OTOLITH CHEMISTRY TO DESCRIBE MOVEMENTS AND LIFE-HISTORY PARAMETERS OF FISHES: HYPOTHESES, ASSUMPTIONS, LIMITATIONS AND INFERENCES

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Abstract   In ever-increasing numbers, researchers wish to extract information based on chemical analyses from otoliths to determine movements and life-history patterns of fish. Such analyses make assumptions about chemical incorporation and interpretation that are beyond those that are important for stock discrimination studies, another common application. The authors aim to clarify the methods of determining fish movement based on natural and artificial otolith chemical tags and review current trends in determining movement using otolith chemistry, otolith sampling methods, and what influences otolith chemistry. Both spatial and temporal variability in water and otolith chemistries, which underpin the assumptions of several methods, are discussed. Five methods for determining movement and migration of fish are outlined: (1) estimates of movement and life-history traits of a single fish group, (2) assessing connectivity among groups using natural chemical tags in otoliths, (3) transgenerational marks to determine parentage and natal origins, (4) profile analysis to define life-history variation within a population and (5) profile analysis to describe movements through different environments. Within each of these methods, background information, specific hypotheses being tested and assumptions and limitations of each technique are provided. Finally,

research directions required to fill current knowledge gaps and enhance the usefulness of otolith chemistry to determine fish movement are identified.

Introduction

Otoliths are proving extremely valuable tools for studying movement and life-history characteristics of teleost fishes. The term 'movement' can be used to describe both small-scale random changes in position or place and large-scale non-random changes from one region to another (migration; Dingle 1996). Irrespective of the scale at which we view movements, identifying and describing movements remains a challenge. Many techniques can be used to identify fish movements (Gillanders et al. 2003), and these can be classified into broad categories of natural tags (e.g., chemical, genetic, and parasitic) and applied tags (passive integrated transponder tags, acoustic tags, artificial chemistry, and archival tags). Natural chemical tags recorded in otoliths have some obvious advantages over applied tags because all fish are marked from early life (many as embryos), the tags are usually permanent, and tags can be related to fish age. The challenge of using natural tags is to translate tag variability into an interpretable pattern of movement. Our aim is to focus on otolith chemical tags, both those that occur naturally and those that are applied artificially. Specifically, these tags have shown great promise for determining fish movements based on the variation in chemical composition, but technical and statistical challenges remain.

Otoliths are paired structures composed of biogenic calcium carbonate, typically in the form of aragonite, deposited on a protein matrix. Forming part of the inner ear of teleost fishes, these structures sit within a membrane filled with endolymph fluid (Campana 1999, Popper & Lu 2000). Otoliths accrete new crystalline and protein material on to their exterior surface daily, and incorporated within these accreted layers are minor and trace elements along with the major constituents of aragonite (C, O, and Ca, Campana 1999) (collectively referred to as 'chemicals'; see 'Glossary'). Hence otoliths provide a chemical chronology over the entire life of a fish. Quantifying and interpreting the chemical composition of fish otoliths is a growing field within fish ecology. The first otolith chemistry papers were published up to 30 yr ago (Gauldie & Nathan 1977, Papadopoulou et al. 1978, Gauldie et al. 1980, Kalish 1989, Radtke 1989). However, investigations of elements in calcified structures of fish pre-date this by several decades (Dannevig 1956, Odum 1957, Sr uptake in bone, Rosenthal 1957). Recently there has been a surge in publications that have used otolith chemistry to determine fish movements (206 papers, 1996–2005, Campana 2005a), three international otolith symposia with dedicated chemistry sessions (Secor et al. 1995a, Fossum et al. 2000, Begg et al. 2005), and several reviews (Campana 1999, Thresher 1999, Elsdon & Gillanders 2003a). There is no doubt that variation in otolith chemistry can be used to explore life-history information. But what does this information tell us? How can we interpret differences in otolith chemistry and infer possible fish movement and life-history traits? Can we develop protocols and procedures, based on the quality and amount of information collected and associated assumptions, to estimate realistic movement and life-history patterns of fish from otolith chemistry? Can we use natural or applied otolith tags to study population dynamics in the same way that we would use other tags?

Campana (2005b) highlighted the applications and assumptions when using otolith chemistry to discriminate among fish groups. An obvious assumption of the approach is that there are characteristic and reproducible markers for each group. All possible groups contributing to the group mixture should be characterized, although statistical techniques that identify uncharacterized groups can be used. The marker also must remain stable over the interval between characterization and mixing. Many researchers wish to go further and extract more information beyond short-term population associations and use otolith chemistry to determine lifetime movements and life-history patterns. In doing so, it is necessary to make a number of assumptions about chemical incorporation and interpretation in addition to those already presented for mixed stock analysis. Determining movements

of fish from otolith chemistry relies on combinations of different chemical signatures (hereafter referred to as a 'tag') being incorporated in different environments. Using this information, we can link groups of fish with similar tags through space (different locations and habitats) and time (different seasons or years) to determine where and when fish moved. If desired, chemical signatures can be examined across an otolith to provide natural tags for several different life-history stages. These chemical profiles can then be related to different environments the fish has lived in; thus, movement patterns of individuals and groups can be deduced. An important assumption is that changes in environments experienced by fish are temporally matched by changes in associated chemical incorporation in the otolith (Secor et al. 1995b, Elsdon & Gillanders 2006a).

Within the current literature, researchers have drawn conclusions about fish movement and life-history behaviours from natural otolith chemical tags, but often using data of varying quality and quantity, and without stating the assumptions that are made when determining fish movements using otolith chemistry. Given this confusion, we have highlighted five methods to determine fish movement from otolith chemistry and their underlying assumptions. All of these methods can describe fish movement and life history. However, the amount of data required and the number of assumptions made differs among methods. Two of these methods make no assumptions about which environmental parameters influence otolith composition, while three do.

The authors aim to clarify the methods of determining movement, and to a lesser degree vital rates, based on otolith chemistry. First, current trends in determining movement using otolith chemistry, otolith sampling methods, and what influences otolith chemistry are reviewed and second, both spatial and temporal variability in water and otolith chemistry that underpin the assumptions of several methods. Third, five methods for determining movement and migration of fish are outlined: (1) estimates of movement and life-history traits of a single fish group, (2) assessing connectivity among groups using natural chemical tags in otoliths, (3) transgenerational marks to determine parentage and natal origins, (4) profile analysis to define life-history variation within a population, and (5) profile analysis to describe movements through different environments, where group(s) refers to all fish with a similar chemical tag. Within each of these methods, the authors provide background information and state the specific hypotheses being tested and clarify which assumptions are made and what limits the interpretability of each method. This should help researchers improve experimental designs and methods of measurement, use proper statistical analyses, and develop more rigorous sets of inferences about fish movement and life histories. Last, research directions required to fill current knowledge gaps and enhance the usefulness of otolith chemistry to determine fish movement are outlined. The examples and discussions given have largely been based on a handful of elements (e.g., strontium and barium) and isotopes (e.g., oxygen and strontium) for which adequate data exist. Assumptions, inferences, and interpretations of the different methods are, however, the same regardless of the chemicals being examined.

Otolith sampling methods

General methods of otolith preparation and analysis on a wide range of instruments (e.g., Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA ICP-MS), electron and nuclear microprobes, synchrotron-based methods, micromilling for stable isotopes) can be found in the existing literature (Campana et al. 1995, 1997, Sinclair et al. 1998, Radtke et al. 1999, Markwitz et al. 2000, Limburg et al. 2007). Briefly, otoliths are dissected from fish and chemical signatures are quantified after either dissolving whole otoliths or sectioning the otolith (Figure 1). Differences in how instruments quantify these chemicals are not the focus of this review. What is of concern, however, is the choice of instrumentation and type of analysis done as this will determine the spatial resolution of chemical tags within the otolith matrix analysed and the resultant information on fish movement. Otolith analyses can be divided into two broad types: those based on whole otoliths or
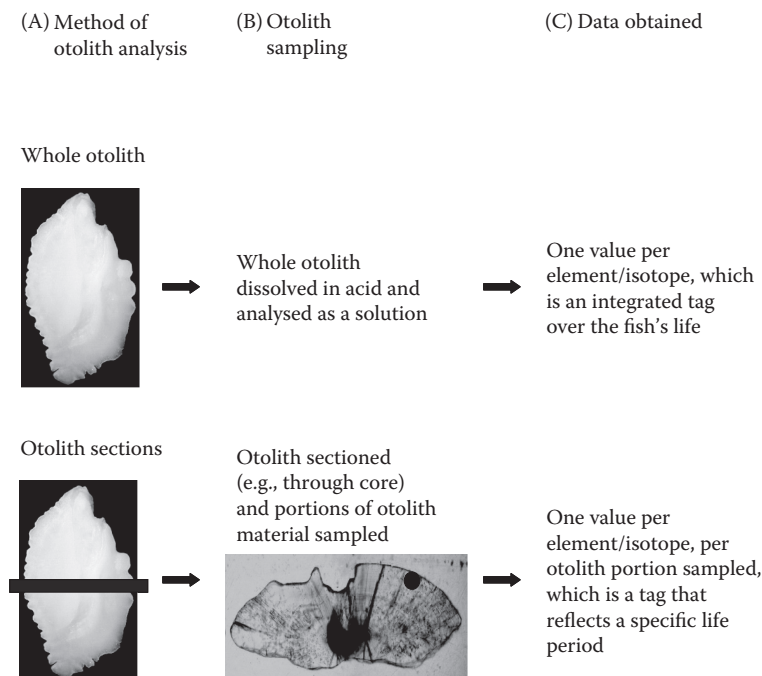
(A) Method of
   otolith analysis

(B) Otolith
   sampling

(C) Data obtained

Whole otolith

Whole otolith
dissolved in acid and
analysed as a solution

One value per
element/isotope, which
is an integrated tag
over the fish's life

Otolith sections

Otolith sectioned
(e.g., through core)
and portions of otolith
material sampled

One value per
element/isotope, per
otolith portion sampled,
which is a tag that
reflects a specific life
period

Figure 1 Two methods of otolith sampling: whole and otolith sections (A, B) and the data obtained from such methods (C).

those based on otolith sections (Figure 1). These two approaches can provide very different types of information and each can be used successfully to answer different questions (as discussed in depth in Campana 2005b).

The analysis of whole otoliths provides a tag that integrates chemical signatures across the entire life of the fish, from embryonic stages to capture (Figure 1). Hence, sampling whole otoliths provides a chemical tag of environments integrated over the fish's entire life, and can serve as a mark of a particular group. Solution based analyses of whole otoliths are often more precise than those obtained using probe based techniques (Campana 1999) and can, therefore, be the preferred option in some applications (Campana et al. 1997, Secor et al. 2001). Whole otolith analyses are particularly good for characterizing chemical tags of groups of fish, then subsequently tracking the movement or mixture of those groups over short periods (Campana et al. 1995, 2000, Gillanders & Kingsford 1996). Unique chemical tags in otoliths of groups of fish will remain distinct and iden- tifiable for a period of time even if the groups move or mix, thereby accreting new otolith material with new chemistry, provided this additional material is minimal. Statistical techniques then allow the proportions of each group in a mixture of fish with unknown origins to be accurately estimated (Campana 1999). Separation of the groups in the mixture ceases to be possible when the composition of new material incorporated after the initial characterization of group signatures alters the chemi- cal tag (Campana 2005b). Thus, whole-otolith chemical tags are unlikely to remain constant over long periods of time, but are stable over shorter intervals of months, seasons, or occasionally years (Kennedy et al. 1997, Campana et al. 2000, Elsdon & Gillanders 2003a, Campana 2005b). In some instances, little otolith growth occurs during periods of mixing and therefore otolith chemical tags can be used to determine life time migration behaviours (e.g., contingent structure, Secor 1999).

