

1 *Running head:* Otolith elemental chemistry in marine fish

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4 CAN OTOLITH ELEMENTAL CHEMISTRY RETROSPECTIVELY TRACK

5 MIGRATIONS IN FULLY MARINE FISH?

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Abstract

Otolith microchemistry can provide valuable information about stock structure and mixing patterns of fully marine fish when the magnitude of environmental differences among areas is greater than the cumulative influence of any „vital effects“. Here, the current understanding of the underlying mechanisms governing element incorporation into the otolith is reviewed. „Hard and soft acid and base“ (HSAB) theory is employed to explore the differences in chemical behaviours, distributions and affinities between elements. Hard acid cations (e.g. Mg^{2+} , Li^+ , Ba^{2+}) tend to be less physiologically influenced and accepted more readily into the otolith crystal lattice but are relatively homogeneous in seawater. Soft acid cations (e.g. Zn^{2+} , Cu^{2+}) on the other hand, exhibit more varied distributions in seawater, but are more likely to be bound to blood proteins and less available for uptake into the otolith. The factors influencing the geographical distribution of elements in the sea, and their incorporation into the otoliths of fully marine fish are reviewed. Particular emphasis is placed on examining physiological processes, including gonad development, on the uptake of elements commonly used in population studies, notably Sr. Finally, case studies are presented that either directly or indirectly compare population structuring or movements inferred by otolith elemental fingerprints with the patterns indicated by additional, alternative proxies. The main obstacle currently limiting the application of otolith elemental microchemistry to infer movements of marine fish appears to lie in the largely homogeneous distribution of those elements most reliably measured in the otolith. Evolving technologies will improve the discriminatory power of otolith chemistry by allowing measurement of spatially explicit, low level elements;

48 however, for the time being, the combination of otolith minor and trace element
49 fingerprints with alternative proxies and (or) stable isotopic ratios can greatly extend
50 the scope of migration studies. Among the otolith elements that routinely occur above
51 instrument detection limits, Ba, Mn and Li are the most likely to prove reliable
52 geographic markers in fully marine species.

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58 KEY WORDS: microchemistry; chemical fingerprint; geolocation; natural tag;
59 movement; trace metals

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INTRODUCTION

An understanding of the spatial structure of fish stocks and the connectivity within and between them is increasingly considered an important pre-requisite for sustainable fisheries management (Pulliam, 1988; Botsford *et al.*, 2009). The concept of “open” marine fish populations with individuals evenly distributed about a homogeneous environment is now largely redundant. Rather, marine habitats and their inhabitants are known to exhibit patchy distributions as a result of spatial and temporal shifts in physical (e.g. hydrodynamics, temperature, salinity) and biological (e.g. productivity, predator abundance) characteristics (Hixon *et al.*, 2002). Identifying such „patches“ is particularly challenging in the open ocean, given its vast, three-dimensional structure and scarcity of geographically and temporally stable boundaries. However, advances in satellite telemetry and remote sensing have greatly increased the sophistication with which water masses and their inhabitants can be linked (Sims *et al.*, 2006) and improved our understanding of the migratory behaviour of individual fish (e.g. Righton *et al.*, 2010; Block *et al.*, 2011).

Historically, catch data and mark-recapture experiments have formed the foundation of our broad understanding of marine fish distributions. However, the resolution of such data tends to be relatively coarse, with patterns inextricably linked to the distribution of the fishing fleets (e.g. Bolle *et al.*, 2005; Righton *et al.*, 2010). The use of pop-up satellite archival tags (PSATs) and electronic data storage tags (DSTs) has increased dramatically over the past decade, with improved

miniaturisation and recording capacity allowing sometimes multi-year recordings of the ambient experience of individual fish (Metcalf & Arnold, 1997; Hunter *et al.*, 2003; Metcalfe *et al.*, 2006; Block *et al.*, 2011). These new technologies have provided exciting new insights into the migratory behaviours and mechanisms that underpin population structuring. Their use, however, is often precluded by cost, battery life, low rates of return and size constraints, with observations largely restricted to the adult phase in top predators.

In order to obtain a full picture of ontogenetic fish movements, a “toolbox” approach is required, where a range of independent techniques providing information at specific spatial and (or) temporal scales can be used to understand connectivity across life-history stages (Begg & Waldman, 1999; Fromentin *et al.*, 2009; Kaplan *et al.*, 2010). A number of natural tags are employed for this purpose, including stable isotopes in soft tissues (Rodgers & Wing, 2008), amino acid signatures (Riveiro *et al.*, 2011), molecular genetics (Cook *et al.*, 2007), parasite loadings (Sequeira *et al.*, 2010), phenotypic markers such as morphometrics (Lawton *et al.*, 2010), colour (Arnegard *et al.*, 1999) and otolith shape (Ferguson *et al.*, 2011), as well as the chemical composition of calcified structures such as otoliths and scales (Campana, 1999). Each method has shown potential for determining population structure and (or) discriminating among resident and migrant fish, however, otolith chemistry has shown the greatest promise for reconstructing lifetime movements. The technique relies on the basic assumption that as the otolith grows, chemical markers from the ambient environment are incorporated into its microstructure, resulting in a „fingerprint“ that reflects, at least in part, the physicochemical properties of the

environment in which it was formed (e.g. Bath *et al.*, 2000; Elsdon & Gillanders, 2003a).

Otoliths are paired crystalline structures located in the inner ear of all bony fish, used for sound reception, maintaining equilibrium and processing directional cues (Popper & Fay, 2011). They display a number of key properties that have resulted in their chemical composition being increasingly exploited in the field of fish spatial dynamics. First, they exhibit unrivalled time-keeping properties. Otoliths develop very early in the fish's life, usually during the embryonic stages, and grow continuously through daily accretions of calcium carbonate (CaCO₃) aragonitic crystals onto a fibroprotein organic matrix (Campana & Neilson, 1985; Tohse & Mugiya, 2002; Payan *et al.*, 2004). Diurnal and seasonal rhythms result in growth bands that have been used in the field of sclerochronology for over a century and provide a baseline for carrying out time-resolved chemical analyses (Campana & Thorrold, 2001). Second, as otoliths are acellular and metabolically inert, they are not reworked or resorbed, even during times of starvation (Campana & Neilson, 1985). Chemical fingerprints are thus retained permanently within the microstructure. Third, while the precise relationships are not always clear, there is no question that otolith composition is affected by environmental conditions. This has been exploited by studies using otolith shape and whole otolith chemistry to infer stock structure, and chemical patterns across growth bands to provide information across life history stages (Elsdon *et al.*, 2008). Fourth, otoliths are common to all teleost species, which is particularly appealing for investigating habitat use in inaccessible fish (e.g. from deep or remote environments), where external tags cannot be applied or are unlikely

to be recovered. Finally, while otolith processing and chemical analyses are not inexpensive, sample acquisition is equivalent to all individuals being tagged and the technique is often significantly cheaper than an equivalent tagging study (Fairclough *et al.*, 2011).

While the last two properties are common to most natural tags, the capacity of otoliths to record (and retain) time-resolved lifetime environmental histories provides unique opportunity for „geolocating“ individual fish in time and space. Accordingly, otolith microchemistry represents a hugely valuable resource: a means to infer past conditions, stock structure, connectivity patterns and individual migrations. Indeed, otolith microchemistry has appeared in almost 700 peer-reviewed papers to date (Web of Science search on 26.09.11, Topic=otolith chemistry OR otolith microchemistry) and growth within the field shows no sign of slowing (Fig. 1). However, the precise mechanisms governing elemental incorporation into the otolith are still not fully understood (reviewed in Campana, 1999) and inconsistent patterns of element incorporation among species and studies undermine the routine application of otolith microchemistry to movement reconstruction (reviewed in Elsdon *et al.*, 2008). Here, we examine the use of otolith microchemistry, specifically minor and trace element concentrations, as a tool for describing the movements of fully marine fish. We review the current understanding of the mechanisms underpinning otolith microchemistry and discuss its application specifically within the marine environment. Finally, we present case studies that have used otolith microchemistry to infer spatial distributions of marine fish and attempted to validate their interpretations using alternative proxies.

