# RELATÓRIOS CIENTÍFICOS E TÉCNICOS

## SÉRIE DIGITAL

# AGE DETERMINATION IN MEAGRE *ARGYROSOMUS REGIUS*

Nuno Prista, José Lino Costa, Maria José Costa and Cynthia Jones

2009

49

Os **RELATÓRIOS CIENTÍFICOS E TÉCNICOS DO IPIMAR** destinam-se a uma divulgação rápida de resultados preliminares de carácter científico e técnico, resultantes de actividades de investigação e de desenvolvimento e inovação tecnológica. Esta publicação é aberta à comunidade científica e aos utentes do sector, podendo os trabalhos serem escritos em português, em francês ou em inglês.

A **SÉRIE COOPERAÇÃO** destina-se, primordialmente, à divulgação de trabalhos realizados com países terceiros no âmbito de programas de cooperação.

A **SÉRIE DIGITAL** destina-se a promover uma Consulta mais diversificada e expedita dos trabalhos na área da investigação das pescas e do mar.

**Edição**
IPIMAR
Avenida de Brasília
1449-006 LISBOA
Portugal

**Edição Digital**
Anabela Farinha / Irineu Batista / Luís Catalan

As instruções para os autores estão disponíveis no sítio web do IPIMAR http://ipimar-iniap.ipimar.pt/ ou podem ser solicitadas aos membros do Corpo Editorial desta publicação

**Capa**
Luís Catalan

**ISSN**
1645-863x

# AGE DETERMINATION IN MEAGRE *ARGYROSOMUS REGIUS*

**Nuno Prista[1,2], José Lino Costa[1], Maria José Costa[1,2] and Cynthia Jones[3]**

[1] Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; [2] Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; [3] Center for Quantitative Fisheries Ecology, Old Dominium University, 800W 46th St., Norfolk, VA 23508, USA

Recebido em 2009.10.16 Aceite em 2009.12.14

## ABSTRACT

The meagre is one of the world's largest sciaenids but its ecology, fishery, and population parameters are scarcely known. In the project "Meagre, *Argyrosomus regius* – biological data towards management and production of a finfish resource" (DGPA-MARE: 22-05-01-FDR-00036), the Centro de Oceanografia of FCUL (Portugal) and the Center for Quantitative Fisheries Ecology of ODU (VA, USA) investigated a set of methodologies to improve meagre age determination along its distribution range. In this study, we provide detailed protocols on the use of otolith thin sections and scale acetate imprints in determining meagre age. For each hard part, we present textual and photographic descriptions of the collection, preparation, and interpretation procedures, and report on the main difficulties met by age readers during age interpretations. We also provide details on the calculations involved in final age assignment to meagre specimens captured on the Portuguese coast. Finally, we discuss the relative importance of scales and otoliths, and their different preparation methods in routine meagre age determination and integrate the procedures into existing knowledge on age determination of other sciaenid species.

**Keywords**: age determination, growth, meagre**,** *Argyrosomus regius*, otoliths, scales.

## RESUMO

### Título: Determinação de idade em Corvina-legítima *Argyrosomus regius*

A corvina-legítima é um dos maiores Sciaenidae do mundo, mas a sua ecologia, pesca, e parâmetros populacionais são pouco conhecidos. No âmbito do projecto "Corvina-legítima *Argyrosomus regius* – dados biológicos para a gestão e produção aquícola de um recurso" (DGPA-MARE: 22-05-01-FDR-00036), o Centro de Oceanografia da FCUL (Portugal) desenvolveu, em colaboração com o Center for Quantitative Fisheries Ecology da ODU (VA, EUA), um conjunto de metodologias destinadas a melhorar a determinação da idade da corvina-legítima ao longo da sua área de distribuição. No presente trabalho apresentam-se os protocolos de determinação de idade a partir de secções finas de otólitos e de impressões de escamas em acetato desenvolvidos durante o projecto. Para cada uma destas estruturas apresentam-se descrições e registos fotográficos das fases de colheita, preparação e interpretação, e são apresentadas as principais dificuldades enfrentadas pelos técnicos durante as leituras de idade. De igual forma, são também apresentados os cálculos necessários à atribuição final de idades a espécimes de corvina-legítima capturados na costa Portuguesa. Finalmente, é discutida a importância relativa de otólitos e escamas, nas suas diferentes formas de preparação, para a determinação de idade de corvina-legítima e as metodologias desenvolvidas são integradas nos conhecimentos existentes sobre determinação de idade noutras espécies de Sciaenidae.

**Palavras Chave:** determinação de idade, crescimento, corvina-legítima, *Argyrosomus regius*, otólitos, escamas.

---

## REFERÊNCIA BIBLIOGRÁFICA

PRISTA, N.; COSTA, J.L.; COSTA, M.J.; JONES, C., 2009. Age determination in meagre *Argyrosomus regius*. ***Relat. Cient. Téc. Inst. Invest. Pescas Mar*****,** ***nº49***, 54 pp.

## TABLE OF CONTENTS

## 1. INTRODUCTION

The meagre (*Argyrosomus regius*, Asso 1801) is one of the world’s largest sciaenids, attaining over 180 cm in total length and 50 kg in weight (Quéméner, 2002; Costa *et al.* 2008). It is a coastal fish (<80 m deep) whose distribution extends from the English Channel to Senegal (including the Mediterranean Sea and Black Sea). Its largest fisheries take place in Mauritania, Morocco, and Egypt, which together comprise over 80% of the *ca.* 10 000 t world annual catch (Quéméner, 2002; FAO, 2009). In European countries, annual meagre landings are generally below 500 t and the fish is of secondary importance in national capture production totals (FAO, 2009). Even so, due to its large size, high ex-vessel prices, and high seasonal availability in inshore and nearshore waters, the meagre constitutes an important target species for many local small-scale multi-gear multi-species commercial fleets and the recreational sector (Quéro and Vayne, 1987; Quéméner, 2002; Silva *et al.*, 2002; Prista *et al.*, 2008). This importance is underscored by the recent development of meagre aquaculture production and by the ecologic value the species presents as a top marine predator in European coastal waters (Quéro and Vayne, 1987; Quéméner, 2002; Jiménez *et al.*, 2005). However, to date the biological characteristics of the meagre have remained scarcely studied worldwide and its fisheries are yet to be routinely monitored or assessed in African and European waters.

Determinations of fish age are an important step of fisheries research and stock assessment because age data is a primary input in the estimation of population vital rates like growth or mortality (e.g., Haddon, 2001). This is particularly so in long lived species where other methods, e.g., length-based approaches, are difficult to apply (Sparre and Venema, 1998). Until recently, the age of meagre had only been studied in North African waters where its long-lived nature was established (maximum age: 15 to 31 years) and its growth first modeled (Tixerant, 1974; Hermas, 1995). However, past research relied on methodologies that were neither detailed nor validated and that are currently considered outdated for sciaenid age determination (namely, break-and-burn of otoliths and analysis of fresh scales). In fact, it is now widely accepted that analysis of otolith thin sections is the most reliable method to determine sciaenid age (Lowerre-Barbieri *et al.*, 1994; Campana and Jones, 1998; VanderKooy and Guindon-Tisdel, 2003; Liao *et al.*, 2008) and that, if scales must be used, they should be imprinted prior to observation to facilitate their interpretation (Matlock *et al.*, 1993; Lowerre-Barbieri *et al.*, 1994; VanderKooy and Guindon-Tisdel, 2003). Furthermore, it is widely recognized that age determinations of any fish species should be based on standardized and validated protocols that assure the validity, replicability and comparability of results across studies and geographical areas (Campana, 2001; Morison *et al.*, 2005).

Recently, Costa *et al.* (2008) made a first evaluation of the main biological characteristics of the meagre captured on the Portuguese coast. Costa *et al*. report was published in Portuguese language and so was of limited availability to the international community; however, it provided the first comprehensive analysis of the meagre growth and age structure in European waters (e.g., new maximum age: 43 years) and involved the development and validation of age determination criteria for meagre otolith thin sections. Nevertheless, because of the need to focus on the estimation of the biological parameters of the species and discuss fisheries management and aquaculture production, the authors did not provide a full account of the age determination protocols they used nor did they detail specifics of meagre otolith interpretation; they also did not report on subsequent research carried out on the use of scale acetate imprints to determine meagre age, which may be useful to assess meagre fisheries in budget-limited situations (Prista *et al.*, 2007).

In this study we provide detailed protocols on the use of otolith thin sections and scale acetate imprints in meagre age determination. These protocols are the basis of the Costa *et al.* (2008) report and present the methodologies currently used to determine the age of meagre on the Portuguese coast. In the protocols, we provide in-depth detail on the specific procedures required to collect, prepare and interpret each meagre hard part. Additionally, we report on the most common difficulties met during meagre age interpretations and provide details and examples on final age assignment. This work is considered important because it updates and substantiates past literature on meagre age determination, promoting the training of hard part readers across several European and North African countries, and contributing to a standardization of age determination procedures across several fields of research, namely fisheries, ecology and aquaculture.

## 2. MATERIALS AND METHODS

The protocols are based on the observation of meagre otoliths ($n = 748$) and scales ($n = 362$) collected from the Portuguese coast from 2000 to 2007. The sample comprised fish from both sexes and included at least 10 otoliths and 10 scales from each month. The fish ranged between 5 cm and 182 cm total length, thus spanning the size range of the species. Otolith samples comprised at least 10 fish for each 10-cm size class between 0 cm and 180 cm, fish over 180 cm being less well represented ($n = 4$). Scale samples comprised at least 10 fish for each 10-cm size class between 20 cm and 180 cm, fish over 180 cm and fish below 20 cm being less well represented ($n = 3$ and $n = 6$, respectively). More detailed coverage of the sampling methodologies can be found in Prista *et al.* (2007) and Costa *et al.* (2008).

The terminology, methods and protocol structure were based on Pentilla and Dery (1988), Schwarzhans (1993), Ericksen (1999), Assis (2000), Panfili *et al.* (2002), VanderKooy and Guindon-Tisdel (2003) and Liao *et al.* (2008), with adaptations and additions as required by meagre specifics. Preparation of the hard parts for observation was carried out according to section 3.2 and section 4.2. Otolith thin sections were observed at 8–40x magnification on a Leica MZ-12 stereomicroscope equipped with hand-adjusted light orientation, pointer unit, and dark-field polarizing filter. Scale imprints were observed on a Bell and Howell R-735 microfiche reader equipped with 20 mm and 29 mm lenses (20x and 32x magnification, respectively). The primary criteria established for age interpretation (as well as any references made to the precision of the age determination methods) resulted from randomized observations of hard-part preparations. These observations were carried out with knowledge of month of capture but without knowledge of any collection detail. Additional interpretation criteria (sections 3.3.5.3 and 4.3.5.3) resulted from observations carried out with knowledge of fish size or after analyzing size-at-age plots. Finally, in agreement with previous work that established the interpretation of otolith thin sections as an accurate means of ageing long-lived sciaenids (Campana and Jones, 1998), a joint analysis of 77 otoliths and matching scales was carried out to check and refine the scale interpretation criteria.

Digital pictures of thin sections (resolution: 150 ppi) were taken at 6.3–25x magnification on a Leica MZ-6 stereomicroscope equipped with a Leica DFC 280 digital camera using Leica Image Manager 500. Digital pictures of scales imprints (resolution: 800 ppi) were taken at 9–50x magnification on a Minolta MS-7000 digital microfilm scanner using IrfanView. Image processing after capture was carried out in Paint.net and was restricted to left–right flipping, resizing and rotation, contrast and brightness adjustments, and minor background clean ups.

## 3. OTOLITH PROTOCOL

### 3.1. Collection

The otoliths used for meagre age determination are the *sagittae*. In meagre, the simplest method to collect these otoliths involves sawing off the top of the fish head (**Fig. 1**). This is accomplished by making two cuts on the fish head – one longitudinal and one transverse – that expose the top part of the brain cavity. The cuts may be done with a strong knife (small specimens) or an electric hand saw (large specimens). The longitudinal cut should run parallel to the frontal plane of the fish and pass slightly above the eyes; the transverse cut should run parallel to the transverse plane of the fish and pass near the insertion of the opercula (**Fig. 1A**). After this, the top of the head should come off easily and the fish brain should be exposed **(Fig. 1B–C).** The *sagittae* are located in the posterior ventrolateral regions of the brain cavity and can be removed with tweezers (**Fig. 1D**). The sawing off method is fast and easy to integrate into schemes involving routine sampling of biometric and reproductive variables. However, it severely damages the appearance of the fish, thus reducing its commercial value.

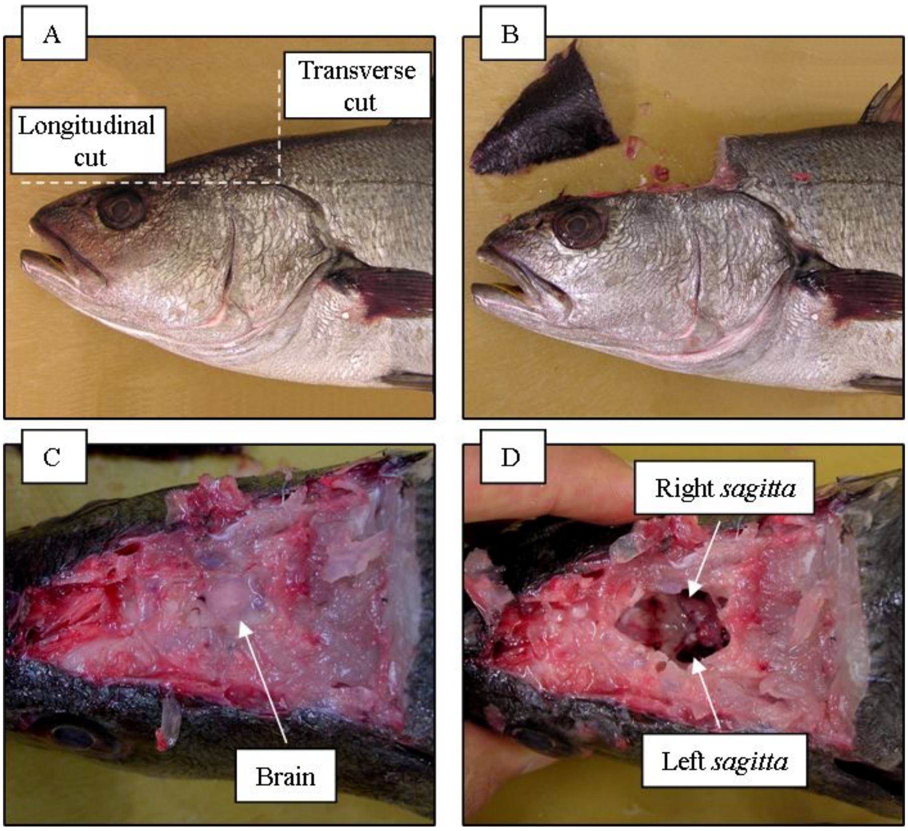

**Figure 1 –** Otolith extraction by sawing off the top of the fish head. See explanation in text.

When it is necessary to avoid loss of commercial value, the *sagittae* are better removed using less damaging techniques (**Fig. 2** and **Fig. 3**). In meagre, the otic capsules are located at the base of the skull, underneath the pharyngeal teeth and near the dorsal insertions of the first gill arches. In smaller meagre, the best way to reach the capsules is through the gill cavity by pulling the operculum open (**Fig. 2A**) and making a small anteroposterior incision at the dorsal insertion of the upper limb of the first branchial arch (**Fig. 2B**). The incision should be just enough to loosen the arch without detaching it, leaving the capsule's surface exposed (**Fig. 2C**). Then, a small lid can be carved out of the capsule using a scalpel or a sharp knife (**Fig. 2D, 2E)** and the otoliths extracted. After the extraction, the bone lid, the gill arches, and the operculum can be put back in their original positions, leaving the external appearance of the fish intact for marketing purposes (**Fig. 2F**).

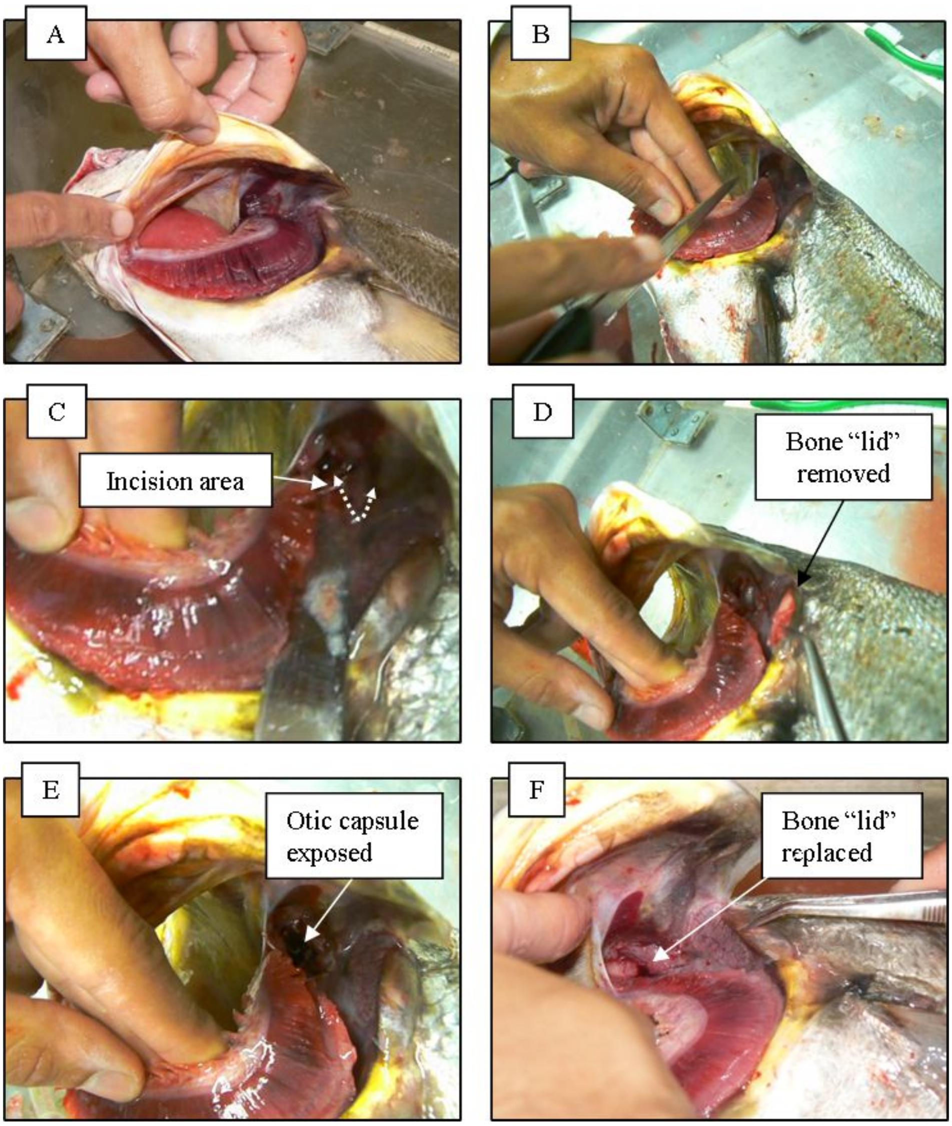

**Figure 2 –** Otolith extraction through the gills (small fish). See explanation in text.

In larger meagre, the opercula and the gill arches are stiffer, so reaching the otic capsules without damaging the appearance of the fish becomes increasingly difficult. In such cases the otic capsules are best reached through the top of the pharynx **(Fig. 3)** than through the top of the first gill arch (**Fig. 2**). This is achieved by making a dorsoventral incision just posterior to the fifth gill arch (**Fig. 3A**). The incision should extend from the dorsal to the ventral insertions of the gill arches, loosening them without detaching them. After that, the gill arches can be lifted against the operculum and a second cut is made around the upper pharyngeal tooth plates' to expose the otic capsules (**Fig. 3B**). An elliptical bone "lid" may then be carved out of the capsule's surface using a strong knife (e.g., an oyster knife) (**Fig. 3C–D**) and the otoliths pulled out inside their sacs (**Fig. 3E**). After the extraction, the bone lid, gill arches, and the operculum can be put back into position to preserve fish market value (**Fig. 3F**).

