All in the ears: unlocking the early life history biology and spatial ecology of fishes

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ABSTRACT

Obtaining biological and spatial information of the early life history (ELH) phases of fishes has been problematic, such that larval and juvenile phases are often referred to as the ‘black box’ of fish population biology and ecology. However, a potent source of life-history data has been mined from the earstones (otoliths) of bony fishes. We systematically reviewed 476 empirical papers published between 2005 and 2012 (inclusive) that used otoliths to examine fish ELH phases, which has been an area of increasing attention over this period. We found that otolith-based research during this period could be split into two broad themes according to whether studies examined: (i) biological objectives related to intrinsic processes such as larval and juvenile age, growth and mortality, and/or (ii) spatial objectives, such as habitat use, dispersal and migration. Surprisingly, just 24 studies (5%) explored a combined biological–spatial objective by simultaneously exploiting biological and spatial information from otoliths, suggesting much more scope for such integrated research objectives to be answered via the use of multiple otolith-based techniques in a single study. Mapping otolith analytical techniques across these two approaches revealed that otolith structural analysis was mainly used to investigate biological processes, while otolith chemical analyses were most often applied to spatial questions. Heavy skew in research effort was apparent across biomes, with most (62%) publications specific to marine species, despite comparable levels of species richness and the importance of freshwater taxa (just 15% of papers). Indeed, around 1% (380 species) of a possible 31400+ extant species were examined in our surveyed papers, with a strong emphasis on temperate marine species of commercial value. Potential model species for otolith-based ELH ecology research are arising, with the eel genus Anguilla (24 studies) and the European anchovy Engraulis encrasicolis (14 studies) attracting more research effort than most other taxa. While there is a preponderance of common techniques (e.g. daily otolith increment counts, increment widths), novel techniques such as transgenerational marking and computed X-ray tomography, are increasingly being applied in published studies. The application of an integrative approach based on a combination of emerging techniques and traditional methods holds promise for major advances in our understanding of ELH fish ecology and to shine light into the ‘black box’ of fish ecology.

Key words: recruitment, larvae, otolith, growth, network analysis, connectivity, isotopes, diadromy.

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I. INTRODUCTION

Demographic processes during the early life history (ELH) of a species are critical to the replenishment, size and structure of adult populations (Hjort, 1914; Begon, Townsend & Harper, 2006). Hjort (1914) first recognised that recruitment (the addition of new individuals into the population) can shape the productivity of fisheries; hence understanding the dynamics of recruitment is vital to fisheries management. Since then, research into the impacts and processes of fish ELH phases has proliferated (Fig. 1), leading to numerous conceptual models explaining recruitment in aquatic ecosystems (marine: Cushing, 1974; Sale, 1977; Doherty, 1983; Jones, 1990; Houde, 2008, freshwater: Vannote et al., 1980; Winemiller, 1989; Humphries, King & Koehn, 1999; Lyle & Poff, 2004). Collecting data on the ELH phases of fishes remains problematic due to their small body size, fragility, high rate of mortality and potential for movements at river catchment or ocean scales (Thorrold et al., 2002; Cowen et al., 2007). This has impeded the application of mark–recapture and telemetry-based tracking techniques, which are commonly applied in terrestrial ecosystems, and in the study of adult aquatic organisms, to track the fate of individuals through space and time (e.g. Diffendorfer, Gaines & Holt, 1995; Sweanor, Logan & Hornocker, 2000; Clobert et al., 2001; Webster et al., 2002). Consequently, a myriad of other approaches have been applied in unravelling the ELH phases of fishes, which continue to be perceived as the ‘black box’ of fish ecology (Leis, 1991; Thorrold et al., 2002; Levin, 2006).

Chronologically explicit information for ELH fish has commonly been sourced from the hard anatomical structures that grow continuously throughout an individual’s life (Campana & Thorrold, 2001). One such structure in bony fishes are the ear stones (otoliths), which are of calcified acellular composition and serve in balance and hearing (Popper, Ramcharitar & Campana, 2005). Otoliths exhibit continuous growth, but differ from other calcified structures such as bones and scales in that they are biogenically inert and hence are subject to minimal to nil metabolic reworking (Campana & Thorrold, 2001). Otoliths grow through accretion of new material onto the external surface of the otolith, thus producing identifiable ‘growth rings’ that are chronologically explicit. As such, otoliths present the equivalent of a ‘flight-box recorder’ in storing biological and chemical information from when the otoliths first form (often before hatching) until an individual dies (Campana & Thorrold, 2001). Extracting biological information from such hard parts goes back centuries (reviewed in Jackson, 2007), but the first use of otoliths to age fishes based upon annuli (structural features within the otolith that form on annual cycles) occurred in the 1890s (Reibisch, 1899; Fig. 1). Once it was realised that otolith growth can be directly proportional to somatic growth, techniques were developed for back-calculating growth (e.g. Lea, 1910; Hickling, 1933). Perhaps the most significant early advance, however, was when Pannella (1971) discovered that fish otolith structure can form on a daily basis, such that age and growth could be reconstructed at daily resolution, which spawned the enormous field of otolith microstructure analysis (Fig. 1). Given that daily
resolution is particularly important for events in the ELH of fishes, this discovery led to an explosion of ELH fish research (reviews: Campana & Neilson, 1985; Campana, 1990, 1999, 2005).

The elemental composition of otoliths can also reveal key information of an individual’s life history (Kalish, 1990; Campana, 1999; Elsdon et al., 2008). Early work revealed environmental origins of inert trace elements and oxygen isotopes that had been deposited in otoliths (Carlström, 1963; Devereux, 1967; Degens, Deuser & Haedrich, 1969). A critical breakthrough occurred in the 1980s when it was found that the abundance of strontium and barium relative to calcium within otoliths (Sr:Ca, Ba:Ca) was linked to the relative environmental abundance of these elements, such that they could be used as tracers of salinity to explore patterns of diadromy (migration between freshwater and marine environments) in fishes (Radtke, 1984; Kalish, 1990; Secor, Henderson-Arzapalo & Piccoli, 1995). This information is stored within the growth rings of the otoliths, revealing temporally explicit patterns of individual habitat use. Advances in analytical instrumentation have provided improved resolution and discrimination of minute changes in the elemental composition of otoliths (otolith chemistry: Campana et al., 1997; Elsdon & Gillanders, 2003; Elsdon et al., 2008), which has facilitated studies of spatial patterns in fish habitat use based upon the chronology of elemental signatures stored in otoliths relative to the geochemistry of their environment (Campana, 1999; Elsdon et al., 2008). This chemical environment–otolith pathway has also been exploited to place artificial chemical marks or ‘tags’ within individual otoliths yielding key insights (Gillanders, 2009). Early work with otolith marking via calcium-binding chemicals that fluoresce under ultra-violet light (i.e. chemical marking that is resolved using light microscopy techniques), such as oxy-tetracycline and calcine, provided key validations of otolith growth and function in relation to fish life-history events (Kobayashi et al., 1964; Campana & Neilson, 1985). Using such fluorescent markers, and others produced via immersion or injection of enriched stable isotopes (Thorrold et al., 2006; Munro et al., 2008) has provided the first means of mass-marking individuals for mark–recapture studies on larval and juvenile life-history phases which are typically too small to mark with physical tags (Jones et al., 1999; Almany et al., 2007).

Publication of otolith research has increased from the late 1980s to 2012 through the development of traditional techniques as well as new technological advances (Fig. 1B). The most recent, comprehensive review of the literature (1998–2004; Campana, 2005) identified that in excess of 100 papers were being published annually, with otolith chemistry being increasingly used (22% of published studies) to study the spatial ecology of fishes (ELH and adult phase combined). Most significantly, 24% of the literature relating to otolith microstructure during that time period examined ELH-phase fish. Otoliths offer a pragmatic and rich source of information regarding larvae and juveniles where there are few alternative means of learning about these fishes, and with the emergence of a prominent literature on this topic, it is timely to review otolith-based applications to early ELH-phase ecology.

