax) for the lowest and/or highest otolith; and the minimum and maximum point in x axis (x_1, $x_1$max; x_2, $x_2$max) for each otolith. The otolith image is then cut using the values of x_1:$x_1$max; y:ymax for the left otolith and x_2:$x_2$max; y:ymax for the right otolith. This step also resizes the image because all images are resized to the otolith proportions. In shape analysis, the otolith side has to be comparable, thus the user can apply a horizontal translation (*i.e.* mirror effect) on the otolith from one side (for this modification, the cut of the image must be reversed, from xmax to x).

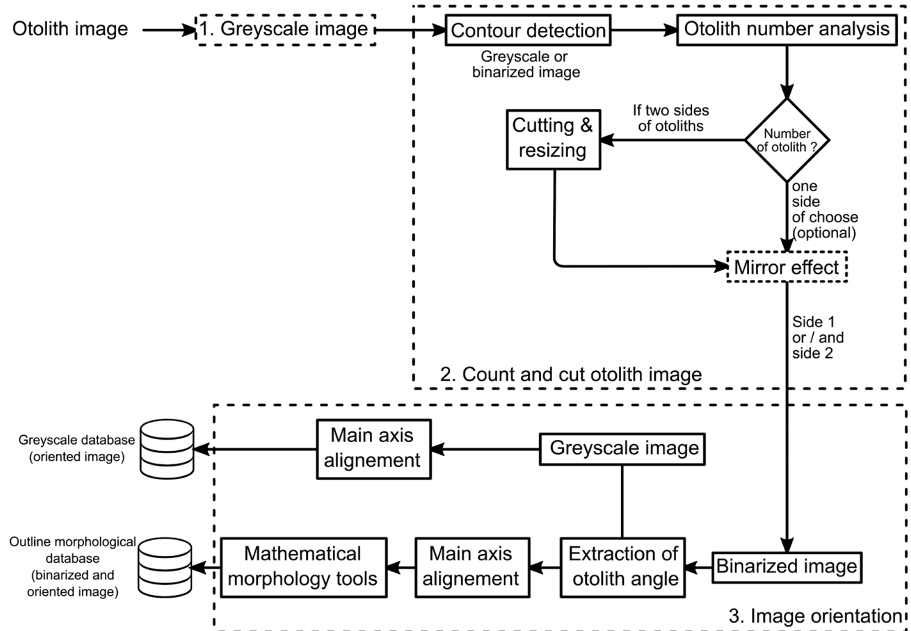


Figure 3. – Proposed processing pipeline for converting otolith images from a conventional ageing database to a standardized shape database format in the R environment (R scripts are presented in the Supplementary data).

The third step performs the otolith reorientation and the extraction of morphological information. The greyscale image is transformed into an image with binary colours (black [pixel value = 0]; white [pixel value = 1]): all pixels with a value superior to 0 are converted into 1. To correct potential conversion problems and to avoid calculation errors of morphological information: (1) smoothing is applied to the contour of the object (*i.e.* the otolith); (2) black pixels are added on the edges of the object to allow better outline detection in the future; and (3) a pixel erosion (with the help of a kernel, used depending on the shape of the otolith, *i.e.* box, disc, diamond) is applied to the image to remove white particles outside the otolith. The orientation angle of the otolith over its longest length (Θ) is then extracted using the "computeFeatures.moment" function. Binarized and greyscale images are reoriented on the 0 axis (rotate at [- Θ]) of the longest length (Fig. 4).

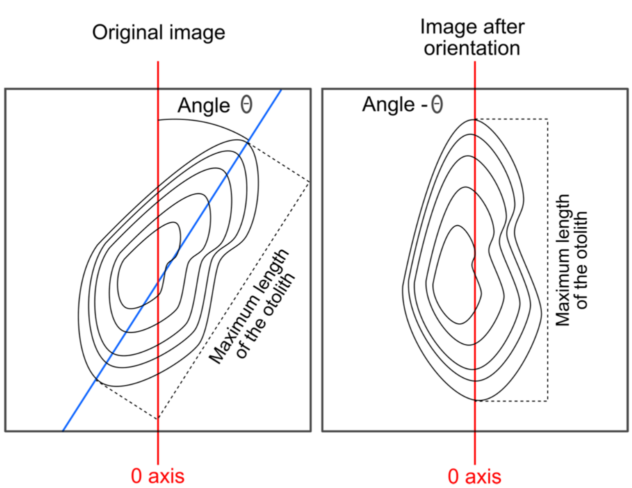


Figure 4. – Automatic otolith image orientation method.

As the binarized image has been correctly oriented, the height of the otolith (in pixels) corresponds to the number of white pixels on the y-axis (on the highest height); the width relates to the number of white pixels on the x-axis (on the widest width); and the area of the otolith relates to the total number of white pixels on the image. Finally, an oriented, binarized image database with metadata on morphometric and resolution (in pixel/cm) information, and another with oriented greyscale images are generated. These databases have standardized images for shape analysis (using oriented, binarized images) and for age analysis (using oriented greyscale images).

