7th INTERNATIONAL OTOLITH SYMPOSIUM

Viña del Mar, Chile
October 9-13, 2023

Book of Abstracts

7TH IOS

IFOP

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RETURN TO INDEX

Chile welcomes you!

On behalf of the Symposium organizers, it is a great pleasure for us to welcome you to Viña del Mar, Chile for the 7th International Otolith Symposium. This scientific event will provide a unique forum for students and scientists to present their new studies, discuss potential new applications, project future perspectives and expand their research networks. In this version, the 7th IOS will receive scientists and graduate students from over 40 countries all around the world.

We also welcome the presence of international experts who will offer keynote conferences and workshops to review and project disciplinary advances as well as address methodological issues on otolith research and other calcified structures.

We express our deep gratitude to all the participants, and we wish you a pleasant, fruitful and memorable stay in Viña del Mar.

Francisco Cerna Troncoso
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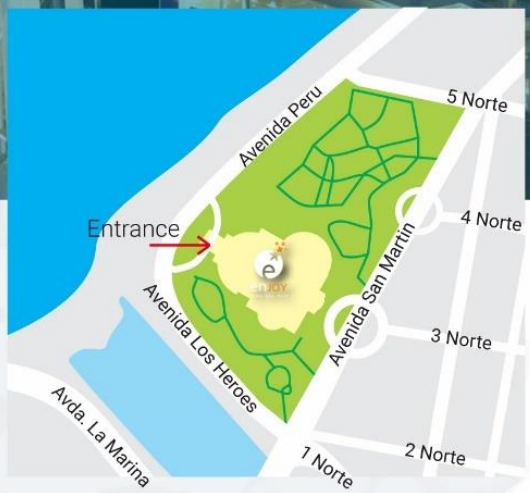
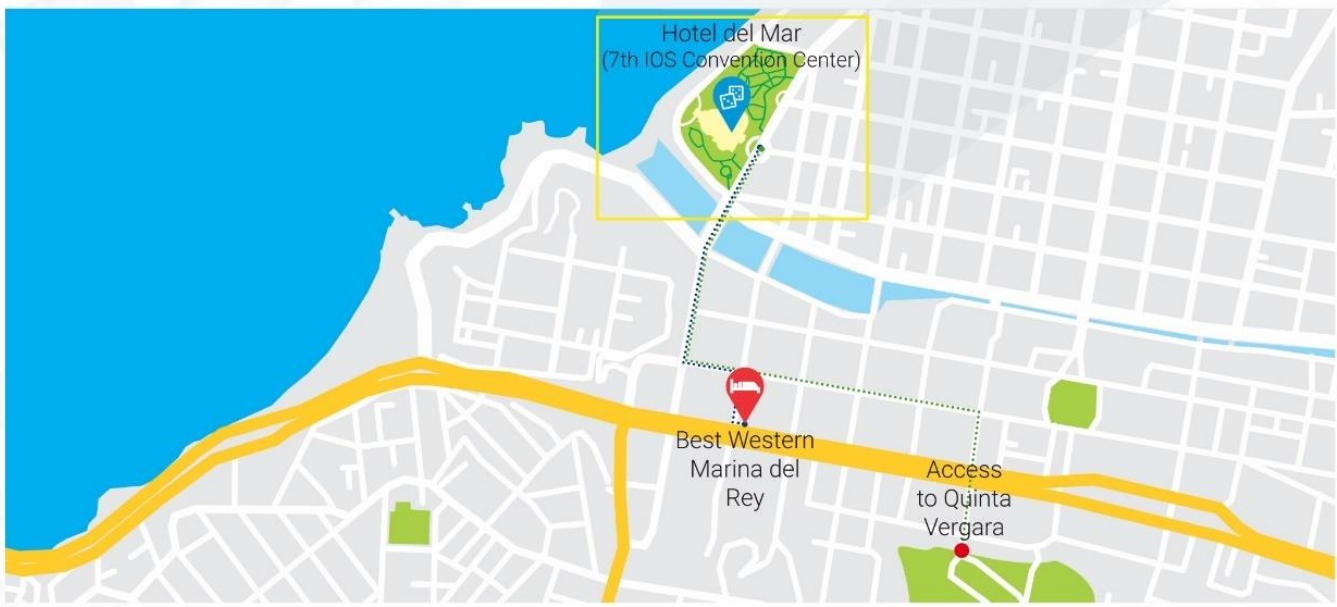
RETURN TO INDEX

Locations and maps

The 7th IOS is held at the Convention Center, located at Avenida Perú, Viña del Mar, Chile (Hotel del Mar).





Addresses

Hotel del Mar
7th IOS Convention Center
Avenida Perú
Viña del Mar

Best Western
Marina del Rey
Banquet hall
Ecuador 299 street
Viña del Mar

Access to
Quinta Vergara
Welcome reception
Errázuriz with
Quinta street
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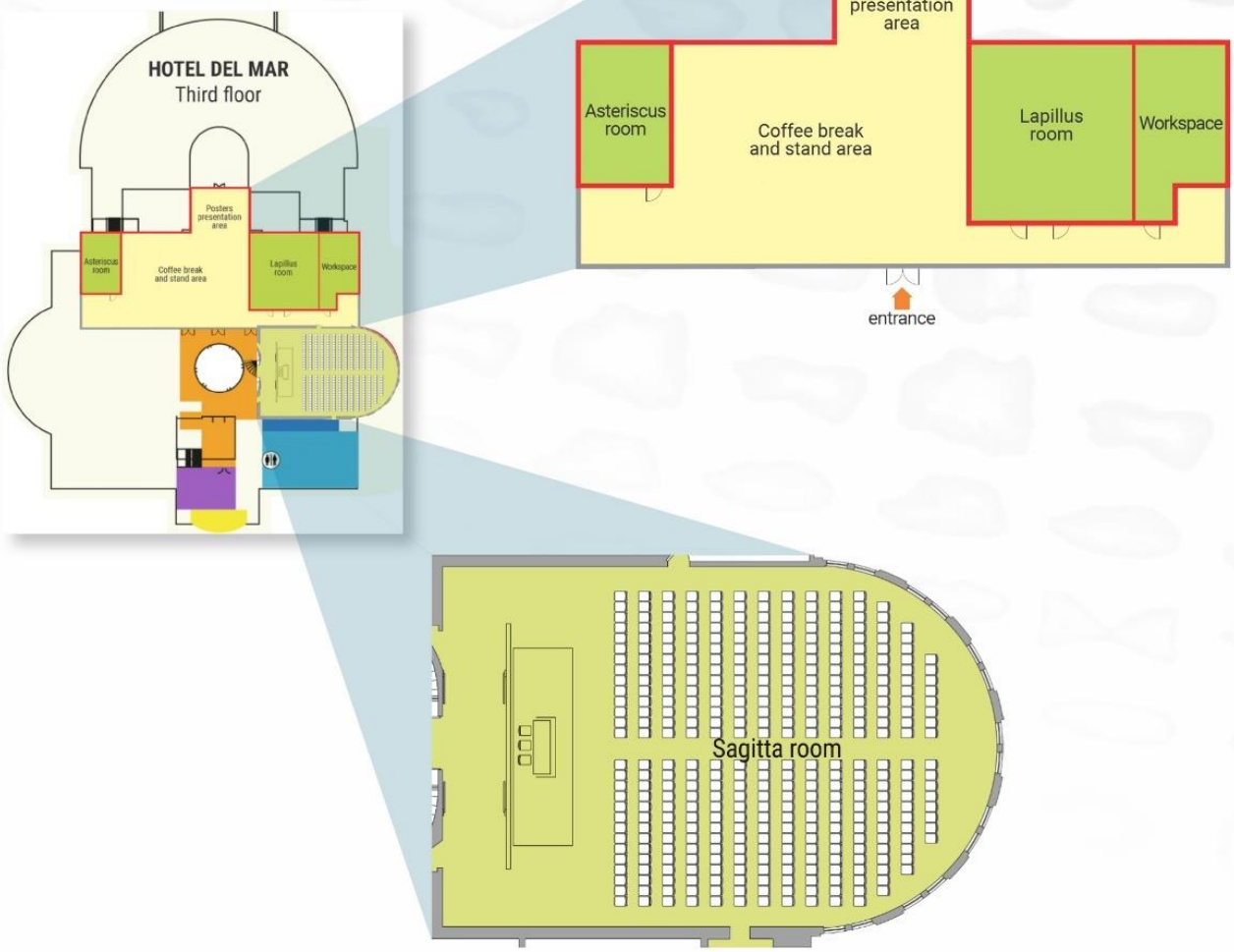
RETURN TO INDEX

Distribution of space
Symposium Meeting Rooms



RETURN TO INDEX

Index

Keynote Conferences

RETUR TO INDEX

Otoliths, metabolism and ecophysiology of marine fishes

Clive N. Trueman[1]
[1]School of Ocean and Earth Science, University of Southampton, UK. trueman@soton.ac.uk

Ocean temperatures are increasing at rates unprecedented in recorded history, and predicting how organisms and ecosystems will respond to global change is probably the most pressing problem faced by ecologists today. The amount of energy expended by an individual fish operating in response to the complex suite of conditions experienced in its natural environment defines both feeding rate and growth potential, whereas the sensitivity of a fishes' physiology to experienced temperature defines its resilience to fluctuating conditions. Metabolic rate varies among individuals within populations and among populations within a species range. Such variation offers potential for selection, adaptation and resilience to climate and environmental change. Metabolic rates also vary predictably with body size and temperature, and most bioenergetic ecosystem or production models are built around assumed metabolic scaling terms. Individual-level field metabolic rate is therefore a critical term to recover, particularly where energy expended can be understood in the context of experienced temperature combined and expressed growth. The otolith offers sclerochronological records of growth with chemical (isotopic) measures of experienced temperature and, as recently recognized, field metabolic rate. This combination of data is unmatched in the animal world, giving us opportunity to address fundamental questions in metabolic and eco-evolutionary theory as well as providing ecophysiological data that can assist predictions of species' response to climate warming.

In this talk I will first outline the physiological mechanism underpinning the stable isotope proxy for field metabolic rate, emphasizing links between respiration, carbonate ion regulation and biomineralization. The ease and relatively low cost of otolith isotope analyses allows collection of ecophysiological datasets at scales rarely possible from alternative approaches. Our current dataset contains estimates of field metabolic rate and experienced temperature in >7500 individual marine teleosts from c.120 species. Our dataset covers 5 orders of magnitude body mass and a temperature range of >30 degrees, including fishes from polar, deep water, and mesopelagic habitats, temperate coastal reefs and tropical pelagic settings. I will draw on this dataset to explore how otolith-derived estimates of field metabolic rate can be used to address ecophysiological questions at scales ranging from macroecological to population and individual levels. At the macroecological level I will show that mass and temperature scaling of field metabolic rate depart from

RETURN TO INDEX

assumptions commonly employed in biophysical models. At species and population levels, I will show how otolith isotope data can be used to construct thermal performance curves for field metabolic rate, identifying thermally limiting temperatures, and the thermal sensitivity of metabolism (and therefore feeding rate). Using a case study from Atlantic bluefin tuna, I will show how such data can be used to predict contrasting futures for fish populations under differing climate and emissions scenarios. I will also present case studies where populations across species' ranges show both genetic metabolic adaptations and phenotypic plasticity, complicating efforts to predict responses to climate change based on species-level physiological data. I will also speculate how community level ecophysiological data may help to predict effects of warming on food web structure and function.

I hope to show that the otolith carbon isotope proxy for field metabolic rate is an extremely exciting chapter in otolith science with applications that are urgently required at a time when ocean temperatures are changing at alarming rates. However, many uncertainties remain to be rigorously tested.

RETURN TO INDEX

From folklore to fisheries and ecosystem-based management – The use of otoliths over the ages

Bronwyn M. Gillanders[1]
[1]School of Biological Sciences, University of Adelaide, SA 5005, Australia

Otoliths were first mentioned in classical literature, where they were believed to provide cures for specific human illnesses and to hold magical powers. Even today, otoliths are still used by some cultures in medicine and as spiritual talisman. Archaeological evidence supports the historical use of fish in indigenous diets based on otolith and bone samples. Not only can the species of fish being targeted be determined, but also fishing strategies, including techniques of fish capture, time of year fish were caught, and where fishing occurred in relation to human settlements. Archaeological otoliths also provide evidence of shifting baselines including how the environment has changed through time along with changes in behaviour and spatial distribution of fish.

In fisheries science, otolith research initially focused on annual age and growth studies, but the proportion of such studies has declined through time as a more diverse range of otolith applications have been investigated. Otolith shape and chemistry are now widely used to investigate population (stock) structure. Expanding the range of otolith applications was aided by significant technological and analytical development that allowed analysis of trace and isotope ratios at increasingly finer temporal resolutions within otoliths. In addition, statistical techniques to analyse large datasets have also evolved. Historically scales were the focus for age and growth studies. Otolith studies dominated fishery sciences in the 20th century, but today a much broader range of analogous archival tissues including eye lenses, vertebrae or opercula are gaining traction, expanding the range of organisms that are investigated beyond just fish. With the increase in not just species information but also environmental information and the broader range of organisms being investigated, the use of such data in ecosystem-based assessments has flourished.

Applications related to biogeochemistry have been diverse especially where the growth information within structures has been incorporated. Many fisheries studies have investigated population structure by analysing trace elements or stable isotopes in otoliths but studies of connectivity and lifetime movements have also revealed an incredible diversity in fish life history and migration strategies, including within populations.

RETURN TO INDEX

Analogous to studies of population structure chemical composition in archival tissues can support investigations of food provenance to determine the harvest location of seafood. Similarly, product authenticity including whether samples are wild versus farmed has been ascertained. Further, stock enhancement, either to help rebuild depleted populations or to provide recreational fishing opportunities, often relies on distinguishing wild fish from hatchery reared fish. In such cases, chemical fingerprints in otoliths are increasingly used to trace whether fish were reared and released into the wild or whether they were natural recruits, thereby enabling the relative contribution of the two groups to be determined and the performance of stock enhancement programs to be ascertained. Linked to growth variations and metabolic changes, the chemical information within otoliths has also been used to assess environmental stress and ecophysiology, often through the lifetime of a fish. Finally, research reconstructing reproductive history and dietary shifts is gaining momentum with potential applications looking into reproductive events and quantifying past food webs.

In this presentation, I will initially focus on the classical literature from Aristotle's time leading into pharmaceutical and other uses of otoliths today. Use of archaeological and modern otoliths will be used to provide perspectives on indigenous peoples, along with fisheries and ecosystem-based management applications, from classical age and growth estimates to understanding how environmental change drives variations in migratory strategies. Finally, a brief perspective on potential future directions will be provided.

RETURN TO INDEX

From individuals to assemblages: using otoliths to understand the ecological impacts of rapid change in aquatic environments

John R. Morrongiello[1]
[1]School of BioSciences, University of Melbourne, VIC 3010, Australia, john.morrongiello@unimelb.edu.au

The world's aquatic environments are experiencing unprecedented rates of environmental change. Rapid warming is having significant direct and indirect impacts on individual fish, which in turn has become manifest at the population and assemblage level. For example, warming-induced shifts in metabolic rate can affect a fish's energy budget while changes in the abundance and composition of the broader assemblage affect the strength and direction of interspecific interactions. Together, these can affect a fish's growth rate and thus the size it attains at critical life history junctures. Likewise, warming can impact on the reproductive phenology of populations and the growing conditions experienced by larvae, which in turn can cause increased variation in recruitment success. Zooming out to the assemblage level, warming is driving shifts in species' distributions and the formation of novel assemblages, with new interspecific interactions.

It is important to remember that rapid warming is occurring against a backdrop of other pervasive anthropogenic impacts that themselves are impacting on individuals, populations and assemblages. River regulation and water abstraction are having acute and chronic impact on the ecology of freshwater and estuarine environments through the impediment of energy flow, degradation of habitat, proliferation of invasive species, and misalignment or cancelling of phenological cues. And in the marine realm, extensive commercial and, more recently, recreational fishing harvest is elevating mortality rates, reducing biomass and imposing sometimes intense phenotypic selection. All these fishing impacts affect the size and growth of fish, how they recruit and how fish interact with each other. Given some of the similarities in how warming and other anthropogenic stressors affect fish, it is plausible that there will be significant synergistic impacts on fish growth and recruitment with flow-on implications for fisheries productivity.

Teasing out the relative importance of warming and other stressors on the ecology of fishes and the fisheries they sustain can be difficult. It requires significant amount of data which is often absent in our hard-to-observe aquatic environments. Otoliths provide fish researchers with an enviable source of biological data. Whilst we have long taken advantage of the periodically formed increments within otoliths to provide an

RETURN TO INDEX

estimate of fish age, it is only in more recent decades that we have begun to dedicate significant time to interpreting the growth information that these increments represent. Otolith-based biochronologies have provided unprecedented capacity to investigate biological patterns at annual to centennial time scales, in lakes and on reefs to across large marine ecosystems.

In this presentation, I will explore how we can use the biological information naturally archived in otoliths to quantify the impacts of climate-induced warming, anthropogenic stressors and natural environmental variation on individual fish, their populations, and the broader assemblages they comprise. I will focus on growth rate and recruitment dynamics as biological responses to change and discuss some of the various statistical models that can be used to further the eco-evolutionary insight provided by otolith data. I will finish by exploring future opportunities for the use of otolith-derived growth and age information, both in terms of furthering our ecological and evolutionary understanding of aquatic systems, and in the management of our marine and freshwater resources.

RETURN TO INDEX

Element Chronicles: Decoding otolith chemistry - what's biomineralization got to do with this?

Karin Hüssy[1]
[1]National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet, 2820 Kgs. Lyngby, Denmark. kh@aqua.dtu.dk

Since the early 1990s, otolith chemistry has gained increasing attention, resulting in a steady rise in annual publications to a notable 100 in the year 2021. These studies have predominantly revolved on elucidating various facets of fish biology and ecology, focusing on questions addressing stock dynamics and environmental reconstruction such as migration patterns, hypoxia and pollution exposure, and connectivity between habitats. At the core of these studies lies an implicit presumption that specific elemental concentrations within the otolith reflect environmental concentrations in a consistent and predictable manner. By assuming such a relationship, the underlying mechanisms of element uptake from the water to their incorporation into the growing otolith are often ignored. When observed chemical patterns within otoliths deviate from this paradigm and are hard to explain, many studies refer to the biomineralization process – the complex interaction between CaCO3 and organic compounds that ultimately result in otolith growth and visual appearance we know so well – as the major "suspect". In this talk, I aim to first summarize the knowledge on otolith composition and biophysical drivers of biomineralization, then present hypotheses on how biomineralization should affect element incorporation, and test the validity thereof with selected case studies. Lastly, I will highlight some of the gaps in our understanding of otolith growth and chemical composition.

I will focus on elements that are generally used for environmental reconstruction: Strontium (Sr), barium (Ba), and manganese (Mn). These elements substitute for Ca during crystal growth or that occur randomly trapped within the crystal lattice. I will also focus on elements under physiological and therefore often neglected: Magnesium (Mg), phosphorus (P) and zinc (Zn). These elements occur primarily protein-bound in the otolith's organic matrix or as co-factor in enzymes important for otolith formation. Uptake of elements predominantly under environmental control should reflect environmental concentrations, without additional effects of salinity. Conversely, uptake of elements under physiological regulation should reflect feeding rate and/or growth, decrease with fish age, and exhibit minimal influence of environmental concentration. For each element I will provide empirical evidence substantiating the validity of these hypotheses using case studies that support – and challenge – these respective hypotheses. These case studies will encompass both spatial and temporal aspects of otolith

RETURN TO INDEX

elemental patterns, underscoring that biomineralization undeniably exerts a modulating influence on the uptake of elements, particularly those related to somatic growth, and conceivably, those substituting for calcium within the crystalline structure.

Interpretation of the ecological significance of patterns from field samples therefore needs to consider the impact of the underlying biomineralization processes of the element in question as well as the physiological mechanisms regulating the availability of ions for inclusion in the growing crystal lattice. I will end the talk by highlighting our major gaps of knowledge and hope to inspire you to challenge the established paradigms in otolith chemistry research through dedicated investigations that target these knowledge gaps.

RETURN TO INDEX

From ghost stories to success stories: the use of otolith chemistry in conservation and management

Anna M. Sturrock[1]
[1]School of Life Sciences, University of Essex, Wivenhoe Park, Colchester UK. anna.sturrock@essex.ac.uk

We are in a period of unprecedented global change, facing rapid loss of habitats and species. Extinction rates are particularly high for migratory species given their complex habitat needs and exposure to broad suites of stressors. But it is the loss of intraspecific diversity, the so-called 'hidden biodiversity crisis' (Des Roches et al. 2021), that represents the aquatic canary in the coalmine, where the truncation of genetic and phenotypic (e.g. size, growth, migration timing) variation within species reduces their ability to persist in an increasingly volatile climate. Here, I showcase how otoliths and other archival tissues provide powerful, but underutilized tools to support fisheries, water and habitat management.

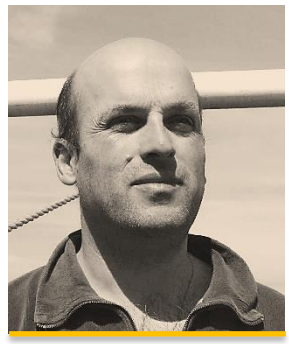
Firstly, I will focus on new approaches to identify the critical habitats supporting endangered and commercial fishes. Policymakers are calling out for science to inform spatial management of marine and freshwater resources, for example identifying areas or habitat types in greatest need of protection for the '30 by 30' global initiative. While adult fish usually receive the most attention because they are harvested and large enough to tag, it is the early life stages that suffer the highest losses and thus offer the greatest benefits from performing targeted habitation protection. Archival tissues offer significant advantages over other tissues and tools by recording the environment, diet and growth of even the earliest life stages, providing unique datasets to test nursery-role hypotheses. Through a series of case studies, I show how otolith and eye lens biochronologies and chemical tracers can allow us to reconstruct individual movement pathways and identify the nursery areas contributing disproportionately to endangered and commercially valuable fishes, both in terms of numbers and biomass. Even when specific localities cannot be resolved, I discuss how otolith chemistry can provide fisheries-relevant information about the phenology of movements and life history events, stock delineations and mixing patterns.

Secondly, I will focus on "the ones that didn't make it", AKA "the ghosts". In fish research, we are rarely sent a body bag to investigate the most likely cause of death. Instead, our sampling efforts are inherently biased towards the survivors. Here, I will explore the use of archival tissues for estimating carryover effects of stressors such as pollutants and hypoxia, as well as understanding patterns in selective mortality. By combining otolith chemistry and strategic sampling across time and space, I demonstrate

RETURN TO INDEX

how we can identify hotspots and drivers of mortality, allowing managers to direct resources more efficiently to promote fish survival, such as through targeted habitat restoration and remediation. Throughout the talk I reference case studies from marine and freshwater systems where otoliths have been pivotal in guiding management and conservation, but I also ask why otolith chemistry is not more widely used in applied sectors, highlighting some of the ongoing knowledge gaps and limitations. Finally, I discuss exciting future directions in otolith research, and champion the use of storytelling and community to stimulate innovation and growth.

RETURN TO INDEX



Swimming across marine isoscapes around South America: new tales about some old coastal fishes

Edwin Niklitschek[1]
[1]Universidad de Los Lagos, Centro i~mar

Stable isotope analyses of δ18O and δ13C values in otoliths have provided valuable insights into the ecology of bony fishes for over 50 years worldwide and over 25 years in South America and Antarctica. These studies have tended to be monospecific, mainly focused on identifying demographic units ("stocks") and, secondarily, on studying migrations and life cycles in coastal species. In this keynote, I briefly summarise this work and present a meta-analysis of otolith isotopic values collected during the last 20 years from nine coastal species distributed around South America: Patagonian sprat, Araucanian herring, Pacific anchovy, Southern hake, Patagonian grenadier, southern blue whiting, Chilean seabass, Southern rays bream and Patagonian blenny. Combining these values with regional oceanographic data and interpolated seawater isoscapes, I attempt to fill further some critical knowledge gaps about these species' ecology and life cycles. Overall, available data supported the assumption that the aragonite fractionation rate of oxygen isotopes in fish otoliths is rather conservative within and between species. Such an assumption implies a close relationship between temperature, salinity and δ18O values, both in otoliths and water, which sustains the use of this isotope for geolocation and the comparative analysis of nursery habitats between species. In some species, however, such applications become limited by the confounding effects of temperature, which follows a latitudinal gradient along the oceanic coast, and salinity, which shows a meridional gradient across the Patagonian Fjords System. Following theoretical expectations, δ13C values showed higher variability between species even within habitat types, suggesting a much looser relationship between otolith and water δ13C values. Although trophic position accounted for some of this variability, most remained unexplained. Given such limitations, understanding the structure, habitat use, connectivity, and movement of these fishes required combining stable isotope analysis with several other sources of information, such as the elemental and microstructural analyses of their otoliths.

This integrative analysis allowed for identifying a diversity of life cycles, particularly within groundfish species, defined by distinct nursery and feeding habitat use patterns, all of which included some intensive use of the Patagonian Fjords Systems. I also made evident the existence of three main nursery hotspots along the west coast of South America, which seemed essential for six of the nine species investigated. Two of these essential habitats are located within the Patagonian Fjords System, which

RETURN TO INDEX

also serves as a major feeding area for juveniles and adults of several species. The demographic contribution of resident self-recruited subpopulations inhabiting this system appeared irrelevant, on the other hand, for some other species like anchovy, pacific sardine and Patagonian grenadier. Nonetheless, the actual contribution of these secondary life cycles to the resilience of such species to natural and anthropically induced catastrophic events remains to be investigated.

In summary, despite its incipient development in South America, otolith science and, particularly, stable isotope analysis have helped identify potentially essential nursery and juvenile feeding areas along the Chilean coast, which deserve further investigation and precautionary protection. These tasks may be critical considering the large variability of natural events such as the ENSO and the increasing magnitude of anthropic-related pressures such as global warming and coastal pollution.

RETURN TO INDEX

THEME I

KEY ASPECTS IN OTOLITH AND CALCIFIED TISSUES FORMATION AND COMPOSITION

RETURN TO INDEX

IOS_021

A rapid technique for otolith removal *from* dried seahorses *Hippocampus ingens*

Sonia Valle[1]*, Analucía Alarco[1]
[1] School of Marine Biology, Universidad Científica del Sur, Lima, Perú;
*svalle@cientifica.edu.pe

Hippocampus ingens, "Pacific seahorse" is currently listed as "vulnerable" on the IUCN Red List due to an estimated population decline as a result of continued fishing pressure and illegal trade. The dried "seahorses" seized from illegal trade are useful material for scientific studies, such as determination of sizes, age ranges, sex, etc. Syngnathid otoliths are very small relative to the size of the fish and are therefore very difficult to extract for studies such as growth line aging, microchemistry or other applications. The goal of this study was to develop a methodology to identify the three pairs of otoliths (*sagitta, asteriscus* and *lapillus*) and extract them from dried individuals of *H. ingens*, from an illegal fishing seizure. We worked with adult individuals of *H. ingens* with an average size of 120 mm TL. We tested a fast and simple method for, first: the detection of the three pairs of otoliths *in situ* from the internal space of the skull (the vestibular apparatus is not detectable in this desiccated state); second: to proceed to the cleaning of each one of the otoliths and, third: the extraction of the three pairs of otoliths and their manipulation for microscopic analysis. The otoliths extracted with this procedure were easily transferred using micro spatulas to excavated glass slides for light microscopy analysis. It was found that the three pairs of otoliths did not differ much from each other in shape (circular). The size (length) of the sagitta is somewhat larger (average 428 µm) than the lapillus (average 408 µm), while the asterisk is slightly smaller (average 308 µm).

Keywords: *Hippocampus ingens*, illegal fishing seizure, Perú

RETURN TO INDEX

IOS_028

The forgotten element: why do we ignore calcium?

Karin E. Limburg[1,2*], Yvette Heimbrand[2], Karin Hüssy[3], Martina Blass[2], Jay Thomas[4], Tomas Naeraa[5].
[1]State University of New York College of Environmental Science and Forestry, Syracuse, NY USA; [2]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden; [3]National Institute of Aquatic Resources, Technical University of Denmark, Kongens Lyngby, Denmark; [4]Earth Sciences, Syracuse University, Syracuse, NY USA; [5]Dept. of Geology, Lund University, Lund, Sweden. *klimburg@esf.edu

Typical analyses of otolith or other carbonate microchemistry use calcium, a major constituent, as an internal standard, setting its value as a constant and ignoring any potential variations. In fact, patterns do occur in Ca deposition, as can be observed either by repeating the analysis, by creating two-dimensional maps of Ca, or both. Here we present evidence of Ca variations in fish otoliths from analyses using synchrotron-based scanning X-ray fluorescence microscopy, electron microprobe analysis, and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). 2-D maps of otoliths created with LA-ICPMS indicate that Ca is elevated where Mg and especially P are low, and vice versa, suggesting that spatial variations in protein deposition may affect concentrations of Ca. We encourage others to examine Ca concentrations in their biomineralized samples to check for variations.

Keywords: Otolith chemistry, calcium variation

RETURN TO INDEX

IOS_ 041

Where is the first «winter» ring? Validating otolith ring formation of juvenile European plaice *Pleuronectes platessa*

Uwe Krumme[1*]
[1]Thünen Institute of Baltic Sea Fisheries, Rostock, Germany. *uwe.krumme@thuenen.de

International age reading guides for plaice refer to the identification of «winter» rings to harmonize the age determination between different institutes but the timing and age of the first increment formation is unvalidated. Validation is crucial to improve the age data of European plaice (*Pleuronects platessa*) used in stock assessments. To better understand patterns in ring formation, length-frequencies and otoliths of juvenile plaice were collected monthly with a scoop net in 2020 from beaches near Rostock, Germany (western Baltic Sea). The length-frequency information was combined with visual categorization (eight otolith types) and ring diameter measurements of whole otoliths to observe the development of the translucent zone (TZ) and opaque zone in otoliths of age-0 and age-1 fish. In addition, recaptures of juvenile plaice bathed in tetracycline and individually marked with liquid latex that had been released in a coastal lake, were used to verify the other approaches. Age-0 fish settled with 2-3 cm from May-July and attained a length range of 4-11 cm in December. Unlike previous assumptions, age-0 fish formed 3 zones until the end of their first year: an opaque core (prior to settlement), a TZ (during warmer water temperatures and faster growth until late summer), and an opaque zone from autumn/winter into spring of the following year (reduced growth). Age-1 fish had two zone changes: the opaque zone of the previous year until late spring, a TZ during the second summer and another opaque zone was again formed from autumn/winter until spring of the following year (age-2). Hence, juvenile plaice apparently form the TZ during summer when they experience best conditions for growth. A mean horizontal core diameter of about 630 µm can help in identifying the first TZ in sectioned otoliths (when the core region is properly sliced) and in whole otoliths (when the surroundings of the core are overgrown by opaque material). Recaptures of chemically marked juvenile plaice confirmed the above described patterns in ring formation and suggest that the widely used «winter» ring terminology is inappropriate to age-read plaice.

Keywords: age validation, *Pleuronectes platessa*, tetracycline, otoliths, length-frequency distribution

RETURN TO INDEX

IOS_042

Age validation of juvenile European flounder *Platichthys flesus*

Uwe Krumme[1]*
[1]Thünen Institute of Baltic Sea Fisheries, Rostock, Germany. *uwe.krumme@thuenen.de

The stock of European flounder in the southern Baltic Sea is the largest commercially used flounder stock in Europe with annual landings >10.000 t, but ageing is uncertain, the age is not validated and there is no age-based assessment. To validate the timing and age of the first increment formation, length-frequencies and otoliths of juvenile flounder were collected monthly with a scoop net in 2020/21 from beaches near Rostock, Germany. The length-frequency information was combined with visual categorization (eight otolith types) and ring diameter measurements of whole otoliths to observe the development of the translucent zone (TZ) and opaque zone in otoliths of age-0 and age-1 fish. In addition, recaptures of juvenile flounder bathed in tetracycline and individually marked with liquid latex that had been released in a coastal lake, were used to verify the other approaches. Age-0 fish settled with 2-3 cm from April-June and attained a length range of 4-10 cm in December. Unlike previous assumptions, age-0 fish formed 3 zones until the end of their first year: an opaque core (prior to settlement), a TZ (during warmer water temperatures and faster growth until late summer), and an opaque zone from autumn/winter into spring of the following year (reduced growth). Age-1 fish had two zone changes: the opaque zone of the previous year until late spring, a TZ during the second summer and another opaque zone was again formed from autumn/winter until spring of the following year (age-2). Hence, juvenile flounder apparently form the TZ during summer when they experience best conditions for growth. A mean horizontal core diameter of about 620 µm can help in identifying the first TZ in sectioned otoliths (when the core region is properly sliced) and in whole otoliths (when the surroundings of the core are overgrown by opaque material). Recaptures of chemically marked juvenile flounder confirmed the above described patterns in otolith ring formation and suggest that the TZ, formed mainly during quarter 3, is a useful reference for determining the first increments in flounder.

Keywords: age validation, *Pleuronectes platessa*, tetracycline, otoliths, length-frequency distribution

RETURN TO INDEX

IOS_053

Limited evidence for species-specific influence on temperature-dependent sensitivity of oxygen stable isotope fractionation in biominerals: a meta-analysis

Olivier Morissette[1*], Clive Trueman[2], Anna Sturrock[3], Audrey Geffen[4] and Kotaro Shirai[5]
[1]Département des sciences fondamentales, Université du Québec à Chicoutimi, Canada; [2]School of Ocean and Earth Science, University of Southampton, UK; [3]School of Life Sciences, University of Essex, UK, [4]Department of Biological Sciences, University of Bergen, Norway, [5]Atmosphere and Ocean Research Institute, The University of Tokyo, Japan. *olivier_morissette@uqac.ca

Water temperature is key to the study of aquatic ectotherm ecology, but precise measurements of individual-based thermal experience remain difficult to acquire. The stable isotope composition of oxygen in biominerals acts as a natural thermometer due to the temperature dependence of isotopic fractionation between water and mineral phases. Coefficients of published temperature-dependent fractionation equations, however, vary among taxa without apparent systematic predictors, implying that species-specific experimental validation may be needed before inferring temperature from biomineral oxygen isotope thermometry. Here we describe a meta-analysis conducted to assess the influence of biological and experimental sources of variation on the coefficients of published isotope thermometry equations. We observed that the thermal sensitivity (equation slope) was insensitive to any biological or experimental factors, while the isotopic spacing between water and biomineral (equation intercept) showed systematic variation. Experimental conditions and phylogeny were the two main sources of variation in equation coefficients, where experiment approaches influenced both equation intercepts and the fit of the linear regression. Our results suggest that the use of common equation slopes and generalized taxa-specific equation intercepts may be appropriate under some circumstances. We additionally suggest that processes related to oxygen balance and osmoregulation may influence equation intercepts, and suggest further experimental work in this area. Finally, our observations provide ground for improvement for future design and reporting of biomineral thermometry experiments.

Keywords: thermal habitat, oxygen stable isotopes, thermometry,

RETURN TO INDEX

IOS_058

4D tomography sheds light on how shape variation leads to differences in otolith motion patterns in three catfish species

Isabelle P. Maiditsch[1]*, Christian M. Schlepütz[1], Qianwei Qu[1], Friedrich Ladich [2], Martin Heß[3], Tanja Schulz-Mirbach[3]
[1]Swiss Light Source, Paul Scherrer Institute, 5232 Villigen, Switzerland; [2]Department of Behavioral and Cognitive Biology, University of Vienna, Vienna Austria; [3]Faculty of Biology, Zoology, Ludwig-Maximilians-University, Planegg-Martinsried, Germany. *isabelle.maiditsch@psi.ch

The interspecific diversity of the morphology of saccular otoliths in teleosts is tremendous. Otophysan fishes display an extraordinary sagitta shape which, in general, is delicate needle-shaped. Some catfishes (Loricariidae, Callichtyidae), however, show deviations from this sagitta shape in possessing a more compact or even angular-shaped morphology. In a former study on goldfish, using (2D + time) radiography, we confirmed the hypothesis that sagittae with an elongateshape show a rotational motion pattern when fish were subjected to pure tones of 200Hz. Based on this former study, we developed and further improved an experimental miniature standing-wave tube-like setup, in order to investigate otolith motion *in-situ* when fishes are exposed to a certain sound field. We used high temporal resolution dynamic 4D tomography to capture these motions in "realtime" at the TOMCAT beamline of the Swiss Light Source. In the current study, we approached the question of how the needle-shaped versus the angular-shaped sagitta morphology results in differences of the motion patterns when subjecting fishes to frequencies of 450-1500 Hz. We chose three catfish species due to differences in the shape of their saccular otoliths; *Kryptopterus vitreolus* (Siluridae) possess the "typical" otophysan sagitta, which is elongate, whereas the one in *Ancistrus dolichopterus* (Loricariidae) is broader than in the former species. In contrast, the sagitta of *Corydoras pygmaeus* (Callichthyidae) is no longer elongate but thick angular-shaped. Preliminary outcomes indicate a complex tilting motion in the sagittae of *Corydoras*, which differs, for example, distinctly from the rotational motion observed for the sagittae of *Kryptopterus*. Further experiments and quantification are in progress and will provide insights into the potential correlation of these different otolith motion patterns, regarding the frequency, the amplitude, and the morphology of the swimbladder and the Weberian ossicles. In all, our study will help to increase knowledge on the role of otoliths in the function of fish inner ears.

Keywords: otophysan fishes, auditory structures, sagitta, motion patterns, synchrotron imaging

RETURN TO INDEX

IOS_059

Can marine multi-stressors make you deaf?

Isabelle P. Maiditsch*[1], Karin E. Limburg[2,3],Tanja Schulz-Mirbach[4]
[1]Swiss Light Source, Paul Scherrer Institute, 5232 Villigen, Switzerland; [2]State University of New York College of Environmental Science & Forestry, Syracuse, NY USA; [3]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden; [4]Department Biology II, Zoology, Ludwig-Maximilians-University, Planegg-Martinsried, Germany.
*isabelle.maiditsch@psi.ch

The Baltic Sea is the world's largest anthropogenic "dead zone," with large areas of low oxygen water that is also warming and increasingly acidic. Cod (*Gadus morhua*) in the Baltic are very stressed, suffering from reduced growth, condition, and infestations by parasites. Recently, cod otoliths have become smoother than during periods of normoxia when fish grew well, losing much of the sculpturing typical of healthy cod otoliths. Does this smoothing affect hearing? To approach this question, we applied a new experimental procedure using dynamic 4D tomography at the TOMCAT beamline of the Swiss Light Source. A miniature standing-wave tube-like setup, developed in a former study, allows us to subject isolated otoliths to pure tones, while capturing motion patterns in "real-time" with 4D tomography. A single otolith is placed within a piece of porous foam and put into the center of the water-filled tube. We will test different frequencies, which ideally cover the range of hearing sensitivity in *G. morhua*. In order to transfer sound to the water body, we will use two miniature inertial shakers, one mounted at each end of the tube. When driving the two shakers 180° out-of-phase, a maximum sound-induced particle motion is created in the center of the tube, where the otolith sample is held in place. Subjecting the otolith to the sound field created by the shakers while performing tomographic imaging with high spatial and temporal resolutions, we compare the 3D motion of the saccular otoliths from fish originating from normoxic habitats (sculpted otolith shape) with those from hypoxic waters (smooth outline). Our experiments are currently in progress and will show if potential differences in the motion patterns due to subtle differences in the otolith outline can be resolved using our approach. If this attempt is successful, this could be an efficient tool to test the implications of ecologically driven modifications of otolith morphology on inner ear function.

Keywords: Otoliths, *Gadus morhua*, sound detection, motion patterns, synchrotron imaging

RETURN TO INDEX

IOS_063

Hatchery-reared Coho Salmon develop less vateritic otoliths in tanks with alternating water flow direction

Leigh Gaffney*[1], Micah Quindazzi[1], Emma Polard[1], Lydia Walton[1], Zoe Molder[1], Francis Juanes[1]
[1]Department of Biology, University of Victoria, Victoria, British Columbia, Canada *lgaffney@uvic.ca

Sagittal otoliths enable salmon to hear and maintain balance. Normally, the sagittal otoliths of salmon are composed of aragonite, a polymorph of calcium carbonate; however, otoliths with inclusions of vaterite, an abnormal polymorph, also occur. Vaterite formation is irreversible and results in deformed otoliths which are larger, lighter, and more brittle. These differences reduce otolith function and cause severe hearing impairment. While vateritic otoliths are rare in wild salmon (< 10%), they are extremely common in hatchery-reared salmon (affecting between 50-80%). Despite long-standing evidence of the occurrence of vateritic otoliths, the cause(s) of vaterite formation in hatchery-reared salmon are largely unknown. One factor that differs between hatchery and wild environments is water flow direction; a factor that influences salmon behaviour and growth. Hatchery salmon are most often reared in round tanks with constant, unchanging water flow directions but there is no scientific evidence to support this choice. The aim of this study was to assess whether changes in tank water flow direction influence vateritic otolith formation in hatchery-reared Coho Salmon (*Oncorhynchus kisutch*), one of the most reared species of salmon. Coho fry (n= 48,000 fish; 0.5g ± 0.1g) were distributed into 6 circular tanks and reared for 52 weeks under standard hatchery conditions of constant water flow direction (2 tanks = clockwise only; 2 tanks = counter-clockwise only) or alternating water flow direction (2 tanks = alternated from clockwise to counter-clockwise every 7 days) until release to the wild as smolts (20g ± 4.5g). Coho Salmon (n=10 fish per tank) were euthanized every month for routine health inspections and otoliths were extracted, photographed, and analyzed for vaterite. We found that vaterite prevalence was significantly reduced (P<0.001) in smolts reared in alternating water flow direction in comparison to smolts reared in constant water flow direction. These results provide the first evidence that alternating water flow direction decreases vateritic otolith formation in hatchery-reared salmon and offer a scientific basis for their recommendation. This research has important implications for fish welfare and the success and efficacy of salmon restocking and conservation programs.

Keywords: Otolith, Hatchery Salmon, Water Flow.

RETURN TO INDEX

IOS_068

Compared analysis of 2D and 3D otolith shape for mainly commercial species (flatfish and roundfish) in the Eastern Channel and the North Sea

Nicolas Andrialovanirina[*1, 2], Aurélie Mateos[3], Émilie Poisson Caillault[2], Sébastien Couette[4,5], Kirsteen MacKenzie[1], Rémi Laffont[5], Kélig Mahé[1]
[1]Ifremer, Fisheries laboratory, Channel and North Sea Fisheries Research Unit, Boulogne-sur-mer, France; [2]Université du Littoral Côte d'Opale, UR 4491, LISIC, Laboratoire d'Informatique Signal et Image de la Côte d'Opale, F-62100 Calais, France; [3]Université d'Artois, Unité BioEcoAgro UMR 1158, Arras, France; [4]Ecole Pratique des Hautes Etudes, PSL Université, Paris, France; [5]Biogéosciences, UMR 6282 CNRS, Université de Bourgogne, 21000 Dijon, France. *nicolas.Andrialovanirina@ifremer.fr

Otolith shape analysis is commonly used in fisheries sciences to validate the relationship with fish length, to discriminate between stock units of fish, and to identify fish species from archeological data or stomach contents. However, all otolith studies have used two-dimensional (2D) images, a partial representation of the whole shape of the otolith. This study presents a comparative analysis of 2D and three-dimensional (3D) otolith shape data for many commercial species (flatfish: *Lepidorhombus whiffiagonis, Scophthalmus maximus, Scophthalmus rhombus, Solea solea, Limanda limanda, Microstomus kitt, Platichthys flesus, Pleuronectes platessa* and roundfish: *Clupea harengus, Sardina pilchardus, Sprattus sprattus, Gadus morhua, Melanogrammus aeglefinus, Merlangius merlangus, Trisopterus luscus, Mullus surmuletus, Eutrigla gurnardus, Chelidonichthys cuculus, Chelidonichthys lucerna, Trachurus trachurus, Zeus faber*) in the Eastern English Channel and the North Sea. 2D otolith images were made with an optical scanner, and 3D otolith images with an X-ray microtomograph. For each species, several specimens were selected from the complete size range. Otolith shape was assessed by analyzing several descriptors from 2D and 3D images from the otolith size parameters (Length, Width, Perimeter, Area and Volume). The relationship between fish length and otolith shape parameters are not the same within species and/or among species for 2D versus 3D data. Moreover, the relationship between 2D otolith size parameters and 3D otolith parameters are different among species. These results demonstrate the importance of 3D otolith shape analysis. In the future, the number of studies using otolith shape analysis in three dimensions must be increased strongly to validate previous studies on 2D images.

Keywords: Otolith Shape, 2D, 3D, Volume, Eastern English Channel, North Sea

RETURN TO INDEX

IOS_077

3D Otoliths models by microphotogrammetry

Lizandro Muñoz*[1], Luis Cid[1]
[1]Sección Edad y Crecimiento, Instituto de Fomento Pesquero, Valparaíso, Chile. *lizandro.munoz@ifop.cl

The shape of the otolith has specific characteristics and morphometric studies have been carried out in different species, but on a two-dimensional scale based on images. The digital advances offer the possibility of working with virtual otoliths, this is possible by the incorporation of microphotogrammetry is a technique that allows obtaining three-dimensional otolith models from two-dimensional images. With the recording and interpretation of sequential photographic images of the same otolith obtained through the use of an image analysis equipment and an adjustable speed slicer, three-dimensional models of otoliths were composed. The objective of this work was to develop a workflow for three-dimensional digital reconstruction with tools and materials that can be replicated in other otolith laboratories. Otoliths of some of the main species of the Chilean commercial fishery were used, such as *Engraulis ringens, Genypterus blacodes, Merluccius australis, Micromesistius australis and Scomber japonicus*. ImagePro Premier, Agisoft PhotoScan and Colmap softwares were used for image processing. As a final result, three-dimensional models of otoliths were obtained by means of microphotogrammetry with the obtaining of several measurements both in two-dimensional and three-dimensional planes and that offer possibilities for further studies, mainly inter and intraspecific morphometric characterization.

Keywords: Otoliths, Microphotogrammetry, 3D models

RETURN TO INDEX

IOS_087

Prevalence of vateritic otoliths in hatchery-reared Coho Salmon varies with hatchery facility

Leigh Gaffney[1], Micah Quindazzi[1], Chloe Kraemer[1]*, Emma Polard[1], Lydia Walton[1], Francis Juanes[1]
[1]Department of Biology, University of Victoria, Victoria, British Columbia, Canada *chloekraemer1@gmail.com

Although Pacific salmonids are of cultural, ecological, and economic importance throughout their range, anthropogenic activities have led to widespread declines in natural populations. To mitigate declines, federal and regional organizations have undertaken large-scale hatchery-rearing of juvenile Pacific salmonids for over a century. Unfortunately, hatchery-reared Pacific salmonids have lower ocean survival in comparison to natural-origin Pacific salmonids, such that hatcheries have proven to be much less effective than initially hoped. The reason(s) for lower hatchery-reared survival rates remain poorly understood but may be due to differences between natural and artificial rearing environments. One substantial, well-documented, difference between hatchery-reared and natural-origin salmonids is the prevalence of abnormal (vateritic) sagittal otoliths. While vateritic sagittal otoliths occur sporadically in natural-origin salmonids (up to 10%); numerous studies report vaterite in 50-80% of sagittal otoliths from hatchery-reared salmonids. Given the importance of sagittal otoliths for the inner-ear function of salmonids, it is important to determine whether the degree of vateritic sagittal otoliths differs with hatchery facility. To date, no large-scale sampling has determined if vateritic sagittal otoliths are consistently more common in certain hatchery facilities, differ between rearing years, nor if the phenomenon is localized or widespread. The aim of our study was to quantify the prevalence of vateritic sagittal otoliths in hatchery-reared Coho Salmon (*Oncorhynchus kisutch*) smolts originating from six different hatchery facilities in southwestern British Columbia, Canada, rearing genetically distinct stocks, over two different rearing years. Our results indicate vaterite prevalence varies significantly with hatchery facility, stock, and rearing year. The Chehalis River and Chilliwack River hatcheries had Coho Salmon stocks with the lowest number of vateritic sagittal otoliths (33% of otoliths contained vaterite) in comparison to all other hatchery facilities (70-95% of otoliths contained vaterite). This research supports efforts to evaluate hatchery rearing-practices and aids in our understanding of how vateritic otoliths may impact the health and marine survival of hatchery-origin Pacific salmonids.

Keywords: Otoliths, sagittae, salmonid enhancement, *Oncorhynchus kisutch*

RETURN TO INDEX

IOS_088

Otolith mineralogy affects otolith shape asymmetry: a comparison of hatchery and natural origin Coho Salmon

Micah Quindazzi[1,2], Leigh Gaffney[1], Emma Polard[1*], Nick Bohlender[3], Will Duguid[1,2], Francis Juanes[1]
[1]Department of Biology, University of Victoria, Victoria, British Columbia, Canada; [2]Pacific Salmon Foundation, Vancouver, British Columbia, Canada; [3]Department of Fisheries and Oceans, Campbell River, British Columbia, Canada
*emma.polard@gmail.com

Many aspects of natural and hatchery origin salmonid genetics, physiology, behaviour, anatomy, and life histories have been compared due to concerns about what effects domestication and hatchery rearing have on salmonid fitness. Fluctuating asymmetry (small, random deviations from perfect left-right symmetry) is commonly used as a proxy measurement for fitness and has been found to impact the sagittal otoliths of hatchery-reared salmonids. Another recently documented difference between hatchery and natural origin salmonids is the significantly higher prevalence of vaterite (an abnormal calcium carbonate polymorph) in the sagittal otoliths of hatchery-reared salmonids. Vateritic otoliths differ substantially from normal, aragonitic otoliths in terms of mass and shape and can artificially inflate any measurement of fluctuating asymmetry if not properly accounted for. Unfortunately, the vast majority of studies conducted on otolith asymmetry to date have failed to take otolith mineralogy, including the presence or absence of vaterite, into account (97% of 388 recent studies failed to account for differences in otolith mineralogy). The aim of this study was to explore the relationship between otolith asymmetry when otolith mineralogy is accounted for. To do this, sagittal otolith pairs from hatchery and natural origin Coho Salmon (*Oncorhynchus kisutch*) originating from three different river systems in British Columbia, Canada, were collected and assessed for overall differences in morphometrics and asymmetry. All sagittal otolith pairs were also assessed for the presence and percent coverage of vaterite and/or aragonite. We found that 59.3% of otoliths from hatchery origin *O. kisutch* were vateritic compared to 11.7% of otoliths from natural origin *O. kisutch*. Otolith mineralogy, rather than origin, was the most significant factor influencing differences in asymmetry for each shape metric. When only aragonitic otoliths were compared, there was no difference in absolute asymmetry between hatchery and natural origin *O. kisutch*. These results indicate the importance of accounting for otolith mineralogy. Hence, we recommend other researchers assess otolith mineralogy when conducting studies regarding otolith morphometrics and otolith asymmetry.

Keywords: Sagittae, *Oncorhynchus kisutch*, morphometrics, vaterite, salmonid enhancement

RETURN TO INDEX

IOS_097

Crystalline polymorphs of otoliths in three Indian freshwater fishes from the river Ganga

Lubna Yasmeen[1], Mohd Sadiq[1], Mohd Hammad[1], Ameer Hamza[1], Begum Razia Nargis[1], Mohammad Afzal Khan[1*]
[1]Fish Biology and Otolith Research Laboratory, Department of Zoology, Aligarh Muslim University, Aligarh-202002 (U.P.), India.
*khanmafzal@yahoo.com

Bio-calcareous ear stones present in fishes are located in the internal ear embedded in endolymph. Due to their unique property of being acellular, metabolically inert and lack of resorption, otoliths contain wealth of information about fish life and environmental history. Biomineralization of otoliths is the process of mineral deposition along with an organic matrix that is directly correlated to metabolic processes. The crystalline nature of otolith is due to the presence of $CaCO_3$ polymorphs namely aragonite, vaterite and calcite. In the present study, confocal micro Raman spectroscopy is employed to investigate the crystalline polymorphs of otolith in three freshwater fishes (*Heteropneustes fossilis, Channa punctata, Labeo bata)* sampled from the Ganga River. Raman signals were collected from core to the margin covering translucent and opaque zones of otolith. In all three fishes, Raman signals showed the strong calcite band intensities of v_1 stretching mode at 1087 cm^{-1} and weak signals of lattice mode at 154.9 cm^{-1}, aragonite band intensities of v_4 vibrational mode at 707 cm^{-1} and lattice mode at 208 cm^{-1}. The vaterite band intensities of lattice mode were recorded in *L. bata* and *C. punctata.* The replacement of calcium by barium was mapped as whiterite ($BaCO_3$) in *H. fossilis* and *C. punctata*. The novel Raman signals were also recorded at 708 cm^{-1} in *H. fossilis*; 156 cm^{-1}, 708 cm^{-1} in *L. bata*; and 156 cm^{-1}, 202.8 cm^{-1}, 204 cm^{-1}, 708 cm^{-1} in *C. punctata.* With the reference of obtained Raman signals and its intensities, it was concluded that all three fishes have calcite otoliths along with traces of aragonite and vaterite polymorphs. This information can be used to understand the biological processes involved in otolith formation and growth.

Keywords: Biomineralization, Confocal micro Raman spectroscopy, *Heteropneustes fossilis, Channa punctata, Labeo bata*.

RETURN TO INDEX

IOS_101

Environmental conditions affect otolith annual increment visibility – a long-term perspective from two Atlantic cod populations

Szymon Smoliński[1]*, Côme Denechaud[2], Audrey J. Geffen[3], Jane A. Godiksen[2]
[1]National Marine Fisheries Research Institute, Gdynia, Poland
[2]Institute of Marine Research, Bergen, Norway
[3]Department of Biological Sciences, University of Bergen, Bergen, Norway
*ssmolinski@mir.gdynia.pl

The optical properties, or macrostructure features, of otoliths, are sensitive to environmental conditions. The visual quality of annual growth increments (e.g. contrast of the translucent and opaque zones) in fish otoliths have implications for the accuracy and precision of the age estimates. Using Atlantic cod (*Gadus morhua*) otoliths as a model, we tested the hypothesis that higher mean annual temperatures and greater seasonal fluctuations in temperature enhance the contrast of the translucent and opaque zone in the otolith annual increment. We used a large dataset of brightness profiles extracted from imaged otolith sections of Icelandic and Northeast Arctic cod (7 629 otoliths and 73 423 annual increments in total), providing a time series of fish growth and age spanning a century (1907-2015). We developed an algorithm to analyze the brightness of the translucent and opaque zones to objectively quantify the contrast between adjacent zones. The algorithm consists of multiple steps, including brightness profile standardization, GAM smoothing, and calculation of the range between the 5th and 95th percentile of the brightness in a given annual increment (translucent and opaque zone). We tested the stability of the algorithm with images acquired under a controlled series of different lighting conditions. The results validate the suitability of the algorithm for long-term assessment of contrast variability in cod otolith sections. Our results show high temporal variability in optical contrast between zones and measurable effects of ocean temperature conditions. Taking into consideration projected temperature rise and amplification of the seasonal cycles of temperature, our findings suggest that the visibility of the annual increments in otoliths can change in the future. Our findings are of importance for the fish aging studies constituting the basis for the research, management, and conservation of fish resources.

Keywords: age reading, increment, contrast, visual, optical, climate, temperature, annulus

RETURN TO INDEX

IOS_104

Coupling otolith and archival tag records to test assumptions underpinning otolith chemistry applications in wild fish

Karin Hüssy[1]*, Stefanie Haase[2], Anna M. Sturrock[3]
[1]National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby, Denmark; [2] Thünen Institute of Baltic Sea Fisheries, Rostock, Germany; [3] School of Life Sciences, University of Essex, Colchester, United Kingdom. * kh@aqua.dtu.dk

Chronological records of elemental concentrations in fish otoliths are a widely used tool to infer the environmental conditions experienced by individual fish. To interpret elemental signals within the otolith, it is important to understand how both external and internal factors impact ion uptake, transport and incorporation. In this study, we have combined chronological records from otoliths and archival data storage tags to quantify the influence of internal (sex, size, age, growth) and external (temperature, depth, salinity) conditions on otolith elemental chemistry of cod (Gadus morhua) in natural settings of the Baltic Sea. Based on known spatial gradients and temporal patterns in environmental and biological drivers, we identified hypotheses on expected otolith element patterns. Otolith concentration of all elements under physiological control (P, Mg, Zn) showed similar responses across environmental and biological variables, with distinct seasonal patterns (lowest concentration in late spring, highest concentrations in winter), a positive correlation with water temperature, higher Zn during spawning and higher P during the feeding season. Otolith concentrations of elements expected to be predominantly under environmental control showed the expected geographic and depth-related trends based on ambient salinity (Ba) and coastal hypoxic events (Mn). However, Sr exhibited unexpected geographic patterns and was unrelated to salinity, even when directly measured by the tag attached to the fish. Otolith Ba, Sr and Mn concentrations also showed pronounced seasonality that were out of phase with each other but to some extent explained by spawning/feeding migrations. These results highlight the need for assessing environmental concentrations and freshwater endmembers before otolith elemental chemistry can be used to reconstruct fish habitat use and environmental histories in the Baltic Sea.

Keywords: Atlantic cod, Data Storage Tags, habitat reconstruction, otolith chemistry

RETURN TO INDEX

IOS_116

Automated 3D Otolith Morphometrics

Francis Juanes[1]*, Micah J. Quindazzi[1], Adam P. Summers[2]
[1]Department of Biology, University of Victoria, Victoria, BC, Canada; [2]Department of Biology, University of Washington, Friday Harbor, WA, USA. *juanes@uvic.ca

The morphometrics of fish otoliths have been commonly used to investigate population structures and the environmental impacts on ontogeny. These studies can require hundreds if not thousands of otoliths to be collected and processed. Processing these otoliths takes up valuable time, money, and resources that can be saved by automation. These structures also contain relevant information in three dimensions that is lost with 2D morphometric methods from photographic analysis. In this study, the otoliths of three populations of Coho Salmon (*Oncorhynchus kisutch*) were examined with manual 2D , automated 2D, and automated 3D otolith measurement methods. The automated 3D method was able to detect an 8% difference in average otolith density, while 2D methods could not. Due to the loss of information in the z-axis, and the longer processing time, 2D methods can take up to 100 times longer to reach the same statistical power as automated 3D methods. Automated 3D methods are faster, can answer a wider range of questions, and allow to automate rather monotonous tasks.

Keywords: µCT, *Oncorhynchus*, ShapeR

RETURN TO INDEX

IOS_120

Comparison of two conventional and one novel otolith chemical age determination method of flounder (*Platichthys spp*) in the Baltic Sea

Martina Blass*[1], Yvette Heimbrand[1], Jan-Erik Johansson[1], Marju Kaljuste[1], Uwe Krumme[3], Karin Limburg[1,2], Zuzanna Mirny[4], Melvin Samson[5], Didzis Ustups[6], Sami Vesala[7], Peter Vingaard Larsen[8], Dace Zilniece[6].
1Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2SUNY College of Environmental Science & Forestry, Syracuse, USA; 3Thünen Institute of Baltic Sea Fisheries, Rostock, Germany; 4Department of Fisheries Resources, National Marine Fisheries Research Institute, Gdynia, Poland; 5INRAE UR EABX, Gazinet Cestas, France; 6Fish Resources Research Department, Institute of Food Safety, Animal Health and Environment (BIOR), Latvia; 7Natural Resources Institute Finland, Helsinki, Finland; 8National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark. *martina.blass@slu.se

Flounder in or from the Baltic Sea (*Platichthys spp*) is traditionally aged by counting annual rings on sectioned and stained otoliths. However, recent age calibration have shown difficulties to identify the first year with acceptable precision. Chemical profiles from the otolith core to the edge reveal lifetime trace elemental variations, reflecting physiological and environmental changes experienced by the fish. We tested a novel chemical aging method developed for Baltic cod to investigate if otolith chemistry could be applied for identifying the first annulus on flounder otoliths. Seasonal patterns in Mg:Ca and Mn:Ca ratios were used to evaluate if the novel chemical aging could give clarity in how to interpret the first annulus. The ICES age reading platform SmartDots was used for the comparison where 12 age readers from 8 countries annotated the first and second annuli on images, as well as on chemical profiles of Mg:Ca and Mn:Ca from 30 flounders. All images of unstained symmetric otolith sections, stained asymmetric otolith sections and chemical profiles of Mg:Ca and Mn:Ca, were in the same size scale to enable comparison of the distance from the core to the first and second annuli for all methods. Generally, annotations were more scattered on the unstained otolith images compared to images of stained otoliths and chemical profiles. Age readers had the highest agreement for the first, second and both annuli on images of chemical profiles (90%, 63%, 60%), followed by stained otoliths (57%, 57%, 43%), and unstained otoliths (36%, 39%, 25%). Experienced age readers had higher agreement than beginners for both stained otoliths (77%, 63%, 60%) and unstained otoliths (57%, 39%, 28%). Full agreement for all methods was reached for only 6 otoliths. The results indicate that chemical profiles can be useful as a tool to identify specifically the first annulus.