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163 **HOW DOES OTOLITH MICROCHEMISTRY SERVE AS AN**

164 **ENVIRONMENTAL TAG?**

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166 CHEMISTRY OF RELEVANT ELEMENTS AND THE MECHANISMS

167 GOVERNING THEIR INCORPORATION INTO THE OTOLITH

168 The mechanisms governing otolith formation are quite different to those
169 controlling bone, shell and coral growth, as the otolith crystallises within the
170 endolymph fluid and is not in direct contact with epithelial tissue or the surrounding
171 water (Payan *et al.*, 2004). Moreover, the mechanism of otolith crystal growth is
172 biologically controlled, and thus likely to differ considerably to inorganic crystal
173 precipitation from a saturated solution (Weiner, 2008). Element incorporation into
174 otolith aragonite is a complex, multistage process, involving the movement of ions
175 from the ambient water into the blood plasma via branchial or intestinal uptake, across
176 inner ear membranes into the endolymph fluid, and finally into the growing surface of
177 the otolith (Payan *et al.*, 2004). As such, elemental discrimination can occur at any of
178 four major interfaces: environment-blood, blood-blood binding proteins, blood-
179 endolymph and endolymph-otolith (Campana, 1999). Changes in element
180 concentrations across these four interfaces provide a tangible means to examine their
181 relative control on elemental fractionation. Here, it is useful to consider the
182 similarities and differences in chemical affinities and behaviours between elements. In
183 the 1960s, chemists developed a concept known as “hard and soft acid and base”
184 (HSAB) theory (Pearson, 1963). HSAB theory categorises reactants as acids or bases,
185 depending on whether they donate or accept electrons within a reaction, and as hard,

soft or intermediate, depending on their polarisability, oxidation state and electronegativity (Fig. 2). Hard acids and bases are typically small ions or molecules with relatively high charge density that are weakly polarisable. Soft acids and bases tend to be large and strongly polarisable. The value of this concept is that acids and bases form strong bonds when binding with similar counterparts (i.e. hard acids form stronger bonds with hard bases and soft acids with soft bases). In a dynamic medium like seawater, and particularly blood plasma, where the number of available ligands outweighs the number of metal ions, strong bonds will tend to dominate the speciation of any elemental ion, and the behaviour of elements can be loosely predicted (Williams, 1971).

So far, 50 elements have been detected in otoliths, at major (Ca, C, O, N), minor (>100ppm: Cl, S, Mg, Na, P, Sr, and K) and trace levels (<100ppm: Ag, Al, As, B, Ba, Bi, Br, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Lu, Mn, Nd, Ni, Pb, Pr, Rb, Sc, Se, Si, Sm, Tb, Tm, U, V, Y, Yb, Zn) (Campana, 1999; Chen and Jones, 2006). However, only seven elements are routinely used to infer past location in fish (Li, Mg, Mn, Cu, Zn, Sr and Ba; Fig. 2), due to their environmental heterogeneity and otolith concentrations often above detection limits. In seawater, hard acid ions such as Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} form strong bonds with OH^- radicals and are mainly found as hydrated free ions (Fig. 2). These ions typically have a residence time longer than the mixing time of the ocean and consequently their concentrations tend to vary with salinity. By contrast, soft and intermediate acid ions such as Cu^{2+} , Zn^{2+} and (to a lesser extent) Mn^{2+} , bind to softer bases in seawater such as Cl^- and CO_3^{2-} . These

bonds are relatively weak, making soft elements reactive and readily removed from seawater (Henderson, 1984).

Little is known about the relative bioavailability of different elements to being taken up in marine fish, but it is likely that they will be fractionated from one another during transport across the gut, gills and skin. Osmoregulation is largely effected across gut membranes, particularly in marine species (Smith, 1930), and maintains relatively constant concentrations of blood Ca, Na, K, Mg and Cl in marine and freshwater fish despite considerable differences in ambient concentrations (Campana, 1999). Once within the circulatory system, the transport, availability and ultimate fate of elements will depend on their chemical characteristics and requirement in metabolic reactions, as well as the composition of the blood fluid. Elements that are constituent parts of functioning enzymes or structural tissues are likely to have relatively long mean residence times in the body due to the continued recycling of enzymes and tissues. By comparison, elements with no major structural or physiological role may be removed rapidly, resulting in low residence times. Exceptions include the quintessentially toxic metals (Cd, Pb, Hg), which have very long residence times due to comparatively ineffective excretion mechanisms (Williams, 1971). The relative timing of elemental changes across an otolith may therefore reflect differences in turnover times and the number of body pools for those particular elements. Elements with short turnover times (e.g. Mn, Ba) may be better suited to studying detailed temporal changes than elements with potentially longer turnover times (e.g. Sr, Pb).

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236 In the blood plasma, soft acid cations such as Cu^{2+} , Zn^{2+} and possibly Mn^{2+} are
237 actively bound to the histidine, methionine and cysteine residues of plasma proteins
238 and used in metabolic reactions or actively transported to the liver where they are
239 excreted (Watanabe *et al.*, 1997). Less than 10% of total blood Cu (Williams, 1971)
240 and less than 1% of Zn (Fletcher & Fletcher, 1980) are estimated to be present as free
241 ions. Hard acid cations (e.g. Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+}) on the other hand, are
242 largely transported as free ions or weakly bound to small molecular complexes,
243 albumins or globulins (Williams, 1971). Calcium (and Ca homologues) may also be
244 strongly bound to carboxyl residues in Ca-binding proteins, allowing plasma Ca to be
245 divided into protein bound (30-40%) and ultrafiltrable fractions (60-70%). The
246 ultrafiltrable fraction is dominated by ionised Ca, meaning that more than 50% of
247 total Ca (and thus probably Sr) is estimated to be present in plasma as the free ion
248 (Mugiya, 1966; Andreasen, 1985; Hanssen *et al.*, 1989). Therefore, hard and soft acid
249 ions are very likely to be fractionated from one another within the blood plasma. Mg
250 may behave somewhat differently to other Group II elements due to an ionic radius
251 that is very small unless hydrated, when it becomes extremely large (Kaim &
252 Schwederski, 1994). Moreover, it has been implicated in stabilising amorphous
253 mineral phases during otolith biomineralisation and, due to its participation in a
254 number of biological processes, is tightly regulated in the body (Weiner, 2008).

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257 Because the speciation of metal ions within blood plasma is dynamic, it is likely
258 that blood chemistry, particularly the composition and relative abundances of blood
259 proteins, will influence the proportion of free ions, and thus affect their ability to cross

the plasma-endolymph membrane barrier (Kalish, 1991). Physiological variation in the composition of blood plasma proteins is more likely to influence the soft acid ions, as they are more strongly associated with blood proteins. However, depletion of all major elements in the endolymph except K (Kalish, 1991) indicates active discrimination against most ion types by the saccular epithelium (Payan *et al.*, 2004).

