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Program SIDEPECHE
Halieutics Information system
Sclerochronology

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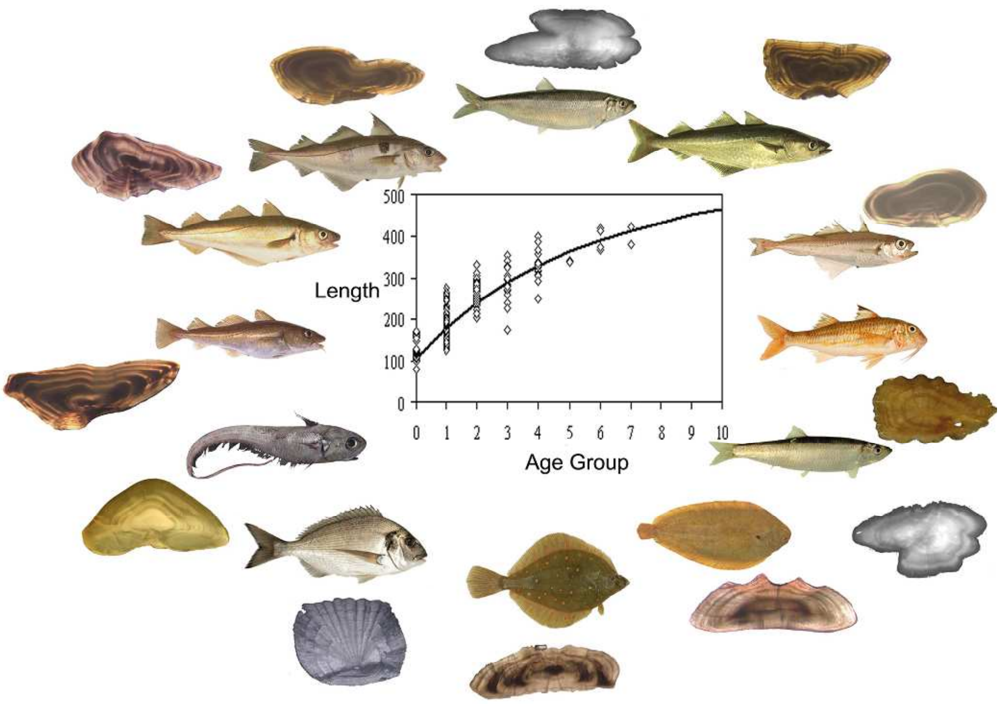
French summary of age estimation procedures



French summary of age estimation procedures

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sommaire

1. Introduction

Estimating the age of fish is one of the most important elements in the study of the population group dynamics. It provides the basis for the calculations necessary to know the growth, the mortality, the recruitment and other fundamental parameters of the fish population (Laurec & Le Guen, 1981).

In order to know the group dynamics of a population, it is necessary to study the age pyramid or, if we use the given name which is etymologically inappropriate, the demographic structure. Knowing the reproduction period, it is possible to easily distinguish groups of animals born in the same year. Each year is thereby associated with what we call an age group. The age group 0 (G0) corresponds to animals younger than 1 year. The group 1 (G1) refers to animals older than 1 year and less than 2 years.

On a European level, the long term management of stocks needs an annual supply of a large amount of information from all the European countries having fishing activity in line with the Data Collection Regulation. In this way, more than 40 000 calcified pieces are analysed each year within the SIDEPECHE programme of IFREMER (Information Systems and Observation Techniques, Economy and Diagnosis for the Evolution of Resources and their uses).

The procedure for estimating age describes each technical stage from the taking of the calcified piece through to the supplying of key sizes/ages to the European work groups. This procedure is specific to each species and even each population. For reasons of Quality within Ifremer (MEQUAPRO project: MEthod, QUality, PROduct), the readers of the calcified pieces drew up some technical, analytical and authorisation protocols[1]. This report is a summary of all these documents :

Bellail, R., 2005. Analyse de la méthode d'estimation de l'âge individuel de la morue en mer celtique (Divisions CIEM VIIe-k), Mars 2005, Rapport interne Ifremer : STH/LBH Lorient, 19p.

Bellail, R., 2005. Analyse de la méthode d'estimation de l'âge individuel du merlan en mer celtique (Divisions CIEM VIIe-k), Septembre 2005, Rapport interne Ifremer : STH/LBH Lorient, 18p.

Bellail, R., 2005. Habilitation des lecteurs de pièces calcifiées pour l'estimation de l'âge individuel de morues de mer celtique (Divisions CIEM VIIe-k), Mars 2005, Rapport interne Ifremer : STH/LBH Lorient, 17p.

Bellail, R., 2005. Protocole technique d'estimation de l'âge individuel de la morue en mer celtique (Divisions CIEM VIIe-k), Mars 2005, Rapport interne Ifremer : STH/LBH Lorient, 35p.

Bellail, R., 2005. Protocole technique d'estimation de l'âge individuel du merlan en mer celtique (Divisions CIEM VIIe-k), Avril 2005, Rapport interne Ifremer : STH/LBH Lorient, 38p.

Bellail, R., 2006. Habilitation des lecteurs de pièces calcifiées pour l'estimation de l'âge individuel de merlan de mer celtique (Divisions CIEM VIIe-k), Février 2006, Rapport interne Ifremer : STH/LBH Lorient, 18p.

[1] intranet site : http://w3.ifremer.fr/isih/affichagePageStatique.do?page=collecte_donnees/echantillonnage_terre/sclerochronologie/sclerochronologie.htm

Dufour, J.L., 2006. Analyse de la méthode d'estimation de l'âge individuel de l'églefin *(Melanogrammus aeglefinus)* de mer du Nord (Divisions CIEM IVb,c), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 18p.

Dufour, J.L., 2006. Analyse de la méthode d'estimation de l'âge individuel du lieu noir *(Pollachius virens)* en mer du Nord (Divisions CIEM IVa), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 19p.

Dufour, J.L., 2006. Analyse de la méthode d'estimation de l'âge individuel du merlan (*Merlangius merlangus*) en mer du Nord et Manche Est (Divisions CIEM IVb, IVc, VIId), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 20p.

Dufour, J.L., 2006. Analyse de la méthode d'estimation de l'âge individuel de la morue (*Gadus morhua*) de mer du Nord et Manche orientale, Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 18p.

Dufour, J.L., 2006. Analyse de la méthode d'estimation de l'âge individuel du tacaud norvégien (*Trisopterus esmarki*) de mer du Nord (Divisions CIEM IVb, c), Octobre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 18p.

Dufour, J.L., 2006. Protocole technique d'estimation de l'âge individuel de l'églefin *(Melanogrammus aeglefinus)* en mer du Nord (Divisions CIEM IVb,c), Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 28p.

Dufour, J.L., 2006. Protocole technique d'estimation de l'âge individuel du lieu noir *(Pollachius virens)* en mer du Nord (Divisions CIEM IVa), Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 35p.

Dufour, J.L., 2006. Protocole technique d'estimation de l'âge individuel du merlan (*Merlangius merlangus*) en mer du Nord et Manche Est (Divisions CIEM IVb, IVc, VIId), Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 37p.

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Dufour, J.L., 2006. Protocole technique d'estimation de l'âge individuel du merlu *(Merluccius merluccius)* de Méditerranée, Octobre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 28p.

Dufour, J.L., 2006. Protocole technique d'estimation de l'âge individuel du tacaud norvégien *(Trisopterus esmarki)* en mer du Nord (Divisions CIEM IVb, c), Octobre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 24p.

Duhamel, E., 2005. Analyse de la méthode d'estimation de l'âge individuel de l'anchois du golfe de Gascogne (Divisions CIEM VIIIa-b), Avril 2005, Rapport interne Ifremer : STH/LBH Lorient, 14p.

Duhamel, E., 2005. Analyse de la méthode d'estimation de l'âge individuel de la sardine du golfe de Gascogne (Divisions CIEM VIIIa-b), Avril 2005, Rapport interne Ifremer : STH/LBH Lorient, 13p.

Duhamel, E., 2005. Protocole technique d'estimation de l'âge individuel de la sardine du golfe de Gascogne (divisions VIIIa-b), Mars 2005, Rapport interne Ifremer : STH/LBH Lorient, 23p.

Duhamel, E., 2005. Protocole technique d'estimation de l'âge individuel de l'anchois du golfe de Gascogne (divisions VIIIa-b), Mars 2005, Rapport interne Ifremer : STH/LBH Lorient, 22p.

Duhamel, E., 2006. Habilitation des lecteurs de pièces calcifiées pour l'estimation de l'âge individuel de la sardine du golfe de Gascogne (Divisions CIEM VIIIa-b), Février 2006, Rapport interne Ifremer : STH/LBH Lorient, 23p.

Elleboode, R., 2007. Protocole technique d'estimation de l'âge individuel de la Daurade royale (*Sparus aurata*) en Méditerranée (Golfe du lion, Division CIEM 37.1.2), Août 2007, Rapport interne Ifremer : DHMMN/RH/BL, 19p.

Gaudou, O., 2005. Protocole technique d'estimation de l'âge individuel de l'églefin en mer celtique (Divisions CIEM VIIb-k), Avril 2005, Rapport interne Ifremer : STH/LBH Lorient, 26p.

Huet, J. & Martin, S., 2008. Protocole technique d'estimation de l'âge individuel du bar (Dicentrarchus labrax) du stock Manche Est (Division VIId), du stock Manche Ouest - mer Celtique (Division CIEM VIIe, h) et du stock golfe de Gascogne (Division CIEM VIIIa, b), Mai 2008, Rapport interne Ifremer : STH/LBH Brest, 13p.

Huet, J. & Morizur, Y., 2008. Analyse d'estimation de l'âge individuel du bar (Dicentrarchus labrax) du stock Manche Est (Division CIEM VIId), stock Manche Ouest - mer Celtique (Division CIEM VIIe, h) et du stock golfe de Gascogne (Division CIEM VIIIa, b), Mai 2008, Rapport interne Ifremer : STH/LBH Brest, 11p.

Le Roy, D., Analyse d'estimation de l'âge individuel du rouget barbet de roche (*Mullus surmuletus*) en Manche orientale et sud mer du Nord (divisions CIEM VIId et IVc), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 21p.

Le Roy, D., Protocole technique d'estimation de l'âge individuel du rouget barbet de roche (*Mullus surmuletus*) en Manche orientale et sud mer du Nord (divisions CIEM VIId et IVc), Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 20p.

Lizaud, O., 2008. Protocole technique d'estimation de l'âge individuel de la cardine (*Lepidorhombus whiffiagonis*) de mer Celtique et du golfe de Gascogne (Divisions VII g, h, j et VIII a,b du CIEM), Novembre 2008, Rapport interne Ifremer : DHMMN/RH/BL, 27p.

Louis, W., 2006. Analyse de la méthode d'estimation de l'âge individuel du grenadier de roche *(Coryphaenoides rupestris)* en Atlantique Nord Boréal, Octobre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 22p.

Louis, W., 2006. Protocole technique d'estimation de l'âge individuel du grenadier de roche *(Coryphaenoides rupestris)* en Atlantique Nord Boréal, Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 23p.

Manten, M.L., Analyse de la méthode d'estimation de l'âge individuel de la plie (*Pleuronectes platessa*) en mer du Nord et Manche Est (Divisions CIEM IVb, IVc, VIId), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 16p.

Manten, M.L., Analyse de la méthode d'estimation de l'âge individuel de la sole de mer du Nord et manche Est (Divisions CIEM IVb,c et VIId), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 17p.

Manten, M.L., Protocole technique d'estimation de l'âge individuel de la plie (*Pleuronectes platessa*) en mer du Nord et Manche Est (Divisions CIEM IVb, IVc, VIId), Octobre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 31p.

Manten, M.L., Protocole technique d'estimation de l'âge individuel de la sole (*Solea solea*) en mer du Nord et Manche Est (Divisions CIEM IVc, VIId), Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 26p.

Nédelec, D., 2005. Analyse de la méthode d'estimation de l'âge individuel du lieu noir de l'Ouest de l'Ecosse (Division CIEM VI a), Mai 2005, Rapport interne Ifremer : STH/LBH, Lorient 27p.

Nédelec, D., 2005. Habilitation des lecteurs de pièces calcifiées pour l'estimation de l'âge individuel du lieu noir de l'Ouest de l'Ecosse (Division CIEM VI a), Mai 2005, Rapport interne Ifremer : STH/LBH Lorient, 7p.

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Verin, Y. & Le Roy, D., 2006. Analyse de la méthode d’estimation de l’âge du hareng (*Clupea harengus harengus*) en Manche Est et mer du Nord (Divisions CIEM IVa-b-c et VIId), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 10p.

Verin, Y. & Le Roy, D., 2006. Analyse de la méthode d’estimation de l’âge du sprat (*Sprattus sprattus sprattus*) en mer du Nord (Divisions CIEM IVa-b-c), Novembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 10p.

Verin, Y. & Le Roy, D., 2006. Protocole technique d’estimation de l’âge du hareng (*Clupea harengus harengus*) en Manche Est et mer du Nord (Divisions CIEM IVa-b-c et VIId), Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 16p.

Verin, Y. & Le Roy, D., 2006. Protocole technique d’estimation de l’âge du sprat (*Sprattus sprattus sprattus*) en mer du Nord (Divisions CIEM IVa-b-c), Septembre 2006, Rapport interne Ifremer : DHMMN/RH/BL, 14p.

2. Calcified pieces used

Within the framework of a European monitoring (DCR), 18 species are treated routinely each year (Fig. 1).

Species		**Stock, ICEs areas (Fig. 2)**	**Calcified piece**
Vernacular name	**Scientific name**		
The North Sea & Eastern Channel			
Herring	*Clupea harengus harengus*	IV, VIId	Otolith
Seabass	*Dicentrarchus labrax*	IV, VIId	**Scale**
Haddock	*Melanogrammus aeglefinus*	IV, IIIa	Otolith
Whiting	*Merlangius merlangus*	IV, VIId	Otolith
Striped Red mullet	*Mullus surmuletus*	IV, VIId	Otolith
Plaice	*Pleuronectes platessa*	VIId	Otolith
Saithe	*Pollachius virens*	IV, IIIa	Otolith
Sole	*Solea solea*	VIId	Otolith
Cod	*Gadus morhua*	VIId	Otolith
The North-eastern ATLANTIC & Western Channel			
Seabass	*Dicentrarchus labrax*	II, V, VI, VII (Exc. D), VIII, IX, X (Exc. A), XII, XIV	**Scale**
Anchovy	*Engraulis encrasicolus*	VIII	Otolith
Cod	*Gadus morhua*	Vb, VI	Otolith
Cod	*Gadus morhua*	VIIe-k	Otolith
Megrim	*Lepidorhombus whiffiagonis*	VII, VIIIab	Otolith
Anglerfish	*Lophius piscatorius & Lophius budegassa*	VII, VIIIabd	**Illicium**
Haddock	*Melanogrammus aeglefinus*	VIIe-k	Otolith
Whiting	*Merlangius merlangus*	VIIe-k	Otolith
Whiting	*Merlangius merlangus*	VIIIab	Otolith
Hake	*Merluccius merluccius*	IIIa, IV, VI, VII, VIIIab	Otolith
Ling	*Molva molva*	II, V, VI, VII (Exc. D), VIII, IX, X, XII, XIV	Otolith
Striped Red mullet	*Mullus surmuletus*	II, V, VI, VII (Exc. D), VIII, IX, X, XII, XIV	Otolith
Saithe	*Pollachius virens*	Vb, VI, XII, XIV	Otolith
Pilchard	*Sardina pilchardus*	VIIIabd	Otolith
Sole	*Solea solea*	VIIe	Otolith
Sole	*Solea solea*	VIIIab	Otolith
The MEDITERRANEAN			
Anchovy	*Engraulis encrasicolus*	1.1, 1.2, 1.3, 2.1, 2.2, 3.1	Otolith

Hake	*Merluccius merluccius*	1.1, 1.2, 1.3, 2.1, 2.2, 3.1	Otolith
Red mullets	*Mullus surmuletus & Mullus barbatus*	1.1, 1.2, 1.3, 2.1, 2.2, 3.1	Otolith
Pilchard	*Sardina pilchardus*	1.1, 1.2, 1.3, 2.1, 2.2, 3.1	Otolith
Gilthead seabream	*Sparus aurata*	1.2, 3.1	**Scale**

Figure 1: Species treated routinely within the DCR framework to estimate age per geographical sector. The calcified piece used is indicated for each species.

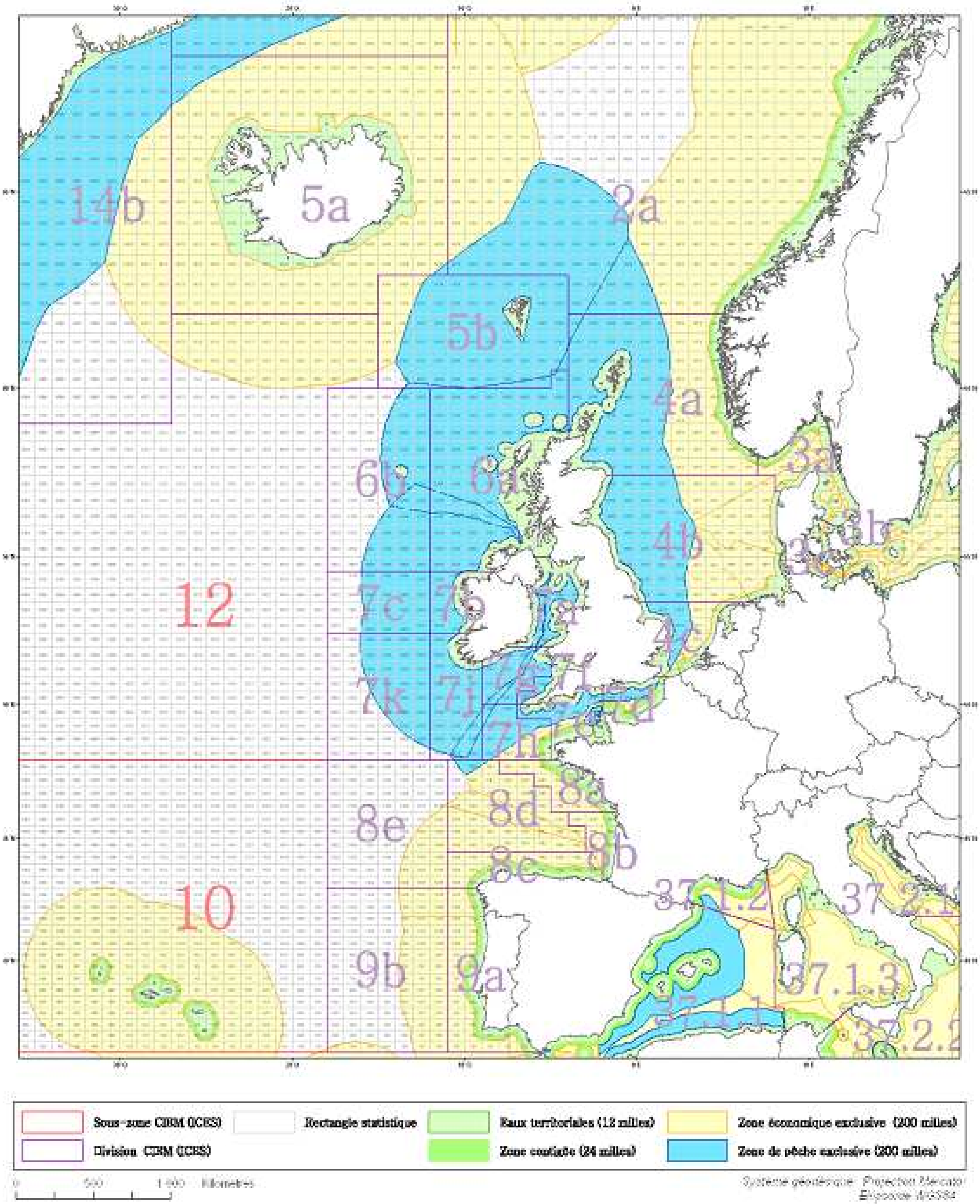


Figure 2 : Map of European waters with the divisions defined by the ICES (International Council for the Exploration of the Sea).

Amongst the species treated routinely, the calcified piece used is always the otolith with the exception of 4 species: Seabass, Seabream and white and black anglerfish. The otoliths are the most widely used calcified pieces because they do not play the role of calcium reservoir as do the scales and the elements of the internal skeleton (Simkiss, 1974). They are, therefore, hardly ever reabsorbed, except in conditions of extreme stress, and, in this way, they keep the complete sequence of recorded growth marks (Panfili *et al.*, 2002). Amongst the 3 pairs of otoliths, for all the species studied, it is always the *sagittae* which are used. They are the biggest.

For the seabass and the seabream, the scale is the calcified piece used because it enables the age for these species to be estimated in the same way as with the otolith. The scales used are of the ctenoïd type.

For the white and black anglerfish, it is the *illicium* or the « fisherman's filament » which is used. However, this calcified piece is very difficult to read in the same way as is the otolith. Estimating the age of the anglerfish is very tricky.

3. Sampling

For the monitoring of the fish populations, samplings are carried out either over the 4 quarters of the year or just once in the year. The samplings are carried out at sea during the scientific campaigns or on land in a fisheries market. When a fish is sampled in order to estimate its age, it is necessary to note the biological parameters (species, individual size and weight, sex, stage of maturity…) and the sampling parameters (date, place of catch, place of sampling…).

3.1. Sampling at sea

Numerous calcified pieces are taken for sampling during scientific campaigns (IBTS, COMOR, CGFS, EVHOE, MEDITS…) in line with the protocols defined by the international work groups coordinating the campaigns such as the group IBTS for the North Sea and the Western approaches to Europe or the MEDITS group for the Mediterranean sea.

During observations at sea on professional boats, sampling of calcified pieces may be carried out when this is programmed by the national sampling plan

3.2. Sampling of commercial landings

Sampling of commercial landings is carried out in fisheries markets (Boulogne-sur-mer, Port-en-Bessin, Roscoff, Brest, Audierne, Lorient, Concarneau, La Rochelle, Les Sables d'Olonne, la Cotinière, l'île d'Yeu, Royan, Sète…). This takes place in the air-conditioned auction halls, sometimes in cold rooms. The fish is usually available for only a short period and is, furthermore, changeable. The sampling needs 2 or 3 people because 1 or 2 people measure the fish with the help of an ichtyometre, and carry out the sampling and the 3rd person records the information and places the calcified pieces in an envelope or in micro tubes (Diagram 3). The fish which is going to be sold is handled with clean, strong rubber gloves.

Figure 3 : Sampling of seabass in auction room.

4. Types of sampling

When the calcified pieces are sampled, it is very important that they are cleaned in order to remove the *mucus* and other organic deposits on them.

Badly cleaned calcified parts may be un-usable.

The calcified pieces may be simply wiped in absorbent paper after their removal.

4.1. Sampling of Otoliths

The otoliths necessitate the opening of the cranium as they are located here. In order to access the cavities which enclose the otoliths there are several slicing methods possible. The frontal section slice is the most common and may be used successfully for all types of fish (whichever the species, the individual size or the cranial morphology). However, a given method is used for a given species further to a phase of adaptation and of technical adjustment. The slicing utensils vary according to the size of the cranium but in general a knife is perfectly suitable. The slice must be made carefully in order to avoid severing the internal ear and the otoliths. Having made the appropriate slice, the otoliths are normally removed with tweezers.

4.1.1. Frontal head section

The fish is held by the eyes between the thumb and the index finger, a slice at 45° is made on the forehead (Fig. 4).

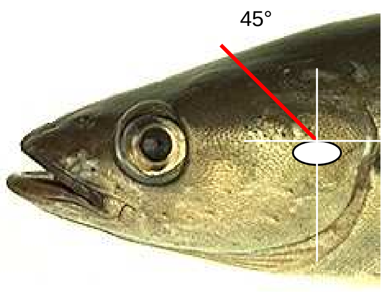


Figure 4 : Position of the cranial frontal slice (red line).

Another technique used for the striped red mullet and the red mullet is an inversed frontal slice (Fig. 5).

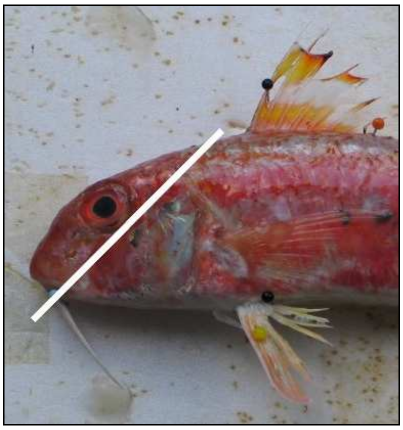


Figure 5 : Position of the inversed cranial frontal slice (white line).

Having opened the cranium and moved the encephalon by turning over the anterior part of the fish head, the two biggest otoliths (the *sagittae*) are easily detected. They are removed with stainless steel tweezers (Fig. 6).

