

# Effects of crystal structure on the uptake of metals by lake trout (*Salvelinus namaycush*) otoliths

Sonia Melancon, Brian J. Fryer, Stuart A. Ludsin, Joel E. Gagnon, and Zhaoping Yang

**Abstract:** This is the first study to report spectroscopic and elemental analysis of aragonite and vaterite growing simultaneously and separately in both the core and the edges of the same otolith. Our investigations focused on understanding differential trace metal uptake, including the influence of the metal itself (i.e., ionic radii), the crystalline structure, and the development state of the fish. Chemistry and crystal structure of sagittal otoliths from lake trout (*Salvelinus namaycush*) were studied using laser ablation combined with inductively coupled plasma mass spectrometry (LA-ICP-MS) and Raman spectroscopy, respectively. Analyses of the composition of vaterite and aragonite growing in the same growth ring show that smaller cations like Mg (0.86 Å) (1 Å = 0.1 nm) and Mn (0.81 Å) were more abundant in the vaterite hexagonal crystal structure, whereas larger cations such as Sr (1.32 Å) and Ba (1.49 Å) were preferentially incorporated in aragonite (orthorhombic). Similarly, the coprecipitation of aragonite and vaterite in cores and edges allowed us to demonstrate that the uptake rates (as determined by element-specific partition coefficients) for Sr and Ba were greater in aragonite than vaterite, whereas those of Mg and Mn were higher in vaterite than in aragonite.

**Résumé :** C'est la première fois qu'on reporte la cristallisation de l'aragonite et de la vaterite séparément dans le cœur et les anneaux du même otolithe. Nos recherches se concentrent sur la compréhension de l'inclusion des métaux dans l'otolithe. L'incorporation peut varier selon la nature du métal (rayon ionique), la structure cristalline et le stade de développement du poisson. Nous avons étudié la chimie et la composition des cristaux de CaCO<sub>3</sub> dans les otolithes sagittales de truites (*Salvelinus namaycush*) par ablation laser couplée avec un spectromètre de masse atomique à plasma induit (LA-ICP-MS) et par spectroscopie Raman, respectivement. Les analyses sur la composition chimique des deux polymorphes de CaCO<sub>3</sub> dans le même anneau d'otolithe, correspondant au même environnement, montrent que les cations de petites tailles tel que Mg (0,86 Å) (1 Å = 0,1 nm) et Mn (0,81 Å) sont plus abondants dans la vaterite, tandis que les cations volumineux comme Sr (1,32 Å) et Ba (1,49 Å) sont préférentiellement incorporés dans l'aragonite. Similairement, la co-précipitation de l'aragonite et de la vaterite dans le cœur et les extrémités de l'otolithe nous a permis de démontrer que les taux d'incorporation (déterminés par les coefficients de partition) de Sr et Ba sont plus élevés dans l'aragonite que dans la vaterite, tandis que ceux de Mg et Mn sont plus élevés dans la vaterite comparativement à l'aragonite.

## Introduction

Otoliths are the ear stones of fish and are mainly composed of CaCO<sub>3</sub>. The continual growth of otoliths is recognizable as concentric rings of alternating opaque and translucent zones. Otoliths are not considered to be subject to resorption, and as such, only ontogenetic and environmental factors should cause changes to their chemical composition (Campana 1999; Halden et al. 2000). Previous work has demonstrated that otoliths of salmonines, including lake trout (*Salvelinus namaycush*), can comprise two different

CaCO<sub>3</sub> crystalline structures, aragonite and vaterite (Campana 1983; Gauldie 1986; Casselman and Gunn 1992), which can differ dramatically in their trace elemental composition (Brown and Severin 1999).

This study focuses on vaterite and aragonite formation in sagittal otoliths of lake trout. Aragonite is the most common crystalline structure in otoliths of teleosts including lake trout, but previous work has shown that both stocked and wild lake trout can have vateritic otoliths (Bowen et al. 1999). The prevalence of vaterite in lake trout otoliths, however, is much greater for stocked fish than for wild fish (59%–86%

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versus 4%–49%, respectively), possibly owing to a physiological response associated with intense stocking stress (Casselman 1986; Bowen et al. 1999; Ludsin et al. 2004).

Brown and Severin (1999) have suggested that one or more genes are responsible for switching the production of proteins that facilitate vaterite or aragonite formation in the growing otolith. More recently, Söllner et al. (2003) reported that the starmaker gene might be responsible for the change in the crystal lattice structure of otoliths in zebrafish (*Brachydanio rerio*). They conducted an experiment in which the activity of starmaker was reduced by the injection of 2–40 ng of modified antisense oligonucleotides, resulting in concentric daily rings that were star-shaped instead of circular. Quite possibly, another environmentally influenced gene is responsible for vateritic otolith transformation in lake trout.

Gauldie (1986, 1996) showed that aragonite replaced vaterite in chinook salmon (*Oncorhynchus tshawytscha*) sagittal otoliths and found that the degree of transformation varied from partial to total replacement. Further, Gauldie (1986, 1996) frequently observed individuals with one otolith that was composed completely of vaterite and the other of aragonite, with relatively few individuals having vaterite in both sagittal otoliths. David and Grimes (1994) observed a similar phenomenon in hatchery-reared juvenile red drum (*Sciaenops ocellatus*). By contrast, Brown and Severin (1999) found regions on the otoliths of the inconnu (*Stenodus leucichthys*) that were comprising a mixture of aragonite and vaterite. However, Campana (1983) was the only author to document the coprecipitation of aragonite and vaterite in different zones of the same otolith, but elemental compositions in the two polymorphic structures were not measured.

Herein, we present chemical and spectroscopic analyses of vaterite and aragonite growth in sagittal otoliths of lake trout in an effort to better understand the crystalline growth process. Specifically, we were interested in learning whether (i) both  $\text{CaCO}_3$  polymorphs grow simultaneously in the same otolith (versus sequentially), (ii) vaterite and aragonite growth occurs in the same region of the otolith, and (iii) growth rate differences exist for these polymorphs, which might influence otolith shape. To do so, we used Raman spectroscopy to identify the distribution of vaterite and aragonite crystal structures in sagittal otoliths. In addition, we used laser ablation – inductively coupled plasma mass spectrometry (LA-ICP-MS) to quantify chemical differences between vateritic and aragonitic portions of otoliths, and to determine partition coefficients ( $K_D$ ) for metals between them, at different growth stages. Ultimately, we discuss potential causal mechanisms to understand differential trace metal uptake between these  $\text{CaCO}_3$  polymorphs, including the influence of the metal itself (e.g., ionic radii), the crystalline structure, and the development state of the fish.

