

An evaluation of the otolith characteristics of *Conger conger* during metamorphosis

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A sample of 20 metamorphosing conger eel *Conger conger* leptocephali were collected from the Minho River, Portugal, in February 1999 and their sagittal otoliths were analysed by scanning electron microscopy. Four different etching agents were applied along both sagittal and frontal sections during otolith preparation to examine the microstructural growth in this species. Otolith growth increments were visible throughout the increment countable zone using all four treatments, but a permanent peripheral diffuse zone, where the daily increments were unclear, appeared on all otoliths, preventing accurate age estimation. To understand more about the nature of the diffuse zone, otoliths of 10 other metamorphosing leptocephali reared in aquaria were marked by immersion in tetracycline hydrochloride. The distance between the fluorescent marks and otolith edge, measured over a fixed period of time, was used to estimate the otolith growth rate. The application of this technique led to an anomalously high estimated otolith growth rate, probably as a result of the capture, marking and handling stress.

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Key words: accessory growth centres; conger eel; leptocephalus; otolith; peripheral diffuse otolith zone.

INTRODUCTION

The conger eel *Conger conger* (L.) is an important fish of the coastal and outer continental shelf marine ecosystems of the north-eastern Atlantic Ocean (Bauchot & Saldanha, 1986). The Mediterranean Sea is thought to be the spawning place for this species, based on the capture of small conger eel leptocephali (Schmidt, 1931). This assumption is supported by the capture of sexually mature specimens of *C. conger* in deep waters south-east of Sardinia (Cau & Manconi, 1983) and by the length and otolith analyses of leptocephali collected in the north and central Atlantic Ocean (Strehlow *et al.*, 1998). Other spawning places, however, have been suggested for *C. conger* in the eastern Atlantic Ocean,

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such as the area between Gibraltar and the Azores (Lythgoe & Lythgoe, 1971; Bagenal & Kenney, 1973; Wheeler, 1985; Hayward & Ryland, 1995). Until now, sexually mature specimens have not yet been caught (Sbaihi *et al.*, 2001; Sullivan *et al.*, 2003), with the exception of a single mature female caught in the Irish Sea (Fannon *et al.*, 1990). Recently, based on the capture of several small and young leptocephali, the conger eel may have another spawning ground near the Azores (Correia *et al.*, 2002a, 2003).

Backcalculated hatching dates from the otolith microstructure of field-caught leptocephali of *C. conger*, suggests a long spawning season, from December to July, with one annual peak occurring in summer (Correia *et al.*, 2002a, 2003; Antunes & Correia, 2003). During the migration from the spawning grounds to the waters of the Atlantic continental slope, the leptocephali grow *c.* 0.38 mm day⁻¹ (Antunes & Correia, 2003), reaching a total length (L_T) of 150 mm (Strehlow *et al.*, 1998). After that the leptocephali start a migration towards the continental shelf and coastal waters that probably induces metamorphosis (Strehlow *et al.*, 1998). It has been suggested that the leptocephali have a long larval life (Bauchot & Saldanha, 1986), taking *c.* 1–2 years to drift inshore and to reach the juvenile elver form (Lythgoe & Lythgoe, 1971; Wheeler, 1985; Strehlow, 1992). The first half of the larval stage (*i.e.* the developing phase) takes between 6 and 9 months (Correia *et al.*, 2002b, 2003). Preliminary studies on metamorphosing leptocephali, however, showed the presence of a peripheral diffuse otolith zone, during which no formation of daily growth increments takes place (Correia *et al.*, 2002b, 2003). For this reason an exact age determination for the metamorphosing conger eel leptocephali by growth increments seems impossible. A similar otolith diffuse zone, where the morphological features are irregular, has also been described in the European eel *Anguilla anguilla* (L.) (Antunes & Tesch, 1997) and in the American conger eel *Conger oceanicus* (Mitchill) (Correia *et al.*, 2004). Tanaka *et al.* (1987), also observed a similar disturbance of the ring arrangement in the marginal region of the otoliths of the Japanese conger eel *Conger myriaster* (Brevoort) but assumed it to be an anomalous, non-permanent structure. Later, Lee & Byun (1996) were able to count very faint increments in this outer otolith zone of metamorphosing leptocephali. The mechanism resulting in such structure is not fully understood, however, several hypotheses have been suggested to explain the formation of this unclear zone in the glass eel otoliths, such as a very slow growth period (Antunes & Tesch, 1997), a stop in the diel vertical migrations of leptocephali (Williamson *et al.*, 1999) and a process of aragonite resorption as part of calcium metabolism (Cieri & McCleave, 2000). Recently, it has been suggested that this otolith zone is an artefact produced during the preparation of *A. anguilla* otoliths as a result of an overetching (Arai *et al.*, 2000).

To test if the peripheral diffuse otolith zone of the metamorphosing conger eel leptocephali is a permanent structure or an artefact resulting from a bad otolith procedure, the effect of different otolith preparation methods, commonly used in anguilliform leptocephalus studies, on the final appearance of the otoliths was studied. Additionally, with the purpose of knowing the rhythm of formation of the peripheral diffuse zone, a fluorescent dye was used as an otolith time marker, on some reared metamorphosing leptocephali.

METHODS

Twenty metamorphosing conger eel leptocephali collected in February 1999, as by-catch of the glass eel fishery, at the mouth of Minho River, north Portugal (41° 30' N; 8° 50') (Correia *et al.*, 2002b), were used in this study. Both right and left sagittae were dissected from the leptocephali, cleaned of adhering tissues, washed with distilled water and air-dried. Left otoliths were mounted on cylindrical stubs with thermoplastic glue, convex side up, and right otoliths were embedded within an epoxy resin block. The excess resin was removed using a low-speed saw. Both otoliths were hand polished in the sagittal (left otolith) and frontal (right otolith) planes with a 2400 silicon carbide abrasive paper and alumina solution (1 : 20) until the core was revealed. During this procedure frequent checks were made on the cores. These were viewed as dark spots under a metallographic microscope (Nikon OPTIPHOT-M). Later, each pair of otoliths was etched with one of four different chemical agents: HCl, CH₃COOH, EDTA and PKb (*i.e.* five pairs of otoliths by etching agent), coated with gold and viewed under a scanning electron microscope (SEM; Jeol JSM 639-1f) at 15 kV. The concentrations and reaction times for each etching agent were established by several trials. The mounting media, polishing procedure and etching agents used in this study have been based on the most common procedures used in otolith studies of anguilliform larvae (Table I). Additionally, the polished otoliths of five leptocephali, coated with carbon, were also examined at 20 kV using a back-scattered detector, instead of the secondary electron detector, a procedure described by Waldron & Gerneke (1997). These unetched otoliths, however, gave poorly contrasted images and were excluded from this study.

A SEM series of overlapping photographs were taken from the core to the edge of each otolith. The number of daily increments was estimated at ×1800 and ×2200 magnifications, to the sagittal and frontal sections respectively. All otolith measurements were carried out according to the procedures of Correia *et al.* (2003). The percentage of the area occupied by the accessory growth centres (AGC) on the total otolith surface was also calculated on the sagittal planes (Correia *et al.*, 2002b). Based on previous data on otolith and larval development studies in closely related species, it was assumed that the growth increments in *C. conger* leptocephali were daily, and that the beginning of the otolith wide increment zone (WIZ) marked the onset of metamorphosis. Ten days were also added to the number of daily increments to account for the time between fertilization and the end of the yolk-sac stage, a period during which no increments were deposited.