Finally, incorporation of an element into the otolith depends on its compatibility to bind directly into the crystal lattice, within interstitial spaces or to the organic matrix (Campana, 1999). Based on HSAB theory, the strong protein binding affinities of soft metals will likely favour their incorporation into the otolith bound to the organic matrix. This is corroborated by spatial heterogeneity in otolith Cu (Milton *et al.*, 2000; Milton & Chenery, 2001b) and an estimated 70-100% of otolith Cu and 40-60% of otolith Zn associated with the protein matrix (Miller *et al.*, 2006). Inclusion within the otolith mineral phase occurs (i) via substitution for Ca, where partitioning will be controlled by ion charge, radius and crystal elasticity influences, (ii) within dislocation sites on the growing crystal surface, where partitioning may be controlled by kinetic influences such as precipitation rate (Reeder & Rakovan, 1999), or (iii) possibly through inclusion in amorphous precursor mineral phases (Weiner, 2008). Fractionation of elements during their incorporation into the otolith is further complicated by additional effects of temperature. It is debatable whether any such temperature effects are direct or indirect however, with many studies attributing them to physiological processes, such as growth rate, which are in turn controlled by ambient temperatures. Walther *et al.* (2010) discuss possible mechanisms underpinning „growth rate effects“ on otolith microchemistry, grouping them into two

broad types: mineralogical kinetic and physiological. Kinetic hypotheses include greater entrapment of trace element impurities into the growing crystal at higher temperatures, due to faster growth and increased numbers of crystal defects (Gaetani & Cohen, 2006). Another „kinetic hypothesis“ relates faster accretion rates with greater supply of Ca^{2+} ions to the endolymph, effectively diluting the other elements within the precipitating fluid (Sinclair, 2005). While crystal growth rate has been shown to influence elemental partitioning during abiotic CaCO_3 formation, biogenic CaCO_3 forms via a transient amorphous phase associated with the organic matrix (Weiner, 2008), thus extrapolating patterns directly from inorganic crystal theory is potentially misleading. Finally, the „physiological hypothesis“ is based on the coupling of somatic growth rates with protein synthesis, with significant changes in growth rate affecting the protein composition of biological fluids and the availability of ions for uptake into the otolith (Kalish, 1991).

INFLUENCES ON OTOLITH MICROCHEMISTRY

In the early days of otolith microchemistry, it was assumed that trace elements would be incorporated into the otolith in proportion to their availability in the surrounding water. For most elements, this is now known not to be the case (Table I). However, positive relationships have been observed between ambient and otolith concentrations for a number of elements, particularly the hard acid elements, Sr and Ba. It is estimated that marine fish derive 83% and 98% of their otolith Sr and Ba from the surrounding water, respectively (Walther & Thorrold, 2006). Besides from a few extreme anomalies (e.g. Elsdon & Gillanders, 2005a), water Sr follows a quasiconservative distribution, allowing otolith Sr/Ca ratios to act as a powerful

marker of movement across extreme salinity gradients. As such, otolith Sr/Ca ratios are fairly routinely applied to reconstructing migrations of diadromous species (e.g. Kalish, 1990; Babaluk *et al.*, 1997; Secor *et al.*, 2001; Thorrold *et al.*, 2001; Gillanders, 2005; Milton & Chenery, 2005; Walther & Thorrold, 2010; Panfili *et al.*, In press). Interpretation of the Sr/Ca signal still requires some caution, however, given considerable interspecific variation in Sr uptake rates (Swearer *et al.*, 2003; Rooker *et al.*, 2004), potential for vateritic inclusions to be misidentified as freshwater excursions (Tzeng *et al.*, 2007) and possibly confounding effects of temperature and physiology (e.g. Kalish, 1991; Elsdon & Gillanders, 2004; Brown & Severin, 2009; Miller, 2011).

Occasionally, elements other than Sr and Ba have exhibited positive correlations between otolith and ambient concentrations (e.g. Geffen *et al.*, 1998; Milton & Chenery, 2001b; Ranaldi & Gagnon, 2008b), but more often than not, no relationship is found or there are contradictions among species and studies (Table I). Some of these contradictions are likely due to species-specific elemental processing, but some may be artefact of reporting method, with studies expressing water chemistry in terms of absolute water concentrations, water element/Ca ratios and (or) salinity. It is still not clear which of these three „measures“ have the greatest influence on otolith chemistry (Bath *et al.*, 2000; Milton & Chenery, 2001b; Elsdon & Gillanders, 2004; Hicks *et al.*, 2010), but is clearly an area requiring further research. Another important consideration is the true bioavailability of the element in question. Reported concentrations tend to represent total dissolved metals, which may be dominated by ligand-bound complexes that cannot cross biological membranes (Campana, 1999).

Also, alternative sources of metals, such as sediment and diet, are rarely discussed but may prove significant for certain elements, such as Cd (Ranaldi & Gagnon, 2009).

As shown by the sheer volume of studies (and contradictions) summarised in Table I, temperature, diet and „vital effects“ such as age, growth rate and gonad maturation, can all influence element incorporation into the otolith, often in multiplicative, complex ways (Kalish, 1991). Given the substantial variation in physiology, biochemistry and otolith morphology among fish species (Söllner *et al.*, 2003; Popper & Fay, 2011), it is perhaps not surprising that interspecific differences in otolith elemental incorporation have been observed. However, within-species differences in elemental processing may also be important, with recent experiments indicating different temperature effects on otolith Mg, Mn and Ba incorporation according to the population from which the fish were sourced (Clarke *et al.*, 2011). Such intrinsic effects on otolith microchemistry have not been previously demonstrated, but have important implications for understanding the underlying mechanisms and theoretically enhance its application for stock discrimination.

Temperature has frequently been cited as a major control on otolith chemistry. Reports of negative relationships between temperature and otolith Sr/Ca ratios, akin to responses of inorganic aragonite and corals, have generally arisen from studies working with larvae of marine, temperate species (Radtke, 1989; Townsend *et al.*, 1992; DiMaria *et al.*, 2010). Otherwise, where significant temperature effects have been observed, the majority of studies have reported positive relationships between

temperature and otolith Sr (Bath *et al.*, 2000; Elsdon & Gillanders, 2002; Martin *et al.*, 2004) and Ba (Miller, 2009). While separating the effects of temperature and growth remains a challenge, a number of studies have reported negative relationships between growth rate and otolith Sr/Ca (e.g. Sadovy & Severin, 1994; Walther & Thorrold, 2010), and more recently, Ba/Ca (Miller, 2009; 2011). Experiments examining temperature or vital effects on otolith elements other than Sr and Ba are scarce, but a negative temperature effect was reported for otolith Mn/Ca ratios (Miller, 2009) and a positive growth rate effect reported for otolith Mg/Ca ratios (Martin & Thorrold, 2005). Recently, increasing numbers of studies have reported interactions between otolith Sr/Ca, Mg/Ca and Ba/Ca ratios, water concentrations and temperature (e.g. Elsdon & Gillanders, 2004; Miller, 2011). Such findings have serious implications, particularly for studies using otolith Sr/Ca ratios to reconstruct migrations among salinity regimes (Martin & Wuenschel, 2006).

APPLICATION OF OTOLITH MICROCHEMISTRY TO RECONSTRUCT FISH MIGRATIONS IN THE MARINE ENVIRONMENT

THE MARINE SYSTEM

The marine system is a dynamic environment with elements constantly added, removed and recycled through biological, physical and chemical processes (Hunter & Boyd, 1999). Yet global averages for seawater element concentrations have remained remarkably conserved, particularly when compared with estuarine and freshwater systems (Turekian, 1968). As such, many marine fish, particularly open ocean pelagics, experience a relatively uniform physicochemical environment with limited

potential for spatial discrimination using inorganic chemical proxies (Proctor *et al.*, 1995), although there are some exceptions (e.g. Ashford *et al.*, 2008). Coastal areas generally offer greater chemical heterogeneity due to upwelling, fluvial and anthropogenic inputs, but often the „pollutants“ that would contribute to such geographic variation (e.g. Ni, Zn) are soft acid metals that are physiologically discriminated against and typically present below detection levels in the otoliths (Hanson & Zdanowicz, 1999). Importantly, unlike estuarine systems (Elsdon & Gillanders, 2005b), Sr/Ca and Ba/Ca ratios are not inversely related in seawater (Ashford *et al.*, 2005), with Sr exhibiting a quasiconservative distribution (Steele *et al.*, 2009) and Ba readily removed from surface waters as barite adsorbed to settling particles (Bruland & Lohan, 2003). Primary productivity is correlated with barite accumulation, resulting in a nutrient-type distribution (Dehairs *et al.*, 1997). At mesopelagic depths, bacterial activity releases the barite, allowing it to accumulate and dissolve, enriching Ba with depth and producing a useful open ocean otolith marker (Ashford *et al.*, 2005). Manganese on the other hand, is a scavenged element and concentrations thus tend to decrease with depth and distance from coastlines and point sources such as hydrothermal vents (Bruland & Lohan, 2003).

