Marine Biology (2003) 142: 777–789
DOI 10.1007/s00227-002-0993-4

A.T. Correia · C. Antunes · E.J. Isidro · J. Coimbra

Changes in otolith microstructure and microchemistry during larval development of the European conger eel (*Conger conger*)

Received: 31 May 2002 / Accepted: 22 November 2002 / Published online: 12 February 2003

Abstract Otolith microstructure and chemical composition (Sr:Ca ratios) of the European conger eel (*Conger conger*) were examined during the larval developmental stages by scanning electron microscopy and wavelength dispersive spectrometer. Back-calculated hatching dates from the otolith microstructure of the developing leptocephali indicate a protracted spawning season from December to June. The early age of our developing specimens captured south of the Azores Islands suggests that the conger eel has another spawning area closer to Azores than the Mediterranean. Otolith increment width, which was relatively constant and narrow in the developing leptocephalus stage, increased sharply at age 170–250 days. Sr:Ca ratios in the otolith, which increased during the developing leptocephalus stage, showed a rapid drop coinciding with the increase in increment width. These coincidental changes were regarded as the onset of metamorphosis for this species. A close linear relationship between the age at metamorphosis and otolith growth rate indicates that the faster-growing larvae metamorphose earlier, suggesting that somatic growth should play an important role in the timing of metamorphosis. As shown in earlier work, the existence of an otolith marginal zone with unclear rings during metamorphosis prevents an accurate estimate of the larval stage duration of this species.

Communicated by S.A. Poulet, Roscoff

A.T. Correia (✉) · C. Antunes · J. Coimbra
Centro Interdisciplinar de Investigação
Marinha e Ambiental,
Rua do Campo Alegre 823,
4150-180 Porto, Portugal

E-mail: acorreia@icbas.up.pt
Tel.: +351-22-6080477
Fax: +351-22-6080479

A.T. Correia · J. Coimbra
Instituto Ciências Biomédicas Abel Salazar da
Universidade do Porto, Largo Abel Salazar 2,
4099-033 Porto, Portugal

E.J. Isidro
Departamento de Oceanografia e Pescas da Universidade dos
Açores, 9901-862 Horta, Portugal

Introduction

The European conger eel (*Conger conger* L., 1758) is a marine benthic fish widely distributed in the NE Atlantic, being found from Norway to Senegal, as well as in the Mediterranean and western Black Sea (Bauchot and Saldanha 1986). However, the knowledge about its early life history, e.g. spawning area(s) and season, distribution and migration of leptocephali is limited (Strehlow et al. 1998; Correia et al. 2002; Antunes and Correia 2002).

Schmidt (1931) caught small conger eel larvae in the Sargasso Sea, Mediterranean and NE Atlantic, suggesting a similar migratory behaviour to the European eel (*Anguilla anguilla* L.), i.e. spawning in the Sargasso Sea, following larval transoceanic migration to European and North African coasts. However, Schmidt's assumption that *C. conger* spawn in the Sargasso Sea was contested by McCleave and Miller (1994). These authors state that the conger eel leptocephali from the Sargasso Sea were *Conger triporiceps*, a species with an overlapping number of myomeres, described later by Kanazawa (1958). Nowadays the leptocephali of both species, i.e. *C. triporiceps* and *C. conger*, are distinguished based on the catch location, either in the West or in the Central and East Atlantic, respectively (McCleave and Miller 1994; Strehlow et al. 1998).

The spawning ground of *C. conger* is presumed to be in the waters south of the Island of Sardinia in the Mediterranean Sea (Cau and Manconi 1983). This study is supported by the length and otolith analyses of leptocephali collected in the North and Central Atlantic Ocean (Strehlow et al. 1998).

It has been suggested that the leptocephali has a long larval life (Bauchot and Saldanha 1986), taking about 1 or 2 years to drift inshore and to reach the juvenile elver form (Lythgoe and Lythgoe 1971; Wheeler 1985;

Strehlow 1992). Back-calculated hatching dates from the otolith microstructure of conger eel developing leptocephali indicate a long spawning season, with one annual peak occurring in summer (Strehlow et al. 1998; Antunes and Correia 2002).

The analysis of metamorphosing larval otolith microstructure suggested that the duration of the developing leptocephalus stage is about 6–9 months. Unfortunately, otoliths of those metamorphosing larvae show a peripheral diffuse zone with unclear rings, which prevents an accurate estimate of the individual metamorphosis duration (Correia et al. 2002).

The knowledge of the timing and duration of metamorphosis is essential for understanding the migration mechanism, geographical distribution and the early life-history strategy of the conger eel. Combination of otolith microstructure and microchemistry has revealed considerable information about the early life history of several anguilliformes fishes, namely the *Anguilla* species (Otake et al. 1994, 1997; Tzeng and Tsai 1994; Cheng and Tzeng 1996; Arai et al. 1997, 1999a, 1999b, 2000b, 2001; Wang and Tzeng 1998, 2000; Marui et al. 2001).

In the present study we examined the ontogenic changes in otolith microstructure and microchemistry (Sr:Ca concentration ratios) that occur during the larval development of *C. conger*. These results form the basis of a discussion about the larval stage duration and the coastal recruitment mechanism for this species.

Materials and methods

Fish collection

A total of 31 fishes were used to examine the otolith growth microstructure and microchemistry: 6 developing leptocephali, 20 metamorphosing larvae and 5 elvers (or juveniles), 4 of which were taken from metamorphosing leptocephali reared in the laboratory.

The developing leptocephali were collected in August 2000 during the cruise of R.V. "Heincke" conducted in the NE Atlantic Ocean (Fig. 1). All larvae were taken with a Young fish trawl (mesh size stretched: 11 mm) between the surface and 800 m depth during the night. Water temperature and salinity of the sampling area ranged from 9.3°C to 24.2°C and from 34.7 to 36.6 psu, respectively.

The metamorphosing specimens were collected by the glass eel fishery at the mouth of the Minho River (41°N; 08°W; see also Fig. 1), north of Portugal, in April 2000. Fishing, using a stow net, took place in the estuarine area during the night, in the period of the new moon during the flood-tide current (Antunes 1994). The water temperature and salinity in this area ranged from 12.2°C to 12.4°C and from 4.6 to 29.8 psu, respectively.

Four metamorphosing conger eels also collected in the Minho River in April 2001 were reared in a 100 l aquarium, under a natural photoperiod and with a water temperature and salinity of 15.0°C and 31.0 psu, respectively. They successfully completed metamorphosis in about 2 months. We determined the end of metamorphosis according to the morphological description by D'Ancona (1931). A small conger eel captured by a fisherman on a sandy beach (Praia da Aguda, north of Portugal, about 100 km south of the Minho River) in November 2000 has also been used.

The external body morphology, pigmentation and myomere counts were used for species identification (Correia et al. 2002). Measurements were made to the nearest 0.1 mm using an ocular micrometer in a binocular dissecting microscope. Measures and counting procedures described by Smith (1989) were adopted. In elvers, only lengths were measured, because their myomeres were not clearly visible. The six developing leptocephali were preserved in 70% ethanol, before the fish measurements. However, the shrinkage caused by the preservation method was not corrected. The ratio of preanal myomeres to total number of myomeres (PAM/TNM) and the ratio of preanal length to total length (PAL/TL) were used to define the developmental stage of conger eel larvae (Tanaka et al. 1987; Yamano et al. 1991; Lee and Byun 1996; Otake et al. 1997; Strehlow et al. 1998; Correia et al. 2002).

