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Strontium Bands in Relation to Age Marks in Otoliths of European Eel *Anguilla anguilla*

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Wann-Nian Tzeng, Kenneth P. Severin, Håkan Wickström and Chia-Hui Wang (1999) Strontium bands in relation to age marks in otoliths of European eel *Anguilla anguilla*. *Zoological Studies* **38**(4): 452-457. Higher-concentration strontium (Sr) bands and hyaline zones in otoliths of European eel, *Anguilla anguilla* (L.), from both brackish waters and freshwater lakes were examined by wavelength dispersive x-ray spectrometry on an electron microprobe and by visible light microscopy, respectively. The positions of higher-concentration (> 0.3 wt %) Sr bands and hyaline zones were identical in otoliths of eels from brackish waters; however, no corresponding higher-concentration Sr bands were discernible in otoliths of eels from fresh water. The higher-concentration Sr bands were deposited when the eels migrated from brackish water to high-saline seawater during winter. The number of hyaline zones in the otoliths corresponds to the age of the eel. Accordingly, higher-concentration Sr bands in otoliths can be used to determine fish age and migratory history in brackish waters.

Key words: European eel, Otolith, Strontium, Annulus.

European eel *Anguilla anguilla* (L.) is the most abundant among 18 species of the genus *Anguilla* in the world (Tesch 1983). It spawns in the Sargasso Sea, and its larvae (leptocephali) drift with the Gulf Stream and North Atlantic Current to the continental shelf of northern Europe, where they metamorphose into glass eels. They become pigmented elvers in estuaries. Their migration from the Sargasso Sea to the estuaries requires 7 to 8 mo (Lecomte-Finiger 1992). The elvers penetrate rivers and streams, and complete their growth stage in fresh water. Some elvers may remain in marine or brackish waters along the coast until maturation (Tsukamoto et al. 1998). Male eels grow in rivers for 3 to 7 yr with a mean of 5 yr, while female eels grow from 4 to 15 yr with a mean of 7 yr (Vøllestad and Jonsson 1986). In late autumn the eels become mature, metamorphose from yellow eels to silver eels, and migrate back to the Sargasso Sea where they spawn and presumably die (Bertin 1956, Tesch 1983).

The otolith of the eel is optically composed of alternating hyaline and opaque zones which are deposited with seasonal changes in fish growth (Sinha and Jones 1967, Hogman 1968, Casselman 1982). Hyaline zones are bright and opaque zones are dark when the otolith is viewed with transmitted light, while opaque zones are bright and hyaline zones are dark when viewed with reflected light (Williams and Bedford 1974). Hyaline zones are deposited during slow growth in winter, and are composed primarily of organic materials with lower amounts of calcium carbonate, while opaque zones are deposited during fast growth in summer, and are composed primarily of inorganic calcium carbonate (Penttila and Dery 1988). The hyaline zones are generally deposited annually, and are usually designated as the annuli.

Strontium (Sr)/Calcium (Ca) ratio in otoliths has been used to study the migratory environmental history of European and Japanese eels (Otake et al. 1994, Tzeng and Tsai 1994, Tzeng 1995, Arai et al. 1997, Tzeng et al. 1997) and other fishes (Radtke et al. 1988, Kalish 1990, Secor 1992, Limburg 1995).

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The ratio is positively correlated with ambient salinity (Tzeng 1996), but negatively correlated with temperature and fish growth rate (Sadovy and Severin 1992 1994, Townsend et al. 1992, Tzeng 1994). Salinity, temperature, and growth rate often change seasonally, suggesting that a temporal relationship may exist between annulus formation and Sr deposition. If this is the case, otolith microchemistry could be used to determine the age and growth history of fish. However, no studies have validated the relationship between annulus formation and otolith microchemistry (Casselman 1982, Proctor et al. 1990, Seyama et al. 1991, Secor 1992, Tzeng et al. 1997).

The objectives of this study were to clarify the temporal relationship between hyaline zone formation and the higher-concentration Sr band deposition in otoliths of the European eel, and to understand the mechanism of Sr band deposition.

MATERIALS AND METHODS

Otoliths of 12 European eels, *Anguilla anguilla*, collected from brackish waters and freshwater lakes of Sweden in a previous study on otolith microchemistry (Tzeng et al. 1997) were used as materials in this study. They were divided into 3 different life history groups according to their otolith microchemistry. Relationships between annuli and microchemistry of sagittal otoliths of the 12 eels were examined, and 1 eel from each group was selected for description (Table 1). The eels of Group 1 were collected from brackish waters off Strömstad on the west coast of Sweden in 1987. The eels of Group 2 were stocked in Lake Ången on the east coast of Sweden at the yellow eel stage after being caught in brackish waters on the west coast of Sweden in 1979, and were recaptured in 1991. The eels of Group 3 were collected from Lake Mälaren on the east coast of Sweden in August 1994.

