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Trace Element Patterns in Otoliths: The Role of Biomineralization

Hüssy Karin [1, *] , Limburg Karin E. [2, 3], De Pontual Helene [4], Thomas Oliver R. B. [5], Cook Philip K. [6, 7], Heimbrand Yvette [3], Blass Martina [3], Sturrock Anna M. [8, 9]

[1] National Institute of Aquatic Resources, Technical University of Denmark, Kongens Lyngby, Denmark;
[2] College of Environmental Science and Forestry, State University of New York, Syracuse, New York, USA;
[3] Department of Aquatic Resources, Swedish University of Agricultural Sciences, Lysekil, Sweden;
[4] IFREMER, Sciences et Technologies Halieutiques, Plouzané, France;
[5] School of BioSciences, The University of Melbourne, Melbourne, Victoria, Australia;
[6] ESRF – The European Synchrotron Radiation Facility, Grenoble, France;
[7] Institute of Physics and Material Sciences, University of Natural Resources and Life Sciences, Vienna, Austria;
[8] Center for Watershed Sciences, University of California, Davis, California, USA;
[9] School of Life Sciences, University of Essex, Colchester, UK

* Corresponding author : Karin Hüssy, email address : kh@aqua.dtu.dk

Abstract :

Otolith chemistry has gained increasing attention as a tool for analyzing various aspects of fish biology, such as stock dynamics, migration patterns, hypoxia and pollution exposure, and connectivity between habitats. While these studies often assume otolith elemental concentrations reflect environmental conditions, physiological processes are increasingly recognized as a modulating and/or controlling factor. In particular, biomineralization—the complex, enzyme-regulated construction of CaCO3 crystals scaffolded by proteins—is believed to play a critical role in governing otolith chemical patterns. This review aims to summarize the knowledge on otolith composition and biophysical drivers of biomineralization, present hypotheses on how biomineralization should affect element incorporation, and test the validity thereof with selected case studies. Tracers of environmental history are assumed to be dominated by elements that substitute for Ca during crystal growth or that occur randomly trapped within the crystal lattice. Strontium (Sr) and barium (Ba) largely comply with the biomineralization-based hypotheses that otolith element patterns reflect environmental concentrations, without additional effects of salinity, but can be influenced by physiological processes, typically exhibiting decreasing incorporation with increasing growth. Conversely, tracers of physiology are assumed to be elements under physiological control and primarily occur protein-bound in the otolith's organic matrix. Physiological tracers are hypothesized to reflect feeding rate and/or growth, decrease with fish age, and exhibit minimal influence of environmental concentration. The candidate elements phosphorus (P), copper (Cu) and zinc (Zn) confirm these hypotheses. Magnesium (Mg) is believed to be randomly trapped in the crystal structure and hence a candidate for environmental reconstruction, but the response to all examined drivers suggest Mg to be coupled to growth. Manganese (Mn) substitutes for Ca, but is also a co-factor in matrix proteins, and therefore exhibits otolith patterns reflecting both environmental (concentration and salinity) and physiological (ontogeny and growth) histories. A consistent temperature response was not evident across

studies for either environmental or physiological tracers, presumably attributable to variable relationships between temperature and fish behavior and physiology (e.g., feeding rate, reproduction). Biomineralization thus has a controlling effect on otolith element concentrations for elements that are linked with somatic growth, but not for elements that substitute for Ca in the crystal lattice. Interpretation of the ecological significance of patterns from field samples therefore needs to consider the impact of the underlying biomineralization processes of the element in question as well as physiological processes regulating the availability of ions for inclusion in the growing crystal lattice. Such understanding will enhance the utility of this technique to address fisheries management questions.

Keywords : Biomineralization, environment, growth, microchemistry, ontogeny, otolith, physiology, salinity, temperature

Introduction

Bony fish have calcium carbonate ear stones, or otoliths, in their inner ears that they use for balance and hearing. Otoliths typically consist of calcium carbonate (~98%), organic matrix (~2%) and small quantities of other elements. Otoliths grow incrementally, where seasonal variations in the ratio of calcium carbonate to organic matrix result in the conspicuous growth bands that have made otoliths such well-known chronometers (Neilson and Geen, 1985; Rice et al., 1985; Høie et al., 2008). The elemental composition of the otoliths often referred to as 'otolith chemistry' can also provide valuable information about the environmental conditions experienced by the fish, and has gained increasing attention as a tool to reconstruct fish stock dynamics, migration patterns, pollution exposure and connectivity between habitats (Campana, 1999; Campana and Thorrold, 2001; Elsdon et al., 2008; Carlson et al., 2017). With improvements in mass spectrometry techniques and decreasing costs associated with these analyses, growing numbers of researchers are measuring a broad suite of elements in otoliths in a bid to answer complex ecological questions (Elsdon et al., 2008). These applications generally build on the assumption that elements are incorporated into the otolith at concentrations roughly proportional to concentrations in the ambient environment (Campana, 1999; Izzo et al., 2018). This is, however, often not the case (Brown and Severin, 2009; Sturrock et al., 2015).

In order to interpret elemental signals within the otolith, it is important to understand how both external and internal factors impact element uptake, transport and incorporation. Fish are multicellular, complex organisms, and the surface of the otolith is several membranes removed from the ambient water. Elements are primarily absorbed from the water and diet across the intestinal wall, and from the water across the gill surface (Watanabe et al., 1997; Campana, 1999; Milton and Chenery, 2001). To maintain blood concentrations within safe physiological limits, fish have evolved sophisticated mechanisms to control ion uptake and excretion rates, and/or to re-route into particular organs and tissues for storage (Watanabe et al., 1997; Bury et al., 2003) (Fig. 1). Elements that remain in the blood plasma (as free ions or bound to proteins or sugar complexes) or are released during tissue breakdown, may then pass through the endolymphatic epithelium into the endolymph and be incorporated into the growing surface of an otolith. Thus, elements face three major

interfaces en route to the otolith: water (or food) – plasma, plasma – endolymph and endolymph – otolith (Kalish, 1989; Campana, 1999). Yet most studies focus on the applicability of otolith chemistry for answering ecological questions and less on the underlying mechanisms and pathways (Fig. 1). Internal processes along this path, such as protein binding, trans-membrane transport, and biomineralization, are increasingly recognized as important factors to consider when interpreting otolith elemental patterns (Elsdon and Gillanders, 2002; Walther et al., 2010; Sturrock et al., 2012; Izzo et al., 2018).

[Figure 1 here]

In order to separate environmental from physiological signals within otolith elemental patterns, it is important to build a mechanistic understanding of ion uptake, transport, and incorporation mechanisms and the factors influencing these processes. One approach involves comparing ion concentration across interfaces (i.e. in the water, blood plasma, endolymph and otolith), but only one such study exists, involving samples collected at different times and from different individuals (Melancon et al., 2009). Other studies have measured ion fractionation across a subset of interfaces, such as between water and blood plasma (e.g. Sturrock et al., 2014), plasma and endolymph (Kalish, 1991a; Payan et al., 1997), endolymph and otolith (Kalish, 1991a), and/or plasma and otolith (Campana, 1999; Sturrock et al., 2015, Kalish, 1989 & 1991a). Cumulatively, these studies suggest that ion discrimination is highly element-specific and can be extremely dynamic, varying among species, systems, and life stagesWith so few studies examining ion processing within the fish (Fig. 1), it is difficult to tease apart the underlying mechanisms and ascertain when environmental signals outweigh physiological 'noise'. In many other taxa, biomineralization plays a critical role in governing elemental concentrations (e.g. Bentov et al. 2009) but is arguably one of the least well understood processes in the field of fish otolith chemistry (Fig. 1). Thus, this review aimed at isolating (not always possible given the scarcity of studies focused on this process) the influence of biomineralization on element incorporation into otoliths.

Gaining a better understanding of the processes driving biomineralization and element incorporation may help clarify inconsistencies between otolith and ambient concentrations reported in the literature (e.g.

Campana, 1999; Walther et al., 2010), spatial heterogeneity in element concentrations across the otolith (Limburg and Elfman, 2010; Limburg et al., 2011, 2015) and elemental differences between crystal types (Melancon et al., 2005). Specifically, this review 1) summarizes the current knowledge on otolith composition and biomineralization, 2) develops conceptual models of bio-physical influences on biomineralization, 3) presents hypotheses on how these processes should affect elemental incorporation, and 4) tests these hypotheses with selected case studies.

Biomineralization

Structure of the endolymphatic epithelium

Fish have three pairs of otoliths (sagitta, lapillus and asteriscus) located in separate chambers in the inner ear. These chambers are interconnected through the semicircular canals but are otherwise a closed system without contact to the surrounding tissues (Mayer-Gostan et al., 1997). The endolymphatic epithelium consists of four types of epithelia: the sensory, transitional, intermediate and squamous epithelium (Mayer-Gostan et al., 1997; Pisam et al., 1998; Saitoh and Yamada, 1989). The otoliths are attached to a gelatinous membrane over the sensory epithelium called the macula (Fig. 2). Embedded in the gelatinous layer are sensory hair cells called kino- and stereocilia which connect to the acoustic nerve. Both the transitional and squamous epithelia contain large numbers of ionocytes (epithelial cells involved in ion transport) and mitochondria-rich cells (Tohse et al., 2004). In the transitional epithelium these cells are larger and more densely organized with high concentrations of endoplasmic reticulum, tubular, and vesicular systems, while the ionocytes in the latter are smaller and irregularly spaced (Pisam et al., 1998). In the transitional and squamous areas, Na^+/K^+-ATPase and carbonic anhydrase rich cells also occur (Mayer-Gostan et al., 1997), while the intermediate area contains less specialized cells (Pisam et al., 1998).

[Figure 2 here]

Calcium carbonate accretion

Sagittal otoliths (the focus of this review) typically comprise aragonite calcium carbonate crystals. The formation of aragonite crystals on the otolith surface requires the presence of Ca^{2+} ions and HCO_3^- in the endolymph. Transport of these two constituents across the endolymphatic epithelium differs considerably. Free Ca^{2+} ions in the blood plasma (typically ~ 40 - 60% of total plasma concentrations, Mugiya, 1966; Andreasen, 1985; Hanssen et al., 1989; Funamoto and Mugiya, 1998) diffuse along a concentration gradient into the endolymphatic epithelium (Mugiya and Yoshida, 1995). From here, the ions are transported into the endolymph by the Ca-binding calmodulin, via a Na^+/Ca^{2+} exchanger driven by Na^+/K^+-ATPase and in particular Ca^{2+}-ATPase, which is activated by the $Ca^{2+/}$-calmodulin complex (Mugiya, 1986; Mugiya and Yoshida, 1995; Cruz et al., 2009) (Fig. 3). Other experiments suggest that Ca^{2+} ions can also move into the endolymph via passive paracellular diffusion process (Tohse and Mugiya, 2001; Payan et al., 2002; Cruz et al., 2009).

The endolymph is supersaturated in CO_3^{2-} ions relative to the blood plasma as a result of active transport across the endolymphatic epithelium (Takagi, 2002), promoting the precipitation of $CaCO_3$ crystals (Payan et al., 1997; Takagi et al., 2000). Elevated concentrations of HCO_3^- in the endolymphatic epithelium are achieved by intracellular activity of carbonic anhydrase, which catalyzes the $CO_2 + H_2O \rightarrow HCO_3^- + H^+$ reaction (Mayer-Gostan et al., 1997; Tohse et al., 2006). HCO_3^- is thereafter transported across the epithelium and into the endolymph through Cl^-/HCO_3^--exchangers driven by HCO_3^-ATPase (Tohse and Mugiya, 2001). Since this reaction results in high H^+ concentrations, $HCO3^-$ transport also strongly depends on the pH regulating Na^+/H^+-exchange maintained via the activity of Na^+/K^+-ATPase, and Cl^- -channels (Payan et al., 1997; Tohse and Mugiya, 2001; Takagi, 2002) (Fig. 3). Importantly, inhibiting mitochondrial activity also aborts transport of Na^+, K^+, Cl^-, Ca^{2+} and HCO_3^- ions across the endolymphatic epithelium (Mugiya, 1986; Payan et al., 1997; Tohse and Mugiya, 2001, 2004). The transport of these major ions into the endolymph thus depends on energy-demanding enzyme activity and is likely tightly coupled to the fish's metabolism. The highest activity of Na^+/K^+-ATPase, HCO_3^--ATPase, carbonic anhydrase and calmodulin has been found in the

transitional and the squamous epithelium (Mayer-Gostan et al., 1997; Beier et al., 2004, 2006; Tohse et al., 2004) (Fig. 2).

Presumably as the result of the spatially heterogeneous distribution of ionocytes, the concentrations of Na^+, K^+, Cl^-, Ca^{2+} and HCO_3^- are not homogenous throughout the endolymph. On the proximal side of the otolith concentrations of Mg^+, PO_4^- and Ca^{2+} are higher, while K^+ and total CO_2 are higher on the distal side (Payan et al., 1999, 2002). In addition, elevated H^+-ATPase activity in the hair cells of the sensory epithelium suggests that $CaCO_3$ biomineralization rates are lower in the sensory macula region on the proximal side of the otolith (Shiao et al., 2005) (for examples, see Fig. 4 and 5). Such spatial gradients in endolymph composition will inevitably influence element:Ca ratios, regardless of the elements' pathways into the endolymph. Consideration of this spatial heterogeneity is thus important when selecting otolith elemental assay locations, and to consistently sample the same otolith axis within (and ideally across) studies.

[Figure 3 here]

Organic matrix

Otoliths contain between 0.1 and 2.3% organic matrix (Morales-Nin, 1986b; Baba et al., 1991; Asano and Mugiya, 1993a; Sasagawa and Mugiya, 1996; Hüssy et al., 2004). The matrix consists of approximately 23% collagens, 29% proteoglycans, and 48% other non-collagenous proteins (Borelli et al., 2001, 2003; Payan et al., 2004). Daily and seasonal changes in the concentration of the organic matrix in the otolith create the otolith growth bands used to reconstruct age and growth rate. Typically, 'opaque' zones (white under reflected light) exhibit higher concentrations of organic matrix and are deposited during the growth season, while 'translucent' zones (dark under reflected light) are more mineral rich and typically deposited during winter (Beckman and Wilson, 2002) (Fig. 4 – 7). Over the years, the number of identified matrix proteins has grown from 14 - 16 (Sasagawa and Mugiya; 1996) to > 380 in adult black bream (*Acanthopagrus butcheri*) (Thomas et al., 2019). Some protein functions are well documented, but many remain unknown. A detailed review of otolith protein biochemistry can be found in Thomas and Swearer (2019); here, just the main components and processes are

highlighted.

The different fractions of the organic matrix are often characterized as “water soluble” (or “soluble”) or “insoluble”. Generally, “soluble” refers to molecules that are solubilized after decalcification with EDTA, and primarily consists of proteoglycans, polysaccharides and non-collagenous proteins (Asano and Mugiya, 1993a; Sasagawa and Mugiya, 1996; Takagi and Takahashi, 1999; Murayama et al., 2000, 2002; Dauphin and Dufour, 2003; Murayama et al., 2004; Kang et al., 2008). Conversely, “insoluble” represents the fraction of the matrix that remains as precipitate after decalcification, and primarily consists of collagenous molecules (Borelli et al., 2001). Here, the two fractions of the matrix are referred to as soluble and collagenous, respectively. Overall, the soluble fraction represents between 15% (Baba et al., 1991), 47% (Asano and Mugiya, 1993a; Sasagawa and Mugiya, 1996) and 65% (Hüssy et al., 2004) of the total organic matrix.