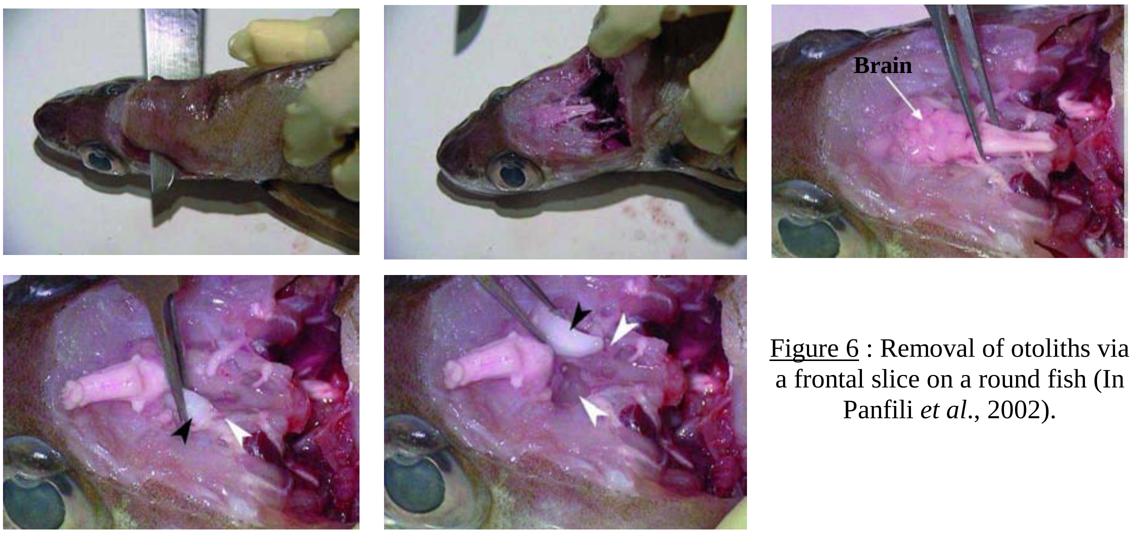


Figure 6 : Removal of otoliths via a frontal slice on a round fish (In Panfili *et al*., 2002).

This type of removal is also carried out on flatfish. In order to remove the otoliths, the fish is kept flat on its stomach and a frontal slice is made. Having spread apart the two parts of the cranium, the otoliths are removed (Fig. 7).

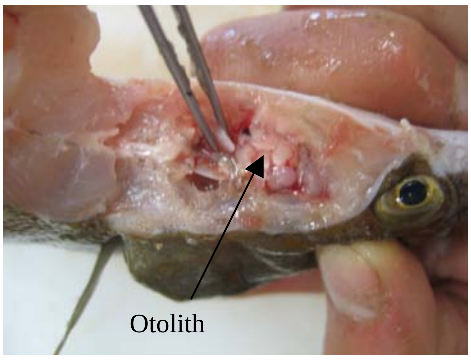


Figure 7 : Removal of otoliths via frontal slice on a flatfish.

4.1.2. Transverse head section

The transversal slice is carried out by separating the body from the head of the fish. This slice is made from the dorsal part towards the ventral part (Fig. 8 & 9).

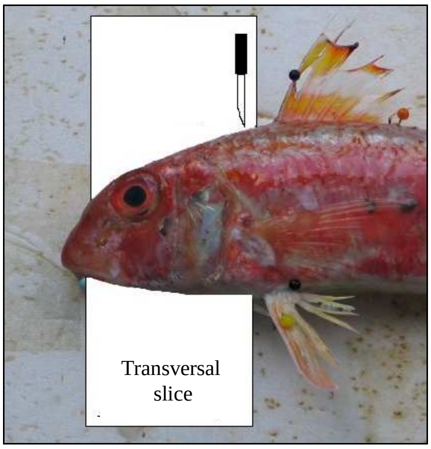
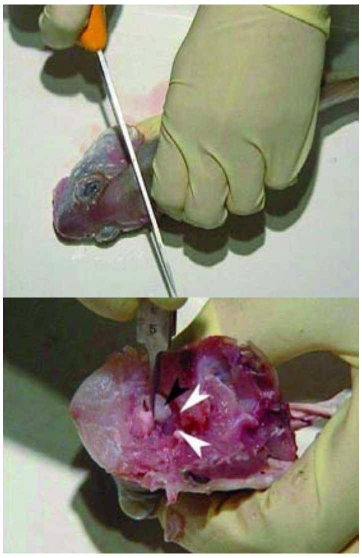


Figure 8 : Position of the transversal slice of the cranium.



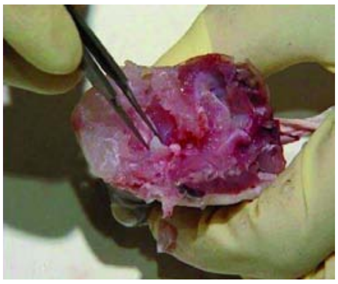


Figure 9 : Removal of otoliths via a transversal slice on a round fish (*In* Panfili *et al.*, 2002).

4.1.3. Ventral head section

On the ventral side, the operculum is a certain distance from the fish head. The gills are therefore visible and the branchial arches are cut on their inner edge. The ventral structure of the bones of the neurocranium appears and all the surrounding tissues must then be withdrawn. The pre-otic bullae are located in the medio-lateral part of the neurocranium. A slight incision in the external part of these bullae opens the internal ear and the *sagittae* may be removed from here (Fig. 10). This technique has the advantage of not damaging the appearance of the fish in the event of it being intended for sale. This technique is used for eviscerated and bled round fish as well as for megrim.

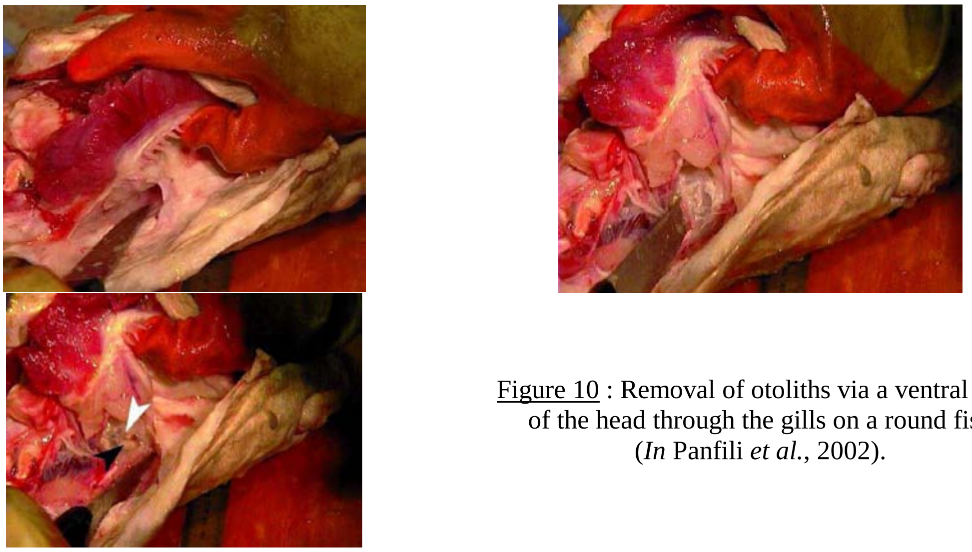


Figure 10 : Removal of otoliths via a ventral slice of the head through the gills on a round fish (*In* Panfili *et al.*, 2002).

4.2. Removal of scales

The scales are most probably the easiest structures to remove. However, it is very important to choose carefully the removal area in order to avoid regenerated scales. For seabass and seabream, the scales are removed under the pectoral fin, an area where regeneration is less frequent and where few visible traces are left for the future commercialisation of the fish (Fig. 11).

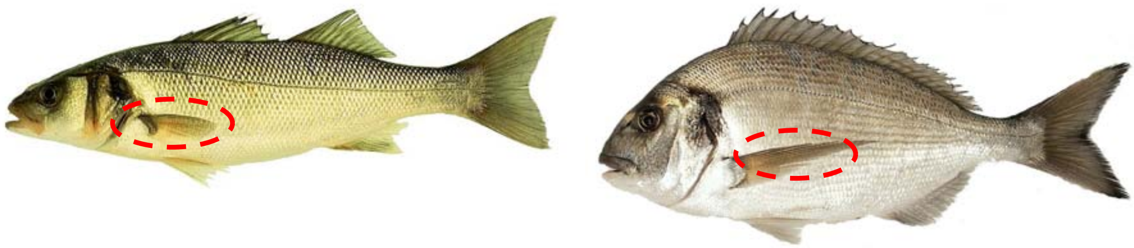


Figure 11 : Removal of scales under the pectoral fin (removal area in red) for seabass and seabream (images : Pierre Porché, IFREMER).

The removal of scales is done with a knife with a clean inox blade or perhaps with tweezers (Fig. 12). The person removing the scales passes the blade firstly from the front to the back in order to remove a maximum of *mucus*, impurities or detached scales which do not necessarily come from the fish, then the blade is wiped with a clean cloth. The person then carries out a movement from the back to the front by placing the blade at 60° and the thumb immobilises the scales which are removed on the blade at the end of this movement. The fish is then descaled over 1 to 3 cm² according to the size of the fish. It is advisable to take enough scales since the proportion of scales which will be readable may vary from 0 to 70% of the total number of scales removed. According to the context, it is advisable to take about 20 scales from a fish which is intended for sale.

Figure 12 : Removal of scales under the pectoral fin of a seabass in fisheries market.

4.3. Removal of the *illicium*

For the black and white anglerfish, the fishing filament also called *illicium* is extracted with a knife and the first 3-4 centimetres from the base are collected. This removal does not deteriorate the appearance of the fish (Fig. 13).

Figure 13 : Removal of the *illicium* on anglerfish (line of the slice in red). The *illicium* is extracted with its base cleaned of soft tissue.

5. Stocking the calcified pieces

The best way to keep the otoliths is to stock them in a completely dry place. After cleaning, they are dried simply by exposing them to the air at room temperature or in a low temperature incubator (Panfili *et al.*, 2002).

There are 2 ways of stocking used at Ifremer (Fig. 14):

- Paper envelopes
- Micro-tubes brand Eppendorf ®

Figure 14 : Paper envelopes and micro-tubes used for stocking the calcified pieces.

These 2 means of stockage present advantages and disadvantages. The paper envelopes, recommended by Williams and Bedford (1974) are very practical during sampling and tidying away (Fig. 15) to keep calcified pieces which are quite big but fragile. For this reason, this is the most frequently-used method at Ifremer. It is necessary to be sure that the calcified piece is at the bottom of the envelope. When the envelopes for a same sampling are gathered together by an elastic band, it is important to be careful that only the upper parts of the envelopes are crushed by the elastic band.

Figure 15 : Stocking the seabass scales in envelopes and files.

The reference of the sample and all the information linked to it (date, length, sex, stage of sexual maturity, species, haul number…) are noted on the envelope before placing the calcified piece inside.

Using micro tubes requires inserting an informative label or sticking it on the outside. The micro tubes have the advantage of being sufficiently rigid to protect the otoliths efficiently from breakages due to handling during the expedition or tidying away.

The stocking areas are best when cool and dark and here, the dry otoliths may be kept indefinitely (Brothers, 1987 *In* Panfili *et al.*, 2002).

It is worth noting that an aquatic environment such as alcohol may be used for stocking. This may be particularly useful for small and fragile otoliths. The concentration of alcohol must be at least 95% (Panfili *et al.*, 2002).

6. Associated biological parametres

When a fish is sampled in order to estimate its age, it is necessary to note the biological parameters (individual size and weight, sex, stage of maturity…) and the sampling information (date, place, …).

All this information and that linked to the age is stocked in a standardised file “biological parameters v4.xls”[2] which is usable irrespective of the place of sample taking (at sea or on land) and which contains the following fields:

- Date
- Ship
- Gear
- Haul number
- Place
- Code_Species
- Area
- Category
- Type_Length
- Increment
- Unity_Size
- Size
- Unity_Weight
- Weight
- State
- Sex
- Maturity_Scale
- Maturity
- Reference_Removal
- Type_Calcified Piece
- Preparation_ Calcified Piece
- Weight_ Calcified Piece
- Age
- Reference_ Calcified Piece
- Observations

[2] The file " biological parameters v4.xls" as well as its manual (Mahé *et al.*, 2009) are available on the intranet site of SIH :
http://w3.ifremer.fr/isih/affichagePageStatique.do?page=collecte_donnees/echantillonnage_terre/sclerochronologie/documentation/documentation_sclero.htm

The files generated by the sample taking sessions are managed in a database which proposes all the links to the halieutical database Harmonie (IFREMER).

For any dispatching of the calcified pieces, it is obligatory to complete and send a file of this type by mail.

If the weight of the otolith is recorded, a weight procedure must first be drawn up .

7. Dispatching of calcified pieces

A lot of calcified pieces are removed by one laboratory and sent to another laboratory to be treated. For this, there are several rules to respect:

- Maximum lead-time: the calcified pieces removed in quarter n must be sent at the beginning of quarter n+1 ; shorter lead-times are preferable in the event of monthly samples.
- Contact : contact the laboratory which will treat the parts before-hand in order to know the name of the person they should be sent to.
- Biological parametres : the file " biological parameters v4.xls" must be filled in (according to the coding in force, see Mahé *et al.*, 2009) and sent by e-mail. It is very important to check the correspondence between the sampling numbers noted on the micro-tubes or the envelopes and those noted in the file "biological parameters".
- Parcels : The calcified pieces are extremely fragile so it is necessary to protect the parcel properly and when paper envelopes are used, they must not be squashed.

8. Types of preparation

In order to study the growth of the fish, it is necessary to identify the calcified piece which is going to used but also the type of preparation to be carried out. There are decision making aids to help (Panfili *et al.*, 2002). The otolith has a tridimensional structure and its shape is unique to each species. According to the species in question, it may be analysed whole, burned, cut, coloured….

8.1. Whole

The calcified pieces which are read whole are the scales and the otoliths which are not very thick.

8.1.1. Scales

At Ifremer, the scale is used routinely in order to estimate the age of 2 fish belonging to the category of perciformes which are seabass and seabream.

After extraction, the scales must be cleaned. The cleaning operation is relatively simple, varying from a direct stockage further to a basic cleaning (wiping on absorbent paper) through to the use of an ultrasound basin. This basin may contain distilled water, tap water, diluted potassium hydroxide, sodium peroxide or trypsin. The duration of immersion in the active solution must be monitored in order to avoid partial destruction (Panfili *et al.*, 2002).

8.1.1.1. Seabass

The preparation steps for seabass scales are described below (*In* Huet & Morizur, 2008) :

- ❖ Remove the scales from the envelope with tweezers and place them on the stand of the binocular magnifying glass.

- ❖ Sort the scales and put to one side the regenerated ones (Fig. 16) in order to keep only the readable scales which are the ones where there is a succession of rings starting from the *nucleus*.

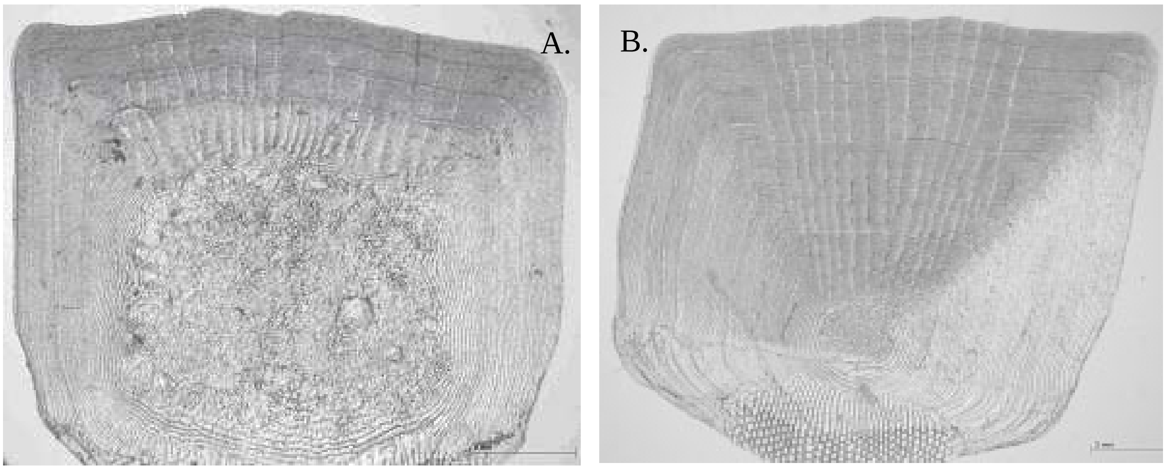


Figure 16 : Difference between a regenerated scale (A.) which does not enable all the growth rings to be seen and a non regenerated scale (B.). The scales are from the same individual.

- ❖ Select 5 readable scales
- ❖ With the tweezers, soak the scale in a small recipient containing a little water to rehydrate and remove any traces of dry mucus. This procedure is often necessary, especially to clean the fish scales which are bigger than 50cm. The water enables the scale to be softened and rehydrated at the same time. Once wet, take the scale between the thumb and the index finger in order to eliminate the *mucus*. This procedure may be repeated twice.
- ❖ Dry the scale on absorbent paper. It is important to dry the fingers also because the drying process is finished by holding the scale between the thumb and the index finger.
- ❖ Place the rehydrated scales between 2 glass strips. The scales are now softer and so may be held in a flat position between the strips more easily for reading (Fig. 17).

Figure 17 : Positioning of 5 scales between 2 glass strips in order to estimate the age.

- ❖ Read the 5 scales as it is possible that one of the scales has a ring which is not visible over the others.

- ❖ If there is any difficulty to estimate the age, it is necessary to select 5 other scales and start the procedure described above again.

Comment : The scales which fall from the stand during the various steps described above must not be retrieved so as to avoid mixing scales from different fish.

8.1.1.2. Seabream

The preparation steps for seabream are described below (*In* Elleboode, 2007) :

- ❖ Remove all the scales from the envelope and place them on a dark surface (Fig. 18).

Figure 18 : Sorting the scales.

- ❖ Sort out the scales into those which are usable, those damaged and those unreadable (regenerated) (Fig. 19).

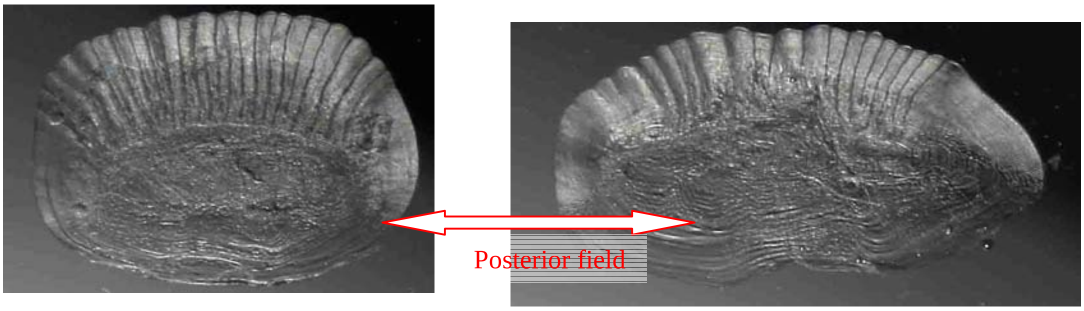


Figure 19 : Scales which are unreadable due to a posterior field which is too big compared to the anterior field.

- ❖ Examine several scales and make a picture which is calibrated and interpreted with the programme TNPC.

8.1.2. Otoliths

The species of fish whose age is estimated using whole scales are the species of small pelagic fish such as anchovies, herring, sardines and sprat and also flatfish such as megrim and lemon sole.

8.1.2.1. The small pelagic fish

The small pelagic fish are practically all collected during scientific campaigns (IBTS, PELGAS, PELMED…). For some of them, the age estimations must be given to the work groups just after the end of the campaign and the examinations are carried out on board the ship.

The preparation steps for the otoliths of small pelagic fish are described below (Duhamel, 2007a ; 2007b ; Vérin & Le Roy, 2006a ; 2006b) :

- ❖ The 2 otoliths are removed. Because of their small size, the pair of *sagittae* is removed. In fact, it is not always easy to distinguish a crystalline otolith from another “readable” otolith during the removal process. Furthermore, one of the 2 otoliths may break being so fragile.
- ❖ After cleaning and removal of soft tissue (wiping on a wet sponge), they are placed directly in the labelled cavities of black plastic strips. Each cavity contains the pair of *sagittae* from an identified fish.
- ❖ The plastic strips and their contents are then placed in a designated space and left a few hours in the open air to complete the drying process.
- ❖ On each strip, the otoliths are placed in the same way in order to facilitate the examination with the binoculars; *sulcus acusticus* at the bottom and convex side visible (Fig. 20).

Figure 20 : Placing the otoliths from small pelagic fish in the cavities of a black plastic strip.

- ❖ The cavities are then filled with transparent resin (Eukitt ; Diagram. 21) which covers the otoliths. The drying time under the hood or in a ventilated area lasts approximately 24 hours.

Figure 21 : Strip with the cavities containing sardine otoliths and filled with Eukitt resin.

8.1.2.2. Flatfish

The otoliths from certain species of flat fish may be analysed without any previous complicated preparation. This is the case for the American plaice, the megrim and the lemon sole.

The preparation steps for whole otoliths for flat fish are described below:

- ❖ The 2 otoliths taken from their storage envelope are immersed in water to rehydrate them.

- They are then cleaned with the help of tongs or needles in order to remove all the remains of rehydrated soft tissue.
- Immersed in water in a flat bottomed and transparent recipient (small glass dish or a polystyrene crystal Petri dish) (Fig. 22), they are examined under the binoculars.

Figure 22 : Whole otoliths of a megrim already cleaned and immersed in water.

8.2. Burnt

For the red mullets and striped red mullets, the burning method described par Christensen (1964) is used.
The result of the burning is that the translucent areas of slow growth (ZCL) take on a distinctly brown colour which makes them more visible (Fig. 23).

Observation in reflected light. Observation in transmitted light. Observation in transmitted light after burning.

Figure 23 : Observations comparing whole otoliths of red mullet before and after burning.

This technique may be carried out on a Bunsen burner or on an electric laboratory hot plate (Fig. 24) or even better on a hot plate with indications of temperature. This is a procedure which had been used since 2007 because it uses a flat heating surface and a controlled temperature.

Figure 24 : Burning the red mullet otoliths.

The steps for burning whole otoliths of red mullets are described below (Le Roy, 2007) :

- Having removed the 2 otoliths from their storage envelope, they are immersed in an aqueous liquid in order to rehydrate them.
- Clean the 2 otoliths with help of tongs or needles in order to remove any remaining soft tissue
- Burn at 250°C for a few seconds for the red mullet. The duration of the burning and the intensity of the browning are controlled and vary according to the size of the otoliths.
- Immersion in water in a flat bottomed and transparent recipient (small glass dish or a polystyrene crystal Petri dish), then examine under binoculars.

8.3. Cut

A lot of species have otoliths (*sagittae*) which are extremely opaque or too thick for their growth marks to be clearly identifiable by transparency. The observation of thin transveral sections, across the nucleus, improves significantly the readability of these marks (Wiedemann Smith, 1968 ; Bedford, 1975 ; 1983). For certain species, if the traditional methods show good results, the observation of the thin transversal sections may be used in order to increase the precision of age estimations and in particular for old fish (Taning, 1938 ; Blacker, 1974 ; McCurdy, 1985, Anonyme, 2004). This is the case for plaice (*Pleuronectes platessa*) (Easey & Millner, 2008). There is no universal standardised method to section the calcified pieces. The procedures as well as the material used to produce simple and/or thin slices vary according to the nature of the application wished for (Mosegaard *et al.*, 1998). The slice of the otolith must pass through the *nucleus* in order to avoid under-estimating the age (Williams & Bedford, 1974).

8.3.1. The material

8.3.1.1. The resin

The resin used is of polyester type. According to the laboratory, it is Crystic R115 PA® from the company Scott Bader or the GTS Pro® from SOLOPLAST-VOSSCHEMIE. They have the same properties, that is plastic qualities. These resins are especially meant for industry and in particular for geology and the metallurgical industry. They present the characteristic of not being breakable once hardened. This is an advantage for the thin strips which resist better to cutting and handling. Previous tests carried out with "standard" inclusion resins have demonstrated the importance of this plastic quality.
The resin is translucent and non transparent. It is pale pink in a liquid state and very pale yellow once hardened. When wishing to colour the resin black, a pigmented paste supplied by STRAND-SCOTT BADER is added in the proportion of 10% of the weight of the resin.
Certain readers prefer to use translucent resin, others prefer black resin (Diagram 25). Both resins having their advantages and disadvantages according to the means of lighting, it is the preparer-reader who chooses. At the sclerochronology centre, all the otoliths are included in translucent resin.