While greater environmental heterogeneity in coastal areas generally increases the discriminatory power of associated otolith signatures, it is, unfortunately, usually at the expense of temporal stability. Indeed inter- and intra-annual differences in water chemistry (Elsdon *et al.*, 2008) and otolith chemical fingerprints (Gillanders, 2002; Swearer *et al.*, 2003; Chittaro *et al.*, 2004; Bergenius *et al.*, 2005; Elsdon & Gillanders, 2006; Walther & Thorrold, 2009) are more the norm than the exception.

For this reason, reference collections of otolith elemental fingerprints for relevant year classes and locations are recommended to elucidate site-specific information from unknown otolith chemistries, particularly for more variable habitats, such as lagoons (Gillanders, 2002; Walther & Thorrold, 2009; Mercier et al 2012).

MARINE SPECIES

Although the physiology of marine, euryhaline and freshwater fishes differs significantly, particularly with regards to osmoregulation (Evans, 1993), there is little to suggest inherent differences in otolith element incorporation mechanisms from laboratory studies (Farrell & Campana, 1996; Walther & Thorrold, 2006). When considering different life history stages, however, ontogenetic effects on otolith Sr/Ca ratios are often observed in species spending at least part of their lifecycle in saltwater (e.g. Fowler *et al.*, 1995a; Walther *et al.*, 2010), particularly during significant metabolic events such as metamorphosis (Arai *et al.*, 2000; de Pontual *et al.*, 2003). Despite water Sr/Ca ratios varying little at salinities above ~8 (Secor *et al.*, 1995; Babaluk *et al.*, 1997; Zimmerman, 2005), Sr/Ca ratios in otoliths of marine fish often exhibit considerable interspecific differences (Kalish, 1989; Hamer & Jenkins, 2007), general increases with age (Kalish, 1989; Walther *et al.*, 2010) and intra-annual fluctuations often larger than those exhibited by diadromous species (Brown & Severin, 2009). Explanations for these patterns have been sought, but to date, no single causal factor has been found, although temperature, age, somatic and otolith growth rate, stress and gonad maturation have all been implicated (Table I).

435 Unfortunately, no experimental work has systematically tested the relationship
436 between ambient temperature and otolith Sr/Ca in adult marine fish across a full
437 reproductive cycle. However, in adult blue grenadier *Macruronus novaezelandiae*
438 Hector 1871, Australian salmon *Arripis trutta* Forster 1801 and bearded rock cod
439 *Pseudophycis barbatus* Günther 1863, growth rate and reproductive investment
440 appear to have a greater effect on otolith Sr/Ca ratios than temperature (Kalish, 1989;
441 Kalish, 1991). Otolith Sr/Ca ratios in female *P. barbatus* correlated significantly with
442 gonadosomatic index and plasma protein concentrations, particularly the relative
443 proportions of albumins and globulins (Kalish, 1991). It was therefore suggested that
444 plasma and endolymph proteins vary in type and prevalence during gonad
445 development, and differences in their metal-binding capacity alter the proportion of
446 free Sr^{2+} ions, thus altering availability and uptake of Sr into the otolith. Similarly,
447 otolith Na, K and Sr concentrations were linked to spawning activity in wild red drum
448 *Sciaenops ocellatus* L. 1766 (Fuiman & Hoff, 1995) and seasonal fluctuations in
449 otolith Sr/Ca ratios were observed in adult Atlantic salmon *Salmo salar* L. 1758 held
450 for >2 years in sea-cages, where salinity was almost constant (32-33) (Clarke &
451 Friedland, 2004). Because the changes in otolith Sr/Ca ratios of the caged *S. salar*
452 appeared to lag behind the changes in water temperature, they were attributed to
453 gonad maturation. However, gonad development is often accompanied by reduced
454 feeding, slower growth rates and changes in ambient water temperature, so there are a
455 number of plausible explanations for the observed otolith Sr/Ca fluctuations.
456 Whatever the explanation, such physiological influences on otolith element ratios
457 have the potential to complicate any element-based interpretations of movement
458 patterns and stock discrimination. It remains to be seen whether purely physiological
459 influences on the trace element composition of otoliths could prove useful. Marked

variations in otolith Sr/Ca ratios within fully marine fish might, for example, indicate onset of sexual maturity or provide a quantitative measure of reproductive investment.

PRACTICAL CONSIDERATIONS

In addition to the variety of exogenous and endogenous factors that can affect otolith chemical patterns, analytical artefacts can also play a role. Instrument performance can significantly affect accuracy and precision (Campana *et al.*, 1997), while otolith treatment can contaminate or remove elements from the microstructure (Gauldie *et al.*, 1998; Proctor & Thresher, 1998; Thresher, 1999; Swan *et al.*, 2006). Instruments are, however, constantly evolving, with the development of improved technologies such as high resolution or sector field inductively coupled plasma mass spectrometry (HR-ICPMS or SF-ICPMS) (Thorrold & Shuttleworth, 2000), and increasing numbers of studies are focusing their attentions on improving decontamination methods (e.g. Campana *et al.*, 2000; Davies *et al.*, 2011).

A significant practical obstacle encountered when attempting to identify movement-related information in otoliths, is the desire to obtain time-resolved chemical data. Current approaches generally achieve this through microsampling or probe-based techniques such as laser ablation ICPMS (LA-ICPMS). Solution analyses can address certain movement-related questions, for example, using whole otolith signatures as natural tags during brief mixing periods (Campana *et al.*, 2000), however, probe-based techniques take advantage of the otolith chronology and offer the attractive option of targeting specific growth increments and life history stages.

The downside to probe-based analyses is that the volume of ablated material is relatively small, reducing detection capabilities and the number of available elements (Campana, 1999). Given the ever-increasing desire for longer element lists and improved discriminatory capabilities, beam diameter and (or) power might be increased to improve detection limits, but this results in wider, deeper pits and averaging over longer time periods. Thus probe-based techniques face a constant struggle between optimising temporal resolution and instrument performance. Pit depth is rarely reported in the literature, but given the three-dimensional structure of otoliths, could have a significant averaging effect, and artificially inflate apparent time lags between environmental changes and responses within the otolith (Jones & Chen, 2003).

Attempting to use otolith chemistry to infer intra-annual movements of adult fish is particularly difficult, as age-related decreases in growth rate (e.g. Fowler *et al.*, 2005) result in the progressive narrowing of growth increments, often to widths well under 30µm. This clearly limits the spatial and temporal resolution attainable for multi-elemental otolith analyses. It also means that few validation studies have focused their attentions on adult fish, with most information inferred from wild-caught adults for which environmental histories are not explicitly known (e.g. Kalish, 1989; Fuiman & Hoff, 1995). The vast majority of empirical studies have employed larval or juvenile fish due to easier and cheaper maintenance, faster growth rates and wider otolith increments. However, given age-related changes in physiology and elemental processing, interpretation of otolith signals in adult fish based on observations from young life-history stages should be treated with caution.