Keywords: Age validation, Otolith chemistry, LA-ICP-M

RETURN TO INDEX

IOS_136

Comparative studies on stable carbon and nitrogen isotope values among tissues and hard structures in bony fishes

Li-Ling Kao[1]*, Mu-Ting Lee[1], Pei-Ling Wang[1], Ming-Tsung Chung[1]
[1] Institute of Oceanography, National Taiwan University, Taipei, Taiwan. *james19322@gmail.com

Trophic ecology explains the energy flow and prey-predator relationship among individuals and species. Trophic levels of species reveal the role that they play in the ecosystem, the variability of trophic levels in the food web over time also indicates the ecosystem stability. In fact, examining the trophic level of an individual is difficult in the field with the traditional method of stomach content analyses because of unidentified prey items and an empty stomach. Compared to stomach content analyses, stable isotope analyses on fish tissues are an alternative approach to studying trophic ecology. However, stable isotope values (i.e. $\delta^{13}C$ and $\delta^{15}N$) could vary among tissues, depending on the biologically controlled fractionation and metabolic influences. Also, the values recorded in metabolic inert structures, such as otoliths, vertebrae, and eye lenses, provide individual ontogenetic information, which cannot be reconstructed with metabolic active tissues, such as muscle tissues. Therefore, this study aims to compare $\delta^{13}C$ and $\delta^{15}N$ values varied among these tissues/structures, assesses the ontogenetic trend derived from metabolic inert structures, and details the advantages and limits of using different tissues/structures. We extracted otoliths, vertebrae, eye lenses and muscle from 1 individuals of 5 Sciaenidae species and established the ontogenetic trend of $\delta^{13}C$ and $\delta^{15}N$ values in otolith organic matter, vertebral collagen, and eye lens. $\delta^{13}C$ and $\delta^{15}N$ shifts among structures were observed, and the temporal resolution of the ontogenetic $\delta^{13}C$ and $\delta^{15}N$ trend was highest using vertebrae followed by eye lenses and otoliths. We provide a protocol for different sample preparation and isotopic analyses and highlight that using different tissues/structures for trophic studies should be carefully evaluated in the future.

Keywords: Otolith, Vertebra, Eye lens, Muscle, Ontogeny, Croaker

RETURN TO INDEX

IOS_144

Metabolic adjustment of fish in an acidified environment: an evaluation using otolith $\delta 1^3C$ and $\delta^{11}B$ values

Ming-Tsung Chung[1]*, Tzu-Yen Liu[1,2], Chi-Yuan Hsieh[1], Yung-Che Tseng,[2], Kuo-Fang Huang[3]
[1]Institute of Oceanography, National Taiwan University, Taipei, Taiwan; [2]Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, I-Lan, Taiwan; [3]Institute of Earth Sciences, Academia Sinica, Taipei, Taiwan.
*mingtsungchung@ntu.edu.tw

Fish's behavioural acclimation and adaptation in response to environmental changes are physiologically regulated but without conclusive evidence of long-term monitoring across the era under anthropogenic influences. One of the noticeable anthropogenic influences is a CO2-induced acidified environment that may affect the metabolism of fish. To investigate the physiological traits that varied under environmental pressure, this study attempts to (1) assess the increase of the metabolic rate in an acidified environment, and (2) evaluate potential proxies used to reconstruct the fish metabolic rate and the environmental pH. We conducted a laboratory-controlled experiment in which black seabream (*Acanthopagrus schlegelii*) was reared at two pH levels (7.6 and 8.1) for a year. We measured their oxygen consumption rate and acid excretion rate monthly throughout the year and analysed two isotope values ($\delta^{13}C$ and $\delta^{11}B$) recorded in otoliths to evaluate the relationship between the ontogenetic trend of $\delta^{13}C$ values and oxygen consumption and to assess the $\delta^{11}B$ variation between and within two pH-level environments. Otolith $\delta^{13}C$ values as metabolic proxy have been validated and observed in a few fish species, but it is the first calibration equation of metabolic proxy and oxygen consumption rate in black seabream. Second, $\delta^{11}B$ values recorded in biogenic carbonates as environmental pH proxy have been widely described in corals and foraminifera, but not applied to otoliths yet. Thus, our study evaluates the possibility of using otolith $\delta^{11}B$ values as environmental pH proxy. We highlight that the novel analytical methods provide great potential to rebuild the individual physiological history under ocean acidification and unravel the adaptive abilities of fish across generations in a changing environment.

Keywords: Oxygen consumption, Acidification, *Acanthopagrus schlegelii*, Acid excretion rate, Isotope proxy

RETURN TO INDEX

IOS_166

Biannual otolith translucent zone formation of Cape hake *Merluccius capensis* in Namibia is regulated by ontogeny and extremes

Margit Wilhelm[1*], Anja van der Plas[2]
[1]Department of Fisheries and Ocean Sciences, University of Namibia, Henties Bay, Namibia; [2]Ministry of Fisheries and Marine Resources, Swakopmund, Namibia; *mwilhelm@unam.na

In this paper the possible factors related to the timing of translucent zone formation were tested with two statistical approaches on Cape hake *Merluccius capensis* otoliths from research survey- and commercial catch samples in the northern Benguela, Namibia. In the first approach, a binomial generalized linear model was used to predict the presence of the first to sixth translucent zones for fish length and maturity stage, area and bottom depth in which the fish were captured. The best model (selected using AICc) was consistently the model in which the presence of a translucent zone was explained by first fish length and second area. Fish caught in the north of Namibia (17-20°S) consistently showed earlier formation of translucent zones and therefore more translucent zones at the same fish lengths than fish from the central and southern areas (21-29°S). This suggests translucent zone formation first is ontogenetic, and second, the additional early formation of translucent zones per fish length, to the area. The results of the second approach suggested that this additional zone formation was linked to warmer temperatures in northern Namibia. In this second approach, timing of translucent zone formation was back-calculated and linked to environmental variables such as sea surface temperature (SST), a change in SST, the SST anomaly, the bottom temperature and bottom dissolved oxygen concentration. Events of translucent zone formation were significantly correlated with periods of warm bottom temperatures, but took place at times of minimum and maximum temperatures as well as peaks in offshore-upwelling-induced productivity. While it is difficult to uncouple the exact influences of environmental or other variables on translucent zone formation with only absence or presence data, this is a first approach for this important commercial resource in Namibia using more than 3000 otoliths. It is therefore hypothesized that the cues for zonation are first endogenous, when adapted to the Namibian specific temperate environment, while the individual variation are responses to localized environmental variability.

Keywords: Otoliths, validation, environmental triggers, *Merluccius*, Benguela

RETURN TO INDEX

IOS_169

Comparison of traditional and otolith chemical age determination methods for eastern Baltic cod (*Gadus morhua*)

Yvette Heimbrand[1]*, Rajlie Sjöberg[1], Peter Johannessen[1], Axel Hjelm[1], Karin Limburg[1,2]
[1]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]SUNY College of Environmental Science & Forestry, Syracuse, New York, USA *yvette.heimbrand@slu.se

Worsening environmental conditions (increasing eutrophication, hypoxia) combined with high fishing pressure, declining prey availability, parasites and predation by seals have led to collapse of eastern Baltic cod (*Gadus morhua*). Biological consequences include a decline in body size and condition, maturation at a smaller size and reduced maximum length. Monitoring of eastern Baltic cod has focused on the central and southern Baltic Sea. Thus, there is a lack of knowledge about the cod north of this area (Åland Sea), where in contrast, catches include large cod with good growth. Accurate knowledge of age and growth are important for assessing the status of fish stocks. Cod are traditionally aged by counting annual growth zones in otoliths. However, unclear, low-contrast growth zones have resulted in unreliable age data. A new chemical age determination method was developed based on interpreting seasonal patterns in the uptake of Mg:Ca and P:Ca in cod otoliths. An age calibration was performed to test the new method and compare cod from the northern and southern Baltic. Three age readers annotated annuli on both otolith images and corresponding chemical profiles of Mg:Ca and P:Ca from 25 cod from each area. The agreement among age readers for images of cod otoliths from the southern Baltic Sea was 66.0% and the precision 24.5%, and for the northern Baltic Sea 54.7% and 17.4% respectively. The agreement improved for otolith chemical profiles, with 72.0% agreement and a precision of 14.8%, for the southern Baltic Sea and even more so for the the northern Baltic Sea with 78.7% and 7.6% respectively. Estimated length at age indicated faster growth for cod in the northern compared to the southern Baltic Sea. Annotations were positioned closer together on chemical profiles than on otolith images where they were more scattered. Age readers generally estimated older ages for otolith images compared to corresponding chemical profiles. These results imply that visual structures in otoliths are more difficult to interpret than seasonal patterns in chemical profiles and that otolith microchemistry can be used as a tool to improve age determination of Baltic cod.

Keywords: Otoliths, Trace elements, LA-ICP-MS, Seasonal patterns, Age estimation

RETURN TO INDEX

IOS_175

A universal metabolic proxy from bone and ear stone

Chi-Yuan Hsieh*[1], Zhu-Yen Liu[1,2], Kotaro Shirai[3], Yung-Che Tseng[2], Chien-Hsiang Lin[4], Pei-Ling Wang[1], Ming-Tsung Chung[1]
[1]Institute of Oceanography, National Taiwan University, Taipei, Taiwan; [2]Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, I-Lan, Taiwan; [3]Atmosphere and Ocean Research Institute, The University of Tokyo, Chiba, Japan; [4]Biodiversity Research Center, Academia Sinica, Taipei, Taiwan. *r11241212@ntu.edu.tw

Metabolic rate, in terms of energy use, is associated with individual fitness, including growth, reproduction, and life history responses to environmental pressure. Thanks to a newly developed approach of otolith $\delta^{13}C$ metabolic proxy, it is possible to estimate the field metabolic rate in marine fishes. However, this approach is not comprehensively studied across species and is limited in bony fishes. Our study aims to expand upon this concept by (1) providing one more validation experiment supporting the relationship between otolith $\delta^{13}C$ metabolic proxy and oxygen consumption ; (2) developing $\delta^{13}C$ metabolic proxy in other calcified structure, a vertebra which is mainly composed of organic matter and hydroxyapatite, but with a minor amount of carbonates in crystal structures; (3) expanding the investigation of $\delta^{13}C$ metabolic proxy among marine fishes and between two calcified structures, otoliths, and vertebrae. To achieve the goal, we cultured black seabreams (*Acanthopagrus schlegelii*) in a controlled environment for a year and measured the oxygen consumption rate monthly, and analysed ontogenetic $\delta^{13}C$ and d18O values in both otoliths and structure carbonates in vertebrae. As result, we (1) compared the established calibration equations of otolith $\delta^{13}C$ metabolic proxy and oxygen consumption rates in black seabream to the one derived from Atlantic cod, which is the only one with experimental validations so far; (2) evaluated the difference of $\delta^{13}C$ metabolic proxy between otoliths and vertebrae in black seabream; (3) evaluated the difference of $\delta^{13}C$ metabolic proxy between otoliths and vertebrae among species, including the tempeature- and mass-dependent trends. With an expansion of the metabolic proxy approach, we can apply this method to more marine vertebrates and answer the unresolved but important questions in metabolic ecology, particularly how marine organisms respond to environmental changes, such as ocean warming, ice melting, acidification, hypoxia, and so on.actividad a la hora de tomar decisiones en su vida diaria. Validando de esta manera las herramientas aplicadas y cumpliendo el objetivo del programa educativo.

Keywords: Stable carbon isotope, Oxygen consumption, Vertebrates, *Acanthopagrus schlegelii,* Carbonate

RETURN TO INDEX

IOS_186

Strontium uptake and expulsion in otoliths: A case study of Greenland Halibut (*Reinhardtius hippoglossoides*) assessing element marking, otolith growth and tag-recapture studies within natural environment

Tracey Loewen*[1], Margaret Treble[1], Rick Wastle[1], James Reist[1], Ravinder Sidhu[2], Kaj Sünksen[3], and Ole A. Jørgensen[3] and Norman Halden[2].
[1]Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Manitoba, Canada; [2]University of Manitoba, Winnipeg, Manitoba Canada; [3]Greenland Institute of Natural Resources, DK-3900 Nuuk, Greenland. *tracey.loewen@dfo-mpo.gc.ca

Elemental marking and recapture techniques were used to document fish growth and otolith accretion rates, and characterise strontium (Sr) uptake and retention periods for Greenland Halibut (*Reinhardtius hippoglossoides)*. Two different concentrations of a strontium-chloride ($SrCl^2$) solution were injected into the intraperitoneal cavity prior to releasing the fish back into their natural environment. Mean fish growth rate was 2.19 $cm.yr^{-1}$ for all 18 individuals or 6.31 $cm.yr^{-1}$ with the elimination of one individual experiencing negative growth. Otolith accretion occurred along different axes in the left and right otolith structures. The right otolith accretion occurred in an anterior-ventral direction, whereas the left grew in posterior-ventral, anterial-dorsal, and proximal directions. The accretion rates were similar between left and right structures along the edge region whereas accretion over the nucleus in the proximal region was slower. Strontium uptake took anywhere from 17 to 242 days and was strongly influenced by otolith accretion rate and dose. In the initial stages of strontium retention, and during the

estimated return-to-background, only dose level played a role in strontium retention. The estimated return-to-background varied from 328-1518 days and one individual in our study did not return to background levels. Storage mechanisms, temperature and growth rates of fish likely contribute to the extended strontium retention period postmarking. Knowing that retention periods post-marking can be long in duration may assist others when interpreting elemental profiles of long-lived species that migrate frequently between marine and freshwater environments on relatively short timeframes. Further research to examine element storage mechanisms and their remobilisation in the context of fish physiology will aid our understanding of element incorporation into otoliths.

Keywords: Otoliths, SrCl , Greenland Halibut, mark-recapture, element-marking

RETURN TO INDEX

IOS_187

Otolith's microchemistry as a tool for identification of (*Acanthocybium solandri*) in Atlantic Ocean

Thays Freitas de Andrade[1],Guelson Batista da Silva[2*]. guelson@ufersa.edu.br
[1]Instituto de Ciencias do Mar, Universidade Federal do Ceará, Fortaleza Brasil;[2] Centro de CiênciasAgrárias – CCA, Universidade Federal Rural do Semiárido, Mossoró, Brasil.

Acanthocybium solandri also known as « Wahoo » is a ocean-pelagic marine specie. Reach depths that can vary between 0-20m and habits subtropical and tropical environments, being distributed in Atlantic, Indian and Pacific Oceans. There are no observations of *A. Solandri* shoals, being an specie with lonely habits although can form little groups of organisms. Despite having exploited stocks in various areas around the world, it is classified as Least Concern (LC) in International Union for Conservation of Nature (IUCN) Red List of Threatened species. Researches using the microchemistry of otoliths has been developed for decades, nonetheless, it is unprecedented with *A. Solandri.* The samples in the present study were collected in three different sites in Atlantic : Brazil (oceanic banks off the northeast coast), Canarian Island and Ivory Coast. The objective of this study was to analyze the microchemistry of *A. Solandri*, using concentrations of Magnesium (Mg), Manganese (Mn), Strontium (St), Barium (Ba) and Lead (Pb) as a way to identify the shaping of distinct fishing stocks. In the results, there was a clear variation in the elements along the three different sites; St had a balanced variation between all the stocks, varying 1000ppm – 2500ppm; Mn variation was 0 – 15ppm, and Ba between 1ppm – 18ppm. These elements followed a very satisfying pattern, although Pb and Mg had bigger variations. Through PCA analyzes it was possible to imply that the elements with more contribuition in otoliths microchemistry are Sr, Mn and Ba. Giving less value to Pb and Mg.

Keywords: Otoliths, Microchemestry, *Odontesthes*, Chilean coast, Sagittae.

RETURN TO INDEX

IOS_196

Understanding the shape plasticity of fish otoliths based on Diffusion Limited Aggregation model simulations

Paulo Duarte-Neto[1,2]*, Joé Edson Gondim[1], Borko Stosic[2]
[1]Graduate Program in Biometrics and Applied Statistics, Universidade Federal Rural de Pernambuco, Recife, Brazil; [2]Department of Stattistics and Informatics, Universidade Federal Rural de Pernambuco, Recife, Brazil;

Application of simulation growth models to describe the growth of natural structures has been gaining momentum over the last decades. When the appropriate model and the minimal set of the most important factors that govern the formation of large structures by aggregation of small subunits (particles) observed in practice are identified, it is often possible to obtain a realistic description of the phenomenon growth under study. Under this premise, we have already proposed the first quantitative and stochastic model that could represent the growth process of otoliths, shedding light on the question: to which extent the shape of otoliths is genetically determined, and to which extent it can be affected by external factors? The proposed general otolith growth model, based on the Diffusion Limited Aggregation (DLA) model, was capable of reconstructing the otolith shape of many species and turned capable of reflecting the biomineralization process of the otoliths. The current proposed modifications to the original DLA model regard several factors, such as particle flux and concentration, type and probability of aggregation, and size and shape of “sensory organs” and “otic capsule”. Each proposed modification is based on the in-situ observations during otolith removal, as well as what is already known about the literature. Thus, it was possible to demonstrate through simulations that the final shape is mainly due to the anatomical configuration where the otolith is located, i.e. the size and shape of the cavity in which the otolith develops and physical impediments within the capsule that prevent aggregation at certain sites. We also observed that the flux does not interfere with the final shape of the otolith and that this factor is not decisive in the shaping process. Still, the structure of the aggregation based on the aggregation type and probability was responsible for the roughness characteristic of the border. With the combinations of these factors, it was possible to simulate many different shapes that the otolith can assume and to describe the growth of the otolith from the early stage to adulthood.

Keywords: Fish otoliths, Growth model, Simulations, Shape.

RETURN TO INDEX

IOS_229

Wavelength dispersion X-Ray Fluorescence (WDFRX) applied to Sr:Ca and Ba:Ca determination in otoliths

Igor Morais[1], Marcos Scapin[2], Juliana Azevedo[3]*
[1]Postgraduate Program in Ecology and Evolution, Institute of Environmental Sciences, Chemical and Pharmaceutical, Federal University of São Paulo, Diadema, Brazil; [2]Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP, São Paulo, Brazil; [3]Institute of Environmental Science, Chemical and Pharmaceutical, Federal University of São Paulo, Diadema, Brazil.
*juliana.azevedo@unifesp.br

Otolith microchemistry has been widely used to investigate fish movements. Sr:Ca and Ba:Ca ratios in otoliths are the main target, as Sr levels are positively correlated with salinity within the estuarine gradient. An inverse profile is observed for Ba concentrations. The aim of this study was to evaluate the use of wavelength dispersive X-ray fluorescence (WDFRX) to determine Sr:Ca and Ba:Ca ratios in lappilus otoliths of the estuarine catfish *Cathorops spixii* (Siluriformes, Ariidae) from the Cananéia-Iguape Estuarine-Lagoon Complex (CIELC), southern coastal area in Brazil. The southern region of the CIELC is better preserved than the northern region because of the strong oceanic influence. In addition, the continental influence and freshwater input are higher in the northern region due to the outflow of the Ribeira de Iguape River. A total of 85 catfish *C. spixii* were collected in August 2018. Age rings (AR) were determined in the lapillus otoliths and the elemental composition (Sr, Ba, and Ca) in the whole otolith was determined by WDFRX. Catfish had a mean AR=5.18±2.79. Fish from the southern region had higher AR than catfish from the northern region ($AR_{Southern}$=6.97±2.56; $AR_{Northern}$=3.52±1.92). Method validation and analytical control were performed by determination of the accuracy (ER%), limit of quantification (LQ), Horwitz test (Hor) and the analysis of a composite standard (S) of CaO, SrF_2 and $BaCO_3$ (Sigma-Aldrich): S1 - Ca (98.00±0.01%), Sr and Ba (1.00±0.01%); S2 - Ca (96.0±0.2%), Sr and Ba (2.00±0.04%). The WDFRX was satisfactory for the analysis of Sr, Ba and Ca. Hor was less than 2 for Ca, Sr and Ba. Accuracy was also satisfactory as the z-score was also less than 2 (S1 and S2). The LQ were sufficient to ensure the Ca, Sr and Ba concentrations in the otolith samples. Thus, the reliability of the proposed method was demonstrated. In general, was observed a significant difference ($p<0.05$) between the Sr and Ba levels in the otoliths of catfish from the southern and northern areas, with higher Sr levels found in *C. spixii* from the more saline and hydrodynamic area (southern – Sr=0.63%, Ba=0.15%; northern – Sr=0.56%, Ba=0.17%).

Keywords: Otolith, Microchemistry, Conservation, Ariidae, Cananéia

RETURN TO INDEX

IOS_245

Eco-densitometry of fish otoliths: advances and perspectives in computerized micro-CT analysis of sagittal otoliths for tropical fish species

Paulo Duarte-Neto[1,2]*, Jonas Vasconcelos-Filho[1], João Valério[1], Borko Stosic[1,2], Wilson Rosa Júnior[1,2], Antonio Antonino[3], Daniel Duarte[3], Rosangela Lessa[4], Francisco Santana[5], Beatrice Ferreira[6].
[1]Graduate Program in Biometrics and Applied Statistics, Universidade Federal Rural de Pernambuco, Recife, Brazil; [2]Department of Statistics and Informatics, Universidade Federal Rural de Pernambuco, Recife, Brazil; [3]Department of Nuclear Energy, Federal University of Pernambuco, Recife, Brazil; [4]Department of Fisheries and Aquaculture, Federal Rural University of Pernambuco, Recife, Brazil, [5]Academic Unit of Serra Talhada, Federal Rural University of Pernambuco, Recife, Brazil; [6]Department of Oceanography, Federal University of Pernambuco, Recife, Brazil. *paulo.duartent@ufrpe.br.

Micro-computed tomography (Micro-CT) has emerged as a valuable non-destructive method for studying the internal structure of various objects, including fish otoliths. Otoliths are essential in marine biology, providing insights into vital aspects such as metabolism, age determination, growth patterns, and fish stock identification. This study presents significant advances in the field of micro-CT analysis of sagittal otoliths for tropical fish species and discusses future perspectives. The research conducted by the LACUNA lab and collaborators focused on exploring and developing protocols and tools for analyzing micro-CT otolith images. In the first stage of our study, fifteen different scan combinations were tested, and optimal scan parameters were determined based on comprehensive analyses of the sinogram, Hounsfield unit distribution, and signal-to-noise ratio. The recommended parameters included 80 kV, 220 µA, and a 0.5 mm aluminum filter, which provided high-resolution images for 3D shape analysis, evaluation of internal and external density distribution, layer-by-layer density segmentation, and potential objective counting of otolith growth rings. Following this analysis, two different techniques were applied to investigate density variations in otoliths. We determined the external 3D curvature of the otolith and utilized the Ball Mapper technique of Topological Data Analysis. Computational efficiency was enhanced by determining the necessary resolution for capturing meaningful density variations comparable to raw data. Topological invariants derived from the network structure enabled the evaluation of graph similarity. By reducing the data by 95%, computational time was significantly reduced when applying topology for high-resolution image analysis. The advancements achieved in this study have promising implications for future research. The optimized micro-CT scan parameters and the application of the Ball Mapper technique and 3D external curvature provide a comprehensive approach to analyzing sagittal otoliths. These techniques enable comparative studies among tropical fish species, detection of ontogenic changes, and exploration of ecological implications related to density variations.

Keywords: Micro-computed tomography (Micro-CT), Fish otoliths, Density variation, Topological Data Analysis

RETURN TO INDEX

IOS_248

Higher CN ratio of collagen in the vertebral centrum during juvenile stage in Japanese flounder (*Paralichthys olivaceus*)

Hiromitsu Kamauchi[1,2]*, Yoshikazu Kato[1,3], Hiroyuki Togashi [4], Yutaka Kurita[4], Yosuke Amano[5], Chikage Yoshimizu[1], Ichiro Tayasu[1]
[1]Research Institute for Humanity and Nature, Kyoto, Japan; [2]Bio-generated Stripe-material Analysis, Nagoya, Japan; [3]Graduate School of Environmental Studies, Nagoya University, Nagoya, Japan; [4]Shiogama Field Station, Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Shiogama, Japan; [5]Fukushima Prefectural Fisheries and Marine Science Research Centre, Iwaki, Japan. *kamauchi@gmail.com

Collagen of fish vertebra is widely using in both the reconstruction of the past environment in archaeology and individual–level estimation of life history. Because organic matter in fishbone including collagen degrade along time, or the vertebral centrum, which is incremental growing part of vertebra, is surrounded by the non-incremental part which also including collagen, extracted collagen from vertebra in the laboratory analysis should be verified weather it can be adopted for the analysis. The molar ratio between carbon and nitrogen of the extracted collagen are widely using for this criterion (2.9 - 3.5 or 3.0 - 3.3) in both anthropology and ecology, however, these criteria were obtained from the meta-analysis of the extraction mainly from the waste part of fish procedure in food industry, therefore those collagens are from the whole vertebra including other than the vertebral centrum of adult individuals. In the present study we modified the cutting out methods of the vertebral centrum in Japanese flounder (*Paralichthys olivaceus*), especially for obtaining more detailed data on the part corresponding to the juvenile. Obtained CN ratio of collagen in the part that corresponding to the juvenile stage of the vertebral centrum were outranged (3.4 - 3.7, on average) to the criteria widely used. Obtained CN ratio gradually decreased along with the time-passing and reached within the widely-using criteria, and each CN ratio were negatively correlated with the stable carbon isotope ratio that was lower in the earlier of juvenile stages that entering bottom life at sandy beach after planktonic larval stage. Therefor observed trend in CN ratio is thought as the natural phenomenon along with the individual growth, neither than the contamination of non-incremental growing part of vertebra. Obtained pattern in CN ratio can be acceptable under consideration of almost not included the juvenile stages as the materials for collagen extraction into the past meta-analysis in the literature. Especially in further ecological research of reconstructing an individual life history from collagen of the vertebral centrum, higher CN ratio should be adopted on the larval stage.

Keywords: Vertebral centrum, Methodology, Collagen, CN ratio, Japanese flounder

RETURN TO INDEX

IOS_250

Heterogeneity of otolith chemical composition from 2D mapping: relationship with biomineralization mechanisms and implications for microchemistry analyses

Hélène de Pontual[1], Kirsteen M. MacKenzie[2], Hélène Tabouret[3], Françoise Daverat[4], Kelig Mahé[2], Christophe Pecheyran[3], Karin Hüssy[5]
[1]IFREMER DECOD (Ecosystem Dynamics and Sustainability), IFREMER, INRAE, Institut Agro, Centre Bretagne, HALGO, LBH, 1625 Route de Sainte Anne du Portzic, CS 10070, 29280 Plouzané, France; [2]IFREMER HMMN, 150 Quai Gambetta, 62200 Boulogne-sur-Mer, France; [3]UMR 5254, Université de Pau et des Pays de l'Adour (UPPA), Avenue de l'Université, BP 576 - 64012 Pau Cedex, France; [4]INRAE, 173 Route de Saint-Jean-de-Luz, 64310 Saint-Pée-sur-Nivelle, France; [5]National Institute of Aquatic Resources, Section for Oceans and Arctic, Technical University of Denmark, Kemitorvet, Building 201, 2800 Kongens Lyngby, Denmark.
*kirsteen.mackenzie@ifremer.fr

Although otoliths are widely used as archives to infer life history traits and habitat use in fishes, their biomineralization process remains poorly understood. This lack of knowledge is problematic as it can lead to misinterpretation of the different types of signals (e.g. optical or chemical) that provide basic data for research in fish ecology, fisheries management and species conservation. Otolith calcification relies on a complex system involving a pericrystalline fluid, the endolymph, which organic and inorganic compositions are spatially heterogeneous for some constituents. This property stems from the particular structure of the calcifying saccular epithelium. In this study, we explored the spatial heterogeneity of elemental incorporation in otoliths for two species of high economic interest, European hake *Merluccius merluccius* (L. 1758) and European sea bass *Dicentrarchus labrax* (L. 1758). Two-dimensional mappings of chemical elements were obtained by UV-fs LA-HR-ICPMS analyses on transverse sections of sagittae. Results highlighted a clear asymmetry between proximal (sulcus) and distal (antisulcus) concentrations for elements such as magnesium Mg, phosphorus P, manganese Mn and potassium K with concentration gradient directions that varied depending on the element. Strontium Sr and barium Ba did not show a proximo-distal gradient. These results are discussed in the light of current knowledge on the endolymph composition and the mechanisms that lead to its compartmentalization, highlighting the need for further research on otolith biomineralization. Operational implications for studies based on otolith chemical composition are also discussed with emphasis on advice for sampling strategies in order to avoid analytical biases, and the need for in-depth analyses of analytical settings before comparing otolith signatures between species or geographical areas.

Keywords: Endolymph, European sea bass, European hake, saccular epithelium, spatial pattern, trace elements.

RETURN TO INDEX

IOS_256

Creation of a publicly available known-age fish structure repository

Martin Hamel[1]*, Dave Buckmeier[2], Jeff Koch[3], Mark Pegg[4]
[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia, USA.*hamel@uga.edu
[2]Texas Parks and Wildlife Department, 2103 Junction Highway, Mountain Home, Texas, USA
[3]Kansas Department of Wildlife and Parks, 1830 Merchant, Emporia, Kansas, USA
[4]School of Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

Accurate determination of fish age from hard structures is a cornerstone of informing fisheries conservation and management. Further, the need to validate age estimates from calcified structures is commonly identified by scientists who attempt age estimation. Pleas for ensuring accuracy of age estimates have been pervasive in recent times, but unfortunately, natural resource agencies charged with managing stocks do not possess resources to build large collections of structures from known-age fish. As such, a conceptual project was developed by members of the American Fisheries Society's (AFS) Fish Management and Education Sections to address the feasibility of developing a publicly available, web-based repository of digital images of known-age reference structures for North American freshwater fishes. We surveyed AFS section members and found broad support for this effort, with over 170 responses gathered from scientists interested in sharing known-age fish structures. In spring of 2023, we began collecting reference structures (e.g., otoliths, spines, fin rays, etc.) containing annual or daily age confirmation, as well as other useful structures, such as those with known time stamps (e.g., OTC marks). We are underway in developing a publicly available web-based application that will display actual images and metadata (e.g., source of structure, preparation method) of these known-age structures. We envision this application being used for training new personnel, quality control, and to advance the science of age estimation.

Keywords: Known-age, Freshwater fishes, Age determination, Age validation

RETURN TO INDEX

IOS_259

Characterization of phosphorous uptake in otoliths using laser-ablation ICP-MS and transmission electron microscopy

Maria Hussain[1]*; Sierra Schlief[1]; Michael Schindler[1]; Kirstin Brink[1]
[1]Department of Earth Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
Hussain9@myumanitoba.ca

Otoliths develop incrementally and are inert. They retain a record of environmental trace elements to which the fish was exposed to throughout life. Few otolith studies have been conducted to understand the uptake of trace elements in fish from the Red River, Winnipeg, MB, Canada and even less to understand the relation between trace element composition and structural features at the nanoscale. As such, this study aimed to (a) use otoliths to understand phosphate uptake in channel catfish (*Ictalurus punctatus*), as a proxy to understand contamination levels in the river, and (b) examine whether phosphate concentrations control nanoscale structural features in otoliths. Laser Ablation Inductively Coupled Mass Spectrometry (LA-ICP-MS) line scans were used to identify areas with phosphate concentration in the ppm range. These areas, with an average value of 600ppm, occurred closer to the nucleus of the otolith. Next, an ion mill was used to thin the phosphorus-rich area to electron transparency, parallel to the LA-ICP-MS line, in order to investigate samples with Transmission Electron Microscopy (TEM). Results showed higher amounts of phosphorous occurred in in discrete nano-size domains in areas with a higher number of aragonite crystal lattice orientations than in those characterized by less crystal lattice orientations. This also includes crystal lattice fringes associated with vaterite and calcite types, not just aragonite. In the aragonite, in the areas with elevated levels of phosphorus, gaps between individual domains were observed and the fringes bend and are discontinuous. This study suggests that the presence of phosphate disrupts aragonite growth, and may have implications for interpreting the mineralization patterns of otoliths.

Keywords: Trace Elements, Nanoscale, Aragonite, Vaterite, Calcite

RETURN TO INDEX

IOS_260

Scratching the surface: high resolution elemental maps of fish biominerals

John A. Mohan[1]*, Benjamin LaFreniere[1], Leonardo Edmondson[1], Michelle Passerotti[2], Alicia Cruz-Uribe[3]
[1]School of Marine and Environmental Programs, University of New England, Maine, USA; [2]NOAA Fisheries Northeast Fisheries Science Center, Apex Predators Program, Rhode Island, USA; [3]School of Earth and Climate Science, University of Maine, Orono, USA. *jmohan@une.edu

The elemental chemistry of fish biominerals are widely used to address ecological questions that can be applied to fisheries management. The applied use of biomineral geochemistry is dependent on understanding the environmental and physiological factors that influence elemental variation in chronologically accreted structures. Laser ablation inductively coupled plasma mass spectrometry is a common approach that requires decisions on laser spot dimensions, discrete spots versus continuous transects, and where to target ablation on the surface of the biomineral. Using discrete spots or lines has limitations, as the full spatial variation of elemental patterns can be missed. High resolution two-dimensional elemental mapping provides an opportunity to fully quantify the extent of spatial elemental variation in biominerals and inform selection of discrete areas of the biomineral. Here, complete elemental maps will be presented and compared among various biominerals and species of fish and sharks. We will explore high resolution maps of otoliths of demersal groundfish white hake (*Urophycis tenuis*), anadromous migratory striped bass (*Morone saxatilis*), pelagic migratory bluefin tuna (*Thunnus thynnus*), and the mineralized vertebral cartilage of highly migratory blacktip sharks (*Carcharhinus limbatus*), regional endothermic shortfin mako (*Isurus oxyrinchus*) and white sharks (*Carcharodon carcharias*). Case studies will be presented to highlight the utility of elemental maps for enhancing age estimates of fish using both otoliths and vertebrae.

Keywords: Otoliths, Vertebrae, 2D mapping, elasmobranch, teleosts

RETURN TO INDEX

IOS_262

Diet effects on tissue-diet fractionation (TDF) factors of stable nitrogen and carbon isotopes in muscle, heart and otolith organic matter

Godiksen JA, Chung MT, Irgens C, Grønkjær P

Reconstruction of the trophic position of a fish can be performed by analysing stable nitrogen and carbon isotopes in otolith protein. Together with existing otolith reference collections this can provide long time-series of stable isotope chronologies and, hence, knowledge on historical changes in food web structure and fish trophic ecology. This study aims to establish the tissue-diet fractionation (TDF) factors of nitrogen and carbon recovered from otolith organic matter, as well as muscle and heart tissue. A controlled diet experiment with two years old cod (*Gadus morhua*) was conducted over an eight-month period. The fish were fed five different diets (cod, herring, capelin, shrimp, and blue mussels), representing lowest to highest trophic levels found in the natural diet of cod. The diet specific TDF's of in muscle tissue varied from 1.78 to 4.62‰ for nitrogen and -1.15 to 4.11‰ for carbon. For heart tissue, the TDFs varied from 2.32 to 5.54‰ for nitrogen and -1.56 to 3.29‰ for carbon and finally, for the otolith protein the TDFs varied from -1.05 to 1.22 ‰ for nitrogen and -1.60 to 2.94‰ for carbon. TDF's were smaller and generally more consistent across diets for otolith protein. Among diets, the fish feed cod showed the highest TDF's. The present study suggest that cod otolith protein stable isotope values are reliable indicators of diet isotope values and can be used to reconstruct contemporary and historical trophic positions of cod.

Keywords: TDF, Otolith, Isotopes, *Gadus morhua*.

RETURN TO INDEX

THEME II

EVOLUTIONARY, BIOLOGICAL AND BIOGEOGRAPHIC CHARACTERISTICS

RETURN TO INDEX

IOS_ 026

Otolith-based ichthyofauna from Cassian Formarion (Carnian), Northern Italy

Maciej Krzysztof Pindakiewicz[1]*

[1]Institute of Paleobiology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland. *mpindakiewicz@twarda.pan.pl

The fossiliferous beds of the Late Triassic Cassian Formation: 237-227 mya (Northern Italy, Southern Alps) is renowned for hosting the most diverse early Mesozoic marine invertebrate fauna known worldwide. It is also known to yield some well-preserved vertebrates fossils, in particular from the base of the formation. The upper part in turn provided only a few chondrichthyan and actinopterygian teeth and scales. The otholiths in the Cassian Formation are excessively rare and only one specimen found at Settsass-Scharte locality has been reported as a possible palaeoniscid by Nützel & Kaim (2014). Our subsequent investigations resulted in the find of additional otoliths in the same locality and several specimens in Picolbach and Misurina Skiliftt localities in the same formation. We compared the Cassian otoliths to their recent and Mesozoic counterparts. We argue that most of the otoliths from Picolbach and Misurina belong to very young, or even larval lepisosteiform neopterygian. Morphology of Late Triassic giglymodian otoliths, suggests an plesiomorphic or basal form of larvae inner ear in this group. Otoliths from Settsass Scharte are more reminiscent of sphaeronchid teleosts rather than palaeoniscids. Otoliths from the Cassian Formation shows similar fish assemblage known, from younger, Polzberg locality in Austria (dated as the Norian : 227-208,5 mya). Therefore, the otolith fauna from the Cassian Formation represents neopterygians and teleosteomorphs domination, and the early stage in teleost diversification.

Keywords: Otoliths, Ginglymodi, Teleosts, Cassian Formation, Polzberg.

RETURN TO INDEX

IOS_027

Paleoecology and diversification pattern of fishes and cephalopods in Mesozoic epicontinental seas based on otoliths and statoliths

Maciej Krzysztof Pindakiewicz[1]*
[1]Institute of Paleobiology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland. *mpindakiewicz@twarda.pan.pl

Majority of research on Mesozoic fishes and cephalopods is based on body macrofossils. The “ear stones” (otoliths in fishes and statoliths in cephalopods) received much less attention. Nevertheless they may prove to be a source of important information on paleoecology and paleogeography of these two groups in sediments where other fossils are rare or absent. Statoliths are well developed structures occurring in cephalopods which are roughly analogous to fish otoliths. We investigated otoliths from the Cassian Formation : 237-227 mya Late Triassic) in Northern Italy, which most likely belong to very young lepisostiform fish and few otoliths, that may represent the oldest teleost fishes. No Triassic statoliths are known to date. The investigated associations of Jurassic “ear stones” display low diversity of otoliths and ubiquity of cephalopod statoliths. Subsequently, we investigated a rich Valanginian (Early Cretaceous) otolith-based ichthyofauna from Wąwał, central Poland. This unique assemblage shows a considerable teleost diversity. This fossil association also reveals a significant shift in the abundance ratio of fish otoliths vs. cephalopod statoliths in fully marine deposits, with otoliths much more abundant than the statoliths. Cephalopod statoliths from the Cretaceous, are unique microfossils that fill the gap in the fossil record between Jurassic and Cenozoic forms, of which few of them are oldest representatives of the Recent groups. The comparison of the Jurassic and the Cretaceous assemblages shows huge ecological shift between teleosts and cephalopods abundance. From Cretaceous localities we found rich, and diverified teleost otolith assemblages with only singular cephalopod statoliths. Despite that fact, both groups had radiation events, which teleosts were more visible in the fossil record. Finding reasons behind these events could also answer why the teleosts are the most common nectonic group of animals in the Recent.

Keywords: Paleoecology, Cephalopods, Otolith, Statolith.

RETURN TO INDEX

IOS_034

Isotope chronologies in eye lenses reveal deficiencies in marine diets linked to reproductive failure in California Salmon, USA

Rachel C. Johnson[1,2]*, Carson Jeffres[2], Steve Litvin[3], Iliana Ruiz-Cooley. I.[4], Jacques Rinchard J. [5], Abbigail Ward[2], Alexandra Chu2, Danhong Li[2], Miranda Bell-Tilcock[2], Matt Emard[2], John Field[1], Nate Mantua[1], Steve Lindley[1], Miles Daniels[1,6], Donald Tillitt[7], Dale Honeyfield[7], Bruce Finney[8]
[1]NOAA Fisheries, Southwest Fisheries Science Center, USA [2]University of California Davis, Center for Watershed Sciences, USA [3]Monterey Bay Aquarium Institute, USA 4. Moss Landing Marine Laboratory, USA [5]State University of New York, Brockport, USA [6]University of California Santa Cruz, USA 7. Retired U.S. Geological Survey, USA [8]Idaho State University, USA

Understanding how factors in one aquatic habitat influences an organism's growth, survival and reproductive success at later life stages is one of the greatest challenges in the conservation and management of migratory fishes. Thiamine Deficiency Complex (TDC) is a nutritional deficiency of thiamine (vitamin B1) that has been observed worldwide in diverse taxa. However, it has recently emerged as a significant stressor to Pacific Salmon linked with high mortalities of early life stages of Chinook salmon in California's Central Valley, USA. TDC is caused by female Chinook salmon not acquiring enough thiamine from their ocean diets to provide needed nutrition in their eggs that their young require for proper development. Here, we use $\delta^{13}C$ and $\delta^{15}N$ in archival tissues (salmon eye lenses) to test the hypothesis that females with low egg thiamine levels fed on a narrow diet of anchovies. Anchovies are known to produce thiaminase an enzyme that destroys thiamine in consumers. Anchovy abundances were found to be anomalously high in 2019-2022. Our work to-date has revealed low levels (3.1 ± 1.5nmol/g) of egg thiamine in endangered winter run Chinook salmon prior to spawning resulting in ~50% thiamine-dependent mortality in 2021 and 2022. Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ measured in sequential eye lens laminae provide a chronology of ocean diets prior to spawning that will be linked to egg thiamine concentrations. TDC is an emerging stressor to California salmon and understanding these aquatic linkages will be critical in assessing causes, impacts, and potential mitigation opportunities.

Key: words: Isotope chronology, Thiamine Deficiency Complex (TDC), California Salmon.

RETURN TO INDEX

IOS_038

Otolith shape variation among ontogenetic stages and cryptic species of the flathead grey mullet *Mugil cephalus* in Taiwan

Qin-Yun Wu[1]*, Chia-Hui Wang[1]
[1]Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan.
*elaine08032001@gmail.com

The cosmopolitan flathead grey mullet *Mugil cephalus* is one of the economically important fish in Taiwan. Three cryptic species previously defined as NWP1, NWP2 and NWP3, were known to occur around the estuaries in Taiwan. Although being clearly distinguished by mitochondrial DNA, their morphological differences were subtle. Otolith shape is known to be intraspecific unique, yet it may be influenced by physiology, fluctuations in environmental condition, and intraspecific ontogeny.

This study aimed at (1) examining the otolith shape variations in cryptic species of mullet between ontogenetic stages by conducting control rearing experiment; (2) justifying the effectiveness of using otolith shapes to identify the cryptic species (NWP1, NWP2). Our samples were categorized into five groups: 1st Rearing experiment, 2nd Continuous marking, 3rd One year-old aquaculture subadult, 4thWild juvenile, and 5th Wild adult. The samples of 1st and 2nd group were marked by oxytetracycline hydrochloride (OTC) and then reared under water temperature at 24±0.3°C and salinity at 15 psu for 20 weeks. Otoliths were extracted and otolith shapes were analyzed by the elliptic fourier analysis (EFA). Results showed that the otolith shape stabilized after being reared for 12 weeks with average age ranging from 125–136 days, but the otolith shape of the 3rd group wasn't stable (post hoc test, $p<0.05$). The otolith shape of the juvenile in the 4th group cannot successfully discriminate cryptic species, but discriminating accurate (>80%) was found in the adult at the 5th group. In conclude, otolith shape varies within ontogeny and environmental changes, which can be used as a sensitive tool for cryptic species identification in the future studies.

Keywords: Elliptic fourier analysis, Ontogeny, Oxytetracycline hydrochloride

RETURN TO INDEX

IOS_ 055

Advantages of the online tools (AFORO) in otolith descriptions of recent first citations in the Mediterranean Sea found in fisheries continuous monitoring (ICATMAR)

Antoni Lombarte[12]*, Laura Recasens[12], Victor Manuel Tuset[3]
[1]Institut de Ciències del Mar-CSIC, Barcelona, Catalonia, Spain; [2] Institut Català de Recerca per a la Governança del Mar, ICATMAR (ICM-CSIC) Barcelona, Catalonia, Spain; [3]Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, Unidad Asociada ULPGC-CSIC, Canary Islands, Spain;. *toni@icm.csic.es

Since 2018, as a consequence of the continuous monitoring of commercial and recreational fisheries developed into ICATMAR framework, the knowledge of the ichthyofauna present in the NW Mediterranean has increased. Four new species were found and described at first time for the Mediterranean Sea (*Polymetme corythaeola*, *Cheilopogon pinnatibarbatus*, *Paracaristius maderensis* and *Antigonia capros*) and two more species for Western Mediterranean waters (*Sudis hyalina* and *Acanthopagrus bifasciatus*). Accurate technics of extraction of the sagittae otolith was used to minimizing damage of the specimens. These otoliths will help in future studies linked to trophic ecology, taxonomy and alien species effects. Our objective is providing anatomical and numerical information (elliptic Fourier, wavelets and morphometrics measurements) using the online system of AFORO, including geographical, ecological and temporal information of these six specimens. The capacity of the online databases to upload continuously new specimens, to update their information and to stablish networks with other database (as GBIF or FishBase) shows a clear advantages in front to classical analogical systems as Guides and Atlas in order to updating and disseminating the information.

Keywords: Otoliths, fisheries monitoring, online databases, alien species, Mediterranean Sea.

RETURN TO INDEX

IOS_ 057

Twenty years of AFORO. New developments and connections to contribute otolith research

Amalia Manjabacas[1], Òscar Chic[1], Emili García-Ladona1, Víctor Manuel Tuset[2], Pere Martí[3], Josep Forest[4], Antoni Lombarte[1]*, [1]Institut de Ciències del Mar, Barcelona, Catalonia, Spain; [2]Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, Unidad Asociada ULPGC-CSIC, Campus de Taliarte, 35214 Telde, Gran Canaria, Canary Islands, Spain; [3]Grup de Processament del Senyal. Universitat of Vic (UVIC-UCC), Vic, Catalonia, Spain; [4]Institut de Recerca en Visió per computador i Robòtica, Universitat de Girona, Girona, Catalonia, Spain; *toni@icm.csic.es

Since 2003, with the aim of addressing different issues related to otoliths and the advancement of technologies, AFORO acronym of Anàlisi de FORmes d'Otòlits (Shape analysis of otoliths), a computational environment with a set of tools including a website for this purpose, has gradually expanded its functionality. This implies different ways to measure otoliths in order to obtain different type of information over a catalogue that contains images of georeferenciated worldwide 7924 specimens corresponding to 2716 species and 274 families of otoliths sagitta in Actinopeterygians fishes. The 2D otoliths shape description using different methods give a comparative view between classical methods (biometrics and Fourier) and the Wavelet transform, used in the automatic classification system of AFORO. Furthermore, the automatic classification system has been improved by incorporating geographical filters. The 3D otoliths description in a few images opens the possibility of classifications using volumetric information, which they show a high performance. To increase utilities, a dynamic table with relationships between otolith and fish length has been added. In order to create an interconnected network, AFORO has been established linkages with database systems as CBMR-ICM, GBIF and recently a bidirectional linkage with Osteobase and FishBase. The diverse information that AFORO offers on the otoliths opens the possibility to approach new studies or to improve the existing ones combining this information.

Keywords: Otoliths, Sagittae, shape analysis, wavelets, automatic taxon identification

RETURN TO INDEX

IOS_062

Correcting incorrect identifications

Bradley Moore[1], Jason Hamill[1], Alan Hart1, Richard Saunders[1], Keren Spong[1]*, Gretchen Skea[2]
[1]National Institute of Water and Atmospheric Research Limited (NIWA), Wellington, New Zealand. [2]Fisheries New Zealand, Ministry for Primary Industries, Wellington, New Zealand. *keren.spong@niwa.co.nz

Three species of jack mackerel (Pisces: Carangidae) are found in New Zealand waters. Two of these are endemic (*Trachurus declivis* (Jenyns, 1841) and *Trachurus novaezelandiae* Richardson, 1843), while a third species, *Trachurus murphyi* Nichols, 1920, first appeared in the mid-1980s. The management of these species relies on accurate species identification and age estimation however identification is known to be problematic, while ageing is costly. We evaluated the performance of using otolith morphometrics to obtain estimates of species identification and age for these three species. Our results suggest that otolith metrics can be used to predict species with a high degree of certainty, and may therefore be used to confirm ongoing or historical species identifications. Otolith metrics also provided a good proxy for age albeit with less certainty, particularly for older individuals.

Keywords: *Trachurus declivis, Trachurus novaezelandiae, Trachurus murphyi*, otolith, jack mackerel

RETURN TO INDEX

IOS_067

Stock identification of red mullet in the Mediterranean Sea: Comparative analysis of otolith shape from 2D and 3D images

Nicolas Andrialovanirina[1, 2]*, Émilie Poisson Caillault[1], Sébastien Couette[3,4], Rémi Laffont[4], Lauriane Poloni[3,4], Camille Lutet-Toti[4,5], Kélig Mahé[2]
[1]Université du Littoral Côte d'Opale, UR 4491, LISIC, Laboratoire d'Informatique Signal et Image de la Côte d'Opale, F-62100 Calais, France; [2]Ifremer, Fisheries laboratory, Channel and North Sea Fisheries Research Unit, Boulogne-sur-mer, France; [3]Ecole Pratique des Hautes Etudes, PSL Université, Paris, France; [4]Biogéosciences, UMR 6282, CNRS, Université de Bourgogne, 21000 Dijon, France; [5]Alma Mater Studiorum - Università di Bologna, 40126 Bologna, Italy. *nicolas.andrialovanirina@ifremer.fr

One of the most commonly used methods for fish stock identification worldwide is otolith shape analysis, which is the result of a combined effect of environmental and genetic factors. Previous studies on otolith shape have relied on two-dimensional (2D) images, showing only a partial representation of the whole shape of the otolith. In a recent study using three-dimensional (3D) images, researchers observed that the otolith of red mullet (*Mullus barbatus*) in the Mediterranean Sea presented a significant asymmetry between the two inner-ear sides (i.e. left and right), which was not detectable through the same analysis from classical 2D images. The present study aims to identify the stocks of red mullet in the Mediterranean Sea using both 2D and 3D images, and investigate whether 3D analysis provides greater accuracy or additional information. Sagittal otoliths of 423 red mullet adults from 21 locations in the Mediterranean Sea were studied using Fourier harmonic descriptors from 2D outlines and spherical Fourier harmonic descriptors from 3D meshes to describe the external shape of the otolith. Several factors were analysed on the otolith shape: the symmetry between right and left otoliths, sexual dimorphism and spatial distribution from a combination of two statistical approaches. The first analysis used hierarchical clustering, which is an unsupervised machine learning method used to group individuals with similar otolith shapes. The second analysis used linear discriminant analysis with jackknifed prediction, which is a supervised machine learning approach used to evaluate the percentage of correct classification. Both the 2D and 3D analyses indicated that the Mediterranean Sea could be separated into three stock units for red mullet with the Eastern, the Central, and the Western components. If the result of stock discrimination was similar from the 2D and 3D images, the accuracy of correct classification was better with 3D images than in 2D (35% correct classification in 2D and 46% in 3D). This difference was explained mostly by the individuals from the western component. This study shows that this type of 3D approach should be developed to increase the accuracy of stock discrimination versus classical 2D studies.

Keywords: Stock identification, otolith shape, Fourier analysis, 2D, 3D, Mediterranean Sea, *Mullus barbartus*

RETURN TO INDEX

IOS_128

The diversity of otolith shapes: an ecomorphological study at a large phylogenetic scale

Arthur Van Damme[1,2]*, Antoni Lombarte[3], Bruno Frédérich[2], Ricardo Betancur-R.[4], Effrosyni Fatira[5], Tanja Schulz-Mirbach[6], Aline Paiva M. de Medeiros[4,7], Eric Parmentier[1], Victor M. Tuset[5].
[1]Laboratory of Functional and Evolutionary Morphology, Freshwater and Oceanic Science Unit of Research, University of Liège, Belgium; [2]Laboratory of Evolutionary Ecology, Freshwater and Oceanic Science Unit of Research, University of Liège, Liège, Belgium; [3]Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain; [4]Department of Biology, University of Oklahoma, Norman, USA; [5]Instituto de Oceanografía y Cambio Global (IOCAG), Universidad de Las Palmas de Gran Canaria (ULPGC), Las Palmas de Gran Canaria, Spain, [6]Department Biologie II, Ludwig Maximilians Universitaet Muenchen, Planegg-Martinsried, Germany; [7]Programa de Pós-Graduação em Ciências Biológicas, Universidade Federal de Paraíba, João Pessoa, Brazil.
*arthur.vandamme@uliege.be

Ecomorphology is the field exploring the links between species morphology and its environment to better understand the adaptative value of a given phenotype. Different studies have now applied this discipline trying to explain intra- and interspecific variability of fishes' otolith shape without pointing towards a consensus involving environmental influence across teleosts. The present work aims to decipher the major drivers of teleosts' otolith diversity. Here, we will test the relationships between various ecological functional traits and otolith shape at a large taxonomic scale. We therefore gathered life history traits and functional characters for 697 fish species. Collected information included species ecology (habitat, mobility, gregariousness, feeding habits, sound production), body characteristics (body shape, mouth position, size class), swimming mode and sensory features (hearing specializations, bioluminescence, barbels). Otolith and sulcus shapes were quantified with landmark-based geometric morphometric methods. Phylogenetic information were retrieved from Betancur *et al*. (2017). Within the scope of this study, three main objectives were addressed: (1) to compare morphological and phylogenetic clusters and assess their discrepancies, (2) to seize the influence of fish ecology and functional traits on their otolith morphology and finally (3) to correlate functional and otolith morphological diversity of clusters and their evolution along the tree of life.

Keywords: Sagittae, Shape, ecology, Functional diversity.

RETURN TO INDEX

IOS_129

Reconstructing fish movements and trophic level shifts throughout the life history with ocean isoscapes

Hsiao, Han-Chun*1, Hsieh, Chi-Yuan1, Lee, Mu-Ting1, Liu, Tze-Yen1,2, Kao, Li-Ling1, Chung, Ming-Tsung1
1Istitute of Oceanography, National Taiwan University, Taiwan; 2Marine Research Station, Academia Sinica, Taiwan.
*r11241211@ntu.edu.tw

Fishes play an important role in marine food webs and energy transfers through trophic levels. However, the interaction between fish growth, movement, and trophic level is complex in the natural environment, so reconstructing ontogenetic trophic levels of marine fishes associated with migration is challenging. Over the past decades, stable isotope values recorded in calcified structures provide a way towards understanding fish movement and trophic ecology. We propose an integrated approach to comprehensively evaluate the trophic position during fish growth. First, we established ocean isoscapes around Taiwan by analysing seawater δ^{18O} values and particulate organic matter (POM) $\delta^{15}N$ values to provide the environmental baseline. Second, we conducted case studies on dolphin fish (*Coryphaena hippurus*) and blackhead seabream (*Acanthopagrus schlegelii*), both commercially important and highly migratory species. The migration routes of individual dolphin fish and blackhead seabream throughout their lifetime were reconstructed by analysing $\delta^{18}O$ of otolith carbonates in comparison to the seawater $\delta^{18}O$ baseline. Moreover, the trophic levels at different life stages were determined by analysing $\delta^{15}N$ of bone organic matter in comparison to the POM $\delta^{15}N$ baseline. Owing to a prior determination of ontogenetic habitat shift, we could more precisely calculate the trophic levels based on the difference between bone and baseline $\delta^{15}N$ values, minimising the bias of spatial variation in baseline values. Combining these data, we detailed the individual life history of dolphin fish and blackhead seabream, demonstrated a comprehensive method using multiple isotope systems with various calcified structures to study the ontogenetic trophic positions of fishes in the marine food web.

Keywords: Otoliths, Bones, Isotope, $\delta^{15}N$, $\delta^{18}O$, *Coryphaena hippurus*, *Acanthopagrus schlegelii*

RETURN TO INDEX

IOS_168

Consistent growth rates and recruitment failure of Namibian West coast steenbras, an over-exploited Sparid confirmed with otoliths, length-frequency-analysis and tag-and-recapture data

Margit R. Wilhelm[1]*, Arariky Shikongo[1], Angelika Veii[1], Ralf Schwamborn[2]
[1]Department of Fisheries and Ocean Sciences, University of Namibia, Henties Bay, Namibia. [2]Oceanography Department, Federal University of Pernambuco (UFPE), 50670-901 Recife, Brazil *mwilhelm@unam.na

West coast steenbras *Lithognathus aureti* is an overexploited coastal Sparid in the northern Benguela Namibia. They are believed to consist of two genetically distinct highly resident populations, one in the north (17-24oS) and one in the south (24-26oS). In this study, we use three sources of data; namely recent age data derived from otoliths (2019-2022) for the northern and southern population, tag-and-recapture data (2004-2011 and 2019-2022) for the southern population, and length-frequency distribution data (2004-2011, 2019-2022 for the southern population and 1995-2018 for the northern population) to confirm consistent growth rates of about 10 cm per year of West coast steenbras from the 1990s until current. We demonstrate that a recruitment failure since the early 2000s in both populations allows for clear definition of cohorts, but puts this species on the endangered' list. We further use the bootstrapped ELEFAN (ELEFAN_boot) to estimate 95% confidence limits of growth rates calculated from all three sources of data. With this we demonstrate a novel approach of estimating confidence limits on growth data and also demonstrate that all three techniques of growth estimation complement each other and sampling for all three should continue on a regular basis.

Keywords: Otoliths, ELEFAN_boot, catch-and-release, length-frequency analysis, Benguela, Sparidae, *Lithognathus,* recruitment failure, bootstrapped ELEFAN

RETURN TO INDEX

IOS_185

The Cenozoic collapse of the West Tethyan biodiversity hotspot: a test using the fish otolith fossil record

Hadeel Saad[1,2]*, Matt Friedman[1,2]
[1]Department of Earth and Environmental Sciences, University of Michigan, Ann Arbor, USA; [2]University of Michigan Museum of Paleontology, University of Michigan, Ann Arbor, USA. *hhsaad@umich.edu

Marine biodiversity hotspots have disproportionately greater species richness than surrounding regions. The species richness and locations of these hotspots are thought to have changed throughout the Cenozoic—transitioning from the West Tethys in the Eocene to the Arabian Peninsula during the Oligocene and finally arriving in the modern-day Indo-West Pacific in the Miocene. Despite extensive research that has examined the geographic shifts in the marine invertebrate biodiversity throughout the Cenozoic, direct paleontological investigation of patterns for teleost fishes remains unexplored. Although the body fossil record for marine teleost fishes is sparse, the record of teleost otoliths represents a tool for testing a hypothesized collapse of the West Tethyan hotspot. Literature data were collected from representing 125 localities dating from the Paleocene through the Miocene and nearly 100,000 individual otolith fossils. Since the relative abundance of species in these faunas are known, the richness of individual assemblages can be meaningfully compared using coverage-based rarefaction (i.e., shareholder quorum subsampling), which standardizes richness estimates based on equal coverage of underlying abundance distributions. Using these statistical methods, local richness shifts from the West Tethys to the Indo-West Pacific during the Paleogene to Miocene interval could not be identified. Furthermore, regional diversity in the West Tethys appears to decline in the late Miocene, rather than the expected early Miocene. These results do not align with the extensive literature on the eastward shift of marine invertebrates into the Indo-West Pacific. It is unclear if teleost fishes made the same migration pattern as other marine organisms or if their shift occurred much later in time.

Keywords: Migration, Paleoecology, Hopping-hotspots

RETURN TO INDEX

IOS_218

Otolith from the deep sea, the case of *Argyropelecus hemigymnus* (Cocco, 1829) from the Strait of Messina (Central Mediterranean Sea)

Gioele Capillo[1]*, Marco Albano[1], Claudio D'Iglio[2], Giovanni Lanteri[2], Sergio Famulari[2], Nunziacarla Spanò[3], Serena Savoca[3]
[1]Department of Veterinary Sciences, University of Messina, Messina, Italy [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy [3]Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, Messina, Italy. *gcapillo@unime.it

Deep sea teleost are often involved in ecological frameworks connecting deep and shallow environments due to their ability to perform diel vertical migrations. The hatchefish *Argyropelcus haemigymnus* (Cocco, 1829) is a mesopelagic teleost species adapted to deep sea environment through several morphological and physiological features, like telescopic eyes, laterally flattened and deep-bodied with ventral series of photophores. *A. haemigymnus* inhabits mean depth between 300 m and 1000 m with a circumglobally distribution and performs diel vertical migrations for trophic and reproductive strategies. In the area of the Messina's Strait this behavior, joint with the intense hydrodynamics and the peculiar orography of the bottom, led to the stranding of specimens at the shoreline, allowing researchers to collect this and other mesopelagic species just by walking on the shore. In this way 50 specimens were collected during spring 2022. Once in the lab specimens were measured, weighted, and sexed and saccular, utricular, and lagenar otoliths extracted. Each otolith was polished from tissue remains using 3% H_2O_2 for 15 minutes and Milli-Q water. Stereomicroscope Zeiss Discovery V8 equipped with Axiocam 208 colour camera was used for two images acquisition (one for each otolith face). Otolith morphological measurements were performed using ImageJ 1.48p software and shape analyses using shape R, an open-access package that runs on R software. Finally, otoliths were observed through SEM analysis with a Zeiss EVO MA10 at the acceleration voltage of 20Kv. The performed analyses allowed to discriminate among the three pairs of otoliths asymmetry and differences among four different size classes. Specifically, morphometric parameters, shape, external textural organization, resulted in intraspecific variability. Data about *A. haemigymnus* otoliths features and intraspecific variabilities improve the basic knowledge about ecomorphological adaptation of teleost to deep sea habitats and such peculiar environment, as the Strait of Messina, can affect their morphology and variability. Moreover, specific data on this species saccular, utricular, and lagenar otoliths are of fundamental taxonomical importance for the contribution of the species in diet of both meso- and epi-pelagic predators, improving knowledge regarding link between deep and shallow ecosystems.