In contrast, chemical tags can be established for different life-history stages, such as larvae, juveniles, and adults, when otoliths are sectioned and sampled on spatial scales that correspond to
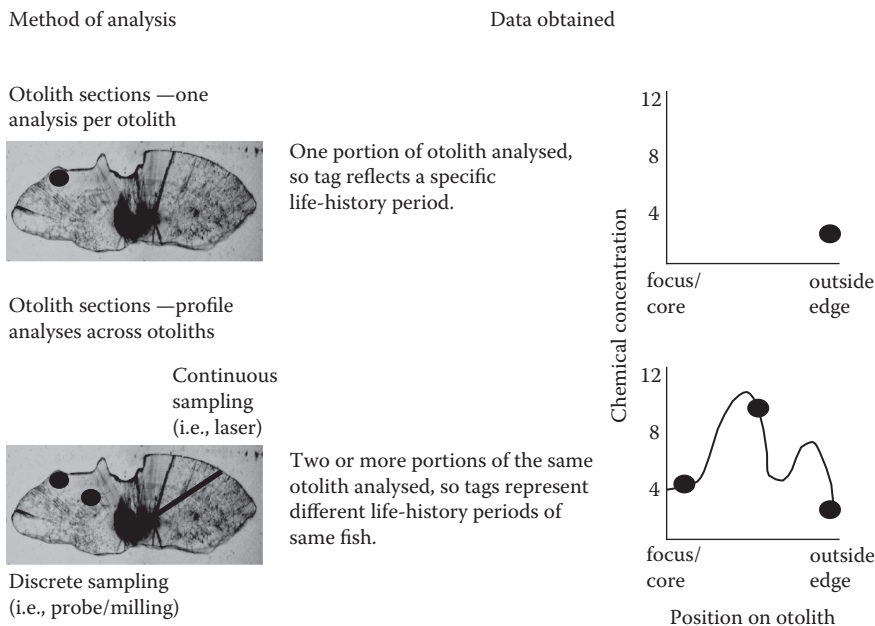
Figure 2 Methods of sampling otolith sections for chemistry: one analysis per otolith, profile analysis across otolith, and the data obtained from such methods.

the time periods of interest (Figure 2). Sampling along sections of the otolith can be accomplished using a range of instruments that can determine chemical tags in otolith material at spatial scales <1 μm that can correspond to temporal scales of <1 day (Markwitz et al. 2000). Analyzing sections of otoliths allows for the detection of small-scale chemical differences in otoliths that would otherwise be averaged out using whole-otolith solution techniques (Fowler et al. 1995a,b, Elsdon & Gillanders 2003a). Thus, for different life-history stages, we can determine unique chemical tags that can be related to different fish groups or environments.

Chemical analyses of otolith sections can be divided into two categories: examination of a single location within an otolith and examination of the chemical profile at two or more locations across a sectioned otolith (Figure 2). In the former, chemistry is determined for a discrete portion of otolith that can then be related to a specific life-history period. We have used the term *profiles* to describe the analysis of two or more different locations along the otolith growth axis; this includes the continuous quantification of chemicals across the otolith surface. X-ray fluorescence, Micro-Proton Induced X-ray Emission (PIXE), electron microprobe, micromilling and mass spectrometry, and LA ICP-MS methods can all produce profiles of chemical tags, which also have been referred to and displayed as transects, trajectories, profiles, scans, chronologies, 2-dimensional maps, and line scans in the literature. The aim of profile analyses is to relate changes in chemistry across otoliths to fish movement.

What influences otolith tags

Information on otolith crystallization is beyond the scope of this review and was comprehensively detailed by Campana (1999). Nevertheless, it is important to discriminate between chemicals that are likely to be good indicators of environmental parameters of specific areas, such as water chemistry, temperature, and salinity, and those chemicals that are controlled by physiology. Chemicals that are incorporated into the otolith via substitution for calcium (i.e., $SrCO_3$) (Bragg 1924) and

included in interstitial spaces (Wyckoff 1964, Faure & Powell 1972, de Vries et al. 2005) are likely to reflect environment parameters. Several element and isotope ratios (i.e., Sr:Ca, Ba:Ca, and Sr and O isotopes) appear to reflect environmental parameters either linearly or non-linearly, and as such they are ideal tracers for determining fish movement (Kennedy et al. 1997, Bath et al. 2000, Limburg et al. 2003, Elsdon & Gillanders 2004, Dorval et al. 2007, Kerr et al. 2007). Elements that are under physiological regulation or those that may leach out of otoliths, including N, K, Cl, Zn, and Cu, are less likely to reflect environmental parameters (Limburg unpublished data, Kalish 1989, Proctor & Thresher 1998, Rooker et al. 2001, Miller et al. 2006).

Several experiments have attempted to identify the sources of elements that are ultimately deposited in otoliths (e.g., Hoff & Fuiman 1995, Farrell & Campana 1996). For example, 83% and 98% of Sr and Ba, respectively, in otoliths were derived from the surrounding water in marine species (Walther & Thorrold 2006) and 88% of Sr was from the surrounding water in freshwater species (Farrell & Campana 1996), with the remaining percentage assumed to be from dietary intake. Similarly, dissolved inorganic carbon (DIC) typically contributes 70–80% to the carbon deposited in otoliths, with the remaining 20–30% coming from metabolic sources (Kalish 1991, Thorrold et al. 1997a, Solomon et al. 2006). It should be noted that not all chemicals will act in similar manners, with chemicals such as strontium representing water chemistry, but sulphur isotopes representing dietary sources.

Environmental processes affecting otolith composition have been reviewed by Campana (1999), Secor & Rooker (2000), and more recently Elsdon and Gillanders (2003a). Relationships between elements and environmental processes are often examined using element-to-Ca ratios, as elements can substitute for Ca in $CaCO_3$, and therefore elemental incorporation will be dependent on Ca concentration. For at least some element-to-Ca and isotope ratios, otolith composition reflects ambient water concentrations, albeit with varying degrees of mass discrimination (Brown & Harris 1995, Farrell & Campana 1996, Bath et al. 2000, Elsdon & Gillanders 2003b, Kraus & Secor 2004, Hobbs et al. 2005, Dorval et al. 2007, Munro et al. 2008). For many elements, however, there is not a clear or consistent relationship between water and otolith chemistry (e.g., Mg, Elsdon & Gillanders 2003b, Wells et al. 2003a, Dorval et al. 2007).

The effect of salinity on otolith chemistry requires careful consideration. Ions will follow a mixing curve between two end-members defined by the element-to-Ca ratio (i.e., ambient Sr:Ca). For instance, Sr:Ca ratios in fully marine environments (salinity = 35) are relatively constant, while freshwater end-members show considerable geographic and temporal variability. In most systems, freshwater Sr:Ca end-members are considerably lower than the global ocean end-member, and this pattern explains why most studies have found a positive correlation between otolith Sr:Ca and salinity (Secor & Rooker 2000). In some systems, however, the freshwater Sr:Ca end-members exceed the marine end-member, leading to a negative correlation between otolith Sr:Ca and salinity (Kraus & Secor 2004, Limburg & Siegel 2006). Underlying differences in water chemistry may therefore explain the various positive, negative, and no effects of salinity on otolith Sr reported in the literature (Fowler et al. 1995b, Hoff & Fuiman 1995, Chesney et al. 1998, Elsdon & Gillanders 2002, 2004, Martin et al. 2004, Martin & Thorrold 2005, Dorval et al. 2007) and points to the importance of critically evaluating water chemistry when studying systems. Salinity effects that are independent of Sr:Ca water concentrations (e.g., due to osmoregulation) have rarely been studied (Kraus & Secor 2004, Zimmerman 2005), but do show some effects (partition coefficients calculated from Elsdon & Gillanders 2004 and published in Martin et al. 2004).

Temperature, independent of its relationship to growth rate, can affect the assimilation of some elements into otoliths. Temperature generally has a positive influence on otolith Sr:Ca (Bath et al. 2000, Elsdon & Gillanders 2002, Martin et al. 2004). The effects temperature has on Ba, Mn, and Mg are varied, with studies detecting positive, negative, and no effects. These differences are, however, based on only a few experiments (Fowler et al. 1995a,b, Hoff & Fuiman 1995, Elsdon &

Gillanders 2002, Martin & Thorrold 2005). The influence of temperature on otolith chemistry is likely to be due to both physiology (Townsend et al. 1992) and kinetic processes (i.e., temperature affecting crystallography, Nielson & Christoffersen 1982).

Interactions between environmental variables can occur when changes in one variable (e.g., salinity) affect the way another variable (e.g., temperature) influences otolith chemistry. Several experiments have documented interactions among environmental variables (Secor et al. 1995b, Elsdon & Gillanders 2002, 2004, Martin & Thorrold 2005). Additional care is therefore required when linking changes in otolith chemistry to water chemistry variations in environments, such as estuaries, where more than one variable can differ.

Variation in water and otolith chemistry

Determining movements of fish based on otolith chemistry is often reliant on knowing the spatial and temporal extent of chemical variation in ambient water and otolith chemistry. Where groups of fish have distinctly different tags in space and time, then connectivity of individuals among those groups can be distinguished without information about water chemistry. Some knowledge of the variation in water chemistry can, nonetheless, aid in evaluating the reliability of natural tags over years and generations. Moreover, using otolith profile analyses to determine movements of fish through different environments relies on knowledge of spatial and temporal differences of water chemistry of those environments. For methods of determining variation in water and otolith chemistry, see 'Detecting variability at different spatial and temporal scales' (p. 321).

Water chemistry

It is important to recognize that water chemistry is likely to vary in space and/or time as chemical concentrations are affected by mixing of water masses, ion exchange between sediments and water, complexation, precipitation, and adsorption (Wilson 1975, Aston 1978, Figures 3 and 4). In inland waters, hydrologic processes, including groundwater transport and retention, play important roles in chemical availability. Together with hydrology and microbial processes, the extent and heterogeneity of parent material (bedrock, soils) determine the availability of elements and isotopes. Although isotopic signatures, such as Sr and O isotopes, are commonly viewed as being reliably stable, these also may differ, especially if water masses of different composition mix, or there are changes in water vapour sources of precipitation or groundwater flow (Fairbanks 1982, Rohling & Bigg 1998). Importantly, correlations between water chemistry and other environmental factors, such as salinity, should not be assumed to be constant.

