Theme session H

Looking backwards to move ahead - how the wider application of new technologies to interpret scale, otolith, statolith and other biomineralised age-registering structures could improve management of natural resources

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In recent years, technical and analytical developments in sclerochronology based on the reading of otoliths, statoliths, spines, vertebrae and other age-registering accretionary hard tissues have contributed increasingly to our ability to assess the life-histories of a wide range of marine organisms, including fish, cephalopods, shellfish and corals. The knowledge gained from such studies, primarily obtained through isotopic and trace element analyses, has important implications for the assessment and management of populations and habitats: new insights are being gained into the biology of species that are data-limited, while conventions are being challenged in species that are data-rich, and our session aimed to reflect this. Moving beyond single species and other, niche analyses, sclerochronology has the potential to provide a much deeper understanding of the interaction between marine life and its environment. Such an understanding is crucial at a time when fisheries management evolves to take account of changing climate and the prevailing environmental conditions. Funding constraints and the concomitant reduced field survey opportunities under austerity make the full and exhaustive utilization of new and historical data collection time-series paramount. This highlights the need to broaden and integrate current advances in sclerochronology to invigorate their applications throughout fisheries and environmental sciences.

With this overarching ambition in mind, Theme Session H (Thursday 22nd-Friday 23rd October) at this year's ICES Annual Science Conference 2016 in Riga, was convened with the aims of broadening understanding, cross-fertilising across different but related areas of sclerochronology and to facilitate the wider uptake of state-of-the-art current approaches. We aimed to address assessment and management applications, from species-specific ageing methods and migration studies, to analytical stock assessments using data collected using novel techniques. Theme session H was by necessity cross-disciplinary and was intended to include technical and ecological studies emphasising new methods and approaches to ageing and use of aging data. We tried to encourage studies from a broad range of species and taxa, and included seabirds and marine mammals, teleost and elasmobranch fishes, cephalopods, gastropod and bivalve molluscs, crustaceans, echinoderms and other invertebrates within our remit.

Specifically, we solicited papers addressing the following topics:

- use of new techniques to assess age or life-history events
- ecological studies based on assessments of age or environmental signals
- Insights into early life history
- assessment of climate change effects on species or populations
- age studies of environmentally important species for which age data are poor or non-existent

In total we accepted forty-three contributions (29 oral presentations, 14 posters). Over half of our contributions (32 in total) were studies of fish, the majority based on aspects of otolith analysis (27 in total). Of these, nine focussed on otolith chemistry, seven addressed incremental analysis and morphometrics, and six applied otolith marking to address the effectiveness of recruitment in stocking studies. Four contributions addressed otolith biochronologies and just one talk focussed directly on pollution monitoring. Beyond otoliths, two contributors discussed fish scale applications, one applied fin-rays, and a poster helpfully summarised the utility of the range of fish tissues available for ageing studies. While these latter contributions emphasised non-lethal sampling strategies, another novel application included the chemical interpretation of proteinaceous eye-lens laminae to determine life-history events.

Having solicited studies from a broad range of species and taxa, the non-fish contributions that comprised the remainder of our session were exclusively molluscan. Of these, we had six contributions focused on cephalopod ageing studies, and a further five contributions focussing on gastropods and bivalves (3 on ageing, 2 on biochronologies). Nonetheless, attendees from each of the three main communities participating within our session were well represented.

To emphasise innovation and cross-fertilisation, presentations from all areas were deliberately mixed within our session, allowing us to offer a diverse and highly stimulating programme to all those present throughout our session. Unlike the schedule however, common topics are pooled here for ease of reporting. Our session attracted top scientists in their fields across our area of remit. Four 'feature' presentations were chosen by the conveners based on novelty or impact, which we felt represented our main themes, and as such, these talks were given a slightly longer time slot (25 minutes, as opposed to 15).

Otolith Chemistry and life-history biology

Otolith chemistry continues to be a driving force in fish population dynamics studies. Our first feature talk (H:207), presented by Bronwyn Gillanders (on behalf of Zoe Doubleday, who unfortunately was unable to join the meeting), provided a fascinating insight into the potential use of sulphur isotopes ($\delta^{34}S$) as biogeochemical markers. After reviewing the scant published literature, new data showing environmental variation in $\delta^{34}S$ recorded by the otoliths of juvenile tank-grown barramundi were shown, noting that concentrations were influenced by both ambient water and by diet. Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS), Inês Farias (H:584) explored more conventional elemental analysis to describe the life history migratory dynamics of the deep-sea black scabbardfish, *Aphanopus carbo*. Gray Redding (H:388) used archived otoliths from the United States, Canada and Iceland, to try and discriminate Northwest Atlantic mackerel contingent population structure and dynamics on the basis of hydrographic differences using otolith $\delta^{18}O$ and $\delta^{13}C$.

