

# Verification of first annulus formation in the illicia and otoliths of white anglerfish, *Lophius piscatorius* using otolith microstructure

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Microincrements in the lapilli otoliths of *Lophius piscatorius* L. were used to validate the age of fish <27 cm TL collected in spring and autumn. Contrary to published estimates of size at age based on illicia by Dupouy *et al.* (1986) and Duarte *et al.* (1997), these small specimens were found to be either 0- or 1-group. Comparison between the macrostructures of illicia and sagittae indicated that a translucent zone is formed in the illicia of most 0-group by autumn but not in sagittae. Hence, earlier estimates of small size at age may have arisen from incorrect interpretation of the time of first translucent zone formation in illicia. In addition to enabling age validation, microstructure provided a means of distinguishing between the translucent material that forms false rings and annuli. Annuli appeared as a sequence of gradually declining increment widths whereas false rings were characterized by an abrupt check in otolith formation followed by increments similar in width to adjacent opaque material. The implications of these findings to age reading and interpretation are discussed.

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## Introduction

In the last two decades, anglerfishes of the genus *Lophius* have become the subject of an important by-catch and target fishery in the North-East Atlantic (Hislop *et al.*, 2001). Although two species, *Lophius budegassa* Spinola and *Lophius piscatorius* Linnaeus coexist over the greater part of the North-East Atlantic (Caruso 1983), the latter predominates north of latitude 55°N in ICES Sub-area IV (North Sea) and Division VIa (West of Scotland) (Hislop *et al.*, 2001). Whilst this species can reach a length of over 150 cm, fish as small as 6 cm are caught as a by-catch of non-directed fisheries.

Attempts to develop an effective stock management strategy have been hindered by a lack of knowledge of the basic biology of anglerfishes. Collection of reliable information on the numbers at age has been made difficult by uncertainty in age estimation. Two calcified

structures are used routinely to estimate age in *Lophius*, the sagittae and the illicium. The latter is formed from the first dorsal finray, and is adapted to act as a “fishing lure”. Whilst sagittae are used for age estimation in most demersal fish species, those of *Lophius* appear particularly difficult to interpret because of multiple ring structures (Tsimenidis and Ondrias 1980; Griffiths and Hecht 1986; Crozier 1989). Several authors have argued that illicia provide a more reliable means of age estimation in *Lophius* because they exhibit fewer secondary growth structures than sagittae (Dupouy *et al.*, 1986; Maartens *et al.*, 1999). However, published growth estimates derived from the two structures differ, particularly with regard to the first few years of life. Attempts to verify the periodicity of increments in either illicia (Dupouy *et al.*, 1986) or sagittae (Crozier 1989) using edge state analysis have not explained the difference in the estimated growth rate between these two structures. Interpretation

is clearly a problem as some readers regard the initial annulus in illicia as a benthic ring, associated with settlement, although no evidence for this interpretation has been given (Anon 1999)<sup>1</sup>.

There is now considerable evidence that microstructural observations can assist in the interpretation of otolith macrostructure. Counts of microscopic daily increments have been used to directly verify that one opaque and translucent zone represents an annulus (Taubert and Tranquilli 1982; Victor and Brothers 1982; Waldron 1994, 1998). Changes in microstructure may also provide a means of distinguishing between seasonal zonations and secondary structures. For example, in temperate species daily deposition can be very compressed or arrested in cold periods (Taubert and Coble 1977; Marshall and Parker 1982). Using light microscopy, an annulus often appears as a discontinuity preceded by increasingly narrow increments (Victor and Brothers 1982). The use of otolith microstructure to study events in the early life history of anglerfish has been described in a previous study (Hislop *et al.*, 2001). In the present study we use microincrements in lapilli otoliths that were first described in Hislop *et al.* (2001) to estimate age and investigate the nature of annuli and secondary structures in the sagittae and illicia of juveniles <30 cm in length.

## Methods

### Sampling

Juvenile anglerfish between 16 and 27 cm total length were collected from routine research vessel surveys in the North Sea and ICES Division VIa during April 1999 (n=8), March 2000 (n=5) and September 1999 (n=7) and 2000 (n=17). Samples were obtained from both bottom otter trawl and scallop dredge. The total length of the fish was measured at sea, whilst the sagittae and lapilli were extracted and the illicia removed on return to the laboratory.

