

Decreased Sr/Ca ratios in the otoliths of two marine eels, *Gymnothorax reticularis* and *Muraenesox cinereus*, during metamorphosis

Y. J. Ling¹, Y. Iizuka², W. N. Tzeng^{3,*}

¹Department of Life Sciences, College of Life Science, National Taiwan University, Taipei 106, Taiwan, ROC

²Institute of Earth Sciences, Academia Sinica, Nankang, Taipei 115, Taiwan, ROC

³Institute of Fisheries Science, College of Life Science, National Taiwan University, Taipei 106, Taiwan, ROC

ABSTRACT: Sr/Ca ratios in the otoliths of 2 marine eels, the moray eel *Gymnothorax reticularis* and the pike eel *Muraenesox cinereus*, decreased greatly when the crystal structure of otolith daily growth increments changed from concentric to radiate form. This decrease is proposed to correspond to the timing of metamorphosis from the leptocephalus to glass eel stage, similar to freshwater eels (*Anguilla* spp.). In freshwater eels, ontogenetic and habitat shifts might influence the decrease. However, marine eels do not migrate into freshwater after completing metamorphosis, so the decrease in these species must be the result of metamorphosis-related physiological, rather than environmental, effects. The mean age at metamorphosis of the moray eels was significantly lower than that of pike eels, and both were lower than that of freshwater eels ($p < 0.05$). Consequently, the spawning ground of marine eels is presumed to be closer than that of freshwater eels to the continental shelf.

KEY WORDS: Otolith · Sr/Ca ratio · Growth increment · Daily age at metamorphosis · *Gymnothorax reticularis* · *Muraenesox cinereus*

—Resale or republication not permitted without written consent of the publisher—

INTRODUCTION

Otoliths are biomineralized aragonitic crystals composed of calcium carbonate with a small quantity of organic matrix (Degens et al. 1969) that occur in the otic vesicles of teleost fishes and play a role in hearing and balance. They are deposited daily in alternating protein-rich and carbonate-rich layers as the fish grow (Pannella 1971). This variable deposition enables the determination of daily fish ages and study of their early growth history.

During otolith formation some trace elements are occasionally co-precipitated with calcium carbonate (Campana 1999). Strontium (Sr) is one of the most common elements replacing calcium (Ca) in otoliths because of its similar ionic radius, valence and chemical activity (Amiel et al. 1973). The incorporation of Sr into the otolith is a complex biogeochemical process that is influenced by physiological and environmental factors

(Kalish 1989, 1991, Radtke & Shafer 1992, Sadovy & Severin 1992). The Sr/Ca ratios in the otoliths of Japanese eels *Anguilla japonica* drastically decrease when the eel larvae pass through a salinity gradient during migration from spawning ground to estuary while simultaneously transitioning from the leptocephalus to glass eel developmental stage (Tzeng & Tsai 1994). Otake et al. (1994) suggested that this sharp decrease in Sr/Ca ratios marks the onset of metamorphosis. However, no direct evidence was given to validate this claim.

To evaluate whether salinity or developmental stage transition is responsible for in the drastic decrease of Sr/Ca ratios during metamorphosis in anguillid eels, we examined Sr/Ca ratios in the otoliths of the moray eel *Gymnothorax reticularis* and the pike eel *Muraenesox cinereus*, marine species that spend their entire lives in the ocean (Shen 1993, Nelson 1994). Both eels metamorphose from leptocephalus to glass eel in sea-

*Corresponding author. Email: wnt@ccms.ntu.edu.tw

water and do not migrate to brackish water after metamorphosis (Devadoss & Pillai 1979, Masuda & Allen 1993). Therefore, if the Sr/Ca ratios in otoliths of the marine eels also decrease greatly at metamorphosis, then we can confirm that the similar decrease in Sr/Ca ratios in the otoliths of freshwater eels is caused by physiological rather than environmental factors.

MATERIALS AND METHODS

Forty moray eels and 31 pike eels were collected from the landing of the local trawler at the fish market in the Da-Shi fishing port, I-Lan Prefecture, northeastern Taiwan from May to September 2002. The fishing ground of the trawl fisheries was around Turtle Mountain Island (Guishandao) off the prefecture (Fig. 1). After collection, the eels were immediately preserved, brought back in ice and then frozen at -20°C in the laboratory. Both species were identified using morphological characteristics (Shen 1993). The total length and body weight were measured to the nearest 0.1 mm and 0.1 g, respectively. Sagittal otoliths were removed for microstructural and microchemical analyses.