## Materials and methods

### Fish and otolith preparation

Analyses were conducted on 13 age-11 lake trout that spent their entire existence in the Allegheny National Fish Hatchery in Warren, Pennsylvania. All fish were from the

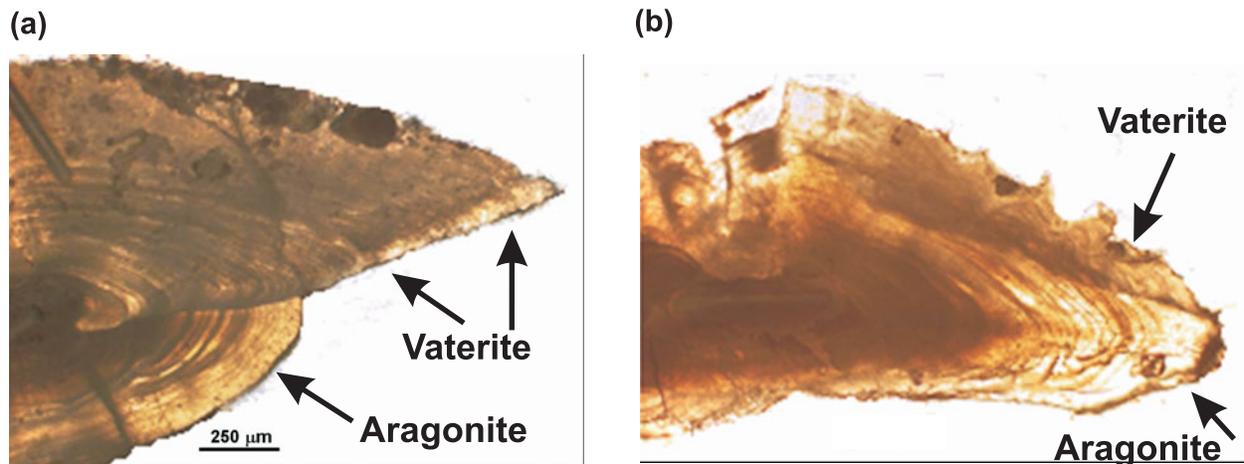
same year class (1992) and were sacrificed and collected at the same time. An additional two hatchery-reared lake trout, collected from the eastern basin of Lake Erie via annual assessment surveys conducted by both state and provincial agencies during 1984–2003 (see Ludsin et al. 2004 and Markham 2004 for sampling details), were also analyzed for this study. Sagittal otoliths from this suite of individuals were provided dry, stored in envelopes.

Sagittal otoliths were embedded in epoxy resin (West Coast Marine<sup>®</sup>) and transverse sections (~350  $\mu\text{m}$  wide) were cut using a Buehler ISOMET<sup>™</sup> saw such that each section contained the full growth chronology (including the core). After mounting sections to a piece of an overhead transparency sheet with Krazy Glue<sup>®</sup>, the upper surface was polished using a combination of 20-, 12-, 1-, and 0.3- $\mu\text{m}$  aluminum oxide 3M<sup>®</sup> lapping film to improve optical quality and to ensure that the otolith core was at the exposed surface of the section. Sections were then randomly mounted (with a small piece of the transparency sheet) onto acid-washed glass slides with Krazy Glue<sup>®</sup> ( $n = 12\text{--}14$  otoliths per slide) and left to dry for 24 h. Slides were then sonicated for 10 min in ultrapure Milli-Q water, rinsed three times in Milli-Q water, dried for 24 h in a HEPA-filtered laminar flow hood in a Class 100 clean room, and then stored in a covered Petrie dish until analysis. These last steps (sonication, rinsing, and drying) were repeated between Raman and LA-ICP-MS use. All postpolishing processing (including storage) occurred in a Class 100 clean room, and only non-metallic instruments were used to handle otoliths.

### Raman spectroscopy

$\text{CaCO}_3$  is found in otoliths in three polymorphic crystalline structures: calcite, vaterite, and aragonite (Truchet et al. 1995; Oliveira and Farina 1996; Tomás and Geffen 2003). The crystal structures are, respectively, rhombohedral, hexagonal, and orthorhombic. Crystal structures of  $\text{CaCO}_3$  in the otoliths can be determined using X-ray diffraction and Raman spectroscopy (Gauldie 1986; Tomás and Geffen 2003). We used Raman spectroscopy, which is the measurement of the wavelength and intensity of scattered light from molecules (Hollas 1998). The Raman scattered light occurs at wavelengths that are shifted from the incident light by the energies of molecular vibrations and rotations that are unique for all chemical complexes. It is a nondestructive technique that quantitatively determines where vaterite and aragonite are located in the otolith (Gauldie et al. 1997). The Raman spectra of each mineral structure are the same, independent of where they are taken, core or edge. The best way to differentiate aragonite from vaterite is the  $1000\text{--}1200\text{ cm}^{-1}$  portion of the spectrum. Raman analyses were conducted on the edges (last 1–2 years of life) of all hatchery-reared lake trout ( $n = 13$ ), and the cores (first 18 months) of three lake trout (one pure hatchery lake trout and the two Lake Erie recaptures) using a Renishaw<sup>®</sup> inVia Reflex Raman spectrometer. The exciting source was a He:Ne laser operating at 633 nm with a power of about 27 mW with a focus of 3.5 mW on the sample. The system was equipped with a charged-coupled device detector. Calibration was performed using a static spectral acquisition on a silicon plate positioned at  $520\text{ cm}^{-1}$ . Because otolith cores are dark (i.e., difficult to see under our microscope), whereas the edges are

**Fig. 1.** Sagittal otoliths from age-11 lake trout (*Salvelinus namaycush*) that spent their entire existence in captivity. (a) Otolith with vaterite growth being greater than aragonite growth; (b) otolith with aragonite growth being greater than vaterite growth.



clear (almost transparent for some), Raman spectra taken on otolith edges were done at higher resolution (50 $\times$ ) than for the cores (20 $\times$ ). Mapping of the transitional zone between aragonite and vaterite growth zones was done at a magnification of 50 $\times$ .

#### LA-ICP-MS

Elemental concentrations were quantified using a purpose-built system composed of a Continuum<sup>®</sup> Surelite<sup>®</sup> I solid-state Nd:YAG laser (wavelength 266 nm, maximum power 40 mJ, pulse rate 20 Hz, pulse width 4–6 ns, laser spot diameter 15  $\mu$ m) coupled to a Thermo-Elemental<sup>®</sup> X7<sup>®</sup> ICP-MS (peak-jumping mode, 10-ms dwell time per isotope). A glass reference standard (National Institute of Standards and Technology NIST 612) with known concentrations of elements was analyzed before and after every 16 samples ( $n = 2$  replicates before and after), which allowed for quantification and correction of instrumental drift. This same standard also was used to determine precision in estimating elemental concentrations. The Ar carrier gas and instrument noise (i.e., background) were analyzed for 60 s before every sample.

Concentrations of the elements were calculated by measuring the following isotopes: <sup>7</sup>Li, <sup>25</sup>Mg, <sup>43</sup>Ca, <sup>44</sup>Ca, <sup>55</sup>Mn, <sup>66</sup>Zn, <sup>85</sup>Rb, <sup>86</sup>Sr, <sup>88</sup>Sr, <sup>137</sup>Ba, and <sup>138</sup>Ba. Each of these elements met a rigorous set of criteria before use in this study. Specifically, for an element to be included in our analyses, it had to be precisely measured, i.e., the average coefficient of variability (i.e., standard deviation/mean  $\times$  100%) for at least one measured isotope, as determined from our National Institute of Standards and Technology samples, had to be less than 10.5% (Gillanders and Kingsford 1996). In addition, its concentration had to be above the limit of detection for 90% of the samples. Details concerning measures of precision, limits of detection, and percentages of samples of above limits of detection are reported elsewhere (Ludsin et al. 2004). A series of ablations were performed at the growing edges, which represent the last 1–2 years of life (last rings). Otolith core concentrations were determined by a single transect of about 200  $\mu$ m that was approximately equidistant on each side of the core (Ludsin et al. 2004). Calcium was used as an internal standard to correct for variations in the amount of material ablated (i.e., ablation yield). The two polymorphs

of CaCO<sub>3</sub>, vaterite and aragonite, do not have the same structure but their chemical composition is identical. Therefore, Ca concentration is the same in both polymorphs (~40% by weight).