All parameters in the algorithm were reported in the table II and were the same in all tested database.

The segmentation of the otolith images (accuracy of the segmentation, good or not) was checked by visualizing the results.

CASE STUDY USING PLAICE, STRIPED RED MULLET AND COD

To apply the standardization process, plaice (*Pleuronectes platessa* Linnaeus, 1758), striped red mullet (*Mullus surmuletus* Linnaeus, 1758) and cod (*Gadus morhua* Linnaeus, 1758) otoliths image database were processed. Data for plaice and striped red mullet were provided by Andria-

Table II. – Optimization of all parameters of the algorithms in each function.

Functions	Parameters	Optimization	Best value
separeOto / separeOto_n1	offset	Number of pixel(s) of accepted overlap in the side of the otolith	0
separeOto / separeOto_n1	ratioArea	For the first 100 images, computes the t3 quantile of the area proportion [1-quantile(area/ image size (Lmat*Hmat), 0.75)]	0.2
separeOto_n1 / calculInfoOto	k (pixel erosion)	shapeKernel parameter requires an image size longer and larger than 60	shapeKernel(c(10,10), type=”box”) shapeKernel(c(5, 5), type=”box”)
separeOto / separeOto_n1	nlevels (contourLines)	Could be between 4-8 to accelerate computation, more than 8 improves the outline precision	8

lovanirina *et al*. (2022). Myers *et al*. (2020) supplied data for cod otolith.

In the French plaice and striped red mullet database, the images were acquired by scanner or binocular microscope using reflected light.

A total of 11,274 routinely acquired images of plaice otolith were processed to obtain 8683 standardized right otolith images (100% of these were well cut). Images could contain a right or left otolith or both. When they contained two otoliths, one could be broken and therefore not retained for analysis. The image processing time varied between 1 and 3 seconds depending on the power of the computer used. Figure 5 shows the standardization process for an otolith image.

For striped red mullet otoliths in the processed photos included burned or unburned, left or right otoliths or both. A total of 1,149 images were standardized to get 918 standardized right otoliths (98% of these were well cut) (example in Fig. 6).

For cod, Myers *et al*. (2020) were used a transmitted light and three exposures for each otolith image (standard,

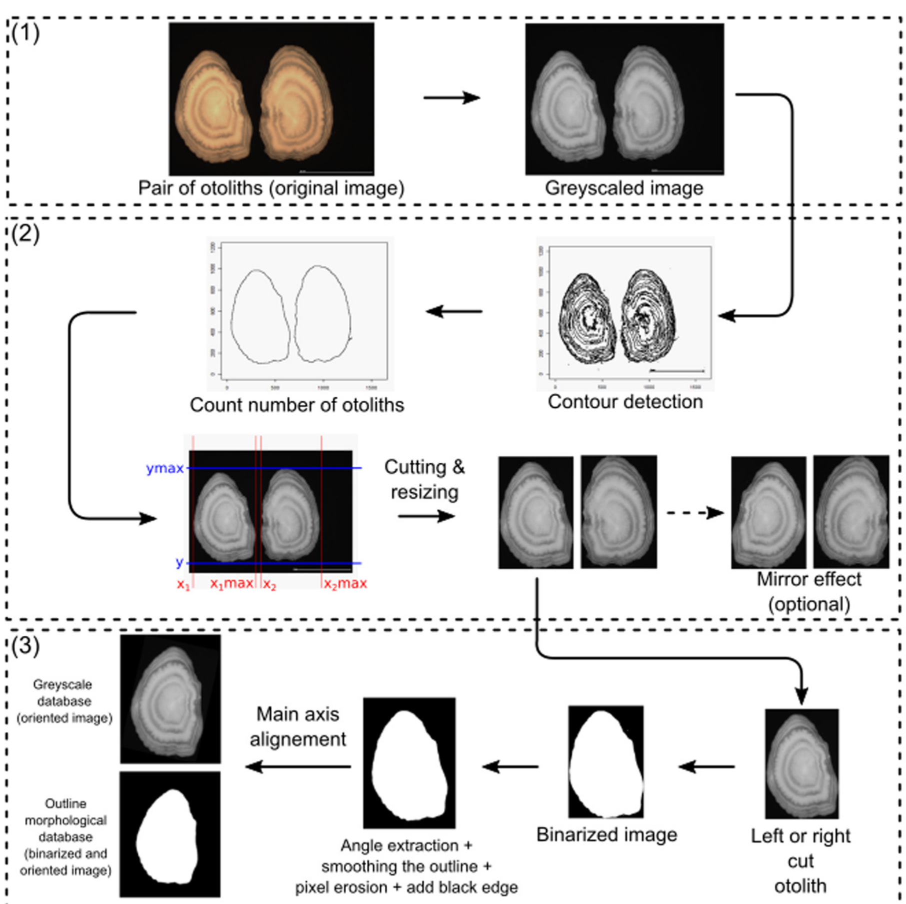


Figure 5. – Otolith image process of plaice (*Pleuronectes platessa*; Linnaeus, 1758) standardization with illustration of three main steps presented in figure 3.