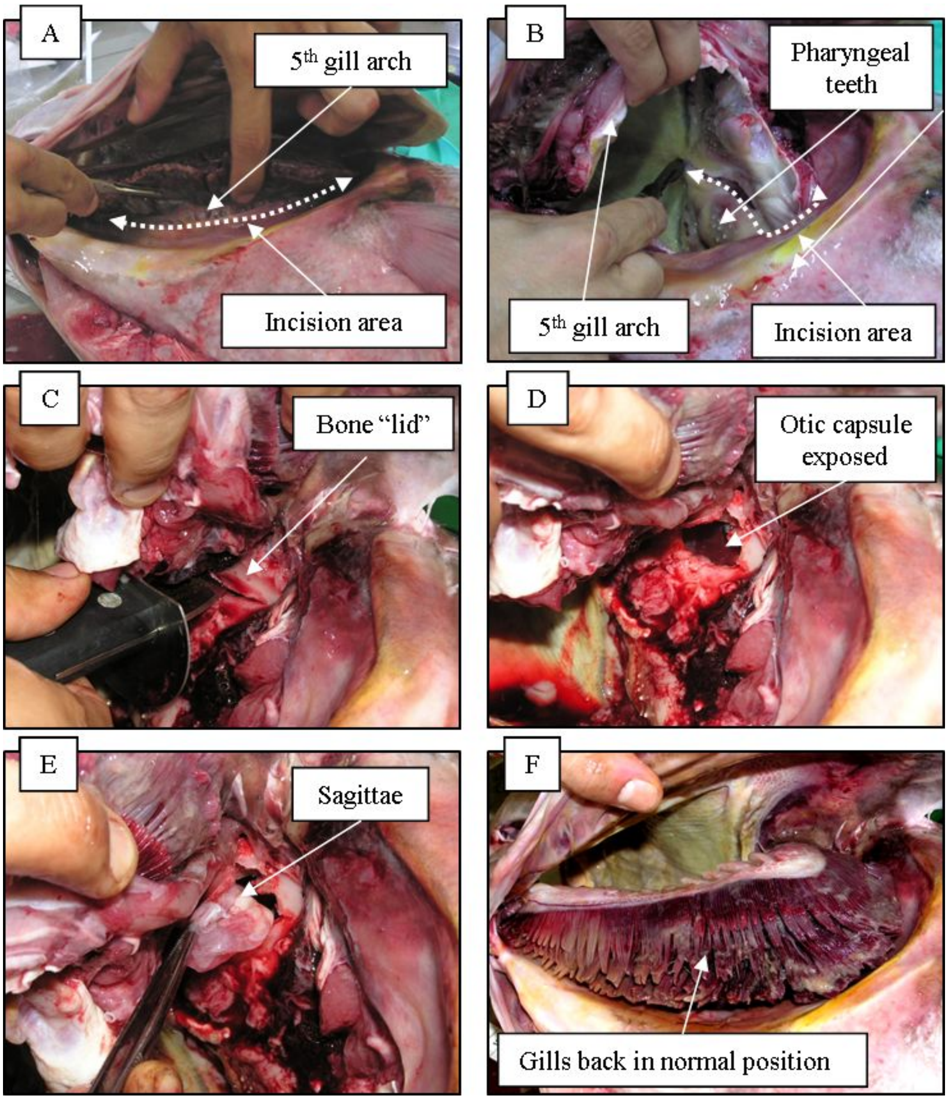

**Figure 3 –** Otolith extraction through the gills (large fish). See explanation in text.

The meagre *sagittae* are large and robust, weighing up to 14 g each. Consequently, they can be freely handled without much risk of breaking. Before storage, any remains of adherent tissue should be removed from the otolith surfaces by scrubbing them with a soft toothbrush under running tap water. The clean otoliths can then be left to dry at room temperature for a few hours and stored in plastic vials.

### 3.2. Preparation

The meagre otoliths are too thick for direct use in age determination. Consequently, thin sections have to be obtained before they can be used to determine fish age. In meagre, otolith thin sections are taken along a specific plane of the otolith body so some familiarization with otolith's external morphology is required to carry out the sectioning procedures.

Meagre *sagittae* present distinct morphological features on their proximal (or inner) and distal (or outer) sides (**Fig. 4)**. The most conspicuous features are a tadpole shaped *sulcus acusticus* on the proximal side (further divided into an anterior *ostium* and a posterior *cauda*) and a conspicuous protuberance termed "umbo" on the distal side[1]. When observed in proximal view, left and right *sagittae* are easy to distinguish: left *sagittae* present the tip of the *cauda* to the right of the observer, and right *sagittae* present it to his left (**Fig. 4)**.

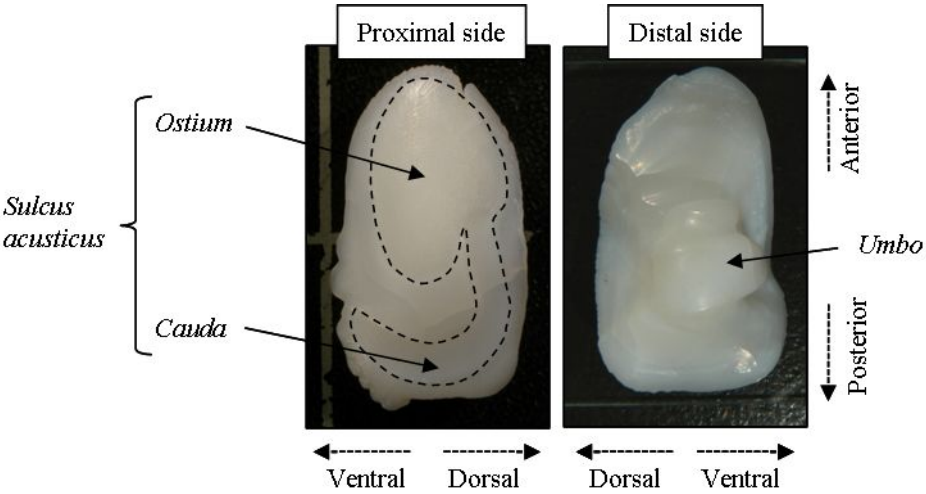

**Figure 4 –** External morphology of a right meagre *sagitta*. The dashed line circumscribes the *sulcus acusticus*.

---

[1] Note: younger meagre present several protuberances instead of a single umbo. These protuberances represent the internal primordia that in older fish appear fused into a single umbo (see Figure 6).

To obtain thin sections, meagre otoliths are mounted, partially embedded in a clear adhesive (e.g., Aremco Crystalbond 509), onto standard microscope slides. For age determination, it makes no difference which otolith (left or right) is mounted but abnormally crystallized otoliths should be avoided (**section 3.3.5.3)**. Otolith embedding in Crystalbond adhesive requires previous softening of the originally solid adhesive sticks over a hot plate. In doing this, care should be taken to keep the adhesive temperature just above its softening point (71ºC) because higher temperatures may crack the otolith surface. Then, a bed of soft Crystalbond is laid on the glass slide and the otolith is placed, distal side downwards, embedded into the adhesive. While doing this, it is important to make sure the otolith is in tilted position, i.e., both its anterior tip and its *umbo* should be in contact with the slide **(Fig. 5A)**, because this improves section quality (see **section 3.3.5.1)**. It is also important to make sure that the adhesive bed completely encompasses the distal side of the otolith **(Fig. 5A)** because this will confer robustness to the mount and reduce otolith breaking during sectioning. Crystalbond adhesive takes a few seconds to harden and can be reheated if it is necessary to readjust otolith position. After embedding, a dorsoventral pencil mark is drawn on the otolith's outer face. This marking should be located at one-third the distance between the posterior margin of the *ostium* and the anterior margin of the *cauda* and indicates the sectioning plane **(Fig. 5B)**.

Meagre otoliths should be sectioned on a low speed saw (e.g., a Buehler IsoMet Low Speed Saw) equipped with a fine-grit diamond-impregnated grinding wheel (e.g., a Norton

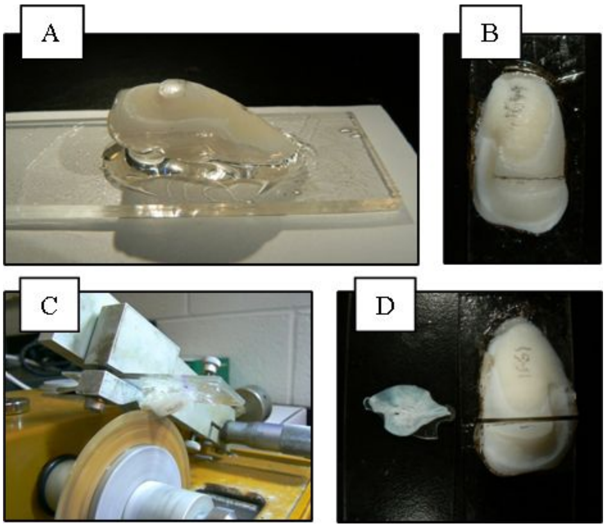

**Figure 5 –** Aspects of otolith preparation. A – embedded otolith; B – marked otolith; C – low speed sectioning; D – overview of sectioned otolith and otolith thin section.

3-in diameter 0.006-in thick 1A1 Diamond Grinding Wheel). Given the large size of many otoliths a “one-blade” saw setup is preferable to a “two-blade + spacer” saw setup. However, the latter may still be used to provide faster sections of smaller otoliths. Under a “one-blade” setup, the otolith slide is positioned so that the grinding wheel runs immediately posterior and parallel to the pencil mark. The saw is then turned on and the otolith is slowly rested on the wheel for sectioning **(Fig. 5C)**. After a few turns, arm weights (up to 75 g) can be added to speed up the sectioning. The first cut should stop when the wheel hits the adhesive bed. At that time the arm is adjusted approximately 0.5 mm in anterior direction and the second cut is performed. When the second cut finishes the thin section is ready and can be removed from the adhesive after slight reheating of the glass slide **(Fig. 5D)**. Overall, the preparation of meagre thin sections may take between 5 and 90 minutes depending on the otolith size and the saw speed and arm loads being used.

Thin sections of meagre otoliths are relatively robust and can be freely handled with tweezers without risk of breaking. Before final mounting, the sections should be cleansed in tap water and any remnants of Crystalbond adhesive should be removed. In general, no further preparation (e.g., polishing, baking or staining) is required. However, at this stage it is important to check the quality of the sections, making sure it is not necessary to perform additional cuts (see **section 3.3.5.1.**). Final section mounting is carried out on clean microscope slides using, e.g., Lerner Laboratories Flo-Texx mounting medium. Flo-Texx requires no cover slip and improves section’s visual appearance while preserving it for long-term use. When Flo-Texx is dry (≈12 hours), the glass slides can be labeled with a diamond scribing pen and stored into their final slide boxes.

## 3.3. Reading

Fish age determination from otolith thin sections requires the interpretation (or reading) of specific patterns occurring on section’s surface. This interpretation requires specific equipment and knowledge of section morphology (**section 3.3.1)** and involves three main steps: annuli interpretation and count (**section 3.3.2**), evaluation of the marginal increment (**section 3.3.3**) and data logging **(section 3.3.4)**. Similar to other fish species, knowledge and training on specific difficulties of the meagre thin sections will improve the quality of final readings (**section 3.3.5**) and ultimately lead to better age determinations.

### 3.3.1. Equipment and terminology

Meagre otolith thin sections should be read on a stereomicroscope under transmitted light. Under such circumstances, opaque structures will appear dark while translucent structures will appear bright. In general, meagre sections are read under low magnification (8–10x), but higher magnifications (20–40x) may be required to evaluate some specific features. As illumination greatly influences the final perception readers get from a thin section, a microscope base that allows manual control of the intensity and orientation of the light source is to be preferred (see **section 3.3.5.2)**. Additionally, whenever possible, the stereomicroscope should also be equipped with a pointer unit (that eases the interpretation of older sections) and a dark-field polarizing filter (which enhances the contrast and improves overall image appearance).

The meagre otolith sections present several internal morphological features which knowledge is required during the readings. Each section can be broadly divided into two main regions: a distal region (that presents several outgrowths) and a proximal region (that presents a conspicuous opaque / translucent banding). These two regions are separated at a proximodistal interface that runs across the section in dorsoventral direction **(Fig. 6).**

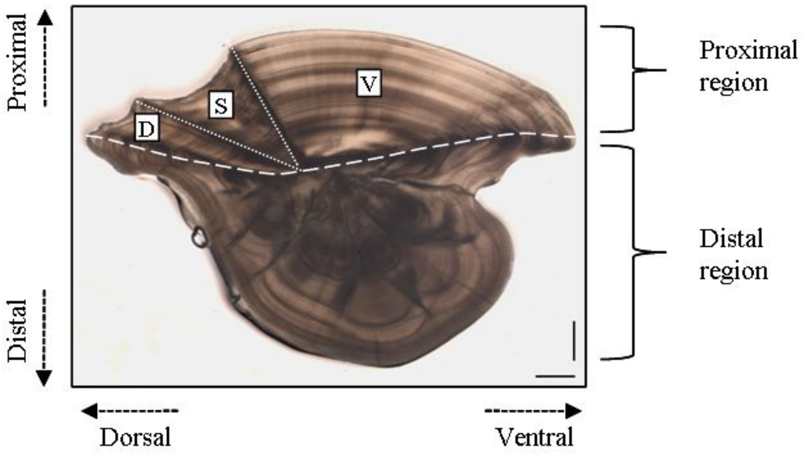

**Figure 6 –** Regions of the meagre otolith thin section. D – dorsal subregion; S – sulcal subregion; V – ventral subregion. The proximodistal interface is indicated by a white dashed line. Scale bars=1 mm, 8x.

The distal region mainly evidences the internal structure of the umbo (**Fig. 4**). Its main feature is a set of accessory primordia that appear as dark outgrowths extending away from the proximodistal interface in distal direction **(Fig. 7)**. In younger fish (less than 3 years old), the primordia are well separated so the distal edge of the section appears bumpy. However, at older ages the primordia appear fused and encompassed by a continuous overgrowth that makes the distal edge appear smooth **(Fig. 4, Fig. 7)**. Overall, the usefulness of the distal region of the section for age determination is low compared to the proximal region. However, at lower magnifications, opaque bands can be observed that span continuously across the primordia and that are related to the banded pattern observed in the proximal region. The most central of these distal bands are sometimes useful to corroborate age interpretations made in the proximal region of the section.

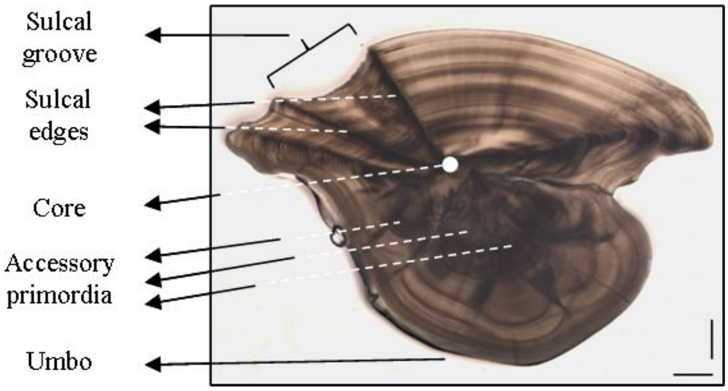

**Figure 7 –** Internal morphology of the meagre otolith thin section. Scale bars=1 mm, 8x.

The proximal region of the meagre otolith presents three main morphological features: the sulcal groove, the sulcal edges, and the core **(Fig. 7)**. The sulcal groove is located in slightly dorsal position along the proximal edge of the section, and shows the concave profile of the otolith *cauda* **(Fig. 4)**. The sulcal edges are two intersecting dark lines that prolong the sulcal groove internally into the proximodistal interface. The core is defined by the intersection of the proximodistal interface and the sulcal edges, and constitutes the region around which the otolith grew. Overall, sulcal groove, sulcal edges, and core constitute the base, legs, and top vertex of an upside-down isosceles triangle that divides the proximal region into three subregions: dorsal, sulcal, and ventral **(Fig. 6)**. Contrary to the distal part of the section, very conspicuous opaque / translucent bands can be observed throughout the entire proximal region of the section. It is the interpretation of these bands that constitutes the heart of the meagre age determination process.

### 3.3.2. Annuli interpretation and count

Determination of fish age from otolith thin sections relies on the interpretation and count of opaque bands that are assumed to form annually at a specific season. These annual opaque bands are termed “annuli” (singular: annulus). The annuli of meagre otolith sections are relatively easy to identify under transmitted light: they are visible in the proximal region, even at low magnification, as continuous concentric opaque (dark) bands that are separated by more translucent (bright) bands. In meagre, annuli exhibit a markedly conspicuous and parallel structure showing up convex in the ventral subregion, concave in the sulcal subregion, and concave to straight in the dorsal subregion. Frequently, central annuli (up to the fifth or sixth from the core) can also be traced across the primordia of the distal region but this becomes increasingly difficult in the peripheral annuli of older specimens.

Adequate interpretation of annuli for purposes of age determination requires the distinction between opaque bands that form annually at a specific season (or “true annuli”) and other opaque bands that may not be laid at annual frequency or that simply should be ignored during age determination (broadly termed “false annuli”). In general, the true annuli of meagre sections are strongly opaque and well-separated by translucent bands throughout the entire section which makes them relatively easy to discriminate. Conversely, false annuli appear as thin inconspicuous opaque bands that either cannot be discriminated throughout the whole proximal area or are suspiciously close to nearby true annuli. In meagre otolith thin sections, false annuli are rare. Consequently precise readings can generally be obtained by any reader that has previously trained with the sections and that is aware of some specifics of their interpretation (see **section 3.3.5**).