Keywords: Hatchefish, Deep Sea teleost, Shape Analysis, Intra-specific variability

RETURN TO INDEX

IOS_220

Intra-specific variability of the saccular, utricular and lagenar otoliths of the garfish *Belone belone* (Linnaeus, 1760) from South-Western Ionian Sea (Central Mediterranean Sea)

Marco Albano[1]*, Gioele Capillo[1], Claudio D'Iglio[2], Sergio Famulari[2], Nunziacarla Spanò[3], Serena Savoca[3]
[1]Department of Veterinary Science, University of Messina, Messina, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Science, University of Messina, Messina, Italy; [3]Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, Messina, Italy. *marco.albano@unime.it

Improving the knowledge base on commercially important pelagic species is necessary to enhance their conservation. Knowledge gaps regarding the biology of many taxa remain within the Mediterranean Sea. In this context, otoliths are essential to assessing teleost's life history traits. *Belone belone* (Linnaeus, 1760) is an epipelagic and predator species recently accepted as the only valid endemic species of this genus in the Mediterranean Sea and the eastern Atlantic Ocean. It inhabits the offshore areas, moving near the coast during spawning periods, representing an important commercial species in the Central Mediterranean Sea. In addition, garfish play a fundamental ecological role as prey and predator in the pelagic domain. Although some biological and ecological aspects of the species were investigated, relatively scarce data are reported on garfish otoliths. The present study aims to analyze the three otolith pairs of garfish from the Ionian Sea through morphological, morphometrical and shape analysis to study the intra-specific variability of *sagittae*, *lapilli* and *asterisci* between different size classes. Seventy-five specimens of *B. belone* with a total length (TL) ranging from 175 to 375 mm were obtained during summer 2022 from a local market, supplied by artisanal fisheries operating in the Ionian Sea. Once in the laboratory, each specimen was weighed, measured, sexed and assigned to a size class according to its TL. Each otolith pair was extracted, processed, and photographed under a stereomicroscope. Otolith images were processed for several measurements and relationships, and shape descriptors were calculated. The morphometrical analysis highlighted how the shape descriptors were in line with the contours' variability detected by shape analysis. Any asymmetry was detected in all otolith pairs. However, significant sagitta parameter variations were found between sex and size classes. Considering the few studies on *sagittae* and that present data are the first reported on *lapilli* and *asterisci* of *B. belone* from the Central Mediterranean Sea, this study represents an essential contribution to the knowledge of this species and its population from this area. Indeed, these morphometrical and shape analysis data could help distinguish separated fish stocks of *B. belone* through comparison with those obtained from future studies.

Keywords: Fisheries, Pelagic fishes, Beloniformes, Shape analysis, Morphometry.

RETURN TO INDEX

IOS_221

Paleoichthyology on the isthmus of Panama

Brigida De Gracia[1]*, Aaron O'Dea[1,2]
[1]Smithsonian Tropical Research Institute, Panamá; [2] SENACYT, SNI, Republic of Panamá.

Formation of the Isthmus of Panama connected North and South America and isolated the Caribbean from the tropical Eastern Pacific. This geological process spanned over 20 million years and culminated in the final separation of the oceans approximately 3 million years ago. Consequently, the Pacific and Caribbean Seas today exhibit distinct characteristics, with the Pacific experiencing strong upwelling regions in stark contrast to the generally oligotrophic Caribbean Sea. In this presentation, we provide an overview of the extensive ichthyological fossil record found on the Isthmus of Panama and how it can be used to explore questions about evolution, ecology and conservation. To facilitate the identification of the fossil otoliths, we curated a comprehensive fish otolith reference collection, consisting of 7,549 otoliths extracted from 173 species of fish captured in coastal shelf, reef, and mangrove habitats in the Pacific and Caribbean. Researchers interested in using this continually expanding reference collection may do so at the Naos Marine Laboratories of the Smithsonian Tropical Research Institute in Panama City. We collected and examined over 400 grab and dredge samples from soft sediments on both sides of the Isthmus. From these samples, otoliths were carefully extracted and identified to at least the family level and often higher. These modern otolith assemblages provide valuable insights into the taxonomic and ecological distinctions within shelf ecosystems on either side of the Isthmus. In addition to these contemporary samples, we have obtained well-dated cores from coral reef matrices on both sides of the Isthmus, allowing us to document changes in reef fish assemblages over the past 7,000 years. Finally, collecting and washing hundreds of bulk samples of Neogene and Pleistocene fossiliferous sediments have revealed thousands of otoliths that offer a unique opportunity to investigate the dynamic evolution of Caribbean fish assemblages over the past 10 million years. By presenting these diverse collections of otoliths, we aim to stimulate evolutionary, ecological, and conservation-related ichthyological research on the Isthmus of Panama.

Keywords: Coral reefs, macroevolution, reference collection, tropical ecology

RETURN TO INDEX

IOS_223

Morphometry of the Sagittae of the Deep-Sea Grenadiers Collected on the Brazilian Continental Slope (*Macrouridae: Gadiformes*)

César Santificetur[1]*, Guilherme R. de Faria[1], Julia Yumi Ibanhez[1], Marcelo R. S. Melo[1]
[1]Laboratório de Diversidade, Ecologia e Evolução de Peixes - DEEP Lab,. Instituto Oceanográfico, Universidade de São Paulo, São Paulo – SP. E-mail: *csantificetur@usp.br

The family Macrouridae, commonly known as rattails or grenadiers, includes 372 valid deep-sea species. The samples were collected on the Brazilian continental slope off São Paulo and Santa Catarina States, on board the N/Oc Alpha Crucis at depths ranging from 200 to 1,500 meters, using bottom trawl nets. Macrourids were some of the most abundant families obtained. The fishes were measured for the total length, pre anal-fin length, and weighted. The left sagittae were extracted from the six most abundant specie: *Cetonurus globiceps* (Vaillant 1884) (n = 5), *Coelorinchus marinii Hubbs 1934* (n = 115), *Hymenocephalus billsam* Marshall & Iwamoto 1973 (n = 80), *Malacocephalus occidentalis* Goode & Bean 1885 (n = 20), *Nezumia aequalis* (Günther 1878) (n = 7), and *Ventrifossa macropogon* Marshall 1973 (n = 9). Four morphometric indices were calculated for each species: circularity, rectangularity, aspect ratio, and the ratio of otolith area to *sulcus acusticus* area. The mean and standard deviation of the morphometric indices for each species were as follows: *C. globiceps* (14.076 ± 0.91; 0.723 ± 0.004; 84.67 ± 5.36; 16.59 ± 3.08), *C. marinii* (16.55 ± 0.74; 0.71 ± 0.04; 60.93 ± 4.54; 31.75 ± 5.13), *H. billsam* (14.44 ± 0.57; 0.70 ± 0.02; 93.33 ± 5.43; 27.16 ± 3.95), *M. occidentalis* (17.01 ± 0.68; 0.72 ± 0.02; 52.58 ± 2.56; 25.75 ± 2.20), *N. aequalis* (14.93 ± 0.55; 0.72 ± 0.02; 21.96 ± 2.22; 26.15 ± 1.59), *V. macropogon* (15.38 ± 0.41; 0.75 ± 0.02; 61.96 ± 2.78; 28.77 ± 2.34). The Kruskal-Wallis and Mann-Whitney tests showed significant differences among the species. The aspect-ratio index effectively differentiated four out of six species, revealing similarity between *C. marinii* and *V. macropogon*, the latter having a higher rectangularity. Circularity distinguished *C. marinii* and *M. occidentalis* from other species, while the area to *sulcus acusticus* area ratio distinguished *C. globiceps* and *H. billsam*.

Keywords: Morphometric index, Southwest Atlantic, N/Oc Alpha Crucis, Otolith

RETURN TO INDEX

IOS_230

Morphometric analysis of otoliths lapillus in *Arius proops* (Siluriformes, Ariidae) from a macrotidal estuary in Brazil

Juliana Azevedo[1]*, Bruno Marassi[2], Larrisa Miranda[2], Elisabete Braga[3]
[1]Institute of Environmental Science, Chemical and Pharmaceutical, Federal University of São Paulo, Diadema, Brazil; [2]Environmental Sciences Undergraduated, Institute of Environmental Sciences, Chemical and Pharmaceutical, Federal University of São Paulo, Diadema, Brazil. [3] Oceanographic Institute, São Paulo University, São Paulo, Brazil. *juliana.azevedo@unifesp.br

Estuaries are coastal ecosystems that are subject to tidal inflow and dominated by physical, chemical, and biological processes. The coastal areas of the municipality of Raposa (Maranhão, Brazil) are used for several socio-economic activities, such as fishing. The Raposa estuary is influenced by the bays of São José and São Marcos and is subject to macrotidal dynamics with amplitudes up to 6.0m. These aspects can promote changes in the water chemistry, resuspension of sediments and, ultimately, changes in fish ecophysiology. Calcified structures, such as otoliths, are widely used in fisheries science to estimate the age and growth of fish. In addition, morphometric data from fish and otoliths are essential for studies in fish ecology, as they provide information on individual development. The aim of the present study was to investigate the sexual variation in otolith dimensions of *Arius proops* from the Raposa estuary. A total of 50 estuarine catfish *A. proops* (Uritinga) were collected in May 2022 and total length (TL) and total weight (TW) were obtained. Sex was determined by identification of the macroscopic, morphological and colour characteristics of the gonads. Catfish were dissected to remove of the pair of lapilli otoliths from the ear capsule and then measured for length (OL), height (OH) and weight (OW). In general, females of *A. proops* were significantly larger than the males ($TL_{females}$=31.7±6.6cm; TL_{males}=23.7±4.6cm; $p<0.05$). The non-linear regression analyses showed that all the measurements performed had a positive and significant correlation ($p<0.05$), with the weight and height of the otolith being the measurements that best responded to the variation in the length and weight of the fish (TL*x*OW: r^2=0.84; TL*x*OL: r^2=0.79; TL*x*OH: r^2=0.81 / TW*x*OW: r^2=0.95; TW*x*OL: r^2=0.84; Tw*x*OH : r^2=0.60). The weight, length and heigth in otoliths of females were higther than of males ($OW_{females}$=0.176±0.099g; OW_{males}=0.086±0.510/ $OL_{females}$=0.649±0.081cm; OL_{males}=0.547±0.089cm/ $OH_{females}$=0.722±0.133cm; OH_{males}=0.558±0.101cm). Therefore, the lapillus otoliths of *A. proops* increase in size and weight throughout the life of the individual.

Keywords: Otoliths, Uritinga, Sex, Raposa estuary, Maranhão

RETURN TO INDEX

IOS_237

Otolith perimeter as a tool in the identification of coexisting species of silversides in Argentina

Santiago Morawick[i1,2], Patricio Solimano[1], Gustavo Thompson[3], Alejandra Volpedo [3,4]*
[1]Centro de Investigaciones y Transferencia de Río Negro, Universidad Nacional de Río Negro, Argentina
[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Río Negro, Argentina
[3] CONICET-Universidad de Buenos Aires. Instituto de Investigaciones en Producción Animal (INPA Buenos Aires, Argentina
[4] Universidad de Buenos Aires. Centro de Estudios Transdisciplinarios del Agua (CETA), Facultad de Ciencias Veterinarias Buenos Aires Argentina
*avolpedo@gmail.com

Argentina has one of the largest numbers of species of the genus *Odontesthes* that coexist in the world (*Odontesthes argentinensis*, *O. platensis, O. smitti, O. nigricans, O. incisa, O. bonariensis* and *O. hatcheri*). These species have an important ecological role in the trophic webs in the region. However, due to the number of species of the genus *Odontesthes*, many times the identification of otoliths in feces and stomach contents of ichthyophages is complex. The objective of this paper is to use otolith perimeter as a morphometric tool for the identification of seven species of silversides that coexist in marine and freshwater environments of Argentina. The sagittae otoliths were extracted from a total of 369 silversides, which were photographed under a stereoscopic magnifying glass and the images were processed using the ShapeR package of the R statistical software. The Wavelet coefficients (CW) and Fourier descriptors (DF) obtained were analyzed using multivariate methods. The results show that with CW the percentage of correctly classified silversides was 71.27%, while with DF it was 81.75% and using CW and DR together, 88.35% was obtained. The PERMANOVA analysis revealed that there are significant differences ($p<0.0001$) between the otolith perimeter of these species. The otoliths of *O. platensis, O. argentinensis, O. smitti* and *O. bonariensis* have elongated shapes, while those of *O. nigricans* and *O. incisa* are rounded. The greatest variations in shape are observed in the antero-posterior axis of the otolith (*rostrum* and *antirostrum*) and at the posterior end. The results found contribute to the identification of these species of silversides in dietary studies of trophic ecology of ichthyophages in the region.

Keywords: Otolith perimeter, *Odontesthes*, Argentina, Sagittae

RETURN TO INDEX

IOS_267

Otolith shape suggests high plasticity at range edge of *Stellifer naso* (Acanthuriformes: Sciaenidae) on the eastern Brazilian coast

Jonas Andrade-Santos[1]*, Rafael Menezes[2], Marcelo Britto[1]
[1]Setor de Ictiologia, Programa de Pós-Graduação em Zoologia, Universidade Federal do Rio de Janeiro, Museu Nacional, Rio de Janeiro, Brazil; [2]Laboratório de Dinâmica de Populações Marinhas (DIMAR), Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife, Brazil. *jonasandrade@ufrj.br

Stellifer naso occurs from Colombia to northeastern Brazil, with its southern range edge likely located near its type location in Cachoeira, Bahia state. Climate change is driving shifts in species' distribution, particularly impacting species with faster life cycles and smaller body sizes. The aim of our study was to investigate whether there was more plasticity of *S. naso* at its expanding range edge, namely, from Paraguaçu River in Bahia state ("Core") to Doce River in Espírito Santo state ("Edge"). We observed significant variation in body morphology in the Edge zones compared to the Mid and Core zones. This resulted from a variation in the body depth, size and proportion of the second and third spine in the dorsal fin. Otolith shape displayed four main groups of variations: pointed, rounded, and both groups including projections/malformations. Species experience accelerated environmental changes at range edges. Such exposure might have led to modifications mirrored by otoliths and body morphology. Further investigations should assess whether similar patterns occur at the northern distribution edge in Colombia, focusing on environmental predictors and traits that contribute to improved persistence at the range edge.

Keywords: Cline variation, Climate change, Species distribution, Stardrums

RETURN TO INDEX

IOS_271

Evolutionary morphology of the saccular otolith at a large phylogenetic scale

Arthur Van Damme[1,2]*, Victor M. Tuset[3], Bruno Frédérich[2], Ricardo Betancur-R.[4], Effrosyni Fatira[3], Tanja Schulz-Mirbach[5], Aline Paiva M. de Medeiros[4,6], Eric Parmentier[1], Antoni Lombarte[7]
[1]Laboratory of Functional and Evolutionary Morphology, Freshwater and Oceanic Science Unit of Research, University of Liège, Belgium; [2]Laboratory of Evolutionary Ecology, Freshwater and Oceanic Science Unit of Research, University of Liège, Liège, Belgium; [3]Instituto de Oceanografía y Cambio Global (IOCAG), Universidad de Las Palmas de Gran Canaria (ULPGC), Las Palmas de Gran Canaria, Spain; [4]Department of Biology, University of Oklahoma, Norman, USA; [5]Department Biologie II, Ludwig Maximilians Universitaet Muenchen, Planegg-Martinsried, Germany; [6]Programa de Pós-Graduação em Ciências Biológicas, Universidade Federal de Paraíba, João Pessoa, Brazil; 7Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain.
*arthur.vandamme@uliege.be

To date, we still lack evidence of the evolutionary forces driving the diversity of sagittae. To better understand the pattern of their shape diversification, we collected the largest dataset of teleost fish otoliths, including 697 species from 68 orders and 309 families. We used geometric morphometric methods to quantify the mesial and dorsal faces. Phylogenetic information was recovered from pruning the consensus time-tree from Betancur et al. (2017). First, we found little evidence of phylogenetic signal on size and shape variation across taxa. Then, we fitted various models of continuous trait evolution to decipher the main dynamic of otolith evolution. Among tested models (e.g. Brownian motion, accelerating and decelerating models), the Ornstein-Uhlenbeck model with a single optimal trait value for all taxa was the best supported. Globally, results from tests of phylogenetic signal and trait modeling reveal that species trait value are uncorrelated. Convergence would be a good candidate to explain such an evolutionary pattern. Next, we assessed morphological integration between sulcus and otolith outline and we surprisingly found high morphological covariation. Finally, we found the accumulation of otolith disparity is unrelated to orders' age but we highlighted a tendency of higher shape (and not size) diversity in clades evolving at faster rates. Our preliminary analyses reveal that the evolution of sagittae is not random. Shape convergence at high taxonomic scales probably highlights morpho-functional constraints. We ask for further studies focusing on eco-morphological hypotheses to reveal more macroevolutionary drivers of in inner ear functional morphology.

Keywords: Sagittae, Shape, Disparity, Phylogenetic signal, Convergence.

RETURN TO INDEX

THEME III

LIFE HISTORY, DEMOGRAPHY AND CONNECTIVITY STUDIES

RETURN TO INDEX

IOS_001

Age determinations and insights at the scale of centuries

Steven Campana[1]*
[1]Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland. *steven.e.campana@gmail.com

Century-scale longevities and time series are no longer rare among some species of marine organisms. Both conventional and unconventional structures and methods are now being used to determine the age of many aquatic species, although some species continue to defy all ageing attempts. However, some ecological and population insights are better tackled with long time series of short-lived species. Drawing upon examples of growth structures from teleosts, sharks, bivalves and whales, spanning collections made over the past millenium, this brief talk highlights some of the successes and failures of recent studies involving the use of either calcified or uncalcified growth structures.

Keywords: Otoliths, Sharks, Whales, Bivalves, Growth synchrony

RETURN TO INDEX

IOS_004

Ecotype diversification as mechanism for coexistence in extreme environments – the case of northern pike (*Esox lucius*) in brackish lagoons

Timo Rittweg[1,2]*, Clive Trueman[3], Michael Wiedenbeck[4], Jan Fietzke[5], Robert Arlinghaus[1,2]
[1]Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Berlin, Germany.
*timo.rittweg@igb-berlin.de
[2]Division of Integrative Fisheries Management, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Invalidenstr. 42, 10115 Berlin, Berlin, Germany
[3]School of Ocean and Earth Science, University of Southampton Waterfront Campus, European Way, SO143ZH Southampton, UK
[4]German Research Center for Geosciences (GFZ) Potsdam, Telegrafenberg, 14473 Potsdam, Brandenburg, Germany
[5]GEOMAR Helmholtz Centre for Oceanography, Wischhofstr. 1-3, 24148 Kiel, Schleswig-Holstein, Germany

Competition for resources fosters the evolution of diverse and often specialized phenotypes, particularly in extreme environments where ecological factors suitable for survival and reproduction vary spatially or temporally. Brackish environments offer extreme conditions for freshwater species, which may respond to the osmoregulatory challenge by developing different habitat use strategies along a gradient between residency in freshwater tributaries, over anadromy to brackish residency. We used high-resolution intra-otolith δ18O isotope ratios and trace elemental concentrations to reconstruct the lifelong realized thermosaline resource utilization of a freshwater predator, the northern pike (Esox lucius), that inhabits brackish lagoons of the southern Baltic Sea at the edge of the species´ salinity tolerance. Age-specific and life-time growth were used as a fitness surrogate. The individual environments experienced throughout life were classified into clusters of ecotypes with a dynamic time warping approach. In a sample of 130 pike collected from lagoons and adjacent tributaries, we detected four distinct ecotypes, a brackish resident type, an anadromous type, a freshwater resident type and an unexpected fourth ecotype that was characterized by freshwater or oligohaline estuarine spawning and subsequent movements into mesohaline conditions (cross-habitat type). 68% of the pike captured in the lagoon ecosystem were of the brackish resident type, suggesting they have undergone evolution to complete the life cycle under mesohaline conditions. 24% were of the cross-habitat type and 8% of were classified as anadromous. Growth of the ecotypes varied significantly between age classes, but life-time growth was similar, suggesting that all ecotypes achieved similar fitness despite significantly different thermosaline niches. Pike have responded to the brackish environment by developing a diversity of ecotypes representing a range of adaptations able to complete a life cycle in an environment offering extreme physiological challenges.

Keywords: Ecological niche, life histories, habitat choice, time-series, growth

RETURN TO INDEX

IOS_006

Temperature effects on otolith shape; results from a long-term experiment

Florian Berg[1]*, Arild Folkvord[2]
[1]Institute of Marine Research, Bergen, Norway; [2]Department of Biological Sciences, University of Bergen, Bergen, Norway.
*florian.berg@hi.no

Otolith shape analysis is a useful tool in population identification of fish since the outline of otoliths varies between distinct populations. Several environmental and genetic factors are known to influence the otolith shape of fish. However, direct evidence of environmental impacts, such as temperature, is missing. Common garden experiment with controlled environmental conditions can help us to create such evidence. Therefore, we conducted a long-term experiment over 3.5 years with spring spawning Atlantic herring (*Clupea harengus*) and reared herring offspring using two fixed temperature levels (7° and 10° C), with seasonal varying light cycle starting either in spring or autumn, and food provided in excess. This experimental setup allowed us to sample fish not only of the same age but also at the same amount of day degrees to investigate the direct effect of temperature. When comparing otolith morphometrics, such as length, width, or area, against the body length of fish there were no significant differences between the two temperature regimes. Comparing the otolith shape outline of fish with similar experienced day degrees, we found significant differences between the temperature regimes. However, we also found differences in the body size and fish from the warmer temperature regime were larger having experienced the same amount of day degrees. Thus, the observed difference in otolith shape cannot solely be explained by temperature differences as such. Further, when only comparing fish with similar amount of day degrees and body size, difference in otolith shape become non-significant. This might demonstrate that temperature has limited direct impact on the otolith shape, but more on the body size which consequently, and indirectly, affects the otolith shape. This long-term experiment demonstrated the variability of otolith shape in different environments, but the final evidence of direct temperature effects is still unresolved.

Keywords: Atlantic herring, *Clupea harengus*, common garden experiment,

RETURN TO INDEX

IOS_007

SmartDots a new tool for age reading exchanges; but is it "smart" to use digital images?

Florian Berg[1]*, Côme Denechaud[1]
[1]Institute of Marine Research, Bergen, Norway. *florian.berg@hi.no

In the last five years SmartDots was introduced as a new tool for age reading exchanges annotating digital images instead of reading physical material. Traditionally, calcified structures used for age reading of fish have been physically exchanged for comparisons. From a reader perspective, this is still the preferred way. However, during these exchanges' samples have been lost or destroyed during shipment between institutes. In this study we compared the age reading results from ten experienced age readers, all ageing the identical individuals by reading 1) the physical otoliths and 2) digital images using SmartDots. In total, 184 Atlantic mackerel (*Scomber scombrus*) otoliths have been read by the 10 readers. The overall ageing agreement among readers was 81.4% for both options, respectively. The modal age between the physical otoliths and digital images varied for 36 individuals (19.6%). There was no indication of over/underestimation in favor of one of the options. In most of the cases the discrepancy was only 1 year different, but for 11 individuals the difference between the modal age from the physical otoliths and the digital images varied up to four-year differences. This study clearly demonstrates that using SmartDots and digital images will not affect the results of age reading exchanges. However, it should be highlighted that all readers work at the same institute and that cross-institutional differences might be different. To successful conduct an exchange in SmartDots it is also of highest importance to use digital images of high resolution and quality.

Keywords: Otoliths, SmartDots, Atlantic mackerel

RETURN TO INDEX

IOS_009

Assessment of spatial and temporal stability of tributary-specific otolith trace element signatures

Olivier Raven[1,3]*, Gerard Closs[1], Jayde Couper[2], Leo Nagelkerke[3]
[1]Department of Zoology, University of Otago, Dunedin, New Zealand; [2]Fish & Game Otago, Dunedin, New Zealand; [3]Aquaculture & Fisheries Group, Wageningen University & Research, Wageningen, The Netherlands. *olivier.raven@outlook.com

Otolith trace element analysis is a powerful tool for studying fish migration between juvenile and adult habitats. To track catchment-scale migration in salmonids, the trace element signature (TES) in otoliths of juveniles is compared with the signature of the core region of adults, allowing adults to be matched back to juveniles from their natal streams. However, for this methodological approach to work the TES from natal streams must remain stable over time, but this has been rarely tested.

To evaluate the spatiotemporal stability of the natal stream TES in otoliths of brown trout, 608 brown trout juveniles were captured from 20 tributaries in the lower Clutha River, New Zealand over a four-year period. Otolith microchemical analysis was performed on the juveniles to obtain their otolith TES, enabling comparisons of the TES within each stream over time.

The findings suggest the TES are generally spatially stable over time, particularly at the scale of major sub-catchments. At the finer scale of individual small streams, greater variation was observed This means that for future studies that compare adults to juvenile natal stream TES, it is not necessary to analyse juvenile otoliths for each year, at least at the sub-catchment scale. Collection of juveniles from key spawning streams across 2-3 years should be sufficient to establish reference TES for each sub-catchment that will facilitate tracking of adult migration over several years.

Keywords: Brown trout, *Salmo trutta*, Otolith, Otolith microchemical analysis, Temporal stability

RETURN TO INDEX

IOS_010

Bombs and fish – Extending the utility of bomb radiocarbon dating to the post-peak decline

Allen H Andrews[1,2,3]*, Francesca Vitale[2], Yvette Heimbrand[2], Caroline Welte[4,5], Jessica Farley[6], J Paige Eveson[6], Kyne Krusic-Golub[7], Alec R Lackmann[8], Ryan N Hupfeld[9], Caroline Durif[10], Francois Roupsard[11], Kei Okamoto[12], Keisuke Satoh[12], Bryan Lockheed[3], Jed Macdonald[11]
astrofish226@gmail.com
[1.] University of Hawaii, Manoa, Department of Oceanography, USA [2]. Swedish University of Agricultural Sciences, Department of Aquatic Resources, Sweden [3].Uppsala University, Department of Earth Sciences, Uppsala, Sweden [4]. Laboratory of Ion Beam Physics, ETH Zürich, Switzerland [5.] Geological Institute, ETH Zürich, Switzerland [6]. CSIRO Environment, Hobart, Tasmania, Australia [7]. Fish Ageing Services Pty Ltd., Queenscliff, Australia [8]. University of Minnesota Duluth, Department of Biology, Minnesota, USA [9]. Large River Investigations Research Station, Department of Natural Resources, Iowa, USA [10]Institute of Marine Research, Austevoll Research Station, Storebø, Norway [11] Pacific Community - SPC, Noumea, New Caledonia [12] National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Yokohama-shi, Japan

Bomb radiocarbon dating was originally developed as a tool for determining or validating the age of marine and freshwater organisms using the rise of bomb-produced radiocarbon in the 1950s and 1960s. Use of this signal is becoming more limited because the organism must have lived through this period to place constraints on age — either the lifespan of recently collected individuals must be approximately 60–70 years or otolith archives from several decades ago must exist for shorter lived species to push birth dates into this informative period. Investigation of the post-peak radiocarbon decline in the Gulf of Mexico as a potential tool in fish age determination or corroboration led to the discovery that indicated the decline was monotonic and could be used as a temporal reference in the western North Atlantic. While the precision associated with an individual age from a measured radiocarbon value was typically lower than determined from the bomb radiocarbon rise period, there were still constraints that limited or eliminated certain age or lifespan scenarios. This was the case for yellowfin and bigeye tuna (*Thunnus albacares* and *T. obesus*) in the North Atlantic and western and central Pacific oceans with lifespans supported to ages exceeding 10 years and reaching 14–18 years with the corroborated age reading protocols. This method was recently extended to fishes of various marine and freshwater environments: 1) Baltic herring and sprat (*Clupea harengus, Sprattus sprattus*) aged to 20 years using a new post-peak Baltic cod (*Gadus morhua*) radiocarbon record, 2) shovelnose sturgeon (*Scaphirhynchus platorynchus*) of the Cedar River in Iowa, USA to an age two times greater than determined from fin rays, and 3) European eel (*Anguilla anguilla*) of Norway where the age of several individuals must have been much greater than estimated from a long-accepted otolith age reading protocol. The potential for this method is considerable but must be assessed from region to region because of differences in radiocarbon decline rates and mixing of remineralized carbon sources, as well as consideration for the age-related questions and an appropriate experimental design.

Keywords: Otolith age estimation, age validation, bomb radiocarbon

RETURN TO INDEX

IOS_011

Age validation of Black Rockfish, Copper Rockfish, and Cabezon using secondary ion mass spectrometry (SIMS) to elucidate seasonal patterns in otolith stable oxygen isotopes

Mark Terwilliger[1]*, Leif Rasmuson[1], Richard Stern[2]
[1]Oregon Department of Fish and Wildlife, Newport, OR, USA; [2]Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, AB, Canada. *mark.r.terwilliger@odfw.oregon.gov

Stock assessors commonly use models that incorporate biological data such as fish length and age to determine the status of fish stocks and how different management scenarios affect stock size. Ages used in assessment models need to be accurate and precise because ageing error can affect many model inputs and potentially result in stock mismanagement. Despite the requirement for sound age data, few studies have investigated accuracy of ages for groundfish captured in coastal nearshore waters off Oregon, USA. In this study, we validate otolith ages for Black Rockfish (*Sebastes melanops*), Copper Rockfish (*Sebastes caurinus*) and Cabezon (*Scorpaenichthys marmoratus)*, three species with recreational and commercial importance to Oregon. Ages obtained by traditional break-and-burn methods were validated using secondary ion mass spectrometry (SIMS) to examine otolith stable oxygen isotope ratios ($\delta^{18}O$) over a fish's lifetime. This technique relies on the inverse relationship that exists between otolith $\delta^{18}O$ and ambient water temperature, and independent counts of $\delta^{18}O$ maxima should be comparable to ages obtained by visual counts of otolith growth marks laid down during cold water periods. Locations of $\delta^{18}O$ maxima in otolith chronologies matched well with locations of visual growth marks in otoliths of all three species, maxima counts were strongly positively correlated with age, and variation in otolith $\delta^{18}O$ decreased with age. However, significant variability in the $\delta^{18}O$ chronologies caused by variability in intra-seasonal upwelling and resulting water temperature variations made maxima counts difficult in several samples. Correct interpretation of chronologies required knowledge of location of the first annulus, the compression of growth zones with age, and an assumption of the seasonal amplitude of the ambient water $\delta^{18}O$.

Keywords: Microchemistry, Temperature, Accuracy, Groundfish, Upwelling

RETURN TO INDEX

IOS_012

Can otolith macrostructures be used to reconstruct individual maturity and spawning?

Côme Denechaud[1]*, Szymon Smoliński[2], Jane Godiksen[1], Audrey Geffen[3]
[1]Institute of Marine Research, Bergen, Norway; [2]National Marine Fisheries Research Institute, Gdynia, Poland; [3]Department of Biological Sciences, University of Bergen (UiB), Bergen, Norway
*come.denechaud@hi.no

Specific changes in the macrostructure of fish otoliths have long been suspected to represent individual maturity and spawning events. In Norway these zones are routinely read for some gadoids, particularly Atlantic cod, but they remain underutilized and poorly understood. Recent experimental work has also suggested a more complex origin than the assumed direct relationship to spawning, highlighting the need for further validation. Here, we first explored the identification and incidence of these so-called spawning zones across multiple Gadidae species and populations exhibiting different life history traits and environmental conditions. Then, we utilized a large archival collection of several hundred thousand otoliths to explore if spawning zone incidence varies through time, and whether they can be used to derive maturity at age estimates in line with those traditionally produced from direct gonad examination. Our results suggest that spawning zones may be a general trait of some species and not limited to certain environments or migratory behaviors as previously proposed for Atlantic cod in Norwegian waters. Estimates of maturity at age derived from otoliths showed temporal fluctuations generally consistent with those from gonad staging. However, differences were observed between the two maturity at age estimates, the direction and magnitude of which depended on the species and populations. Northeast Arctic cod exhibited a clear offset of one or two years consistent with known high trial run and skip-spawning occurrence in the first two years following the onset of maturity, which is suspected to reflect a stabilizing of energy partitioning toward reproduction. The relationships were harder to evaluate in other species and populations, which may reflect the lower readability and interpretability of their spawning zones as perceived by experienced age readers. While spawning zones may have a high potential for reconstructing maturity from archives, our results highlight the need for a better understanding and validation of the physiological processes behind their incidence and formation.

Keywords: otolith checks, growth & reproduction, energy allocation, Gadidae

RETURN TO INDEX

IOS_016

Relationship between habitat use and condition of European eel (*Anguilla anguilla*) in small estuaries of the eastern English Channel

Jérémy Denis[1], Kélig Mahé[2], Hélène Tabouret[3], Khalef Rabhi [1], Kévin Boutin [1], Mamadou Diop [1], Rachid Amara [1]*
rachid.amara@univ-littoral.fr
[1]Univ. Littoral Côte d'Opale, Univ. Lille, CNRS, IRD, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F-62930 Wimereux, France.
[2] IFREMER, Fisheries Laboratory, 150 quai Gambetta, BP 699, F-62321 Boulogne-sur-Mer, France.
[3] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, F-64053 Pau, France.

The European eel is a facultatively catadromous species that occupies a variety of freshwater, brackish and marine habitats during the growth phase. Although brackish habitats are considered important for eels, knowledge of habitat use and the implications for life history strategies is still limited, especially in the estuaries. The aim of the study was to characterise and compare the habitat use and condition of European eels during their growth phase in six small and medium-sized estuaries of northern France. Eels were collected during four sampling periods (i.e. March, May, July and October 2019) using fyke nets along the salinity gradient (i.e. three sampling stations per estuary) in six estuaries located along the French coast in the eastern Channel (Slack, Wimereux, Liane, Canche, Authie and Somme estuaries). Five condition indices, including Fulton condition factor K, lipid content, hepatosomatic index, annual growth rate and health status based on the abundance and prevalence of lesions and parasitism, were measured at the individual scale on 119 eels to explore variation with local habitat characteristics and habitat use strategies. Environmental history and movements were reconstructed from the Sr:Ca and Ba:Ca ratios of otoliths from eel samples (n = 37) in two contrasting estuaries. Sr:Ca and Ba:Ca ratios were used to distinguish three sectors of estuarine residence and showed that all estuaries had a high proportion of resident eels (81%). Resident eels in the estuaries showed clear differences in condition depending on the sector of residence. While resident eels in the lower part of estuaries showed higher lipid storage, eels in the upper part exhibited higher growth. Eels assigned to the middle part of estuaries showed better condition K and health, in response to a more diverse diet of freshwater and marine prey. The differences in condition observed according to habitat use appear to be related to variations in their diet composition, which in turn depends on local hydro-morpho-sedimentary characteristics.

Keywords: Otoliths, Microchemestry, environmental history, growth, lipids, health, trophic status.

RETURN TO INDEX

IOS_017

Escaping the black box: explicit annotation of otolith growth rings with deep learning

Troels A. Bojesen[1], Côme Denechaud[2]*, Ketil Malde[2]*
[1]Department of Informatics, University of Bergen (UiB), Bergen, Norway; 2Institute of Marine Research, Bergen, Norway
*come.denechaud@hi.no

In the last two decades, the emergence and development of machine-trained algorithms for identifying image features have sparked renewed interest for the automation of otolith age reading, a task that is central to fish population monitoring. Attempts at developing deep learning models for age estimation across different species have shown promising results that could complement human expertise as the systems are further tuned. However, their adoption has been limited by the opacity of the underlying processes, as machine-trained models often operate like a "black box" with little legibility. Recently, research in explainable AI have resulted in methods that can unveil the parts of the input used by the model to make its prediction, but their outputs can be hard to interpret and may differ from the visual and biologically relevant clues used by human readers. In this study, we explored an alternative approach. Instead of predicting a single age estimate, our method mimics the behavior of a reader by first identifying the origin of the otolith and placing an annotation. It then proceeds iteratively by estimating the next annotation conditional on the previous ones, while simultaneously estimating the probability to end the sequence. This results in an estimate of fish age that can be easily verified by a human, as the algorithm produces an annotated image similar to annotations produced by experts. The algorithm was trained on approximately 4000 annotated images of Atlantic cod otoliths. Our results showed a high predictive accuracy in line with or better than recently developed deep learning methods. Examination of the probability distributions for annotation marks confirmed that the iterative method led to a model behaving more in line with human readers by highlighting the ring structures in its predictions. When multiple expert readers were given a random blind sample of both human and machine-generated images, our results showed that the predicted annotations had a generally high credibility although their quality varied with otolith clarity and readability.

Keywords: fish aging, machine learning, automation, Atlantic cod

RETURN TO INDEX

IOS_018

Life history traits and migration of wild mangrove snapper (*Lutjanus argentimaculatus*) in the waters off Taiwan

[1]Yeh, Yun-Ting, Nien, Yu-Ling, Lu, Chien-Yu, Chiang, Wan-Chen, Wang, Chia-Hui
sherryyayeh@gmail.com

Lutjanus argentimaculatus is one of the fisheries species, and also a key target species in the stock enhancement program in Taiwan. However. So far only few researches have focused on the fishery biology of wild population in Taiwan coastal water. To understand the life history traits and migration, current study collected the wild mangrove snapper monthly between March 2015 and November 2018. A total of 601 fish was collected, including 146 females, 113 males, and 342 sex unrecorded specimens. Overall, sex ratio (female :male) ratio is 0.56. The total length ranged from 12.2 – 97.2 cm and the age ranged from 0+ - 42+ years old. The growth parameters of all samples are L_{∞}=88.89cm、k=0.16、t_0=-1.40, by von Bertalanffy growth equation (VBGE). Meanwhile, trace elements in the otoliths, Sr/Ca and Ba/Ca ratios, were measured by LA-ICP-MS. The elemental time series profiles was used to understand the migration of the mangrove snapper. Two movement patterns were discovered. A portion of specimens are amphidromous, that lived in fresh water at a younger age, then move into the seawater. Few specimens showed the movement that go back and forth from fresh to marine water. Another pattern individuals stayed in the sea for their whole life. Investigate the growth and movement pattern in consideration with age data in different region will be done for consequent fishery management and stock evaluation in Taiwan.

Keywords: Lutjanus argentimaculatus, Life history, Otolith.

RETURN TO INDEX

IOS_020

A multi-scale approach to assess homing and straying rates of Atlantic salmon (*Salmo salar*) in the New Aquitaine region

Anthony Fontaine[1]*, Matthias Vignon[2], Hélène Tabouret[1], Alexandre Holub[3], Christophe Pécheyran[1], David Nachon[4], Josu Huarte[5], Gilles Bareille[1]
[1]University of Pau and the Adour Region, E2S UPPA, CNRS, IPREM, MIRA, Pau, France; [2]University of Pau and the Adour Region, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France; [3]MIGRADOUR, Gan, France, [4]University of Santiago de Compostela, Department of Zoology, Santiago de Compostela, Spain; [5]Fish Management Area, Pamplona-Irunea, Navarra, Spain.
*anthony.fontaine@univ-pau.fr

Although straying can be seen as a direct loss of potential spawners from a populational perspective, the dispersion of some individuals towards non-native watersheds is essential when considering the metapopulation. On one hand, a strong homing rate participates in maintaining a well-adapted population in a given environment and maximizes reproductive success. On the other hand, connectivity among distinct populations is crucial to sustain sufficient genetic and phenotypic diversity, especially in a global change context. In the Bay of Biscay, most of Atlantic salmon (*Salmo salar*) populations are largely supported by local restocking programs. As a result, management strategies are promoting the populational level and do not necessarily take into account the importance of connectivity. However, considering the higher straying rate for individuals from hatcheries compared to wild salmons, in addition to the expected increase in dispersal due to climate change, it is critical to assess the level of homing and straying among salmon populations in a multi-scale framework. In our study, we collected sagittal otolith of returning adults from 2009 to 2018 in the Adour and Garonne-Dordogne basins as well as six watersheds or hydrographical regions along the Bay of Biscay (from North to South: South Brittany, Allier, Nivelle, Bidasoa, Asturias and Galicia). Using microchemistry profiles, we built a two-step statistical model (random forest) to predict the geographical origin of sampled fish. The first step ("basin-wide") consists in providing a watershed or a region of origin for every fish. The second step ("sub-catchment") is designed to assign a sub-basin or a river of origin. The combination of these two steps allowed us to infer the level of intra and inter-basins straying and to investigate the extent to which distinct populations exchanges individuals. Additionally, our preliminary results help us identifying the watersheds determined as "donors" of individuals, which are essentials for managers, stakeholders and policymakers.

Keywords: Connectivity, Microchemistry, Fish movement.

RETURN TO INDEX

IOS_024

Grow slow, die young? Testing the bigger-is-better hypothesis using larval and age-1 capelin (*Mallotus villosus*) otoliths

Gabrielle Perugini[1]*, Hannah Murphy[2], Dominique Robert[3], Maxime Geoffroy[1]
[1]Center for Fisheries Ecosystems Research, Marine Institute of Memorial University of Newfoundland, St. John's, NL A1C 5R3, Canada; [2]Northwest Atlantic Fisheries Center, Fisheries and Oceans Canada, St. John's, NL A1C 5, Canada; [3] Institut des sciences de la mer, Université du Québec à Rimouski, Rimouski, QC G5L 3A1, Canada. *gabrielle.perugini@mi.mun.ca

Capelin (*Mallotus villosus*) is a key forage species in the Newfoundland and Labrador (NL) ecosystems, transferring energy from lower-level plankton to higher-level vertebrate predators. In 1990-91, the Newfoundland capelin stock collapsed and has yet to recover. Since 1991, recruitment of age-2 capelin into the stock is positively correlated with survival during the first two weeks post-hatch. In NL, larval survival of capelin can be driven by both bottom-up forcing (i.e., availability of *Pseudocalanus* spp. as larval prey) and top-down processes (i.e., predation). It has been suggested that selective predation by Atlantic herring (*Clupea harengus*) on capelin larvae found in high densities may be a mechanism for explaining recruitment failure of capelin in the Barents sea, however, this is unknown for NL capelin. The bigger-is-better hypothesis predicts that larger and faster growing fish larvae are less vulnerable to predation than their smaller, slower growing conspecifics, which results in increased survival of fast-growing larvae. In this study, we used otolith-derived ages to compare growth rates of the 2021-2022 capelin larval cohorts to surviving 2021-22 cohorts at age-1, to test if fast-growing larvae evade predation and dominate the age-1 population. Future work will then assess the diet of juvenile Atlantic herring sampled in the vicinity of capelin spawning beaches in Trinity Bay, NL to test the hypothesis that slow-growing capelin larvae are predated upon more frequently. We expect that these findings will provide insight into the drivers of capelin larval survival and may contribute to Fisheries and Oceans Canada's capelin forecast model in support of an ecosystem-based approach to fisheries management.

Keywords: Otoliths, Recruitment, Larval Survival, Growth, *Mallotus villosus*

RETURN TO INDEX

IOS_025

Ageing different calcified structures; does the structure matter?

Florian Berg[1]*, Eydna í Homrum[2] , Julie Coad Davies[3], Stina Bjørk Stenersen Hansen[3], Erling Kåre Stenevik[1]
[1]Institute of Marine Research, Bergen, Norway; [2]Faroe Marine Research Institute, Tórshavn, Faroes; [3]DTU Aqua, National Institute of Aquatic Resources, Denmark. *florian.berg@hi.no

Calcified structures, such as otoliths or scales, are regularly used for the ageing of fish. This age information is the backbone of fisheries stock assessment to estimate the stock size. Thus, small discrepancies in the ageing might have huge impacts on the final assessment estimates. For some Atlantic herring stocks (*Clupea harengus*), more specifically Norwegian spring spawners, both otoliths and scales are commonly used for age estimation by different institutes. In this study, we conducted an age reading calibration exercise among several readers from different institutes ageing both calcified structures of the same individuals. Age readers were only reading structures they are experienced on and read routinely, i.e. some were reading both. Modal ages for each individual and calcified structure were estimated to directly determine any difference between readers estimates. In total 255 herring were aged by 15 different readers with an overall agreement of 79.7% and 88.4% for otoliths and scales, respectively. In 21.9% (N = 56), there was a mismatch between the modal age of otoliths vs. scales. For 13 individuals the modal age of otoliths was 1-2 years older. However, the agreement among these individuals was below 65% for both structures. In contrast, the modal age of 43 herring was older for the scales reading (1-8 years differences). The agreement for the readings of these 43 herring was only 52.3% and 71.1% for otoliths and scales respectively. This mismatch between otoliths and scales mostly occurs for herring of ages 10 or older. This age reading calibration and direct comparison of age readings from two calcified structures demonstrated the need for age validations for different calcified structures. A resulting discrepancy of >20% between the commonly used calcified structures for age reading might have a non-neglectable effect on the following stock assessment. Further studies, to investigate the effects of this discrepancy, especially among older herring, is needed to provide reliable data for the stock assessment.

Keywords: Otoliths, scales, Atlantic herring, *Clupea harengus*, Norwegian spring spawning herring

RETURN TO INDEX

IOS_030

Novel scale chemistry of *Salmo salar* L.

Michelle von Ehr[1]*, Yvette Heimbrand[1]
[1]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden.
*michelle.vonehr@gmail.com

Baltic salmon (*Salmo salar L.)* are valued for their ecological, economic, and cultural benefits. They adapt to various natural challenges, such as changes in salinity, currents and temperature but are exposed to further stressors, including anthropogenic structures, climate change and diseases. In terms of diseases, Baltic salmon suffer from maternally transmitted M74 reproductive disorder, which causes yolk-sac fry mortality due to thiamine deficiency of maternal fish. To determine underlying causes for thiamine deficiency, trace elemental analyses of otoliths and scales of Baltic salmon have been performed and exposed both environmental and physiological patterns. Otolith chemistry is an established wide-spread method to determine major life-history events in fish. However, this is a lethal method wherefore developing non-lethal sampling analyses of alternative structures, such as scales, is of great importance. Here, chemical analyses using laser-ablated coupled plasma mass spectrometry was applied on scales and otoliths of 170 female Baltic salmon, thereby studying the alignment of chemical profiles among both structures. Comparison between two chemical age readers, interpreting seasonal patterns in Mg:Ca, found 73% and 70% agreement for otoliths and scales, respectively. Further comparison of both structures to each other revealed 51 – 58% agreement precision between both readers. The chemical analyses additionally reflect trace elemental patterns evincing salinity (Sr:Ca) and fresh- and saltwater transition (Ba:Ca) in both structures, thereby revealing that scale chemistry can be applied for migration studies. Egg thiamine concentrations were measured using chromatography and the analysed Sr:Ba ratio in scales from severely thiamine deficient salmon (thiamine concentration < 0.4 nmol/g) showed significantly lower mean levels than healthier fish (> 8 nmol/g) throughout the whole life cycle, indicating that severely deficient salmon use coastal habitats more than healthier fish. The Sr:Ba ratio in scales correlated negatively to trace elements known for tracking pollution (Pb, U, Zn, and Cu). Additionally, a significant increase of B over life in the otoliths of severely deficient fish was discovered. Scale Sr:Ba shows novel promising results as a non-lethal sampling alternative for tracking M74 in Baltic salmon, with potential as a biomarker already early in life.

Keywords: Otoliths, Scales, Laser-ablation coupled inductively plasma mass spectrometry, *Salmo salar* L., M74

RETURN TO INDEX

IOS_032

Cataloguing otoliths of mesopelagic and bathypelagic fishes: a tool for species identification

Lydia Walton*1, Micah Quindazzi[1], Catherine Stevens[2]
[1]Department of Biology, University of Victoria, Victoria, British Columbia, Canada; 2School of Earth and Ocean Sciences, University of Victoria, Victoria, British Columbia, Canada. *lydiawalton14@uvic.ca

Identifying fish to species level is important for fisheries research because it ensures the accuracy of catch data reported by fishing vessels and informs best management strategies for harvested taxa. Currently, visual and genetic methods are the most common techniques used for identifying fish species, but these can be time-consuming and costly. In the case of meso- and bathypelagic fishes, these methods may also lead to incorrect identifications as many closely related species look morphologically identical, and baselines do not always exist for the genetic samples. Otolith shape analysis, using geometric morphometrics, is an inexpensive alternative that can be used to identify large numbers of fish samples quickly and with relatively high accuracy. The objective of this study was to compare taxonomic identification methods for meso- and bathypelagic fishes as they occupy a critical role in marine food webs and the ocean's biogeochemical cycle. We present a catalogue of sagittal otoliths from meso- and bathypelagic fishes off West Coast Vancouver Island and Haida Gwaii and show that otolith shape analysis is a useful tool for species identification. The right sagittal otoliths of 22 species belonging to 11 families of fish were photographed, and two images for each sagitta were included in the catalogue, one with the sulcus side down and one with the sulcus side up. For most species, multiple individuals were photographed to demonstrate intraspecific variability in otolith shape. We compared three methods of fish identification: visual identification by trained researchers, genetic analysis using the COI-5P marker, and otolith shape analysis using the geomorph package in R (n = 216 individuals). The results of the genetic analysis were assumed to be the most accurate measure of taxonomic identification and were used as a baseline to compare the results of the visual and otolith shape analyses. Our results showed that visual identification in the field was correct only 23% (49/216 fish correctly identified) of the time when compared to genetic analysis, while otolith shape analysis was correct 91% (197/216 fish correctly identified) of the time. Thus, otolith shape analysis represents a more accurate and cost-effective method for identifying deep-sea fishes.

Keywords: Geometric morphometrics, Sagittae, Fish taxonomy, Deep-sea, Fisheries management

RETURN TO INDEX

IOS_033

Where deep-sea fish live and grow - Tracking life history patterns of Pacific grenadier (*Coryphaenoides acrolepis*) with otolith microchemistry

Adrian Castillo[1]*, Leticia M. Cavole[2], Allen H. Andrews[3], Octavio Aburto-Oropeza[1], Chris J. Russo[4]
[1]Scripps Institution of Oceanography, University of California, San Diego, United States, [2]Department of Wildlife, Fish and Conservation Biology, University of California, Davis, United States, [3]Oceanography, University of Hawai'i, Mānoa, United States, [4]College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, Oregon. *acastillo@esf.edu

Pacific grenadier (*Coryphaenoides acrolepis*) are deep-sea fishes of the family Macrouridae commonly found in the north Pacific Ocean along the continental shelf, with previous work validating the annual periodicity of their otolith growth rings. Their life history is not well understood; however, otolith microchemistry offers a promising methodology for tracking ontogeny across individual samples. Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was used to analyze the microchemistry of 118 mature Pacific grenadier otoliths. Samples were collected offshore of the Baja California Peninsula and near Isla Guadalupe, Mexico, between the years of 1967 and 1971. Twelve elemental isotopes (^{7}Li, ^{11}B, ^{24}Mg, ^{26}Mg, ^{55}Mn, ^{31}P, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{86}Sr, ^{138}Ba, and ^{208}Pb) were quantified as ^{43}Ca ratios using LA-ICPMS on otolith sections ranging from the core to the edge. Transects were divided into larval, post-settlement, and mature stages to classify potential differences in environment and physiology across ontogeny. Age estimates were consistent with previous work and Von Bertalanffy growth curve parameters were revised with the inclusion of older and larger individuals. The ^{43}Ca ratios of ^{31}P, ^{63}Cu, and ^{66}Zn decreased with distance from the core of the otolith, which could be due to a decrease in metabolic activity and somatic growth with age. The ^{86}Sr and ^{138}Ba ratios both steadily increased through ontogeny, indicating movement to more saline waters with higher ambient concentrations of those isotopes across depth. The ^{55}Mn, ^{86}Sr, and ^{138}Ba ratios were elevated within the post-settlement stage, suggesting a shift in environment that may reflect the hypoxic conditions of the oxygen minimum zone. Samples collected near Isla Guadalupe exhibited greater ^{7}Li, ^{55}Mg, ^{65}Cu, and ^{208}Pb ratios, while samples found off Baja California were greater in ^{11}B, ^{55}Mn, and ^{86}Sr ratios, indicating differences between the offshore continental shelf and near island populations. Isotopic ratios of Pacific grenadier otoliths varied only slightly between the continental shelf of Baja California and the insular depths of Isla Guadalupe collection sites, with statistical clustering accurately reclassifying 62.5% of samples. Overall, otolith microchemistry appears to be an effective tool to detect ontogenetic and regional patterns of Pacific grenadier and other deep-sea fishes.

Keywords: Deep sea, LA-ICPMS, *Coryphaenoides*, Southern California Bight, Hypoxia

RETURN TO INDEX

IOS_036

Investigating the optimal growth temperature of a sub-tropical deepwater snapper, *Pristipomoides multidens*, from Gascoyne Bay, Australia

Clement Z. W. Ng[1]*, Júlio G. Gonzalez[1], Muhammad Faiq[1], Corey B. Wakefield[2], Stephen J. Newman[2], Ong J. L. Joyce[1]
[1]Asian School of the Environment, Nanyang Technological University, Singapore; [2]Western Australian Fisheries and Marine Research Laboratories, Department of Primary Industries and Regional Development, Government of Western Australia, Australia. *NG0002NT@e.ntu.edu.sg.

Somatic growth is an important driver of fish population dynamics. Alterations in growth rates can not only affect individual fitness via changes in mortality rates and reproduction schedules but impact a suite of demographic parameters and fisheries stock productivity. Additionally, growth may also be modified by extrinsic environmental stressors, including ocean temperature. While there has been a growing body of literature examining how local and regional factors influence fish growth, many of these factors are derived from the ocean surface due to the lack of long-term environmental factors at depth. Using environmental proxies acquired from the sea surface layer to explain the growth of deepwater marine fish species may be biologically inappropriate, as they do not reflect the actual conditions fish are located. The aim of our study was to identify extrinsic variables and the depth ranges these factors were most strongly related to the annual growth variation of a commercially valuable deepwater snapper (*Pristipomoides multidens*) from the Gascoyne Bay, Australia. *Pristipomoides multidens* was chosen as our model species due to its extensive depth distribution ranging between 40 and 360 meters, limited movement (sedentary) of adults, and protracted longevity. We hypothesized that annual growth of *P. multidens* would be best explained by temperature and salinity conditions between 100 to 150 meters, as fish are commonly encountered at those depths. Annual otolith increment widths, which are proxies for fish growth, were first analyzed using a series of linear mixed-effects models, to identify the intrinsic (individual or population-specific) and extrinsic (environmental) drivers of annual otolith growth variation. Temperature and salinity-at-depth data was then extracted from the Australian Integrated Marine Observing System (IMOS) database, from CTDs (Conductivity-Temperature-Depth) deployed around the coastline of Australia and analyzed using sequential t-test analysis (STARS), to establish the depths in which somatic growth was most strongly correlated with the environmental data. Our findings highlight the importance of using environmental factors that are biologically relevant to individual species, as the failure to assess variables at the appropriate spatial or temporal scale may downplay the true ecological response to climate change.

Keywords: Otoliths, Growth, *Pristipomoides multidens*, Modelling, Temperature, CTD.

RETURN TO INDEX

IOS_037

Migrations of Ariid Catfishes in the Mekong River

An V. Vu[1,2]*, Lee J. Baumgartner[2], Martin Mallen-Cooper[2,3], Julia A. Howitt[4], Gregory S. Doran[4], Karin E. Limburg[5], Bronwyn M. Gillanders[6], Cameron M. Kewish[7], Juliane Reinhardt[7], Ian G. Cowx[8]
[1]Fisheries Ecology and Aquatic Resources Division, Research Institute for Aquaculture No.2, Vietnam. *vvu@csu.edu.au
[2]Gulbali Institute, Charles Sturt University, Australia
[3]Fishway Consulting Services, Australia
[4]School of Agricultural and Wine Sciences, Charles Sturt University, Australia
[5]College of Environmental Science and Forestry, State University of New York, USA
[6]Southern Seas Ecology Laboratories and the Environment Institute, School of Biological Sciences, The University of Adelaide, Australia
[7]Australian Synchrotron, Australia
[8]Hull International Fisheries Institute, University of Hull, UK

Ariid catfishes (Family: Ariidae) are important species for capture fisheries in the Lower Mekong River Basin (LMB). Some Mekong ariids ranked among the top ten species in terms of catch. However, their migrations are not well understood. This study examined potential migration patterns of three important ariid catfishes (*Cephalocassis borneensis, Arius maculatus*, and *Osteogeneiosus militaris*) in the LMB using otolith microchemistry. They are mouthbrooding fish species that incubate their eggs and larvae for protection. Several trips were carried out in the LMB, covering more than 2000 km of river to sample these three species. Lapilli otoliths of these species were removed, set in resin, sectioned, and polished until the core was exposed. Key trace elements (magnesium, manganese, calcium, strontium, and barium) in otolith sections were quantified by two analytical techniques: Laser Ablation – Inductively Coupled Plasma – Mass Spectrometry, and X-ray fluorescence microscopy. We found that one species (*C. borneensis*) completed its life cycle in the freshwater zones of the Mekong River, but occasionally (17% of specimens) connected to areas of higher salinity (estuarine brackish water). The other two species (*A. maculatus* and *O. militaris*) showed complex migration patterns, with three different migration strategies identified. Although *A. maculatus* and *O. militaris* experienced a wide range of environments (fresh, brackish, and marine), they mainly lived in brackish and marine waters, with limited excursions into fresh water. Our findings provide critical information for better assessment and management of ariid catfishes in the LMB.

Keywords: Ariid catfishes, fish migration, Mekong River, otolith microchemistry, trace metals.

RETURN TO INDEX

IOS_039

Updating the growth parameters of the Whitemouth croaker, *Micropogonias furnieri* (Desmarest 1823), in the Southwester Atlantic

Daniele Costa Batalha[1,4], Estevan Luiz da Silveira[2], Antônio Olinto Ávila-da-Silva[2], André Martins Vaz-dos-Santos[3,4]*
[1]Programa de Pós-Graduação em Engenharia e Tecnologia Ambiental – PPGETA. Universidade Federal do Paraná; [2]Instituto de Pesca (SAA-SP/APTA). Av. Bartolomeu de Gusmão, 192 CEP 11030-906 Santos – SP – Brasil; [3]Programa de Pós-Graduação em Aquicultura e Desenvolvimento Sustentável – PGADS; [4]Laboratório de Esclerocronologia Universidade Federal do Paraná. Rua Pioneiro, 2153 CEP 85950-000 Palotina – PR – Brazil. *danielebatalha7@gmail.com

The Whitemouth croaker, *Micropogonias furnieri* is one of the most important fishery resources in the Southwestern Atlantic (23°-28ºS), the main sciaenid target of industrial fishing in the area. Under constant fishing pressure, best management practices depend on fishing monitoring. The study goal was to update the life history parameters of the croaker, in this case the von Bertalanffy growth model (VBGM). Monthly samplings in commercial landings were taken from August 2021 to September 2022. Specimens were measured in terms of total length and otolith cross-sections were prepared following routine techniques. Three blind readings were performed, quality tools to evaluate readings were applied and then the growth parameters were estimated. A total of 520 croakers (50.96% males, 47.12% females) with a total length range 196-690 mm were analyzed. The otoliths present a complex ring pattern in view of its shape and the successive translucent and opaque zones. Age bias plot among readings revealed no tendency in the analysis. Comparisons among readings in terms of number of rings resulted in an average percent error of 3.2% and coefficient of variation of 4.1%. Individuals from 0 to 61 years of age could be observed, predominating relatively young croakers (0 to 10 years, 86.89%). The mean age ± standard error was 8.73 ± 0.00186 years. The growth parameters of VBGM (L_∞, k, t_0) by sexes and grouped were: 534.045 mm, 0.11237 yr^{-1}, -3.79515 yrs. (Females), 529.417 mm, 0.1666 yr^{-1}, -2.6324 yrs. (Males), and 532.964 mm, 0.1404 yr^{-1}, -3.26334 yrs. (Grouped). There was no difference between-sex models ($P > 0.05$). Although presenting a low growth rate, *M. furnieri* has shown resilience in face of fishing pressure, probably due to a relatively adaptable life cycle. The prevalence of young individuals (and reduced number of older) in the landings calls attention for short-medium-long term effects on the stock sustainability.

Keywords: demersal fish, trawlers, Fishing, Brazil.