Spatial differences in water chemistry have often been derived via predictive relationships between chemicals and salinity, where actual chemical concentrations are not measured. For example, a common viewpoint is that water Sr is positively and Ba negatively correlated to salinity. However, many freshwater locations have water Sr:Ca ratios greater than that of seawater (Limburg 1995, Wells et al. 2003a, Kraus & Secor 2004), and ambient Ba levels can be linked to particulate sediments (Li & Chan 1979). It is therefore important to assess spatial patterns in water chemistry both within and among locations (e.g., rivers, estuaries, and reefs). Although published studies on spatial scales of water chemistry (e.g., Ca, Sr, Ba) variability are limited (see Table 1 for a review; Figures 3 and 4), significant variation in water chemistry has been detected at spatial scales ranging from tens of metres to hundreds of kilometres (Figures 3 and 4). Variation in ambient water chemistry is likely to be system dependent, based on tides, water movements, hydrogeology, precipitation, and upwelling (Figure 4), but geographic separation does not guarantee useful differences in water chemistry among locations. There is clearly a need to understand variation in water chemistry in specific systems to aid interpretations of fish movement through environments.
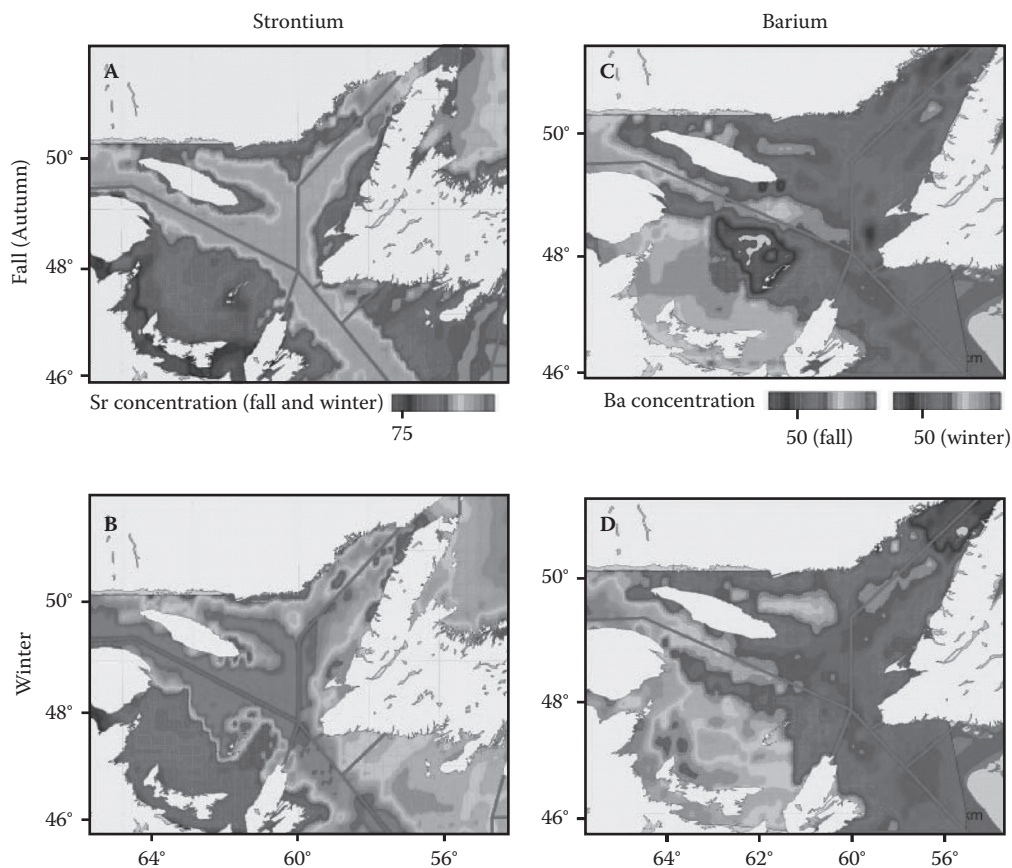
Figure 3 (See also Colour Figure 3 in the insert following p. 250.) Variation in dissolved strontium and barium concentrations in the Gulf of St. Lawrence, Canada, showing spatial variation (within each plot) and temporal variation between seasons for strontium, comparing fall (A) with winter (B); barium, comparing fall (C) with winter (D). Values in parts per million. (S.E. Campana unpublished data.)

Water chemistry at any site typically varies over time (Figure 3). Some knowledge of temporal variation in water chemistry is particularly important if fish movements are to be related to habitats over time. Sampling of temporal variability is generally done by taking replicate samples at fixed time intervals (Surge & Lohmann 2002), and those samples are assumed to represent areas and times beyond the point of sample collection. Two studies have shown little variation in Mg, Mn, Ca, Sr, and Ba from estuarine waters over monthly and seasonal scales (Dorval & Jones 2005, Elsdon & Gillanders 2006b) (Table 1). However, water chemistry in dynamic environments with large tidal ranges may vary over seasonal (Figure 3) and shorter timescales (days, tidal cycles, Elsdon & Gillanders 2006b). For example, large differences between samples collected on different days have been detected for Ca, Mn, Sr and Ba within three small (<10 km) tidal estuaries, where variation on scales of days accounted for up to 64% of the total variation of scales of days, weeks, months, and seasons (e.g., Ca, Ba, Elsdon & Gillanders 2006b). Similarly, water samples collected 1–2 h apart from a single site can have differences in Mn concentration as large as 3.3× (average 1.73× from 85 paired samples), with an average difference of 29.48 μg 1^{-1} (average sample 46.42 μg 1^{-1}) (United States Geological Survey data, Hackensack River, New Jersey). Oxygen isotopes in freshwater systems are responsive to floods and droughts and may change over seasons and years (Fairbanks
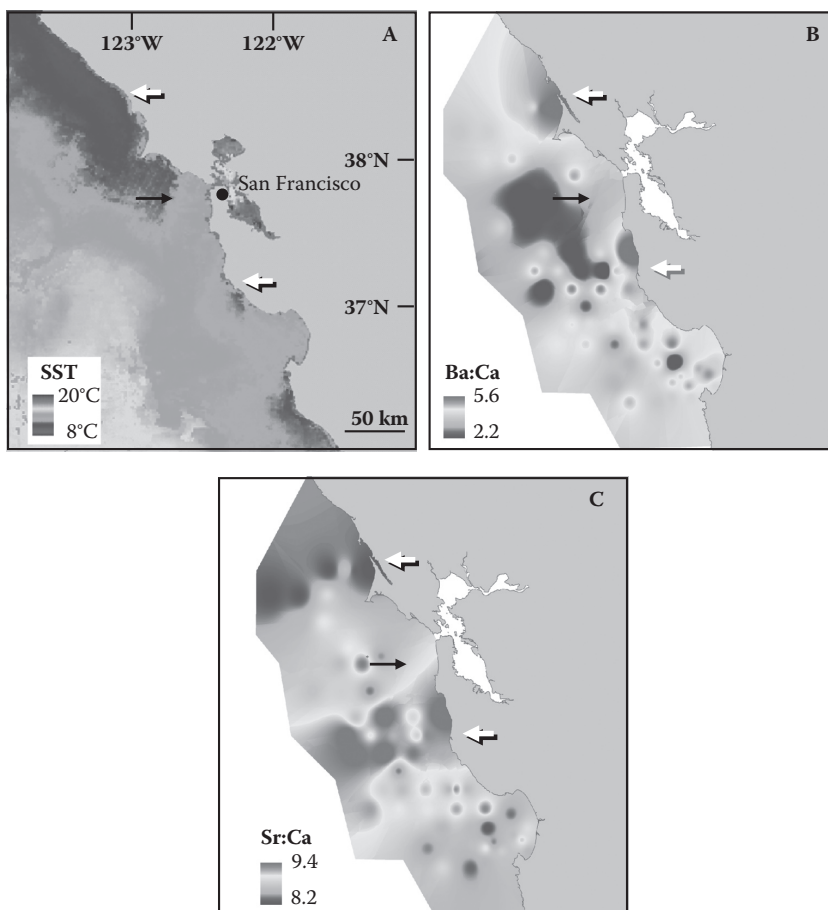
Figure 4 (See also Colour Figure 4 in the insert.) (A) Sea surface temperatures (SSTs) collected during the upwelling season along central California (5–19 June 2006; National Oceanic and Atmospheric Administration Coast Watch program) and representing (B) the Ba:Ca ratios (µmol mol^{-1}) and (C) Sr:Ca ratios (mmol mol^{-1}) collected during the same upwelling season (B. Wells & K. Stierhoff unpublished data, figure prepared by K. Stierhoff). White arrows represent regions of active upwelling and the black arrow represents infusion of saline, warmer waters from farther off shore into the region. *Note:* Variability in Ba:Ca is derived largely from the distribution of upwelling in the region and will vary with the degree and timing of upwelling.

1982, Kerr et al. 2007). Thus, to detect and interpret temporal variation within estuaries it is useful to examine a range of temporal scales and ideally these should be nested (see 'Detecting variability at different spatial and temporal scales'). An appropriate experimental design is not necessarily an expensive process and can lead to a greater understanding of the systems in which we work.

Otolith chemistry

Otolith chemistry of individuals or groups of fish living in different environments may differ if they reside in environments long enough to incorporate a detectable chemical tag. Detailed reviews of spatial and temporal variation in otolith chemistry exist elsewhere (Gillanders et al. 2001, Gillanders 2002a), and therefore we present only a brief synopsis of these and more current literature.

Table 1 Summary of published and unpublished data that examined spatial and temporal scales of variation in ambient chemistry that are potentially useful to investigate movements of fish (organized from smallest to largest scales)

Sampling design	Scale of investigation	Location of study	Chemicals analysed	Differences	Source
Spatial scale investigations					
1 estuary, 8 locations	1–20 km	East coast, Australia	Mn (also Cd, Ni, Cu, Zn)	Significant difference among stations	Hatje et al. (2003)
5 estuarine habitat 'locations', 6 sites per location	5–90 km	East coast, USA	Sr, Ba, Mn, Mg	Analysed by 'habitats'; significant differences for Sr, Ba, Mn, Mn among habitats	Dorval & Jones (2005)
1 bay (San Francisco Bay), 24 sites dispersed through bay	5–140 km	West coast, USA	Mn	Significant differences on scale of sites, with sites grouping out spatially, observed from spatial mapping	Roitz et al. (2002)
2 estuaries, 2 locations in each	10–480 km	East coast, Australia	Sr, Ba, Mn, Mg, Li	No significant differences for Sr, Mg, Li; significant differences for Ba, Mg among sites	Gillanders (unpublished data)
4 locations (3 bays and open coastal), 4–10 sites per location	10–760 km	South-east coast, Australia	Ca, Sr, Ba, Mn, Mg	Analysed by locations; significant differences for all among locations, 1 bay different from other locations	Hamer et al. (2006)
1 river, 2 tributaries	20 km	East coast, USA	Ca, Sr, Ba, Mn, Mg	Significant differences for Ca, Sr, Mg; no significant differences for Ba, Mn	Walther & Thorrold (unpublished data)
12 rivers, 1 location per river	20–900 km	East coast of USA and Canada	Ca, Sr, Ba, Mn, Mg	Significant differences for locations for all chemicals	Walther & Thorrold (unpublished data)
36 rivers	60 km	Idaho, USA	Ca, Sr, Ba, Mg	Significant differences between locations	Wells et al. (2003a)
Temporal Scale Investigations					
Within years					
1 river, 1 location	85 collections, paired samples collected 1–2 h apart	East coast, USA	Mn	Significant differences among samples collected hours apart, average difference ×1.73; observations of means	[a]USGS NWIS data (http://waterdata.usgs.gov/nwis)
2 estuaries, 1 location per estuary	2 collections over 2 tides	South coast, Australia	Ca, Sr, Ba, Mn	Significant differences among tides for Ca, Sr, Ba, Mn at one location	Elsdon & Gillanders (2006b)
1 estuarine location	3 nested scales of tidal cycles, 3 tidal stage (flood, slack, ebb), 4 h	East coast, Australia	Mn (also Al, Fe, Cu, Cr, Pb, Zn)	Significant differences among hours for Mn; no significant differences for tidal cycle or tidal stage	Hatje (2003)