Finally, there are also practical considerations regarding the choice of statistical methods used to analyse the complex, often skewed, multivariate data produced by most otolith elemental analyses (Elsdon *et al.*, 2008). Analyses within chemical profiles from individual fish suffer from issues of non-independence, but this can be overcome by using repeated measures of analysis of variance (RM-ANOVA) (e.g. Clarke *et al.*, 2010) or mixed model analyses (e.g. Hamilton & Warner, 2009). Habitat discrimination based on multivariate elemental fingerprints is a common aim among otolith studies. Recent work comparing the classification accuracy of different statistical methods indicated that machine learning methods such as „random forest“ exhibited greater classification efficiency with fewer assumptions than equivalent discriminant function analyses (Mercier *et al.*, 2011).

CASE STUDIES

To date, though many studies have used otolith chemistry to examine population structure and (or) movements of marine or partially marine fish, few have attempted to corroborate their results with additional, alternative proxies. As discussed by Begg & Waldman (1999), a more „holistic approach“ to stock identification instils greater confidence in the results, but also provides support for (or challenges to) the spatial distributions implied by each marker. Here, we present examples of studies that have used otolith microchemistry to infer population structure or movements of adult

marine (or partially marine) fish, and attempted to corroborate their results, either directly or indirectly, with additional, alternative proxies.

OTOLITH CHEMISTRY vs. GENETIC TAGS

Very few studies have directly compared chemical and molecular tags for describing spatial distributions of fish populations. One such study found that both microsatellite DNA and trace element concentrations at the otolith margin (Mg, Mn, Zn, Sr and Ba) inferred c.65% site fidelity for adult black rockfish *Sebastes melanops* Girard 1856, a value also independently suggested by mark-recapture studies (Miller *et al.*, 2005). This study provided rare quantitative support for the use of probe-based techniques such as LA-ICPMS to elucidate population structuring in an exclusively marine fish. Possible movement patterns were not explored in this work, but otolith chemical profiles may have indicated temporally-resolved differences between the resident fish and recent immigrants. Microsatellite and otolith elemental markers were also integrated in an attempt to determine dispersal patterns of the anadromous rainbow smelt *Osmerus mordax* Mitchill 1814 (Bradbury *et al.*, 2008). While the two techniques were not formally compared, they produced corroborative results with molecular markers indicating high local recruitment and otolith Sr and Ba profiles implying limited dispersal among estuaries.

A number of stock discrimination studies have also anecdotally compared results from otolith chemistry and molecular studies. While none have reported contradictory results, nearly all have observed higher spatial complexity using otolith

560 microchemistry than their genetic equivalent. For example, otolith margin
561 microchemistry (Li, Mg, Mn, Sr and Ba) of the southern garfish *Hyporhamphus*
562 *melanochir* Valenciennes 1847, an estuarine and nearshore species, inferred 6 „semi-
563 discrete, population components“, contrasting with the 2 management units suggested
564 by molecular markers (Steer *et al.*, 2009). The difference was attributed to a „stepping
565 stone model“ of exchange among neighbouring subpopulations, as misclassified fish
566 were generally assigned to adjacent or proximal sites. Similarly, whole otolith
567 fingerprints (Ba, Cd, Cu, K, Pb, Sr, Zn, Mg, Na and S) of orange roughy *Hoplostethus*
568 *atlanticus* Collett 1889, a fully marine species, implied stock separations that
569 corroborated those indicated by genetic markers and parasite loads, but inferred an
570 additional grouping within an area previously classified as a single stock (Edmonds *et*
571 *al.*, 1991). These examples raise an important point – while otolith microchemistry
572 may resolve groups of fish with shared environmental histories and thus reveal spatial
573 structuring undetectable by other methods, these groups may mix on spawning
574 grounds and still be best managed as a single unit. Thus, terming such works „stock
575 discrimination“ can be somewhat misleading, for the point at which groups of fish
576 with shared environmental histories should be considered and managed as separate
577 „stocks“ is difficult to define and depends on a number of factors specific to the
578 system, species and subunits in question (Campana, 2005). Otolith core chemistry has
579 also been used to infer shared (e.g. Fowler *et al.*, 2005) or multiple (e.g. Tanner *et al.*,
580 2012) spawning grounds in marine species. A significant challenge remains however,
581 to demonstrate that shared core chemistries reflect common origin, rather than similar
582 water chemistries or physiological overprinting (maternal or intrinsic). Encouragingly,
583 element concentrations in the otolith nuclei of two marine species, Scotia Sea icefish
584 *Chaenocephalus aceratus* Lönnberg 1906 and Patagonian toothfish *Dissostichus*

eleginoides Smitt 1898, corroborated population boundaries inferred by a number of additional markers, including growth rates, morphometrics, parasite loadings and genetics, but again, suggested finer scale population structuring than the other methods (Ashford *et al.*, 2006; Ashford *et al.*, 2010).

OTOLITH CHEMISTRY vs. OTOLITH SHAPE

While otolith shape has a predominantly genetic basis, it is susceptible to local conditions and, after significant time in contrasting environments, can differ significantly among regions and stocks (Campana & Casselman, 1993). In an estuarine and nearshore species, the mullet *Argyrosomus japonicus* Temminck & Schlegel 1843, otolith margin Na, Mg, Sr, Ba concentrations classified fish to capture region with high success (94%), while otolith morphometrics produced slightly lower classification scores (83%), but supported the subdivisions suggested by the elemental fingerprints (Ferguson *et al.*, 2011). The relative time periods represented by each marker is of interest, with otolith margin chemistry representing the most recent material and implying short term geographic separation, while the differences in otolith shape implying limited mixing over longer time periods. Similar methods were used to examine spatial distributions of the roundnose grenadier *Coryphaenoides rupestris* Gunnerus 1765, a deep sea macrourid (Longmore *et al.*, 2010). Here, classification scores were considerably lower using shape descriptors (43%) cf. otolith microchemistry (92%), implying possible mixing events prior to the most recent otolith growth. Ontogenetic patterns in otolith Li, Mn, Cu, Zn and Ba concentrations and stable isotopic ratios ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) suggested a shallower larval phase for this

deep sea species, which may facilitate longer term regional genetic drift, but further corroborated relatively static population units (Longmore *et al.*, 2011).

OTOLITH CHEMISTRY vs. MORPHOMETRICS

Similar to otolith shape, variations in fish morphometrics can be genetically and (or) environmentally induced (e.g. Silva, 2003). In a study of adult sea perch *Helicolenus percooides* Richardson 1842 in Fiordland, New Zealand, differences in whole otolith microchemistry (Li, Mg, Sr and Ba), morphometric characters, length-at-age and muscle $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ composition implied limited movement between the four fjords under study (Lawton *et al.*, 2010). In the same region, consistent differences in otolith marginal chemistry (Beer *et al.*, 2011) and tissue isotopes (Rodgers & Wing, 2008) of blue cod *Parapercis colias* Forster 1801 implied even finer scale population structuring, with limited movement between inner and outer fjord habitats, results that were corroborated by mark-recapture studies (Carbines & McKenzie, 2004). In contrast, morphometric variations in the shad hilsa *Tenuulosa ilisha* Hamilton 1822, an anadromous species, were not corroborated by patterns in otolith core and margin chemistries (Li, Na, Mg, Al, Mn, Zn, Sr, and Ba) or allozyme markers (Milton & Chenery, 2001a; Salini *et al.*, 2004). Rather, the localised variability in morphometrics was attributed to susceptibility to local conditions, while the widespread variability in chemical and molecular markers was attributed to extensive mixing. While it has been suggested that a lack of detectable differences in otolith microchemistry provides little information (Campana *et al.*, 2000), the authors argued that the areas in question were characterised by significant differences in water

chemistry, and the manner in which the otolith and molecular markers covaried indicated highly mobile individuals and a well-mixed population.

