Otolith in Chinese Characters

耳

A pictograph means hearing and balancing organs of vertebrates.

石

A pictograph means that there is a stone under the cliffs, concentrated of mineral mixtures.
Welcome to Ilha Formosa!

On behalf of the Symposium conveners, it is our great pleasure to welcome you to Keelung, Taiwan, for the 6th International Otolith Symposium.

The Series of International Otolith Symposium has travelled widely since 1993, and we are delighted to be the first Asian country to act as host to the IOS. Formosa, as you know, means “Beautiful Isle”, and we are sure you will be entranced by our rich Oriental culture and friendly society, both exotic and familiar at the same time, and we know you will fall in love with our food - one of the finest cuisines in the world.

The programme includes six themed sessions and four workshops, giving you ample opportunity to share and review the advances into otolith research across the last quarter century. We are proud to offer you a varied program of top class presentations and speakers, and we aim to further build the reputation of the Symposium as the gold standard event for fish otolith research.

We wish you an enjoyable, profitable, and memorable stay in Taiwan.

Chia-Hui Wang & Li-Shu Chen

Chia-Hui Wang
Department of Environmental Biology & Fisheries Science, National Taiwan Ocean University

Li-Shu Chen
Exhibition & Education Division, National Museum of Marine Science and Technology
**Venue and Maps**

Address of venue: No.367, Beining Rd., Zhongzheng Dist., Keelung City, Taiwan

- **Main Exhibition Building**
- **Museum Dormitory**
- **Education Center (Conference Hall)**
- **IMAX Theater**

- **Evergreen Hotel Keelung**
- **TRA NMMST Station**
- **K-Hotel Keelung**

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**Symposium Meeting Rooms**

- **Poster Exhibition Hall**
- **Banquet**
- **Room 1**
- **Room 2**
- **Room 3**
- **Organization Office**
- **Library**

- **Toilet**

- Museum area is completely non-smoking.
National Taiwan Ocean University

No.84 in BRICS&Emerging Economies Rankings 2015
No.76 in top 100 Asia University Ranking of The Times Higher Education World University Rankings 2012-2013
NTOU has 7 colleges which house a total of 22 undergraduates, 11 graduate institutes, 27 master programs and 20 PhD program.

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Address: No.2, Pei-ning Road, Keelung, Taiwan
Tel: +886-2-24622192 ext. 1068~1069, 1221~1224
Fax: +886-2-24634786
Email: oia.ntou@mail.ntou.edu.tw
http://english.ntou.edu.tw/bin/home.php

National Museum of Marine Science & Technology

National Museum of Marine Science & Technology (NMMST) is situated in Badouzi, Keelung city. It is more than just a museum. It epitomizes balance, combining modern design and scenic landscapes and including an award-winning Museum Complex, breathtaking highland viewpoints, and numerous parks and trails.

Formerly the site of the Pei-Pu Steam Power Station, which was constructed in 1937 during the Japanese occupation of the island, the Main Exhibition Building retains the power station’s existing foundation and structure, which can still be seen from inside the museum today.

We welcome you to visit and discover for yourself everything that the NMMST has to offer, it is certainly a destination that is not to be missed!

Exhibitions

- Marine Environment Gallery
- Marine Science Gallery
- Naval Architecture and Ocean Engineering Gallery
- Fishery Science Gallery
- People and the Sea Gallery
- Wonders of the Deep Sea Gallery
- Kid’s Exploration Zone
- Deep Sea Theater (2nd Special Exhibition Gallery)

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General Information

Oral Presentations & 5 Minutes Talk
Oral Presentations are allotted 17 minutes for each presentation, and 3 minutes for questions or discussion at the end of the presentation. Five Minute Talks are short presentations, for a maximum of 5 minutes, without question/discussion time at the end. Please submit the files of your presentation to the Symposium Desk on the day before your presentation, preferably on a suitable memory stick.

• For example, if your presentation is arranged on 18th April, please go to Symposium Desk and share your presentation file with us on the 17th April.

Our IT facilities for the presentations include both Microsof® Windows and MacOS operating systems. Please check carefully in advance that your presentation works on the presentation computer when load files. In general personal computers cannot be used for the presentation unless the presenter has the approval of Symposium Committee.

A staff member will let you know that your presentation time is up 3 minutes before the end of your Oral Presentations, and 1 minute before the finish of 5 Minute Talks. A presentation remote control with laser pointer will be available. Symposium staff will be present in your presentation room to help you set up and get ready for your big talk.