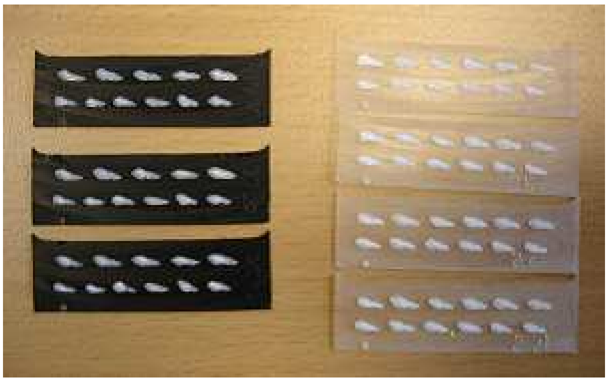


Figure 25 : Otolith slices from Celtic sea whiting included in black resin and in translucent resin.

The resin is pre-accelerated and needs only a catalyser to be used. The catalysers used are BUTANOX® (2% of weight of resin) or MEKP MEC ® (1 to 2% of weight of resin) (Tab. 1). When the temperature of the preparation room is under 18°C an accelerator (STRATYL®) is added in the proportion of 0.1g per 100g of resin.

Tableau 1 : Accelerator and catalyser used according to resin.

Resin	Possible accelerator	Catalyser
Crystic R115 PA®	STRATYL®	BUTANOX®
GTS Pro®	No	MEKP MEC ®

8.3.1.2. The moulds

The moulds used for the inclusion of the calcified pieces for the resin are generally made of polished aluminium with engraved markers (markers at regular intervals) (Fig. 26). The aluminium is heavier than stainless steel but is less resistant to scratches and knocks.

A film of de-moulding agent (silicone oil in spray form) is placed on the surface of the mould before pouring in the liquid resin. This makes it easier to extract the blocks of hardened resin.

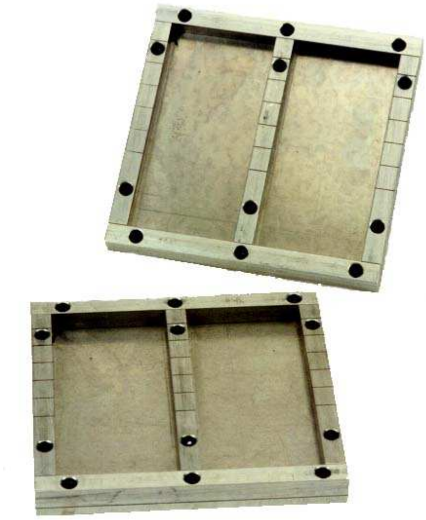


Figure 26 : Inclusion mould made of polished aluminium engraved with markers.

8.3.1.3. The saw

There are 2 types of saws, according to the speed.
For routine use, the fast speed precision saws generate a production speed which is higher than with slow saws (Almeida & Sheehan, 1997). These saws enable a large number of otoliths to be treated with blades 0.4 mm thick. The high speed saws are used for anglers and black-bellied anglers, for haddock, saithe, sole, plaice, pouts, whiting and cod.
The slow speed saw is used to produce strips which are only 0.2 mm thick from otoliths of roundnose grenadier, a very slow growth species.

High speed saws

Since 2003, the automatic Servocut A250® Escil saw (Fig. 27) has been used in all the Ifremer laboratories (Boulogne-sur-mer, Lorient and La Rochelle). The speed of the disc varies from 400 to 4000 rpm^{-1}. The speed generally used is 1500 rpm^{-1}. The saw is equipped with diamond-tipped discs. The table is motorised and mobile in X and in Y. It is set to hold the blocks produced by the mould used. In order to significantly gain time, the saw is used in semi automatic mode, which means that only the X axis is active and motorised, and the disc is inclined manually in order to make a diving slice with the help of a lever.
At Boulogne-sur mer, a modification has been made to this saw in order to adjust the depth of the slice. An adjustable guard has been added to prevent the thin slice breaking off totally at the end of the sawing. A small part of the slice stays attached to the block and the operator only has to detach it manually with a sharp cut. In this way, the slice does not break off suddenly and risk being projected into the bin or being broken by the speed of the rotating blade.

Figure 27 : Automatic high speed ESCIL Servocut A250® saw.

Further to the construction of the Sclerochronology site at Boulogne-sur-mer, two new saws have been in use since September 2008. They are the Escil automatic Brillant 250® saws (Fig. 28). This model is modified in accordance with the specifications necessary for sawing blocks of resin with very fragile calcified pieces.

This machine offers the advantages of use in semi-automatic mode. It is equipped with a speed which varies from 250 to 3000 rpm^{-1}. The speed may be selected according to the species being treated.

For a similar quality, the Brillant 250 is much faster than the Servocut A250.

Figure 28 : Automatic high speed Escil Brillant 250®.

Slow speed saws

The otolith slices for the roundnose grenadiers are carried out with the precision saw Isomet® from Buehler (Fig. 29), equipped with a diamond tipped or resinoid disc. The slicing speed may vary from 0 to 300 rpm^{-1}

<u>Figure 29</u> : Isomet® slow speed saw from Buehler.

Since the construction of the Sclerochronology site at Boulogne-sur-mer, a new saw has been used. This is the automatic Escil Brillant 221® saw (Fig. 30).

<u>Figure 30</u> : Escil automatic variable speed Brillant 221® saw (400 to 6000 rpm).

For a similar quality, the Brillant 221 is much faster than the Isomet® from Buehler.

8.3.2. The production steps for thin otolith strips

The slicing method used is that described by Bedford (1983) and modified by Souplet and Dufour (1983). This technique is similar to the one used in England by the CEFAS (Easey & Millner, 2008).

8.3.2.1. Inclusion of the otoliths

The aluminium moulds are already covered in a de-moulding film (silicone oil in spray form) then an initial layer of polyester resin (pre-accelerated) mixed with the catalyser (and with the colouring agent in order to obtain a black resin if necessary) is poured into the bottom of the moulds (in each compartment, see Fig. 26) in order to obtain a layer 3-4mm thick.

At 18°C, 1h30 is necessary to completely polymerize a mixture of resin catalysed to 2% of its weight. In these conditions, the resin becomes solid but still sticky in 30 minutes and the user has one hour to position the otoliths on top.

The otoliths are placed manually or with tweezers, convex side against the resin, and « stuck » by lightly pressing with the fingers or the tong. The otolith slice passing obligatorily through the *nucleus* in order to be able to observe all the life history of the fish, the *nuclei* are aligned. A digital positioning system (digital video camera linked to a LCD screen) is used in order to facilitate the alignment of the small sized otoliths (a monitor with a marker line is linked to a camera; Fig. 31).

Figure 31 : Digital positioning system of the otoliths.

For each block, a spaghetti marker is used which enables the right hand side of the line of otoliths to be distinguished from the left hand side. When the mould is completely filled with the positioned otoliths, a layer of catalysed resin is poured liberally over the top to cover them entirely and to obtain a total thickness of resin more than 5 mm(Fig. 32). Each block is labelled with a piece of paper at the bottom of the block (Fig. 32). The de-moulding of the hardened blocks containing the otoliths may be carried out after the complete polymerization after 24 hours in an incubator at 25°C.

Figure 32 : Inclusion des otolithes de lieu noir dans la résine.
Inclusion of saithe otoliths in resin.

8.3.2.2. Slice

The automatic high speed saw (Brillant250® from ESCIL) is used in semi-automatic mode which means that only the X axis is active and motorised and the disc is inclined manually in order to make a diving slice by means of a lever. An adjustable guard which limits the depth of the slice has been added in order to prevent the thin slice being totally broken off at the end of the sawing. The operator simply has to break off the slice. In this way, the slice does not break off suddenly and risk being projected into the bin or being broken by the speed of the rotating blade (Fig. 33).

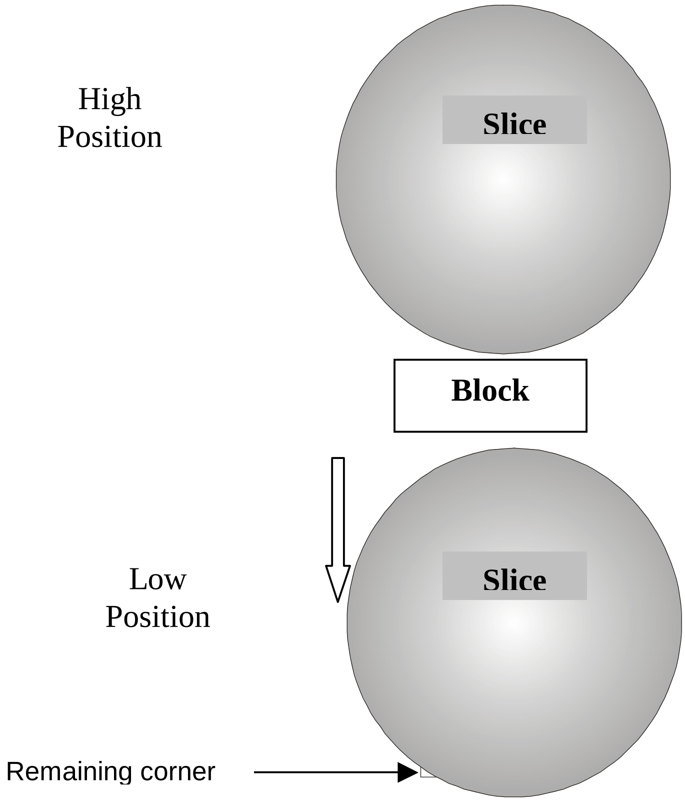


Figure 33 : Otolith slicing.

The saw is equipped with a 150 mm diameter diamond tipped disc, 0.5mm thick and with a bore of 32 mm. The table is motorised and mobile in X and Y. It is adjusted in order to hold the blocks produced by the mould used.

The slicing process follows the following steps :

1. The table is set at zero on the X axis
2. the block is placed on the jaws of the clamp (without tightening)
3. The blade is lowered to the surface of the resin and the block is moved in order to position the blade in front of the first marker of the block (distance of the marker equivalent to the thickness of a slice)
4. the jaws of the clamp are tightened
5. the lid is closed and the process started
6. press lightly and continuously on the handle until the stop
7. let go of the handle, the small block which is cut remains connected to the rest of the block by a small corner
8. adjust the X axis according to the size of the otoliths, from the smallest to the largest
9. start sawing again to obtain the first strip, which is also attached to the block
10. repeat operations 8 and 9

11. the two strips and the small block may then be removed
12. move the block towards the next marker and repeat all the process as from operation 3.

With this type of saw, the average thickness of the strips is 0.4mm. In this way, for each row of otoliths included in the resin, it is possible to make 3 or 4 successive slices according to the species. These slices pass through the opaque central structure of each otolith and at least one of them passes through the *nuclei* (Fig. 34).
Experience has shown that two strips are enough in order to obtain an usable strip, ie. one that passes through the *nuclei*.

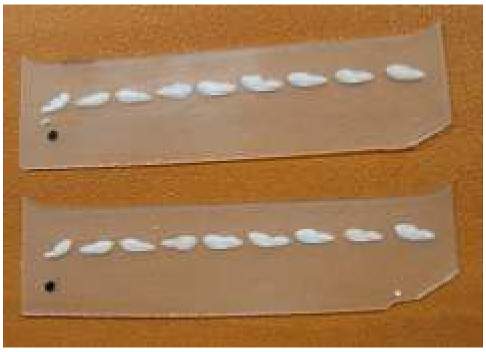


Figure 34 : Successive thin strips from a same row of otoliths (Celtic sea whiting) included in tranlucent resin.

For certain difficult species, such as the roundnose grenadier, it is necessary to make slices which are thinner, about 0.2mm thick. For this, a slow speed saw for example the Isomet® from Buehler is used or the Brillant221® from ESCIL which operates in the same way as described above.

8.3.2.3. Staining

The colouring is a preparation method which reveals thin chromophil growth lines, comparable, sometimes, to those obtained after burning. This method is used for sole otoliths (*Solea solea* ; Fig. 35).

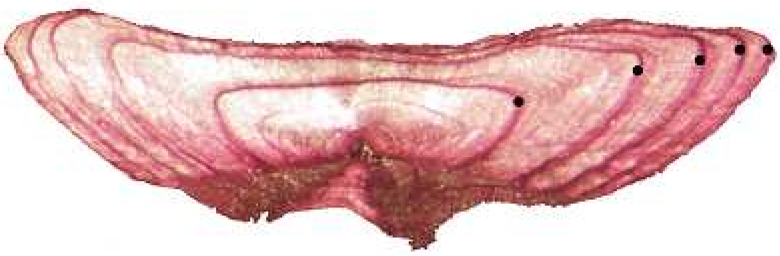


Figure 35 : Sole otolith sliced then stained in neutral red (translucent growth rings stained and marked by black points)

Having cut the otolith, the thin strip obtained is immersed in a colouring agent which has had acid added to it. The staining agent is neutral red (Neutral Red Solution) from the company Sigma. For 100ml of staining agent, 0.5ml of acetic acid dosed at 100% is added. Having sliced the otolith, the thin strip obtained is immersed in a staining agent which has had acid added to it. For 100 ml of staining agent, 0.5 ml of pure acetic acid (concentration>99%) is added. The strips are immersed for 20 to 25 minutes. They are then rinsed with a little water then dried on absorbent paper.

This slicing and then staining technique was developed by Bouain & Siau (1988). The staining reaction time is very variable, depending on the species and the size of the otoliths : a few minutes are enough to obtain good preparations, but preliminary tests are necessary in each case.

8.4. Summary of the preparation per species

In 2008, 25 species were monitored routinely by France within the DCR. Table 1 shows the calcified piece and its preparation method for each species.

Table 1 : Calcified piece and its preparation method for age estimation in species treated routinely in France.

Species	Type of calcified pieces	Preparation method
Seabream, seabass	scale	Whole scale
White, black or red anglerfish	*illicium*	Thin slice of 0,4 mm
Roundnose grenadier	otolith	Thin slice of 0,2 mm
Haddock, Saithe, Norway pout, Whiting, Cod, Plaice, Turbot, Pout, Hake	otolith	Thin slice of 0,4 mm
Sole	otolith	Thin slice of 0,4 mm then colouring or not
Megrim	otolith	Whole otolith
Red mullet, Striped red mullet, red gurnard, tub gurnard	otolith	Whole burnt otolith
Herring, Sprat, Anchovies and Pilchard	otolith	Whole otolith included in resin

9. Observation techniques

The observation of calcified pieces preparation may be carried out directly with a microscope or a binocular magnifying glass. However with the progress in imaging, Ifremer has developed a system for acquiring and treating pictures used for computer assisted age and growth estimation (Panfili *et al*., 2002).

9.1. Digital Processing for Calcified Structures (TNPC)

Since 2007, routine age estimation has been carried out with the TNPC software (Digital Processing for Calcified Structures ; Fig. 36) developed by Ifremer with the company Noesis.

Figure 36 : TNPC® software developed by Ifremer with the company Noesis (for more information : http://www.ifremer.fr/lasaa/TNPC/Tnpc4_2English.htm).

This system of acquiring and treating pictures consists of 3 units (Fig. 37) :

- ❖ A picture acquisition unity (camcorder or scanner) : the camcorder must be high resolution. The characteristics of the camcorders used are the following : SXGA 1392 x 1040 pixels with a resolution of 1 450 000 CCD pixels (e.g. : Sony 910).
- ❖ A central treatment, storage and visualisation unity: a computer with a big random access memory (minimum 2 Go) for the digital analysis with 2 screens (screen 1: visualisation of calcified piece; screen 2: biological parameters or picture analysis).
- ❖ A software unity : TNPC software for the acquisition and treatment of calcified pieces (Fablet & Ogor, 2005).

Figure 37 : Computer assisted system for estimating age and growth.

The acquisition and the treatment of the pictures of calcified pieces absolutely must respect a standardised procedure (use of TNPC software: Mahé *et al*., 2006).

This system of computer assisted age and growth estimation is used routinely for acquiring and interpreting the growth structures and their storage.

- Acquiring the picture : the pictures of the calcified pieces are made directly with the TNPC software (Fig. 38). The pictures are stocked per sample in an Access database. Each picture is obligatorily calibrated. The format of the pictures is im6 which enables the recording of all the components linked to the picture. A file called « Biological parameters » is filled out (Mahé *et al*., 2007a) and associated with the database created. When acquiring the pictures it is possible to carry out a pre-treatment (optimisation of movement, increase of contrast, reduction of noise, detection of edges…).

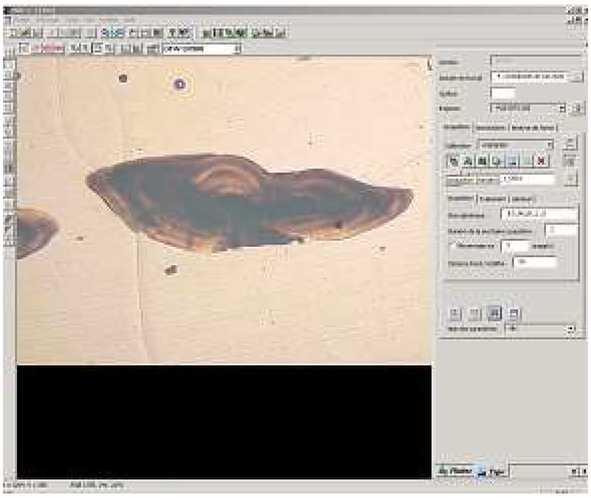


Figure 38 : Acquisition of pictures directly with the TNPC software (*In* Mahé *et al*., 2006).

- Interpretation of growth structures: estimating the age is carried out by the reader with the help of radials (Fig. 39) which are integrated directly into the picture database created previously. Each radial is recorded with 3 files (rad, pro and iid), which enables the distances between the *nucleus*, the growth rings and the edge of the otolith to be measured.

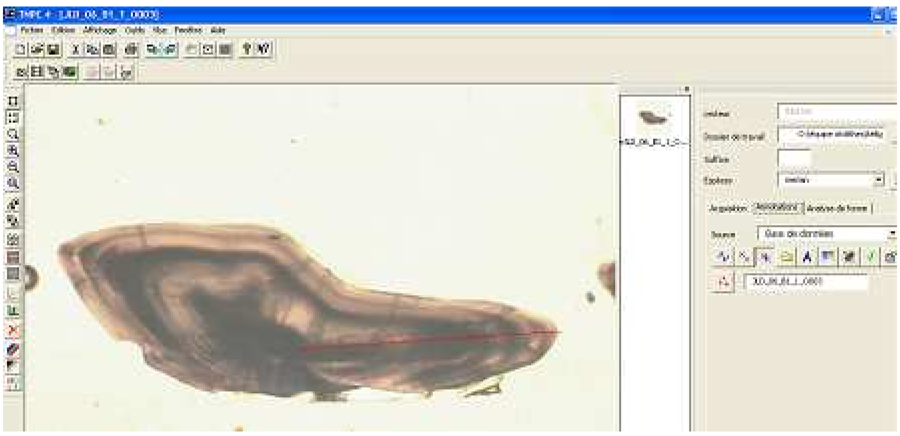


Figure 39 : Interpretation of a calcified piece using the TNPC software (growth rings identified by the marker lines ; *In* Mahé *et al*., 2006).

The TNPC software offers interpretation assistance for growth structures with the growth law noticeable on a calcified piece according to the position of the rings which is compared to those on other calcified pieces already interpreted and with the position of the rings on the grey scale along the radial.

- Archiving the pictures and associated information : the calibrated pictures and the associated information (biological parameters and interpretation of growth structures) are saved (Mahé *et al*., 2007b) and this enables the filing to be done without losing the quality which is perhaps not the case for the calcified pieces.

9.2. Observation liquid

The use of a light-enhancing milieu is recommended for all types of observation, with or without previous preparation of the calcified pieces. Such a milieu is a liquid or a hardening product (resin) which enables the contrast and the visualisation of the growth marks to be improved. The milieu often has the same optic density as that of the calcified piece, or a similar density, thereby enabling the light to penetrate more easily, in the aim of revealing the most internal structures. Several light-enhancing liquids are available: the most frequently used is probably water, then there are the saline solutions (more isotonic), followed by alcohol, water-alcohol mixtures (in different proportions), glycerine, a glycerine-alcohol mixture (30:70) and the immersion oils. The essential oils (clove, rosemary, pine, cedar, camomile etc.) also give good results (*In* Panfili *et al*., 2002).

Routinely, when the calcified pieces (otoliths and scales) are whole and devoid of previous preparation, the observation liquid is water. Inversely, the thin slices of otoliths are observed immersed in glycerine, paraffin oil or alcohol (Fig. 40).

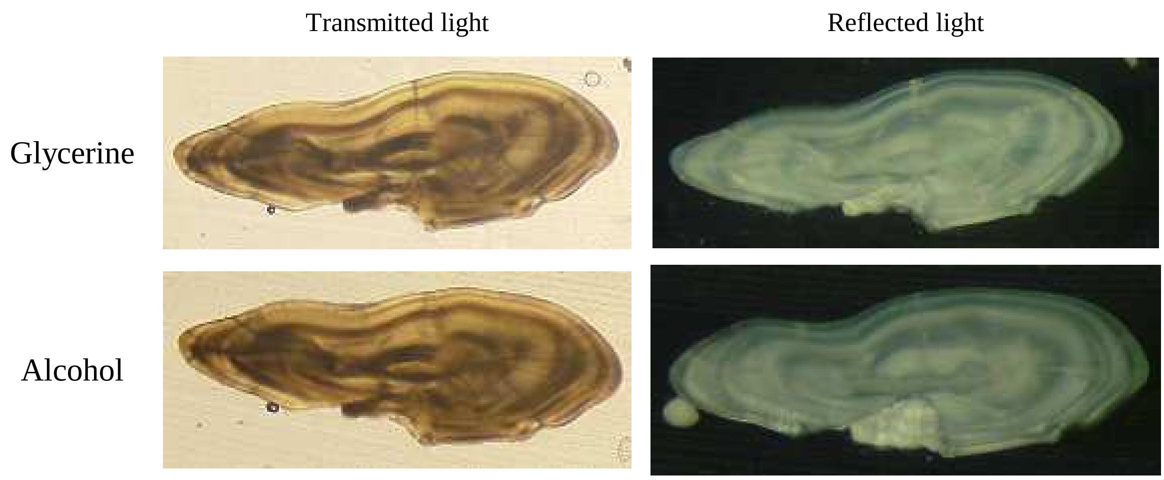


Figure 40 : Observation of a thin whiting otolith slice without light-enhancing liquid, with glycerine and alcohol.

The use of glycerine lightens the surface. The marks of the slice are softened and when examined in reflected light, the contrast is improved. After examining and before archiving, it is necessary to clean the strips carefully with water in order to remove all traces of glycerine which may make the strips unusable in the future due to loss of contrast between the observable growth areas. The use of paraffin oil makes the cleaning operation easier due to the lower level of viscosity.

9.3. Magnification

For most of the calcified pieces treated routinely, it is not possible to observe the seasonal growth structures with the naked eye.
For the whole otoliths and the thin strips, the magnifying glass binoculars used are equipped with a lens shot x 1 and a zoom varying from x6 to x50. A lens shot x 0.5 is sometimes necessary in order to observe the totality of a scale.

9.4. Light

We routinely use sources of white and cold light (temperature from 2500 to 3200°K).
The episcopic lighting produces light which is reflected by the surface of the piece observed. The diascopic lighting produces light which is transmitted across the piece observed.
The appearance of seasonal growth will depend on the type of lighting used (Fig. 41):

- ❖ In transmitted light, the translucent growth areas are light and the opaque areas are dark.
- ❖ In reflected light, the translucent growth areas which are not very reflective are dark and the opaque marks which are very reflective are light.

The fact that the growth marks are described as “light” or “dark”, according to the mode of lighting, may lead to a certain confusion and for this reason, the terms “opaque” and “translucent” should always be employed (Casselman, 1974, 1983 ; Williams & Bedford, 1974; Panfili *et al*., 2002).

Reflected light :

- The opaque areas appear clear
- The translucent areas appear dark

Ring 1
Ring 2
Ring 3

Transmitted light

- The opaque areas appear dark
- The translucent areas appear clear

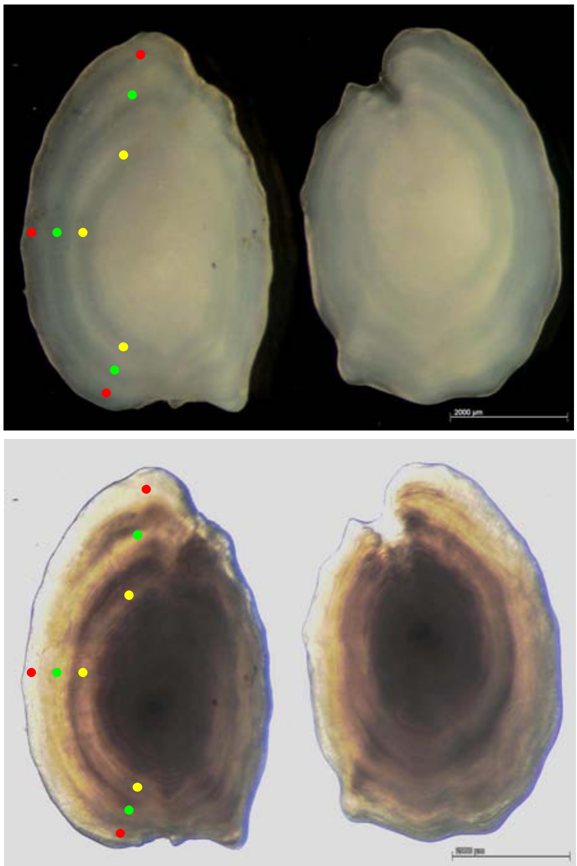


Figure 41 : Visualization of opaque and translucent areas according to the type of light used (reflected light : A ; transmitted light: B).