Adopting an experimental approach, Jasmin Martino (H:286) raised juvenile snapper (*Chrysophrys auratus*) under four different temperature regimes to examine the relationship between physiology (metabolic rate) and otolith $\delta^{13}C$. The ultimate aim here is to understand physiological histories to decipher past population influences and predict future responses in an otolith time series spanning forty-five years. In an elegant study

combining the ambient environmental experience of North Sea plaice *Pleuronectes platessa* (as recorded simultaneously by archival tags and otoliths from the same fish), Audrey Darnaude (H:219) showed spatial and temporal variability in the acuity of otolith $\delta^{18}O$ for providing fishery-independent estimates of fish location. Karin Hüssy (H:54) reported on current difficulties on matching otolith elemental patterns as a means of age estimation for cod in different areas of the Baltic. Despite extensive environmental differences between the three study areas, the element concentrations of Cu, Zn and Rb were strongly correlated in all individuals with similar correlations in all three areas, suggesting the same incorporation mechanisms for these three elements independent of environmental concentrations.

As a 'local' example of the application of otolith chemistry, Didzis Ustups from the Fish Resources Research Department in Riga described how elemental analysis was being used to address stock identity questions concerning Baltic flounder, *Platichthys flesus*. Other examples of otolith chemistry in the early stages of development from current PhD students included posters from Jen Lewis (H:379), who was applying elemental analysis to study range-expansion in gilthead seabream (*Sparus aurata*), Tom Stamp (H:532), who aims to use strontium stable isotope ratios to infer juvenile European Sea Bass (*Dicentrarchus labrax*) estuarine fidelity and movement within high estuarine habitats, and Coley Hughes (H:542) who was using a random forests model to back-cast adult striped bass (*Morone saxatilis*) otoliths to determine critical coastal nursery habitats.

In her own presentation, Bronwyn Gillanders (H:246) demonstrated how chemistry (Ba:Ca and Sr:Ca) and growth properties of calcified structures can provide data that reflect the biology and ecology of organisms and variability of their habitats sometimes over centennial time scale. By correlating environmental data with seasonally resolved chemical chronologies, element-environment regression functions were developed and used to reconstruct environmental conditions based on elemental information from archaeological otoliths, thereby revealing change over decadal and centennial time scales.

Finally, as an example of an underdeveloped application of otolith chemistry, Audrey Geffen (H:530) gave us a fairy-tale complete with a wicked witch (decommissioned oil rigs as a source of radioactive contaminants). Tusk (*Brosme brosme*) otoliths revealed significant uptake of lead in contaminated versus uncontaminated areas, suggesting a possible role of otolith chemistry to detect and monitor anthropogenic impacts. Following the use of bomb radiocarbon to validate the age of Mediterranean pink dentex, *Dentex gibbosus* Sergio Vitale (H:561) then applied Excimer Laser Ablation ICPMS (ELA-ICP-MS) to look at otolith elemental distribution. An emergent function of these results was the suggestion that increasing B and decreasing levels of Pb in the dentex otoliths correlated with the introduction of 'unleaded' fuel, as further example of a potential role for otoliths in monitoring environmental health.

Otolith Biochronologies

Gaining a better understanding of the relative importance of, and interaction between, natural and anthropogenic drivers in shaping marine systems, John Morrongiello (H:104) presented 19-year biochronologies (1980-1999) for three south-east Australian populations of a site-attached temperate reef fish, purple wrasse (*Notolabrus fucicola*).

Using otolith growth as a biological indicator, he showed that fishing and temperature can have a synergetic impact on marine populations via within-individual responses. Synchrony of climate and fish growth patterns based on multidecadal otolith biochronologies were also the subject of a poster by Szymon Smoliński (H396), based on observations of the short-lived European flounder (*Platichthys flesus*). Short term biochronologies and trace element analyses were also employed by Hector Andrade and colleagues (H:454) to examine the effects of climate upon cod life history and habitat use across a temperate-arctic gradient. One of our early career scientists, Joyce Ong (H:110) provided an outstanding account of a cross-dating approach that clearly linked the responses of marine and terrestrial ecosystems to the El Niño Southern Oscillation (ENSO) phenomenon. Work of this nature potentially allows accurate predictions of cross-system biological responses, with significant national implications, notably for the future of fisheries in developing nations.