Sampling locations	Sampling design	Location	Elements	Results	Reference
1 bay location	Roughly weekly sampling over 100 days	Antarctica	Mn	Significant differences among times — observations from table in paper	Grotti et al. (2001)
3 estuaries, 1 location per estuary	16 collections on nested scale of 2 seasons, 2 months, 2 wk, 2 days	South coast, Australia	Ca, Sr, Ba, Mn	Significant differences among seasons for Sr (3 estuaries) and Mn (1 estuary); no significant differences for months; significant differences among weeks for Ca, Sr, Mn (2 estuaries); significant differences among days for Ca, Sr, Ba (3 estuaries), Mn (2 estuaries)	Elsdon & Gillanders (2006b)
5 estuarine habitat 'locations', 6 sites per location	6 collections, 2 per month for 3 months	East coast, USA	Sr, Ba, Mn, Mg	Significant differences for Sr among months; no significant differences for Ba, Mn, Mg among months	Dorval & Jones (2005)
1 estuary, 8 locations	3 seasons (summer, winter, summer)	East coast, Australia	Mn (also Cd, Ni, Cu, Zn)	Significant differences among seasons for Mn	Hatje et al. (2003)
1 bay (San Francisco Bay), 24 sites dispersed through bay	3 seasons (winter, summer, spring)	West coast, USA	Mn	Significant differences among times at individual sites, observed from table	Roitz et al. (2002)
16 rivers	Single collections from each river compared between two seasons	Idaho, USA	Ca, Sr, Mg	No significant differences detected	Wells et al. (2003a)
6 rivers	One collection during summer flow compared to fall flow collections	Idaho, USA	Ca, Sr, Mg	Variation between seasons was not significant	Wells et al. (2003a)
Among years					
2 locations (bay and coastal)	4 collections on nested scales of 2 seasons within 2 yr	South-east coast, Australia	Ca, Sr, Ba, Mn, Mg	Analysed as 'time' differences; significant differences in Ca, Sr, Mn, Mg; no significant difference in Ba	Hamer et al. (2006)
9 estuaries	4 collections on nested scale of 2 yr, 2 months within years	East coast, Australia	Sr, Ba, Mn, Mg, Li	No significant differences for Sr (time or estuary); significant differences in Ba, Mn with estuary and month (year); significant differences for Mg between years	Gillanders (unpublished data)
>25 locations from Lake Ontario to the Hudson River mesohaline estuary	Single collections in synoptic surveys, 2 yr running	New York, USA	Ca, Sr, Na, Ba, Mn	Element:Ca ratios geographically consistent between a very wet and very dry year	Limburg & Siegel (2006)

[a] United States Geological Survey National Water Information System (USGS NWIS)

Spatial variation in otolith tags has been investigated in at least 30 papers (Gillanders et al. 2001). Studies have typically assessed differences in otolith chemistry in several locations along extensive coastlines (>1000 km) (Campana et al. 1994, Edmonds et al. 1999). Several studies have, however, examined more than one spatial scale using a nested design by sampling fish at replicate sites (scales of hundreds of metres to kilometres) within several locations separated by at least 10 km (Thorrold et al. 1997b, Campana 1999, Gillanders et al. 2001, Patterson et al. 2004). Spatial discreteness of otolith tags has been detected for sites separated by as little as several metres (Gillanders & Kingsford 2000, Kingsford & Gillanders 2000, Wells et al. 2003a) and for locations over broader spatial scales (up to 1200 km; Secor & Zdanowicz 1998, Thorrold et al. 1998, Campana 1999). Other studies have found no differences in chemical tags among locations separated by as much as 3000 km (Proctor et al. 1995, Kalish et al. 1996). The distinctiveness of otolith chemical tags among groups is likely to depend on (1) individuals occupying environments with sufficiently different physico-chemical properties, (2) the amount of time fish spend in an environment before being captured, and (3) the selection of elements and isotopes to be assayed. Distance among locations is not a good predictor of the magnitude of difference in otolith chemistry as otolith chemistry will reflect differences in environmental properties and the scales at which these vary (see previous section, 'Water chemistry').

Temporal differences in otolith chemistry have been investigated in at least 15 publications (see Gillanders 2002a). The majority of papers have investigated otolith tag differences on either annual (Campana et al. 2000, Rooker et al. 2001, Dorval & Jones 2005, Kerr et al. 2007, Walther et al. 2008) or monthly scales (Hamer et al. 2003) to assess the stability of tags from locations among different annual cohorts. If tags are stable among years, then matching unknown chemical tags to known tags from specific cohorts to determine connectivity is not necessary. Unfortunately, this is rarely the case (Milton et al. 1997, Rooker et al. 2001, Gillanders 2002a, Hamer et al. 2003, Dorval & Jones 2005). A general finding among published papers was that otolith tags showed significant interannual variation. Temporal variability of otolith tags therefore likely reflects differences in temperature, salinity, and water chemistry (Figures 3 and 4). Sampling fish using appropriate designs (i.e., a nested design that incorporates temporal and spatial aspects of the system) that also accommodates for variation in fish growth rates by sampling the population in a representative fashion can go a long way to identifying sources of variation.

Methods for determining movements and life-history measurements

In the following section we refer to a 'population' of fish as all fish of the same species within an area of interest that have the potential to mix (as opposed to a reproductive population), and a 'group' refers to fish with similar otolith tags within a population. We refer to 'location' as an area that groups of fish inhabit and 'region' as the area inhabited by a population and containing more than one location (see 'Glossary').

Method 1: Estimates of movement and life-history traits of a single fish group

Background

In this method, we aim to determine movement and life-history traits, including survival, mixing rates, and recruitment, within a single group of fish. For estimates of movement and life-history traits of more than one group of fish see Method 2. The method is based on establishing baseline chemical tags (natural or applied) for fish groups of interest. This method only requires that the group of

interest possesses a tag that is unique from all other groups. Fish of unknown group membership are then assigned to the group using chemical tags in otoliths. Otolith chemistry can be quantified using solution techniques or by sampling sectioned otoliths (Figure 1). The analysis of whole otoliths is particularly relevant to this method if a group of fish has been segregated long enough to possess a unique tag, and these fish subsequently mix with other groups (Campana 1999, 2005b).

For natural tags, it is important to identify all group tags to evaluate the uniqueness of the group being examined. Deliberately applied otolith chemical tags can be generated by spiking rearing water with unnaturally high levels of a common element (e.g., Sr, Brown & Harris 1995), an element typically in low concentrations (e.g., lanthanides, Ennevor & Beames 1993), a fluorescent bone-seeking chemical (e.g., tetracycline, Jones et al. 1999), or an unnatural isotopic ratio of a common element (e.g., Ba, Thorrold et al. 2006). The key to artificially applied tags is that the tag is substantially different from that which would occur naturally.

To use otolith tags to address questions of mortality, movement, mixing rates, and recruitment the basic assumptions of mark-recovery studies should be met. Specifically, Brownie et al. (1978) and Schwarz & Arnason (1990) identified up to eight assumptions that must be met, or compensated for, to use tags to their full potential. These assumptions are relevant to all methods. We discuss each of these assumptions relative to natural and applied tags (see Table 2).

Table 2 The five methods of determining fish movements based on otolith chemistry and the assumptions that are made for each method

Assumption	Method 1[a]	2	3	4	5
1. Marked sample is representative of the target group, and only the target group	X	X	X	X	X
2. Age of individuals is correctly identified	X	X	X	X	X
3. There is no tag loss	X	X	X	X	X
4. Survival rates are not affected by the handling or tagging itself	X	X	X	X	X
5. The period of recovery is correctly identified	X	X	X	X	X
6. Tagging does not alter fish behaviour	X	X	X	X	X
7. The fate of a given tag is random	X	X	X	X	X
8. All tagged individuals of an identifiable class in the sample have the same recovery rates	X	X	X	X	X
9. Segregation of individuals to incorporate unique tags		X	X	X	X
10. Growth differences among groups of fish are quantified		X	X	X	X
11. Similar methods are used to detect tags		X	X	X	X
12. The maternal tag source is identified			X		
13. Estimating tag retention in the mother and period of tag transmission			X		
14. Otolith chemistry of individuals within groups can be differentiated into identifiable contingents				X	
15. Sampling of fish and their profiles is representative of all profiles that exist				X	
16. Independence of sampling along a profile				X	X
17. Otolith chemistry changes predictably with environmental parameters				X	X
18. Interactive effect of environmental parameters on otolith chemicals are known					X
19. Ontogenetic effects on otolith chemistry are known (if reconstructions bridge different life-history periods)					X
20. Spatial and temporal variation in environmental parameters are quantified					X
21. Correlations between otolith crystallization and chemistry are known					X

[a] The summary does not distinguish between natural tags and applied tags; see Method 1 in text for further details.

Hypotheses and assumptions

If one group possesses a tag that is distinctly different from all other tags, then that tag can be used to trace fish that move among groups.

Assumption 1: Marked sample is representative of the target group, and only the target group
In any study it is critical that the sample fish are representative of the group for which inferences are to be made.

Natural tag   There are two concerns related to natural tags. First, it is possible that the natural mark occurs on only a subset of fish from a group if, for instance, individuals used to determine baseline signatures are from a small, but environmentally distinct habitat within a location. Therefore, fish should be sampled from multiple areas to ensure the signature is characteristic of the whole group and not only a subset. Second, it is possible that the sampled natural tag represents multiple groups that are not of interest. It is critical that the natural tag from an identified group be compared to all possible other groups to ensure it only characterizes the group of interest. This second concern cannot be addressed with statistical techniques used to identify uncharacterized groups as uncharacterized groups may have different tags. Determining the direction of fish movement among these groups becomes impossible if two geographically isolated groups have the same tag. Thus, if during the course of sampling, it is determined that two or more groups of fish possess the same tag, then the spatial or temporal resolution of the hypothesis requires alteration. Ultimately, the hope is that among-group variation exceeds within-group variation, thus providing discrete spatial and temporal resolution of group tags.

In addition to sampling the spatial extent of group tags it is important to quantify the degree of temporal variation in tags to match the scale of the question being addressed. One typical method is to collect otolith tags from each group throughout their residency in a particular location. If individuals leave a habitat or location over time (e.g., salmon emigration from a natal watershed), then sampling must occur before fish start to leave. On a longer time frame, studies have shown that location-specific tags often vary interannually. Therefore, baseline tags must be quantified for each cohort of interest unless chemical tags remain the same over time (see 'Variation in water and otolith chemistry').

Applied tag   Typically, in applied marking studies an appropriately chosen subsample of fish is marked (e.g., tagging on spawning grounds, in the hatchery), and the tag cannot be confused with natural groups.

Assumption 2: Age of individuals is correctly identified
If the age of a fish is incorrectly identified and the elemental tag of that group varies over time, then the tag in the recovered fish may be classified incorrectly.

Natural tag   It is critical to know the age of unknown fish so that we can correctly identify individuals to specific groups using the appropriate baseline data (see, for example, Gillanders 2002b). Age is also critical for the estimation of cohort- and age-specific rate estimation and can commonly be obtained from otoliths.

Applied tag   To estimate cohort- and sex-specific rates of a group with an applied tag it is important to know the age of the tagged fish and use a tag specific to that age group, so that we can correctly identify fish to specific groups using their otolith tags.

