

Misidentification of the migratory history of anguillid eels by Sr/Ca ratios of vaterite otoliths

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ABSTRACT: Most fish otoliths are composed of aragonite, but occasionally they form a mosaic with its polymorph, vaterite. We found an unusual mosaic structure of extremely low strontium to calcium (Sr/Ca) ratio in the aragonite otoliths of European eels *Anguilla anguilla*. The mosaic crystal structure in the eel otolith appeared opaque under reflected light after EDTA etching and was confirmed to be a vaterite crystal by Raman spectroscopy. Analysis by laser ablation inductively coupled plasma mass spectrometry indicated that the elements sodium (Na), strontium (Sr) and barium (Ba) were lower in abundance and magnesium (Mg) and manganese (Mn) were higher in the vaterite than in the aragonite. Otoliths with vaterite inclusions were found in 48.1% of the 108 eels examined. If the mosaic vaterite in otoliths is not identified and avoided, the migratory environmental history of fish could be misidentified when otolith elemental signatures are used as biological tracers.

KEY WORDS: European eel · Otolith microchemistry · Carbonate polymorphs · Raman effect · EPMA · Laser ablation ICP-MS

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INTRODUCTION

Fish otoliths (ear stones) are biomineralized crystals, mainly composed of CaCO_3 , with a minor organic matrix (Degens et al. 1969). Their functions are connected with fish hearing and balance. Otolith increments are rhythmically deposited on daily and annual schedules, which allow the determination of daily and annual fish ages (Pannella 1971). At least 31 elements are deposited in the otoliths in proportion to their concentrations in the ambient water experienced by the fishes (Campana 1999). Otoliths are metabolically inert. Depositions of these elements in the growth increments of the otolith represent a permanent record

of the environmental conditions experienced by the fish at a particular time. Thus, temporal changes in the elemental composition in the otoliths can be used as a biological tracer to study the past migratory environmental history of the fishes (e.g. Tzeng & Tsai 1994).

The deposition of elements in the otolith is a complex biogeochemical process influenced by the ambient water (Campana 1999), the physiology and ontogeny of the fish (Kalish 1989, Tzeng 1996), and by the otolith crystal structure (Brown & Severin 1999, Melancon et al. 2005). In nature, CaCO_3 has 3 polymorphs: calcite, aragonite and vaterite, which differ in their crystal structures (Oliveira & Farina 1996, Klein & Hurlburt 1999). Aragonite is metastable in ambient conditions,

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but most otoliths are precipitated in this phase. Otoliths of salmonids, including Chinook salmon *Oncorhynchus tshawytscha* (Gauldie 1986), coho salmon *O. kisutch* (Gauldie et al. 1997), inconnu *Stenodus leucichthys* (Brown & Severin 1999) and lake trout *Salvelinus namaycush* (Melancon et al. 2005), can consist of both aragonite and vaterite crystalline phases, with different elemental compositions (Melancon et al. 2005). A mosaic crystal co-precipitated in the aragonite otolith of the European eel that contained extremely low strontium to calcium (Sr/Ca) ratios was found in a previous study (Shiao et al. 2006) and was suspected, but not confirmed, to be a vaterite crystal.

Sr/Ca ratios in otoliths have been widely used to study the habitat use and migratory environmental history of Japanese eel *Anguilla japonica* (e.g. Tzeng & Tsai 1994, Kotake et al. 2004), European eel *Anguilla anguilla* (e.g. Tzeng et al. 1997, 2000, Daverat et al. 2006), New Zealand temperate eels (Marui et al. 2001) and American eel *Anguilla rostrata* (e.g. Jessop et al. 2006). Based on the temporal change of otolith Sr/Ca concentration ratios, the habitat-use types of yellow-stage eels were classified as freshwater residents with lower otolith Sr/Ca ratios, seawater residents with higher otolith Sr/Ca ratios, and estuarine residents with otolith Sr/Ca ratios that varied between those of freshwater and seawater residents (Tzeng et al. 2002). These reflect the phenomena known as semi-catadromous (Tzeng et al. 2000) or facultatively catadromous migration (Tsukamoto & Arai 2001). If the vaterite in the aragonite otolith were not identified, it would be possible to misinterpret the habitat-use behavior or migratory model of the eel based on its otolith chemical signature. Unfortunately, no studies document the existence of vaterite in eel otoliths despite the many otolith microchemistry studies made to infer the migratory environmental history of eels.

The present study examines the existence of vaterite in the eel otolith by Raman spectroscopy. An optical microscope and SEM (scanning electron microscope) were used to examine the growth pattern of vaterite, and the elemental compositions of vaterite and aragonite in otoliths were analyzed using EPMA (electron probe microanalysis) and laser ablation ICP-MS (inductively coupled plasma mass spectrometry). The difference in elemental composition between vaterite and aragonite was explained according to the coordination number and effective ionic radius of the elements in the 2 CaCO_3 polymorphs. A possible mechanism for the biomineralization of vaterite is given.

MATERIALS AND METHODS

The otoliths of 108 silver- and yellow-stage European eels *Anguilla anguilla*, collected in 2003 from Baltic Sea coastal waters, the Curonian Lagoon and the freshwater Lake Baluošai in Lithuania by Shiao et al. (2006), were examined for the presence of vaterite. Sr/Ca ratios were measured from the primordium to the edge of the otolith at 10 μm intervals with EPMA (Jeol, JXA-8900R) (Shiao et al. 2006). Synthesized aragonite (CaCO_3) and strontianite ($\text{Sr}_{0.95}\text{Ca}_{0.05}\text{CO}_3$; NMNH R10065) were used as calibration standards. From the 52 otoliths identified as having vaterite inclusions based on Sr/Ca ratios and optical microscope observations, 9 were randomly selected to further confirm the existence of vaterite with Raman spectroscopy. Additionally, they were examined for differences in growth increment and elemental composition between aragonite and vaterite regions by SEM and laser ablation ICP-MS, respectively (Table 1). Then, 5 of the 9 otoliths were selected to illustrate the differences in optical/SEM images and elemental composition patterns between aragonite and vaterite.