### Preparation of calcified structures

Microincrements are only present in otoliths and it is difficult to prepare sagittae because of their multiple planes of growth. Consequently, we used the lapilli to investigate the formation of the first annulus. Lapilli were mounted onto glass slides using a methacrylate adhesive (Loctite Glass Bond), and ground with a 2500 grit metallographic grinding paper disc lubricated with 1 µm alumina slurry, using a lapping wheel. After grinding, lapilli were cleaned in 70% ethanol and cleared in immersion oil before viewing. Increments were counted

at × 1000 magnification along the axis demonstrating the most unambiguous incremental sequence, from primordium to the outer edge. Increment counts were repeated at least 3 times, and the median value used to represent age, reducing the influence of single aberrant counts. Increment counts were cross-checked between two experienced readers and between-reader differences were <10% of counts. We assumed that the first increment was formed on the day of hatching. Illicia were soaked in water and any adherent soft tissue was rubbed off. Both illicia and sagittae were embedded in Trylon Clear Embedding resin. A transverse section, 0.8 mm thick, was cut with a Buehler slow speed saw fitted with double blades at 150 rpm. The illicia were cut at a right angle to where the stem thins approximately 1–5 mm above the basal bulb (see Duarte *et al.*, 1997). Once sectioned, they were viewed with transmitted light and read at × 67 magnification. Sagittae were cut along the sagittal plane and the sections were polished with a commercial polishing fluid (Silvo) and then mounted using Eukitt. The sagittae were viewed with reflected light at × 34 magnification.

### Measurements of calcified structures

An annulus is one of a series of concentric zones on a structure that may be interpreted in terms of age (Kalish *et al.*, 1995). Two zones are formed within a year, the opaque and translucent, the former restricts the passage of light when compared with the latter. The opaque zone is generally associated with a period of high growth rate. Different terminology has been used for the zones found in otolith and bone, although in general the translucent annulus found in illicia corresponds to that of the translucent zone in otoliths. Therefore, in this paper we use the term translucent zone for both structures.

The frequency and width of zones along a fixed radius was compared between the three calcified structures using TNPC 3.1 Visilog software (Noesis Ltd). All otolith measurements were conducted on the sagittal plane along the maximum radius from core to rostral region. Figure 1 illustrates the zones and radii along which measurements were taken from the otoliths and illicia from the same fish. In order to compare the frequency and width of zones between structures all measurements were standardized relative to the radius of the structure measured. A sign rank test was used to test for individual differences in the frequency of zones between structures. Microincrements were used to estimate age, hatch date, age and date at translucent zone formation and the pelagic-demersal transition. The location of opaque and translucent material at the microstructural level was distinguished by alternating illumination between reflected and transmitted light. Differences in accretion rate during the formation of

<sup>1</sup>Anon 1999. 3rd International Ageing Workshop on European Anglerfish. IPIMAR, Lisbon, 8–12 March, 1–24+ Annexes. Unpublished Manuscript.