Assumption 3: There is no tag loss
Otoliths are considered chemically inert such that, once material is accreted, it is not reworked. Although this may be true for ions substituting for Ca in the aragonite structure, elements that are

weakly bound in interstitial spaces of the otolith may leach out during storage (e.g., Zn, Limburg unpublished observations). Tag loss or alteration may also occur from the method of preservation (Milton & Chenery 1998, Proctor & Thresher 1998, but see Campana et al. 2000). Some elements are more affected than others, depending on where they are incorporated in otoliths, and caution should be taken to determine if there are preservation effects on elements or isotopes of interest, or alternatively the same methods can be used for all fish. Delayed removal of otoliths may also affect their integrity. For example, freezing of brown trout (*Salmo trutta trutta*, Linnaeus, 1783) heads resulted in partial erosion of otoliths, possibly due to contact with acids in the braincase (Limburg unpublished observations). The eroded otoliths showed differential accumulations of Sr and Zn that were likely due to post-mortem transport of the elements. Tag loss due to the methods of preservation may be reduced or eliminated by using consistent methods of otolith extraction and storage for all fish or by using elements or isotopes not subject to preservation effects. Extraction methods have included ultraclean procedures for otolith removal and storage or rigorous decontamination procedures following extraction and storage (e.g., Thresher 1999, Secor et al. 2001).

Tag loss can occur due to the method of chemical sampling. If the original chemical tag is identified on a sectioned otolith at a defined spot (e.g., the core) using a probe-based assay, then otoliths from fish sampled subsequently must be analysed using the same analytical approach and the same region (Gillanders 2002b) (also see Method 2, Assumption 11). Different principles apply to the analysis of whole otoliths, for which the tag represents the entire lifetime of the fish up until the time of capture. Any otolith growth subsequent to that point has the potential to change the tag because new material will be added, which may have different chemistry. Therefore, it is important that the use of whole-otolith tags be restricted to relatively short periods of time after otoliths are taken for tag characterization, during which the whole-otolith composition does not change appreciably due to subsequent growth (e.g., otolith weight does not increase by more than 5%, Campana et al. 1995). Thus, a spatially restricted otolith sampling procedure, such as laser ablation, microprobe or milling small portions of otoliths, offers a reliable methodology for reducing tag alteration if subsequent otolith growth is an issue.

Assumption 4: Survival rates are not affected by the handling or tagging itself

Natural tag   The otoliths of all members of the population are tagged naturally *in situ*; therefore, this assumption is typically not violated.

Applied tag   Handling and tagging of fish may cause immediate or delayed mortality. Violation of this assumption, wherein tagged fish have higher mortality than non-tagged fish, would lead to an underestimation of mortality rates and incorrect interpretation of fish movement. Unfortunately, the effects of long-term survival rates due to chemical tagging are largely unknown, although studies of fluorescent tags do exist (e.g., Tsukamoto et al. 1989).

Assumption 5: The period of recovery is correctly identified
The time between tagging and recovery (fish collection) should be correctly identified, so that life-history measurements, which are intrinsically time related, can be estimated correctly. Hence, estimates of recovery periods are necessary to determine movement rates or survival based on appropriate cohort tags.

Assumption 6: Tagging does not alter fish behaviour
If tagged fish associate with themselves more than with untagged fish of the same population there will be overdispersion of the data (observed variance is greater that the theoretical variance of the model) and migration and life-history measurements will be biased.

Natural tag   Natural tags are not affected by the action of the study, so this assumption does not apply.

Applied tag   Applying a tag may result in changes in behaviour and associations among fish, such as schooling and migration (McKinnell et al. 1997, Hay & McKinnell 2002), and tagged fish may not mix with untagged fish, for example if fish are tagged in a school of similar size or age. Thus, it is important to consider and, if possible, correct for behavioural effects of tagging.

Assumption 7: The fate of a given tag is random

Natural tags   This assumption simply implies that the likelihood of collecting a tag, and therefore an individual fish, is random between collection periods. We see no reason why natural chemical tags in fish would violate this assumption, unless there is a delayed or cumulative reaction to elemental exposure or for some reason fish from different tag groups associate differently compared to others.

Applied tags   For applied tags, any alteration in behaviour, such as clustering of tagged fish, and associations among fish (previous assumption) are likely to affect the collection of a tag.

Assumption 8: All tagged individuals of an identifiable class in the sample have the same recovery rates

Natural tags   In terms of sampling, all individuals must be collected by the same sampling gear with the same catchability and effort (C.M. Jones personal communication). Sample sizes should not be fixed, but rather reflect differences in population densities in most cases.

Applied tags   For applied tags, the recovery rates are usually taken as a nuisance parameter and not investigated further. There may, however, be concern if the distribution and mixing rate of the fish are size dependent as opposed to age specific, which may occur when applied tags affect fish growth. This is important for mortality and production estimation and for inferring movements of a whole group.

Limitations and inferences

Using natural chemical tags as a mark-recovery tool provides a means to reliably 'mark' many more fish than conventional applied tags at zero cost. However, the development of appropriate statistical models to interpret recoveries quantitatively is in its preliminary stages (C.M. Jones personal communication). This method, however, holds much promise if all of the relevant assumptions are addressed since inferences about movement and life-history measurements can be quite powerful. In the meantime, the advantage of applied tags, such as altering otolith chemistry, is that normal mark-recapture procedures can be followed and quantitative estimates developed for movement and survival using well-known statistical techniques. By addressing assumptions, otolith tags can answer the same range of questions as traditional tag-recovery studies (e.g., mortality, relative contribution of tagged groups, and distribution).

The most important difference between measuring movement and survival by use of natural and applied otolith tags is that for natural tags it is necessary to determine the number of tagged fish within a group through an independent survey (C.M. Jones personal communication). To infer population-level movements of fish among groups it is important to recognize that estimates of the number of fish marked, the survivorship of fish in different groups, and the recovery rates of groups are needed. With estimates of these values, mark-recovery calculations from natural tags can be used to estimate true population movements (e.g., estuary X contributes 80% of the recruits to the

population) (C.M. Jones personal communication). Without group-specific estimates of survivorship, descriptions of movement can be reported as relative rates given the representative sampling done, as have been previously published (Thorrold et al. 1998, Gillanders 2002b).

Example: Applied elemental tagging fish in early life

Ennevor and Beames (1993) demonstrated that lanthanide elements could be used to mark the otoliths of coho salmon fry (*Oncorhynchus kisutch*, Walbaum 1792). Likewise, Schroder et al. (1995) used artificially elevated strontium concentrations to mark chum and sockeye salmon fry with success. Swearer et al. (1999) and Jones et al. (1999) present two examples of applying these techniques in which the degree of self-recruitment of reef fish was determined by natural (Swearer et al. 1999) and applied (Jones et al. 1999, 2005) tags. Jones et al. (1999) applied otolith tags to a known portion of damselfish (*Pomacentrus amboinensis*, Bleeker 1868) embryos at Lizard Island (Great Barrier Reef) that were later identified in recruited juveniles. With knowledge of the proportion of embryos marked and the proportion of juveniles with the applied tag, Jones et al. (1999) determined that as many as 60% of the damselfish that recruited to the region were from local sources as opposed to the products of long-distance dispersal.

Method 2: Assessing connectivity among groups using natural chemical tags in otoliths

Background

Method 2 extends the previous section in that more than one group of fish is marked and, in subsequent sampling, each group is recovered (Table 2). From this information, the contribution of different groups to a mixed population is determined. Here we examine a specific case in which the researcher uses natural chemical signatures in otoliths to describe connectivity of individuals and groups in an effort to evaluate group mixing, movements among groups, and natal homing. We discuss only natural tags in otoliths, as they are the more complicated extension of traditional mark recovery, but the method could be easily extended to using different applied chemical tags (e.g., Munro et al. in press). We do not discuss issues related to describing mortality and productivity as they follow the same assumptions as the previous methodology and are applied to multiple populations simultaneously.

Hypotheses and assumptions

Otolith chemical tags can be used to estimate connectivity among groups if groups possess distinctly different chemical tags and those groups subsequently mix.

Assumptions for Method 1 should be addressed (Table 2), in addition to:

Assumption 9: Segregation of individual groups to incorporate unique tags
All groups of fish must be chemically distinguishable from one another, or estimates of movement among groups are not possible. Logically, this implies that groups have been segregated in different locations (or habitats) during the same life-history stages, or at least at some life-history stage in the case of whole-otolith tags. Furthermore, it is assumed that enough otolith material is incorporated during the time period when the groups were segregated so that these differences can be detected (see also Assumption 3 for statistical tag loss, if fish have only a small portion of their otolith tagged from one location). If groups cannot be distinguished, then all fish with similar tags should be treated as a single group in any analyses.

Assumption 10: Growth differences among groups of fish are quantified

Differences in growth rates are not important for establishing tag differences; however, they do become important if recaptured fish from different groups have had different growth rates and whole otoliths are analysed. Therefore, it is important that fish size and age within each group are known, and where possible, fish of similar size and age are collected between regions (Thorrold et al. 1998, Wells et al. 2003b). If there is a substantial difference in growth between groups of fish it may be necessary to account for such differences. Growth differences among groups can be used as a covariate in order to determine if correlations between otolith tags and growth occurred (Gillanders et al. 2001).

Assumption 11: Similar methods are used to detect tags

Similar methods of chemical analyses should be used to estimate tags in all groups; otherwise variation in analytical methods can lead to false identification of group-specific tags (Campana et al. 1997). Thus, the same region of the otolith must be sampled for all groups of fish. We also recommend that the same analytical protocol (instruments, standards, etc.) is used for all groups, as different methods may result in different estimates of chemical concentrations (Gunn et al. 1992, Campana et al. 1997, Secor et al. 2002, Elsdon & Gillanders 2003a, Ludsin et al. 2006). It is, however, possible to use different analytical techniques between the assessment of the baseline and unclassified dataset, so long as they can be standardized and compared. For instance, Thorrold et al. (1998) examined baseline variation in juvenile weakfish (*Cynoscion regalis*, Bloch and Schneider 1801) using solution-based ICP-MS of otoliths from major groups of weakfish along the Atlantic coast. Adults collected from the mixed population were, however, examined using laser-ablation ICP-MS to analyse the otolith region formed during the juvenile period (that period examined using solution-based techniques earlier) (Thorrold et al. 2001). The data were normalized so that relative differences in location-specific tags were not affected by sampling method.

Limitations and inferences

If all groups are not included in the baseline dataset, errors may be made when estimating the mixed-stock composition (Campana et al. 2000, Gillanders 2005). Specifically, if a group is not included in the baseline dataset, but recovered subsequently, it may be misclassified to another known group, depending on how distinctive its tag is and on the statistical test used (Gillanders 2005). Methods to deal with undescribed groups as a contributor to the mixed population do exist (Smouse et al. 1990, Pritchard et al. 2000). Excluding or consolidating groups that make up a small proportion of the mixed population (e.g., a given river mouth from a larger estuary, Thorrold et al. 1998) may improve the estimates of group proportions in the mixed population (Fabrizio 2005).

The identification of multiple group tags to assess connectivity is a powerful technique. The analysis of connectivity can also be used to describe the proportion of a population that results from self-recruitment. In addition to addressing assumptions of Method 1 and those outlined in this section, it is important to recognize the limitation of inferring connectivity and self-recruitment if only a subset of groups from the entire population is identified. If only subsets of groups are examined then it is important that there are no substantial overlaps in tags among groups, which could bias estimates. It is difficult to assess if this has occurred unless all groups are sampled, which requires adequate fish sampling (see 'Variation in water and otolith chemistry'). Conversely, if a number of groups have unique tags that are not represented by other groups, then connectivity of a subset of the population can be assessed. Again, to infer connectivity as a proportion of the actual population it is necessary to determine the relative number of tagged fish within groups (Jones et al. 1999), and the survivorship of fish within particular groups or locations, so that mark-recapture calculations using natural tags can be done (C.M. Jones personal communication).