OTOLITH CHEMISTRY vs. APPLIED TAGS

Mark-recapture studies provide the most irrefutable evidence of individual movement patterns, but to our knowledge, no study has directly compared tag-derived geolocations in a marine species with otolith material deposited during the same period. In Australia, age-related changes in otolith Sr/Ca and Ba/Ca profiles for snapper *Pagrus auratus* Forster 1801, a fully marine species, were related to emigration away from a common spawning ground (Fowler *et al.*, 2005). As water Sr and Ba concentrations were not formally examined, there was the possibility that such shared otolith chemistries reflected intrinsic ontogenetic effects, rather than common origin (Fowler *et al.*, 1995a; de Pontual *et al.*, 2003; Tanner *et al.*, 2011). However, substantial differences in oceanographic conditions and terrestrial inputs were predicted among the water masses in question, and the interpretations were supported by behavioural inferences from mark-recapture studies. Meanwhile, in a different part of Australia, the same species was shown to persist in a far more complex set of population units; findings inferred by whole otolith microchemistry, mark-recapture studies, morphometrics and genetics (Edmonds *et al.*, 1989; Edmonds *et al.*, 1995). Such intra-specific plasticity in population structuring emphasise the benefits of using a “toolbox” approach while highlighting the potential hazards of extrapolating observed species distributions to adjacent areas.

To examine movement-related questions, there is a basic need for time-resolved information. Most studies address this using probe-based otolith analyses, but Campana *et al.* (2000) used averaged whole otolith fingerprints (Mg, Sr, Ba, Mn and Li) to track spawning stock aggregations of cod *Gadus morhua* L. 1758 during brief mixing periods. A maximum likelihood-based stock mixture analysis estimated the relative contribution of reference stocks to summer feeding and over-wintering fish, based on the assumption that source signatures would remain identifiable so long as the mixing period was shorter than the time taken for new otolith growth to overprint reference signatures. By repeated sampling over time it was possible to test this assumption, and elemental fingerprints were found to be stable over a 2-3 year period and some elements even over a decade. The results of this study were supported by previous tagging work, but were unambiguous in their own right, taking advantage of substantial sample sizes (~2500 fish) sampled across a large geographic area over a number of years (Campana, 2005). It should be noted that despite efforts to sample similar size ranges, significant relationships between element concentrations and otolith mass were still observed (positive for Sr; negative for Mn and Mg) and needed detrending, again stressing the importance of size and age in otolith studies (Campana *et al.*, 2000).

DISCUSSION

It is encouraging that of most of the studies published so far have reported congruence between the spatial distributions implied by otolith trace element

fingerprints and by alternative tags, both natural and artificial (e.g. Miller *et al.*, 2005). By taking advantage of the superior detection capabilities associated with whole otolith solution analyses (e.g. Campana *et al.*, 2000), stock discrimination and mixed stock analyses can be carried out with relative confidence (Campana & Gagne, 1995; Ferguson *et al.*, 2011). In addition, otolith trace elemental profiles and fingerprints have shown good potential to infer ontogenetic and inter-annual changes in the distribution of marine fish stocks and source-sink connectivity patterns (Fowler *et al.*, 2005; Clarke *et al.*, 2010). However, with regards using otolith microchemistry to track the seasonal migration patterns of individual, fully marine fish (e.g. Hunter *et al.*, 2004), further validation is required. Only very few studies to date have attempted to use intra-annual otolith chemistries to obtain geolocations of individual fish across time and space (e.g. Mercier *et al.*, 2012). As discussed by Elsdon *et al.* (2008), the number of assumptions increases markedly with the spatial complexity of the question being asked. In the marine realm, the low spatial variability among the hard acid cations most likely to be incorporated into the otolith as a function their availability would certainly appear to hinder the widespread application of otolith microchemistry for geolocation. For example, for *D. eleginoides* captured in the open ocean, otolith element concentrations typically varied by just 1-3 standard deviations (Ashford *et al.*, 2007). Once the inherent error and skew found in most trace elemental data has been accounted for, the likelihood for otolith chemical profiles to reveal individual migrations with great precision or accuracy are slim. Other factors too appear discouraging, such as the relatively poor detection capabilities of probe-based analyses and possible confounding effects of temperature, ontogeny and physiology.

Otolith-derived positional information can be further improved by adding stable isotopes (e.g. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) to the chemical fingerprint (Thorrold *et al.*, 2001; Ashford & Jones, 2007; Tanner *et al.*, 2011). However, it is essential that the physicochemical characteristics of the area in question are well described, as subtle differences in salinity can significantly alter water (Harwood *et al.*, 2008) and otolith (e.g. Surge & Walker, 2005) $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signatures. The geographical and temporal scales of migration are clearly of great importance. Geographic differences in otolith chemistries are often observed among groups of marine fish, implying that even if there is insufficient information to couple element concentrations with specific locations, there may be potential for separating groups of fish with shared migration histories (known as 'contingents', see Secor, 1999; Secor *et al.*, 2001; Elsdon *et al.*, 2008). *In situ* validation studies coupling migration pathways and environmental conditions derived from DST records with concomitant otolith chemistries will further help to elucidate whether otolith trace element concentrations can successfully reconstruct or infer individual movements in exclusively marine species (e.g. Hunter & Darnaude, 2004).

Interpretation of signals in physiologically-influenced otolith elements, such as Mg and most importantly, Sr, has been identified as an area urgently requiring further investigation. As discussed, otolith composition is influenced by the ambient environment as well as by a suite of physiological processes that may alter the relative abundances of elements between water and their eventual incorporation into the otolith. To better understand these trends, validation experiments examining elemental uptake across realistic temperature regimes and maturity states would be of

great use (Walther *et al.*, 2010), as would quantifying the time taken for environmental changes to be reflected in the growing otolith (Miller, 2011). However, stock discrimination studies often include phenotypic traits, such as size and growth rate, to discriminate among groups of fish. If such traits also generate distinctive, reproducible otolith chemistries, their use as population markers can be considered legitimate (Campana *et al.*, 2000). While this assumption may well hold for averaged whole otolith chemical fingerprints, determining the causal effect of intra-annual signals within individual otoliths is particularly complex, and interpretations could easily be confounded by interactions between environment and physiology. However, when attempting to obtain spatial information from any „form“ of otolith microchemistry, it must be emphasised that the influence of physiology should be minimised by controlling for sex, size and age when selecting individuals for analysis.

CONCLUSIONS

In order to track individual fish migrations using otolith microchemistry, knowledge of the distributions of elements in the region of interest must be balanced with an understanding of the physiological influences on otolith chemistry over the time period of interest. Limited contemporary understanding of the mechanisms controlling elemental incorporation into the otolith restricts the application of elemental data for positioning fish in time and space (Elsdon *et al.*, 2008), while limited environmental variability in the elements most reliably measured in otoliths presents an additional significant challenge for geolocating fully marine species. The

otolith elements most likely to act as useful spatial indicators within the open ocean will vary among species, systems and scales, and as detection capabilities of probe-based analyses improves, will be augmented by spatially explicit, low level elements such as rare earths (Arslan & Paulson, 2003). Based on their behaviour as „hard“ or „intermediate“ acid cations, their potential for environmental heterogeneity, and their positive relationships with ambient concentrations, it seems likely that Ba, and perhaps Mn and Li, will prove to be the most useful and reliable otolith elements in studies of marine fish movements in the coming years. Nevertheless, the importance of complementing laboratory experiments with *in situ* validation studies cannot be underestimated. Combining otolith trace element profiles with other geolocation tools such as archival tag data and genetics (Begg & Waldman, 1999; Fromentin *et al.*, 2009) will support and greatly extend the overall utility of otolith chemistry for retrospectively describing migrations and mixing patterns of fully marine fish.