Otolith preparation

Sagittal otoliths were extracted, cleaned and embedded in epoxy resin. The otoliths were then ground by hand through the sagittal section with 600, 1200 and 2400 silicon carbide abrasive paper to expose the core. After that, they were polished with 6, 3 and 1 μm

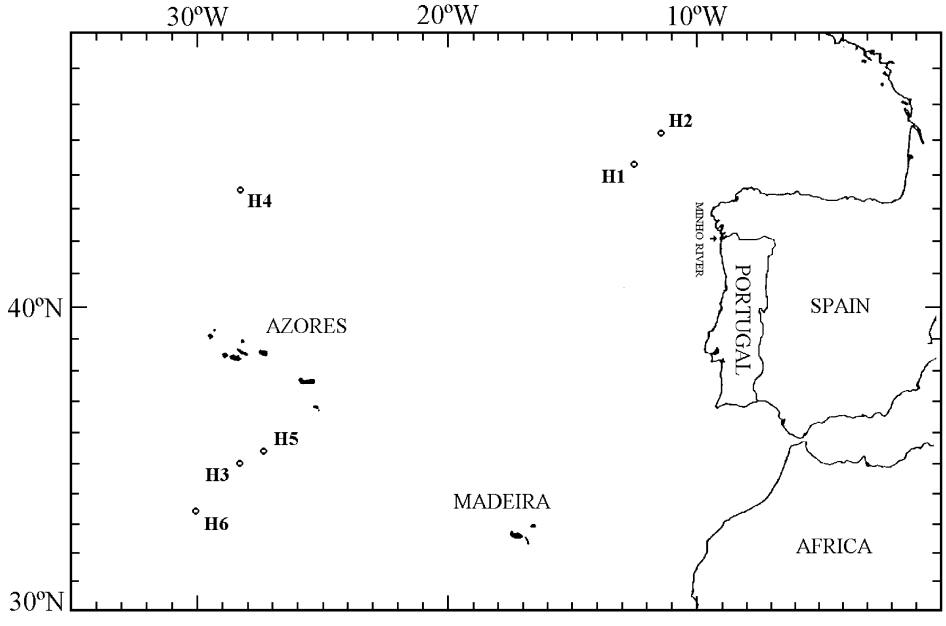

Fig. 1 *Conger conger*. Horizontal distribution of the six conger eel developing leptocephali collected by the R.V. "Heincke" in the NE Atlantic in August 2000 (*H1–H6* specimens, see Table 3 for details)

diamond pastes and finally with alumina solution (1:20) on an automated polishing wheel (Struers, Planapol V). The surface of the otolith to be examined must be highly polished to prevent large diffractions of x-rays and subsequent analytical error (Radtke 1989). Finally, they were cleaned with absolute ethanol in an ultrasonic bath for 5 min and rinsed with deionised water, prior to analysis.

Otolith x-ray analysis

For electron microprobe analysis the otoliths were gold coated in a high-vacuum evaporator. Strontium (Sr) and calcium (Ca) concentrations (% dry weight) were measured along the longest axis of each otolith using a wavelength dispersive x-ray electron microprobe (CAMEBAX SX 50). Accelerating voltage and beam current were 15 kV and 10 nA, respectively. The electron beam was focused on a point approximately 2 μm in diameter, with intervals of 5 and 10 μm, for the developing/metamorphosing and elver specimens, respectively. The counting time was 60 s (30 s per element, 20 s for the measurement of the counts in the corresponding peak and 10 s for measuring background contribution). Apatite [$Ca_5(PO_4)_3$] and celestite ($SrSO_4$) were used as standards. The intentional bombardment by an electron beam with increased absorbed current voltages created a slight burn depression at the sampled location, serving as a convenient marker (Tzeng and Tsai 1994). Thus, microprobe measurement points, which were seen as burn depressions on the otolith surface (see Fig. 4B), were assigned to otolith growth increments. The results are presented as the amount of Sr divided by the amount of Ca times 1000. The averages of successive data for Sr and Ca concentrations pooled for every ten successive growth increments were used for the life-history transect analysis.

Otolith increment analysis

Following electron microprobe analysis, the otoliths were repolished with alumina solution (1:20) to remove the coating, etched for 5–10 s with 0.05 M HCl and vacuum coated with gold for scanning electron microscope observation (SEM, Jeol JSM 630-1F) at 15 kV.

In most marine fishes, the deposition of the first daily increment occurs during their first exogenous feeding, when larvae have completed yolk-sac absorption (Lough et al. 1982; McGurk 1984; Tzeng and Yu 1988). In the Japanese eel the yolk sac was completely absorbed 4–6 days post-hatching (Umezawa et al. 1989). Like in other leptocephalus age estimates (Tzeng 1990; Tzeng and Tsai 1992; Cheng and Tzeng 1996; Wang and Tzeng 1998), 5 days were added to the number of daily increments, although no increments were deposited in the core during the yolk-sac stage.

We assumed the growth increment in the larval otolith of conger to be daily, although daily deposition has not been validated in this species. We base this assumption on the results of several related anguilliformes species, e.g. *Conger myriaster* (Mochioka et al. 1989), *Anguilla japonica* (Tsukamoto 1989; Umezawa et al. 1989; Umezawa and Tsukamoto 1991), *A. rostrata* (Martin 1995), *A. celebesensis* (Arai et al. 2000a) and *A. marmorata* (Sugeha et al. 2001), which have been shown to have daily depositions.

All otolith measurements were carried out according to the procedures of Correia et al. (2002). The otolith radius and increment width were measured along the maximum otolith radius. Average widths of every ten successive increments, from the first feeding check (FFC) to the end of the increment countable zone (ICZ) were used for otolith growth analysis (Fig. 2).

Based on previous data on otolith microstructure and Sr:Ca concentration ratios in several anguilliformes leptocephali (see "Discussion"), the age at metamorphosis was calculated from the number of daily growth increments in the otolith of the metamorphosing larvae, counted from the outer core (FFC) to the otolith point where otolith increment width suddenly became wide and the Sr:Ca ratios drastically decreased (onset of metamorphosis), including the additional 5 days of the yolk-sac larval stage.

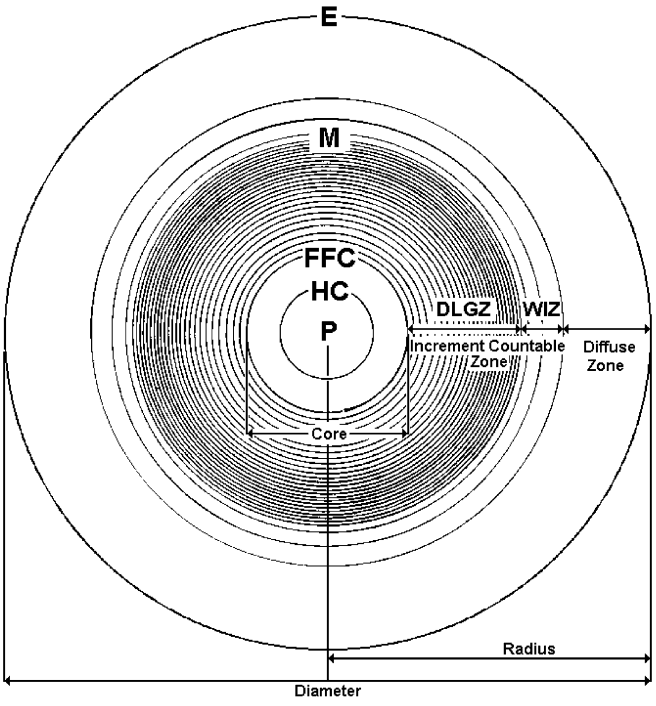

Fig. 2 *Conger conger*. Schematic diagram of the distinct zones and measurements in the otoliths of the metamorphosing leptocephalus (*P* primordium; *HC* hatch check; *FFC* first feeding check; *M* metamorphosis; *E* edge; *DLGZ* developing leptocephalus growth zone; *WIZ* wide increment zone)

The duration from the beginning of metamorphosis to the time of capture, and thus the total age of the larvae, was impossible to establish, because increments were not visible in the diffuse zone (DZ) of the metamorphic otoliths (Correia et al. 2002; present study).

Statistical analysis

All statistical analyses were carried out according to the procedures described in Zar (1996). Differences among the three larval stages for some otolith parameters (core diameter and core Sr:Ca concentration ratios) were examined by a nonparametric test (Kruskal–Wallis). Significance of the correlation coefficient and regression slope were tested, respectively, by a Fisher's *Z*-transformation and by an analysis of variance (ANOVA). We used a level of significance (α) of 0.05. Data are presented as ranges and mean values ($\pm$ standard deviation).