## Results

#### Microscopic observations

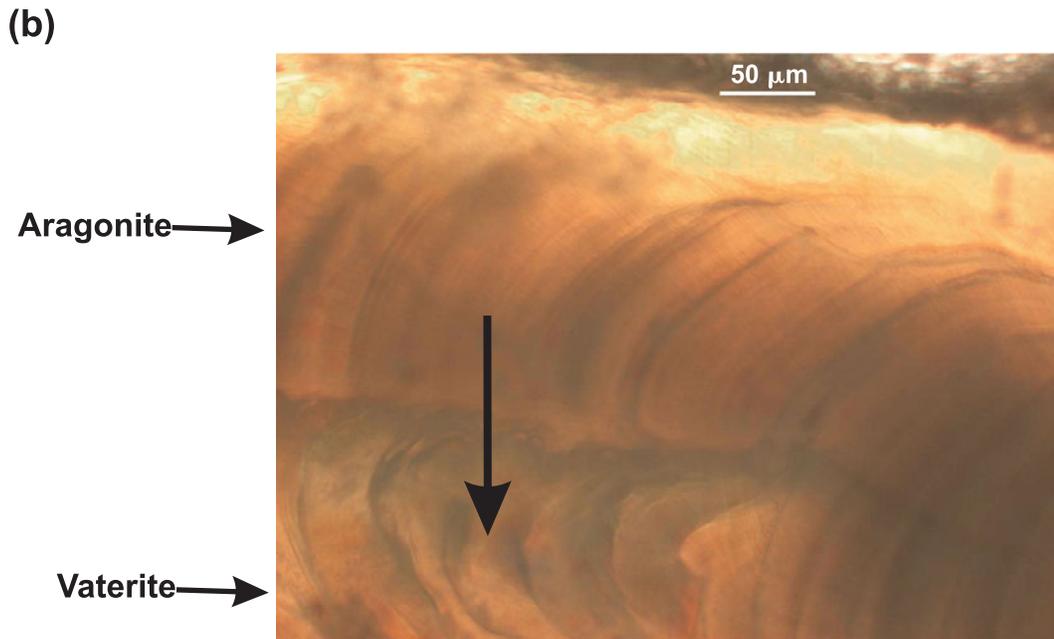
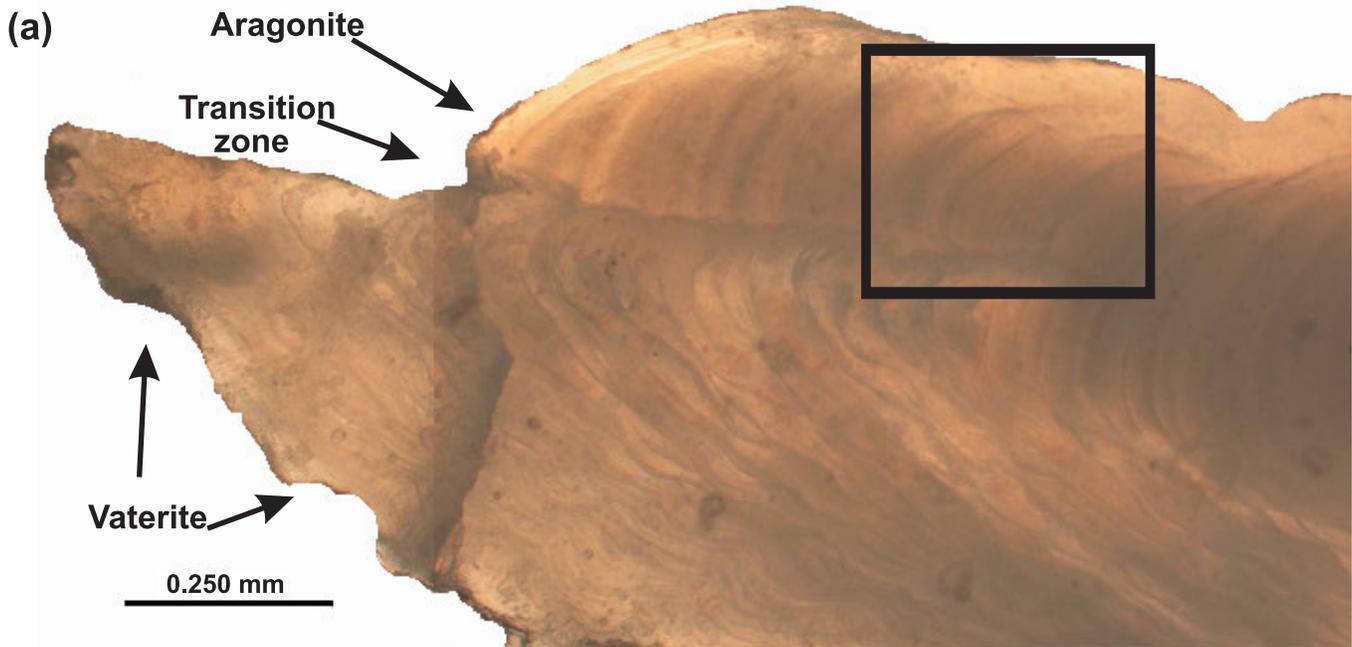
Microscopic observations of individual sagittal otoliths demonstrated that aragonite and vaterite grow simultaneously in the otolith, but in different locations that are separated by an easily identifiable transition zone. Interestingly, growth rates of these polymorphs differed, even in the same growth rings. In most instances, vaterite growth rates exceeded those of coprecipitating aragonite, as evidenced by larger vateritic areas (Fig. 1a). Although relatively uncommon, it also was possible for aragonite growth to be greater than vaterite growth (Fig. 1b). In addition to these growth zone and growth rate differences, clear differences in the structure of growth bands were evident microscopically. In general, vateritic portions of the otolith demonstrated excessive waviness, which made aging impossible (Fig. 2). By contrast, growth bands in the aragonitic zone of the otolith were less wavy and more uniform, making aging easier (Fig. 2). These two crystalline polymorphs also had different optical properties, as the opacity of vaterite was greater than that of aragonite (Fig. 2a). Further, the contact (transition) zone between aragonite and vaterite growth, over 20–30  $\mu$ m, did not optically look like pure aragonite or pure vaterite (Fig. 2b).

Sagittal cores were of three different types: pure aragonite (the most dominant case), pure vaterite, and a combination of the two (Fig. 3). Under transmitted light, aragonitic and vateritic cores are quite dark and microscopic observations could not help distinguish their crystal composition. Sketches of the different otolith types are presented beside the photographs to facilitate identification (Fig. 3).