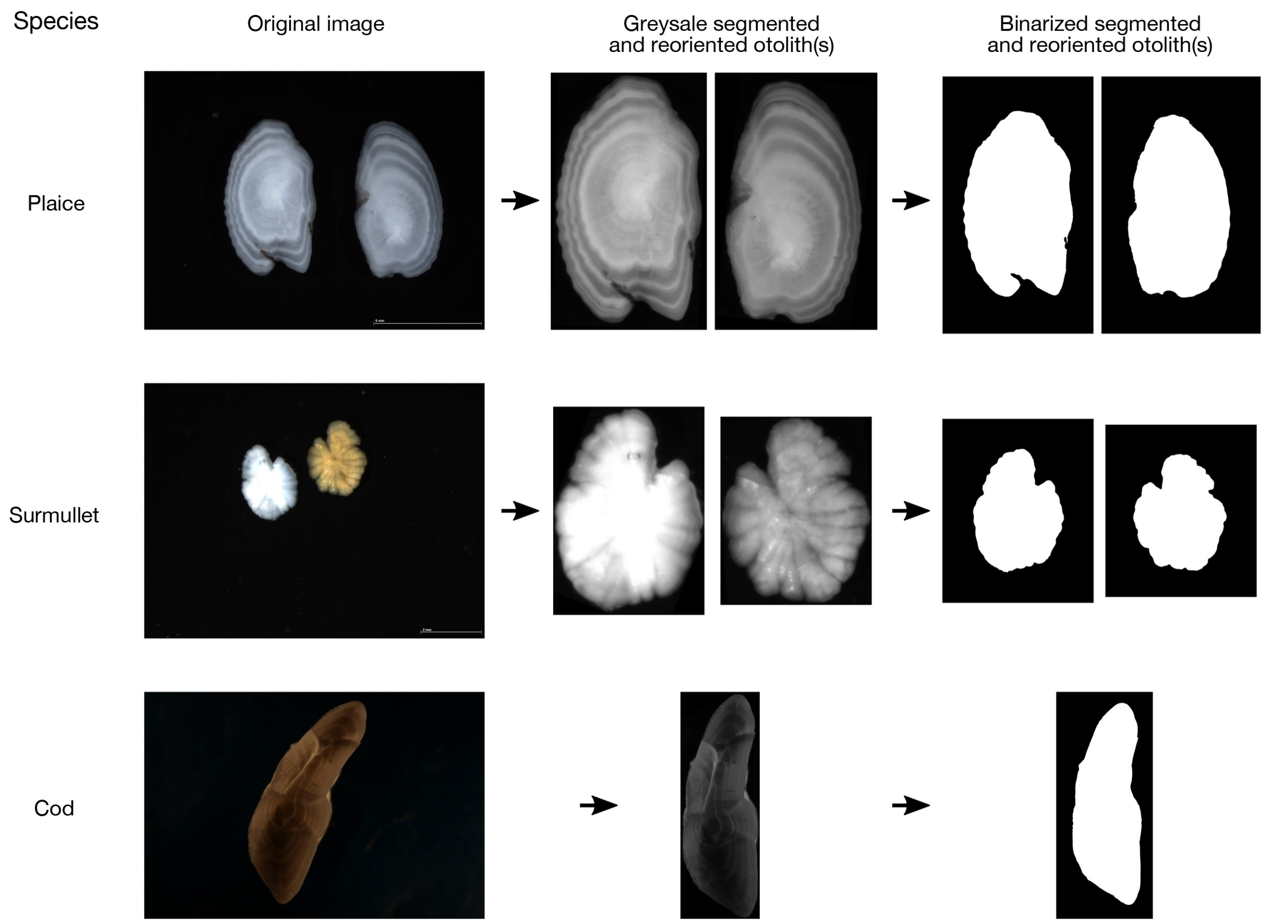


Figure 6. – Example of otolith image standardization for the three species.

decreased, and increased exposure). If the exposure was not decreased, the background was not uniform. A total of 1,055 images of cod otoliths were processed on three types of exposure (standard, increased and decreased). For each image, there was only one side of the otolith. The images with a decreased exposure were the best cut with 97% success (Fig. 6) (compared to 47% for standard exposures and 6% for increased exposures).

