

Sagitta microstructure of European conger eel, *Conger conger* (L.), leptocephali compared with leptocephali of the eel, *Anguilla anguilla* (L.)

Carlos Antunes¹⁾; Alberto Teodorico Correia^{1, 2)}

¹⁾ Centro Interdisciplinar de Investigação Marinha e Ambiental, Porto, Portugal

²⁾ Instituto Ciências Biomédicas Abel Salazar, Porto, Portugal

Abstract

Sagitta microstructure of the European conger eel (*Conger conger*) was analysed. The study used twenty-one pre-metamorphic leptocephali collected from the Iberian continental slope and the Bay of Biscay, and two metamorphosing larvae captured on the continental shelf near to the Minho River entrance. The total length and the radii of the sagittae of the leptocephali ranged from 85 to 133 μm and from 113 to 260 μm , respectively. Daily rings were visible along the entire length of the sagitta radii of the pre-metamorphic leptocephali, with an average of 277 rings. A diffuse zone, where no rings were visible, was present in the sagittae of the two larvae which had already metamorphosed. The fact that conger eel larvae grow well up until metamorphosis, could be the reason for the clear visibility of most of the daily rings in the pre-metamorphic leptocephali. This could be useful, when comparing other species, for example the European eel, *Anguilla anguilla*, whose sagittae present a diffuse zone prior to metamorphosis.

Kurzfassung

Die Mikrostrukturen der Sagittae von einundzwanzig, am Iberischen Kontinentalschelf und in der Bucht von Biskaya gesammelten, pre-metamorphosierten Leptocephali des Europäischen Meeraals (*Conger conger*) und von zwei, auf dem Kontinentalschelf nahe der Rio-Minho-Mündung gefangenen, metamorphosierenden Larven, wurden analysiert. Die Totallängen und die Radien der Leptocephalus-Sagittae variierten von 85 bis 133 μm und von 113 bis 260 μm . Tagesringe waren entlang der gesamten Längen der Sagitta-Radien bei den pre-metamorphosierten Leptocephali sichtbar und im Durchschnitt konnten 277 Ringe gezählt werden. Eine diffuse Zone, in der keine Ringe sichtbar waren, war in den Sagittae der zwei, bereits metamorphosierten Larven vorhanden. Die Tatsache, dass die Larven des Meer-aals bis zur Metarmorphose hin gut wachsen, könnte der Grund für die gute Sichtbarkeit der meisten Tagesringe der pre-metamorphosierten Leptocephali sein und beim Vergleich mit anderen Arten, zum Beispiel dem Europäischen Aal *Anguilla anguilla*, nützlich sein, dessen Sagittae eine diffuse Zone vor der Metarmorphose aufweisen.

Introduction

Daily growth increments (rings) have been shown to occur in the otoliths of anguilliform fishes. They have been demonstrated in the artificially hatched larvae of *Anguilla japonica* during the first 6 days of life (Umezawa *et al.* 1989) and in the elvers (Umezawa and Tsu-

kamoto 1991), as well as in the otoliths of leptocephali and the metamorphic stages of *Conger myriaster* (Mochioka *et al.* 1989). In the European eel, *Anguilla anguilla* (L.), determining the age of glass eels is problematic because a diffuse zone is present prior to metamorphosis; this is a region of the sagitta where the morphological features are irregular (Antunes and Tesch 1997). However, increments in the sagittae of premetamorphic European conger eel, *Conger conger* (L.), leptocephali are easily counted (Strehlow *et al.* 1998).

The interpretation of the “daily rings” of the economically important genus *Anguilla*, as well as those of other lesser known Anguilliformes, is important because the number and morphology of the rings can help increase understanding of their life history. The other alternative method of investigation is bio-oceanographic sampling, which is difficult and expensive for these deep sea fish.

The Mediterranean Sea is thought to be a spawning area for European conger eel, indicated by catches containing small, 9 to 20 mm leptocephali (Schmidt 1931). Schmidt's (1931) assumption that *C. conger* could also spawn in the western Sargasso Sea was contested by McCleave and Miller (1994). They suggested that the small leptocephali from the Sargasso Sea, which had a relatively high number of myomeres, were *Conger triporiceps* and that occurrence of *C. conger* leptocephali is restricted to the eastern and central Atlantic. However, nothing is known about the spawning location and time of metamorphosis of *C. conger* (Strehlow *et al.* 1998).