In meagre, true annuli (hereafter termed annuli for sake of simplicity) are counted in outward direction from the core to the proximal margin along four predefined axes: the sulcal groove axis (located in the middle of the sulcal subregion), the sulcal edge axis (located along the ventral side of the ventral sulcal edge), the midventral axis (located near the middle of the ventral subregion) and the ventral interface axis (located in the ventral subregion along the proximal side of the proximodistal interface) **(Fig. 8)**. Counts are occasionally performed along the dorsal interface axis (located on the dorsal subregion, along the proximal side of the proximodistal interface) but essentially to corroborate readings obtained on other axes **(Fig. 8)**. The sulcal edge axis is generally found the most useful axis to count meagre annuli. However, annuli should be routinely examined on all axes before a final annuli count is assigned to the specimen (see **section 3.3.5**). In doing this, it is useful to have a pointer unit coupled to the stereomicroscope because it eases the tracing of the putative annuli across the different axes and facilitates the counting of the numerous annuli of older meagre. Some examples of final meagre annuli counts are shown in **Figures 9 and 10**.

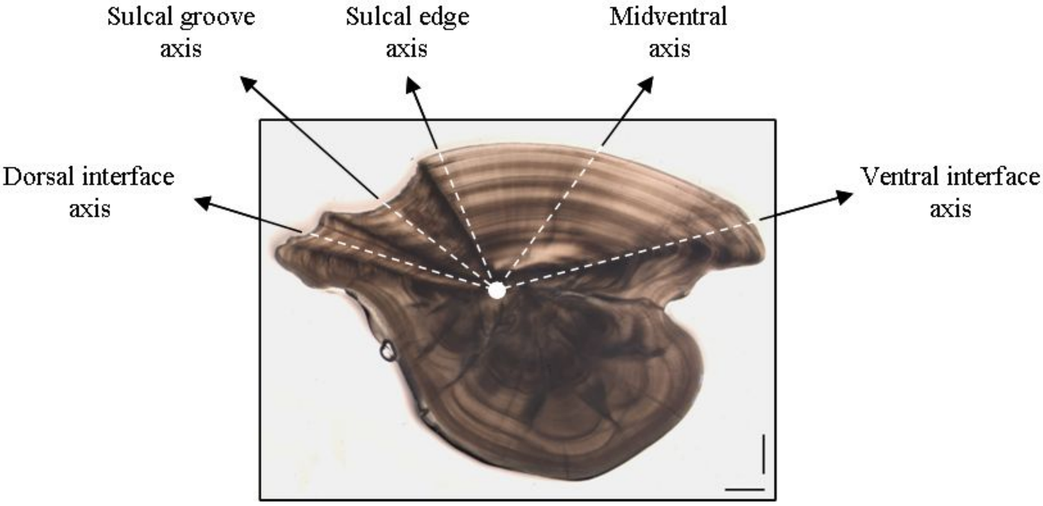

**Figure 8 –** Axes of the otolith section where the annuli are counted. Scale bars=1 mm, 8x.

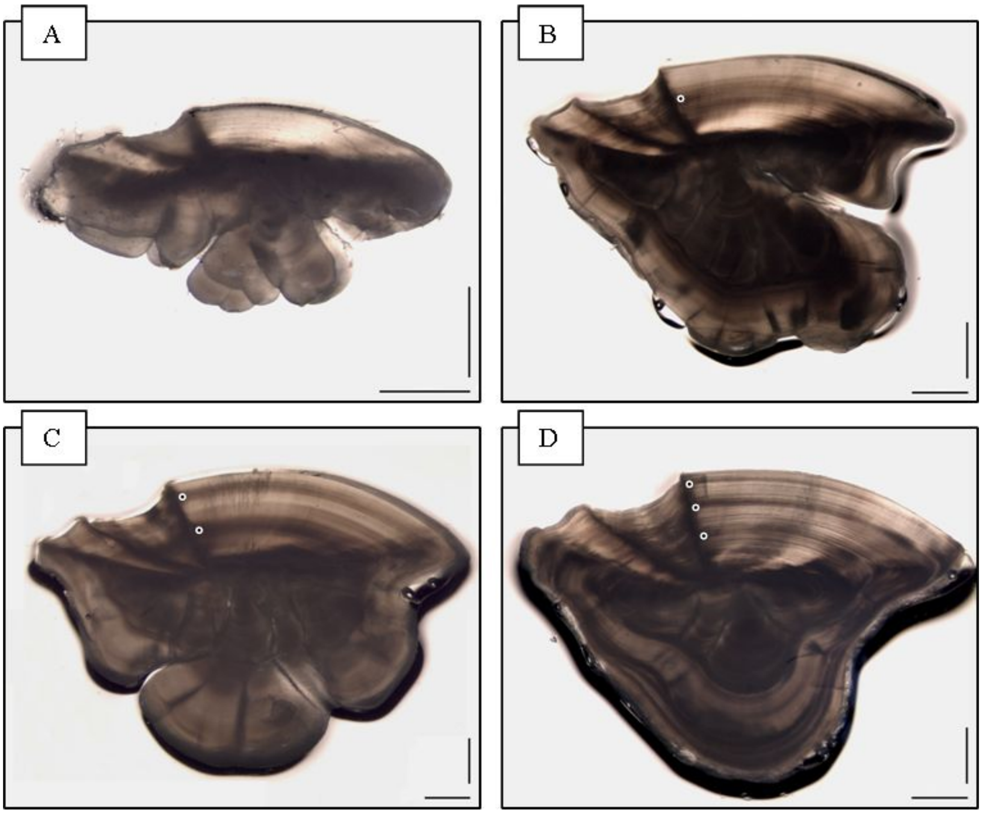

**Figure 9 –** Annuli counts in younger meagre. A – 0 annulus; B – 1 annulus; C – 2 annuli; D – 3 annuli. The white dots along the sulcal edge axis indicate the annuli. Scale bars=1 mm, 20x (A), 12.5x (B), 10x (C), 12.5x (D).

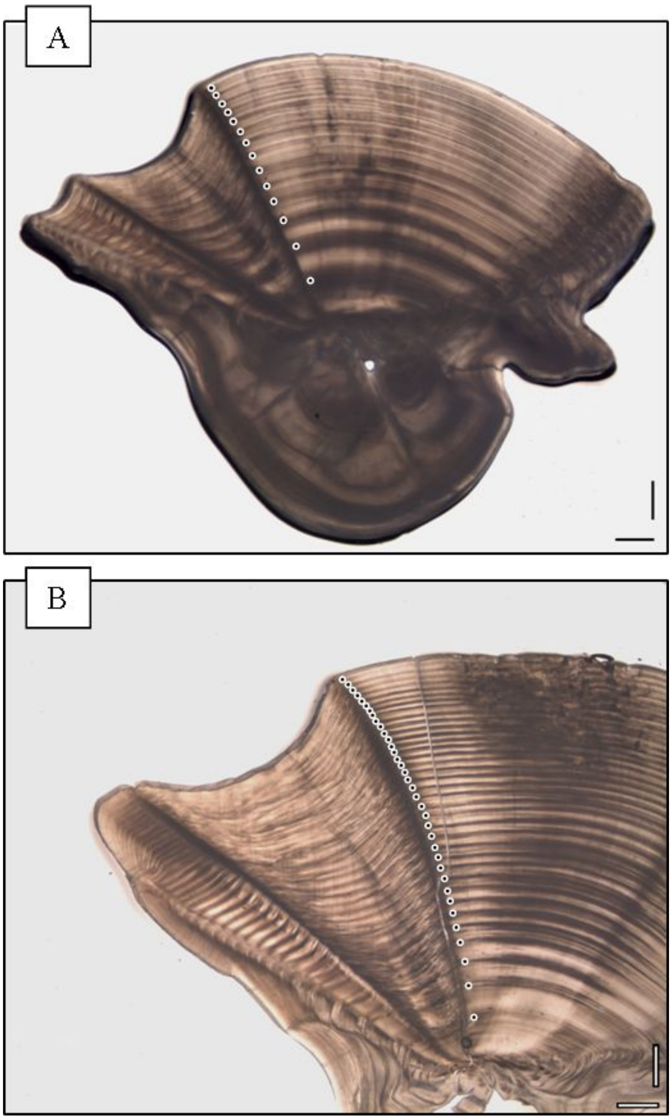

**Figure 10 –** Annuli counts in older meagre. A – 14 annuli; B – 36 annuli. The white dots along the sulcal edge axis indicate the annuli. Scale bars=1 mm, 6.3x.

### 3.3.3. Marginal increment analysis

The marginal increment (MI) is the distance between the last annulus and the margin of the otolith. It corresponds to the otolith growth that took place between the time of the deposition of the last annulus and the time of fish capture. In routine age determinations, the marginal increments of the sections are evaluated qualitatively, but if necessary, corroboratory measurements may be taken along the sulcal edge axis. The following categorical scale is suggested for rapid evaluation of the marginal increment of the meagre otolith thin sections **(Fig. 11)**:

**Type I** – An annulus is clearly observable along the margin of all reading axes. No translucent marginal increment is observed or, if so, it is inconspicuous **(Fig. 11A)**.

**Type II** – A narrow translucent marginal increment is observed between the last annulus and the otolith margin **(Fig. 11B)**. The width of the marginal increment is generally <50% the width of the last annual increment (LAI), i.e., <50% the distance between the last annulus and the previous one.

**Type III** – A wide translucent marginal increment is visible between the last annulus and the margin **(Fig. 11C–D)**. It is expected that a new annulus will form soon. The beginnings of this new annulus may be visible along some reading axes but, if so, are inconspicuous. The width of the marginal increment is generally >50% the width of the last annual increment.

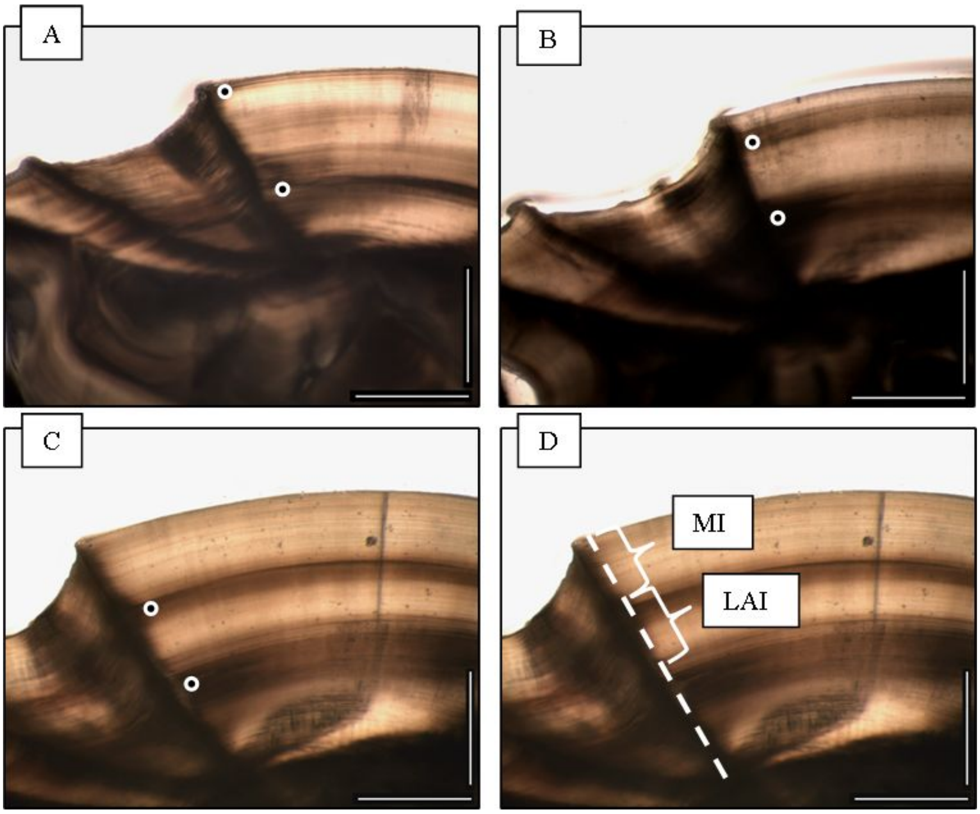

**Figure 11 –** Marginal increment analysis of meagre otoliths. A – type I margin; B – type II margin; C – type III margin; D – Measurements. The white dots indicate the annuli. Figure D displays the measurement axis (dashed line), the marginal increment (MI), and the last annual increment (LAI). Scale bars = 1 mm, 25x.

### 3.3.4. Data collection and data logging

During routine age determinations, meagre otolith sections should be read in random order without knowledge of fish size. Providing readers with knowledge of month of capture is optional but will prevent unnecessary mistakes in marginal increment evaluations[2]. Data from otolith readings can be entered into tables similar to **Table I**. During the readings, the “Age notation” column is commonly filled immediately according to **section 5.1**. Notes should always be kept on doubtful section interpretations.

**Table I –** Example of datasheet for logging otolith readings. Boldface indicates information available to reader. Italics indicate the data entered during hypothetical readings. The “Age notation” column is filled according to **section 5.1**

| Specimen | Month of capture | Annuli count | Margin type | Age notation | Notes |
|---|---|---|---|---|---|
| **036** | **8** | *4* | *II* | *4+4* | |
| **198** | **2** | *18* | *III* | *18+19* | |
| ⋮ | | ⋮ | ⋮ | ⋮ | |
| **075** | **6** | *9* | *I* | *9 (9)* | |

### 3.3.5. Difficulties in annuli interpretation

Compared to some other fish species, the annuli of well-prepared meagre thin sections are clearly distinguishable against a well-lit background and therefore relatively easy to interpret. Also, false annuli are rare and, when present, they can generally be readily distinguished from true annuli based on aspects such as opacity, width, or continuity (see **section 3.3.2**). Consequently, otolith readings tend to be precise even when older fish are included in the sample. Even so, practice shows that substantial improvements to the accuracy and precision of the final age determinations are be achieved with increased staff awareness and training on specific aspects of the meagre thin sections. Three main aspects should be considered in that training: a) preparation-related issues (**section 3.3.5.1**), b) observation-related issues (**section 3.3.5.2**), and c) more meagre-specific issues (**section 3.3.5.3**).

#### 3.3.5.1 Preparation-related issues

Well-prepared sections are fundamental for accurate and precise readings. Consequently, it is important to check the quality of the thin sections before mounting them into their final glass

---

[2] Note: knowledge of month of capture should not be provided to readers if the periodicity and season of annulus deposition are being established at the same time as the age readings are done.

slides. A well-prepared thin section is interpretable along all reading axes and presents clearly outlined annuli and a sulcal subregion that accurately defines the otolith core (**Fig. 9, Fig. 10**). Common preparation-related imperfections found in meagre thin sections are a) excessive opacity or brightness, b) the presence of an ill-defined core, and/or c) the presence of an *ostium* blotch:

a) Excessive opacity or brightness: Excessive opacity and excessive brightness impair annuli identification by making it difficult to distinguish between opaque and translucent bands. Excessive opacity occurs when meagre thin sections are cut wider than 0.5 mm and to correct it the section must be polished until a ≈0.5 mm width is attained. Most frequently, the grinding can be done manually over a flat surface using, e.g., 3 µm Buehler Fibrmet discs. Conversely, excessive brightness occurs when meagre thin sections are cut narrower than 0.5 mm. Excessive brightness is rarer than excessive opacity because narrow sections often break during sectioning. To solve it a new thicker section must be made. In doing it, care should be taken not to obtain a section that presents other ill-preparation issues such as an ill-defined core or a large *ostium* blotch.

b) Ill-defined core: The presence of an ill-defined core usually impairs the identification of more central annuli of the section. This is particularly the case of the first and the second annulus which are located nearer to the core. Two types of core ill-definition may take place: a "blunt sulcal tip" (i.e., the sulcal vertex appears rounded instead of sharp and ends before the proximodistal interface) or a "tornado sulcal tip" (i.e., the sulcal vertex appears twisted in ventral direction and does not directly intercept the proximodistal interface) (**Fig. 12)**. In general, only one type of ill-definition will be found in a section and most frequently, it will be detectable only on one of its sides. When so, the section can be mounted with the best-prepared side facing upwards as reliable interpretations can still be drawn from it. However, if that is not the case, core ill-definition is indicative that the sectioning took place at a wrong location of the otolith surface (**Fig. 5B**) and a new section must be prepared. In doing this, evidence may be gathered from the ill-prepared section that will help determine the position of the new section: if a "blunt sulcal tip" was present, the new section should be taken further away from the *ostium* (i.e., closer to posterior edge of the otolith); if a "tornado sulcal tip" was present, the new section should be taken closer to the *ostium* (i.e., closer to anterior edge of the otolith).

c) *Ostium* blotch: A common problem found in meagre thin sections is the presence of a broad dark blotch in the ventral subregion (**Fig. 12**). The blotch is caused by the section cutting across the internal extension of the *ostium*, a region that presents different light transmission properties from adjoining areas. Most frequently, the presence of this blotch impairs age interpretations along the sulcal edge and midventral axes, but the extent of this impairment generally depends on the effective position and tilt of the sectioning plane. There are two possible causes for the *ostium* blotch: it may be caused by insufficient tilting of the otolith when originally embedded in Crystalbond (**Fig. 5A**) or it may result from the pencil marking having been misplaced on the otolith surface (**Fig. 5B**). When the former happens, and readings are judged to be severely impaired, it is necessary to prepare the other *sagitta*. When the latter happens, the blotch is generally found associated to a “blunt sulcal tip” (**Fig. 12**) and a new section, taken from a slightly posterior position, is generally sufficient to improve readability (see “ill-defined core”).

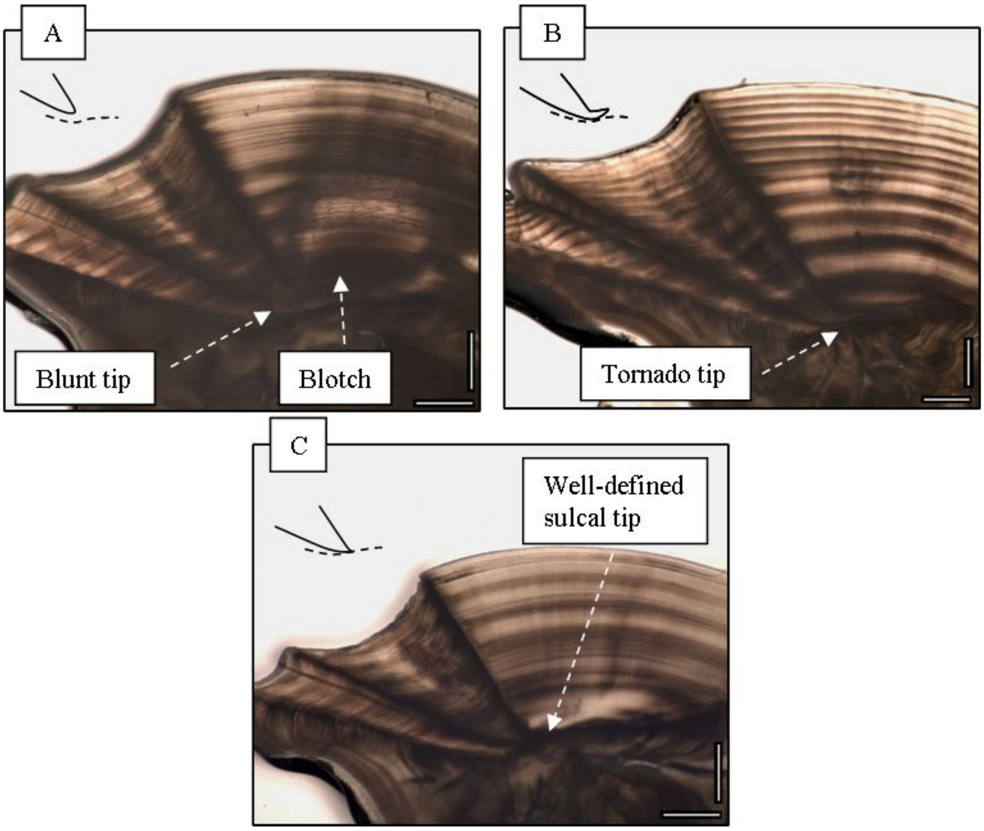

**Figure 12 –** Quality checking of otolith thin sections. A – blunt sulcal tip and *ostium* blotch; B – tornado sulcal tip; C – well-prepared otolith section. Inset drawings show the position of the tip relative to interface. Scale bars = 1 mm, 12.5x (A), 10x (B), 12.5x (C).