RETURN TO INDEX

IOS_040

Thermal and metabolic histories of inaccessible cephalopods revealed from statolith stable isotopes: a case approach on *Sepioteuthis lessoniana*

Chun-I Chiang[1]*, Ming-Tsung Chung[2], Pei-Ling Wang[2], Jen-Chieh Shiao[2], Chia-Hui Wang[1,3]
[1]Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan; [2]Institute of Oceanography, National Taiwan University, Taipei, Taiwan; [3] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan. *xyzoo123@gmail.com

Thermal histories describe ambient temperature experienced by cephalopods from birth to death, reflecting their habitats and distributions. In addition, studying the metabolic ecology of cephalopods is a fundamental step in understanding their responses to environmental change. These can be reconstructed using ontogenetic oxygen and carbon isotopes ($\delta^{18}O$ and $\delta^{13}C$ values) in statoliths of *Sepioteuthis lessoniana* adults, which were collected from northeastern and southwestern Taiwan during 2017–2019. The experienced temperatures among interannual and geographical populations varied from 15°C to 34°C, associated with seasons, geographic locations, and environmental conditions. A spatial interpolation method was applied to model the probabilities of distribution in these two populations. Overall, the two geographical populations adopted distinct ontogenetic movements and distribution due to ocean currents and diverse topography. Particularly, the northeastern population at a seasonal cohort level showed a southward shift between the two year-period (2017–2018 and 2018–2019) due to an El Niño event. The distribution overlapping at the adult stage supports an assumption of population connection in Taiwan. Furthermore, the proportion of metabolically derived carbon deposited on the statoliths ranged between 0.56 and 0.75, suggesting a relatively higher level of metabolic rate even compared to the reported proportions in various cephalopod and teleost species. It is possibly due to overwintering movement, high feeding rate and energy consummation during the reproductive period. The approach with statolith stable isotopes provides insights into the field observation of the distribution and metabolic performance of *S. lessoniana* and the progress in relating its population dynamics to environmental variability, which is important for the sustainable management of squid fisheries.

Keywords: Bigfin reef squid, Oxygen and carbon isotopes, Experienced temperature, Ontogenetic distribution, Metabolic proxy

RETURN TO INDEX

IOS_ 046

Can New Zealand White shark be aged using a microCT based vertebral animation?

Brittany Finucci[1], Caoimhghin Ó Maolagáin[1], Thomas Barnes[1]*
[1]National Institute of Water and Atmospheric Research, 305 Evans Bay Parade, Hataitai, Wellington, Aotearoa/New Zealand.
Presenting author *Tom.Barnes@niwa.co.nz.

White shark (*Carcharodon carcharias*) is wide-ranging throughout most waters of New Zealand (NZ), from the northern Kermadec region to the Campbell Plateau and northern Macquarie Ridge in the south. Life history parameters for this species are not well defined in NZ but are vital for estimating population status for conservation purposes. In a preliminary study, both standard Xray and microCT imaging techniques were used to estimate age and growth from vertebral banding patterns for NZ white sharks for the first time. Vertebrae were obtained over a 30-year period (1991–2021). The final sample (n=38) included 20 females, 12 males, and six unsexed sharks ranging from 1.52 to 5.36 m total length (TL). Vertebrae were difficult to read, particularly for older sharks. There was strong agreement between readers for age estimates of vertebrae for young specimens but large disagreement for older sharks. Using the video-based reconstruction and animation of one of our larger specimens, we are asking symposium participants to assist with putative annual band pattern interpretation. Participants can view the microCT based video on the laptop stationed below our poster and attempts to assign an age can be entered onto a spreadsheet, with comments invited. Overall results will be displayed at the close of the symposium.

Keywords: White shark, *Carcharodon carcharias*, ageing, vertebra, Xray, microCT, video analysis.

RETURN TO INDEX

IOS_048

Integrating machine learning with otolith isoscapes: reconstructing connectivity of a marine fish over four decades

Kohma Arai[1]*, Martin Castonguay[2], Vyacheslav Lyubchich[1], David H. Secor[1]
[1]Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD, USA; 2Fisheries and Oceans Canada, Institut Maurice-Lamontagne, Mont-Joli, QC, Canada. *karai@umces.edu

Natural markers recorded in biogenic tissue such as stable isotopes have been employed as a powerful tool to uncover the life history and connectivity of marine fishes. Geographic natal assignments often require categorizing the spatial domain through a nominal approach, which can introduce bias given the continuous nature of these tracers. Stable isotopes predicted over a spatial gradient (i.e., isoscapes) allow a probabilistic and continuous assignment of origin across space, although applications to marine fishes remain limited. Here, we present a new framework that integrates nominal and continuous assignment approaches by (1) developing a machine-learning multi-model ensemble classifier using Bayesian model averaging (nominal); and (2) integrating nominal predictions with continuous isoscapes to estimate the probability of origin across the spatial domain (continuous). We applied this integrated framework to predict the geographic origin and connectivity of the Northwest Atlantic mackerel (*Scomber scombrus*), a migratory pelagic fish comprised of northern and southern components that have distinct spawning sites off Canada (northern contingent) and the US (southern contingent), and seasonally overlap in US fished regions. The nominal approach based on otolith carbon and oxygen stable isotopes ($\delta^{13}C/\delta^{18}O$) yielded high contingent classification accuracy (84.9%). Contingent assignment of unknown-origin samples revealed prevalent, yet highly varied contingent mixing levels (12.5–83.7%) within the US waters over four decades (1975–2019). Nominal predictions were integrated into mackerel-specific otolith oxygen isoscapes developed independently for Canadian and US waters. The combined approach identified geographic nursery hotspots in known spawning sites, but also detected geographic shifts over multi-decadal time scales. This framework can be applied to other marine species that form carbonate biominerals (e.g., otoliths) to understand migration and connectivity at high spatial resolution, relevant to management of unit stocks in fisheries and other conservation assessments.

Keywords: Stable isotopes, Atlantic mackerel, Migration, Spatial structure, Fisheries management

RETURN TO INDEX

IOS_049

Using otolith strontium isotopes to assess portfolio effects in Californian Chinook salmon

Authors: Kohma Arai[1,2]*, Anna Sturrock[3], Malte Willmes[4], George Whitman[2], Mikaela Provost[5], Carson Jeffres[2], Rachel Johnson[2,6]
[1]Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD, USA; [2]University of California Davis Center for Watershed Sciences, Davis, CA, USA; [3]University of Essex, Colchester, UK; [4]Norwegian Institute for Nature Research, Trondheim, Norway; [5]University of California Davis, Wildlife Fish and Conservation Biology, Davis CA, USA; [6]NOAA Fisheries, Southwest Fisheries Science Center, Santa Cruz, CA, USA.
*karai@umces.edu

Life-history diversity confers population stability and resilience through asynchronous population responses to varying environmental conditions (i.e., portfolio effects). Such population diversity has been reported in Central Valley (CV) Chinook salmon from the Sacramento-San Joaquin River system (USA), which forms the backbone of a large ocean salmon fishery in California. However, little is known about the population composition and abundance of juveniles leaving the freshwater Delta and entering the San Francisco Estuary, a gauntlet that all CV salmon populations must transit in order to reach the ocean. Here, we used otolith strontium isotope ratios ($^{87}Sr/^{86}Sr$) to understand the life history patterns and origin of juvenile fall-run Chinook salmon entering the estuary from 2014 and 2022, sampled by mid-water trawl across a wide range of hydrologic conditions. Time series analyses and assignment models were used for natal-origin assignment, size at natal river exit, duration of freshwater rearing, and population composition estimates. We explore shifts in composition and life history diversity over time, and test whether contributions from different populations displayed asynchronous dynamics. Future work will combine these data with annual estimates of juvenile production generated via trawl efficiency studies to enable us to build more sophisticated demographic models. Findings will inform stock-recruitment relationships and assessment models, relevant to the population persistence of this important species.

Keywords: Strontium isotopes, Life-history diversity, Fisheries management, Migration

RETURN TO INDEX

IOS_054

Habitat use and migratory patterns of Atlantic halibut in the Gulf of St. Lawrence

Charlotte Gauthier[1]*, Dominique Robert[2], Jonathan Fisher[3] and Pascal Sirois[1]
[1]Department of Fundamental Science, University of Quebec at Chicoutimi, Chicoutimi, Canada; [2]Rimouski Institute of Marine Sciences, University of Quebec at Rimouski, Rimouski, Canada; [3]Centre for Fisheries Ecosystems Research, Fisheries and Marine Institute, Memorial University of Newfoundland, St. John's, Canada. *charlotte.gauthier1@uqac.ca

Atlantic halibut (*Hippoglossus hippoglossus*) is currently the groundfish with the highest commercial value per unit weight in the Gulf of St. Lawrence (GSL), Canada. Despite a collapse in the 1950s and subsequent decades of low abundance, landings of Atlantic halibut have steadily increased since the early 2000s, with 2020 recording the highest catch in the last 60 years at over 1400 tons. Following this strong return, improving our knowledge of the habitat use of Atlantic halibut in the GSL is crucial to ensure the long-term sustainability of the stock. From 2013 up to 2018, 114 Pop-up Satellite Archival Tags (PSAT) were deployed on adult halibut throughout the GSL. Prior reconstructions of movement tracks with a geolocation model revealed that spawning occurs in the deep channels of the GSL, and PSAT detachment locations revealed summer site fidelity. To extend analyses across life-stages, we used otolith chemistry to gain information on halibut early-life history and reconstruct migratory movements from birth to capture. Transects of 200 otoliths of juveniles and adults were analysed by LA-ICP-MS (^{88}Sr, ^{138}Ba and ^{24}Mg). The end of each transect, representing capture site, was used to distinguish elemental fingerprints from shallow (<100 m) and deep (>100 m) waters, and split moving window analyses were used to infer migratory patterns. Our results suggest that the stock of Atlantic halibut in the GSL is constituted of multiple contingents. In general, halibut captured in the south of the GSL tend to migrate every year while halibut from the north migrate from shallow to deeper waters for the first years of their life before settling in deeper waters. Overall, our findings highlight the occurence of partial migration which has valuable implications for population structure, productivity, and resilience, and will define the conditions needed for enhanced management outcomes.

Keywords: Otolith chemistry, partial migration, contingents, *Hippoglossus hippoglossus*, split moving window

RETURN TO INDEX

IOS_056

Chasing the great migrant: deciphering the connectivity of yellowfin tuna in the Indian Ocean using otolith stable isotopes

Iraide Artetxe-Arrate[1]*, Iker Zudaire[1]: Patricia Lastra-Luque[1], Jessica Farley[2], Naomi Clear[2], David Dettman[3], Shoaib Abdul Razzque[4], Iñigo Krug[1], Umair Shahid[4]: Gorka Merino[1], Hilario Murua[5], Igaratza Fraile[1]
[1]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Sukarrieta, Spain; [2] CSIRO Oceans and Atmosphere; Hobart, Australia; [3]Environmental Isotope Laboratory, Department of Geosciences, University of Arizona, Tucson, USA; [4]WWF Pakistan, Karachi, Pakistan; [5]International Seafood Sustainability Foundation, Washington DC, USA. * iraide.artetxe@azti.es

Yellowfin tuna (*Thunnus albacares*) is a large migratory species that inhabits the tropical and subtropical waters of the Pacific, Atlantic and Indian Oceans. It supports the second largest tuna fishery worldwide, and in the Indian Ocean, it is considered to be overfished to the maximum sustainable yield limits, with the western region accounting for the ~75% of the catches. This overfishing presents a significant challenge to fisheries management, requiring effective measures to restore and maintain the stock at sustainable levels. For stock assessment purposes in the Indian Ocean, a single stock of yellowfin is assumed by the Indian Ocean Tuna Commission (IOTC). However, the degree of connectivity and mixing rates are still unknown. This study uses otolith oxygen and carbon stable isotope composition ($\delta^{18}O$ and $\delta^{13}C$) of young-of-the-year and juvenile yellowfin tuna from "known" nursery areas in the equatorial Indian Ocean (West, Central and East) to establish a reference baseline of isotopic signatures. This baseline was then used to determine the origin of older yellowfin tuna individuals captured in three locations in the western Indian Ocean: offshore Pakistan, Seychelles, and Reunion. Preliminary results indicated that although some mixing of yellowfin tuna in the western Indian Ocean occurs, each fishing area received different relative contributions of individuals originated from the different nursery areas. Findings of otolith stable isotope composition of yellowfin tuna in the western Indian Ocean can provide a more comprehensive understanding of the species' spatial structure and connectivity beyond the current assessment of a single stock in the ocean basin. This information is essential for developing effective and sustainable management strategies, as species response to management decisions cannot be accurately predicted if the boundaries that characterise the stock do not reflect the reality. To that aim, advancing collaborative scientific and sampling designs in highly migratory species such as yellowfin tuna should be encouraged.

Keywords: population connectivity; stock structure; otolith microchemistry; tuna; stock assessment.

RETURN TO INDEX

IOS_061

Otolith microchemistry and spatial stream network models: investigating natal origins and freshwater habitat use for Broad Whitefish (*Coregonus nasus*) in Arctic, Alaska

Jason C. Leppi[1]*, Daniel J. Rinella[2], Randy J. Brown[3], Karen J. Spaleta[4]
[1]The Wilderness Society, Anchorage, Alaska, United States of America; [2]Fish and Wildlife Field Conservation Office, U.S. Fish and Wildlife Service, Anchorage, Alaska, United States of America; [3]Fairbanks Fish and Aquatic Conservation Office, U.S. Fish and Wildlife Service, Fairbanks, Alaska, United States of America; [4]Alaska Stable Isotope Facility, Water and Environmental Research Center, Institute of Northern Engineering, University of Alaska Fairbanks, Fairbanks, Alaska, United States of America;
*jason_leppi@tws.org

Conservation of Arctic fishes is challenging partly due to our inability to track fish through time and space, which constrains our understanding of movement patterns and habitat use. Broad Whitefish (*Coregonus nasus*) is an important subsistence species for Alaska's Arctic Indigenous communities, yet little is known about natal origins, migration patterns, and freshwater habitat use. Using laser ablation Sr isotope otolith microchemistry and spatial stream statistical models developed for the Colville River watershed, we analyzed Broad Whitefish $^{87}Sr/^{86}Sr$ chronologies (n = 61) to identify natal origins and reconstruct movements and habitat use across the freshwater larval and juvenile rearing periods. $^{87}Sr/^{86}Sr$ in otolith natal regions suggests that multiple areas were used for spawning and that individuals from a given spawning area had roughly similar life histories. Otolith $^{87}Sr/^{86}Sr$ from freshwater rearing regions was distinct from that in natal regions, suggesting that most individuals moved downstream to rear after hatching. Otolith $^{87}Sr/^{86}Sr$ from early life stages suggest that freshwater habitat use changed in association with age, seasons, and life history strategies. Our research provides new insights into complex patterns of larval and juvenile habitat use by Broad Whitefish and highlights the importance of maintaining a diverse and connected portfolio of habitats across the Colville River watershed. This information will help managers and conservation planners better understand the risks of anthropogenic impacts and help conserve this vital subsistence resource.

Keywords: Subsistence, Colville River, Conservation, Stream model

RETURN TO INDEX

IOS_065

Simple non-destructive method of individual cohort determination of young Barents Sea cod for condition analyses

Arild Folkvord[1,2]*, Julie Skadal[1], Christian Irgens[1,3], Øyvind Langnes[1], Elena Eriksen[2]
[1]Department of Biological Sciences, University of Bergen, Bergen, Norway. [2]Institute of Marine Research, Bergen, Norway;
[3]Rådgivende Biologer, Bergen, Norway, *arild.folkvord@uib.no

Assignment of individual cohort affiliation is essential when documenting individual condition dynamics. Even though the size overlap between the youngest annual cohorts typically is low, accurate clarification of cohort affiliation of fish in overlapping size ranges is needed to separate the slowest growing fish of the older cohort from the fastest growing fish of the younger cohort as these fish are assumed to have experienced different condition dynamics. We used simple non-destructive otolith shape and morphological analysis of Barents Sea cod juveniles sampled from two independent surveys for cohort assignment. This enabled us to follow the changes in condition of the cod over the winter season without having to section otoliths for age determination of fish in the overlapping size range. The accurate individual cohort assignment revealed a more variable size dependent condition status than expected, indicating different life history strategies to cope with survival challenges during winter at high latitudes.

Keywords: Atlantic cod, *Gadus morhua*, cohort determination, condition

RETURN TO INDEX

IOS_066

Migration history of Pacific cod around Hokkaido, Japan, using 14C analysis of otoliths

Keito Aonuma[1]*, Kozue Ando[1], Yosuke Miyairi[1], Masayuki Chimura[2], Tomonori Hamatsu[2], Osamu Sakai[2], Yusuke Yokoyama[1]
[1]The Atmosphere and Ocean Research Institute (AORI), the University of Tokyo, Kashiwa, Japan; 2Kushiro Field Station, Fisheries Resources Institute, Japan Fisheries Research and Education Agency (FRA), Kushiro, Japan. *aonuma-keito168@g.ecc.u-tokyo.ac.jp

Pacific cod (Gadus macrocephalus) is an important fishery resource in the North Pacific Ocean and the Sea of Okhotsk. From a conservation perspective, their stock structure has been estimated using morphological differences and the relationship between catches among different areas. Local migration histories have been studied in several areas using tagging however, there have been no studies of migration histories over larger areas. This study reports on the use of 14 C in otoliths to estimate the migration history of Pacific cod around Hokkaido. Otoliths have been widely used as indicators of the migratory history of fish. For Pacific cod living in deep waters, traditional approaches to analysing migration history present difficulties, as installing sensors and loggers requires fish capture and can result in damage to the fish due to decompression.

Analysing migration history using otoliths has the advantage of not requiring such prior capture. In this study, Pacific cod caught in four areas around Hokkaido, Japan were analysed. Otoliths were graphitised using a stepwise acid dissolution method, and the graphite was analysed by AMS (Accelerator Mass Spectrometry). As a result, individuals which never left a particular area of the sea throughout their lives and those that moved between multiple areas of the sea were identified. Notably, the majority of Pacific cod caught in the Sea of Japan remained in that sea throughout their lives. The results showed that some of the individuals captured in other areas had extremely low Δ 14 C values of nearly -700‰. Such low Δ 14 C values are unlikely based on 14 C concentrations in typicalsea waters, suggesting that the fish may have migrated to the waters with a carbon supply from solid earth. This study confirms the suitability of a recently developed radiocarbon-based method for estimating the migration history of Pacific cod.

Keywords: Accelerator Mass Spectrometry, Stepwise acid dissolution method, Radiocarbon analysis, *Gadus macrocephalus*

RETURN TO INDEX

IOS_070

Pattern for otoliths readings of *Chloroscombrus chrysurus* in the South-eastern Brazilian Bight

Kathleen Angélica Rautenberg[1], Eduardo Luis Cupertino Ballester[1,2], Dimitris Politikos[3], Paulo Ricardo Schwingel[4], André Martins Vaz-dos-Santos[2,5]*
[1]Post-graduate Program of Zoology, Federal University of Paraná, Brazil; [2]Post-graduate Program in Aquaculture and Sustainable Development, Federal University of Paraná, Brazil; [3]Hellenic Center for Marine Research, Greece. [4]University of Vale of Itajaí, Brazil; [5]Sclerochronology Laboratory, Federal University of Paraná. Rua Pioneiro, 2153 CEP 85950-000 Palotina – PR – Brazil.
*kathleenrautenberg@live.com

Subtropical species usually present complexes patterns in the otolith's rings due to environmental and biological plasticity. The Atlantic bumper, *Chloroscombrus chrysurus*, is a small pelagic fish that inhabits shallow and estuarine waters in larval and juvenile stages; the adults form dense shoals in coastal waters and in the continental shelf. It is targeted by the industrial purse seine fleet in the South-eastern Brazilian Bight (SBB, 22ºS-28º40'S). To establish a pattern for the macrostructural analysis, 485 sagitta of individuals (160-400 mm TL) sampled between 2006-2010 in the SBB were analysed. The whole otoliths were photographed under stereomicroscope, then the number of rings were counted (NR, from the core to the begin of each translucent zone), and measurements were done (length, Lo; height, Ho, radius, Ro; ring radius, Rn, all in millimetres and the two last from the core to the posterior edge). Techniques in line were used to describe otoliths radius and rings measurements. The otolith length (Lo) varied from 2.33 to 7.54 mm (average±s.d.: 5.592±0.717 mm), the otolith height (Ho) from 1.98 to 5.74 mm (2.204±0.201 mm) and the Ro from 1.82 to 3.74 mm (2.790±0.330). NR varied from one to seven, and the Rn varied as follows: 1st: 1.036±0.128 mm; 2nd: 1.509±0,149 mm; 3rd: 1.959±0.171 mm; 4th: 2.327±0.184 mm; 5th: 2.637±0,192 mm; 6th: 2.888±0,203 mm; and 7th: 3.282 mm. The constancy graph (TL vs Rn) and boxplots showed low overlap in Rn, revealing precision in ring positions, indicating that macrostructural analysis can be used to age and growth. Nevertheless, a fitted power model TL vs Ro (a=0.084, b=0.6265, r^2 =0.6448) presented relative high variability. Despite the establishment of a reading pattern, further ring's validation (including the 1st by microstructural analysis) and back-calculation techniques will refine the current results for accuracy in the age determination of *C. chrysurus*.

Keywords: whole otoliths, radius, Atlantic bumper, purse seine.

RETURN TO INDEX

IOS_072

Blue Belt programme – Preliminary results on age-based life-history traits of four species from Ascension Island

Valerio Visconti[1]*, Ian Woodgate[1], Suzy End1, Maura Smith[1, 2], Tiffany Simpson, Paul Whomersley[1].
[1]Cefas, Lowestoft, United Kingdom; [2]Fisheries and Conservation department, Ascension Island. *valerio.visconti@cefas.gov.uk

The Blue Belt is the UK Government's flagship international marine conservation Programme that assists several UK Overseas Territories (UKOTs) in creating and maintaining healthy and productive ecosystems. Ascension Island is one of those territories; an isolated volcanic island in the equatorial waters of the South Atlantic Ocean, a hotspot of pelagic biodiversity that attracts higher abundances of commercially targeted species within its EEZ. Apart from big pelagic (e.g. yellowfin tuna, bigeye tuna etc.), Ascension Island hosts a mixture of fish species (133, including 11 endemics) that can be found in a limited area of shallow waters. The inshore fishing pressure is largely recreational and constrained by the small size of the island population. It targets mainly rockhind grouper, moray eel, and glasseye snapper. Only recently, an Inshore Fisheries Management Strategy has been proposed and consulted on to secure social, economic and environmental objectives. However, little is known regarding the stock status and population dynamics of the targeted species. Here, we present the preliminary results on age-based life-history traits of four species from Ascension Island waters: glasseye snapper *Heteropriacanthus cruentatus*, moray eel *Gymnothorax moringa*, wahoo *Acanthocybium solandri* and squirrelfish *Holocentrus adscensionis*. Fish samples were obtained from the recreational fishery and collected during specific surveys between 2014 and 2021, and the pair of otoliths (sagittae) were extracted and preserved dry or in ethanol. A thin transverse section (250-400 µm) from each of the 295 specimens was prepared for age determination and subsequently read by two expert readers under transmitted light. Annual growth increments were identified for each species and age-determination protocols were established. Preliminary age-based results revealed various life spans across species with the glasseye snapper attaining 9 years, moray eel 23 years, squirrelfish 21 years and wahoo 8 years. While the generated model of growth and parameters showed consistency with previously studied species (e.g. wahoo), the present study represents the first attempt at ageing unstudied species. Overall, the results of this research provide valuable age-based information essential to support future conservation actions, monitoring and management programs in Ascension Island.

Keywords: glasseye snapper, moray eel, wahoo, squirrelfish, life-history

RETURN TO INDEX

IOS_074

Should I stay or should I go: Resident flounder in an Arctic lake inferred by otolith chemistry and genetics

Susanne E. Tanner[1]*, Thais Ferreira-Araújo[2], Petri Kemppainen[3], Bronwyn M. Gillanders[4], Paolo Momigliano[3], Patrick Reis-Santos[4]
[1]MARE - Marine and Environmental Sciences Centre/ARNET - Aquatic Research Network Associated Laboratory, Department of Animal Biology, Faculty of Sciences, University of Lisbon, Lisbon, Portugal; [2]Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland; [3]School of Biological Sciences, Faculty of Science, The University of Hong Kong, Hong Kong; [4]Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, Adelaide, Australia; *setanner@ciencias.ulisboa.pt

Plasticity in life-history traits allows species to adapt to varying conditions throughout their distribution range as well as to stochastic variations in their local environments. Furthermore, plasticity can increase species' adaptation potential to rapidly changing environmental conditions as well as resilience to exploitation, ecosystem transience and global change. European flounder (*Platichthys flesus*) is known to exhibit plasticity in several traits, ranging from timing and duration of spawning period to differential movement behaviors along the marine-freshwater gradient. Here, we used otolith chemical profiles and genetic markers to investigate movement patterns and genetic diversity of adult and sub-adult flounder sampled near the northern limit of its distribution in the freshwater lake Pulmanki (Finland) and two nearby fjords (Norway). Otolith chemical *composition* in core-to-edge transects (Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca) was determined using LA-ICP-MS and SNP markers were genotyped. Otolith chemical transects showed several distinct patterns. For fish captured in Pulmanki lake, most fish displayed a consistent freshwater signature throughout their life (stable low Sr:Ca and elevated Ba:Ca) but otolith chemical transects also indicated movement from marine or brackish waters to the lake. For individuals sampled in the fjords signatures were more variable and ranged from fully marine to fully freshwater. Overall, a time-series clustering algorithm identified clearly distinct clusters from the otolith chemistry life history profiles. Genetic data provided evidence for weak genetic differentiation between Pulmaki lake and fjord samples.The majority of fish from Pulmanki lake showed genotypes with no admixture to neighbouring populations, while the remaining Pulmanki and fjord samples showed admixed genotypes. Integrating information from both markers suggested that part of the Pulmaki flounder are resident in the lake and may even spawn there given the non-admixed genotypes, however for several individuals from Pulmaki lake and the fjords the information obtained using the two markers is contradictory (e.g., otolith freshwater signature and admixed genotype). Different scenarios that can explain the observed movement patterns and genetic differentiation are explored.

Keywords: *Platichthys flesus*, movement patterns, genetic diversity, otolith chemical transects, SNP markers

RETURN TO INDEX

IOS_075

Combining multiple tissues to unravel estuarine habitat use in a euryhaline fish

Benjamin D. Walther[1]*, Ethan J. Taulbee[1], Louisa E. Torrance[1]
[1]Department of Life Sciences, Texas A&M University-Corpus Christi, Corpus Christi, USA; *benjamin.walther@tamucc.edu

Abstract: The combined use of chemical signatures in multiple structures in mobile fishes can help provide a comprehensive understanding of movement and habitat use across dynamic estuarine gradients. We first conducted a literature review to assess prior studies that have analyzed pairwise combinations of otolith chemistry, tissue stable isotopes, and scale elemental or isotope chemistry. The taxa and analytes investigated are diverse but reveal critical gaps in both field-based and experimental evaluations of signature incorporation dynamics. We then applied combined otolith, scale and muscle tissue analyses to understand the oligohaline movement patterns and dietary interactions of a euryhaline species Red Drum (*Sciaenops ocelltus*) in subtropical estuaries in the northwestern Gulf of Mexico. These systems are subject to restricted freshwater inflow punctuated by fold events and prolonged droughts. Red Drum showed evidence of limited movement into oligohaline waters and instead exhibited a high degree of site attachment at a sub-bay scale. Signatures in both scales and muscle were strongly driven by proximity to freshwater inflow sources indicating differential impacts of terrestrial subsidies. This case study demonstrates the flexibility and utility of analyzing multiple structures in fishes inhabiting complex estuarine systems.

Keywords: Otoliths, Scales, Muscle isotopes, Migration, Sciaenidae.

RETURN TO INDEX

IOS_076

Stable isotope composition of otolith nuclei as a tool to assign spawning components of Baltic cod (*Gadus morhua*)

Sven Stötera[1]*, Uwe Krumme[1], Nils Andersen[2], Franziska Maria Schade[1,3]
[1]Thünen Institute of Baltic Sea Fisheries, Alter Hafen Süd 2, 18069 Rostock, Germany; [2]Leibniz -Laboratory for Radiometric Dating and Stable Isotope Research, University of Kiel, Germany; [3]Agriculture and Fisheries Research Institute for Mecklenburg-Vorpommern, Dummerstorf, Germany; *sven.stoetera@thuenen.de

In the Baltic Sea, stock assessment and ecological understanding of the western and eastern cod (*Gadus morhua*) stock is hampered due to ample mixing. The stocks spawn at different seasons in basins with distinct isotopic characteristics of the water bodies which should be reflected in the otolith nuclei during the juvenile period when cod display a rather localized habitat use. Therefore, we aimed at assessing the stable isotope composition in Baltic cod otolith nuclei as a potential indicator of spawning ground origin to facilitate stock assignment of cod in the Baltic Sea. Stable isotope oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) were obtained from otolith nuclei of adult spawning Baltic Sea cod females of five spawning components from the Belt Sea, Arkona Sea and Bornholm Sea to establish spawning component baselines. Subsequently, the isotopic composition of otolith nuclei of juvenile cod sampled along a west-east axis were compared to the baseline to assign the spawning origin. Two spawning component baselines were established, a western-spring (Belt Sea, Arkona Sea) and an eastern spring/summer (Arkona Sea, Bornholm Sea) group. $\delta^{13}C$ lacked any spatial pattern. Based on $\delta^{18}O$, all juvenile cod otoliths could be assigned to either the western or eastern spawning component baseline suggesting that $\delta^{18}O$ in the nucleus of the otolith can be used as proxy for stock assignment. In addition, to connect the baselines and the assigned juvenile cod to the two cod stocks, the baselines were assigned to either stock by otolith shape analysis. Despite thorough selection of the baseline otoliths by spawning time and area, the shape analysis of spawning component baseline otoliths displayed an uncertainty of 12% (Belt Sea) and 13% (Bornholm Sea) in stock assignments. The results also suggested a mixing of juvenile western and eastern Baltic cod in the eastern Arkona and western Bornholm Sea. The $\delta^{18}O$ signature of adult and juvenile otolith nuclei reflected the spatial differences between the basins, however, due to the uncertainties in the baselines, individual cod could not be assigned unambiguously to their stock component. This suggests that validation by methods independent of the environmental influence such as genetic analysis is required.

Keywords: Otoliths, nucleus, stable isotopes, spawning components, stock discrimination

RETURN TO INDEX

IOS_079

Insights from tetracycline-marked otoliths of Baltic Sea flounder stored for over 40 years

Dace Zilniece[1]*, Tatjana Baranova[1] , Didzis Ustups[1], Maris Vitins
[1]Institute of Food Safety, Animal Health and Environment BIOR, Latvia. *dace.zilniece@bior.lv

We re-examine otoliths from Baltic Sea flounder (Platichthys flesus and Platichthys solemdali) marked with tetracycline hydrochloride (TCH) in two mark-recapture experiments in 1976 and 1981. The otoliths have been stored for over 40 years at room temperature in dry and dark conditions. We verify that most marks are still visible on whole otolith samples and match images taken immediately after recapture, confirming that TCH is suitable for very-long-term chemical marking of otoliths. Furthermore, in otoliths sliced in accordance with the age reading method recommended by ICES, the marks are visible even more clearly than in the original samples, reaffirming the utility of otolith slicing. Next, we examine the pattern of opaque and hyaline zone formation outward from the marks, which represents the time between capture and recapture (several weeks to five years). Given this pattern and the capture and recapture dates, we validate the assumptions about otolith growth zone formation that underlie the standard age determination method in flounder. Finally, we measure the radii of the otoliths to the edge and to the TCH mark and combine these data with the original records on the body length of the fish at capture and recapture to examine the relationship between otolith growth and body length growth.

Keywords: Mark-recapture; Tagging; Chemical marking; Age determination

RETURN TO INDEX

IOS_081

Investigating *Aphanopus spp.* in the Atlantic waters using otolith contour: converging population hypotheses

Joana Vasconcelos[1,2]*, José Luis Otero-Ferrer[3], Alba Jurado-Ruzafa[4], Antoni Lombarte[5], Carlos Hernández-González[4], Mafalda Freitas[2,6], Víctor M. Tuset[7]
[1]BIOCON, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain; [2]MARE - Marine and Environmental Sciences Centre/ARNET - Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Funchal, Portugal; [3]Biostatech, Advice, Training and Innovation in Biostatistics (Ltd.), Santiago de Compostela, Spain; [4]Centro Oceanográfico de Canarias (IEO-CSIC), Santa Cruz de Tenerife, Spain; [5]Departamento de Recursos Marins Renovables, Institut de Ciències del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 37–49, 08003 Barcelona, Catalonia, Spain [6]Direção de Serviços de Monitorização, Estudos e Investigação do Mar, Direção Regional do Mar, Funchal, PORTUGAL.; [7]Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, Unidad Asociada ULPGC-CSIC, Canary Islands, Spain. *joanapatricia.reis@ulpgc.es

The black scabbardfish (*Aphanopus carbo*) and the intermediate scabbardfish (*A. intermedius*) coexist from the North to South Atlantic waters. These sympatric species are landed as a single mixed resource since no external differentiation is noticeable to discern both species. In the past decade, a great effort was made into separating these two scabbardfishes using both genetic and body morphometric analyses. A total of 492 otoliths from Madeira archipelago were used in a previous analysis to discriminate both species using the contour analysis with wavelets functions. The classifier Support Vector Machine (SVM) identified *Aphanopus* species with a 100% level of accuracy. Considering the former sample as a training-set for the species differentiation, 1,546 otoliths collected in 1990, 2003 and 2021 from Madeira archipelago and 897 otoliths of from Morocco (collected in 2005 and 2006) and Mauritania (collected in 2007) were analyzed to accurately distinguish both species. Our results show the possible co-existence of oceanic and shelf platform populations of *A. carbo* and *A. intermedius*. Finally, a thorough revision of previous published studies is urgently needed since they may contain mixed individuals/specimens of both *Aphanopus* species.

Keywords: Otolith contour analysis, wavelets, *Aphanopus* spp., population connectivity, Atlantic Ocean.

RETURN TO INDEX

IOS_082

Moving matters: Growth implications of movement and environmental variation for a potamodromous fish

Joshua Barrow[1]*, Jian Yen[2], John Koehn[2], Brenton Zampatti[3], Ben Fanson[2], Jason Thiem[4], Zeb Tonkin[2], Wayne Koster[2], Gavin Butler[5], Ryan Woods[6], Steven Brooks[7], Arron Strawbridge[8], John Morrongiello[1] *Joshua.barrow@unimelb.edu.au
[1]School of BioSciences, University of Melbourne, Parkville, Victoria, 3010, Australia [2] Applied Aquatic Ecology, Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, 123 Brown St, Heidelberg, Victoria, 3084, Australia.[3] Commonwealth Scientific and Industrial Research Organisation (CSIRO), Locked Bag No. 2, Glen Osmond, SA 5064, Australia. [4]Department of Primary Industries, Grafton Primary Industried Institute, 16 Experiment Farm Road, Trenayr, NSW 2460, Australia. [5]Department of Primary Industries, Narrandera Fisheries Centre, PO Box 182, Narrandera, NSW 2700 Australia. [6]Department of Environment and Science, Ecosciences Precinct, GPO Box 5078, Brisbane, Qld, 4001, Australia. [7]Department of Agriculture and Fisheries, GPO Box 46, Brisbane, Qld, 4001, Australia. [8]Inland Waters and Catchment Ecology Program, SARDI Aquatic Sciences, PO Box 120 Henley Beach, SA 5022 Australia.

Movement is a critical activity for many fish species that facilitates important life history processes, including dispersal, access to spawning grounds for reproduction, and improving foraging opportunities. Movement behaviours vary considerably among species and among individuals. Partial migration is one strategy, where some individuals within a species remain resident in one location, and others move, in some cases, large distances. In our studies, we aimed to identify associations between movement and growth of golden perch *Macquaria ambigua*, a potamodromous and partially migratory fish native to the Murray-Darling Basin in eastern Australia. We used growth and microchemistry information that is naturally archived in otoliths to analyse movement and growth patterns over the course of individuals lives. Our first analysis aimed to identify differences in growth between individuals that moved, and those that remained resident. Next, we focussed on directional juvenile *movements* to examine whether the direction in which individuals moved was linked with differences in growth, and subsequently if growth of each dispersal direction was affected by river discharge. Finally, we investigated the environmental drivers of growth, and examined whether including finer scale movement data, thus increasing the resolution of environmental predictors would increase the performance of fish growth models. First, we found that individuals that moved had increased growth, particularly in the first eight years of life, which was when movements were more likely to occur. Next, we identified that juvenile growth was enhanced by moving downstream, compared to upstream and resident individuals. There was a negative effect of birth year summer discharge on growth of all dispersal types and a positive effect of birth year spring discharge on growth of downstream dispersers. Finally, we found that increasing the resolution of environmental predictors is initially beneficial for model performance, but there was no clear difference between the performance of the current modelling technique, using conditions from the location of capture, and our new method, using environmental conditions from locations where individuals lived. Our studies further our understanding of the associations between movement and growth and provide a pathway for future growth modelling for mobile fishes.

Keywords: Otoliths, Microchemistry, Stable isotopes, Dispersal, Movement, Partial Migration, River regulation.

RETURN TO INDEX

IOS_083

Life history chronology of anadromous Coregoninae fishes of subsistence harvest importance in Arctic Alaska

Kevin M. Fraley[1]*, Jason C. Leppi[2], Randy J. Brown[3]
[1]Wildlife Conservation Society, Fairbanks, Alaska, USA; [2]The Wilderness Society, Anchorage, Alaska, USA; [3]United States Fish and Wildlife Service, Fairbanks, Alaska, USA. *kfraley@wcs.org

In Northwest Alaska, Coregoninae fishes such as inconnu (*Stenodus leucichthys*; Iñupiaq: *Sii*) and humpback whitefish (*Coregonus pidschian*; Iñupiaq: *Iqalupiaq*) are important subsistence harvest species for rural and Indigenous Alaskans in both coastal and inland communities. Inconnu in particular are an important resource, with over 25,000 harvested annually in Kotzebue Sound. The migratory patterns and preferred habitats of these fishes are well-documented for the freshwater spawning stage of their life cycle, but their estuarine and coastal marine movement patterns and habitat use are largely unknown. Recent reports of inconnu being found more than 250 km away from their normal coastal range have amplified calls to characterize life history patterns of anadromous whitefishes in the region, in part to describe how they may be responding to climate change effects. To ascertain this, we collected sagittal otoliths from twenty-two adult inconnu and fourteen adult humpback whitefish from coastal lagoons within Cape Krusenstern National Monument. Using laser ablation isotope microchemistry, we analyzed strontium (^{88}Sr, $^{87}Sr/^{86}Sr$) across each otolith core-to-edge chronology to infer movements and habitat use during the lives of each fish. Preliminary results indicate that inconnu from two separate spawning stocks spend the majority of their lives in brackish environments, returning to freshwaters to spawn multiple times after reaching maturity. Similar patterns were seen for humpback whitefish, although members of this species appeared to occupy full saltwater habitats more often than inconnu. Analyses are ongoing, but these data represent novel information about the life history patterns of these important subsistence-harvested fishes.

Keywords: Otoliths, microchemistry, whitefishes, coastal lagoons, Alaska

RETURN TO INDEX

IOS_084

Diet journals recorded in eye lenses reveal critical habitats supporting an endangered salmon

Carson Jeffres[1]*, Miranda Bell-Tilcock[1] and Anna Sturrock[3], Rachel Johnson[1,2]
[1]University of California Davis, Center for Watershed Sciences, Davis, California, USA; [2]National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center, Santa Cruz, California, USA. [3]University of Essex, Wivenhoe Park, Colchester, UK. *cajeffres@ucdavis.edu

The function of floodplains to produce abundant food resources that results in accelerated growth for fishes is well studied. However, one of the largest unknowns is whether individuals that access floodplains relative to other freshwater habitats function as nurseries translating to disproportionate survival and a greater number of fish that are recruited to adulthood. Here, we used stable isotopes ($\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$) in sequential lamina in salmon eye lenses to identify juvenile use of floodplain ($\delta^{34}S$ <5‰) versus riverine food webs ($\delta^{34}S$ >5‰) in adult endangered Sacramento River winter-run Chinook salmon in California (USA). We compared the proportion of adults that used floodplains as juveniles in 3 years with contrasting timing and duration of hydrologic connection. Results reveal that the vast majority of natural origin winter run adults (84-99%) that survived to spawn used floodplain food resources relative to fish that solely relied on river-derived food resources. This high level of floodplain use in the adults was surprising given some years fish had seemingly limited floodplain access. Otolith strontium isotopes from the same fish were used to discriminate between rearing time in different freshwater rivers during outmigration. However, without the associated lens data, otolith chemistry was unable to detect in-river vs. off-channel rearing patterns. Timing of migration among salmon runs, flow events, and off-channel inundation all play important roles in determining the extent to which juvenile salmon have access to floodplain resources. This study demonstrates the applicability of this technique as a new tool to quantify population-level benefits of floodplain habitats for juvenile salmon to adult recruitment. It also highlights the ability to use $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ in individual eye lens lamina to identify specific rivers and/or floodplains based on their unique isotopic signature.

Keywords: Eye lenses, Stable isotopes, Salmon, California, Floodplains

RETURN TO INDEX

IOS_085

Anchoveta *Engraulis ringens* along the Chilean coast: Management units, demographic units and water masses: Insights from multiple otolith-based approaches

Cristóbal Garcés[1]*, Edwin J Niklitschek[2], Guido Plaza[3], Francisco Cerna[4], Mathieu Leisen[5], Pamela Toledo[2], Fernando Barra[6]
[1]Escuela de Graduados, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile; [2]Centro i~mar, Universidad de los Lagos, Puerto Montt, Chile; [3]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile; [4]Instituto de Fomento Pesquero, Valparaíso, Chile; [5]Géosciences Environnement Toulouse , Université de Toulouse, Toulouse, France; [6]Andean Geothermal Center of Excellence, FCFM, Universidad de Chile, Santiago, Chile *garcesbima@gmail.com

Engraulis ringens is a small pelagic fish that inhabits the Humboldt Current System where it supports one of the most productive ecosystems in the world. Three management units (MUs) are used for stock assessment and fishery regulations of anchoveta *Engraulis ringens* in Chilean coast (>65% of *E.ringens* latitudinal range): MU-I [18–25)°S, MU-II [25–32)°S and MU-III [32–42)°S. To evaluate whether these three managements units correspond to separate demographic units, as well as to estimate mixing rates and spatial distribution of these demographic units, we combined three types of otolith natural markers: isotopic signatures ($\delta^{13}C$ and $\delta^{18}O$), elemental compositions (Na, Mg, Mn, Sr and Ba) and microstructural indexes. All markers were determined in nuclear and marginal otolith's regions of juveniles and adults fish from cohorts 2012 and 2015. Differences in core region markers indicated spatial segregation between three environmentally distinct nursery areas, probably related to Subtropical Water (MU-I), Subantarctic Water (MU-III) and the Subtropical Convergence (MU-II). Comparison of core and marginal regions, from juvenile and/or adult fish, suggested that adult fish remained nearby nursery areas, separated from fish nursed in other MUs. Nonetheless, ontogenetic migrations from warmer (offshore) nursery habitats to cooler (deeper/more coastal) feeding habitats occurred within MUs. In summary, our results support the consideration of the three management units as three separate demographic units. Estimated mixing rates indicated MU-II received contributions of 31% from MU-I and 3% from MU-III, while complete segregation existed between MU-I and MU-III. Such mixing rates between MU-I and MU-III seem large enough to justify its further consideration by stock assessment models and management procedures.

Keywords: Humboldt Current, Stock assessment, Stable isotopes, Elemental compositions, Microstructural indexes

RETURN TO INDEX

IOS_091

Evaluating otolith increment deposition rates in bigeye tuna (*Thunnus obesus*) and yellowfin tuna (*T. albacares*) tagged in the Atlantic Ocean

Kyne Krusic-Golub[1]*, Lisa Ailloud[2,3]
[1]Fish Ageing Services, 28 Swanston Street Queenscliff, Victoria 3225, Australia *kyne.krusicgolub@fishageingservices.com
[2]International Commission for the Conservation of Atlantic Tunas, Secretariat Calle Corazón de Maria 8 28002 Madrid, Spain
[3]Present address: Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA 75 Virginia Beach Drive Miami, Florida 33149

A recent Atlantic-wide tag-recapture experiment run by the International Commission for the Conservation of Atlantic Tuna was an opportunity to directly validate otolith increment deposition rates for bigeye tuna (*Thunnus obesus*) and yellowfin tuna (*T. albacares*) in the region. Age and time at liberty were estimated by using annual and daily increment counts for sectioned otoliths from sampled fish previously injected with oxytetracycline and later recaptured. The use of annual increment counts resulted in greater age estimates than those from daily increment counts, for fish >55 cm straight fork length (SFL). Use of daily increment counts led to underestimation of time at liberty in fish >55 cm SFL at recovery, compared with known times at liberty. In contrast, predictions based on annual increment counts are accurate across the entire size range of sampled fish, validating the notion that increments are deposited annually. We therefore recommend that counting annual increments be the preferred method for aging yellowfin and bigeye tuna from the Atlantic Ocean and that the use of daily increments for aging be limited to young of the year. Aging fish accurately is important for stock assessments in which data on age and growth play an increasingly essential role in examining population dynamics. It is crucial that otolith reading practices and analyses based on age data reflect the most up-to-date recommendations for age estimation.

Keywords: Otolith, Oxytetracycline, ICCAT, Annual increments, Daily increments

RETURN TO INDEX

IOS_093

Can otolith transition zones benefit the management of exploited fisheries? A New Zealand fishery example

Thomas Barnes[1,2]*, Matthew Dunn[1], Peter Horn[1], Caoimhghin Ó Maolagáin[1], Keren Spong[1]
[1]National Institute of Water and Atmospheric Research, 305 Evans Bay Parade, Hataitai, Wellington, Aotearoa/New Zealand
[2]Institute for Marine and Antarctic Studies, Hobart, Tasmania, Australia

Otoliths provide a wealth of information which via stock assessment models and other analysis assist management of exploited fish stocks. Multiple types of otolith features are routinely applied to research and assessments (e.g. increment width, age estimation etc.). However, there are other less utilised otolith features such as transition zones (TZs) also available to fisheries scientists. Transition zones are where otolith growth zone widths change because energy allocation switches from somatic growth to include gamete production. TZs therefore mark the age of sexual maturity. For orange roughy (ORH), an exceptionally long-lived deepwater species in New Zealand (NZ), otolith readers routinely identify and record the age at TZ. We give examples of TZs on orange roughy otoliths and analyse the TZ age. Analysis showed the median TZ (~30 years) can differ (~±5 years) between spatially separated stocks. In NZ, ORH maturity has been estimated within stock assessment models, rather than using the TZ. The TZ age is often substantially lower than model estimates. The difference might indicate skipped spawning in younger fish, but we (briefly) show how model maturity estimates can be driven by other, seemingly unrelated, data. The difference between TZ and model estimates might therefore be an indicator of structural problems in the assessment model, rather than requiring a biological explanation. Transition Zones and other less utilised otolith features may therefore benefit other fisheries research and monitoring promoting more accurate management of exploited fish stocks.

Keywords: Otoliths, sexual maturity, stock structure, stock assessment, deepwater, fisheries management

RETURN TO INDEX

IOS_095

Utilizing alizarin red S in multi coding of three different strains of brown trout stocked in Lake Inari, Finland

Sami Vesala[1]*, Teuvo Niva[1], Nico Alioravainen[1], Sari Raineva[1]
[1]Natural Resources Institute Finland; *sami.vesala@luke.fi

In 2000's Lake Inari, large arctic lake in Northern Finland, has supported fisheries with total annual catch of 140t, from which about 50 t is by caught by professional fishermen. Lake has been regulated for hydro power production since 1950's, which caused strong decrease in fish catches. For compensating catch losses, a massive stocking program was implemented in 1976. Only local stocks of Arctic charr (*Salvelinus alpinus),* brown trout (*Salmo trutta*) and whitefish (*Coregonus lavaretus*) are used for stocking. Moreover, brown trout broodstocks at the hatchery include three genetically different strains. All species used in stocking reproduce in Lake Inari also naturally.

Alizarin Red S (ARS) marking of all young fish has used in Inari hatchery regularly since 2004 for separating stocked fish from wild ones. Moreover, for brown trout we have developed multi coding system for identifying the three different strains. Multi coding is based on repeated marking during different stages of development of fish. First marking at eyed egg stage produce smaller mark than that of newly hatched larvae, and repeated treatments can be done until the fish is stocked.

The stocking success of whitefish, brown trout and Arctic charr is monitored annually from samples collected from both commercial and recreational fishery and non-fishery dependant sampling. The sample data is combined with catch statistics inquired from local people. At the lab, all otoliths are checked for ARS under stereo fluorescence microscope to separate stocked and wild fishes. Ageing of fish is used for growth analysis and year class structure. The results show that in brown trout at younger age groups the proportion of stocked fish is high, but in older age groups the wild individuals more abundant. Utilizing ARS has been proved to be a cost effect method for estimate productivity of large-scale stockings in relation to wild fish production.

Keywords: Otoliths, alizarin, fishery management, productivity, stoking

RETURN TO INDEX

IOS_100

Near-infrared spectroscopy of otoliths in the discrimination of the Baltic herring stock components

Karolina Jonko-Sobuś[1], Szymon Smoliński[2]*, Marina De Gea Neves[3]
[1]Department of Food and Environmental Chemistry, National Marine Fisheries Research Institute, Gdynia, Poland
[2]Department of Fisheries Resources, National Marine Fisheries Research Institute,
Gdynia, Poland
[3]Department of Physical Chemistry, University of Duisburg-Essen, Essen, Germany
*ssmolinski@mir.gdynia.pl

Herring (*Clupea harengus*) is one of the most important species in the Baltic Sea. During its annual migration herring groups from distinct spawning areas meet on the same feeding ground. Since these distinct groups are often characterized by different biological parameters and population dynamics, proper assignment of the stock components is crucial for the stock assessment and sustainable management of these living marine resources.

There are numerous methods for the identification of individuals originating from distinct areas. Although classical methods are well-established, i.e. comparison of the otolith shape or morphometrics features, the development of a less time-consuming alternative would be useful.

Non-invasive spectroscopic techniques, including Near Infrared Spectroscopy (NIR), gains more interest in environmental science. In combination with chemometrics, NIR can be used to detect subtle differences and gain in-depth information on the otoliths, which “fingerprints” can provide valuable information on the individual origins.

Herring otoliths have been collected in the southern Baltic Sea in the year 2020. Presented preliminary studies apply the handheld NIR scanner in combination with a PLS-DA calibration model to distinguish otoliths from different Baltic herring components. Measurements included 142 specimens along with reference data. The discrimination method based on chemometric analysis of spectroscopy spectra of two distinguished groups of herring (so-called northern and southern components) obtained discrimination accuracies >82%. These promising preliminary results demonstrate the potential of the NIR technique as a fast screening tool. Incorporation of this method in the sampling routines can provide near-at-sea estimates of the contribution of different groups of herring in mixed scientific or commercial catches.

Keywords: Baltic herring, mixing, near-infrared spectroscopy, otolith

RETURN TO INDEX

IOS_102

Micro-X-ray fluorescence image analysis of otoliths to distinguish wild-born and stocked whitefish in the Baltic Sea

Viktor Finnäs[1]*, Jan-Olof Lill[2], Timo Saarinen[3], Christer Lindqvist[4], Yvette Heimbrand[5], Erkki Jokikokko[6], Henry Hägerstrand[1]
[1]Environmental and Marine Biology, Åbo Akademi University, Turku, Finland; [2]Accelerator Laboratory, Åbo Akademi University, Turku, Finland; [3]Department of Geography and Geology, University of Turku, Turku, Finland; [4]Cell Biology, Åbo Akademi University, Turku, Finland; [5]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Öregrund, Sweden; 6Natural Resources Institute Finland, Keminmaa, Finland. * viktor.finnas@abo.fi

Whitefish in the Gulf of Bothnia, northern Baltic Sea, occur as two sympatric ecotypes, anadromous river-spawning and sea-spawning whitefish, which cannot reliably be differentiated based on morphology. To counteract the decline in the whitefish stocks, around ten million freshwater-reared one-summer-old fingerlings are annually stocked along the Finnish coast. Wild-born river-spawning whitefish and whitefish stocked as fingerlings retain a low concentration of strontium (Sr) ~ 200–1700 µg/g in the central region of the otolith, reflecting the low concentration found in the freshwater environment where they spend the first stage of their life. This can be contrasted with otoliths from wild-born sea-spawning whitefish in the Gulf of Bothnia which obtains a strontium concentration of ~ 2600–7700 µg/g, reflecting the salinity gradient in this area. The aim of the present work was to demonstrate a method for differentiating wild-born individuals of the two ecotypes and whitefish stocked as fingerlings using image analysis of strontium distribution maps of otoliths. Wild-born river-spawning whitefish were sampled during the spawning run in River Tornionjoki. To represent whitefish stocked as fingerlings, a sample caught during the spawning run in River Kemijoki was selected which in previous research was determined to be comprised of stocked individuals. The third sample was caught whilst spawning in the sea near the Åland Islands, representing wild-born sea-spawning whitefish. The distribution of strontium in the otoliths was mapped with µ-XRF. Using ImageJ, an open-access software, the maps were converted to 16-bit grayscale and a suitable threshold was selected to define the border between the Sr-poor and Sr-rich regions in the otoliths. Finally, the area of the Sr-poor region was measured using the wand-tracing tool of the software. The area of the Sr-poor region in otoliths from wild-born river-spawning whitefish had a mean area of 0.18 ± 0.2 mm^2. The corresponding area in otoliths from whitefish stocked as fingerlings was 2.3 ± 0.3 mm^2. Otoliths from wild-born sea-spawning whitefish lacked a Sr-poor region altogether. Measuring the area of the Sr-poor regions in whitefish otoliths provides a useful method to differentiate whitefish of different provenance and to determine the contribution from stocking.

Keywords: *Coregonus lavaretus* L., µ-XRF

RETURN TO INDEX

IOS_103

Using otolith 87Sr/86Sr to determine fish provenance and migration history across a large river basin: approaches, management utility and future considerations

Brenton Zampatti[1]*, Benjamin Fanson[2], Lee Baumgartner[3], Gavin Butler[4], Steven Brooks[5], David Crook[6], Katherine Doyle[3], Alison King[1], Wayne Koster[2], Roland Maas[7], Aleksey Sadekov[8], Peter Scott[8], Arron Strawbridge[9], Jason Thiem[6], Zeb Tonkin[2], Phillipa Wilson[10], Jon Woodhead[7] and Ryan Woods[11]

[1]Commonwealth Scientific and Industrial Research Organisation (CSIRO), Adelaide, Australia; [2]Arthur Rylah Institute for Environmental Research, Melbourne, Australia; [3]Gulbali Institute, Charles Sturt University, Albury, Australia; [4]Department of Primary Industries, Grafton, Australia; [5]Electrofishing Services, Wamuran, Australia; [6]Department of Primary Industries, Narrandera, Australia; [7]School of Geography, Earth and Atmospheric Sciences, The University of Melbourne, Melbourne, Australia; [8]Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Perth, Australia; [9]South Australian Research and Development Institute (SARDI), Adelaide, Australia; [10]Australian Institute of Marine Science, Indian Ocean Marine Research Centre, The University of Western Australia, Perth, Australia; [11]Department of Environment and Science, Brisbane, Australia. *brenton.zampatti@csiro.au

Globally, the effective conservation of riverine fishes requires an understanding of when and where key life history processes (spawning, recruitment, and movement) occur. To aid this, the structural and chemical properties of otoliths provide a unique means to recount a fish's life in time and space. Relating otolith chemistry to the chemistry of a fish's surrounding environment, in conjunction with otolith chronological properties, has become an increasing popular tool to elucidate the spatial history of individuals. The approach, however, relies on a detailed understanding of spatio-temporal variability in ambient water chemistry or catchment geology, and accurate methods to quantitatively ascribe otolith chemistry to these factors.

Here, we used otolith 87Sr/86Sr to study aspects of the autecology and population dynamics of a wide-ranging, pelagophilic fish (golden perch, *Macquaria ambigua*) in Australia's Murray-Darling Basin (>1 x 106 km2), one of the worlds most regulated river basins. Using >700 water samples across 7 years we developed a whole-of-basin 87Sr/86Sr isoscape using a Bayesian spatial network model for a braided stream system. We then developed a Markov chain probabilistic assignment approach to assign natal origin and lifetime regional locations, based on known capture location, to 87Sr/86Sr profiles from 712 individual golden perch. Development of the approach has also highlighted limitations which, if addressed, will improve confidence in predictions.

The approach allowed us to investigate provenance and recruitment, and the movement of individuals (including ontogenetic variability) from rivers across the Basin. In turn, we have related spawning and recruitment to spatially reconciled river flow and developed probabilistic models of flow-related movement to inform management of golden perch at the basin-scale. This research is guiding the management of river flows and the mitigation of barriers to fish movement to achieve population-level outcomes, including the rehabilitation of populations following prominent fish kills. Our investigations further demonstrate the utility of otoliths as a tool to understand the lifetime movements of fish across the riverscape and the application of this knowledge to inform actions that will aid the conservation of imperilled riverine fishes.

Keywords: Murray-Darling Basin, dendritic networks, connectivity, recruitment, flow

RETURN TO INDEX

IOS_106

Validating true winter zones in European eel (*Anguilla anguilla*) otoliths, using oxygen isotopes

Rickard Yngwe [1]*, Jennie Strömquist[1], Malin Hällbom[1], Yvette Heimbrand[1]
[1]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden. *rickard.yngwe@slu.se

The European eel (*Anguilla anguilla*) living in the northern part of Europe is a slow growing species that can get up to 30 years old before starting its long journey back to the Sargasso Sea. During winter, near freezing temperatures can occur, and during this period, eels are hibernating. True annuli are believed to form as translucent zones when the water is cold during winter, followed by an opaque zone corresponding to fast growth in summer. However, during summer there can be disturbances that cause severe stress for the eels, such as low water temperatures, shortage of food, and other disturbances in the eels' habitat. This can create "false" annuli, or "stress checks". The irregular growth patterns and various checks in the otoliths can be difficult to interpret for an accurate age assignment. Calibrations between age readers have shown low level of agreement compared to other species. Thanks to various tagging experiments, there is a large number of otoliths from eels of known age. The results from an age calibration of 161 eels of known age, with ten age readers, showed a total agreement of <50%. In order to validate true annuli in eel otoliths without known age, stable oxygen isotope analyses were performed using a secondary ion mass spectrometry (SIMS). Otolith $\delta^{18}O$ reflects the ambient water temperature and salinity. Hence, micro chemical analyses of otolith $\delta^{18}O$ provide detailed information of the water temperature at the time of the otolith formation, which can be used for age validation.

Keywords: Microchemistry, Age validation, δ18O

RETURN TO INDEX

IOS_107

Identification of otolith chemical marking and implications for age determination of European eel (*Anguilla anguilla*)

Rickard Yngwe *[1], Jennie Strömquist[1], Malin Hällbom[1], Yvette Heimbrand[1]
[1]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden. *rickard.yngwe@slu.se

The European eel (*Anguilla anguilla*) is severely threatened. Catches have declined by over 90 % during the last 50 years. Factors behind this negative development of the stock consists of changes in climate and oceanic currents, overfishing, pollutants, anthropogenic obstacles restricting migration, such as dams and hydropower related mortality. A management plan was produced in Sweden in 2007, due to a regulation issued by the European Union. The plan included measures for the recovery of the European eel considering regional and local conditions. One part of the Swedish management plan is restocking glass eels. Since 2009 all glass eels restocked in Sweden are quarantined and bathed in a strontium chloride ($SrCl_2$) solution. This procedure creates a chemical mark in the otoliths, which incorporates high levels of Sr into the calcium carbonate structure. During the last decade, as a stock assessment strategy, otoliths have been checked for Sr marking to assess the proportion of restocked versus naturally recruited eels in Swedish waters. Eels are age determined by counting the number of annual growth zones, formed by translucent winter zones and opaque summer zones. Otoliths from restocked eels often display a false ring from the Sr marking during the quarantine period that can easily be mistaken for a true winter zone, which is problematic in age determination. Therefore, otolith micro chemical analyses were performed to determine if these otolith ring formations were true winter zones or caused by the Sr marking.

Keywords: Strontium, Microchemistry, Age validation

RETURN TO INDEX

IOS_108

Importance of estuaries for the horse-eye jack (*Caranx latus*) in northeast Brazil

Júlio Guazzelli Gonzalez[1]*, Thierry Frédou[2], Paulo José Duarte-Neto[3], Maylis Labonne[4], Audrey M. Darnaude[4]
[1]Asian School of the Environment, Nanyang Technological University, Singapore; [2]Departamento de Recursos Pesqueiros e Aquicultura, Universidade Federal Rural de Pernambuco, Recife, Brazil; [3]Departamento de Estatística e Informática, Universidade Federal Rural de Pernambuco, Recife, Brazil; [4]MARBEC, Marine Biodiversity, Exploitation & Conservation, Université de Montpellier, CNRS, Ifremer, IRD, Montpellier, France. *julio.gonzalez@ntu.edu.sg

This study evaluated whether inshore estuaries are key juvenile habitats for the horse-eye jack (*Caranx latus*), supporting the exploited marine stocks of this tropical species. For this, otoliths from 143 juveniles were analyzed to develop a dataset of multi-elemental fingerprints for the main juvenile habitats of *C. latus* in northeast Brazil, namely seven estuaries and the coastal zone. This dataset was then confronted with the signatures in the juvenile part of the otolith of 40 sub-adults and adults captured at sea to identify their origin. Because otolith multi-elemental concentrations largely overlapped for some of the estuaries investigated, the maximum overall discrimination accuracy between them was only 58%. However, grouping neighbouring estuaries according to their level of freshwater inputs (saline or brackish) allowed us to increase it to 80%. In both cases, correct re-assignment rates for the coastal zone were above 94%, and strontium contributed most to juvenile habitat discrimination. The remaining elements (B, Ba, Co, P, Rb, Sr and Zn) allowed distinguishing between estuarine habitat types, for which correct re-assignment rates were 70-88%. Juvenile fingerprints in sub-adult and adult otoliths revealed that most of them (75%) had spent their first year in an estuary, principally saline (32.5%). Thus, at least in northeast Brazil, inshore habitats are key in maintaining *C. latus* marine stocks. This connectivity at the land-sea interface is likely to happen for other marine species and should be considered for sustainable management of coastal fisheries in the tropics.