Procedures of preparing the otoliths, including embedding, sectioning, polishing, and coating, for microprobe quantitative analysis of Sr and Ca and x-ray mapping of Sr concentration are described in Tzeng et al. (1997). After microprobe analysis, otoliths were repolished to remove carbon coating, etched with EDTA to enhance the hyaline zones (annuli), and photographed with both transmitted and reflected light microscopes. The positions of annuli were compared with those of the higher-concentration Sr bands in the x-ray map.

RESULTS

Brighter areas in the Sr maps indicate higher Sr concentrations (Fig. 1a, c, e). Sr content increased from the primordium and reached the highest (> 0.8 wt %) at metamorphosis when the leptocephalus metamorphosed to glass eel. Beyond the elver stage, Sr contents dramatically decreased, and the patterns of distribution of Sr concentrations differed among otoliths from the 3 groups.

Sr concentrations in Group 1 otoliths beyond the elver mark averaged approximately 0.3 wt %. The otoliths revealed 5 distinct higher-concentration Sr bands (> 0.3 wt %) and 5 dark hyaline zones (Fig. 1a, b). The positions of the 5 high-Sr bands and the 5 hyaline zones in the otoliths were identical. The hyaline zones were dark while opaque zones were bright, irrespective of being viewed with transmitted or reflected light, although it was noted that hyaline zones of otoliths were bright in transmitted light (Penttila and Dery 1988). The 1st opaque zone was wider than others, indicating that the eel grew faster in the 1st year. Sr levels of the 2nd band were low, and the corresponding hyaline zone was relatively faint. On the other hand, Sr levels of the 4th band were higher, and the corresponding hyaline zone was clearer, indicating that the slow growth period was longer.

Group 2 otoliths had 2 different levels of Sr concentration beyond the elver mark (Fig. 1c). Sr concentration in the inner layer averaged approximately 0.3 wt %, which was significantly greater than that in the outer layer (< 0.05 wt %). There are 9 high-Sr bands and 9 hyaline zones in the inner layer, and their positions were identical between Sr bands and hyaline zones (Fig. 1c, d). In the outer layer there are