The otoliths were embedded in epofix resin, ground and polished with sandpapers of 1500 and 2400 grit until the primordia were exposed. They were then washed with distilled water, dried at 80°C in an oven overnight and coated with carbon. The Sr and Ca concentrations were measured from the otolith primordium to the edge by Electron Probe Microanalyzer (EPMA, JEOL JXA-8900R) with an electron beam condition of 15 kV and 5 nA, beam size 10 μm , at a measuring interval of 10 μm .

After EPMA analysis, 23 moray and 18 pike eel otoliths were randomly selected for microincrement analysis. They were repolished to remove the carbon coating, etched with 0.05 M HCl for 30 s, and then dried in the oven and coated with gold for scanning electron microscope (SEM) analysis to reveal the growth microincrements. The growth microincrements in the otoliths of these 2 marine eels were assumed to be deposited daily, as for other eels (Mochioka et al. 1989, Umezawa et al. 1989). The daily age of the eels at metamorphosis was determined from the growth microincrements counted from the primordium to the growth check (GC), where Sr/Ca ratios decreased greatly and the growth microincrements changed from a circular to a radiate pattern. The GC was assumed to be deposited at metamorphosis, similar to Japanese eel *Anguilla japonica* (Cheng & Tzeng 1996). The maximum radius from the primordium to the GC (R_m) was divided by the corresponding daily age (T_m) to estimate the mean daily otolith growth rate (G_m) of the eels in the leptocephalus stage. The mean incremental



Fig. 1. Sampling area of moray eel *Gymnothorax reticularis* and pike eel *Muraenesox cinereus* in waters around Turtle Mountain Island (Guishandao), northeastern Taiwan

widths were measured from the primordium to the GC with an increment interval of 5 and evaluated to reveal their early life history.

The differences in age at metamorphosis, R_m and G_m between the 2 species were examined by analysis of variance (ANOVA). Data on Japanese eels *Anguilla japonica* (Cheng & Tzeng 1996) were adopted to enable further comparisons with the 2 marine eels.

RESULTS

Otolith microstructure and age at metamorphosis

The general otolith microstructure was similar for moray eel and pike eel (Fig. 2). Concentric rings appeared from the primordium to the growth check (GC). Beyond the GC, the growth pattern changed to radiate and the growth increments became unclear and uncountable. The GC was considered to be deposited at metamorphosis. The patterns of otolith increment width and age at metamorphosis were different for these 2 marine eels (Fig. 3).