The otolith growth rate in the peripheral diffuse zone (DZ) was studied using wild-caught laboratory-reared metamorphosing leptocephali ($n = 10$) also collected in the Minho River in June 2000 and April and May 2001, immediately after entry into estuarine waters. After collection, the larvae were transported to the laboratory in aerated containers and reared in a 100 l tank, under an artificial photoperiod (12L : 12D) and with a water temperature and salinity of 15° C and 31, respectively. After 1 day acclimation the leptocephali were anaesthetized with 2-phenoxyethanol (250 µl ml⁻¹), weighed (±0.01 g) and measured (±0.1 mm). All the specimens have been classified according to the staging scheme of Bell *et al.* (2003). The preanal length : total length ($L_{PA} : L_T$) has been also adopted as a larval developmental stage criterion (Otake *et al.*, 1997; Strehlow *et al.*, 1998; Bell *et al.*, 2003). On the 7th and 14th days, the leptocephali were placed into a 5 l aerated seawater aquarium containing 400 mg l⁻¹ of tetracycline hydrochloride (C₂₂H₂₄N₂O₈.HCl; Sigma Chemical Co.) for 24 h in the dark to prevent light-degradation of the fluorescent chemical. It was assumed that there was no lag-time between exposure to the treatment and incorporation of the chemical into the otoliths (Pitcher, 1988). This treatment did not cause any mortality. Near the end of the captive experiment the larvae were fed twice a day with live marine polychaetes. The leptocephali were sacrificed *c.* 1 month after capture, when they had metamorphosed into glass eels and elvers. The left sagittae were removed, cleaned and fixed on microscope glass slides using cyanoacrylate glue. The otoliths were then ground with 2400 silicon carbide paper and alumina solution (1 : 20) until the core was revealed. All otoliths were kept in darkness to avoid photo-degradation of tetracycline (Lorson & Mudrak, 1987). The detection of the fluorescent band was carried out by viewing the

TABLE I. Review of the most common procedures from scanning electron microscope otolith studies on anguilliform larvae

Authors	Species	Medium	Plane	Polishing	Etching	Coating
Antunes & Tesch (1997)	<i>Anguilla anguilla</i>	TG	S	2400 mesh sand paper and aluminium paste	0.5% HCl (6–8 s)	Au
Antunes & Correia (2003)	<i>Conger conger</i>	TG	S	2400 mesh sand paper and aluminium paste	0.5% HCl (6–8 s)	Au
*Arai <i>et al.</i> (1997)	<i>Anguilla japonica</i>	ER	?	6 and 1 µm diamond pastes	0.05 M HCl (10–30 s)	Pt-Pd
Bishop <i>et al.</i> (2000)	<i>Paraconger caudilimbatus</i> <i>Artosoma baleareicum</i> <i>Gymnothorax saxicola</i> <i>Ophichthus gomesii</i>	ER	S	?	0.10 N HCl (?)	?
Castonguay (1987)	<i>Anguilla anguilla</i> <i>Anguilla rostrata</i>	ER	S	Fine grain sandpaper	5% EDTA (2–3 min)	Au
Cieri & McCleave (2000)	<i>Anguilla rostrata</i>	TG	S	12, 9 and 3 µm metal lapping films and 0.05 µm polishing fluid	5% EDTA (2 min)	Au
Correia <i>et al.</i> (2002a, b)	<i>Conger conger</i>	TG	S	2400 silicon carbide paper and alumina suspension	0.5% HCl (8 s)	Au
*Correia <i>et al.</i> (2003)		TG	S	600, 1200 and 2400 silicon carbide papers with 6, 3 and 1 µm diamond pastes and aluminium solution	0.05 M HCl (10 s) 0.05 M HCl (10 s)	Au
Lecomte-Finiger & Yahyaoui (1989)	<i>Anguilla anguilla</i>	PR	?	600, 1000 and 1500 grit papers	5% EDTA (1 min)	Au
Lee & Byun (1996)	<i>Conger myriaster</i>	PR	S	600, 800 and 1200 grit silicon carbide papers with 1 and 0.3 µm alumina powders	0.3% HCl (3–5 s)	Au

Mochioka <i>et al.</i> (1989)	<i>Conger myriaster</i>	ER	?	Waterproof abrasive paper	0.05 N HCl (?)	Au
*Otake <i>et al.</i> (1994, 1997)	<i>Anguilla japonica</i> <i>Conger myriaster</i>	ER	S	Polishing papers and 1 µm diamond paste	0.1 N HCl (?)	Au
Shiao <i>et al.</i> (2001)	<i>Anguilla australis</i>	ER	?	?	0.05 M HCl (15 s)	Au
Sugeha <i>et al.</i> (2001)	<i>Anguilla marmorata</i>	ER	?	6 and 1 µm diamond pastes	0.05 M HCl (10–30 s)	?
Tabeta <i>et al.</i> (1987)	<i>Anguilla japonica</i>	ER	F	Whetstone	0.5% HCl (?)	Au
Tanaka <i>et al.</i> (1987)	<i>Conger myriaster</i>	ER, TG	S	Number 1200 and Number 12000 emery papers	1% HCl (1–3 s)	?
Tsukamoto (1989)	<i>Anguilla japonica</i>	ER, TG	S	Number 1200 and Number 12000 emery papers	1% HCl (1–3 s)	?
Tzeng (1990)	<i>Anguilla japonica</i>	SP	F	1000–2000 mesh polishing papers and alumina paste	5% EDTA (3 min)	Au
Umezawa <i>et al.</i> (1989)	<i>Anguilla japonica</i>	ER	S, F, T	Rubber stone and 2000–8000 grit emery papers	0.1 N HCl (10–20 s)	Au-Pd
Present study	<i>Conger conger</i>	ER, TG	S, F	2400 silicon carbide paper and alumina suspension	0.5 M HCl (10 s) 5% EDTA (2 min) 5% CH ₃ COOH (1 min) #PKb (30 min)	Au

TG, thermoplastic glue; ER, epoxy resin; PR, plastic resin; SP, spurr; S, sagittal; F, frontal; T, transverse; ?, procedure not documented; *, included also microchemistry analysis; #, according to Shiao *et al.*, 1999.

otolith under a Leitz DMRBE microscope (Leica Microsystems) fitted with an ultraviolet (UV) light (568 nm). The presence and location of the fluorescent tetracycline marks were confirmed under transmitted light at $\times 100$ magnification. The otolith growth rate was estimated by measuring the maximum radius between the first and the second tetracycline marks, and between the latter mark and the otolith edge and by dividing by the time elapsed. The lengths of the anterior-posterior and dorsal-ventral axes of the otoliths were also measured.

Statistical analyses were performed using *t*-tests and procedures described by Zar (1996). A level of significance of 0.05 was used. Data are presented as ranges and means \pm s.d.

RESULTS

SIZE, DEVELOPMENTAL STAGE AND MORPHOLOGY

The L_T and developmental stage indicator ($L_{PA} : L_T$) of the 20 metamorphosing leptocephali captured in February 1999 ranged from 104.0 to 133.0 mm (117.1 ± 6.5 mm) and 0.39 to 0.54 mm (0.44 ± 0.04 mm), respectively.

The L_T and wet mass (M) of the 10 laboratory-reared metamorphosing leptocephali at capture were 109.3 ± 6.6 mm and 1.374 ± 0.373 g, respectively. The $L_{PA} : L_T$ at the beginning of the rearing experiment was 0.42 ± 0.02 . The average L_T and M of the glass eels and elvers at the end of the experiment were 80.4 ± 8.2 mm and 0.640 ± 0.212 g, respectively, *i.e.* there was a substantial decrease in M (53%) and L_T (26%). The $L_{PA} : L_T$ had a mean value of 0.36 ± 0.01 (Table II).

During the experimental period the leptocephali body became shorter, thicker and lower. The eyes started to elongate in the anterior-posterior direction and the definitive teeth replaced the larval dentition. A series of internal melanophores appeared along the spinal cord, extending from the tail forward. External melanophores appeared dorsally on the head and on the latero-caudal region. The blood became pigmented and some internal organs, like the heart, swim-bladder and gall bladder, were now visible. At the end of the experiment, the pigmentation formed above and below the midlateral line became more denser.

OTOLITH MORPHOLOGY

The sagittae of the metamorphosing conger eels were subelliptical in shape, convex on their distal side and somewhat concave proximally (Fig. 1). The longest axis of the otoliths was along the anterior-posterior direction, while the shortest axis was along the proximal-distal direction (Fig. 2). The otolith growth rate was different in each direction, being faster in the anterior-posterior direction due to larger increment width there (Fig. 2). Increment width was the narrowest at the distal side and the growth increments were interrupted on the proximal side [Fig. 2(b)].