The microstructure of eel otoliths etched with 5% ethylenediamine tetraacetic acid (EDTA) was examined with both optical microscopy and SEM (Jeol, JSM-6360LV). The CaCO_3 crystal structure of the otolith may have 3 different polymorphs: calcite is rhombohedral, vaterite is hexagonal and aragonite is orthorhombic (Klein & Hurlburt 1999). The mosaic vateritic crystals in the eel otolith were distinguished from the aragonite with a microRaman spectrometer (Jobin Yvon, Lab Ram HR) that collected the unpolarized Raman signals under backscattering (180°) geometry (Gabrielli et al. 2000). The spectra were excited by the 514.5 nm line of an argon laser and recorded

Table 1. *Anguilla anguilla*. Biological information on 9 selected European eels used for otolith microstructure examination by scanning electron microscope and microchemistry analysis by inductively coupled plasma mass spectrometry, and crystal structure analysis by Raman spectroscopy. Migratory patterns of freshwater residents (1), seawater and brackish-water residents (2) and interhabitat shifters (3) follow Shiao et al. (2006)

Specimen code	Sampling site (period)	Origin	Sex	Length (cm)	Weight (g)	Age (yr)	Migratory pattern
1	Curonian Lagoon (Jun to Aug 2003)	Wild	F	62.7	566	11	3
2		Wild	F	63.8	460	10	3
3		Wild	F	68.8	780	8	3
4		Wild	F	73.1	1135	12	3
5	Baltic Sea (Jun to Sep 2003)	Wild	F	67.0	1400	12	3
6		Wild	F	70.5	905	11	3
7		Wild	F	72.0	893	11	2
8		Restock	F	66.6	613	13	1
9		—	F	59.5	473	—	—

at room temperature with an Olympus SLM Plan 20× microscope objective with ~17 mW power focused on the sample (spot size ca. 4 µm in diameter). Wave numbers were accurate to $\pm 1 \text{ cm}^{-1}$ as determined from plasma emission lines.

The elemental compositions of vaterite and aragonite were analyzed and compared by a high-resolution ICP-MS (Finnigan, Mat Element 2) equipped with a laser microprobe (Merchantek, LUV 266 Nd:YAG UV). The laser was pulsed at 20 Hz through the primordium at a speed of $25 \mu\text{m s}^{-1}$ along 2 different transects on the sagittal plane of the otolith. One of the 2 transects passed through the vaterite crystal zone, the other transect through the aragonite (Fig. 2). Each data point took about 2.46 s and represented the chemical composition of a 37 µm section of the transect. Six elements, sodium (^{23}Na), magnesium (^{24}Mg), calcium (^{44}Ca), manganese (^{55}Mn), strontium (^{88}Sr) and barium (^{138}Ba), were detected. The temporal changes of the 'element to Ca ratios' along the 2 transects on each otolith were compared between aragonite and vaterite. To avoid the effect of ontogenetic shift on the comparison of element to Ca ratios (Tzeng 1996), the mean values of the 5 element to Ca ratios for the comparison between aragonite and vaterite were calculated from the elver check to the edge of otolith. The difference in mean values between vaterite and aragonite was tested by 1-way analysis of variance (ANOVA) and then by multiple comparison using the Tukey test (Sokal & Rohlf 1995).

RESULTS

Morphology, Sr/Ca ratios and prevalence of vaterite in otoliths

The mosaic vaterite crystal in the otolith appeared opaque, and could be easily discriminated from normal aragonite crystal under reflected light after polishing and etching with EDTA (Fig. 1), but was not easily recognized

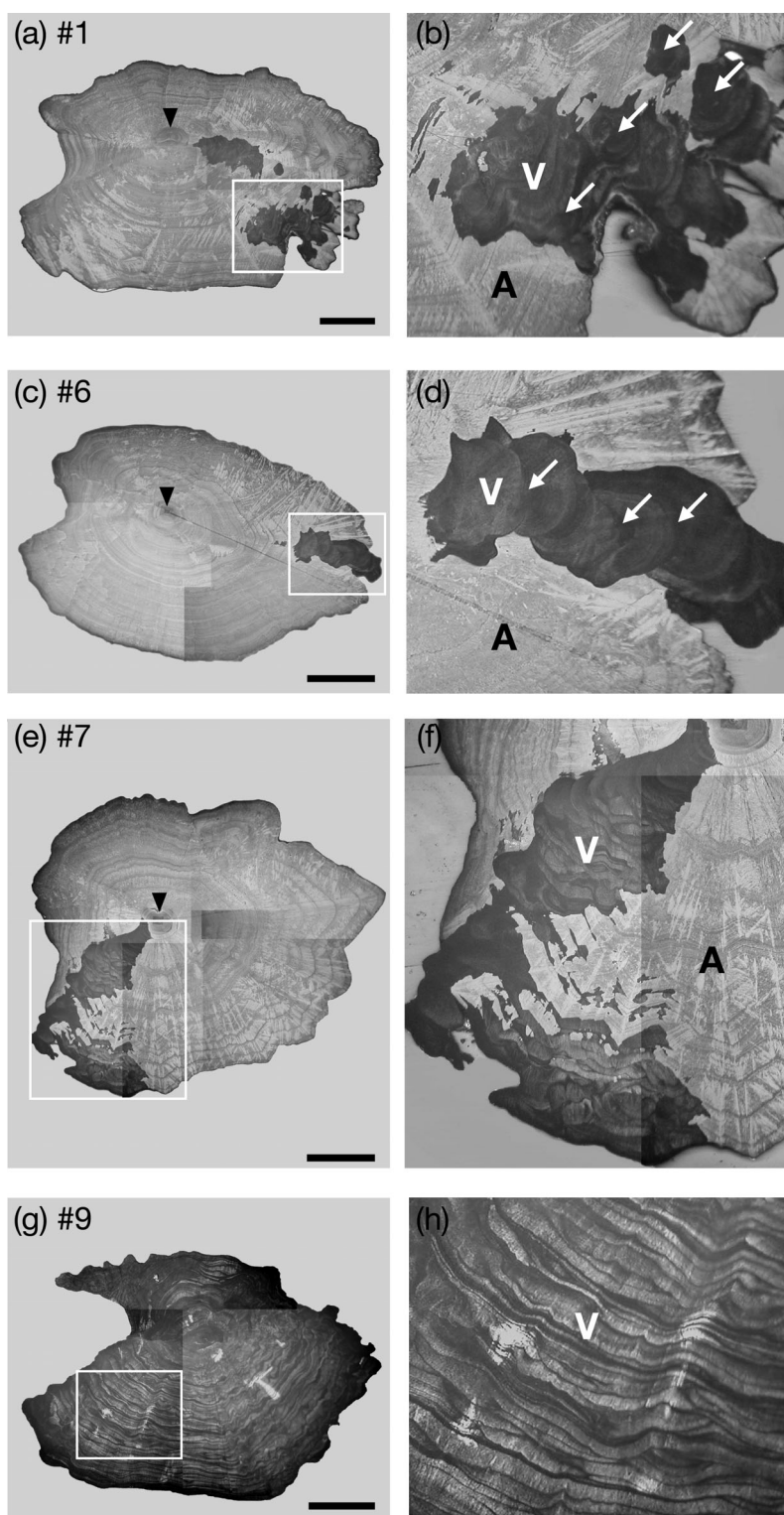


Fig. 1. *Anguilla anguilla*. Opaque mosaic vaterite structure (V) in the aragonite (A) otoliths of 4 European eels collected in Lithuanian waters in 2003. (Specimens 1, 6, 7 and 9 are described in Table 1). (a to h) All photographs were taken by reflected light microscope after the otoliths were etched by EDTA; Panels b, d, f and h are magnifications of Panels a, c, e and g. Arrowhead: primordium; arrows: new primordia. Scale bars = 500 µm