#### Aragonite and vaterite growth relationships

The characteristics of the transition zone and vateritic cores were investigated by Raman spectroscopy and LA-ICP-MS. Raman study on the structural forms of CaCO<sub>3</sub> in

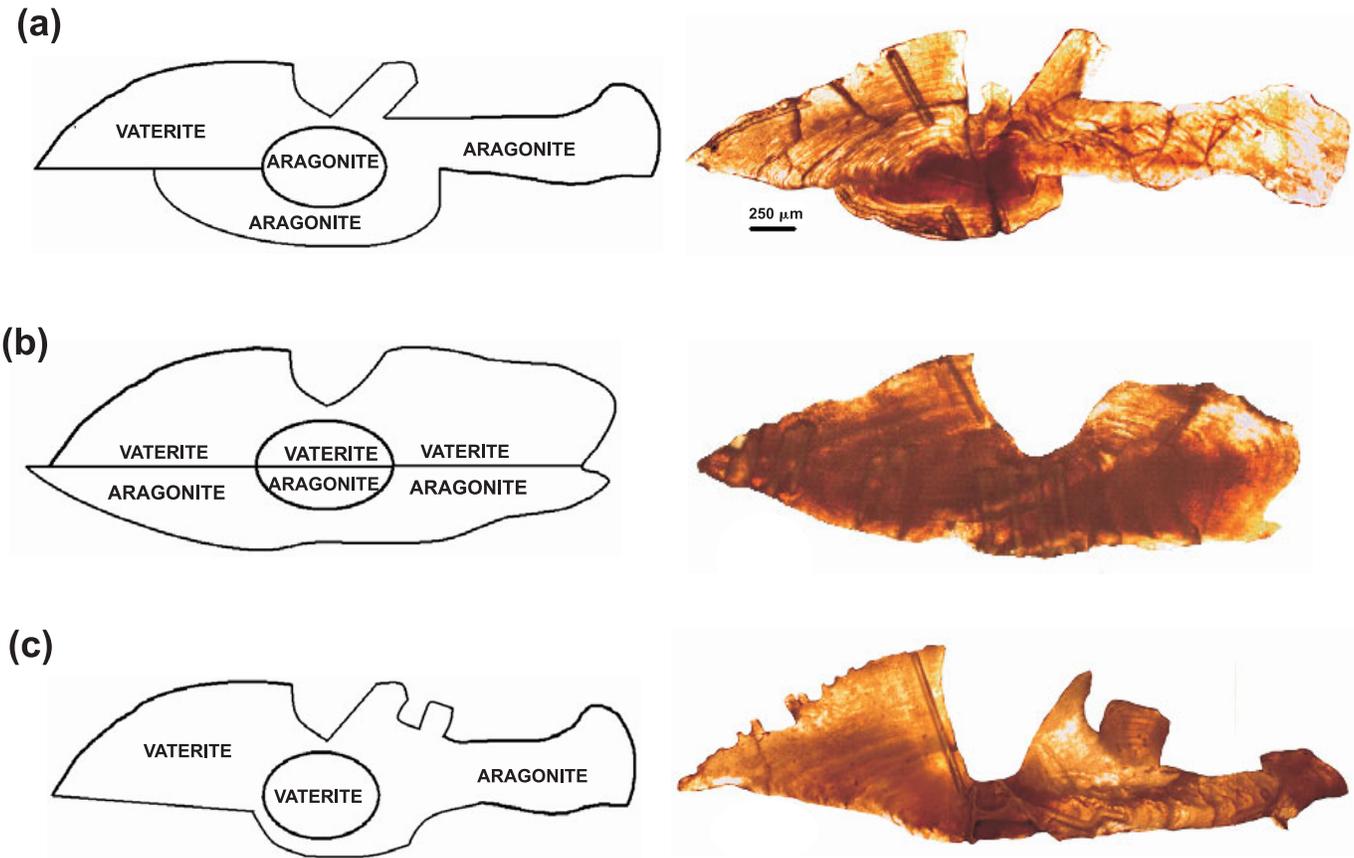
**Fig. 2.** (a) Transition (contact) zone between aragonite and vaterite for an otolith of an 11-year-old hatchery-reared lake trout (*Salvelinus namaycush*) under transmitted light. Notice the “waviness” of the growth bands in vaterite as compared with aragonite. (b) The box in a is shown in more detail. The arrow in b represents the direction of the Raman mapping done on the boundary zone.



lake trout sagittae substantiated the microscopic analyses, which indicated aragonite and vaterite growing at the same time in otoliths (Figs. 4a–4c). Three distinct characteristics associated with group vibration, a change in the dipole moment of the molecule (Gans 1971; Truchet et al. 1995), were used by Gauldie et al. (1997) to distinguish vaterite from aragonite. We used the same ones here, which were lattice mode, symmetric stretching, and in-plane bending. Lattice modes of vaterite were characterized by two peaks in closer

proximity to one another (peaks at 152 and 205  $\text{cm}^{-1}$ ) relative to aragonite with peaks at 106 and 302  $\text{cm}^{-1}$  (Fig. 4a). Second,  $\nu_4$ , the in-plane bending of  $\text{CO}_3^{2-}$ , was characterized by a narrow doublet instead of a broader doublet for the Raman spectrum of aragonite (Fig. 4b). Finally, vaterite was characterized by triplet bands (1075, 1081, and 1090  $\text{cm}^{-1}$ ) for symmetric stretching,  $\nu_1$ , of C—O from  $\text{CO}_3^{2-}$  bonded to Ca (Fig. 4c), whereas aragonite had only a single band (1084  $\text{cm}^{-1}$ ). The bands  $\nu_2$  and  $\nu_3$  were not visible, as their

**Fig. 3.** Vateritic formations in lake trout (*Salvelinus namaycush*) otoliths. (a) Aragonitic core with the transition zone one side and the aragonite edge on the other; (b) vateritic and aragonitic core with the transition zone on both sides; (c) vateritic core with one vateritic and one edge aragonitic edge.



intensities are too weak and fluorescence overshadowed their peaks. In any case, they were not needed to differentiate the two polymorphs. Analysis of the transition zone, dividing areas of aragonite and vaterite growths, demonstrated that a thin zone (20–30 μm) comprising a mixture of aragonite and vaterite separates the crystal polymorphs. This was evident from a gradual shift from a single peak to broad triplet in symmetric stretching as one moved from aragonite into vaterite (Fig. 4d). Additional Raman analysis of otoliths from the remaining 12 lake trout demonstrated that most cores were composed entirely of aragonite. In one instance, however, the entire core was vaterite, while two others had cores that were half aragonite and half vaterite.