Poster Format
Posters should be a maximum of 90 cm (width) x 180 cm (height). Poster stands and push-pins will be provided. Please set up your poster between 11:50 AM and 13:30 PM on the 16th April, and remove it after 13:30 PM on the 20th April. Any posters still posted after 5pm on the 20th April will unfortunately have to be disposed of. Symposium staff will be in the Poster Exhibition Gallery to assist you. Presenters must be available for public discussion during 16:30 – 19:00 PM for the Poster Session on the 17th April.

Accommodations
EVERGREEN LAUREL HOTEL (KEELUNG)
No. 62-1, Zhongzheng Rd., Keelung, 202, Taiwan
Mandarin: 長榮桂冠酒店 (基隆)
基隆市中正路62-1號

K Hotel Keelung
No.7, Yi 1st Rd., Jhongjheng Dist., Keelung City 202, Taiwan
Mandarin: 柯達飯店基隆
基隆市義一路7號

National Museum of Marine Science & Technology (NMMST) Dormitory Hall
No.52, Ln. 396, Beining Rd., Zhongzheng Dist., Keelung City, Taiwan
Mandarin: 國立海洋科技博物館學員宿舍
基隆市中正區北寧路396巷52號

Shuttle Bus Timetable
Shuttle Bus schedule is as follows: All guests attending the 6th IOS2018 can take free shuttle buses between the Evergreen Laurel Hotel & K Hotel and National Museum of Marine Science & Technology (NMMST) during the 15th–20th April. First stop is K Hotel then followed at Evergreen Laurel Hotel.
A metered taxi can be called through hotel receptions.
Wifi service in venue

Free Wi-Fi is provided in the museum area (including dormitory), and symposium rooms. It's open Wi-Fi, and no password is required.

Wi-Fi server name: FreeWiFi_Nmmst & NMMSTfree

App download

The 6th IOS2018 App can be downloaded to your mobile device free of charge. Download at the Google Play store, by searching for: IOS2018, or from the Apple Store, searching for TWIOS2018. Information includes in App: symposium information, programs, abstracts and update news, and it allows delegates to arrange their own personalised agenda.

Android:  

IOS:  

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Message Board
All messages will be placed on the message board located near the Symposium Desk. Please check the board regularly. Unfortunately we can take no responsibility for undelivered messages.

Lunch
Lunch boxes with a variety of main dishes to suit your dietary preferences will be provided, and vegetarian lunch box will be available. All foods will have labels to explain the ingredients. To minimise waste and plastic pollution, guests are asked to provide their own tableware (fork, spoon, chopsticks, etc.).

Morning and Afternoon Breaks
With the exception of the afternoon of the 17th and 18th April, coffee breaks will take place at 10:30 – 11:00 AM and 15:10 – 15:40 PM every day. Please enjoy our sweet desserts, traditional snacks, and the seasonal fruits of Taiwan. Again, bring your own tableware for environmental protection.

Special Requirements
If you have any special requirements (dietary, medical etc.), please go to the Symposium Desk or let any symposium staff know. We will do all that is necessary to accommodate you, and make your stay with us as special and rewarding as possible.

Insurance Policy in Venue (NMMST)
Museum insurance can cover your medical expenses if injured in the museum area (including in museum buildings, outdoor, parks and dormitory). Please inform any of our museum or symposium staff if you have or encounter an accident, we will deal with first aid and further assistance should you require it.

Symposium Proceeding
An special issue will be published in Marine and Freshwater Research in early 2019. The deadline of manuscript submission is end of June, 2018.
More updated information please check the symposium webpage.

Travel Information

Currency Exchange
We recommend that you exchange in your local bank prior to arriving in Taiwan, or over the Bureau de Change counter at TPE or TSA airport when you arrive in Taiwan. ATMs are commonplace in downtown Keelung, and most convenience stores (7-Eleven, Family Mart etc.) - most of which allow international withdrawal services.

Mobile Phone Service
You can purchase temporary phone SIM cards at the following locations:
1. In the Arrival Halls in Terminal 1 and 2 of TaoYuan International Airport (TPE) (opening hours 08:00~21:00hrs.) For more information visit: www.taoyuan-airport.com/english/store3_1/1106
2. 7-Eleven convenience stores (there are two stores close to the venue - ask a member of staff for directions). For more information: www.7-11.com.tw/ibonmobile/english1_1.html

Visiting the Museum
Every participant of the Symposium can visit the NMMST’s museum exhibits, including the IMAX Theater, once each free of charge. Please present your symposium badge to the staff in the Ticketing Office for exchanging the museum ticket before entering.
For opening hours please refer to: www.nmmst.gov.tw/enhtml/content/253

Travel Suggestions
Please Note: These tours are run by travel agencies and local community associations, and we are not responsible for bookings, refunds or cancellations.