Results

Size and developmental stage

The morphometric and meristic values obtained for the developing/metamorphosing leptocephali and elvers are presented in Tables 1 and 2, respectively.

The length of the six developing leptocephali ranged from 49.0 to 96.0 mm. In these specimens the PAM/TNM (PAL/TL) values kept an almost constant value of

Table 1 *Conger conger*. Morphometric and meristic characters of the developing ($n = 6$)/metamorphosing($n = 20$) conger eel leptocephali

Parameter	Abbreviation	Range (mm)	Mean ± SD (mm)	Mode
Total length	TL	49.0–96.0/113.0–142.0	70.2 ± 20.7/126.8 ± 6.8	
Predorsal length	PDL	44.0–57.0/32.0–57.0	50.5 ± 9.2/44.2 ± 6.9	
Preanal length	PAL	43.0–82.0/49.0–68.0	60.5 ± 17.5/60.3 ± 5.6	
Head length	HL	3.8−6.1/7.8−9.3	4.8 ± 1.1/8.5 ± 0.4	
Body depth	BD	4.0–9.0/6.4–9.4	6.2 ± 1.9/7.4 ± 0.7	
Eye diameter	ED	0.8–1.8/1.8–2.2	1.2 ± 0.4/2.0 ± 0.1	
Total number of myomeres	TNM	156–161/154–160		158/156
Last vertical blood vessel	LVBV	59–61/–		61/−
Predorsal myomeres	PDM	77–79/33–60		−/49
Preanal myomeres	PAM	121–126/50–75		126/61

Table 2 *Conger conger*. Sampling date, length, otolith size and developmental stage of the elvers used in this study. Note: for the four reared elvers, MA01, the lengths were final size

Specimen	Sampling date	Total length (mm)	Preanal length/ total length	Otolith radius (μm)	Otolith diameter (μm)
AGUN00	13 Nov 2000	210.0	0.38	420	833
MA01(12)	26 Apr 2001	88.5	0.38	712	1300
MA01(14)		82.0	0.35	689	1222
MA01(16)		87.0	0.37	667	1222
MA01(17)		96.0	0.38	789	1411

0.80 ± 0.01 (0.86 ± 0.01). The length of the 20 metamorphosing conger eels ranged between 113.0 and 142.0 mm, with a mean of 126.8 ± 6.8 mm. However, for this metamorphic stage the PAM/TNM (PAL/TL) ratio ranged from 0.32 to 0.48 (0.41–0.54) and presented an average value of 0.41 ± 0.04 (0.48 ± 0.04). The four laboratory-reared larvae had a mean length at capture of 133.0 ± 8.7 mm and an elver size of 88.4 ± 5.8 mm at the end of metamorphosis. The developmental stage (PAL/TL) of the metamorphosing leptocephali at the beginning of the rearing experiment was 0.48 ± 0.01. For the elvers (including the wild-caught specimen) the PAL/TL presented a mean value of 0.37 ± 0.01.

Otolith microstructure

Otoliths in the developing leptocephali were circular, and the growth increments were visible in all sections from the core to the edge (Fig. 3A). The core, constituted of well-calcified substances arranged in a radial form, was observed as a deep hole in the otolith. The primordium located in the core centre was substantially composed of an organic material and appeared as a deep hole after HCl etching. Two rings delimited the core area. The first, being visible as a deep circular groove surrounding the hole, corresponds to hatching (hatch check, HC). The second deeply etched ring, which separated the first growth increment and the core, probably marks the first feeding of the larva (first feeding check, FFC). These checks were postulated to be the HC and the FFC, since their morphology was similar to those in other anguilliformes fishes (Lecomte-Finiger and Yahyaoui 1989; Antunes 1994; Wang and Tzeng 1998, 2000; Arai et al. 2001). Outside this second check, in an outwards direction, daily growth increments were observed (Fig. 3B). The otolith diameter and radius of the six developing leptocephali ranged from 96 to 226 μm and from 56 to 153 μm, respectively.

The otoliths of metamorphosing leptocephali, with a less rounded shape, presented other permanent structures, like a peripheral diffuse zone (DZ), where the rings were unclear, and some accessory growth centres (AGC) (Fig. 4A). The otolith's increment countable zone (ICZ) presented an inner portion similar to that of the developing leptocephali (developing leptocephalus growth zone, DLGZ); however, its final portion presented a series of wide rings (wide increment zone, WIZ) that became less clear and disappeared when entering the DZ (Fig. 4B). The diameter and radius presented average values of 361 ± 36 and 210 ± 20 μm, respectively. The radial distance from the primordium to the end of the DLGZ ranged between 90 and 161 μm, and was close to the above-mentioned maximum otolith radius (153 μm) in developing leptocephali.

A clear change in otolith microstructure of the conger eel was observed in the elver stage (Fig. 5A). The ground and etched otoliths of the young juveniles were elliptical; the shape changed with growth. The growth of otoliths in the anterior–posterior direction was faster than in other directions. The microstructure of the otolith from the primordium to end of the DZ was also similar to that of the otoliths of metamorphosing eels (Fig. 5B). Several growth checks were observed in the otolith. The otolith diameter and radius of the reared conger eels presented mean values of 1289 ± 89 and 714 ± 53 μm, respectively. The wild young conger eel presented a diameter and radius of 833 and 420 μm, respectively.

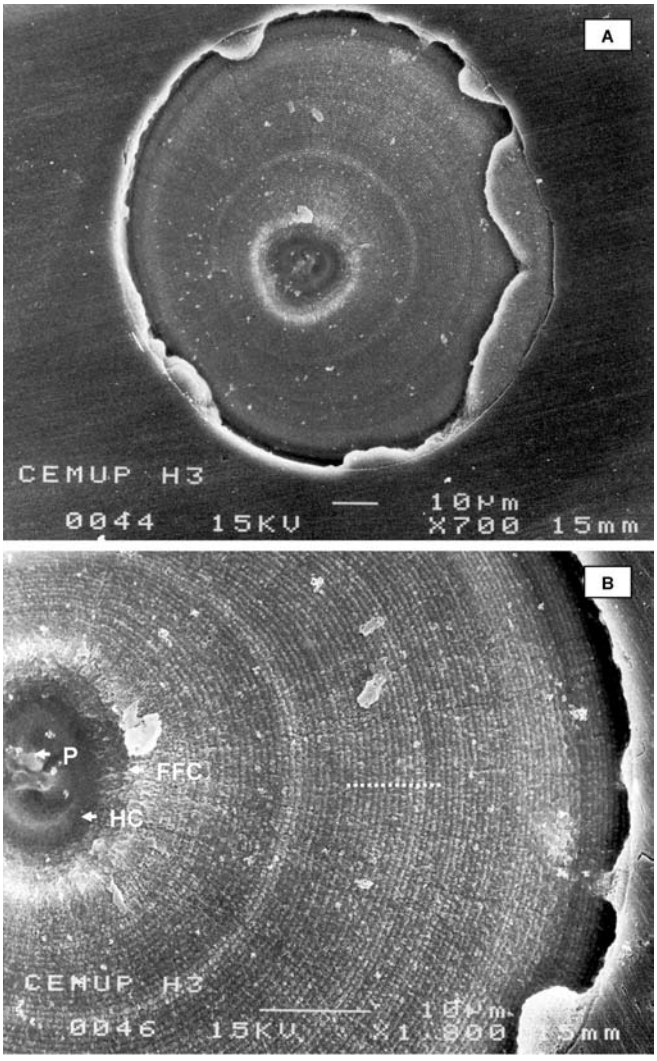

Fig. 3A, B *Conger conger*. SEM microphotographs showing the otolith microstructure of a developing stage leptocephalus (54.0 mm length): **A** whole view, **B** radius (*P* primordium; *HC* hatch check; *FFC* first feeding check; *white dots* daily growth increments)