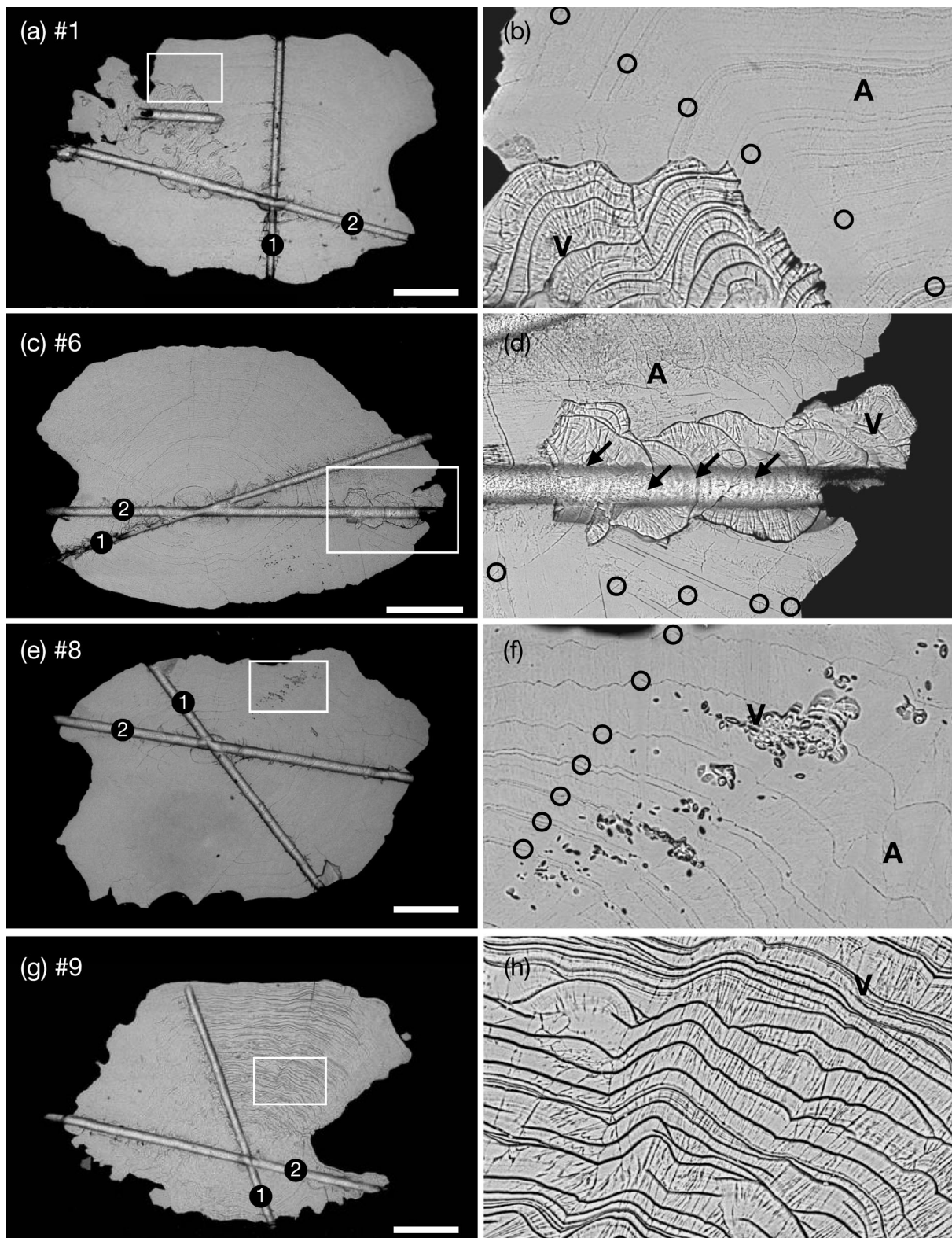


Fig. 2. *Anguilla anguilla*. (a to h) Scanning electron microscope photographs of the microstructure of vaterite (V) and aragonite (A) in the otoliths of 4 European eels (Specimens 1, 6, 8 and 9 are described in Table 1). Panels b, d, f and h are magnifications of Panels a, c, e and g. Open circles: annuli; arrows: new primordia. Transects 1 and 2 are trajectories through the primordium by laser ablation inductively coupled plasma mass spectrometry. Scale bars = 500 μm

without etching, irrespective of whether transmitted or reflected light was used. The mosaic vaterite structures in the otolith were irregular in shape with multiple primordia (Fig. 2a–d) and varied from small scattered points (Fig. 2e,f) to entirely vaterite (Fig. 2g,h). In comparison with aragonite, growth increments in the mosaic vaterite were wider and more irregular (Fig. 2b,d,f,h), indicating that the vaterite crystal grew faster than did aragonite. The fast-growing vaterite appears to contain many discontinuous growth increments, which complicates the discrimination of the annuli for age determination.

Fig. 3 shows an otolith with vaterite inclusions identified by EPMA. The Sr map of the otolith indicated that Sr content was lower in vaterite than in aragonite (Fig. 3a,b). The Sr/Ca ratios in the otolith were similar

in the core area from primordium to elver check for all 3 transects, increased from 12×10^{-3} in the primordium to a peak of 18×10^{-3} at a distance approximately 100 μm from the primordium during metamorphosis from leptocephalus to glass eel, and then dramatically decreased (Fig. 3a,c), a pattern similar to that found in the Japanese eel (Tzeng & Tsai 1994). Beyond the elver stage, the Sr/Ca ratios remained at a higher level, around 5×10^{-3} , along the 1st transect of aragonite (Fig. 3c, Transect 1), which indicated that at the yellow stage this eel lived in seawater (Shiao et al. 2006). However, when transects crossed the opaque area (vateritic crystals) the Sr/Ca ratios decreased to almost zero (Fig. 3a,c, Transects 2 and 3), and the eel could be misinterpreted to have been a freshwater resident.