The sagitta microstructure of conger eel leptocephali at two different larval phases was analysed and an initial estimation of larval age, based on otolith microstructure was carried out. Knowledge of the microstructure of conger eel leptocephali sagittae was used to aid identification of the phase of *A. anguilla* leptocephali. A comparison was made with the same phase of growth in otoliths of *A. anguilla* in which the daily rings were obscured by the diffuse zone.

Materials and methods

Twenty-one premetamorphic conger eel leptocephali were captured using an Isaacs-Kidd mid-water trawl during an expedition of the R.V. Heincke to the Bay of Biscay and the Portuguese coast between 26th May and 5th July 1989 (Table 1). Two metamorphic conger eel leptocephali were collected in April 1998 and November 1998 as by-catch of the glass eel fishery at the mouth of Minho River.

The number of myomeres present in the pre-metamorphic leptocephali was counted for species identification. In the metamorphic larvae the total number of myomeres (TNM) and the number of myomeres to the origin of the anal fin (MA) were counted, and the MA/TNM ratio calculated. The ratio was used as an indicator of the stage of development (Tanaka *et al.* 1987).

Both right and left sagittae were dissected from the leptocephali, polished in the sagittal plane with 2400 mesh sand paper and aluminium paste until the core was revealed. Then they were etched for 8 s with a 0.5 % solution of HCl, coated with gold and viewed under SEM (Jeol JSM 35 C) at 15 kV. The diameter (D), radius (R), width of rings in the countable zone (CZ) and diffuse zone (DZ) width were measured (Figure 1). In the diffuse zone of metamorphic leptocephali there were small fragments of ring structure and the narrower spacing of these rings was used for calculating the number of days in that part of the otolith.

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Table 1: Age determination by daily rings and radius of otoliths of *Conger conger* leptocephali caught in the East Atlantic and in the Minho River (leptocephali data modified from Strehlow *et al.* 1998).

Area	n	Capture date	Position	L_T (mm)	Radius of otolith (μm)	Number of daily rings
Bay of Biscay	7	26.5.1989	47° N/ 7° W	109	172	368
				121	191	364
				133	178	299
		4.6.1989	44° N/ 9° W	120	133	273
				109	135	288
				102	143	253
North coast of Portugal	11	3.6.1989	41° N/ 9° W	125	167	301
				98	140	269
				89	147	273
				114	180	313
				123	148	262
		2.6.1989		110	203	313
				108	183	334
				108	119	269
		30.5.1989		102	133	250
				105	126	250
Northern Iberian Basin	3	28.5.1989	43° N/ 13° W	116	142	315
				109	183	385
				96	130	263
				91	113	208
Minho River	2	23.4.1998	41° N /8° W	85	147	230
				128	195	483
				133	246	736

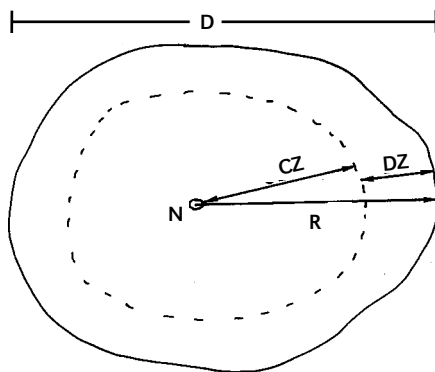


Figure 1: Schematic otolith measurements of conger eel leptocephali. N = nucleus; D = diameter; R = radius; CZ = zone of countable rings; DZ = diffuse zone.

For premetamorphic leptocephali, the daily growth increments by length of leptocephali (TGL) and by width of sagittae (TGS) were calculated from the total length (L_T), and the radius distance with age ratios, respectively.

Results

The microstructure of conger eel larval otoliths showed well defined structures: the nucleus (N), the ring-countable zone (CZ) and the diffuse zone (DZ) (Figure 1).