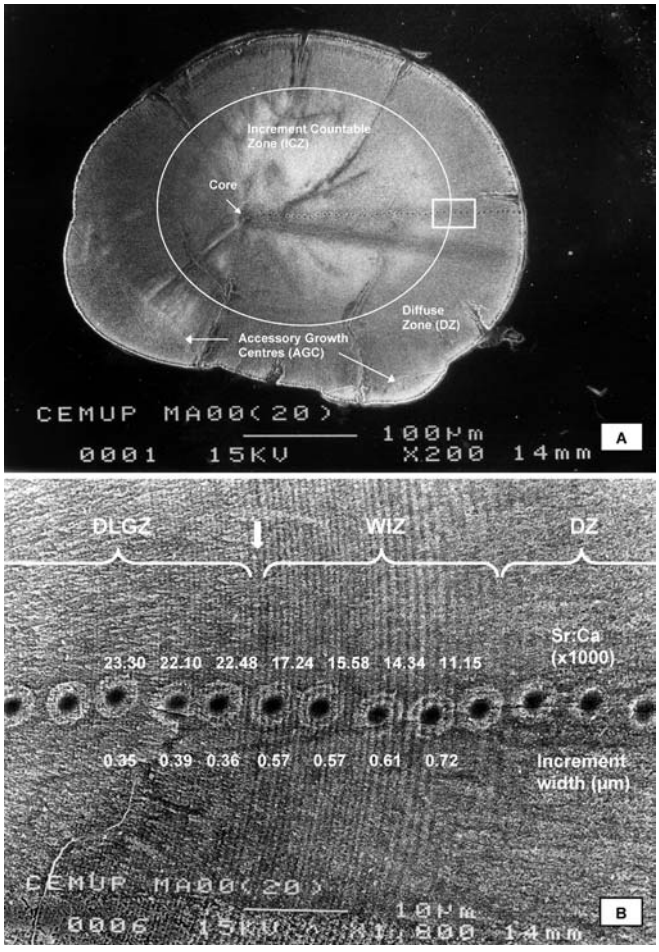

Fig. 4A, B *Conger conger*. SEM microphotographs showing the otolith of a metamorphosing leptocephalus (length: 117.0 mm; preanal length/total length: 0.50): **A** whole view showing the distinct otolith zones, **B** detail of the otolith zone indicated in a box in A (*DLGZ* developing leptocephalus growth zone; *WIZ* wide increment zone; *DZ* diffuse zone). Note: the *numbers above and below burn depressions* indicate the Sr:Ca ratios and increment width values, respectively. The *arrow* represents the onset of metamorphosis. See also Fig. 2

There was good correlation between the diameter (D) and radius (R) of the otolith for the developing leptocephali ($r^2 = 0.99$, $n = 6$, $P < 0.05$), metamorphic larvae ($r^2 = 0.66$, $n = 20$, $P < 0.05$) and elvers ($r^2 = 0.99$, $n = 5$, $P < 0.05$). There was a good relationship between otolith size (D and R) and fish length for the developing stage ($r^2 = 0.96$, $n = 6$, $P < 0.05$ and $r^2 = 0.97$, $n = 6$, $P < 0.05$, respectively); however this correlation did not exist during the metamorphosing stage ($r^2 = 0.08$, $n = 20$, $P = 0.22$ and $r^2 = 0.00$, $n = 20$, $P = 0.74$). There was no correlation between otolith size, expressed as D ($r^2 = 0.20$, $n = 6$, $P = 0.36$) or R ($r^2 = 0.21$, $n = 6$, $P = 0.36$), and the PAL/TL ratio of the developing-stage leptocephali. During metamorphosis, PAL/TL was negatively correlated with D ($r^2 = 0.20$, $n = 20$, $P < 0.05$), but not with R ($r^2 = 0.02$, $n = 20$, $P = 0.53$). The cores presented an overall mean value of 21.9 ± 1.4 µm, and no significant differences were found among the different larval stages ($P > 0.05$).

Otolith growth and Sr:Ca ratios change patterns

In the otoliths examined, changes in the Sr:Ca concentration ratios were due mainly to the variation in the amount of Sr, since the Ca content was stable during larval development ($30.8 \pm 3.9\%$).

Patterns of change in the otolith increment widths and Sr:Ca ratios in the developing leptocephali along the life-history transect, from the core to the edge, are shown in Fig. 6. Otolith increment widths increased between the FFC and age 20–40 days, thereafter gradually decreasing and becoming constant toward the edge. The average increment width was 0.54 ± 0.19 µm. Otolith Sr:Ca ratios tended to rise from the core toward

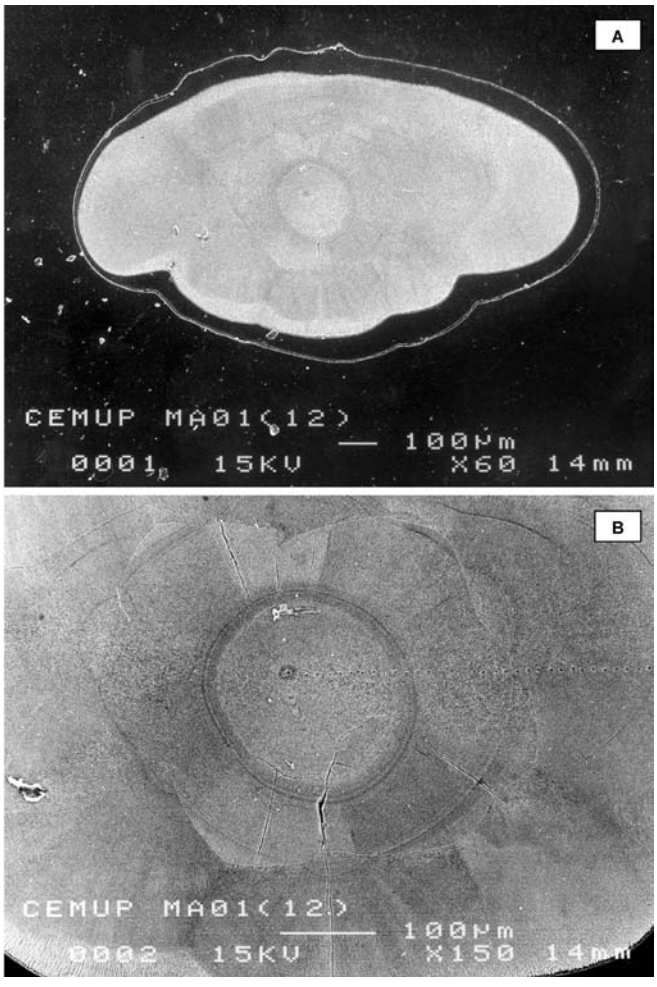

Fig. 5A, B *Conger conger*. **A** Elliptical shape of the otolith of an elver (total length: 85.5 mm), **B** detail of the central enlarged zone

the edge. A slight drop in the ratio was found from age 20–60 days. The minimum ratio was recorded in the core (7.1×10^{-3}), with the maximum values ($10.2–24.0\times10^{-3}$) occurring in the outermost regions.

Figure 7 shows patterns of change in otolith increment width and Sr:Ca ratios along the life-history transect of the metamorphosing larvae. The patterns were characterised by drastic changes in both increment width and Sr:Ca ratios in the outer region of the otolith. Otolith increment widths and Sr:Ca ratios showed profiles similar to those in developing leptocephali. However, in the final portion of the ICZ, otolith increment width sharply increased (WIZ) at the age of 170–250 days, until the microincrements disappeared in the otolith DZ. The mean number of increments in the DLGZ and ICZ was 215 ± 24 and 256 ± 28, respectively. The duration between the onset of width increase and peak width (i.e. the number of daily increments in the WIZ) was 39–91 days (45 ± 14 days). Sr:Ca ratios reached a maximum level (20.9×10^{-3}) at the end of the otolith DLGZ, decreasing abruptly thereafter, simultaneous with the increase in growth increment width (onset of metamorphosis). The minimum value was recorded in the core (7.1×10^{-3}). After metamorphosis the Sr:Ca ratios decreased, but never reached the minimum values obtained in the core.

The pattern of change in the Sr:Ca ratios of otoliths was consistent between the five elvers and was similar for both laboratory-reared and field-caught conger eels (Fig. 8A, B). Sr:Ca concentration ratios were lowest in the primordium (6.3×10^{-3}) and at the edge of the otolith ($4.7–6.1\times10^{-3}$) in elvers. Profiles of increment widths and Sr:Ca ratios along the otolith ICZ of the elvers were similar to those observed in metamorphosing eels (Fig. 8C).