Water chemistry information is not needed to evaluate connectivity. Nevertheless, an initial examination of water chemistry across the land or seascape may provide useful information on potential variability in otolith signatures (Wells et al. 2003a, Limburg & Siegel 2006). Dorval & Jones (2005), Dorval et al. (2007) and Elsdon & Gillanders (2006b) demonstrate examples of using nested designs to determine variability in water chemistry. See also 'Detecting variability at different spatial and temporal scales'.

Example: Mixed composition and natal homing of weakfish along the Atlantic coast

Thorrold et al. (2001) described connectivity among groups of weakfish spawning in estuaries along the Atlantic coast of the United States using natural otolith tags. Baseline elemental signatures of young-of-the-year juveniles were quantified from five major groups along the Atlantic coast using a nested design within each of the five estuarine systems. Fish were collected from the same five areas during the spawning season 2 yr later, and the degree of connectivity between source regions on spawning grounds as well as natal homing was described on a cohort-specific basis. A further step of determining the actual connectivity in a population, as opposed to describing the proportion of self-recruitment from the sampled fish, would require estimates of the number of fish possessing each of the different tags (C.M. Jones personal communication). See also Campana (1999) and Campana et al. (2000) for estimating connectivity between spawning groups and Gillanders (2002b) for estimating connectivity between juvenile and adult fish.

Method 3: Transgenerational marks to determine parentage and natal origins

Background

Otolith transgenerational marking refers to the incorporation of a tag from the mother to the egg that is subsequently incorporated into the otolith primordia of the progeny. Several research questions can be addressed using transgenerational marks, such as estimating self-recruitment, individual and group fitness, and natal origin if, for example, one evaluates stock mixing when both diadromous and non-diadromous forms contribute to a group. These techniques are an extension of Method 2 (Table 2).

The approach is based on the observation that oocyte chemistry reflects that of the mother at least up to the point after fertilization when the chorion (the membrane surrounding the embryo) hardens and becomes impermeable to dissolved ions. Maternal chemistry will therefore be recorded in the composition of the embryonic otoliths. Tags for transgenerational marks can be both natural (i.e., reflecting the mother and the environment she has lived in) and artificial (i.e., female exposed to enriched stable isotopes or elements). It is unclear at this point whether the incorporation of elements is linearly or non-linearly related to the ambient and ovarian concentrations. However, otolith cores appear to be chemically distinct from material deposited after hatching (Brophy et al. 2004), and oocyte chemistry can reflect artificial tags of the mother for Ba (Thorrold et al. 2006) and natural tags of the environment in which the mother has lived for Sr (Kalish 1990, Rieman et al. 1994).

Hypotheses and assumptions

Chemical tags within females pass to the egg during oocyte development and are subsequently incorporated into embryonic otoliths.

Assumptions for Methods 1 and 2 (Table 2), in addition to:

Assumption 12: The maternal tag source is identified

Natural tag   Ambient water chemistry influences the composition of body fluids within a female and developing egg, albeit with some degree of physiological discrimination likely for most elements. Therefore, analysis of water samples can be used to establish that maturing females are likely to pass on a unique signature from their environment. In the study of anadromy and life history, for example, the chemistry of fresh and marine waters needs to differ significantly; this often occurs, but not always. However, without a complete record of waters to which females have been exposed or a representative sample of an otolith from those environments, little can be said about natal origins or habitat. Rieman et al. (1994) presents an example of how to ground truth and use natural maternal tags.

Artificial tag   This concern is alleviated if unnatural levels of elements or isotopic ratios are used since they cannot be found in nature.

Assumption 13: Estimating tag retention in the mother and period of tag transmission

Natural tag   If the fish undergoes long migrations or residence in different waters (Rieman et al. 1994) the oocyte chemistry and the tag deposited in the embryonic otolith may be unreliable as an indicator of natal origin. However, once the egg is fertilized and the chorion hardens, the otolith's internal environment and therefore the tag are likely to be stable.

Artificial tag   If females are artificially tagged only once, yet they spawn multiple times, then the tag in the egg is likely to degrade with each spawning event (Thorrold et al. 2006). If females are artificially tagged in advance of spawning, then loss of the tag becomes an important consideration when estimating the proportion of eggs produced by a female that will carry the unique tag. Whether tag degradation is related to the number of spawning events (egg production) or simply the duration of spawning season is unclear (but see Thorrold et al. 2006).

Limitations and inferences

The use of otolith chemistry to determine natal origin, and therefore life history, is a powerful technique, but comes with easily violated assumptions. To determine if a given system is appropriate a few steps must be taken. First, it is necessary to demonstrate that potential water chemistries experienced by the female are indeed distinct. Second, it is important to examine the signatures of known-origin fish to confirm that there are tag differences in transgenerational signatures. Third, experiments should be done to determine the effect of time exposed to varying environments on the oocyte tag (Thorrold et al. 2006).

Examples: Determining resident and anadromous origins of salmon and maternal transmission

Natural tag   One use of transgenerational marks is to determine whether salmon (*Oncorhynchus* spp., Walbaum, 1792) in coastal rivers are from anadromous sources or resident sources or have themselves switched life histories from their maternal parent (Rieman et al. 1994, Limburg et al. 2001). For example, Rieman et al. (1994) proposed that if the prehatch region of an otolith had high Sr then the egg most likely developed when the female lived in marine waters, indicating that the parent was anadromous. Furthermore, if Sr in the otolith core was similar to that in later-formed otolith material (i.e., both high Sr, indicating a marine tag), then it was likely the fish was also anadromous. Rieman et al. (1994) demonstrated that sockeye salmon otoliths could be used to determine if the maternal parent of an individual fish was anadromous or resident (low Sr in otolith core, similar values to material deposited later). Limburg et al. (2001) also demonstrated that anadromous mothers could give rise to resident offspring and vice versa in Baltic Sea trout (*Salmo trutta*,

Linnaeus, 1758). These studies assume that high Sr:Ca corresponds to marine waters, which may not always be the case (e.g., Kraus & Secor 2004) and therefore requires testing.

Artificial tag   Newer methods include injecting developing females with enriched isotopes (e.g., Ba, Thorrold et al. 2006) or unique elemental tags, such that the developing eggs incorporate the tag, which is then deposited in embryonic otoliths. Thorrold et al. (2006) demonstrated that an individual female fish could be injected with an enriched barium isotope, and that the signature was transferred to the egg and progeny. Similar results have been found for freshwater species (e.g., Gillanders et al. unpublished data).

Method 4: Profile analysis to define life-history variation within a population

Background

Profile analysis of natural otolith tags can define differences in movement patterns of individuals within a population, for example, different contingents (*sensu* Hjort 1914, Clark 1968, Secor 1999), where 'contingents' refers to groups of fish with similar patterns of life-history behaviours. The use of profile analysis to identify contingents relies on establishing relationships between otolith tags and environmental parameters. However, interpretations can be limited to quantifying variability in patterns without necessarily reconstructing the exact movements of fish (this is dealt with in Method 5). In such cases, the descriptions of fish movements are limited to outlining that different contingents occupied different environments across seasons and years of their life (e.g., Fowler et al. 2005), but does not necessarily identify the location of the environments (this is dealt with in Method 5) (Table 2).

Contingents are typically characterized by similarities of ontogenetic and lifetime patterns of otolith chemical concentrations (for example, of Sr:Ca or Ba:Ca) in individuals within a group. Typically, coarse generalizations can be made, such as identifying cohorts of precocious emigrating anadromous fishes or cohorts of anadromous fishes that forage and overwinter within freshwater or in an estuary, instead of migrating to sea. The relative proportions of different cohorts that appear in an unbiased sample of adults (for example, in a spawning run) may reflect the importance of different habitats, or combinations of habitats, for survival and ultimately contribution to the spawning population.

Hypotheses and assumptions

Profiles of otolith chemistry can infer contingents if groups of fish have different chemical profiles that reflect life-history strategies and possible movements among habitats or areas.

Assumptions for Methods 1 and 2 (Table 2), in addition to:

Assumption 14: Otolith chemistry of individuals within groups can be differentiated into identifiable contingents

Classification of contingents is reliant on determining similar patterns in chemical tags across otoliths. Environments must, therefore, be sufficiently homogeneous that they influence the tags of all members of a given contingent in a similar manner. Furthermore, for the contingent to be uniquely distinguishable from other contingents, these patterns must covary among individuals within the contingent in a way that is interpretable from an ecological or life-history perspective.

Assumption 15: Sampling of fish and their profiles is representative of all profiles that exist

As with most tag studies, it is assumed that individuals caught and the contingents identified from those fish are representative of all contingents that exist. The relative proportions of fish belonging

to different contingents within a sample can then be identified. If sampling has been limited, then conclusions should be limited to within-group descriptions of contingents. Descriptions of contributions of contingents to a population require representative sampling of that population (e.g., sampling mixed contingents of an anadromous species on a spawning ground).

Assumption 16: Independence of sampling along a profile
The repeated sampling of several locations within a single otolith raises concerns over the independence of measurements in subsequent statistical analyses. Methods to address non-independent data through time are discussed separately (see 'Further data and statistical considerations', p. 321) and efforts should be made to address hypotheses in light of non-independent sampling.

Assumption 17: Otolith chemistry changes predictably with environmental parameters
When describing movements of fish using profiles of otolith tags, it is assumed that otolith chemicals change with environmental parameters, such as water chemistry, temperature, or salinity. Thus, it is important to quantify how otolith tags change with environmental parameters using experimental manipulations or carefully designed field tests. Chemicals under physiological or genetic control (Geffen et al. 1998, Halden et al. 2000) are not suited for reconstructing environments. Nevertheless, if a link can be established between water chemistry, temperature, or salinity, then it should be possible to describe the environments where fish have lived. This still does not give an indication of the geographic location of the different environments unless specific locations have unique characteristics that are reflected in the otolith tag (see, for example, Dorval et al. 2007). Importantly, links between otolith tags and environmental parameters are often species specific and therefore should be determined for each species of interest (Gillanders & Kingsford 2003).

Limitations and inferences

Identification of contingents based on otolith chemistry profiles does not require fine-scale linkage between otolith chemistry and environmental parameters. Rather, the current method relies on environmental gradients to define large-scale movements (e.g., freshwater to marine water habitats). Contingents are sometimes defined based upon profile patterns alone without verification of the underlying otolith chemistry environmental relationships, but as indicated below (Assumption 19) seasonal or ontogenetic changes in physiological state could result in incorrect interpretations of changes in a particular natural tag. Because contingent definition emphasizes movements between habitats or areas, other approaches such as telemetry can serve as strong corroborative evidence of contingent behaviours (Brenkman et al. 2007, Wingate & Secor 2007). Still, the ability to assign contingents to particular habitats at particular times in their lives will be curtailed because water chemistry shows important temporal-spatial variability, which is unknown. To deduce patterns of specific habitat use requires that chemical profiles be related to spatial and temporal data on environmental parameters (this is described in Method 5).

Example: Contingents of Hudson River striped bass from otolith Sr:Ca

Secor et al. (1995b, 2001) and Zlokovitz et al. (2003) aimed to determine contingents of striped bass (*Morone saxatilis*, Walbaum, 1792) using otolith Sr and Sr:Ca profiles across sectioned otoliths. Striped bass were collected in the Hudson River and their otoliths dissected and sectioned. Otoliths were analysed for Sr and Ca with a series of 5-µm point samples across the otoliths using an electron microprobe. Data were adjusted for otolith growth using marginal increment analyses to account for differences in annual increment widths among years. Based on known relationships between otolith Sr:Ca and salinity in their system, Secor et al. (1995b) classified striped bass to different life-history behaviours: resident, mesohaline, and ocean contingents (Figure 3, Secor 1999). Contingents were related to habitats characterized by different salinities. In particular, the striped

bass of the resident contingent showed evidence of high exposure rates to freshwater sources of polychlorinated biphenyls (PCBs). Contingent membership also has consequences for growth, age at maturation, and population persistence (Kraus & Secor 2005). Still, contingent designations only define a coarse set of movements; more precise spatial and temporal movements can be inferred from chemical profile analysis (see Method 5).