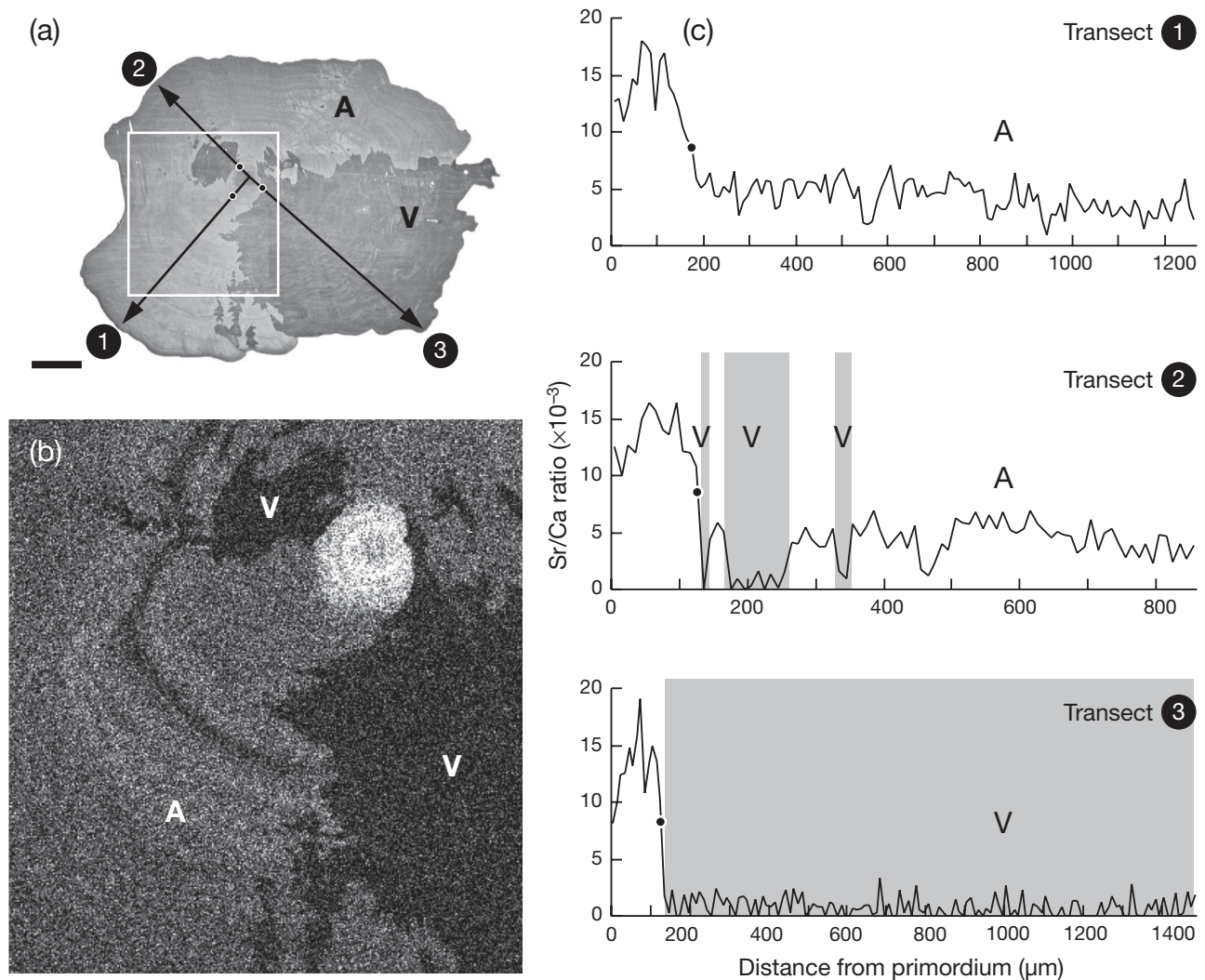


Fig. 3. *Anguilla anguilla*. (a) European eel otolith; the eel was collected from the Baltic Sea adjacent to Lithuania (length: 64.0 cm, weight: 634 g, age: 11 yr). Comparison of Sr content (b) and Sr/Ca ratios (c, Transects 1 to 3) between aragonite (A) and the mosaic vaterite (V) by electron probe microanalysis for the otolith shown in Panel a. The Sr content was highest in the core area of the otolith (bright), medium in the aragonite (grey) and lowest in the mosaic vaterite (dark grey) (Panel b). Black dots in Panels a and c: locations of the elver check. Scale bar = 500 μm

The percentage of otoliths with vaterite inclusions was very high in the European eel collected from Lithuanian waters, occurring in approximately 48.9% of naturally recruited eels collected from the Baltic Sea and Curonian Lagoon and in 45.0% of restocked eels collected from Lake Baluošai. Overall, vaterite inclusions occurred in 48.1% of 108 eels examined (Table 2).

Raman spectra of aragonite and vaterite

The Raman shifts of the lattice modes (i.e. the bands below 350 cm^{-1}), which represent the cation-related vibrations, differed significantly between vaterite and aragonite (Fig. 4a); the peak intensity of lattice modes appeared at 152 and 205 cm^{-1} for aragonite and at 119 and 302 cm^{-1} for the vaterite crystals. Similarly, the wave frequency of both CO_3^{2-} internal modes ν_1 and ν_4 also differed significantly between aragonite and vaterite crystals. The ν_1 mode of CO_3^{2-} caused a strong single Raman band in aragonite (1084 cm^{-1}), but resulted in 2 bands in the vaterite crystals (1075 and 1090 cm^{-1}) (Fig. 4b). In addition, the in-plane bending of CO_3^{2-} (the ν_4 mode), caused a doublet at 702 and 705 cm^{-1} in aragonite, and

caused the bands at 740 and 751 cm^{-1} in the vaterite crystals (Fig. 4c). Based on the assignments for the Raman bands of aragonite and vaterite, and the correlation between Raman bands and the crystal structure of vaterite (Gauldie et al. 1997, Gabrielli et al. 2000), the eel otolith undoubtedly contained vaterite crystals.