In recent decades, otolith microstructure and chemistry techniques have emerged as popular means of investigating multiple aspects of fish demography, and have included the very recent development of trans-generational marking of otoliths within the maternal source (Fig. 1B). Over a decade has passed since Campana & Thorrold (2001) called for an integration of otolith structure and chemistry analysis techniques to provide spatially resolved biological information on fishes. While this directive has the potential to drive major advances in the field of fish ELH biology and ecology, to the best of our knowledge there has not yet been a review examining whether these two fields of otolith microstructure and chemistry are being integrated, and the consequences of such integration.

Here we examine how the suite of available otolith-based techniques are being applied in the recent literature (2005–2012) to meet the huge challenge of understanding the ELH of fishes (for Methods, see online Appendix S1), which has been a rapidly moving field over this period in terms of applying old and new technology for novel data collections. Specifically, we explore the extent that otolith-based technologies may be driving major advances in this arena by: (i) examining what biological and spatial information is extracted from otoliths, both in isolation and simultaneously to answer research objectives on fish ELH, (ii) identifying geographical patterns in the publication of ELH otolith research, model systems and species that are at the forefront of ELH otolith research, and (iii) highlighting emerging analytical techniques and current knowledge gaps in fish ELH biology and ecology that may be addressed through greater application and/or integration of multiple otolith-based techniques to unlock the ELH biology and spatial ecology of fishes.

II. LINKS BETWEEN RESEARCH OBJECTIVES AND OTOLITH TECHNIQUES

By far the most common topic for ELH otolith-based papers between 2005 and 2012 were questions of a biological nature, which is perhaps a reflection of the relatively low cost and long-established nature of the technologies commonly applied in this field of research (sensu Campana, 2005, Fig. 1). Most (68.4%) studies explored biological questions (e.g. role of age and growth in fish ELH), while 84 (17.6%) studies explored spatial objectives, relating to interest in dispersal and
habitat use, with a clear separation between the use of otolith structural analysis and otolith chemical analysis for biological or spatial objectives, respectively (Fig. 2). However, a relatively small proportion (14%) explored both biological and spatial objectives within the same study (Fig. 2). Although Campana & Thorrold (2001) called for a greater integration of otolith structural and chemical analyses over a decade ago, just 24 of the 476 papers examined here (5%) adopted such an approach to exploring both biological and spatial objectives in fish ELH ecology. While 47 studies within marine biomes explored both biological and spatial objectives, 35 of these employed only otolith structural analyses of fishes sampled across defined habitat zones to explore biological processes through space (Fig. 2). Most were single-species studies (just four multi-species studies, Table 1), which is perhaps a reflection of the technical workload associated with this integrative approach. Indeed, workload, funding and the availability of appropriate technology appear to be likely barriers to explain the low adoption of multiple techniques within the same study.

Despite a wealth of available otolith-based techniques (Campana, 2005, Figs 1 and 3) most studies employed just a few common techniques. Most common in ELH otolith-based research during 2005–2012 were counts of daily otolith growth increments (Fig. 3), as was the case between 1998 and 2004 (Campana, 2005). However, otolith chemistry has emerged in recent years as the fifth most commonly employed technique in ELH fish publications (Fig. 3). Some biases in techniques are apparent across biomes, with rarer techniques under-represented in freshwater fish studies compared to marine and estuarine/diadromous fishes (Fig. 3). Investigations of natural uptake and storage of elemental ratios (Sr:Ca, Ba:Ca) were exclusively applied to estuarine/diadromous fishes, reflecting a focus on movement across the marine/freshwater interface (Campana, 1999; Gillanders, 2005). Despite the dominance of the commercial Clupeidae and Engraulidae in the broader ELH literature, they do not predominate within such integrated otolith-based studies; suggesting that the phenomenon of model species relevant to certain regions and/or concepts (e.g. marine protected area planning) is driving novelty in otolith-based research over and beyond commercial importance.

While most studies tended to explore biological objectives using otolith structural analyses, or use otolith chemical analyses to explore spatial questions, there were several notable exceptions. For instance, nine papers employed otolith structural analyses to explore the spatial ecology of ELH phase fishes. Three papers (Brophy, Danilowicz & King, 2006; Burke, Brophy & King, 2008a,b) used distinctive otolith structural features to distinguish between sympatric spring and autumn spawning Clupea harengus populations in the Irish and Baltic seas. Similarly, other studies used internal otolith structure or otolith external morphology to distinguish between demographic units of interest within populations (e.g. Oresland & Andre, 2008; Stransky et al., 2008). The application of otolith-shape analyses to identify individual stocks of commercial fish species has been readily applied in adult fishes (Campana & Casselman, 1993; Begg & Brown, 2000; Berg...
Table 1. Summary of 24 studies published between 2005 and 2012 that explored both biological and spatial objectives in early life history phase fishes, using both otolith structural and chemical analyses