Collagenous matrix

The collagenous matrix fraction primarily consists of an inner-ear specific collagen called *Otolin*-1 (Degens et al., 1969; Dunkelberger et al., 1980; Davis et al., 1995; Takagi and Takahashi, 1999; Murayama et al., 2002, 2004). *Otolin*-1 is required for the anchoring of otoliths over the sensory epithelium (Dunkelberger et al., 1980), acts as a nucleation site for crystal formation (Lundberg et al., 2006; Petko et al., 2008) and appears to stabilize the scaffolding of the otolith matrix (Murayama et al., 2002, 2005). Furthermore, it has been suggested by Hołubowicz et al. (2017) that *Otolin*-1 molecules trimerize to facilitate otolith matrix molecule interactions. More recent studies have found that the collagenous fraction of the matrix also contains the sialophosphoprotein *Starmaker*, and its functional homologs (Söllner et al., 2003; Tohse et al., 2008; Bajoghli et al., 2009; Thomas et al., 2019). The phosphorylation of these proteins facilitates calcium binding (Wojtas et al., 2012), thereby creating nucleation sites for crystal growth (Söllner et al., 2003; Tohse et al., 2008; Thomas et al., 2019). Immunohistochemical staining of the endolymphatic epithelium revealed that collagen synthesis occurs in a spatially limited area, within the marginal zone of the sensory epithelium (Takagi and Takahashi, 1999; Murayama et al., 2004) (Fig. 2). Conversely, within the otolith, collagen concentrations are

highest on the proximal side over the sulcus (Takagi and Takahashi, 1999; Murayama et al., 2004), but also occur at low concentrations throughout the otolith (Murayama et al., 2004).

Soluble matrix

The soluble matrix consists of a wide range of polysaccharides, acidic proteins, lipoproteins and glycoproteins (Baba et al., 1991; Sasagawa and Mugiya, 1996; Murayama et al., 2000; Thomas et al., 2019). The most abundant glycoproteins are Otolith Matrix Protein, *OMP*-1 and *Secreted protein acidic and rich in cysteine* (*Sparc*, also known as *Osteonectin*) and *Neuroserpin* (Murayama et al., 2000, 2005; Kang et al., 2008; Thomas and Swearer, 2019). Sub-units of these glycoproteins have calcium-binding capacity (Wright, 1991a; Asano and Mugiya, 1993a; Sasagawa and Mugiya, 1996; Kang et al., 2008; Thomas et al., 2019). These molecules form large complexes with other glycoproteins and fibrous proteins such as collagen and may also bind cations. The abovementioned *OMP-1* for example, is a transferrin glycoprotein that acts as an inhibitor of carbonic anhydrase, has a strong calcium binding capacity and also provides binding sites for other proteins in the otolith organic matrix (Murayama et al., 2005; Petko *et al.*, 2008). *Neuroserpin* is a key component of the organic matrix and likely regulates increment growth given that it is also a protease inhibitor (Kang et al., 2008; Thomas et al., 2019). *Sparc* is a multifunctional protein, able to bind Ca and collagen, as well as enhance plasmin activity (Murayama et al., 2000, 2005; Kang et al., 2008; Thomas and Swearer, 2019). Immunohistochemical staining of the endolymphatic epithelium revealed that soluble matrix (*OMP*-1) synthesizing cells occur throughout the transitional and squamous epithelium but not in the sensory epithelium (Takagi and Takahashi, 1999; Takagi et al., 2000; Murayama et al., 2002; Suzuki et al., 2004) (Fig. 2). In the otolith, the highest soluble matrix staining intensities occur along the otoliths' longest growth axes, revealing the structures of daily growth increments (Takagi and Takahashi, 1999). Polysaccharides are primarily represented as glycosaminoglycans (mucopolysaccharides) attached to a proteoglycan core (e.g. decorin, biglycan) serving a structural function by stabilizing the matrix (Asano and Mugiya, 1993b).

Processes affecting otolith biomineralization

The following paragraphs review extrinsic and intrinsic drivers that may affect the accretion of otolith calcium carbonate and matrix proteins to set the background for hypotheses on how these may affect otolith elemental composition. Owing to the scarcity of studies separating soluble and collagenous fractions, only the total organic matrix is considered. The impact of each extrinsic (salinity, temperature, oxygen) and intrinsic (ontogeny, growth/feeding, maturation) driver is summarized in Table 1.

Extrinsic processes

Salinity: Overall, salinity on its own (independent of ambient ion concentrations) does not appear to have a major impact on otolith biomineralization (Umezawa and Tsukamoto, 1991; Chen et al., 2008; Hüssy et al., 2009), but species-specific effects may occur, with one study suggesting reduced otolith biomineralization rates at higher salinities in an experimental setup covering naturally occurring salinity levels in an estuarine species (*Micropogonias undulatus*) (Peterson et al., 1999).

Temperature: Temperature has a profound impact on all physiological processes. Metabolic rate is thus known to increase by a factor of 1.9 to 2.6 with a 10°C. increase in temperature (Schurmann and Steffensen, 1997). Since transport of most major otolith constituents relies on energy-dependent processes (see above), otolith biomineralization has been found to be tightly coupled to metabolic rate (Mosegaard et al., 1988; Wright, 1991b; Wright et al., 2001; Yamamoto et al., 1998; Hüssy and Mosegaard, 2004). Temperature has thus a direct effect on the precipitation rate of the mineral fraction, with crystal growth approximately doubling with every 10°C. increase within the optimal temperature range for that species (Hüssy and Mosegaard, 2004; Fablet et al., 2011), a value within the range observed for total metabolic rate. It is generally assumed that this results from kinetic effects and increased crystal growth rates; however, temperature also exerts a significant effect on matrix synthesis. For example, in juvenile *Gadus morhua*, total matrix protein concentrations decreased linearly with temperature, either due to reduced matrix synthesis, increased calcium carbonate accretion, or an interaction between the two (Hüssy et al., 2004). Interestingly, concurrent with this decrease

in total protein incorporation, the proportion of soluble proteins increased with temperature (Hüssy et al., 2004).

Oxygen: Anaerobic stress induced by exposure to low oxygen (hypoxic) water can significantly reduce biomineralization rates, potentially via cortisol-induced changes in altering Ca^{2+} transport rates across the saccular epithelium (Walther et al. 2010). Extreme anaerobic stress could even lead to otolith resorption, although formation of a prominent opaque check on the otolith during hypoxic periods has suggested that matrix synthesis is less impaired than growth of the mineral fraction (Mugiya and Uchimura, 1989).

Intrinsic processes

Ontogeny: Fish size and/or age are among the strongest drivers of otolith protein content, with protein concentration approximately 10 times higher in juveniles than adults. For example, proteins typically represent 0.3% to 2.3% of otolith weight in juveniles (Morales-Nin, 1986a; Baba et al., 1991; Asano and Mugiya, 1993a; Sasagawa and Mugiya, 1996; Hüssy et al., 2004), and potentially up to 10% (Degens et al., 1969), but only 0.16 to 0.2% in adult fish (Degens et al., 1969; Morales-Nin, 1986a; Baba et al., 1991). The proportion of soluble to collagenous protein fractions is also subject to ontogenetic change, overall decreasing with age and size (Morales-Nin, 1986a; Baba et al., 1991; Asano and Mugiya, 1993a; Davis et al., 1995; Sasagawa and Mugiya, 1996; Hüssy et al., 2004). The concurrent decrease in opacity thus suggests that the soluble matrix fraction has a particularly large impact on the visual appearance of the otolith.

Feeding/growth: The impact of feeding rate and somatic growth rate on the organic matrix content and elemental composition of otoliths is not well understood. It is well known that feeding rate can affect the visual appearance of otoliths, with reduced feeding resulting in lower opacity (Neilson and Geen, 1985; Rice et al., 1985; Hüssy and Mosegaard, 2004; Høie et al., 2008) and a lower protein content (Mugiya, 1965; Mugiya and Muramatsu, 1982; Watabe et al., 1982; Seyama et al., 1991). Protein synthesis is directly related to feeding rate, and protein synthesis rates in different tissues are highly correlated (Houlihan et al., 1989). Starvation, the most extreme example of reduced feeding, does have a significant effect on endolymph chemistry in that

prolonged starvation does not seem to affect endolymph CO_2 and pH and Ca^{2+} concentrations (Payan et al., 1998), but decreases concentrations of "calcium-binding matrix" by 70% (Guibboldini et al., 2006). Therefore, it is highly plausible that feeding rate could also affect blood, endolymph and otolith protein concentrations. This was not observed in juvenile cod, although the range of feeding rates may not have been large enough (Hüssy et al., 2004).

Maturation: Sexual maturation and spawning are energy-demanding processes that are often coupled with reduced feeding rates and long migrations to spawning grounds, resulting in translucent “spawning checks” from decreased otolith growth (Williams and Bedford, 1974; Rijnsdorp and Storbeck, 1995; Francis and Horn, 1997; Zuykova et al., 2009). The physiological processes involved in this check formation are presumably similar to the ones describe under “Feeding rate”. Gonad development is also often accompanied by changes in blood protein composition to re-route essential elements into reproductive tissues (Sturrock et al. 2012, 2014). Depending on the extent of reproductive investment, the length of the spawning season, and the distance to the spawning grounds, the impact of maturation and spawning on otolith element availability and incorporation could be considerable.

[Table 1 here]

Trace elements

Otolith element composition

To date, 50 elements have been detected in fish otoliths, including the major elements calcium (Ca), carbon (C), oxygen (O) and nitrogen (N), minor elements (>100 ppm) such as sodium (Na), strontium (Sr), phosphorus (P), magnesium (Mg), potassium (K), chloride (Cl) and sulfur (S), and most other elements at trace levels (< 10 ppm) (Campana, 1999; Sturrock et al., 2012). Many of these trace elements are required for metabolic reactions and processes associated with growth and reproduction (Watanabe et al., 1997 and

references therein; Bury et al., 2003). Fish that are hypertonic to ambient water ion concentrations (freshwater environments) primarily absorb elements across the gill surface, while fish that are hypotonic (marine environments) primarily absorb elements across the gut wall and excrete excess salt and ammonia from their gills. Most studies have suggested that water represents the main source of ions to the fish, but it should be noted that most studies have focused on Sr and Ba (Kalish, 1991b; Campana, 1999; Watanabe et al., 1997; Milton and Chenery, 2001; Walther and Thorrold, 2006; Doubleday et al., 2013). It is critical that fish maintain blood ion concentrations within safe physiological limits (often vastly different to ambient concentrations), resulting in evolution of a myriad of physiological mechanisms to regulate ion uptake, excretion, storage and recycling (Watanabe et al., 1997; Bury et al., 2003).

Among the biochemically-important elements, magnesium (Mg) is a co-factor of adenosine triphosphate (ATP) and contributes to the phosphorylation of enzymes including *Starmaker* (Wojtas et al., 2012). Iron (Fe) is an essential building block of red blood cells, plays an active role in oxidation/reduction reactions such as lipid oxidation, is involved in electron transport associated with cellular respiration, and is present in the endolymph protein serotransferrin (Thomas and Swearer, 2019). Copper (Cu) and Selenium (Se) are involved in the activity of many essential enzymes and are required elements in oocyte formation in vertebrates (Sturrock et al. 2013). Manganese (Mn) is necessary for the activation of specific enzymes, in particular for brain functioning, lipid and carbohydrate metabolism and protein synthesis. Mn is also a co-factor of a biomineralization protein, *Extracellular serine threonine protein kinase FAM20C* (Tagliabracci et al., 2012). This protein is found in otoliths, and likely acts to phosphorylate *Starmaker* homologs (Thomas et al., 2019). Zinc (Zn) is a cofactor for many metalloenzyme reactions involved in metabolism, essential for vitellogenesis (yolk formation) in teleosts (Sturrock et al. 2013, 2014), and a co-factor in many matrix metalloproteinases and carbonic anhydrase (essential for the conversion of carbon dioxide to bicarbonate ions during mineralization, Fig. 3) (Thomas et al., 2019). Consequently, Zn has a strong influence on fish somatic growth by regulating the digestibility of protein and carbohydrate.

Pathways from plasma to endolymph

The mechanisms and pathways used by trace and minor elements to move from the blood plasma into the endolymph are not well understood (Fig. 1). In plasma, 40 - 60% of total Ca occurs as free ions (Mugiya, 1966; Andreasen, 1985; Hanssen et al., 1989; Funamoto and Mugiya, 1998) which enter the endolymph via active transport assisted by calmodulin, as well as via passive diffusion along a concentration gradient (see paragraph on Ca incorporation above and Fig. 3). For other elements, only one comparable study exists: Payan et al. (2002) showed *in vitro* that movement of Sr across the endolymphatic epithelium is passive and occurs primarily on the proximal side of the otolith in contact with the macula. A study examining blood chemistry of the goldfish, *Carassius auratus*, suggested that approximately 1/3 of total plasma Sr was diffusible free ions with the remaining 2/3 being protein-bound (Funamoto and Mugiya, 1998). Other elements that likely occur in the plasma primarily as hydrated free ions include Li^{+}, Mg^{2+} and Ba^{2+} (Campana, 1999; Sturrock et al., 2012). Transport of these ions across the endolymphatic epithelium presumably also occurs primarily via passive diffusion along a concentration gradient, however hydrated Mg^{2+} ions are ~25% larger than their unhydrated form, and ~ 50% larger than the hydrated Ca^{2+} ions, and may thus behave quite differently to other Group II ions. Thiophilic ('sulfur-loving') elements such as Cu, Zn and Mn, occur primarily bound to plasma proteins (Fletcher and Fletcher, 1980; Watanabe et al., 1997) and therefore presumably require active transport across the endolymphatic epithelium. Lower concentration of most major elements (Ca, Na, Sr, Cl, Mg) in the endolymph relative to the plasma (Kalish, 1991a; Payan et al., 1997, 1999) suggests active discrimination against those elements across the endolymphatic epithelium (Payan et al., 2004). With a pronounced enrichment in the endolymph, K seems to be the only exception (Kalish, 1991a; Payan et al., 1997).

Trace elements in the crystal lattice

Elements can be incorporated into the otolith either 1) randomly trapped in the crystal lattice, 2) substituted for Ca on the growing crystal surface, or 3) bound to organic matrix constituents. In a recent paper, Thomas et al. (2017) paired otolith and endolymph samples to determine concentrations and enrichment factors for an

array of elements. Ba and Sr have a similar ionic radius to Ca and interact with the same protein types as Ca, and thus compete for Ca binding sites in the lattice and occur in the carbonate fraction. Li, K, and Rb only appear in the endolymph and the carbonate fraction of the otolith with no enrichment, suggesting that they are randomly trapped in the crystal lattice. Thomas et al. (2017) observed that also Mn only appeared in the carbonate fraction, but with a high enrichment in the otolith suggesting it may substitute for Ca. In freshwater mussels, Soldati et al. (2016) confirmed that Mn^{2+} ions are incorporated into the shell aragonite by substituting for Ca^{2+} in the inorganic fraction of the $CaCO_3$ complex. A similar incorporation mechanism for Mn in otoliths is assumed given that they are also predominantly aragonite. Magnesium appears only in the carbonate fraction without any enrichment from endolymph to otolith (Thomas et al., 2017). Thomas et al. (2017) suggest that Mg is more likely trapped randomly in the crystal lattice, corroborating observations in the coral literature (Finch and Allison, 2008). It is likely that Mg^{2+} ions behave differently to other Group II metals given that it has a much smaller radius than Ca^{2+} and thus is not likely to compete for Ca-specific binding sites. The radius of the hydrated form of Mg^{2+}, however, is much larger than the hydrated form of Ca^{2+}, which could impact both transport and incorporation mechanisms (Kaim and Schwederski, 1994). It is important to bear in mind that the incorporation mechanisms of elements caught in interstitial spaces are difficult to assess, owing to the micro-channel architecture of the otolith which can allow elements to move in and out of the otolith after initial deposition (Gauldie et al., 1998; Proctor et al., 1998). Transition metals such as Fe, Zn, Cu and Ni are typically found in the otolith bound to metalloprotein complexes associated with the soluble matrix fraction (Izzo et al., 2016; Thomas et al., 2017). Miller et al. (2006) estimated that 70 - 100% of otolith Cu and 40 - 60% of otolith Zn are bound to the organic matrix, with carbonic anhydrase and matrix proteins *OMP*-1, *MMP2* playing a critical role in their incorporation (Thomas and Swearer, 2019).