Premetamorphic leptocephali

In the premetamorphic leptocephali, otolith growth increments were visible in all sagittal sections from the nucleus to the edge (Figure 2).

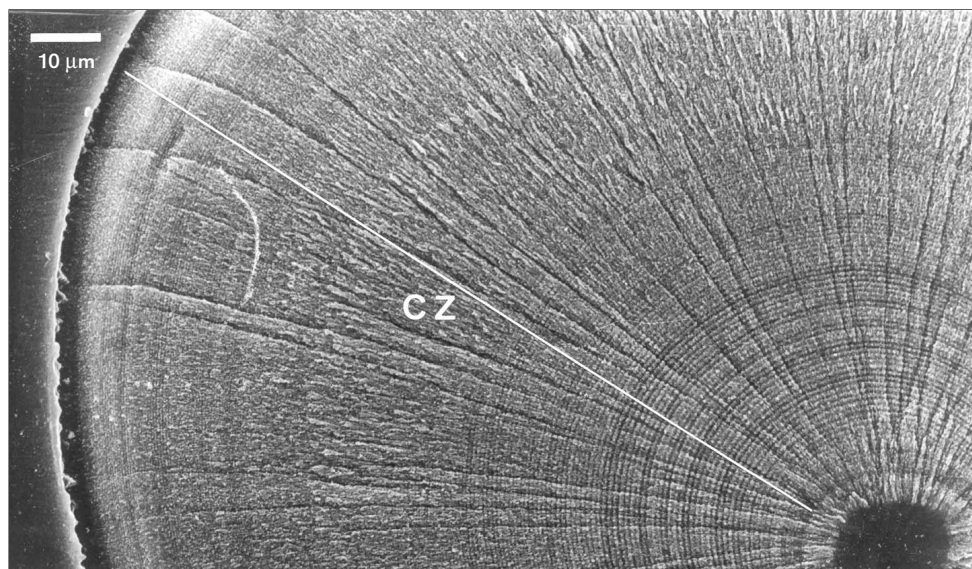


Figure 2: Sagitta of premetamorphic *Conger leptocephali*, May 1989, sampled on the Atlantic continental slope. N = nucleus, CZ = zone of countable rings.

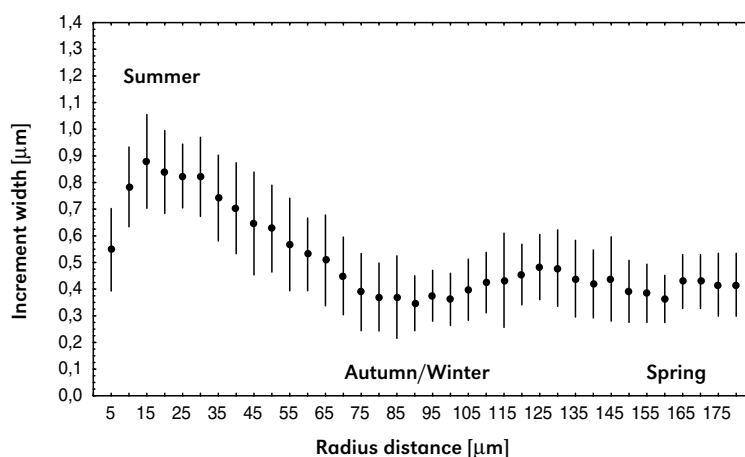


Figure 3: Sagitta increment width versus distance of radius from the nucleus (mean value \pm s).

The increment width along the sagittal radius showed a characteristic curve (Figure 3). The width increased ($0.5 \mu\text{m} - 0.9 \mu\text{m}$) from the nucleus up to a distance of $15 \mu\text{m}$ and then decreased ($0.4 \mu\text{m}$) up to a distance of $85 \mu\text{m}$. Beyond $85 \mu\text{m}$ there was little variation (0.4 to $0.6 \mu\text{m}$) in increment width to the edge of the otolith.

An average of 277 increments (equivalent to about 9 months) were counted from the nucleus to the edge, giving the date of hatching in the summer, if it is assumed that each increment corresponds to one day (Figure 4).