### 3.3.5.2 Observation-related issues

Two main observation-related issues must be considered when reading meagre otolith thin sections: parallax errors and issues related to the orientation of the transmitted light. Both these issues impact the readability of the sections by changing the final image that readers observe through the stereomicroscope lens. To prevent or ameliorate them it is important to include specific practices into the reading routines.

Parallax errors: The 0.5 mm thickness of meagre otolith sections is important to obtain nicely contrasted annuli but it also makes annuli counts more susceptible to parallax errors. Parallax errors occur because a reader observes a section as combination of three-dimensional details that are present across the section's width and not just the details present on the section's upper surface. As a consequence, the image obtained from the section is highly dependent on the observation angle and so are the annuli counts and the marginal increment evaluations made. In fact, when readings are done at directions not parallel to the width of the annuli, the latter tend to look wider than they really are and may even appear fused to adjoining annuli. Additionally, it is also common that marginal increment evaluations done at directions that are oblique to otolith surface become confounded by the margins' own width, revealing an opaque margin when in fact the margin is translucent. To avoid these types of parallax errors, readers must search for a reading plane that is as parallel as possible to the plane of the annuli and to the plane of the margin before performing the final annuli counts and marginal evaluations. That plane is section-specific and very dependent on the exact tilt and positioning of the sectioning plane. Consequently, the best way to find it requires readers to observe each section tilted at different angles while looking for the orientation that provides them with the narrower annuli, wider interannuli spaces, and the narrower otolith margin.

Transmitted-light orientation: Transmitted-light microscope bases may provide for a fixed-light orientation or allow for hand-adjusted control of light orientation. Different light orientations provide for different directions from which the light waves interact with the three-dimensional structure of the otolith sections. These different directions can change the reader's perception of the section by, e.g., making annuli less apparent or providing emphasis to false annuli, and consequently interfere with age interpretation. Because of this, it is preferable to read meagre otolith sections on a microscope base that allows for hand-adjusted control of light orientation since this will allow readers to obtain crispier images. Additionally, it is also advantageous to have a dark-field polarizing filter attached to the stereomicroscope objective. Dark-field polarizing filters confer a dark appearance to the bright background of transmitted-light observations, substantially reducing glare and enhancing the image contrast, thus enhancing overall section readability (**Fig. 13**).

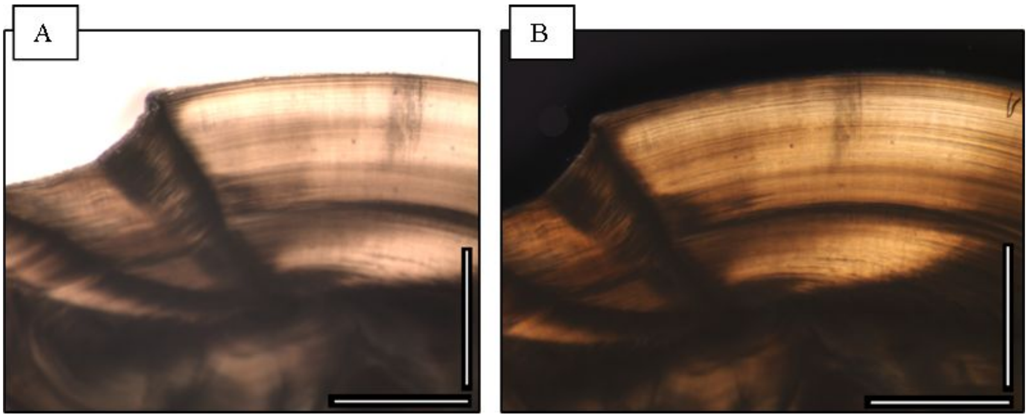

**Figure 13 –** Effect of dark-field polarization on otolith section readability. A – without filter; B – with filter (a Nikon dark-field polarizing filter was used). Scale bars = 1 mm, 30x.

### 3.3.5.3 Other issues

Three types of difficulties are generally reported by readers when they are first introduced to meagre otolith thin sections: a) difficulties in the identification of the first annulus, b) difficulties related to annulus splitting, and c) difficulties related to abnormal otolith crystallization:

a) Difficulties in first annulus identification: To the less experienced reader, the identification of the first annulus is the main difficulty met when interpreting meagre sections. In many specimens the first annulus is difficult to discriminate along the sulcal groove axis because it is close to the core and appears masked by the filamentous appearance of the sulcal subregion. Often, this is not a major difficulty because the annulus will still show up sufficiently opaque and distant from the proximodistal interface along the remaining axes to be clearly outlined **(**e.g., **Fig. 9)**. However, cases exist where first annulus identification remains troublesome along the remaining axes. When this happens, three main issues are found to be the cause:

- Annulus “brightness”: In some specimens, the first annulus appears brighter than usual and presents little contrast to adjacent translucent bands **(Fig. 14)**. Usually, this happens along the sulcal edge axis or midventral axis, and is particularly noticeable when an *ostium* blotch, even of small size, is present near the core **(**see

**section 3.3.5.1)**. In these cases, to verify if an annulus effectively exists near the core, the ventral interface axis should be examined: if present, the annulus will show up as a strongly opaque bend backwards that penetrates the distal region; if not, the bend will not be observed and the annulus should be searched for farther away from the otolith core **(Fig. 14)**.

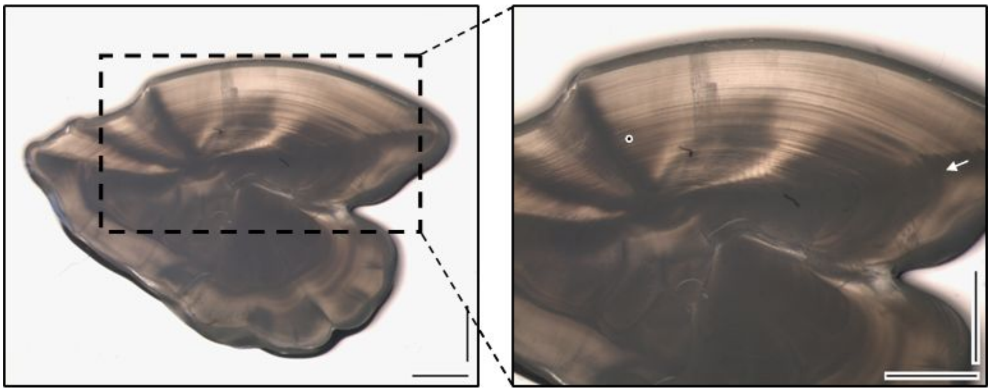

**Figure 14 –** First annulus "brightness". The white dot indicates the first annulus. The white arrow indicates the bend of the annulus towards the distal region. Scale bars = 1 mm, 12.5x (left), 20x (right).

- <u>Annulus "rippling"</u>: In some specimens, a set of concentric opaque "ripples" occurs near the otolith core which causes the first annulus to be mistaken as several distinct annuli **(Fig. 15)**. In most such cases, the first annulus will remain clearly identifiable along the ventral interface axis and readings can proceed. However, even if not, practice shows that the first annulus can be confidently assigned to the entire set of ripples and that regular counts should be resumed at the second annulus.

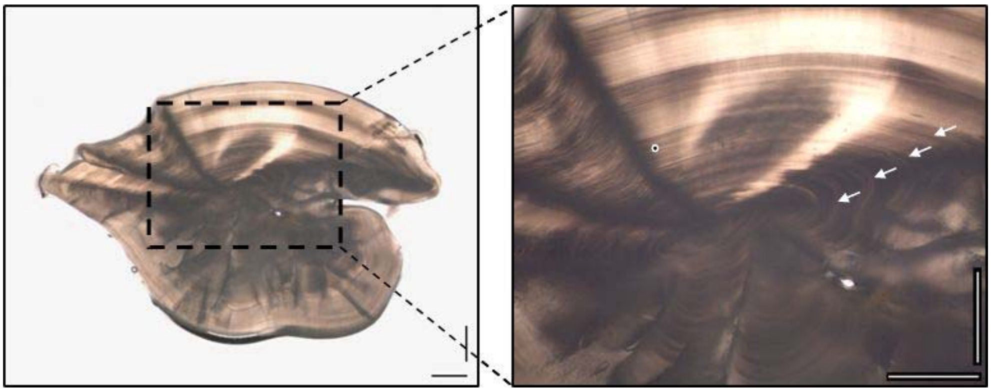

**Figure 15 –** First annulus rippling. The white dot indicates the first annulus. The white arrows indicate the ripples. Scale bars = 1 mm, 8x (left), 20x (right).

- <u>“Dent”</u>: In some specimens, a dark mark similar to a dent occurs near the otolith core. To inexperienced readers this dent resembles a very early first annulus **(Fig. 16)**. However, the dent results from sectioning imperfections generated at the interception of the sectioning plane with the internal structure of the *ostium*. Consequently, it should not be counted as the first annulus and readers should look farther away from the core for better evidence of this annulus.

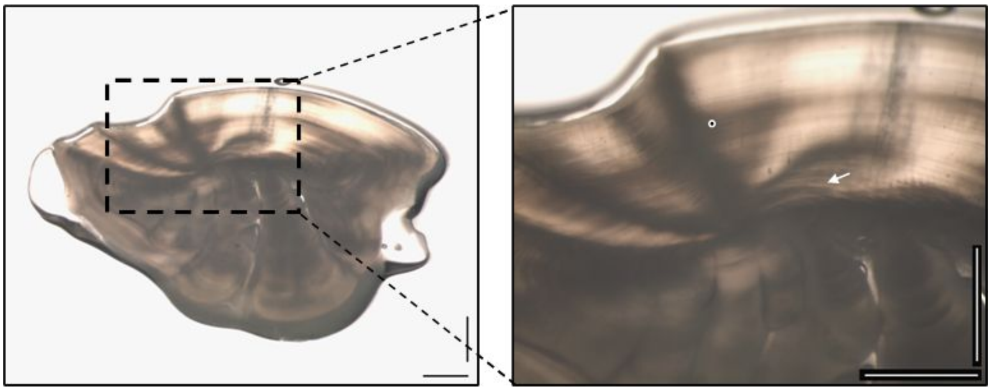

**Figure 16 –** Dent. The white dot indicates the first annulus. The white arrow indicates the dent. Scale bars = 1 mm, 10x (left), 25x (right).

b) <u>Difficulties due to annulus splitting:</u> In meagre, split annuli are relatively frequent between the third and the ninth annulus (**Fig. 17**). Annuli are generally found to split into two distinct branches near the ventral sulcal edge. The two branches then run parallel to each other – separated only by a thin translucent band – throughout the ventral subregion and rejoin only near the ventral interface axis. Because annulus splitting does not usually extend to all reading axes, it is generally detected when annuli counts from different axes are compared. However, to completely resolve the issue, readers should trace down the branches of each putative split annulus to check if they effectively rejoin at the ventral interface axis. In doing this, it is particularly advantageous to have the stereomicroscope equipped with a pointer unit because this will ease the tracking down of the split branches across the large ventral subregion. Once all split annuli have been identified, readers should obtain the final counts by doing some “jumping around” between the different axes, i.e., by counting each annulus at the axis (or axes) where the annulus was not observed to split.

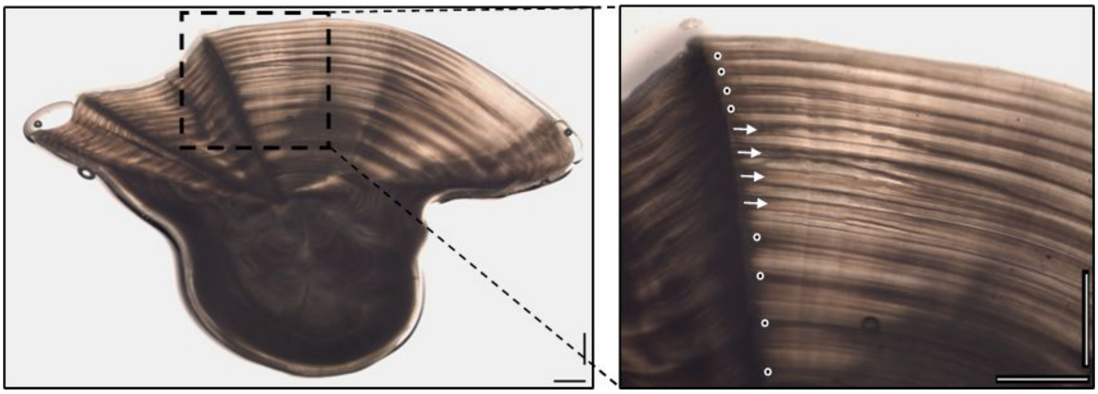

**Figure 17 –** Annulus splitting. The white dots indicate regular annuli. The white arrows indicate four annuli that split near the ventral sulcal edge. Scale bars = 1 mm, 6.3x (left), 20x (right).

c) <u>Abnormal otolith crystallization:</u> Some meagre otoliths evidence abnormal crystallization in one (or both) of the otoliths. Abnormal crystallization is caused by major crystallization of calcium carbonate as vaterite crystals (instead of the usual aragonite crystals) and results in otoliths that are lighter than usual, externally very irregular, and internally very translucent (**Fig. 18**). When abnormal crystallization occurs, it generally extends from a specific point in the interior of the section all the way to its periphery, and it is clearly noticeable on the otolith surface (**Fig. 18A**). To circumvent it, it is advisable to select for sectioning the normal (or the less impacted) *sagitta*. However, if necessary, an attempt may still be made at sectioning abnormal otoliths because their annuli are generally still interpretable along some of the reading axes (**Fig. 18B**). When sectioning abnormally crystallized otoliths, lower saw speeds and lighter arm loads should be used because the otoliths are brittle and break easily if too much pressure is exerted on them.

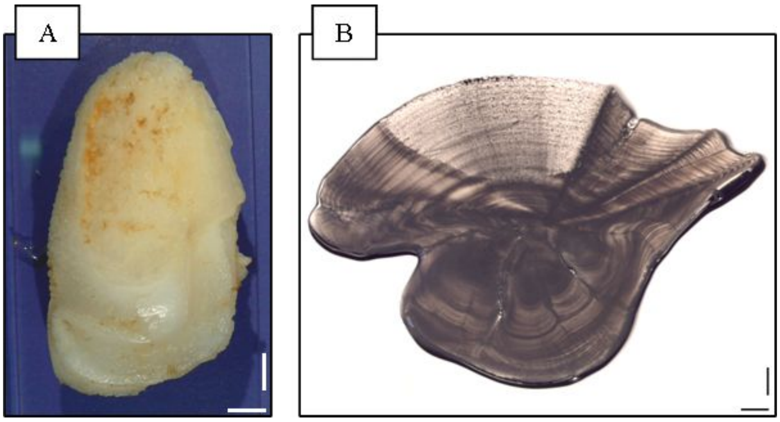

**Figure 18 –** Abnormal crystallization in meagre otoliths. A – whole otolith; B – otolith section. A and B were taken from different fish. Scale bars = 5 mm (A), 1 mm (B), 6.3x (B).

# 4. SCALE PROTOCOL

## 4.1. Collection

Meagre scales are generally collected from the left side of the fish from the region located between the first dorsal fin and the lateral line **(Fig. 19)**. In general, 10 to 15 scales are collected from each fish. In meagre, the scales are tightly embedded in the dermis so they are not easy to release from the fish body. The simplest method to collect scales involves rubbing a knife over the skin surface, in successive posterior to anterior movements, while exerting pressure to insert its blade underneath the scales. This method releases many scales, making them "jump" out of the skin, but also damages the external appearance of the fish. In many cases, the latter has to be avoided because specimens will enter the commercial circuit. When so, scales should be collected one-by-one with tweezers **(Fig. 19)**. Collecting scales with tweezers takes more time but causes no loss of commercial value because the fish skin can be brought back to its original appearance with a gentle rub in posterior direction.

**Figure 19 –** Aspects of the collection of meagre scales. The shaded rectangle indicates the area of collection.

During field sampling, meagre scales are commonly put inside labeled paper envelopes without much cleaning. Back in the lab, they should be cleaned before being stored. For this, scales are first immersed in water for a few minutes to soften and separate and then rubbed individually between the thumb and the index finger to remove dirt and adherent tissues. If necessary, a soft toothbrush may be used but excessive pressure should be avoided because it will scratch the scale surface. Once clean, meagre scales may be left to dry at room temperature until stored into their final paper envelopes.

## 4.2. Preparation

Meagre scales are frequently too thick to be directly evaluated under a stereomicroscope or microfiche reader. Consequently, their external surface should be imprinted into acetate slides before readings take place. Before imprinting, some preliminary sorting and selection of scales is generally required. In sorting and selection, preference should be given to scales that present continuous margins and a roughly similar shape and size. Rectangular scales, slightly wider than longer, are common in the collection area and among the easiest to interpret. However, most importantly, the scales selected for imprinting must not present signs of regeneration **(Fig. 20)**. In general, regenerated scales can be distinguished from nonregenerated scales before being pressed on the basis of their extreme flexibility and their inconsistent microstructure when observed under a common lens. Flexibility and microstructure are, however, hard to evaluate in smaller scales or when regenerated portion is small. Consequently, besides careful sorting, it is good practice to always imprint a larger number of scales than the number necessary to determine the fish age (3–5 scales) as this will ensure that enough nonregenerated scales are present on the final slides.

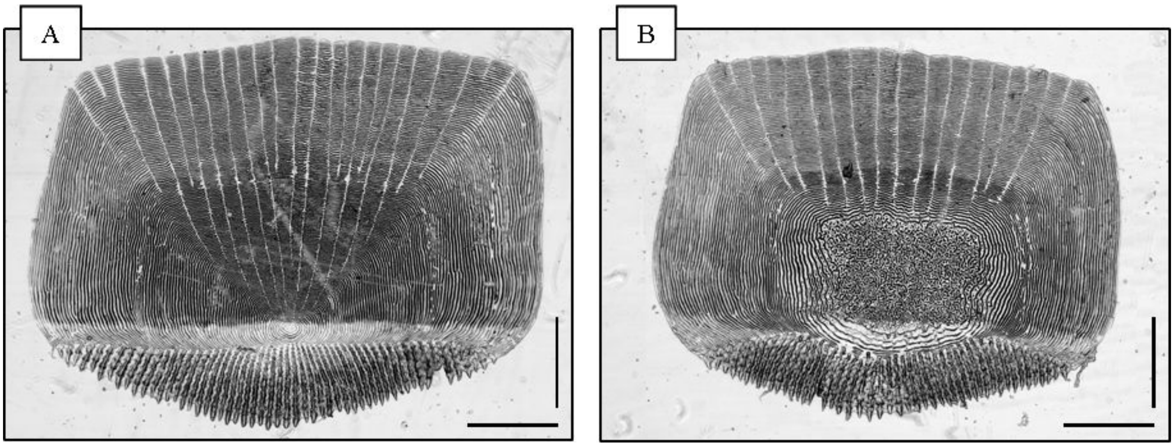

**Figure 20 –** Scale regeneration. A – nonregenerated scale, B – regenerated scale (same fish). Scales were imprinted into acetate slides. Scale bars = 1 mm, 50x.