Effects of biomineralization on trace element patterns

Based on extrinsic and intrinsic influences on otolith biomineralization (Table 1), transport mechanisms of

elements into the endolymph (see above) and the manner of elemental incorporation - randomly trapped, substituted for Ca, or matrix-bound (see above) - hypotheses for how the same drivers might concurrently influence otolith element concentration patterns are developed. Thereafter case studies exploring each hypothesis are provided, contradictory findings reviewed, and the extent to which biomineralization processes are likely to influence otolith elemental composition discussed. The reviewed literature covers both experimental and field studies across a range of species and life stages, as summarized in Table 2. The case studies are accompanied by illustrations consisting of optical images of the otoliths and 2D elemental maps for four different species, arapaima (*Arapaima* sp.) (Fig. 4), sea perch (*Helicolenus percoides*) (Fig. 5), Atlantic cod (*Gadus morhua*) (Fig. 6), Maraena whitefish (*Coregonus maraena*) (Fig. 7) and European perch (*Perca fluviatilis*) (Fig. 8), representative of taxonomically differing species.

[Table 2 here]

Tracers of environmental history

For otolith chemistry to effectively record the environmental history of a fish (operationally defined here as water element concentrations, salinity, temperature, dissolved oxygen), concentrations in the otolith should reflect ambient environmental conditions in a consistent and predictable manner (Campana, 1999). Sr, Ba, and Mn are often identified as promising environmental tracers since they substitute for Ca (Doubleday et al., 2014; Thomas et al., 2017). A number of elements that are likely randomly trapped in the crystal lattice (Mg, Li, K, Rb, Al, Pb, Cd, K, Li, Fe, Cu, Zn) may also exhibit environmental sensitivity and thus serve as useful geographic markers (Miller et al., 2006; Izzo et al., 2016; Thomas et al., 2017). Here, the focus will be on the tracers most frequently cited in the otolith literature: Sr, Ba, Mn and Mg.

Hypotheses for how bio-physical factors should influence otolith environmental tracers

Our hypotheses for environmental tracers are broadly underpinned by the assumption that these elements

substitute for Ca or are randomly trapped in the $CaCO_3$ lattice, and that their transport mechanisms into the endolymph are similar to those for Ca. In each case, hypotheses of how that driver would influence otolith biomineralization and concurrently element concentrations are developed, assuming that (1) all other drivers remained constant, and (2) upstream processes (e.g. uptake rate, blood chemistry) played only a minor role.

- Environmental concentrations: Increasing otolith element:calcium ratios (E:Ca) with increasing environmental concentrations.
- Salinity: No relationship between salinity (independent of ambient concentrations) and otolith E:Ca.
- Temperature: No relationship between temperature and otolith E:Ca.
- Oxygen: No relationship between water oxygen saturation and otolith E:Ca.
- Ontogeny: No relationship between fish age and otolith E:Ca.
- Food and growth: No relationship between feeding rate and otolith E:Ca, or somatic growth rate and otolith E:Ca.
- Maturation: No relationship between maturation and otolith E:Ca.

The impact of each driver on E:Ca patterns is discussed via case studies, on an element-by-element basis. The outcome of these literature reviews is summarized in Table 3, highlighting the extent to which the case studies agreed with these hypotheses (green signifies agreement, red a lack thereof, yellow indicates mixed responses). In the following sections, E:Ca ratio will be referred to as for example otolith Sr.

Case study: Strontium (Sr)

Strontium (Sr) represents the otolith elemental marker most frequently used for environmental reconstructions; applied since the mid-1990s to track fish movements across salinity gradients (Limburg, 1995; Secor et al., 1995). Sr concentrations are typically lower in freshwater and higher and fairly constant in the ocean (Walther and Limburg, 2012), although freshwater concentrations depend on bedrock geology in the drainage basins. Indeed, Sr concentrations in freshwater endmembers sometimes even exceed marine concentrations (Gillanders, 2005), emphasizing the importance of characterizing endmembers on a system-

by-system basis before applying this tool. As both Sr and Ca are conserved with salinity, water Sr/Ca ratios typically exhibit minimal variation at salinities above 8 psu but increase sharply from 0 to ~8 psu (e.g. Lin et al., 2007; Hicks et al., 2010), providing a useful tool to track fish movements between fresh and brackish waters (Kraus and Secor, 2004).

Environmental concentration: Strong positive relationships between water and otolith Sr/Ca have been documented in many experimental studies (Bath et al., 2000; Elsdon and Gillanders, 2003, 2004, 2005a; Kraus and Secor, 2004; Miller, 2011). In these experimental settings (typically using larval or juvenile life stages), ambient concentrations explain between 70 - 85% (Bath et al., 2000; Elsdon and Gillanders, 2005b) and > 95% (Elsdon and Gillanders, 2003; Hicks et al., 2010; Reis-Santos et al., 2013) of otolith Sr, supporting the hypothesis of a positive relationship between water and otolith Sr concentrations (Table 3). The otolith elemental maps in Figures 4 and 6-8 show patterns in otolith Sr that likely result from movements between areas of differing water concentrations, driven by variation in bedrock geology (Fig. 4) and salinity-related environmental concentrations (Fig. 6-8). Note that the cod (Fig. 6) is from the Kattegat and the whitefish (Fig. 7) and perch (Fig. 8) from the Baltic Sea. Both areas are unlike 'typical' marine and coastal settings (salinity > 30psu) polyhaline, exhibiting salinities between 15 and >30 in the Kattegat, and as low as 1 psu in the Baltic Sea.

Positive relationships between endolymph vs. otolith Sr/Ca (Kalish, 1989) and plasma vs. otolith Sr/Ca (Sturrock et al. 2015) support the hypothesis that Sr and Ca ions exhibit broadly similar behaviors and transport mechanisms within the fish. Multiple studies – typically those involving adult fishes in exclusively marine settings (> 30 psu) - have however reported large variations in otolith Sr despite constant environmental concentrations (e.g. Fig. 5), highlighting that differences in otolith Sr within or among individuals do not always reflect differences in environmental concentrations (Hoff and Fuiman, 1995; Brown and Severin, 2009; Sturrock et al., 2015). These deviations suggest that physiological processes altering the relative concentrations of Sr and Ca in the blood (e.g. differences in uptake or removal rate) or their relative availability

to cross the saccular epithelium (e.g. changes in blood protein concentrations or binding capacity) could complicate environmental signals in otolith Sr (Kalish, 1991a; Sturrock et al., 2014, 2015).

Salinity: As Sr is conserved with salinity and most laboratory experiments create salinity gradients by diluting seawater, it is difficult to separate the effect of ambient concentration from salinity. Here, only studies using experimental salinities with Sr-adjusted water concentrations and where partition coefficients were reported are included. While the strongest influence of salinity on otolith Sr were typically related to environmental concentrations (e.g. see above and Fig. 6-8), minor salinity effects have been reported, ranging from positive (Elsdon and Gillanders, 2004; Zimmerman, 2005; Lin et al., 2007; Hicks et al., 2010; Miller, 2011; Reis-Santos et al., 2013) to negative (Elsdon & Gillanders, 2002; Martin and Wuenschel, 2006). Other studies have found no systematic effect (Elsdon and Gillanders, 2002; Stanley et al., 2015) (Table 3).

Temperature: The influence of temperature on otolith Sr in controlled laboratory experiments is consistently lower than the effect of changing environmental concentrations, typically explaining only a few percent of the observed variation, and often interacting with salinity. Response direction varies from negative (Townsend et al., 1992; DiMaria et al., 2010), to positive (Bath et al., 2000; Clarke et al., 2011; Miller, 2011; Barnes and Gillanders, 2013; Reis-Santos et al., 2013), but most studies find no systematic effect (Kalish, 1989; Secor et al., 1995; Gallahar and Kingsford, 1996; Tzeng, 1996; Elsdon and Gillanders, 2004; Walther et al., 2010) (Table 3). Concurrent with this, Clarke and Friedland (2004) and Sturrock et al. (2015) did not observe a direct temperature effect on otolith Sr in Atlantic salmon (*Salmo salar*) or plaice (*Pleuronectes platessa*) pen-reared for 1 to >2 years, despite large temperature ranges (1.5-12.2^{o}C and 4-15 ^{o}C, respectively). Stanley et al. (2015) observed that at low temperatures, Sr incorporation rates decreased with increasing temperature, but remained constant when approaching the optimal temperature for *Gadus morhua*, while Elsdon and Gillanders (2002) found a U-shaped relationship between temperature and Sr incorporation rates. The observed response of Sr incorporation to temperature may – in part - therefore depend on the temperature range examined and the optimal temperature range for the species. Among-species comparisons suggest that temperature does not have a systematic effect on Sr incorporation into otoliths, but correlations with growth rate could explain some

of the apparent temperature signals in otolith Sr (Sadovy and Severin, 1994; Secor et al., 1995; Campana, 1999). Now, two decades later, these issues are still being debated (Walther et al., 2010; Izzo et al., 2018).

Oxygen: The effect of hypoxia on otolith Sr seems to depend on the severity and periodicity of the exposure event and is somewhat inconsistent. In the one case study found, constant and moderate hypoxia exposure was unrelated to otolith Sr in *Micropogonias undulates*, while periodic hypoxia was negatively related to otolith Sr in males but not females (Mohan et al., 2014).

Ontogeny: In flatfish, otolith Sr can exhibit ontogenetic decreases during larval and juvenile stages, independent of ambient concentrations (de Pontual et al., 2003). Generally, however, systematic changes in otolith Sr with fish age (independent of habitat use) is difficult to assess, as long-term rearing experiments are rare. One exception is Elsdon and Gillanders (2005b), who found no change in otolith Sr over the first two years of life in *Acanthopagrus butcheri*. In field samples of *Merluccius merluccius* otolith Sr decreased over the first three years of life (Morales-Nin et al., 2005). But most studies on marine species report increases in otolith Sr with fish age, particularly over the first few years of life and following onset of sexual maturation (Kalish, 1989; Secor and Rooker, 2000; Fowler et al., 2005; Jessop et al., 2008; Brown and Severin, 2009; Avigliano et al., 2015; Hughes et al., 2016; Siskey et al., 2016; Grammer et al., 2017). Whether or not such patterns indicate ontogenetic changes fish growth and ion processing vs. ontogenetic shifts in habitat use remains to be seen, however, system-specific differences in trends suggests an important environmental component (Sadovy and Severin, 1992; Secor and Rooker, 2000; Elsdon and Gillanders, 2005a; Fowler et al., 2005; Jessop et al., 2002, 2008; Brown and Severin, 2009).

Food and growth: In laboratory-reared juvenile fish from different taxa, neither food ration (Walther et al., 2010) or composition (Hoff and Fuiman, 1995; Lin et al., 2007; Marohn et al., 2009) affected otolith Sr. Man studies have also reported no relationship between somatic growth rate and otolith Sr (Kalish, 1989; Secor et al., 1995; Bath et al., 2000; DiMaria et al., 2010), however, others have observed negative relationships (Lin et al., 2007; Stanley et al., 2015) (Table 3) and variation in effect strength among life stages (Clarke and Friedland, 2004; Walther et al., 2010). In adult pen-reared plaice, *Pleuronectes platessa* (Sturrock et al., 2015)

and field samples of *Haemulon plumieri* and *Epinephelus guftatus* (Sadovy and Severin, 1992, 1994), otolith Sr was negatively related to otolith growth rate. Seasonal signals with higher otolith Sr levels typically occurring in opaque zones (Siskey et al., 2016) suggest a link between otolith Sr and growth and/or maturation. Such growth and/or maturation related patterns may occur in both freshwater fish (Fig. 4) and strictly marine fish (Fig. 5)

Maturation: Evidence from field and pen-reared mature marine fishes suggest that seasonal peaks in otolith Sr coincide with peak spawning (Kalish, 1991a; Granzotto et al., 2003; Clarke and Friedland, 2004; Sturrock et al., 2015). In Sturrock et al. (2015), otolith Sr was positively related to gonadosomatic index in the females, with the peak occurring during the spawning period reflecting changes in plasma Sr/Ca that resulted from asynchronous cycles in plasma Sr and Ca concentrations (plasma Sr peaking about two months later than Ca).

[Figure 4 and 5 here]

Case study: Barium (Ba)

Ba has a greater bioavailability to fish in freshwater than in marine environments, as it predominantly occurs in its free form in freshwater and bound to other compounds in saline waters (Turner et al., 1981). Ba is removed from the water through scavenging by biological organisms or precipitation of barite (Paytan and Griffith, 2007). Consequently, Ba concentration patterns show a nutrient-like distribution that is strongly related to environmental salinity with depletion in surface waters, in particular in areas of high productivity (Elsdon and Gillanders, 2005a; Walther and Limburg, 2012). Highest Ba concentrations generally occur at salinities between 5 and 20 psu (Walther and Limburg, 2012).

Environmental concentration: Otolith Ba concentration almost exclusively reflects ambient concentrations (Bath et al., 2000; Milton and Chenery, 2001; Elsdon and Gillanders, 2002, 2003, 2004, 2005b; Martin and Thorrold, 2005; Miller, 2009, 2011; Hicks et al., 2010; Reis-Santos et al., 2013; De Vries et al., 2005). Water Ba/Ca explains between 80% (Elsdon and Gillanders, 2005b; Miller, 2009; Reis-Santos et al., 2013) and 90 -

98% of otolith Ba (Bath et al., 2000; Elsdon and Gillanders, 2003; Hamer et al., 2006; De Vries et al., 2005) supportingthe hypothesis that biomineralization is not impairing a coupling between environmental concentration and otolith Sr (Table 3). There is no evidence for strong physiological control of Ba uptake into the blood (Sturrock et al., 2014), however, a decreasing partition coefficient with increasing environmental concentration, suggest saturation or limitation related transport mechanisms (Bath et al., 2000). As for Sr, the elemental maps show patterns in Ba exposure relating to local geochemistry (Fig. 4) or reflecting known coastal/offshore migrations across salinity gradients (Fig. 6-8).

Salinity: Similar to Sr, Ba incorporation can be subject to a minor positive effect of environmental salinity, albeit much smaller than the effect of environmental concentration and temperature (Elsdon and Gillanders, 2002, 2004; Martin and Thorrold, 2005; Martin and Wuenschel, 2006; Reis-Santos et al., 2013; Stanley et al., 2015) (Table 3). Other studies found no effect of salinity on otolith Ba (Elsdon and Gillanders, 2002; Hicks et al., 2010) or a slightly negative effect (Miller, 2011). Reported differences between freshwater and marine field samples seem exclusively driven by environmental availability and not by salinity *per se* (Elsdon and Gillanders, 2005a).

Temperature: Temperature has been reported to have small positive effects on otolith Ba (Elsdon and Gillanders, 2002, 2004; Miller, 2009; Barnes and Gillanders, 2013; Reis-Santos et al., 2013; Stanley et al., 2015), no effect (Martin and Thorrold, 2005; Clarke et al., 2011; Martino et al., 2017), and sometimes negative effects in larval (DiMaria et al., 2010) as well as adult fish (Sturrock et al., 2015). So there is somewhat contradictory evidence to support the hypothesis that temperature has no effect on otolith Ba (Table 3). The interactions between temperature and growth rate (see below) and between environmental Ba concentration, salinity and temperature make it difficult to separate their effects, potentially complicating interpretation of field data (Miller, 2011; Barnes and Gillanders, 2013).

Oxygen: Constant hypoxia had no effect on otolith Ba, while periodic hypoxia exposure had a negative effect in *Micropogonias undulates* (Mohan et al., 2014).

Ontogeny: As with Sr, few studies exist documenting ontogenetic patterns in otolith Ba incorporation for individuals with known rearing histories, but two such studies reported no change in otolith Ba with age over several years (Elsdon and Gillanders, 2005b; Hamer et al., 2006). In field samples, extensive variation in otolith Ba has been observed among areas (Elsdon and Gillanders, 2005a; Hughes et al., 2016), among individuals captured in the same location (Hamer et al., 2006), but none of these studies reported a consistent pattern with age. One study addressing this question specifically reported a consistent increase in otolith Ba with age (Grammer et al., 2017), suggesting either that ontogenetic patterns only occur in some species, or that they are typically masked by environmental signals (Table 3) (Fig. 4, 6-8).