<table>
<thead>
<tr>
<th>Biome</th>
<th>Study</th>
<th>Species</th>
<th>Study focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Aldanondo et al. (2010)</td>
<td>Engraulis encrasicolus</td>
<td>Explores the relationship between dispersal history and growth using otolith microchemistry and otolith microstructure analysis</td>
</tr>
<tr>
<td>M</td>
<td>Ben-Tzvi et al. (2008)</td>
<td>Chromis viridis</td>
<td>Explores dispersal pathways and potential sources of recruits, based upon otolith microchemistry profiles and pelagic larval durations</td>
</tr>
<tr>
<td>M</td>
<td>Di Franco et al. (2011)</td>
<td>Diplodus sargus sargus</td>
<td>Explores differences in pelagic larval durations, hatch dates and otolith microchemistry among widespread populations</td>
</tr>
<tr>
<td>M</td>
<td>Fowler et al. (2005)</td>
<td>Pagrus auratus</td>
<td>Examines individual movements via changes in otolith Sr:Ca and Ba:Ca profiles and annuli</td>
</tr>
<tr>
<td>M</td>
<td>Hoie &amp; Folkvord (2006)</td>
<td>Gadus morhua</td>
<td>Relates oxygen isotope ratios to annual increment formation in otoliths to confirm seasonal timing of increment formation</td>
</tr>
<tr>
<td>M</td>
<td>Hamer et al. (2011)</td>
<td>Pagrus auratus</td>
<td>Examines the influence of Port Phillip Bay, Australia, being a major source of recruitment, using otolith Sr:Ca and Ba:Ca profiles and annual age</td>
</tr>
<tr>
<td>M</td>
<td>Hamilton (2008)</td>
<td>Thalassoma bifasciatum</td>
<td>Explores how oceanic residence (defined using otolith microchemistry) influences larval size and condition (using otolith microstructure) at settlement</td>
</tr>
<tr>
<td>M</td>
<td>Hamilton et al. (2008)</td>
<td>Thalassoma bifasciatum</td>
<td>Explores the relationship between survival, growth and recruitment source (nearshore versus offshore) using otolith microchemistry and microstructure analysis</td>
</tr>
<tr>
<td>M</td>
<td>Kingsford, Patterson &amp; Flood (2008)</td>
<td>Pomacentrus coelestus</td>
<td>Examines the relationship between water chemistry, crystallography and otolith increment width with microstructure and microchemistry analysis</td>
</tr>
<tr>
<td>M</td>
<td>Shiao et al. (2009)</td>
<td>Thunnus maccoyii</td>
<td>Explores the ontogeny of thermal environment and diet with stable carbon and oxygen isotopes, at specific life-history points, using otolith microstructure analysis</td>
</tr>
<tr>
<td>M</td>
<td>Shima &amp; Swearer (2009)</td>
<td>Forsterygion latillum</td>
<td>Examines the ontogeny of thermal environment and diet with stable carbon and oxygen isotopes, at specific life-history points, using otolith microstructure analysis</td>
</tr>
<tr>
<td>M</td>
<td>Shima &amp; Swearer (2010)</td>
<td>Forsterygion latillum</td>
<td>Examines the relationship between survival, growth and recruitment source (nearshore versus offshore) using otolith microchemistry and microstructure analysis</td>
</tr>
<tr>
<td>E/D</td>
<td>Arai, Chino &amp; Kotake (2009)</td>
<td>Anguilla japonica and A. anguilla</td>
<td>Examines the variability in habitat use (marine, estuarine, fresh water) of A. japonica and A. anguilla (single specimen) in Tokyo bay, using otolith Sr:Ca profiles, and microstructure analysis</td>
</tr>
<tr>
<td>E/D</td>
<td>Chang et al. (2006)</td>
<td>Rhinogobius giurinus</td>
<td>Explores age and growth trajectories of larvae with otolith microstructure analysis, and explores potential dispersal among three Taiwanese estuaries with microchemistry analysis</td>
</tr>
<tr>
<td>E/D</td>
<td>Chen &amp; Tzeng (2006)</td>
<td>Megalops cyprinoides</td>
<td>Examines the relationship between survival, growth and recruitment source (nearshore versus offshore) using otolith microchemistry and microstructure analysis</td>
</tr>
<tr>
<td>E/D</td>
<td>Chen et al. (2008)</td>
<td>Megalops cyprinoides</td>
<td>Examines the relationship between survival, growth and recruitment source (nearshore versus offshore) using otolith microchemistry and microstructure analysis</td>
</tr>
<tr>
<td>E/D</td>
<td>Daverat et al. (2011)</td>
<td>Anguilla anguilla and Platichthys flesus</td>
<td>Examines the relationship between habitat use (freshwater, upper estuarine, lower estuarine) defined using Sr:Ca and annual age, and abundance of environmental contaminants present in body tissues</td>
</tr>
<tr>
<td>E/D</td>
<td>de Albuquerque, Miekeley &amp; Muelbert (2010)</td>
<td>Micropogonias furnieri</td>
<td>Compares the Ba:Ca and Sr:Ca profiles with annual age in an isolated freshwater population and a naturally occurring population</td>
</tr>
<tr>
<td>E/D</td>
<td>Hsu et al. (2009)</td>
<td>Mugil cephalus</td>
<td>Identifies the production of a growth check at the time of estuarine settlement, using otolith microstructure analysis and Sr:Ca profiles</td>
</tr>
<tr>
<td>E/D</td>
<td>Kuroki et al. (2008)</td>
<td>Anguilla anguilla and A. rostrata</td>
<td>Examines the relationship between survival, growth and recruitment source (nearshore versus offshore) using otolith microchemistry and microstructure analysis</td>
</tr>
<tr>
<td>E/D</td>
<td>Labonne et al. (2009)</td>
<td>Ethmalosa fimbriata, Sarotherodon melantheron, Tilapia guineensis</td>
<td>Examines the relationship between survival, growth and recruitment source (nearshore versus offshore) using otolith microchemistry and microstructure analysis</td>
</tr>
<tr>
<td>E/D</td>
<td>Volk et al. (2010)</td>
<td>Oncorhynchus tsawyutchtsa</td>
<td>Examines the relationship between survival, growth and recruitment source (nearshore versus offshore) using otolith microchemistry and microstructure analysis</td>
</tr>
<tr>
<td>F</td>
<td>Dufour et al. (2008)</td>
<td>Alosa pseudoharengus</td>
<td>Examines changes in otolith oxygen isotope ratios with age in Lake Michigan, USA</td>
</tr>
<tr>
<td>F</td>
<td>Reichert et al. (2010)</td>
<td>Perca flavescens</td>
<td>Explores the effects of pelagic habitat use in recruits, using otolith microchemistry and microstructure analysis</td>
</tr>
</tbody>
</table>

Biomes: E/D, estuarine/diadromous; F, freshwater; M, marine.
Fig. 3. Number of species-studies that employ specific otolith techniques, by biome. Numbers above each column represent the total number of occurrences. Otolith chemistry techniques marked with ‘*’. Techniques with less than 10 occurrences may represent emerging techniques. ARS, alizarin; OTC, oxy-tetracycline; MicroCT, computed X-ray tomography; SEM, scanning electron microscopy.

Further research examining the underlying mechanisms that drive changes in otolith shape is required to understand fully how otolith shape can be used to delineate spatial processes in ELH fishes, after removing confounding effects caused by non-spatial processes (e.g. Gagliano & McCormick, 2004).

III. ADOPTING AN INTEGRATED APPROACH

Researchers are increasingly being motivated to answer complex questions requiring information on multiple aspects of fish biology and ecology in order to understand their response to emerging threats such as climate change (Munday et al., 2008; Bell et al., 2013). The application of multiple otolith-based analytical techniques within a given study, while restricted (Fig. 4), has been particularly revealing for fish ELH biology and ecology. In most cases, daily ageing of fish has readily accompanied increment-width analyses, microstructure and otolith morphology examinations (Fig. 4). This may be explained by the relative convenience of preparing otoliths for these different purposes for minimal additional cost. Conversely, it is understandable that daily ageing has not been combined with otolith chemistry, Sr:Ca, Ba:Ca and stable isotope analyses, given the need for additional analytical instruments (e.g. mass-spectrometers) and data analysis. However, a promising trend has emerged in the combined use of otolith structural and chemical analyses to explore either biological and/or spatial questions in fish ELH.

A modest number of studies (33) have examined otolith structural features to identify life-history events and also applied chemical analyses to resolve spatial movement across different habitats or environments. For example, Berumen et al. (2010) explored the potential for otolith chemistry analyses to unravel larval dispersal of a tropical coral reef fish, the orange clownfish Amphiprion percula. Specifically, they located the settlement checkmark on the otolith thereby delineating the chronological record of the pelagic larval phase from the post-settlement juvenile phase. As such, they were able to demonstrate that the probable dispersal environment around Kimbe Island, Papua New Guinea experienced by pelagic larval A. percula has minimal geochemical variation, limiting the efficacy of contemporary otolith chemistry analyses to define natal sources of recruits. Similar effects have been observed in freshwater ecosystems (Pangle, Ludsin & Fryer, 2010), however improvements in instrument sensitivity and statistical analyses, coupled with greater application of elemental and isotopic isoscapes should see increased spatial resolution across all environments (Bowen, 2010). Hoover, Jones & Grosch (2012) examined Sr:Ca and Ba:Ca in addition to daily otolith growth increments to estimate the point of estuarine ingress of juvenile
Atlantic croaker *Micropogonias undulatus*. They revealed that estuarine ingress occurs at 68–85 days of age, and was unrelated to body size, refuting the critical-body-size hypothesis. Improvements in laser ablation mass spectrometry will enable spatially explicit chemical analyses of otoliths, delineating changes in otolith chemistry at the temporal scale of days (e.g. Macdonald, Shelley & Crook, 2008). Resolving the sensitivity of otolith chemistry to spatiotemporal variation in water geochemistry will be critical to facilitating future studies of fine-scale movements by ELH phase fishes and advancing our understanding of ELH fish ecology (e.g. Ben-Tzvi et al., 2007; Macdonald & Crook, 2010).