#### Microchemistry of aragonite and vaterite

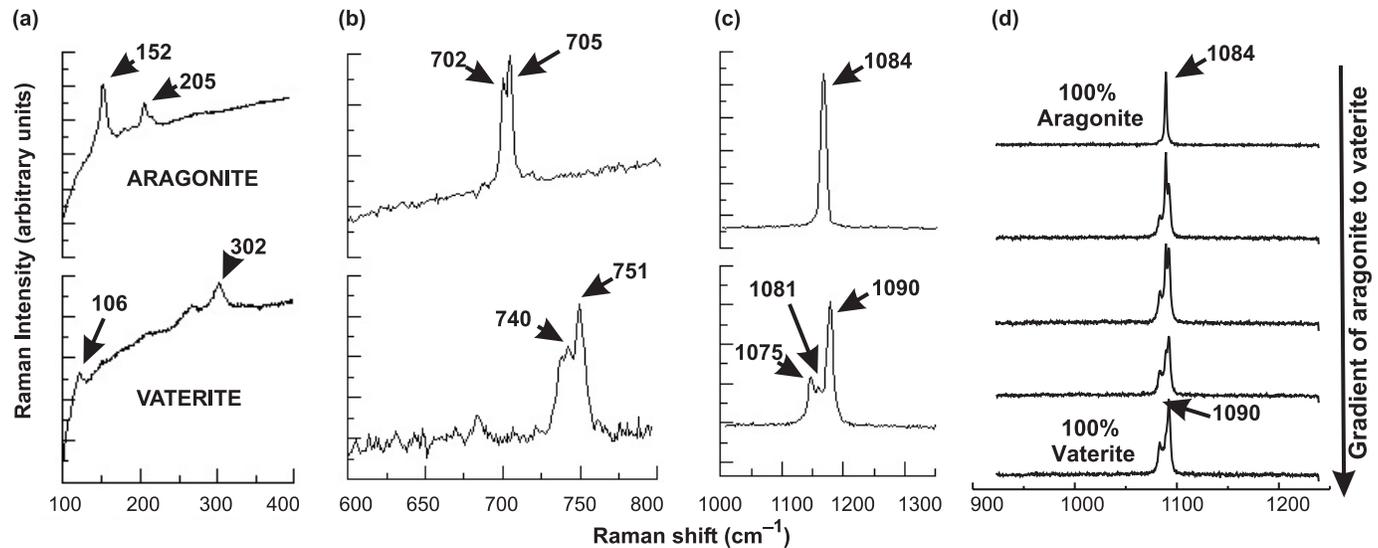
The microchemistry of aragonite and vaterite growing simultaneously in sagittae was quantified using LA-ICP-MS. This technique is very sensitive, detecting low concentrations of trace metals (parts per billion) in environmental matrices, and permits determination of temporal concentrations (Bellootto and Miekeley 2000). Vateritic core data were obtained from two types of fish: (i) two hatchery-reared fish that were released and recaptured in Lake Erie (note that the core is representative of hatchery existence, given that these individuals spent their first 18 months in the hatchery; see Ludsins et al. 2004) and (ii) one individual that spent its en-

tire existence in the hatchery. Microchemical analyses of otolith edges were from fish that resided in the hatchery since birth ( $n = 13$ ).

To quantify differences in average concentrations between aragonitic and vateritic edges in our hatchery lake trout ( $n = 13$ ), paired  $t$  tests were conducted for each element. Significant differences existed between mineral types for all elements (paired  $t$  test: all  $|t| \leq 3.5$ , all  $p < 0.004$ ) but Zn ( $t = -0.07$ ,  $p = 0.94$ ). In the core, Mg levels in vaterite were 30-fold higher and Mn levels were five times higher than in aragonite, whereas Sr levels were 10-fold higher and Ba levels were 20-fold higher in aragonite than in vaterite (Table 1). There was a similar trend for the edges; concentrations of Mg were 40-fold higher in vaterite and about 20-fold lower for Sr as compared with aragonite. Manganese was 10-fold more abundant in vaterite and Ba 40-fold more concentrated in aragonite (Table 1).

An LA-ICP-MS transect through the transition zone demonstrated chemical differences (Fig. 5) that correspond well to the Raman results (see Fig. 4d). There was a gradual but not abrupt transition from one mineral type to the other. Pure aragonite was characterized by high Sr and Ba and low Mg and Mn, whereas vaterite demonstrated the opposite trend (also see Table 1). The arrow in Fig. 2b shows the perpendicular trajectory of the Raman and ICP-MS laser transects through the aragonite–vaterite boundary zone separating si-

**Fig. 4.** Wave numbers from the Raman spectroscopy analysis of aragonite and vaterite portions of a hatchery-reared lake trout (*Salvelinus namaycush*) that spent its entire existence in the Allegheny National Fish Hatchery (this is the same individual as in Fig. 2a). The patterns presented, in arbitrary units plotted against Raman shift ( $\text{cm}^{-1}$ ), were typical of 12 other broodstock analyzed with Raman spectroscopy. (a) Lattice modes; (b) in-plane bending ( $\nu_4$ ) of C—O from  $\text{CO}_3^{2-}$  bonded to Ca; (c) symmetric stretching ( $\nu_1$ ) of  $\text{CO}_3^{2-}$ ; (d) Raman mapping (symmetric stretching only) through the boundary zone (as shown in Fig. 2b), demonstrating the transition from aragonite to vaterite for the same sample.



**Table 1.** Mean metal concentrations in the cores and edges of lake trout (*Salvelinus namaycush*).

Cation radius ( $\text{\AA}$ ) <sup>a</sup>	Edge				Core		
	Vaterite > aragonite ( $n = 7$ )		Vaterite < aragonite ( $n = 6$ )		Aragonite ( $n = 25$ )	Vaterite ( $n = 3$ )	
	Aragonite	Vaterite	Aragonite	Vaterite			
Li	0.90	1.1±0.8	1.0±0.5	0.5±0.2	0.5±0.1	0.18±0.03	0.6±0.2
Rb	1.66	0.05±0.01	0.027±0.005	0.05±0.01	0.027±0.002	0.12±0.01	0.09±0.03
Mn	0.81	0.26±0.03	3.4±0.2	0.34±0.05	4.6±0.4	2.0±0.2	6.3±0.1
Mg	0.86	20±5	757±32	21±4	768±38	19±2	514±54
Zn	0.88	4.4±0.5	4.5±0.9	5±1	4.6±0.8	34±2	51±6
Ca	1.14	1 000 000	1 000 000	1 000 000	1 000 000	1 000 000	1 000 000
Sr	1.32	202±12	11±1	170±16	11±2	666±32	60±5
Ba	1.49	1.3±0.1	0.03±0.01	1.5±0.1	0.03±0.01	14.7±0.6	0.89±0.05

**Note:** Edge results are divided based on the crystal growth vaterite > aragonite or vaterite < aragonite ( $\mu\text{g metal}\cdot\text{g Ca}^{-1} \pm 1$  SE). Elements are arranged by ionic charges (+1 for Li and Rb, +2 for the rest) and then by cation radii sizes. All edge and core (aragonite only) concentrations were from individuals that spent their entire existence in the hatchery. The vaterite core concentrations were derived from two hatchery-reared fish that were recaptured in Lake Erie (i.e., the core chemistry reflects the hatchery environment) and a single individual that resided solely in the hatchery.

<sup>a</sup>Shannon (1976).

multaneous growth of aragonite and vaterite. Some variation within each polymorph may be due to the fact that we passed through two annuli while analyzing this portion of the otolith.