Food and growth: The bulk of otolith Ba originate from the surrounding water and not from the diet (Walther and Thorrold, 2006; Lin et al., 2007). Consequently, otolith Ba does not seem to vary with prey type (Lin et al., 2007; Marohn et al., 2009). Prey quantity and growth on the other hand do seem to have an effect on otolith Ba, with low rations (Walther et al., 2010) and slow growth (Miller, 2011; Sturrock et al., 2015) leading to higher otolith Ba concentrations, where growth may explain 20% of the observed variation in otolith Ba (Walther et al., 2010) (Table 3). Studies on larval fish did not find a significant relationship between otolith Ba and growth rate (Bath et al., 2000; Martin and Thorrold, 2005). As with Sr, it is notoriously difficult to separate the effects of temperature, diet and growth, in that higher temperatures typically result in increased feeding and growth rates, which may explain the negative ration and growth effects observed by (Walther et al., 2010).

Maturation: There are apparently no studies reporting maturation effects on otolith Ba.

[Figure 6 here]

Case study: Manganese (Mn)

Manganese is the 25th element in the periodic table; it is a transition metal with multiple oxidation states. Of these, Mn^{2+}, Mn^{3+}, and Mn^{4+} are among the most commonly found in nature. Mn is one of the most abundant elements in Earth's crust (Reddy et al., 2008) and is far more abundant in soils, inland waters, estuaries, and

coastal seas than in open oceans (Aguilar and Nealson, 1998; Limburg et al., 2011; Van Hulten et al., 2016). In the former, Mn is found in the mg/kg range, and in the latter, often at sub-ng/kg levels, particularly at depth. An active redox participant, Mn cycles between dissolved (Mn^{2+}, Mn^{3+}) and particulate (Mn^{4+}) phases in sediments and waters as a function of pH and dissolved oxygen, with lower pH and oxygen favoring the dissolved forms (Slomp et al., 1997; Trouwborst et al., 2006). Dissolved Mn^{2+} can however linger in solution even when oxygen returns and may be available for uptake at low, but not zero, dissolved oxygen (Pakhomova et al., 2007). Mn is also involved as a co-factor in many enzymes (Thomas and Swearer, 2019).

Environmental concentration: Laboratory experiments have had dubious outcomes when manipulating environmental concentrations of Mn. Miller (2009) reported no relationship between otolith Mn and water concentration and Elsdon and Gillanders (2003) found only a weakly negative relationship. Sturrock et al. (2015) reported a significant positive relationship between water and otolith Mn concentrations of pen-reared adult *Pleuronectes platessa*, noted that total plasma Mn concentrations in the same fish were unrelated to either. In the wild, relationships have been shown to be positive (Thorrold and Shuttleworth, 2000; Dorval et al., 2007; Mohan et al., 2012). Consequently, otolith Mn has been applied in the field to track hypoxia exposure (Limburg et al., 2011, 2015; Payne Wynne et al., 2015; Mohan and Walther, 2016; Altenritter et al., 2018; Altenritter and Walther, 2019; Limburg and Casini, 2018) (Table 3). In the figures used here, the nursery areas of arapaima, cod and whitefish are known to suffer from frequent hypoxia which is reflected in elevated levels of otolith Mn (Fig. 4, 6, 7), while the seasonal migrations of adult cod and whitefish to hypoxic coastal habitats resulted in annual exposures to high Mn (Fig. 6 and 7).

Salinity: Salinity *per se* does not appear to have a strong effect on otolith Mn (Elsdon and Gillanders, 2002; Martin and Thorrold, 2005; Martin and Wuenschel, 2006), except for potential increases in incorporation rates at the highest salinities (Stanley et al., 2015; Mazloumi et al., 2017). Similarly, field observations suggest that Mn is taken up regardless of salinity (Dorval et al., 2007; Limburg et al. 2015) (Table 3).

Temperature: Temperature effects on otolith Mn reported in laboratory experiments have been variable, ranging from no effect (Elsdon and Gillanders, 2002; Martin and Wuenschel, 2006; Clarke et al., 2011),

negative (Miller, 2009) and positive effects (Stanley et al., 2015; Mazloumi et al., 2017), but only at the highest experimental temperatures (Martin and Thorrold, 2005). Conversely, Elsdon and Gillanders (2002) observed minimum otolith Mn incorporation at intermediate temperatures (Table 3).

Oxygen: Declines in dissolved oxygen change the redox state of aquatic environments (Reddy and DeLaune, 2008). When oxygen is used up, Mn reduction occurs and it is this divalent Mn, which forms at very low levels of oxygen (i.e., close to anoxia), which substitutes for Ca^{2+} in aragonite (Soldati et al., 2016). It is thus oxygen-dependent environmental concentrations that make most Mn available for biomineralization in hypoxic environments (Limburg et al., 2015) (Table 3). Mohan et al. (2014) did not observe increased otolith Mn after prolonged and periodic exposure to hypoxia and attributed this to only minor increases in the Mn levels of the water related to the experimental hypoxia treatment. Limburg et al. (2015) argued that it may be difficult to replicate redox conditions *in vitro*, and thus truly mimic environmental conditions.

Ontogeny: Information on ontogenetic patterns in otolith Mn is scarce in the literature. In the laboratory, otolith Mn decreased with age during juvenile stages (Miller, 2009; Clarke et al., 2011). In field samples of a wide range of species, Mn concentrations are often elevated in the otolith core suggesting maternal transfer (Brophy et al., 2004; Ruttenberg et al., 2005). Multiple species also exhibiting declining concentrations over their lifetime, at a decreasing rate with age (Friedrich and Halden, 2010; Limburg et al., 2015; Hughes et al., 2016; Limburg and Casini, 2018). This pattern is consistent with the expectations for elements that represent tracers of growth (Table 3).

Food and growth: There are only few studies explicitly tracking multiple trace elements from diet into otoliths, and they concur that there are no differences in otolith Mn uptake between diets of differing Mn concentrations (Buckel et al., 2004; Marohn et al., 2009). In terms of growth effects, conflicting results are reported, with some studies reporting no growth effect on otolith Mn (Martin and Thorrold, 2005; Clarke et al., 2011), others reporting a positive growth effect (Limburg et al., 2011; Limburg et al., 2015; Stanley et al., 2015; Sturrock et al., 2015) (Table 3). To disentangle impacts of environmental concentration and physiology, Limburg and

Casini (2018) proposed a heuristic model of Mn uptake that involved both exogenous biogeochemical processes that make Mn^{2+} available, and endogenous growth processes regulating the rate of uptake.

Maturation: There are apparently no studies reporting effects of maturation on otolith Mn.

[Figure 7 here]

Case study: Magnesium (Mg)

Magnesium is the ninth most abundant element in the universe. In aquatic environments, Mg reacts with water much like Ca and, consequently, contributes to water alkalinity. Mg is conserved with salinity, with environmental concentrations ranging from <50 mg l^{-1} in freshwater to 1350 mg l^{-1} in seawater, where Mg is the second-most-abundant cation (Cox, 1989). Mg is also one of the most abundant elements in the tissues of all living organisms, where it forms an essential part of many enzymes, for example through its interaction with phosphate in basic nucleic acid chemistry. It occurs naturally in combination with a range of other elements or in free ionic form (Cox, 1989). In fish plasma, Mg concentrations are strongly correlated with plasma protein levels (Sturrock et al., 2014). Fish can take up Mg from either water or diet (Shearer and Åsgård, 1992).

Environmental concentration: The few studies that examined the rate of Mg incorporation in relation to environmental concentration agree that otolith Mg does not seem to be influenced by water concentrations (Wells et al., 2003; Marohn et al., 2009; Miller, 2011; Woodcock et al., 2012). Otolith Mg therefore does not conform to being a tracer of environmental concentrations (Table 3).

Salinity: As hypothesized, salinity does not seem to influence otolith Mg (Elsdon and Gillanders, 2002, 2003; Martin and Thorrold, 2005; Martin and Wuenschel, 2006; Barnes and Gillanders, 2013; Stanley et al., 2015). In a study on larval galaxiids, Hicks et al. (2010) observed a negative effect of salinity on otolith Mg but only across a limited range of low salinities. In nature, the species in that study typically spend their first 6 months at sea, and may therefore not be adapted to rearing at the low salinities of this experiment.

Temperature: As with other elements, the effect of temperature on otolith Mg varied across studies, ranging from no effect (Elsdon and Gillanders, 2002; Martin and Wuenschel, 2006; DiMaria et al., 2010; Clarke et al., 2011) to strongly positive (Elsdon and Gillanders, 2003; Miller, 2011; Barnes and Gillanders, 2013; Stanley et al., 2015; Sturrock et al., 2015; Mazloumi et al., 2017). Only Martin and Thorrold (2005) reported a negative effect of temperature on otolith Mg (Table 3). These inconsistencies are not explained by life stage, as both response groups included both larval, juvenile and adult fishes. Field samples of a wide range of species suggest a positive effect of temperature on otolith Mg. Limburg et al. (2018) observed a decreasing amplitude in seasonal otolith Mg (see below) with latitude, which corresponded well with modelled temperature-driven metabolic rates, which suggests that Mg incorporation is not independent of temperature during biomineralization as hypothesized.

Oxygen: Variable periods of exposure to hypoxia did not affect otolith Mg (Mohan et al., 2014). But Limburg and Casini (2018) found that during periods of severe hypoxia exposure (as indexed by elevated Mn/Ca ratios in cod otoliths), otolith Mg declined significantly.

Ontogeny: The limited information available on ontogenetic patterns indicate that otolith Mg increases sharply during the juvenile stage of most species in both experimental and field samples (Ruttenberg et al. 2005; Clarke et al., 2011) and then attenuates slowly over the rest of the fish's life (Morales-Nin et al., 2005), concurrent with a decrease in amplitude between growth seasons (see below) (Hughes et al., 2016; Hüssy et al., 2016; Grammer et al., 2017; Limburg et al., 2018). From a biomineralization perspective, patterns related to ontogeny would not be expected. The illustrations used here do not show large declines in otolith Mg, presumably owing to the young age of the individuals (Fig. 6-8).

Food and growth: Only a few studies examined dietary effects on otolith Mg, and the general consensus is that uptake of otolith Mg from the diet is limited (Marohn et al., 2009; Woodcock et al., 2012). With respect to growth effects, the general pattern emerging from published studies is that otolith Mg is unrelated to somatic growth in larval fish (Martin and Thorrold, 2005; DiMaria et al., 2010; Clarke et al., 2011) but positively related to growth rate in juvenile and adult fish across a wide range of taxa (Hamer and Jenkins, 2007; Stanley

et al., 2015; Sturrock et al., 2015; Limburg et al., 2018) (Table 3). In field samples of adult fishes from a wide range of species, otolith Mg exhibits pronounced seasonality (Grammer et al., 2017), where minima in otolith Mg transects from core to edge correspond to visually-identified winter growth zones (Limburg et al., 2018). Similar patterns also are evident in the cod, perch and whitefish examples (Fig. 6-8). The amplitude in Mg differences between summer and winter growth zones decreases with age (Hüssy et al., 2016; Limburg et al., 2018), mirroring somatic growth patterns. Additionally, Limburg and Casini (2018) found that cod with a high body condition at capture had significantly higher otolith Mg throughout their lives compared with cod exhibiting low capture condition. Protein synthesis is directly related to feeding rate, and protein synthesis rates in different tissues are highly correlated (Houlihan et al., 1989). These observations suggest that Mg incorporation into the otolith is thightly coupled to otolith matrix protein incorporation and do therefore not support the hypothesis of feeding and growth-indepent element incorporation during biomineralization.

Maturation: There are apparently no studies reporting effects of maturation on otolith Mg.

[Figure 8 here]

Tracers of physiology and growth

Contrasting with elements typically used as environmental tracers, the incorporation of thiophilic ("sulfur loving") elements into fish otoliths is more likely to occur via protein binding – both in the plasma and the otolith – and are thus likely to be under greater physiological control. The majority of published studies using otolith elemental composition focus on reconstructing environmental histories of fish. In this context, elements under physiological control are less useful. Stock discrimination studies are the exception, as stocks may exhibit different phenotypes as a function of genetic and/or environmental differences. Here, physiological tracers can enhance such studies by amplifying among-stock differences in otolith multi-elemental fingerprints, but as with all such studies, well-described and cohort-matched reference libraries are a critical first step. Generally, however, information on matrix-bound elements, including Cu, Zn (Miller et al., 2006;

Izzo et al., 2016; Thomas et al., 2017) Fe, Mn (Izzo et al., 2016), Co, Ni and P (Thomas et al., 2017), is rather limited and does not allow us to address all hypotheses outlined below. Here, the focus will be on the tracers that are known to be co-factors in many enzymes and proteins: P, Zn and Cu.

Hypotheses for elemental patterns of physiological tracers

Based on extrinsic and intrinsic influences on otolith biomineralization and the manner of elemental incorporation into the crystal lattice, hypotheses for how the same drivers might concurrently influence otolith element concentration patterns are developed. These hypotheses are based on the assumption that physiological tracers occur bound to proteins in plasma and otolith and that synthesis of matrix proteins is linked to feeding rate and body tissue synthesis.

- *Environment*: No relationship between environmental concentration and otolith E:Ca ratio
- *Salinity*: Insufficient data available
- *Temperature*: Decreasing otolith E:Ca ratio with increasing temperature and Ca:matrix ratio.
- *Oxygen*: Insufficient data available
- *Ontogeny*: Decrease in otolith E:Ca ratio with fish age
- Food and growth: Increasing otolith E:Ca ratio with higher feeding rate and with faster growth
- *Maturation*: Decrease in otolith E:Ca ratio during maturation

Case study: Phosphorus (P)

Phosphorus is an essential element in every single biological process on earth, accounting for 2–4% of the dry weight of most cells (Karl, 2000). P is a highly reactive element and therefore seldom found as a free ion in aquatic environments, but rather as dissolved organic and inorganic compounds (Suzumura et al., 2012; White and Dyhrman, 2013). Despite P being the fifth most frequently occurring element in otoliths (Campana, 1999), it has received very little attention in the literature, likely because the assumption has been that otolith

concentrations are driven by physiology and matrix concentrations, while most applications are interested in environmental reconstructions. In otoliths, P presumably occurs in the proteoglycans making up 29% of the total matrix (Borelli et al., 2001, 2003; Payan et al., 2004) and possibly via phosphorylation of amino acid residues in other matrix proteins as well, which makes it a likely tracer of growth patterns. According to the hypotheses, higher concentrations of P would be expected in young fish and a seasonally varying signal related to growth.

Environmental concentration: Otolith concentrations are not correlated with environmental concentrations (Campana, 1999) as hypothesized for physiological tracers.

Salinity: In larval galaxiids a positive effect of salinity explained 33% of otolith P (Hicks et al., 2010).

Ontogeny: Begg et al. (1998), the only authors who examined otolith P in relation to age albeit for stock discrimination purposes, found decreasing concentrations of otolith P with fish size, while P concentrations in four-year old *Gadus morhua* (K. Hüssy, unpublished data) are clearly increasing with fish age (Fig. 9). Contradictory patterns in adult *Helicolenus percoides* aging up to 30 years old (A. Sturrock unpublished data) suggest that there is no consistent relationship between ontogeny and otolith P.

Food and growth: No published studies document seasonal P patterns in otoliths, but in other calcified structures (fin spines) lowest P incorporation occurs during late fall/winter with a peak in spring (Stevenson and Secor, 2000). Similar patterns are evident in the illustrations from Atlantic cod and perch (Fig. 6 and 7), but not in whitefish (Fig. 8), suggesting taxon-specific differences in biomineralization and matrix synthesis rates. Pilot studies on adult *Gadus morhua* and *Helicolenus percoides* suggest that seasonal patterns in P concentration correspond to seasonal growth zones (Fig. 9) (K. Hüssy and A. Sturrock, unpublished data). The example in Fig. 9 furthermore suggests that higher otolith P occurs in faster growing fish. The little evidence available in the literature and the examples thus suggest that patterns in otolith P are linked to biomineralization-related matrix protein incorporation as hypothesized.

There are apparently no studies reporting effects of temperature, oxygen, or maturation on otolith P.