CONCLUDING REMARKS

The otolith is a calcified part, which is very often used to estimate the age of fish; many laboratories have thus a lot of image databases of otoliths in order to develop age structured models for stock management. However, images taken by each laboratory are not always using the same image acquisition protocol, this results in non-standardized image databases (see material). The objectives of this study were to develop a method to standardize otolith images and applying it on three species otolith images, originating from two laboratories. The method was able to standardize images with different acquisition processes, two types of illumination (reflected and transmitted), various species, and with burned and unburned otoliths. It was very effective on images where there is a strong contrast between the background of the image and the otolith. On the opposite, when the background is brighter (case of standard exposure and enhanced cod otolith images) the detection of the otolith is difficult using our algorithm. In the case of an image with an illuminated background, it is then necessary to use a decreased exposure. When the otolith is darker (case of 3% of cod and 2% of unburned red mullet otolith samples), an increased exposure of the otolith could be considered.

The method takes into account the cut and orientation of the otoliths as well as the translation of one side of otolith for studies of otolith asymmetry for example. All recent research into the shape of the otolith and the process of biomineralization, taking into account potential differences between the two ears, requires the standardized images as proposed in this paper to analyse the internal or external patterns of otoliths. Several methods of classification/learning have become well-developed in recent years with the emergence of mathematical and statistical tools and, particularly, artificial intelligence (*i.e.* deep learning). This latter approach allows the development of innovative topics, such as automatic age reading, but requires a very large number of images (at least several thousand). It is therefore very important to standardize the tools used for otolith image analysis, such as our method for standardizing routinely acquired otolith images. This standardization step is essential before any automated analysis (analysis of the internal and external structures of otoliths) since routinely acquired otolith images can present several variations (see Methods), as has been discussed in several previous studies (Easey and Millner, 2008; Mahé *et al.*, 2009; Vitale *et al.*, 2019; VanderKooy *et al.*, 2020). Currently, there is a debate about the best standard image formats to use, but several methods remain in current use. Acquisition by scanner is, however, increasingly used, allowing the acquisition of multiple standardized otolith images (Gonçalves *et al.*, 2017; Moore *et al.*, 2019; Mahé *et al.*, 2021). Moore *et al.* (2019), for example, acquired otolith images using a scanner and developed a machine learning technique for the automatic ageing of fish.

Recently, otolith research on automatic methods for species identification or automatic ageing using a deep learning approach around has developed strongly (Stock *et al.*, 2021; Moen *et al.*, 2018; Ordoñez *et al.*, 2020; Politikos *et al.*, 2021; Vabø *et al.*, 2021; Benzer *et al.*, 2022). Other research domains have also shown that image standardization is an important step in computer vision or deep learning (Lee *et al.*, 2017; Folmsbee *et al.*, 2019; Misztal *et al.*, 2020). For otoliths, Fisher and Hunter (2018) and Myers *et al.* (2020) developed automation of otolith image interpretations. Our protocol, a preliminary step to automated ageing studies using a very large number of images, should facilitate access to standardized images and thus the development of these automation approaches. Our method could be standardized with one image per second from mathematical algorithms (*i.e.* machine time), which is faster than the classic method with the time of four minutes realized by an expert. In France, over 40000 images are analyzed each year in the Ifremer Center for Sclerochronology. These images could be used for other scientific purposes such as stock identification, automatic ageing, species discrimination and investigation of the potential drivers controlling otolith morphogenesis. Using our protocol, however, the preliminary choices of light sources (transmitted or reflected) and image formats during the acquisition stage make it necessary to treat these batches of images differently. Deep learning could, from a different perspective, also be used to sort images with different light sources and with various presentations of otoliths (*e.g.* broken or overlapped otoliths).

The principle of the method presented here is to standardize otolith images acquired routinely with microscopes or scanners, but this method can be applied in other areas to standardize images with a uniform background. In fisheries science, for example, images of fish eggs acquired with a microscope could be standardized before analysis; Duan *et al.* (2019) has already developed a method to automatically segment fish eggs.

Acknowledgements. – The authors thank technicians, researchers, and students who helped with the samples, and the Sclerochronology team of IFREMER for image acquisition and ageing data. The authors would especially thank Kirsteen MacKenzie for valuable help in editing this manuscript.
The authors are also grateful for the insightful comments and recommendations offered by the anonymous peer reviewers at this manuscript. Their comments improved very well the quality of this paper.
This study was supported by the French National Research Agency (ANR-21-EXES-0011) and the Data Collection Framework(DCF; EC Reg. 199/2008, 665/2008; Decisions 2008/949/EC and2010/93/EU). This work was supported by the Institut Français de Recherche et d'Exploitation de la Mer and the ULCO University (doctoral support to N. ANDRIALOVANIRINA, 2021-2024).