10. Description of observable structures

Examining the calcified piece involves identifying the seasonal growth structures and the interpretation thereof which leads to the estimation of the age in years of the calcified piece[3].

Certain inevitable interpretation criteria must be identified straight away: for example, the exact place of the birth mark, the first growth mark, the transition areas on the calcified piece, or the nature of the edge of the calcified piece. The biological information available on the species given in literature must be used at first in order to define these criteria empirically. They may be compared with each other in order to establish an “alphabet” (identification of the growth marks) and to determine the “grammatical rules” (interpretation criteria based on existing knowledge) in order to attribute an age (Sych, 1974).

The coherence of the age estimation process must then be determined. This means that it must be possible to constantly identify the same growth structures. The repeatability of the estimation procedure (internally) must be determined and the ages estimated must be calibrated (externally) with other experts (Chapter 12).

10.1. Identification of the structures

The age estimation process requires the reading axis (*nucleus*-edge) to be defined in the first place then the seasonal structures along this axis to be identified.

Seasonal growths, also called seasonal areas, marks, rings or *annuli*, are often distinguishable because they have differences of opaqueness and of organic matrix rates. In transmitted light, the opaque areas are dark and the translucent areas are luminous and, in reflected light, the opaque areas are luminous and the translucent areas are dark (Fig. 41 & 42).

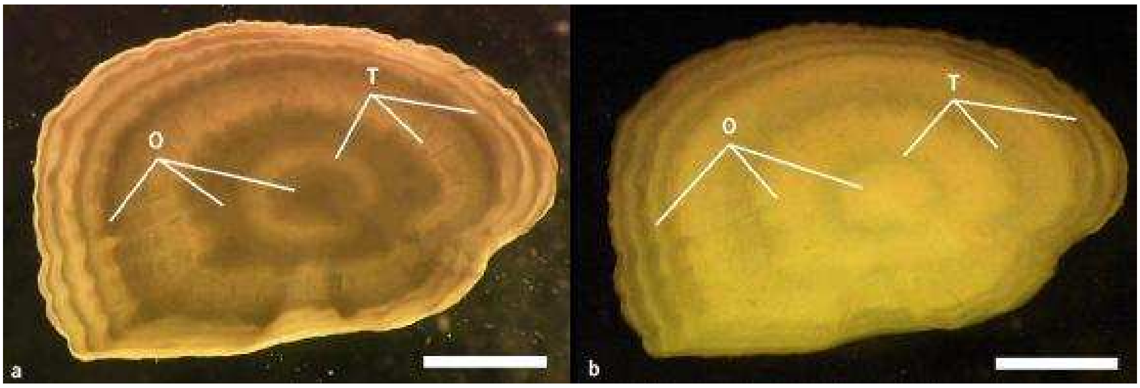


Figure 42 : Whole otolith of common plaice, *Pleuronectes platessa* (47 cm TL), showing the opaque areas (O) and translucent areas (T) observed in transmitted light (a) and in reflected light on a black background (b). The opaque areas are dark in transmitted light and luminous in reflected light, and it is the opposite for the translucent areas. Scale = 2 mm. (*In* Panfili *et al*., 2002).

The identification of the seasonal growth areas is sometimes difficult, due to the presence of double rings, of discontinuities or of other structures which correspond to non cyclical events (migration reproduction…). The identification is therefore based on their clearness, their continuity over the entire calcified piece, their thickness and their

[3] the term « reading » is commonly used to estimate the age based on the identifiation of the growth structures observed.

width. The marks should theoretically decrease in width from the centre of the calcified piece until its edge, in relation with the reduction in the rate of growth with age.

10.2. Interpretation of the structures

Having identified all the growth marks of the calcified piece, the following information is necessary in order to attribute an age to an individual:

- The date of capture ;
- The individual date of birth ;
- The growth marks considered ;
- The nature of the edge of the calcified piece.

Conventionally, the birth date is fixed at the 1st January as the birth date for all the individuals (Williams and Beford, 1974). Converting the count of growth marks into an age value involves relating the formation date of the marks considered to the date of individual capture and the date of birth (Fig. 43).

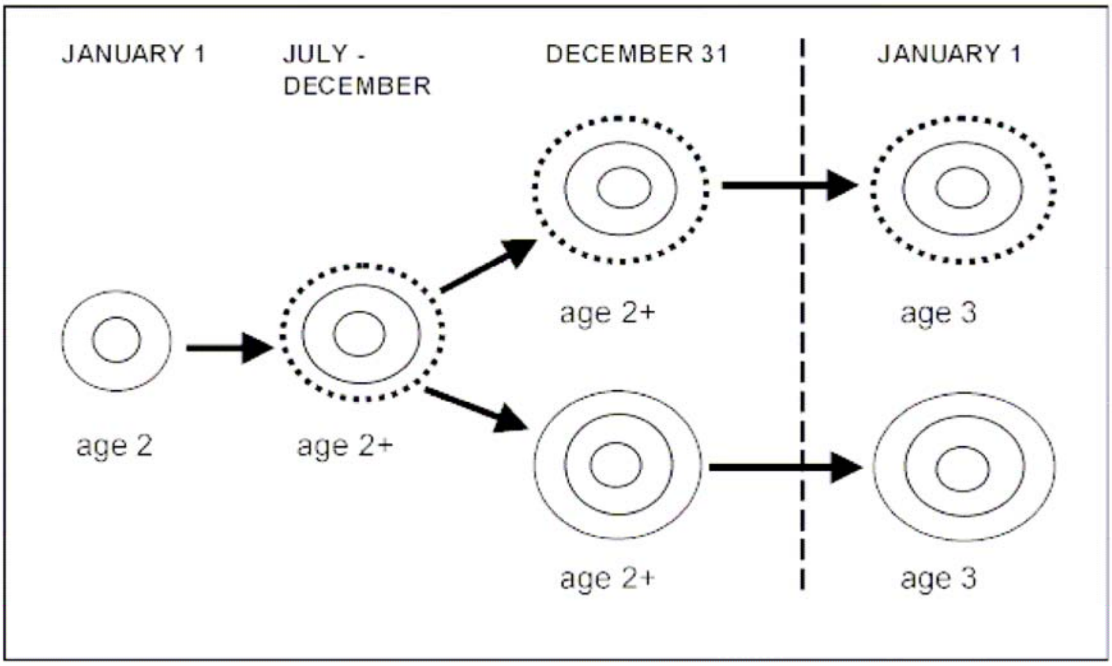


Figure 43 : This image represents an otolith displaying growth steps from 1st January for one year. The black continuous lines represent the translucent rings which alternate with the wide white areas representing the opaque areas. The dotted lines indicate an opaque ring which is not finished. This image demonstrates the different observable possibilities during one year as from 1st January (*In* Anonymous, 2006).

In this way, the age estimation of an individual may be calculated from the following decision tables (Tab. 2 *In* Bellail, 2005).

Tableau 2 : List of n translucent areas, including the edge. The count begins at 1 (1st seasonal translucent area formed after the opaque area in the year of birth).

Nature of the edge	*Quarter 1*	*Quarter 2*	*Quarter 3*	*Quarter 4*
Wide Translucent	***Age = n***	***Age = n***	***Age = n–1***[4]	***Age = n-1***
Narrow Translucent	***Age = n***	***Age = n***	***Age = n-1***	***Age = n-1***
Wide Opaque	***Age = n+1***	***Age = n+1***	***Age = n***	***Age = n***
Narrow Opaque	***Age = n+1***	***Age = n+1***	***Age = n***	***Age = n***

Each year is therefore associated with what we will call an age group. The age group 0 (G0) corresponds to animals of less than 1 year. The group 1 (G1) refers to animals of more than one year and less than two years.

[4] There is no more indecision when comparing the width of the marginal growth with the homologous area which precedes it, having recorded the rate of growth of the otoliths during the first semester of the year considered or having previously examined the otoliths taken for sampling at the end of the year.

10.3. Examples

10.3.1. Whiting

The birth period is in spring. The otolith therefore begins naturally by an opaque growth area around the *nucleus*. Then the translucent area starts from September. For an otolith of one year from the month of June, we may find the structure of an otolith from group 0 plus an opaque ring on the periphery. For an otolith of 2 years from the month of June, we may still find the previous structures plus a translucent ring and an opaque ring forming (Fig. 44).

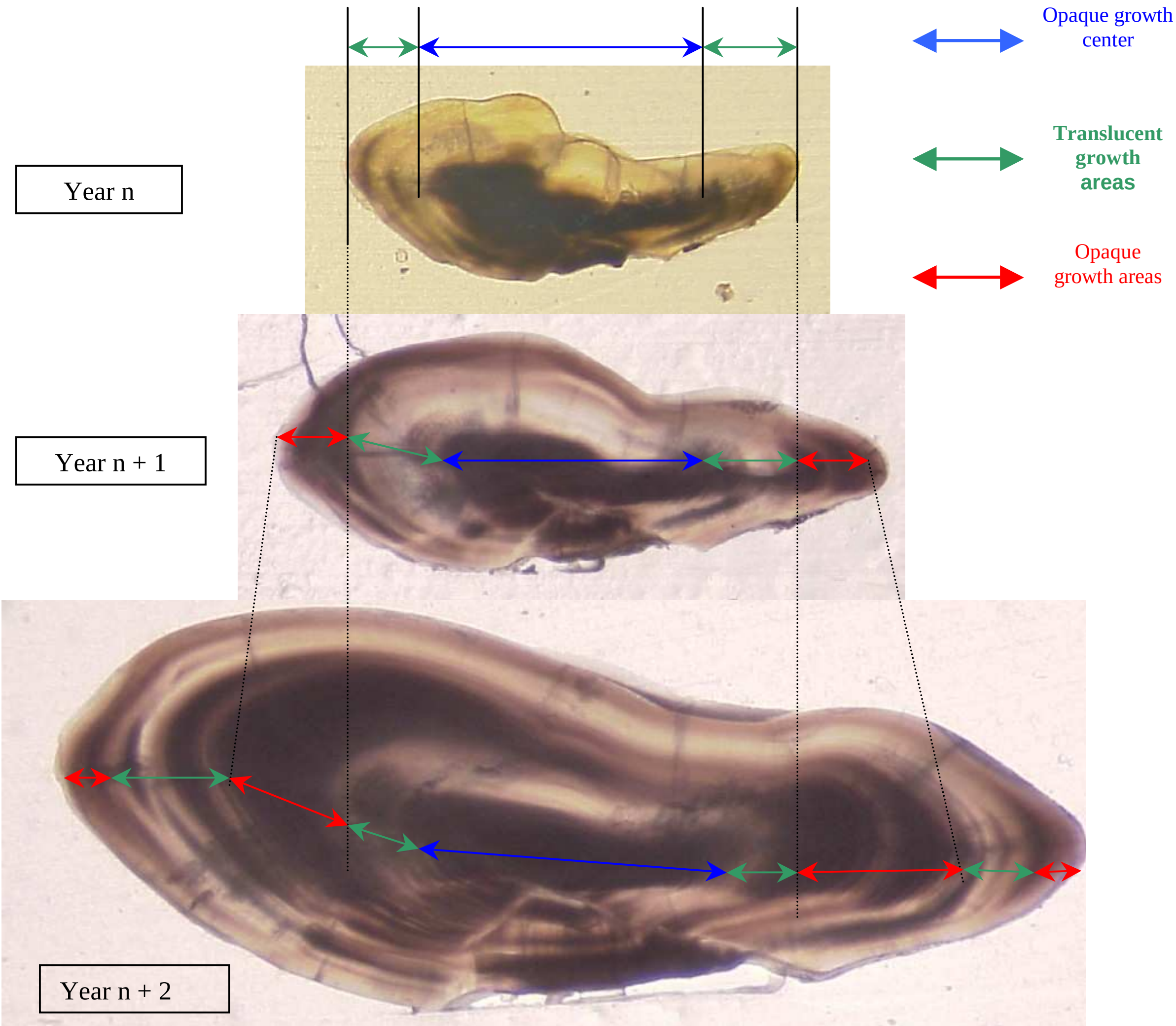


Figure 44 : 3 otolith slices in transmitted light from the first 3 years of the life of a whiting.

The whiting otolith slices display rings which are not linked to the growth also called false rings (Fig. 45). These small opaque areas were called "Humphries'shadow", during a workshop in 1987 in Dublin, in reference to our English former colleague Colan Humphries who presented orally this particularity. The figure 44 shows these false rings.

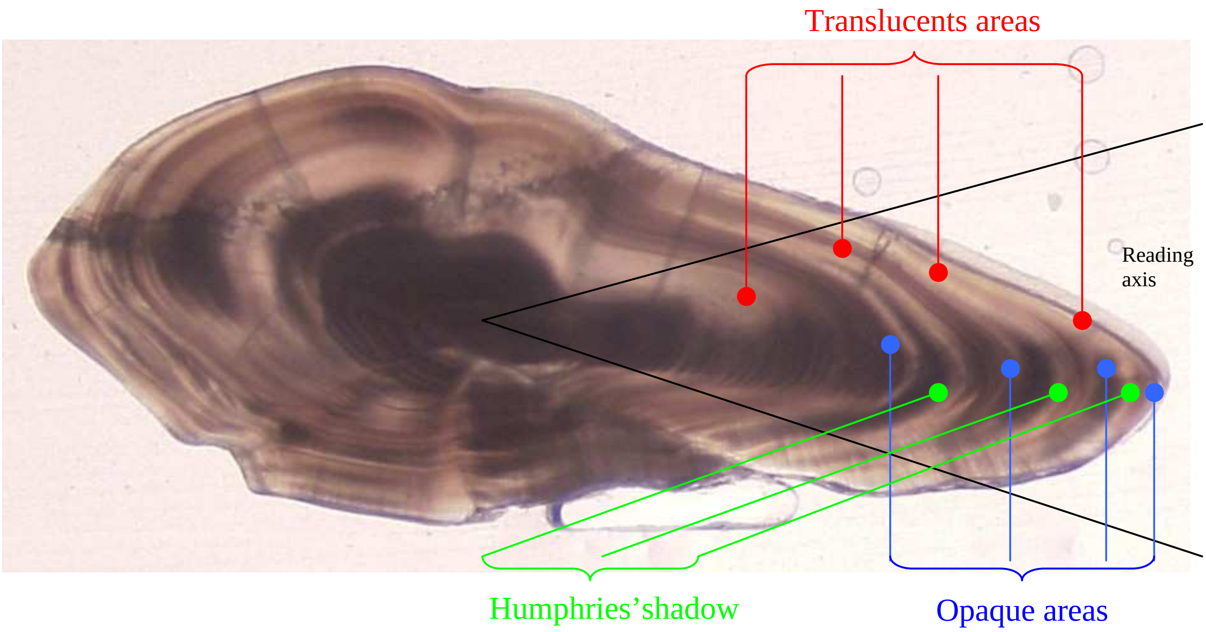


Figure 45 : Age estimation by counting the hyaline rings (whiting of 4 years).

10.3.2. Megrim

According to the recommendations from the work groups on megrim (Anon., 1991, 1995 & 1997), the reading areas used require having 2 whole otoliths (Fig. 46).

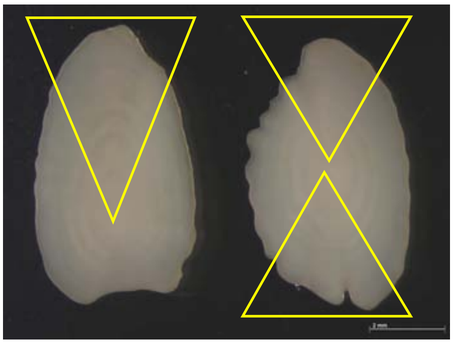


Figure 46 : Reading areas on the right and left whole otoliths of megrim.

The reproduction period being in the spring, the centre of the otolith consists of an opaque material which corresponds to a growth area around the *nucleus*, from the beginning of the fish's life until the end of the autumn or the beginning of the winter. Then the translucent matter is formed during the winter.
The growth areas diminish during the life of the fish which signifies a slowing of the growth rate with age (Fig. 47).

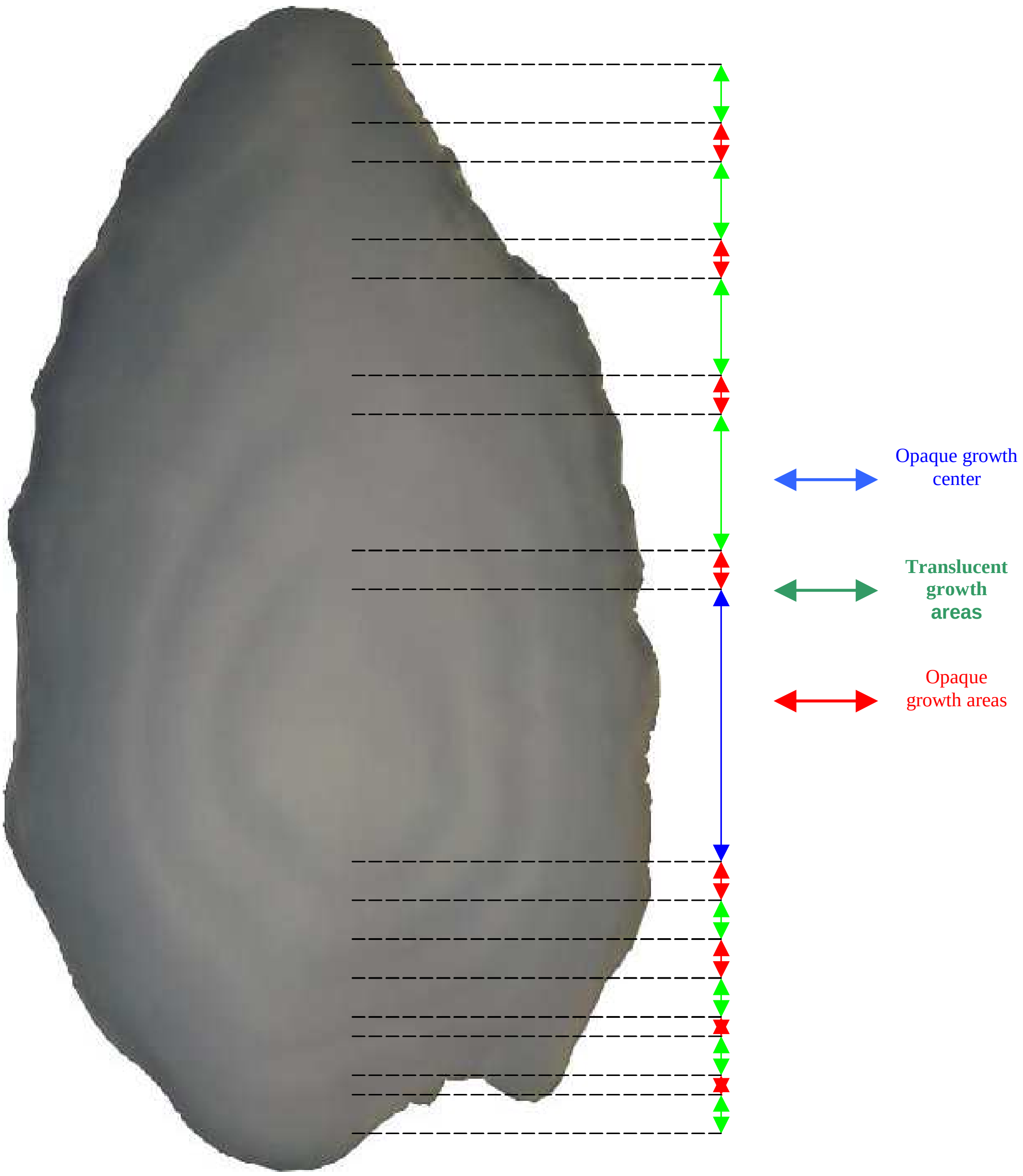


Figure 47 : Vizualisation of the appearance of rings around the *nucleus* on a megrim whole otolith, in reflected light, displaying successively alternate opaque and translucent areas.

The structures which are observable for an age group n are also observable for an age group n+1 (Fig. 48).

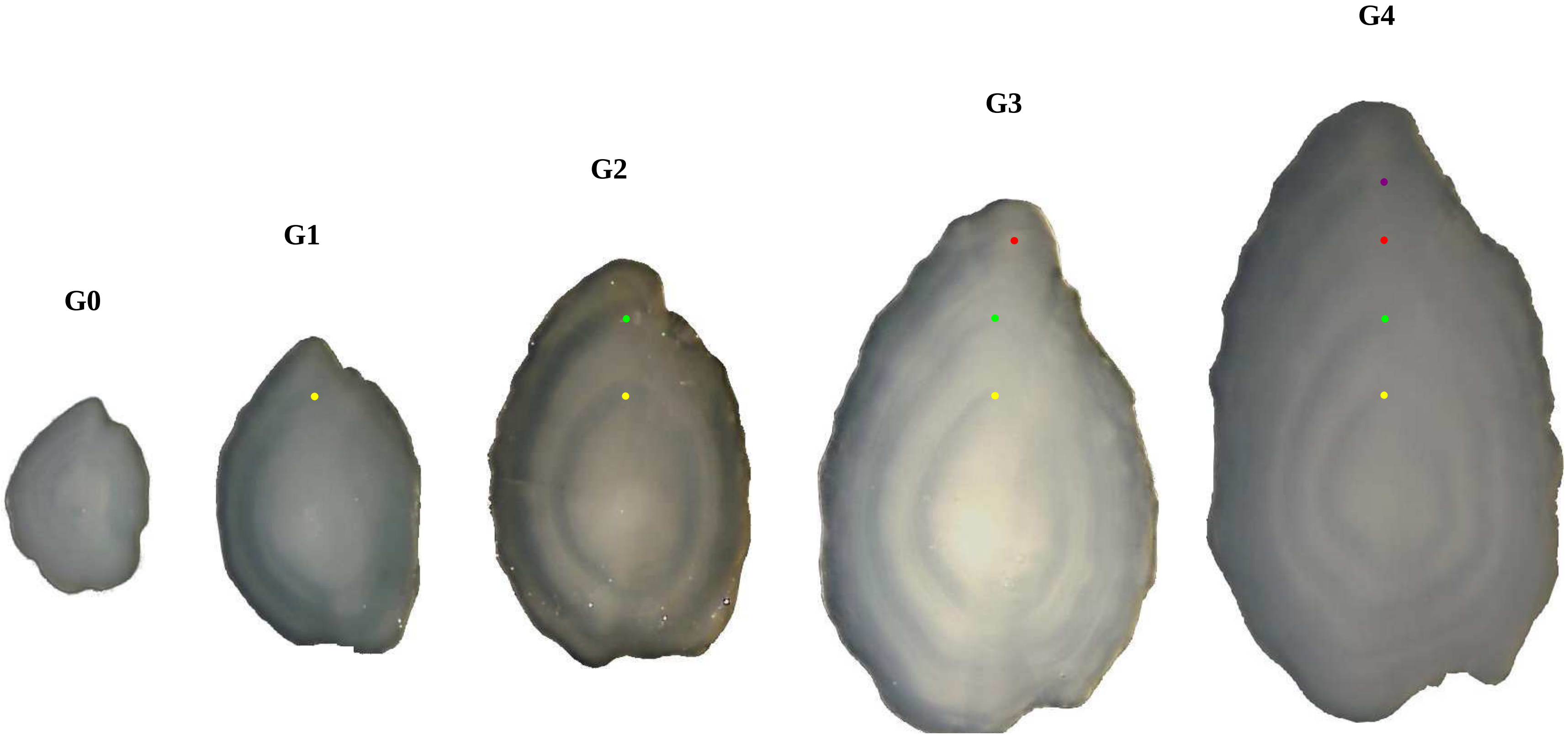


Figure 48 : Pictures of megrim otoliths from age groups 0, 1, 2, 3 and 4 in quarter 4.

10.3.3. Seabream

For the seabream scales, the reading is carried out in transmitted light. The age estimation may generally be carried out over a large reading area, because the rings can usually be monitored for the whole surface of the scale (Fig. 49).