Meagre scales may be imprinted onto transparent cellulose acetate slides (25 mm x 75 mm x 0.5 mm) using a heated press (e.g., a Carver Laboratory Heated Press Model C) **(Fig. 21A)**. Scales of older fish tend to be large and thick and consequently require larger and thicker acetate slides to be imprinted (e.g., 30 mm x 75 mm x 1 mm). In general, between 2 and 10 scales can be imprinted on each slide and between 1 and 4 acetate slides can be pressed at each press operation. Before pressing, the scales should be aligned with their external side (i.e., the side that appears rougher and less reflective to light) kept in contact with the acetate slide **(Fig. 21B)**. Then, the slides are inserted between a pair of portable platens and transparency-film coverslips and put to press (**Fig. 21C**). Under such a setup, the standard conditions for pressing meagre scales involve pressing for 7 min., at a pressure of 109.5 MPa, and temperature of 75°C. However, slight adjustments to time, pressure, and temperature may be required to achieve adequate imprints across the entire thickness range of the meagre scales (see **section 4.3.5.1**).

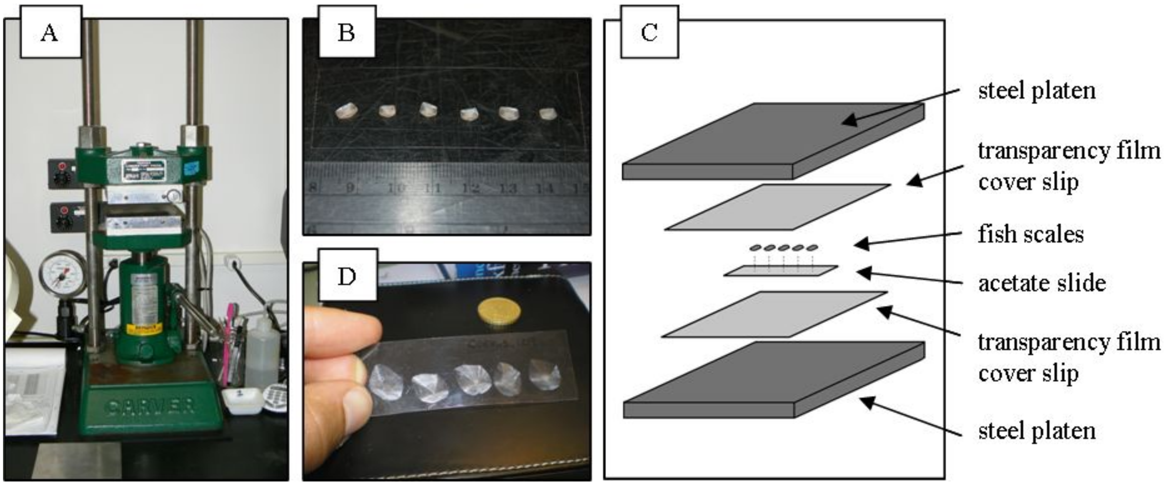

**Figure 21 –** Aspects of scale preparation. A – Carver Laboratory Heated Press Model C; B – meagre scales ready to be pressed (photo courtesy of Christina Morgan); C – pressing setup; D – meagre scale imprints ready for storage.

After pressing, meagre scales are generally found adhered to the acetate slide. Gentle pulls with tweezers can be used to release them as long as care is taken not to scratch the imprint with the tweezers' tips. Then, before storage, it is good practice to perform a preliminary quality check on the imprints to guarantee that all scales have been adequately pressed (**see section 4.3.5.1**). At this time, if necessary, new scales can be readily imprinted and future delays avoided. However, because previously pressed scales tend to be brittle, curved up, and/or cracked, a new set of scales must be prepared. When imprint quality is found appropriate, final scale slides are labeled with a permanent marker and stored inside microscope slide boxes until readings are done (**Fig. 21D**).

## 4.3. Reading

Age determination from fish scales involves the interpretation (or reading) of a set of markings on the scale surface that are faithfully depicted on the acetate imprints. Interpreting those markings requires specific equipment and knowledge of scale morphology (**section 4.3.1)** and involves three main steps: the interpretation and counting of annuli (**section 4.3.2),** an evaluation of the marginal increment **(section 4.3.3)** and data logging **(section 4.3.4)**. Meagre scales, particularly from older fish, are difficult to interpret even to experienced readers. Consequently, adequate scale collection and preparation, and full awareness and training on specific patterns and details of the meagre scales, are fundamental to the age determination process (**section 4.3.5**).

### 4.3.1. Equipment and terminology

Acetate imprints of meagre scales are read on a microfiche reader. Common microfiche readers work on transmitted light and the acetate imprints show up inverted on a screen. Because the meagre scales exhibit a large variability in size, the microfiche reader should be equipped with lenses that provide for somewhat different magnifications in the range of 10x to 30x.

Well-prepared acetate imprints faithfully depict the morphology of the external surface of the scales. When an imprint is observed at low magnification, the thicker areas of the original scale (namely its center portion) appear darker and the thinner areas (namely its periphery and margins) appear brighter. At higher magnification, the crests and ridges of the original scale appear as dark lines, whereas the grooves show up as bright lines. In the body region where samples are collected, the scales are ctenoid and their margins show up undulated (anterior margin), straight (lateral margins), and prickly (posterior margin). Four main regions can be defined on the scale surface – a “posterior field”, two “lateral fields” (dorsal and ventral) and an “anterior field” **(Fig. 22)**.

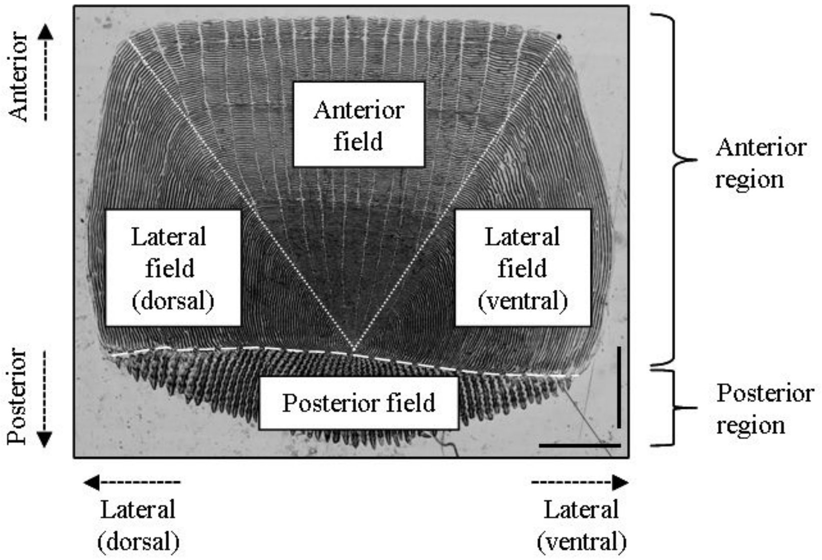

**Figure 22 –** Morphology of the meagre scale. The anteroposterior interface is indicated by a white dashed line. Scale bars = 1 mm, 40x.

The posterior field presents a spiny appearance and corresponds to the part of the scale that is directly exposed to the environment. This field is separated from the remaining fields of the scale by an interface (the "anteroposterior interface") that broadly divides the scale into a posterior region and anterior region **(Fig. 22)**. The spiny appearance of the posterior field results from long segmented tube-like structures (the "*ctenii*") that extend from the interface to the posterior margin of the scale **(Fig. 23)**.

The lateral and anterior fields are both located in the anterior region of the scale. Both fields exhibit a markedly parallel appearance and correspond to parts of the scale that, while in the fish body, are largely concealed underneath neighboring scales. The lateral and anterior fields present thin concentric ridges (the "*circuli*") which run from one lateral field to the next across the anterior field. In meagre, all *circuli* are centered in the same region (the "focus") that is located in medial position near the anteroposterior interface **(Fig. 23)**. Even so, the appearance of *circuli* changes drastically from the lateral to the anterior fields: in the lateral fields, *circuli* run in anteroposterior direction and are continuous; in contrast, in the anterior field, *circuli* run in dorsoventral direction and are divided into numerous segments (the "platelets") by a set of radial grooves that stem outwards from the focus towards the anterior margin (the "*radii*") **(Fig. 23)**. For simplicity of this protocol, we termed the part of a *circulus* that appears segmented on the anterior field as "segmented *circulus*" and its nonsegmented part, located along each lateral field, as "continuous *circulus*" **(Fig. 23)**.

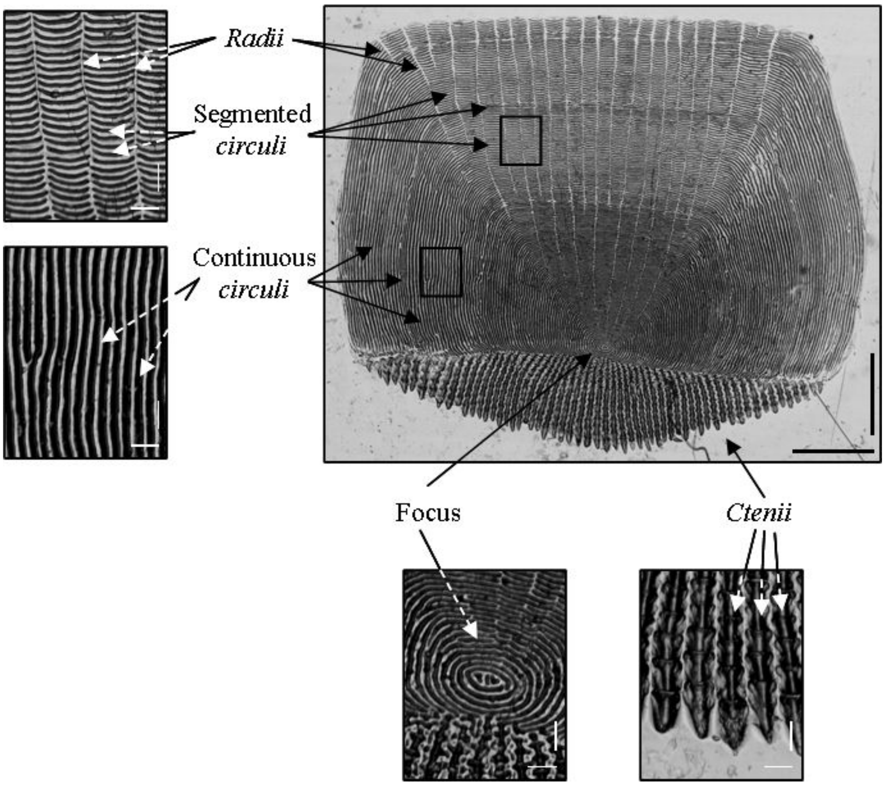

**Figure 23 –** Morphology of the meagre scale. Each segment of a "segmented *circulus*" is termed a "platelet". Scale bars = 1 mm (main figure), 0.1 mm (details), 40x (all).

### 4.3.2. Annuli interpretation and count

The use of scales in age determination relies on the interpretation and count of specific scale markings that are assumed to form at annual intervals (termed "annuli"). Ctenoid scale annuli are relatively narrow continuous concentric bands that extend across the lateral and anterior fields of the scale. In meagre, scale annuli encompass small groups of homogenous-looking *circuli* that can be discriminated from adjoining, closely-resembling, nonannulus *circuli* using specific structural criteria (termed "primary criteria"). The primary criteria used to identify annuli in meagre scales are: a) *circuli* "disruption", b) *circuli* "straightening out", and c) *circuli* "compaction" **(Fig. 24)**[3]:

a) <u>*Circuli* "disruption"</u>: The vast majority of continuous *circuli* do not suffer any significant interruption. However, the continuous *circuli* that belong to an annulus

---

[3] Note: the meagre "*circuli* disruption" and "*circuli* straightening out" bear some resemblance to the "cutting over" marks (also known as "crossing over" marks) observed in, e.g., summer flounder and striped bass scales (Pentilla and Dery, 1988; Liao *et al.*, 2008).

present disruptions to their continuity which resemble small strings, or aggregations, of white spaces within the continuous parallel pattern of the lateral fields **(Fig. 24A)**.

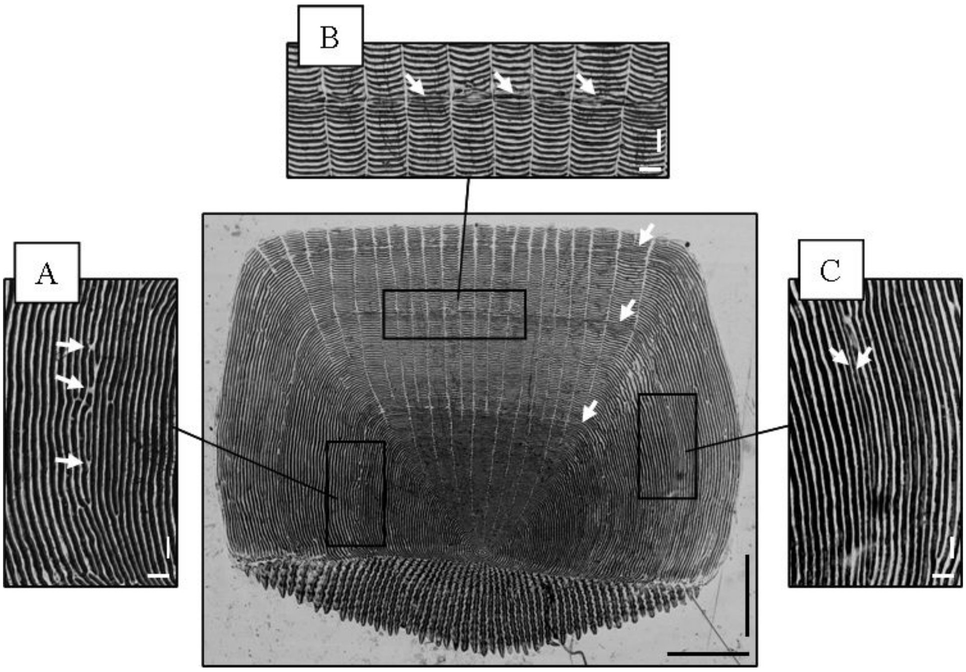

**Figure 24** – Aspects of primary criteria used in annuli interpretation. White arrows in central picture indicate annuli position. White arrows in lateral pictures indicate disruption (A), straightening out (B) and compaction (C). Scale bars = 1 mm (main figure), 0.1 mm (details), 40x (all).

b) <u>*Circuli* “straightening out”</u>: The vast majority of segmented *circuli* are composed of concave platelets. However, at an annulus, the platelets of one or more *circuli* become straight (or, in older fish, highly irregular) instead of concave, which causes the annulus to resemble a string of whitish nodules extending across the anterior field **(Fig. 24B)**.

c) <u>*Circuli* “compaction”</u>: At an annulus, both continuous and segmented *circuli* appear more compact than in adjoining areas due to a reduction in inter-*circuli* distances. In the lateral fields, this compaction is generally noticed as a band of continuous *circuli* that looks somewhat darker and more compact than the surroundings areas **(Fig. 24C)**. In the anterior field, *circuli* compaction generally takes place immediately before and / or after the straigtening out of segmented *circuli* and also provides a contrasting darker appearance to the annulus region when compared with adjacent regions **(Fig. 24B)**.

In meagre scales, a group of *circuli* is considered an annulus when it matches all primary criteria and can be traced throughout the lateral and anterior fields of the scale. If these two characteristics (criteria match and traceability) are not met, then the group of *circuli* belongs to an interannuli region or to some other type of distinct scale feature that should be ignored for effects of age determination (broadly termed “false annulus”). In meagre scales, false annuli are relatively frequent but can generally be distinguished from true annuli because they match only one criterion and they cannot be traced throughout the anterior regions of the scale. However, despite their apparent objectivity, the use of primary criteria and traceability to identify scale annuli is not always clear-cut. The reason for this is that some criteria are easier to observe in some parts of the scale (or in some annuli) than others. That is the case of, e.g., *circuli* compaction (that is sometimes easier to verify in the lateral fields than in the anterior field) and *circuli* disruption (which is more evident in central annuli than in peripheral ones). To circumvent these and other annuli identification difficulties, it is generally acceptable to extend the annulus definition to any group of *circuli* that meets, at least, two primary criteria while remaining traceable throughout the scale. However, should this broader definition be used and annulus identification must be supplemented with a few corroboratory criteria to reduce the increased risks of assigning true annuli to false annuli (**see section 4.3.5.3).**

In meagre scales, annuli are counted from the focus to the periphery along specific axes **(Fig. 25)**. Because some criteria are more observable along some reading axes than along other, some “jumping around” between different axes may be necessary as counts proceed, particularly in older scales. In general, the anteroposterior axis and the two anterolateral axes are the most useful to count meagre annuli, but the final count of each scale should be based on a consensus among the counts obtained on the different axes. Finally, the annuli count of each specimen is based on a consensus among the counts attained in at least three scales among the several imprinted for that particular fish. In the selection of the latter set of scales, it is important to exclude regenerated scales (because they may not show all annuli) and scales which counts differ markedly from the remaining (because they may have originated from different fish) (see **section 4.3.5.1)**.

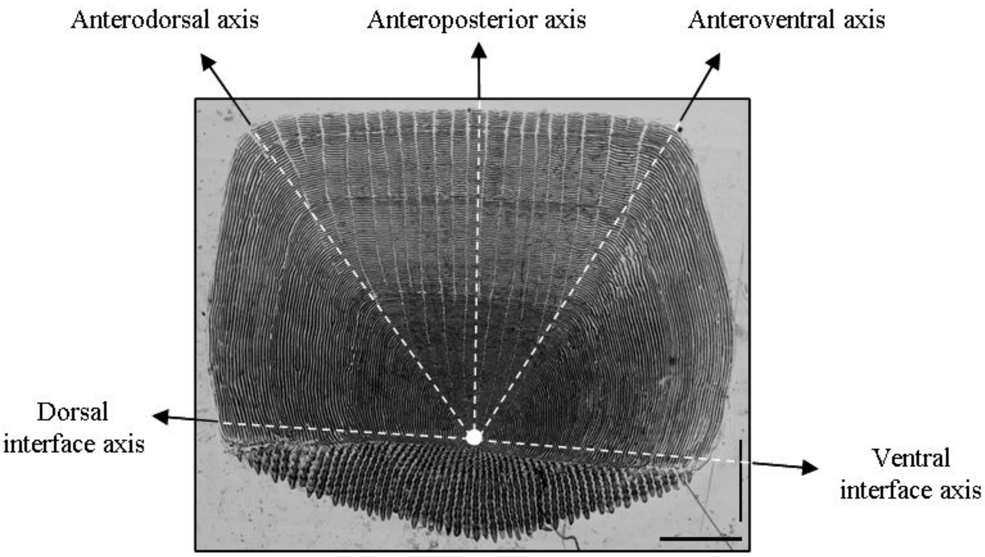

**Figure 25 –** Axes of the meagre scale where the annuli are counted. Scale bars = 1 mm, 40x.

Some examples of final annuli counts in meagre scales are shown in **Figures 26 and 27**.