[Figure 9 here]

Case study: Zinc (Zn) and Copper (Cu)

Zinc and copper occur at low concentrations in most marine, estuarine and fresh waters. They are essential trace elements, but can be toxic to vertebrates even at low concentrations (Jarvinen and Ankley, 1999). In aquatic environments, Zn and Cu occur largely in complexes bound to dissolved organic matter and only a minor fraction occurs in the inorganic divalent form (Zn^{2+} and Cu^{2+}) (Holcombe and Andrews, 1978; Gardner and Ravenscroft, 1991; Mayer et al., 1994). The degree of complexation increases from marine to freshwater (Sylva, 1976), where particularly hard and alkaline freshwater systems have higher complexation capacity (Holcombe and Andrews, 1978; Gardner and Ravenscroft, 1991). The bio-availability of Zn and Cu depends on their occurrence as free ions that can be absorbed across the gills (Milton et al., 2000) and decreases therefore from marine to freshwater environments (Sylva, 1976). Most published studies examining the occurrence of these metals in otoliths have focused on their utility as tracers of contaminated water sources (Friedrich and Halden, 2010; Ranaldi and Gagnon, 2010).

Environmental concentration: Otolith Zn does not seem to be influenced by water concentrations in either in the laboratory (Ranaldi and Gagnon, 2008) or in the field. The latter is generally inferred from a lack of differences among otoliths obtained from sampling sites featuring variable environmental concentrations (Thorrold et al., 1997; Hanson and Zdanowicz, 1999; Milton et al., 2000; Arai et al., 2007). Similarly, otolith Cu incorporation was not related to environmental concentrations in field caught fish (Hanson and Zdanowicz, 1999; Milton et al., 2000). These studies therefore support the hypothesis that otolith Zn and Cu patterns are independent of environmental concentration. When ambient concentrations are high, however, Cu uptake and incorporation rates may increase in areas with enriched water concentration (Milton and Chenery, 2001).

Salinity: Information on the influence of salinity on otolith Zn and Cu is scarce. Hicks et al. (2010) found no relationship between otolith Zn and salinity as expected from a physiological tracer but observed a positive relationship between otolith Cu and salinity. Unfortunately, water concentrations were not reported leaving some doubt as to the underlying mechanism. In a mining area featuring high levels of water Cu contamination, Cu concentrations in the otoliths of adult *Lates calcarifer* did not rise during freshwater residency, despite the concentration of dissolved Cu in the water being several times greater than in the lower estuary and adjacent coast (Milton et al., 2000).

Ontogeny: Otolith Zn generally increases sharply from hatch, peaks during juvenile stages, then decreases steadily with distance to the core in adult fish (Papadopoulou et al., 1978; Arai et al., 2007; Ranaldi and Gagnon, 2010; Avigliano et al., 2015; Hüssy et al., 2016), supporting the hypothesis of a link between organic matrix and Zn incorporation during biomineralization. Otolith Cu seems subject to an ontogenetic pattern in some fishes (Hüssy et al., 2016). In flounders and gadoids, both Zn and Ca tend to be markedly elevated in the core region (Jackman et al., 2016; K. Limburg, M. Samson, unpublished obs.).

Food and growth: Marohn et al. (2009) found no influence of diet on otolith Zn, whereas Ranaldi and Gagnon (2008) showed that dietary Zn represented the major source of Zn to fish liver and otoliths, with no significant effect of water concentration. The importance of dietary Zn sources to fish is further suggested by the fact that food represents ca. 80 % of total Zn across different fish tissues (Willis and Sunda, 1984). Consistent with food-dominated uptake mechanisms, a number of studies on adult fish have reported seasonality in otolith Zn patterns that appears to relate to fish growth. Adult *Pleuronectes platessa* exhibited seasonal cycles in otolith Zn that correlated most strongly with temperature and water Zn concentrations, and seasonal cycles in otolith Cu that were negatively related to condition and temperature (Sturrock et al., 2015). In a range of species within the families of Salmonidae, Esocidae and Gadidae, otolith Zn correlates with visually-identified seasonal growth zones, with minimum Zn concentrations occuring during translucent winter zones (Halden et al., 2000; Halden and Friedrich, 2008; Friedrich and Halden, 2010; Limburg and Elfman, 2010) – see also Fig. 6 and 7. In other families, e.g. Percidae, this pattern is less defined (Friedrich and Halden, 2010) (Fig. 8), or

even non-existent, e.g. Osmeridae (Limburg and Elfman, 2010). These family-related differences in Zn incorporation suggests that biomineralization effects are accompanied by phylogeny-related mechanisms regulating Zn uptake and/or transport into the endolymph. Cu-enhanced diet did not lead to increased otolith Cu (Milton and Chenery, 2001).

Maturation: In adult *Pleuronectes platessa*, otolith and blood plasma Zn concentrations decreased in mature females during the spawning season and were negatively related to female gonadosomatic index (Sturrock et al., 2015), suggesting the rerouting of Zn to the ovaries during vitellogenesis, a phenomenon observed across a broad range of vertebrates (Sturrock et al. 2013).

There are apparently no studies reporting effects of temperature, or oxygen on otolith Zn or Cu.

[Table 3 here]

Conclusions and future perspective

This review has synthesized the current understanding of otolith biomineralization processes, and linked uptake and incorporation patterns of various elements to different drivers. Hypotheses for how elements are expected to behave were proposed, separating them into elements traditionally assumed to be primarily influenced by environmental concentrations vs. elements thought to be primarily under physiological control. The literature was then reviewed to seek evidence supporting or refuting these hypotheses (summarized in Table 3, expressed as responses to increasing strength of each driver). Overall, roughly equal numbers of studies that supported, did not support, or fully contradicted the posed hypotheses were observed. For some of the physiologically-regulated elements, an absence of studies examining the effects of particular drivers (e.g. salinity, oxygen) on their incorporation rates into otoliths was notable. As technologies advance, allowing researchers to measure low-concentration elements with higher confidence, performing experiments to address these knowledge gaps should be a high priority, particularly as physiological reconstructions may help

to determine sub-lethal effects of climate change and other stressors on wild fish populations.

Tracers of environmental history

The elements most frequently used to reconstruct fish environmental histories are Sr, Ba, Mn and Mg. When considering biomineralization mechanisms, these elements were hypothesized to be incorporated in equilibrium with environmental concentration, without effects of salinity, temperature, ontogeny, feeding rate, growth and maturation. Ba largely complies with these hypotheses, with some inconsistencies with respect to temperature and growth. Sr typically complies with these hypotheses in early life stages, but exhibits greater inconsistency, particularly in mature fish, that appear to relate to a combination of temperature, metabolic rate, growth and/or maturation. Some of the reported temperature effects may to some extent be attributable to the experimental setup and temperature range used. In studies covering the largest temperature ranges, the relationship between otolith element concentrations and temperature was often non-linear. This suggests that temperatures close to the upper/lower thresholds of thermal tolerance limits impose greater challenges on physiological processes, affecting element uptake, transport or biomineralization directly. Future experimental work should try to cover the full range of temperatures occurring in the species natural habitat to better describe these relationships in order to better predict species and life stage-specific responses to future climate change and other stressors.

Inconsistencies in reported element incorporation often occurred in response to fish growth. Since uptake of unbound Sr and Ba from plasma into the endolymph and the otolith is considered to be under minimal physiological control, changes their relative availability resulting from seasonal changes in protein composition or binding capacity seems a likely reason for the observed inconsistencies, but warrants further study. Physiological processes such as growth and maturation can exert a strong influence on the uptake and regulation of Ca, Sr, Mn, Cu, Zn, Se and Pb ions in fish blood plasma (Sturrock et al., 2014), while otolith Sr, Zn (and potentially Se and Cu) may relate to reproductive investment (Sturrock et al., 2015). Further experiments to examine element sources and transport pathways across a range of species, systems, life stages, and phenotypes will be important if researchers are to separate physiological and environmental signals in otolith chemistry and to realize the many potential applications that this unique biomineral may offer. In

particular, a better understanding of the factors influencing blood chemistry (e.g. ion uptake, excretion, re-routing, recycling) and elemental availability (e.g. protein concentrations, types, and binding capacities) will help to shed light on many of the inconsistencies among studies. Given the clear potential for ontogeny, growth and maturation to influence element uptake and processing in fish, it is important to try to use comparable phenotypes (e.g. fish of similar age, sex, size) and to cohort-match wherever possible when comparing patterns from the laboratory and field.

Incorporation of Mn and Mg into otoliths, which are often considered as tracers of environmental history, followed hypothesized patterns only to a limited degree, indicative of considerable physiological control on uptake mechanisms.

Tracers of physiology

Elements proposed as tracers of physiology and growth are essential co-factors in many metalloenzymes and needed for a range of physiological processes, including otolith biomineralization. Potential candidates are Zn, Cu, and P – and as this review suggests, also Mg. When considering biomineralization mechanisms, the incorporation of these elements into the otolith organic matrix is hypothesized to be largely independent of environmental concentrations, to decrease with fish age and temperature, and increase with feeding rate and growth. Knowledge of the organic matrix's composition, the interactions of the different molecules, their element binding capacity, and importance for biomineralization has grown rapidly in recent years. Unfortunately, this group of elements has to date received limited attention in the literature; therefore, not all hypotheses could be tested, but where available, patterns of Mg, Zn, Cu and P typically seem to conform to these hypotheses. Mg seems to behave like a growth tracer in its response to all well-documented drivers (environmental concentration, ontogeny, feeding and growth), except for temperature. Future studies should revisit the role of Mg in the synthesis of matrix constituents and the biomineralization process - and their respective link to somatic growth. As tracers of growth, elements like Mg, Zn, Cu and P may provide a wealth of information, particularly in species and stocks where such information is not available from visual growth zones. Future studies focusing on quantifying the impact of physiological processes such as growth, maturation, stress etc. on otolith matrix protein synthesis and elemental composition are therefore strongly

recommended. Development of modelling tools able to combine biomineralization processes and experimental results (e.g. Fablet et al., 2011) should be considered to improve the understanding of biomineralization, to better correct for introduced or lost signals relating to instrument performance, and to enhance the scientists ability to correctly interpret elemental signals in the otoliths of wild fishes.

Tracers of environment and physiology

Mounting evidence suggests that most otolith trace elements are to varying extent affected by both exogenous and endogenous drivers, and that patterns of incorporation are explained by a combination of both. As a paradigm of this new thinking, manganese appears to be sensitive to both external (environmental) concentrations and internal (physiological) regulation, behaving like an environmental tracer with respect to environmental concentrations and a growth tracer with respect to ontogeny and feeding rate and growth (Table 3). While Mn may substitute for Ca in the aragonite lattice, its importance as a co-factor in different kinases (Thomas and Swearer, 2019) presumably results in a growth signal in otolith Mn concentrations. Future experiments should take into account the form of Mn made available and ambient redox potential (in addition to concentration, temperature, and salinity), and more work is needed to understand and separate out phylogenetic, trophic, and growth effects. And in general, process-based models (cf. Fablet et al., 2011; Limburg and Casini, 2018) should incorporate both internal (e.g. bioenergetics) and external (biogeochemical) drivers.

Summary

This review has highlighted that biomineralization does not, as suggested in the literature, exert a strong influence on the incorporation of otolith elements that exclusively substitute for Ca, largely supporting their use as tracers of environmental concentrations and fish movements. On the other hand, for elements reported as being "under physiological control" in the literature, biomineralization has a profound impact on element incorporation. Upstream processes, in particular uptake of elements into the plasma, transport across the endolymphatic epithelium, and endolymph composition, may have a much stronger impact on their incorporation into the otolith and thus need to be a focus of future studies examining element incorporation

patterns.

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Data availability statement

This work has not produced any data.

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Figure captions

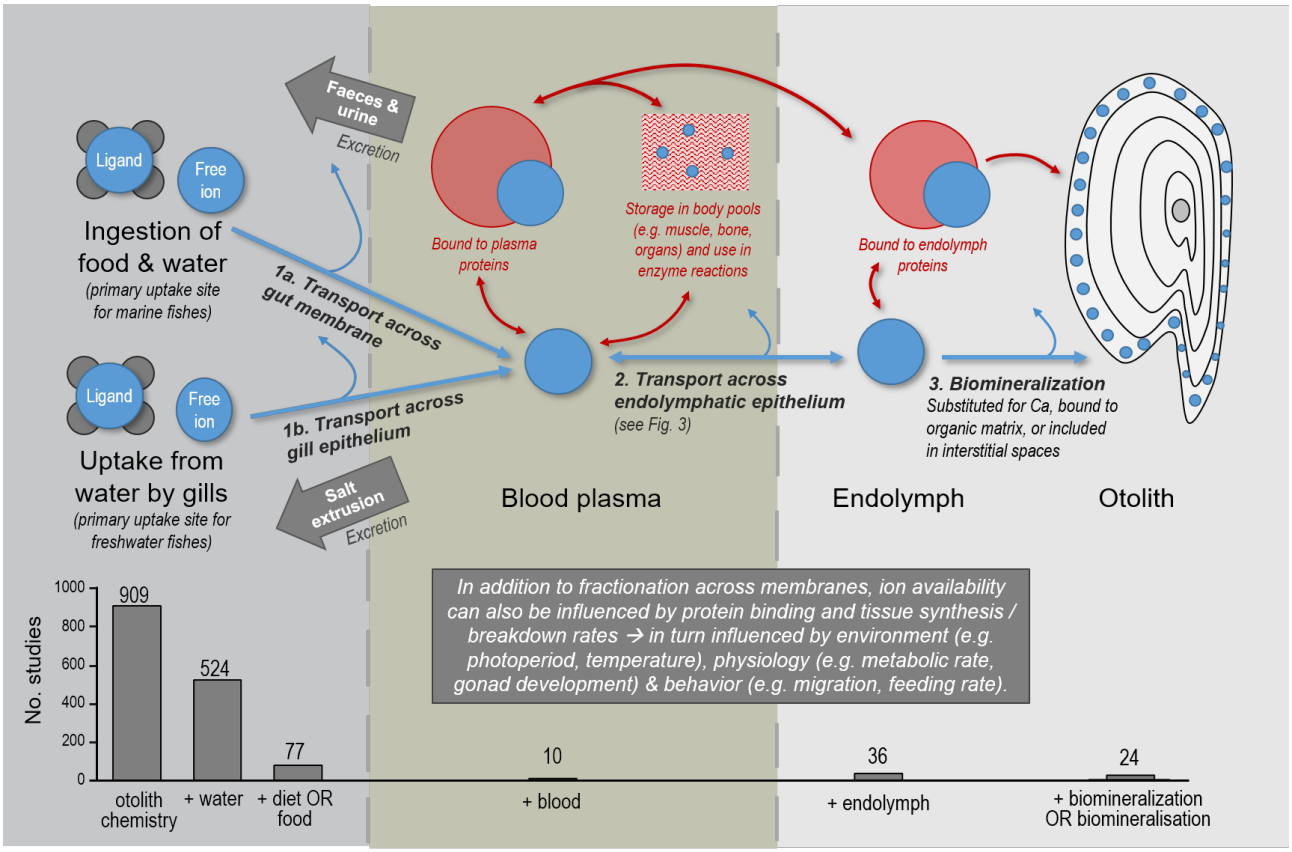

Fig. 1. Schematic representation of the main ion pathways into an otolith, primarily involving uptake across gill and gut membranes into the blood plasma, transport across the endolymphatic epithelium into the endolymph, then incorporation into the growing crystal lattice of the otolith (adapted from Campana 1999). Discrimination or active uptake can alter ion concentrations across each of these interfaces, while protein binding and re-distribution among body pools can alter their availability within the fish. The bars at the bottom show the results of a Web of Science search performed Dec 10th 2019 (Topic = "otolith chemistry", "otolith chemistry water", etc), that highlighted the scarcity of otolith studies focusing on element processing within the fish, with most studies focused on uptake mechanisms and the relationship between otolith and ambient concentrations.