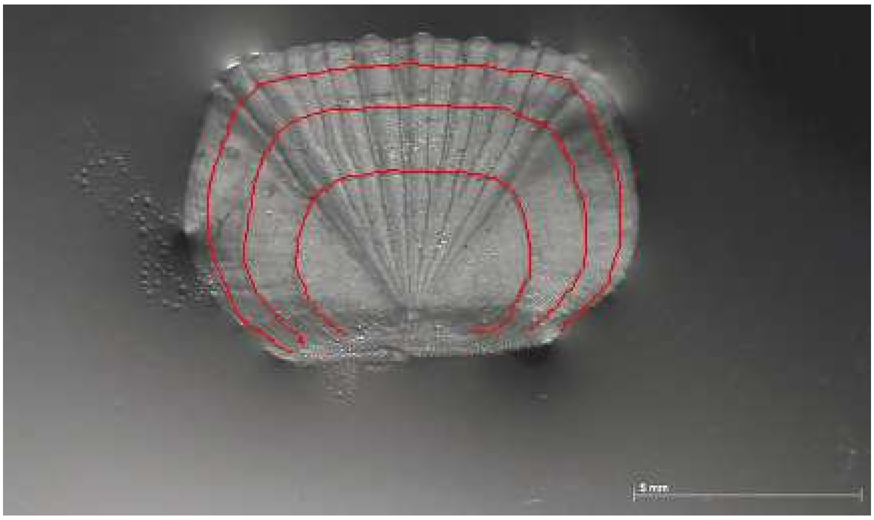


Figure 49 : Seabream scales with identification of growth rings.

However, the reading area is restricted with the biggest distance being between the edge and the growth centre of the scale (Fig. 50).

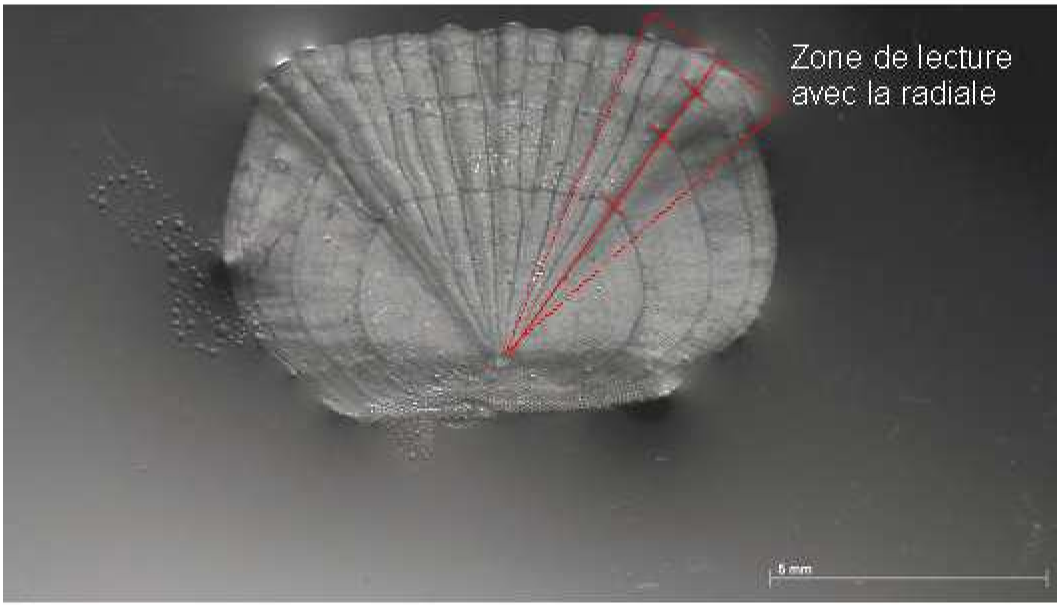


Figure 50 : Preferential reading area on seabream scales.

The birth period is at the end of winter, the scale starts naturally by a growth area in the spring, then the growth slows down and a winter mark appears (Fig. 51).

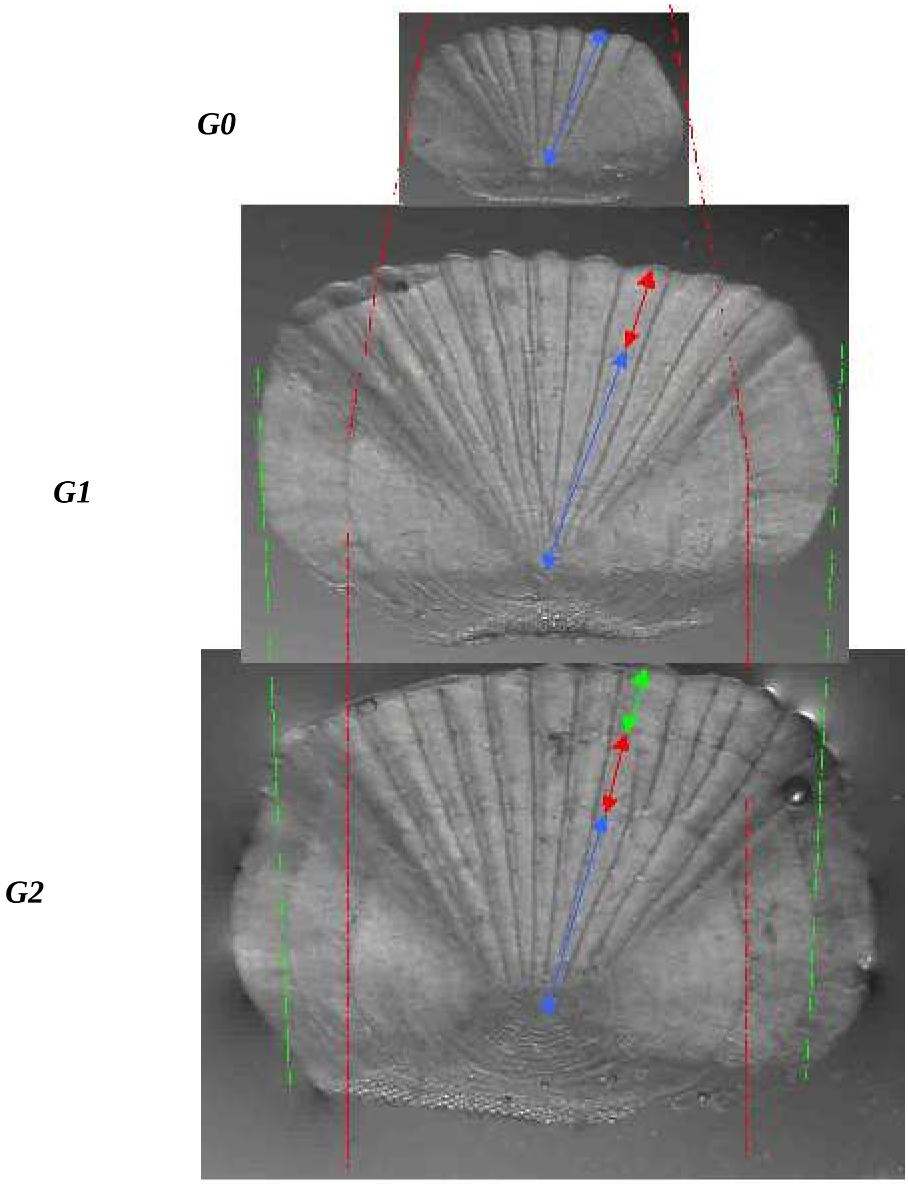


Figure 51 : Scales of the first 3 age groups of seabream (second semester).

10.3.4. Haddock

For the haddock, the otolith slice is interpreted in the dorsal area as shown in figure 52.

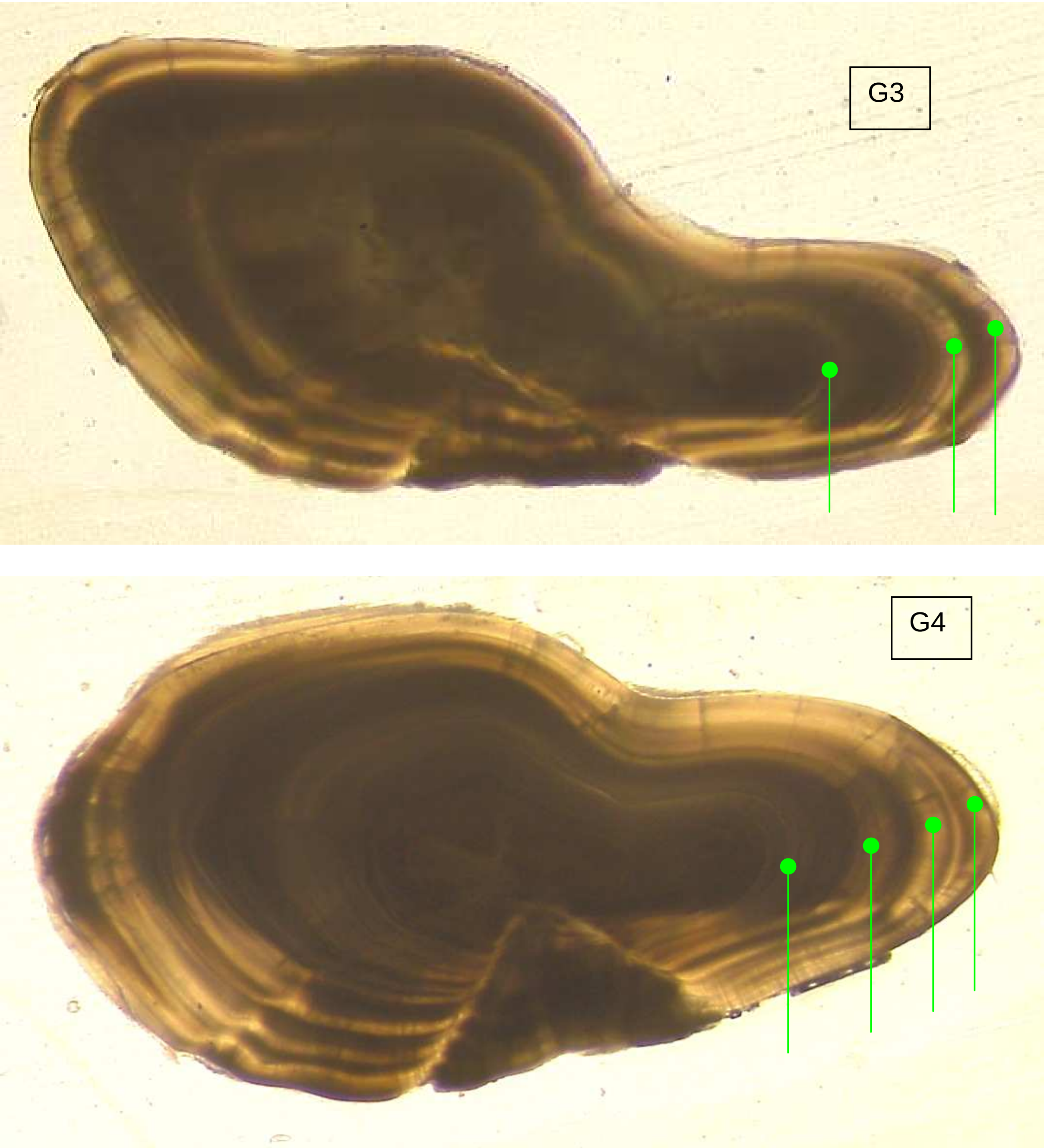


Figure 52 : Age estimation for haddock otolith slices taken in 2nd quarter in transmitted light (in green, the translucent areas).

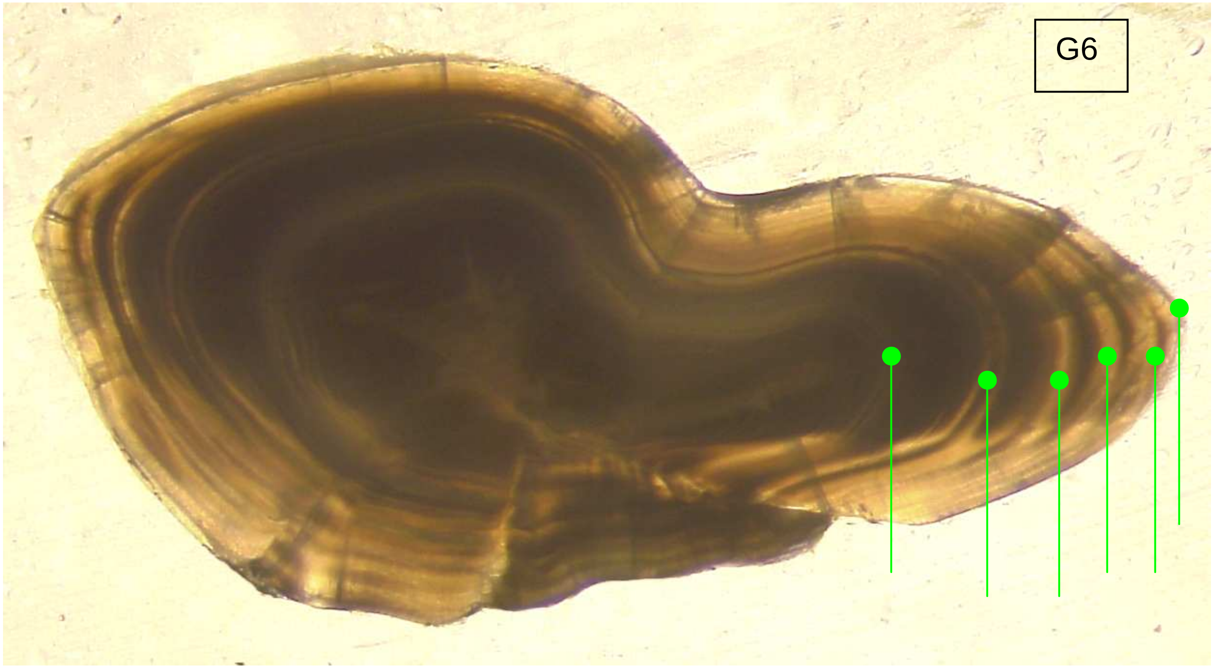


Figure 52 (cont.) : Age estimation for haddock otolith slices taken in 2nd quarter in transmitted light (in green, the translucent areas).

10.3.5. Saithe

The structures (*sulcus*, *nucleus*, opaque and translucent rings) are clearly visible on the saithe slices (Fig. 53).

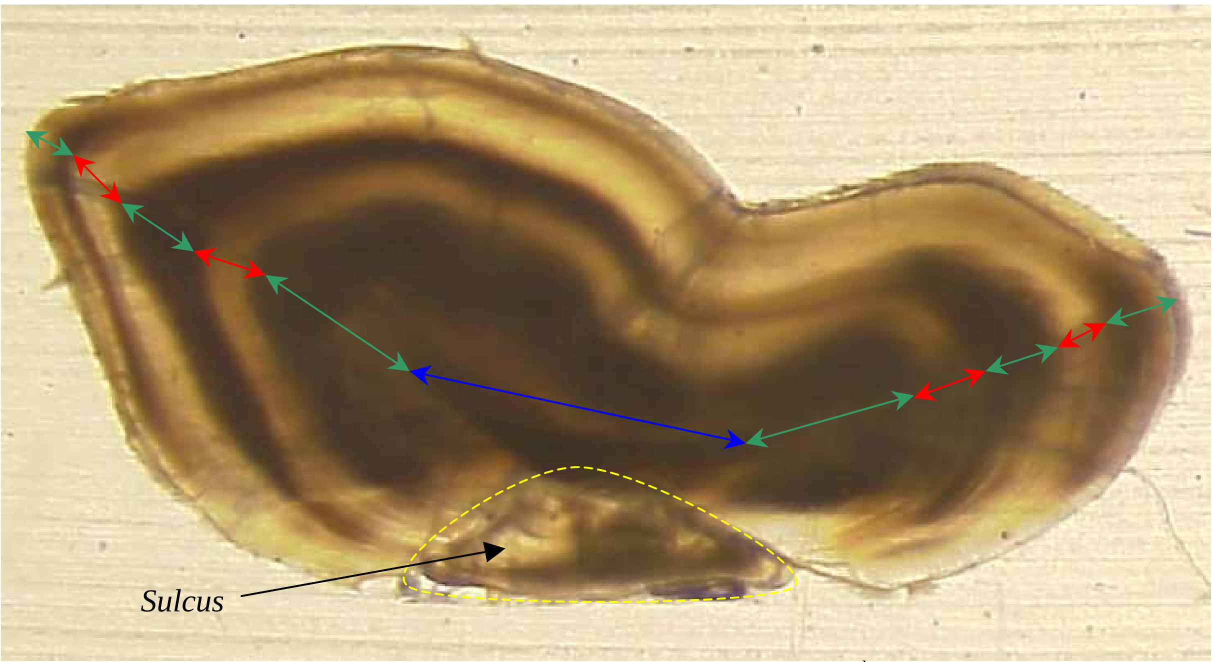


Figure 53 : Interpretation of a thin otolith slice of 2 year old saithe (taken in 3rd quarter) in transmitted light (blue: *nucleus* ; green: opaque ring, red: translucent ring).

10.3.6. Striped Red mullet

For the observation of the red mullet otolith, there are 3 preferred reading axes (Fig. 54).

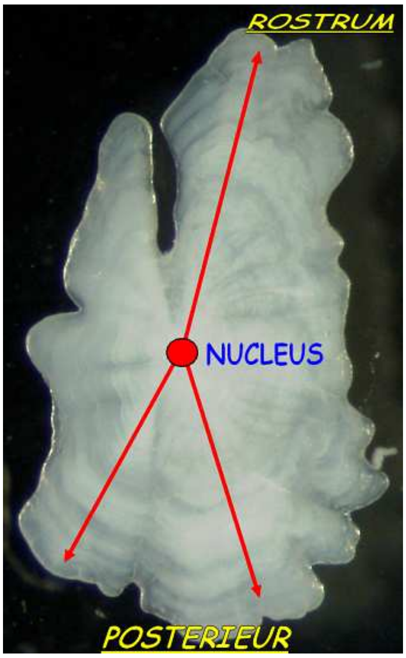


Figure 54 : Preferred reading axes for a striped red mullet whole otolith in reflected light.

The opaque rings are more easily observable after burning (Fig. 55).

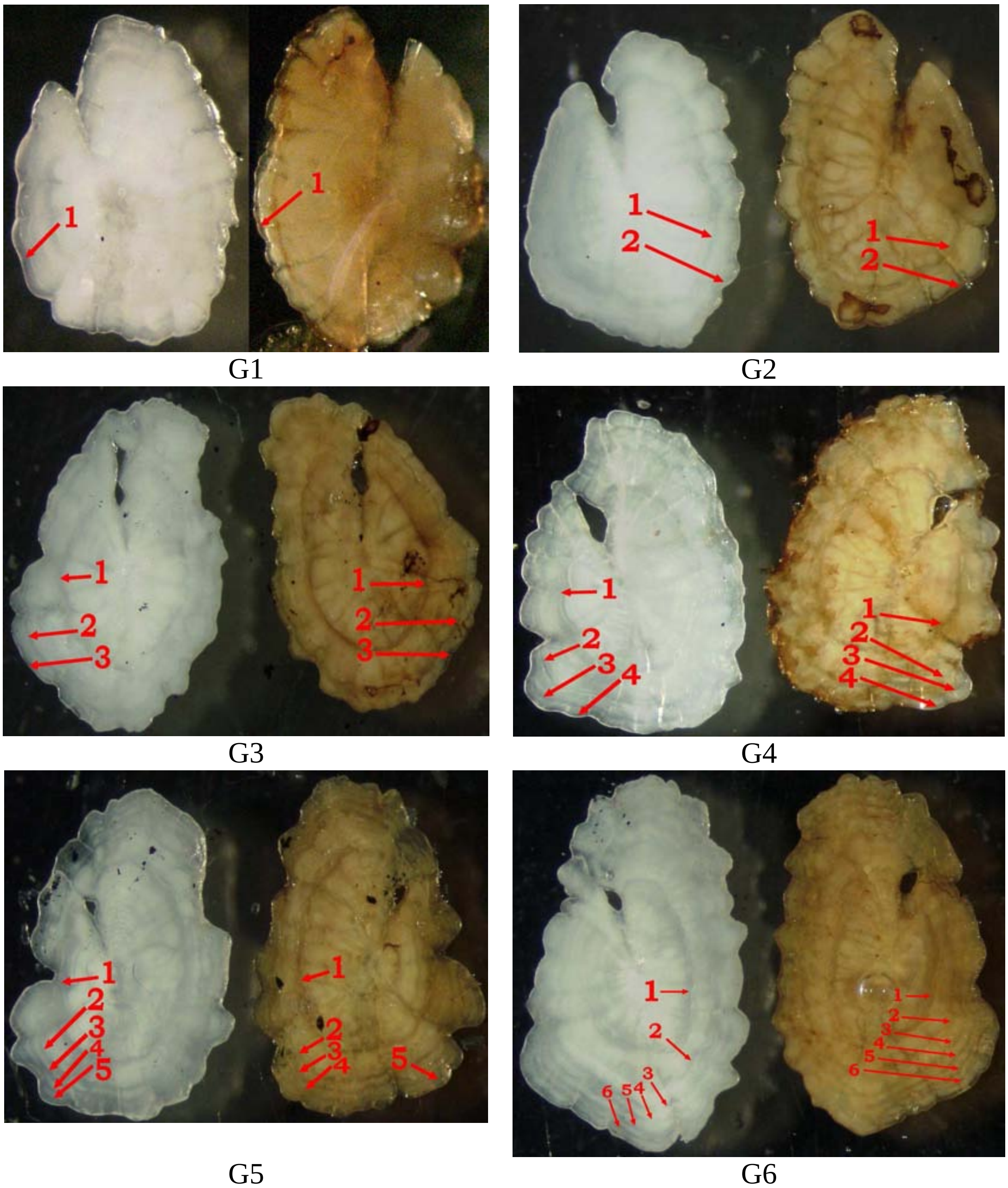


Figure 55 : Age estimation (age group G1 to G6) on red mullet whole otoliths in reflected light before and after burning (taken in 1st semester). The opaque rings counted are in red.

11. Supply and Archiving of age estimations

11.1. Age estimation and associated biological parameters

Since the 1st January 2008, when taking calcified pieces, a standardised Excel file (format of European project COST) has been filled in according to a defined agreement (Mahé *et al*., 2007a). When estimating the age, this is finalised (Table. 3).

Tableau 3 : Example of completion of standard age information archiving file with associated biological information.

Date	Ship	Gear	Haul_Number	Place	Species	Species_Code
-1	-1	-1	-1	-1	Common sole	3121
-1	-1	-1	-1	-1	Common sole	3121
30/09/2004	101987	-1	-1	XBL	Plaice	3115
15/10/2004	278970	OTB	10	CGFS	Plaice	3115
28/09/2005	273888	OTB	-1	UCH	Common sole	3121
11/10/2005	101717	GTR	-1	LCN	Common sole	3121

Area	Division	Year	Quarter	Category	Description	Increment	Size_Unity	Size	Weight_Unity
MCE	7d	2003	1	-1	-1	-1	cm	-1	-1
MCE	7d	2003	2	3	Total length	1	cm	29	gr
MCE	7d	2004	3	2	Total length	0.5	cm	36	gr
MCE	7d	2004	4	-1	Total length	1	cm	30	gr
MCO	7e	2005	3	3	Total length	1	cm	31	gr
MCE	7d	2005	4	3	Total length	1	cm	30	gr

Weight	State	Sex	Maturity_Scale	Maturity	Reference_Taking
-1	-1	-1	-1	-1	-1
216	Full	M	7	1	P1-MED-MB-023
500	Emptied	-1	5	4	P1-MED-MB-024
266	Full	M	7	2	P1-MED-MB-025
294	Full	I	7	2	P1-MED-MB-026
320	Full	F	7	2	P1-MED-MB-027

Type_CP	Preparation_CP	Weight_CP	Age	Reference_CP	Observations
O	whole	0.005	2	JLD_06_B1_C1_O001	-1
-1	-1	-1	-1	-1	Broken otolith
-1	-1	-1	-1	-1	-1
E	Sliced	0.0056	2	JLD_06_B1_C1_O003	-1
O	Sliced	0.0093	4	JLD_06_B1_C1_O004	-1
I	Sliced	0.005	2	JLD_06_B1_C1_O005	-1

At the moment, the file used is the version 4.

All these files are stocked within an Access database which uses the referential Harmonie (Base Access Bargeo ; Fig. 56) which enables all this information to be managed and the key size/age information to the European work groups in the various formats requested.