No significant differences were found in mean Sr:Ca concentration ratios in the core among different larval stages ($P>0.05$). The core Sr:Ca ratio presented an overall mean value of 7.0×10^{-3}.

Age and hatching time

The ages of the six developing leptocephali ranged from 69 to 260 days (Table 3). Conger eel hatching dates, back-calculated from estimated daily ages, indicated that the spawning season lasted 7 months (from early December 1999 to end of June 2000).

A strongly significant correlation was found between otolith size (D and R) and age of the developing leptocephali ($r^2=0.98$, $n=6$, $P<0.05$ and $r^2=0.98$, $n=6$, $P<0.05$, respectively). There was also a linear relationship between length and age of the developing leptocephali ($r^2=0.99$, $n=6$, $P<0.05$).

Age at metamorphosis

Based on previous data on otolith microstructure and Sr:Ca ratios in some anguilliformes fishes, namely the *Anguilla* species (see ''Discussion''), the age at the otolith point where increment width showed a drastic increase coincidental with a marked decrease in Sr:Ca ratios (Figs. 2, 4B and 6) was regarded as the onset of metamorphosis for this species (i.e. age at metamorphosis). The duration of the developing leptocephalus stage ranged between 170 and 250 days, with an average value of 215 ± 24 days. The age at metamorphosis was negatively correlated with the mean increment widths of the DLGZ ($r^2=0.56$, $n=20$, $P<0.05$) (Fig. 9).

Discussion and conclusions

The number of myomeres of conger eel larvae was counted for species identification and was in agreement with the data reported by several authors (D'Ancona 1931; Schmidt 1931; Castle 1970; Strehlow et al. 1998; Correia et al. 2002). The external body morphology and pigmentation pattern of conger eel leptocephali has been described in detail by D'Ancona (1931) and Correia et al. (2002). The PAM/TNM ratio has been adopted as

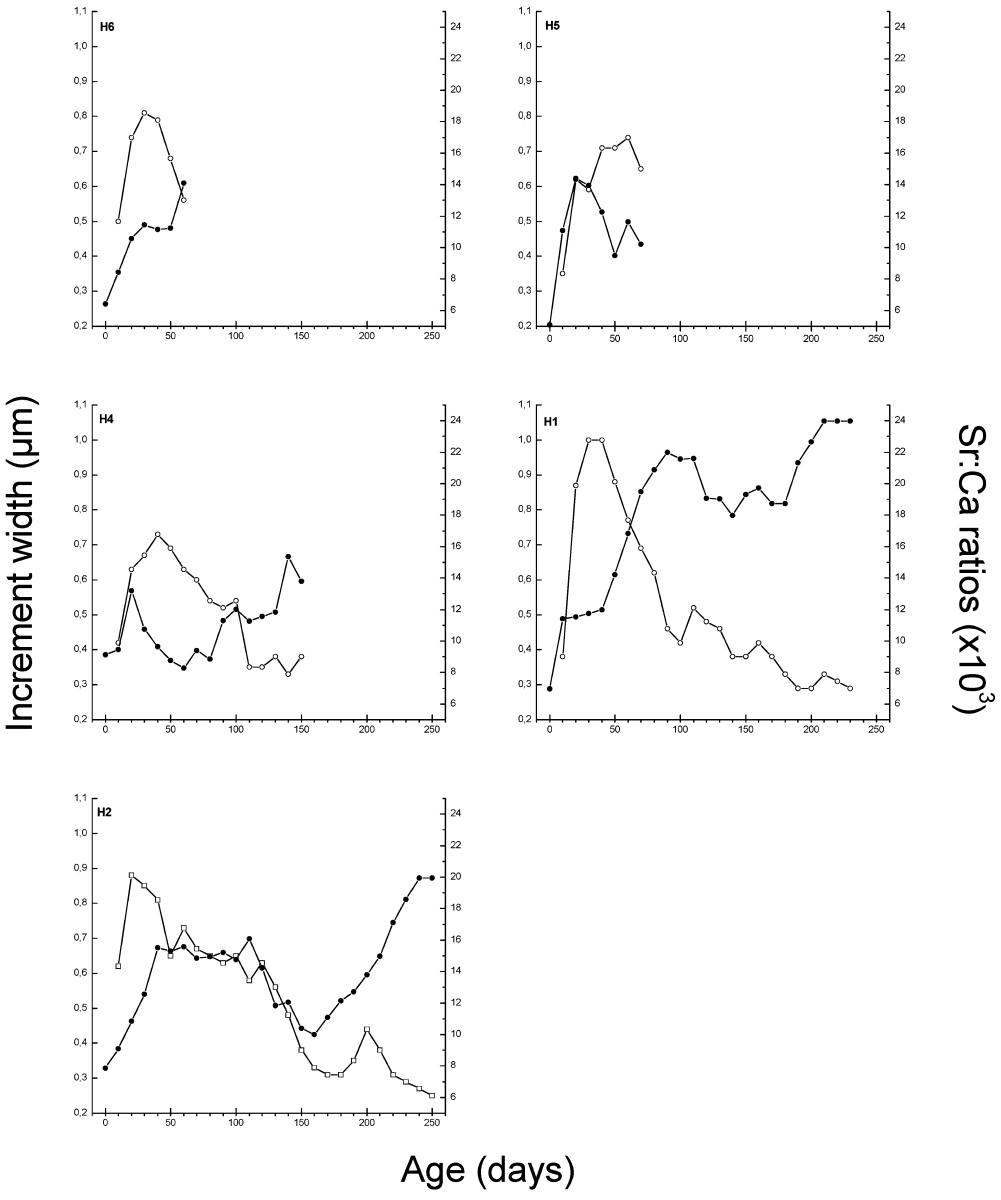

Fig. 6 *Conger conger*. Profiles of the otolith increment width (*open circles*) and Sr:Ca concentration ratios (*closed circles*) measured from the core (age 0) to the edge in the developing leptocephali. *Each point* represents the average of data for every 10 days (*H1–H6* specimens, see Table 3 for details)

a criterion for differentiating the developmental stages of conger eel leptocephali (Tanaka et al. 1987; Lee and Byun 1996; Otake et al. 1997; Strehlow et al. 1998; Correia et al. 2002), because it is difficult to define the developmental stages of conger eel larvae from body proportions, external morphology and pigmentation alone (Tanaka et al. 1987). The PAL/TL index has been used with the same purpose, since it is correlated with the PAM/TNM ratio (Yamano et al. 1991; Otake et al. 1997; Correia et al. 2002), and has the advantage of being more easily obtainable and less affected by counting errors (Correia et al. 2002). The PAL/TL and PAM/TNM mean values obtained for the developing leptocephali (0.86 and 0.80, respectively), metamorphosing larvae (0.48 and 0.41, respectively) and elvers (0.37) were within the ranges recorded by other authors (Strehlow 1992; Correia et al. 2002). It has recently been suggested that *Conger conger* leptocephali begin to metamorphose at PAL/TL (or PAM/TNM) values between 0.86 and 0.55 (or 0.77 and 0.42) (Correia, unpublished data). Lee and Byun (1996) reported, for instance, that *C. myriaster* leptocephali began to metamorphose at PAM/TNM values between 0.82 and 0.74.