Table 1. Life history of 3 European eels used in this study

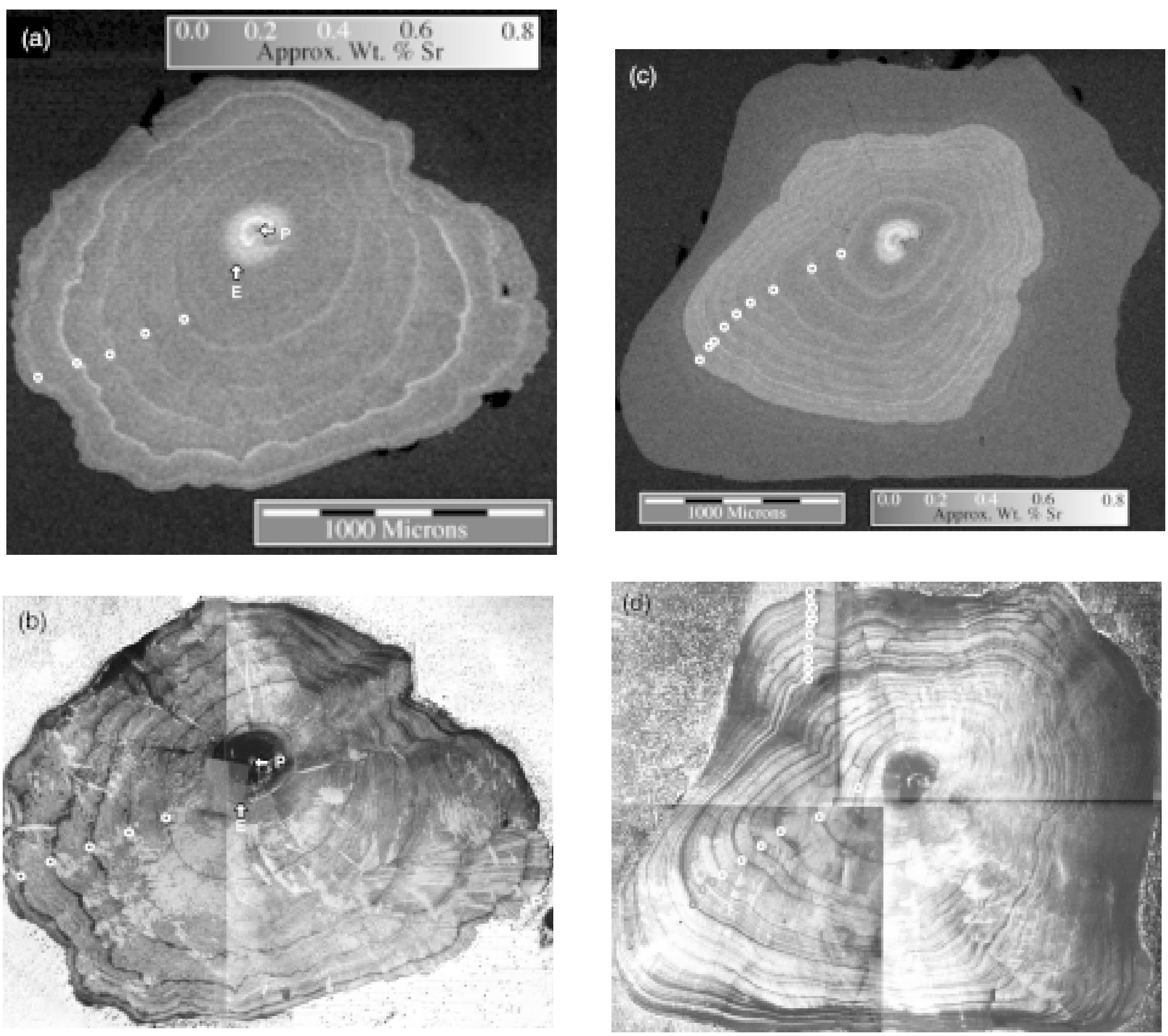
Group no.	Sampling date	Sampling site	Salinity	Sex	Total length (mm)	Body weight (g)
1	9 July 1987	Strömstad	Brackish water	♀	393	96
2	1 July 1991	Lake Ången	Brackish and fresh water	♀	760	820
3	30 Aug. 1994	Lake Mälaren	Fresh water	♀	680	461

11 more-or-less distinct hyaline zones, but there are no corresponding high-Sr bands. These indicate that the eel lived in brackish water for 9 yr and in fresh water for 11 yr. In the boundary between the high and low levels of Sr concentration, the growth of the otoliths seems to be retarded, and thus Sr bands and hyaline zones are narrow and complicated. This may lead to an underestimation of the numbers of Sr bands and/or hyaline zones.

Sr concentration in Group 3 otoliths averaged less than 0.05 wt %. Other than the area around the nucleus, there were no areas of high Sr levels even though there were 14 distinct hyaline zones visible with light microscopy (Fig. 1e, f).

DISCUSSION

Regardless of later life history, the nucleus of any eel is deposited during a marine life phase. The nuclei of the otoliths of all 3 eels are hyaline and also have high Sr contents, as do those of other eels (Otake et al. 1994, Tzeng and Tsai 1994, Arai et al. 1997, Wang and Tzeng 1998). In addition, all the higher-concentration Sr bands correspond to narrow hyaline zones. This is similar to what Seyama (1991) found in the otolith of a red emperor (*Lutjanus sebae*). However, the mechanism of the formation of higher-concentration Sr bands in otoliths is not clear. Incorporation of Sr into otoliths is a compli-



(Caption see next page)

cated biogeochemical process influenced by abiotic factors such as temperature, salinity, and water chemistry, as well as by biotic factors such as genetics, developmental stage, growth rate, food, and physiological condition of the fish (Dodd 1967, Yamada et al. 1979, Kalish 1989, Gallahar and Kingsford 1992, Radtke and Shafer 1992, Sadovy and Severin 1992, Otake et al. 1994, Tzeng and Tsai 1994, Tzeng et al. 1997).