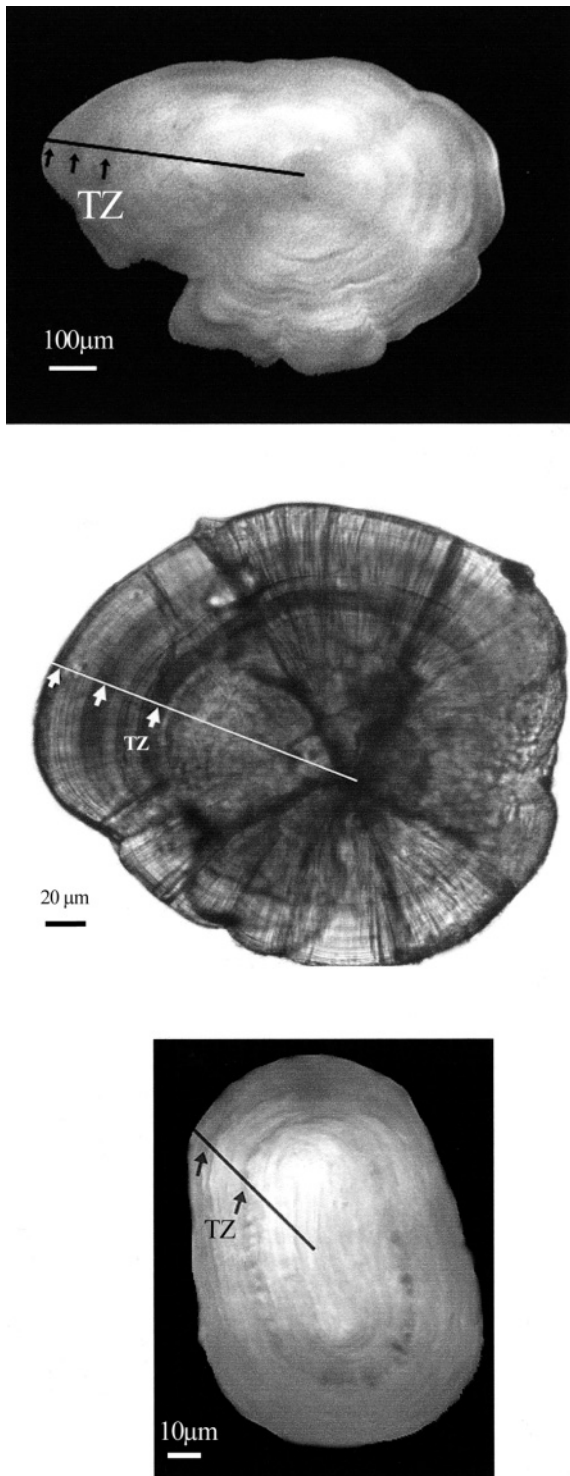


Figure 1. Photographs of the three calcified structures used in the present study, showing the translucent zones (TZ) measured (arrows). (A) Sagitta viewed with reflected light. (B) lapilli viewed with transmitted light. (C) Illicia viewed with transmitted light. All structures are from the same individual.

opaque and translucent material were examined from measurements of average daily increment width for five increment sequences.

## Results

### Comparison of macro- and microstructures in lapilli

Whilst translucent zones varied in width, a distinction could be made between wide and narrow translucent zones (subsequently referred to as WTZ and NTZ, respectively). Of the 13 fish caught in the spring, 12 had a WTZ near the margin of the lapilli and one fish caught in April 1999 had recently resumed opaque zone (OZ) formation. This completed WTZ corresponded to a sequence of 50 increments. Based on the number of increments at which translucent material first appeared, these WTZs began to form in January (1 fish), February (8 fish) or early March (4 fish). WTZs were characterized by a decline in increment width over several days to weeks. The increment widths of WTZs were significantly smaller than those in the adjacent opaque zone and often began with a discontinuity or interruption in the otolith sequence (median increment width in WTZ =  $1.11 \mu\text{m}$  cf. OZ =  $1.48 \mu\text{m}$ ; sign test  $p < 0.001$ ; Figure 2a). Microscopic examination of NTZs revealed that these structures at most corresponded to a sequence of 15 microincrements and were sometimes associated with an initial discontinuity (Figure 2b). The increment width in the five opaque increments preceding or following these NTZ did not differ significantly from those around in the NTZ (sign test  $p = 0.77$ ). The check associated with the pelagic-demersal transition (Hislop *et al.*, 2001) appeared as a very narrow opaque zone ( $\sim 5 \mu\text{m}$ ) that was smaller than most NTZs (Figure 3). Given the size of this structure it is unlikely to be confused with a seasonal growth zone.

### Age and growth compositions from microincrements

The range in size of the fish was similar in both spring and autumn collections from the two years. A composite plot of total length versus number of microincrements is presented in Figure 4. The relationship between length and age was approximately linear and indicated an average growth rate of  $0.91 \text{ mm d}^{-1}$  ( $r^2 = 0.79$ ;  $p < 0.001$ ;  $\text{TL} = 29.884 + 0.911 \text{ age}$ ). There was no significant difference in length at age between spring and autumn seasons (ANCOVA  $r^2 = 0.801$ ; intercepts  $F_{1,33} = 2.474$ ;  $p = 0.125$ ; slope  $F_{1,33} = 2.191$ ;  $p = 0.148$ ). The variation around the regression line was very large with a 200-d-old fish having a 95% confidence interval of 179–244 mm TL.