Keywords: Marine fish, Transitional waters, Otolith microchemistry, Juvenile habitat.

RETURN TO INDEX

IOS_109

The use of otolith ageing and microchemistry to disentangle European hake connectivity and its application to understand its recruitment dynamics

Sílvia Pérez-Mayol[1]*, Mariña Fabeiro[2], Ignacio A. Catalán[1], Sonia Rábade[2], Beatriz-Morales-Nin[1], Fran Saborido-Rey[2]
[1]Department of Marine Ecology, Institut Mediterrani d'Estudis Avançats (IMEDEA, CSIC-UIB), Esporles (Balearic Islands), Spain; [2]Department of Marine Ecology and Resources, Instituto de Investigacións Mariñas (IIM, CSIC), Vigo, Spain. *silvia@imedea.uib-csic.es

European hake (*Merluccius merluccius*) is a highly exploited fish species in Spanish waters. Although many studies were addressed to reach a better understanding of its biology, there are still knowledge gaps related to its recruitment dynamics. Therefore, management policies cannot be correctly adopted to maintain this valuable resource, which is currently considered overexploited. The North Atlantic (NA) and South Atlantic (SA) European hake stocks show differences in their genetic composition, and several studies have demonstrated strong differences in their reproduction dynamics, which are modulated by environmental factors and fishing. European hake on the Eastern Atlantic are managed considering two stocks corresponding to NA and SA ICES divisions, which include the Spanish Gulf of Biscay and the Galician Coast, respectively. There is evidence to suggest that these two separately managed stocks could have some degree of spatial overlap. Our specific objective is to estimate the degree of connectivity between the two stocks using sclerochronology, combining both otolith age analysis and the otolith chemical microcomposition. Thus, otoliths of 97 European hake (mature females from commercial landings and juveniles from different surveys) from both stocks were aged (daily) and analysed by LA-ICPMS. Trace element analysis consisted of transects from core-to-edge of isolated spots of 30 µm diameter, quantifying a suite of 14 isotopes (up to 8113 shots between otoliths and reference materials). While otolith edge microcomposition could not be used to identify capture origin (i.e., stock affiliation), chemical differences were found for cores, at least for juveniles of both stocks. Moreover, ontogenetic differences in chemical composition were found in mature hake from NA, in addition to larger sizes in comparison to mature hake from SA. These results suggest that fish of the two Spanish areas studied experience different environmental conditions during hatching/early larval stages and juvenile stages, and that they have a different geographical origin of where adults are captured. In conclusion, there is a subpopulation structure at the juvenile level that seems to be maintained also on adults.

Keywords: *Merluccius merluccius*, Atlantic divisions, Stocks, Subpopulations, LA-ICPMS

RETURN TO INDEX

IOS_110

Migration and habitat use of the Japanese grenadier anchovy *Coilia nasus* in the Ariake Sea, Japan

Tianying Li[1,2]*, Yulina Hane[3], Hiroki Takenaka[1,2], Ryoshiro Wakiya[1,2], Tomihiko Higuchi[2], Kento Goto[4], Kotaro Shirai[2], Kazuki Yokouchi[5], Shingo Kimura[1,2], Hikaru Itakura[2]
[1]Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Japan; [2]Atmospheric and Ocean Research Institute, University of Tokyo, Japan; [3]Graduate School of Agricultural and Life Sciences, University of Tokyo; [4]Fukuoka Fisheries and Marine Technology Research Centre; Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Japan
*1286690836@edu.k.u-tokyo.ac.jp

The Japanese grenadier anchovy *Coilia nasus* is a species distributed in the western Pacific that can live up to four years and grow to a maximum length of approximately 410 mm. In China, it is one of the most expensive fish, with the anadromous variety reaching prices up to US$1500/kg. In Japan, it is only found in the Ariake Sea and its inflowing rivers. While adult fish migrate annually from the sea to freshwater areas to spawn in the summer, juveniles migrate to the sea in October to overwinter. However, the specific timing of their initial downstream migration and the extent of their spatial migration in the Ariake Sea are still poorly understood, despite the species experiencing a continuous decline in fishery catch. Previous studies have categorized four groups with different initial migration patterns based on otolith Sr/Ca ratios. However, Sr/Ca ratios cannot differentiate between residence in brackish water environments such as estuaries and coastal seawaters, and they cannot infer absolute values for the salinity that the fish experienced. An alternative approach is to use oxygen stable isotopes in the otoliths ($\delta^{18}O_{otolith}$) to estimate salinity. The $\delta^{18}O$ values of brackish water in estuarine regions are primarily dependent on mixing conditions between freshwater and seawater. This leads to large variations in $\delta^{18}O_{otolith}$ in fish that migrate across freshwaters, estuaries, and seawater. Reconstruction of salinity by $\delta^{18}O_{otolith}$ is based on linear isotopic fractionation relationship between $\delta^{18}O_{otolith}$ values, temperature, and $\delta^{18}O_{water}$. The species-specific fractionation equation is derived from tank rearing experiments, and otolith are drilled with a micromill accordingly to the growth ring from edge to core at a depth of 50 μm, with the powders collected for $\delta^{18}O_{otolith}$ measurement. By comparing the highest salinity reconstructed with the salinity data from monthly observations by prefecture authorities, the possible residence range of the fish in the Ariake Sea can be inferred. This method allows for a better understanding of the fish's movement patterns in brackish environments. Understanding the migration patterns is important for developing management plans that can help to protect them from further decline.

Keywords: Oxygen stable isotope, Otoliths, Salinity reconstruction

RETURN TO INDEX

IOS_111

Age validation of yellowfin tuna in the Indian Ocean using post bomb radiocarbon chronologies

Patricia L. Luque[1*], Igaratza Fraile[1], Steven E. Campana[2], Jessica H. Farley[3], Kyne Krusic-Golub[4], Naomi Clear[3], Paige Eveson[3], Iraide Artetxe-Arrate[1], Iker Zudaire[1], Hilario Murua[5], Gorka Merino[1]
[1]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Pasaia, Gipuzkoa, 20110, Spain; [2] Faculty of Life and Environmental Sciences, University of Iceland, Reykjavic, Iceland; [3]CSIRO Oceans and Atmosphere, Hobart, Tasmania 7000, Australia; [4]Fish Ageing Service, Queenscliff, VIC 3225, Australia; [5]International Sustainability Seafood Foundation (ISSF), Pittsburg, PA, United States *plastra@azti.es

Yellowfin tuna (*Thunnus albacares*) stock assessments use age-structured models, therefore, accurate methods for ageing the catch are required. Age estimation techniques need to be validated at the population level to ensure accuracy. However, otolith-based age estimates of yellowfin tuna in the Indian Ocean have not yet been validated. The current study provides the first age validation work done for Indian Ocean yellowfin tuna using the post-bomb period of the bomb radiocarbon (^{14}C) chronometer. A ^{14}C reference chronology based on accelerator mass spectrometry assays of known-age yellowfin tuna otoliths was consistent with published regional coral records, with all showing similar rates of decline during 2000 to 2019 study period. After back-calculating the birth years of sub-adult and adult yellowfin tuna from otolith annual increment counts, otolith core $\Delta^{14}C$ values were projected over the observed decline slope of the reference chronology. No systematic offsets were observed between the birth years of validation and reference samples, supporting the otolith increment age determination methodology between the ages of 2 and 10 years. The next challenge in this promising field of research is to have a larger reference chronology that will provide greater precision in validation results.

Keywords: ^{14}C, *Thunnus albacares*, otoliths, age validation

RETURN TO INDEX

IOS_112

Biphasic versus monophasic growth curve equation, an application to common sole in the northern and central Adriatic Sea

Francesco Masnadi[1],[2],; Pierluigi Carbonara[3]; Fortunata Donato[1]; Laura Sabatini[1],[4]*; Giulio Pellini[1]; Massimiliano Cardinale[5]; Giuseppe Scarcella[1]
laura.sabatini@irbim.cnr.it*
[1]National Research Council, Institute for Marine Biological Resources and Biotechnology (CNR IRBIM), Ancona, Italy; [2]Department of Ecology, Environment and Plant Sciences (DEEP), Stockholm University, Stockholm, Sweden; [3]COISPA Tecnologia & Ricerca – Stazione Sperimentale per lo Studio delle Risorse del Mare, Bari, Italy; [4]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy; [5]Swedish University of Agricultural Sciences, Department of Aquatic Resources, Institute of Marine Research, Lysekil, Sweden.

Traditionally, growth patterns are described as constant throughout life using von Bertalanffy's equation. However, a change in growth due to a reallocation of energy during an individual's lifespan is to be expected. Following this hypothesis, back-calculation measurements obtained from SoleMon survey data were used to fit and compare monophasic and biphasic growth curves for common sole in the northern and central Adriatic Sea. Moreover, individual variability in growth was considered through nonlinear mixed-effect models in which the individual parameters were considered as a random effect. The analyses performed in this study revealed systematic age-specific discrepancies in the monophasic curve and demonstrated that the fit of the biphasic curve was superior (Δ AIC: 329; Δ BIC: 310), confirming the theory that growth in size would decrease as a consequence of reproductive effort. Finally, since common sole is routinely assessed using models that rely on growth to derive assessment estimates and related management reference points, a stock assessment simulation was performed to compare the two growth alternatives. The results showed how the biphasic alternative was preferable to the conventional alternative and how the use of the monophasic pattern would result in an overly optimistic view of stock status (+40% in SSB/SSB_{target} and -35% in F/F_{target} compared to the biphasic pattern), thereby increasing the risk of overfishing.

Keywords: Back-calculation, Growth comparison, Mixed-effect modeling, Stock assessment effects, *Solea solea*

RETURN TO INDEX

IOS_113

Stable isotope ratios in otoliths and eye lenses reveal population connectivity of sardine in the western North Pacific and its marginal seas

Tatsuya Sakamoto[1],[2]*, Amélie Simon[3], Kotaro Shirai[4]
[1]Instituto Português do Mar e da Atmosfera, Portugal; [2]Japan Fisheries Research and Education Agency, Japan; [3]Universidade de Lisboa Instituto Dom Luiz, Portugal; [4]Atmosphere and Ocean Research Institute, University of Tokyo, Japan. *tatsfish@gmail.com

Sardine in the western North Pacific and the Sea of Japan supports one of the largest sardine fisheries in the world. Although the sardine is assumed to consist of two self-sustaining subpopulations in the Pacific and the Sea of Japan, biological evidence for this structure is severely limited. In 2014 and 2019, sardine schools in the Sea of Japan, which come to coastal areas each spring to spawn, were barely observed, and fishery catches and abundances of eggs declined to about one-tenth of the previous year. In 2015 and 2020, however, schools of sardines appeared in spring, containing age-1 fish hatched the previous year as usual. These observations not only indicated significant shifts in sardine migration patterns in 2014 and 2019, but also called into question the origin of the recruits in the Sea of Japan. Here, based on biogeochemical approaches, we show that sardine in the Sea of Japan do not form a self-sustaining subpopulation and most adult fish there likely originate from the Pacific. Analyses of stable oxygen isotope ratios in otoliths from over 350 individuals and stable carbon and nitrogen isotope ratios in eye lens cores from over 1900 individuals showed that isotope values were remarkably different between juveniles and adults in the Sea of Japan and similar between juveniles in the Pacific and adults in the Sea of Japan. Sea surface temperature analysis also showed that the southern Sea of Japan had been hit by severe marine heatwaves in the summer of 2013 and 2018. Unusually high summer sardine catches were observed near the boundary between the Sea of Japan and the Pacific Ocean in the summers of 2013 and 2018, implying that the heatwave caused Pacific immigrants to return to the Pacific, and not enter Sea of Japan again in 2014 and 2019.

Keywords: Otoliths, Eye lenses, Stable isotope, Sardine

RETURN TO INDEX

IOS_115

Otolith shape analysis as an effective tool for stock identification of two commercially important marine fishes, *Helicolenus dactylopterus* and *Merluccius merluccius*, in the Northeast Atlantic and the Mediterranean

Ana Vaz[1], Milene Alexandra Guerreiro[1], Jorge Landa[2], Ioannis Thasitis[3], Hannipoula Olsen[4], Giuseppe Scarcella[5], Laura Sabatini[5,6], Sergio Vitale[7], Estanis Mugerza[8], Kélig Mahé[9], Patrick Reis-Santos[10], Susanne E. Tanner*[11], Christoph Stransky[12], Miguel Pardal[1], Filipe Martinho[1]
[1]Centre for Functional Ecology - Science for People & the Planet, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, Coimbra, Portugal; [2]Instituto Español de Oceanografía, Centro Oceanográfico de Santander (IEO, CSIC), Santander, Spain; [3]Fisheries Resources Division, Department of Fisheries and Marine Research, 2033 – Nicosia, Cyprus; [4]Faroe Marine Research Institute, Faroe Islands; [5]Institute for Biological Resources and Marine Biotechnologies - National Research Council of Italy (IRBIM-CNR), Ancona, Italy; [6]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy; [7]Institute for Biological Resources and Marine Biotechnologies - National Research Council of Italy (IRBIM-CNR), Mazara del Vallo, Italy; [8]AZTI, Sustainable Fisheries Management, Basque Research and Technology Alliance (BRTA), Sukarrieta, Bizkaia (Basque Country), Spain; [9]IFREMER – Channel and North Sea Fisheries Research Unit, Center Manchmer du Nord, Boulogne sur mer, France; [10]Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, Australia; [11]MARE – Marine and Environmental Sciences Centre/ARNET – Aquatic Research Network Associated Laboratory, Department of Animal Biology, Faculty of Sciences, University of Lisbon, Portugal; [12]Thünen Institute of Sea Fisheries, Bremerhaven, Germany.

Stock identification studies are essential to understanding fish population structure and connectivity across wide geographical areas, and thus contribute to efficient fisheries management. The blackbelly rosefish, Helicolenus dactylopterus, and European hake, Merluccius merluccius, are two economically important marine fishes in European waters, but there are still gaps in knowledge regarding their present stock structure. Our objective was to assess the ability of otolith shape to define stock structure for the two species along the Northeastern Atlantic Ocean and the Mediterranean Sea, based on samples from eight and seven areas, for blackbelly rose fish and European hake, respectively. Otolith shape analysis was obtained through Wavelet analysis, and canonical analysis of principal coordinates provided significant evidence for different population units with a clear separation between the Atlantic and Mediterranean populations for both species. However, separate analyses of Atlantic and Mediterranean were necessary for better discrimination at a smaller spatial scale. Random forest procedures indicated that the discrimination power of otolith shape varied with species and locations: for blackbelly rosefish, various Atlantic populations were more evident than for European hake, highlighting distinct connectivity patterns of both species. Overall, we demonstrated the usefulness of otolith shape to delineate stocks of these two species with distinct life history traits across a broad spatial region from the mid-Atlantic isles to the polar regions, as well as in the Mediterranean. Moving forward, it will be key to align our growing understanding of population structure with our increasing knowledge on species' biological traits to ensure management units reflect population structure.

Keywords: Otoliths, Microstructure, Dicentrarchus labrax, Platichthys flesus, Solea solea

RETURN TO INDEX

IOS_117

Extent of anadromy among pike (*Esox lucius*) in eastern Baltic Sea and coastal fresh water bodies

Mehis Rohtla[1]*, Roland Svirgsden[1], Imre Taal[1], Lagle Matetski[1], Kateriina Rumvolt[1], Mari-Liis Põlme[1], Markus Vetemaa[1]
[1]Estonian Marine Institute, University of Tartu, Tartu, Estonia. *mehis.rohtla@ut.ee

Pike (*Esox lucius*) inhabiting the Baltic Sea region has recently received considerable scientific interest because of an ecosystem regime shift that has resulted in increased prey fish and decreased predatory fish abundances. Being a top aquatic predator and an important catch in recreational and commercial fisheries (country dependent), there is a strong need to improve the population status of pike. Pike display three life-history tactics in the Baltic Sea region: freshwater residency, anadromy and brackish water residency. However, the relative and absolute proportions of these tactics are mostly unknown, but seem to vary across the Baltic Sea based on available data. Identification of these proportions will allow to advise stock managers and direct conservation and restoration needs. We sampled young-of-the-year pike (n=510) from 34 coastally-connected streams or lakes, and adult pike (n=562) from 28 locations in the coastal sea and quantified their otolith strontium to calcium ratios to explore the extent of pike anadromy and brackish water residency in eastern Baltic Sea. The results demonstrated that freshwater spawning (anadromy) is strongly prevalent (~90%) among Baltic Sea sampled pike, and brackish water spawning is rare (~10%), although historically it was widespread according to anecdotal evidence. Anadromous pike migrated up to 11 river-kilometers (rkm) upstream, although the mean was ~4 rkm among different locations. Progeny of anadromous mothers also prevailed in freshwater spawning grounds which were accessible to anadromous individuals and within 11 rkm from the sea. Our results demonstrate the current strong prevalence of freshwater spawning compared to brackish water spawning among Baltic Sea dwelling pike, and stress the importance of conservation and restoration actions in fresh water to support the pike stock in the coastal sea.

Keywords: Otolith, microchemistry, Sr:Ca, Estonia.

RETURN TO INDEX

IOS_118

Life-history of Atlantic bluefin tuna (*Thunnus thynnus*) revealed by otolith chemistry

Igaratza Fraile[1], Iraide Artetxe-Arrate*[1], Haritz Arrizabalaga[1], Patricia L. Luque[1], Natalia Diaz-Arce[1], Naiara Rodriguez-Ezpeleta[1], Christophe Pecheyran[2], Fanny Claverie[2], Jay Rooker[3], Deirdre Brophy[4], Yohei Tsukahara[5], Jose-Luis Varela[6], Simeon Deguara[7], Robert Allman[8], Beverly Barnett[8], Piero Addis[9]
[1]AZTI, Marine Research, AZTI Basque Research and Alliance, Herrera kaia portualdea z/g, 20110 Pasaia, Spain; [2]UPPA IPREM, Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux, Université de Pau et des Pays de l'Adour/CNRS, 2 avenue P. Angot, 64053 Pau cedex 9, France; [3]Department of Marine Biology, Texas A&M University at Galveston, 200 Seawolf Parkway, Galveston,TX 77553, USA; [4]Marine and Freshwater Research Centre, Atlantic Technological University, Dublin road, Galway, H91 T8NW, Ireland; [5]Japan Fisheries Research and Education Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa, 236-8648, Japan; [6]Department of Biology, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI·MAR), Av. República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain; [7]AquaBioTech Group, Central Complex, Naggar Str., Mosta, MST 1761, Malta; [8]National Marine Fisheries Service, Southeast Fisheries Science Center, 3500 Delwood Beach Rd, Panama City, FL 32408, U.S.A.; [9]Department of Animal Biology and Ecology, University of Cagliari Viale Poetto 1, I-09126 Cagliari, Italy. *irartetxe@azti.es

The migratory nature of Atlantic bluefin tuna (*Thunnus thynnus*) presents challenges to its management. The understanding the biological and environmental factors influencing trans-Atlantic movements is fundamental for the management and conservation of this species. Atlantic bluefin tuna undertake long-distance migrations from the main spawning grounds in the Gulf of Mexico (GOM) and the Mediterranean Sea (MED) to the rich feeding grounds in the North Atlantic Ocean, where mixing occurs. Although understanding the movement patterns of this species is key to understand its behavior and essential to develop management plans that ensure conservation, incorporating this complex spatial structure into the stock assessment of Atlantic bluefin tuna is challenging, partly due to the lack of sufficient information on habitat use and stock mixing. In this study, otolith microchemistry across ontogeny is used to examine the migratory patterns of individuals captured in the feeding grounds for which the potential source population was previously determined through genetics. Chronological profiles of four major trace elements (Mg, Mn, Sr and Ba) were measured by femtosecond laser ablation-ICPMS across the otolith growth axis. Overall, Mg and Mn concentrations were high in the young-of-the-year period and decreased to low levels after one year of age. Instead, Sr and Ba concentrations increased after age one and displayed sinusoidal temporal patterns, with peaks correlating with opaque bands, likely reflecting migratory patterns between water masses of distinct physico-chemical properties. Patterns across ontogeny were investigated and compared between individuals genetically assigned as arising from the MED and GOM populations, shedding light into the habitat use of ABFT from either eastern or western populations. Our results will be used to inform the stock assessment process about the migratory behaviour of the Atlantic bluefin tuna in relation to biological and environmental factors and will thus contribute to developing efficient management plans.

Keywords: Migratory, Atlantic Ocean, stock origin, trace elements, bio-chronology

RETURN TO INDEX

IOS_119

Comparing shape R packages using wavelet functions for connecting fish populations

Joana Vasconcelos[1]*,[2], José Luís Otero-Ferrer[3], Alba Jurado-Ruzafa[4], Amalia Manjabacas[5], Victor M. Tuset*[6], Antoni Lombarte[5]
[1]BIOCON, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain; [2]MARE - Marine and Environmental Sciences Centre/ARNET - Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Funchal, Portugal; [3]Biostatech, Advice, Training and Innovation in Biostatistics (Ltd.), Santiago de Compostela, Spain; [4]Centro Oceanográfico de Canarias (IEO-CSIC), Santa Cruz de Tenerife, Spain; [5]Department de Recursos Marins Renovables, Institut de Ciències del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 37–49, 08003 Barcelona, Catalonia, Spain; [6]Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, Unidad Asociada ULPGC-CSIC, Canary Islands, Spain; *vtuset@ulpgc.es

Otolith shape analysis has been widely used to delineate structure of populations of marine fish species, mostly based on Elliptic Fourier descriptors. Alternatively, the wavelet transform of the radii length improves the otolith contour interpretation, quantifying the otolith irregularities and determining accurately their position. This last method was initially applied and developed by the AFORO team in 2005, that incorporated it on their website (http://aforo.cmima.csic.es/) and later through the commercial software Age&Shape. Several studies successfully used wavelets for species and stocks identification. Though, Fourier analysis remains in use as it is an open-source software. The worldwide application of the mathematical R environment allowed the development of a package named *shapeR* for the otolith shape analysis in a free access mode. However, we have detected inconsistencies in the philosophy of the use of wavelets in published works. We are currently developing a new R package (*HardShape*) based on previous AFORO works. The present study compares protocols and outputs of both packages using the blue jack mackerel *Trachurus picturatus* from the Canaries and Madeira archipelagos as a case study. *ShapeR* works with radii distances that provides 10 wavelet using the Daubechies least-asymmetric wavelet. *HardShape* defines the noprmalized contour distance from equidistant points using the detail signal of an “à trous” cubic B-spline wavelet. These differences have great effects on the classification system success. In addition, we tested the use of different variables in the standardization of the wavelet coefficients (fish or otolith length) and parametric and non-parametric algorithm classification systems using both packages. More useful information, both in statistics and morphology, is provided by *HardShape*. Also, high resolution samples are easier to manipulate and less time consuming. We are currently trying to make this new *HardShape* R package available for the study of all types of shapes with singular irregularities such as hard structures (e.g., otoliths, limpet shells, bones, among others).

Keywords: Otolith contour analysis, Wavelets, *shapeR*, *HardShape*

RETURN TO INDEX

IOS_121

Reconstructing juvenile common snook ontogenetic movement in estuaries of the Abrolhos Bank using otolith chemistry

Marcelo Soeth[1,2], Mauricio Hostim-Silva[1], Mario Vinicius Condini[1], Felippe Alexandre Daros[4,*]
[1]Marine Fish Ecology Laboratory, North University Center of Espírito Santo, Federal University of Espírito Santo, São Mateus, Brazil; [2]Postgraduate Program in Coastal and Oceanic Systems, Federal University of Paraná, Paraná, Brazil; [3]Instituto Meros do Brasil, Curitiba, Paraná, Brazil; [4]Fish Biology and Ecology Laboratory, São Paulo State University (UNESP – Registro), SP, Brazil, * felippe.daros@unesp.br

Fish movement ecology affects population structure and their resilience to disturbance events and fishing pressure and therefore has been of particular interest to sustainable fisheries management. In the present study, otolith elemental fingerprints were used to reconstruct juvenile common snook ontogenetic movement in four estuaries of the Abrolhos Bank, Brazil: Caravelas (E1), São Mateus (E2), Ipiranga (E3), and Rio Doce (E4). Otolith sampling (n = 60) of juvenile (standard length, mean ± SD = 252.4 ± 54 mm) common snook *Centropomus undecimalis* (Bloch, 1792) took place between January and February 2019. Otolith elemental ratios (Sr/Ca and Ba/Ca) were recorded from the core to the otolith edge using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Typically, the otolith Sr/Ca ratio is positively related to salinity in coastal systems, whereas a high otolith Ba/Ca ratio is indicative of estuarine or freshwater masses. Therefore, a salinity index ($SI = \frac{Sr/Ca\ mg\ g^{-1}}{\mu g\ g^{-1}}$) was calculated to improve the detection of fish movement through a range of saline environments. The SI values on the otolith edges (50 µm) were assumed to be representative of the capture environment. Otolith SI values for common snook individuals from E2, E3 e E4 were often between the thresholds expected for fish residing in oligohaline (59.1%) and freshwater (29.5%). In contrast, otolith SI values from E1 indicated that juveniles spent most of their life (64.1%) in mesohaline-polyhaline waters. Spawning of common snook is known to occur in salinities higher than 24, however, only 33% of individuals showed SI values at the beginning of the otolith profile indicating polyhaline-euhaline waters. Low variability and low near-core SI values were consistent with an early ingress from early life stages in low salinity environments. Additionally, otolith chemistry revealed that juvenile common snook has a limited role in the population mixing between estuaries of the eastern Brazilian coast.

Keywords: Natural tag, Sagittae, *Centropomus*, Eastern Brazilian coast.

RETURN TO INDEX

IOS_122

Prediction of early life events in Brook Trout (*Salvelinus fontinalis*) using a combination of otolith microstructure and developmental index

Michael Hayden[1]*, Todd Dubreuil[2], Jennifer H. Fair[2], Matthew J. O'Donnell[2], Benjamin H. Letcher[2]
[1]Department of Environmental Studies, Antioch University New England, 40 Avon St., Keene NH 03431, USA; and [2]U.S. Geological Survey, Eastern Ecological Science Center, Turners Falls, MA 01376, USA *mhayden@usgs.gov

Brook Trout is a cold-water species native to streams of Eastern North America whose reliance on cold, clean water and pristine habitat requires managers to prioritize streams for protection. While a significant factor in establishing year-class strength, little is known about how environmental variables like temperature influence the timing of early life events (spawn, hatching, and emergence) and Young-of-Year (YOY) survival. We collected brook trout YOY and ambient water temperature in seven headwater streams in Western Massachusetts, USA, to estimate spawn and hatch dates from otolith microstructure analysis. We assessed 1) the reliability of hatch mark, 2) combining otolith microstructure with a developmental index to estimate early life events and 3) applicability of this method to estimate start of emergence for three of our study streams. To create a reference for the otolith analysis on wild fish, otoliths were first collected from lab-raised YOY with known spawn and hatch dates. A reliable hatch mark was not found because there were multiple dark rings encircling the primordia. We developed a new method by counting back to the first ring formation, corresponding to eye-up, then used a temperature-based development index to estimate spawn and hatch dates. Lab otolith-derived estimates were an average of one-week past observed spawn and hatch dates, which is consistent with other published literature. When applied to stream YOY, estimated start of emergence was averaged eight days past field observations. There was some variability between sites with a standard deviation of +/- 6 days. We hypothesize that the variability in the estimated emergence date for wild fish was caused by using ambient water temperature as opposed to actual redd developmental temperatures. Therefore, we plan to refine data on redd temperatures using oxygen stable isotope analysis.

Keywords: Otolith Microstructure, Developmental Index, Salmonid, Brook Trout, Otolith Microchemistry

RETURN TO INDEX

IOS_123

Studying growth drivers in strong year-classes of redfish (*Sebastes spp.*) from the Gulf of St. Lawrence, Canada based on otolith-derived biochronology

Lola Coussau[1]*, Dominique Robert[1], Olivier Morissette[2], Pascal Sirois[2]
[1]Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, QC, Canada; Département des sciences fondamentales, Université du Québec à Chicoutimi (UQAC), Chicoutimi, QC, Canada, *lola.coussau@uqar.ca

After 25 years under moratorium, the redfish (*Sebastes spp*.) fishery is about to reopen in the Gulf of St. Lawrence, Canada (GSL). Due to unprecedented recruitment of three consecutive year-classes: 2011, 2012, and 2013, redfish biomass is currently estimated to represent about 80% of the total fish biomass in the GSL. As redfish stock presents highly sporadic recruitment, the 1980s year-classes were the last major recruitment event that contributed to redfish stock before the collapse. The present study proposes to investigate the growth of the last two high recruitment periods (1980s and 2010s) that emerged under contrasting environmental conditions and developed under different competition regime. The Von Bertalanffy growth parameters revealed that individuals from the 1980s year-classes experienced higher growth rate and reached larger sizes than individuals from the 2010s year-classes. The intrinsic and extrinsic drivers of redfish growth were investigated through linear-mixed effect models applied to time series of otolith increment width covering the 1982-1987 and 2012-2018 periods. After accounting for the growth age-dependent effects, density-dependence was identified as the main regulator of redfish growth. We hypothesized that the growth of the 2011-2013 year-classes was slowed by a substantial competition for food caused by the extraordinary recruitment. The trend of increasing temperatures in the deep waters of the GSL, where redfish live, was positively correlated with growth. In contrast, higher temperature of the cold intermediate water layer (CIL), a layer that redfish larvae need to cross to reach surface waters where they feed, presented negative effect on redfish growth. This relation was most likely explained by the CIL interaction with the dynamics of the copepod *Calanus hyperboreus,* an important prey of redfish. Examination of correlation relationships between increment widths revealed evidence of carry-over effect and compensatory growth process in the fast- and slow-growing year-classes, respectively. Ultimately the conclusions of this study will improve the understanding of the redfish stock dynamic in the GSL, with potential implications for the management of this future large-scale commercial fishery.

Keywords: Otolith increments, Fish growth, Mixed-effect models, Density dependence, Temperature.

RETURN TO INDEX

IOS_124

Can otolith microchemistry and morphometry be used jointly to enhance the prediction of Atlantic salmon (*Salmo salar*) origins?

Anthony Fontaine*[1], Hugo Gallier[2], Matthias Vignon[2], Hélène Tabouret[1], Gilles Bareille[1]
[1]University of Pau and the Adour Region, E2S UPPA, CNRS, IPREM, MIRA, Pau, France; [2]University of Pau and the Adour Region, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France.*anthony.fontaine@univ-pau.fr

In scientific literature, a substantial amount of research has investigated the performance of otolith microchemistry versus otolith morphometry to assess fish natal origin. While the two methods are usually used independently as they provide their own information, only a few studies have considered using them simultaneously. When reconstructing fish life history (i.e. natal origins and life strategies), especially the juvenile stage for migratory species, otolith microchemistry is predominant. However, the use of these natural biomarkers may sometimes be limited as the chemical signatures among habitats may be too similar. Alternatively, morphometric analyses could provide additional or complementary information since it has been demonstrated that otolith shape was influenced by genetics, the environment but also the interaction between these two variables. In our study, otoliths from Atlantic salmon (*Salmo salar*) returning to spawning grounds were sampled from 2009 to 2018 in the Adour and the Garonne-Dordogne basins (France), in addition to individuals from six basins or hydrographical locations along the Bay of Biscay (from North to South: South Britany, Allier, Nivelle (France), Bidasoa, Asturias and Galicia (Spain)). Individual otolith shape was described using the elliptic Fourier analysis (EFA) and the microchemical profile using a femto-laser ablation linked to an inductively coupled plasma mass spectrometry (fs-LA-ICP-MS). The objectives of our study are (i) to compare the results obtained via otolith shape and microchemistry in order to predict a natal (i.e. wild or hatchery) and a geographic origin of salmon, (ii) to investigate the complementarity of shape analysis when microchemistry is limiting and (iii) to study the relevance of combining the two methods within the same dataset to assign an individual origin.

Keywords: Discriminant analyses, Salmonids, Anadromy, Elliptic Fourier Analysis

RETURN TO INDEX

IOS_125

Fin spine chemistry advances knowledge of Atlantic bluefin tuna's early life history migrations

Patricia L. Luque*[1], Saburo Sakai[2], Gorka Bidegain[3,4], Iraide Artetxe Arrate[1], Pedro Morais[5,6], Fanny Claverie[7], Christophe Pecheyran[7], Igaratza Fraile[1], Hilario Murua[8], Haritz Arrizabalaga[1].
[1]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Pasaia, Spain; [2]Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Biogeochemistry Program, Yokosuka, Japan; [3]University of the Basque Country (UPV/EHU), Department of Applied Mathematics, Bilbao, Spain; [4]University of the Basque Country (UPV/EHU), Research Centre for Experimental Marine Biology and Biotechnology (PiE), Plentzia, Spain; [5]Sustainable and Smart Aquaculture Collaborative Laboratory. Av. Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal; [6]Institute of Environment, Florida International University, Miami, FL, United States; [7]Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux UMR 5254 Université de Pau et des Pays de l'Adour 2, avenue du Président Angot 64053 Pau Cedex 09, France; [8]International Sustainability Seafood Foundation (ISSF), Pittsburg, PA, United States.*plastra@azti.es

The assessment of population connectivity and quantification of demographic exchange between populations is critical for the management of migratory fish. Electronic tags are great tools that may disclose 3D movements of fish and environmental characteristics; however, the number of deployed tags still constraints our knowledge. Fish hard structures also present unique insights into the life of migratory fish with the advantage of scientists being able to analyze more individuals. Here, we explore if fin spine chemistry can expand our knowledge about the Atlantic bluefin tuna (ABFT) *Thunnus thynnus* (Linnaeus, 1758) stocks in the Atlantic. The juvenile movement pattern is complex, and longstanding questions remain about the timing, duration, and fraction of the population that makes trans-oceanic migrations between the western Atlantic stock, which spawns in the Gulf of Mexico, and the eastern Atlantic and Mediterranean Sea stock, which spawns in the Mediterranean Sea and from the Mediterranean Sea to the Bay of Biscay. We performed multi-element chemistry analyses of fin spine to investigate whether trace elements (Mg, Mn, Li) and oxygen isotope composition ($\delta^{18}O$) measured across the fin spine growth transects reliably distinguish ambient water characteristics to 1) infer transoceanic and/or local migrations, 2) identify when the movement between Atlantic and Mediterranean waters occurs to better inform the stock assessment and management of ABFT. The chemistry of ABFT fin spines set apart individuals caught across the eastern Atlantic and Mediterranean Sea. The Mn and $\delta^{18}O$ nonparametric regression models showed a fluctuating pattern across annuli that mirrors the seawater characteristic of where the fish lived. In particular, the intra- and interannual $\delta^{18}O$ variation over the lifetime of ABFT had enough resolution to detect early-life migrations between the Atlantic and the Mediterranean. In conclusion, we demonstrated that fin spine chemistry is a reliable tool to characterize residency and unravel movements between ecosystems with strong $\delta^{18}O$ gradients. The management of this iconic fish species in the north Atlantic now benefits from a tool that greatly expands our understanding of their spatial population dynamics.

Keywords: *Thunnus thynnus*, Fin spines, Microchemistry, Stable Isotopes, Migration, Stock delineation.

RETURN TO INDEX

IOS_130

Otolith microchemistry reveals contrasting intra-stock structuring in two demersal species off the Iberian Peninsula

Georgios Kerametsidis[1,2,*], Sílvia Pérez-Mayol[3], Esther Abad[4], Jorge Landa[5], Encarnación García[6], Miguel Vivas[6], Antonio Esteban[6], Antonio Punzón[5], Angelique Jadaud[7], Vincent Rossi[8], Beatriz Morales-Nin[3], Manuel Hidalgo[1]
[1]Centro Oceanográfico de Baleares (IEO-CSIC), Palma, Illes Balears, Spain; [2]University of the Balearic Islands, Palma, Illes Balears, Spain; [3]IMEDEA (UIB-CSIC), Department of Marine Ecology, Esporles, Illes Balears, Spain; [4]Centro Oceanográfico de Vigo (IEO-CSIC), Vigo, Pontevedra, Spain, [5]Centro Oceanográfico de Santander (IEO-CSIC), Santander, Cantabria, Spain; [6]Centro Oceanográfico de Murcia (IEO-CSIC), Lo Pagan, Murcia, Spain; [7]MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Sète, France, [8]Mediterranean Institute of Oceanography (UM 110, UMR 7294), CNRS, Aix Marseille Univ., Univ. Toulon, IRD, Marseille, France.
*georgios.kerametsidis@ieo.csic.es

Stock structure is of central importance for the sustainable management of commercial fisheries. Revealing discrepancies between real biological and management population units has long been a major area of research as such situations inhibit the accurate assessment of exploited stocks. In recent years, stock structure research has moved beyond delimiting boundaries to better comprehend the complexity of population structures within already established stock units. To identify regional intra-stock connectivity across regional gradients off the Iberian Peninsula, we quantified trace element (^{7}Li, ^{23}Na, 24Mg, ^{25}Mg, ^{28}Si, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{88}Sr, ^{137}Ba, ^{138}Ba) concentrations by LA-ICPMS in sagittal otoliths of adult individuals of two highly important demersal resources, the four-spot megrim (*Lepidorhombus boscii*) and the red mullet (*Mullus barbatus*) in the NE Atlantic and NW Mediterranean Sea, respectively. To account for distinct ontogenetic phases that might differently affect horizontal movement patterns, elemental concentrations were measured in the core area (natal region), near the first demersal growth increment (transition from pelagic to demersal stage) and in the edge (catch region) of the otolith. Two techniques were used to assess the discrimination capacity among the different locations, a classic method (Quadratic Discriminant Analysis - QDA) and a machine learning algorithm (Random Forest - RF). Unsupervised RF was used as an additional clustering method to explore how samples cluster spatially without a predefined spatial scale. Differences were detected in some elements among different locations but, for both species, the differences were always less pronounced in the core area. The classification success was higher for the red mullet than for the four-spot megrim using the predefined locations which indicate higher degree of connectivity for the latter mainly during the early-life stage dispersal. For red mullet, a heterogeneous pattern of connectivity was identified within the subunits that were examined. Our study confirms the feasibility of otolith microchemistry as a tool to reveal the spatial structural complexity of marine populations and to investigate connectivity within stock boundaries.

Keywords: Trace elements, Connectivity, Random Forest, *Lepidorhombus boscii*, *Mullus barbatus*

RETURN TO INDEX

IOS_131

First in-situ measurements of field metabolic rate in wild Atlantic mackerel

Yuan Tian Chou[1,2], Bob Marsh[2], Ewan Hunter[3], Clive Trueman[2]
[1]Eastern Marine Biology Research Center, Fisheries Research Institute, COA, Taiwan; [2]Ocean and Earth Science, University of Southampton, UK; [3]Centre for Fisheries and Aquatic Science (AFBI), Belfast, UK. *ytc1m19@soton.ac.uk

The effect of temperature on animal phenotypic and life history traits plays a crucial role in population dynamics, community structure, and evolutionary ecology. Metabolic rate (and therefore food and oxygen requirement) also dictates habitat suitability and growth rate and informs bioenergetic models. While laboratory-based respirometry has provided insights into thermal sensitivity, the current understanding primarily relies on extremes of metabolic rates. In natural settings, processes like feeding and individual variability might decouple from temperature. This discrepancy between laboratory and field conditions calls for a comprehensive exploration of fish metabolism, crucial for ecological and evolutionary insights. However, direct measurements of field metabolic rates (FMR) in marine ecosystems pose challenges, motivating the novel use of otoliths for FMR estimation. We outline a four-step approach to assessing realized thermal sensitivity of FMR in juvenile mackerel *(Scomber scombrus*) from the UK waters. By analyzing otoliths' stable isotopes, experienced temperatures and FMR reconstructions were derived, allowing insights into the mass and temperature scaling of field metabolism in these pelagic fish within UK waters. The findings emphasize body weight as a primary determinant of FMR, highlighting its significant influence. Notably, though individual variability in mass-scaled FMR is prominent, the link between experienced temperature and FMR is found to be inconsequential. This suggests that factors beyond temperature contribute substantially to individual variations in FMR. Interestingly, the results indicate a low thermal sensitivity in juvenile *Scomber scombrus* residing in Scottish waters. The wide-ranging individual FMR variation suggests a robust aerobic capacity under current conditions, potentially affording resilience against temperature fluctuations. However, the adaptability of aerobic scope to future changes in temperature or food availability remains a crucial consideration. The innovative utilization of otoliths as a proxy for FMR offers a promising monitoring tool to assess the ecological and physiological vulnerability of wild fish populations. This study provides valuable insights into the intricate interplay between temperature, metabolism, and individual variability, with implications for understanding and managing the impacts of environmental change on marine ecosystems.

Keywords: Otoliths, $\delta_{13}C$, otolith, metabolic rate, Atlantic mackerel

RETURN TO INDEX

IOS_132

Otolith trace elemental discrimination between Baltic cod (*Gadus morhua*) ecotypes

Yvette Heimbrand[1],*, Karin Limburg[1,2]
[1]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]SUNY College of Environmental Science & Forestry, Syracuse, New York, USA *yvette.heimbrand@slu.se

The Åland Sea is an area in the northern Baltic Sea proper where cod (*Gadus morhua*) are large with good growth and body condition. In the southern Baltic Sea, however, the opposite is true. Here, the alarming status of the cod stock led to emergency measures and a closing of the commercial fishery. Because the monitoring of Baltic cod historically focused mainly on the central and southern parts of the Baltic Sea, there is little knowledge about the cod in the Åland Sea. Cod from both areas are managed as one single population, and genetic analyses have not yet been able to confirm whether that is correct or if there are in fact two distinctly different populations. Trace elemental signatures in cod otoliths from both areas were therefore analysed to evaluate migration pattern and if it was possible to separate Åland cod from the southern Baltic Sea cod based on the otolith chemical composition. The results indicated that there are two ecotypes, one stationary and one migratory type of cod in the Åland Sea. Furthermore, that it was possible to separate cod of age class 1–4 caught in the Åland Sea from cod caught in the south-eastern Baltic Sea based on the otolith trace elemental composition. Boron proved to be the most promising trace element to use as a unique chemical biomarker for identifying Åland cod, with often ten times higher levels in Åland cod otoliths compared to otoliths from southern Baltic cod. The cause of the elevated boron concentrations is still unknown, but likely indicates a large, non-point source.

Keywords: Boron, Baltic Sea, Fish populations, Microchemistry, LA-ICP-MS

RETURN TO INDEX

IOS_134

Otolith microchemistry as a tool to analyse the recruitment of coastal northern pike (*Esox lucius*)

Roland Svirgsden[1],*, Mehis Rohtla[1], Lagle Matetski[1], Mari-Liis Põlme[1], Jürgen Karvak[2], Markus Vetemaa[1]
[1]Estonian Marine Institute, University of Tartu, Tartu, Estonia
[2]Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia
*roland.svirgsden@ut.ee

Northern pike (*Esox lucius*) is a freshwater origin predatory fish that also inhabits coastal zones of the brackish Baltic Sea. It has high value for fisheries and an important ecological role as a predator in coastal ecosystems. In coastal sea pike has two sympatric forms: brackish water resident and anadromous. Depending on area, one form is often more dominant than other, but the exact proportion is usually unknown. To make sound management decisions, it is necessary to know the relative importance of different spawning grounds for coastal pike populations. In this study we assessed the potential of otolith microchemistry to determine the contribution of potential pike spawning and nursery sites on Saaremaa Island (Estonia) to the Baltic pike recruitment. Young-of-the-year (YOY) were obtained from five out of eight pre-selected potential natal sites. Adult pike were sampled from the coastal sea. Multi-elemental signature (Sr:Ca, Ba:Ca and $^{87}Sr:^{86}Sr$) from otolith natal region was used as a natural tag for identifying hatching biome and also specific natal site. Sr:Ca values from otolith core were used to determine the maternal origin (i.e. resident or anadromous) of the sampled YOY. The quadratic discriminant function analysis had a successful reclassification rate of 87% for the juvenile pike sampled from potential natal sites. Based on these classification functions, adult pike sampled from the coastal sea were assigned to their possible natal areas. Altogether 62% of adult fish originated from the freshwater natal sites considered in this study, 10% hatched in brackish water and 28% originated from unknown freshwater sites. Among the juvenile pike sampled from the freshwater reproduction areas 43% were the progeny of anadromous pike and 57% were classified as progeny of freshwater resident pike. Proportion of anadromous progeny varied markedly between sites (0–100%). These results stress the importance of different northern pike forms in maintaining the pike stocks in the coastal sea and adjacent fresh waterbodies. We conclude that otolith microchemistry is a valuable method for studying northern pike recruitment in the eastern Baltic Sea.

Keywords: Natal origin, Multi-elemental signature, Mother peak, Baltic Sea, Estonia

RETURN TO INDEX

IOS_140

Validation of annual periodicity in otoliths of European anchovy (*Engraulis encrasicolus*) in the Gulf of Cadiz (Western Iberian Peninsula)

Carmen Hernández[1]*, Clara Dueñas-Liaño[1], Jorge Tornero[2], Jorge Landa[1], Fernando Ramos[2]
[1]Instituto Español de Oceanografía (IEO, CSIC). Centro Oceanográfico de Santander, Spain. [2]Instituto Español de Oceanografía (IEO, CSIC). Centro Oceanográfico de Cádiz, Spain. *carmen.hernandez@ieo.csic.es

The Spanish waters of the Gulf of Cadiz (GoC, ICES Subdivision 9a South) is one of the core areas of the distribution of European anchovy in the western Iberian stock (Div. 9a). Although previous validation studies exist for this species in other areas, GoC anchovy shows a protracted spawning period which could cause that anchovies from different spawning batches (say spring, summer and autumn-born) could show different growth patterns. Taking this aspect into account, growth validation studies are here focused on a population level. The periodicity of the otolith increment formation in anchovy is here validated through the otolith marginal increment (MIA) and the otolith edge analysis (EA). Furthermore, the temporal link between the reproductive cycle (gonadosomatic index-GSI) and the somatic conditions (condition factor-CF) is evaluated with the otolith edge formation and the patterns of monthly increments. A total of 3038 otolith from 2015 and 2016 were analyzed. Otoliths were aged following standardized criteria and a statistical modelling approach were here adopted to assess the coherence of the two methods, MIA and EA, in order to validate the first annulus formation. The otolith study for the two years analysed showed that the annulus formation resulted mostly complete for this species in the GoC by April. Both methodologies in two different yearly cycles converged toward the same result, thus confirming the annulus identification for the first year class. The GSI showed a spawning peak in July, according to the spawning season. The results obtained in the present study, based on the combined use of MIA and EA, and supported by statistical modelling and translucent zones completion frequency distribution, evidenced temporally coherent patterns providing a more robust validation of the first annulus formation in the European anchovy in the GoC.

Keywords: otolith formation, age determination, marginal increment, otolith edge.

RETURN TO INDEX

IOS_141

Age determination of Atlantic horse mackerel (*Trachurus trachurus*) in Western stock using otoliths

Carmen Hernández[1]*, Clara Dueñas-Liaño, Jorge Landa
[1]Instituto Español de Oceanografía-CSIC. Centro Oceanográfico de Santander, Spain. *carmen.hernandez@ieo.csic.es

The otoliths of Atlantic horse mackerel (*Trachurus trachurus*) are a challenge in age estimation due to their annual growth zones may contain double or multiple translucent zones, false rings or checks that may be similar to true annual increments (annuli). Moreover, the presence of a preannual increment that surrounds the increment of the first year (juvenile increment) can be misinterpreted as the first annulus. The potential for inaccurate ageing of this species is high resulting in wide variation in age estimates. Otoliths from Western stock horse mackerel present different visual appearance from otolith of other two Northeast Atlantic stocks, indicated that false increments and annuli can only be identified if concurrent measurements of growth zone widths are available. Whole otoliths of that Western stock from two consecutive years (2021-2022) were examined in this study and the radii of the suspected annual growth zones and false rings were measured. The age was estimated by identifying and counting annuli and the marginal increment widths were also measured and used to identify ages of fish. Our results confirmed the presence of false rings on the otolith structure which resembles the appearance of annuli. Mean otolith size, mean increment width and mean age at the first three annual growth were presented. This study contribute to help set up a more accurate annual age estimation criterion. This is essential for the stock assessment process of this species, given the problematic of its annual age estimation.

Keywords: Northeast Atlantic, false rings, increments width, juvenile increment.

RETURN TO INDEX

IOS_142

Using otolith shape indices for a quick-easy diagnostic of *Opisthonema oglinum* geographic variation

André Martins Vaz-dos-Santos[1]*, Kathleen Angelica Rautenberg[1], Cristiane Gallego Augusto[2], Eduardo Luis Cupertino Ballester[3], Paulo Ricardo Schwingel[4], Alberto Teodorico Correia[5]
[1]Sclerochronology Laboratory (LABESC), Federal University of Paraná, Palotina, Brazil; [2]Federal Institute of São Paulo, São Paulo, Brazil; [3]Prawn Culture Laboratory (LABCAR), Federal University of Paraná, Brazil; [4]Aquatic and Fisheries Ecosystem Laboratory, Universidade do Vale do Itajaí, Brazil; [5]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto (UP), Porto / Escola de Ciências da Vida e do Ambiente (ECVA), Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
*andrevaz@ufpr.br

Otolith shape indices were disregarded for species identification. Nevertheless, they are still useful for intraspecific/population diagnosis, providing a low-cost and quick-easy tool for diverse uses. Even with technological facilities on hand (such as mobile phones), some advances are still far from routine both in the lab and in the field (e.g., fish landings), mainly in developing countries. The thread herring *Opisthonema oglinum* is one of the most important fishery resources in the Southeastern Brazilian Bight (22°-29°S). Aiming to evaluate its geographic variation between two opposite areas (Rio de Janeiro, RJ: 23°04'S, 44°,03'W and Santa Catarina, SC: 26°05'S, 48°18'W), otolith samples were obtained from commercial landings between June and October of 2011 (individuals between 227-254 mm TL, 25 for each area). Otoliths were measured and the relative proportions (OL/TL, OH/TL, OL/OH), and the shapes indices (form factor, roundness, circularity, rectangularity and ellipticity) were calculated and subjected to bivariate and multivariate statistics by area. Graphically, relative proportions involving otolith height showed different patterns between RJ and SC, although only OL/OH were significant (t-test, P=0.021). Remarkable differences in shape indices were recorded only for rectangularity and ellipticity (t-test, P<0.05). Herrings from Rio de Janeiro have proportionally larger otoliths than those from Santa Catarina. Linear discriminant analysis using principal components presented 68% of correct reclassification (76% RJ, 60% SC). MANOVA after Doornik-Hansen test (P=0.08) confirmed between-areas differences (F=3.242, P=0.0304). Summarizing, relative proportions and shape indices were useful to detect geographic variation in *O. oglinum* already detected with other techniques (shape, elemental signatures), reinforcing the metapopulation structures for the species in the area.

Keywords: Atlantic thread herring, Brazil, sagitta, purse seine.

RETURN TO INDEX

IOS_143

Reconstructing spatial production patterns of salmon in large Alaska watersheds

Ben Makhlouf[1], *, Daniel Schindler[1], Tim Cline[2], Lisa Seeb[1], Liz Lee[3], Diego Fernandez[4]
University of Washington School of Aquatic and Fishery Sciences, Seattle, USA[1], Montana State University Department of Ecology, Bozeman, USA[2], Gene Conservation Lab, Alaska Department of Fish and Game, Juneau, USA[3], University of Utah Department of Geology and Geophysics, Salt Lake City, USA[4], bmak29@uw.edu*

The use of $Sr^{87/86}$ in salmon otoliths has emerged as a tool for reconstructing spatial patterns of movement and provenance in remote Alaskan watersheds. In doing so, otolith-based methods augment traditional research methods for prioritizing conservation efforts and designing climate adaptation strategies. To do so requires continued advancement in quantifying basic physiological mechanisms and resolving limitations in current frameworks. We addressed two of these research needs by 1) integrating genetic data with otolith strontium isotopes to improve precision of geographic assignments and 2) quantifying physiological turnover of otolith Sr87/86 in response to shifting water chemistry and diets with unique strontium characteristics.

Current approaches for using otolith strontium isotopes for assigning natal locations in Alaskan watersheds use a probabilistic Bayesian framework developed on the relationship between otolith δ-values and watershed isoscapes. In some cases, redundancy in isotope values across the landscape lead to substantial assignment uncertainty. We demonstrated that by adding data from genetic stock identification to this existing framework, we can resolve a portion of this redundancy and improve precision of geographic assignments for Chinook Salmon in the Yukon River basin.

We also quantified the effect of diet and water chemistry changes on otolith δ-values, which are critical considerations for assessing movement patterns of fish throughout watersheds. To do so, we conducted a controlled experiment in which juvenile coho salmon were exposed to a switch in water chemistry. We examined the subsequent change in otolith δ-values and quantified two contributing turnover pools with half-lives of 7.87 and 76.42 days and a negative relationship between growth and turnover rate. In addition, we also observed a small contribution of diet to otolith δ -values when fish were switched onto a unique diet that was not influenced by growth rate.

Taken together, these advancements improve otolith stable isotope methods by advancing statistical frameworks, augmenting with new data sources, and quantifying biologically relevant turnover in fish strontium pools. In doing so, we continued to improve the utility of otolith-based methods both for Alaska salmon as well as for fisheries broadly.

Keywords: Otoliths, Microchemistry, Strontium, Bayesian model, turnover

RETURN TO INDEX

IOS_145

Validation of Annual Growth Zone Formation in Gray Triggerfish (*Balistes capriscus*) Dorsal Spines, Vertebrae, and Otoliths

Walter D. Rogersa*, Jennifer C. Pottsb, Troy C. Rezekc Amanda R. Rezekd
a. NOAA Fisheries Southeast Fisheries Science Center, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516, USA. Jennifer.Potts@noaa.gov. ORCID ID: 0000-0002-4961-034X
b. Cooperative Institute for Marine and Atmospheric Studies, University of Miami, in support of NOAA Fisheries Southeast Fisheries Science Center, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516, USA. Walt.Rogers@noaa.gov. ORCID ID: 0000-0002-1371-2918.
c. CSS-Inc., Under Contract to NOAA National Centers for Coastal Ocean Science, 101 Pivers Island Road, Beaufort, NC 28516, USA. Troy.Rezek@noaa.gov
d. NOAA Fisheries Southeast Fisheries Science Center, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516, USA. Amanda.Rezek@noaa.gov

Uncertainty in age estimates from dorsal spines has been a persistent issue in stock assessments of gray triggerfish *Balistes* capriscus. This study sought to validate the annual deposition of growth zones on dorsal spines, vertebrae, and otoliths of gray triggerfish through chemical marking. Fish (n=101) were collected from offshore habitats and held in an aquaculture facility. 74 adult fish were chemically marked with a 50 mg/kg body weight injection of calcein, and reared for an average of 527 days post-marking. Upon completion of the rearing period, fish were sacrificed and first dorsal spines, vertebrae, and otoliths were extracted and sectioned. Narrow, slow-growth zones (annuli) were enumerated for spines (n=96), vertebrae (n=94), and otoliths (n=48) and ranged from 0-11 annuli for spines and vertebrae, and 1-12 annuli for otoliths. Age bias plots showed strong agreement between spine and vertebra annuli counts for all observed ages, while counts of spines and vertebrae appeared to underage beginning at age 4 when compared to otolith annuli counts. Tests of symmetry indicated that the annuli counts between paired age structures were not biased ($p > 0.05$). Growth zones were observed distal to calcein marks in all of the age structures, and expected number of these zones were observed in 91% of spine, 90% of vertebrae, and 100% of otolith sections. Marginal increment analysis of ageing structures indicated that annuli form in spring and summer months. Percentages of slow-growth zones deposited on the margins peaked in May for vertebrae (80%), June for spines (58%), and July (14.3%) for otoliths. Results from this study validate the annual deposition of growth zones in three gray triggerfish ageing structures, but further consideration needs to be taken when ageing fish older than age-4.

Keywords: *Balistes capriscu*s, Otolith, Vertebrae, Annuli.

RETURN TO INDEX

IOS_150

"Jesstimation": a new method to estimate the fractional age of species with protracted spawning

Jessica Farley[1]*, Paige Eveson[1], Kyne Krusic-Golub[2], Naomi Clear[1], Francois Rouspard[3], Caroline Sanchez[3], Simon Nicol[3], John Hampton[3]
[1]CSIRO Environment, Hobart, Australia; [2]Fish Ageing Services, Queenscliff, Australia; [3]Pacific Community (SPC), Nouméa, New Caledonia. *Jessica.farley@csiro.au

Accurate estimates of age and growth are critical for age-structured stock assessments and the development of effective management advice. Counting daily or annual growth increments in otoliths is generally considered the most accurate method for estimating the age of fish, providing it is validated. The number of annual growth increments counted provides an integer estimate of the fish's age, so it is common to try estimating a more precise fractional age by accounting for the time elapsed since the fish's last birthday. Estimating a fractional age for species with protracted spawning seasons, however, can be challenging because the individual birthdates are unknown. Moreover, it can be further complicated if the timing of annual increment formation is variable or unknown in relation to a fish's birthdate. Here we present an improved method to convert counts of annuli into fractional ages using a combination of opaque zone counts, daily ageing of young of the year (YOY) samples and otolith measurements, with application to western Pacific bigeye tuna (*Thunnus obesus*), a species that can spawn throughout the year in the tropics. This method negates the use of a single assumed birth date for all fish and a uniform increment formation period and provides a more accurate conversion of zone counts to age estimates, particularly for species with variable spawning times.

Keywords: Satittae, marginal increment, decimal age, age-at-length, tropical tuna.

RETURN TO INDEX

IOS_158

Modelling the growth of Chilean jack mackerel (*Trachurus murphyi*) considering the effect of age-specific sample size

Lilian Cisterna[a,b], Sebastián I. Vásqueza,[c], Aquiles Sepúlveda[a], Evelyn Sanhueza[a]
[a]Instituto de Investigación Pesquera, Talcahuano, Chile; [b]Programa de Doctorado en Ciencias con mención en Manejo de Recursos Acuáticos Renovables (MaReA), Universidad de Concepción, Chile; [c]Programa de Doctorado en Oceanografía, Universidad de Concepción, Chile

Growth modelling is essential to inform fisheries management but is often hampered by sampling biases and imperfect data. Additional methods such as interpolating data through backcalculation may be used to account for sampling bias but are often complex and time consuming. Focusing on Chilean jack mackerel (CHJM), here we present an approach to improve plausibility in growth estimates when there is an age-specific imbalance in the sample size. In addition we implemented an approach based on Bayesian fitting growth models using Markov Chain Monte Carlo (MCMC) with informative priors on growth parameters. Considering the recent validation of the daily periodicity of the microincrements in CHJM otoliths, the readings of annual rings in the otoliths were complemented with reading of daily increments for individuals younger than two years of age with the aim of improving the estimation of the parameter L_0. Parameter estimates for the von Bertalanffy growth function confirmed age-specific sample size bias as an important source of uncertainty. The parameters estimated with the corrected database showed less difference between the adjustment methods (frequentist and Bayesian). A methodology based on sampling without replacement by age group is proposed to correct the imbalance in the sample size. Although some differences were observed between the periods evaluated, the Bayesian analysis produced more biological reliable estimates for both L_∞ and L_0. The growth rate coefficient, K, varied according to the estimation of the other parameters, being higher when L_∞ was smaller and L_0 higher. Considering this approach, the von Bertalanffy growth parameters were estimated as L_0 = 12.20 L_∞ = 67.43 and K = 0.14 for the entire period evaluated.