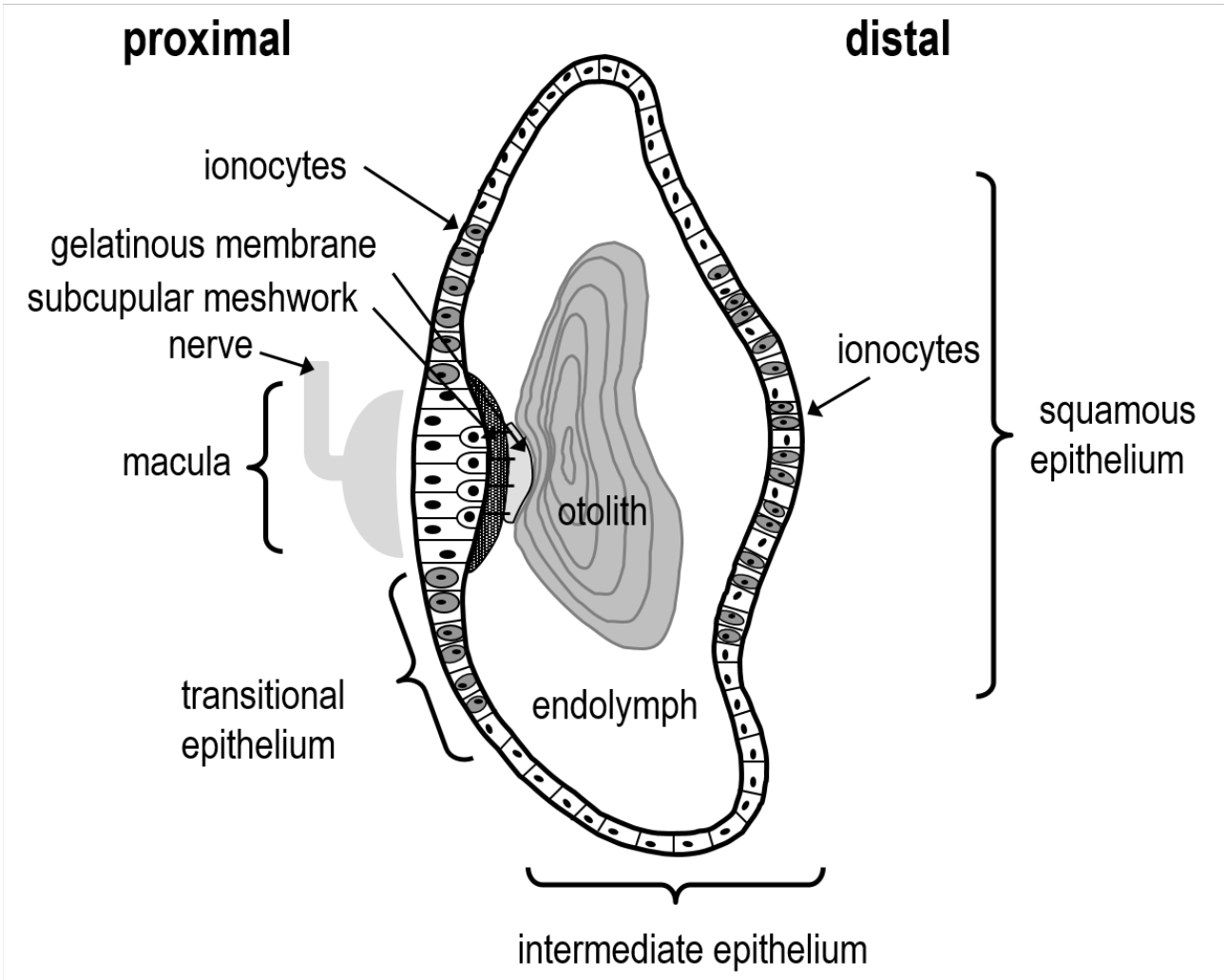

Fig. 2. Schematic model of a cross section across otolith and endolymphatic epithelium, representing different cell types and their spatial distribution patterns within the epithelium (modified from Saitoh and Yamada (1989), Mayer-Gostan et al. (1997), Pisam et al. (1998)).

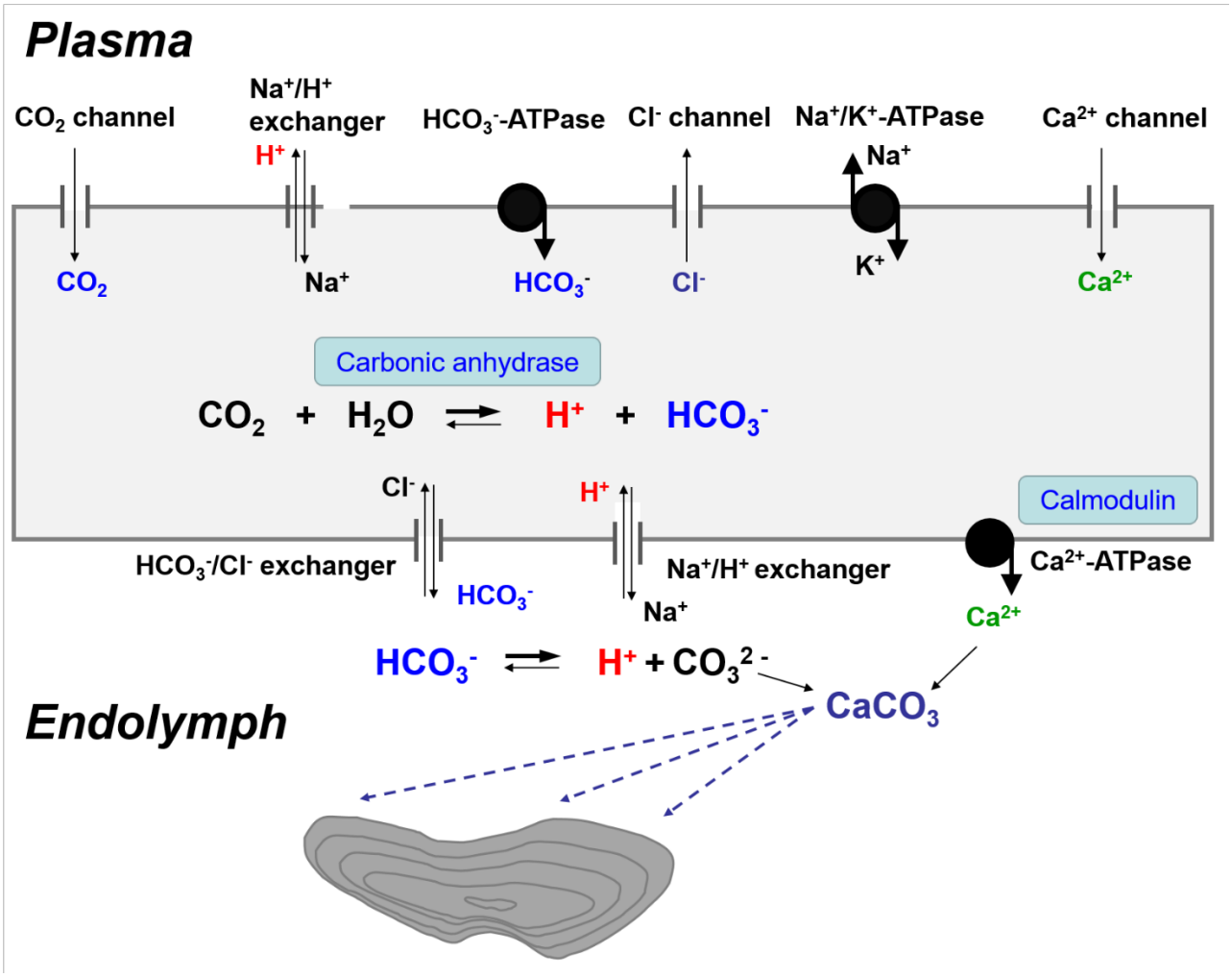

Fig. 3. Schematic representation of processes involved in transporting Ca^{2+} ions and CO_3^{2-} across the endolymphatic epithelium from plasma into the endolymph (modified from Mayer-Gostan et al. (1997), Payan et al. (2004), Tohse et al. (2006) and Tohse and Mugiya (2001)).

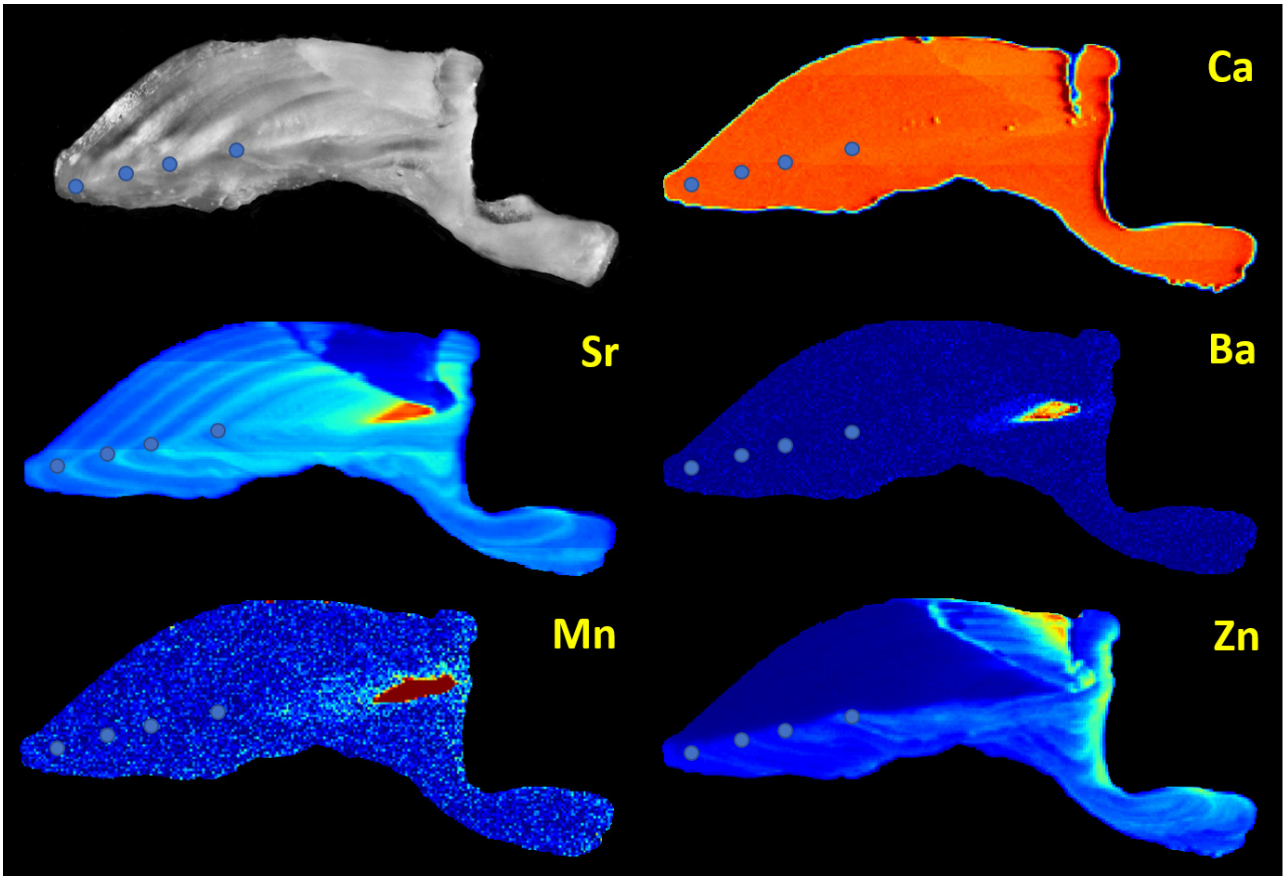

Fig. 4. Optical image of otolith viewed under reflected light and 2D maps of Ca, Sr, Ba, Mn and Zn of *Arapaima* sp. (160 cm standard length) was collected in November 2008 by C. Watson and D.J. Stewart from Inkapati Head Pond near Apoteri, adjacent to the Essequibo River mainstream in Guyana (4°7.027 N, 58°29.531 W). The optical image shows transversal section through the sagitta viewed under reflected light, sulcus at the top of the image, ventral axis on the left. Shading indicates element concentration, ranging from low (dark blue) to high (bright red) and blue dots indicate visually identified translucent zones. Arapaima is an air-breathing freshwater species. This individual likely spent its juvenile stage in a hypoxic and/or acidic nursery area featuring elevated Sr and Ba water concentrations, and its adult life in rivers and lakes. . The collection area features complex bedrock geology, but the stressful rearing conditions may have also influenced element incorporation in the otolith core area. Analyzed by Scanning X-Ray Fluorescence Microscopy at the Cornell High Energy Synchrotron Source (Photo and elemental maps: K. Limburg)

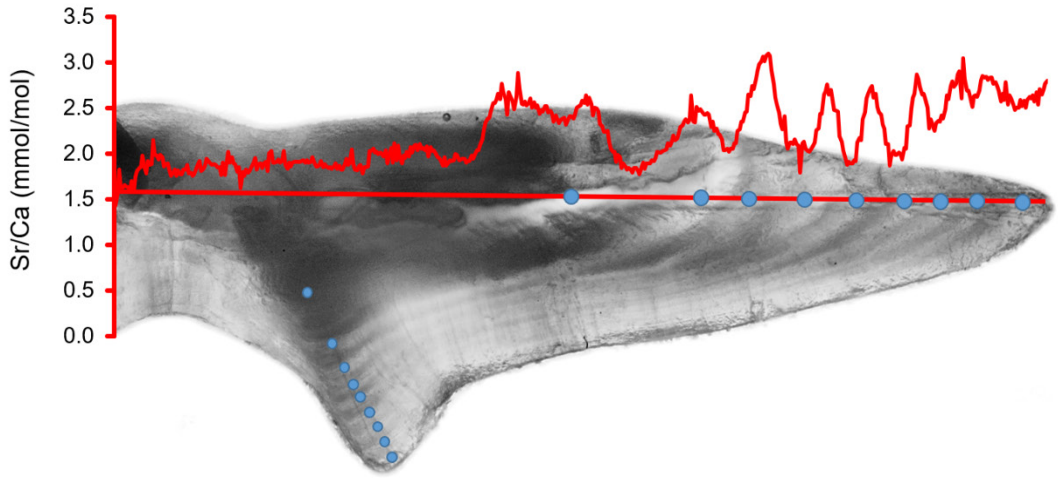

Fig. 5: Sagittal otolith and Sr/Ca profile of a 9-year old adult male sea perch (*Helicolenus percoides*), 30 cm long and weighing 486 g, caught in February 2007 from 40m deep at the Akatore Pinnacle, New Zealand (46°09'37"S, 170°20'52" E). The transverse section was imaged under transmitted light. Blue dots indicate visually-identified translucent winter growth zones. Sea perch are benthic, do not undertake extensive movements, and are exclusively marine, experiencing salinities > 30 psu across their entire lifetime. Analyzed by line scan on an Agilent 7500 laser ablation-inductively coupled plasma mass spectrometer at the Research School of Earth Sciences, Australian National University, Canberra. (Photo and elemental profile: A. Sturrock)