Hyaline zones are formed during the slow-growth phase of the otolith. Generally, otolith growth is proportional to somatic growth, and seasonal growth of eels is lower in low-temperature periods (Bruun 1963, Sinha and Jones 1967, Campana and Neilson 1985, Tzeng et al. 1994). Thus, higher-concentration Sr bands corresponding to hyaline zones in otoliths of brackish water eels are deposited during low temperature periods when fish growth is slow. Sr concentrations are higher in seawater than in brackish water (Tzeng and Tsai 1994). Sr/Ca ratios in otoliths of eels are negatively correlated to ambient temperature, and highly positively correlated to salinity (Tzeng 1994 1996). Temperatures in the coastal waters of Sweden decrease sharply during winter. At this time eels generally migrate from coastal waters to deeper water for overwintering (Tesch 1983). Salinities are higher in deeper water than in coastal waters in the studied area (Tomczak and Godfrey 1994). These facts may indicate that higher-concentration Sr bands corresponding to hyaline zones in otoliths of brackish water eels are deposited at low temperatures during winter when eels migrate from brackish water to high-saline deep water (Fig. 1a-d). However, Sr contents are lower and no regular higher-concentration Sr bands corresponding to the hyaline zones are observed in otoliths of freshwater eel (Fig. 1c-f). This indicates that fresh water does not provide enough strontium for incorporation during the formation of hyaline zones, and the effect of temperature and growth rate on the formation of the higher-concentration Sr bands is not obvious.

Hyaline zones apparently are formed once a year, which was validated in a previous study (Tzeng et al. 1994). Higher-concentration Sr bands are deposited synchronously with the hyaline zones in otoliths of eels from brackish waters. This suggests that higher-concentration Sr bands in otoliths can be used to estimate the age of brackish water eels.

In conclusion, the higher-concentration Sr bands in otoliths are deposited when eels migrate from brackish water to high-saline sea water. The deposition of higher-concentration Sr bands is synchronous with the hyaline zones that are formed once a year. Thus, the higher-concentration Sr bands in otoliths can be used to determine the age of eels in brackish water and their migratory history.

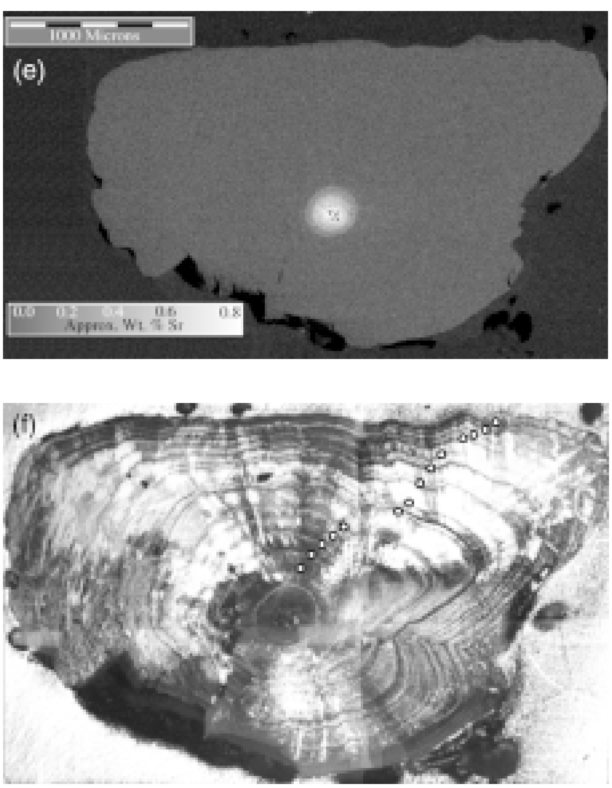


Fig. 1. Higher-concentration Sr bands in Sr x-ray maps (a, c, and e) and hyaline zones in visible-light photographs (b, d, and f) of otoliths of 3 European eels (a and b, Group 1; c and d, Group 2; e and f ,Group 3; E, elver mark; P, primordium; circles, annuli). Groups 1, 2 and 3 refer to table 1. The Sr maps a, c, and e are modified from Tzeng et al. 1997.

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歐洲鰻耳石上之高鍶環與年輪之關係

曾萬年[1] Kenneth P. Severin[2] Håkan Wickström[3] 王佳惠[1]

利用電子微探儀及光學顯微鏡，分析歐洲鰻耳石的高濃度鍶環與年輪之關係。結果發現來自鹹淡水的歐洲鰻的耳石，有很明顯的高鍶環出現，高鍶環的位置與年輪的位置一致。但是，來自湖泊者，則沒有高鍶環出現。推測高鍶環可能是鰻魚於冬季從鹹淡水迴游到高鹽度的海水時所形成的。由年輪的數目可以知道鰻魚的年齡。因此，由高鍶環的數目可以推測鰻魚在鹹淡水時的年齡及其迴游履歷。

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