Keywords: *Trachurus murphyi*, Growth modelling, Markov Chain Monte Carlo (MCMC), Otolith

RETURN TO INDEX

IOS_159

Connectivity between the Saguenay Fjord and the St. Lawrence Estuary (Canada) of groundfish populations exploited by a winter recreational fishery

Rachel Mailhot[1]*, Dominique Robert[1], Pascal Sirois[2]
[1]Institut des Sciences de la Mer, Université du Québec à Rimouski, Rimouski, Canada; [2]Départment des sciences fondamentales, Université du Québec à Chicoutimi, Saguenay, Canada *rachel.mailhot@uqar.ca

An important winter recreational groundfish fishery takes place on the Saguenay Fjord (Canada). Atlantic redfish (*Sebastes mentella*), Atlantic cod (*Gadus morhua*) and Greenland halibut (*Reinhardtius hippoglossoides*) are the main species targeted by anglers. Previous studies based on genetics and phenotypic traits indicated that the Saguenay groundfish stocks would be sink populations of the Estuary and Gulf of St. Lawrence. Considering the status of the three species in the St. Lawrence (endangered species for Atlantic cod, commercially exploited for Greenland halibut and imminent reopening of the commercial fishery for redfish), the understanding of the mechanisms of stock renewal in the Saguenay Fjord is essential to ensure a sustainable winter recreative fishery. The general objective of this project is to determine the connectivity between the Saguenay Fjord and the St. Lawrence Estuary of the main groundfish species exploited by the winter recreational fishery using otolith trace elements analysis. More specifically, the study aims to evaluate when in their life cycle Atlantic redfish, Atlantic cod and Greenland halibut would enter the Saguenay, and then to determine if these populations are resident in the Fjord or if they migrate between the Saguenay and the St. Lawrence. A comparison of two periods will also be carried out to assess the stability of migratory patterns over the last two decades. Concentrations of 38 trace elements will be measured in 50 otoliths for two periods (2008 and 2023) and for each of the three species (total of 300 otoliths) by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). We are expecting, for the three species, that the stock renewal in the Saguenay would be dependent on the migration of juveniles from the St. Lawrence and that the fish would remain residents of the Fjord afterward.

Keywords: Connectivity, Otolith microchemistry, *Gadus morhua, Sebastes mentella, Reinhardtius hippoglossoides*

RETURN TO INDEX

IOS_160

Coastal upwelling influences population structure of dusky grouper: an integrative approach based on otolith chemistry and muscle stable isotopes

Cristiano Albuquerque[1], Mario Condini[2] Susanne Tanner[3], Patrick Reis-Santos[4], Tatiana Saint'Pierre[5], Elisa Seyboth[6], Bianca Possamai[7], David Hoeinghaus[8], Alexandre Garcia[9]
[1]Universidade Federal Rural do Semi-Árido, Mossoró, Brazil; [2]Universidade Federal do Espírito Santo, São Mateus, Brazil; [3]Universidade de Lisboa, Portugal; [4]The University of Adelaide, Australia. [5]Pontifícia Universidade Católica do Rio de Janeiro, Brasil; [6]University of Technology, Cape Town, South Africa; [7]University of Vermont, Burlington, USA; [8]University of North Texas, Denton, USA. [9]Universidade Federal de Rio Grande, Rio Grande, Brazil.

The dusky grouper (*Epinephelus marginatus*) is an overfished and threatened fish species, that occurs across a broad region influenced by two major upwelling systems in the Southwestern Atlantic. We combined otolith chemistry and muscle stable isotope analysis to examine the stock structure of *E. marginatus* and its association with the two upwelling systems (the Cabo Frio and the Cabo Santa Marta). The samples were taken from shallow coastal waters of the Southwest Atlantic Ocean, covering the southeastern and southern Brazilian coasts, among Macaé (22ºS), Santos (24ºS), Florianópolis (27ºS), and in Rio Grande (32ºS). When analyzed independently, otolith chemistry (Sr:Ca, Ba:Ca and Zn:Ca) differentiated the southern (32ºS) collection areas from the remaining regions (27º, 24º, 22ºS). Whereas the stable isotopes analyses ($\delta^{13}C$ and $\delta^{15}N$) also separated the southern sampling site (32ºS) from the others. However, combined analysis of both natural markers suggests that there are three stocks of *E. marginatus* coherently structured across the two upwelling systems, namely: North (north of Cabo Frio); Center (between upwelling regions); and South (south of the Cabo Santa Marta system). This combined approach, leveraging information from distinct natural tags, and reflecting changes in water chemistry and food webs, improved our ability to discriminate *E. marginatus* stocks, and allowed us to enhance our understanding of how major upwelling systems can influence the structuring of fish populations along the southwestern Atlantic Ocean.

Keywords: Population structure, *Epinephelus marginatus*, Southwestern Atlantic, Epinephelidae, Vulnerable species.

RETURN TO INDEX

IOS_161

Age and growth from daily microstructures in otoliths of the Jaguar guapote, *Parachromis managuensis* (Günther, 1867), a fish introduced in the Brazilian semi-arid region

Amanda Resende[1,2,3], Francisco Santana*[1,2,3], Rosangela Lessa[3]
[1]Laboratório de Dinâmica de Populações Aquáticas (DAQUA), Unidade Acadêmica de Serra Talhada (UAST), Universidade Federal Rural de Pernambuco (UFRPE), Serra Talhada - PE, Brazil; [2]Programa de Pós-Graduação em Biodiversidade e Conservação, Unidade Acadêmica de Serra Talhada (UAST), Universidade Federal Rural de Pernambuco (UFRPE), Serra Talhada - PE, Brazil; [3]Laboratório de Dinâmica de Populações Marinhas (DIMAR), Departamento de Pesca e Aquicultura (DEPAq), Universidade Federal Rural de Pernambuco (UFRPE), Recife - PE, Brazil. *framarsantana@yahoo.com.br

Age and growth studies in bony fish are the basis of fisheries biology and population dynamics studies. The present study aimed to determine the age and growth of the jaguar guapote, *Parachromis managuensis*, a fish introduced in the semi-arid region of Pernambuco, Brazil. The specimens were collected monthly between December 2015 and November 2016, totalizing 617 specimens captured, of which *sagittae* otoliths were collected in 258. The individuals presented total lengths (*TL*) between 26 and 240 mm. The data were worked in a grouped way, since there was no significant difference between the sexes for analysis of the growth curves. The microstructures counted in the otoliths were validated, with bathing of individuals in Alizarin Red S, and it was verified the daily periodicity. Ages ranged from 0.07 to 1.22 years, and longevity was estimated at 1.45 years. The parameters estimated according to the von Bertallanfy growth model were: L_{∞} = 198.59 mm *TL*; k = 2.58 day^{-1} and t_0 = 0.02 year, demonstrate rapid growth of the species. The *P. managuensis* tends to grow faster and found favorable conditions for its population in the study site. Among the age determination methodologies, the one granted by reading the microstructures in otoliths proved to be adequate for the study, since it proved to be proportional to the growth in length of the species. P. managuensis has a short life cycle with growth strategies that aim to accelerate its dynamics, which may affect local native species.

Keywords: Freshwater, *Cichlidae*, Chemical validation.

RETURN TO INDEX

IOS_162

What can one otolith tell you? Moving otolith science's storyline from research to application

David Secor
University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, Solomons, Maryland, US; secor@umces.edu

The otolith science and application story arc comprises plot (design), exposition (sampling), sub-narratives (serial events and their consequences), and resolution (application). This storyline is sometimes obscured by individual fish stories. An epitome was my early career attempt to tell a story about Coelacanth ecology from a single otolith, including a population growth model, estimates of maturity and an ontogenetic habitat shift, and revision of Pauly's fish growth-gill surface area model. Advisedly, the paper was withdrawn. Still, individual fish stories persist in the literature. I evaluated progress in developing full story arcs by scoring the literature for project goal (development versus application), sampling design (stratification, design targets, power analysis); sample size (total N; maximum and minimum subsample N); and integration of individual and group variance (repeated measures, covariance models, time series analysis, AI applications). The volume of literature caused me to examine only otolith chemistry studies, for which I examined those I co-authored (N=42) and a stratified random subsample of papers over the past 30 years (N=280). Findings showed progress in developing full story arcs in otolith science, particularly in increased exposition (orders of magnitude increased sample sizes) and resolution. Progress in design elements was modest. Further, fish stories persist, which suggests that either they hold value and/or we are not doing full service to otolith science's storyline.

Keywords: Application, Connectivity, Design, Microchemistry

RETURN TO INDEX

IOS_163

SmartDots – creating a link between fish ageing and stock assessment modelling

Julie Coad Davies*[1], Karen Bekaert[2], Carlos Pinto[3], Wim Allegaert[2], Kevin De Coster[2], Patricia Gonçalves[4], Jane Aanestad Godiksen[5], Côme Denechaud[5], Annelie Hilvarsson[6], Cindy van Damme[7], Ulrika Beier[7], Colin Millar[3] and Alfonso Pérez Rodríguez[8]
[1]DTU Aqua, National Institute of Aquatic Resources, Lyngby, Denmark; [2]ILVO, Flanders Research Institute for Agriculture, Fisheries and Food, Ostend, Belgium; [3]ICES International Council for Exploration of the Seas, Copenhagen, Denmark; [4]IPMA Portuguese Institute for Sea and Atmosphere, Lisbon, Portugal; [5]Institute of Marine Research, Bergen, Norway; [6]Department of Aquatic Resources at the Swedish University of Agricultural Sciences, Lysekil, Sweden; [7]Wageningen University and Research, Wageningen, Netherlands; [8]Spanish Oceanographic Institute (IEO), Cádiz, Spain.*email: joco@aqua.dtu.dk

The estimation of the age composition of a stock is fundamental to the evaluation of its status as age data underpins growth and mortality rates, maturity patterns and eventually stock size. Routine ageing is conducted in age reading laboratories across the globe with the aim to deliver age data for stock assessment purposes, for a set of samples that are representative of survey and commercial catches. Fish age is commonly estimated from the observed patterns of the growth structures laid down in the hard structures like otoliths or scales. Age estimation is a subjective process and thus variance between and within readers is expected, this can potentially lead to bias and imprecisions in the estimated age composition of a population. The ICES SmartDots platform was developed in 2018 as a tool to support quality assurance procedures aimed at achieving a high level of age data quality. SmartDots age calibration events are image-based interactive exercises which can be accessed online by multiple expert and trainee age readers. All registered data is available in a standardized format in the connected reporting environment with R-scripts producing statistical outputs indicating levels of age reader accuracy and precision. Recent efforts have been focused on incorporating the output (either the raw empirical data or the so-called Age Error Matrices (AEM)) from these exchange events into the stock assessment process. Currently three of the most widely used stock assessment models in the ICES community, SAM, Gadget and SS3 are capable of incorporating ageing error information. The ultimate aim of an ICES SmartDots event is to improve the reliability of the stock assessment. Various fora, from workshops and exchanges to working groups have identified the need for cross disciplinary cooperation between age readers, data quality managers, stock assessors and model developers as being necessary if this goal is to be reached. A case study is presented which aims to cover the entire process, from the exchange event to the assessment process. Improving the reliability of the assessment, while being the ultimate goal will nevertheless enhance the efficiency of monitoring programs and support better informed sustainable fisheries management practices.

Keywords: Otoliths, Calibration, Image-based, Age reading error, Data quality

RETURN TO INDEX

IOS_164

Habitat use plasticity by the dog snapper (*Lutjanus jocu*) across the Abrolhos Bank shelf, eastern Brazil, inferred from otolith chemistry

Rafael Menezes[1,2], Perimar E.S. Moura[3], Alexandre C.A. Santos[3], Leonardo E. Moraes[4], Mario V. Condini[5], Ricardo S. Rosa[2], Cristiano Q. Albuquerque*[6] cristiano.albuquerque@ufersa.edu.br
[1]Laboratório de Dinâmica de Populações Marinhas (DIMAR), Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife-PE, Brazil; [2]Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Universidade Federal da Paraíba, João Pessoa-PB, Brazil; [3]Programa de Pós-Graduação em Ecologia e Biomonitoramento, Universidade Federal da Bahia, Salvador-BA, Brazil; [4]Centro de Formação de Ciências Ambientais, Universidade Federal do Sul da Bahia, Porto Seguro-BA, Brazil; [5]Programa de Pós-Graduação em Oceanografia Ambiental, Universidade Federal do Espírito Santo, Vitória-ES, Brazil; [6]Centro de Biociências, Universidade Federal Rural do Semiárido, Mossoró-RN, Brazil

Reef fishes move between adjacent coastal systems throughout their ontogeny, but these movements are complex and often poorly understood. In this study, we examined how the dog snapper (*Lutjanus jocu*) uses marine and estuarine habitats across the Abrolhos Bank shelf (eastern Brazil) through the analysis of elemental signatures in otoliths. Eighty-three specimens were sampled in an estuary and inner-shelf marine system. Ba/Ca, Sr/Ca, Mg/Ca and Mn/Ca ratios were measured by LA-ICPMS along the otolith growth axis to cover the entire lifetime of the fish. Change-point analysis, principal component analysis and BRT modeling were used to identify habitat-use patterns of the species. Otolith Ba/Ca ratios performed better than other elements for tracking *L. jocu* movements between estuarine and marine habitats, which may result from small variations in salinity and high turbidity levels, as these characteristics are typical of the Abrolhos Bank. Two contrasting habitat-use patterns were identified for *L. jocu*: i) marine residents (56% of sampled fish) - fish that remain in marine systems throughout their lifetime; and ii) marine migrants (37%) - juveniles inhabit estuaries and move to marine systems with age. These categories were successfully re-classified by the leave-one-out technique, which suggests that they are coherent and reliable. Given the findings of diversified habitat-use patterns in many other fish species, *L. jocu* habitat requirements also appear to be quite flexible. As the dog snapper is facing overexploitation in the region, this plasticity in habitat use should be considered in future fisheries management.

Keywords: Cross-shelf migration, Habitat connectivity, Ba/Ca ratios, Lutjanidae

RETURN TO INDEX

IOS_165

Mixed stocks of the dog snapper (*Lutjanus jocu*) along the northeast region of Brazil revealed by otolith shape analysis

Rafael Menezes[1,2], Rosângela P.T. Lessa[1], Ricardo S. Rosa[2], Leonardo E. Moraes[3], Ronaldo B. Francini-Filho[4], Cristiano Q. Albuquerque*[5]
[1]Laboratório de Dinâmica de Populações Marinhas (DIMAR), Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife-PE, Brazil; [2]Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Universidade Federal da Paraíba, João Pessoa-PB, Brazil; [3]Centro de Formação de Ciências Ambientais, Universidade Federal do Sul da Bahia, Porto Seguro-BA, Brazil; [4]Centro de Biologia Marinha, Universidade de São Paulo, São Sebastião-SP, Brazil; [5]Centro de Biociências, Universidade Federal Rural do Semiárido, Mossoró-RN, Brazil *cristiano.albuquerque@ufersa.edu.br

Widespread fish populations tend to exhibit multiple stocks, which can be either spatially connected (mixed stocks) or segregated (single stocks). In the western Atlantic, the dog snapper (*Lutjanus jocu*) occurs from Massachusetts/USA to Santa Catarina/Brazil and around the four Brazilian oceanic islands. As the species is commercially exploited throughout Brazil, we aimed to identify *L. jocu* stocks across the northeast region, using the otolith shape as a natural marker of stocks. Eighty-eight specimens were acquired from the artisanal fleets across Maranhão (MA), Ceará (CE), Paraíba (PB) and southern Bahia (BA) in order to cover the whole extension of the northeastern Brazilian coast. Elliptical Fourier descriptors were used for outlining the otolith shapes of *L. jocu* from each sampling area. A high similarity in the otolith shape contour was observed across MA, CE, PB and BA, with slight variations in *post-* and *anti-rostrum* rims of otoliths from the MA and BA. Such results were corroborated and validated by the Linear Discriminant Analysis and leave-one-out re-classification technique, respectively, suggesting spatial connectivity of *L. jocu* stocks along the extension, with partial isolation in Maranhão and southern Bahia. The connectivity of *L. jocu* stocks may result from their high-dispersal capacity and habitat-generalist behavior, which enable the species to use shallow and mesophotic reefs as corridors for crossing ecologically distinct environments along their occurrence range. Further studies integrating otolith chemistry and genetic analysis may clarify such results, thus assisting the establishment of stock-based management for the dog snapper along the northeast region of Brazil.

Keywords: Fish stock, Stock identification, Spatial connectivity, Lutjanidae

RETURN TO INDEX

IOS_167

Stock structure of Atlantic tomcod in the St. Lawrence River (Canada) as revealed by otolith elemental fingerprints

Louis Landry-Massicotte[1],[2], Philippe Brodeur[2], Émilie Paquin[2], Pascal Sirois*[1]
[1]Chaire de recherche sur les espèces aquatiques exploitées, Université du Québec à Chicoutimi, Chicoutimi (QC) Canada;
[2]Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs du Québec, (QC) Canada. *pascal_sirois@uqac.ca

Atlantic tomcod (*Microgadus tomcod*) is an anadromous fish species that reproduces during the winter in freshwater tributaries of the St. Lawrence River and drifts towards the estuary after hatching. It is an important forage fish species in the St. Lawrence Estuary and it is also exploited by a winter recreational fishery during migration to spawning grounds. Historically, the Atlantic tomcod population used several tributaries of the St. Lawrence River for spawning, but since its significant decline in the 1980s, at least two tributaries are known to be used for reproduction. We do not know the contribution of these spawning grounds to the tomcod population. The main objective of this project is to determine the number of spawning grounds contributing to the Atlantic tomcod recruitment and to assess their relative contribution to the St. Lawrence Estuary stock. To achieve this objective, we measured the concentration of trace elements in the otolith cores of juveniles and adults captured in the St. Lawrence Estuary and on the otolith margins of adults captured on spawning grounds. Results of the elemental fingerprints in otolith cores showed two sources of Atlantic tomcod that appeared to correspond to the two known spawning sites, namely the Sainte-Anne and Batiscan Rivers. Trace elements measured on the otolith margins of adults captured on the spawning grounds also tended to demonstrate distinct fingerprints between the two source rivers. Results revealed that the Sainte-Anne River was the main source of Atlantic tomcod in the St. Lawrence Estuary and the Bastican River exhibited a lower contribution to the stock. This study shows the importance of defining fish stock structure in the context of sustainable fisheries management.

Keywords: *Microgadus tomcod*, Large-river ecosystem, Natal sources, Otolith chemistry

RETURN TO INDEX

IOS_171

Size-age variation of otolith morphology of *Chloroscombrus chrysurus* Linnaeus 1766 in the Southwestern Atlantic

Ana Cláudia Rojas de Souza[1], Estevan Luiz da Silveira[2,3], Antônio Olinto Ávila-da-Silva[2], André Martins Vaz-dos-Santos[1,3]*
[1]Laboratório de Esclerocronologia Universidade Federal do Paraná. Rua Pioneiro, 2153 CEP 85950-000 Palotina – PR – Brazil; [2]Instituto de Pesca (SAA-SP/APTA). Av. Bartolomeu de Gusmão, 192 CEP 11030-906 Santos – SP – Brasil; [3]Programa de Pós-Graduação em Aquicultura e Desenvolvimento Sustentável – PGADS; [4] Laboratório de Esclerocronologia Universidade Federal do Paraná. Rua Pioneiro, 2153 CEP 85950-000 Palotina – PR – Brazil. *andrevaz@ufpr.br

The Atlantic bumper, *Chloroscombrus chrysurus*, is a halieutic resource for purse seine fleets in the Southwestern Atlantic (23°-28ºS). Under the context of gas and oil exploration in the area, Brazil, CENPES/PETROBRAS developed the *Santos Project: Environmental Characterization of the Santos Basin* for monitoring the main fisheries resources. Otoliths are well-known as natural tags of life cycle. In order to describe the otolith's changes along the ontogeny, commercial landings were monthly sampled during a year (Aug/2021/Sep2022). At laboratory bumper otoliths were measured and the age was attributed by macrostructural analysis. Relative proportions (OL/TL, OL/OH, OW/TW) were calculated, the size effect was removed, and then they were tested by size (130-390 mm) and age classes (zero-8 years-old) means of Kruskal Wallis test (bivariate) and PERMANOVA test (multivariate). Individually, the 207 analyzed presented differences for OW/TW by size classes ($H=91.14$, $P=4.69\times10^{-11}$), for OL/TL by age classes ($H=17.55$, $P=0.0249$), and for OW/TW by age classes ($H=71.69$ $P=2.29\times10^{-9}$). Both PERMANOVAs did not indicate differences ($F_{size}=1.259$, $P_{size}=0.2058$; $F_{age}=1.291$, $P_{size}=0.2488$). OW/TW by size represented three otolith phases of mass gain: 130-170 mm, 180-260 mm, and then 270-390 mm. By age, both relative proportions evidenced a first otolith growth phase (zero to 2-years-old), followed by a mid- phase (3-6-years-old), and then the last phase (7-8-years-old). Size and age phases did not exactly match, although some correspondence is clear, evidencing at least three distinct growth phases. Up-dated and accurate techniques (geometric morphometrics) already in course will clarify these relationships, improving the comprehension of the ontogenetic development of *C. chrysurus* otoliths.

Keywords: pelagic fish, purse seine, ontogeny, Brazil.

RETURN TO INDEX

IOS_173

Age determination of Atlantic cod using Fourier-transform near infrared spectrometry

Aaron Adamack*[1]; Kelly Antaya[1]; Sudagar Dhaliwal[2]; Karen Dwyer[1]; Shelley Lang[1]; Christina Bourne[1]
[1]Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, St. John's, Canada [2]Bruker Ltd. Canada, Milton, Canada.
*aaron.adamck@dfo-mpo.gc.ca

Aging fish otoliths is an important but time consuming and costly component of the fisheries stock assessment process. A method for aging otoliths using Fourier transform near-infrared spectrometry (FT-NIR) has recently been developed by researchers in Australia and the United States. This method is potentially faster and less expensive than our existing method of estimating ages from otoliths by counting their growth rings. Using FT-NIR spectrometry, a beam of near infrared light is reflected off of the otolith and the frequencies of near infrared light absorbed by the otolith are recorded, resulting in a spectral signature for that otolith. Spectral signatures for Atlantic cod (*Gadus morhua*) with ages determined by manually counting their rings can be used to generate a partial least squares (PLS) regression model which can subsequently be used to estimate the ages of otoliths with unknown ages. We describe the development of a PLS regression model for aging otoliths from NAFO Divisions 2J3KLNOPs for Atlantic cod aged 1 to 12+. We examine the accuracy and precision of the method and provide a comparison of the time cost for aging cod using this new method compared to the manual counting of otolith growth rings. Additionally, we investigate the potential for using FT-NIR spectrometry to assist in identifying fish species using their otoliths. If effective at differentiating some common species in the North-West Atlantic, this technology may simplify hard part analysis when determining the diet composition of marine predators, such as seals. While these new methodologies have significant potential for speeding up the process of determining fish ages or identifying species while reducing operating costs, substantial work is required before the methodologies can be operationalized.

Keywords: Otoliths, FT-NIR, *Gadus morhua*, Northwest Atlantic, species identification

RETURN TO INDEX

IOS_178

Natal Origins & Population Structure of a Recolonizing Salmon Population

Lauren G. Hitt*[1,2], Malte Willmes[3,4], Mackenzie C. Miner[1,2], George E. Whitman[2], Rachel Johnson[2,5], Dennis E. Cocherell[1], Nann A. Fangue[1], and Andrew L. Rypel[1,2]
[1]Department of Wildlife, Fish, & Conservation Biology, University of California, Davis, Davis, CA, USA; [2]Center for Watershed Sciences, UC Davis, Davis, CA, USA; [3]Institute of Marine Sciences, University of California Santa Cruz, Santa Cruz, 5CA, USA; [4]Norwegian Institute for Nature Research, Trondheim, Norway; 5National Marine Fisheries Service, Southwest Fisheries Science Center, Santa Cruz, CA, USA. *renhitt@gmail.com

California's Chinook salmon populations (*Oncorhynchus tshawytscha*) have seen severe population declines and extirpations as a result of extensive damming and management of inland California waterways, which obstruct anadromous migration pathways. Studying how Chinook salmon populations can recover in dam-altered waterways will be an important tool in the continued management and conservation of imperiled California salmon populations. After adopting restorative dam-management practices, Putah Creek, a tributary of the Sacramento River, has consistently seen cohorts of spawning Chinook salmon since 2014, more than fifty years after the waterway was dammed and made unavailable to salmon. We studied sagittal otoliths collected from the carcasses of five cohorts of adult Chinook salmon observed spawning in Putah Creek to evaluate whether this waterway has developed an established, natal salmon population or to what degree the yearly spawning cohort is inflated by hatchery-origin salmon straying into Putah Creek. We counted annuli growth bands to estimate annual ages for each fish and evaluate changes in population age structure among cohort years and relationships between annual age and fish size. We analyzed the microchemistry of the natal regions of the sagittal otoliths using plasma mass spectrometry to generate $^{87}Sr/^{86}Sr$ isotope ratios for each fish. We then matched these values to known Sr isotope ratios for other Central Valley rivers and hatcheries using a classification and regression trees model to make the highest likelihood natal origin assignments. To differentiate overlapping Sr signals between Putah Creek and another wild California population, we analyzed trace elements of the otoliths of fish with uncertain classifications to identify unique signals for each waterway. We found that 3-year-old fish were the predominant age class in all spawning cohorts and that the proportion of wild-origin fish spawning in Putah Creek increased from less than 2% in the first cohort to more than 20% in the most recent cohort. These findings provide valuable insight into the population dynamics and spawner contributions in recolonization events, providing a blueprint for the establishment of salmon populations in newly available habitat.

Keywords: *Oncorhynchus*, Restoration, Annuli, Microchemistry, California Central Valley

RETURN TO INDEX

IOS_180

Some aspects on the life history of the Bali sardine in Southeastern Luzon, Philippines

Kaola Alezandra Pamonag*[1], Kim P. Nuñez[1], Alexanra Bagarinao-Regalado[1,2], Wilfredo L. Campos[1,2]
[1]OceanBio Laboratory, Division of Biological Sciences, College of Arts and Sciences, University of the Philippines-Visayas, Miag-ao, Iloilo, Philippines; [2]Faculty of the Division of Biological Sciences, College of Arts and Sciences, University of the Philippines-Visayas, Miag-ao, Iloilo, Philippines. *ktpamonag@up.edu.ph

Sardinella lemuru dominates the small pelagic fisheries in the inner waters of Southeastern Luzon, Philippines, therefore correct and updated estimates of their age and growth are vital to improving management interventions. The daily deposition of otolith rings has been used to determine the age of tropical fishes and allows direct estimates of their developmental rates provided that the measurements are done accurately. However, the magnification and resolution of light microscopes are limited and could lead to age underestimation. This is common among older fishes with slower growth rates and deposit densely packed increments (<1um), which typically exceeds the resolution capacity of compound light microscopes (1-2.5 um). In this study, 60 otoliths of fish ranging in size from 9.2 – 17.6 cm SL were examined to derive age estimates using light microscopy. Of these, 19 were counted in parallel employing scanning electron microscopy to quantify the extent of underestimation. Results showed that SEM and LM age estimates ranged from 90-336d and 89-310d, respectively. Regression analysis showed an increase in variability between SEM and LM counts at 126 days, signifying an underestimation. These results may then be used to validate population age structure and growth models.

Keywords: Otolith microstructure, *Sardinella lemuru*, Light Microscopy, Scanning Electron Microscopy, Philippines

RETURN TO INDEX

IOS_183

A Comparison of seasonal Early Life Growth in *Sardinella gibbosa* from the Visayan Sea, Philippines

Raphael De la Rosa*[1], Wilfredo Campos[2], Alexanra Bagarinao-Regalado[3], Luke Felix[4], Marivic Pepino[5]
[1]College of Arts and Sciences, University of the Philippines Visayas, Miagao, Philippines; [2]Division of Biological Sciences, University of the Philippines Visayas, Miagao, Philippines; [3]Division of Biological Sciences, University of the Philippines Visayas, Miagao, Philippines; [4]OceanBio Lab, University of the Philippines Visayas, Miagao, Philippines; [5]OceanBio Lab, University of the Philippines Visayas, Miagao, Philippines. *rmdelarosa@up.edu.ph

Sardinella gibbosa is the dominant species of the sardine fisheries in the Visayan Sea, central Philippines and knowledge regarding its growth and development during its early life is essential in maintaining its population in the fishing ground. Several factors such as food, temperature and salinity contribute to the survival of fish larvae, and these factors can be influenced by seasonal conditions. In this study, early life growth in *S. gibbosa* during the Southwest (June – Early October) and Northeast (Late October - February) monsoon seasons in the Philippines were compared for the years 2017 to 2021. A total of 134 previously aged *S. gibbosa* otoliths with hatch dates within the monsoon months were used in this study. Daily increment measurements for the first 40 days of individuals hatched within the same monsoon season of the same year were pooled and used to construct growth curves based on the mean cumulative increase in their otolith radius. These were then related to existing data on the environment, catch and fecundity. Results show that there is a seasonal difference in early life growth (P: 5.94E-124) but not between years in both the Southwest (P: 0.38) and Northeast (P: 0.052) monsoon seasons. Measurements of mean cumulative increment widths ranged from 0.229 - 0.302 mm during Northeast monsoon while it ranged from 0.239 - 0.287 mm during the Southwest monsoon, where values are higher for most years. As for slope values indicative of growth rates, values ranged from 0.0067 - 0.008 during the Southwest monsoon and ranged from 0.0062 - 0.0082 during the Northeast monsoon. Higher values of mean cumulative increment widths during the Southwest monsoon coincides with its relatively higher temperature since higher temperatures tend to promote faster larval growth. Relating the results to catch data, monthly trends on the catch was difficult to relate with early life seasonality due to the bias caused by the closed season in the Northeast monsoon. As for fecundity, higher values for individuals hatched during the Southwest monsoon may be explained by the timing of their maturation (4.5 months) situated on the peak spawning months during the Northeast monsoon.

Keywords: *S. gibbosa*, Early growth, Monsoon seasons, Visayan Sea, Philippines

RETURN TO INDEX

IOS_189

Tracking Galapagos sailfin grouper using otolith chemistry

Solange Andrade-Vera*[1], Jose Marin Jarrin[2], Malte Willmes[3], Leticia Cavole[4]
[1]Charles Darwin Research Station, Charles Darwin Foundation, Puerto Ayora, Galapagos, Ecuador; [2]Telonicher Marine Lab, Department of Fisheries Biology, Cal Poly Humboldt, Trinidad, California, USA; [3]Norwegian Institute for Nature Research, Trondheim, Norway; [3]University of California Davis, Davis, California, USA. *solange.andrade@fcdarwin.org.ec

The Galapagos sailfin grouper *Mycteroperca olfax,* locally known as bacalao, is an economically, ecologically and socio-culturally important species in the Galapagos Archipelago, and regional endemic. This species is currently overexploited by the artisanal fishing sector; however, key biological and ecological gaps remain regarding its life history strategies and connectivity patterns. A previously untested tool for bacalao that may begin to elucidate these ecologically relevant traits is the analysis of various minor, trace and major elements in otoliths. To do so, we used a laser-ablation inductively coupled mass spectrometer to analyze the chemistry of otoliths from 92 adult specimens collected from rocky reefs sites in 9 islands and islets in 2016, distributed in the western and central-southeastern bioregions of the archipelago. We analyzed a set of environmentally and physiologically-controlled elemental ratios (Li:Ca, Mg:Ca, Mn:Ca, Cu:Ca, Zn:Ca, Rb:Ca, Sr:Ca, Ba:Ca and Pb:Ca) recorded at the edge of their otoliths, and tested the predictive accuracy of four statistical models to discriminate individuals at the island and bioregion spatial scales. The models included: random forest (RF), linear discriminant analysis (LDA), mixture and flexible discriminant analysis (MDA), and classification and regression trees (CART). We observed greater spatial variation and reclassification rates (~65%) at the bioregion scale than at the island scale (~50%). In addition, it appears that environmentally-controlled elements were slightly more important to separate adults than physiologically-controlled elements. Relatively low reclassification rates at the island spatial scale suggest (1) dynamic oceanography, with high mixing rates and relatively homogeneous water masses throughout the archipelago, and/or (2) high rates of movement and connectivity among adult individuals, diluting spatial patterns imprinted in their otoliths. The latter hypothesis would have implications for the conservation of the species, suggesting that high fishing efforts on a given island could have negative cascading effects into both adjacent and distant locations. In order to better understand their population structure and movement patterns, future can combine monitoring efforts with genetics and tagging studies. This will provide essential information to the decision makers for the conservation of this species.

Keywords: *Mycteroperca olfax*, marine reserve, ecoregions, bacalao, endemic.

RETURN TO INDEX

IOS_193

Otolith Strontium Isotopes Elucidate Diverse Life Histories in Fishes of the San Francisco Estuary, California, United States

Levi S. Lewis*[1], Malte Willmes[2], Leticia Cavole[1], James Hobbs[1]
[1]Department of Wildlife, Fish, and Conservation Biology, University of California Davis, Davis, California, United States
[2]Norwegian Institute for Nature Research, Trondheim, Norway
*lslewis@ucdavis.edu

Delta Smelt (*Hypomesus transpacificus*), Longfin Smelt (*Spirinchus thaleichthys*), and Chinook Salmon (*Oncorhynchus tshawytscha*) are imperiled migratory fishes that are native to the San Francisco Estuary, California, United States. Each species has experienced significant population declines due to numerous interacting anthropogenic stressors; however, key details regarding diversity in their migratory life histories have remained unknown. Here we applied Sr-isotope geochemical analysis to reconstruct the life histories of these fishes to inform on-going conservation and management efforts. Results indicate that Delta Smelt exhibit complex migratory behaviors, including resident and migratory phenotypes that vary in relation to regional climate. In contrast, Longfin Smelt appear semi-anadromous, exhibiting broad variation in salinity during the early life history. Last, we show that anadromous Chinook Salmon returning to spawn after a population collapse, and those spawning within restored streams, are predominantly hatchery-origin fish that have strayed from several different watersheds and hatcheries. Results of these geochemical analyses are shining new light on the life histories and habitat needs of imperiled fish populations in the San Francisco Estuary, information that is key for developing effective fisheries conservation and management actions.

Keywords: Delta Smelt, Longfin Smelt, Chinook Salmon, Salinity, Migration, microchemistry

RETURN TO INDEX

IOS_194

The role of moonlight on larval growth of inanga and other fishes

Jeff Shima[1],*, Mark Kaemingk[2], Conor Neilson[3], and Steve Swearer[4]
[1]School of Biological Sciences, Victoria University of Wellington, [2]Department of Biology, University of North Dakota, [3]Palantir Technologies, [4]School of BioSciences, University of Melbourne, * Jeffrey.shima@vuw.ac.nz

The life experiences of larval fishes that develop at sea remain a mystery at the best of times, and nocturnal interactions are rarely considered. We hypothesise that moonlight drives a dynamic trophic cascade between surface-dwelling larvae of amphidromous inanga (*Galaxias maculatus*) and a community of diel vertical migrants (e.g., myctophids and copepods) that comprise their predators and food. To partially test this hypothesis, we (1) estimate daily larval growth rates from otoliths of inanga and several other species; (2) quantify age-independent lunar periodicity; and (3) evaluate spatial variation in in lunar-periodic larval growth (across multiple sites in New Zealand and southern Australia for inanga; and from tropical to temperate sites for other reef fish larvae). We find that most samples exhibit compelling patterns of lunar periodicity in larval growth. For inanga and several other species, larval growth rates appear to be maximal near the waning moon and minimal near the waxing moon. However, these patterns are heterogeneous among locations, suggesting that the nocturnal interactions that potentially underlie these patterns (i.e., interactions with myctophids) may also vary. We discuss the fundamental implications of this hypothesis (for recruitment dynamics, evolution of marine life histories, climate change, and fisheries).

Keywords: Otoliths, Microstructure, Larval growth, Lunar rhythms, *Galaxias maculatus*

RETURN TO INDEX

IOS_198

Age and growth of the blue shark in the Southwest Atlantic

Francisco Marcante Santana*[1,2], Santiago Montealegre-Quijano[3], Fabrice Duponchelle[4], Rodrigo Risi Pereira Barreto[5], Jacques Panfili[4] and Rosângela Lessa[1]
[1]Laboratório de Dinâmica de Populações Marinhas (DIMAR), Departamento de Pesca e Aqüicultura (DEPAq), Universidade Federal Rural de Pernambuco (UFRPE), Recife - PE, Brazil; [2]Laboratório de Dinâmica de Populações Aquáticas (DAQUA), Unidade Acadêmica de Serra Talhada (UAST), Universidade Federal Rural de Pernambuco (UFRPE), Serra Talhada - PE, Brazil; [3]Universidade Estadual Paulista (UNESP), Departamento de Recursos Pesqueiros e Aquicultura, Registro – SP, Brazil; [4]MARBEC, Université de Montpellier, Institut de Recherche pour le Développement (IRD), Montpellier, France; [5]Departamento de Engenharia de Pesca e Ciências Biológicas (DEPB), Centro de Educação Superior da Região Sul, Universidade do Estado de Santa Catarina (UDESC), Laguna – SC, Brazil. *framarsantana@yahoo.com.br

Age and growth data of the blue shark, *Prionace glauca*, were compared for the the oceanic region of the southwest Atlantic (SWA), using vertebrae from three different periods from equatorial zone (northeastern Brazil), between 1999 and 2020, and 2004 to 2006, from the subtropical zone (southern Brazil). These data did not present significant temporal or spacial differences in proportionality pattern between blue shark size, vertebrae radius and growth parameters, and therefore whole sample was treated together, allowing us to estimate the age and growth for the entire SWA. A sample of 1437 blue sharks (95.4 to 310 cm total length – *TL*, 64.5% males and 35,5% females) had their vertebrae analized. We found an annual pattern in growth marks formation, with minimum marginal increment and percentage of translucent rings at the edge in August. There was no significant difference in growth parameters between males and females, being estimated for both sexes, according to the von Bertalanffy model, as L_{∞} = 290.4 cm *TL*, k = 0.203 year^{-1} and t_0 = -0.978 year^{-1}. The age structure of *P. glauca* catches in the SWA include from neonates of less than one year old, to large adults of 13 years old. Fishing recruitment was estimated at 5 years old for females and 4 years old for males. Age at maturity was estimated at 6.5 years old for both sexes. Thus, our data indicate that catches of blue sharks in the SWA are composed mostly by juveniles being 63% females and 72% of males. Although blue shark has a relative high reproductive capacity among elasmobranhs, catches of young *P. glauca* may affect the entire population of the SWA, thus decreasing the breeding stock, and therefore making this species vulnerable to overfishing.

Keywords: *Prionace glauca*, Age structure, Vertebrae, Sharks, Brazil.

RETURN TO INDEX

IOS_200

Unveiling the role of the NW Patagonian Fjords as a refuge for the persistence of fish populations in the face of disturbances

Pamela Toledo*[1,2], Edwin Niklitschek[1], Cristóbal Garcés[3], Ximena Valenzuela[4]
[1]Centro i~mar, Universidad de Los Lagos, Puerto Montt, Chile; [2]Universidad Austral de Chile, Puerto Montt, Chile; [3]Programa de Doctorado en Ciencias Mención Ecología y Evolución, Universidad Austral de Chile, Valdivia, Chile. [4]Universidad Austral de Chile, Campus Patagonia, Coyhaique, Chile. *pamela.toledo@ulagos.cl

Several studies have highlighted the importance of estuarine zones as spawning, nursery, and breeding areas for many species. In this work, we investigate the importance of the NW Patagonian Fjords (NWPF) for the long-term persistence of several fishes intensively exploited in the southeastern Pacific Ocean. Given the current and future disturbance scenarios these populations are facing (over-fishing, over-exploitation, pollution, climate change), knowing and protecting the diversity of life-cycles might be key to ensure their long-term persistence. Within this scenario, we focus on four fish species categorized as over-exploited or depleted by the Chilean fisheries authority: Pacific sardine *Strangomera bentinki*, Pacific anchovy *Engraulis ringens*, Patagonian grenadier *Macruronus magellanicus* and Southern hake *Merluccius australis*. We combined two types of natural markers in juvenile and adult otoliths: isotopic signatures ($\delta^{13}C$ and $\delta^{18}O$) and elemental composition (Na, Mg, Mn, Sr, and Ba) to estimate the relative contribution of the NWPF as a spawning, nursery or juvenile feeding habitat for each of these species. Our main results indicate that all four species present resident and migratory contingents that use the NWPF at some point in their life-cycle. Nonetheless, the relative contribution of this habitat to the adult fraction of the population changed dramatically between species. The NWPF nursery area provided a marginal contribution (<6%) to Pacific sardine and anchovy adult fractions being heavily exploited in oceanic waters further north. Even within the NWPF, self-recruitment was unexpectedly low (<17%). While the NWPF also represented a secondary nursery area for Patagonian grenadier (10-35% of adult oceanic samples), it was intensively used by these fish as subadults (43% of adult oceanic samples). The NWPF was, instead, the dominant nursery area being used by Southern hake (77 and 92% of oceanic and estuarine adult samples, respectively), NWPF resident fish were also more frequent in this species (41%). Despite these uneven contributions, the NWPF would play a key role in the long-term persistence of these species. Either by providing an essential habitat for Patagonian grenadier and southern hake or serving as a relict and refuge to over-exploitation and global perturbations affecting Pacific sardine and anchovy in oceanic waters.

Keywords: Microchemestry, Pacific sardine, Pacific anchovy, Patagonian grenadier, Pacific hake

RETURN TO INDEX

IOS_204

Mapping the environmental strontium isotopic ratio in South America to reveal the life history of freshwater fishes

Esteban Avigliano*[1], Ming-Tsung Chung[2,3], Marc Pouilly[4], Kuo-Fang Huang [2,3], Miguel Casalinuovo[5†], Jael Dominino[5], Natalia Silva[6], Sebastian Sánchez[6], Juan F. Facetti[7], Cristhian Clavijo[8], Jorge Pisonero[9], Ana Méndez[9], Pablo Scarabotti[10], J. D. Caffetti[11], A. V. Volpedo[1]

[1]Instituto de Investigaciones en Producción Animal (INPA-CONICET), Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, (C1427CWO) Buenos Aires, Argentina. *estebanavigliano@conicet.gov.ar; [2]Institute of Earth Sciences, Academia Sinica, Taipei 115, Taiwan; [3]Institut de Recherche pour le Développement (IRD), UMR BOREA (MNHN, CNRS, IRD, SU, UNICAEN, UA), F75005 Paris, France; [4]IRD (Institut de Recherche pour le Développement), UMR BOREA (MNHN, IRD, CNRS, UPMC), F75005 Paris, France; [5]Fundación Río Ctalamochita, Argentina; [6]CONICET- Universidad Nacional del Nordeste. Instituto de Ictiología del Nordeste; [8]Museo Nacional de Historia Natural (11100) Montevideo, Uruguay; [9]Departamento de Física, Facultad de Ciencias, Universidad de Oviedo, Federico García Lorca, no. 18, 33007 Oviedo, Spain; [10]Instituto Nacional de Limnología (CONICET-UNL), (S3001XAI) Santa Fe, Argentina; [11]Instituto de Biología Subtropical (IBS-UNaM-CONICET), Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Argentina.

The $^{87}Sr/^{86}Sr$ isotope ratio has turned out to be a useful natural tag in freshwater fishes to reveal migratory patterns in environments with geological heterogeneity. Here, we map the environmental $^{87}Sr/^{86}Sr$ ratio in 15 Atlantic and Pacific basins of South America (Argentina, Brazil, Chile, Paraguay and Uruguay), incorporating novel and recently published data from 84 sampling sites. In the vast La Plata Basin (3,170,000 km^2, Argentina, Brazil, Paraguay and Uruguay), 7 areas with different isotopic ratios have been discriminated (0.7083-0.7230), while in Patagonia (Argentina and Chile) 7 groups of basins have been discriminated (0.7045-0.7240). In the vast La Plata Basin, temporal variation was evaluated using invasive bivalve shell (*Limnoperna fortunei*) as $^{87}Sr/^{86}Sr$ biomonitors. Significant positive linear relationships were found between shell edge and water for $^{87}Sr/^{86}Sr$ (relationship 1:1, R^2=0.96-0.97, p<0.005) in summer and winter, suggesting that the *L. fournieri* shell is a good tool to monitor the spatio-temporal variation in water $^{87}Sr/^{86}Sr$. The otolith core-to-edge $^{87}Sr/^{86}Sr$ profile was analyzed in seven native (*Pseudoplatystoma corruscans* (Surubí), *Luciopimelodus pati* (Patí), *Salminus brasiliensis* (Dorado) and *Prochilodus lineatus* (Sabálo), *Oxydoras kneri* (Armado), *Hemisorubim platyrhynchos* (Tres Puntos), *Zungaro jahu* (Manguruyú)) and introduced (*Oncorhynchus tshawytscha* Chinook) species, which was contrasted with the water baseline for lower habitat use and natal origin. This analysis revealed migrations of up to 2,000 km for some fish species. The environmental baseline turned out to be a powerful tool to study the life history of fish, although it is necessary to increase the number and frequency of sampling to enhance the spatio-temporal resolution. We propose to incorporate biomonitors (shell) as a temporary evaluation method.

Keywords: Otolith, Neotropical fish, Migration, Natal homing

RETURN TO INDEX

IOS_205

Unpacking complexity of longitudinal movement patterns of small-bodied riverine fish using otolith microchemical analyses

Konrad Górski[1,2]*, Rodrigo Ramírez-Álvarez[2], Aurélien Vivancos[3], Daniel Ruzzante[4] and Evelyn Habit[3]
[1]Instituto de Ciencias Marinas y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile; [2]Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile; [3]Departamento de Sistemas Acuáticos, Facultad de Ciencias Ambientales y Centro EULA – Chile, Universidad de Concepción, Concepción, Chile; [4]Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada *Konrad.Gorski@uach.cl

Understanding of fish longitudinal movement patterns is fundamental to elucidate drivers of their life-history variation, recruitment and habitat colonisation. Still, movement of small-bodied fish remains poorly understood often due to methodological challenges frequently associated with their small body size and often cryptic nature. There is history of use of otoliths as time-resolved record of physiological aspects linked to growth and the environment of fish and otolith microchemical analyses are an emerging tool to assess movement patterns of small-bodied fish for which other movement assessment tools such as tagging are difficult to apply. In this talk, we present applications of recursive partitions and machine learning methods applied to multi-elemental composition of otoliths to assess movement patterns of resident (Chilean darter *Percilia irwini* and juvenile rainbow trout *Oncorhynchus mykiss*) and amphidromous (puye *Galaxias maculatus*) small-bodied fish species. We show how such analyses allow reconstruction of life-long movements patterns and identification of recruitment habitats. Furthermore, presented examples shade light on how anthropogenic alterations of connectivity of riverine corridors may affect natural movement patterns and population recruitment of such small-bodied fish. Finally, we discuss advantages and limitations of otolith microchemical analyses as a tool to study movement of small-bodied fish.

Keywords: Otoliths, Microchemistry, *Percilia*, *Galaxias*, *Oncorhynchus*, Fresh water

RETURN TO INDEX

IOS_207

Otoliths as tools to understand changes and patterns in fishes from South America to North America

Leticia Cavole[1]*
[1]Wildlife, Fish and Conservation Biology, University of California Davis, Davis, USA. *lmcavole@udavis.edu

South and North American fisheries (hereafter "American") are highly diverse and complex, just like the societies that utilize them. As a Latina who now works in the United States, navigating fisheries and otolith research has been both immensely valuable and challenging. Here, I provide an overview of several projects I have been involved in throughout my academic career, as well as the applications of my "otolithic" research. As an undergraduate in Brazil, I studied the age and life history of whitemouth croaker, which represents one of the most important demersal fisheries in the Southwest Atlantic. I validated the periodicity of daily growth increments in juveniles using a marking experiment, while for adults, I used the annual bands of ~ 5,000 specimens to examine the impact of industrial fisheries on their population dynamics. This work revealed a loss of the largest and oldest individuals, and a reduction in the age of first maturity for whitemouth croaker. During my master's research, I developed a novel approach to estimate the ages of brazilian codling and generated growth curves and reproduction parameters, which provide vital information for future management practices. While pursuing my doctorate in the USA, I jumped into the intricate world of otolith chemistry. With the aid of various collaborators and various multipurpose instruments (LA-ICPMS, SIMS, X-ray fluorescence), I examined shallow- and deep-water fishes from the Eastern Tropical Pacific Ocean and Oxygen Minimum Zones. Otolith chemistry revealed connectivity patterns and thermal histories for yellow snappers in Baja California and Galapagos, and hypoxia histories for long-lived deep-sea fishes off the Southern California Bight. Now, at my post graduate research, I continue to unravel the unique life history strategies, habitat uses, and phenotypic variation of the Delta Smelt - a forage fish vital to improving water policies in the San Francisco Estuary. Immersed in otolith research, I have met people, places, and fishes with incredible lives and natural histories. As an early-career scientist, I hope this knowledge will contribute to unraveling natural and anthropogenic changes and patterns in American fishes, as well as to protecting its incredible diversity of shapes, colors, and life strategies.

Keywords: Age and growth, Microchemistry, Brazil, Ecuador, Mexico, USA

RETURN TO INDEX

IOS_208

Age and growth of the Acoupa Weakfish, *Cynoscion acoupa* of the Brazilian Amazonian Coast, through micro and macrostructures in otoliths

Francisco Marcante Santana[1,2,3], Zafira da Silva Almeida[4](in memorian), Cícero Diogo Lins de Oliveira[1,2,3], Amanda Graziele Araújo Resende[1,2,3], Antônio Carlos Leal de Castro[5] and Rosângela Lessa[1].
[1]Laboratório de Dinâmica de Populações Marinhas (DIMAR), Departamento de Pesca e Aqüicultura (DEPAq), Universidade Federal Rural de Pernambuco (UFRPE), Recife - PE, Brazil; [2]Laboratório de Dinâmica de Populações Aquáticas (DAQUA), Unidade Acadêmica de Serra Talhada (UAST), Universidade Federal Rural de Pernambuco (UFRPE), Serra Talhada - PE, Brazil; [3]Programa de Pós-Graduação em Biodiversidade e Conservação, Unidade Acadêmica de Serra Talhada (UAST), Universidade Federal Rural de Pernambuco (UFRPE), Serra Talhada - PE, Brazil; [4]Laboratório de Pesca e Ecologia Aquática (LabPEA), Departamento de Biologia (DBio), Universidade Estadual do Maranhão (UEMA), São Luís – MA, Brazil; [5]Departamento de Oceanografia e Limnologia (DEOL), Universidade Federal do Maranhão (UFMA), São Luís – MA, Brazil. *framarsantana@yahoo.com.br

The Acoupa Weakfish, *Cynoscion acoupa*, is one of the most heavily exploited fish species on the northern Brazil, however, there are few studies that allow generating information for the sustainable management and conservation of this fishing resource. The aim of the present study was to estimate age and growth using micro and macrostructures in otoliths and length frequency distribution analysis (LFA), in a sample of 255 specimens (13 to 107 cm in total length - *TL*) collected on the coast of the Maranhão State between 2007 and 2008. Validation of microstructures using alizarin dyes demonstrated daily deposition of microstructures whereas macrostructures were validated as annual according to monthly marginal increment analysis. A zone of daily microstructures from the core was observed in the otolith until 328 days (0.87 year) and after this zone, the first annual macrostructure was formed. There were no significant differences between growth of males and females using the von Bertalanffy model (VBGM), resulting in k = 0.13 year^{-1}, L_{∞} = 142.95 cm *TL* and t_0 = -0.45 year, with ages ranged from 0.3 to 10.2 years. The growth coefficient estimated by LFA was very high (k = 0.28). Results indicated that *C. acoupa* of the coast of the Maranhão has a slower growth and higher longevity if compared to other Scianidae species. Almost the half of the sample was made up of juveniles (53.58%), overall the species endures worrying conservation context according to regional assessments in Brazil mainly due to increasing fishing pressure.

Keywords: Chemical validation, otolith, Length frequency distribution.

RETURN TO INDEX

IOS_215

The contribution of Artificial Intelligence on otolith research: State-of-the-art, perspectives and challenges

Dimitris Politikos[1],*, Kathleen Angélica Rautenberg[2], Eduardo Luis Cupertino Ballester[2,3], Paulo Ricardo Schwingel[4], André Martins Vaz-dos-Santos[3,5]
[1]Hellenic Center for Marine Research, Greece; [2]Post-graduate Program of Zoology, Federal University of Paraná, Brazil; [3]Post-graduate Program in Aquaculture and Sustainable Development, Federal University of Paraná, Brazil; [4]University of Vale of Itajaí, Brazil; [5]Sclerochronology Laboratory, Federal University of Paraná. Rua Pioneiro, 2153 CEP 85950-000 Palotina – PR – Brazil.
dimpolit@hcmr.gr / andrevaz@ufpr.br

Data on fish ageing are vital for extracting knowledge about the biological traits (age at maturity, reproduction, recruitment, mortality) and status of fish stocks. Every year, marine institutes around the world read systematically thousands of otolith images, collected within the frame of their national fisheries data collection program, to estimate the age of all commercial species, following common protocols and validation methods to reduce bias and uncertainties among expert readers. Manual fish age-reading is however costly and time-consuming due to specialised staff and labour work needed to detect annual zones formation in thousands of otoliths. Artificial intelligence (AI) has emerged over the last years as a promising tool to address this issue. The purposes of this study are threefold. First, we provide a concise review of the of published research that uses AI to process otolith images and morphometric data for attaining age estimation. Second, we illustrate the applicability of deep learning on estimating automatically fish age using a novel dataset of 1,055 otolith images of *Chloroscombrus chrysurus* (155-400 mm TL) from the South-eastern Brazilian Bight (SBB, 22ºS-28º40'S). Final, we present the potential of a free web-tool, called DeepOtolith (http://otoliths.ath.hcmr.gr/) to facilitate automated fish ageing in laboratories. The perspectives and future challenges related to data collection, processing and implementing deep neural networks on otolith imagery are also highlighted.

Keywords: Otoliths, Deep learning, Age estimation, AI web platform

RETURN TO INDEX

IOS_217

Tracking migration routes of juvenile chum salmon using otolith intrinsic tracer

Yuxiao Gou[1]*, Tomihiko Higuchi[1], Kotaro Shirai[1], Kentaro Honda[2], Takatoshi Higuchi[1,3], Takaaki K. Abe[1,4], Takashi Kitagawa[1]
[1]The Atmosphere and Ocean Research Institute, The University of Tokyo, Chiba, Japan; [2]Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Hokkaido, Japan; [3]CRESCO, Muséum National d'Historie Naturalle, Dinard, France; [4]College of Bioresource Science, Nihon University, Kanagawa, Japan *gouyuxiao@aori.u-tokyo.ac.jp

Chum salmon *Oncorhynchus keta* is a key anadromous fish species for indigenous peoples around Pacific Rim. Throughout the round-trip migration of this species, primary offshore migration of juvenile is the most challenging stage and the serious decline during when could potentially determine the final returning number and year class of matures, thus a call for a better understanding of the migration ecology during their early ocean life stages. To integrate the oceanic performance of migrants, tracking their migration routes is usually the very first process.

In this situation, isotope composition in specific tissues, e.g., vertebrate, eye lens and otolith, is thought to be a useful intrinsic tracer for tracking long-term migration routes without limitations of study areas. Among the approaches, oxygen isotope values in fish otolith (δ18Ootolith) are mostly widely introduced in case that: (1) otolith increments reflect an entire lifetime interval of fish; (2) the oxygen isotope fractionation between otolith and ambient water exists strong temperature dependency, which is proposed to be species-specific; (3) after increments deposited, there are no metabolically reworked or resorption, promising high chemical stability; (4) this approach produces fish sampling only once, without any recoveries or recapture effort.

In this study, a spatiotemporal model, modified by temperature dependency of δ18Ootolith fractionation on chum salmon with a hydrodynamic model-based δ18Oseawater isoscape, was developed and applied to the Japan-originated juvenile chum salmon. The reconstructed migration routes successfully reproduced the known migration behaviors of Japanese species individuals, e.g., Okhotsk Sea entrance and departure, overwintering in the western North Pacific, movement towards Bering Sea, and produced some counterintuitive routes, which did not utilize the Okhotsk Sea during migration for southern Japan-originated individuals.

We sought the configuration of the migration routes provides evidence for the possible route selection strategies of this species, so as to evaluate the biological performance related to the environmental integration and ecological consequences in variable scenarios.

Keywords: Chum salmon, Migration route, Otolith Stable isotope, North Pacific

RETURN TO INDEX

IOS_226

Daily growth and natal origin of capelin (*Mallotus villosus*) stock in Icelandic waters

Thassya Christina dos Santos Schmidt*, James Kennedy
Marine and Freshwater Research Institute, Hafnarfjöður, Iceland.
*thassya.dos.santos.schmidt@hafogvatn.is

Growth in early larval stages is critical for survival and future recruitment success of any fish species. In general, survival is higher in faster growing individuals in comparison to those with slower growth, with environmental factors influencing growth and thus survival. Capelin is a small-bodied forage fish species of the North-Atlantic Ocean with the Iceland-East Greenland-Jan Mayen stock representing the most important (both ecologically and economically) pelagic fish stock in Iceland. Spawning takes place between March and May, mostly in coastal areas south and west of Iceland. In our study, we aim to gather information to better understand the recruitment processes in capelin, examining daily growth in different areas and attempt to trace the natal origin using chemical analysis of the otoliths. The timing makes this study particularly interesting, with samples being collected during two years of low (2017 and 2018) and two years of high recruitment (2019 and 2020). During the study years, capelin larvae were collected around the Iceland shelf in May, and the sagittal otoliths were extracted. A subset of the otoliths was photographed in order to count daily increments while others were subjected to microchemistry analysis. These analyses are in progress, but preliminary results show a positive and nonlinear relationship between larvae size and otolith size, and between larvae size and number of increments. So far, in 2020 the mean daily growth was 0.25 mm day^{-1}, and ranged from 0.22 to 0.32 mm day^{-1} among five pre-established areas (southwest, northwest, north, east, and south). We observed that some older larvae (more than 40 increments) have most likely reached the nursery areas in western and northern Iceland by May, but we also found newly hatched larvae in all areas, especially in the eastern Iceland, a previously undocumented spawning ground.

Keywords: Daily growth, Microchemistry, Spawning grounds, forage species, recruitment

RETURN TO INDEX

IOS_228

Habitat use of the common snook *Centropomus undecimalis* in lagoon systems of the southwest Atlantic Ocean inferred by otolith core and edge elemental signatures

Paulo Almeida[1,2], Marcus Costa[1], Rebeca Coutinho[1], Ana Mendez[3], Jorge Castro[4], Cassiano Monteiro-Neto[1] & Alberto Teodorico Correia[2,5,6,*]
[1]Departamento de Biologia Marinha, Universidade Federal Fluminense (UFF), Niterói, Rio de Janeiro, Brasil; [2]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Matosinhos, Portugal; [3]Scientific and Technological Resources (SCTs), University of Oviedo, Mieres, Spain; [4]Department of Physics, Faculty of Science, University of Oviedo, Oviedo, Spain; [5]Departamento de Biologia e Ambiente (DeBA), Escola de Ciências da Vida e do Ambiente (ECVA), Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal; [6]Departamento de Produção Aquática (DPA), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto (UP), 4050-313 Porto, Portugal. * corresponding author: atcorreia.ciimar@gmail.com

The common snook, *Centropomus undecimalis*, is an important fishery resource in the Southwest Atlantic Ocean. It is a demersal and catadromous fish, however its habitat residency is not fully understood in the Brazilian Atlantic waters, which make the species vulnerable to overfishing and anthropic impacts. The present study aimed to identify patterns of habitat use by *C. undecimalis* juveniles captured in the lagoon systems of Itaipu (ITA), Maricá (MAR), Saquarema (SAQ) and Araruama (ARA), located in the Eastern Rio de Janeiro state, Brazil. Sixty individuals (15 individuals per site) were collected between November 2019 and March 2020. Individuals from the same age group (3 years old), following age estimation by counting the annual growth increments, were used. Elemental signatures of the otolith's cores and edges were obtained using laser ablation inductively coupled-plasma mass spectrometry (LA-ICP-MS). Data were analyzed using univariate and multivariate statistics to assess the degree of separation among individuals regarding the natal origin (otolith cores) and moment of capture (otolith edges) from the sampling sites, and differences between otolith cores and edges. Elemental signatures exhibited distinct local patterns, driven by Ba/Ca, Li/Ca and Sr/Ca in the otolith cores and Ba/Ca, Li/Ca, Mn/Ca and Sr/Ca in the edges. A low reclassification was recorded for the otolith cores values (overall: 45%; by lagoon: 53% SAQ, 53% MAR, 47% ITA and 27% ARA) were founded. However, a highest overall reclassification was observed for the otolith edge (overall: 65%; by lagoon: 73% ITA, 73% SAQ, 60% MAR, and 53% ARA). Differences between otolith core and edge signatures were detected in Ba/Ca, Cu/Ca, Li/Ca, Mn/Ca and Sr/Ca ratios. An ontogenetic habitat shift pattern was detected, with similar origin of 0^+ individuals due to marine water influence, and moderate discrimination of groups of 3^+ juveniles associated to environmental characteristics of the lagoon systems. The hereby results reinforce the importance of habitat's conservation and the connectivity maintenance.

Keywords: Centropomidae; sagittae; natural tags; chemical analyses.