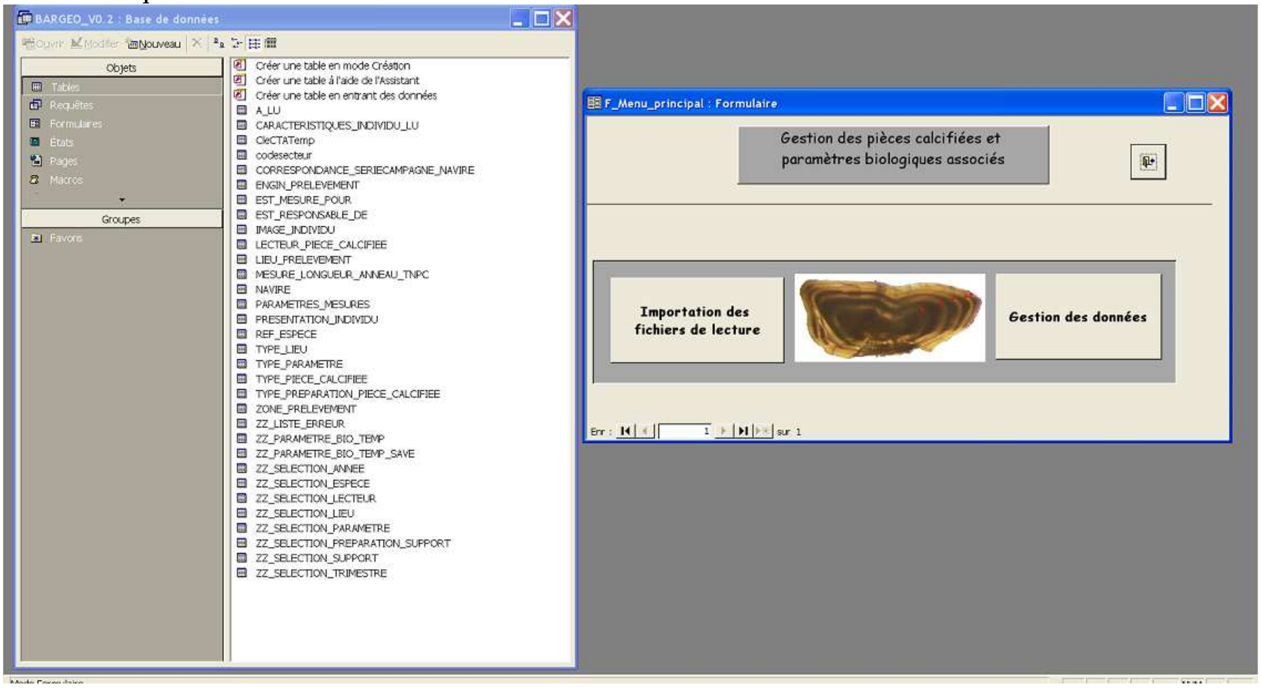


Figure 56 : Management database for calcified pieces and associated biological parameters.

11.2. Calibrated and interpreted pictures

Since the 1st January 2008, the pictures and the interpretations thereof have all been stocked on a network disc according to a defined protocol (Mahé *et al*., 2007b).

12. Reading quality

The repeatability of the estimation procedure (internally) must be determined and the ages estimated must be calibrated (externally) with other experts (European experts and the 2nd French reader). In the same way, the validation of the growth rings formation enables the reading precision to be increased.

The reader's experience is an essential factor in order to be able to reduce the different possible bias. However, in the case of a particular species, experienced readers can also give incorrect results for a new species.

Having reached a certain level of expertise in estimating age, precautionary measures must be taken in order to keep this knowledge and to avoid the perfected method being deteriorated or changed with time. This is an essential phase in order to ensure an optimal quality for the age estimation procedures.

12.1.1. Two French readers per stock

It is necessary to have 2 readers per species (Tab. 4) because if one reader is absent, he may be replaced by the second. However, for this, it is necessary to carry out independent double readings for each of the species/stocks currently studied in order to estimate the precision existing between 2 readers or amongst several readers. Access to the pictures on a network disc facilitates the double readings.

Table 4 : List of stocks followed by France with the 1st and 2nd readers identified.

Stocks	First Reader	Second Reader
Mediterranean anchovy	Jean-Hervé Bourdeix	Erwan Duhamel
Anchovy VIII	Erwan Duhamel	Patrick Grellier
Larvae anchovy VIII	Patrick Grellier	
Seabass VIId-h, VIII	Jérôme Huet	Stéphane Martin
Anglerfish VII-VIII	Joël Dimeet	
Megrim VII, VIIIa,b	Océann Lizaud	Romain Elleboode
Mediterranean gilthead seabream	Romain Elleboode	Kélig Mahé
Haddock IVb,c	Jérôme Félix	Jean Louis Dufour
Haddock VII b-k	Jérôme Félix	Jean Louis Dufour
Roundnose grenadier	Romain Elleboode	
Herring IVc, VIId	Jean Louis Dufour	Jérôme Félix
Saithe IV	Jérôme Félix	Jean Louis Dufour
Saithe VIa	Jérôme Félix	Jean Louis Dufour
Whiting IVbc, VIId	Jean Louis Dufour	Robert Bellail
Whiting VIIe-k	Jean Louis Dufour	Robert Bellail
Hake IIA-VIIIab	Jean Louis Dufour	Robert Bellail
Mediterranean hake	Jean Louis Dufour	
Cod 3Ps	Jean Louis Dufour	
Cod IVbc, VIId	Jean Louis Dufour	Robert Bellail
Cod VIIe-k	Jean Louis Dufour	Robert Bellail
Plaice IVbc, VIId	Marie Line Manten	Romain Elleboode
Mediterranean striped red mullet	Romain Elleboode	Kélig Mahé
Mediterranean red mullet	Romain Elleboode	Kélig Mahé
Striped red mullet IVbc, VIId	Romain Elleboode	Kélig Mahé
Mediterranean pilchard	Jean-Hervé Bourdeix	Erwan Duhamel
Pilchard VIII	Erwan Duhamel	
Sole IVbc, VIId	Romain Elleboode	Anne Boiron
Sole VIIe	Romain Elleboode	Anne Boiron
Sole VIIIab	Anne Boiron	Romain Elleboode
Sprat IVbc, VIId	Jean Louis Dufour	Jérôme Félix
Norway pout IV	Jean Louis Dufour	

12.1.2. European Exchanges and Workshops

During these exchanges and workshops, the samples used were not validated therefore the « true age » is not known. In this way, the work groups demonstrate the precision of age estimation between readers but not the accuracy (Secor *et al*., 1995 ; Panfili *et al*., 2002 ; ICES, 2007) (Fig. 57).

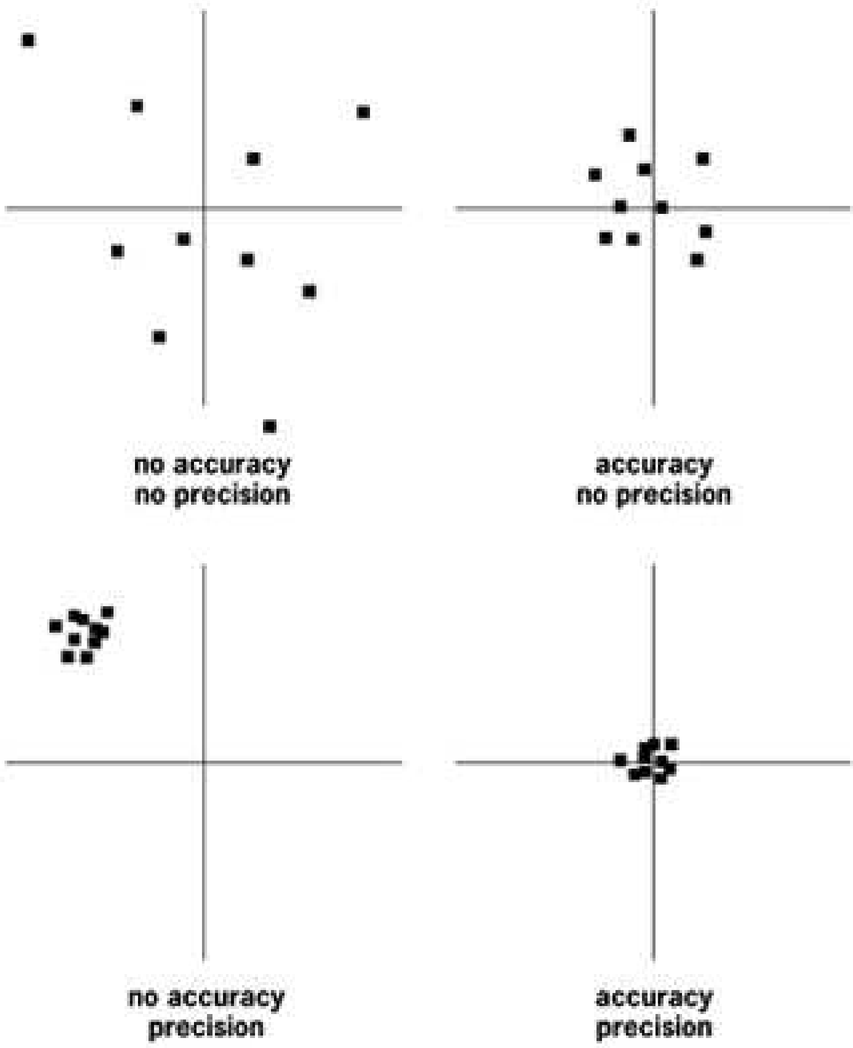


Figure 57 : Accuracy and precision in the sclerochronological studies. The age estimation results (black boxes) are marked in relation to the true age value (intersection of axes X and Y). The accuracy corresponds to the proximity to the real value whereas the precision corresponds to the proximity of repeated measures (*In* Panfili *et al*., 2002).

The summary of results of the exchanges which French readers took part in is shown in table 5.

Tableau 5 : List of exchanges and workshops which French readers took part in. For each exchange, the species, the year, the method of preparation of the calcified piece and the area concerned are indicated. For each reader, the percentage of agreement with other readers and the external bias are noted.

Species	Year	Mode of preparation	Area	Reader 1	Reader 2	Reader 3
Red mullet	2008	Whole otolith	The Mediterranean	Romain Elleboode Agreement : 38,3% bias: -0,67	Kélig Mahé Agreement : 55,0% bias: -0,4	Didier Le Roy Agreement : 45% bias: -0,62
Striped red mullet	2008	Whole otolith	Eastern Channel (VIId)	Romain Elleboode Agreement : 87,3% bias: -0,08	Kélig Mahé Agreement : 95,2% bias: 0,05	Didier Le Roy Agreement : 87,3% bias: 0,13
Striped red mullet	2008	Burnt whole otolith	Eastern Channel (VIId)	Romain Elleboode Agreement : 87,3% bias: -0,03	Kélig Mahé Agreement : 88,9% bias: 0,06	Didier Le Roy Agreement : 84,1% bias: 0,14
Whiting	2005	Otolith slice	Eastern Channel (VIId) North sea (IV)	Jean Louis Dufour Agreement : 88,9% bias: 0,04	Robert Bellail Agreement : 93% bias: 0,01	
Whiting	2005	Burnt otolith	Eastern Channel (VIId) North sea (IV)	Jean Louis Dufour Agreement : 79,4% bias: 0,03	Robert Bellail Agreement : 73,2% bias: -0,13	
Saithe	2008	Otolith slice	North sea (IV)	Jérome Félix Agreement : 92,9% bias: 0,06	Jean Louis Dufour Agreement : 98,7% bias: -0,01	Romain Elleboode Agreement : 94,8% bias: -0,02
Roundnose grenadier	2008	Otolith slice	Western Scotland (VI)	Romain Elleboode Agreement : 37,5% bias: -0,30	Jean Louis Dufour Agreement : 28,9% bias: 0,66	
Anglerfish	2004	Illicium slice	Channel (VII)	Joël Dimeet Agreement : 32,4% bias: 0,74		
Anchovy	2006	Whole otolith	Bay of Biscay (VIII)	Erwan Duhamel Agreement : 92,8% bias: 0,03	Patrick Grellier Agreement : 85,4% bias: 0,13	
Pilchard	2005	Whole otolith	Bay of Biscay (VIII)	Erwan Duhamel Agreement : 92,5% bias: -0,05		
Sprat	2004	Whole otolith	North sea (IV)	Yves Vérin Agreement : 71,3% bias: -0,24	Jean Louis Dufour Agreement : 79% bias: 0,02	
Cod	2008	Otolith slice	North sea (IV)	Jean Louis Dufour Agreement : 76,9% bias: 0,14	Robert Bellail Agreement : 90,7% bias: -0,07	
Plaice	2003	Otolith slice	Eastern Channel (VIId)	Marie Line Manten Agreement : 85,6% bias: 0,00	Jean Louis Dufour Agreement : 84,7% bias: -0,01	
Plaice	2003	Whole otolith	Eastern Channel (VIId)	Marie Line Manten Agreement : 81% bias: -0,18	Jean Louis Dufour Agreement : 82,5% bias: -0,16	
Sole	2008	Otolith slice	Eastern Channel (VIId)	Romain Elleboode Agreement : 91,6% bias: 0,04		
Sole	2007	Otolith slice	Bay of Biscay (VIII)	Anne Boiron Agreement : 84,6% bias: 0,19		

12.1.3. Validation of the growth ring formation

There are 2 methods :

- Qualitative method: This method consists in evaluating the presence or the absence of a given growth mark (according to the optical properties of the growth material formed) on the edge of a calcified piece and in expressing the results in the form of percentages for the population studied.
- Quantitative method: This method consists in measuring the distance separating the last growth marks on the edge of a calcified piece. The measuring axis chosen and the description of the marks used must be rigorously standardized.

Amongst the species routinely monitored, the monitoring of the growth mark formation has been carried out for striped red mullet, whiting and pilchards. However, the standardization of pictures with measures will enable a similar monitoring for all species treated.

12.1.3.1. Striped red mullet

The periodicity of the appearance of growth marks on the otoliths has been checked by calculating the growth between the last and the next to last stria or the marginal increment (M.I.). The periodicity of the appearance of rings on the red mullet otoliths has been checked using a sample of 93 fish, 84 of which are females. The variations in the marginal increment of the otolith according to the time in females and males are comparable (Fig. 58). In this way, from May until the end of December, there is an active period of increment during which the distance between the last winter ring and the edge of the otolith increases. Inversely, from January to May, the marginal increment is slowed down. These observations confirm the identification of the translucent rings as winter marks and their possible use as annual marks, in determining age.

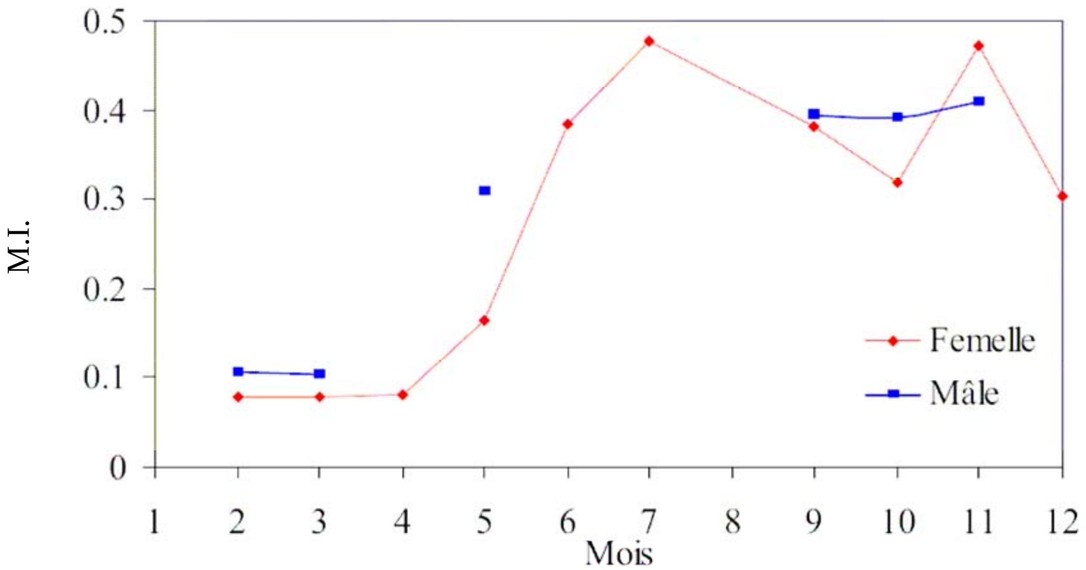


Figure 58 : Marginal increment (M.I.) of striped red mullet otoliths in the East Channel during the year 2004 (*In* Mahé *et al*., 2005).

12.1.3.2. Whiting

The analysis of the marginal growth according to the qualitative method on the dorsal edge of the Celtic sea whitings' otoliths (VII f,g,h) for an indirect validation of the age estimations started in January 1996 and remains practically continuous except in June 2004 when sampling of the commercial landings was not carried out. We notice that the growth model used (an opaque seasonal area and a translucent seasonal area are formed each year) is globally checked with a few inter-annual fluctuations (Fig. 59). The variations of 10% are only a reflection of the fluctuations linked to different tides sampled in VII f,g,h.

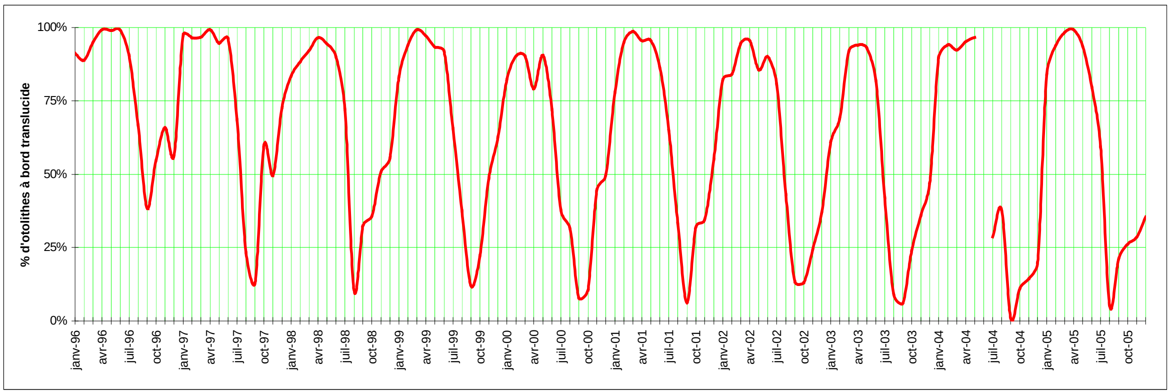


Figure 59 : Annual monitoring of the growth (% per month of the otoliths which have a translucent edge) of Celtic sea whiting otoliths from the monthly sampling from commercial landings. All ages mixed (from 1 to 11 years). 15608 otoliths examined.

The average annual cycle between 1996 and 2005 shows that from January to July, the dorsal edge of the otoliths is mainly translucent. The maximum number of opaque edges is observed in September (diagram 60). The experimental information shows that more than 80% of the otoliths display a translucent edge in January whereas there are only about 50% in December. This is the result of a combination of the contributions of each cohort at the beginning of the year and the end of the year. The oldest fish are more abundant in the 1st semester, during the reproduction period, and then relatively rare in the landings. Conversely, the fish of 1 year appear in the landings only in July at the earliest and those fish of 2 years are more abundant in the 2nd semester. A third factor which is very fluctuating is the variable proportion of the takings not landed and this factor is also influential but unknown. From one tide to another, according to the market opportunities, the tradable fish will or will not be kept and landed. The results from the analysis of otoliths only from the commercial landings are therefore a reflection of the changeable working practices.

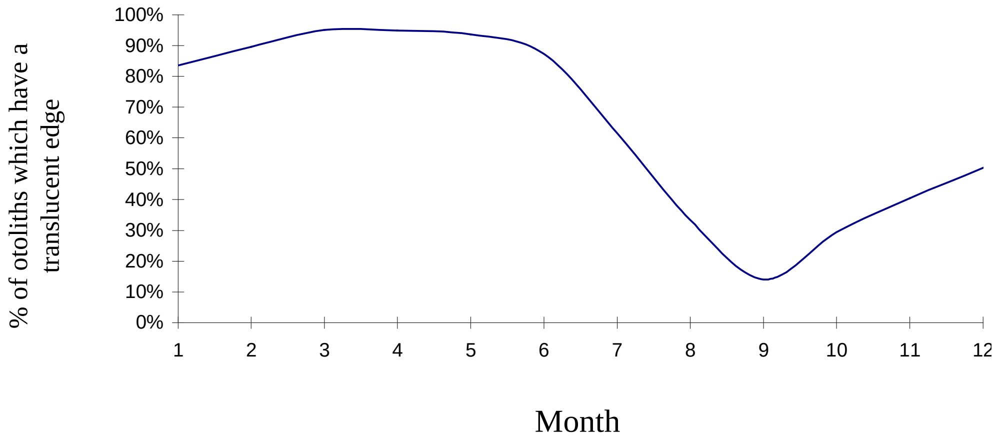


Figure 60 : Average annual growth cycle for Celtic sea otoliths per age group (data from 1996 to 2005). Percentage per month of the otoliths which have a translucent edge.

The analysis according to the age groups shows an increase in the proportion of otoliths with a translucent edge in May for fish of 2 years after an early renewal of annual opaque growth (Fig. 61). This phenomenon could be linked to the first sexual maturity of young whitings. The information concerning sexual maturity which was gathered during the EVHOE 2002 campaign showed that 13% of 1 year old whitings are mature, 97% of 2 year old whitings and 100% of 3 year olds or older (Bellail, 2003).

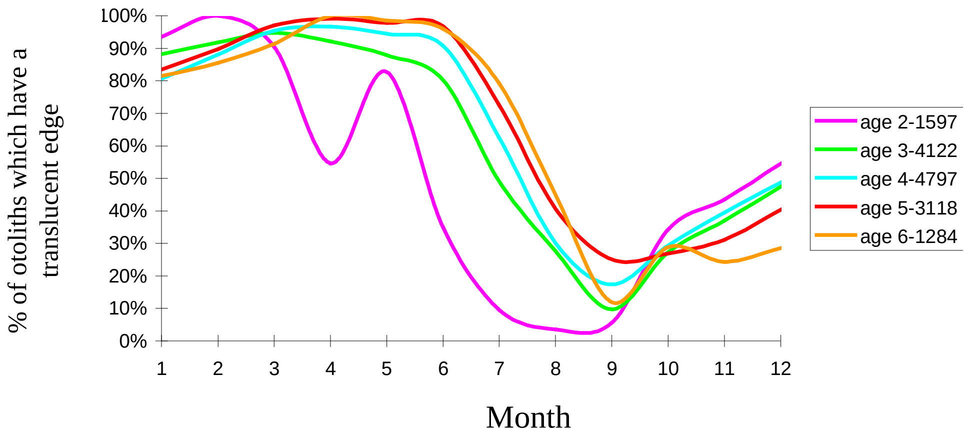


Figure 61 : Average annual growth cycle for Celtic sea whiting otoliths.

The analysis of the otoliths taken on a same abundant cohort, class 1999, monitored from 2000 to 2005 (Fig. 62) also shows that as from April the marginal growth of the otolith is opaque in about 35% of the observations at 2 years, followed by a non periodical translucent edge in May in about 85% of observations. For older fish, more than 50% of the otoliths display an opaque edge from July-August with quite a constant maximum in September.

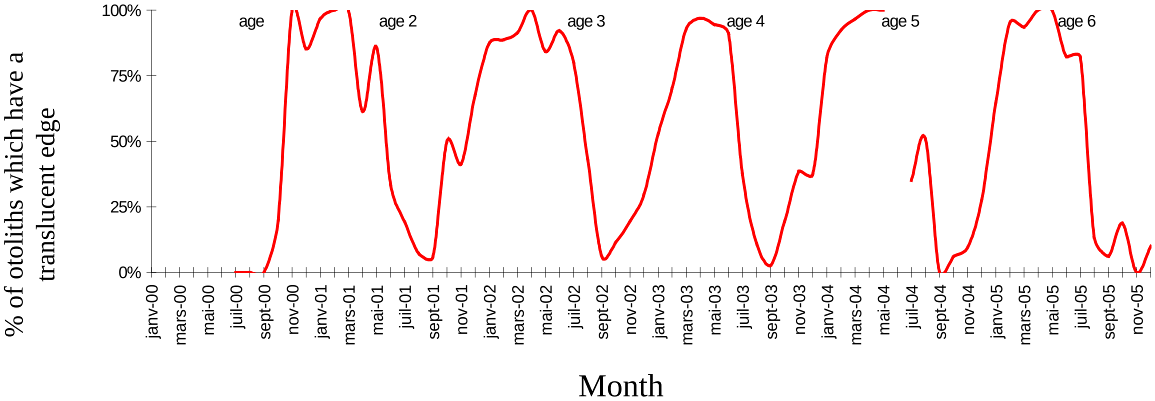


Figure 62 : Annual growth cycle for Celtic sea whiting otoliths from class 1999.

In practice, these observations show that in the 1st semester, the otoliths display mostly a translucent dorsal edge with the exception of certain otoliths of 2 years which display a thin translucent and supernumerary area formed in April-May and preceded by an opaque area which is considered incomplete. Furthermore, the youngest otoliths (1 and 2 years) have practically finished their annual growth cycle before the end of the calendar year.

12.1.3.3. Pilchard

The analysis of the marginal growth according to the qualitative method for the pilchard otoliths was carried out in May to December 2005 in the bay of Biscay (1341 individuals sampled). It is important to point out that the definition of an otolith edge as being opaque or translucent is not easy due to the fineness of the edge which results in a low reliability of the results.