Age validation and otolith morphology

Demonstrating progress within well-established ageing techniques, Kelig Mahé and colleagues investigated the variability of estimated age using Marginal Increment Analysis (MIA) across a range of potential explanatory factors and across a variety of species (H:99). Using generalized linear mixed effect models, this study clearly underlined that the influence of sex, sampling year and area on the relationship between age and total length is species-dependent. Dariusz Fey and colleagues (H:414) presented a poster showing the first results of age validation and growth rate of larval and early juvenile pike (*Esox lucius* L.) using both sagittal and lapillar otoliths.

In ongoing work looking at the potential for applying image-recognition in processing otoliths, James Mapp (H:302) provided encouraging news for expert otolith readers in Fisheries Laboratories everywhere, with human readers outperforming automated outlining methods in all aspects of boundary based stock classification. Morphological changes of otoliths during the growth of three species of icefish were also the subject of a poster by Ryszard Traczyk, although in this instance, the changes were related to the life habits of the study fish. Two further posters detailing automated shape recognition routines were ultimately not forthcoming (H:68, H:303).

Otolith applications in recruitment studies

Daniel Zapf's work (presented by Roger Rulifson, H:538) on juvenile river herring (*Alosa* spp.) effectively used otolith elemental fingerprints in the first year of life to identify essential nursery habitats in Albemarle Sound. Watershed fingerprints were defined primarily by the elements Sr, Ba, Mn & Mg. David Secor (H:189) demonstrated how the mass chemical marking of hatchery raised striped bass larvae was used to assess estuarine retention and subsequent production of these fish. The spatial and demographic fates of both naturally produced and introduced larvae were tracked by successive surveys, and linked recruitment success with increased nursery volume. Similarly, Håkan Wickström (H:257) has recently been using a similar approach to gage the success (or otherwise) of Swedish restocking programmes for eel *Anguilla anguilla,* a stock in serious decline throughout Europe. Also currently in progress, Adam Lejk (H:626) described testing on

alizarin immersion as a means of marking of juvenile sea trout *Salmo trutta* m. *trutta* L. otoliths, now being deployed to assess a current stocking program in the southern Baltic.

Non-lethal sampling and alternative age-registering structures in fishes

While otoliths continue to be the main focus of fish ageing studies, otolith removal requires the death or sacrifice of the donor specimen. Sampling does not generally pose a problem for abundant species in routine survey and sampling, however it is problematic for rare, endangered and long-lived fishes. The poster by Ori Tzadik and colleagues (H:549) provided a neat summary of the pros and cons associated with to the use of alternative structures, with discrimination between trace-element analysis for inorganic matrices and stable-isotope analysis for organic matrices. Structures with high turnover rates or those that are metabolically active will not effectively record elemental or isotopic compositions over time.

The theme of "decapitation is lethal" was further pursued in our third feature presentation by Ben Walther (H:75), who successfully applied both inorganic (e.g. strontium and barium concentrations) and organic (e.g. carbon and nitrogen isotope ratios) proxies to reconstruct migration and dietary histories from scales of the Atlantic Tarpon *Megalops atlanticus*. His combined approach showed that trans-haline migrations by tarpon were associated with ontogenetic trophic shifts. With sequential sub-sampling made possible by scale architecture, this study is an encouraging example of scales being used as a non-lethal alternative to monitor fish migrations across chemical gradients, with the further possibility of citizen-science engagement. Vidar Moen (H:625) looked at post-depositional changes in naturally incorporated trace elements in the scales of anadromous Atlantic salmon (Salmo salar L.), but the aim here was to discriminate wild fish from aquaculture escapees.

Non-lethal fin-ray sampling for trace element analysis to evaluate essential nursery habitats of the critically endangered Atlantic Goliath Grouper, *Epinephelus itajara*, was featured in Ori Tzadik's main presentation (H:547). This was a convincing example of elemental fingerprint identification of essential juvenile habitat with a high degree of classification accuracy.