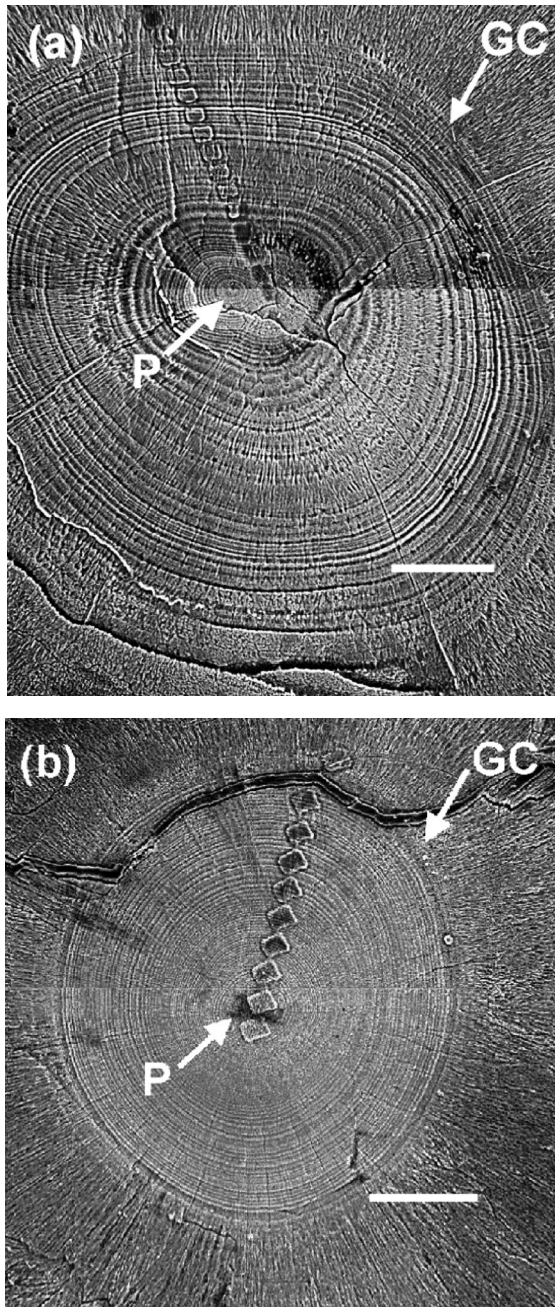


Fig. 2. (a) *Gymnothorax reticularis*, and (b) *Muraenesox cinereus*. SEM microphotographs showing changing pattern and growth check (GC) of daily growth increments in otoliths of moray eel (total length [TL] = 464.09 mm, body weight [BW] = 144.6 g) and pike eel (TL = 497.47 mm, BW = 189.05 g). P = primordium. Square spots indicate where Sr/Ca ratios were measured by EPMA. Scale bars = 50 μm

In moray eels, the otolith increment width increased rapidly until the mean age at metamorphosis and decreased beyond that age. The minimum age at metamorphosis in moray eels ranged from 24 to 48 d. The discrepancy in age at metamorphosis among indi-

viduals led the standard error in increment width to increase from the 24th day. In pike eels increment width remained relatively constant, and the increase was not obvious before the mean age at metamorphosis, 65 d. The minimum age at metamorphosis in pike eels was 38 d, but most were between 50 and 70 d. The increment width was relatively larger for the specimen metamorphosed at the 103rd day.

Variations in Sr/Ca ratios

The otolith Sr/Ca ratios of the moray eel increased from ca. 7.5×10^{-3} in the primordium to a maximum of 9.5×10^{-3} around the GC and then decreased to 4.0×10^{-3} (Fig. 4). The timing of the decrease in Sr/Ca ratios coincided with the appearance of the GC (Fig. 2a), after which the ratios fluctuated between 4.0 and 9.0×10^{-3} . The Sr/Ca ratios in pike eel otoliths increased similarly from 8.0×10^{-3} in the primordium to a peak of 9.0×10^{-3} at the GC, then decreased sharply to ca. 4.0×10^{-3} , after which they fluctuated between 3.0 and 6.5×10^{-3} (Fig. 4). Thus, the Sr/Ca ratios in the otoliths of both marine eels decreased greatly at the GC, which was assumed to be deposited during metamorphosis.

Comparison of age at metamorphosis and growth rates

The mean daily age of moray eels at metamorphosis was 37 d, which was significantly smaller than that of pike eels (66 d), and both were significantly smaller than that of Japanese eels *Anguilla japonica* (116 d) ($F = 60.05$, $p < 0.001$). Conversely, the mean maximum otolith radius from the primordium to the GC for moray eels was 98.1 μm , which was significantly larger than that of pike eels (66.1 μm) ($F = 36.32$, $p < 0.001$) but similar to that of the Japanese eel (105.5 μm) ($p > 0.05$). Furthermore, the mean otolith growth rate of moray eels before GC ($2.7 \mu\text{m d}^{-1}$) was significantly larger than that of pike eels ($1.0 \mu\text{m d}^{-1}$) (Table 1) and Japanese eels ($0.9 \mu\text{m d}^{-1}$) ($F = 14.98$, $p < 0.001$) but showed no significant difference between pike eels and Japanese eels ($p > 0.05$).