The otolith morphology of *C. conger* leptocephali has already been described during its developing (Antunes 1994; Antunes and Correia 2002) and metamorphosing stages (Correia et al. 2002), and is similar to that observed in other anguilliformes species (Lecomte-Finiger and Yahyaoui 1989; Antunes 1994; Wang and Tzeng 1998, 2000; Arai et al. 2001). Assuming that the increments are deposited on a daily basis, ages of the developing leptocephali were estimated as 69–260 days old after hatching. The estimated hatch dates, back-calculated from sampling dates and ages of the developing leptocephali, ranged from December 1999 to June 2000. Antunes and Correia (2002) reported that the hatching season occurs

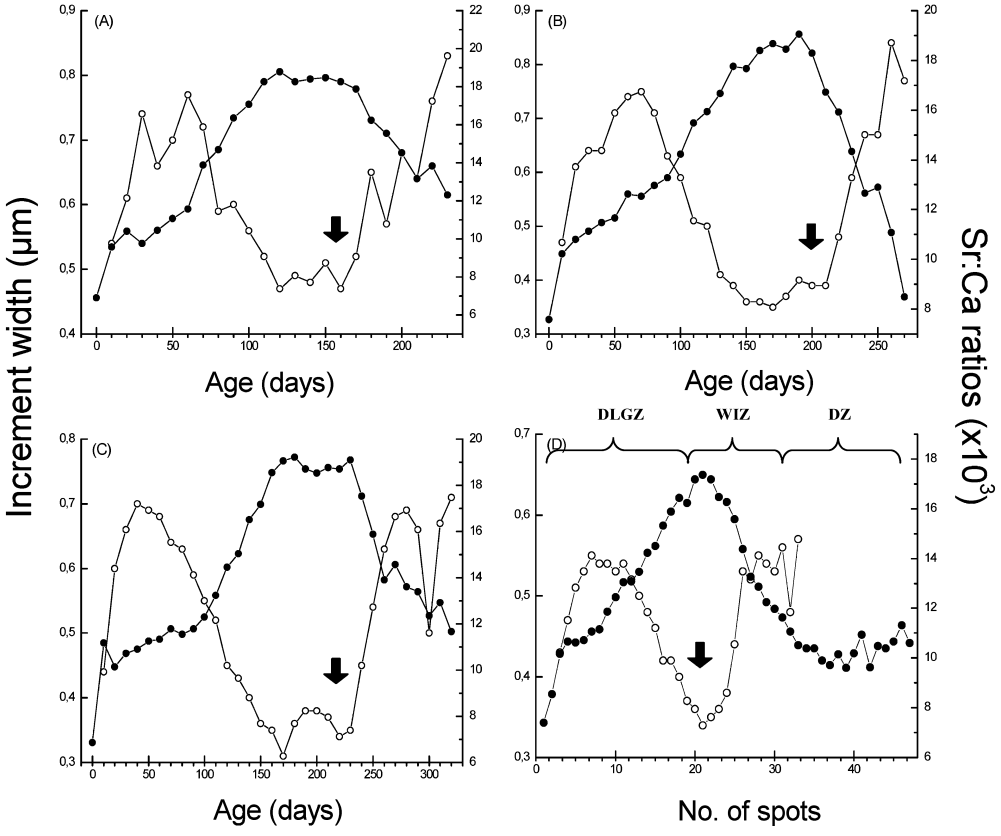

Fig. 7A–D *Conger conger* metamorphosing larvae. **A–C** Profiles of the otolith increment width and Sr:Ca concentration ratios measured from the core (age 0) to the end of the increment countable zone. Specimens were grouped by the time when coincidental changes in increment width and Sr:Ca ratios occurred (**A**: 170–190 days, $n=4$; **B**: 200–220 days, $n=7$; **C**: 230–250 days, $n=9$). **D** Mean values for width increment and Sr:Ca ratios for total specimens ($n=20$), measured from the core to the otolith edge (*DLGZ* developing leptocephalus growth zone; *WIZ* wide increment zone; *DZ* diffuse zone; *black arrows* approximate locations of metamorphosis). See also Figs. 2 and 4

from April to October. The estimated values for the hatching season were slightly different among these studies, all suggesting an extended spawning season for the conger eel. A long spawning season has been reported in the Japanese eel (Tabeta et al. 1987; Tsukamoto 1990; Tsukamoto and Umezawa 1990), and it has been proposed that this might be due to multiple populations of adult *Anguilla japonica* prolonging the duration of the spawning season (Tsukamoto 1990).

The spawning ground, ecology and migration of leptocephali in the ocean are poorly known at present. Strehlow et al. (1998), based on spatial and temporal distribution of conger eel developing leptocephali collected during several oceanographic cruises, suggested that spawning occurs in the Mediterranean Sea between July and September. Around November, after a short growth period, the larvae (>30 mm) start migration in a NW direction, namely to southern Portugal and Spain, spreading throughout the eastern and central zones of the Atlantic. Here, conger eels experience a new period of growth, lasting until the beginning of summer (130–150 mm and a maximum of 165 mm length), at which time they resume migration. It is supposed that this bout of migration, in the direction of the coastal waters of the continental slope, with a possible return to the Mediterranean, induces metamorphosis. However, it is difficult to explain how some specimens reached the Azores area based on this larval migration pathway.

Let us assume, for instance, that our smaller developing specimens collected south of the Azores (49.0, 54.0 and 54.0 mm long and 69, 81 and 85 days old, respectively) were in fact born in the Mediterranean Sea (Cau and Manconi 1983). They would have had about 2–3 months to travel the approximately 3000 km from the spawning ground to the catch location. This means that these larvae would have to swim about 35–44 km day^{-1} against the prevailing circulation pattern of the NE Atlantic (Klein and Siedler 1989; Käse and Krauss 1996; Johnson and Stevens 2000). If conger eel leptocephali migrate actively and directly, for example, at 10 km day^{-1}, as proposed for *A. anguilla* larvae (Antunes and Tesch 1997), they would only be able to cover a distance of about 690–890 km from the spawning ground. Based on the age of our smaller developing leptocephali and the water current systems off the Azores, we propose that conger eel might also spawn in or near the Azores archipelago. However, further information on the geographical distribution, age and growth of *C. conger* is necessary in order to determine the spawning area(s) of this species and its migration pathways to the European coast and to the Azores.

Several studies have indicated that past environmental history of anguilliformes fishes can be reconstructed from analysis of otolith Sr:Ca concentration ratios. Changes in otolith Sr:Ca ratios have been considered to be related to environmental factors such as water temperature (Tzeng 1994), salinity (Tzeng and Tsai 1994) and water mass (Otake et al. 1994), as well to endogenous physiological factors (Tzeng 1996; Otake et al. 1997; Arai et al. 1997).

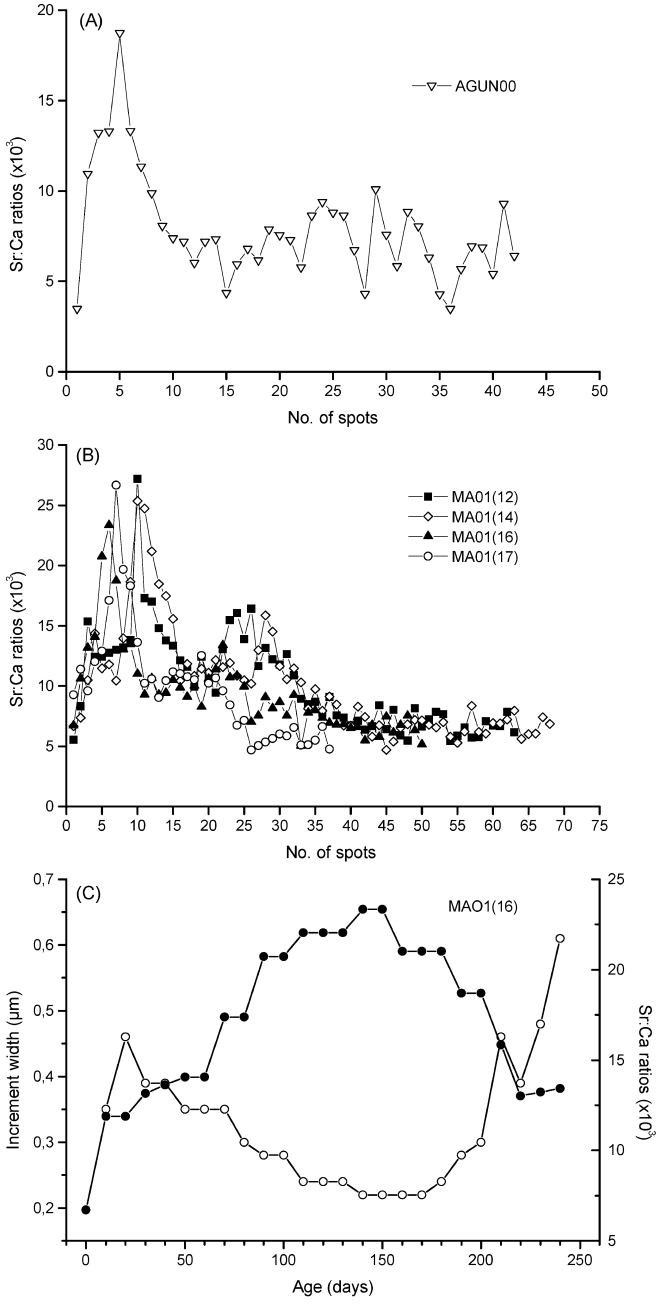

Fig. 8A–C *Conger conger*. **A, B** Sr:Ca concentration ratios measured from the primordium to the otolith edge, respectively, for the wild and reared elver specimens. **C** Changes in otolith increment width (*open circles*) and Sr:Ca ratios (*closed circles*) in the increment countable zone of one elver (*abbreviations*, see Table 2)