Conversely, even fewer (4) studies applied both otolith structural and chemical analyses to explore primarily biological objectives. Munday *et al.* (2011) explored otolith structure and microchemistry in relation to the potential impacts of ocean acidification, which is currently an active area of research. This study revealed that while otolith structure and chemistry were largely unaffected due to the experimental increases in $\text{PCO}_2$, larval behaviour was significantly affected. Rowell *et al.* (2008) compared otolith increment widths and $\delta^{18}\text{O}$ in recently collected and historic otoliths from aboriginal shell middens of the endangered totoaba, *Totoaba macdonaldi* collected from the Colorado River estuary to examine the effects of river diversion on their growth in the ELH phase. They revealed that growth rates of fish have decreased since river diversion, compared to fossil otoliths originating 1000–5000 years ago (Rowell *et al.*, 2008). This they relate to the changes in estuarine habitat, reflected in otolith $\delta^{18}\text{O}$ profiles. Interestingly, Hidalgo *et al.* (2008) explored $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ isotope ratios within otoliths to explore the habitat use and trophic ecology of larval and juvenile European hake *Merluccius merluccius* around the Balearic Islands. Changes in otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were employed to infer selective thermal habitat use and vertical migration patterns in larval hake, and changes in feeding patterns with ontogeny, respectively. Previous studies had usually analysed otolith $\delta^{18}\text{O}$, and had explored broad-scale changes in distribution or dispersal, rather than small-scale changes in thermal environment, likely relating to the greater variation in $\delta^{18}\text{O}$ along horizontal gradients, relative to vertical gradients (Schmidt, Bigg & Rohling, 1999). Recent advances in preparation techniques for separating organic and dissolved inorganic carbon for otolith $\delta^{13}\text{C}$ analyses (McMahon *et al.*, 2011; Grønkjær *et al.*, 2013) will further enhance capacity to explore changes in trophic ecology and metabolism throughout ontogeny in fishes. Further advances in otolith chemical analyses may increase the suite of techniques available to study both biological and spatial processes within ELH-phase fishes. A single study (Andrews *et al.*, 2012) employed bomb-radiocarbon dating in conjunction with otolith microstructure analysis to age adults and juveniles of the long-lived crimson jobfish *Pristopomoides filamentosus*. With sufficient confidence in bomb-radiocarbon dating, there is scope for ageing long-lived fish without otolith microstructure validation. However, this is unlikely to be a prominent feature of ELH fish research as bomb-radiocarbon dating is primarily applied to resolving timeframes in the order of decades, rather than days. Conversely, the development of $\delta^{13}\text{C}$ isotope analyses of otoliths may catalyse growth in the employment of otolith chemical analyses to other, primarily biological questions (e.g. trophic ecology).

A particularly powerful approach seems to have been the integration of biological and spatial questions within single studies examining fish ELH ecology. Specifically, both otolith microstructure and chemistry technology have been combined to provide key autecological insights (Table 1). For instance, a study of freshwater yellow perch *Perca flavescens* (Reichert *et al.*, 2010) employed otolith chemistry analyses and otolith microstructure analysis to explore pelagic larval duration (PLD) within Lake Erie (US/Canada border) revealing important implications of this behaviour for recruitment success. Likewise, Dufour *et al.* (2008) employed $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ analyses in combination with otolith microstructure analysis to explore the thermal ecology and habitat use of ELH alewife *Alosa pseudoharengus* within Lake Michigan, USA, revealing that movements between near-shore and river-mouth habitats was rare during the ELH phase, and that larvae prefer warmer-than-average environments. Both these studies centred on fish in lentic systems (large lakes) providing an important opportunity for comparing the pelagic larval phases of freshwater and marine fishes (Janssen *et al.*, 2014). Further research in pelagic freshwater settings is required before general patterns and processes in ELH survival, growth and dispersal pathways can be comprehended across biomes. Indeed this is a promising area for fish and aquatic ecosystem research and management.

Diadromous and estuarine fishes were often the subject of research adopting a range of otolith technologies, with some exciting advances arising from studies that integrate chemistry and microstructure techniques. Most studies employed otolith elemental (Sr:Ca, Ba:Ca) ratios, which can reflect changes in salinity, to identify movements between marine and freshwater environments (Fowler, Gillanders & Hall, 2005; Gillanders, 2005; Hamer *et al.*, 2011). Validation of this approach has included Chen & Tzeng (2006) and Chen *et al.* (2008) exploring the metamorphosis of tarpon *Megalops cyprinoides* in laboratory-based studies to confirm that otolith chemistry and microstructure accurately record changes in water chemistry and metamorphosis in this diadromous species. Such studies provide a powerful confirmation of the efficacy of otolith-based inferences regarding habitat use and the timeframe over which metamorphosis occurs, and demonstrates a subtle shift in focus from the majority of research conducted on...
diadromous fishes. Going beyond defining the existence of diadromy or estuarine residence, such studies integrate multiple techniques to link intrinsic biological processes chronologically (e.g. growth) to patterns of habitat use. This approach provides scope for exploring the relative role and implications of subtle shifts in the timing, intensity and spatial distribution of environmental factors in shaping fish ELH ecology (Limburg, 2001; Liberoff et al., 2014). We suggest that future research on estuarine and diadromous fishes could use these studies as examples of how to approach questions regarding the causes and consequences of diadromy and estuarine habitat use in ELH-phase fishes.

Marine fishes were some of the first subjects for the application of otolith chemistry (Campana, 1999, Fig. 1), and in recent times, the integration of this with more traditional otolith microstructure analysis has provided key insights. Most marine fish species have a pelagic larval phase that varies in duration from days to months and can range over broad oceanic scales (Cowen & Sponaugle, 2009; Shanks, 2009). Otolith-based research has provided key advances in understanding this larval phase, challenging long-held paradigms about open population dynamics in reef fishes (Caley et al., 1996) by demonstrating high levels of self-recruitment via larval otolith marking and chemistry analyses (Jones et al., 1999; Swearer et al., 1999). Within 2005–2012, studies of marine fishes adopting otolith microchemistry analysis (abundances of elements within otoliths) have revealed how ELH spatial ecology is linked to aspects of their biology (e.g. Ben-Tzvi et al., 2008; Aldanondo et al., 2010; Shima & Swearer, 2010, Table 1). For example, Hamilton, Regetz & Warner (2008) explored the effect of local retention versus long-distance dispersal (defined via otolith chemistry analysis) on the growth rates and condition of newly settled Thalassoma bifasciatum (Table 1). Recently, the costs and benefits of dispersal and self-recruitment have been a primary area of focus, with the emergence of several studies providing direct linkages between dispersal history and individual fitness (e.g., Shima & Swearer, 2009, 2010). This highly integrated approach to ELH research, which employs a broad range of both otolith structural and chemical analyses has produced profound insights into the ‘black box’ of fish ecology, and may be considered the forefront of ELH fish research. We contend that less-developed research arenas could benefit greatly by adopting the approaches used in these studies.

IV. BIOMES AND GEOGRAPHICAL REGIONS

Research effort has been unequally applied across latitudes and biomes. A majority of studies were conducted in temperate regions, at latitudes greater than 23.5° (Fig. 5). In the marine biome, 184 (62%) studies were conducted in temperate waters, there were 58 (53.2%) estuarine/diadromous studies and 26 (36.1%) freshwater studies. Conversely, 39 (13.1%) marine studies were conducted in tropical waters at latitudes less than 23.5°. In freshwater ecosystems, proportionally fewer studies (6.9%) were undertaken in tropical latitudes. A similar pattern in research effort focussed on temperate, northern hemisphere organisms has been reported previously on research examining phylogeography (Behereregayar, 2008). A considerable proportion of studies in the marine biome (22.2%), estuarine/diadromous biome (28.4%) and freshwater biome (56.9%) failed to report the latitude at which the study was conducted. However, many of these studies that failed to report latitude were laboratory-based (54.2%). The high incidence of solely laboratory-based freshwater studies (36.1%) may be due to the relative ease of maintaining and breeding freshwater fishes in captivity, relative to marine (13.1% of studies) and estuarine/diadromous (6.4% studies) species. More broadly, the pattern of greater research effort in temperate regions may be explained by the focus on commercial species of the northern hemisphere, and the relationship between economic prosperity and latitude, fuelling research at higher latitudes (Collen et al., 2008). Clearly, opportunities exist to expand research on fish ELH into low-latitude ecosystems where high levels of biodiversity, economic development and a multitude of threatening processes exist (Brook, Sodhi & Bradshaw, 2008; Dudigeon, 2011).

Intriguingly, there was unequal effort dedicated towards biological objectives across biomes, with 71.4% of publications originating in the marine biome focussing solely on biological objectives (Fig. 2). The marine biome was best represented, with 297 (62.1%) of all papers examining marine species. Estuarine/diadromous species were represented by 109 (22.8%) studies, while freshwater species were the least represented, totalling 72 (15.1%) papers. The striking dominance of literature originating from the marine biome that focussed on biological objectives presents an exciting opportunity for synthesis and exploration of how otolith-based research may be applied to understand the ELH ecology of fishes at a fish community or ecosystem level. The dominance of biological otolith-based studies in the marine biome may be explained by the relative commercial value of marine species (FAO, 2012) and the requirement for biological data to manage commercial fisheries.