#### Otolith elemental partitioning

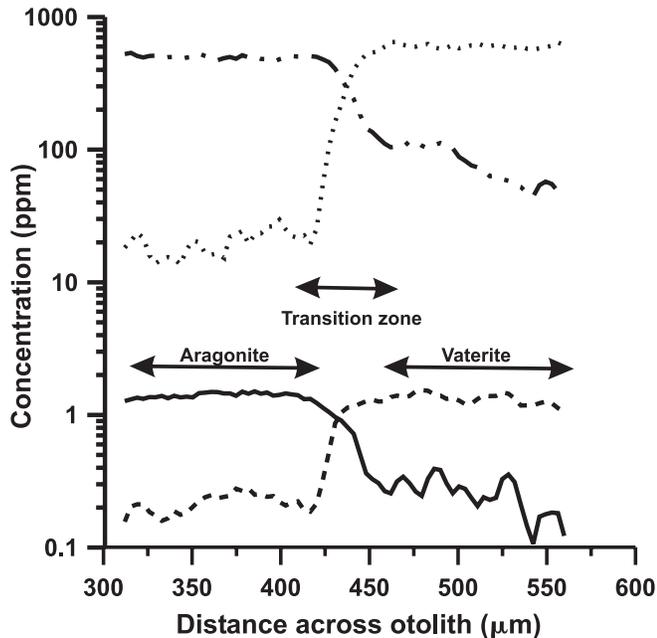
Partition coefficients represent the concentrations of elements in one phase as compared with the concentration in a coexisting phase. Typically, researchers calculate the ratio between elemental concentrations in water relative to those in the otolith, generally aragonite crystals (Bath et al. 2000; Elsdon and Gillanders 2003). However, with aragonite and vaterite growing simultaneously, and separately, in the same

otolith, we were provided with the unique opportunity to calculate partition coefficients for the two mineral types using the following equation:

$$(1) \quad K_D = [\text{metal}/\text{Ca}]_{\text{aragonite}} / [\text{metal}/\text{Ca}]_{\text{vaterite}}$$

where  $K_D$  is the partition coefficient between the concentration of a metal in aragonite relative to its concentration in vaterite. For this determination, all metals were standardized against Ca before analysis. The average partition coefficients determined for the edges and the cores are presented in Table 2. As expected, based on mean concentrations in Table 1, uptake rates of Sr and Ba were higher in aragonite than in vaterite (i.e., coefficients greater than 1) in both the edge and

**Fig. 5.** Transition from aragonite to vaterite in trace metal concentrations perpendicular to the transition zone between aragonite and vaterite boundary (such as in Fig. 2b) of a hatchery-reared lake trout (*Salvelinus namaycush*). Elemental concentrations were quantified along this transect using laser ablation with inductively coupled plasma mass spectrometry. Barium is represented by the solid line, Mg by the dotted line, Mn by the dashed line, and Sr by the dashed-dotted line.



the core of aragonitic portions of the otolith, whereas the opposite was true for Mg and Mn (Table 2). These trends also were evident from plots of metal concentrations in vaterite versus aragonite (Fig. 6). Specifically, those elements with slopes greater than 1 indicate preferential uptake by aragonite relative to vaterite. Both Sr and Ba had slopes in excess of six (Figs. 6f and 6g, respectively). By contrast, both Mg and Mn had slopes much less than 1 (both less than 0.07), indicating preferential uptake by vaterite relative to aragonite (Figs. 6b and 6c, respectively). Lithium demonstrated near equal portioning between mineral types (slope = 1.42) (Fig. 6a). Also, with the exception of Zn, which demonstrated no obvious relationship between aragonite and vaterite (Fig. 6d), all elements were positively (but not necessarily significantly) related in vaterite and aragonite, which makes sense given that these minerals were reflecting the same water chemistry.

## Discussion

Previous investigations involving salmonines, including lake trout, have documented the existence of vaterite in otoliths that also contain aragonite. Our investigation is unique because we demonstrate through both elemental and spectroscopic analyses that both vaterite and aragonite can grow in otoliths simultaneously. Interestingly, these two minerals grow at different locations within the otolith, separated by a well-demarcated transition zone that consists of a blend of these two crystalline polymorphs. Further, from microscopic

**Table 2.** Values of partition coefficients ( $K_D \pm 1$  SE) for the edge and core of hatchery-reared lake trout (*Salvelinus namaycush*).

$K_D$ (aragonite/vaterite)	Ionic charge	Cation radius <sup>a</sup>	Edge ( $n = 13$ )	Core ( $n = 3$ )
Li	+1	0.90	0.79±0.09	1.1±0.6
Rb	+1	1.66	2.0±0.3	3±1
Mn	+2	0.81	0.08±0.01	0.46±0.01
Mg	+2	0.86	0.027±0.004	0.043±0.006
Zn	+2	0.88	1.2±0.2	0.8±0.1
Sr	+2	1.32	17±1	10±1
Ba	+2	1.49	42±7	13.0±0.8

**Note:** Elements are listed by ionic charge and cation radius sizes.  
<sup>a</sup>Shannon (1976).

observations, there are growth rate differences between vateritic and aragonitic portions of the otolith with vaterite growth being generally greater than aragonite growth. Below, we explain the likely mechanisms responsible for differences in chemical composition as well as growth rates.

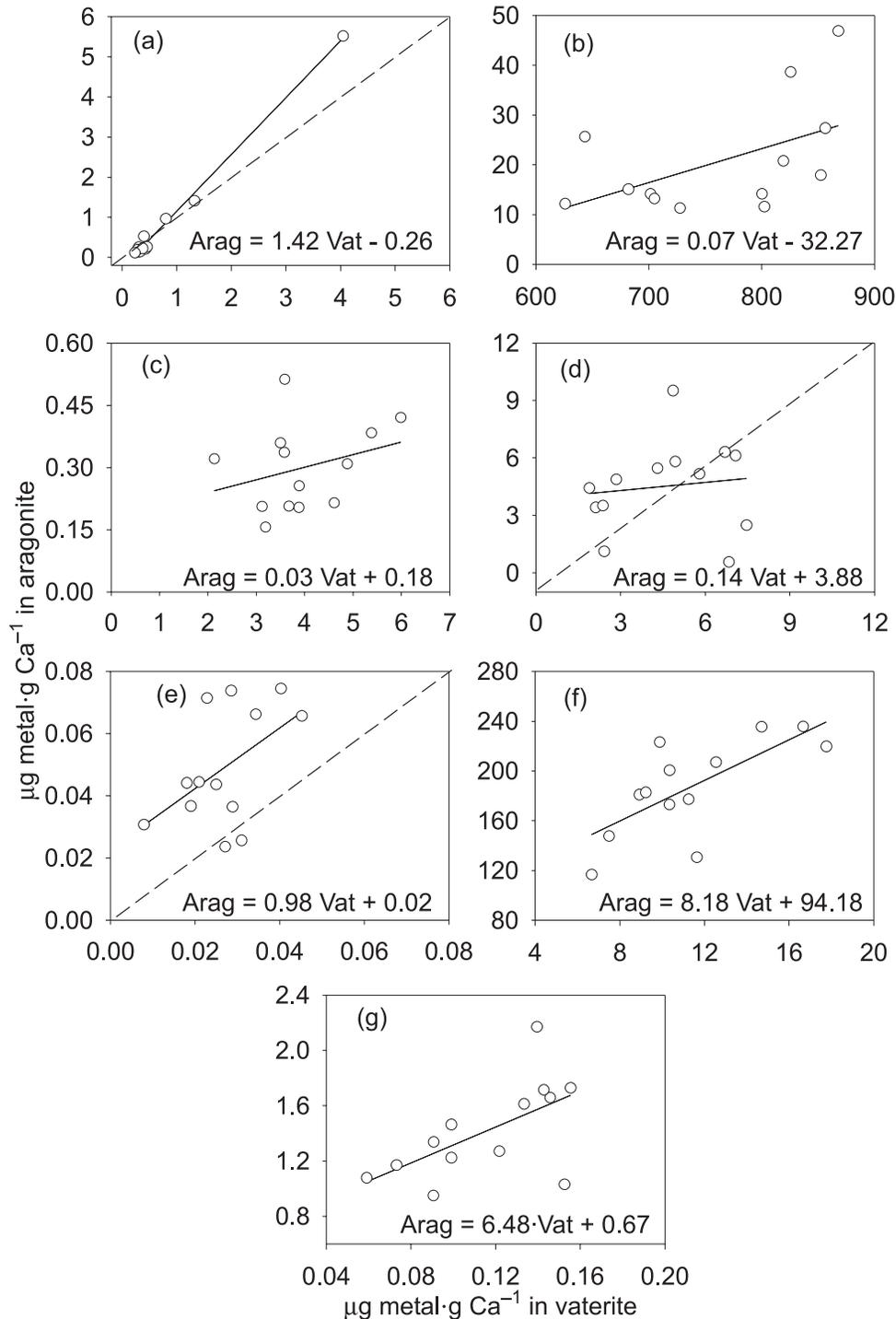
## Microchemistry of aragonite and vaterite