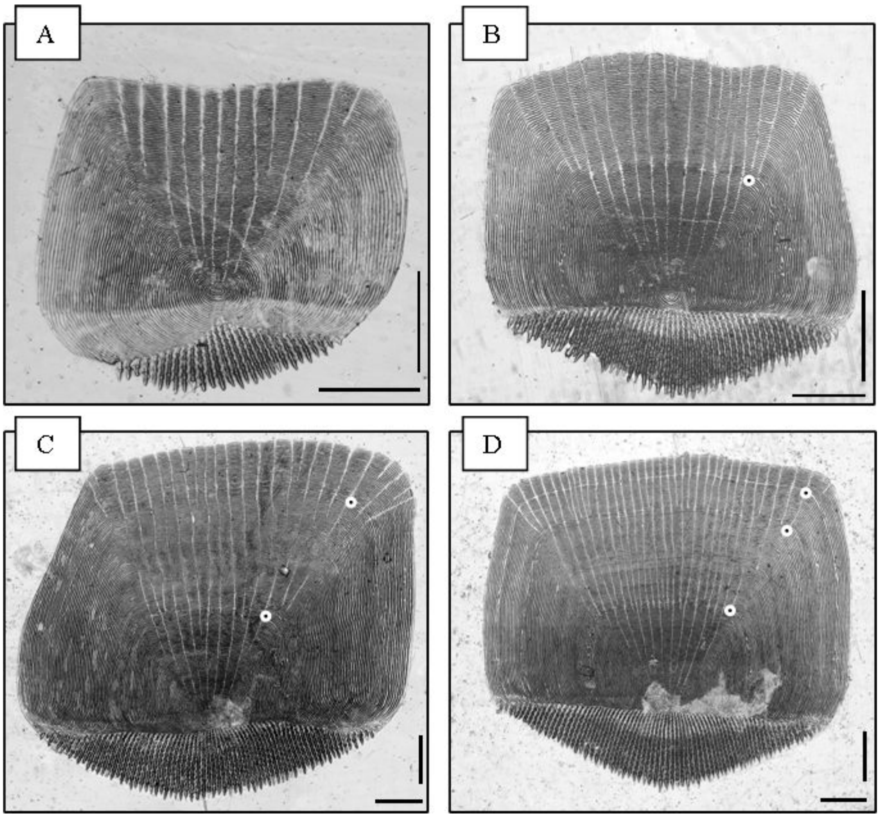

**Figure 26 –** Annuli counts in younger meagre. A – 0 annulus; B – 1 annulus; C – 2 annuli; D – 3 annuli. The white dots along the anterodorsal axis indicate the annuli. Scale bars=1 mm, 50x (A), 50x (B), 25x (C), 30x (D).

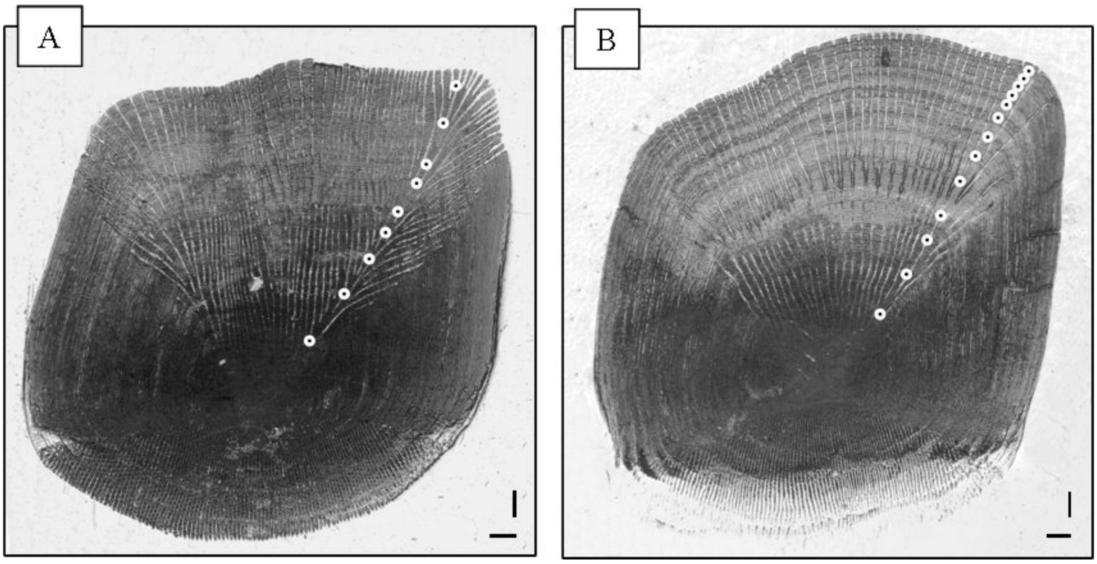

**Figure 27 –** Annuli counts in older meagre. A – 9 annuli; B – 13 annuli. The white dots along the anterodorsal axis indicate the annuli. Scale bars=1 mm, 11x (A), 9x (B).

### 4.3.3. Marginal increment analysis

The marginal increment (MI) is the distance between the last annulus and the margin of the scale. It corresponds to the scale growth that took place between the time of the deposition of the last annulus and the time of fish capture. In routine age determination, the marginal increment of meagre scales is evaluated qualitatively, but if necessary corroboratory measurements may be taken along the anteroposterior axis. The following categorical scale is suggested for evaluating the marginal increment of the meagre scales **(Fig. 28)** [4]:

**Type I** – the last annulus is located at the scale margin or very near to it. It is not expected that a new annulus will form soon. The marginal increment is <25% the width of the last annual increment (LAI), i.e., <25% the distance between the last annulus and the previous one.

**Type II** – the last annulus is located relatively distant to the margin. The marginal increment width is 25%–75% of the last annual increment width.

**Type III** – the last annulus is located very distant to the margin. It is expected that a new annulus will form soon. The marginal increment width is >50% of the last annual increment width.

---

[4] Note: in this classification, the overlapping percentages of type II and type III margins reflect some inherent difficulties of meagre scale interpretation in the Portuguese coast (compare with **section 3.3.3** on meagre otoliths). Amongst other, these difficulties are related to a large variability in the interannuli distances and to a long annulus deposition period (**section 5**).

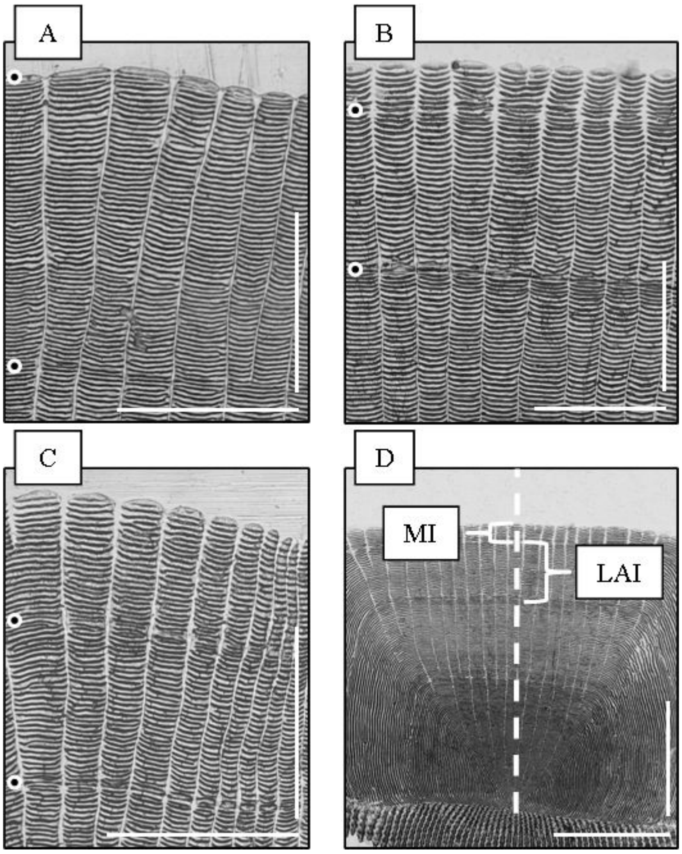

**Figure 28 –** Marginal increment analysis of meagre scales. A – type I margin; B – type II margin; C- type III margin; D – measurements. The white dots indicate the annuli. Figure D displays the measurement axis (dashed line), the marginal increment (MI) and the last annual increment (LAI). Scale bars=0.1 mm (A-C), 1 mm (D), 50x (A), 40x (B-D).

### 4.3.4. Data collection and data logging

During routine age determinations, meagre scale imprints should be read in random order without knowledge of fish size. Providing readers with knowledge of month of capture is optional but will prevent unnecessary mistakes in the marginal increment evaluations[5]. The final consensus on annuli counts and margin evaluations may be entered into tables similar to **Table II**. During the readings, the "Age notation" column is commonly filled immediately according to **section 5.1**. Notes should always be kept on doubtful scale imprint interpretations.

---

[5] Note: knowledge of month of capture should not be provided to readers if the periodicity and season of annulus deposition are being established at the same time as the age readings are done.

**Table II –** Example of datasheet for logging scale readings. Boldface indicates information available to reader. Italics indicate the data entered during hypothetical readings. The “Age notation” column should be filled according to **section 5.1**

| Specimen | Month of capture | Annuli count | Margin type | Age notation | Notes |
|---|---|---|---|---|---|
| **036** | **10** | *4* | *II* | *4+4* | |
| **078** | **2** | *18* | *III* | *18+19* | |
| ⋮ | | ⋮ | ⋮ | ⋮ | |
| **011** | **8** | *9* | *I* | *9 (9)* | |

### 4.3.5. Difficulties in annuli interpretation

When interpreting meagre scales it is frequent for disagreements to occur at within-sample level (i.e., between the several scales pressed for a specific fish), at within-reader level (i.e., between readings obtained by a single reader on different occasions), and at between-reader level (i.e., between readings obtained by multiple readers). Additionally, it is not infrequent for annuli counts obtained from scales to be substantially different from annuli counts obtained from the otolith sections of the same fish. This is so, even when experienced readers are involved and results essentially from difficulties in standardizing scale preparation and in objectively applying the primary criteria used in meagre scale annuli identification (**see section 4.3.2**). Even so, practice shows that the final age estimates obtained at all reading levels can be much improved, particularly in younger fish, if the personnel involved in meagre scale collection, preparation and interpretation is given training on specific issues of the meagre scales. This training should cover: collection- and/or preparation-related issues (**section 4.3.5.1**), observation-related issues (**section 4.3.5.2**), and more meagre-specific issues (**section 4.3.5.3**).

#### 4.3.5.1 Collection- and/or preparation-related issues

Contrary to otoliths, scales are external structures that can easily detach from the fish body and “contaminate” nearby fish. Consequently, if scale collection is careless there is a high probability that each paper envelope will contain scales from more than one fish. If that occurs, it will become increasingly difficult to establish a consensus between the readings of the several scales imprinted for each fish and the quality of age determinations will decrease.

To avoid sample contamination, field personnel should always clear the fish skin from already detached scales before collecting the samples. Additionally, the scales should always be collected in large numbers as this will reduce the proportion of alien scales in each sample. These two practices are relatively obvious but should be routinely stressed to field samplers as this will prevent unnecessary errors in the final ages.

Scale interpretations become increasingly difficult when dealing with acetate slides that contain imprints of scales of markedly different size, shape, or thickness. The main reason for this is that imprints on such slides tend to show up unevenly pressed. Additionally, it is generally hard to achieve consensus among differently-shaped scales. Most of these difficulties can be avoided with increased standardization of the collection and preparation procedures. The size and shape of the imprints can be made more homogeneous if large samples are collected and scales are removed from the target area instead of regions too close to the dorsal fin or the lateral line. Additionally, during preparation it is important to take time to carefully select the most similarly-looking scales from each sample. Stressing such simple practices will seem unnecessary to many field and lab technicians and implementing them will generally increase the time spent in collection and preparation of the scales; however, these will be largely rewarded with less reading time and an overall improvement of the age determinations.

The quality of final age determinations is highly dependent on the quality of the acetate imprints. Consequently, before acetate slides are stored into their final slide boxes it is good practice to carry out a preliminary check on the quality of the imprints. A well-prepared scale imprint presents well-resolved *circuli* (both at the center and periphery), clearly defined anterior and lateral margins, and no cracks (e.g., **Fig. 26, Fig. 27**). If that is not the case, the imprint should be considered unsatisfactory and pressing should be repeated in a new set of scales with readjusted press settings. In doing this, lower temperatures, shorter pressing times, and lower pressures will provide for lighter markings; however, if excessively low, they will also cause insufficient pressing of the margins and lead to imprints with heterogeneous appearance (**Fig. 29**). In contrast, higher temperatures, longer pressing times, and higher pressures will provide for stronger markings and clearer marginal contours; however, if excessively high, they will also cause cracks and/or blurred *circuli* thus troubling annuli interpretations (**Fig. 30**). In meagre, adequate pressing is particularly hard to achieve in larger and thicker scales, which frequently do not show up well-pressed at the first attempts. Consequently, particularly at the beginning of a study, it is important to collect a larger-than-average number of scales from the bigger fish (e.g., over 20 scales) in order to ensure that enough scales are available to obtain good imprints. Later on, with increased technician expertise, this number can generally be dropped down to the 10 to 15 scales typical of the routine collection protocol **(section 4.1**).

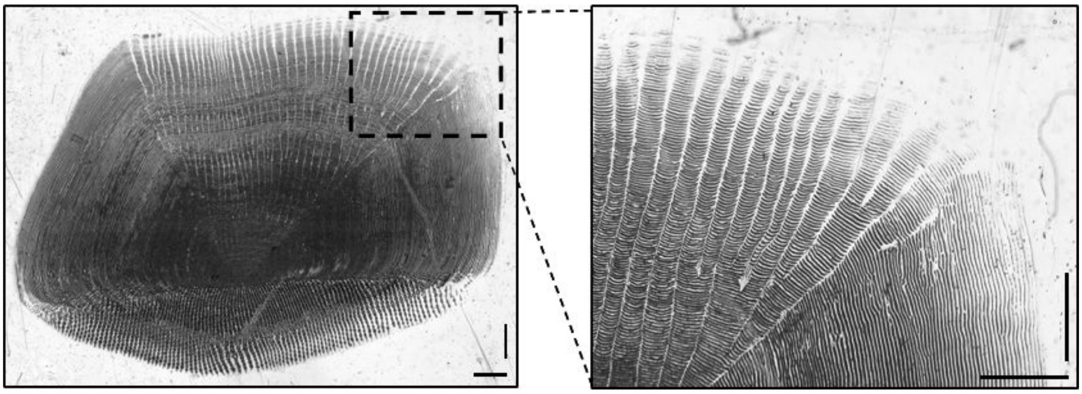

**Figure 29 –** Aspects of a badly-prepared scale imprint due to insufficient temperature, pressure and time. Scale bars = 1 mm, 16x.

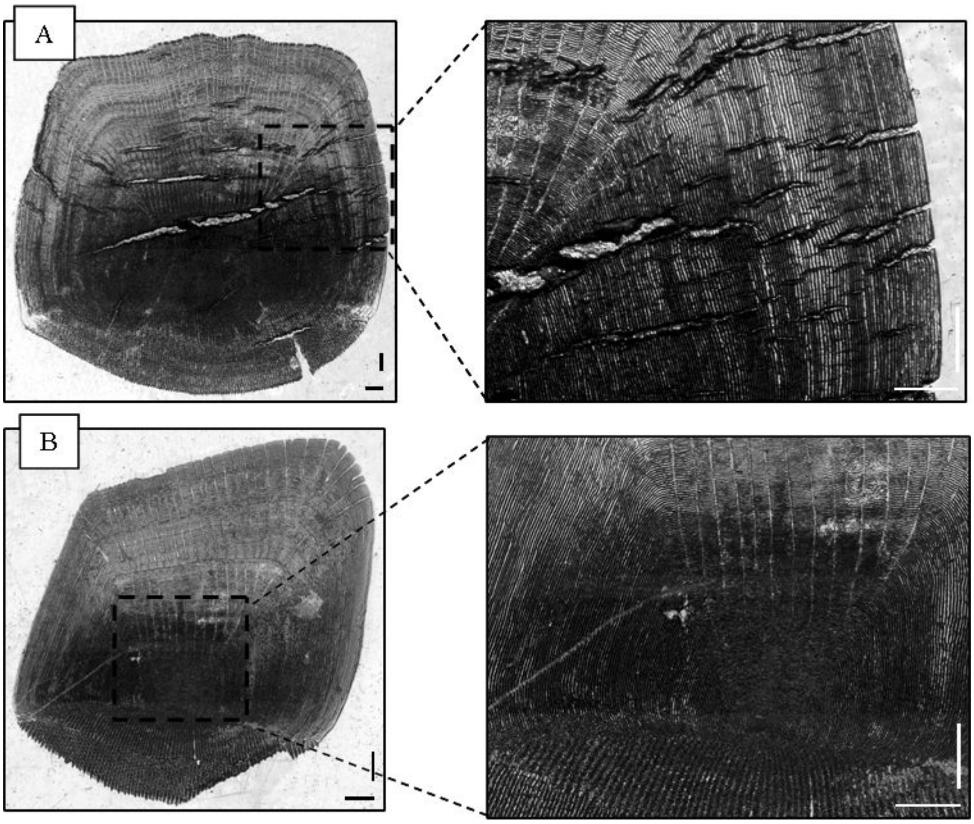

**Figure 30 –** Aspects of badly-prepared scale imprints due to excessive temperature, pressure and time. A – cracks, B – blurred center. Scale bars = 1 mm, 9x (A), 16x (B).

#### 4.3.5.2 Observation-related issues

The annuli of meagre scale imprints are searched for within a high-resolution grayscale image that bears hundreds of similarly looking concentric *circuli*. Analyses of this type of images are difficult and tiresome to the human eye making scale annuli counts susceptible to optical illusions and eye-weariness biases. Two simple practices may be adopted that reduce these negative impacts by aiding in pattern recognition, reducing eye weariness, and/or helping to maintain reader's motivation during scale readings. The first practice involves readers routinely alternating between focused and unfocused images, and between close-up observations (e.g., 30 cm from screen) and more distant observations (e.g., 1 m from screen), as doing this will help reveal obscure annuli and reduce effort and time spent in annuli search. The second practice involves readers taking frequent breaks during the reading sessions (e.g., every 1–1.5 hours) because this helps reducing eye weariness and sustaining reader's motivation, improving consistency across the usually long periods of exposure to microfiche reader illumination.