All specimens exhibited the same otolith growth pattern, including the presence of a peripheral otolith diffuse zone. The core, located in the central part of the otoliths, was visible as a deep hole (primordium, P) surrounded by a dark circular groove (hatch check, HC) and a crystalline crown, which was in turn surrounded by a deeply etched ring (first feeding check, FFC) [Fig. 3(a)]. The core had an overall mean \pm s.d. diameter of 22 ± 1 μ m and no significant

TABLE II. Total length L_T , mass (M), preanal length ($L_{PA} : L_T$), developmental stage, major otolith length (L_O) and otolith growth rate (G_O) of those specimens used for tetracycline marking at the beginning and at the end of the rearing experiment

Specimen	Beginning of experiment						End of experiment						
	Date	L_T (mm)	M (g)	$L_{PA} : L_T$	Stage*	Date	L_T (mm)	M (g)	$L_{PA} : L_T$	Stage	L_O (μm)	G_O ($\mu\text{m day}^{-1}$) first rearing week	G_O ($\mu\text{m day}^{-1}$) remaining rearing period
1	2 June 2000	107.0	1.050	0.41	Transforming, M1	5 July 2000	77.0	0.533	0.36	Elver, M6	950	9.5	3.5
2	2 June 2000	110.0	1.348	0.45	Transforming, M1	11 July 2000	76.0	0.498	0.36	Elver, M5	1260	10.2	5.0
3	2 June 2000	114.0	1.277	0.41	Transforming, M1	11 July 2000	66.0	0.383	0.36	Elver, M5	1200	10.2	6.7
4	2 June 2000	100.0	0.889	0.41	Transforming, M1	11 July 2000	80.0	0.647	0.36	Elver, M5	1240	10.3	4.2
5	2 June 2000	100.0	1.001	0.42	Transforming, M1	11 July 2000	76.0	0.512	0.36	Elver, M5	1210	10.3	4.5
6	2 June 2000	110.0	1.245	0.44	Transforming, M1	14 July 2000	75.0	0.490	0.36	Elver, M6	1240	8.9	5.0
7	26 April 2001	112.0	1.734	0.43	Transforming, M1	5 June 2001	88.5	0.838	0.36	Elver, M6	1300	10.3	3.5
8	26 April 2001	120.0	1.986	0.40	Transforming, M1	5 June 2001	82.0	0.558	0.35	Elver, M6	1220	8.9	4.0
9	24 May 2001	116.0	1.863	0.43	Transforming, M1	15 June 2001	92.0	1.004	0.38	Glass eel, M4	1300	10.2	5.8
10	24 May 2001	104.0	1.351	0.42	Transforming, M1	15 June 2001	91.0	0.941	0.37	Glass eel, M4	1250	9.6	6.2

*, metamorphosing.

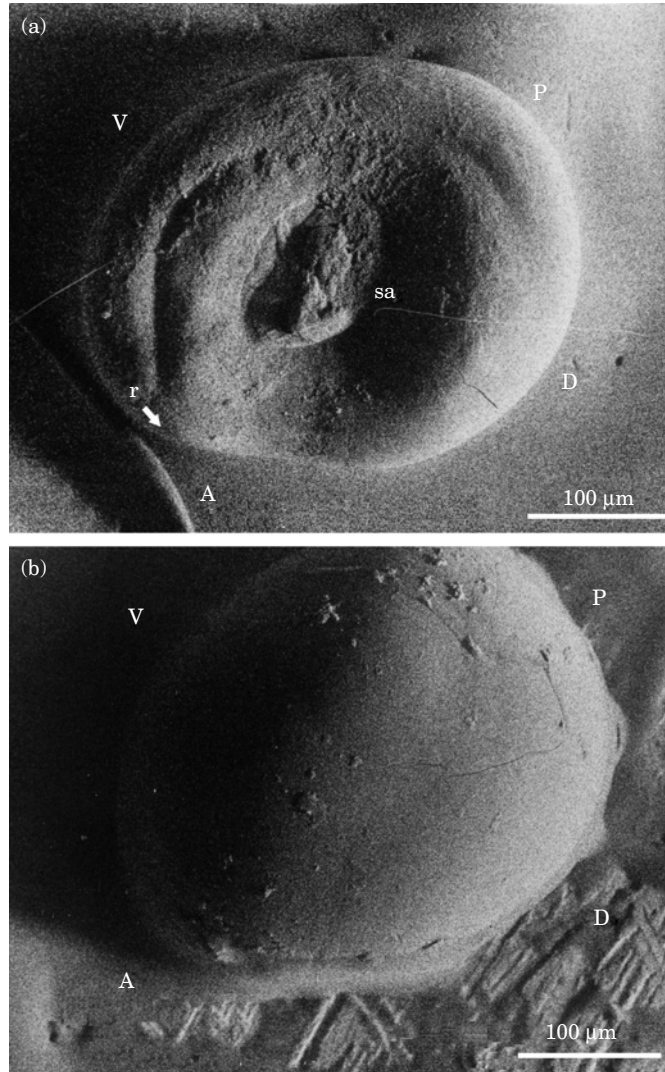


FIG. 1. Scanning electron microscope micrographs showing the external features of the sagittae from a metamorphosing leptocephalus of *Conger conger* (115.0 mm L_T ; $L_{PA} : L_T$ 0.44). (a) Proximal side of the right otolith. (b) Distal side of the left otolith. A, anterior; P, posterior; D, dorsal; V, ventral; r, rostrum; sa, sulcus acusticus.

differences were found between sagittal and frontal planes (paired t -test, $P = 0.44$). Beyond the FFC a series of growth increments were distinctive along the increment countable zone (ICZ). No increments were visible in the DZ [Figs 4(b) and 5]. The majority of the otoliths (75%) presented one to seven AGC along the edge (Fig. 2), which occupied 3.2–21.8% of the otolith cross-sectional surface area.

The increment width showed a characteristic profile along the ICZ (Fig. 4). From the FFC to the first 30–60 days, increments were wide ($0.68 \pm 0.11 \mu\text{m}$)

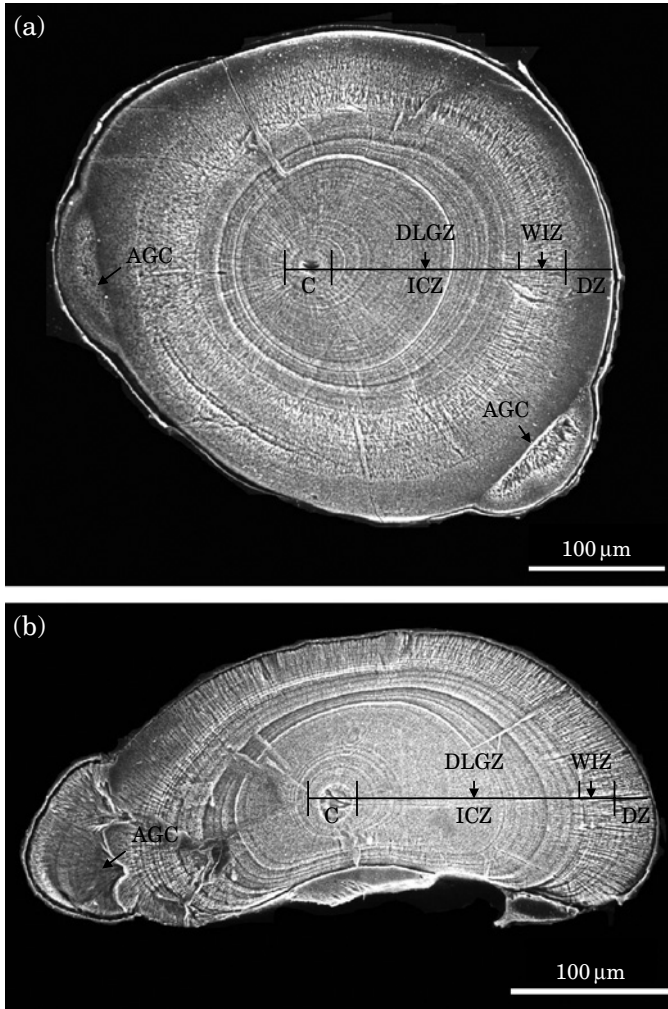


FIG. 2. Scanning electron micrographs showing the microstructure of an otolith from a metamorphosing leptocephalus of *Conger conger* (122.0 mm L_T ; $L_{PA} : L_T$ 0.46) on a (a) sagittal and (b) frontal plane. C, core; DLGZ, developing leptocephalus growth zone; WIZ, wide increment zone; ICZ, increment countable zone; DZ, diffuse zone; AGC, accessory growth centre.