The Sr:Ca concentration ratios and their variation in conger eel larvae are similar to those reported in other anguilliformes leptocephali (Otake et al. 1994, 1997; Tzeng 1994, 1996; Tzeng and Tsai 1994; Arai et al. 1997, 1999a, 1999b, 2000b, 2001; Wang and Tzeng 1998, 2000; Marui et al. 2001). Sr:Ca ratios were lowest in the primordium and at the edge of the otolith. Some authors (Tzeng and Tsai 1994; Wang and Tzeng 2000) suggested that the low Sr:Ca ratio in the otolith primordium of

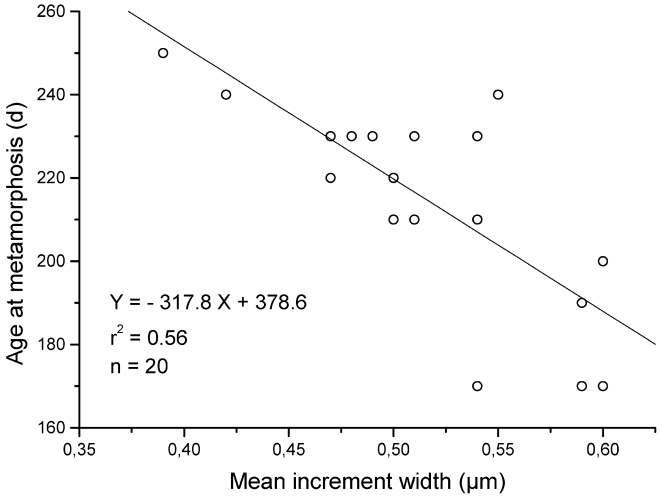

Fig. 9 *Conger conger* metamorphosing larvae. Scatter diagram of age at metamorphosis versus otolith mean increment width. Regression line represents a least-square fit of the linear equation ($P < 0.05$)

temperate eels (*A. japonica* and *A. rostrata*) is probably due to the freshwater, maternal origin of the yolk sac. Our results, in conjunction with the data obtained by Otake et al. (1997) for *C. myriaster*, show that this feature is not restricted to the catadromous species, as it appears to be common in all anguillid leptocephali, including entirely marine species. We think that the low Sr content in the core reflects its chemical composition, since it is substantially composed of organic material, probably fibroprotein otolin, instead of the typical aragonite matrix. Several investigators (Tzeng 1994; Tzeng and Tsai 1994; Wang and Tzeng 2000) also proposed that the drop in Sr in the eel at and after metamorphosis reflects the sudden change in salinity of the environment of the migratory eel (i.e. the entry into freshwater habitats less rich in Sr). In the case of conger eel, however, we agree with Otake et al. (1997), who have proposed that the drastic decrease in Sr in the otolith matrix during late metamorphosis reflects the mobilisation of body minerals for rapid bone development.

The change in the pattern of otolith growth increments in the metamorphosing eels, as previously described by Correia et al. (2002), could be divided into four phases. The first phase is characterised by a pronounced increase in the width of daily rings (until a maximum of 0.80 µm), from the FFC to about 30 days. A second phase shows a gradually decreasing increment width, until a relatively constant minimum value (0.35–0.45 µm) is reached, at about 160–180 days. This period of narrow rings can be quite extensive (170–250 days), depending on the specimen. These two phases overlap with those of the developing leptocephali. In a third phase, the increment width changes dramatically and becomes wide (to a maximum of 0.65–0.90 µm) and less clear, until the microincrements disappear, at about 200–320 days, when entering into the fourth phase

Table 3 *Conger conger*. Length, otolith radius, sampling date, location, age andhatching date of the leptocephali collected by the R.V. ''Heincke'' in August 2000 (*, specimens used for electron microprobe analysis)

Specimen	Sampling date	Catch position	Total length (mm)	Otolith radius (μm)	Age (days)	Hatching date
H6*	11 Aug 2000	33°56′N;30°08′W	49.0	56	69	4 Jun 2000
H5*	12 Aug 2000	35°39′N; 27°39′W	54.0	67	81	24 May 2000
H3	12 Aug 2000	35°00′N;28°36′W	54.0	60	85	20 May 2000
H4*	4 Aug 2000	43°57′N; 28°32′W	76.0	93	163	24 Feb 2000
H1*	16 Aug 2000	44°29′N;12°59′W	92.0	139	240	21 Dec 1999
H2*	17 Aug 2000	45°11′N; 11°46′W	96.0	153	260	2 Dec 1999

(diffuse zone). These last two fluctuations are not found in the otolith of developing leptocephali, in which increment widths remained narrow throughout. Furthermore, Sr:Ca concentration ratios in the otolith show a drastic decrease coincidental with the beginning of the third phase of otolith incrementation, whereas the Sr:Ca ratio does not drop in the larger developing leptocephali.

There is no clear correlation between any of the otolith growth phases (first, second, third and fourth) in metamorphosing conger eel leptocephali and morphological or ecological events. Arai et al. (2001) proposed that the inflection in otolith growth ca. 30 days from hatching, might be related to favourable somatic growth after successfully switching their nutritional source from yolk material to exogenous feeding, which probably occurs after 10 days based on observations that artificially hatched eel larvae exhausted their yolk material during this period (Yamauchi et al. 1976; Tanaka et al. 1995). In the Japanese conger eel, *C. myriaster*, it has been reported that otolith increment widths increased at the onset of metamorphosis (Tanaka et al.1987; Mochioka 1989; Lee and Byun 1996; Otake et al. 1997), which coincided with a drop in otolith Sr:Ca ratios (Otake et al. 1997). Such relationships between otolith growth, Sr:Ca ratios and metamorphosis seem to be typical in anguilliformes fishes, and have been reported for several species, e.g. in *Anguilla* species (Otake et al. 1994; Tzeng and Tsai 1994; Cheng and Tzeng 1996; Arai et al. 1997, 1999a, 1999b, 2000b, 2001; Wang and Tzeng 1998, 2000; Marui et al. 2001). Leptocephali contain large amounts of gelatinous extracellular matrix composed of sulphated glycosaminoglycans (GAG) (Pfeiler 1991) that have a high affinity for Sr (Nishizawa 1978). It has been proposed that the rapid GAG breakdown during metamorphosis (Pfeiler 1999) causes a loss of corporal Sr, resulting in a drastic decrease of otolith Sr content and consequently Sr:Ca ratios (Otake et al. 1997). These considerations lead to the conclusion that the marked increases in otolith increment widths, coincidental with a dramatic decrease in Sr:Ca ratios, herald the onset of metamorphosis.

Since the number of daily increments from the otolith core to the beginning of the WIZ represents the duration of the developing leptocephalus stage, our results confirm an earlier study (Correia et al. 2002), which indicated that the European conger eel takes about 6–9 months from hatching to reach the onset of metamorphosis. The age at metamorphosis and the duration of the leptocephalus stage appear to be important factors determining the long-distance dispersal of the Japanese eel (Cheng and Tzeng 1996). Wang and Tzeng (2000) showed that the differences in leptocephalus stage duration and growth rate are the principal factors determining the segregation of migrating American (*A. rostrata*) and European (*A. anguilla*) eels.