REFERENCES

ANDRIALOVANIRINA N., HACHÉ A., MAHÉ K., COUETTE S. & POISSON CAILLAULT E., 2022. – Data applied to automatic method to transform routine otolith images for a standardized otolith database using R. SEANOE. https://doi.org/10.17882/91023

BENZER S., GARABAGHI F.H., BENZER R. & MEHR H.D., 2022. – Investigation of some machine learning algorithms in fish age classification. *Fish. Res.*, 245. https://doi.org/10.1016/j.fishres.2021.106151

CADRIN S.X. & DICKEY-COLLAS M., 2015. – Stock assessment methods for sustainable fisheries. *ICES J. Mar. Sci.*, 72: 1-6. https://doi.org/10.1093/icesjms/fsu228

CADRIN S., KERR L. & MARIANI S., 2013. – Stock Identification Methods: Applications in Fishery Science. Second Edition. Elsevier Academic Press, Amsterdam.

CAMPANA S.E. & THORROLD S.R., 2001. – Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.*, 58: 30-38. https://doi.org/10.1139/f00-177

CARDINALE M., DOERING-ARJES P., KASTOWSKY M. & MOSEGAARD H., 2004. – Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Can. J. Fish. Aquat. Sci.*, 61: 158-167. https://doi.org/10.1139/f03-151

CASSELMAN J.M., 1987. – Determination of age and growth, *In*: The Biology of Fish Growth (Weatherley A.H. & Gill H.S., eds), pp. 209-242. New York.

CHRISTENSEN J.M., 1964. – Burning of otoliths, a technique for age determination of soles and other fish. *ICES J. Mar. Sci.*, 29: 73-81. https://doi.org/10.1093/icesjms/29.1.73

CHUNG M.T., TRUEMAN C.N., GODIKSEN J.A., HOLMSTRUP M.E. & GRØNKJÆR P., 2019. – Field metabolic rates of teleost fishes are recorded in otolith carbonate. *Comm. Biol.*, 2: 1-10. https://doi.org/10.1038/s42003-018-0266-5

DELERUE-RICARD S., STYNEN H., BARBUT L., MORAT F., MAHÉ K., HABLÜTZEL, P.I., HOSTENS K. & VOLCKAERT F.A.M., 2019. – Size-effect, asymmetry, and small-scale spatial variation in otolith shape of juvenile sole in the Southern North Sea. *Hydrobiologia*, 845: 95-108. http://dx.doi.org/10.1007/s10750-018-3736-3

DUAN Y., LI D., STIEN L.H., FU Z., WRIGHT D.W. & GAO Y., 2019. – Automatic segmentation method for live fish eggs microscopic image analysis. *Aquacult. Eng.,* 85: 49-55. https://doi.org/10.1016/j.aquaeng.2019.01.004

EASEY M.W. & MILLNER R.S., 2008. – Improved methods for the preparation and staining of thin sections of fish otoliths for age determination. *Sci. Ser. Tech. Rep*. 143, 16.

FISHER M. & HUNTER E., 2018. – Digital imaging techniques in otolith data capture, analysis and interpretation. *Mar. Ecol. Prog. Ser.*, 598: 213-231. https://doi.org/10.3354/meps12531

FOLMSBEE J., JOHNSON S., LIU X., BRANDWEIN-WEBER M. & DOYLE S., 2019. – Fragile neural networks: the importance of image standardization for deep learning in digital pathology. *In*: Medical Imaging 2019: Digital Pathology, pp. 222-228. Presented at the Medical Imaging 2019: Digital Pathology. SPIE. https://doi.org/10.1117/12.2512992

FOSSUM P., KALISH J. & MOKSNESS E., 2000. – Foreword. *In*: 2nd International Symposium on Fish Otolith Research and Application, Bergen, Norway, 20-25 June 1998. *Fish. Res.,* 46(1-3): 1-2. https://doi.org/10.1016/S0165-7836(00)00126-0

GONÇALVES P., MAHE K., ELLEBOODE R., CHANTRE C., MURTA A., AVILA DE MELO A. & CABRAL H., 2017. – Blue whiting otoliths pair's symmetry side effect. *Int. J. Fish. Aquat. Stud*., 5: 6-9.

HILBORN R. & WALTERS C.J., 2013. – Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. 575 p. Springer Science & Business Media.

HÜSSY K., LIMBURG K.E., DE PONTUAL H., THOMAS O.R.B., COOK P.K., HEIMBRAND Y., BLASS M. & STURROCK A.M., 2021. – Trace element patterns in otoliths: the role of biomineralization. *Rev. Fish. Sci. Aquacult*., 29: 445-477. https://doi.org/10.1080/23308249.2020.1760204

ICES, 2011. – Report of the Workshop of National Age Readings Coordinators (WKNARC). 5-9 Sep. 2011, Boulogne-sur-Mer, France. ICES CM 2011/ACOM:45, 175.

ICES, 2020. – Stock Identification Methods Working Group. *ICES Sci. Rep*., 2: 38.