DISCUSSION

In general, the otolith microstructure and the Sr/Ca ratios around the GC in the otoliths of both moray eels and pike eels were similar to those around the metamorphosis check of anguillid eels such as *Anguilla japonica* (Tzeng 1990), *A. rostrata* and *A. anguilla*

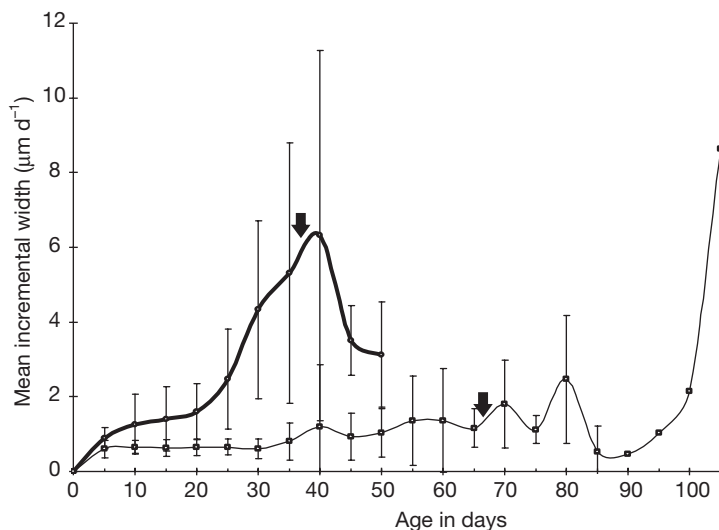


Fig. 3. *Gymnothorax reticularis* and *Muraenesox cinereus*. Mean (\pm SD) daily growth increment width measured along maximum otolith radius from primordium to GC for moray eels ($n = 18$, thick line) and pike eels ($n = 15$, thin line). Arrows = mean daily age at GC of 37 and 66 d, for the 2 species respectively

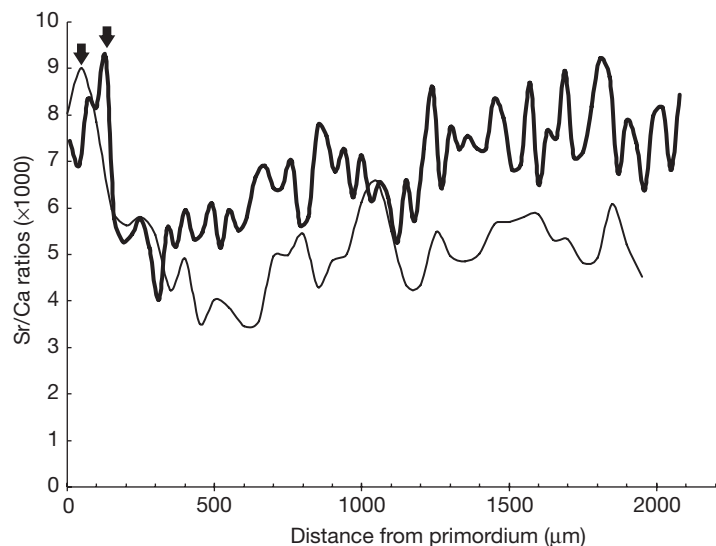


Fig. 4. *Gymnothorax reticularis* and *Muraenesox cinereus*. Temporal changes in Sr/Ca ratios, composed of averages of 3 measured values from primordium to otolith edge for moray eel (thick line) and pike eel (thin line). Arrows = GC, for the 2 marine eels shown in Fig. 2

(Wang & Tzeng 2000) and marine eels such as *Conger conger* (Correia et al. 2003), *C. oceanicus* (Correia et al. 2004) and *C. myriaster* (Lee & Byun 1996, Otake et al. 1997). Thus, we believe that the GC in the otoliths of the moray and pike marine eels will have been deposited during the metamorphosis from leptocephalus to elvers.

Japanese eels and both marine eels all have a similar temporal change in otolith Sr/Ca ratios, which greatly decrease at metamorphosis. After metamorphosis,

Japanese eels migrate toward an estuary where salinity is lower (Tzeng & Tsai 1994, Cheng & Tzeng 1996). Consequently, environmental effects on the large decrease in otolith Sr/Ca ratios cannot be excluded because for Japanese eels they decreased as salinity decreased (Tzeng 1996, Tzeng et al. 2002). In contrast, the marine-resident moray eels and pike eels did not migrate toward an estuary after metamorphosis. Thus, the large decrease in otolith Sr/Ca ratios of both marine eels at metamorphosis may be attributed to physiological rather than environmental effects. This hypothesis was also strengthened by the finding that seawater-resident freshwater eels did not enter freshwater but their Sr/Ca ratios around the GC in the otolith of the eel decreased (e.g. Tzeng et al. 2000).