Whether metamorphosis is triggered by some environmental stimulus or occurs spontaneously at a certain age or size is unknown (Smith 1989). For some species we know that metamorphosis is initiated when pelagic leptocephali migrate to inshore waters, suggesting that the shallow, near-shore environment is somehow involved in the triggering mechanism (Pfeiler et al. 1990). However, in other species, metamorphosis takes place in the open ocean, indicating that factors other than proximity to shore are involved (Pfeiler 1999). Arai et al. (2001) suggested that the environmental sea temperature and somatic growth should play important roles in the timing of metamorphosis in eels.

The existence of a diffuse zone without distinct rings, in the marginal portion of the otoliths of the metamorphic leptocephalus stage, prevents accurate estimate of the duration of the larval stage in this species (Antunes and Correia 2002; Correia et al. 2002; present study). Tanaka et al. (1987) observed a disturbance of the ring arrangement in the marginal region of the otoliths of Japanese conger eel leptocephali, with a microstructure similar to our diffuse zone. However, these authors assumed it to be an anomalous, non-permanent structure, and did not characterise it as a normal structure produced during the process of metamorphosis. Mochioka et al. (1989) also reported that the otoliths of *C. myriaster* during metamorphosis grew rapidly and with irregularities on the outer surface. Lee and Byun (1996) mentioned that the otolith increments of the outer opaque zone in the metamorphic larvae of Japanese conger eels were not always easily identifiable under light microscopy or scanning electron microscopy. They supposedly solved this problem, by first examining the opaque zone when the ground plane reached the outer margin, and thereafter polishing the otoliths until the core plane was exposed. Curiously, they never showed any picture of this ''opaque zone'' with clear increments to the otolith edge. Recently, Otake et al. (1997) described the otolith morphology of the developing and metamorphosing stage of *C. myriaster* leptocephali, and also reported some difficulties in consistently etching all

increments from the core to the edge. These descriptions suggest some subjectivity in the examination of otolith microstructure in the leptocephali of Japanese conger eel.

A diffuse otolith zone was also described in European eel leptocephali, before the onset of metamorphosis (Antunes and Tesch 1997). Antunes and Tesch (1997) and Williamson et al. (1999) suggested that this otolith portion marks a period of very slow growth and is made up of many daily growth rings that are too thin to be distinguished and counted. Based on this assumption, Antunes and Correia (2002) estimated a total age of about 483 and 736 days, for two metamorphosing conger eels collected in Minho River on April and November 1998, respectively. They used the average increment width of small fragments of ring structure in the diffuse zone to calculate the number of days in that unreadable portion of the otolith. Williamson et al. (1999) suggested that during this period of life the leptocephali have very little food intake and grow very slowly. So, to conserve energy the eel leptocephali may remain at depths of about 300 m for a long period of time and stop performing diel vertical migrations. Umezawa and Tsukamoto (1991) showed that conditions of prolonged starvation and/or cold temperatures could stop the deposition of daily rings in *A. japonica*. Tabeta et al. (1987) suggested that the daily vertical migration in the leptocephalus stage might be one of the environmental conditions that cause the formation of the daily growth rings. Arai et al. (2000a) suggested that the formation of the diffuse (unclear) increment area in *A. anguilla*, demonstrated by Antunes and Tesch (1997), may simply be due to a technical problem (e.g. overetching), since Lecomte-Finiger (1992) observed clear concentric rings throughout the otolith of this species including the leptocephalus and metamorphic stages. Recently, Cieri and McCleave (2000) proposed that the diffuse zone could be produced by a process of calcium resorption in the otolith periphery during metamorphosis of the eel, as part of the calcium metabolism as skeletal elements are being formed.

We can conclude by saying that the mechanism behind the formation of this structure is not yet understood. If this otolith portion is a visual artefact, resulting from the technical preparation of the otolith, or a different interpretation of the microstructural growth pattern, or even a permanent structure caused by an environmental or physiological stimulus remains open to discussion.

We recently proposed (Correia et al. 2002), based on indirect evidence (duration of the developing leptocephalus stage, time of capture and developmental stage of metamorphosing conger eels) that the duration of the larval phase for this species is about 2 years. However, it seems that the exact age is impossible to determine from counting daily growth increments, and the values presented for the duration of metamorphosis and for total larval age, unfortunately, remain speculative for the conger eel.

The estimated duration of metamorphosis in Japanese conger eel is variable among investigators: 23–31 days (Kubota 1961); 22 days at temperatures of 18–22°C (Asano et al. 1978); 53–75 days at temperatures of 10–16°C (Lee and Byun 1996); and 71 days at temperatures of 11–15°C (Otake et al. 1997). Our specimens, captured in April 2001, successfully completed metamorphosis in about 2 months at 15°C. However, it is plausible that captive specimens may accelerate metamorphosis (Butler et al. 1996). It is well known, for example, that the stress hormone cortisol plays an important role on the metamorphic process of leptocephali (Yamano et al. 1991). As well as the temperature of the sampling area, the difficulty or ambiguity of identifying the beginning and completion of metamorphosis may also result in inaccurate estimation of its duration (Otake et al. 1997).

The diameter and radius of the larval otolith are highly correlated during larval development, suggesting that these otolith measures are helpful in describing the otolith growth rate. The significant correlation between otolith radius (or diameter) and estimated age of the developing larvae suggested the potential use of otolith size to represent fish age. However, during metamorphosis otolith and body growth appear to be uncoupled in conger eel, as shown by Lee and Byun (1996) and Correia et al. (2002). Otolith size from the reared elvers (diameter range: 1222–1300 μm) was twice that of the wild specimen (833 μm), an unexpected result. However, some preliminary studies on reared specimens using fluorescent dyes as otolith time markers have suggested an anomalously high growth rate of the otolith (Correia, unpublished data), probably as a result of the stress to fish. These observations suggest that the real daily growth rhythm in the otolith diffuse zone cannot be calculated by examining reared larvae.

The number of daily growth increments from the outer core to the onset of metamorphosis (i.e. age at metamorphosis) was negatively correlated with mean increment width, suggesting that fast-growing leptocephali metamorphosed earlier. Several authors (Tsukamoto and Umezawa 1990; Tzeng 1990; Cheng and Tzeng 1996) observed the same phenomenon in *A. japonica* and suggested that the time taken for migration from the oceanic spawning ground to coastal waters was probably shorter for the fast-growing larvae. In general, fast-growing larvae metamorphose early and swim quickly to the preferred habitat (Hunter 1972; Miller et al. 1988). In contrast slow-growing larvae, which are unable to swim as fast as the fast-growing ones, prolong the duration of the larval stage and delay metamorphosis and recruitment to the estuary (Victor 1986). Furthermore these larvae are more vulnerable to predation, which may cause poor recruitment (Tzeng 1990). Several studies (Tsukamoto 1990; Tsukamoto and Umezawa 1990; Wang and Tzeng 1998; Arai et al. 1999a, 1999b, 2000b, 2001; Marui et al. 2001) have also reported a positive relationship between age at recruitment and age at metamorphosis in temperate and tropical eels, suggesting that early-metamorphosing

larvae were recruited to coastal habitats at a younger age.

The presence of metamorphosing conger eel larvae in the northern coastal waters of Portugal has been recorded from late October to mid-June, because they sometimes enter the mouth of the Minho River, by tidal transport, and are caught by the Portuguese glass eel fishery (Correia et al. 2002). These authors showed that the largest larvae, in an advanced metamorphic stage, are recruited early to northern Portuguese coastal waters. Thus, the migrating mechanism of the conger eel can be summarised as follows: the larvae with a faster growth rate metamorphose and recruit earlier to coastal areas, probably at a younger age, and with a larger size and an advanced developmental stage. This larval recruitment pathway appears to be common in anguillid fishes.

Acknowledgements The Portuguese Foundation for Science and Technology (Research Project Praxis XXI:2/2.1/MAR/1728/95) supported this study. The author has a PhD grant (BD 18082/98) from the same sponsor. We thank Dr. J. Wilson for English language assistance, and Dr. F. Ramos and Dr. P. Piedade for help in preparing and analysing the otolith microchemistry, respectively.

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