Method 5: Profile analysis to describe movements through different environments

Background

Describing fish movement through environments using natural otolith tags relies on an established link between environmental parameters (salinity, temperature, water chemistry) and otolith chemistry (see 'What influences otolith tags', p. 301). Satisfying the many assumptions concerning the effects of environmental variability on otolith chemistry is no easy task (Table 2). Many studies have not addressed these assumptions adequately. For instance, Campana (2005a) notes that several palaeo-reconstructions of past environments using otolith chemistry did not verify the relationship between water and otolith chemistry and therefore may have produced erroneous results. Once correlations between otolith chemistry and environmental variables are established there remain several further assumptions that need to be evaluated before we are able to describe fish movements. Perhaps the most important overlooked scenario is that a change in otolith chemistry may represent a change in the environment surrounding a stationary fish. Hence, knowing the variability of environmental parameters in space and time is integral to describing movement. It is this last assumption that can cause misinterpretations of fish movements.

Hypotheses and assumptions

Chemical profiles can be used to infer environmental and habitat occupancy of fish if relationships between otolith chemistry and environmental parameters are known.

Assumptions for Methods 1, 2, and Assumptions 16 and 17 (Method 4) (Table 2), in addition to:

Assumption 18: Interactive effects of environmental parameters on otolith chemicals are known
In addition to establishing a link between otolith chemistry and environmental parameters, it is important to recognize that several parameters may simultaneously affect chemical tags. For example, if temperature and water chemistry both influence otolith composition (Fowler et al. 1995a,b, Elsdon & Gillanders 2004), then simultaneous changes of these may have interactive effects on otolith chemistry (when one environmental parameter influences the effect of another). Investigations have shown that interactions can occur and they should be considered (Secor et al. 1995b, Elsdon & Gillanders 2002, 2004). For example, environmental reconstructions based on otolith $\delta^{18}O$ (and corals, foraminiferans, sclerosponges) are influenced by both temperature and the oxygen isotope composition of ambient waters. When environmental parameters do interact to affect otolith tags, then reconstructions need to tease apart these effects or they may misinterpret movements using only one variable. It may, therefore, be necessary to examine the spatial and temporal changes of the interacting parameters (see also otolith crystallization in Assumption 21) within the water bodies being examined.

Assumption 19: Ontogenetic effects on otolith chemistry are known (if reconstructions bridge different life-history periods)
Otolith tags can differ with life-history stages (larval, juvenile, subadult, and adult otolith growth) and metamorphosis (Toole et al. 1993). If profiles of chemical tags bridge life-history stages, then

tag differences may represent ontogenetic effects and not changes in environmental parameters. Fish with obvious metamorphic stages, such as eels (Arai et al. 2000, 2002, Correia et al. 2003), are particularly prone to ontogenetic changes in otolith chemistry that could be misinterpreted as large-scale movements. Rearing fish in constant, or at least known, environments during ontogenetic (Fowler et al. 1995b, Elsdon & Gillanders 2005a) and physiological changes (e.g., osmoregulation, Zimmerman 2005) can elucidate such effects.

Assumption 20: Spatial and temporal variations in environmental parameters are quantified
To determine fish movement from otolith tags, it is often assumed that differences in otolith chemistry profiles represent fish movement, when in fact they could represent a change in the environment around a stationary fish. Thus, to infer fish movement, it is necessary to demonstrate that changes in otolith profiles must have resulted from a movement between habitats. Few descriptions of fish movement using otolith chemistry have acknowledged this issue (Kraus & Secor 2004, Elsdon & Gillanders 2006a). Long-term stability of environmental parameters should not be assumed, especially in dynamic environments (Table 2, Figures 3 and 4). Examining spatial and temporal variation in environmental parameters can provide a solution to this problem (see 'Further data and statistical considerations'), as otolith tags can be compared to environmental parameters in space and time to determine where fish were during periods of tag incorporation (e.g., Elsdon & Gillanders 2006a). The best descriptions of fish movement would be expected in environments with stable water chemistry, temperature, and salinity (e.g., Elsdon & Gillanders 2005b). Barring such constancy, researchers may also be able to infer movement on fairly coarse spatial scales, such as among estuarine salinity zones (e.g., Kimura et al. 2000), if underlying chemical gradients are sufficiently different.

Assumption 21: Correlations between otolith crystallization and chemistry are known
Links between environmental properties or physiology and otolith chemistry are undoubtedly correlated to otolith crystallization rate. Examples where otolith crystallization has been correlated to otolith chemistry include Sr:Ca changes with ontogeny (Tzeng 1996), different effects of temperature at low versus high salinity (Elsdon & Gillanders 2002), and annual variations in Sr:Ca (Sadovy & Severin 1992). Correlations between crystallization rate and otolith chemistry have been discussed in Campana (1999), although there are limited data on the chemical processes that are responsible. It is important, however, to realize that differences in chemical tags along profiles can be caused by variation in crystallization rate and might not necessarily represent differences in environmental properties (see also Assumptions 17–19). This may, in turn, result in similar misinterpretations as those described for Assumption 20. Since otolith crystallization rate is often highly correlated with the somatic growth rate of the fish, comparisons of somatic growth rate among the groups or stages of interest can usually point to potential misinterpretations.

Limitations and inferences
Done correctly, the determination of fish movement by relating otolith chemical profiles to environmental parameters provides valuable information. Determining precise fish movements using profile analysis is perhaps the ultimate goal of many studies, but it is difficult to achieve in practice. Few inferences about precise fish movements can be made if assumptions on how environmental parameters affect otolith tags and the spatial and temporal variation in environmental parameters are not met. Studies that do not meet assumptions, particularly about the spatial and temporal extent of variation in environmental parameters, may misinterpret fish movements. For instance, a migration inferred as an estuarine-to-marine movement could in fact represent an estuarine-to-freshwater movement if Sr:Ca in the freshwater end-member is higher than the marine Sr:Ca values (Kraus

& Secor 2004). To what extent misinterpretation of fish movements has occurred in the existing literature is unknown.

Example: Estuarine residency determined using otolith Sr:Ca and temporal collection of water Sr:Ca

Elsdon & Gillanders (2006a) linked otolith Sr:Ca of the estuarine fish black bream (*Acanthopagrus butcheri,* Munro 1949) to temporal collections of water Sr:Ca to determine if fish were resident or migrants to particular sites. Water Sr:Ca was collected on nested spatial scales of months, weeks, and days in summer and winter at two estuarine locations. The sampling design allowed for the detection of coarse (month and week) and fine (day) temporal scales of variation (Elsdon & Gillanders 2006b). Water Sr:Ca variation was used, along with known correlations, to predict otolith Sr:Ca of a stationary 'resident' fish at each location. Fish were collected at each location corresponding to the last water sample, so otolith Sr:Ca of wild fish could be compared to the predicted stationary 'resident' fish. Otoliths of fish were analysed using LA ICP-MS to generate profiles of Sr:Ca across the otolith. Fish were classified as residents if their otolith Sr:Ca matched that of the predicted concentrations of a stationary 'resident' fish or as migrants if their otolith Sr:Ca did not match predicted values (Figures 3 and 4, Elsdon & Gillanders 2006a).

Further data and statistical considerations

Statistical techniques for examining natural and applied otolith tags include univariate and multivariate approaches. Simple guidelines already exist both for general statistics of otolith data (Campana 2005b) and for more specific statistics used to determine connectivity among groups (Method 2, Gillanders 2005). We do not cover what has already been published, but instead highlight three areas that require clarification: detecting variability at different spatial and temporal scales, non-independence of repeated measurements within sectioned otoliths, and limitations in using natural tags to assess philopatry and movement rates, as well as some further data considerations.

Detecting variability at different spatial and temporal scales

Detecting and interpreting variation in otolith tags and environmental parameters in space and time is required to advance interpretations of movement via Methods 1, 2, and 5. It is critical that otolith and water chemistries vary at scales appropriate to the hypotheses and questions being addressed. Many papers investigating patterns in space and time do so by collecting data using either fixed or haphazard sampling designs (i.e., sampling water chemistry once a month for several years to determine 'monthly' or 'seasonal' patterns). Although fixed sampling designs provide useful information, the data acquired may only have relevance for a particular point in space or time. This is especially true if variation occurs at scales smaller than those being investigated. It is therefore useful to examine variation in space and time using a nested or hierarchical sampling design. Such designs are commonly described in the ecological literature (Underwood 1997, Quinn & Keough 2002, Gotelli & Ellison 2004), but they are less commonly used to describe variation in environmental parameters. Nested designs entail collecting data on several scales of space or time, such as taking replicate sampling of water chemistry (1–2 m apart), within sites (kilometres apart), within locations (tens of kilometres apart) (Dorval & Jones 2005), or sampling water chemistry on different tidal cycles within replicate days, weeks, months, and seasons (Elsdon & Gillanders 2006b).

Data obtained using nested designs can be analysed using nested analysis of variance (ANOVA; either ANOVAs for univariate or multivariate ANOVAs [MANOVAs] for multivariate analyses) to determine scales of significant differences. In addition, data can be examined using variance components (Vaughan & Corballis 1969, Graham & Edwards 2001) to determine the proportion of the total

variation attributed to different scales. Variance components have not been widely used in ecological research, perhaps due to differing views about their applicability to mixed-model and orthogonal ANOVA designs. Nested designs are, however, ideally suited to variance component calculations.

Repeated measurements on an otolith

Quantifying chemical tags within two or more specific portions of a single otolith raises questions of independence of sampling and repeated measurement on a subject. Primarily, investigations of movement, such as Method 2, are best done using independent otoliths. For example, otoliths of juvenile fish caught at specific locations are used to establish chemical tags for those locations (Campana 2005b), and then otoliths of adult fish are analysed in the juvenile portion of otolith growth to establish the locations of juvenile occupation. In this respect, the otoliths used to establish tags and those for which movements are determined are independent.

If otolith data consist of chemical profiles, individual measurements within that profile (of the same fish's otolith) are not independent. Therefore, repeated measures of analysis of variance (RM-ANOVA) can be used to investigate differences among groups of fish over time, where otoliths of individual fish have been repeatedly sampled. It is important to recognize that a group of fish may contain individuals that have different otolith tags if fish movements do not completely overlap. The method requires that individual fish within a group have similar chemical tags across their otoliths (i.e., tag (across the otolith) × individual term is non-significant, Underwood 1997). Thus in applications of RM-ANOVA, chemical profiles have been classified into age or size bins (Secor & Piccoli 1996, Kimura et al. 2000). Mixed model RM-ANOVA can provide a more efficient means to extract information from profile data by estimating degrees of freedom from fitted covariance structures, rather than assumed covariance (Jones 2000, Kimura et al. 2000). RM-ANOVA is a useful way to group together individuals with similar life-history patterns (Method 4) or movements (Method 5). If individuals have similar tag patterns through time (tag (across the otolith) × individuals (group); not significant), then this could be used as grounds for grouping fish with similar profiles. Regardless of how data are treated, questions of data independence when examining otoliths using profile analyses should be acknowledged and statistically addressed.