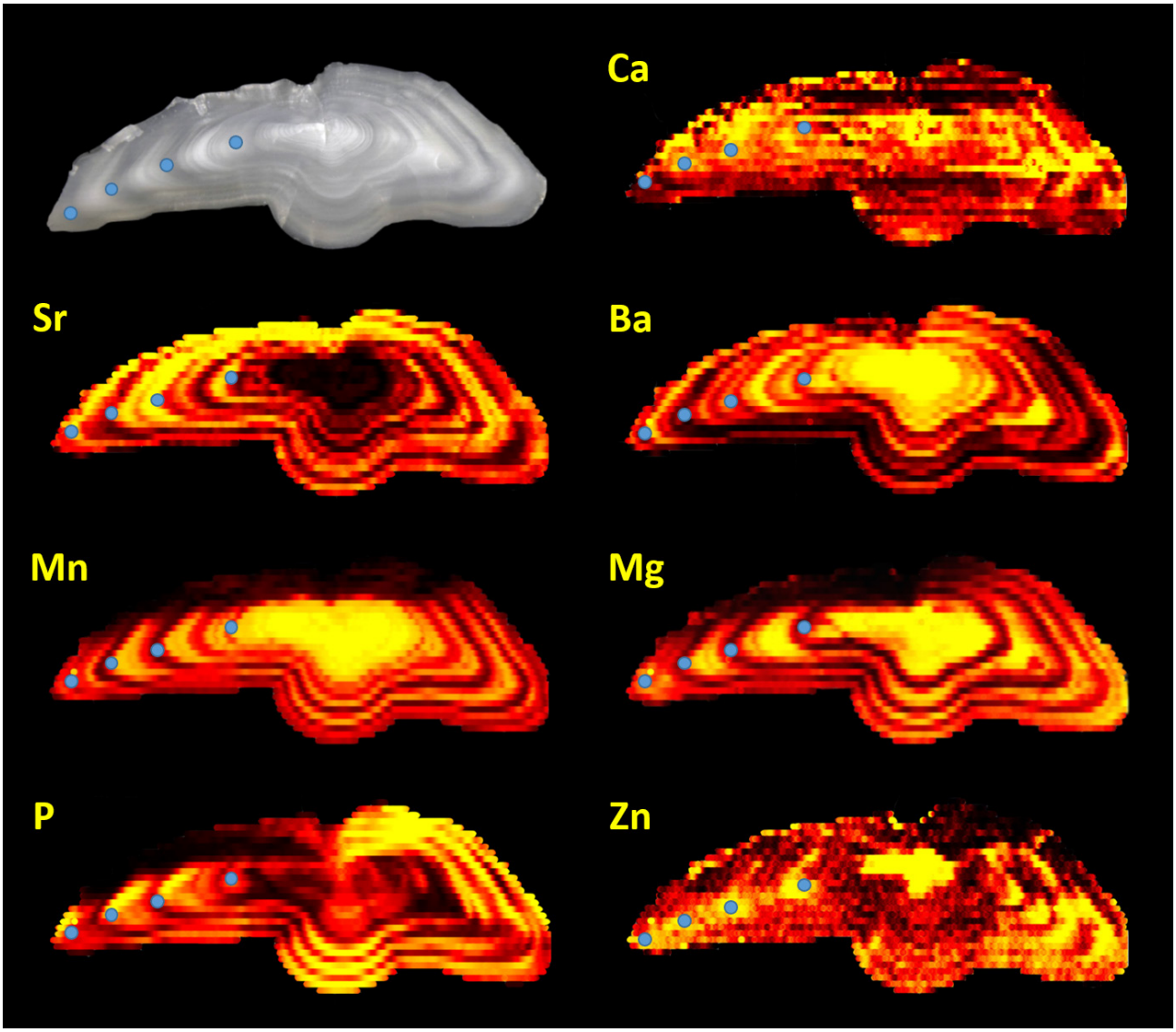

Fig. 6: Optical image of sagittal otolith and 2D map of Ca, Sr, Ba, Mn, Mg, P, and Zn of an Atlantic cod (*Gadus morhua*), a 4-year old male, 56 cm long and weighing 1952 g, caught in February 2005 in the Kattegat (57°10 N, 11°30.5 E). Image shows transverse section with the sulcus at the top and dorsal axis on the left viewed under reflected light. Blue dots indicate visually-identified translucent winter growth zones. Shading indicates element concentration, ranging from low (dark brown) to high (yellow). Juvenile cod in the Kattegat inhabit shallow, nearshore waters characterized by salinities of 15 - 25 psu and frequent hypoxic conditions then move further offshore with age, where they experience salinities of > 30 psu. Adults perform seasonal migrations between coastal feeding areas and offshore spawning areas. Analyzed at the Analyte G2 Excimer Laser Ablation System at Lund University, Sweden. (Photo and elemental maps: Y. Heimbrand)

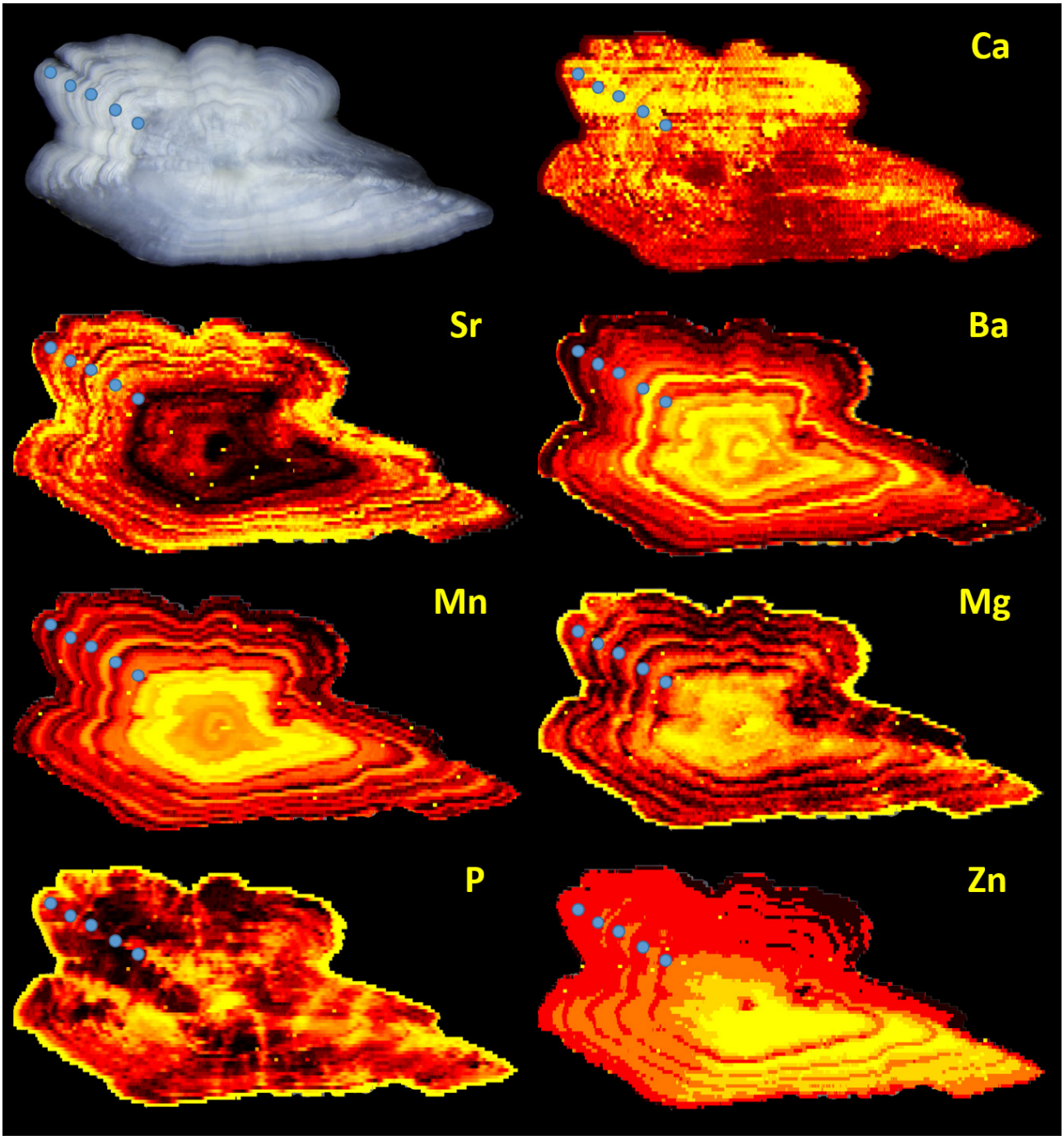

Fig. 7: Optical image of sagittal otolith and 2D map of Sr, Ba, Mn, Mg and Zn of a whitefish (*Coregonus maraena*). The whitefish was a 5-year old male, 24.8 cm long and weighing 133 g, caught in the northern Bothnian Bay (Baltic Sea, 65°15 N, 21°44 E) in 2016. Image shows sagittal section with the rostrum to the right viewed under reflected light. Blue dots indicate visually-identified translucent winter growth zones. Shading indicates element concentration, ranging from low (dark brown) to high (yellow). There are two different ecotypes of whitefish in the Bothnian Bay, river-spawners and sea-spawners. River-spawners (such as the example shown here) migrate up rivers to spawn and spend their first time in freshwater before migrating out to sea; conversely, sea-spawners spend their entire lives at sea. Analyzed at the Analyte G2 Excimer Laser Ablation System at Lund University, Sweden. (Photo and elemental maps: M. Blass)

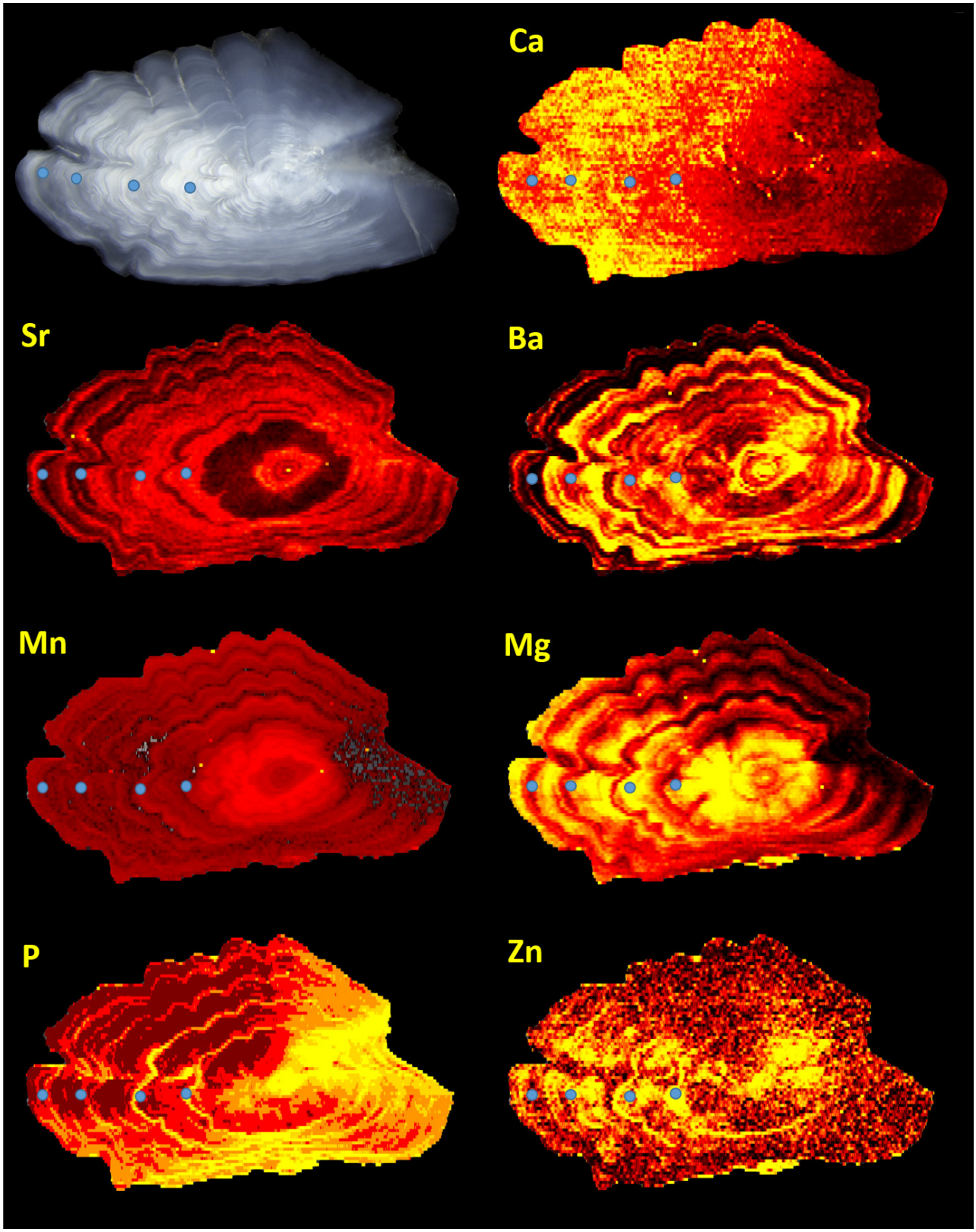

Fig. 8: Optical image of sagittal otolith and 2D map of Sr, Ba, Mn, Mg and Zn of a Baltic perch (*Perca fluviatilis*). The perch was a 4-year old female, 26.1 cm long and weighing 212 g, caught in the northern Bothnian Bay (Baltic Sea, 65°03 N, 21°31 E) in 2007. Image shows sagittal section with the rostrum to the right viewed under reflected light. Blue dots indicate visually-identified translucent winter growth zones. Shading indicates element concentration, ranging from low (dark brown) to high (yellow). Baltic perch are

distributed in distinct and resident populations throughout the coast of the Bothnian Bay, spawning either at the coast or a short distance upstream in rivers. This individual spent its juvenile phase in freshwater then migrated to the coast as an adult, carrying out annual spawning migrations between river and coastal habitats after reaching sexual maturity. Analyzed at the Analyte G2 Excimer Laser Ablation System at Lund University, Sweden. (Photo and elemental maps: M. Blass)

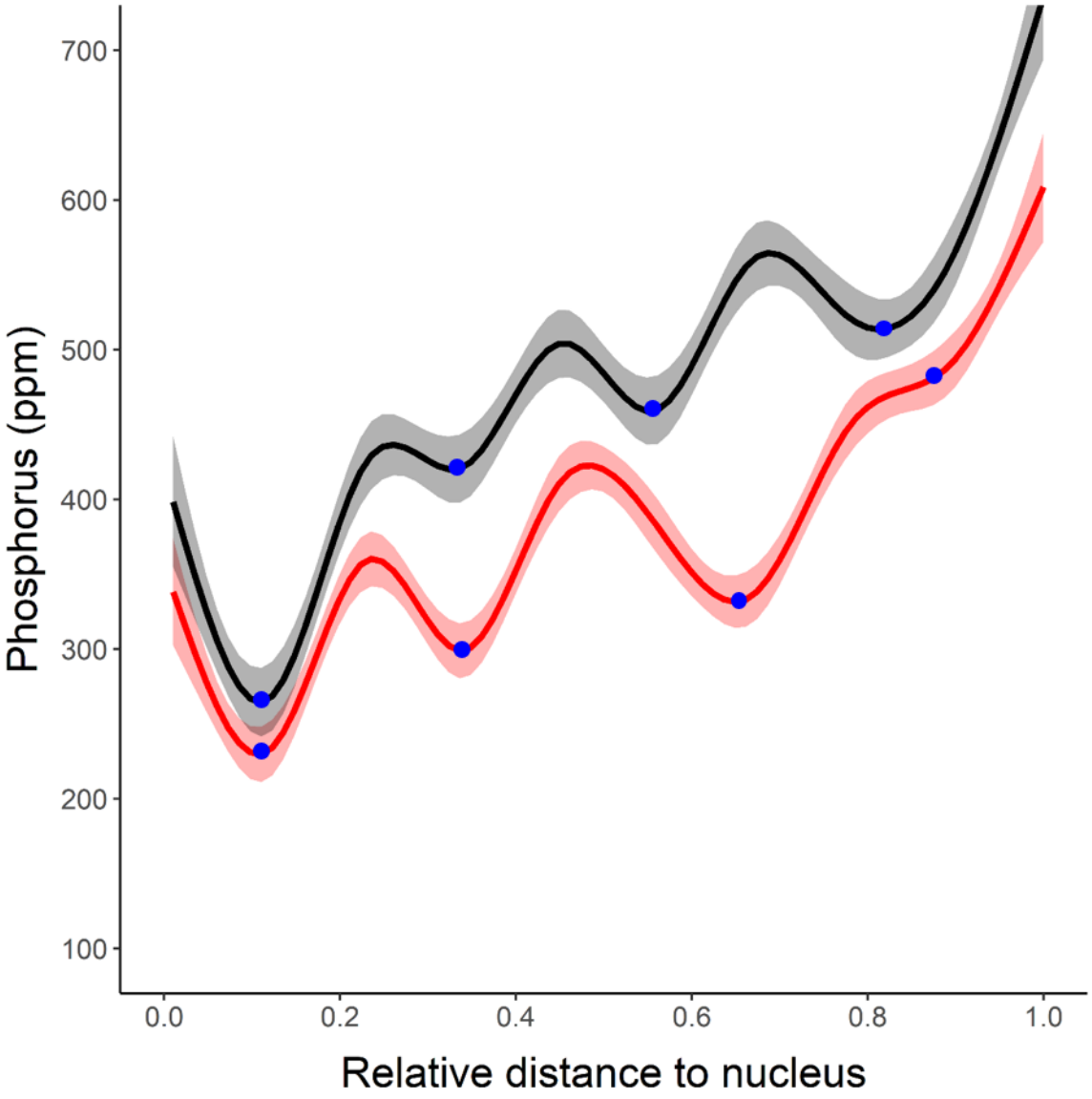

Fig. 9. Phosphorus profiles from the nucleus to the edge of 4-year old Atlantic cod from the Kattegat (Denmark) sampled in December 2016. Good growth (black): The 5 individuals with the largest size at age 4 (78.0 ± 3.0 cm) out of 30 individuals; Poor growth (red): The 5 individuals with the smallest size at age 4 (51.1 ± 4.4 cm). Profile lengths were standardized by dividing the distance of each measurement by the total length of the profile. Blue dots identified profile minima that correspond to visually identified translucent winter growth zones. Values shown are Loess-smoothed mean ppm with confidence intervals.