The number of increments until the end of the decreasing width zone at $85 \mu\text{m}$ from the nucleus, corresponded to a total of 109 ± 26 days (mean \pm s). From back-calculation,

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Figure 4: Back-calculated hatch dates of pre-metamorphic conger eel larvae.

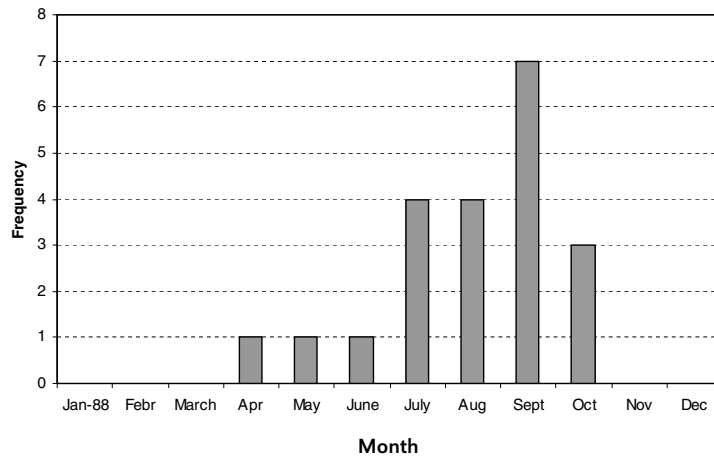
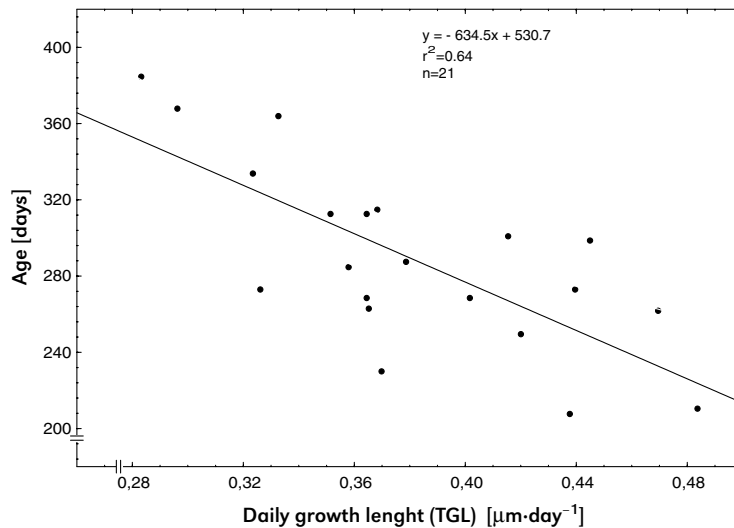


Figure 5: The relationship between age and average daily growth length (TGL) of pre-metamorphic leptocephali.



November was determined as the period with smallest increment widths. A significant correlation was found between the assumed age of leptocephali and the age where increment width ceased decreasing (lowest point) ($r^2 = 0.37$; $P < 0.05$; $n = 21$), as well as between sagitta diameter and age ($r^2 = 0.71$; $P < 0.05$; $n = 21$).

Significant correlation was found between L_T and sagitta diameter ($r^2 = 0.23$, $P < 0.05$, $n = 21$), sagitta radius ($r^2 = 0.16$, $P < 0.05$, $n = 21$) and age ($r^2 = 0.22$, $P < 0.05$, $n = 21$). The average daily growth increment of leptocephali (TGL) after hatching was $0.38 \pm 0.05 \text{ mm}\cdot\text{day}^{-1}$ ($n = 21$), while the average daily growth of sagittae (TGS) was $0.53 \pm 0.06 \text{ }\mu\text{m}\cdot\text{day}^{-1}$.

A significant negative correlation was found between age and TGL ($r^2 = 0.64$, $P < 0.05$, $n = 21$) (Figure 5) but not between age and TGS ($r^2 = 0.14$, $P = 0.24$, $n = 21$) (Figure 6). The regression of age and L_T was: $x = 69 + 1.956 L_T$ (Figure 7). Thus, if summer is the time when they hatch, they will be 30 mm at the first winter, and when they reach 160 mm they will be approximately 380 days old (13 months).