(phase I) [Fig. 3(a)]. Then they narrowed and gradually decreased, until a relatively constant minimum value ($0.42 \pm 0.09 \mu\text{m}$) was reached, at *c.* 170 days (phase II). This internal portion of the ICZ that includes phases I and II was named the developing leptocephalus growth zone (DLGZ). Between 180 and 260 days, depending on the specimen, the increments abruptly widened to a maximum of $0.86 \pm 0.17 \mu\text{m}$ (phase III). This wide increment zone (WIZ) lasted *c.* 30–70 days. After that the increments became less clear and disappeared, which corresponds to the onset of the DZ (phase IV) [Fig. 3(b)]. The width of the peripheral otolith diffuse zone ranged from 16 to $84 \mu\text{m}$ ($50 \pm 19 \mu\text{m}$) and showed a weak but significantly negative correlation with the $L_{PA} : L_T$ ratios

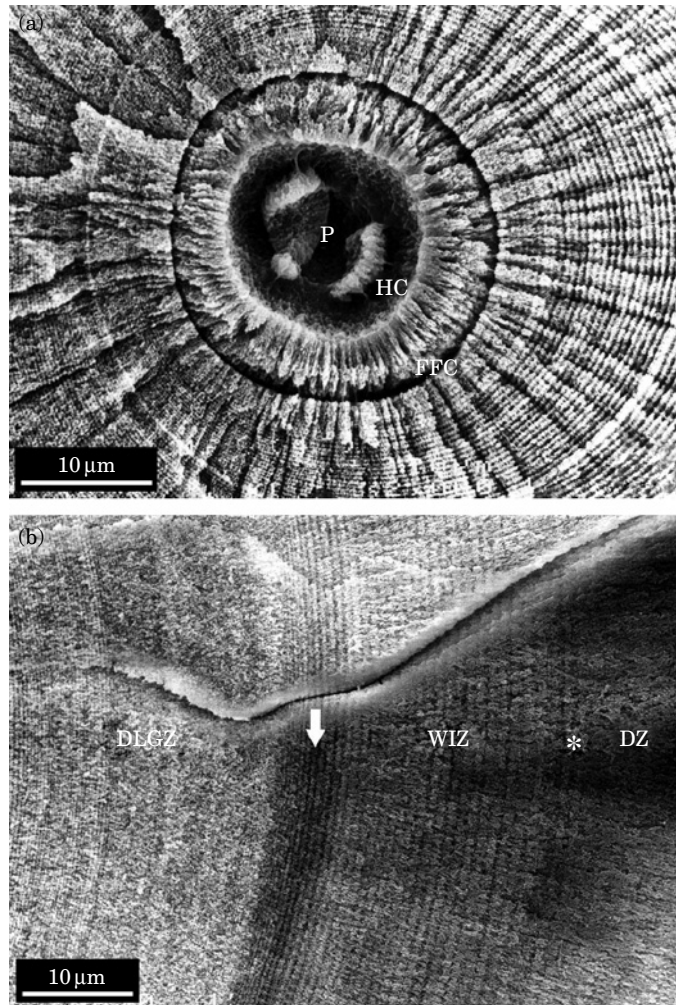


FIG. 3. Scanning electron micrographs showing some details of the otolith microstructure of a metamorphosing *Conger conger* leptocephalus (120.0 mm L_T ; $L_{PA} : L_T$ 0.42). (a) Area surrounding the core. P, primordium; HC, hatch check; FFC, first feeding check. (b) Transition area between the developing leptocephalus growth zone (DLGZ), wide increment zone (WIZ) and diffuse zone (DZ). \downarrow , abrupt change of the increment width that probably marks the onset of metamorphosis; *, last countable daily growth increment. Both otoliths are on the sagittal plane.

($r^2 = 0.23$, $n = 20$, $P < 0.05$). As the DZ grew larger, the AGC were formed (phase V).

There were no significant differences either in diameter or radius between left (sagittal plane) and right (frontal plane) otoliths (paired t -tests, $n = 20$, $P = 0.87$ and $n = 20$, $P = 1.00$, respectively). The diameter and radius of the otolith ranged from 361 to 494 ($427 \pm 41 \mu\text{m}$) and 202 to 278 ($240 \pm 20 \mu\text{m}$), respectively. No significant differences in increment counts were found between left and right otoliths (paired t -test, $n = 20$, $P = 0.72$). Therefore, these data

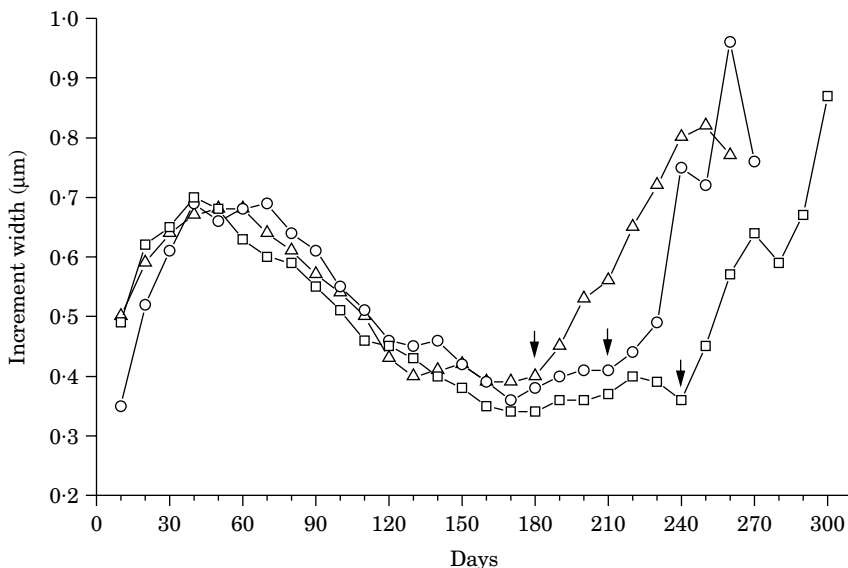


Fig. 4. Mean increment widths from the first feeding check (age 0 days) to the end of the otolith increment countable zone. The specimens were grouped according to the time when the daily increments abruptly increased (\blacktriangledown), which probably marks the onset of metamorphosis (Δ , 180–200 days, $n = 10$; \circ , 210–230 days, $n = 4$; \square , 240–260 days, $n = 6$).

were averaged for each conger eel. The number of growth increments in the otolith DLGZ, WIZ and ICZ ranged from 185 to 265 (219 ± 26), 30 to 70 (41 ± 11) and 227 to 312 (264 ± 28), respectively. The mean growth rate of the otolith in the DLGZ and WIZ was 0.51 ± 0.15 and $0.65 \pm 0.18 \mu\text{m day}^{-1}$, respectively.

OTOLITH PREPARATION

Otoliths from the metamorphosing conger eel *leptocephali* etched with HCl, CH_3COOH , EDTA and PKb revealed similar results, in both sagittal and frontal sections. Although all etching agents gave a good enhanced visibility of individual daily growth increments, EDTA produced the best contrast of daily increments. PKb was time-consuming (long-term enzyme digestion) and expensive. The increments were broader (paired t -test, $n = 20$, $P < 0.05$) and were counted more clearly in the sagittal section comparatively with the frontal section (Fig. 2). The otoliths did not form a complete growth sequence throughout the early lifetime of this species (Fig. 5). Daily growth increments were clearly discernible in the ICZ, but a peripheral area of poorly defined increments (DZ) was present in all samples. There were also morphological changes in the manner in which the increments had been deposited, resulting in a change in the direction of crystal growth obscuring the increments.