#### 4.3.5.3 Other issues

The major difficulties met in reading meagre scales cannot be directly avoided because they are related to the long life span of the fish, to the slow growth of its scales at older ages, and to the large thickness of the scales collected from older specimens. In fact, the annual scale increments of meagre older than 10 years are small (frequently less than 0.5 mm), which causes the most peripheral annuli to appear very compact ("crowded") near the scale margin. This crowding effect takes place throughout the whole anterior field of the scale and makes primary criteria like *circuli* compaction and *circuli* disruption difficult to evaluate. Additionally, the scales of older meagre tend to be very thick (commonly over 0.30 mm, up to 0.75 mm) making it particularly difficult to obtain good imprints (see **section 4.3.5.1**). Altogether, these aspects lead to increased subjectivity in the discrimination of scale annuli from younger to older fish. In fact, readers commonly report objective scale readings only up to the tenth annulus. Thereafter, scale annuli counts are deemed increasingly subjective and frequently found to underestimate otolith-derived ages up to a factor of 2, even if readers relax the application of the primary criteria and, e.g., begin to count every straightening-out region visible in the anterior field.

Even if the difficulties of reading older meagre scales cannot be avoided, the interpretations of scales of younger fish can be made precise and comparable to otolith-derived ages. However, for this to happen it is important that readers are aware of some specific patterns of the meagre scales. One such pattern is annulus splitting. Annulus splitting involves the branching of single annulus into two (or, more rarely, three) distinct branches and if unaccounted for can lead to an overestimation of the annuli counts of younger fish. Splitting generally takes place in the anterior field of the scale and shows up as a set of distinct straightening-out and compaction areas that are very close to one another and resemble different annuli (**Fig. 31**). However, a closer look at these putative annuli generally confirms that they are distinct branches of a single annulus that effectively rejoin in the lateral fields of the scale. Consequently, the only effective way to prevent the errors caused by undetected annulus splitting is through increased practice and training in the analysis of meagre scale patterns. In doing this, it is particularly important to ensure that the habits of systematically tracing annuli across the entire anterior region and systematically comparing the readings obtained from different axes are well-included into the reading routines.

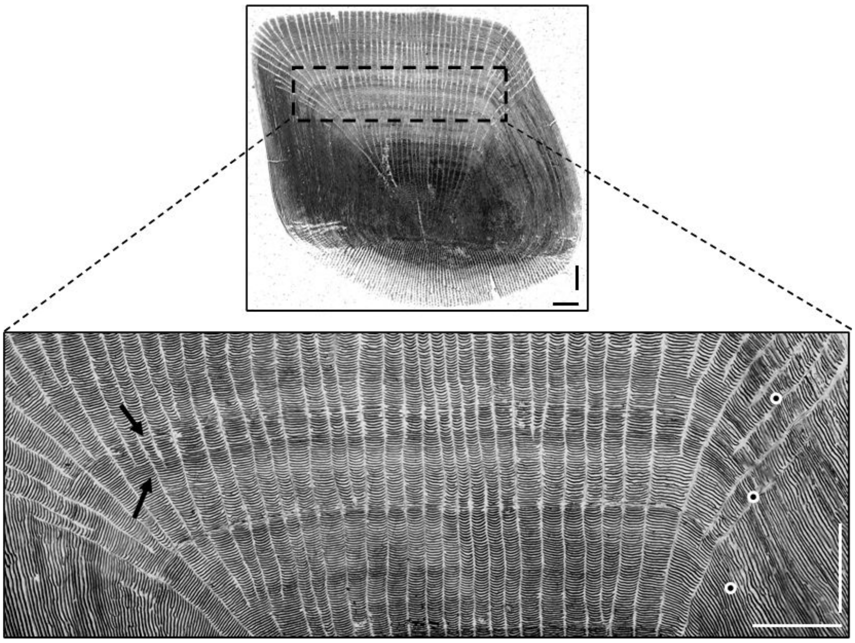

**Figure 31 –** Annulus splitting. The arrows indicate a doubled annulus. The white dots indicate the actual annuli. Scale bars = 1 mm, 16x (above), 30x (bottom).

To counteract the major difficulties felt in applying the primary criteria of annulus identification, a set of secondary (or corroboratory) criteria exists. These criteria are not to be used singly to assign annuli but along with the primary criteria have been found to improve the reliability of the scale interpretations, particularly when the primary criteria are not met by all annuli or along the entire course of every single annulus. The corroboratory criteria used in meagre readings can be broadly divided into a) criteria used to identify the first annulus and b) other criteria used in annulus identification:

a) Criteria used to identify the first annulus: The segmented *circuli* of the first annulus are frequently too narrow and compact for the straightening-out effect to be clearly observable. Additionally, the center portion of the thicker scales frequently appears dark and blurred, troubling annuli identification in that part of the scale. In such cases, three types of evidence have been found to aid in first annulus identification. The first evidence comes from the observation that the *circuli* compaction tends to be much larger before the first annulus than immediately after it, particularly in the anterior field. This difference in *circuli* compaction creates a contrast between the region located just before the first annulus and the region immediately after it that can be used to corroborate the annulus when primary criteria are not conclusive **(Fig. 32)**. The second evidence comes from a similar observation but in the posterior field of the scale, where the first annulus is frequently evidenced by a semicircular band of lighter *ctenii* that contrasts the darker appearance of more peripheral regions **(Fig. 32)**. Finally, it has also been observed that the first annulus is found along the anteroposterior axis of the scale at a distance of 1.3–2.6 mm from the focus (average: 1.9 mm). Consequently, taking some measurements along the anteroposterior axis is frequently useful in narrowing the region where *circuli* are inspected for primary criteria match.

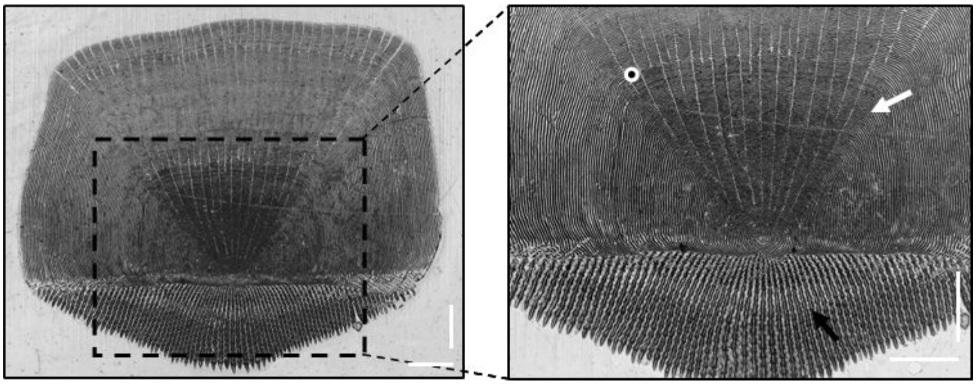

**Figure 32 –** Aspects of corroboratory evidence for the first annulus. The white dot indicates the first annulus. The white arrow indicates center compaction. The black arrow indicates the lighter posterior band. The first annulus is at a distance of 2.5 mm from the focus. Scale bars = 1 mm, 20x (left), 40x (right).

Other criteria used in annulus identification:

- "Dark margin": An annulus forming at the margin of the scale is generally difficult to ascertain because it is rarely observable along the whole anterior and lateral fields of the scale and because primary criteria like *circuli* "straightening out" or "*circuli* disruption" are hard to apply without comparing *circuli* appearance to more peripheral regions. In those cases, practice shows that if a) a large marginal increment is observed beyond the last clearly observed annuli, b) there are some signs of *circuli* straightening out along the anterior margin, and c) a very dark *circulus* can be seen outlining the lateral fields of the scale, then an annulus should be assigned to the scale margin (**Fig. 33**).

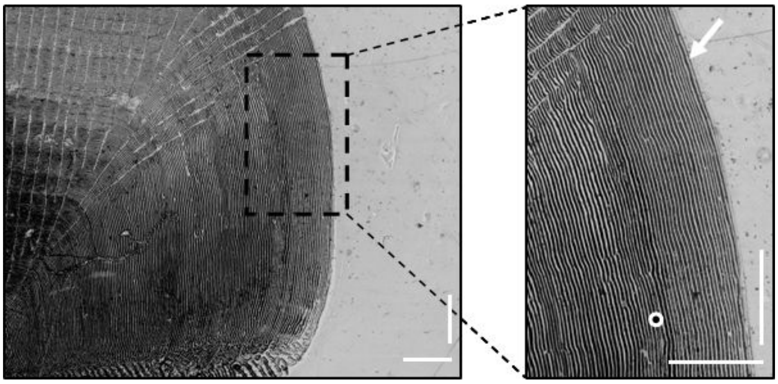

**Figure 33 –** Aspects of corroboratory evidence for marginal annulus. The white dot indicates the last clearly visible annulus. The white arrow points to the dark margin that evidences that a new annulus is just forming. Scale bars = 1 mm, 20x (left), 50x (right).

- "Annulus protrusion": At the anteroposterior interface, most continuous *circuli* halt their course and do not penetrate the posterior region of the scale. However, at an annulus they are frequently observed to protrude into the posterior field, traversing the interface as straight dark lines **(Fig. 34)**. Practice shows that such "annulus protrusion" into the posterior field is useful for corroborating intermediate annuli when primary criteria do not verify along the full extent of the anterior region of the scale.

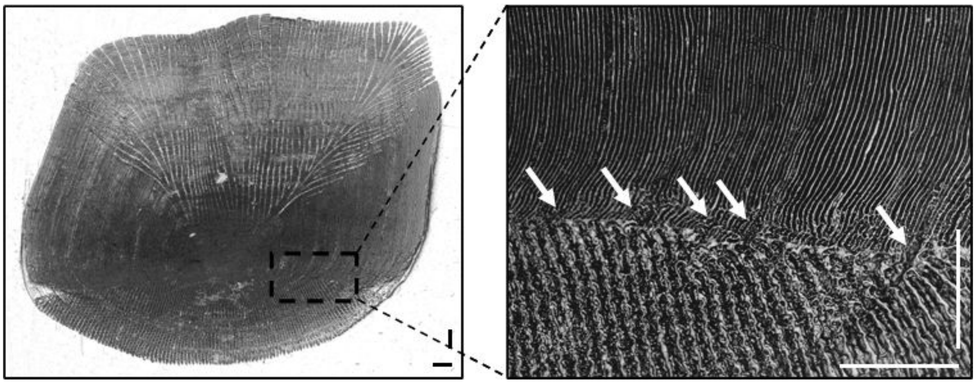

**Figure 34 –** Aspects of other corroboratory evidence for meagre annuli. The white arrows indicate annulus protrusion areas. Scale bars = 1 mm, 11x (left), 40x (right).

- Within-sample "scale regularities": Several regularities occur in the annuli structure of scale imprints taken from a single meagre specimen. The most important of these is that the imprints – even if presenting slightly different sizes and shapes – tend to have correlated interannuli distances and/or split annuli at similar locations. Consequently, when reading meagre scales it is good practice to start by taking an overall look at the several imprints to identify the main scale patterns before carrying out more detailed analyses on individual scales.

- Between-sample "scale regularities": The absolute annual growth of both meagre and its scales is very variable. Consequently, it is generally incorrect to assume that an annulus (other than the first) should sit at any specific distance from the scale focus. However, similar to other fish, there is an overall trend toward successively shorter interannuli distances from the center to the periphery of the scales. Considering this radial trend may be useful to corroborate some doubtful annuli interpretations: e.g., if a peripheral annuli is thought to sit very far from a previous one, it is probable that one or more annuli may have been missed; conversely, if a central annuli sits very close to a previous one, it is possible that it is a split branch and not a true annuli[6].

---

[6] Note: it is important to bear in mind that despite the long-term decrease in interannuli distances, large variability in interannuli distances still occurs in the short-term. An example of this is that, in meagre, it is not infrequent for the third annuli to be found very close to the second. For this reason, annuli corroboration based on interannuli distances should be used only in scales that bear at least 10 annuli and, particularly, never as a sole criterion to assign the most marginal annuli.

## 5. AGE ASSIGNMENT

The information required to determine the final age of meagre is: a) hard part reading data (annuli counts and marginal increment evaluations), b) stock-specific information (annulus deposition periods and spawning season), and c) sample-specific information (date of capture). In Portugal, marginal increment analyses indicate that otolith annuli are laid down from March to June and scale annuli are laid down from April to September (Costa *et al.*, 2008, N. Prista, *unpub. data*). Additionally, reproductive studies indicate that the meagre spawns from March to July with a peak in May and June (Costa *et al.*, 2008). Using this information, three types of age-related results can be calculated for each meagre specimen: age group (**section 5.1**), year class (**section 5.2**), and biological age (**section 5.3**).

### 5.1. Age group

The age group of a fish is the number of calendar years the fish lived until it was captured. To determine age group, data on hard part readings (annuli counts and marginal increment evaluation), on the month of capture, and on the annulus deposition season of the hard part under analysis are required. The meagre age groups are determined using January 1 as a standard birth date (i.e., a fish born in May 2000 will be assigned to age group 0 if captured until December 2000, to age group 1 if captured during 2001, to age group 2 if captured in 2002, and so on). The following procedure is used to determine age group:

- Fish captured between January 1 and the beginning of the annulus deposition season: The fish are generally assigned an age notation of $x + (x + 1)$, where $x$ is the number of annuli in the otolith or scale. Their age group is $x + 1$.

  Examples (Portuguese coast):

  Otoliths: Any fish captured between January 1st and February 28/29th with three annuli and a translucent margin (type II/III), should be assigned an age notation 3 + (3 + 1), i.e., 3 + 4. The fish is age group 4.

  Scales: Any fish captured between January 1st and March 31st with five annuli and type II/III margin, should be assigned an age notation 5 + (5 + 1), i.e., 5 + 6. The fish is age group 6.

- Fish captured between the end of the annulus deposition season and the end of the year: The fish are generally assigned an age notation of $x + x$, where $x$ is the number of annuli counted in the otolith or scale. Their age group is $x$.

Examples (Portuguese coast):

<u>Otoliths</u>: Any fish captured between July 1st and December 31st with three annuli and a translucent margin (type II/III), should be assigned an age notation of 3 + 3. The fish is age group 3.

<u>Scales</u>: Any fish captured between October 1st and December 31st with five annuli and a type II/III margin, should be assigned an age notation 5 + 5. The fish is age group 5.

- <u>Fish captured during the annulus deposition season:</u> The fish are assigned an age notation $x$ ($x$), $x + x$, or $x + (x + 1)$, depending on the development of the hard-part margin: if an annulus is visible at the margin (type I), the notation is $x$ ($x$) and the fish age group is $x$; if little growth has taken place beyond it (type II), the notation is $x + x$ and the fish age group is $x$; if substantial growth is visible beyond the last annulus (type III) the notation is $x + (x + 1)$ and the fish age group is $x + 1$.

Examples (Portuguese coast):

<u>Otoliths</u>: A fish captured between March 1st and June 30th with three annuli: if the last annulus is on the edge (type I) or there is little growth beyond it (type II) the fish should be assigned an age notation 3 (3) or 3 + 3, respectively, and belongs to age group 3; if, however, significant growth occurred beyond the last annulus or a new annulus is anticipated to be forming soon (type III) the fish should be assigned an age notation 3 + 4 and belongs to age group 4.

<u>Scales</u>: A fish captured between April 1st and September 30th with five annuli: if the last annulus is on the edge (type I) or there is little growth beyond it (type II) the fish should be assigned an age notation 5 (5) or 5 + 5, respectively, and belongs to age group 5; if, however, significant growth occurred beyond the last annulus and a new annulus is anticipated to be forming soon (type III) the fish would be assigned an age notation 5 + 6 and belongs to age group 6.

### 5.2. Year class

The year class is the year when the fish was born (e.g., 1997 year class). Year class ($YC$) is calculated as $YC = CY - AG$, where $CY$ is the year of capture and $AG$ is the age group:

Example: a fish captured in 2004 and age group 3 is from the 2004 – 3 = 2001 year class.

### 5.3. Biological age

Biological age is the time elapsed from fish birth to fish capture. To determine biological age, information on the fish age group and capture date is required. Furthermore, it is necessary to assume a common birthday for all fish in the stock (June 1 in Portuguese waters). Biological age ($BA$) is generally expressed in months and calculated as $BA = 12 \text{ x } AG - (BD - CD)$, where $AG$ is the age group, $BD$ is the month of birth and $CD$ is the month of capture, with minor corrections being needed only in larval fish:

Example (Portuguese coast):

A fish belonging to age group 4 and captured in February is 12 x 4 – (6 – 2) = 44 months.

## 5.4. Examples

In **Table III**, readings and final age assignments are presented for all otolith sections and scales imprints displayed in the current study.