IWATA H. & UKAI Y., 2002. – SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Hered*., 93: 384-385. https://doi.org/10.1093/jhered/93.5.384

L'ABÉE-LUND J.H., 1988. – Otolith shape discriminates between juvenile Atlantic salmon, *Salmo salar L*., and brown trout, *Salmo trutta* L. *J. Fish Biol*., 33: 899-903. https://doi.org/10.1111/j.1095-8649.1988.tb05538.x

LEE H., TAJMIR S., LEE J., ZISSEN M., YESHIWAS B.A., ALKASAB T.K., CHOY G., & DO S., 2017. – Fully automated deep learning system for bone age assessment. *J. Digit. Imaging*, 30: 427-441. https://doi.org/10.1007/s10278-017-9955-8

LIBUNGAN L.A. & PÁLSSON S., 2015. – Shape R: An R package to study otolith shape variation among fish populations. *PLOS ONE*, 10: e0121102. https://doi.org/10.1371/journal.pone.0121102

LOMBARTE A. & LLEONART J., 1993. – Otolith size changes related with body growth, habitat depth and temperature. *Environ. Biol. Fish*., 37: 297-306. https://doi.org/10.1007/BF00004637

LOMBARTE A., CHIC Ò., PARISI-BARADAD V., OLIVELLA R., PIERA J. & GARCÍA-LADONA E., 2006. – A web-based environment for shape analysis of fish otoliths. The AFORO database. *Sci. Mar.*, 70: 147-152.

MAHÉ K., 2019. – Sources de variation de la forme des otolithes : implications pour la discrimination des stocks de poissons. PhD Thesis, 273 p. Université du Littoral Côte d'Opale (ULCO), école doctorale – Sciences de la Matière, du Rayonnement et de l'Environnement.

MAHÉ K., BELLAIL R., DUFOUR J.L., BOIRON-LEROY A., DIMEET J., DUHAMEL E., ELLEBOODE R., FELIX J., GRELLIER P., HUET J., LABASTIE J., LE ROY D., LIZAUD O., MANTEN M.L., MARTIN S., METRAL L., NEDELEC D., VERIN Y., & BADTS V., 2009. – French summary of age estimation procedures. 79 p. Ifremer

MAHÉ K., FAVE S. & COUTEAU J., 2011. – TNPC User guide. 83 p. Ifremer – Département Halieutique Manche Mer du Nord, Laboratoire Ressources Halieutiques de Boulogne.

MAHÉ K., COPPIN F., VAZ S. & CARPENTIER A., 2013. – Striped red mullet (*Mullus surmuletus* Linnaeus, 1758) in the eastern English Channel and southern North Sea: growth and reproductive biology. *J. Appl. Ichthyol*., 29: 1067-1072. https://doi.org/10.1111/jai.12266

MAHÉ K., MACKENZIE K., IDER D., MASSARO A., HAMED O., JURADO-RUZAFA A., GONÇALVES P., ANASTASOPOULOU A., JADAUD A., MYTILINEOU C., RANDON M., ELLEBOODE R., MORELL A., RAMDANE Z., SMITH J., BEKAERT K., AMARA R., DE PONTUAL H. & ERNANDE B., 2021. – Directional bilateral asymmetry in fish otolith: a potential tool to evaluate stock boundaries? *Symmetry*, 13: 987. https://doi.org/10.3390/sym13060987

MILLE T., 2015. – Sources de variation intra-populationnelle de la morphologie des otolithes : asymétrie directionnelle et régime alimentaire. PhD Thesis, 222 p. Université de Lille 1 – Sciences et Technologies.

MISZTAL K., POCHA A., DURAK-KOZICA M., WĄTOR M., KUBICA-MISZTAL A. & HARTEL M., 2020. – The importance of standardization – COVID-19 CT & Radiograph Image Data Stock for deep learning purpose. *Comput. Biol. Med*., 127. https://doi.org/10.1016/j.compbiomed.2020.104092

MOEN E., HANDEGARD N.O., ALLKEN V., ALBERT O.T., HARBITZ A. & MALDE K., 2018. – Automatic interpretation of otoliths using deep learning. *PLOS ONE*, 13: e0204713. https://doi.org/10.1371/journal.pone.0204713

MOORE B., MACLAREN J., PEAT C., ANJOMROUZ M., HORN P. & HOYLE S., 2019. – Feasibility of automating otolith ageing using CT scanning and machine learning.(New Zealand Fisheries Assessment Report 2019/58. 23 p. https://doi.org/10.13140/RG.2.2.29670.16960

MYERS S.C., THORSEN A., SMOLIŃSKI S., AANESTAD GODIKSEN J., MALDE K., & HANDEGARD N.O., 2020. – An efficient protocol and data set for automated otolith image analysis. *Geosci. Data J*., 7: 80-88. https://doi.org/10.21335/NMDC-1826273218

NEVES A., SEQUEIRA V., FARIAS I., VIEIRA A.R., PAIVA R. & GORDO L.S., 2011. – Discriminating bluemouth, *Helicolenus dactylopterus* (Pisces: Sebastidae), stocks in Portuguese waters by means of otolith shape analysis. *J. Mar. Biol. Ass. U. K*., 91: 1237-1242. https://doi.org/10.1017/S002531541000189X