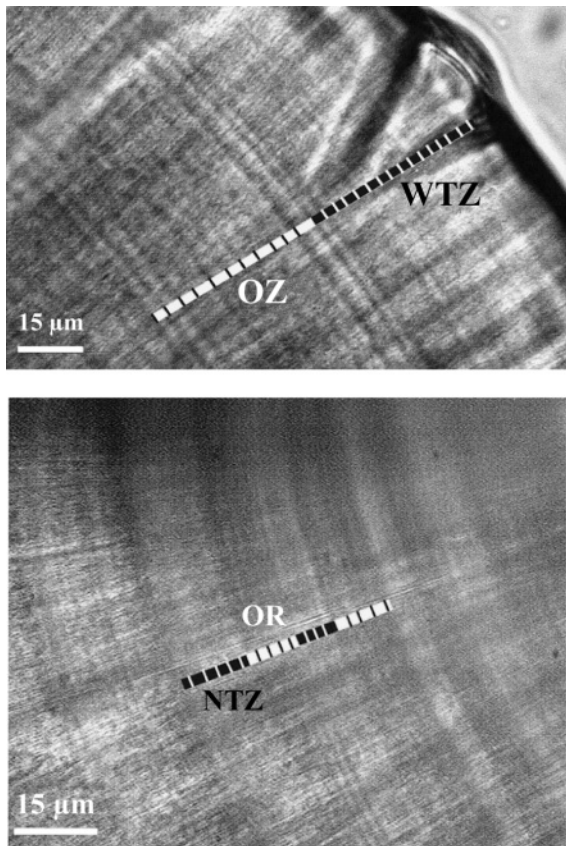


Figure 2. Microstructure of translucent zones under transmitted light: (A) showing the opaque zone (OZ) (white bar) and wide translucent zone (WTZ) (black bar) and the associated decline in the width of increments (=lines); (B) a sequence of narrow translucent zones (NTZ) (black bar) with increments (=lines) of similar width to those in adjacent opaque regions (OR) (white bars).

### Comparison of macrostructure between lapilli, sagittae and illicia

Changes in the opacity of lapilli reflected those in the sagitta, both in terms of the frequency and relative position of translucent zones along a radius (see Figure 1). Therefore, changes in lapilli appearance and microstructure provide a suitable proxy for interpreting changes in sagitta macrostructure.

In sagittae, the 95% ranges in the width of WTZ and NTZ were 4–4.7 and 2.6–3.1% of the total radius, respectively. In illicia the 95% ranges for WTZ and NTZ were 6–8 and 2.5–4 % of the total radius, respectively. In contrast to the two otoliths, there was a significant difference in the frequency of both types of translucent zones between sagittae and illicia (sign test NTZ  $p < 0.0001$ ; WTZ  $p = 0.0001$ ). Sagittae of individuals 17–30 cm TL caught in September tended to have no WTZs, whilst similar sized individuals caught in March and April tended to have one close to the outer margin.

Illicia of individuals caught in September tended to have approximately one WTZ, whilst 1-group caught in March tended to have two. All fish caught in September had one or more NTZs in both sagittae and illicia.

### Discussion

As annual age in northern temperate species is assigned in terms of a 1 January birthdate, the small *Lophius* caught in spring and autumn can be considered to be of different annual ages despite the similarity in their length and daily age range. Consequently, those caught in spring could be regarded as 1-group whilst those caught in September and having hatched between calendar day 3 and 92 would be regarded as 0-group. The present study demonstrates that many of the translucent zones found in sagittae, lapilli and illicia of *Lophius piscatorius* currently classified as 0- and 1-group must be secondary structures. Moreover, the study of lapilli shows that the changes in optical density observed macroscopically can arise from different processes at the microstructural level. Importantly, WTZs, which appear to represent true annuli, are characterized by a gradual decline in increment width. This pattern is consistent with previous descriptions of the translucent zone in a number of other species (Pannella 1980; Victor and Brothers 1982). In contrast, NTZs do not appear to correspond to any long-term decline in the otolith accretion rate. Whilst the reason for this difference between NTZs and WTZs in otoliths was not studied, evidence from other studies suggests that feeding may have been important. Rearing experiments have shown that increment width may not be affected by starvation for sometime after feeding has ceased (Wright *et al.*, 1990; Mugiya & Tanaka, 1992). However, the otolith increments of starving fish appear faint due to a reduction in the matrix template (Eckman and Rey 1987; Titus and Mosegaard 1991). As such, the NTZs observed in anglerfish otoliths may represent periods when the fish are either starving or moving to find better feeding opportunities, as has been suggested by Crozier (1989).