Pfeiler (1984, 1986, 1991) found large amounts of glycosaminoglycans (GAG) in the extracellular gelatinous matrix of leptocephali. GAG have high affinity for Sr (Nishizawa 1978), and thus the higher content of GAG in the leptocephali might promote the uptake of Sr in the body and subsequent deposition in otoliths. Pfeiler (1986) divided the leptocephalus stage into 2 phases: phase 1, growth of fish length; phase 2, metamorphosis from leptocephalus to glass eel, in which the body length does not increase and may even decrease. Bishop et al. (2000) further partitioned phase 1 into 2 subphases based on metabolic energy use. Subphase 1, use of metabolic energy mostly for body growth; subphase 2, energy storage, in which the extracellular gelatinous matrix, including GAG, is accumulated to meet the huge energy requirements for metamorphosis. Thus, the amount of GAG in the body reaches its maximum before metamorphosis and consequently also the Sr/Ca ratios in the otoliths of leptocephali. Metamorphosis consumes large amounts of extracellular gelatinous matrix and subsequently causes a decrease in GAG in the body.

The decrease in the corporal GAG leads to a reduction in a fish's ability to absorb Sr, and thus the Sr/Ca ratios in leptocephalus otoliths dramatically decrease during metamorphosis, as previous suggested by Otake et al. (1997).

The huge release of energy from the breakdown of GAG at metamorphosis from leptocephalus to glass eel would cause an enhanced ossification to meet the demands for recombination and reconstruction of body structures. This might also be a factor leading to increased otolith growth rates, represented by the wider

Table 1. *Gymnothorax reticularis* and *Muraenesox cinereus*. Mean (\pm SD) total length (TL) and body weight (BW), and comparisons for daily age at metamorphosis (T_m), maximal radius (R_m) from the primordium to the growth check (GC) and mean otolith growth rate (G_m) before the GC between moray and pike eels. Numbers in brackets are the sample sizes for otolith and microchemistry analyses

	<i>G. reticularis</i> ^a	<i>M. cinereus</i> ^b	Significant difference
Sample size	43 (23)	31 (18)	
TL (mm)	441.2 \pm 53.6	520.7 \pm 57.9	
BW (g)	110.2 \pm 39.0	181.9 \pm 85.8	
T_m (Day)			
Mean \pm SD	37 \pm 7	66 \pm 16	b > a (p < 0.01)
Range	24–48	38–102	
R_m (μ m)	98.1 \pm 28.6	66.1 \pm 9.9	a > b (p < 0.01)
G_m (μ m d ⁻¹)	2.7 \pm 0.4	1.0 \pm 0.2	a > b (p < 0.01)

incremental widths during otolith growth around the GC. Additionally, Yamono et al. (1991) found that thyroid hormones increased in metamorphosing, and this increase may lead to the rapid otolith growth rate at metamorphosis (Shiao & Hwang 2004).

Pfeiler (1986) indicated that the amount of NaCl in the body fluid of leptocephali decreased at metamorphosis. The metamorphic leptocephali had lower and less variable osmolarities of body fluid (Hulet & Robins 1989), and the osmoregulation-related cells, the chloride-type cells, were found in the integuments of the phase II leptocephali of *Albula* sp. (Pfeiler & Lindley 1989), suggesting that the ability to osmoregulate becomes stronger in metamorphosis. This may also cause the decrease in Sr uptake and subsequent deposition in the otolith. Consequently, it is possible that not only does the GAG break down but the enhanced osmoregulation influences the variability in otolith Sr/Ca ratios of eels during metamorphosis.

The daily ages at metamorphosis of moray eels and pike eels were significantly smaller than that of Japanese eels (Cheng & Tzeng 1996). The age at metamorphosis and growth rate of leptocephali of Japanese eels plays an important role in determining their dispersal distance when leptocephali are transported by oceanic currents from the oceanic spawning ground toward the nursery ground (Cheng & Tzeng 1996, Wang & Tzeng 1998, 2000, Shiao et al. 2002). Moray eels metamorphosed at a younger age than did pike eels and Japanese eels, which implies that the spawning grounds of moray eels and pike eels are less distant than that of Japanese eel. It might also be very difficult for moray and pike leptocephali to drift as far as Japanese eel leptocephali, which hatch in and migrate from the west of the Mariana Islands (Tsukamoto 1992). Pike eels and moray eels may spawn near the continental shelf and

migrate a much shorter distance than freshwater eels. McCleave (1993) proposed that leptocephali that metamorphose at a young age might favor short-distance migration, while those that are older at metamorphosis might favor migration. Thus, instead of a distant dispersal, the moray eel that metamorphoses at a much younger age than Japanese eels might 'choose' to stay closer to the continental shelf grounds. This implies the adoption of different migration and metamorphosis strategies.