RETURN TO INDEX

IOS_235

Comparative study on *sagittae* shape, morphometry, and age structures from two populations of *Scorpaena porcus* (Linnaeus, 1758) inhabiting the Strait of Messina (Central Mediterranean Sea) and Split area (North-Central Mediterranean Sea)

Claudio D'Iglio[1]*, Sergio Famulari[1], Marco Albano[2], Serena Savoca[3], Gioele Capillo[2], Josipa Ferri[4]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy; [2]Department of Veterinary Sciences, University of Messina, Messina, Italy; [3]Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, Messina, Italy; [4]Department of Marine Studies, University of Split, Split, Croatia.
*cladiglio@unime.it

Improve the knowledge base on the ecomorphological adaptation of teleost species to different environments, trying to reconstruct how environmental pressures can shape *sagittae,* is essential for conservational porpoises, evolutionary evaluations, and population dynamics' studies. Here is provided a comparative study between *sagittae* of *Scorpaena porcus* (Linnaeus, 1758) from the Strait of Messina (Messina, Italy, Central Mediterranean Sea) and Split area (Split, Croatia, Adriatic Sea, North Central Mediterranean Sea). A total of 90 samples (45 from Messina and 45 from Split), with a total fish length ranging from 65 to 240 mm, have been collected from two totally different environments for depths and physiochemical features: tidal ponds, along the northern Sicilian coast of the Messina Strait (50 cm of maximum depth), and the infralittoral rocky-sandy plane of Split (40 m of maximum depth). Total fish length, weight and sex of each specimen were assessed. Once extracted, left *sagittae* were analyzed using shape and morphometric analysis, evaluating the age, the total otoliths radius and the radius of each formed annulus. Results showed an overall different morphology of *sagittae* between the two populations. Samples from Messina were characterized by a most elongated and slender shape, and a most regular serration of margins than those from Split. The latter exhibited a wider *sagitta,* with a most enhanced anti-rostrum and longer rostrum than those from Messina. Morphometrical analysis highlighted several shape indices' differences, in line with the contours' variability between populations detected by shape analysis. The age structures and growth dynamics were also different, with a slower growth showed by Split specimens. Results have confirmed the reliability of *sagittae* to detect the intra specific variability between populations of *S. porcus* from different geographical area, an essential tool for stock assessment and population studies. Data on the morphometrical and morphological variability between the two studied populations can improve the knowledge base on the ecomorphological adaptation of teleost species to different habitats. Further analyses are required to fully understand how *sagittae* could be shaped by different environmental pressures in Mediterranean ecosystems.

Keywords: Otoliths, Shape analysis, age, populations differences, *Sagittae*

RETURN TO INDEX

IOS_236

Eco-morphology of sagittal otoliths in five Macrouridae species from Central Mediterranean Sea

Serena Savoca[1]*, Claudio D'Iglio[2], Marco Albano[3], Sergio Famulari[2], Nunziacarla Spanò[1], Gioele Capillo[3]
[1]Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, Messina, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy; [3]Department of Veterinary Sciences, University of Messina, Messina, Italy. *ssavoca@unime.it

Macrouridae represents in the Mediterranean Sea an essential component of the bathyal community in continental slope environments and a dominant component for abundance below the 1000 m depth. Despite their low commercial value, they are one of the major components of by-catch in deep-seas shrimp fisheries. The increasing impact of fisheries activities have led to a greater attention on monitoring the effects of the over-exploitation on deep-sea fish assemblage and habitats. In this context, otoliths can be a strategic tool to assessing their life history, stock composition, conservation and management actions. Herein, we investigated the intra and inter specific sagittal otoliths variability in five species of grenadiers. Samples, coming from Tyrrhenian Sea, were obtained by professional fisherman, during October and November 2022. A total of 144 individuals (20 *Coelorinchus caelorhincus*, 35 *Coryphaenoides guentheri*, 24 *Hymenocephalus italicus*, 24 *Nezumia aequalis*, 40 *Nezumia sclerorhynchus*) were analyzed. After species identification and specimen measurement, pairs of sagittae were collected, cleaned, washed and stored dry. Images of left and right sagittae were captured and measurements were recorded using ImageJ software. Specific morphological parameters were analyzed using univariate and multivariate analysis to detect differences between the right and left sides of the otolith specimens within and between the species. The correlation between the variable and fish size was tested. Otolith shapes analysis was performed using shape R package. Wavelet coefficients were used to analyze shape variation among species using ANOVA and LDA. All species showed variations of parameters such as sagitta weight, area, perimeter, length and width, including sulcus, cauda and ostium parameters. Significant dissimilarities were found between specimens belonging to *Nezumia* sp *vs C. caelorhincus* and *C. guentheri vs H. italicus*. The latter species resulted characterized by more rounded sagittae. Shape analysis and LDA confirmed this result. No bilateral asymmetry was detected, except for the values of CL/SL % in *C. caelorhincus* and SS/OS% in *N. sclerorhynchus*. These data could improve the knowledge on these species' ecology, for a better management and conservation of marine environment and resources, being Macrouridae species essential for the well-functioning of the entire Mediterranean deep-sea ecosystems.

Keywords: Sagitta, Shape analysis, Grenadiers, deep environments, Tyrrhenian Sea

RETURN TO INDEX

IOS_240

Mugil liza as sentinel's species in Southeast-South America aquatic ecosystems

Rafael Schroeder[1,2], Esteban Avigliano[3], Alejandra V. Volpedo[3], Roberta Callicó Fortunato[3], Pedro Barrulas[4], Felippe A. Daros[5], Paulo R. Schwingel[6], Martin C. Dias[7] and Alberto T. Correia*[2,8,9], atcorreia.ciimar@gmail.com
[1]Laboratório de Estudos Marinhos Aplicados. Escola Politécnica. Universidade do Vale do Itajaí (UNIVALI). Rua Uruguai 458, Centro 88302-901, Itajaí, Brasil; [2]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR). Terminal de Cruzeiros do Porto de Leixões. Avenida General Norton de Matos S/N, 4550-208 Matosinhos, Portugal; [3]CONICET-Universidad de Buenos Aires. Instituto de Investigaciones en Producción Animal (INPA); Facultad de Ciencias Veterinarias; Av. Chorroarín 280; C1427CWO, Buenos Aires, Argentina; [4]Laboratório HERCULES - Herança Cultural, Estudos e Salvaguarda. Largo Marquês de Marialva 8, 7000-809 Évora, Portugal; [5]Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP). Campus de Registro. R. Nelson Brihi Badur, 430; Vila Tupi; Registro, SP, Brasil; [6]Laboratório de Ecossistemas Aquáticos e Pesqueiros. Escola Politécnica. Universidade do Vale do Itajaí. R. Uruguai 458, Centro 88302-901. Itajaí, Brasil; [7]Oceana Brasil; endereço: SIG Quadra 1, Lote 985, Sala 251, Centro Empresarial Parque Brasília, Brasília, CEP 70610-410; [8]Departamento de Biologia e Ambiente (DeBA), Escola de Ciências da Vida e do Ambiente (ECVA), Universidade de Trás-os-Montes e Alto Douro (UTAD), Quinta dos Prados, 5000-801Vila Real, Portugal; [9]Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto (ICBAS-UP). Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.

The Lebranche grey mullet *Mugil liza* is an important commercial species off southern Brazil. Recently, a multidisciplinary approach using scale counts, body morphometrics, otolith shape analysis and micro-chemistry established connective patterns between adult fish captured between Rio Grande do Sul and São Paulo (25ºS-34ºS), Brazil with juveniles sampled in the northern Patagonic shelf (35ºS-40ºS), Argentina. Despite the economic importance little is known about the environmental conditions in spawning areas and early life cycle. This study was built under the hypothesis that otolith microchemistry of the core (natal origin) and edge (moment of capture) regions reconstructed over time can be used as a pollution biomarker to trace significant shifts in element/Ca concentrations in otoliths sampled between Brazil and Argentina (25ºS-40ºS). *M. liza* migrates northwards from nursery areas to spawn in the open ocean, and the larvae are carried back southwards by coastal marine currents to nursery areas, where they spend most of their life cycle (~5 years) until reaching sexual maturity. This particular characteristic makes *M. liza* suitable as a sentinel species. The hypothesis was tested in generalized additive models from a comparison of the element/Ca ratios (Ba, Cd, Cu, Fe, Li, Mg, Mn, Ni, Pb, Sr, and Zn) from the otolith core and edge among years. For the otolith core analyses, it was detected significant progressive increases in four element/Ca ratios (Cd, Fe, Li, and Mn) in mullets born between 2004 and 2014. In the otoliths' edge five element/Ca ratios (Ba, Cd, Li, Sr, and Zn) show significant increases for mullets sampled between 2011 and 2017. The increasing values of Cd/Ca and Fe/Ca are possibly associated with agricultural and industrial activities. The present study showed that otolith microchemistry can be used as a sclerochronological biomarker of urbanization impacts in Southeast-South America aquatic ecosystems.

Keywords: Otolith microchemistry; nursery areas; spawning areas; natural tags; urbanization impacts.

RETURN TO INDEX

IOS_243

Variability in early life growth of the goldstripe sardinella, *Sardinella gibbosa*, in the Visayan Sea and the role of environmental factors

Alexanra Bagarinao-Regalado*, Wilfredo L. Campos, Raphael de la Rosa, Kaola T. Pamonag, Renmill Lambid and Rhyxy Mamon
Oceanbio lab, Division of Biological Sciences, College of Arts and Sciences, University of the Philippines Visayas, Miagao, Iloilo
aabagarinao@up.edu.ph

The abundance of sardine populations has been strongly linked to recruitment strength which mainly depends on the influence of various factors affecting the early life stages. Understanding these factors is essential if we are to manage sardine resources effectively. Sardines is one of the major fishery resources in the Philippines, with *Sardinella gibbosa* comprising the bulk of the catches in various major sardine fishing grounds like the Visayan Sea. Otolith increments were analyzed to examine the early life growth of *Sardinella gibbosa* in the Visayan Sea from a 5-yr period (2016-2021). Based on the fitted regression, the mean growth rate of individuals ranges from 0.26 – 0.74 mm day^{-1}. Growth varied significantly within the season, between seasons in a year, and between years. These differences in early life growth appear to be influenced by environmental and climatic conditions. Cohorts hatched during the northeast monsoon months (dry and cold) display significant variability in growth compared to those hatched during the southwest monsoon months (wet/rainy). Moreover, growth greatly varied during strong/weak El Niño/La Niña years compared to neutral/moderate ENSO years.

Keywords: Otolith, Sardines, Recruitment, ENSO

RETURN TO INDEX

IOS_244

Not only sagittae: evaluation of intra-specific differences of lapilli and asterisci of *Scorpaena porcus* (Linnaeus, 1758) from the Strait of Messina (Italy – Central Mediterranean Sea)

Sergio Famulari[1]*: Claudio D'Iglio[1]: Marco Albano[2]: Serena Savoca[3]: Nunziacarla Spanò[3]: Josipa Ferri[4]: Gioele Capillo[2]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy; [2]Department of Veterinary Sciences, University of Messina, Messina, Italy; [3]Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, Messina, Italy; [4]Department of Marine Studies, University of Split, Split, Croatia.
*serfamulari@unime.it

Over the last decades, the importance of studying otoliths morphology and morphometry has been widely recognized as a valuable tool to investigate several biological and ecological aspects of teleost species and their adaptations to marine environments. Compared to *sagittae*, the scientific interest and knowledge of *lapilli* and *asteriscii* are still limited, due to their generally small size and difficult extraction procedures. As a consequence, their intra and interspecific variability is almost completely unexplored. In the present study, attention was addressed to specimens of *Scorpaena porcus* (Linnaeus, 1758) from the Strait of Messina (Messina, Italy, Central Mediterranean Sea). A total of 49 samples with body length between 65 and 230 mm were collected from tidal ponds located along the northern Sicilian coast of the Strait of Messina. Total length (mm) and body weight (g) of each specimen were measured and sex was assessed. Pairs of *lapilli* and *asterisci* were manually extracted, cleaned from tissues, and photographed using a stereomicroscope with a built-in digital camera. Images thus collected were binarized for outline detection and statistical and shape analysis were subsequently performed using open-source software packages that run on the R platform. Results achieved showed moderate intra-specific differences among specimens analyzed, concerning both *lapilli* and *asterisci*. For each type of otolith, a comparison was made between males and females, showing a more elongated and slender shape in the latter case. Slight differences were also detected between left and right otoliths, with the right ones characterized by a wider shape and less regular margins. Despite the well-known importance of studying *sagittae, lapilli* and *asterisci* can also be considered useful tools to evaluate intra-specific differences, as shown by the results here provided that represent a baseline for future studies. Further analyses are undoubtedly required to fill the lack of knowledge on morpho-functional adaptation mechanisms of bony fishes to several habitats.

Keywords: Otoliths, Shape Analysis, Morphology, Variability.

RETURN TO INDEX

IOS_246

Subpopulational structure of *Pogonias courbina* in two lagoon systems in Southwest Atlantic Ocean inferred by shape and elemental signatures of otoliths

Paulo Almeida[1,2], Marcus Costa[1], Ana Ribeiro[1], Agostinho Almeida[3], Rui Azevedo[3], Cassiano Monteiro-Neto[1] & Alberto Correia[2,4,5,*]
[1]Departamento de Biologia Marinha, Universidade Federal Fluminense (UFF), Niterói, Rio de Janeiro, Brasil; [2]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Matosinhos, Portugal; [3]Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; [4]Departamento de Biologia e Ambiente (DeBA), Escola de Ciências da Vida e do Ambiente (ECVA), Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal; [5]Departamento de Produção Aquática (DPA), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto (UP), 4050-313 Porto, Portugal. atcorreia.ciimar@gmail.com

The black drum, *Pogonias courbina*, is an estuarine and coastal demersal fish, widely distributed in the Western Atlantic Ocean. It is a vulnerable fish in the IUCN Red List of Threatened Species, because overfishing. Thus, the present study aimed to understand the population structure of *P. courbina* in two lagoon systems in the Southwest Atlantic Ocean. A total of 60 individuals were collected in the lagoons of Saquarema (SQ) and Araruama (AR) between November 2019 and April 2020. Thirty individuals per location from the same age group (2 years old), following age estimation by counting the annual growth increments, were used. The otolith shape was evaluated using Elliptical Fourier descriptors (EFD). Multi-elemental signatures (MES) of the whole otoliths were obtained using solution-based inductively coupled plasma mass spectrometry (SB-ICP-MS). Data were analyzed using univariate and multivariate statistics to assess the degree of separation between individuals from both lagoons. EFD analysis showed differences between lagoon systems, with overall reclassification rate of 90% (80% SQ and 100% ARA). The MES exhibited distinct patterns between lagoon systems, mainly driven by differences in Ba/Ca, Co/Ca, Li/Ca, Mg/Ca, Ni/Ca, Sr/Ca and Zn/Ca ratios. Overall reclassification rate for MES was of 100% (100% SQ and 100% ARA). The overall reclassification rate obtained using both EFD and MES of otoliths was of 90% (80% SQ and 100% ARA). The results founded corroborates for the discrimination of the subpopulations of *P. courbina* in the two lagoon systems of Eastern Rio de Janeiro, based on the shape and elemental signatures. An almost complete discrepancy in the otolith shape and chemistry indicates a restricted connectivity between *P. courbina* of the Saquarema and Araruama lagoon systems. The differences founded can be related to environmental conditions of the lagoon systems, where Saquarema receive a larger freshwater contribution and have more water exchange with the sea, and Araruama a hypersaline lagoon because of the low precipitation rates in the region. The low connectivity observed suggests that the lagoon systems consist in distinct habitats for. *courbina*, indicating that these subpopulations should be managed independently.

Keywords: Scianidae; Fourier descriptors; natural tags; chemical analyses.

RETURN TO INDEX

IOS_249

Segmental isotope analysis of the vertebral centrum reveals the spatiotemporal population structure of adult Japanese flounder *Paralichthys olivaceus* in Sendai Bay, Japan

Yoshikazu Kato[1,2,3], Hiroyuki Togashi[3], Yutaka Kurita [3], Yutaka Osada[4], Yosuke Amano [5], Chikage Yoshimizu[1], Hiromitsu Kamauchi*[1,6], Ichiro Tayasu[1]
[1]Research Institute for Humanity and Nature, Kyoto, Japan; [2]Graduate School of Environmental Studies, Nagoya University, Nagoya, Japan; [3]Shiogama Field Station, Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Shiogama, Japan; [4]Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Yokohama, Japan; [5]Fukushima Prefectural Fisheries and Marine Science Research Centre, Iwaki, Japan; [6]Bio-generated Stripe-material Analysis, Nagoya, Japan. *kamauchi@gmail.com

To identify the origin of various fishes and reconstruct their migration history at the individual level, isotope analysis is a powerful alternative to artificial tagging. We used a novel individual-based methodology to reconstruct individual migratory and/or trophic shifts associated with growth based on isotopic data in the vertebral centrum of adult Japanese flounder *Paralichthys olivaceus* in Sendai Bay. We measured carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in muscle tissues, and conducted a segmental isotope analysis of bulk $\delta^{13}C$ ($\delta^{13}C_{bulk}$), bulk $\delta^{15}N$ ($\delta^{15}N_{bulk}$), and $\delta^{15}N$ of glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine ($\delta^{15}N_{Phe}$) in vertebral collagen. The $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values for bone collagen revealed an increase in trophic position and a shift to lower trophic baselines ($\delta^{15}N_{Base}$: indicative of $\delta^{15}N$ values of primary trophic sources) for most individuals. For both $\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$, we detected significant positive correlations between values for muscle and the outermost section of vertebral collagen. A nonlinear time-series analysis of $\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$ suggested that a combination of intrinsic (the timing of migration from the nursery to deep offshore areas in juveniles) and extrinsic (habitat and/or food qualities) factors influence the isotopic chronology. A segmental isotope analysis revealed the segregation of individuals among sampling sites at all life stages and changes in trophic positions and $\delta^{15}N_{Base}$ values during growth. Our results suggest that the *P. olivaceus* population in Sendai Bay has both temporal and spatial structure. The temporal structure may be caused by variation in the timing of migration from the nursery to the deep offshore area in juveniles, and the spatial structure may be explained by individual variation in habitat preferences.

Keywords: Vertebral centrum, Ecology, Collagen, Northwest Pacific coast, Japanese flounder

RETURN TO INDEX

IOS_253

Life-history Traits of Twaite Shad (*Alosa fallax*) in the Scheldt Estuary

Karen Bekaert[1]*, Giuliano Lecompte[2], Raf Vanneste[2], Pieterjan Verhelst[3]
[1]Flanders Research Institute for Agriculture, Fisheries and Food, Ostend, Belgium; [2]Faculty of Science, Ghent University, Ghent, Belgium; [3]Research Institute for Nature and Forests, Brussels, Belgium. *karen.bekaert@ilvo.vlaanderen.be

Twaite shad (*Alosa fallax*) is an anadromous fish that used to be widespread along the coasts and rivers of the North Atlantic from Iceland to Morocco, through the Mediterranean and the Baltic sea. Populations collapsed during the 20th century due to human impacts such as dam constructions, overexploitation and pollution. After 100 years of absence, twaite shad is spawning in the Scheldt estuary again, but few is known about the composition and life-history traits of this population.

To get more insight in the population, mature adults were caught during spawning season (April – May) in the Scheldt estuary from 2020 to 2022 and life-history traits such as age at first maturity, growth rate, age composition and length-weight relationships were investigated. Therefore, scales from adult twaite shad were gathered and analyzed. Age and distances were determined through digital processing of images by examining the structures on the scales using the SmartDots software. Seawater and freshwater rings were identified.

In total, 160 fish with a length between 320 and 500 mm were analyzed over the 3 years. Female shad attained ages between 3 and 11 years. Male shad were significantly younger with ages ranging from 5 to 7 years, but both sexes showed a majority of 5 and 6-year old fish. Age reading precision between different readers was acceptable. Females were significantly larger than males and median age at first spawning was 4 years for females and 3 years for males. Age composition of shad populations seem to follow a latitudinal gradient where fish grow older towards Northern latitudes. Our results match this gradient. The older ages of twaite shad towards the North might be linked to their iteroparous lifestyle in contrast to the semelparous southern populations. Also the ages at first maturity correspond with studies on other twaite shad populations in Europe.

This study gives a first insight in the life history traits of the twaite shad population in the Scheldt estuary. It will be important to continue the monitoring of this vulnerable population in the future.

Keywords: scales, age reading, maturity, growth

RETURN TO INDEX

IOS_254

Larval growth and mortality rates of *Engraulis ringens* in Northern Chile

Miguel Araya, Jessica Peñailillo, Carlos Pacheco
Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile. maraya@unap.cl

The larval growth rate is dependent on environmental conditions and can have a major impact on fish larval mortality. The growth rate and larval mortality of the anchovy *Engraulis ringens* are estimated for winter 2019 and summer 2020 in the Chipana sector. Ages are estimated by reading the microincrements present in the otoliths. The larvae were between 1 and 16 mm in winter and between 1 and 12 mm in summer, which is equivalent to age in days between 3 and 18 days. The growth for both periods behaved linearly, LE = -0.22 + 0.88 t (winter), LE = 0.84 + 0.71 t (summer), the slopes of both adjustments are significantly different. The birth dates for winter shows a peak on September 6, 2019, while for summer births are spread over a greater number of days, between January 12 and 21, 2020. The mean growth rate for winter larvae was between 0.01 and 0.78 mm/day, with an average of 0.39 ± _0.233 mm/day and for summer between 0.1 and 0.86 mm/day with an average of 0.41 ± _0.214 mm/day. The effect of somatic growth rate on the relationship between otolith size and larval length was not detected for both sampling periods. The relationship between the growth rate and the standard length shows two phases for both sampling periods, behaving linearly. The daily production rate of larvae was much higher in the summer season. The estimated mortality values were 0.43 day-1 for winter and 0.53 day-1 for summer. The two estimates do not show significant differences.

Keywords: Mortality, larvae*, Engraulis ringens*, growth rates

RETURN TO INDEX

IOS_255

Fjords linkage to early growth and survival of Patagonian sprat, *Sprattus fuegensis*, along southwest Patagonia in austral spring 2019

Erika López-Soto*[1,2], Gillian Ord[2], Manuel I. Castillo[3,4,5], Guido Plaza[6], Mario A. Cáceres[3], María Teresa González[7], Maritza Sepúlveda[8], Alicia Guerrero[8], Andrea Piñones[5,9], Mauricio F. Landaeta[1,3]
[1]Laboratorio de Ictiología e Interacciones Biofísicas (LABITI), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; [2]Programa Magíster en Oceanografía, Universidad de Valparaíso y Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile; [3]Centro de Observación Marina para Estudios de Riesgos del Ambiente Costero (COSTA-R), Universidad de Valparaíso, Valparaíso, Chile; [4]Laboratorio de Oceanografía Física y Satelital (LOFISAT), Escuela de Biología Marina, Facultad de Ciencias del Mar y de Recursos Naturales, Universidad de Valparaíso, Valparaíso, Chile; [5]Centro de Investigación Oceanográfica COPAS Sur-Austral, Universidad de Concepción, Concepción, Chile; [6]Laboratorio de Esclero-Cronología, Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile; [7]Instituto de Ciencias Naturales Alexander von Humboldt, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile; [8]Laboratorio de Ecología de Mamíferos Marinos (LECMMAR), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; [9]Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile. *erikalopez.erika@gmail.com

Patagonian sprat, Sprattus fuegensis, is an economically and ecologically important small pelagic fish inhabiting the continental shelf, fjords and channels of Patagonia, southern tip of South America. It is a fast-growing species that reproduces by partial spawning during the austral spring. Patagonian sprat larvae are usually distributed in the mixed layer of the water column, exposed to large variations in physical and biological conditions. We aimed to evaluate the effects of different environmental conditions on early life history traits of S. fuegensis. Characterisation of oceanographic features and larval growth was carried out in the austral spring of 2019, during a bio-oceanographic cruise between 49°54'S and 53°55'S. This area is directly influenced by freshwater inputs from river discharge and ice melt from the Northern and Southern Ice Fields, the largest glaciers in the Southern Hemisphere. Larval growth was estimated from otolith microstructure analysis; the daily growth rate was 0.20 ± 0.05 mm d-1 for larvae between 9 and 19 days post-hatch. S. fueguensis larvae grew similarly in partially stratified (7 - 50 J m-3) and stratified waters (50 - 100 J m-3), whereas in mixed waters (< 7 J m-3) larval growth was significantly slower (0.16 mm d-1). Mortality rates showed significant differences according to the stratification of the water column, being higher in partially stratified waters (24% of daily loss), followed by stratified areas (14%) and lower in mixed zones (12%). Therefore, there was a trade-off in the early life history of this small pelagic fish; fish larvae hatched or advected to water parcels over the continental shelf experienced slower growth but lower daily mortality, while those living in fjords and channels grew faster but had higher daily mortality. Future studies need to address the effects on early developmental patterns of S. fuegensis in the context of climate change, which will alter the coastal waters off Patagonia.

Keywords: Otoliths, Larvae, Icefield, Stability, Potential Energy Anomaly

Funding: CIMAR 25, Anillo AT220033

RETURN TO INDEX

IOS_258

Impact of annuli validation in age structure and growth parameters. The case of *Trachurus murphyi*

Camilo Rodríguez-Valentino*1, Francisco Cerna1, Lizandro Muñoz1, Guillermo Moyano1[1]Age and Growth Laboratory, Sea Institute of Peru. Lima, Peru. *lusca@imarpe.gob.pe

1Sección de Edad y Crecimiento, Instituto de Fomento Pesquero, Valparaíso, Chile; *camilo.rodriguez@ifop.cl

Age determination in commercially important species is fundamental for the application of stock assessment models which allow estimation of recruitment, mortality and other stock condition characteristics, impacting the decision making processes regarding management measures. Recently, Trachurus murphyi's annuli were validated through otolith microstructure analysis, length frequency mode progression and bomb radiocarbon analysis estimating a higher growth rate the first 2 years and slowing down after the 3rd year. This new annuli interpretation changed the historic age structures, allowing the observation of young Age Groups (AG) entering the fishery that previously were unaccounted, and an increase in the AG historic mean weights. Length at first maturity (L50), Von Bertalanffy growth parameters and Zhang & Megrey natural mortality were estimated using the jack mackerel otolith reference collection, reading otolith transversal slices. L50 was the parameter affected the most, estimated in ~1 year, which is 1.5 years earlier than previously reported. The jack mackerel's Von Bertalanffy growth parameters have been estimated many times by different authors with a wide range of estimates (L∞: 46 – 75 cm, K: 0.09 – 0.17), our estimations fall within this range with L∞ at 57 cm and the growth parameter K at 0.17. Zhang & Megrey natural mortality was estimated in 0.25 year-1 which is slightly higher than 0.23 year-1 previously estimated and currently in use for stock assessment models.

Keywords: Annuli, Trachurus, Validation, Growth parameters, Age structure

RETURN TO INDEX

IOS_268

Determination of age and growth in fish of the pacific pomfret (*Brama australis*) in South Central and Southern Chile

Guillermo Moyano Altamirano, Jorge Contreras Herrera. jorge.contreras@ifop.cl
Sección edad y crecimiento, Instituto de Fomento Pesquero (IFOP)

The main life history parameters of the Pacific pomfret (*Brama australis*) of samples from the industrial (trawl) and artisanal vessels (longline and gillnet) that operate in south-central and southern off Chile (35 ° 00 'S and 57° 16 'S) during the period 2012 to 2021, represented by individuals between 25.0 to 58.0 cm fork length (FL). The growth parameters were obtained from the annuli reading in *sagittal* otoliths, where the location of the first annulus was verified through daily microincrements analysis. The highest growth rates occurred during the first year of life, reaching a size of 30.13 cm FL at a daily rate of 0.55 mm*day^{-1}. The catches of the different fishing gears were mostly represented by individuals of 4 years of age. The average length of sexual maturity ($L_{50\%}$), corresponding to 37.7 cm FL was reached at age 3. Finally, otolith shape indexes analysis showed 3 different morpho-types, which are not associated with capture zones, fishing gears, sex or length of the individual.

Keywords: *Brama australis*, Life history, Annuli reading.

RETURN TO INDEX

IOS_269

Microstructural characteristics of the sagitta otolith of the common eel, *Ophichthus remiger*, from northern Peru

Luis Usca[1]*
[1]Age and Growth Laboratory, Sea Institute of Peru. Lima, Peru. *lusca@imarpe.gob.pe

The microstructural characteristics of the sagittae otoliths of the common eel *Ophichthus remiger*, collected in northern Peru, the area with the highest landings of this resource, are presented. The following have been identified: a single primordium, a hatching mark, a first feeding mark and a first growth zone that corresponds to the leptocephalus larval stage that lasts between 20 and 60 days, a second very marked growth zone with several discontinuities that would correspond to the metamorphosis period, the radius fluctuates between 50 and 150 µm and a time between 80 and 100 days, a third zone that coincides with the formation of the first translucent zone that would be reaching between 180 and 200 days. The growth rate is different for the three zones, in the first zone an increase rate of ≈ 1 µm is observed, then the metamorphosis zone with a higher growth rate between 1.5 to 2.0 µm, and a third zone with a higher growth rate. slow 1 to 1.4 µm. Checks are incremental bands with homogeneous growth at a rate < 1 µm. These characterizations allow a better understanding of the ecological behavior of this species, mainly the metamorphosis phase and its duration as determining factors for the geographical distribution of this species and the recruitment processes.

Keywords: Metamorphosis, leptocephalus, daily increment, growth rate, checks.

RETURN TO INDEX

IOS_270

Characterization of the otolith microstructure and description of growth in juveniles of *Merluccius gayi peruanus*

Sandra Obando*[1]. Jorge Mostacero[1]
[1], [1]Age and Growth Laboratory, Sea Institute of Peru. Lima, Peru. *sobando@imarpe.gob.pe

Peruvian hake is the main demersal resource that supports Peru's bottom trawl fisheries. Given its intense exploitation in the past, proper resource management is necessary. Both ecological and age/growth information about this species are important for understanding population dynamics and improving fishery management. However, little is currently known about the life history and growth of Peruvian hake at early ages. It is known in different hake species that they have a pelagic life phase during their larval stage and the demersal life phase during their juvenile stage. A gradual settling period occurs between these phases, which is associated with their metamorphosis process. These life cycle changes are registered on the otolith in response to variations of environmental conditions, feeding, or genetic factors. To describe the life history of hake, the microstructure technique was applied to sagittae otoliths from 63 juvenile hake measuring 40-160 mm in total length, collected during the cruise for demersal resources assessment in the northern Peru in the years 2000, 2005 and 2011. The otolith microstructure was analyzed, considering the microincrements at each characteristic mark of the pelagic and settling phase. The data obtained in this study are compared with the currently described data in other species of the genus to achieve a better interpretation of the growth of this species in its early life stages. Juvenile individuals presented between 69 and 250 daily increments, with the pelagic phase lasting approximately 2 months and the metamorphosis phase lasting around 1 month. After 20 days of this latter phase, the formation of rings hialine is observed until reaching the first annulus. This study characterizes the microstructure of Peruvian hake otoliths and, based on this technique, estimates for the first time the duration of the early stages of its life.

Keywords: daily increments, metamorphosis, settlement, sagittae.

RETURN TO INDEX

IOS_273

Fitness metrics for migratory and resident Delta Smelt

Leticia Maria Cavole[1],*, Bruce G Hammock[2], Tomofumi Kurobe[2], Swee J. Teh[2], Malte Willmes[3], Christian Denney[1], James Hobbs[1], Brian Mahardja[4], Andrew Schultz[5], Levi S. Lewis[1]
[1]Department of Wildlife, Fish and Conservation Biology, University of California Davis, Davis, CA, USA; [2]Department of Anatomy, Physiology, and Cell Biology, University of California Davis, Davis, USA; [3]Norwegian Institute for Nature Research, Trondheim, Norway; [4]United States Bureau of Reclamation, Bay-Delta Office, Science Division, Sacramento, CA, USA; [5]U.S. Geological Survey, Southwest Biological Science Center, Grand Canyon Monitoring and Research Center; *Presenting Author: lmcavole@ucdavis.edu

Fishes have evolved diverse migratory life history strategies to persist within dynamic environments. Understanding how life history variation influences growth and reproductive fitness can be key to the effective conservation of imperiled species. The Delta Smelt (*Hypomesus transpacificus*) is an imperiled forage fish that is endemic to the San Francisco Estuary (SFE). This semi-anadromous fish exhibits a complex life history, with migrant and resident phenotypes that can be identified using otolith strontium isotope analysis. Here, we used generalized additive models (GAMs) to examine how overall and size-adjusted differences in body size (e.g., length and weight) and reproductive metrics (e.g., gonad weight, clutch size, and oocyte size) vary among the major Delta Smelt life history phenotypes. Overall, migratory (MIG) and brackish-water resident (BWR) fish exhibited greater body length, total weight, gonad weight, and clutch size relative to freshwater residents (FWR). Size-adjusted models, analogous to body condition, gonadosomatic index, and mass-specific clutch size, were also greater for fish with the MIG and BWR phenotype. Thus, fish with MIG and BWR life-history phenotypes appeared larger, healthier, and more fecund than FWR fish. Similar patterns have been observed in partially anadromous salmonids, where more diverse life history portfolios are believed to enhance population resilience and stability in dynamic and unpredictable environments. Given that the contemporary Delta Smelt population is dominated (~80%) by the MIG phenotype, it appears that the fitness benefits of migration currently outweigh those of either brackish or freshwater residency in the highly modified San Francisco Estuary.

Keywords: otoliths, strontium isotopes, phenotypes, reproduction, Delta Smelt

RETURN TO INDEX

IOS_274

Age analysis of hoki (*Macruronus magellanicus* Lönnberg, 1907) from the Chilean coast, using sagittae otoliths, with emphasis on the description of growth rings

Héctor Hidalgo Valdebenito[1]
[1]Sección de Edad y crecimiento, Instituto de Fomento Pesquero, Valparaíso, Chile
hector.hidalgo@ifop.cl

This paper presents the validation and determination of the age of hoki (Macruronus magellanicus) using whole sagittae otoliths of specimens captured on the Chilean coast (43°S-57°S) during the years 2012 to 2016. The pair of otoliths was submerged in a Petri dish with running water and observed with a stereoscopic microscope with 10X magnification and reflected light. The observations of these structures allowed us to identify the opaque and hyaline growth zones, including the one present at the edge of the otolith. The age was verified through the periodicity detection in the formation of the opaque or hyaline ring at the otoliths' edge. The monthly distribution of the type of ring present at the edge of the otolith showed a greater development of hyalines between April and September (with a peak of 70% in July-August), and opaque ring maximums between October and March (with a peak of 76% in December). Annuli types of the species were characterized up to specimens with 20 years of age. The average radius of the hyaline growth rings analyzed for five years were compared with those reported in Chile by Aguayo & Gili (1984), and in Argentina by Giussi (1996), finding that there are no significant differences, at least up to ring 10. This result allows us to confirm that the age estimation criterion of the hoki population compared to Chile has not changed in time, and it also shows a pattern of growth rings similar to that identified for the Atlantic population off the Argentine coast.

Keywords: Hoki, otoliths, growth rings, age, Chilean coast

RETURN TO INDEX

IOS_275

Corroborating otolith age using oxygen isotopes and comparing outcomes to scale age: Consequences for estimation of growth and reference points in northern pike (*Esox lucius*)

Timo Rittweg*[1,2], Clive Trueman[3], Elias Ehrlich[1], Michael Wiedenbeck[4], Robert Arlinghaus[1,2]
[1]Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Berlin, Germany.
*timo.rittweg@igb-berlin.de
[2]Division of Integrative Fisheries Management, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Invalidenstr. 42, 10115 Berlin, Berlin, Germany
[3]School of Ocean and Earth Science, University of Southampton Waterfront Campus, European Way, SO143ZH Southampton, UK
[4]German Research Center for Geosciences (GFZ) Potsdam, Telegrafenberg, 14473 Potsdam, Brandenburg, Germany

Accurate age estimates are crucial for assessing the life-histories of fish and providing management advice, but validation studies are rare for many species. We corroborated age estimates with annual cycles of oxygen isotopes (δ18O) in otoliths of 86 northern pike (*Esox lucius*) from the southern Baltic Sea, compared results with visual age estimates from scales and otoliths, and assessed bias introduced by different age-estimation structures on von Bertalanffy growth models and age-structured population models. Age estimates from otoliths were accurate, while age estimates from scales significantly underestimated the age of pike older than 6 years compared to the corroborated reference age. Asymptotic length (L_∞) was larger, and the growth coefficient *k* was lower for scale ages than for corroborated age and otolith age estimates. Consequentially, scale-informed population models overestimated maximum sustainable yield (*MSY*), biomass at *MSY* (B_{MSY}), relative frequency of trophy fish (≥ 100 cm), and optimal minimum length limit but underestimated fishing mortality at *MSY* (F_{MSY}). Using scale-based ages to inform management regulations for pike may therefore result in conservative management and lost yield. The overestimated asymptotic length may instill unrealistic expectations of trophy potential in recreational anglers targeting large pike, while the overestimation in MSY would cause unrealistic expectations of yield potential in commercial fishers.

Keywords: age estimation, secondary ion mass spectrometry, stable oxygen isotopes, yield, δ18O

RETURN TO INDEX

IOS_276

Fast shape changes prior to settlement for a temperate cryptobenthic fish: an approach using geometric morphometrics and otoliths

Frances Páez-Collao[1]; Yanara Figueroa-González[1], Guido Plaza[2], Hugo A. Benítez[3], Mauricio F. Landaeta* [1,4,5,*]
[1]Laboratorio de Ictiología e Interacciones Biofísicas (LABITI), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Chile
[2]Laboratorio de Esclerocronología, Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Chile
[3]Laboratorio de Ecología y Morfometría Evolutiva, Centro de Investigación de Estudios Avanzados del Maule, Universidad Católica del Maule, Chile
[4]Centro de Observación Marino para Estudios de Ambiente Costero (COSTA-R), Universidad de Valparaíso, Chile
[5]Millenium Nucleus for Ecology and Conservation of Temperate Mesophotic Reef Ecosystems (NUTME), Valparaíso, Chile,
*email: mauricio.landaeta@uv.cl

Important morphological and ecological modifications occur during the transition between pelagic and demersal phases in marine fish. However, still is unknown how fast these shape changes may occur. We studied the shape changes of a common cryptobenthic fish, the triplefin *Helcogrammoides chilensis* during the habitat shift from pelagic larvae to recently settled individuals, in rocky shores of central Chile during austral summer 2020 and 2021. Shape changes were analyzed utilizing landmark-based geometric morphometrics, while age was estimated using sagittal otolith microstructure analysis. There was an important overlap in the size (length and weight) between older larvae and recently settled individuals (between 20-25 mm *SL*, and 0.08-0.17 g), nonetheless, the head shape and paired fins were clearly different between stages. Pelagic larvae (46-88 days post-hatch) had a shorter pectoral fin base, frontal mouth opening, and eyes located at the level of the tip of the upper jaw, meanwhile, recently settled (80-112 days post-hatch) had wider, vertical positioned pectoral fins, mouth displaced to a vertical position, and eyes positioned upper and forward. Larvae experienced faster growth rates than settlers (0.24 *vs.* 0.02 mm d^{-1}, respectively), and the pattern of ontogenetic shape changes decreased two orders of magnitude after settlement. It is plausible that after settlement most of the fish's energy was derived to a rearrangement of the body structure, and the increment of the body pigmentation, as an adaptation as cryptobenthic juvenile to the new benthic environment.

Keywords: Otoliths, geometric morphometrics, *Helcogrammoides*, Chile, settlement

RETURN TO INDEX

IOS_278

First attempt to age the coldwater coral *Desmophyllum dianthus*

Rafaella Mella[1], Guido Plaza[1]; Juan Pablo Espinoza[2]; Darinka Pecarevic[2]
[1]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Chile; [2]Fundación San Ignacio de Huinay, Chile

Desmophylium dianthus is a cosmopolitan coral distributed widely in deep waters of all oceans but exceptionally occurs in shallow waters in fjords in southern Chile. Yat some life history traits such as age and growth patterns has not been addressed for this species. The current research aimed to determine the periodicity of formation of growth bands of the exoskeleton of the cold-water coral *Desmophyllum dianthus*, in the Comau fjord, in southern Chile (42°22' 10"S 72°27'18"O), through the relationship between the ring width chronology with interannual variations of the sea surface temperature (SST) and chlorophyll-a (Chl-a). Growth series were detrend to remove the effect of age by fitting spline curves. Both the crossdating and standardization processes were carried out using the statistical software dplR, available for the R statistical program. Once the series were standardized, they were averaged to obtain a master chronology of the study area and to compare it with the annual average time series of SST (TSM) and (Chl-a). The analysis of the simple linear model between the annual means showed that Chl-a explained 80% of the variability of the annual growth of the coral for a fraction of the series. On the other hand, the LMM effects demonstrated that the annual fluctuations of Chl-a had a significant effect on the variations in coral growth, becoming the first evidence that the macro-bands recorded at the base of the corallites would have an annual periodicity. Conversely, mean annual SST was not a significant factor for growth of D. dianthus in the Comau Fjord. The present study demonstrated the potential of the exoskeleton of the D. dianthus to record annual growth bands, which may be promising for future applications in sclerochemistry for this species.

Keywords: Exoskeleton, Sea surface temperature, Chlorophyll-a, linear mixed effects model, intercorrelation

RETURN TO INDEX

IOS_279

Interannual changes in the otolith shape of the anchoveta (*Engraulis ringens*), as related to changes in Chlorophyll a and SST fluctuations

Valentina Palta*[1], Guido Plaza[1]; Francisco Cerna[2]
[1]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Chile; [2]Instituto de Fomento Pesquero, Chile

In the current study, the influence of the sea surface temperature (SST) and the concentration of chlorophyll-a (Chl-a) on the morphometry of sagittal otoliths of anchoveta *Engraulis ringens* in northern Chile was evaluated and quantified using size and shape indices (area, circularity, rectangularity, roundness and ellipticity), along with contour analysis using normalized elliptical Fourier descriptors (NEFDs). A collection of sagittal otoliths of fish ranging between 10.5 and 15.5 cm in total length, collected between 18oS and 24oS, for the years 1973, 1982, 1995, 1996, 1998, 1999, 2004, 2005, 2008, 2010, 2011, 2013, 2014, 2016, 2017, 2020 and 2021. NEDFs were obtained using the SHAPE version 1.3 program and the ShapeR library. The SST and Chl-a were obtained from MODIS Aqua with a temporal resolution of 1 month and a spatial resolution of 4 km. The data was converted to a numerical format using the Panoply program. GAMS in which fish length was included as a covariate showed significant interannual variations in area, perimeter, and ellipticity. A PERMANOVA analysis based on 9 principal components of the original NEFDs matrix, demonstrated that the contour of the sagittal otoliths varied significantly among years. The variations of the average annual SST showed the warm and cold cycles, associated with the ENSO cycle, whereas the chl-a showed two large cycles of higher productivity, one between the years 2002-2003 and other one after the El Niño event 2015-2016. A general linear model showed that only Chlo-a had a significant effect on the interannual variability of otolith size and shape. This research is a contribution to the understanding the life cycle issues of anchovy, a key forage species with a great ecological and commercial importance in a productive ecosystem in the southern Pacific Ocean.

Keywords: Anchovy, otolith, size indices, Fourier elliptic descriptors, northern Chile

RETURN TO INDEX

IOS_280

Age and Growth of newly settled Tripterygiid with bipartite life cycle in Chile

Jose Ágreda-Arango[1]*, Mauricio F. Landaeta[2], Guido Plaza[3], Jorge E. Contreras[4]
[1]Magíster en Oceanografía, Escuela de Ciencias del Mar, Universidad de Valparaíso, Valparaíso, Chile
[2]Laboratorio de Ictiología e Interacciones Biofísicas (LABITI), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile
[3]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[4]Instituto de Fomento Pesquero (IFOP), Valparaíso, Chile

A proper development in the early life of fishes with bipartite life cycle is crucial for the habitat change, and it also has an impact in the species life history and population dynamics. Four cohorts of newly settled of *Helcogrammoides chilensis* were compared, to assess age, size, and growth. Newly settled were collected biweekly (n = 109) between March and December 2021 using hand net, then sacrificed with a benzocaine 20% overdose. Each individual was weighed and measured, and their otoliths were removed. Age, microincrement width and pelagic larval duration data were obtained. Hatching backcalculation allowed separating and grouping into four categories corresponding to the four seasons. Regarding the length-weight relationship, winter and spring cohorts showed negative allometry, while summer and autumn showed positive allometry. Age-length relationship showed that growth just after settlement was slow and similar among seasons, fluctuating between 0.017 and 0.069 mm d-1. Global PLD mean ± SD was 92 ± 19 days. Regarding the cohorts, spring PLD was between 65 and 92 days (79 ± 9 days), summer PLD was between 63 and 125 days (83 ± 14 days), autumn PLD was between 73 and 143 days (116 ± 16 days), and winter PLD was between 74 and 132 days (101 ± 15 days). In particular, autumn and winter cohorts showed significant differences in their PLD compared to the other seasons, and spring and summer cohorts did not show significant differences between themselves. Age range of spring cohort was between 80 and 107 days with mean 91 ± 8 days, summer cohort was between 79 and 154 days with mean 110 ± 20 days, autumn cohort was between 74 and 154 days with mean 128 ± 18 days, and winter cohort was between 95 and 138 days with mean 116 ±11 days. Finally, the early life conditions are not homogeneous throughout the year, suggesting an influence of the environmental variability typical of temperate regions.

Keywords: Otoliths, *Tripterygiidae, Helcogrammoides*, Chilean coast, Sagittae

RETURN TO INDEX

IOS_281

Contrasting growth chronologies of the threefin hake (*Micromesistius australis*) using linear mixed-effects models and a tree-ring based approach (dplR)

Claudeth Asencio-González[1]* & Guido Plaza[1], Francisco Cerna[3]
[1]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile; [2]Instituto de Fomento Pesquero, Chile

Recently linear mixed effect models (LMM) have become a powerful tool to elaborate growth chronologies in fishes, because this approach can deal with the effect of extrinsic and intrinsic factors affecting growth. As result standardized chronologies can be generated which can be used to relate them with potential environmental or climatic drivers. Alternatively, growth chronologies can also be elaborated using more classic tree-ring approaches used to create white noise series. In the current study the first growth chronology was elaborated for *Micromesistius australis* in the southeastern Pacific Ocean, using 601 sagittal otoliths collected during the years 1997 to 2019. Otoliths were digitized, and growth chronologies were constructed from them based on the measurement of widths of seasonal and annual increments. Chronologies covering a range of years from 1983-2019 were elaborated, using LMM and the tree-ring-based approach (dplR). For dplR, an autoregressive model (AR1) was applied to construct the chronology. Growth index derived from both approaches synchronized in capturing the upward and downward long-term trend when they were smoothed. However, LMM and dplR based-series did not match when they were contrasted in the high frequency mode (r=0.40; p>0.05).

Keywords: Chronologies, dplR, LMM, otoliths, Southeast Pacific Ocean.

RETURN TO INDEX

THEME IV

PAST AND RECENT AQUATIC ECOSYSTEM ECOLOGY AND CLIMATE CHANGE INDICATORS

RETURN TO INDEX

IOS_015

Highly necessary and obvious effectiveness of Fishing Ban on the Yangtze River demonstrated by a "Indicator" species *Coilia nasus*

Jian Yang*, Tao Jiang, Hongbo Liu, Xiubao Chen
Laboratory of Fishery Microchemistry, Freshwater Fisheries Research Center, Chinese Academy of Fishery, Wuxi 214081, Jiangsu Province, China. *jiany@ffrc.cn

The Yangtze (Changjiang) River is the third largest river in the world with a vast catchment and huge water system that provides numerous ecosystem services. In the past decades this river has suffered a severe loss of fishery resources especially by overfishing. Therefore, an unprecedented national policy of Fishing Ban on the Yangtze River had launched since 2019 in China for fisheries ecosystems restoration, and the effective evaluation of the policy has become an urgent demand in turn. As a long distance anadromous fish, estuarine tapertail anchovy *Coilia nasus* is qualified to be one of "Indicator", "Flagship" and "Umbrella" species in the Yangtze River. Based on discovery of a spawning site and natal homing habit for a certain stock of *C. nasus* in river-connected Poyang Lake (ca. 1000 km upstream of the Yangtze's mouth) through our previous otolith microchemistry studies, an evaluation was conducted for effect of the Fishing Ban by the data of catch per unit effort (CPUE) and ratios of anadromous to freshwater resident *C. nasus* (confirmed by otolith microchemical fingerprints corresponding to freshwater, brackish water and sea water) sampled at the spawning site in the Poyang Lake during the similar fishing season over an eight-year term before (2014-2018) and after (2019-2022) implementation of the policy. From 2014 to 2018, the CPUE was 0.27±0.37~3.16±3.89 [$\times 10^{-5}$, ind./(h·m)] or 0.38±0.52~4.42±5.45 ind./d, and the individual ratio was 0.08%~5.68% for anadromous *C. nasus*. In contrast, from 2019 to 2022, CPUE increased to 26.43±12.50~466.67±246.10 [$\times 10^{-5}$, ind./(h·m)] or 12.50±10.61~176.00±112.37 ind./d. The corresponding ratio increased to 31.66%~92.03%. These unique results demonstrate that a large amount of anadromous *C. nasus* can migrate back to the Poyang lake for reproduction due to benefits under the Fishing Ban (i.e., fish resource of the Yangtze River is obviously recovering), and provide strong evidence of highly necessary and obvious effectiveness of Fishing Ban on the Yangtze River.

Keywords: Otolith microchemistry, Estuarine tapertail anchovy, Yangtze ecosystem, Resource recovery, Indicator species, Fishery

RETURN TO INDEX

IOS_019

Fossil otolith δ18O and δ13C reveals the process leading to a local extirpation of demersal fishes in deep time

Konstantina Agiadi[1]*, Iuliana Vasiliev[2], Geanina Butiseacă[3], George Kontakiotis[4], Danae Thivaiou[4], Evangelia Besiou[4], Stergios Zarkogiannis[5], Efterpi Koskeridou[4], Assimina Antonarakou[4], Andreas Mulch[2]
[1]Department of Palaeontology, University of Vienna, Austria; [2]Senckenberg Research Biodiversity and Climate Research Centre, Frankfurt am Main, Germany; [3]Institute of Archaeological Sciences, Eberhard Karls Universität Tübingen Germany; [4]Department of Historical Geology and Paleontology, National and Kapodistrian University of Athens, Greece; [5]Department of Earth Sciences, University of Oxford, UK. *konstantina.agiadi@univie.ac.at

Revealing the processes leading to extinctions is one of the most important questions in Palaeontology, even though these are documented well in the fossil record. Otoliths are commonly preserved as fossils in marine and lake sediments, and their isotopic record faithfully records palaeoenvironmental and palaeobiological conditions. In order to understand how extinction at any spatial scale may take place, we investigate as an example the fish fauna of the Mediterranean in the Late Miocene. The restriction of the Mediterranean–Atlantic marine connection over the latest stage of the Miocene, the Messinian (7.25 to 5.33 million years before the present), which led to a salinity crisis and the deposition of the youngest salt giant on Earth, impacted the composition and structure of marine biota in unprecedented ways, leading to massive local extirpation of marine organisms from the basin and a major turnover event. By analyzing the stable oxygen and carbon isotopic composition of the otoliths of two carefully selected, common fish species, the pelagic extinct species *Bregmaceros albyi* and the benthic *Lesueurigobius friesii*, we were able to infer not only the sea surface and bottom salinity and oxygenation conditions, but also the fishes' metabolic response to the paleoceanographic changes in the Eastern Mediterranean from 7.2 to 6.5 million years. The high salinity and stratification of the Mediterranean water column during this interval hampered the ability of the marine fishes to grow, particularly those dwelling in the sea bottom. Initially, high temperature and salinity induced an increase in the metabolism of fishes across the water column. Subsequent warming increased salinity and stratification further intensifying the environmental stress for bottom-water fishes. To cope with these extreme conditions, demersal fishes increased their metabolism despite low food availability, which implies that fishes metabolized their own tissue to survive. The disappearance of demersal fishes in the study area after this point suggest that the above process, driven by environmental change, led to their local extirpation. Our study promotes the importance of otoliths stable isotopic analyses as tools for reconstructing complex paleoenvironmental histories.

Keywords: Biogeochemistry, stable isotopes, carbon, oxygen, Miocene, Mediterranean

RETURN TO INDEX

IOS_023

Acidification effect on fish otoliths shape: adaptive capacity to long-term effects

Kélig Mahé*[1], David Mazurais[2], Kirsteen M. MacKenzie[1], Théo Coutin[1], Olivier Mouchel[2], Arianna Servili[2], Bruno Ernande[3,4], José Luis Zambonino-Infante[2]
[1]Ifremer, Fisheries laboratory, Channel and North Sea Fisheries Research Unit, Boulogne-sur-mer, France; [2]Ifremer, Univ Brest, CNRS, IRD, LEMAR, F-29280 Plouzané, France; [3]MARBEC, Univ. Montpellier, IFREMER, CNRS, IRD, Montpellier, France; [4]Evolution and Ecology Program, International Institute for Applied Systems Analysis (IIASA), Laxenburg; *kelig.mahe@ifremer.fr

Climate change exposes ocean and coastal ecosystems to changing environmental conditions. Among climatic impact-drivers, ocean acidification (OA) modifies the biomineralisation of marine calcifiers and calcified structures such as otoliths, which are currently used in fisheries sciences as an efficient tool for stock discrimination. The goal of this study was to assess experimentally the influence of OA on European seabass (*Dicentrarchus labrax*) otolith morphogenesis during a very long period covering the larval to adult stage, contrary to previous studies that have generally focused on the larval phase. The individuals were reared from 0 to 2833 days post-hatching (dph) in sea water temperature following the natural fluctuations of the English Channel, with pCO_2 of 650 µatm (corresponding to pH 8.0, usually observed), and another pCO_2 of 1660 µatm (corresponding to pH 7.6, acidification level predicted for year 20100 in RCP8.5 global change scenario), with 2 replicates for each experimental condition. To follow the development of mean otolith shape during the experiment, the individuals per condition and replicate were sampled twice (at 318 dph and 2833 dph). Shape analysis was carried out on both sagittal otoliths of each sampled fish. Firstly, otolith morphometric measurements, shape factors and normalized elliptical Fourier coefficients describing outline shape were extracted using image analysis. The effects of OA on otolith shape variation with respect to time were then estimated using linear mixed effect models accounting for variation across replicates. For all shape analyses, the evolution of otolith descriptors changed significantly from 318 to 2833 dph. Ocean acidification had a significant effect on otolith shape for the elliptical Fourier descriptors and for two shape factors (form-factor and roundness). The interaction between OA and time, however, was not significant for the otolith shape. In conclusion, this study shows that otolith shape can be affected in the long-term by ocean acidification and that fish could show adaptive capacity to the long-term effects of this climatic impact-driver.

Keywords: Otolith Shape, CO_2, Long-term experiment, Elliptic Fourier Descriptors, *Dicentrarchus labrax*, Climate change

RETURN TO INDEX

IOS_029

Marked recent declines in boron in Baltic Sea cod otoliths – a bellwether of incipient acidification in a vast hypoxic system?

Karin E. Limburg*[1,2], Yvette Heimbrand[2], Karol Kuliński[3]
[1]State University of New York College of Environmental Science and Forestry, Syracuse, NY USA; [2]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden; [3]Institute of Oceanology of the Polish Academy of Sciences, Sopot, Poland. *klimburg@esf.edu

Abstract: Ocean acidification is spreading globally as a result of anthropogenic CO_2 emissions, but the Baltic Sea has until recently been thought to be relatively well-buffered by terrigenous inputs of alkalinity from its watershed. We discovered a 3- to 5-fold decline in boron (as B:Ca) in otoliths of eastern Baltic Sea cod (EBC) between the late 1990s and 2021. B:Ca is positively proportional to pH in carbonates, as B in the form of borate is taken up in the $CaCO_3$ matrix. Examining a time series of EBC otoliths, we found varying levels of B:Ca since the 1980s, with the most recent years at an all-time low during this period. This trend correlates with declines in pH and dissolved oxygen, but not with changes in salinity. We examined possible physiological influences on B:Ca by including a collection of healthy Icelandic cod as an out-group. Icelandic cod otoliths showed strongly positive correlations of B:Ca with physiologically regulated P:Ca; this was not the case for EBC. Finally, B:Ca in EBC otoliths is anti-correlated to some extent with Mn:Mg, a proposed proxy for hypoxia exposure. This negative relationship is hypothesized to reflect the dual phenomena of hypoxia and acidification as a result of decomposition of large algal blooms. Taken together, the otolith biomarkers Mn:Mg and B:Ca suggest a general increase in both hypoxia and acidification within the Baltic intermediate and deep waters in the last decade reflected in cod otoliths.

Keywords: Otolith chemistry, B:Ca, ocean acidification, hypoxia

RETURN TO INDEX

IOS_043

How does fish behavior influence exposure to environmental mercury or hypoxia?

Jacob Oster*[1], Jessica Myers[2], Jeremy Conkle[2], Benjamin Walther[1]
[1]Department of Life Sciences, Texas A&M University-Corpus Christi, Corpus Christi, USA; [2]Department of Physical and Environmental Sciences, Texas A&M University-Corpus Christi, USA; *joster@islander.tamucc.edu

Red Drum (*Sciaenops ocellatus*), Sourthern Flounder (*Paralichthys lethostigma*), and Atlantic Croaker (*Micropogonias undulatus*) are important parts of the saltwater recreational fishery in Texas and much effort has gone into the protection and management of these species. While these three species use the estuaries as nursery habitat and migrate offshore as adults, not much is known about the variation in the timing of this offshore movement or the subsequent behavior once offshore. In addition, stress from exposure to hypoxia and mercury may change their migration and feeding patterns. Therefore, our research is investigating variation in migration and feeding behaviors in Red Drum, Southern Flounder, and Atlantic Croaker captured near Matagorda Bay, Texas. Red Drum and Southern Flounder carcasses were collected from recreational anglers in Port O'Conner, Texas. Atlantic Croaker were collected from a bait shop in Palacios, Texas. Otoliths and muscle were taken from each fish. Otoliths were thin sectioned and analyzed for trace elements, including calcium, strontium barium, magnesium, phosphorus, and manganese. Muscle tissue from each fish was analyzed for total mercury concentration. Here we present preliminary insights into the life history of these three species. This data suggests that individuals with high mercury concentrations in muscle tend to have otolith trace element profiles with elevated strontium to barium ratios, indicative of higher residence time in estuarine habitat. More time spent in estuaries may coincide with exposure to hypoxia, as indicated by manganese to magnesium ratios in the otolith. In addition, fish with high manganese to calcium ratios tended to have lower phosphorus to calcium ratios, indicating fish exposed to hypoxia may have reduced feeding rates.

Keywords: Otoliths, Microchemistry, Migration, Texas, *Sciaenidae*.

RETURN TO INDEX

IOS_044

Reconstructing long-term growth of deep-water snappers throughout the Pacific using otolith chronologies

Joseph Widdrington[1]* Patrick Reis-Santos[1], John Morrongiello[2], Jed Macdonald[3], Simon Nicol[3], Bronwyn Gillanders[1]
[1]Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, SA, Australia; [2]School of BioSciences, University of Melbourne, Melbourne, Victoria, Australia; [3]Oceanic Fisheries Programme, The Pacific Community, Noumea, New Caledonia *joseph.widdrington@adelaide.edu.au

Tropical deep-water snappers underpin significant commercial, ecological and cultural importance throughout the Pacific Ocean. However, little is known about their life-history, and the lack of historical data limits our ability to predict the current and future effects of global change on population dynamics and productivity. This is of particular concern as environmental processes such as the El Nino Southern Oscillation (ENSO) are increasing in intensity and severity, and have widespread impacts throughout the Pacific region. Here, we aim to provide the first investigation into long-term growth trends of commercially important deep-water snappers across various Pacific Ocean economic exlusion zones (EEZ's). We used the information stored in fish otoliths to reconstruct long-term records of growth for three deep-water snapper species; *Etelis carbunculus*, *Etelis boweni* and *Etelis coruscans* (1960's-2010's). We developed a series of increasingly complex linear mixed-effects models to partition the variation in otolith growth among intrinsic and extrinsic factors such as ENSO, Pacific Decadal Oscillation (PDO), and boundary current strength. Results illustrate variations in growth among species and are discussed in the context of how large-scale and regional climate or environmental factors influence synchronous or nonsynchronous responses across the Pacific. Overall, understanding how deep-water snappers respond to environmental variability is central to developing sustainable fisheries and management strategies under climate change.

Keywords: Otoliths, Biochronologies, *Etelis*, Pacific, Climate change

RETURN TO INDEX

IOS_047

The effects of climate change on spatial-temporal distribution and early life history of three cryptic mullet species in Taiwan

Yu-Ling Nien*, Chia-Hui Wang
Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan.
*lucy71724@gmail.com

Mugil cephalus is a worldwide species, while previous studies have reported three cryptic mullet species in the northwestern Pacific (NWP1-3) and the juveniles coexisting in the estuaries of Taiwan. The habitat of the three cryptic mullets are partially overlapped, and their preferred temperatures are different. The distribution of NWP1 was found in temperate areas, NWP2 in temperate to tropical areas, and NWP3 in tropical areas. Several studies showed that the catch of mullet decreased and fishing grounds shifted due to sea surface temperature fluctuated, indicating that the population dynamics of mullet was under regulation of climate change. This study was then tried to analyze the effect of climate change on the species composition and parameters during early-life history of mullet. Mullet juveniles were collected from normal, El Niño and La Niña years, and the spatial-temporal variation of species composition and dynamic changes of mullet juveniles entering estuaries were investigated by species fast screening and otolith daily increment counting. The results found that the NWP2 was the most abundant species, and was the dominant species in several months and estuaries. The abundance of NWP1 were lower than NWP2, but its proportion was relatively higher in La Niña, and rare in El Niño. NWP3 had the lowest abundance than the other two species, while the spatial-temporal distribution expanded in El Niño. Based on otolith daily increment analysis, the back-calculated hatching month distribution showed variated among years. The results showed that NWP2 had a consistent hatching period among years, and its hatching months partially overlapped with those of other species. The hatching months of NWP1 were earlier during La Niña year compared to other years, while during El Niño, the peak of hatching month of NWP1 was delayed, and the hatching duration of NWP3 were extended. We speculated that the hatching duration variations were influenced by annual sea surface temperature, which further affect inter-annual species composition and recruiting dynamic in the estuaries. In our study, we found that the lower SST in La Niña was beneficial to NWP1 abundance, whereas the higher SST in El Niño was beneficial to NWP3.