The size at age relationship determined from counts of microincrements for the lapilli indicate that *Lophius piscatorius* may attain a size of 27 cm TL by September of its first year, and that 1-group caught in March and April may be a similar size. Studies of *Lophius piscatorius* in Greek waters (Tsimenidis and Ondrias 1980) and the Irish Sea (Crozier 1989) based on counts of presumed annuli in sagitta indicated similarly high growth rates. However, much slower growth rates have been reported for this species from the Bay of Biscay/Celtic Sea (Dupouy *et al.*, 1986) and Iberian waters (Duarte *et al.*, 1997) based on illicia readings. Importantly, differences between illicia and sagitta zone formation observed in the present study may explain

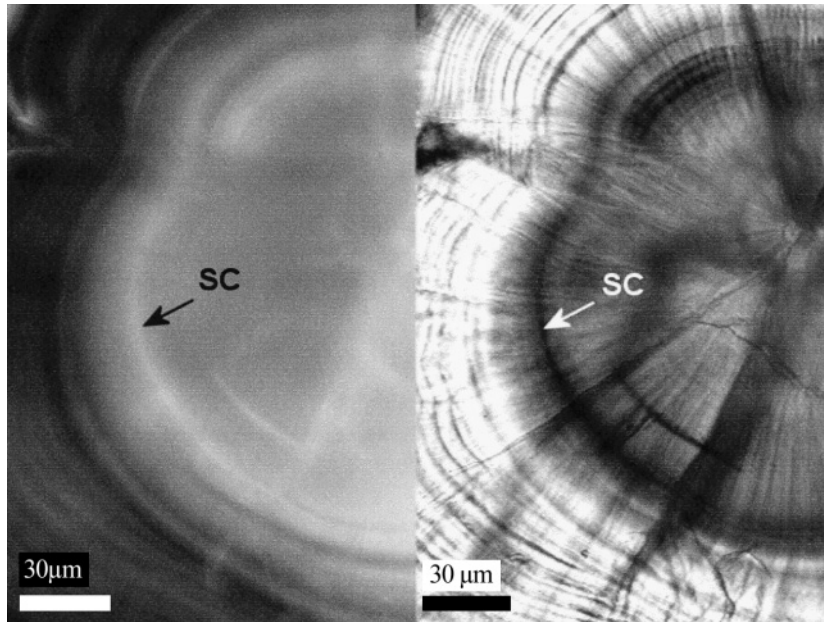


Figure 3. Microstructure of pelagic-demersal transition check (SC) in lapilli viewed with both (a) reflected light and (b) transmitted light.

why readings based on illicia have led to reports of slow early growth rates. This study shows that illicia form a translucent zone sometime between April and September in their first year whilst the sagittae generally do not. Clearly a fish with no translucent zone in the sagitta will be read as 0-group whilst an illicia could be read as an 0-group or 1-group depending on the readers knowledge of when the first translucent zone is formed. Few studies have given descriptions of the criteria used in assigning age. However, it is evident from the

annotated illustrations of illicia given by Dupouy *et al.* (1986) and Duarte *et al.* (1997) that they ascribed each translucent zone as an annual increment, making fish as small as 10 cm a 1-group. This interpretation problem appears to be prevalent amongst all illicia based age readings. Such studies report similar growth rates and an absence of 0-group in catches, despite the presence of many fish between 7 and 28 cm TL (Landa *et al.*, 1998; Quincozes *et al.*, 1998; Landa *et al.*, 2001). If we account for the apparent interpretation problem in these studies by subtracting 1 from the reported ages, we see that the mean length is broadly consistent with those derived from sectioned sagittae from both Crozier (1989) and the present study (i.e. Dupouy *et al.*, 1986, 13 cm; Landa *et al.*, 2001, 20 cm).