Finally, Jennifer Granneman and colleagues (H:128) provided an intriguing account of how the 'onion layer'-like laminae comprising the eye-lenses of red snapper *Lutjanus campechanus*, collected from the Gulf of Mexico can be applied to identify isotopic histories. The metabolically inert optical proteins deposited in fish eye lenses form in successive, concentric circles (laminae) in much the same way that annuli form in otoliths. Although still in progress, this work demonstrated how cross-referencing between otolith trace-metal and eye lens isotope profiles could be indicative of the approximate locations of trace-metal exposure and other environmental signals signalling life history events.

Age-registering structures and cephalopod research

Session H was attended by around a third of active cephalopod specialists working on problems related to aging squids, octopods and cuttlefish, including several acting and ex-members of Cephalopod International Advisory Council (CIAC) – a body of 18 people elected once in three years to stimulate, accelerate and influence the direction of cephalopod research, to provide help and advice on aspects of cephalopod biology, including those

relevant to the management of the increasingly important cephalopod fisheries and to disseminate information on past and current research (https://cephalopod.wordpress.com/council-2/).

Our second feature presentation from Graham Pierce (H:426) gave an exhaustive account of all aspects of life-history revealed by extensive investigations of statoliths and gladii of the "iconic" commercial species *Doryteuthis gahi* and *Loligo forbesi*. Fjodor Lishchenko (H:83) and Alexander Arkhipkin (H:125) both provided accounts of the generalities of using of age registering structures in cephalopod molluscs; the first presenter emphasised the technical peculiarities involved in materials' processing, whereas Arkhipkin concentrated on interpretation of the observed growth rings.

Statoliths have traditionally been used for ageing squid and cuttlefish. Anastasiya Lishchenko (H:146) presented an analysis of age and growth of the Commander squid from the Kurile Islands, based on statolith readings. Embryogenesis in this species lasts for half of the annual life cycle – a very unusual situation for a commercial species. During discussion, other cephalopod specialists present made known further observations for additional taxa. Of particular note was discussion around the periodicity of formation of statolith growth rings: Experiments in which feeding was withheld suggest that ring formation in statoliths is led by feeding cycles rather than diurnal environmental cycles.

In octopods however, the statoliths are 'chalky' and do not exhibit growth rings. Consequently, the ageing of octopods has not been possible until recently. Richard Schwarz (H:377) and Catalina Perales-Raya (H:204) presented their recent findings on ageing using cephalopod beaks, which are becoming increasingly important in ageing studies, particularly for octopods. Both presenters expressed concerns that in some situations (particularly outside optimum temperatures) the octopods can produce less than one ring per day, sometimes in the region of 10 rings in 20 days.

Growth increments in bivalve molluscs

In our final feature presentation, Paul Butler (Bangor University) demonstrated the usefulness of absolutely dated chronologies built from annual growth increments within the shells of several bivalve species (including the heavily researched *Arctica islandica* that might live for several centuries) for reconstructing past environmental conditions (H:474). He went on to briefly report on the success of the ongoing 'Annually Resolved Archives of Marine Climate Change' (ARAMACC) project which is attempting to build several key chronologies for sites around Europe as well as answering key issues about general biology and shell formation of important bivalve species. This is being achieved through the combination of 10 PhD studentships and several Post-Doctoral positions at 8 collaborative institutions across Europe. One of the ARAMACC students (Stella Alexandroff, Bangor University), presented a poster on how a shell chronology in combination with calibrated shell isotope data had reconstructed seawater temperatures from the 4th millennium BP (H:473). Paul then described some collaborative work from David Reynolds (Cardiff University) which explained the potential of using networks of shell chronologies from several areas to look for broad scale signals and changes in oceanographic conditions.

The idea of using several combined chronologies echoed Joyce Ong's (University of Western Australia) presentation (H110), in which tree, otolith and coral chronologies were

successfully used to predict serious negative impacts with increasing strength and frequency of ENSO events (H:110). Sara Pace (University of Southern Mississippi), a student of Roger Mann (Virginia Institute of Marine Science, also present), highlighted the regular recruitment of *A. islandica* into an important commercial fishery, following increased recruitment between 110 and 160 years ago (H:398).