OOMS J., 2021. – magick: Advanced Graphics and Image-Processing in R. https://cran.r-project.org/web/packages/magick/index.html

ORDOÑEZ A., EIKVIL L., SALBERG A.B., HARBITZ A., MURRAY S.M. & KAMPFFMEYER M.C., 2020. – Explaining decisions of deep neural networks used for fish age prediction. *PLOS ONE*, 15: e0235013. https://doi.org/10.1371/journal.pone.0235013

PALMER A.R., 2016. – What determines direction of asymmetry: genes, environment or chance? *Philos. Trans. R. Soc. B: Biol. Sci.*, 371: 20150417. https://doi.org/10.1098/rstb.2015.0417

PANFILI J., DE PONTUAL H., TROADEC H. & WRIGH P.J. (eds), 2002. – Manual of Fish Sclerochronology. 464 p. Brest, France: Ifremer – IRD.

POLITIKOS D.V., PETASIS G., CHATZISPYROU A., MYTILINEOU C. & ANASTASOPOULOU A., 2021. – Automating fish age estimation combining otolith images and deep learning: the role of multitask learning. *Fish. Res.*, 242: 106033. https://doi.org/10.1016/j.fishres.2021.106033

RANDON M., PAPE O.L., ERNANDE B., MAHÉ K., VOLCKAERT F.A.M., PETIT E.J., LASSALLE G., BERRE T.L. & RÉVEILLAC E., 2020. – Complementarity and discriminatory power of genotype and otolith shape in describing the fine-scale population structure of an exploited fish, the common sole of the Eastern English Channel. *PLOS ONE*, 15: e0241429. https://doi.org/10.1371/journal.pone.0241429

REIBISCH J., 1899. – Ueber die Eizahl bei *Pleuronectes platessa* und die Altersbestimmung dieser Form aus den Otolithen. *Nebst Bemerkung zu vorstehender Arbeit von Dr. V. Hensen*, pp. 233-248. Wiss. Meeresunt (Kiel).

SMITH M.K., 1992. – Regional Differences in Otolith Morphology of the Deep Slope Red Snapper *Etelis carbunculus*. *Can. J. Fish. Aquat. Sci.*, 49: 795-804. https://doi.org/10.1139/f92-090

STEVENSON D. & CAMPANA S., 1992. – Otolith microstructure examination and analysis. *Can. Spec. Publ. Fish. Aquat. Sci.*, 117:135. https://doi.org/10.13140/RG.2.2.22258.61127

STOCK M., NGUYEN B., COURTENS W., VERSTRAETE H., STIENEN E. & DE BAETS B., 2021. – Otolith identification using a deep hierarchical classification model. *Comput. Electron. Agric.*, 180: 105883. https://doi.org/10.1016/j.compag.2020.105883

VABØ R., MOEN E., SMOLIŃSKI S., HUSEBØ Å., HANDEGARD N.O. & MALDE K., 2021. – Automatic interpretation of salmon scales using deep learning. *Ecol. Informatics,* 63: 101322.

VANDERKOOY S., CARROLL J. & ELZEY S, GILMORE J. & KIPP J., 2020. – A Practical Handbook for Determining the Ages of Gulf of Mexico and Atlantic Coast Fishes, Third Edition. Gulf States Marine Fisheries Commission & Atlantic States Marine Fisheries Commission. GSMFC No. 300.

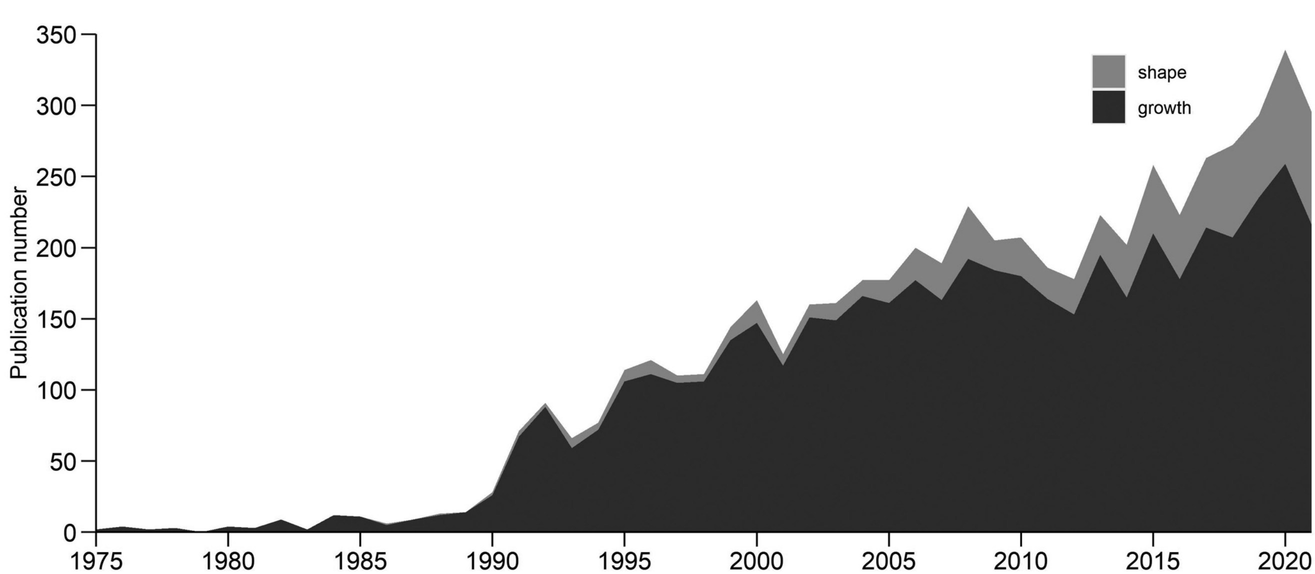
VIGNON M., 2012. – Ontogenetic trajectories of otolith shape during shift in habitat use: Interaction between otolith growth and environment. *J. Exp. Mar. Biol. Ecol.*, 420-421: 26-32. https://doi.org/10.1016/j.jembe.2012.03.021