The results for all ages mixed together give a growth model with a periodicity of 1 year and the formation of an opaque ring in summer (July to September) and a translucent ring in winter (Fig. 63).

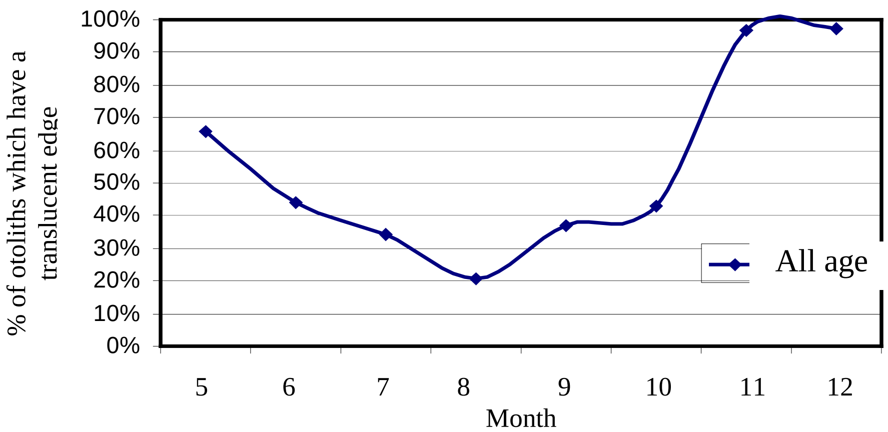


Figure 63 : Development in the nature of the edge of the pilchard otoliths for age groups of 1 to 5 in the Bay of Biscay in 2005.

It may observe that the growth takes place according to the age of the individuals : in the month of May, the young pilchards (1 year) have already resumed their growth (only 14% of translucent edges) whereas the otoliths of older individuals (5 years) all show a translucent edge which is proof of the fact that their growth has not yet resumed (Fig. 64).

This series of information on the nature of the edge must be completed in order to have a complete set of information on one year.

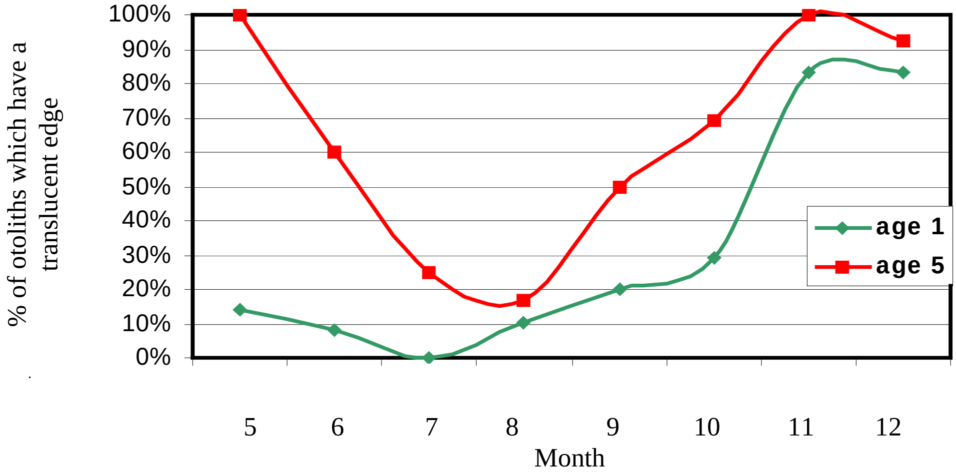


Figure 64 : Development of the nature of the pilchard otolith edge for age groups of 1 to 5 years in the Bay of Biscay in 2005.

13. Conclusion

This report is a summary of 42 reports drawn up on the techniques, the analyses and the authorizations of the methods used in order to estimate the age of fish by using calcified pieces and thereby supply information to the European work groups for stock evaluation. Annually, in France, nearly 50 000 calcium pieces are monitored by Ifremer staff.

The otolith is the calcified piece which is the most used. However, for certain species, the scales are usable and enable good results to be obtained. This is the case for seabream and seabass. Furthermore, currently, *illicium* is used to estimate the age of anglerfish but this is cause for debate within the European scientific community… For all the other species monitored, the otolith is used. For certain species of small pelagics (anchovies, herring, sprat and pilchard), the otolith may be interpreted directly. This is possible because these are species with a very short life expectancy so the otoliths show few growth marks and are not very thick. For the red mullets and the gurnards, the whole otoliths are burnt before being interpreted.

For most of the otoliths treated routinely, they are sliced. This technique was described by Bedford in 1983. Since, modifications and the material development described in this report have enabled numerous improvements to be made on the speed of the process as well as on the precision.

The picture analysis developments carried out at Ifremer with the TNPC software over the past few years enable us to take calibrated and interpreted pictures of all the calcified pieces routinely treated. This is a great development in the treatment and archiving of the calcified pieces.

The standardization of the methods, of the information management, of the pictures and the interpretations is an integral part of the Quality process in the treatment of this information. In the same way, the number of European exchanges and workshops, which is markedly increasing at the moment, will lead to a better inter-calibration of the European readings for a given species.

The routine digital treatment of the calcified pieces will facilitate the inter-calibration exercises between readers and will validate the formation of growth rings for all species but that will have to be completed in the future by studies which will enable the accuracy of the age estimations to be analysed.

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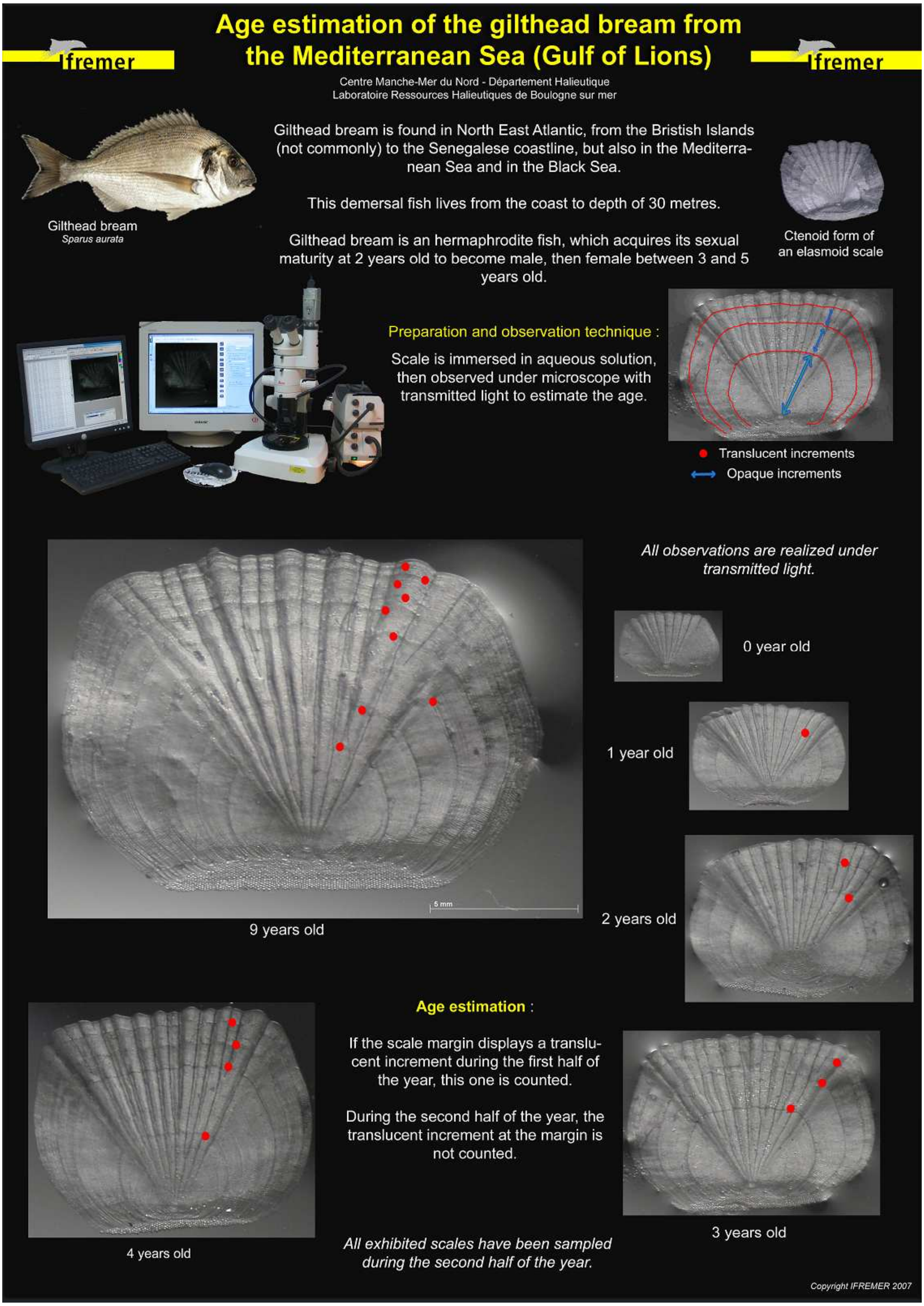
List of the illustrations

Appendix 1: Synthetic posters on the preparation and the interpretation of calcified pieces for several species

The species presented are as follows:

- Gilthead seabream
- Haddock
- Herring and sprat
- Saithe
- Whiting
- Cod
- Plaice
- Striped red mullet
- Sole


Age estimation of the gilthead bream from the Mediterranean Sea (Gulf of Lions)
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Gilthead bream
Sparus aurata
Gilthead bream is found in North East Atlantic, from the Bristish Islands (not commonly) to the Senegalese coastline, but also in the Mediterranean Sea and in the Black Sea.
This demersal fish lives from the coast to depth of 30 metres.
Gilthead bream is an hermaphrodite fish, which acquires its sexual maturity at 2 years old to become male, then female between 3 and 5 years old.
Ctenoid form of an elasmoid scale
Preparation and observation technique :
Scale is immersed in aqueous solution, then observed under microscope with transmitted light to estimate the age.
Translucent increments
Opaque increments
All observations are realized under transmitted light.
0 year old
1 year old
2 years old
5 mm
9 years old
Age estimation :
If the scale margin displays a translucent increment during the first half of the year, this one is counted.
During the second half of the year, the translucent increment at the margin is not counted.
3 years old
4 years old
All exhibited scales have been sampled during the second half of the year.
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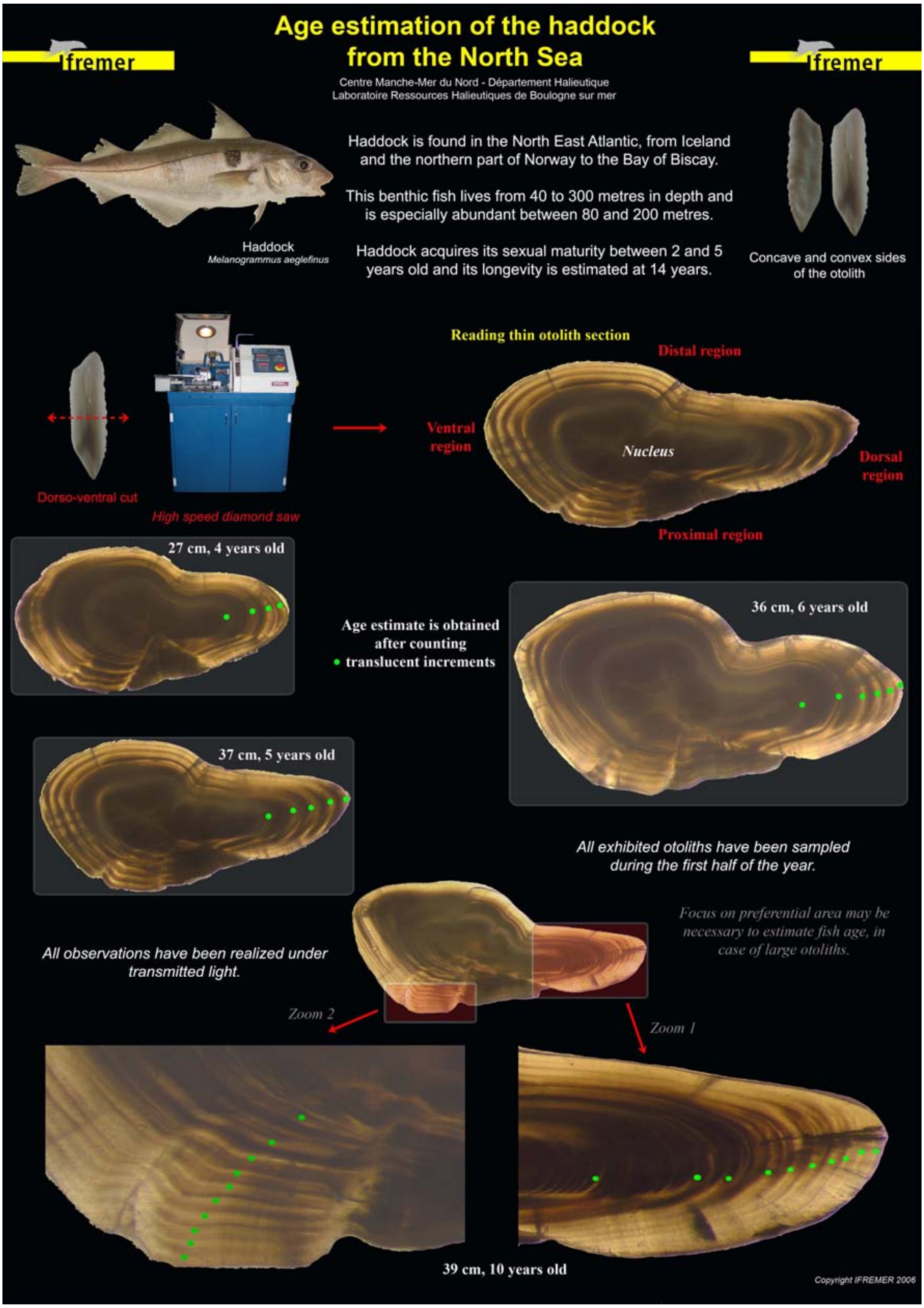
Age estimation of the haddock
from the North Sea
Ifremer
Ifremer
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Haddock is found in the North East Atlantic, from Iceland and the northern part of Norway to the Bay of Biscay.
This benthic fish lives from 40 to 300 metres in depth and is especially abundant between 80 and 200 metres.
Haddock acquires its sexual maturity between 2 and 5 years old and its longevity is estimated at 14 years.
Haddock
Melanogrammus aeglefinus
Concave and convex sides of the otolith
Dorso-ventral cut
High speed diamond saw
Reading thin otolith section
Distal region
Ventral region
Nucleus
Dorsal region
Proximal region
27 cm, 4 years old
Age estimate is obtained after counting
• translucent increments
36 cm, 6 years old
37 cm, 5 years old
All exhibited otoliths have been sampled during the first half of the year.
Focus on preferential area may be necessary to estimate fish age, in case of large otoliths.
All observations have been realized under transmitted light.
Zoom 2
Zoom 1
39 cm, 10 years old
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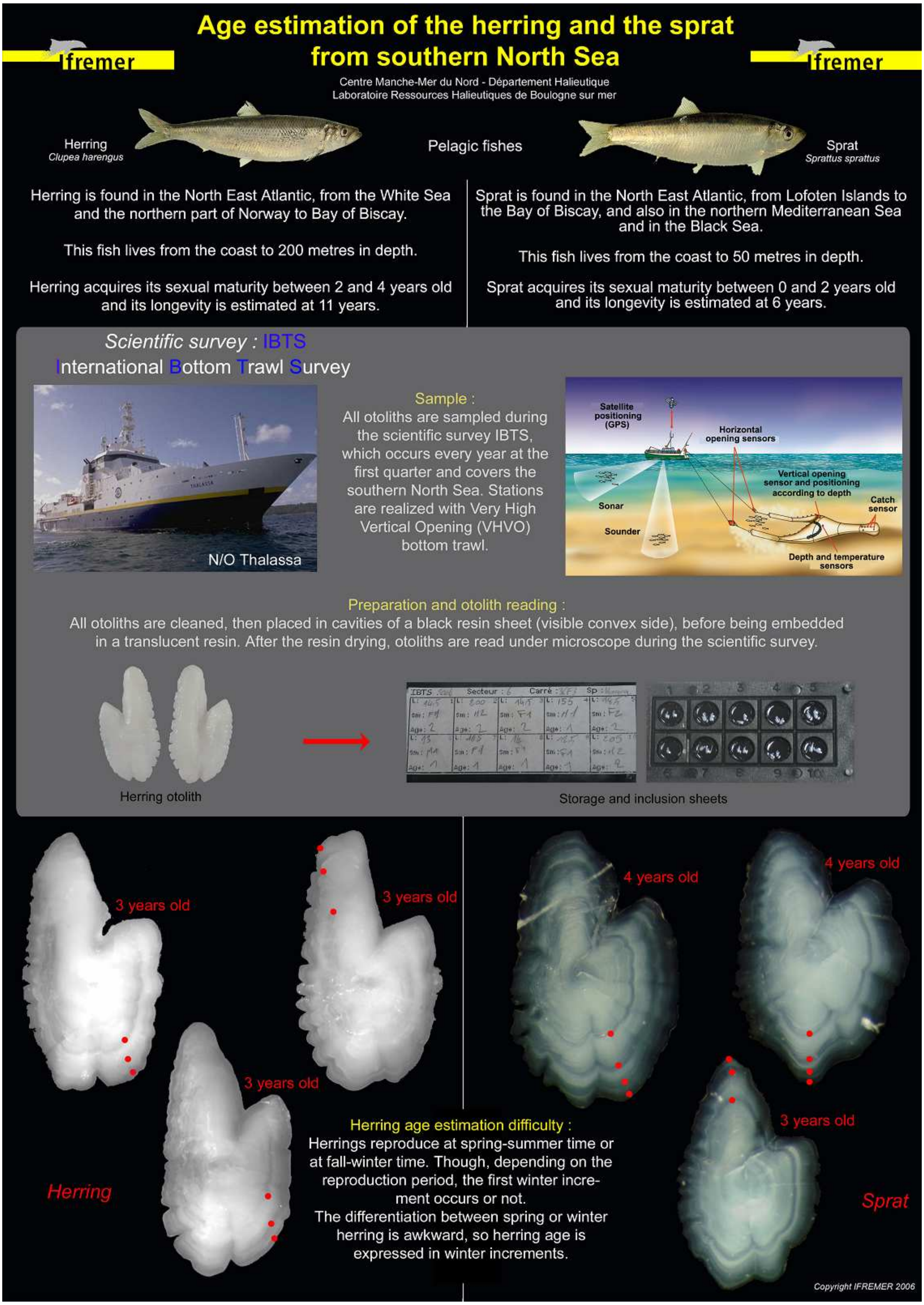
Age estimation of the herring and the sprat
from southern North Sea
Ifremer
Ifremer
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Herring
Clupea harengus
Pelagic fishes
Sprat
Sprattus sprattus
Herring is found in the North East Atlantic, from the White Sea and the northern part of Norway to Bay of Biscay.
This fish lives from the coast to 200 metres in depth.
Herring acquires its sexual maturity between 2 and 4 years old and its longevity is estimated at 11 years.
Sprat is found in the North East Atlantic, from Lofoten Islands to the Bay of Biscay, and also in the northern Mediterranean Sea and in the Black Sea.
This fish lives from the coast to 50 metres in depth.
Sprat acquires its sexual maturity between 0 and 2 years old and its longevity is estimated at 6 years.
Scientific survey : IBTS
International Bottom Trawl Survey
N/O Thalassa
Sample :
All otoliths are sampled during the scientific survey IBTS, which occurs every year at the first quarter and covers the southern North Sea. Stations are realized with Very High Vertical Opening (VHVO) bottom trawl.
Satellite positioning (GPS)
Horizontal opening sensors
Vertical opening sensor and positioning according to depth
Catch sensor
Sonar
Sounder
Depth and temperature sensors
Preparation and otolith reading :
All otoliths are cleaned, then placed in cavities of a black resin sheet (visible convex side), before being embedded in a translucent resin. After the resin drying, otoliths are read under microscope during the scientific survey.
Herring otolith
Storage and inclusion sheets
3 years old
3 years old
3 years old
Herring
4 years old
4 years old
3 years old
Sprat
Herring age estimation difficulty :
Herrings reproduce at spring-summer time or at fall-winter time. Though, depending on the reproduction period, the first winter increment occurs or not.
The differentiation between spring or winter herring is awkward, so herring age is expressed in winter increments.
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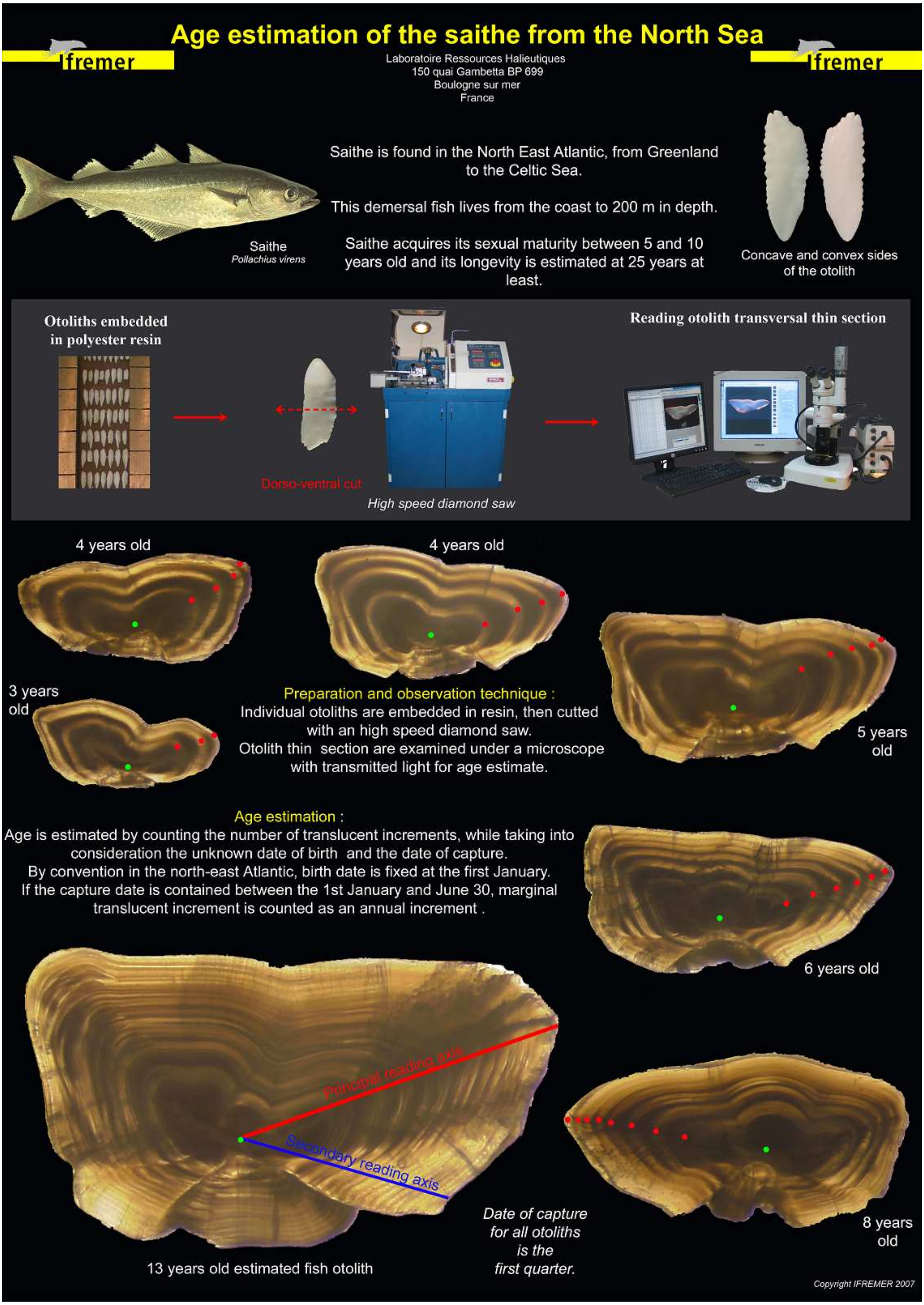
Age estimation of the saithe from the North Sea
Ifremer
Laboratoire Ressources Halieutiques
150 quai Gambetta BP 699
Boulogne sur mer
France
Ifremer
Saithe is found in the North East Atlantic, from Greenland to the Celtic Sea.
This demersal fish lives from the coast to 200 m in depth.
Saithe acquires its sexual maturity between 5 and 10 years old and its longevity is estimated at 25 years at least.
Saithe
Pollachius virens
Concave and convex sides of the otolith
Otoliths embedded in polyester resin
Dorso-ventral cut
High speed diamond saw
Reading otolith transversal thin section
4 years old
4 years old
3 years old
5 years old
Preparation and observation technique :
Individual otoliths are embedded in resin, then cutted with an high speed diamond saw.
Otolith thin section are examined under a microscope with transmitted light for age estimate.
Age estimation :
Age is estimated by counting the number of translucent increments, while taking into consideration the unknown date of birth and the date of capture.
By convention in the north-east Atlantic, birth date is fixed at the first January.
If the capture date is contained between the 1st January and June 30, marginal translucent increment is counted as an annual increment .
6 years old
Principal reading axis
Secondary reading axis
13 years old estimated fish otolith
Date of capture for all otoliths is the first quarter.
8 years old
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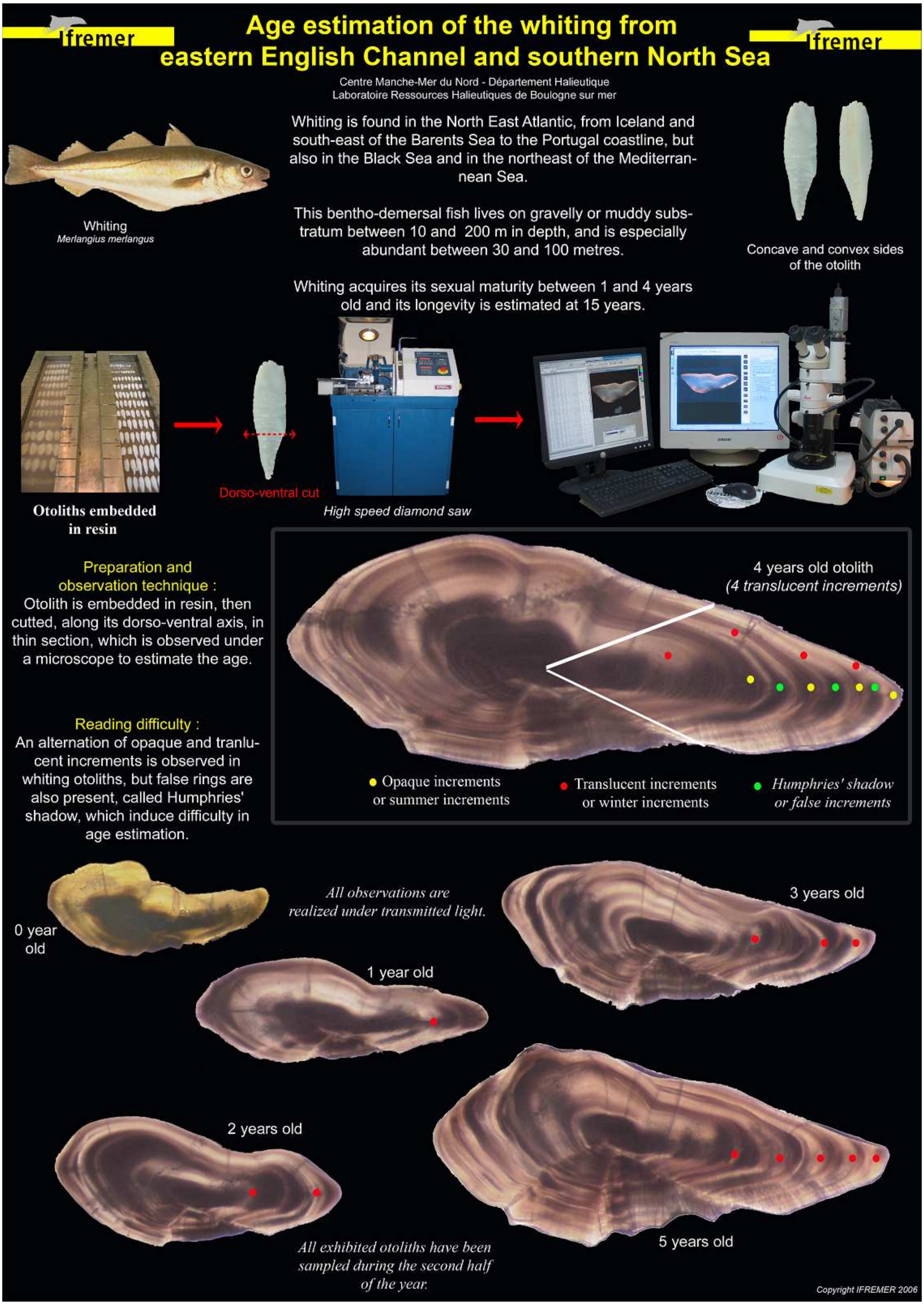
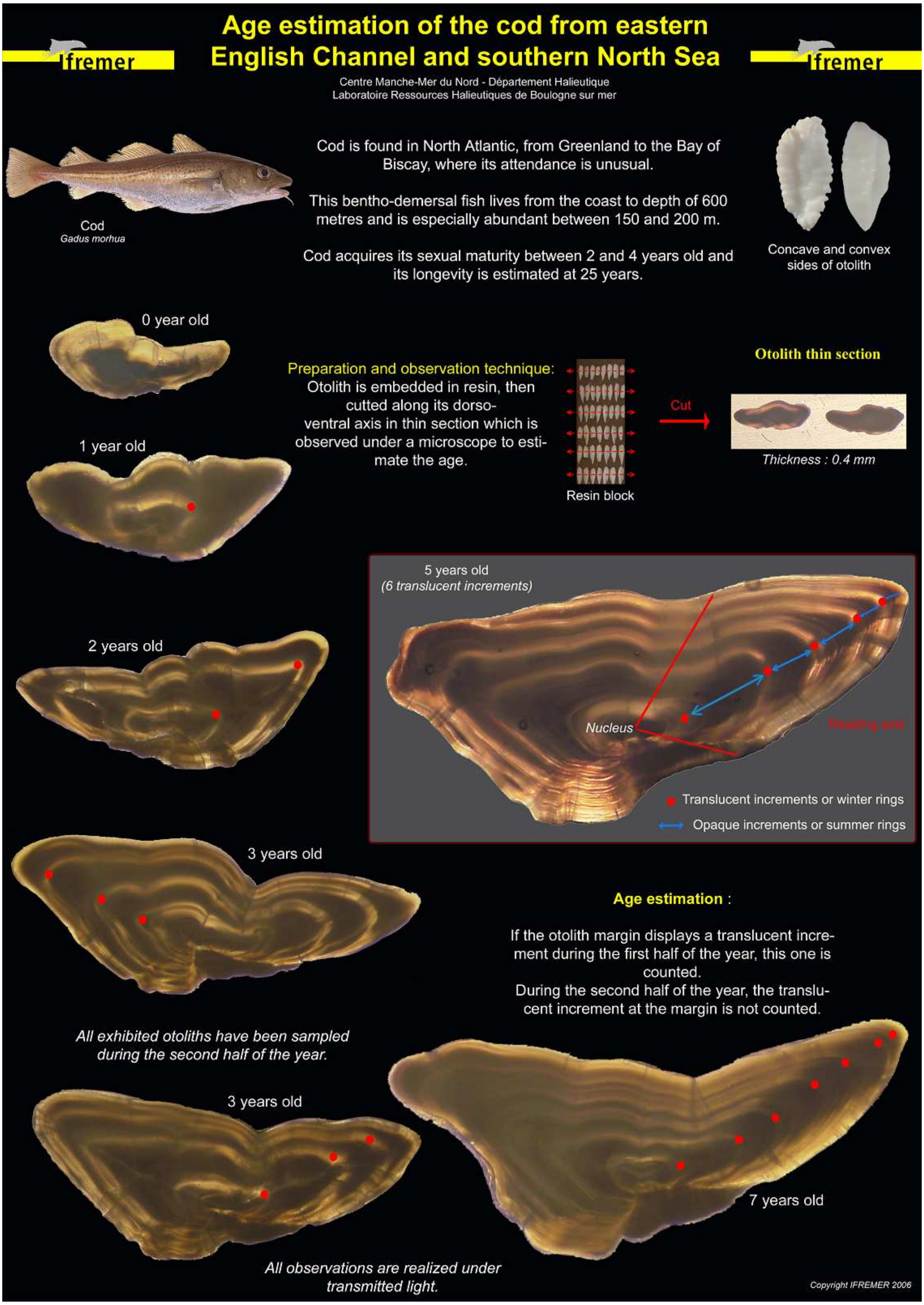
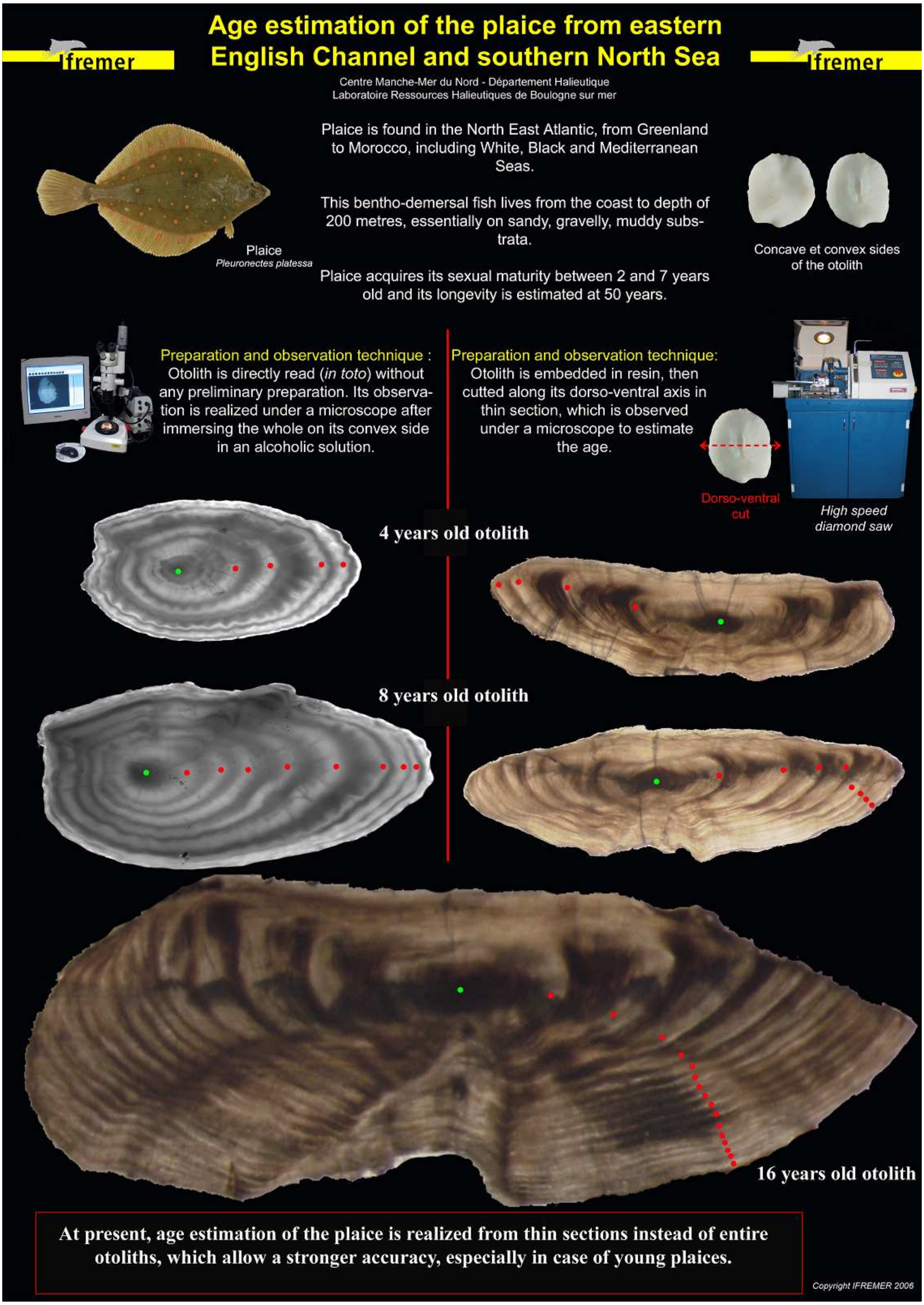