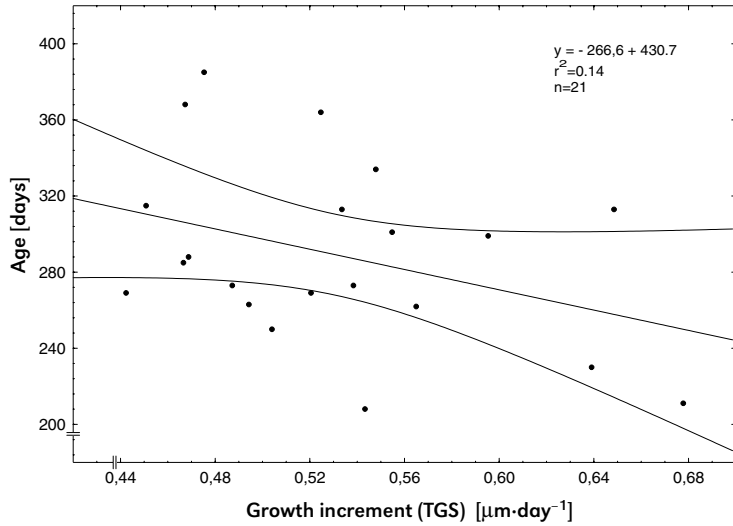


Figure 6: The relationship between age and average daily growth increment (TGS) of pre-metamorphic leptocephali sagitta.

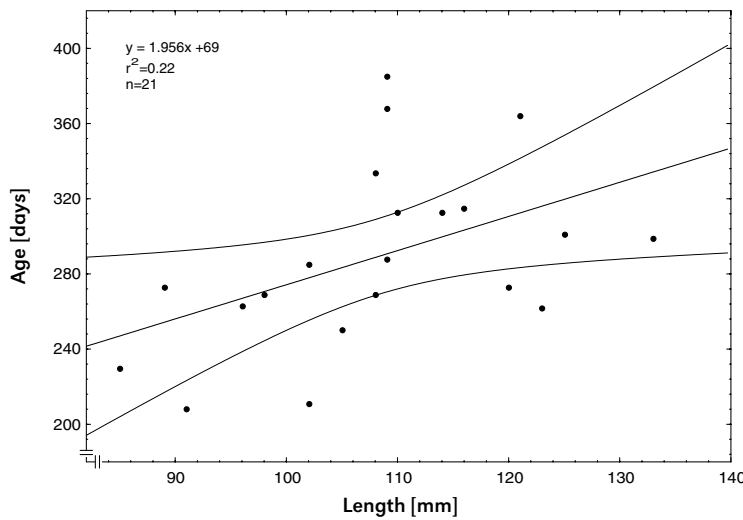


Figure 7: The relationship between age and length of pre-metamorphic leptocephali.

Metamorphic leptocephali

The lengths of the two leptocephali at metamorphosis were 128 and 133 mm (April and November 1998, respectively). The number of myomeres counted to the origin of the anal fin (MA) was 65 in April and 63 in November. The total number of myomeres (TNM) was 160 in April and 155 in November, giving MA:TNM = 0.41 for both leptocephali. These values indicated that the leptocephali were at metamorphosis, compared with those obtained for premetamorphic leptocephali (0.71) by Strehlow *et al.* (1998).

The sagittae had radii of 195 and 260 µm and diameters of 366 and 380 µm, respectively. The increment widths were similar to those on the sagittae of premetamorphic stages (Figure 8). The difference was the presence of a diffuse zone beyond 180 µm (Figure 9). The

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Figure 8: Sagitta increment width versus distance of radius from the nucleus of metamorphic leptocephali.