Digging deeper, it appears that papers exploring biological objectives overwhelmingly employed otolith structural analyses in the marine (99.1%), estuarine/diadromous (98.2%) and freshwater (100%) biomes. Likewise, the majority of studies exploring spatial objectives used otolith chemistry analysis in the marine (43.5%), estuarine/diadromous (51.6%) and freshwater biomes (85.7%). The relatively lower application of otolith-based research to explore spatial
objectives in marine systems compared to estuarine/diadromous and freshwater ecosystems reflects the greater potential for chemical analyses to reveal movements in the estuarine and freshwater biomes, where variation in water chemistry across spatial and temporal scales is greater than in many marine ecosystems (Gillanders, 2005; Kerr, Secor & Kraus, 2007; Bowen, 2010). Of those studies exploring both biological and spatial objectives, a moderate proportion simultaneously employed both structural and chemical analyses (23.4% marine, 41.7% estuarine/diadromous and 100% fresh water; for further discussion, see Section V). Otolith structural analyses were most commonly applied to explore objectives related to age and growth of ELH fishes.

By contrast, studies conducted in the estuarine/diadromous biome displayed a fundamentally different pattern to marine studies, with proportionally more papers focused on solely spatial or both biological and spatial objectives, with a greater diversity of application of otolith techniques (Fig. 2). A considerable proportion (32.5%) of the estuarine/diadromous literature was focussed on exploring habitat use and movement within or across the freshwater/marine interface, to confirm estuarine and diadromous life histories, respectively. Within freshwater systems, telemetry [radio, acoustic and passive integrated transponder (PIT)] has seen a recent revolution, with increasing research effort exploring the spatial ecology of fishes (Cooke et al., 2013). However, ELH-phase individuals are mostly too small to insert telemetry tags, precluding the use of this technology to explore ELH spatial ecology. Otoliths remain a source of ELH spatial data, but are not limited by behavioural modification due to tagging, or restricted observational timeframes due to short battery life. Potentially, as the movement patterns

Fig. 5. Proportion of species within the database whose status was classified as either commercial (FAO, 2013), threatened (IUCN, 2013) or both commercial and threatened, or neither, by (A) marine, (B) estuarine/diadromous and (C) freshwater biome. Distribution of studies conducted in temperate, tropical, both tropical and temperate or undefined regions by (D) marine, (E) estuarine/diadromous and (F) freshwater biome.
of these species are defined, studies may shift towards exploring primarily biological objectives, reflecting current trends in marine studies.

Recent otolith-based studies of ELH estuarine/diadromous fishes has largely focussed upon truly diadromous species, particularly amphidromous species (Figs 6 and 7), which in many cases are transient and/or ephemeral residents of estuaries during just their ELH phase(s). Surprisingly, we found comparatively less effort direction towards taxa that facultatively use estuaries during ELH phase growth and development. This is despite estuaries being identified as key nurseries for a suite of estuarine, marine and freshwater species of both commercial and ecological importance (Beck et al., 2001; Gillanders et al., 2003; Elliot et al., 2007). For instance, numerous recreational and commercial fisheries are supported by species that occupy estuaries for much of their life cycle, including their early development phases [e.g. croakers Micropogonias spp. (Sciaenidae); Searcy, Eggleston & Hare, 2007a,b; Hoover et al., 2012]. Emerging data suggest the temporal extent of estuarine occupation by ELH fishes may have been underestimated in the past, which may have important implications for the management and protection of estuaries as key habitats for both the purposes of nursery habitats and connectivity corridors for fishes spanning multiple biomes (e.g. Beck et al., 2001; Closs, Hicks & Jellyman, 2013). Artificial and natural marking (including otolith chemistry analyses) have been identified as potential sources of spatial information to advance our understanding of these crucial nursery habitats (Gillanders et al., 2003), yet application of otolith structural analyses either in isolation, or in tandem with other techniques, appears to be limited in the estuarine context within recent years. As such, estuaries appear to be a prime biome for future applications of otolith-based studies of ELH-phase fishes.

V. TAXONOMIC PATTERNS AND EMERGING MODEL TAXA

Applications of otolith technology have not been equally distributed across ELH fish taxa, yet may provide some indication of emerging model taxa at the forefront of this discipline. Effort was not clearly related to levels of inter-family diversity, with the highest number of studies in marine species for the damselfishes (Pomacentridae), herrings (Clupeidae) and anchovies (Engraulidae) (Fig. 6), despite the total number of species in these families being relatively low (390, 194 and 148 species, respectively). Commercial status seems to be a key driver, with both the family Clupeidae and Engraulidae consisting of notable commercial species central to several major fisheries globally. Some of the most speciose marine fish families were very poorly represented, although at least one species was examined in each of the Gobiidae (cf. 1693 species), Serranidae (cf. 540 species) and Labridae (cf. 521 species). In the estuarine/diadromous biome, the two most speciose families, the Gobiidae (40 studies) and Cichlidae (12 studies) have mixed fortunes. A notable exception were the Anguillidae (18 species) achieving an average of 1.33 studies per species. Despite the low species diversity in this family, considerable research has been conducted, primarily aiming to reveal the long and complex ELH phases of these fishes.

Commercially important species were over-represented and may help explain many of the observed patterns in the literature. A third of all species encompassed by the current review were classified as commercially important, with relatively equal weighting across marine (30.9%), estuarine/diadromous (41.7%) and freshwater (28.8%) biomes suggesting that commercial status may be driving major research efforts towards understanding recruitment processes in fisheries. This is supported by commercial species (33% of species represented) being present in 46.7% of all studies conducted, and averaged 2.42 studies per commercial species, compared to 1.36 studies each for non-commercial species. On the other hand, threatened species were not equally represented across biomes, with the marine (4.7%) and estuarine/diadromous (4.8%) groups having considerably less threatened species than the freshwater (23.7%) biome (Fig. 5). Perhaps this is not surprising, given the vulnerability of freshwater ecosystems to human impacts (Dudgeon et al., 2006; Heino, Virkkala & Toivonen, 2009; Strayer & Dudgeon, 2010; Vörösmarty et al., 2010), and the smaller distributions that freshwater fish populations exhibit (Myers, Mertz & Bridson, 1997; DeWoody & Avise, 2000). There is possibly an interplay between the lethal nature of otolith-based research limiting application on threatened populations, and a counter-acting force of greater research effort being applied to enhance management of threatened populations and species. However, those species listed as threatened represented 3.6% of all studies, and averaged 1.27 studies per species, compared to the average of 1.72 studies per non-threatened species, suggesting restricted research effort on the ELH ecology of threatened fishes. The necessity to sacrifice individuals for otolith-based work is potentially a major limitation in researching threatened species; however greater appreciation of the wealth of information obtainable from otoliths and how this information could be used to enhance management of threatened fishes may yield worthy trade-offs on a case-by-case basis. For instance, the information gain from sacrificing ELH samples may prove particularly attractive when focussing on threatened species that exhibit high fecundity but low adult standing stock.