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Table I – Review of significant and non-significant (NS) effects of different influences (water chemistry, temperature, diet, vital and intrinsic effects) on otolith element concentrations and their partition coefficients, focusing on fish that spend at least part of their lifetime in seawater; the few fully freshwater studies are indicated (FW). Elements have been loosely ordered into hard and soft acids, with Mn and Zn ions behaving as something of intermediaries.

Element	vs. <i>(including Me/Ca, absolute concentrations & salinity)</i>	Water chemistry	Temperature	Other <i>(including diet, vital and intrinsic effects)</i>
Sr		POSITIVE - in the laboratory (Gallahar & Kingsford, 1996; Tzeng, 1996; Bath <i>et al.</i> , 2000; Milton & Chenery, 2001; Elsdon & Gillanders, 2003; Martin <i>et al.</i> , 2004; Elsdon & Gillanders, 2005a; Zimmerman, 2005; Walther & Thorrold, 2006; Hicks <i>et al.</i> , 2010; Macdonald & Crook, 2010; Miller, 2011)	POSITIVE (Kalish, 1989; Fowler <i>et al.</i> , 1995b; Hoff & Fuiman, 1995; Bath <i>et al.</i> , 2000; Elsdon & Gillanders, 2002; Elsdon & Gillanders, 2004; Martin <i>et al.</i> , 2004; Martin & Wuenschel, 2006)	Somatic growth rate: NEGATIVE (Sadovy & Severin, 1992; 1994; de Pontual <i>et al.</i> , 2003) Otolith precipitation & somatic growth rate: NS or weakly negative (Bath <i>et al.</i> , 2000; Martin <i>et al.</i> , 2004; DiMaria <i>et al.</i> , 2010)) Reproduction: SIGNIFICANT (Kalish, 1989; Kalish, 1991; Fuiman & Hoff, 1995; Friedland <i>et al.</i> , 1998; Clarke & Friedland, 2004) Stress: POSITIVE (Kalish, 1992; Townsend <i>et al.</i> , 1992). <i>In the latter it was proposed that stress caused by low temperature reduces ability to discriminate against Sr</i> Age/size: POSITIVE (Kalish, 1989; Fowler <i>et al.</i> , 1995b; Fuiman & Hoff, 1995; Proctor <i>et al.</i> , 1995; Fowler <i>et al.</i> , 2005; Steer <i>et al.</i> , 2009); NS (Elsdon & Gillanders, 2005a) Ontogeny: SIGNIFICANT (Toole <i>et al.</i> , 1993; Fowler <i>et al.</i> , 1995a; Tzeng, 1996; de Pontual <i>et al.</i> , 2003); NS (Elsdon & Gillanders, 2005a) Intrinsic (among species): (Kalish, 1989; Swearer <i>et al.</i> , 2003; Rooker <i>et al.</i> , 2004; Martin & Wuenschel, 2006; Hamer & Jenkins, 2007) Diet : POSITIVE (Limburg, 1995; Gallahar & Kingsford, 1996; Buckel <i>et al.</i> , 2004); NS (Hoff & Fuiman, 1995; Farrell & Campana, 1996; Sanchez-Jerez <i>et al.</i> , 2002; Walther & Thorrold, 2006)
		POSITIVE - in the field (oft-inferred rather than measured) (Thorrold <i>et al.</i> , 1997b; Babaluk <i>et al.</i> , 1997; Secor & Rooker, 2000; Milton & Chenery, 2005; Tzeng <i>et al.</i> , 2005)	NEGATIVE (Radtke <i>et al.</i> , 1990; Townsend <i>et al.</i> , 1992; DiMaria <i>et al.</i> 2010) NS (Gallahar & Kingsford, 1996)	
			INTERACTION WITH WATER CHEMISTRY (Elsdon & Gillanders, 2002; Elsdon & Gillanders, 2004; Martin & Wuenschel, 2006; Miller, 2011)	
	<i>See also Secor & Rooker review (2000)</i>			
Ba		POSITIVE vs. water Ba/Ca and (or) NEGATIVE vs. salinity (Bath <i>et al.</i> , 2000; Elsdon & Gillanders, 2002; Elsdon & Gillanders, 2003; 2004; de Vries <i>et al.</i> , 2005; Elsdon & Gillanders, 2005a; Elsdon & Gillanders, 2005b; Martin & Thorrold, 2005; Martin & Wuenschel, 2006; Walther & Thorrold, 2006; Miller, 2009) POSITIVE -in field studies (Thorrold <i>et al.</i> , 1997; Elsdon & Gillanders, 2005a; Elsdon & Gillanders, 2005b; Dorval <i>et al.</i> , 2007) INTERACTION WITH Sr (de Vries <i>et al.</i> , 2005) NS - in field (Forrester, 2005)	POSITIVE (Elsdon & Gillanders, 2002; Elsdon & Gillanders, 2004; Miller, 2009) NEGATIVE (DiMaria <i>et al.</i> , 2010) NS (Bath <i>et al.</i> , 2000; Martin & Thorrold, 2005; Martin & Wuenschel, 2006) INTERACTION WITH WATER CHEMISTRY (Elsdon & Gillanders, 2002; Elsdon & Gillanders, 2004; Miller, 2011)	Somatic growth rate: NEGATIVE (Miller, 2011) , NS (DiMaria <i>et al.</i> , 2010) Ontogeny: NS (Elsdon & Gillanders, 2005a; Hamer <i>et al.</i> , 2006) Diet: POSITIVE (Sanchez-Jerez <i>et al.</i> , 2002; Buckel <i>et al.</i> , 2004) ; NS (Walther & Thorrold, 2006) Intrinsic (within species): SIGNIFICANT (Clarke <i>et al.</i> , 2011)

Mg	NS (Elsdon & Gillanders, 2002; Martin & Thorrold, 2005; Hamer <i>et al.</i> , 2006; Miller, 2011)	NS (DiMaria 2010)	Somatic & otolith growth rate: NEGATIVE (Martin & Thorrold, 2005), NS (DiMaria 2010) Diet: NS (Hoff & Fuiman, 1995) Intrinsic (within species): SIGNIFICANT (Clarke <i>et al.</i> , 2011)
Li	NEGATIVE vs. water Li/Ca, but POSITIVE vs. salinity (Milton & Chenery, 2001) POSITIVE (Hicks <i>et al.</i> , 2010)		
Rb	NEGATIVE (Hicks <i>et al.</i> , 2010)		
K & Na		NEGATIVE (Hoff & Fuiman, 1995)	Reproduction: SIGNIFICANT (Fuiman & Hoff, 1995) Diet: NS (Hoff & Fuiman, 1995)
Mn	NS/NEGATIVE, particularly in the core (Hanson & Zdanowicz, 1999; Brophy <i>et al.</i> , 2003; Elsdon & Gillanders, 2003b; Brophy <i>et al.</i> , 2004; Miller, 2009) POSITIVE – in field (Dorval <i>et al.</i> , 2007), POSITIVE but NS – in field (Hamer <i>et al.</i> , 2006) NS (cf. sediment concentrations) (Hanson & Zdanowicz, 1999)	NEGATIVE (Miller, 2009)	Diet: POSITIVE (Sanchez-Jerez <i>et al.</i> , 2002) ; NS (Buckel <i>et al.</i> , 2004) Intrinsic (within species): SIGNIFICANT (Clarke <i>et al.</i> , 2011)
Zn	POSITIVE (Arai <i>et al.</i> , 2007) POSITIVE (FW)? (Halden <i>et al.</i> , 2000) NS (Thorrold <i>et al.</i> , 1997b; Hanson & Zdanowicz, 1999; Ranaldi & Gagnon, 2008b)		Reproduction? [†] POSITIVE (FW)? (Halden <i>et al.</i> , 2000) Diet: POSITIVE (Ranaldi & Gagnon, 2008b)
Cu	NS (cf. sediment concentrations) (Hanson & Zdanowicz, 1999) POSITIVE (Milton & Chenery, 2001b)		Reproduction? [†] Diet: NS (Milton & Chenery, 2001)
Pb	POSITIVE (Ranaldi & Gagnon, 2008a; Milton & Chenery, 2001b); POSITIVE for 2 spp; NS for plaice (Geffen <i>et al.</i> , 1998)		Diet: NS (Milton & Chenery, 2001).
Hg	POSITIVE for 2 spp; NS for plaice (Geffen <i>et al.</i> , 1998)		
Se	POSITIVE (Lochet <i>et al.</i> , 2010); POSITIVE (FW) – in field (Limburg <i>et al.</i> , 2010)		Reproduction? [†]
Fe	POSITIVE (Ranaldi & Gagnon, 2008)		Age: NEGATIVE (Papadopoulou <i>et al.</i> , 1980)
Cd	POSITIVE (Ranaldi & Gagnon, 2009)		Diet: POSITIVE (Ranaldi & Gagnon, 2009);
Al	POSITIVE – (Mugiya <i>et al.</i> , 1991)		
Others	Cr, Ni: NS (cf. sediment concentrations) (Hanson & Zdanowicz, 1999)		Age: NEGATIVE for Cs, Co and Ag (Papadopoulou <i>et al.</i> , 1980)