Table 2. *Anguilla anguilla*. Percentage occurrence of otolith with vaterite inclusions by location, origin and life-history pattern of European eels collected in Lithuania. Numbers in parantheses are sample sizes

	Baltic Sea	Curonian Lagoon	Baluošai Lake	Total
Origin				
Naturally recruited	60.4 (48)	35.0 (40)	–	48.9 (88)
Restocked	–	60.0 (10)	30.0 (10)	45.0 (20)
Life-history pattern				
Freshwater residency	–	60.0 (10)	30.0 (10)	45.0 (20)
Seawater and brackish-water residency	61.5 (26)	–	–	61.5 (26)
Interhabitat shifter	59.1 (22)	35.0 (40)	–	43.5 (62)
Total	60.4 (48)	40.0 (50)	30.0 (10)	48.1 (108)

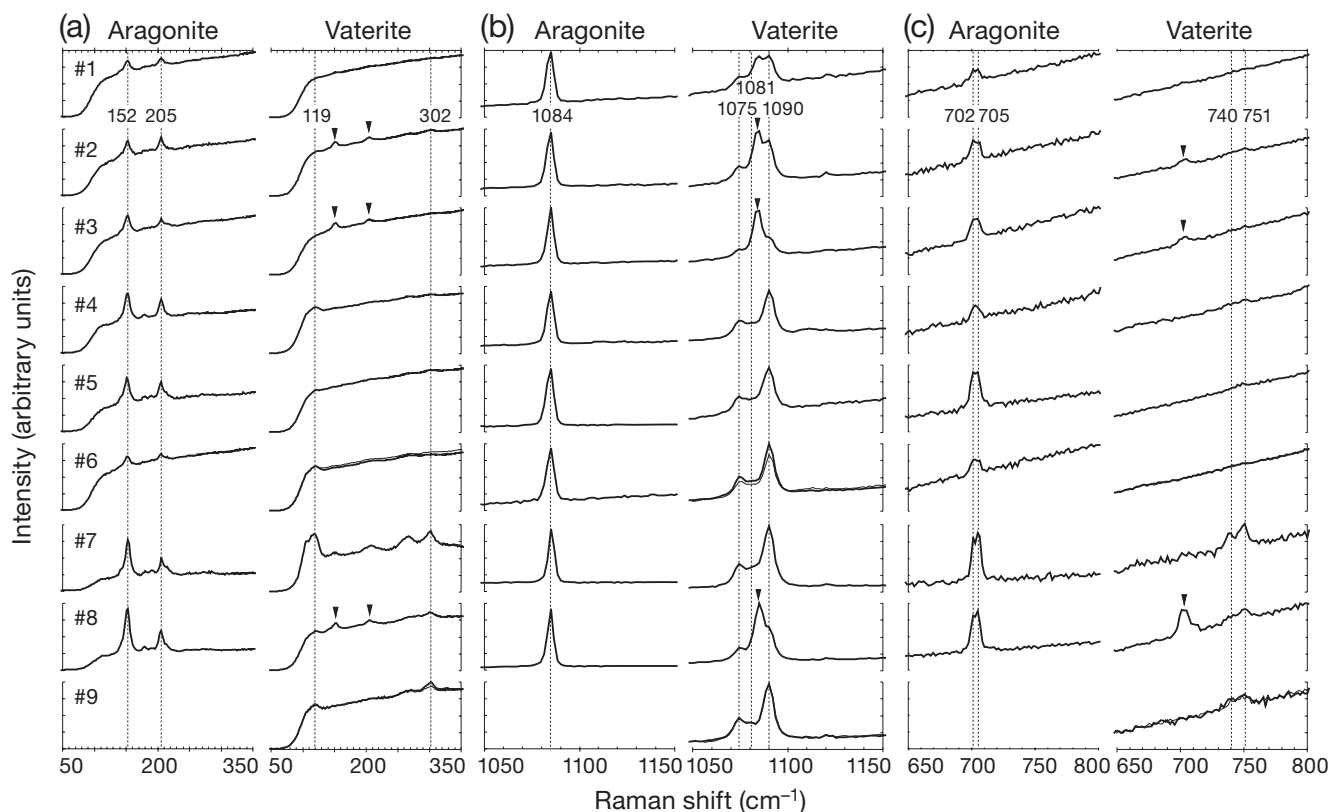


Fig. 4. *Anguilla anguilla*. Raman spectra of aragonite and vaterite collected from portions of the 9 European eel otoliths referred to in Table 1. (a) Lattice modes, (b) symmetric stretching (ν_1) of CO_3^{2-} and (c) in-plane bending (ν_4) of CO_3^{2-} . Arrowheads indicate that the sample of vaterite was mixed with aragonite

Comparison of the elemental composition between aragonite and vaterite

Profiles of the 5 element (Mg, Mn, Na, Sr and Ba) to Ca concentration ratios along the 2 transects (diameters) in otoliths of the 4 eels analyzed with laser ablation ICP-MS all showed that Mg/Ca and Mn/Ca ratios distinctly increased and Na/Ca, Sr/Ca and Ba/Ca ratios decreased in the vateritic crystals in comparison with those of aragonite (Fig. 5).

Mean Mg/Ca and Mn/Ca concentration ratios in the otoliths for the 4 eels (1, 6, 7 and 9) were approximately 16 to 50 and 3.8 to 4.3 times higher, but Na/Ca, Sr/Ca and Ba/Ca ratios were approximately 2.0 to 2.1, 7.1 to 7.4 and 6.9 to 7.0 times lower in vaterite than in aragonite (all differences were significant at the $p < 0.01$ level; Table 3). In addition, none of the 5 mean element/Ca ratios differed significantly between the 2 transects in the aragonite in each of the otoliths 1, 6 and 7 ($p > 0.05$). On the other hand, in the vaterite of #9, while the Sr/Ca and Ba/Ca ratios between the 2 transects did not differ significantly ($p > 0.05$), the Na/Ca, Mg/Ca and Mn/Ca ratios between the 2 transects were significantly different ($p < 0.01$) (Table 3). This indicated that the impurities in the vaterite crystal of #9, such as the cations Na, Mg and Mn, were not homogeneously distributed (Fig. 5d).