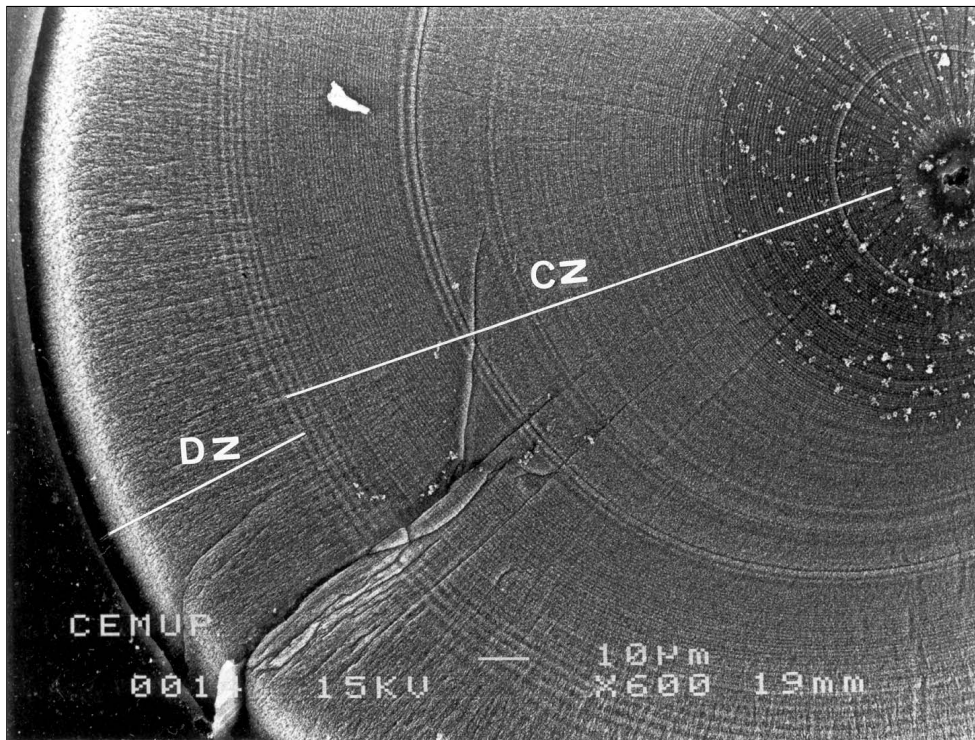
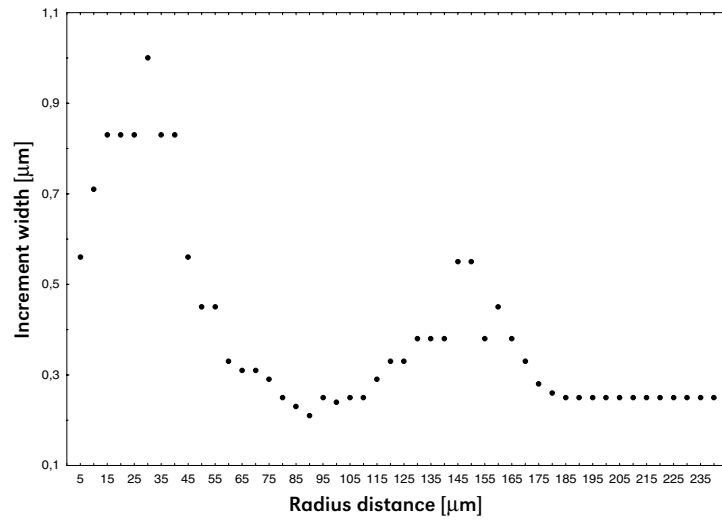


Figure 9: Sagitta of metamorphic *Conger* leptocephalus captured at the Minho River mouth. N = nucleus, CZ = zone of countable rings, DZ = diffuse zone.