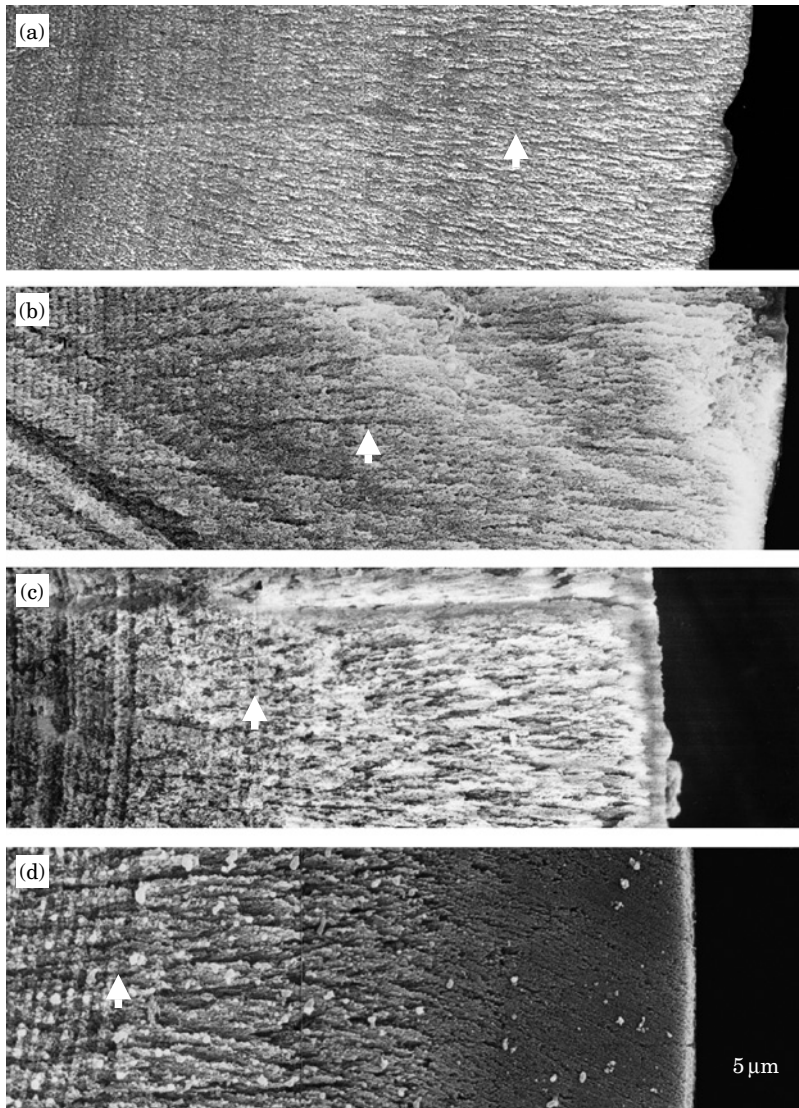


FIG. 5. Scanning electron micrographs showing the peripheral zone (*i.e.* end of the WIZ and beginning of the DZ (see Fig. 3)) of four otoliths from metamorphosing *Conger conger* leptocephali on the sagittal plane treated with different etching agents: (a) HCl, (b) CH₃COOH, (c) EDTA and (d) PKb. \triangle , the last countable daily growth increment and the beginning of the otolith peripheral diffuse zone.

TETRACYCLINE MARKING

Tetracycline appeared as a distinct bright-yellow ring when viewed under ultraviolet light [Fig. 6(a)]. Incorporation of a fluorescent mark occurred in the otoliths of all conger eel leptocephali. The tetracycline mark was also clearly visible as a slight check when viewed with a transmitted light microscope [Fig. 6(b)]. The otolith DZ growth rate was higher in the first rearing week

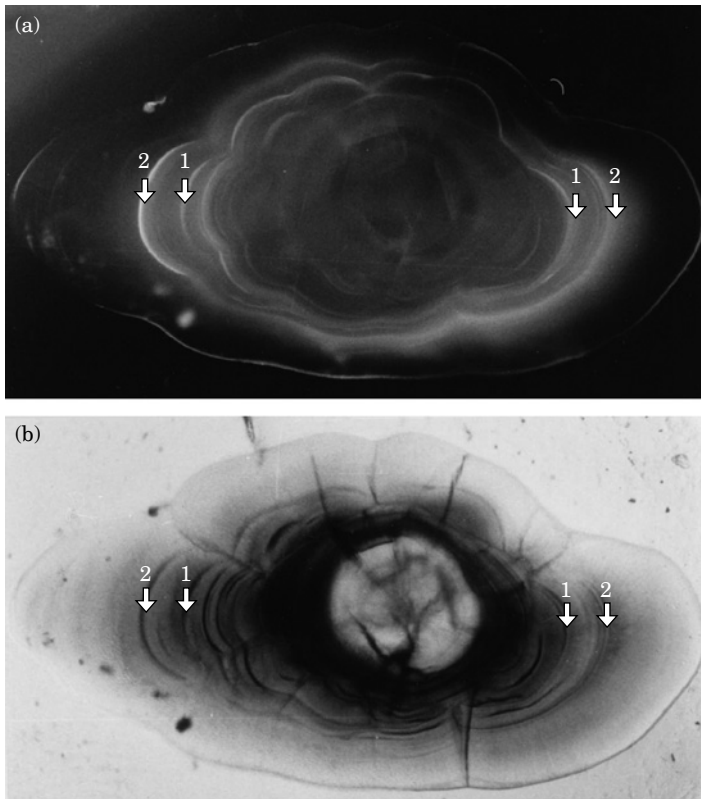


FIG. 6. (a) UV light photograph of a sagittae from a *Conger conger* eelver (80.0 mm L_T ; $L_{PA} : L_T$ 0.36) treated with tetracycline *in vivo*. (b) Photograph of the same sample under normal light. \downarrow 1, \downarrow 2, the location of the first and second tetracycline marks, respectively.

($9.8 \pm 0.6 \mu\text{m day}^{-1}$) compared to the remaining period ($4.8 \pm 1.1 \mu\text{m day}^{-1}$) (paired *t*-test, $n = 10$, $P \leq 0.05$). Otoliths showed an elliptical shape and measured 1217 ± 100 and $672 \pm 56 \mu\text{m}$, on the anterior-posterior and dorsal-ventral axes, respectively (Table II).

DISCUSSION

Conger eel leptocephali are occasionally caught in the mouth of the Minho River (northern Portugal), from late October until the middle of June, while they are undergoing metamorphosis (Correia *et al.*, 2002b). The metamorphosing leptocephali collected in this study were at the same developmental stage and within the same length range previously reported by Correia *et al.* (2002b, 2003). The changes in morphology, pigmentation and dentition through metamorphosis were similar to those previously described by D'Ancona (1931). There was also a substantial decrease in mass and size during metamorphosis, which is typical in leptocephalus larvae (Pfeiler, 1986).

Otolith increments of various closely-related anguilliforms have been found to be deposited daily in their leptocephalus, glass eel and elver stages (Mochioka *et al.*, 1989; Tsukamoto, 1989; Umezawa *et al.*, 1989; Umezawa & Tsukamoto, 1991; Martin, 1995; Arai *et al.*, 2000; Cieri & McCleave, 2001; Sugeha *et al.*, 2001; Shinoda *et al.*, 2004). An otolith growth increment refers to a bipartite structure composed of an incremental zone (calcium carbonate) and a discontinuous zone (proteinaceous organic matrix) formed over 24 h (Mugiya *et al.*, 1981). The age at which daily increment formation is initiated varies widely among fish species (Morales-Nin, 2000). Based on the examination of similar published otoliths images of other anguilliform leptocephali (Lecomte-Finiger & Yahyaoui, 1989; Wang & Tzeng, 1998, 2000; Arai *et al.*, 2000, 2001), it was assumed that the FFC, which marks the onset of daily growth deposition, is probably related to the first exogenous feeding, when larvae complete yolk-sac absorption (Correia *et al.*, 2002a, b, 2003). Horie *et al.* (2002) recently showed that hatching occurred in 84 h (*c.* 3.5 days) after artificial insemination in *C. myriaster* and that mouth opening occurred on day 7 after hatching. On the assumption that *C. conger* could have a similar larval development, 10 increments were expected to be deposited from fertilization until yolk-sac absorption. It is recommended, however, that increment periodicity for *C. conger* be validated in the future, since an appropriate validation of the periodicity of otolith increments formation is still essential for a correct interpretation of the otolith microstructure (Geffen, 1992).

The growth history of the metamorphosing conger eels was reflected in the changes of width of the otolith increments. In an earlier study (Correia *et al.*, 2002b), otolith increment width, which was relatively constant and narrow in the developing leptocephalus stage, was shown to increase sharply at age 170–280 days. At the same time, the Sr : Ca ratios in the otolith, which increased during the developing leptocephalus stage, showed a rapid drop coinciding with the increase in increment width (Correia *et al.*, 2003). These coincidental changes have been regarded as the onset of metamorphosis and are typical for several anguilliform fish species, including *C. myriaster* (Otake *et al.*, 1997) and *C. oceanicus* (Correia *et al.*, 2004). Based on this assumption, the age of leptocephali in the first part of its larval phase (developing leptocephalus stage) was estimated at 219 ± 26 days (*i.e.* the average number of days in the DLGZ), including the 10 days hatching and yolk-sac period adjustment. Although the rapid drop in Sr : Ca ratios might be associated with decreasing Sr levels in the body as a result of catabolism of Sr-rich sulphated glycosaminoglycans (GAG) during metamorphosis (Otake *et al.*, 1997), no explanation has been given for the larger growth increments recorded in the otolith WIZ. According to D'Ancona (1931), in *C. conger* growth continues for a time after metamorphosis begins, before the decrease in length starts, so that the early metamorphosing leptocephali ('younger semilarvae') are larger than the late premetamorphic leptocephali ('fully developed larvae'). Based on this observation, the WIZ could be produced by a last short growth period of the leptocephali (lasting 30–70 days, *i.e.* the number of days of the WIZ), before the beginning of the body shrinkage, which should occur at 150 mm L_T (Strehlow *et al.*, 1998). Although leptocephali probably stop feeding at the onset of metamorphosis (Pfeiler, 1986), they may have some remaining energy reserves, which enable them to continue growing for