Growth increments in gastropod molluscs

Fishery scientists have struggled in recent years to assess whelk stocks due to the inability to measure key components of the population dynamics, such as natural mortality and growth rate. Two of our contributions addressed the commercially important whelk *Buccinum undatum*. Vladimir Laptikhovsky (CEFAS, UK) showed how trawled whelk shells, often being occupied by hermit crabs, can be used to retro-actively estimate the rate of natural mortality (H:74). Phil Hollyman (Bangor University/CEFAS) described the development and validation of statoliths' use for age determination in *B. undatum* (H:277). This work combined several field, laboratory and geochemical experiments to confirm an annual periodicity to visible growth rings within statoliths. The addition of natural mortality estimates and population age structures will allow fisheries scientists to successfully perform analytical stock assessment on this species for the first time.

Theme Session H Synopsis

Overall, Theme Session H provided a broad and rich cross section of current applications using age-registering biomineralised structures of marine organisms. In general terms, the breadth of contributions satisfied the ambition of our call, however we note that our portfolio was representative rather than fully exhaustive. As was anticipated, most contributions were restricted to studies of teleost fish, cephalopod and gastropod molluscs, therefore our call for a broader range of species and taxa did not materialise. However, we were successful in attracting papers addressing all of our principal topics, with clear examples of: new techniques to assess age or life-history events (e.g. H:128, H:207,H:277); ecological studies based on assessments of age or environmental signals (e.g. H:75, H474, H219); Insights into early life history (e.g. H:204, H:538, H:547); assessment of climate change effects on species or populations (e.g. H:104, H474, H:110) and; age studies of environmentally important species for which age data are poor or non-existent (e.g. H:146, H:377, H:584).

Our contributions demonstrated clear evidence of progress and innovation within the field, even in established areas. The blossoming of elemental and isotopic analyses in determining life-history events and population structure continues to grow, however there is often still a fundamental underlying lack of understanding of both sampling protocols and the underlying chemistry, as clearly identified in our final discussion session. A good example was the discussion of the results of Hüssy et al. (H:54). The requirement for an informed choice of elements in experimental protocols investigating biomineralised structures was a theme echoed by several key delegates. A discussion around the avoidance of 'scatter-gun' approaches for techniques such as elemental fingerprinting resulted from observations that many elements have already been discounted as being unsuitable for geochemical analyses, as established in long-standing, peer-reviewed literature. It was noted however, that

improved understanding of basic elemental incorporation and subsequent interpretation could only be attained through more fundamental experimental studies. We did see evidence of a wider uptake of elemental and isotopic data, specifically within fisheries management e.g. using chemical gradients in ageing where growth increments are unclear, but also with considerable potential to be implemented more broadly in marine environmental management e.g. by using otoliths as monitors of environmental quality. We suggest therefore that while the interpretation, validation, cross-referencing and application of biogeochemical markers continues to improve and proliferate, there is still some way to go before the full realisation of potential in this field.

The ageing of cephalopod and gastropod molluscs in particular has some way to go before reaching the same level of maturity as fish ageing studies. During the final discussion, Alexander Arkhipkin emphasised the ongoing requirement for cross-validation between different structures, particularly for gastropods, where reliance on any one structure may result in biased observations. Richard Schwarz then proposed that a dedicated, laboratory-based cephalopod age reading workshop was necessary. This idea was supported by other cephalopod scientists as it would provide a platform for specialists to co-process and read samples of statoliths, beaks and gladii to unify methodology and interpretation of growth rings, and to provide cross-validation of results. Marianne Robert further supported the idea of using shell age structure in thanatocoenoses to estimate natural mortalities of commercial shellfish.

Conclusion

Overall, our session provided a broadly comprehensive snapshot of state-of-the art in the wider application of new technologies to interpret scale, otolith, statolith and other biomineralised age-registering structures to improve the management of natural resources. We demonstrated innovation, but were we successful in demonstrating cross-fertilisation and wider uptake? We had some examples of cross-validation between techniques and/or structures, an open area where big advances can still be achieved relatively easily. We also observed some convergence between disciplines in our discussion sessions to better interpret existing results e.g. the poorly defined elemental-based increments in Baltic cod being reinterpreted using image analysis techniques. We also saw evidence of exciting early-stage research into analogous structures (eye-lenses) in both fishes and cephalopods, with clear synergies. Given that our respective communities rarely co-convene, we see this as a valuable starting point, and given the high turnout and the attendance of a very respectable number of top-level scientists, operational scientists and early-career scientists, and the mooting of a follow-on, broad-based cephalopod ageing workshop, we consider our Theme Session to have been a success.