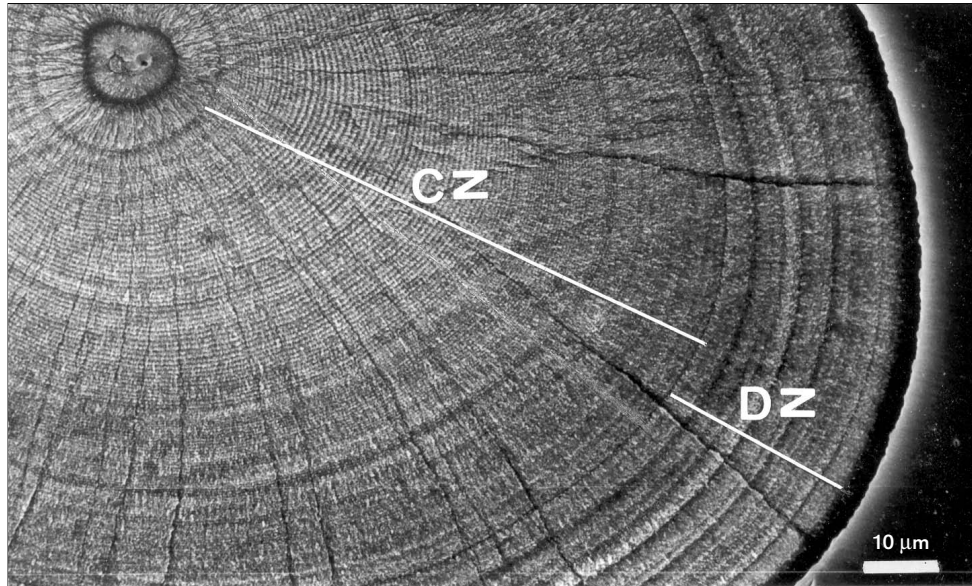


Figure 10: Sagitta of *Anguilla leptocephalus*, stage I, May 1989, captured on the Atlantic continental slope. N = nucleus, CZ = zone of countable rings, DZ = diffuse zone.

estimated total number of increments after hatching, was 483 (April 98) and 736 (November 98), calculated using the narrow “rings” in the D.Z. For these otoliths, a period of 120 days was measured from the first ring until the point where increment width stopped decreasing. It is also important to note that after the 400th ring, a zone appeared which is difficult to read.

Figure 10 shows a sagitta of a 75 mm *Anguilla leptocephalus* prior to metamorphosis (stage I), collected on the same cruise along with premetamorphic conger eel leptocephali. However, at this stage it was possible to detect the presence of the diffuse zone.

Discussion

Beyond the nucleus, it was assumed that the periodic structures were the daily growth rings, as demonstrated in other Anguilliform species (Mochioka *et al.* 1989; Umezawa *et al.* 1989; Umezawa and Tsukamoto 1991). Based on this assumption, the average total number of increments for the premetamorphic leptocephali was 277, which corresponds to a summer spawning season and agrees with the observations on leptocephali of a smaller body size in the Mediterranean Sea (Schmidt 1931; Strehlow *et al.* 1998).

Increment width increases (reaching 0.9 μm) until the otolith radius (R) is 15 μm , corresponding to summer. The width then decreases to 0.4 μm , when R = 80 μm (120 days by back-calculation). This is probably caused by the lower temperatures during autumn and winter. The increasing temperature in spring probably explains the next increase in the width of the increments and the continuous larval growth until the length at metamorphosis is reached (160 mm).

Older larvae are bigger. For the premetamorphic leptocephali, the correlation between age and the point at which increment width stops decreasing of is probably due to the influence of environmental factors.

The daily growth increment (TGL) of premetamorphic leptocephali ($0.38 \text{ mm}\cdot\text{day}^{-1}$) is similar to that of small *Anguilla* larvae, as described by Castonguay (1987). However, for the *Anguilla* leptocephali of 75 mm length, captured in May 1989, the TGL was only $0.24 \text{ mm}\cdot\text{day}^{-1}$ (Antunes 1994). These larvae were near to their maximum length prior to metamorphosis and the sagittae already showed the “diffuse zone”. The TGL is still lower (around 0.2) in the smallest *Anguilla* sp. (Boetius and Harding 1985; Kleckner and McCleave 1985; Tesch 1998).

Otake *et al.* (1997) considered that the onset of metamorphosis for *C. myriaster* occurs around about the fourth month (110 rings). In the present study, *C. conger* commenced metamorphosis later, *c.* 9.5 months (120 mm L_T).