sometime. In fact, the number of increments in various fishes appears to be unaffected by food deprivation at least when body energy reserves are sufficient to enable limited skeletal growth to occur (Marshall & Parker, 1982; Campana, 1983; Volk *et al.*, 1984; Neilson & Geen, 1985). Some studies have shown that the ability of the otolith to reflect changes in food abundance and growth fluctuations of larger larvae and juveniles may lag by 1 week (Moksness *et al.*, 1995), 2 weeks (Molony & Choat, 1990; Folkvord *et al.*, 2000) or even 3 weeks (Neilson & Geen, 1985). The existence of this lag period could be attributable to real physiological events, such as the mobilization and exhaustion of the energy reserves (Molony & Choat, 1990). Later on during metamorphosis, the wider increments became poorly contrasted and disappear, when entering the DZ (Correia *et al.*, 2002b, 2003; present study). In this otolith zone, the formation of distinct ring structures seems to stop, which may be a result of poor growth conditions, since the metamorphosing leptocephali exhausted their energetic reserves allocated for growth and started to shrink. Some unknown physiological conditions associated with the later stages of metamorphosis, however, may be responsible for the peripheral otolith diffuse zone. Recently, Shiao & Hwang (2004) suggested that the abrupt increase of the daily growth increment width at the onset of metamorphosis in leptocephalus larvae can be the result of an elevation of the T_3 plasma levels. It might be also possible that the WIZ zone reflects such a phase with low somatic growth due to starvation, but high metabolism which produces, in this case, wide and distinct rings. It is also plausible that starvation could induce a high stress level increasing for example plasma cortisol. Furthermore, the relative influences of endogenous and exogenous factors on the scaling of the increment width and the observed transitions in increment-width patterns remains unclear.

The conventional methods generally apply diluted HCl or EDTA to etch the sectioned otoliths for SEM observations (Campana & Neilson, 1985). Sometimes these methods do not discriminate easily between the incremental and discontinuous zones. These two chemicals are expected to dissolve only $CaCO_3$, but in practice the proteinaceous discontinuous zones are dislodged prior to the highly calcified incremental zones (Mugiya *et al.*, 1981; Watabe *et al.*, 1982). An alternative is the use of a proteinase K buffer (PKb), which removes the organic matrix leaving the calcified structure almost intact to reveal conspicuous daily increments (Shiao *et al.*, 1999). In the present study, EDTA was found to be the most efficient and effective etching agent and there were no significant differences in size and morphological features of the otoliths in the two section planes. Due to ontogenetic changes in accretion rates onto different portions of the otolith, the anterior-posterior direction of the otolith seemed the most appropriate for examining daily growth increments, with increments on proximal and distal portions of the otolith becoming narrow and difficult to discern. Independent of the section plane and etching agent used, all otoliths recorded a peripheral diffuse zone, showing that it was not an optical artefact resulting from the otolith preparation, but a permanent structure of the late metamorphosing conger eel leptocephali.

Several authors reported some problems during the examination of the otolith microstructure of *C. myriaster* leptocephali during metamorphosis (Tanaka *et al.*, 1987; Mochioka *et al.*, 1989; Lee & Byun, 1996; Otake *et al.*, 1997), but

none of them has identified the existence of a peripheral diffuse zone. Lee & Byun (1996), for instance, were able to count very faint increments in the outer otolith zone (they called it the opaque zone) of metamorphosing leptocephali using a special grinding procedure. Curiously, none of these studies showed a picture of the otolith peripheral zone with clear daily increments. Based on this information two other hypotheses cannot be discarded to explain this unclear area in *C. conger*: 1) that chemical alternatives used for etching may have been unsuccessful to distinguish increments in the otolith diffuse zone or 2) that slight species specific or environmental differences prevented increments to be detected in the peripheral otolith zone. Lee & Byun (1996) also proposed that the otolith opaque zone represents the period of metamorphosis, but they did not use any fully developed elvers to confirm if otolith increment deposition resumed at the end of metamorphosis. In the present study only otolith light microscope images of elvers reared in captivity were analysed to estimate the otolith growth rate, with the purpose of knowing how much lifetime represents the diffuse zone. The existence of large diffuse zones and several growth checks in otoliths of elvers, including one wild-caught specimen, however, has recently been observed (Correia *et al.*, 2003). The time when the diffuse zone ends and clear daily increments start to form again is unknown at present. Although a hypothesis has been suggested here for the origin of this structure, the mechanism behind the formation of this structure, be it an environmental or a physiological stimulus, is not yet clearly understood. Future attempts should be made to determine the pathway of the DZ deposition.

Marking otoliths is probably the best method for validation of growth rhythms in otoliths for species which cannot be reared from larvae to adults in the laboratory (Cermeño *et al.*, 2003). Tetracycline is the most commonly used marking agent and is known to be incorporated into calcifying tissues in fishes during growth, although generally with high mortality rates (Geffen, 1992). Tetracycline was successful in inducing a mark in the otoliths of all conger eels exposed, with no mortality observed during and following immersions. During the first controlled period (first captive week), the otolith growth rate was *c.* 10 $\mu\text{m day}^{-1}$. During the last captive period which lasted between 15 and 30 days, however, the otolith growth rate was slow, *c.* 5 $\mu\text{m day}^{-1}$. In both cases, the daily otolith growth rate was much higher than the largest increment growth rate (*c.* 1.20 $\mu\text{m day}^{-1}$) reported for this species (Correia *et al.*, 2002b, 2003). Results from this study indicate that under artificial conditions otolith growth rate in metamorphosing conger eel leptocephali was higher than in nature. Indeed, it has recently been reported that reared metamorphosing leptocephali had larger otoliths than wild specimens (Correia *et al.*, 2003). Studies on the larval development of *C. myriaster* have shown that metamorphosis is accompanied by changes in cortisol and thyroid hormones (T_3 and T_4) (Yamano *et al.*, 1991). The thyroid hormones could influence, for example, the skeletal development of larvae, causing anomalies affecting the caudal fin and cranium (Power *et al.*, 2001). Shiao & Hwang (2004) have recently demonstrated that exogenous T_3 can increase the otolith growth rate of the metamorphosing leptocephalus tarpon *Megalops cyprinoides* (Broussonet). Stressed fishes show typical endocrine responses characterized for example by an elevation of the plasma cortisol (Donaldson, 1981). Tissue cortisol concentrations in the Japanese flounder

Paralichthys olivaceus Temminck & Schlegel appear to vary depending on the rearing conditions (Tanaka *et al.*, 1995). Cortisol may have a synergistic action with thyroid hormones (TH) in the metamorphosis of flatfish larvae (De Jesus *et al.*, 1991). These findings suggest that the artificial rearing conditions and the handling stress, may produce a disequilibria of the endocrine control of metamorphosis, producing an anomalous high grow rate of the otolith.

The presence of AGC on the otolith surface of conger leptocephali is common in a late stage of metamorphosis (Correia *et al.*, 2002b, 2003; present study). These structures appear with or after metamorphosis from larvae to juvenile in several fish species (Modin *et al.*, 1996; Fischer, 1999; Brown *et al.*, 2001), including other conger species (Lee & Byun, 1996; Correia *et al.*, 2004). Conger eels undergo habitat, morphological and physiological changes at the larval to juvenile transition (Yamano *et al.*, 1991). During metamorphosis they change from a larval pelagic to a juvenile benthic stage (Bell *et al.*, 2003). At the same time, by the end of metamorphosis, leptocephali switch to oral feeding (Rasquin, 1955; Mercado & Ciardelli, 1972). Such behaviour and habitat changes are probably accompanied by significant shifts in ambient temperature and salinity, and food availability. These variables are known to influence the microstructural features of the otoliths and could explain the occurrence of AGC in conger eels.