Set against a backdrop of more than 31000 recognised extant species within the Teleostei infraclass (Eschmeyer & Fong, 2013), during the period 2005–2012, the ELH
Fig. 6. Number of valid species (diversity) listed in the Catalog of Fishes (Eschmeyer & Fong, 2013) by family in the (A) marine, (B) estuarine/diadromous and (C) freshwater biomes. Top 20 families ranked by number of species-studies, broken down by biological, spatial or both objectives (research objectives) within the database by (D) marine, (E) estuarine and diadromous and (F) freshwater biomes. Top 20 families ranked by number of species-studies, broken down by structural, chemical or both structural and chemical analyses (research techniques) within the database by (G) marine, (H) estuarine/diadromous and (I) freshwater biome.
Fig. 7. Number of studies per species in (A) marine, (B) estuarine/diadromous and (C) freshwater biomes, by biological, spatial or both biological and spatial objectives (research objectives). Number of studies per species in (D) marine, (E) estuarine/diadromous and (F) freshwater biomes, by otolith structure, chemical or both structural and chemical analyses (research techniques).
phases of just 380 species were studied using their otoliths. Furthermore, research effort was dedicated mostly to 81 species, which were the focus of 50% of all studies. Within the marine biome, 14 (70%) of the 20 most-studied species were commercially important targets of wild fisheries, with good representation by members of the Engraulidae (two species) and Clupeidae (five species) families (Fig. 7). Within the top five most-studied marine species, 71.4% of studies examined biological objectives, with otolith structural analyses present in 96.8% of these studies (Fig. 7), of which the primary focus was ELH age and growth. However, a few other species have received high levels of research effort to answer a range of biological and ecological questions. One of these ‘model species’ was Thalassoma bifasciatum (10 studies), which was the subject of a spread of biological (50%), spatial (30%) and both biological and spatial (20%) study objectives and a similarly diverse use of otolith technologies (structural = 50%, chemical = 10%, both structural and chemical analyses = 40%). Both Thalassoma bifasciatum and Forsterygion lapillum have been used to understand the consequences of ELH dispersal and fitness on recruitment and population dynamics (Swearer, 2009). The use of both otolith structural and chemical analyses to explore the biological consequences (otolith increment widths) of differing dispersal histories (otolith chemistry). These taxa join other marine ‘model species’, such as clownfishes of the genus Amphiprion and damselfishes of genus Pomacentrus, who have been the subject of multiple otolith-based studies measuring dispersal pathways, connectivity and extent of self-recruitment in fish populations (e.g. Jones et al., 1999, 2009; Almany et al., 2007). Shifting research effort towards large-bodied species, exhibiting broadcast spawning and longer PLDs will further enhance our understanding of connectivity in marine populations, with benefits for marine park design and fisheries management.

Diadromous Anguilla species received particularly high amounts of research attention for a given taxon, likely due to a combination of commercial, conservation and academic motivations. Twenty-five percent, corresponding to 5 of the 20 most studied species from the estuarine/diadromous biome, were from the genus Anguilla (Fig. 7). Anguilla anguilla and Anguilla japonica are both listed as commercial species with A. anguilla being the focus of conservation efforts within Europe (ICES WGEEL, 2006; Cucherousset et al., 2007). Additionally, the life history of Anguilla species is recognised as one of the most intriguing life cycles in the natural world, having been speculated on by philosophers and biologists alike for centuries (e.g. Aristotle 350 BC in Kuroki, Righton & Walker, 2014). Estimating the lengthy and variable pelagic larval duration has been fundamental to discerning the long-distance migration of adults, and coupled with oceanography and hydrodynamic modelling, has led to the identification of likely spawning locations of adult eels (Tsukamoto, Aoyama & Miller, 2011). Furthermore, the examination of PLD in sympatric populations of A. rostrata and A. anguilla in Iceland has identified that temporal separation on distant spawning grounds is the likely factor that maintains genetic separation between the two species (Kuroki et al., 2008). Given that a majority of the Anguilla species have had recent research attention in addition to the long history of ELH research on this group, we are rapidly obtaining a fuller understanding of the range and diversity of ELH dispersal trajectories of the Anguilla species, which is fundamental to understanding the evolution of this genus (Araki et al., 2001; Tsukamoto, Aoyama & Miller, 2002). This is a complex problem, and has been tackled using a combination of otolith structural and chemical analyses. For instance, of the four studies on the European eel, Anguilla anguilla, none examined solely a biological objective, with all four studies employing both otolith structure and chemistry to examine their objectives (Fig. 7).

Recently, amphidromous gobies have received research attention including from an ELH perspective. Of the top 20 most-studied species in the estuarine/diadromous biome, 15% (three species) were amphidromous gobies, two of which were from the genus Stenopterus and the other Cotelurus acutipinnis. The evolution and maintenance of amphidromy has received considerable debate in recent decades (McDowall, 1987; Closs et al., 2013; Feutry et al., 2013; Watanabe et al., 2014). The examination of PLDs to infer potential dispersal trajectories and otolith chemistry analyses to determine potential source populations will further aid our understanding of this unique fauna and its evolution (Lord et al., 2010, 2012). The possibility of natal homing in many populations suggests that disparate dispersal strategies may occur, which may be facilitated by larval settlement behaviour, driven by olfactory cues, and plasticity in settlement times (Miles et al., 2014). Greater application of otolith chemistry analyses and otolith marking techniques (chemical marking, or transgenerational marking) is poised to advance this area of study.

No obvious model fish species for otolith-based research of ELH within freshwater environments emerged from the current review. Of the 20 most-studied freshwater species, a majority were from the families Cyprinidae (7 species) and Salmonidae (4 species) (Fig. 7). Furthermore, the genus Coregonus was relatively well represented, with three species present within the top 20 most-studied freshwater species. However, just 14 species within the freshwater biome were represented by more than a single study between 2005 and 2012. The zebrafish Danio rerio is a model species for understanding vertebrate developmental processes (Haffter et al., 1996; Knapik et al., 1998) and there has
also been a call for zebrafish to be used as a model organism in aquaculture-based research (Ribas & Piferrer, 2013). All studies of zebrafish retrieved in the current literature review focussed on biological objectives related to otolith growth and development, and relied solely on otolith structural analyses (Fig. 7). Notably, all of these studies were laboratory-based (e.g. Bang & Grønkjær, 2005; Cruz et al., 2009). If marine studies provide some indication of what will make a model species or at least a popular species for ELH otolith study, it might be that commercial or even recreational angling freshwater fishes will emerge over time. Further, the linear networks of rivers and the success of telemetry to study the movement of adult-phase fishes may well be combined with otolith techniques to understand biological processes across life-history phases in freshwater fishes.

One possible model freshwater species for examining ELH did emerge within the surveyed (2005–2012) literature: Australian smelt Retropinna semoni. Recruitment of R. semoni has been explored in relation to river hydrology in southeastern Australia in the form of multiple studies. Early research (Tonkin, King & Ramsey, 2008a; Tonkin, King & Robertson, 2008b; Tonkin, Ramsey & King, 2008c) examined the influences of food and temperature on the growth of ELH R. semoni, and the applicability of growth reconstruction using daily increments within otoliths. More recently, field studies have employed age and growth reconstructions from otoliths of juvenile R. semoni to explore the influence of differing hydrological events on recruitment in this species, and evaluated multiple theoretical models relating features of the hydrograph and thermal environment to recruitment (Tonkin et al., 2011; Humphries et al., 2013). Similarly, research on Perca flavescens in the USA has revealed that growth of larvae that undertake a pelagic larval phase within the marine-like environment within large lakes tends to be greater than benthic/shore hugging larvae, suggesting that a pelagic phase may be adaptive, and demonstrates the plasticity of the ELH phase in this species (Weber, Detttmers & Wahl, 2011; Janssen et al., 2014; see Section III). Furthermore, this research demonstrates that approaches to ELH research applied in marine systems may be equally valid in specific freshwater ecosystems. Clearly, otolith-based research into ELH phases of freshwater fishes can inform us on the massive diversity of life-history strategies that exist within the diverse freshwater teleost fauna, providing similar insights to those revealed in marine systems. The application of emerging techniques (described in Section VI) to freshwater fishes is likely to lead to considerable advances in our understanding of freshwater fish ecology with benefits for both fisheries and conservation.

Investing effort to identify potential model taxa within freshwater ecosystems may allow identification of key knowledge gaps, and key concepts that have been tested using otolith-based technologies in marine and estuarine/diadromous research systems. Fortuitously, many studies from the marine and estuarine/diadromous literature could serve as templates for this development of our understanding of fish ELH ecology in freshwater systems (Table 1). Indeed, the adoption of cutting-edge approaches and techniques originally applied in marine systems (e.g. transgenerational marking, see Section VI) has started to emerge in the freshwater context and is likely to drive rapid advances in our understanding of the ELH ecology of freshwater fishes.