The mean otolith daily growth increment width and its variability in moray eels during early development are more apparent than for pike eels, indicating that the growth rates of moray eels may be higher and more variable. Moreover, moray eels may inhabit a more variable shallow-water marine environment that is nutrient-rich but fluctuates greatly in environmental conditions such as water temperature due to the influence of a nearby continent. Thus moray eels may have evolved to a shorter larval duration, and higher and varied larval growth in order to pass from a pelagic to a benthic habitat as soon as possible.

Acknowledgements. This survey was supported by a Summer Students Program of the National Science Council, ROC (No. 91CFA0100082). We thank Miss C. Y. Lin, College of Life Science, National Taiwan University (NTU), and Ms. S. J. Jih, College of Agriculture, NTU, for their help with SEM photography and the fishermen at the Da-Shi fishing port for providing samples. We are also grateful to all our colleagues at the Fishery Biology Laboratory, Institute of Fisheries Science, NTU, for providing technical and skills assistance for otolith preparation and analysis. Special thanks to Mr. B. M. Jessop and 4 anonymous reviewers for their useful comments in the early drafts of the manuscript.

LITERATURE CITED

- Amiel AJ, Friedman GM, Miller DS (1973) Distribution and nature of incorporation of trace elements in modern aragonite corals. *Sedimentology* 20:47–64
- Bishop RE, Torres JJ, Crabtree RE (2000) Chemical composition and growth indices in leptocephalus larvae. *Mar Biol* 137:205–214
- Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar Ecol Prog Ser* 188:263–297
- Cheng PW, Tzeng WN (1996) Timing of metamorphosis and estuarine arrival across the dispersal range of the Japanese eel *Anguilla japonica*. *Mar Ecol Prog Ser* 131:87–96
- Correia AT, Antunes C, Isidro EJ, Coimbra J (2003) Changes in otolith microstructure and microchemistry during larval development of the European conger eel (*Conger conger*). *Mar Biol* 142:777–789
- Correia AT, Able KW, 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. *Mar Biol* 145:477–488
- Degens ET, Deuser WG, Haedrich RL (1969) Molecular structure and composition of fish otoliths. *Mar Biol* 2:105–113