Keywords: mullet, species composition, otolith, climate change

RETURN TO INDEX

IOS_050

Otolith morphometrics in a changing Arctic - Challenges and new insights in the population structure of Atlantic cod

Lisa Spotowitz[1]*, Torild Johansen[2], Agneta Hansen[2], Erik Berg[2], Christoph Stransky[3], Markus Brand[1], Philipp Fischer[1]
[1]Alfred Wegener Institute for Polar and Marine Research, Helgoland, Germany
[2]Institute for Marine Research, Tromsø, Norway
[3]Thünen Institute of Sea Fisheries, Bremerhaven, Germany
*lisa.spotowitz@gmail.com

The Arctic is facing increasing water temperatures, leading to a northward shift of Atlantic species into Arctic waters. Arctic marine ecosystems are therefore subject to substantial changes in species distribution and occurrence. Climate warming induced long- and short-term fluctuations in water temperature affect the distribution of invading boreal species locally within the Arctic environment. Svalbard, an archipelago located in the Arctic Ocean, is a region strongly affected by warming. Annual sea ice cover is in continuous decrease, whereas water temperatures are on the rise, which makes the local fjord systems more and more suitable for boreal species. Atlantic cod has been observed in Svalbard waters since the 1880s, their distribution in the past was strongly related to water temperature and thereby the effects of temporary warming events like the Early Arctic warming. The largest known stock of Atlantic cod is the migrating Northeast Arctic cod (NEAC) that is distributed along the Norwegian coast, the Barents Sea, and off Svalbard. By literature, Atlantic cod in Svalbard waters is generally known to belong to the NEAC ecotype. A stationary coastal cod (CC) spawns together with NEAC at the Lofoten and at several areas along the Norwegian coast. Our study provides evidence, that the climate-induced changes in Svalbard waters have led to the establishment of a local Svalbard coastal cod (SCC) population in Svalbard fjords. The population structure of Atlantic cod was investigated using otolith structure, single nucleotide polymorphic (SNP) markers and the locus Pantophysin (*Pan* I). Inner and outer otolith shape analysis was used to differentiate Atlantic cod ecotypes and indicates a discrepancy in distinct identification due to environmental adaptation. The combination of morphometric and genetic analyses revealed that in addition to both NEAC and CC, 0-group and adult CC differ significantly from those along the Norwegian coast, indicating a separation into a local Svalbard coastal cod population. This observation indicates ongoing borealization processed in a changing Arctic with severe consequences for the local Arctic community.

Keywords: Otolith shape analysis, SNP, *Gadus morhua*, Svalbard, Climate change

RETURN TO INDEX

IOS_051

Otoliths by the millions: A community resource

Katherine P. Maslenikov*[1]: Luke Tornabene[1]: Thomas Helser[2]
[1]University of Washington, School of Aquatic and Fishery Sciences and Burke Museum of Natural History and Culture, Seattle, WA, USA; [2]National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Seattle, WA, USA.
*pearsonk@uw.edu

An enormous collection of otoliths is available to researchers through the University of Washington Fish Collection. For decades the United States National Oceanic and Atmospheric Administration (NOAA) has collected otoliths across the northeastern Pacific Ocean and Bering Sea in support of their fisheries surveys. Similarly, NOAA Observers aboard commercial fishing vessels routinely collect otoliths as part of their biological sampling. Funding from the National Science Foundation in 2012 allowed this historic collection of otoliths to be transferred from NOAA to the University of Washington, with continuing collections transferred on a yearly basis. The collection includes 2.5 million pairs of otoliths dating back to 1976 and is comprised of 83 species in 41 genera and 17 families. Approximately 60% of the collection has been used for age determination studies by the NOAA Alaska Fisheries Science Center (AFSC) Age and Growth Laboratory. The collections grow by 50,000-70,000 pairs annually, thus space constraints necessitated additional funding from the Institute of Museum and Library Services (IMLS) in 2019 to install additional compactor shelving to allow for many years of growth. A collection of this size is a tremendous resource to researchers interested in otolith microchemistry. The sheer number of available samples allows for destructive sampling. Many of the Rockfish species (*Sebastes* spp.) in the collection are known to live over 100 years, and have been collected routinely for almost 50 years, allowing for ocean chemistry data to be recovered well back into the 1800's. Technological advances are allowing otoliths to be used in many exciting new ways. The collection supports ongoing research at the AFSC Age and Growth Laboratory. It is our hope that this collection will be of use to many and that we may foster greater access and collaboration.

Keywords: Otoliths, Microchemistry, Museum Collection, Sagittae

RETURN TO INDEX

IOS_064

Growth response of an estuarine fish to past and present environmental conditions

Koster G. Sarakinis[1]*, Patrick Reis-Santos[1], John Morrongiello[2], Qifeng Ye[3], Jason Earl[3], Bronwyn M. Gillanders[1]
[1]Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, SA 5005, Australia; [2]School of BioSciences, University of Melbourne, Melbourne, Victoria, Australia; [3]South Australian Research and Development Institute Aquatic Sciences, Adelaide, West Beach, South Australia 5024, Australia. *koster.sarakinis@adelaide.edu.au

Understanding how animal growth varies in response to changing environmental conditions at different spatial scales can help predict population responses to global climate change and support the management of wild populations. Here, we assessed the growth response of black bream, *Acanthopagrus butcheri*, to environmental conditions in estuaries across southern Australia. Specifically, we investigated the large and small-scale spatial variability in growth patterns over a multi-decadal period using otolith growth chronologies and increasingly complex linear mixed-effects models to partition the effects of intrinsic and extrinsic factors (e.g., age, freshwater inflow, sea surface temperature, El Niño-Southern Oscillation). By constructing long-term ecological datasets, we were able to identify major drivers of growth variation across the species distribution range and highlight patterns in growth variability among heterogeneous estuaries and climatic regions. Our understanding of growth variability can support the sustainable management of exploited black bream populations while maximising their resilience to fishing and climate pressures. Overall, our findings provide a foundation to understand the drivers of growth variations in complex estuarine environments and can be a proxy for assessing similar estuarine dependent species. As we move forward, we will investigate whether the growth patterns identified here can also be linked to partial migration in the species.

Keywords: Sagittae; Otoliths; Growth chronology; *Acanthopagrus butcheri*; Climate change

RETURN TO INDEX

IOS_069

Using otoliths to understand how marine heatwaves affect fish growth

Jessica Randall*[1]; Bryan Black[2]; Stephen Swearer[1]; Emily Fobert[1]; John Morrongiello[1]
[1]School of BioSciences, University of Melbourne, Melbourne, Victoria, Australia [2]College of Science, University of Arizona, Tucson, Arizona, USA *jrrand@student.unimelb.edu.au

The increased frequency and intensity of global marine heatwaves in the past century have led to greater awareness of how these extreme events can shape aquatic ecosystems. While we know that these acute warming events can cause a wide range of impacts, most commonly researchers focus solely on the lethal effects of extreme heat on marine life. Few studies have considered the sub-lethal impacts of heatwaves on marine fish such as growth, which can play a critical role in shaping ecosystem and fishery production. We aim to assess the impact of marine heatwaves on Southeast Australian fish and fisheries by examining the immediate and legacy effects of exposure to extreme heat on wild fish growth. Specifically, we 1) identified patterns in fish growth responses to heatwaves among species groups and life history stages and 2) if growth was impacted, examined how long growth was altered by heatwaves both during and after the event. We used satellite-derived daily sea surface temperatures from 1981-present to identify heatwaves and calculate a suite of physical parameters describing each event. Then, we related these heatwave attributes to individually resolved estimates of fish growth from otoliths. Effect sizes from all 15 taxa were then combined in a meta-regression to determine which heatwave event attributes best explained juvenile and adult growth variation. Overall, we found that many species experienced depressed growth during and in the year after a marine heatwave, with this effect most pronounced in juvenile life stages. In particular, the acute intensity of a heatwave, and the number of sequential heatwaves in a year were most related to growth depression. Our ability to anticipate the sensitivity of fish growth to heatwaves is not only important for predicting responses under future climate events but is also essential for understanding the legacy effects of these events which may alter food webs and assemblages well after the event subsides.

Keywords: Climate warming, Biochronologies, Sub-lethal impacts, Australia, Extreme events

RETURN TO INDEX

IOS_073

Sciaenidae paleoecology of the Castilletes Formation

Natasha Travenisk Hoff*[1], Michel Michaelovitch de Mahiques[2], June Ferraz Dias[3], Carlos Jaramillo[4], Orangel Aguilera[5]
[1]Programa de Pós-Doutorado, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil; [2]Departamento de Oceanografia Física, Química e Geológica, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil; [3]Departamento de Oceanografia Biológica, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil; [4]Smithsonian Tropical Research Institute, Panama; 5Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, Niterói, Brasil. *tashahoff@gmail.com

The Sciaenidae is an important fish group inhabiting temperate to tropical shallow coastal waters and estuaries worldwide. Eighty fossil species have been found in tropical America suggesting that the region was a significant center of sciaenid diversity for at least the last 20 million years. The Castilletes Formation (16.7-14.2 Ma) is located in the Guajira Peninsula (Colombia), cropping out along the eastern margin of the Cocinetas Basin. Paleontological studies have been carried out for organisms, including invertebrates, mammals, elasmobranchs, fish, reptiles, and vegetation. Here, we evaluated the Sciaenid paleoecology in the Castilletes Formation by analyzing 22 samples from 15 locations collected in the Castilletes Formation during several expeditions between 2010 and 2014. Samples were sieved and screen washed (mesh sizes: 0.5 and 2 mm) for subsequent picking of specimens. The fossil Sciaenidae assemblage consisted of 1361 otoliths from 22 taxa belonging to 12 genera (*Atractoscion, Cynoscion, Equetulus, Frizzellithus, Paranebris, Pareques, Plagioscion, Pogonias, Polycirrhus, Protolarimus, Protosciaena,* and *Umbrina*). Of 14 recorded species, two represented 74.5% of all otoliths: *E. davidandrewi* (759, 55.8%), registered in all samples, and *F. longecaudatus* (255, 18.7%). These and two more species (*E. fitch* and *P. henrici*) were extinct during the Langhian and Tortonian periods. Unidentified specimens of *Equetulus* could represent a new species. The Castilletes Neogene Sciaenidae assemblage provides new insights into the ecology of this important demersal family inhabiting the fluviodeltaic environment of the northwestern margin of South America before the uplift of the Panamanian isthmus.

Keywords: Otoliths, Paleoichthyology, Sciaenids, Colombia, Neogene.

RETURN TO INDEX

IOS_080

Using Stable Isotopes to investigate growth variability in juvenile Baltic Sea herring (*Clupea harengus* L.)

John Taylor*[1], Gustav Andersson[1], Andrius Garbaras[2], Agnes ML Karlson[1,3]
[1]Department of Ecology, Environment, and Plant Science, Stockholm University, Stockholm, Sweden; [2]Center for Physical Sciences and Technology, Vilnius, Lithuania; [3]Stockholm University Baltic Sea Centre, Stockholm, Sweden. *john.taylor@su.se

In fish, growth during the first year of life is directly related to survival, yet, this crucial life stage is often overlooked. This study aimed to investigate the variability in growth of young-of-year (YOY) herring (*Clupea harengus*) during summer in the rapidly changing Baltic Sea, where nitrogen-fixing cyanobacteria form extensive blooms and are expected to increase in duration and magnitude along with a warming climate. YOY herring were sampled monthly during July-September from two coastal areas on the eastern Swedish coast. Growth was back-calculated using otolith daily rings. White muscle tissue was analyzed for bulk $\delta^{13}C$ and $\delta^{15}N$ as well as compound specific analysis of $\delta^{15}N$ in amino acids with the aim to trace the distinctly depleted ^{15}N signal of cyanobacteria in fish in the end of summer, despite larger size of fish at that time. Fish sizes ranged from 28-78 mm and growth rates were higher in larger fish. Overall, the fish in September had lower bulk $\delta^{15}N$ values, indicating contribution of cyanobacterial nitrogen to fish growth, but no correlation with growth or condition on individual level was found within the time periods studied. Spatio-temporal fluctuations in temperature and salinity will be investigated to explain variation in both growth and isotope trends. The preliminary results of this study show that growth of YOY herring is inconstant in time and space and that using stable isotopes coupled with environmental data can explain some of the observed variation. These findings can aid in understanding the decreasing size and condition of Baltic herring stocks.

Keywords: Climate change, Young of year, Otoliths, Growth, Cyanobacteria

RETURN TO INDEX

IOS_086

Fish in warm water: growth of brown trout across thermal and hydrological gradients in Australia

Patricia Y. Koh*[1], Matthew West[1], Jarod, P. Lyon[2] John R. Morrongiello[1]
[1]School of Biosciences, University of Melbourne, Australia.
[2] Arthur Rylah Institute for Environmental Research, Heidelberg, Australia.

Freshwater fish are particularly vulnerable to the impacts of human-induced climate change due to the dendritic and fragmented nature of their environment. In Australia, weather conditions are projected to become more extreme and unpredictable with declining rainfall and warming temperatures altering the hydrological and thermal regimes of many south-eastern waterways. Freshwater fish with preferences for consistent streamflow and cooler water temperatures are especially at risk as changing environmental conditions reduce the amount of suitable habitat and shift their ranges to higher elevations. We examined how variable environmental conditions have affected the somatic growth of brown trout, *Salmo trutta*, a non-native coldwater fish species of high environmental, economic and social importance in south-eastern Australia. Otoliths were collected from brown trout across 14 wild populations encompassing a range of climatic and hydrological conditions. We used these otoliths to create growth biochronologies to model the growth dynamics of brown trout with seasonal temperature and seasonal river flow as key variables. We found that annual trout growth displayed distinct spatial and temporal variation which could be attributed to a mix of short- and longer-term hydrological and climatic variables. In particular, trout grew faster when there was a warmer and drier than average winter but grew slower when there was a warmer and wetter than average winter. Our findings indicate that the growth of brown trout is highly sensitive to environmental change, suggesting both reduced resistance and resilience of populations and fishery productivity to a predicted warmer and drier future.

Keywords: climate change, growth, freshwater, *Salmo trutta*

RETURN TO INDEX

IOS_092

Otoliths on acid: a meta-analysis of ocean acidification effects on otoliths

Patrick Reis-Santos*[1], Christopher Izzo[2], Bronwyn Gillanders[1]
[1]Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, SA 5005, Australia; [2]FRDC - Fisheries Research and Development Corporation, Kent Town, SA 5071, Australia. *patrick.santos@adelaide.edu.au

Ocean acidification is intrinsically linked to rising levels of atmospheric carbon dioxide (CO_2) with seawater pH decreasing as ocean water absorbs increasing CO_2. Levels of atmospheric CO_2 have surged since pre-industrial values, causing an average decrease of 0.10 pH units in global seawater. Depending on mitigation and reduction actions, atmospheric CO_2 values are forecast to continue to rise, leading to further reductions in seawater pH of 0.15 (RCP 4.5) or 0.30 units (RCP 8.5) by the year 2100. These changes pose challenges to marine organisms, though recent evaluations suggest negative or deleterious effects on marine calcifiers may be less than expected. In fish, there are conflicting results regarding the effects of acidification on otolith growth, size and chemistry. Because otoliths play a crucial role in balance, hearing and movement, any detrimental morphologic or development impacts in these calcified structures may have broad physiological or long-term health and survival implications, triggering population-level responses. Here, we used a systematic review approach to compile the literature related to ocean acidification and fish otoliths, and undertake a meta-analysis to identify global trends in the effects of ocean acidification on fish otoliths (and where available analogous internal hard structures such as statoliths). We used a mixed-effects approach that allowed for study level covariates (e.g., wild vs lab, salinity, temperature, exposure time, pH range), and this multidimensional approach enabled us to explore mechanistic and compensatory processes, and how impacts of extreme conditions differ to other acidification scenarios. Ultimately, disentangling the influence of acidification on otoliths will aid in evaluating the extent to which key traits or intrinsic factors may mediate long-term adaptation to global change.

Keywords: Otoliths, Climate change, Ocean acidification, Growth, Chemistry,

RETURN TO INDEX

IOS_096

Biobank, database and collection of samples in Natural Resources Institute Finland

Sami Vesala*[1], Anu Kantola[1], Oili Tarvainen[1]
[1]Natural Resources Institute Finland; *sami.vesala@luke.fi

Natural Resources Institute Finland (Luke) is a governmental institution that was founded in 2015 combining three former institutions, agricultural, forestry and game and fisheries research institutes. The former institutes had a massive archive of previously collected sample sets, which will be selectively centralized to specified mutual Biobank repositories of Luke during ongoing development project.

Previously the samples were managed locally by individual researchers or research teams by various processes and versatile manners. Now Luke is creating mutual guidelines, according to which we sort out scientifically important samples that may be reused after the initial use by researchers who collected the samples.

The fish sample sets consist a large variety of different calcified structures such as otoliths and scales and they form long-time series including several species, from a wide spatial range and variety of habitats. New methods such as stable isotope analyses or trace element studies provides a versatile opportunities to reuse these samples for further research from wide perspective.

The Luke biobank will consist of long-term or permanently stored samples, as well as a digital sample tracking solution and a metadata database, which enables organization-level lifecycle management of the samples. Registration of samples and monitoring of the sample flow from the collection point to analysis and further to sample storage will be implemented by QR -identification methods. The metadata of the samples accumulates in the database, for which the maintenance and user interfaces will be created. A documented and systematically managed sample set creates the basis for Biobanking.

Our goal is to have a valuable and versatile natural resource sample reserve of Natural Resources Institute Finland (Luke) preserved in a high-quality manner, and to form a Biobank that is nationally, and internationally interesting. The sample management approach will be implemented in the Biobank project (2022-25).

The sample set of the Biobank is continuously replenished when fresh samples are collected from nature and research environment.

Keywords: Archived samples, otoliths, Stable isotopes, trace elements

RETURN TO INDEX

IOS_099

Otolith biochronology for the long-term reconstruction of growth and stock dynamics of fish

Szymon Smoliński[1]*, Julita Gutkowska
[1]Department of Fisheries Resources, National Marine Fisheries Research Institute, Gdynia, Poland
*ssmolinski@mir.gdynia.pl

Biological time series covering long periods are essential to evaluate previous responses of organisms to the alterations in the environment and to develop proxies of past environmental conditions. In this project, we reconstructed the variation in the growth of European sprat (*Sprattus sprattus*) in the Baltic Sea over the last multiple decades based on the measurements in archived otoliths. The width of otolith annual increments was used as an approximation of fish somatic growth. We assessed the relationships between fish growth and environmental factors, considering ongoing hydrological changes and variations in the sprat stock size. We estimated the spawning stock biomass (SSB) prior to the period of available historical data (back to 1956) based on the obtained information on growth variation in the past. This estimation was based on the strong relationships between SSB and sprat growth, salinity, and temperature (R2=0.62), and the model was calibrated using data from the more recent past when official information on the SSB is available. This study provides new multidecadal data, giving insights into environmental factors affecting the growth of Baltic sprat. This research demonstrates the potential of otolith-based biochronology for the provisioning of independent indices of the historical fish stock size.

Keywords: otoliths, sclerochronology, Baltic Sea, temperature, climate, pelagic fish, growth, ecology, population dynamics, intraspecies competition, modeling

RETURN TO INDEX

IOS_ 105

Growth and feeding ecology studies on two cephalopod species off southwestern Taiwan waters

Chia-Hui Wang*[1,2], Chun-I Chiang[1], Pei-Ling Wang[3], Ming-An Lee[1,2]
[1]Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan; [2]Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan.; [3]Institute of Oceanography, National Taiwan University, Taipei, Taiwan; *chwang99@mail.ntou.edu.tw

Cephalopod species, *Euprymna berryi* and *Abralia multihamata* are two commonly fisheries species in southwestern Taiwan waters. The age and growth, reproductive biology and tropic ecology information is needed for further fisheries management. Present study collected specimens of the two species from trawling bycatch, measured the morphological and reproductive parameter, and analyzed the age and growth parameters using statolith daily increment. Stable isotopic ratio of $\delta^{13}C$ and $\delta^{15}N$ in muscle was also analyzed for trophic position calculation and its coordination in the food web in the ecosystem of Taiwan Bank. The results showed the age of *E. berryi* ranged between 60-112 days, and no significant difference of growth rate was found between sex (0.27 ± 0.04 mm/day). Daily age of *A. multihamata* ranged from 44-78 days and female (0.74 ± 0.10 mm/day) grew faster than male (0.52 ± 0.07 mm/day). The two species hatched all year round with no clear peak month. Trophic position of *E. berryi* was 3.61 ± 0.47 that calculated based on the mesoplankton at the same area as baseline ($\delta^{15}N$: 5.53 ± 0.96 ‰; $\delta^{13}C$: -21.83 ± 0.49 ‰), and the result of dietary breadth indicated the food sources includes various benthic species including crustaceans and small fishes. Present study provided cephalopod population dynamic information in southwestern Taiwan waters.

Keywords: Statolith, Daily Growth Increment, Stable Isotopes, Tropic Position, Population Dynamic

RETURN TO INDEX

IOS_127

Taxonomic and ecological shifts in Caribbean reef fish communities since the Holocene explored in otolith assemblages

Aaron O'Dea[1], Brigida De Gracia[1], Chien-Hsiang Lin[2], Jessica Lueders-Dumont*[1,3] jald@berkeley.edu
[1]Smithsonian Tropical Research Institute, Panamá; [2]Biodiversity Research Center, Academia Sinica, Taipei, Taiwan; [3]University of California, Berkeley, USA.

The Holocene fossil record of Caribbean coral reefs offers stark and compelling evidence that coral communities have degraded into the Anthropocene. In contrast we know much less about how coral reef fish communities have changed over the same time period. In this study, we describe the discovery of more than 5000 fossil reef fish otoliths in mid-Holocene (~7ka) and modern reefs in Bocas del Toro, Panama and the Dominican Republic and discuss their utility to reconstruct baseline conditions of reef fish communities. To identify these otoliths we built a reference collection of modern reef fish otoliths that is housed at Smithsonian Tropical Research Institute in Panama. Coupled surveys on reefs found otoliths in surface sediments reflected the abundances of living fishes on the same reefs, demonstrating that otolith assemblages retain ecological information with some interesting caveats. Both fossil and modern otolith assemblages were found to be dominated by small, cryptobenthic (e.g. Gobiidae and Apogonidae) and epipelagic (e.g. Engraulidae) fishes with high reproductive turnover. Analysis of the taxonomic composition of the otolith assemblages showed that modern reef fish communities are entirely distinct from their mid-Holocene counterparts, reflecting the patterns we see in the coral communities. However, when otoliths were assigned to functional guilds, modern and pre-human reefs were found to be indistinguishable. This result, if taken at face value, implies that while community membership has changed over time, functional roles have persisted into the Anthropocene. However, size-frequency analyses of the otolith assemblages and δ15N data from the otoliths suggest subtle but potentially significant changes in the trophic and functional structure of modern reefs that could have significant implications for the energetic roles of coral reef fishes today.

Keywords: Cryptobenthos, Isthmus of Panama, coral reefs, shifting baselines

RETURN TO INDEX

IOS_135

On the growth of goldband snapper (*Pristipomoides multidens*) in the Indo-Pacific

Júlio Guazzelli Gonzalez*[1] Clement Z. W. Ng[1], Muhammad Faiq Bin Mohd Saleh[1], Corey B. Wakefield[2], Stephen J. Newman[2], Ong J. L. Joyce[1]
[1]Asian School of the Environment, Nanyang Technological University, Singapore; [2]Western Australian Fisheries and Marine Research Laboratories, Department of Primary Industries and Regional Development, Government of Western Australia, Australia. *julio.gonzalez@ntu.edu.sg

Growth is a key process modulating the fitness of fish populations. Understanding the factors that drive fish growth is essential to ensure food security in regions under high exploitation rates. This is the case in Southeast Asia, one of the most exploited regions around the globe but with limited knowledge on the growth of species targeted by fisheries. In this study, we investigated the growth of a highly prized deepwater fish in Southeast Asia, the goldband snapper (*Pristipomoides multidens*), and compared it with other localities in the Indo-Pacific. To this end, otolith samples of *P. multidens* were gathered from specimens captured at different areas in Southeast Asia (Indonesia and Malaysia) between 2020 and 2023. These samples were complemented and compared with otolith collections of *P. multidens* specimens caught in Western Australia, at Pilbara (1998) and Gascoyne regions (2007-2020) respectively. All otolith samples (approximately 1400 samples) were aged, and the width of each growth band was recorded (increment in mm). This allowed us to create a chronological database of growth increment measurements for the species at varying latitudes and spanning from 1983 to 2022. Because the species is highly sought-after at younger ages, our analysis was focused on the early years of life (up to 6 years). A series of linear mixed models was thus applied to assess the effect of intrinsic (within-individual or population level) and extrinsic (environmental) factors on the growth of the species and identify key drivers. Despite the sedentary behavior of *P. multidens*, resulting in segregated stocks within the region, we expected that fish would respond similarly to extrinsic factors. However, unique features of each location may play a role modulating the intensity of this relationship. These results can support local management strategies to ensure sustainable exploitation rates of this fisheries resource in future harvesting and climate scenarios.

Keywords: Australia, Biochronology, Deepwater fish, Otolith, Southeast Asia.

RETURN TO INDEX

IOS_147

Exploring the relationship between hypoxia and mesopelagic fish growth using otolith increments: a case study from western Norwegian fjords

Francesco Saltalamacchia*[1,2], Martine Røysted Solås[1,2], Arild Folkvord[1,3], Anne Gro Vea Salvanes[1,2]
[1]Department of Biological Sciences, University of Bergen, Bergen (Norway), [2]Bjerknes Centre for Climate Research, Bergen (Norway), [3]Institute of Marine Research, Bergen (Norway), *francesco.saltalamacchia@uib.no

In the last decade, warming of the North Atlantic Ocean has contributed to reduced ventilation and subsequent oxygen depletion in the mesopelagic zone of Masfjorden, a fjord in western Norway. Semi-enclosed systems such as fjords are prone to rapid and intense oscillations in their physical parameters, and provide unique conditions to study the effects of climate change on fish populations. Using this natural infrastructure, we aimed to assess whether low-oxygen conditions affect the growth and recruitment of one of the key mesopelagic fish species in the North Atlantic, the glacier lanternfish *Benthosema glaciale*. Biological samples and environmental information were collected at fixed depth intervals over several years (before, during and after the hypoxic event) in Masfjorden and in nearby fjords. In this study, we compare growth patterns in each area and for multiple cohorts, and model otolith annual increments as a function of the key environmental drivers oxygen, temperature and food availability at the time of increment formation. We also evaluate recruitment based on cohort analysis. Our preliminary analyses show that the species is quite resilient to abrupt and prolonged changes in environmental conditions, and might have evolved mechanisms to cope with low-oxygen zones. Ocean warming and deoxygenation are pressing concerns in coastal areas worldwide, and the mesopelagic zone is one of the most understudied marine regions in the world. Since mesopelagic fish play a key role in marine ecosystems across the globe and are considered a potential food source for the future, it is crucial to assess their vulnerability to climate-induced changes.

Keywords: deoxygenation, fjord, growth, mesopelagic zone, otolith increments, recruitment

RETURN TO INDEX

IOS_149

Cod otoliths document accelerating climate impacts

Yvette Heimbrand*[1], Karin Limburg[1,2], Karin Hüssy[3], Tomas Naeraa[4], Monica Mion[1] & Michele Casini[1,5]
[1]Department of Aquatic Resources, Swedish University of Agricultural Science, Uppsala, Sweden; [2]SUNY College of Environmental Science and Forestry, Syracuse, New York, USA; [3]National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby, Denmark; [4]Department of Geology, Lund University, Lund, Sweden, [5]Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, Italy, *yvette.heimbrand@slu.se

Expanding global deoxygenation of marine habitats affects fish and other aquatic organisms. The anoxic and hypoxic areal extents in the Baltic Sea have increased over time, resulting in a loss of feeding and spawning grounds for the demersal Baltic cod (*Gadus morhua*). Fish otoliths can serve as chronological biomarkers as the uptake of trace elements reflects life time physiological and environmental changes. We used Baltic cod as "canaries in the coal mine" and their otoliths to track changes in hypoxia exposure, salinity, growth and metabolic status over time. Baltic cod otoliths from the normoxic Neolithic Stone Age period were used to create a baseline for modern Baltic cod otoliths from 1927-2019. Intensifying anoxia in the brackish Baltic Sea was correlated with increasing levels of otolith Mn:Mg. That ratio decreased during major saline inflows from the North Sea, which were proxied by otolith Sr:Ca. Mean annual otolith Mg:Ca, which correlates with growth and metabolism, increased with higher dissolved oxygen percent saturation. Both peaked in the 1990s, when highest growth rates were observed. In 2010s, coinciding with the most intense period of hypoxia in the Baltic Sea, a dramatic decline in growth was documented that clearly separates from all other decades. Long time series of otolith chemical data provide a historic perspective of physiological responses in fish linked to environmental changes, which can also help to forecast future climate impacts on fish.

Keywords: Hypoxia, Otolith microchemistry, Trace elements, *Gadus morhua*, LA-ICP-MS,

RETURN TO INDEX

IOS_153

Cold fish in hot water – growth and field metabolic rates of juvenile Atlantic cod exposed to peak summer temperatures

Peter Grønkjær*[1], Jonathan Christensen[1], Kris-Emil Mose Jørgensen[1], Freja Holm Dahl[1], Halvor Knutsen[2,3], Per Erik Jorde[2], Ming-Tsung Chung[4]
[1]Department of Biology, Aarhus University, Aarhus, Denmark; [2]Institute for Marine Research, Flødevigen, Norway; [3]Centre for Coastal Research, University of Agder, 4604 Kristiansand, Norway; [4]Institute of Oceanography, National Taiwan University, Taipei, Taiwan

Increasing ocean temperatures expose coastal nursery grounds to more frequent episodes of extreme temperatures. These events may increase maintenance metabolic costs of coastal fishes to an extent that reduces food intake, growth and, ultimately, survival. Here, we combine the analysis of otolith daily increment widths and stable carbon and oxygen isotopes to explore the effects of peak summer temperatures on the growth and field metabolic rate (FMR) of 106 age-0 Atlantic cod in the southern Norwegian archipelago. The fish were sampled using beach seine 3-4 weeks after having experienced maximum summer temperatures. FMR integrated over ca 2 weeks before capture was estimated based on $\delta^{13}C$ values of otolith aragonite obtained from the edge of the otolith. FMR displayed the expected pattern of an increase with increasing temperatures. However, FMR abruptly increased to near maximum metabolic rate in most fish experiencing temperatures close to their thermal limits (~15°C), indicating metabolic stress. The life-long growth trajectories of the cod showed 10 day averaged growth rates varying between 0.07 and 1.65 mm/day. The typical trajectory showed an increase in growth rates that closely followed the increasing ocean temperatures during spring and summer. However, 78% of the fish experienced a growth rate collapse, defined as a growth rate less than 25% of the maximum experienced growth rate of that fish, when exposed to temperatures higher than ca. 13 °C. Moreover, only 13% of these fish were able to recover, defined as an increase to 75% of maximum experienced growth rate, when temperature again decreased. Of the fish experiencing growth collapse, the majority were also fish displaying severe metabolic stress. Our results suggest that increasing ocean temperatures, including episodic marine heatwaves, have a large effect on the metabolic rates of coastal fish, which subsequently translates into reduced growth rates and, potentially, survival.

Keywords: Growth, Field metabolic rate (FMR), Atlantic Cod, Otolith.

RETURN TO INDEX

IOS_155

Using otolith biochronologies from sardine (*Sardinops sagax*) in the Benguela to identify ecosystem shifts

Faye Brinkman1, Margit Wilhelm[1,2], Yonela Geja[3] & Carl D. van der Lingen[3,4]
[1]Department of Fisheries and Aquatic Sciences, University of Namibia, Henties Bay, Namibia; [2]Sam Nujoma Marine and Coastal Resources Research Centre (SANUMARC), University of Namibia, Henties Bay, Namibia; [3]Branch: Fisheries Management, Department of Forestry, Fisheries and the Environment (DFFE), Cape Town, South Africa; [4]Department of Biological Sciences and Marine Research Institute, University of Cape Town, Cape Town, South Africa. fbrinkman@unam.na

The sardines *Sardinops sagax* has shown substantial changes in population size over the past 70 years in the northern and southern Benguela (Namibia and South Africa, respectively). A large population in the Benguela that initially supported high catches collapsed in the early 1960s, recovered in the 1990s, and then collapsed again. In this study, we developed two robust otolith biochronologies of annual increment width to infer long-term growth patterns of sardine using otoliths archived in South Africa from 1962 to 2019 and in Namibia from 1975 to 2021. Linear mixed effect models were applied to separate intrinsic (within individual) and extrinsic (by year – e.g. population density, environment) factors to estimate mean annual growth. Sequential t-test of regime shifts (STARS) analysis performed on the best linear unbiased predictor (BLUP) of the year effect on fish growth indicated four regimes with two alteration points in the southern Benguela. The first alteration occurred in 1986 from well-below average growth to well-above average growth, and the second in 2006 from well-above average growth to average growth the last alteration occurred in 2016 from average growth to below average growth. These three regimes correspond with periods of low, high, and low biomass, respectively; i.e. well-above average growth rates occurred during the high biomass period and vice versa. However, STARS analysis performed on the BLUP of the year effect in the northern Benguela demonstrated one regime with a constant average growth. Moreover, predicted annual growth significantly correlated positively with SST in winter through spring (ρ= 0.459, n=37, $p<0.01$) and zooplankton abundance (ρ=0.556, n=36, $p<0.01$) in the southern Benguela. Whereas, in the northern Benguela, predicted annual growth significantly correlated positively with upwelling index (ρ=0.345, n=40, $p<0.05$) and negatively with the SST in spring (ρ=-0.368, n=39, $p<0.05$). However, predicted annual growth did not significantly correlate with biomass in the Benguela. Therefore, growth is climate driven and together with overfishing resulted in a bottom-up trophic cascade. Furthermore, the study demonstrates the capacity for developing otolith increment methods to identify ecosystem shifts in the Benguela ecosystem.

Keywords: Regime shift, Sardine, *Sardinops sagax*, Time series, Ecosystem

RETURN TO INDEX

IOS_156

Trophic Level Comparisons of Archaeological and Modern Cod (*Gadus morhua*) in the Western Atlantic Ocean

Kenya Ripley-Dunlap*[1]; Jessica Lueders-Dumont[1]; India Ingemii[1]; Bess Ward[1]; Daniel Sigman[1]. kenyar@princeton.edu
[1]Princeton University

Understanding diet changes in a popular fishery species like cod (*Gadus morhua*) is increasingly important to determine the effects of anthropogenic change on marine food webs. To characterize differences in the populations' diet, nitrogen isotope composition (δ15N) in archaeological (17th century) and modern cod otoliths was compared. First, all otoliths were cleaned to remove unbound organic nitrogen and contaminants. δ15N was then determined through oxidation and subsequent bacterial conversion to nitrous oxide that was quantified by gas-chromatography isotope ratio mass spectrometry. Micromilled modern and archaeological samples are from the Gulf of Maine, in the Western Atlantic. The precision of this technique allowed us to measure otolith pieces less than half a milligram. This means that we were able to measure δ15N content of juvenile and adult stages of the fish's life. Archaeological cod otoliths were from the island of Smuttynose and modern cod otoliths were from modern fisheries-independent surveys in the Massachusetts waters of the Gulf of Maine. Because fisheries are expected to have preferentially removed larger fish, archaeological cod are expected to be larger than modern cod. Therefore, due to connections between trophic level and size resulting from gape limitation of small individuals, the isotopic composition of nitrogen in otoliths from archaeological cod should also be greater than modern cod. While there was a small difference in the mean δ15N of the two populations, the results were not statistically significant when controlling for fish size. Our micromilled samples from the otolith core suggest no change in isotopic baseline over this time period, consistent with previously published records. This points to the conclusion that archaeological and modern cod take up an equivalent role in the food web between the 17th century and today. This was a surprising result, and may bode well for recovery if collapsed populations are allowed to rebound. There are other factors at play.. Older and larger cod are likely to eat organisms higher up in the food chain; therefore, the relatively fewer large and older cod today suggests that changes in size structure alone may have had significant impacts on the structure and functioning of marine food webs in the Gulf of Maine. The equivalent trophic level of modern and archaeological cod, when controlling for fish size, may suggest that declines in fish size, and not changes in trophic level-at-size, may be the greatest anthropogenic impact on cod diet in the Gulf of Maine.

Keywords: Otolith, Nitrogen isotopes, Cod fisheries

RETURN TO INDEX

IOS_172

Growth rate extremes of a Sciaenid in an ocean-warming hotspot

Charmaine E Jagger[1,2], Margit R Wilhelm*[2], Warren M Potts[3]
[1]Ministry of Fisheries and Marine Resources, National Marine Information and Research Centre, Swakopmund, Namibia; [2]University of Namibia, School of Agriculture and Fisheries Sciences, Department of Fisheries and Ocean Sciences, Henties Bay, Namibia; [3]Rhodes University, Department of Ichthyology and Fisheries Science, Grahamstown, South Africa.
*mwilhelm@unam.na

In this study we developed a 25-year otolith biochronology for *Argyrosumus inodorus*, a cool water Sciaenid in the northern Benguela off Namibia, making up the highest catches in the recreational shore-angling fishery. The linear mixed-effects models fitted to the *A. inodorus* otolith biochronology indicated that the best linear unbiased predictor of growth was significantly positively correlated ($r = 0.580$, $n = 25$, $p < 0.05$) with mean Sea Surface temperatures (SSTs) in July of the year of formation and significantly negatively correlated ($r = -0.473$, $n = 25$, $p < 0.05$) with mean summer SSTs (October to November y-1) in the area 20-24◦S, 12-14◦E. Thus, faster annual growth was observed during warmer winters (up to 15℃) and slower growth was noted when exposed to warmer summer temperatures above 17℃. These findings provide evidence for the narrow temperature tolerance of *A. inodorus*, a loser in this rapidly warming environment. We would therefore expected future narrowed distribution ranges and consequently temporary higher catch rates and recruitment failures.

Keywords: Otoliths, ocean warming, tolerance range, *Argyrosomus*, Benguela, biochronology, Sciaenidae

RETURN TO INDEX

IOS_176

Effects of lifetime hypoxia exposure on fish mercury uptake and food web structure

Hadis Miraly[1], N. Roxanna Razavi[1], Matthew Altenritter[2], Elizabeth Duskey[1,3], Richard T. Kraus[4], Ann Marie Gorman[5], and Karin E. Limburg*[1,3]
[1]State University of New York College of Environmental Science and Forestry, Syracuse, NY USA; [2]State University of New York College at Brockport, NY USA; [3]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden; [4]US Geological Survey, Lake Erie Biological Station, Huron, Ohio USA; [5]Ohio Department of Natural Resources, Fairport Harbor, Ohio USA. *hmiraly@esf.edu

Climate warming is producing large impacts on aquatic ecosystems. Among other effects, warming is one of the main drivers of oxygen loss in aquatic ecosystems (hypoxia; operationally defined as < 2 mg O_2/ L). Hypoxia can alter habitat for fish and thereby affect trophic interactions. Additionally, anoxia favors microbial methylation of mercury (MeHg), and that compound can persist in hypoxia, resulting in potential for increased Hg transfer up the food web. Using mass spectrometry, we analyzed fish ear stones (otoliths), eye lenses and muscle tissues to assess the long-term effects of hypoxia exposure on food web relationships by tracking changes in fish stable isotopes and Hg contents through time in Lake Erie. Lake Erie is a one of the Great Lakes that experiences extreme seasonal hypoxia, the Central Basin of Lake Erie is the most hypoxic zone of the lake. Lake Erie Walleye (*Sander vitreus*, n = 30), Yellow Perch (*Perca flavescens*, n = 40), and Round Goby (*Neogobius melanostomus*, n = 40) were collected to cover an ecological gradient from most heavily dependent on localized, benthic habitat (Round Goby) to least (Walleye). Mn:Ca ratios in Yellow Perch and Round Goby otoliths were higher in the hypoxic Central Basin compared to the more normoxic Western Basin. Sulfur stable isotopic ratios ($\delta^{34}S$) can also serve as an indicator of hypoxia due to a large negative fractionation from sulfate reduction. We measured $\delta^{34}S$ in eye lenses and found significantly lower $\delta^{34}S$ in Central Basin Yellow Perch and Round Goby; in agreement with our otolith chemical proxy. Also, we found that fish with hypoxia exposure history showed significantly higher eye lens [Hg]; this was not detectable by muscle tissue [Hg]. Overall, our results showed that eye lenses, made of pure protein, can complement the chemical data from otoliths and can be used to study fish Hg, hypoxia, and trophic history. This novel study demonstrates the strength of a combining complementary analyses of different chronometric body parts to explore the influence of hypoxia exposure on food web relationships and Hg accumulation patterns.

Keywords: Stable isotopes, Eye lenses, Otoliths, Lake Erie, Hypoxia

RETURN TO INDEX

IOS_188

Morphotypes of fish otoliths presented in marine sediments of the continental slope of the São Paulo state (Brazil)

Natasha Travenisk Hoff*[1], Beatriz de Castro Siqueira[2], June Ferraz Dias[3], Orangel Aguilera[4], Marcia Caruso Bícego[5], Michel Michaelovitch de Mahiques[5]
[1]Programa de Pós-Doutorado, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil; [2]Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil; [3]Departamento de Oceanografia Biológica, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil; [4]Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, Niterói, Brasil; [5]Departamento de Oceanografia Física, Química e Geológica, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil. *tashahoff@gmail.com

Brazilian paleoichthyological and bioarchaeological studies are restricted to demersal species and coastal zones, such as the Miocene Pirabas Formation and Quaternary shell mounds. Brazilian mesopelagic fishes, such as Myctophids, are totally unknown from Miocene to the Quaternary. Here, we evaluated the occurrence of otoliths' morphotypes in 37 samples from a marine sediment core of the continental slope off the São Paulo state. Samples were sieved (mesh size: 0.350 mm) for subsequent picking of specimens in a stereomicroscope. Each otolith was photographed, characterized through morphometrics measures, and visually classified into the morphotypes, which were described according to shape indexes (circularity - CI; ellipticity - EL; form factor - FF; rectangularity - RE; roundness - RO; aspect ratio - AR). Significant differences among morphotypes were tested using uni- and multivariate analyses. From the 294 otoliths found, 189 were classified among six morphotypes: A (oval shape, developed rostrum and antirostrum, higher values of otolith length, CI, and RE); B (oval shape, anterior region double-peaked, underdeveloped antirostrum); C (discoidal or hexagonal shape); D (discoidal or hexagonal shape, lower EL and AR, higher RO); E (oval shape, developed rostrum, underdeveloped antirostrum, serrated ventral margin, lower FF and RO values, higher EL and AR values); F (oval shape; underdeveloped rostrum and antirostrum, higher FF values; lower values of otolith length, CI, and RE). Significant differences between C, D, and E from other morphotypes were detected for RO, EL, and AR. Morphotypes B and C were the most abundant (29.9 and 24.6%, respectively). Most otoliths belong to the Myctophidae family, the main mesopelagic fish group, playing an important role in organic carbon exportation from the surface to deeper zones. They present worldwide distribution, great abundance, biomass, and diversity. Consequently, their otoliths predominate in marine sediments collected at depths greater than 200 m, which justifies the greater abundance of specimens we found. Once Myctophidae fossil otoliths are of difficult identification and present a large lack of knowledge worldwide, shape morphometry may be a good possibility for this kind of analysis and comparison with recent otoliths.

Keywords: Paleoichthyology, Myctophidae, Otolith morphometry, Morphotypes, Marine sediments.

RETURN TO INDEX

IOS_190

Otolith collections: a new challenge to Southeastern Brazil

June Ferraz Dias[1], Monica Angélica Varella Petti[2], Renata Ramos Gomes[3], Raphael Machado Martinelli[4], Natasha Travenisk Hoff[5], Carolina Siliprandi[6], Jaermeson Silva Santos Júnior[4]
[1]Departamento de Oceanografia Biológica, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brasil; [2]Coleção Biológica Edmundo Ferraz Nonato (ColBIO), Depto de Oceanografia Biológica, IOUSP, São Paulo, Brasil; [3]Programa de Pós-Graduação em Oceanografia, IOUSP, São Paulo, Brasil; [4]Bacharelado em Oceanografia, IOUSP, São Paulo, Brasil; [5]Programa de Pós-Doutorado, IOUSP, São Paulo, Brasil; [6]Coleção de Otólitos de Peixes Teleósteos da Região Sudeste-Sul do Brasil (COSS-Brasil)

The organization of biological collections is essential for its preservation and services as lending and identification of materials, in addition of training human resources and disseminating scientific knowledge. In this sense, the Edmundo Ferraz Nonato's Biological Collection of the Oceanographic Institute -USP (ColBIO) is a reference for storing samples of plankton, ichthyoplankton and organism's tissues, as well as georeferenced oceanographic data associated with these samples. Biological collections dedicated to otoliths are few and Brazil is home to COSS Brazil (Collection of Teleosts Fish Otoliths from the Southeastern-Southern Brazil). COSS stores a total of 51923 otoliths corresponding to 201 species from Southwest-South Atlantic, and offers an online catalogue of otoliths, also sheltered by IOUSP. Due to their intrinsic characteristics of preserving their structure and composition, otoliths have been used in studies of several areas of knowledge, including recent approaches (current species) and past approaches (shell midden or sediments otoliths). This work presents a new set of otoliths, from fish captured in the project "Ecology of the nektonic fauna of the Brazilian continental shelf in the region between Cabo de São Tomé (22º04' S) and Torres (29º21'S)" - FAUNEC, carried out along 1975, as well as the data referring to each specimen. This project represents a milestone in studies on marine fish biodiversity in Brazil. The number otoliths range from 1700 to 1 pair per species, from 110 species, with more than 14000 otoliths. Now, the information contained in the onboard files and biological files is being typed and the otoliths checked, cleaned, and organized. It is intended that this collection can be integrated into COSS Brazil. The otoliths from the FAUNEC project are kept in the Prof. Carmen Rossi-Wongtschowski space, named in honor of the creator of COSS-Brazil. Its educational, cultural, and scientific potential involves the possibility of applying information on environmental conditions from the recent past on the Southeastern coast of Brazil.

Keywords: Otoliths, FAUNEC project, Southwest Atlantic

RETURN TO INDEX

IOS_201

Nitrogen isotopes from modern and fossil Myctophidae otoliths track changes in nutrients fueling Caribbean food webs since the Miocene

Jessica A. Lueders-Dumont*[1],[2],[3], Aaron O'Dea[2], Brigida de Gracia[2], Daniel M. Sigman[3], Seth Finnegan[1]
[1]Department of Integrative Biology, UC Berkeley, USA; [2]Smithsonian Tropical Research Institute, Ancon, Gamboa, Panama;
[3]Department of Geosciences, Princeton University, Princeton, NJ, USA. *jald@berkeley.edu

Characterizing the source of nutrients fueling primary productivity and food webs is a major topic of study in the modern ocean and can be pursued by comparing isotopic measurements of dissolved nutrients in seawater, particulate organic matter, and consumer tissues. On paleo time scales, the nitrogen isotopic composition (d15N) of organics bound in planktonic foraminifera tests from open ocean sediment cores has been used to identify changes in large-scale oceanographic circulation and nutrient drawdown during the Holocene (10,000 y) and across the Cenozoic (70 Ma). The fossil-bound d15N approach has not yet been applied to investigate nutrient patterns supplying food webs on continental shelves, which are among the most productive regions of the global ocean. By measuring the d15N of fossil and modern otoliths (d15Noto) from a cosmopolitan group of mesopelagic fishes (Myctophidae) across multiple spatial and temporal scales, we find that d15Noto can be used as a tracer of the nutrients fueling productivity on continental shelves. Using otoliths from modern marine surface sediments on both sides of the Panama Isthmus, we find clear d15Noto differences that match the known d15N difference between the Caribbean and Eastern Pacific today. With a fossil study focused on a 'natural experiment', the uplift of the Panama Isthmus and the resulting closure of the Central American Seaway (CAS), we find that myctophid d15Noto records the decline of Pacific-sourced nutrients in the Caribbean basin from the late Miocene to the Plio-Pleistocene. Our study shows that myctophid d15Noto can serve as a tracer for the pelagic (open ocean) isotopic baseline. We previously found that d15Noto of predatory fishes records trophic level, given information about the ecosystem's baseline d15N. Myctophid d15Noto will complement d15N measurements of other taxa by providing isotopic baseline information, increasing our ability to study nutrient supply and food webs in prehistoric ecosystems.

Keywords: Fossils, Lanternfish, Pacific Ocean, Atlantic Ocean, Productivity

RETURN TO INDEX

IOS_206

Archaeological otolith analysis: a tool for understanding past and present fishing practices in the Yucatan Peninsula

Ariana Solis Gómez*[1], Maribel Badillo Alemán[2], Alfredo Gallardo Torres[2], Nayeli Jiménez Cano[3], Nidiyare Hevia-Montiel[4], Jorge Pérez González[4], Emiliano Vásquez Morales[5], José Antonio Martínez Pérez[5], Xavier Chiappa-Carrara[2]
[1]Posgrado en Ciencias del Mar y Limnología, UNAM-Campus Yucatán, Mérida, Mérida Yucatán, México; [2]Laboratorio de Biología de la Conservación; Facultad de Ciencias, UNAM-Parque Científico Tecnológico de Yucatán, Mérida, Yucatán, México; [3]UMR 7209 Archéozoologie - Archéobotanique. Sociétés, pratiques et environnements, Muséum National d'Histoire Naturelle, París, Francia; [4]Unidad Académica del Instituto de Investigaciones en Matemáticas Aplicadas y en Sistemas en el Estado de Yucatán, UNAM-Parque Científico Tecnológico de Yucatán, Mérida, Yucatán, México; [5]Laboratorio de Zoología. Facultad de Estudios Superiores Iztacala, UNAM, Tlalnepantla, Estado de México, México. *arianagmz@comunidad.unam.mx

Fish remains recovered from several archaeological sites show that fishing has been a key subsistence activity for the inhabitants of the Yucatan Peninsula region since ancient times. However, this practice has affected natural populations by overexploiting and depletion, causing decreases in fish size and changes in species composition and distribution. Fish remains from the archaeological record offer the possibility to analyze these changes over time in order to understand human interaction with fishery resources and their implications for current fishing practices. Amongst others, otoliths are structures that can be recovered from the archaeological record and are very useful considering their species-specific characteristics, making them ideal for studies of various kinds. Analysis of otoliths provides information for understanding the fishing practices and can help in the implementation of sustainable fishing strategies. The objective of this work was to determine morphological and morphometric characteristics of fish otoliths suitable for a correct identification of species and to obtain allometric parameters to relate otolith and fish size, allowing to estimate the size of specimens found in the archaeological record. Three types of analysis were used: traditional morphometry, geometric morphometry, and invariant morphometric descriptors using otoliths from contemporary specimens and otoliths from prehispanic settlements in the Mayan area. These analytical approaches allowed correct taxonomic identifications; the use of each of these methods will depend on the type of data available and the objectives of the research. The otoliths from the archaeological record belong to species from three fish families: Sciaenidae (*Micropogonias undulatus*, *Micropogonias furnieri*, *Cynoscion nebulosus*, and *Bairdiella ronchus*), Haemulidae (*Haemulon plumierii*), and Ariidae. Minimum, maximum, and average size of otoliths from the archeological record were larger than those belonging to present-day specimens, especially for species of the Scianidae. Estimated fish sizes reveal that fish caught by prehispanic fishermen correspond to organisms that reached or approached the sexual maturity size. These data provide an overview of fishing practices and their impact on the composition and size structure of fish populations along the coast of the Yucatan Peninsula.

Keywords: Otoliths, archaeological record, Scianidae, Ariidae, Haemulidae

RETURN TO INDEX

IOS_209

Otolith oxygen isotopes unravel mysteries of Eastern School Whiting (*Sillago flindersi*) and Stout Whiting (*Sillago robusta*) along the east coast of Australia

Melissa Tan*[1], Karina Hall[2], Brendan Kelaher[1]
[1]National Marine Science Centre, Southern Cross University, Coffs Harbour, NSW Australia;
[2]NSW Department of Primary Industries (Fisheries), National Marine Science Centre, Coffs Harbour, NSW Australia.
*m.tan.19@student.scu.edu.au

Ocean warming is a growing concern for marine species and ecosystems, as well as the sustainability of fisheries. Trawl whiting, including eastern school whiting (*Sillago flindersi*) and stout whiting (*Sillago robusta*), are commercially important along the east coast of Australia, with an estimated yearly catch of approximately 3000 tonnes. However, critical information about the early life history and seasonal movements of these fish remains largely unknown, which can have significant implications for effective stock management. This study employs otolith oxygen isotope ratios ($\delta^{18}O_{otolith}$) as a proxy for thermal preferences, a useful tool for assessing fish movement and habitat use. We developed a species-specific fractionation model for Sillaginidae based on a controlled-temperature experiment; and applied the model to $\delta^{18}O_{otolith}$ analyses of wild-caught *S. flindersi* and *S. robusta* collected across their full distribution range. For wild-caught fish, core and edge portions of thin-sectioned otoliths were sampled using a micromill, to obtain approximately 60 to 80 µg of calcium carbonate powder. Powdered samples were analysed for $\delta^{18}O_{otolith}$ with a Kiel IV carbonate device coupled with a ThermoMAT253 isotope ratio mass spectrometer (IRMS). The $\delta^{18}O_{otolith}$ values derived from the core samples represent the water temperature experienced during the fish's juvenile stage, while the values from the edge samples reflect the water temperature averaged over 1-2 years prior to the time of capture as an adult. Notably, we observed a latitudinal trend in $\delta^{18}O_{otolith}$ values obtained from the otolith edge, with values differing substantially across fish from different locations, yet exhibiting a smaller spread of values within a location. In contrast, core values were relatively similar for fish across all locations, yet demonstrated a larger spread of values within a location. These findings have important implications for the use of $\delta^{18}O_{otolith}$ values as a proxy for water temperature and the interpretation of such data in the context of thermal preferences and spatial distributions under projected climate change.

Keywords: Otoliths, oxygen isotopes, Sillaginidae, fisheries, climate change.

RETURN TO INDEX

IOS_210

Sixty-year biochronology for Golden Redfish *Sebastes norvegicus* based on otolith growth-increment widths in the Northeast Arctic

Christine Lucas*[1], Bryan Black[2], Hector Andrade Rodriguez[3],[4]
[1]Fluvial Ecology Laboratory, Department of Biological Sciences, CENUR Litoral Norte, Universidad de la República, Paysandú, Uruguay; [2]Laboratory of Tree Ring Research, University of Arizona, Tucson, Arizona, USA; [3]Insitute of Marine Research, Tromsø, Norway, [4]Akvaplan-niva AS, Tromsø, Norway
*clucas@litoralnorte.udelar.edu.uy

Aquatic biochronologies contribute valuable information for understanding growth-climate relationships in marine ecosystems. The golden redfish *S. norvegicus* is a long-lived, late-maturing species of commercial interest. The northeast Atlantic stock is currently severely depleted despite efforts to limit direct and indirect fishing mortality. Nevertheless, there are some positive recovery signs from the immature portion of the stock as biomass has increased since 2011. Whether such increase can be attributed to favorable environmental conditions or to effective fishing regulations protecting the species remains to be studied. We constructed a sixty-two-year biochronology for the golden redfish, based on annual growth-increment widths of sagittal otoliths of 41 individuals (27-47 y old) from Arctic Norway. Preparation was critical to resolving growth-increment boundaries and involved thin-sectioning individuals to >0.5 mm thickness, polishing with successively finer lapping film followed by 3-micron diamond paste, and then viewing with transmitted light. Annual growth increments were crossdated and at least two transects were measured per otolith, excluding the first 6-18 years of juvenile growth. The master chronology extended from 1955-2017, was dominated by interannual variability, and had an inter-series correlation of 0.43. Low growth in the years 1966, 1979, 1987 and 1995 plus exceptional growth years in 1960, 1983, 1990, 1993 and 1998 aided in crossdating and underscored population-wide patterns of growth synchrony. The redfish chronology positively correlated with Norwegian Sea surface temperatures between Jan and Apr, suggesting that warmer waters from the late winter through early spring favored more rapid somatic growth. This research demonstrates the potential for biochronology development in redfish in the eastern Atlantic Ocean, complementing previous research on rockfish (Sebastes spp.) chronology development in the Pacific Ocean.

Keywords: Biochronologies, Crossdating, Otoliths, Norwegian Sea, Rockfish, *Sebastes*

RETURN TO INDEX

IOS_265

Local and global environmental drivers of growth chronologies in a demersal fish in the south-eastern Pacific Ocean

Guillermo Moyano, Guido Plaza, Francisco Cerna and Ariel A. Muñoz

Upwelling and the El Niño "Southern Oscillation" (ENSO) are recurrent climatic phenomena in the southeastern Pacific Ocean that severely affect the reproduction and growth of pelagic fish populations. However, there are not long-term growth data from demersal fish populations to test these interconnections in a long-term analysis. For this reason, a first extensive growth chronology was reconstructed from the annual growth of sagittal otoliths as a proxy for somatic growth for the cardinalfish (*Epigonus crassicaudus*). Adult fish ranging from 35 to 40 cm in fork length and from 39 to 63 years in age were collected off Chilean waters. The master chronologies were estimated for the period from 1974 to 2014, using the regional curve standardization approach (RCS) and linear mixed models (LMMs). Growth indexes derived from both approaches followed a similar trend and were positively correlated with the Humboldt Current Index (HCI) and negatively with ENSO, Pacific Decadal Oscillation and sea surface temperature. LMMs showed that a 75% of growth variability was explained by the age of increment formation and HCI was the environmental index that most significantly affected the annual growth of cardinalfish followed by the sea surface temperature in spring. A reduced growth phase from 1974 to 1996 contrasted with a higher growth period from 1997, matching the 1997/1998 climatic regime shift, demonstrating that the enhanced growth for cardinalfish was associated with upwelling of nutrient rich water to the surface, triggering an increase of the primary and secondary productivity during the prevalence of a cold regime period in the Humboldt Current System. The consistency between RCS and LMM methods was indicative that both approaches are promising to evaluate the influence of environmental drivers on the growth condition of a demersal fish population in a highly productive marine ecosystem.

Keywords: Growth chronology, RCS, LMMs, Otolith, *Epigonus crassicaudus*

RETURN TO INDEX

IOS_282

Contrasting otolith morphology and age-growth patterns between Holocene and modern populations of *Sciaena deliciosa*

Osneider Palomino-Bolaños[1]*, Guido Plaza[1], Carola Flores[2], Jimena Torres[3]
Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile[1]; Universidad Austral, Valdivia-Chile[2]; Universidad de Magallanes, Punta Arenas-Chile[3].

The age, growth and morphometry of the external face and acoustic sulcus of adult Sciaena deliciosa (Roncacho) were compared for adult fish belonging to the middle Holocene (~4245 ky BP) and modern epoch (2020-2022). Age and growth patterns were addressed by analyzing annual increments, whereas changes in otolith morphology were evaluated using geometric methods. Archaeological samples were obtained from sediment profiles from Caleta Vitor 1, 7 and Morro 5 in Arica, whereas modern samples were collected from Chinchorro beach and fishing terminal in Arica. Modern and archaeological sagittal otoliths were digitized with stereoscopic magnifier with incandescent light, then fixed in resin blocks and sectioned with low speed saw. Fish lengths in both epochs were backcalculated using the proportional BHP method and for archaeological samples, total lengths (TL) were backcalculated based on the methodology proposed by Disspain et al., (2017). For geometric analysis 10 landmarks were identified for the external face and 8 for the acoustic sulcus. Ages ranged from 21 to 30 cm, and from 21 to 26 cm for archaeological and modern populations, respectively. A growth curve (Von Bertalanffy) was fitted for both periods to the age-backcalculated TL relationship. Kimura's likelihood ratio test showed significant differences (P<.0001) in the three growth parameters between both periods: modern (Linf=28; K=0.31; t0=-0.58) and archaeological (Linf=25; K=0.6; t0=0.2). No significant differences were found in the shape of the external face and acoustic sulcus (P<.0001) of the otoliths. There was a 1.34% of total allometry with no significant differences in the morphometry of the external face of the otoliths (P: 0.0973). A similar result was found for the acoustic sulcus where the allometry reached a 1.27%.

Keywords: sciaenidae, otoliths, growth, morphometry, coastal, increase, sulcus

RETURN TO INDEX

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