[†] Crucial in vertebrate reproductive processes (Versieck & Cornelis, 1989; Bedwal & Bahuguna, 1994; Watanabe *et al.*, 1997); possible effects on otolith chemistry unknown

1 **FIGURE LEGENDS**

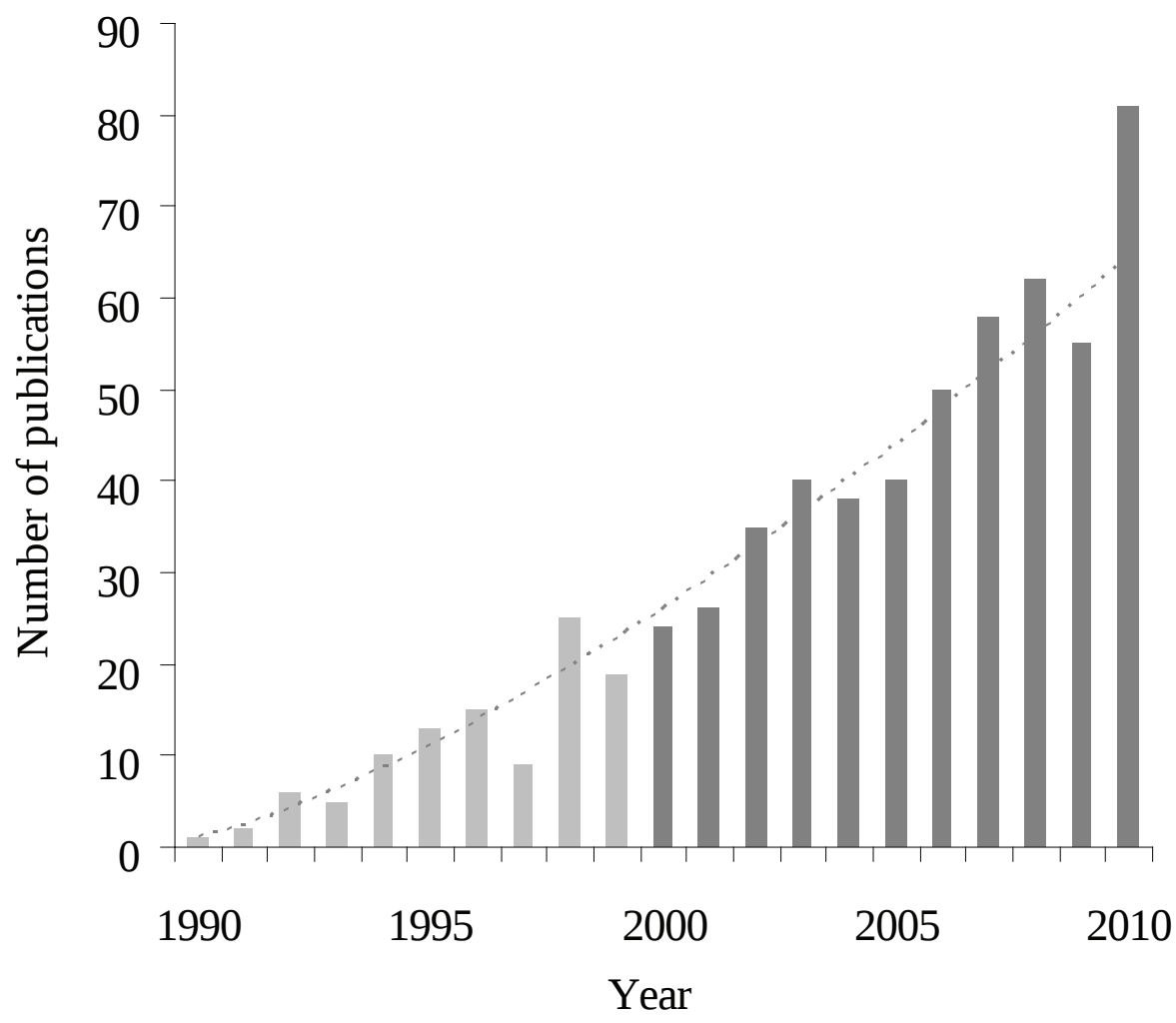
2

3 **Figure 1** Yearly numbers of publications featuring otolith chemistry over the
4 past two decades (Web of Science search on 26.09.11, Topic=otolith chemistry OR
5 otolith microchemistry). Pale grey bars indicate years overlapping with Campana &
6 Thorrold (2001). A power curve closely fits the data ($y = 0.91x^{1.40}$, $r^2 = 0.97$),
7 although the sharp rise in 2010 can be explained, in part, by two special issues of
8 Environmental Biology of Fishes dedicated to “Proceedings of the 4th International
9 Otolith Symposium”

10

11 **Figure 2** Schematic summarising the chemical behaviour of key elements used
12 in otolith research. „Hard“ acid elements such as Mg, Sr, Ca and Ba are typically held
13 as hydrated ion complexes in both seawater and blood and their relative
14 concentrations will be passed to otoliths with less influence from blood protein
15 chemistry. By contrast, „soft“ acid elements such as Cu and Pb are strongly bound to
16 organic ligands and have a greater affinity for protein-binding in blood. The practical
17 outcome is that while soft ions hold greater promise for geolocating in marine
18 settings, they are also the ions most susceptible to physiological fractionation in the
19 body. Figure is based on “hard and soft acid and base” (HSAB) theory and data in
20 Williams (1971), Henderson (1984) and Kaim & Schwederski (1994).

Figure



Figure

Hard soft acid base (HSAB)	Element	Type of distribution [†]	Ocean residence time (yrs)	Ion radius	Charge	Electronegativity
Hard	Li	c	10 ^{^8}	0.76	1+	0.98
	Mg	c	10 ^{^8}	0.72	2+	1.31
	Ca	almost c	10 ^{^8}	1	2+	1
	Sr	almost c	10 ^{^8}	1.18	2+	0.95
	Ba	n	10 ^{^4}	1.35	2+	0.89
Intermediate	Mn	s	10 ^{^4}	0.67	2+	1.55
	Zn	n	10 ^{^4}	0.74	2+	1.65
Soft	Cu	s + n	10 ^{^4}	0.77	2+	1.9

[†] c = conservative, s = scavenged, n = nutrient-like (after Steele et al., 2009)