A. Bus Tours
15th April 2018
North Coast Geological and Art Tour (08:00-16:30hrs, one-day tour): NT$2600
Pick-up→Yehliu Scenic Area (1.5hrs)→Juming Museum (2hrs)→Laomei Green Reef (1hr)→Return

18th April 2018
Northeast Coast Gold Mine and Old Street Tour (13:00-18:00hrs, half-day tour): NT$1900
Pick-up→Jinguashi Geological Park (Yinyang Sea, Remains of 13 stratolothic levels, Golden Waterfall) (1hr)→Gold Museum (1.5hrs)→Jiufen Old Street (2hrs)→Return
• The fee includes English guide, Taiwan Tour Bus fare, tickets, insurance and tips for guide and driver (By convention in Taiwan, you do not need to pay additional tips).
• Minimum Persons for this tour is 10. If not, it will be canceled.

B. Fishing Village Tour

18th April 2018
Baoduzi fishing village guide (13:00-17:00hrs, half-day tour): NT$ 600
Fishing village & Mazu Temple & Catches handling house (2hrs) → Traditional squid cuisine DIY (1hr) → Traditional cast net experience (1hr) → Return

• The fee includes an English-speaking guide, fishing materials, insurance and tips for guide and driver (By convention in Taiwan, you do not need to pay additional tip).

C. How to book a tour

Bus Tours:
Please send your name, contact info and number of people to the travel agency (wing@edison.com.tw, edisonsnts@ms6.hinet.net) by e-mail and c.c. to ios2018tw@gmail.com. Be sure to make your subject title: “6th International Otolith Symposium 2018”. The travel agency will reply to you and request the payment by credit card. The deadline to book onto a bus tour is 5th April.

Fishing Village Tour
You can order and pay directly at the Symposium Desk between the 15th and 17th April during the Symposium.

Enjoying local cuisine in Keelung
You can go to the link (https://goo.gl/TQiDqN) to learn about the food, restaurants and stalls we recommended around the venue, and get directions immediately in your Google Maps.

Symposium Events

Registration
You can register at any one of our three registration sites, during the following times:
1. Museum Dormitory: Sunday 15th April, 15:00 – 19:00 (Welcome Reception)
2. Museum IMAX Theater: Monday 16th April, 08:00 – 11:00 (Opening Ceremony)
3. Museum Education Centre Conference Hall: 16th – 20th April, 08:30 – 17:00 (Symposium Desk)

Sunday 15th April 17:00 – 19:00 PM
Welcome Reception
The Museum Dormitory is located in the old dormitory of the North Thermal Power Plant of Taiwan. This ecologically harmonious building combines the beauty of the natural environment with the history of our rich railway culture. Our Welcome Reception will take place in the Lounge Room of the Museum Dormitory. With light refreshments, music and wine, this is a lovely way to start the Symposium should you arrive early enough.

Monday 16th April 08:20 – 09:10 AM
Opening Ceremony
The Opening Ceremony will be held in the IMAX Theater in main Museum. Our opening show, entitled “Pakalunay”, will start at 08:20hrs. Pakalunay is a traditional symbolic ritual of the Amis, the indigenous people of Taiwan, and represents the rite of passage when the young become adults - but only after they accept the necessary skills and knowledge from their elders. We feel this is fitting for the Symposium, which is our platform to share experiences about fish otolith research, and learn from one another. We invite you to join the Amis people to dance the Pakalunay before our Opening Ceremony.
Monday 16th April 18:00 – 20:00 PM
Student Night
Organizers:
Tzu-Yun Ching, National Taiwan Ocean University, Taiwan
Joyce Ong, Rutgers University, USA

We will be hosting a student night party in the Lounge Room of first floor of the Museum dormitory, including a few exchange games for the students and even a fashion show at the end of the evening. We would love the students to prepare some marine theme clothing to wear to the party and help build a wonderful, maritime, atmosphere. This will be a great way for students to meet and get to know one another, as well as a super chance to discuss their own fields of study. We hope that life-long relationships and collaborations will come from this symposium, so this is a great way to fan the flames of friendship for the future. The fee for the party is 300 NTD for food and drinks - the friends for life are free!