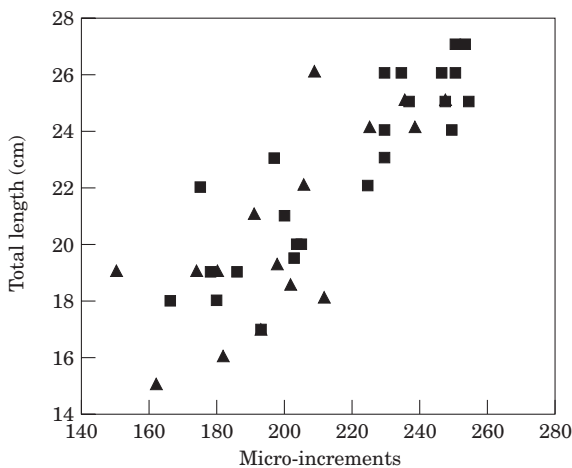


Figure 4. Relationship between length and microincrements of demersal juvenile *Lophius piscatorius*. Symbols refer to data from March and April (▲) and September (■).

Several studies have attempted to identify length modes corresponding to young age-classes (see Dupouy *et al.*, 1986; Duarte *et al.*, 1997), although these are generally difficult to define. An example of the lack of any distinct modal class associated with recruiting fish can be seen from the catch composition of the North Sea Scottish Bottom Trawl quarterly surveys (Figure 5). From the present study it is clear that this lack of a distinct seasonal mode in recruitment and or settlement can be explained by the combination of protracted spawning period and variation in early growth rate. Assuming an embryonic phase of around 20 days (Hislop *et al.*, 2001), the number of microincrements in the lapilli imply that fish caught in spring hatched between August and November, whilst those caught in

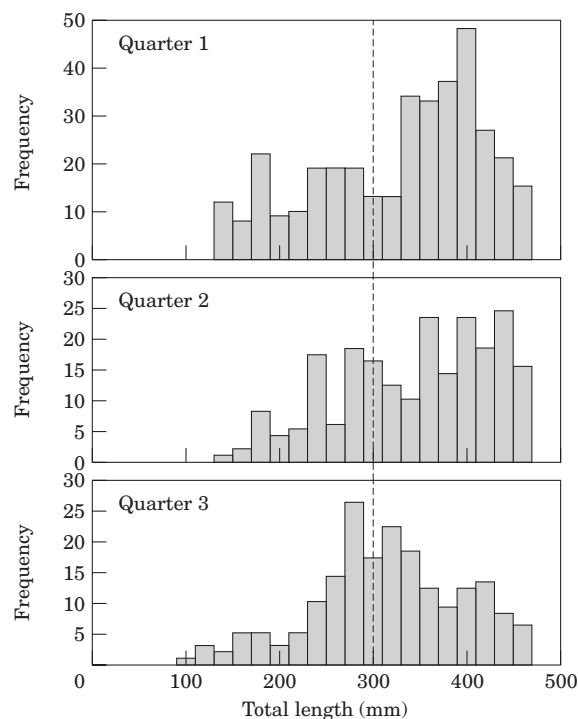


Figure 5. Combined length compositions of *Lophius piscatorius* from Scottish quarterly Bottom Trawl surveys for the years 1991–1997 and quarters 1 (Jan–Mar), 2 (Apr–Jun) and 3 (Jul–Sep). The dashed line represents the maximum size of specimens used in this study.

September hatched between December and March. This extensive range in hatch date covers both the period when females have been recorded in spawning condition (November–May; Afonso-Dias and Hislop 1996) as well as observations on the appearance of eggs (February–early August; Fulton 1903; Bowman 1920; Tåning 1923).

This study has clear implications for the choice and interpretation of increments in the calcified structures of *Lophius*. Incorrect interpretation of illicia structures in the past may have led to inaccurate estimates of growth rate. Consequently, future readers need to be aware that the first annulus (TZ) in illicia does not correspond to a year.

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