DISCUSSION

Ecological implications of otolith microchemistry

This is the first confirmation that vaterite can exist in the aragonite otoliths of the European eel *Anguilla anguilla*. The vaterite crystal has a strikingly different elemental composition compared with the portion of the otolith made of the usual aragonite polymorph crystal (Fig. 5, Table 3), which is consistent with observations in other species for the same type of study (Gauldie 1986, Brown & Severin 1999, Tomás et al. 2004, Melancon et al. 2005). The Sr/Ca concentration ratios in anguillid eel otoliths have been widely used to infer their migratory life histories between seawater and freshwater. However, no studies mention the effect of crystal structure on the Sr/Ca or other element concentration ratios in eel otoliths (e.g. Tzeng et al. 1997, 2000, 2002, Marui et al. 2001, Kotake et al. 2004, Daverat et al. 2006, Jessop et al. 2006). Whether European eels were collected from the Baltic Sea or Curonian Lagoon, mean Sr/Ca ratios of mosaic vaterite crystals in otoliths (0.43×10^{-3} to 0.83×10^{-3}) were much lower than those of aragonite (3.02×10^{-3} to 5.11×10^{-3}) (Table 3), and also lower than those used to discriminate between freshwater and sea residents of anguillid

eels during the growth phase of the yellow stage (4.0×10^{-3}) (e.g. Tzeng et al. 2002). This indicated that the effect on crystal structure resulted from more than ambient water salinity on the Sr/Ca ratios in the eel otolith (Tzeng 1996). If the vaterite is not discriminated from the aragonite, a serious misinterpretation of eel life history may result. For example, the eel in Fig. 3 that was collected from the Baltic Sea, a brackish water body with salinities from 4.9 to 6.8 psu (Shiao et al. 2006), may be misidentified as having lived in freshwater if Sr/Ca ratios were measured passing through vaterite in the otolith. Specimen 9 had an entirely vaterite otolith, for which the age, the origin of the eel (naturally recruited or restocked), and the migratory life-history pattern (freshwater, seawater, or inter-habitat shifter) were not determinable (Table 1) because the growth increment and Sr/Ca concentration ratios in the vaterite otolith differed from those in the normal aragonite otolith (Figs. 1, 2 & 5, Table 3).

The percentage of otoliths with vaterite inclusions was very high, occurring in approximately 48% of 108 eels examined (Table 2). Recently, populations of American, European and Japanese eels have declined to levels of concern, possibly as a result of habitat loss (Dekker 2003). The identification of vaterite in their otoliths is, therefore, very important because otolith chemistry is increasingly used to investigate the habitat use and migratory history of eels. The prevalence of otoliths with vaterite inclusions differs greatly among species. For example, diadromous fishes such as inconnu *Stenodus leucichthys* had a prevalence of 10% ($n = 266$) (Brown & Severin 1999), and lake trout *Salvelinus namaycush* had a prevalence of 59 to 86% for stocked fish and 4 to 49% for wild fish (Bowen et al. 1999). The prevalence of otoliths with vaterite inclusions might reflect stress conditions, especially among reared fishes (e.g. Bowen et al. 1999). The prevalence of vaterite in otoliths of the wild European eels in this study was considered to be high and did not differ among habitats (Table 2). Variations in temperature or salinity may be two of the factors controlling otolith crystalline growth in marine fishes. Water temperature and salinity varied greatly with season where the European eels were collected. A recent study on the synthesis of the 3 polymorphs of CaCO_3 (calcite, aragonite and vaterite) using dicarboxylates as templates at different temperatures indicated that temperature plays an important role in the synthesis of the CaCO_3 polymorphs (Mukkamala et al. 2006). The effect of this kind of stress on the prevalence of vaterite in European eel otoliths should be validated by the reconstruction of the environmental history of the eel in association with the identification of vaterite formation based on otolith microchemistry.

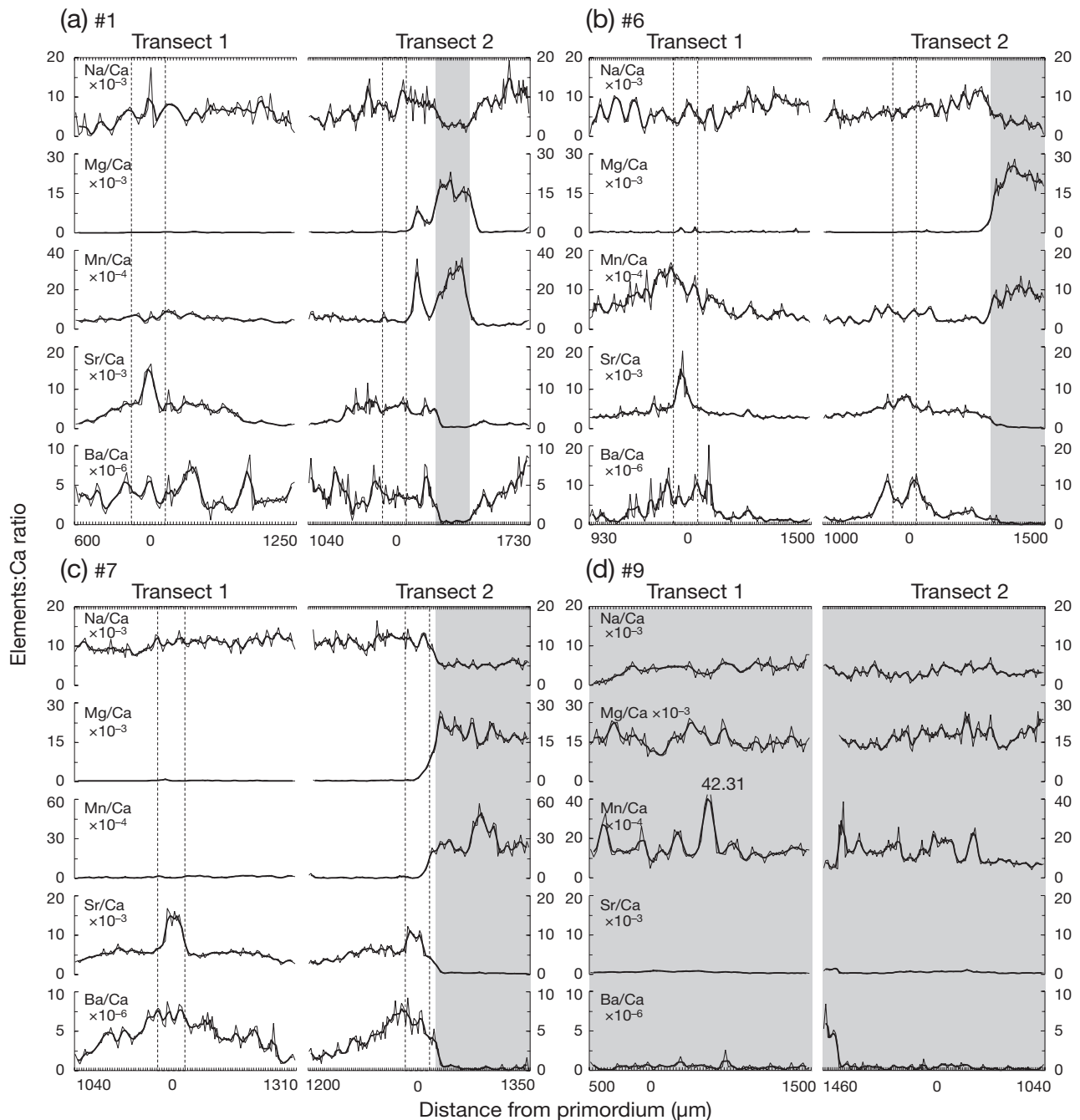


Fig. 5. *Anguilla anguilla*. Comparison of the 5 element to Ca concentration ratios scanned as in Fig. 2 by laser ablation ICP-MS across aragonite (Transect 1) and vaterite (shaded area in Transect 2) in otoliths of the 4 European eels (Specimens 1, 6, 7 and 9 are described in Table 1). The core area from the primordium (Position 0) to the elver checks approximately 150 μm on either side of the primordium was excluded in the comparison to avoid the effect of ontogenetic noise. Thicker lines are smoothed by 3 points' moving averages and the thinner ones are not smoothed