VI. EMERGING TECHNIQUES

Perhaps most intriguing are five analytical techniques which have the potential to provide major breakthroughs in ELH fish ecology, but that have rarely been used in studies to date. Application of rarer techniques, such as C and O isotopes, asymmetry and computed X-ray tomography (CT scanning) have not occurred in conjunction with many of the other, more common analysis techniques, possibly reflecting that research using these techniques is still at the descriptive stage, and applications answering questions in broader biological or ecological contexts remain to be conducted. Many of these technologies, such as fluctuating asymmetry (FA), natural isotope ratio analysis and bomb-radiocarbon analysis, were developed many decades ago (Fig. 1). FA has long been seen as a useful proxy for fitness, as increasing FA is related to the interplay between developmental stability and environmental stress (Leary & Allendorf, 1989; Allenbach, 2011), and has been linked to recruitment success (Lemberget & McCormick, 2009). However, the ability to detect FA has been controversial and recently has seen a decline in application (Dongen, 2006). Applications of natural isotope ratios such as $^{87}\text{Sr}/^{86}\text{Sr}$ in ELH contexts is likely to rise as analytical instrumentation sensitivity improves, enabling chronologically explicit analysis of minute quantities of otolith material, a prerequisite for analysing ELH otoliths of most fishes (e.g. Ben-Tzvi et al., 2007; MacDonald et al., 2008; Walther & Limburg, 2012). Developments in ecogeochronology to elucidate movements will fuel further expansion in this field of research, and will benefit from greater integration of otolith chemistry and isotope analyses (Bowen, 2010; McMahon, Hamady & Thorrold, 2013). Finally, bomb-radiocarbon analysis is a tool for age validation that has been used since the late 1980s when it was first discovered that radioactive $^{14}\text{C}$ was stored within the otoliths of fishes, and can be used to confirm the age of long-lived fishes (Kalish, 1993). Given that bomb-radiocarbon analysis is best suited to questions that are of considerable longevity (such as the lifespan of long-lived fishes), we see the application of bomb-radiocarbon to be of limited application to ELH-phase fishes. While these techniques have existed for decades, transgenerational marking and CT scanning have recent origins (Fig. 1).
(1) Transgenerational marking

Transgenerational marking is a very recent technique, and enables the mass marking of larval fish, via maternal transmission of enriched stable isotopes of barium and/or strontium via the yolk of the egg (Thorrold et al., 2006; Starrs et al., 2014). Transgenerational marking has been validated on multiple species of fish, and has been found to have minimal impacts on both females and their resultant offspring (Williamson, Jones & Thorrold, 2009; Roy et al., 2013; Starrs et al., 2014). Transgenerational marking has been applied in a single field study, to explore the contribution of self-recruitment in populations of clownfish (Amphiprion percula) and butterfly fish (Chaetodon vagabundus), in Kimbe Bay, Papua New Guinea (Almany et al., 2007). Transgenerational marking facilitates mass marking of larvae during the embryonic phase, and overcomes many of the logistical problems associated with mass marking of ELH-phase fishes (Jones et al., 1999; Elsdon et al., 2008). While marking a large component of larval production may still be costly and logistically prohibitive, careful choice of study species and systems may demonstrate this technique to be highly suitable for exploring connectivity in small aquatic systems, such as in upland stream networks. An integrated approach may involve combining otolith microstructure analysis with transgenerational marking to explore the prevalence and fitness consequences of natal dispersal and philopatry in fishes. Further research is required to confirm if the impacts of transgenerational marking are negligible and explore the biological significance of observed changes in larval morphology and growth (Starrs et al., 2014), while development of multiple batch-markers through application of multiple enriched stable isotopes and manipulating molar fractions will broaden the potential applications of transgenerational marking in both laboratory and field studies. The complementary approach of immersion marking ELH-phase fishes in enriched stable isotopes baths can also be applied within mass-production facilities to provide opportunities to explore the effects of restocking on conservation and fisheries-related objectives (Munro et al., 2008; Woodcock et al., 2011).

(2) MicroCT

CT scanning is a powerful technique for examining hard structures in situ, and is a core technique in both the research and treatment of bone-related injuries and disease in humans (Kalender, 2006). CT scanning involves the repeated X-ray scans of an object in a complete 360° arc, to produce a composite three-dimensional (3D) image of a structure. CT scanning has been applied to fish otoliths (Hamrin et al., 1999), to provide a 3D image of adult Gadus morhua otoliths, with potential for enhancing stock discrimination based on otolith shape. However, as CT resolution improves, coupled with advances in post-production analysis software, the ability to examine the 3D structure of ELH otoliths in situ is rapidly approaching. For example, Bignami et al. (2013) used a MicroCT system to explore the effects of ocean acidification on larval cobia Rachycentron canadum otolith size, shape and density in situ. An exciting future development will be to use MicroCT scanning to examine otolith microstructure in situ, eliminating the need for otolith extraction. This will depend on using MicroCT scanning to detect the subtle differences in otolith density corresponding to otolith growth increments (reflecting a shift in relative deposition rates of calcium carbonate and the organic matrix within the otolith; Morales-Nin, 2000). Rapid mass-scanning of individuals prior to otolith extraction (e.g., for chemical analyses) could reveal specific demographic units of interest based upon specific otolith features, and therefore significantly reduce processing costs. Identifying check marks on the otoliths of individuals exposed to environmental stressors (such as thermal pollution or starvation) may facilitate studies on the long-term impacts of these critical processes on growth and reproduction. Specifically, CT scanning may assist in obtaining sufficient sample sizes from wild populations that experienced starvation during their ELH phases, thereby providing insight into the prevalence and consequences of compensatory growth (Ali, Nicienza & Wooton, 2003). Application to live, anaesthetised fish would require rapid, high-resolution MicroCT scanning, and careful quantification of possible impacts of electromagnetic radiation exposure during scanning. This would enable repeated measures of otolith growth from individuals and presumably quantification of the determinants of growth. Furthermore, the potential to obtain critical life-history data without the need for euthanasia has major implications for the research and management of rare and threatened species (see Section V). Coarse-resolution scanning may have applications to examining annuli formed within otoliths, however MicroCT scanning resolution approaching 1 μm voxel size is technically feasible (Metscher, 2013), which may be suitable for examining daily growth increments in many (but not all) ELH-phase fishes. Advances in this area could lead to both faster data acquisition through reductions in processing time, and reduced costs associated with otolith microstructure analysis.

VII. FUTURE DIRECTIONS

Currently, a suite of otolith-based analytical techniques exists to extract a wide range of biological and spatial information from the otoliths of ELH-phase fishes (Fig. 3). As has been highlighted throughout this review, relatively few studies adopt multiple technologies, nor examine both the spatial and biological information available within otoliths to examine aspects of fish ELH. Exploring broader ecological objectives should be a
focus for future otolith-based research on the ELH ecology of fishes, especially if we are to understand the impacts of climate change on patterns of population size, replenishment and connectivity (Munday et al., 2008; Bell et al., 2013). Changes in dispersal patterns, habitat use and behaviour will all operate to mediate the impacts of climate change, yet to appreciate fully these mediating effects, identifying changes in spatial ecology will be essential to understanding the true impacts of climate change on populations (Munday et al., 2008). We acknowledge that studies combining otolith-based techniques with other, non-otolith-based research (e.g. genetics, parasitology, and behaviour) can yield deep insights in ELH fish ecology as much as the integrated otolith studies identified here (e.g. White & Warner, 2007; Grutter et al., 2010; Correia et al., 2012). Notwithstanding, studies integrating otolith structural and chemical analyses in concert represent an excellent approach to identifying the influence of critical environmental conditions for the movement, growth, condition and survival of ELH fish populations. New research techniques will likely be invented and older techniques refined as analytical instrumentation improves in precision, and consequently creativity and innovation will occur. However, at this moment we see scope for targeted application of currently available techniques to answer research objectives of both a biological and spatial nature. Ideally, the application of these techniques in tandem will allow novel combinations of spatial and biological data to provide new insights into key questions. Disparate applications of otolith-based technologies in different biomes, geographical regions and taxa also provide some clues to both key knowledge gaps and emerging model systems that may provide key advances in this arena.