VIGNON M., MORAT F., GALZIN R. & SASAL P., 2008. – Evidence for spatial limitation of the bluestripe snapper *Lutjanus kasmira* in French Polynesia from parasite and otolith shape analysis. *J. Fish Biol.*, 73: 2305-2320. https://doi.org/10.1111/j.1095-8649.2008.02070.x

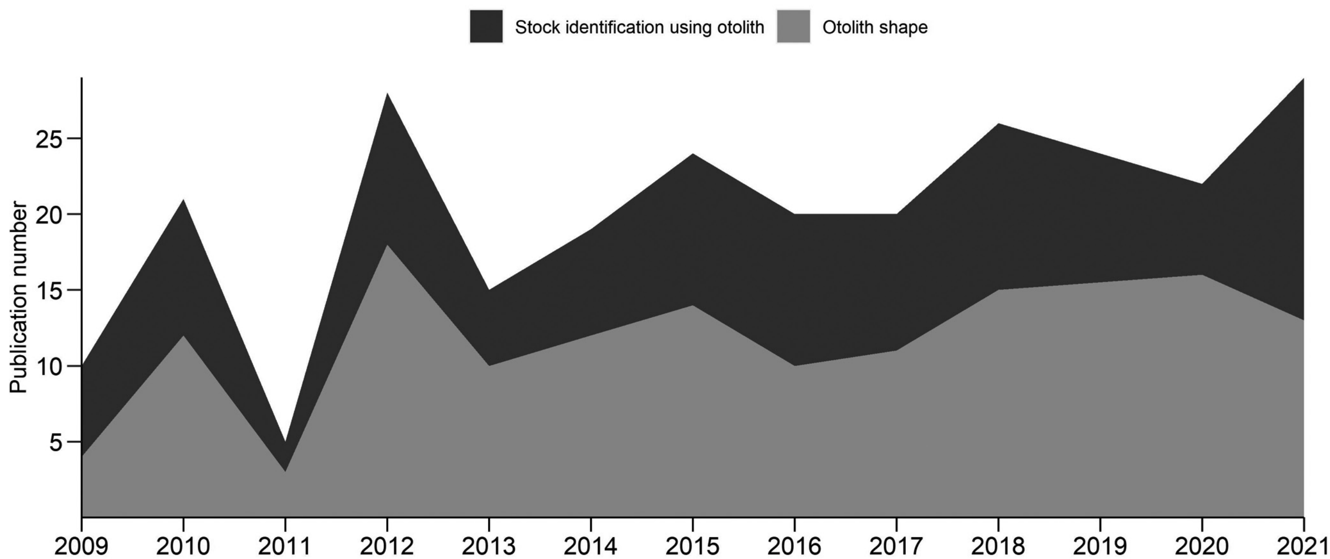
VITALE F., CLAUSEN L.W. & CHONCHÚIR G.N. (eds), 2019. – Handbook of fish age estimation protocols and validation methods. *ICES Coop. Res. Rep.*, 346. http://doi.org/10.17895/Ices.pub.5221

WILLIAMS T. & BEDFORD B.C., 1974. – The use of otoliths for age determination. *In*: The Ageing of Fish (Bagenal T.B., ed.) Proc. of an International Symposium. Unwin Brothers Ltd. 234 p.

Supplementary data



Supplementary figure 1. – Studies number in Web of Science about otolith growth and otolith shape.



Supplementary figure 2. – Studies number in the Stock Identification Methods Working Group (SIMWG) about stock identification using otolith and shape of otolith.