- Devadoss P, Pillai PKM (1979) Observations on the food and feeding habits of the eel *Muraenesox cinereus* (Forskål) from Porto Novo. Indian J Fish 26:244–247
- Hulet WH, Robins CR (1989) The evolutionary significance of the leptocephalus larva. In: Böhlke EB (ed) Fishes of the Western North Atlantic. Memoir No. 1, Part 9, Vol 2. Sears Foundation for Marine Research, New Haven, CT, p 669–677
- Kalish JM (1989) Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J Exp Mar Biol Ecol 132:151–178
- Kalish JM (1991) Determination of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of reared rock cod *Pseudophycis barbatus*. Mar Ecol Prog Ser 74:137–159
- Lee TW, Byun JS (1996) Microstructural growth in otoliths of conger eel (*Conger myriaster*) leptocephali during the metamorphic stage. Mar Biol 125:259–268
- Masuda H, Allen GR (1993) Meeresfische der Welt—Groß Indopazifische Region. Tetra, Herrrenteich Melle
- McCleave JD (1993) Physical and behavioural controls on the oceanic distribution and migration of leptocephali. J Fish Biol 43:24–273
- Mochioka N, Tabeta O, Kanda T (1989) Daily growth increments in otoliths of the conger eel *Conger myriaster* leptocephali. Working Group on Eel, European Inland Fisheries Advisory Commission (EIFAC). Porto: 1–2 (cited in Correia et al. 2002)
- Nelson JS (1994) Fishes of the world, 3rd edn. Wiley, New York, p 104–116
- Nishizawa K (1978) Marine algae from a viewpoint of pharmaceutical studies. Jpn J Phycol 26:73–78
- 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*. Mar Ecol Prog Ser 112:189–193
- Otake T, Ishii T, Nakahara M, Nakamura R (1997) Changes in otolith strontium: calcium ratios in metamorphosing *Conger myriaster* leptocephali. Mar Biol 128:565–572
- Pannella G (1971) Fish otoliths: daily growth layers and periodical patterns. Science 173:1124–1127
- Pfeiler E (1984) Glycosaminoglycan breakdown during metamorphosis of larval bonefish *Alba*. Mar Biol Lett 5:241–249
- Pfeiler E (1986) Toward an explanation of the developmental strategy in leptocephalus larvae of marine teleost fishes. Environ Biol Fish 15:3–13
- Pfeiler E (1991) Glycosaminoglycan composition of anguilliform and elopiform leptocephali. J Fish Biol 38:533–540
- Pfeiler E, Lindley V (1989) Chloride-type cells in the skin of the metamorphosing bone fish (*Albula* sp.) leptocephalus. J Exp Zool 250:11–16
- Radtke RL, Shafer DJ (1992) Environment sensitivity of fish otolith microchemistry. Aust J Mar Freshw Res 43: 935–951
- Sadovy Y, Severin KP (1992) Trace elements in biogenic aragonite: correlation of body growth rate and strontium levels in the otoliths of the white grunt, *Haemulon plumieri* (Pisces: Haemulidae). Bull Mar Sci 50:237–257
- Shen SJ (1993) Fishes in Taiwan. National Taiwan University, Taipei, p 97–119
- Shiao JC, Hwang PP (2004) Thyroid hormones are necessary for teleostean otolith growth. Mar Ecol Prog Ser 278: 271–278
- Shiao JC, Tzeng WN, Collins A, Iizuka Y (2002) Role of marine larval duration and growth rate of glass eels in determining the distribution of *Anguilla reinhardtii* and *A. australis* on Australian eastern coasts. Mar Freshw Res 53:687–695
- Tsukamoto K (1992) Discovery of the spawning area for Japanese eel. Nature 356:789–791
- Tzeng WN (1990) Relationship between growth rate and age at recruitment of *Anguilla japonica* elvers in a Taiwan estuary as inferred from otolith growth increments. Mar Biol 107:75–81
- Tzeng WN (1996) Effects of salinity and ontogenetic movements on strontium: calcium ratios in the otoliths of the Japanese eel *Anguilla japonica* Temminck and Schegel. J Exp Mar Biol Ecol 199:111–122
- Tzeng WN, Tsai YC (1994) Changes in otolith microchemistry of the Japanese eel *Anguilla japonica* during its migration from the ocean to the rivers of Taiwan. J Fish Biol 45: 671–684
- Tzeng WN, Wang CH, Wickström H (2000) Occurrence of the semi-catadromous European eel *Anguilla anguilla* in the Baltic Sea. Mar Biol 137:93–98
- Tzeng WN, Shiao JC, Iizuka Y (2002) Use of otolith Sr:Ca ratios to study the riverine migratory behaviors of Japanese eel *Anguilla japonica*. Mar Ecol Prog Ser 245:213–221
- Umezawa A, Tsukamoto K, Tabeta O, Yamakawa H (1989) Daily increments in the larval otolith of the Japanese eel *Anguilla japonica*. Jpn J Ichthyol 35:440–444
- Wang CH, Tzeng WN (1998) Interpretation of geographic variation in size of American eel *Anguilla rostrata* elvers on the Atlantic coast of North America using their life history and otolith aging. Mar Ecol Prog Ser 168:35–43
- Wang CH, Tzeng WN (2000) The timing of metamorphosis and growth rates of American and European eel leptocephali: a mechanism of larval segregative migration. Fish Res 46:191–205
- Yamano K, Tagawa M, De Jesus EG, Hirano T, Miwa S, Inui Y (1991) Changes in whole body concentrations of thyroid hormones and cortisol in metamorphosing conger eel. J Comp Physiol B 161:371–376

Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

Submitted: November 23, 2004; Accepted: July 17, 2005
Proofs received from author(s): November 11, 2005