Ifremer
Age estimation of the whiting from eastern English Channel and southern North Sea
Ifremer
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Whiting
Merlangius merlangus
Whiting is found in the North East Atlantic, from Iceland and south-east of the Barents Sea to the Portugal coastline, but also in the Black Sea and in the northeast of the Mediterranean Sea.
This bentho-demersal fish lives on gravelly or muddy substratum between 10 and 200 m in depth, and is especially abundant between 30 and 100 metres.
Whiting acquires its sexual maturity between 1 and 4 years old and its longevity is estimated at 15 years.
Concave and convex sides of the otolith
Otoliths embedded in resin
Dorso-ventral cut
High speed diamond saw
Preparation and observation technique :
Otolith is embedded in resin, then cutted, along its dorso-ventral axis, in thin section, which is observed under a microscope to estimate the age.
Reading difficulty :
An alternation of opaque and tranlucent increments is observed in whiting otoliths, but false rings are also present, called Humphries' shadow, which induce difficulty in age estimation.
4 years old otolith
(4 translucent increments)
Opaque increments or summer increments
Translucent increments or winter increments
Humphries' shadow or false increments
0 year old
All observations are realized under transmitted light.
3 years old
1 year old
2 years old
5 years old
All exhibited otoliths have been sampled during the second half of the year.
Copyright IFREMER 2006


Ifremer
Age estimation of the cod from eastern English Channel and southern North Sea
Ifremer
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Cod
Gadus morhua
Cod is found in North Atlantic, from Greenland to the Bay of Biscay, where its attendance is unusual.
This bentho-demersal fish lives from the coast to depth of 600 metres and is especially abundant between 150 and 200 m.
Cod acquires its sexual maturity between 2 and 4 years old and its longevity is estimated at 25 years.
Concave and convex sides of otolith
0 year old
1 year old
Preparation and observation technique:
Otolith is embedded in resin, then cutted along its dorso-ventral axis in thin section which is observed under a microscope to estimate the age.
Resin block
Cut
Otolith thin section
Thickness : 0.4 mm
5 years old
(6 translucent increments)
Nucleus
Reading axis
Translucent increments or winter rings
Opaque increments or summer rings
2 years old
3 years old
Age estimation :
If the otolith margin displays a translucent increment during the first half of the year, this one is counted.
During the second half of the year, the translucent increment at the margin is not counted.
All exhibited otoliths have been sampled during the second half of the year.
3 years old
7 years old
All observations are realized under transmitted light.
Copyright IFREMER 2006


Ifremer
Age estimation of the plaice from eastern English Channel and southern North Sea
Ifremer
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Plaice
Pleuronectes platessa
Plaice is found in the North East Atlantic, from Greenland to Morocco, including White, Black and Mediterranean Seas.
This bentho-demersal fish lives from the coast to depth of 200 metres, essentially on sandy, gravelly, muddy subs-trata.
Plaice acquires its sexual maturity between 2 and 7 years old and its longevity is estimated at 50 years.
Concave et convex sides of the otolith
Preparation and observation technique : Otolith is directly read (in toto) without any preliminary preparation. Its observa-tion is realized under a microscope after immersing the whole on its convex side in an alcoholic solution.
Preparation and observation technique: Otolith is embedded in resin, then cutted along its dorso-ventral axis in thin section, which is observed under a microscope to estimate the age.
Dorso-ventral cut
High speed diamond saw
4 years old otolith
8 years old otolith
16 years old otolith
At present, age estimation of the plaice is realized from thin sections instead of entire otoliths, which allow a stronger accuracy, especially in case of young plaices.
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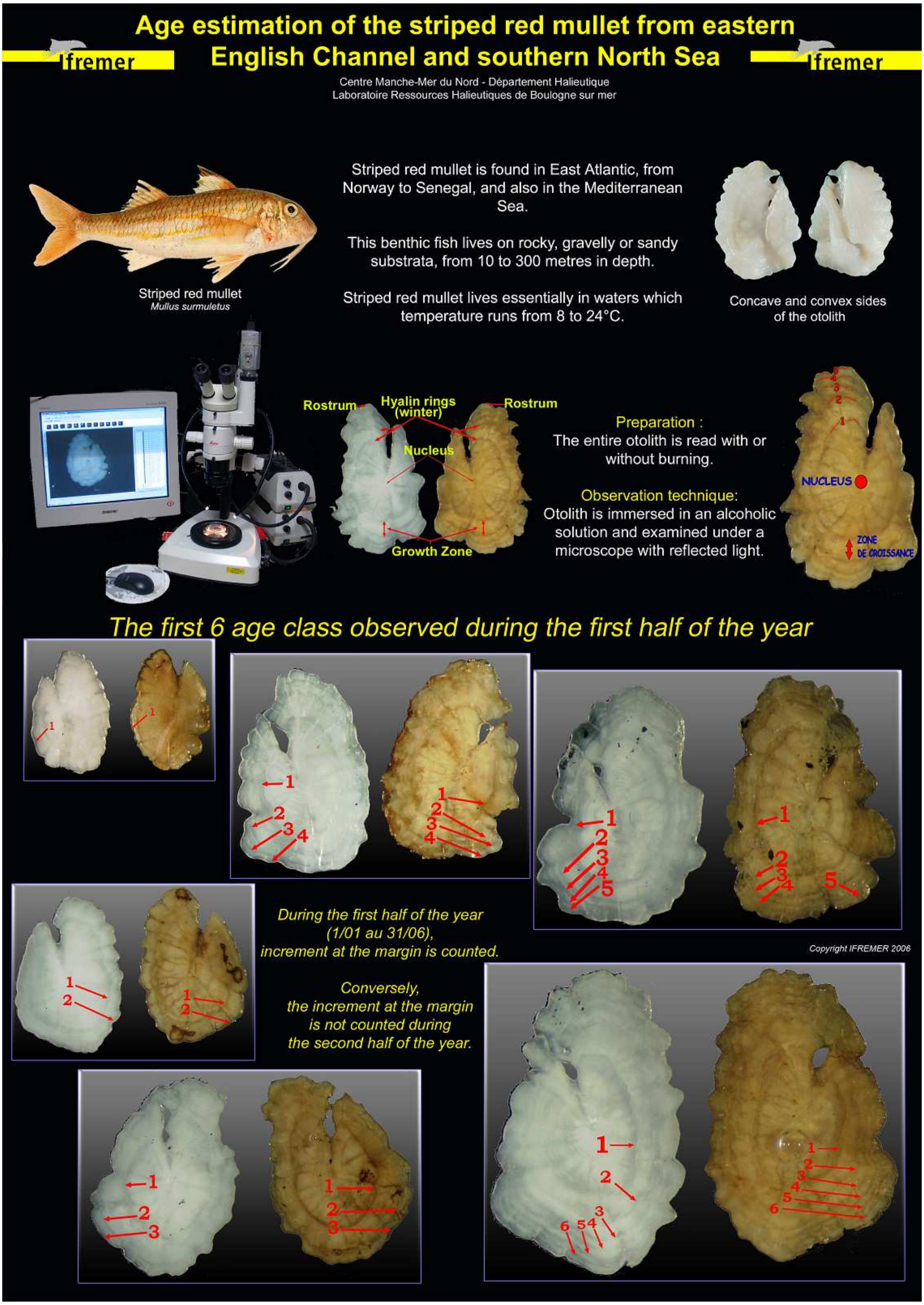
Ifremer
Age estimation of the striped red mullet from eastern English Channel and southern North Sea
Ifremer
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Striped red mullet is found in East Atlantic, from Norway to Senegal, and also in the Mediterranean Sea.
This benthic fish lives on rocky, gravelly or sandy substrata, from 10 to 300 metres in depth.
Striped red mullet lives essentially in waters which temperature runs from 8 to 24°C.
Striped red mullet
Mullus surmuletus
Concave and convex sides of the otolith
Rostrum
Hyalin rings (winter)
Rostrum
Nucleus
Growth Zone
Preparation :
The entire otolith is read with or without burning.
Observation technique:
Otolith is immersed in an alcoholic solution and examined under a microscope with reflected light.
NUCLEUS
ZONE DE CROISSANCE
The first 6 age class observed during the first half of the year
During the first half of the year (1/01 au 31/06), increment at the margin is counted.
Conversely, the increment at the margin is not counted during the second half of the year.
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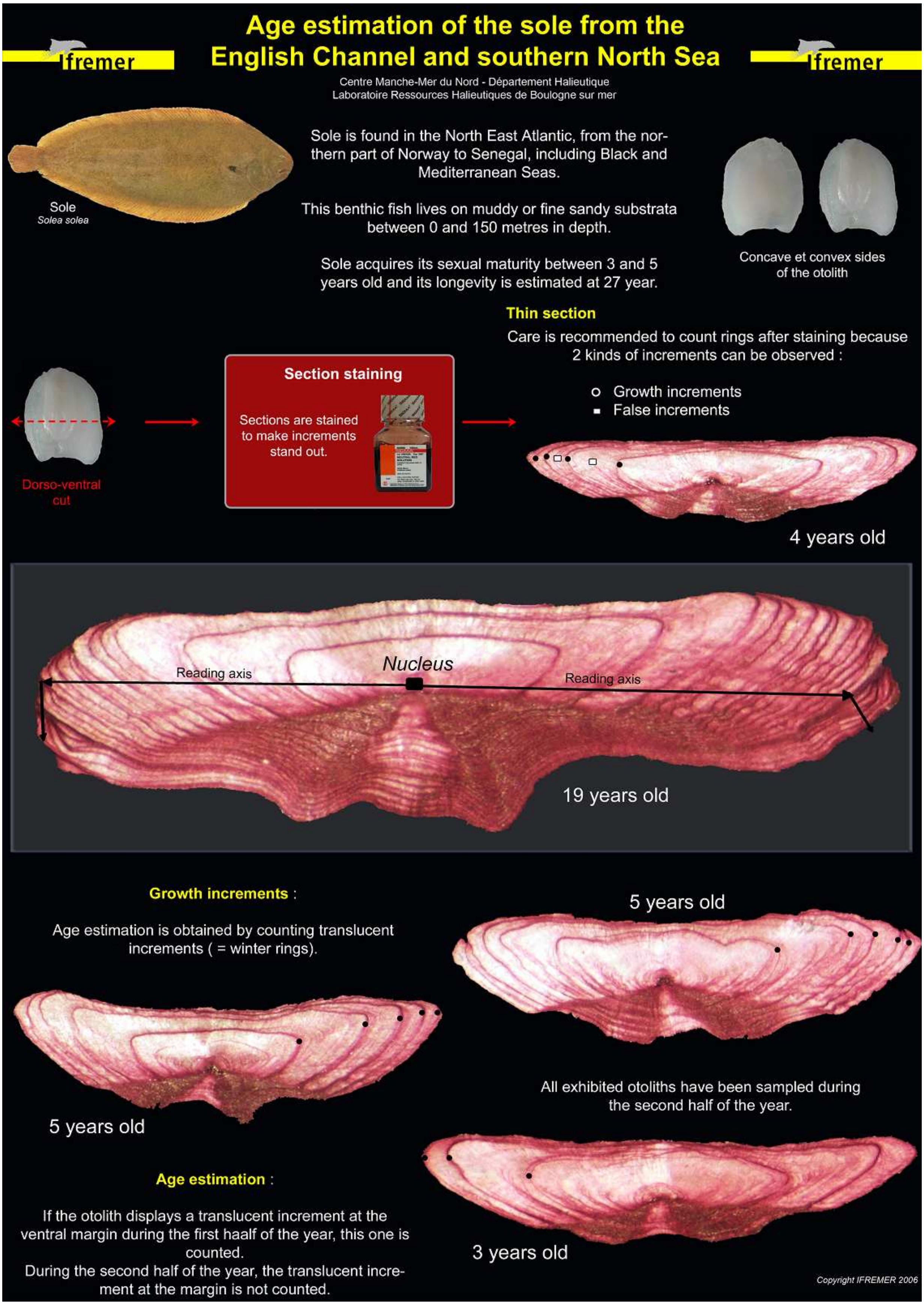
Age estimation of the sole from the English Channel and southern North Sea
Ifremer
Ifremer
Centre Manche-Mer du Nord - Département Halieutique
Laboratoire Ressources Halieutiques de Boulogne sur mer
Sole
Solea solea
Sole is found in the North East Atlantic, from the northern part of Norway to Senegal, including Black and Mediterranean Seas.
This benthic fish lives on muddy or fine sandy substrata between 0 and 150 metres in depth.
Sole acquires its sexual maturity between 3 and 5 years old and its longevity is estimated at 27 year.
Concave et convex sides of the otolith
Thin section
Care is recommended to count rings after staining because 2 kinds of increments can be observed :
Growth increments
False increments
Dorso-ventral cut
Section staining
Sections are stained to make increments stand out.
4 years old
Reading axis
Nucleus
Reading axis
19 years old
Growth increments :
Age estimation is obtained by counting translucent increments (= winter rings).
5 years old
5 years old
All exhibited otoliths have been sampled during the second half of the year.
Age estimation :
If the otolith displays a translucent increment at the ventral margin during the first haalf of the year, this one is counted.
During the second half of the year, the translucent increment at the margin is not counted.
3 years old
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