Some taxa have received high levels of research attention on their ELH, notably a small number of commercially important species (Fig. 7), and these are emerging as powerful model species upon which we have multiple sources of information and datasets to answer key questions. For instance, the wealth of research on Anguilla has revealed that each species of Anguilla has a characteristic ‘migration loop’ however, facultative diadromy calls into question the relative importance of diadromy, and how this complex behaviour, and speciation in this genus may have evolved (Tsukamoto et al., 2002, 2011). Similar data-rich species Engraulis encrasicolis, Clupea harengus and Gadus morhua offer unique opportunities to examine ecological processes across broad spatial and temporal gradients. By drawing upon the research effort of multiple groups working in different regions, targeted meta-analyses could combine the results of these studies to delve into the effects of long-term environmental changes on the ELH ecology of these important species (sensu Payne et al., 2013), and reduce uncertainty in the estimation of biological parameters (Myers & Mertz, 1998). Long-term datasets such as these will be invaluable for discerning changes in population dynamics, and may in turn serve insightful as surrogates for understanding the biology and ecology of less-studied species (and ecosystems).

Lessons should also be taken from some of the heavily studied systems to challenge our understanding of other biomes and regions. For instance, major developments within marine biomes have provided some examples of how otoliths can yield breakthroughs in fish ELH in other systems. The marine biome has received the majority of research effort (62%), and this has resulted in several key insights into levels of self-recruitment (Jones et al., 1999; Almany et al., 2007), the importance of larval and juvenile body condition on growth and survival (Vigliola & Meekan, 2002; Meekan et al., 2006; Hamilton et al., 2008) and the influence of maternal effects on recruitment (Wright & Gibb, 2005), which all give insight into the diversity of life-history strategies observed in marine fishes. Given the relatively slow uptake of such techniques within freshwater biomes relative to marine and estuarine/diadromous systems, this appears to be a key area for future work. While the application of otolith chemistry is highly suited to questions within freshwater environments where natural geochemical variation occurs over smaller spatial scales (Bowen, 2010), actual applications are infrequently represented in recent literature. Applied in conjunction with otolith structural analyses, researchers could explore the relative prevalence of the above-mentioned factors in shaping the ELH ecology of freshwater fishes.

Despite the research effort applied in marine ecosystems, a number of important areas and species have not received recent attention in the ELH literature. For instance, deep-sea fishes are highly vulnerable to overfishing due to being long lived, low fecundity and often with restricted distributions on sea mounts (Koslow et al., 2000; Cailliet et al., 2001; Roberts, 2002; Devine, Baker & Haedrich, 2006). Furthermore, studies on deep sea fishes are few (Morales-Nin & Panfili, 2005). Studying the otoliths of these fishes has been traditionally problematic due to slow growth and longevity, making the delineation of annuli difficult (Morales-Nin & Panfili, 2005). While daily increments have been observed in the otoliths of deep sea fishes (Morales-Nin, 1990), research employing otolith chemical analyses (e.g. $\delta^{18}$O profiles; Mulcahy et al., 1979) may help to reveal aspects of ELH ecology that cannot be revealed solely from otolith microstructure analysis. A broader consideration of the types of information that can be obtained from otoliths will be critical to identifying new approaches to studying the ELH ecology of deep sea fishes, and indeed, fishes in other poorly known marine ecosystems.

While estuarine/diadromous fishes have had a great breadth of research exploring biological and spatial objectives, a clear focus in this ELH literature has been the diadromous Sicydiine gobies and the eel family
Anguillidae. For the freshwater-spawning (amphidromous) gobies, exploring natal homing in larvae may be possible by exploring the chemistry of otolith cores, and linking them to specific freshwater environments, or using marking approaches, such as in-stream enriched stable isotope labelling (e.g. Macneale, Peckarsky & Likens, 2005). Conversely, for the Anguillidae with their extensive marine migration before spawning, the validity of using leptocephali otolith cores to delineate adult habitats is questionable. The longevity of injected enriched stable isotopes in female fishes may offer some prospects for transgenerational marking, however, alternatives such as enriched isotope implants which provide a means of controlling the release of enriched stable isotopes prior to spawning, may enable mass-marking of offspring to facilitate research examining the natal homing behaviour in eels, gobies and other diadromous species. New approaches in ecogeochemistry such as the application of isoscapes and employing multiple chemical analyses including Sr:Ca, Ba:Ca, $\delta^{18}$O, $\delta^{13}$C, $^{87}$Sr/$^{86}$Sr and other tracers may help to unravel the relative costs and benefits of diadromy (including partial and facultative migration; Kerr & Secor, 2010; for a review, see Walther & Limburg, 2012) on recruitment (Hobbs et al., 2010; Walther & Limburg, 2012; McMahon et al., 2013). Insight into these costs and benefits hold the answers to understanding the evolution of diadromy (Tsukamoto et al., 2002; Closs et al., 2013; Libeoff et al., 2014).

VIII. CONCLUSIONS

(1) Otolith research, centred on ELH phases of fishes can be split into two broad categories; those studies that are primarily focussed on examining biological processes, such as age and growth, and those studies that are examining spatial processes, such as habitat use and dispersal. A relatively small proportion of studies (13.7%) attempt to examine both simultaneously.

(2) Techniques applied to study otoliths also fall into two broad categories. Otolith structural analysis examines the visible structures in otoliths, and primarily relates to daily and annual growth increments, check marks and otolith morphology. Conversely, otolith chemistry analysis examines the chemical properties of otoliths, including the relative abundances of elements and stable isotopes, as well as being a vector for artificial marking agents. Importantly, otolith structural analysis is overwhelmingly used to answer biological questions in fish ELH, whereas otolith chemistry analysis holds great power in answering spatial questions in ELH-phase fishes.

(3) Otolith research on fish ELH has recently been most heavily focussed on marine species, with studies examining biological objectives with otolith structural analyses being the most dominant form of research undertaken. Both freshwater and estuarine/diadromous species science lag behind in terms of research effort; however estuarine/diadromous species are receiving a greater diversity of research effort in terms of applications of otolith analysis techniques, than freshwater species. The ELH of freshwater species is relatively neglected in terms of overall research effort, however, the heterogeneity in natural geochemical properties of freshwater ecosystems is highly suitable for delineating habitat use and movement ecology of freshwater fishes, and presents exciting opportunities for future research.

(4) Over the period 2005–2012, otolith research of ELH fishes was based on published studies of just 380 species. A sizeable proportion (33%) were commercial species, with the families Clupeidae, Engraulidae, and Gadidae being well represented (18.3% of studies). A majority of the research has been focussed on temperate species (56.3%), with the tropical fish fauna relatively neglected in comparison (12.5%), however, nil reporting of latitude (26.2% of species-studies) may be biasing this result. A clear split was evident between those species that are of considerable commercial value and those that are studied to explore specific questions regarding ELH biology and ecology. Study of the genus Anguilla stood out in terms of both research effort (24 studies) and the diversity of research techniques used.

(5) Daily ageing of ELH-phase fishes was the most common otolith analysis technique applied, followed by measurements of daily otolith increment widths. MicroCT and transgenerational marking are emerging techniques, and present exciting opportunities to obtain new spatial and biological data on ELH-phase fishes. Opportunities exist in combining techniques to collect complementary information from otoliths to explore current and new research objectives.

(6) Future research in ELH ecology needs to integrate further biological and spatial objectives within studies, as the means to do so currently exist. Technological advances will continue to drive research at the forefront of otolith research in well-studied systems; however, there remain considerable opportunities to apply existing techniques in under-studied systems. Greater research effort on non-commercial and freshwater species in general will better place us to test key theories and manage fish populations across a broader spectrum of environments and biomes.

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X. REFERENCES
References marked with asterisk have been cited within the supporting information.


Otolith-based research in fish biology & ecology


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**XI. SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article.

**Appendix S1. Methods.**