Role of intrinsic factors in vaterite growth

Although mosaic vaterite was frequently found in aragonite otoliths, the bio-mineralization process of vaterite is unclear. Otoliths are composed of CaCO_3

with a minor organic matrix and are surrounded by endolymphatic fluid that contains ions and soluble proteins thought to mediate the precipitation of the otolith (Falini et al. 2005). The presence of ionic (K^+ and Na^+) and proteomic gradients along the proximal–

Table 3. *Anguilla anguilla*. Comparison of mean \pm SD (range, number of values calculated) element to Ca ratios for aragonite and vaterite segments in Transects 1 and 2 of the otoliths of 4 European eels, Specimens 1, 6, 7 and 9 in Fig. 5. 9_1 & 9_2 are the Transects 1 & 2 of the whole vaterite specimen #9

Element/Ca	Specimen code	Transect 1 Aragonite A ₁	Transect 2 Aragonite A ₂	Transect 2 Vaterite V	Tukey HSD test
Na/Ca ($\times 10^{-3}$)	1	5.28 \pm 2.06 (0.47–9.10, 59)	7.72 \pm 2.77 (2.35–14.87, 80)	3.26 \pm 1.06 (2.08–6.47, 18)	A ₁ = A ₂ > V
	6	6.74 \pm 2.26 (1.74–11.65, 95)	6.77 \pm 2.12 (4.01–11.61, 68)	3.70 \pm 1.06 (1.94–5.54, 25)	A ₁ = A ₂ > V
	7	10.42 \pm 1.34 (7.57–13.34, 79)	10.97 \pm 1.33 (6.82–13.17, 47)	5.32 \pm 0.52 (4.41–6.51, 44)	A ₁ = A ₂ > V
	9_1	–	–	4.04 \pm 1.36 (0.50–6.53, 72)	V ₁ > V ₂
	9_2	–	–	3.40 \pm 1.00 (1.05–5.58, 111)	
	Subtotal	7.62 \pm 2.85 (0.47–13.34, 233)	8.17 \pm 2.79 (2.35–14.87, 195)	3.90 \pm 1.26 (0.50–6.53, 270)	A ₁ = A ₂ > V
Mg/Ca ($\times 10^{-3}$)	1	0.15 \pm 0.09 (0.05–0.42, 59)	1.47 \pm 2.50 (0.03–11.17, 80)	16.03 \pm 2.23 (12.59–20.21, 18)	A ₁ = A ₂ < V
	6	0.38 \pm 0.13 (0.14–0.88, 95)	0.47 \pm 0.81 (0.10–5.43, 68)	20.46 \pm 3.77 (8.99–25.45, 25)	A ₁ = A ₂ < V
	7	0.42 \pm 0.08 (0.29–0.63, 79)	1.29 \pm 3.12 (0.34–15.94, 47)	18.50 \pm 2.67 (13.94–24.85, 44)	A ₁ = A ₂ < V
	9_1	–	–	15.89 \pm 2.83 (10.14–22.61, 72)	V ₁ < V ₂
	9_2	–	–	17.09 \pm 2.81 (12.21–24.91, 103)	
	Subtotal	0.34 \pm 0.15 (0.05–0.88, 233)	1.08 \pm 2.30 (0.03–15.94, 195)	17.25 \pm 3.15 (8.99–25.45, 262)	A ₁ = A ₂ < V
Mn/Ca ($\times 10^{-4}$)	1	5.25 \pm 1.31 (3.22–9.06, 59)	5.78 \pm 5.00 (1.70–28.75, 80)	23.14 \pm 7.09 (9.16–32.27, 18)	A ₁ = A ₂ < V
	6	6.36 \pm 3.42 (2.16–15.93, 95)	2.82 \pm 1.07 (1.51–5.64, 68)	8.93 \pm 1.40 (5.94–11.38, 25)	A ₁ = A ₂ < V
	7	1.00 \pm 0.53 (0.19–2.06, 79)	1.78 \pm 4.85 (0.07–20.73, 49)	28.48 \pm 8.34 (19.00–49.83, 44)	A ₁ = A ₂ < V
	9_1	–	–	15.71 \pm 6.12 (7.91–40.15, 72)	V ₁ > V ₂
	9_2	–	–	12.45 \pm 5.01 (5.35–29.18, 111)	
	Subtotal	4.26 \pm 3.31 (0.19–15.93, 233)	3.76 \pm 4.38 (0.07–28.75, 197)	16.32 \pm 8.56 (5.35–49.83, 270)	A ₁ = A ₂ < V
Sr/Ca ($\times 10^{-3}$)	1	3.60 \pm 1.98 (0.75–6.91, 59)	3.02 \pm 2.03 (0.84–7.54, 80)	0.83 \pm 1.00 (0.34–4.10, 18)	A ₁ = A ₂ > V
	6	3.73 \pm 0.92 (2.65–7.29, 95)	4.44 \pm 1.28 (2.49–8.11, 68)	0.57 \pm 0.42 (0.26–2.02, 25)	A ₁ = A ₂ > V
	7	5.11 \pm 0.89 (2.89–6.87, 79)	4.93 \pm 1.35 (2.50–7.26, 49)	0.43 \pm 0.23 (0.24–1.63, 44)	A ₁ = A ₂ > V
	9_1	–	–	0.57 \pm 0.19 (0.29–0.96, 72)	V ₁ = V ₂
	9_2	–	–	0.55 \pm 0.28 (0.25–1.46, 111)	
	Subtotal	4.16 \pm 1.43 (0.75–7.29, 233)	3.99 \pm 1.83 (0.84–8.11, 197)	0.56 \pm 0.37 (0.24–4.10, 270)	A ₁ = A ₂ > V
Ba/Ca ($\times 10^{-6}$)	1	3.76 \pm 1.37 (1.83–7.32, 59)	3.98 \pm 1.47 (1.13–8.15, 80)	0.55 \pm 0.47 (0.20–2.08, 18)	A ₁ = A ₂ > V
	6	3.02 \pm 2.62 (0.63–11.67, 95)	3.25 \pm 2.48 (0.88–11.08, 68)	0.50 \pm 0.39 (0.06–1.38, 25)	A ₁ = A ₂ > V
	7	4.08 \pm 1.51 (0.94–6.83, 79)	3.83 \pm 1.83 (1.37–7.87, 49)	0.41 \pm 0.35 (0.07–2.12, 44)	A ₁ = A ₂ > V
	9_1	–	–	0.46 \pm 0.25 (0.04–1.21, 72)	V ₁ = V ₂
	9_2	–	–	0.64 \pm 1.08 (0.00–5.96, 111)	
	Subtotal	3.57 \pm 2.06 (0.63–11.67, 233)	3.69 \pm 1.97 (0.88–11.08, 197)	0.53 \pm 0.74 (0.00–5.96, 270)	A ₁ = A ₂ > V