Table 1 Summary of processes affecting otolith biomineralization.

	Process	Direction	CaCO3	Organic matrix	Ca:matrix ratio
Extrinsic	**Salinity**	increasing	no effect [2]	n/a	n/a
	Temperature	increasing	increasing	decreasing	increasing
	Oxygen	increasing	increasing	n/a	n/a
Intrinsic	**Ontogeny** [1]	increasing	decreasing	decreasing	increasing [2]
	Growth/food	increasing	increasing [2]	increasing	decreasing [2]
	Maturation	present	decreasing [2]	decreasing [2]	increasing [2]

n/a indicates that no information is available in the literature

[1] Refers to the fish's entire lifespan

[2] Inferred from visual appearance of otolith and otolith growth measurements, but not quantification of matrix and $CaCO_3$

Table 2 Overview of studies referred to in this review, including which species, life stages and elements were analyzed, and whether they were based on laboratory experiments or field samples. Drivers examined: Environmental concentrations (E), salinity (S), temperature (T), ontogeny (O), food and growth (F/G), and oxygen (O_2), with salinity and temperature levels examined in brackets.

Reference	**Species**	**Life stage**	**Elements**	**Drivers**
Altenritter et al. (2018) [f]	*Micropogonias undulatus*	Juveniles	Ba, Mn	E
Avigliano et al. (2015) [f]	*Percophis brasiliensis*	Adults	Sr, Zn	O
Barnes and Gillanders (2013) [e]	*Argyrosomus japonicus*	Juveniles	Sr, Ba, Mg	S (10-30-40-50), T (16-20-24)
Bath et al. (2000) [e]	*Leiostomus xanthurus*	Larvae	Sr, Ba	E, T (20-25), F/G
Begg et al. (1998) [f]	*Scomberomorus queenslandicus, Scomberomorus munroi*	Sub-adults	Sr, Ba, Mn, Mg, Li, Fe, Na, P, S	ontogeny
Brown and Severin (2009) [f]	81 freshwater, diadromous and marine species	Adults	Sr	S (ambient)
Buckel et al. (2004) [e]	*Pomatomus saltatrix*	Juveniles	Sr, Ba, Mn, Mg, Na, K	F/G
Clarke and Friedland (2004) [fe]	*Salmo salar*	Adults	Sr	T (1.5-12), O, F/G
Clarke et al. (2011) [e]	*Menidia menidia*	Juveniles	Sr, Ba, Mn, Mg	T (15-21-27), F/G, O
de Pontual et al. (2003) [f,e]	*Solea solea*	Juveniles	Sr, Na, K	S (ambient, experimental 33-34), O
DeVries et al. (2002) [e]	*Acanthopagrus butcheri*	Juveniles	Sr, Ba	E
DiMaria et al. (2010) [e]	*Gadus macrocephalus*	Larvae	Sr, Ba, Mg	T (2-5-8), F/G
Dorval et al. (2007) [f]	*Micropogonias undulatus*	Juveniles	Sr, Ba, Mn, Mg, In, La	E, S (ambient 17 to 22), T (ambient 13 to 30)
Elsdon and Gillanders (2002) [e]	*Acanthopagrus butcheri*	Juveniles	Sr, Ba, Mn, Mg	E, S (5-17-30), T (12-16-20-24-28)
Elsdon and Gillanders (2003) [e]	*Acanthopagrus butcheri*	Juveniles	Sr, Ba, Mn	E
Elsdon and Gillanders (2004) [e]	*Acanthopagrus butcheri*	Juveniles	Sr, Ba	E, S (5-32), T (17-26)
Elsdon and Gillanders (2005b) [f]	*Acanthopagrus butcheri*	Juveniles, adults	Sr, Ba	E, S (ambient 2 to 35), T (ambient 17 to 27), O
Elsdon and Gillanders (2005a) [f]	*Acanthopagrus butcheri*	Adults	Ba	S (ambient 2 to 35), O
Forrester (2005) [f]	*Gillichthys mirabilis*	Juveniles	Mn, Cu	E
Fowler et al. (1995) [e]	*Microspogonias undukatus*	Juveniles	Sr, Ba, Mn, Mg, Zn, Cu, Na, Fe, Ni, Co, Rb, B	O

Fowler et al. (2005) [f]	*Pagrus auratus*	Adults	Sr, Ba	O
Friedrich and Halden (2010) [f]	*Esox Lucius, Sander vitreus*	Adults	Sr, Ba, Mn, Zn, Cu, Ni, C, Pb	F/G, O
Gallahar and Kingsford (1996) [e]	*Girella elevata*	Juveniles	Sr	T (18-29)
Grammer et al. (2017) [f]	*Helicolenus percoides*	Adults	Sr, Ba, Mg, Na, Li	O
Halden et al. (2000) [f]	*Salvelinus alpinus*	Adults	Sr, Zn	F/G
Halden and Friedrich (2008) [f]	*Salvelinus alpinus, Coregonus clupeaformis, Salvelinus namaycush, Esox lucius, Stizostedioan vitreum, Catostomus Commersoni, Coregonus alpenae, Oncorhynchus mykis*	Adults	Sr, Ba, Mn, Zn, Na, Li, Rb, Cs	F/G
Hamer and Jenkins (2007) [f]	*Pagrus auratus, Platycephalus bassensis*	Juveniles	Sr, Ba, Mn, Mg	F/G
Hamer et al. (2006) [f]	*Pagrus auratus*	Adults	Sr, Ba, Mg	E
Hanson and Zdanowicz (1999) [f]	*Micropogonias undulatus*	Juveniles	Sr, Ba, Mg, Na, K, Li, Rb, Ga	E
Hicks et al. (2010) [e]	*Galaxias maculatus, Galaxias argenteus*	Larvae	Sr, Ba, Mn, Mg, P, Zn, Cu, S, Li, B, Al, Rb, Pb	E, S (2-5-10-20-34)
Hoff and Fuiman (1995) [e]	*Sciaenops ocellatus*	Juveniles	Sr, Mg, Na, K	E, S (10-30-32-38-40), T (21-23-27-30-34), F/G
Hughes et al. (2016) [f]	*Arripis trutta*	Adults	Sr, Ba, Mn, Mg, Na, Li	O
Jessop et al. (2002) [f]	*Anguilla rostrata*	Late juveniles	Sr	O
Jessop et al. (2008) [f]	*Anguilla rostrata*	Adults	Sr	O
Kalish (1989) [e]	*Arripis trutta, Macruronus novaezelandiae*	Juveniles	Sr, Na, K, S	E, T (13-16-19-22), O, F/G
Kennedy et al. (2002) [f]	*Salmo salar*	Adults	Sr	E
Kraus and Secor (2004) [e]	*Morone americana*	Juveniles	Sr	E
Limburg and Elfman (2010) [f]	*Salmo salar, Coregonus lavaretys, Esox Lucius, Osmerus eperlanus*	Adults	Zn	F/G
Limburg et al. (2015) [f]	*Platichthys flesus, Gadus morhua, Perca flavescens, Micropogonias undulatus*	Adults	Sr, Mn	E, O_2, O

Limburg et al. (2018) [f]	*Platichthys flesus, Gadus morhua, Reinhardtius hippoglossoides*	Juveniles, adults	Mg	T (ambient), F/G, O
Lin et al. (2007) [e]	*Anguilla japonica*	Juveniles	Sr	S (0-5-15-25-35), F/G
Marohn et al. (2009) [e]	*Anguilla anguilla*	Juveniles	Sr, Ba, Mn, Mg, Zn, Cu, Na, Rb	E, F/G
Martin and Thorrold (2005) [e]	*Leiostomus xanthurus*	Larvae, juveniles	Ba, Mn, Mg	E, S (15-25), T (18-20-23-26), F/G
Martin and Wuenschel, 2006) [e](	*Lutjanus griseus*	Juveniles	Sr, Ba, Mn, Mg	S (5-15-25-35-45), T (18-23-28-33)
Mazloumi et al. (2017) [e]	*Sillaginodes punctatus*	Juveniles	Sr, Ba, Mn, Mg	S (30-40), T (16-19-22-25)
Martino et al. (2017) [e]	*Lates calcarifer*	Juveniles	Sr, Ba, Mn, Mg, Li, B	T (26-30-34), CO_2
Miller (2011) [e]	*Oncorhynchus tshawytscha*	Juveniles	Sr, Ba, Mg	E, S (0-5-10-14), T (9-12-15), F/G
Miller (2009) [e]	*Sebastes melanops*	Juveniles	Ba, Mn	E, T (7.4-13), O
Milton and Chenery (2001) [e]	*Lates calcarifer*	Juveniles	Sr, Cu, Pb	E, F/G
Milton et al. (2000) [f]	*Lates calcarifer*	Adults	Sr, Ba, Mn, Mg, Zn, Cu, Li, Rb, Pb	E, S (ambient)
Mohan et al. (2012) [fe]	*Morone saxatilis*	Juveniles	Sr, Ba, Mn, Mg	E
Mohan et al. (2014) [e]	*Micropogonias undulatus*	Juveniles	Sr, Ba, Mn, Mg, Na	O_2
Mohan and Walther (2016) [f]	*Micropogonias undulatus*	Juveniles	Ba, Mn	E, S (ambient 32 to 36), O_2
Morales-Nin et al. (2005) [f]	*Merluccius merluccius*	Adults	Sr, Ba, Mg, Mn, Zn, Rb, Pb	O
Payne Wynne et al. (2015) [f]	*Alosa aestivalis*	Adults	Sr, Ba, Mn	E, S (ambient 0 to 35), O
Papadopoulou et al. (1978) [f]	*Scomber japonicus colias*	Adults	Zn	ontogeny
Ranaldi and Gagnon (2008b) [f]	*Acanthopagrus butcheri*	Adults	Ba, Mn, Zn, Al, Cd	E, F/G, O
Ranaldi and Gagnon (2008a) [e]	*Pagrus auratus*	Juveniles	Zn	E
Reis-Santos et al. (2013) [e]	*Dicentrarchus labrax*	Juveniles	Sr, Ba	E, S (10-20-30), T (21-25)
Sadovy and Severin (1992) [f]	*Haemulon, pulmieri*	Adults	Sr	O, F/G
Sadovy and Severin (1994) [f]	*Epinephelus guftatus*	Adults	Sr	F/G
Secor and Piccoli (1996) [f]	*Murone saxatilis*	Adults	Sr	O
Secor and Rooker (2000) [f]	*Morone saxatilis*	Adults	Sr	O
Secor et al. (1995) [e]	*Morone saxatilis*	Juveniles	Sr	S (0-5-10-15-20-30), T (15-25), F/G

Siskey et al. (2016) [f]	*Thunnus thynnus*	Adults	Sr	G, O
Stanley et al. (2015) [e]	*Gadus morhua*	Juveniles	Sr, Ba, Mn, Mg	S (25-28.5-32), T (5-8.5-12), F/G
Sturrock et al. (2015) [fe]	*Pleuronectes platessa*	Adults	Sr, Ba, Mn, Mg, Zn, Cu, K, Li, Rb, Se, Pb	E, S (30-35), T (4-15), F/G
Thorrold et al. (1997) [f]	*Micropogonias undulatus*	Juveniles	Sr, Ba, Mg, Zn	E
Townsend et al. (1992)	*Clupea harengus*	Juveniles	Sr	T (ambient 2 to 17)
Tzeng (1996) [e]	*Anguilla japonica*	Juveniles	Sr	S (0-10-25-35), T (23-28)
Walther and Thorrold (2006) [e]	*Fundulus heteroclitus*	Juveniles	Sr, Ba	F/G
Walther et al. (2010) [e]	*Acanthochromis polyacanthus*	Adults	Sr, Ba	T (26-28-31), F/G
Willis and Sunda (1984) [e]	*Gambusia affinis, Leiostomus xanthurus*	Adults	Zn	F/G
Woodcock et al. (2012) [e]	*Bidyanus bidyanus*	Juveniles	Mg	E, F/G
Zimmerman (2005) [e]	*Oncorhynchus tshawytscha, Oncorhynchus kisutch, Oncorhynchus nerka, Oncorhynchus mykiss, Salvelinus alpinus*	Juveniles	Sr	S (0.1-6.3-12.7-18.6-25.5)

[e] Laboratory experiments
[f] Field samples
[fe] Field experiment

Table 3 Predicted relationships between bio-physical drivers and otolith element:calcium ratios (E:Ca) for tracers of water chemistry and growth (B), based on their effects on otolith biomineralization (Table 1).

A. Environmental tracers

	Driver	Predicted relationship	Sr	Ba	Mn	Mg	P	Zn & Cu
Extrinsic	Environmental conc.	Positive	positive	positive	positive	none	none	none
	Salinity	None	none	none	none	none	n/a	n/a
	Temperature	None	none	contradictory	contradictory	contradictory	n/a	n/a
	Oxygen	None	none [d]	none [d]	indirect [a]	none [d]	n/a	n/a
Intrinsic	Ontogeny [b]	None	contradictory	none	negative	negative	contradictory	negative
	Food & growth	None	contradictory	contradictory	contradictory	positive	positive [c]	positive [c]
	Maturation	None	contradictory	none [d]	none [d]	none [d]	n/a	negative (Zn) [d]

B. Tracers of growth

	Driver	Predicted relationship	Sr	Ba	Mn	Mg	P	Zn & Cu
Extrinsic	Environmental conc.	None	positive	positive	positive	none	none	none
	Salinity	- [e]	n/a	n/a	n/a	n/a	n/a	n/a
	Temperature	Negative	none	contradictory	contradictory	contradictory	n/a	n/a
	Oxygen	- [e]	n/a	n/a	n/a	n/a	n/a	n/a
Intrinsic	Ontogeny [b]	Negative	contradictory	none	negative	negative	contradictory	negative
	Food & growth	Positive	contradictory	contradictory	contradictory	positive	positive [c]	positive [c]
	Maturation	None	contradictory	none [d]	none [d]	none [d]	n/a	negative (Zn) [d]

The predicted response of elemental uptake is indicated as a function of increasing driver strength. This table also summarizes the responses observed in the literature (Table 2), and the extent to which they support or refute our hypotheses (green: support, shaded green: mostly support, red: refute, orange: contradictions among studies, white: few or no studies, 'n/a' represents examples with too few studies to form or test predictions). A lack of support indicates either that the element in question is a poor tracer of water chemistry or growth, or that biomineralization alone cannot explain the observed patterns, indicating the importance of upstream processes such as uptake rates and protein binding.

[a] Indirect effect: Decreased oxygen liberates Mn^{2+} from sediments, thereby increasing water concentrations

[b] Refers to the fish's entire lifespan

[c] Correspondence between elemental patterns and visual otolith growth patterns

[d] Only a single study available

[e] Too few studies to make predictions