Limitations in using natural tags to assess philopatry and movement rates

Otolith chemistry is increasingly used as a tag to quantify movement and philopatry without careful attention to underlying assumptions and limitations. There is a rich and well-established statistical literature that deals with the use and misuse of tags (Brownie et al. 1978, Seber 1982, Williams et al. 2002) that can guide investigators in using tagging data properly. Unfortunately, these statistical approaches have only been developed for applied tags and the theory and methods of use for natural tags is in its infancy (C.M. Jones personal communication). Until these methods are better developed, we caution investigators to be circumspect in their attempts to develop rate estimates with natural otolith tags.

Conclusion and future research needs

We have highlighted five methods that use otolith chemical tags to determine movement and life-history measurements of fish. Each of these methods addresses a discrete and different hypothesis about fish movements. The first three methods are primarily based on reconstructing movements by linking groups of fish in space and time. The last two methods are based on analyzing chemicals across otoliths and relating these patterns to possible movements. Method 4 allows inferences related to ontogenetic and lifetime movement modalities (i.e., contingents). Method 5 permits more

spatially and temporally precise estimates of residency and movement. By using one of these five methods, and addressing the corresponding assumptions, inferences and interpretations of fish movement using otolith chemistry can be maximized.

Three major knowledge gaps require further investigation. First, knowledge of factors influencing otolith chemistry is still limited, making it difficult to generalize environmental effects among species. Advancing our knowledge base in this area will help us understand why otolith tags differ, and if they are likely to differ in predictable ways. Second, it is important to know the spatial and temporal scale of variation in both otolith tags and environmental properties. Some excellent data on spatial and temporal variability in otolith chemistry exist. Less emphasis has been placed on examining variability in environmental properties, specifically for dissolved elements that are useful in otolith studies (i.e., Sr and Ba). Obtaining these basic patterns is imperative to determining movements of fish based on all methods. Third, natural otolith chemistry holds great promise to address issues of philopatry and movement, but the ability to fulfil this promise will depend on the clarification of proper statistical models and techniques.

Technological advances have made chemical analyses of otoliths more routine. Technology will continue to improve, and it is foreseeable that we will be able to quantify chemicals with greater accuracy and precision. These advances have the potential to increase our understanding of fish movement; however, the interpretation of data will still rely on a thorough understanding of the assumptions presented here.

Acknowledgements

Glossary

Chemical: Collective term used to represent elements (i.e., Ca, Sr), isotopes (i.e., δ^{13}C, δ^{18}O), and other trace compounds (elements in trace levels of abundance, such as rare earth elements).

Contingent: A term first used by Hjort (1914) and later by Clark (1968) to describe groups of fish with similar migration pathways.

End-member(s): Water masses with distinct chemical properties between which gradual changes occur, for example, mixing of two chemical end-members (freshwater and saltwater) along an estuary, where a gradual chemical change is detected from one end-member to the other.

Group(s): Fish within a population that have similar otolith chemical tags. These fish can be considered to have occupied similar areas or had similar movement patterns.

Location: The area inhabited by a group(s) of fish. A region is made up of more than one location.

Migration: One type of movement, commonly associated with a response to changing resources (Dingle 1996).

Movement: Change in location from one place to another. There are several subcategories of movement, such as foraging, commuting, territorial, and ranging, as outlined in Dingle (1996).

Philopatry: Tendency of a fish to stay in or return to its home area.

Populations (of fish): A unit of fish of the same species within a given area that has the potential to mix. Two units of fish of the same species that occupy non-overlapping areas (i.e., Atlantic and Indian Oceans) and do not mix would be considered separate populations.

Profile: The quantification of chemicals in two or more different portions of one otolith. These have also been referred to in the literature as transects, trajectories, profiles, scans, chronologies, and line scans, but for the purpose of this review have been unified in name. The main use of profile analyses in otoliths is to determine differences in chemical tags that relate to different life-history periods, be that days, weeks, months, or years.

Region: The area inhabited by units of fish at a population level. This gives no scale to the area, but suggests that it is large enough to incorporate groups of fish with different otolith tags, and therefore would contain more than one location.

Tag: The chemical concentrations (univariate or multivariate) in otoliths that can be used as an identifiable measurement of fish movement. Tags can be of natural origin (chemicals in natural abundances) or deliberately applied (chemicals in altered abundance or unnatural isotopic ratio, or an element typically in low concentration (e.g., lanthanides)). For whole-otolith solution analyses, one tag is estimated, which is the integrated signature of the otolith. For sectioned otoliths, one or more tag(s) can be estimated that relate to different life-history periods of the fish.

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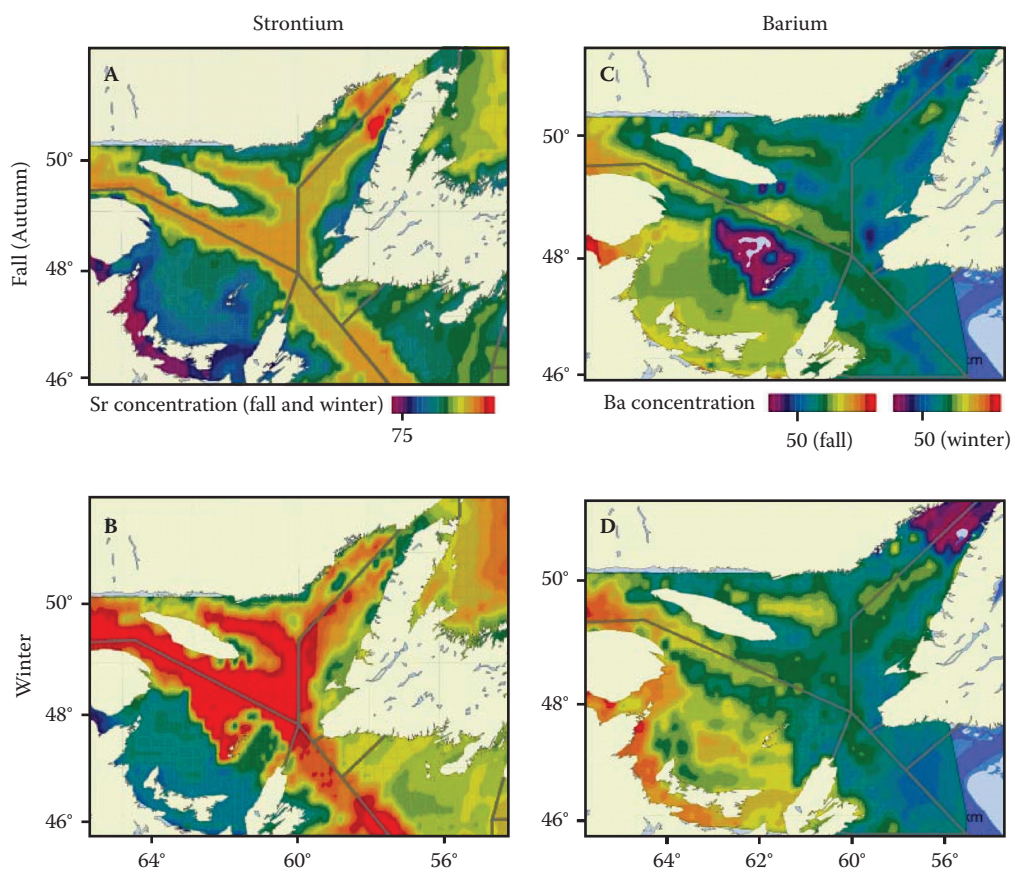
Colour Figure 3 (Elsdon et al.) Variation in dissolved strontium and barium concentrations in the Gulf of St. Lawrence, Canada, showing spatial variation (within each plot) and temporal variation between seasons for strontium, comparing fall (A) with winter (B); barium, comparing fall (C) with winter (D). Values in parts per million. (S.E. Campana unpublished data.)
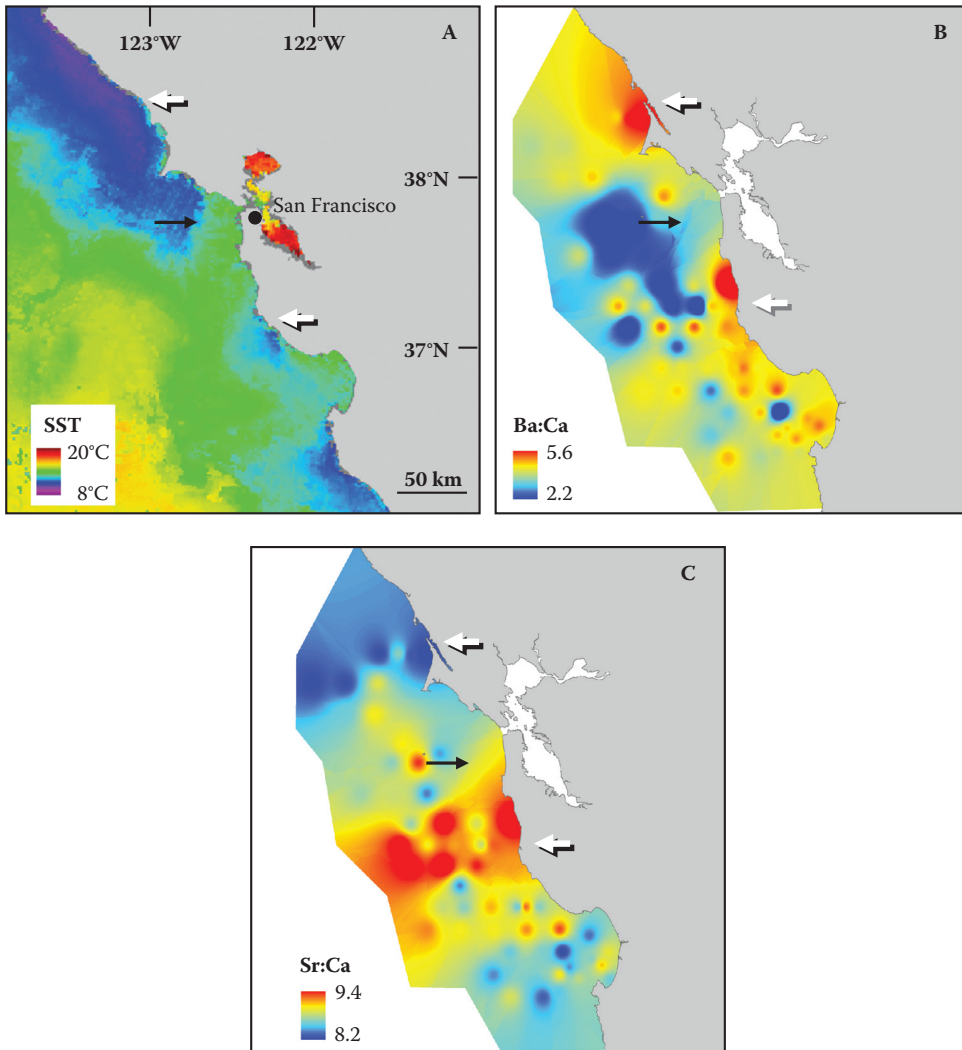
Colour Figure 4 (Elsdon et al.) (A) Sea surface temperatures (SSTs) collected during the upwelling season along central California (5–19 June 2006; National Oceanic and Atmospheric Administration Coast Watch program) and representing (B) the Ba:Ca ratios (μmol mol⁻¹) and (C) Sr:Ca ratios (mmol mol⁻¹) collected during the same upwelling season (B. Wells & K. Stierhoff unpublished data, figure prepared by K. Stierhoff). White arrows represent regions of active upwelling and the black arrow represents infusion of saline, warmer waters from farther off shore into the region. *Note:* Variability in Ba:Ca is derived largely from the distribution of upwelling in the region and will vary with the degree and timing of upwelling.