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References

- Antunes, C. & Correia, A. T. (2003). Sagitta microstructure of the European conger eel, *Conger conger* (L.), leptocephali compared with leptocephali of the eel, *Anguilla anguilla* (L.). *Archive of Fishery and Marine Research* **50**, 227–237.
- Antunes, C. & Tesch, F. W. (1997). A critical consideration of the metamorphosis zone when identifying daily rings in otoliths of the European eel, *Anguilla anguilla* (L.). *Ecology of Freshwater Fish* **6**, 102–107.
- Arai, T., Limbong, D. & Tsukamoto, K. (2000). Validation of otolith daily increments in the tropical eel *Anguilla celebesensis*. *Canadian Journal of Zoology* **78**, 1078–1084.
- Arai, T., Otake, T. & Tsukamoto, K. (1997). Drastic changes in otolith microstructure and microchemistry accompanying the onset of metamorphosis in the Japanese eel *Anguilla japonica*. *Marine Ecology Progress Series* **161**, 17–22.
- Arai, T., Aoyama, J., Ishikawa, S., Miller, M. J., Otake, T., Inagaki, T. & Tsukamoto, K. (2001). Early life history of tropical *Anguilla* leptocephali in the western Pacific Ocean. *Marine Biology* **138**, 887–895.
- Bagenal, T. B. & Kenney, A. R. (1973). *Identification of British Fishes*. Amersham: Hulton Educational Publications.
- Bauchot, M. L. & Saldanha, L. (1986). Congridae. In *Fishes of the Northeastern Atlantic and the Mediterranean* (Whitehead, P. S. P., Bauchot, M. L., Hureau, J. C., Nielsen, J. & Tortonese, E., eds), pp. 567–574. Paris: Unesco.
- Bell, G. W., Witting, D. A. & Able, K. W. (2003). Aspects of metamorphosis and habitat use in the conger eel, *Conger oceanicus*. *Copeia* **2003**, 544–552.
- Bishop, R. E., Torres, J. J. & Crabtree, R. E. (2000). Chemical composition and growth indices in leptocephalus larvae. *Marine Biology* **137**, 205–214.
- Brown, A. L., Busby, M. S. & Mier, K. L. (2001). Walleye pollock *Theragra chalcogramma* during transformation from the larval to juvenile stage: otolith and osteological development. *Marine Biology* **139**, 845–851.

- Campana, S. E. (1983). Feeding periodicity and the production of daily growth increments in the otoliths of steelhead trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). *Canadian Journal of Zoology* **61**, 1591–1597.
- Campana, S. E. & Neilson, J. D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **42**, 1014–1032.
- Castonguay, M. (1987). Growth of American and European eel leptocephali as revealed by otolith microstructure. *Canadian Journal of Zoology* **65**, 875–878.
- Cau, A. & Manconi, P. (1983). Sex ratio and spatial displacement in *Conger conger* (L., 1758). *Rapport et Proces-Verbaux des Réunions de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* **28**, 93–96.
- Cermeño, P., Uriarte, A., Murguía, A. M. & Morales-Nin, B. (2003). Validation of daily increment formation in otoliths of juvenile and adult European anchovy. *Journal of Fish Biology* **62**, 679–691. doi: 10.1046/j.0022-1112.2003.00056.x
- Cieri, M. D. & McCleave, J. D. (2000). Discrepancies between otoliths of larvae and juveniles of the American eel: is something fishy happening at metamorphosis? *Journal of Fish Biology* **57**, 1189–1198. doi: 10.1006/jfbi.2000.1381
- Cieri, M. D. & McCleave, J. D. (2001). Validation of daily otolith increments in glass-phase American eels *Anguilla rostrata* (Lesueur) during estuarine residency. *Journal of Experimental Marine Biology and Ecology* **257**, 219–227.
- Correia, A. T., Isidro, E. J., Antunes, C. & Coimbra, J. (2002a). Age, growth, distribution and ecological aspects of *Conger conger* leptocephali collected in the Azores, based on otolith analysis of premetamorphic specimens. *Marine Biology* **141**, 1141–1151.
- Correia, A. T., Antunes, C. & Coimbra, J. (2002b). Aspects of the early life history of the European conger eel (*Conger conger*) inferred from the otolith microstructure of metamorphic larvae. *Marine Biology* **140**, 165–173.
- Correia, A. T., Antunes, C., Isidro, E. J. & Coimbra, J. (2003). Changes in otolith microstructure and microchemistry during the larval development of the European conger eel (*Conger conger*). *Marine Biology* **142**, 777–789.
- Correia, A. T., Able, K. W., Antunes, C. & Coimbra, J. (2004). Early life history of the American conger eel (*Conger oceanicus*), as revealed by otolith microstructure and microchemistry of metamorphosing leptocephali. *Marine Biology* **145**, 477–488.
- D'Ancona, U. (1931). Uova, larve e stadi giovanili di Teleostei. *Fauna e Flora del Golfo di Napoli* **38**, 94–156.
- De Jesus, E. G., Hirano, T. & Inui, Y. (1991). Changes in cortisol and thyroid hormone concentrations during early development and metamorphosing in Japanese flounder (*Paralichthys olivaceus*). *General and Comparative Endocrinology* **82**, 369–376.
- Donaldson, E. M. (1981). The pituitary-interrenal axis as an indicator of stress in fish. In *Stress and Fish* (Pickering, A. D., ed.), pp. 11–48. London: Academic Press.
- Fannon, E., Fahy, E. & O'Reilly, R. (1990). Maturation in female conger eel, *Conger conger* (L.). *Journal of Fish Biology* **36**, 275–276.
- Fischer, P. (1999). Otolith microstructure during the pelagic, settlement and benthic phases in burbot. *Journal of Fish Biology* **54**, 1231–1243. doi: 10.1006/jfbi.1999.0956
- Folkvord, A., Blom, G., Johannessen, A. & Moksness, E. (2000). Growth-dependent age estimation in herring (*Clupea harengus* L.) larvae. *Fisheries Research* **46**, 91–103.
- Geffen, A. J. (1992). Validation of otolith increment deposition rate. *Canadian Special Publication of Fisheries and Aquatic Sciences* **117**, 101–113.
- Hayward, P. J. & Ryland, J. S. (1995). *Handbook of the Marine Fauna of Northwest Europe*. Oxford: Oxford University Press.
- Horie, N., Utoh, T., Yamada, Y., Okamura, A., Zhang, H., Mikawa, N., Akazawa, A., Tanaka, S. & Poka, H. (2002). Development of embryos and larvae in the common Japanese conger *Conger myriaster*. *Fisheries Science* **68**, 972–983.
- Lecomte-Finiger, R. & Yahyaoui, A. (1989). La microstructure de l'otolithe au service de la connaissance du développement larvaire de l'Anguille européenne *Anguilla anguilla*. *Comptes Rendus de L'Académie des Sciences, Série III: Sciences de la Vie* **308**, 1–7.