Two primary factors should influence the incorporation of elements into aragonite versus vaterite. First, we would expect less substitution of metals with a +1 ionic charge (e.g., Li, Rb) in the  $\text{CaCO}_3$  matrix of otoliths than metals with a +2 ionic charge (e.g., Mn, Mg, Zn, Sr, Ba), given that calcium is a +2 cation and crystals must be electrically neutral. Second, the relationship between the ionic radii relative to Ca versus potential replacement ions also should be important, with ions whose ionic radii is most similar to Ca (e.g., Mg, Sr) being favored over those that are not (e.g., Rb) (Table 3).

The coprecipitation of aragonite and vaterite in otolith cores and edges allowed us to directly measure concentrations in both polymorphs and then calculate the partition coefficients (uptake rates) at different development stages (core = larval to fry, edge = adult). Regardless of relative growth rates between vaterite and aragonite, Sr and Ba were higher in edges comprising aragonite than in those comprising vaterite, whereas the opposite was true for Mg and Mn. By contrast, the variation in Rb and Zn between these two polymorphs was minimal. Similar differences (and non-differences) were evident in otolith cores.

Aragonitic otoliths preferentially ( $K_D > 1$ ) incorporated large cations such as  $\text{Sr}^{2+}$  (1.32 Å) (1 Å = 0.1 nm),  $\text{Ba}^{2+}$  (1.49 Å), and  $\text{Rb}^{1+}$  (1.66 Å), whereas smaller cations like  $\text{Mg}^{2+}$  (0.86 Å) and  $\text{Mn}^{2+}$  (0.81 Å) were favored ( $K_D < 1$ ) in vaterite. These results can be explained by the crystal structure of vaterite and its reduced ability to incorporate larger cations (i.e., Sr, Ba) without serious crystal distortion (Casanova et al. 2004). In addition, the length of the metal–oxygen bond in  $\text{MCO}_3$  in both crystal structures supports this conclusion: an average of 2.46 Å is found in vaterite compared with approximately 2.53 Å for aragonite (Taylor et al. 1993; Hasse et al. 2000). Zinc and Li partition coefficients were close to 1 for both the core and the edge, indicating that their uptake was the same in both crystal structures and is largely independent of the age of fish. As expected, the

**Fig. 6.** Concentrations of metal ( $\mu\text{g metal}\cdot\text{g Ca}^{-1}$ ) in aragonite (Arag) versus vaterite (Vat) from otolith edges of hatchery-reared lake trout (*Salvelinus namaycush*). (a) Lithium ( $R^2 = 0.99$ ,  $p < 0.001$ ); (b) Mg ( $R^2 = 0.27$ ,  $p = 0.07$ ); (c) Mn ( $R^2 = 0.09$ ,  $p = 0.32$ ); (d) Zn ( $R^2 = 0.02$ ,  $p = 0.69$ ); (e) Rb ( $R^2 = 0.26$ ,  $p = 0.08$ ); (f) Sr ( $R^2 = 0.50$ ,  $p < 0.01$ ); (g) Ba ( $R^2 = 0.35$ ,  $p = 0.03$ ). Broken lines, shown on some plots, are one-to-one lines. Regression equations and regression broken lines are shown.



concentrations of trace metals in both  $\text{CaCO}_3$  polymorphs also were strongly influenced by differences in their ionic radii relative to that of Ca, with the closest +2 metals to Ca being preferentially incorporated. These quantitative results for pure vaterite and pure aragonite follow the same trends as obtained by previous researchers using whole otoliths that

were dominantly vaterite or aragonite (Gauldie 1986, 1996; Tomás and Geffen 2003).

Trace metal abundances of otoliths from fish raised in the hatchery environment can vary significantly with ontogeny. Specifically, Ludsin et al. (2004) reported significant concentration differences between the edge (age 2 to age 11)

**Table 3.** Metals size differences in ionic radii from  $\text{Ca}^{2+}$  in the  $\text{CaCO}_3$  crystal.

Metal	Ionic charge	Radius difference from $\text{Ca}^{2+}$ (Å)
Li	+1	0.24
Rb	+1	0.52
Sr	+2	0.18
Zn	+2	0.26
Mg	+2	0.28
Mn	+2	0.33
Ba	+2	0.35

**Note:** Elements are classified by ionic charge and cation radius sizes.

and core (first few months) of these same hatchery-reared individuals for both Zn and Mn. These authors were uncertain of the causal mechanisms but suggested the potential role of a changing crystal structure or perhaps a shift in diet or metabolism (physiology).

An additional possibility that needs to be explored for changing element concentrations is whether element concentrations are affected by the growth rate of the crystals. The results for vaterite > aragonite and vaterite < aragonite are similar for most elements. Lithium, which had similar partition coefficients for both crystal growth rate groups, showed distinctly different concentrations for the two groups (two times the concentration of Li in vaterite growth > aragonite compared with vaterite growth < aragonite). Except for this element, the dimensional differences of  $\text{CaCO}_3$  crystals are not influencing the concentration results. One explanation for this difference is that Li may have a different mechanism of incorporation in otoliths than the other elements studied. The Li uptake may differ for the two polymorphs owing to metals being trapped in the  $\text{CaCO}_3$  crystal structure as opposed to substituting for  $\text{Ca}^{2+}$ . Indeed, previous work with aragonitic otoliths has demonstrated that metals can bind to proteins or be trapped in the growing crystal lattice (Milton and Chenery 2001). To date, however, no similar work has been conducted with vateritic otoliths.