The preanal length of the different larval stages is an indicator of their developmental stage. Strehlow (1992) observed a MA/TNM ratio of 0.77 for larvae in the premetamorphic stage (that includes the specimens recorded in this study). In the leptocephali captured in the Minho River, this ratio was 0.41. *Anguilla* sp. exhibited similar tendencies (Schoth 1982). Tanaka *et al.* (1987) used this method to describe premetamorphic and juvenile development for *C. myriaster*, and found values of 0.84 and 0.23, respectively.

In the sagitta of metamorphic larvae, the area of the second decrease in increment width corresponded to about the 400th ring. The rings in this area were so thin that it was not possible to count them and the area was termed the “diffuse zone”. This zone is probably laid down in late summer and early autumn and could be due to the leptocephali having nearly completed their growth before the commencement of metamorphosis.

Sagitta analysis showed relatively older larvae coming from the Bay of Biscay compared with those from the northern coastal region of Portugal. This was similar to the larvae of *A. anguilla* (Antunes and Tesch 1997) which for other ecological reasons, were even younger at higher latitudes.

Sometimes, metamorphic conger leptocephali are caught in the European glass eel fishery (November to April), in the marine area next to the mouth of the Minho River. There are similar reports for *C. oceanicus*, which is found in the coastal and estuarine regions of the Chesapeake Bay between May and August, and commences metamorphosis at 160 mm (Hardy 1978). *C. myriaster* has been recorded in coastal waters between November and July, at the end of its metamorphosis (Tanaka *et al.* 1987).

The comparison between *A. anguilla* and *C. conger* is interesting. Analyses of the microstructure of the sagittae of conger eel leptocephali before and during metamorphosis were used as a reference for the description of the larval phases of *Anguilla* leptocephali. Most larval phases of *A. anguilla* are probably found within the diffuse zone of the sagitta, as it attains a considerable length in the first seven months of life with only a little further growth prior to metamorphosis (Antunes and Tesch 1997). In *C. conger*, using the present data, the half time of its larval phase could be easily identified in the sagitta, and the reason for this could be the fact that the spawning season is in summer. Note that the maximum larval growth rate is expressed at the end of the following summer.

Data from J. Schmidt (in: Boetius and Harding 1985) indicate that the spawning peak for *A. anguilla* takes place in March/April. The number of rings in the countable

zone (CZ) is about 200 (Antunes 1994). This number corresponds well with the number of days from spawning time to the beginning of winter. The leptocephali may also have reached higher latitudes and lower temperatures. These events could cause a slowdown in growth to the extent that daily rings are no longer formed, giving way to the diffuse zone. This development is favoured by a slowdown in growth, and faster growth is not resumed during the next summer (Antunes and Tesch 1997). In *A. anguilla*, the diffuse zone of the sagittae appears in the premetamorphic stage, as seen in larvae captured in May/June and October (Antunes 1994; Antunes and Tesch 1997). The appearance of this diffuse zone could coincide with autumn/winter and the non-deposition of visible increments (rings) following these seasons and could be due to the fact that *A. anguilla* had reached the length (60 to 70 mm) at which the metamorphosis starts. In *C. conger*, the fact that during its first winter the larva is only 30 mm (metamorphosis at 160 mm), leaves considerable potential for continuing growth, and narrow winter increments are formed and not a diffuse zone, as in *A. anguilla*. Broader increments are resumed in the next growth phase in spring.

The possible reason why increment counting is harder from the 400th ring could be that the larva's length is close to 160 mm, *i. e.* close to metamorphosis. The diffuse zone could be produced in summer-autumn and the growth rate reduction associated, later on to metamorphosis, could contribute to difficulties in estimating the duration of this phase. Recording the onset of metamorphosis and its duration is problematic. The diffuse zone could appear before metamorphosis starts, which is similar to *A. anguilla*.

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Authors' addresses: Carlos Antunes; Centro de Investigação Marinha e Ambiental, Rua dos Bragas, 4050-123 Porto, Portugal, e-mail: cantunes@icbas.up.pt; Alberto Teodorico Correia, Centro de Investigação Marinha e Ambiental, Rua dos Bragas, 4050-123 Porto, Portugal and Instituto Ciências Biomédicas Abel Salazar, Largo Prof. Abel Salazar, 2 4099-003 Porto, Portugal.

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C. Antunes; A. T. Correia