- Lee, T. W. & Byun, J. S. (1996). Microstructural growth in otoliths of conger eel (*Conger myriaster*) leptocephali during the metamorphic stage. *Marine Biology* **125**, 259–268.
- Lorson, R. D. & Mudrak, V. A. (1987). Use of tetracycline to mark otoliths of American shad fry. *North American Journal of Fisheries Management* **7**, 453–455.
- Lythgoe, J. & Lythgoe, G. (1971). *Fishes of the Sea: the Coastal Waters of the British Isles, Northern Europe and the Mediterranean*. London: Blandford Press.
- Marshall, S. L. & Parker, S. S. (1982). Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 542–547.
- Martin, M. H. (1995). Validation of daily increments in otoliths of *Anguilla rostrata* (Lesueur) elvers. *Canadian Journal of Zoology* **73**, 208–211.
- Mercado, S. J. E. & Ciardelli, A. (1972). Contribución a la morfología y organogénesis de los leptocefalos del sábalo *Megalops atlanticus* (Pisces: Megalopidae). *Bulletin of Marine Science* **22**, 153–184.
- Mochioka, N., Tabeta, O. & Kanda, T. (1989). *Daily Growth Increments in Otoliths of the Conger Eel Conger myriaster Leptocephali*. Porto: Working Group on Eel, European Inland Fisheries (EIFAC).
- Modin, J., Fagerholm, B., Gunnarsson, B. & Pihl, L. (1996). Changes in otolith microstructure at metamorphosis of plaice, *Pleuronectes platessa* L. *ICES Journal of Marine Science* **53**, 745–748.
- Moksness, R., Rukan, K., Ystanes, L., Folkvord, A. & Johannessen, A. (1995). Comparison of somatic and otolith growth in North Sea herring (*Clupea harengus* L.) larvae: evaluation of growth dynamics in mesocosms. In *Recent Developments in Fish Otolith Research* (Secor, D. H., Dean, J. M. & Campana, S. E., eds), pp. 119–134. Columbia, SC: University of South Carolina Press.
- Molony, B. W. & Choat, J. H. (1990). Otolith increment widths and somatic growth rate: the presence of a time-lag. *Journal of Fish Biology* **37**, 541–551.
- Morales-Nin, B. (2000). Review of the growth regulation process of otolith daily increment formation. *Fisheries Research* **46**, 53–67.
- Mugiya, Y., Watabe, N., Yamada, J. M., Dunkelberger, D. G. & Shimizu, M. (1981). Diurnal rhythm in otolith formation in the goldfish, *Carassius auratus*. *Comparative Biochemistry and Physiology A* **68**, 659–662.
- Neilson, J. D. & Geen, G. H. (1985). Effects of feeding regimes and diel temperatures cycles on otolith increment formation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. *Fishery Bulletin* **83**, 91–101.
- Otake, T., Ishii, T., Nakahara, M. & Nakamura, R. (1994). Drastic changes in otolith strontium:calcium ratios in leptocephali and glass eels of Japanese eel *Anguilla japonica*. *Marine Ecology Progress Series* **112**, 189–193.
- Otake, T., Ishii, T., Nakahara, M. & Nakamura, R. (1997). Changes in otolith strontium:calcium ratios in metamorphosing *Conger myriaster* leptocephali. *Marine Biology* **128**, 565–572.
- Pfeiler, E. (1986). Towards an explanation of the developmental strategy in leptocephalus larvae of marine fishes. *Environmental Biology of Fishes* **15**, 3–13.
- Pitcher, C. R. (1988). Validation of a technique for reconstructing daily patterns in the recruitment of coral reef damselfish. *Coral Reefs* **7**, 105–111.
- Power, D. M., Llewellyn, L., Faustino, M., Nowell, M. A., Björnsson, B. Th., Einarsdottir, I. E., Canario, A. V. M. & Sweeney, G. E. (2001). Thyroid hormones in growth and development of fish. *Comparative Biochemistry and Physiology C* **130**, 447–459.
- Rasquin, P. (1955). Observations on the metamorphosis of the bonefish, *Albula vulpes* (Linnaeus). *Journal of Morphology* **97**, 77–117.
- Sbahi, M., Fouchereau-Peron, M., Meunier, F., Elie, P., Mayer, I., Burzawa-Gerard, E., Vidal, B. & Dufour, S. (2001). Reproductive biology of the conger eel from the south coast of Brittany, France and comparison with the European eel. *Journal of Fish Biology* **59**, 302–318. doi: 10.1006/jfbi.2001.1642
- Schmidt, J. (1931). Eels and conger eels of the North Atlantic. *Nature* **128**, 602–604.

- Shiao, J. C. & Hwang, P. P. (2004) Thyroid hormones are necessary for teleostean otolith growth? *Marine Ecology Progress Series* **278**, 271–278.
- Shiao, J. C., Tzeng, W. N., Collins, A. & Jellyman, D. J. (2001). Dispersal pattern of glass eel stage of *Anguilla australis* revealed by otolith growth increments. *Marine Ecology Progress Series* **219**, 241–250.
- Shiao, J. C., Tzeng, C. S., Leu, C. L. & Chen, F. C. (1999). Enhancing the contrast and visibility of daily growth increments in fish otoliths etched by proteinase K buffer. *Journal of Fish Biology* **54**, 302–309. doi: 10.1006/jfbi.1998.0866
- Shinoda, A., Tanaka, H., Kagawa, H., Ohta, H. & Tsukamoto, K. (2004). Otolith microstructural analysis of reared larvae of the Japanese eel *Anguilla japonica*. *Fisheries Science* **70**, 339–341.
- Strehlow, B. (1992). Untersuchungen an leptocephali und adulten exemplaren der Ordnung Anguilliformes aus dem Iberischen Becken und dem seegebiet vor Nordwestafrika. Doctoral thesis, University of Rostock, Rostock.
- Strehlow, B., Antunes, C., Niermann, U. & Tesch, F. W. (1998). Distribution and ecological aspects of leptocephali collected 1979–1994 in North and Central Atlantic. I. Congridae. *Helgolander Meeresuntersuchungen* **52**, 85–102.
- Sugeha, H. Y., Shinoda, A., Marui, M., Arai, T. & Tsukamoto, K. (2001). Validation of otolith daily increments in the tropical eel *Anguilla marmorata*. *Marine Ecology Progress Series* **220**, 291–294.
- Sullivan, S. O., Moriarty, C., Fitsgerard, R. D., Davenport, J. & Mulcahy, M. F. (2003). Age, growth and reproductive status of the European conger eel, *Conger conger* (L.) in Irish coastal waters. *Fisheries Research* **64**, 55–69.
- Tabeta, O., Tanaka, K., Yamada, J. & Tzeng, W. N. (1987). Aspects of the early life history of the Japanese eel *Anguilla japonica* determined from otolith microstructure. *Nippon Suisan Gakkaishi* **53**, 1727–1734.
- Tanaka, K., Tabeta, O., Mochioka, N., Yamada, J. & Kakuda, S. (1987). Otolith microstructure and ecology of the conger eel (*Conger myriaster*) larvae collected in the Seto Inland Sea, Japan. *Nippon Suisan Gakkaishi* **53**, 543–549.
- Tanaka, M., Tanangonan, J. B., Tagawa, M., De Jesus, E. G., Nishida, H., Isaka, M., Kimura, R. & Hirano, T. (1995). Development of the pituitary, thyroid and interrenal glands and applications of endocrinology to the improved rearing of marine fish larvae. *Aquaculture* **135**, 111–126.
- Tsukamoto, K. (1989). Otolith daily increments in the Japanese eel. *Nippon Suisan Gakkaishi* **55**, 1017–1021.
- Tzeng, W. N. (1990). Relationship between growth rate and age at recruitment of *Anguilla japonica* elvers in a Taiwan estuary as inferred from otolith growth increments. *Marine Biology* **107**, 75–81.
- Umezawa, A. & Tsukamoto, K. (1991). Factors influencing otolith increment formation in Japanese eel, *Anguilla japonica*, elvers. *Journal of Fish Biology* **39**, 211–223.
- Umezawa, A., Tsukamoto, K., Tabeta, O. & Yamakawa, H. (1989). Daily growth increments in the larval otolith of the Japanese eel, *Anguilla japonica*. *Japanese Journal of Ichthyology* **35**, 440–444.
- Volk, E. C., Wissmar, R. C., Simenstad, C. A. & Eggers, D. M. (1984). Relationship between otolith microstructure and the growth of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. *Canadian Journal of Fisheries and Aquatic Sciences* **41**, 126–133.
- Waldron, M. E. & Gerneke, D. A. (1997). Comparison of two scanning electron microscope techniques for examining daily growth increments on fish otoliths. *Journal of Fish Biology* **50**, 450–454. doi: 10.1006/jfbi.1996.0308
- Wang, C. H. & Tzeng, W. N. (1998). Interpretation of geographic variation in size of American eel *Anguilla rostrata* on the Atlantic coast of North America using their life history and otolith ageing. *Marine Ecology Progress Series* **168**, 35–43.
- Wang, C. H. & Tzeng, W. N. (2000). The timing of metamorphosis and growth rates of American and European eel leptocephali: a mechanism of larval segregative migration. *Fisheries Research* **46**, 191–205.

- Watabe, N., Tanaka, K., Yamada, J. & Dean, J. M. (1982). Scanning electron microscope observations of the organic matrix in the otolith of the teleost fish *Fundulus heteroclitus* and *Tilapia nilotica*. *Journal of Experimental Marine Biology and Ecology* **58**, 127–134.
- Wheeler, A. (1985). *The World Encyclopedia of Fishes*. London: Macdonald.
- Williamson, G. R., Stammers, S., Tzeng, W. N., Shiao, J. C., Prokhorchik & Lecomte-Finiger, R. (1999). Drifting and dreaming: is this how the baby eels cross the Atlantic Ocean? *Ocean Challenge* **9**, 40–45.
- Yamano, K., Tagawa, M., Jesus, E. G., Hirano, T., Miwa, S. & Inui, Y. (1991). Changes in whole body concentrations of thyroid hormones and cortisol in metamorphosing conger eel. *Journal of Comparative Physiology B* **161**, 371–375.
- Zar, J. H. (1996). *Biostatistical Analysis*, 3rd edn. London: Prentice-Hall.