### Possible mechanisms causing vaterite growth

#### *Role of extrinsic factors*

Discrimination of  $\text{CaCO}_3$  polymorphs can be done qualitatively, given that vaterite growth zones have bands that are optically relatively “wavy” relative to the more evenly spaced and uniform coprecipitating aragonite growth zones. We also observed that vaterite and aragonite are deposited in the normal growth increments as the layers crystallize chronologically, which is what would be expected given the basic model of otolith growth. In contrast, Brown and Severin (1999) documented regions comprising a mixture of aragonite and vaterite that were not limited to growth bands, as they diagonally crossed otolith daily increments. These authors explained that their results did not follow the basic model of otolith growth based on their assumption that some genes switched production of proteins causing a mixture of aragonite and vaterite. Other researchers have found that otoliths of fish regularly grow vaterite around an aragonitic core following normal otolith increment deposition (Gauldie

et al. 1997; Bowen et al. 1999; Tomás and Geffen 2003). One vateritic and one aragonitic otolith have also frequently been observed in the same fish (Gauldie 1986; David and Grimes 1994).

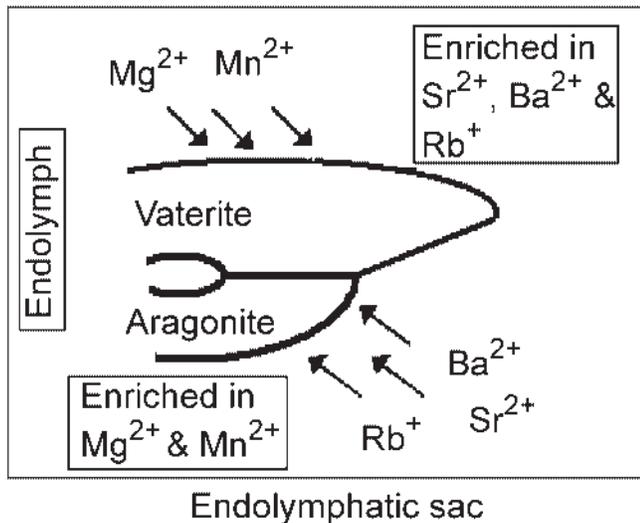
As for the cause of vateritic growth, we are not certain. Presently, it is assumed that stocking stress or possibly residing in a hatchery environment is responsible for this change in the crystal structure of the otolith (Casselman 1990; Casselman and Gunn 1992; Bowen et al. 1999). However, determining whether it is the hatchery environment itself or the act of stocking that caused vaterite to form in otoliths has remained elusive. Clearly, based on the fact that we found vaterite in adult lake trout that never left the hatchery, as well as in the cores of recaptured fish deposited before they left the hatchery, indicates that stocking itself might not be the root cause. The fact that several investigators (Casselman 1986; Ludsins et al. 2004) have documented vaterite formation in lake trout fingerlings and adults that were of wild origin also demonstrates that the hatchery environment is not solely responsible for vaterite formation. As such, perhaps it is not the specific environment (e.g., hatchery versus wild) that is so important but how stressed that individual is in that environment. Natural environments can be relatively more stressful for some individuals than others, which may explain the occurrence of vaterite in some wild individuals. The fact that vaterite is more typical of hatchery-reared individuals than wild ones (Casselman 1990; Casselman and Gunn 1992; Bowen et al. 1999) only indicates that a hatchery environment (or the act of stocking) may be more stressful than a natural setting. Clearly, this is a research area deserving more attention. Perhaps additional research might identify an environmentally influenced gene similar to *starmaker*, which controls crystal lattice formation in zebrafish (Söllner et al. 2003), that triggers vaterite otolith transformation in salmonines such as lake trout.

#### *Role of intrinsic factors*

Previous hypotheses on vaterite formation were always related to hatchery stress (Gauldie 1986; David and Grimes 1994; Bowen et al. 1999). The presence of a vateritic core and two mixed vaterite–aragonite cores suggests that causes of vaterite crystallization are more complicated than previously described, as it is occasionally found early in the larval stage. Pote and Ross (1991) found that each otoconia polymorph, from several species of vertebrates, had unique proteins associated with it. Researchers also have suggested that a gene could influence the production of some proteins facilitating the production of vaterite or aragonite in a growing otolith (Oliveira and Farina 1996; Brown and Severin 1999). If specific proteins control vaterite or aragonite formation, we should expect to find only vaterite or aragonite crystals precipitating at one time in the endolymphatic sacs. In this study, both phases were found to form simultaneously on either side or wing of the otolith.

One possibility to explain the simultaneous growth of aragonite and vaterite in otoliths is the possibility of the organic matrix to differ from one zone of the otolith to the other (Williams 1984; Strong et al. 1986). From these observations, it is believed that the presence of a gradient in the endolymph chemical composition influences the uptake of

**Fig. 7.** Crystallization process of otolith. Arrows portray elements that are mainly incorporated in each crystalline polymorph (vaterite versus aragonite) from the endolymph. Text boxes indicate what is enriched in the residual fluid after elemental incorporation into the otolith. Differential incorporation of metals into the otolith portions consisting of vaterite versus aragonite would theoretically cause a gradient in the endolymph that might influence future otolith concentration changes.



metals by the otolith and perhaps the crystal structure as well. Indeed, ionic ( $K^+$ ,  $Na^+$ ) and proteomic gradients have been observed within the endolymph along the proximal–distal axis (Payan et al. 1999; Borelli et al. 2001). Further, Payan et al. (1999) documented that nonuniformity in the composition of the endolymph of rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*) can influence the biomineralization process of the otoliths. They concluded that the chemical gradient is partially maintained through the sagittal otolith acting as a physical barrier to the diffusion of ions and proteins. This new information on endolymph composition needs to be taken into consideration as a factor in determining otolith concentration changes (such a scenario is portrayed in Fig. 7).

### Implications

This study identified vaterite and aragonite growing separately, but simultaneously, on the edges and the cores of lake trout otoliths. This characteristic had only been documented once before (Campana 1983) and raises several questions concerning their origin(s) and highlights the need to investigate the partitioning of trace metals between endolymph and otoliths for a better understanding of uptake mechanisms. Stress might be a cause of vaterite replacing aragonite during otolith growth but cannot explain what causes vateritic or half aragonitic – half vateritic cores or the two phases growing simultaneously. Is a mixture of proteins responsible for the occurrence and geometry of  $CaCO_3$ ? Is the endolymph gradient (ionic and proteomic) influencing the crystallization process? These questions are unanswered and complicate the understanding of polymorphic growth mechanisms in otoliths.

Our work also has implications for future otolith microchemistry work involving salmonines such as lake trout.

Based on our research, otoliths can vary considerably in crystalline structure, which in turn will cause variation in otolith elemental composition. Given the lack of a proportional (linear) relationship between the chemistry of coprecipitating vaterite and aragonite for some elements, it is clear that aragonite and vaterite do not incorporate all elements from the water in the same way. Previous work has demonstrated that aragonitic otolith chemistry reflects that of the water (e.g., Thorrold et al. 1998; Bath et al. 2000; Gillanders and Kingsford 2000). If this is always true, it appears that vateritic portions of the otolith likely do not reflect water chemistry equivalently. Thus, the use of vateritic portions of otoliths in traditional microchemical studies (e.g., stock discrimination, migration history tracking) would be limited. As such, care must be taken when dealing with otoliths that contain vaterite. Certainly, the criteria for discriminating between these two crystalline polymorphs would be valuable to future otolith microchemistry efforts by allowing investigators to avoid vateritic areas.

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