distal axis within the endolymph of the inner ear of fish suggests that non-uniformity in the elemental composition of the endolymph may influence the biomineralization process of the otoliths (Borelli et al. 2001). However, we found that most of the vaterite precipitated in eel otoliths was irregular in shape and lacking orientation in precipitation (Figs. 1 to 3). Thus, the formation of vaterite in eel otoliths may not be due to normally irregular ionic and proteomic gradients in the endolymph. On the other hand, we found that the crystallization of vaterite was nucleated at different growth points (multiple primordia) (Figs. 1 & 2). The active uptake of cations is influenced by enzyme activity during the embryological development of the respiratory system (Brophy et al. 2004). Elevated Mg and Mn levels in vaterite otoliths of European eels occurred in newly formed primordia, suggesting a correlation with enzyme activity during fast development. Vaterite appeared to grow faster than aragonite

(Fig. 2), which may imply that vaterite was more quickly regenerated to repair the otolith when aragonite was damaged. The factors affecting otolith damage are not clear; one possible reason may be otolith absorption induced by anaerobic stress (Mugiya & Uchimura 1989). Vaterite occurs where mineralized tissue has been repaired in certain gastropods (Kamhi 1963). If this occurs in the eel otoliths, the preexisting aragonite will be mosaic, with vaterite resulting from the repair of the otolith. On the other hand, the water-soluble proteins of the organic matrix in otoliths are characterized by a high content of acidic amino acids that can control CaCO₃ morphology and polymorphism (Falini et al. 2005). Thus, changes in any of the factors intrinsic to the fish, such as anaerobic stress, enzyme activity and the protein composition of the organic matrix in the otolith all have the potential to influence the formation of vaterite in the otolith. These possibilities all remain to be validated.

Why elemental composition differs between vaterite and aragonite

One explanation for the observed differences in elemental composition between vaterite and aragonite (Fig. 5, Table 3) is that Mg^{2+} and Mn^{2+} preferentially enter the Ca^{2+} sites in vaterite, while Na^+ , Sr^{2+} and Ba^{2+} preferentially enter the Ca^{2+} sites in aragonite. The coordination number (CN) of a cation differs between aragonite and vaterite, which have different crystal structures. Calcium in aragonite is 9-coordinated, but it is 6-coordinated in vaterite (Kamhi 1963). The ionic radius of a cation in a crystal depends on its CN (Shannon 1976). The effective radii of Mg^{2+} , Mn^{2+} , Ca^{2+} , Na^+ , Sr^{2+} and Ba^{2+} at CN = 6 (and 9) are 0.072, 0.083, 0.100 (0.118), 0.102 (0.124), 0.118 (0.131) and 0.135 (0.147) nm, respectively. The oxygen anion has been generally considered to have a radius of 0.140 nm, regardless of its CN (Shannon 1976). The 6-coordination of a cation is possible only when the ratio of the cationic and anionic radii is in the range 0.414 to 0.732 (Klein & Hurlburt 1999). For the case of vaterite, only Mg^{2+} and Mn^{2+} meet this rule. Thus, Mg^{2+} and Mn^{2+} can enter the lattice of vaterite during crystallization. The ratio of the cationic and anionic radii must be >0.8 for the formation of a 9-coordination cation, limiting ion entrance to the aragonite lattice to Na^+ , Sr^{2+} and Ba^{2+} . It is thought that Na^+ preferentially enters the lattice of aragonite because the ionic radii ratio of Na^+ to O^{2-} (0.729 at CN = 6) is close to the stable upper limit of vaterite (0.732). This explanation is consistent with our data (Fig. 5, Table 3) and with previous studies (Brecevic et al. 1996, Lenaz et al. 2006). In addition, Ca^{2+} also preferentially enters the aragonite lattice rather than vaterite because the ionic radii ratio of Ca^{2+} to O^{2-} (0.714 at CN = 6) is also close to the stable upper limit of vaterite. For this reason the Ca in the vaterite crystal is less stable than that in the aragonite, and thus was easily chelated when the otolith was etched with EDTA and more organic matrix was left for vaterite than for aragonite crystals (Fig. 2). The organic matrix reflects light poorly. Consequently, the mosaic vaterite structure in the otolith appeared opaque when viewed under reflected light with the optical microscope (Fig. 1). Alternatively, some of the elements such as Cu and Zn, but not Mg and Mn may combine with protein of the organic matrix rather than replacing the inorganic $Ca^{2+}CO_3^{2-}$ in the otolith (Miller et al. 2006). Thus, the increase of Mg/Ca and Mn/Ca ratios in the vaterite compared with aragonite may be primarily attributed to the coordination chemistry effect of the crystals of different calcium carbonate polymorphs.

In conclusion, the Na/Ca, Sr/Ca and Ba/Ca concentration ratios were higher and Mg/Ca and Mn/Ca were lower in aragonite than in vaterite. Otolith repair is a

possible cause for the mosaic vaterite formation. The difference in elemental composition between vaterite and aragonite can be attributed to the different coordination numbers of the cation in substitution for Ca^{2+} in the $CaCO_3$ polymorphs. The prevalence of otoliths with mosaic vaterite was high, approximately 48% of the eels examined. If mosaic vaterite is not distinguished from aragonite, the fish migratory history may be misidentified when otolith elemental signatures are used as biological tracers.

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