**Table III** – Full set of readings and age assignments of the otolith and scales depicted in sections 3 and 4. Fish total length is provided for indicative purposes.

| Figure | Specimen ID | Total length (cm) | Struct. | Date of capture | Annuli | Margin | Age notation | Age group (years) | Year class | Biological age (month) |
|---|---|---|---|---|---|---|---|---|---|---|
| 6–8, 12C | CORV_0194 | 92 | Otolith | 01-07-2005 | 5 | II | 5+5 | 5 | 2000 | 61 |
| 9A | CORV_1846 | 17 | Otolith | 03-09-2003 | 0 | III | 0+0 | 0 | 2003 | 3 |
| 9B | CORV_0913 | 41 | Otolith | 10-01-2005 | 1 | III | 1+2 | 2 | 2003 | 19 |
| 9C | CORV_0123 | 61 | Otolith | 23-08-2004 | 2 | II | 2+2 | 2 | 2002 | 26 |
| 9D | CORV_1769 | 41 | Otolith | 20-06-2006 | 3 | II | 3+3 | 3 | 2003 | 36 |
| 10A | CORV_0334 | 157 | Otolith | 01-08-2005 | 14 | II | 14+14 | 14 | 1991 | 170 |
| 10B | CORV_0216 | 182 | Otolith | 13-10-2005 | 36 | II | 36+36 | 36 | 1969 | 436 |
| 11A, 13 | CORV_0072 | 35 | Otolith | 07-03-2002 | 2 | I | 2 (2) | 2 | 2000 | 20 |
| 11B | CORV_1672 | 59 | Otolith | 19-07-2006 | 2 | II | 2+2 | 2 | 2004 | 25 |
| 11C, 11D | CORV_1401 | 81 | Otolith | 16-02-2006 | 2 | III | 2+3 | 3 | 2003 | 32 |
| 12A | CORV_0257 | 126 | Otolith | 17-08-2005 | 8 | II | 8+8 | 8 | 1997 | 98 |
| 12B | CORV_0188 | 152 | Otolith | 13-10-2005 | 12 | III | 12+12 | 12 | 1993 | 148 |
| 14 | CORV_0092 | 38 | Otolith | 11-01-2005 | 1 | III | 1+2 | 2 | 2003 | 19 |
| 15 | CORV_1658 | 86 | Otolith | 15-05-2006 | 3 | III | 3+4 | 4 | 2002 | 47 |
| 16 | CORV_1657 | 51 | Otolith | 15-05-2006 | 1 | III (a) | 1+2 | 2 | 2004 | 23 |
| 17 | CORV_1231 | 148 | Otolith | 09-07-2005 | 13 | II | 13+13 | 13 | 1992 | 157 |
| 18B | CORV_0435 | 161 | Otolith | 20-06-2005 | 13 | II | 13+13 | 13 | 1992 | 156 |
| 20 | CORV_0749 | 39 | Scale | 18-04-2005 | 1 | III | 1+2 | 2 | 2003 | 22 |
| 22–25, 28B, 28D | CORV_1764 | 53 | Scale | 20-06-2006 | 3 | II | 3+3 | 3 | 2003 | 36 |
| 26A | CORV_0607 | 22 | Scale | 28-10-2000 | 0 | III | 0+0 | 0 | 2000 | 4 |
| 26B | CORV_0768 | 42 | Scale | 13-10-2004 | 1 | III | 1+1 | 1 | 2003 | 16 |
| 26C | CORV_0599 | 59 | Scale | 13-08-2004 | 2 | II | 2+2 | 2 | 2002 | 26 |
| 26D | CORV_1772 | 51 | Scale | 20-06-2006 | 3 | II | 3+3 | 3 | 2003 | 36 |
| 27A, 34 | CORV_0024 | 144 | Scale | 19-06-2004 | 9 | II | 9+9 | 9 | 1995 | 108 |
| 27B | CORV_0334 | 157 | Scale | 01-08-2005 | 13 | III | 13+14 | 14 | 1991 | 170 |
| 28A | CORV_0843 | 71 | Scale | 23-08-2004 | 4 | I | 4 (4) | 4 | 2000 | 50 |
| 28C | CORV_0064 | 79 | Scale | 06-06-2004 | 3 | III | 3+4 | 4 | 2000 | 48 |
| 29 | CORV_1203 | 100 | Scale | 25-04-2005 | 4 | III | 4+5 | 5 | 2000 | 58 |
| 30A | CORV_0004 | 162 | Scale | 06-08-2005 | 12 | II | 12+12 | 12 | 1993 | 146 |
| 30B | CORV_0368 | 109 | Scale | 08-11-2005 | 4 | II | 4+4 | 4 | 2001 | 53 |
| 31 | CORV_1446 | 95 | Scale | 17-05-2006 | 5 | III | 5+6 | 6 | 2000 | 71 |
| 32 | CORV_0036 | 61 | Scale | 22-08-2004 | 2 | II | 2+2 | 2 | 2002 | 26 |
| 33 | CORV_0338 | 111 | Scale | 10-08-2005 | 6 | I | 6 (6) | 6 | 1999 | 74 |

(a) Note: the observation plane does not account for parallax errors making the section look like a margin I.

## 6. DISCUSSION

The European meagre has recently become the focus of increased scientific attention. On the one hand, the species is considered a promising species for European aquaculture (Angelini *et al.*, 2002; Quéméner *et al.*, 2002) and there has been increased interest in studying the biology of its wild populations to optimize aquaculture production (Quéméner, 2002; Jiménez *et al.*, 2005; Costa *et al.*, 2008); on the other hand, some concerns have been raised on the conservation status of the meagre populations in France, Spain, and Portugal, which have sparked research on the meagre fisheries and population parameters (Quéméner, 2002; Muñoz *et al.*, 2006; Prista *et al.*, 2007; Costa *et al.*, 2008; Prista *et al.*, 2008). Concomitantly, appendix VII of Council Decision 2008/949/EC recently established minimum age-sampling requirements for the European meagre (50 fish per 1000 t landed) which, given the geographic distribution of the species, will prompt routine sampling of commercial meagre landings along ICES Subareas XIa and VIIIa–c. Similar efforts and concerns have also taken place in Northern Africa – namely in Egypt, Mauritania, Senegal, and Morocco – where the species constitutes a more significant resource for local economies and also represents a promising candidate for aquaculture production (Hermas, 1995; Bebars *et al.*, 1997; Quéméner, 2002; El-Shebly, 2007). Altogether, these aspects make relevant the existence of validated standardized protocols for age determination of the species as only these will provide the quality and comparability of results required for progress in research, assessment and management at both national and international levels. The otolith and scale protocols presented in this study constitute a first step towards that faster progress as they detail the procedures involved in the collection and preparation of the meagre hard parts, specify the criteria used in the readings, and highlight many aspects and difficulties that should be addressed in reader's training across the species geographical range.

Sampling meagre hard parts for age and growth determination is a difficult task. In Europe, adult meagres are absent or rare in most marine fishery-independent surveys (Quéro and Vayne, 1987, Fátima Cardador, INRB/IPIMAR, *pers. comm.*) and commercial landings are low, seasonal, and size specific (Quéméner, 2002; Prista *et al.*, 2008). Additionally, the meagre is marketed round at local ports (i.e., neither beheaded nor gilled or gutted) and presents a high commercial value (large specimens may cost over 400 € ex-vessel) which makes otolith collection expensive and scale collection a delicate task (Quéméner, 2002; Prista *et al.*, 2007). Such situations markedly contrast those of other large sciaenids in, e.g., the Eastern United States, whose carcasses (and body parts) can be obtained from commercial and recreational fisheries at relatively low cost (Liao *et al.*, 2008); they also largely justify the comparative scarcity of meagre age and growth research in European waters and the need to consider alternative sampling techniques and alternative hard parts in determining the age of European meagre.

In Portugal, Prista *et al.* (2007) have shown that it is possible to obtain representative samples of meagre otoliths from the fishery, at low cost, by means of commercial mark-recapture. However, given to the large geographical extent of the Portuguese meagre fisheries (Prista *et al.*, 2008) and the large size range of its landings (42–184 cm), it is unlikely that commercial mark-recapture can solely provide for routine long-term samples unless the meagre fishery becomes a management priority. Scales have long been used in fish age determination for an array of reasons, including the fact that they are easier to collect, can be collected without jeopardizing the fish commercial value, and generally present lower costs and preparation times than otolith thin sections (VanderKooy and Guindon-Tisdel, 2003). However, unlike otoliths, fish scales suffer from regeneration, erosion, or resorption, all of which complicate and bias age interpretations (Ericksen, 1999; VanderKooy and Guindon-Tisdel, 2003). Additionally, scale patterns are in general much harder to interpret (Lowerre-Barbieri *et al.*, 1994; Liao *et al.*, 2008) and much more prone to underage older fish than otolith thin sections (Lowerre-Barbieri *et al.*, 1994; Panfilli *et al.*, 2002; VanderKooy and Guindon-Tisdel, 2003). The latter is the case of scales from meagre and other long-lived species (e.g., striped bass *Morone saxatilis*), where annuli must be searched for within visually complex *circuli* patterns, and where substantial annuli crowding takes place at the periphery of older scales (Lowerre-Barbieri *et al.*, 1994; Liao *et al.*, 2008). However, even if suboptimal, scales may be worth considering in the sampling of, at least, some segments of the fishery and/or size classes.

A detailed comparison between scales and otoliths as hard parts used for meagre age determination was beyond the objectives of the current study and will be addressed elsewhere. Otolith thin sections are indubitably the most valid and effective method of determining sciaenid ages across the entire size range of the species (Beckman *et al.*, 1989; Lowerre-Barbieri *et al.*, 1994; Griffiths and Hecht, 1995; Campana and Jones, 1998; VanderKooy and Guindon-Tisdel, 2003; Liao *et al.*, 2008). However, readings of scale imprints have generally been considered sufficiently reliable to age the younger fish of the stock (Matlock *et al.*, 1993; Lowerre-Barbieri *et al.*, 1994). In meagre, even if more expensive to sample and time consuming to prepare, otolith thin sections should also be preferred to scale acetate imprints on basis of their easier interpretation and better performance in older fish. However, scales may constitute a valid alternative for meagre age determination if the research or assessment context involves samples composed of smaller fish (e.g., recruitment studies). In fact, a reasonable agreement (>90%) between otolith and scale readings is generally observed in fish younger than 4 years and smaller than 60 cm, even if underestimations of over 10 annuli are common in fish older than 10 years and larger than 160 cm (N. Prista, *unpub. data*).

In the current study, we obtained transverse thin sections of meagre otoliths and observed them under transmitted light without further processing. In doing this, we adapted the standard protocols used to determine sciaenid ages in the Eastern USA (Beckman *et al.*, 1989; Lowerre-Barbieri *et al.*, 1994; Campana and Jones, 1998; VanderKooy and Guindon-Tisdel, 2003; Liao *et al.*, 2008), but departed from other existing studies on meagre (Tixerant, 1974; that used the break-and-burn technique and reflected light) and other *Argyrosomus* (e.g., Griffiths and Hecht, 1995; that used longitudinal thin sections and reflected light). These departures were motivated by preliminary analyses carried out on different preparation procedures, where aspects such as sectioning speed (low speed vs. high speed), sectioning plane (longitudinal vs. transverse), and postsectioning enhancement procedures (polishing and baking) were examined in terms of the relative improvement they brought to the readability and processing times of the otolith thin sections (N. Prista, *unpub. data*). The outcomes of these analyses indicated that, even if lower-speed single-otolith setups presented longer sectioning times (e.g., when compared to high-speed multiple-otolith setups), they provided for a better control of the sectioning plane and yielded easier to interpret sections that required no postsectioning enhancement. Additionally, they also indicated that readings obtained from longitudinal and transverse sections were alike and, consequently, that no readability advantage occurred in longitudinal sections that could justify the longer times they take to prepare. Quite on the contrary, it was found that transverse sectioning, when properly carried out, actually minimized the *ostium* blotch which is found limiting the interpretation of longitudinal sections (e.g., Griffiths and Hecht, 1995) thus providing an improvement to the overall readability of the otolith thin sections.

## 7. ACKNOWLEGMENTS

The authors would like to thank all fishers and fish merchants involved in the collection of meagre otoliths and scales. Thanks are also due to João Calado and several FCUL students that actively collaborated in fish sampling, and to Catarina Moreira and Sónia Gabriel that assisted in capturing the pictures in section 3.1. Additional thanks are due to CQFE and its personnel, namely Eric Robbilard and Karen Underkoffler (for initial contributions on hard part preparation procedures), Srujan Baddam and Billy Culver (for processing hard parts), Christina Morgan (for comments on an earlier draft of the manuscript and age interpretation criteria), and Joey Ballenger (for comments on age interpretation criteria and collaboration in hard part readings). We also thank Rafael Duarte, António Marçal, and the INRB/IPIMAR for their collaboration in high-speed saw tests; Nuno Branco and the Arquivo Nacional da Torre do Tombo for their collaboration in the capture of scale images; and Carlos Assis for his collaboration in the capture of otolith images. Finally we thank Eduardo Soares, Fátima Cardador, and Maria Manuel Martins for their reviews and inputs on the final draft. Funding for the present work was provided by FCT grant BD/12550/2003 to N. Prista, research project CORV (DGPA-MARE: 22-05-01-FDR-00036) to M. J. Costa, and research project VMRC-F1-126-R-5 to C. M. Jones.

## 8. REFERENCES

ANGELINI, M.; GILMOZZI, M.; LANARI, D.; GIORGI, G., 2002. Allevamento dell'ombrina bocca d'oro, *Argyrosomus regius* (Asso). *In:* D. LANARI, E. TIBALDI (Eds.), *Ricerca per lo sviluppo dell'acquacoltura toscana. Risultati conseguiti.* ARSIA Pubblicazione Speciale, Associazione Piscicoltori Italiani, Verona, Italy, pp. 13–38.

ASSIS, C. A. S., 2000. Estudo morfológico dos otólitos *sagitta*, *asteriscus* e *lapillus* de Teleósteos (*Actinopterygii*, *Teleostei*) de Portugal Continental. Sua aplicação em estudos de filogenia, sistemática e ecologia. PhD Thesis, Faculdade de Ciências da Universidade de Lisboa, 1004 p.

BEBARS, M. I.; LASSERRE, G.; LAM HOAI, T., 1997. Analyse des captures des pêcheries marines et lagunaires d'Egypte en liaison avec la construction du haut barrage d'Assouan. *Oceanologica Acta* 20: 421–436.

BECKMAN, W. D.; WILSON, C. A.; STANLEY, A. L., 1989. Age and growth of red drum, *Sciaenops ocellatus*, from offshore waters of the northern Gulf of Mexico. *Fishery Bulletin* 87: 17–28.

CAMPANA, S. E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* 59: 197–242.

CAMPANA, S. E.; JONES, C. M., 1998. Radiocarbon from nuclear testing applied to age validation of black drum, *Pogonias cromis*. *Fishery Bulletin* 96: 185–192.

COSTA, M. J.; CABRAL, H.; COSTA, J. L.; PRISTA, N.; LOPES, J. C., 2008. Corvina-legítima *Argyrosomus regius* – Dados biológicos para a gestão e produção aquícola de um recurso". Final Report, DGPA-MARE: 22-05-01-FDR-00036, Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, 125p.

EL-SHEBLY, A. A.; EL-KADY, M. A. H.; HUSSIN, A. B.; HOSSAIN, M. Y., 2007. Preliminary observations on pond culture of meagre, *Argyrosomus regius* (Asso, 1801) (Sciaenidae) in Egypt. *Journal of Fisheries and Aquatic Science*, 2: 345–352.

ERICKSEN, R. P., 1999. Scale Aging Manual for Coastal Cutthroat Trout from Southeast Alaska. Special Publication 99–4, Alaska Department of Fish and Game, Anchorage, Alaska, USA, 44p.

FAO, 2009. Capture Production 1950–2007. FISHSTAT Plus: Universal software for fishery statistical time series. Version 2.3. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FAO, Rome, Italy.

GRIFFITHS, M. H.; HECHT, T., 1995. Age and growth of South African dusky kob *Argyrosomus japonicus* (Sciaenidae) based on otoliths. *South African Journal of Marine Science*, 16: 119–128.

HADDON, M., 2001. Modelling and Quantitative Methods in Fisheries. Chapman and Hall, Boca Raton, Florida, USA, 406p.

HERMAS, J., 1995. Contribuition à l'étude des débarquements et de la biologie du maigre *Argyrosomus regius* (Asso, 1801) au port d'Agadir. PhD Thesis, Université Ibnou Zohr, Morocco, 113p.

JIMÉNEZ, M. T.; PASTOR, E.; GRAU, A.; ALCONCHEL, J. I.; CÁRDENAS, S., 2005. Revisión sobre el cultivo de esciénidos en el mundo y presentación del Plan Nacional de Cría de corvina (*Argyrosomus regius*). X Congreso Nacional de Acuicultura, Gandia, Spain.

LIAO, H.; JONES, C. M.; MORGAN, C.; BALLENGER, J. C., 2008. Virginia – Chesapeake Bay Finfish Ageing in 2007. Final Report to Virginia Marine Resources Commission. Center for Quantitative Fisheries Ecology, Old Dominium University, Norfolk, Virginia, USA, 122p.

LOWERRE-BARBIERI, S. K.; CHITTENDEN Jr., M. E.; JONES, C. M., 1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. *Fishery Bulletin*, 92: 555–568.

MATLOCK, G. C.; COLURA, R. L.; McEACHRON, L. W., 1993. Direct validation of black drum (*Pogonias cromis*) ages determined from scales. *Fishery Bulletin*, 91: 558–563.

MORISON, A. K.; BURNETT, J.; MCCURDY, W. J.; MOKSNESS, E., 2005. Quality issues on the use of otoliths for fish age estimation. *Marine and Freshwater Research*, 56: 773–782.

MUÑOZ, J. L.; BRUZÓN, M. A.; JIMÉNEZ, M. A.; ZURITA, F.; GARCÍA, M. M.; CATALÁN, I. A.; ALCONCHEL, J. I.; PÉREZ, F., 2006. Contribución al estudio comparado de las tallas mínimas de seis especies de peces y crustáceos (Mediterrâneo-Atlántico). *In:* J. A. HERNANDO, J. C. MANZANO (Eds.), *Avances en la Investigación Pesquera y Acuícola en el Âmbito del Estrecho de Gibraltar*, Observatório de Pesca Andalucía–Marruecos, Facultad de Ciências del Mar y Ambientales, Universidad de Cádiz, Puerto Real, Spain, pp. 97–189.

PANFILI, J.; DE PONTUAL, H.; TROADEC, H.; WRIGHT, P. J. (Eds.), 2002. Manual of Fish Sclerochronology. Ifremer-IRD, Plouzané, France, 464p.

PENTILLA, J.; DERY, L. M. (Eds.), 1988. Age Determination Methods for Northwest Atlantic Species. NOAA Technical Report NMFS 72, U.S. Department of Commerce, Seattle, Washington, USA, 135p.

PRISTA, N.; COSTA, J. L.; COSTA, M. J.; JONES, C. M., 2007. New methodology for studying large valuable fish in data poor situations: commercial mark-recapture of meagre *Argyrosomus regius* in the southern coast of Portugal. ICES CM 2007/O:43, ICES, 18p.

PRISTA, N.; JONES, C. M.; COSTA, J. L.; COSTA, M. J., 2008. Inferring fish movements from small-scale fisheries data: the case of *Argyrosomus regius* (Sciaenidae) in Portugal. ICES CM/K:19, ICES, 19p.

QUÉMÉNER, L., 2002. Le Maigre Commun (*Argyrosomus regius*). Biologie, Pêche, Marché et Potentiel Aquacole. Ifremer, Plouzané, France, 31p.

QUÉMÉNER, L.; SUQUET, M.; MERO, D.; GAIGNON, J.-L., 2002. Selection method of new candidates for finfish aquaculture: the case of the French Atlantic, the Channel and the North Sea Coasts. *Aquatic Living Resources*, 15: 293–302.

QUÉRO, J. -C.; VAYNE, J. -J., 1987. Le maigre, *Argyrosomus regius* (Asso, 1801) (Pisces, Perciformes, Sciaenidae) du Golfe de Gascogne et des eaux plus septentrionales. *Revue des Travaux de l'Institut des Peches Maritimes*, 49: 35–66.

SCHWARZHANS, W., 1993. A Comparative Morphological Treatise of Recent and Fossil Otoliths of the Family Sciaenidae (Perciformes). Verlag Dr. Friedrich Pfeil, Munich, Germany, 245p.

SILVA, L.; GIL, J.; SOBRINO, I., 2002. Definition of fleet components in the Spanish artisanal fishery of the Gulf of Cádiz (SW Spain ICES division IXa). *Fisheries Research*, 59: 117–128.

SPARRE, P.; VENEMA, S. C., 1998. Introduction to Tropical Fish Stock Assessment. *FAO Fisheries Technical Paper*, 306.1, Rev. 2, FAO, Rome, 407p.

TIXERANT, G., 1974. Contribuition à l'étude de la biologie du maigre ou courbine sur la côte Mauritanienne. PhD Thesis, Université d'Aix–Marseille, France, 123p.

VANDERKOOY, S.; GUINDON-TISDEL, K. (Eds.), 2003. A practical handbook for determining the ages of Gulf of Mexico Fishes. Gulf States Marine Fisheries Commission. Publication Number 11, Ocean Springs, Mississippi, USA, 125p.