Tuesday 17th April 16:30 – 19:00 PM
Poster Presentations
Our Poster Sessions will take place Tuesday afternoon, through to the early evening, and we ask that presenters are asked to make themselves available to discuss their presentations with the public during this time. We’ll have refreshments, including delicious Taiwanese beer and a selection of local cuisine, provided for you in the Poster Exhibition Hall. At the same time, SmartDots soft will be demonstrated by the development team beside their poster (PO-03).

Thursday 19th April 12:20 – 13:30 PM
SmartDots demo at Room 2
SmartDots is a training and quality assurance tool, based on otolith images, which was first developed for in-house purposes and which is now a complete software platform used for international age reading workshops and exchanges, management of associated data and standardised reporting of results. Further development of features will take place in 2018 based on user requirements and feedback. By demonstrating SmartDots to the wider otolith community which will be present at the IOS2018 participants will be given the opportunity to experience the tool hands-on and the development team will benefit from exposure to and feedback from a diverse group of otolith experts and trainees.

Thursday 19th April 18:30 – 21:00 PM
Bandou Banquet
Our banquet will take place near the base of electric generator and cooling waterway of the North Thermal Power Plant, one of the heritage locations on our main site. Unlike normal banquets, we are holding a traditional Taiwanese “Bandou” banquet, where the host would hire restaurant chefs to his residence, and cook delicious dishes to specially invited attendees. We’ll have open-plan seating in this historic space, and you will share in fabulous food while being entertained by two Taiwan folk arts: The Drum Dance and Electric-Techno Neon Gods. Online registration is required in advance, and guests are asked to wear their name badge during the event. Come along and enjoy this special spring banquet with us!
Dress code: Casual

Friday 20th April 17:00 – 17:30 PM
Closing Ceremony
The Closing Ceremony for the Sixth International Otolith Symposium takes place in the Conference Hall, and will include the prize-giving for our Best Student Presentation Awards, sponsored by The Ichthyological Society of Taiwan. This is a fabulous opportunity to round off the event in style, and join us in celebrating the success of the Symposium.
Keynote Speakers

**Dr. Ming-Tsung Chung**
Department of Bioscience, Aarhus University, Aarhus, Denmark

09:10 AM on Monday, April 16, 2018
Abstract Code: K1

Ming-Tsung Chung is interested in developing innovative analytic skills in biominerals such as fish otolith and cephalopod statolith/outer shell, and mostly focused on geochemical approaches for answering ecological questions. Currently, he is working on the reconstruction of fish field metabolic rate by using otolith carbon isotope values, and investigate fish physiological and behavioural adaptation responding to increasing temperature. His research is aimed to predict fish population dynamic under environmental pressure and explore potential mechanism underpinnings.

**Dr. Tanja Schulz-Mirbach**
Department of Earth & Environmental Sciences, Ludwig Maximilians University, Munich, Germany

09:50 AM on Monday, April 16, 2018
Abstract Code: K2

Tanja Schulz-Mirbach’s research focuses on the functional morphology in the ear of teleost fishes mainly in structure-function relationships between otolith shape, inner ear structures like sensory epithelia, ancillary auditory structures, and hearing abilities. She is interested in questions of whether adaptations to divergent environmental conditions lead to differences in otolith shape and texture and may thus affect hearing abilities, and if hearing specializations are traceable in fish ear morphology. For her studies, she applies multiple tools such as electrophysiological methods to evaluate fish hearing in terms of detectable frequency range and auditory sensitivities as well as different microscopic techniques (scanning electron microscopy, confocal microscopy), immunohistochemistry, synchrotron radiation imaging, and 3D reconstructions based on microCT imaging and serial histological sectioning. As she is also working at the interface of biology and paleontology, she is involved in studies dealing with the evolution of otolith shape diversity in teleost fishes. Her research activities into functional morphology therefore aim to identify adaptations or specializations—where existent— which may help to interpret fossil otoliths from a physiological view point, as otoliths are generally the only remains of teleost ears. In order to better understand the emergence of solid otoliths in teleost fishes contrasting the numerous tiny otoconia found in the inner ears of most other vertebrate groups and the evolution of otolith shape diversity, her current work focuses on the characterization of the very basics of otolith motion in-situ and factors like otolith weight and shape influencing this motion. These basic data are implemented in a recent cooperative project in advanced modelling of otolith motion in the fish ear.
Dr. Audrey J. Geffen
Department of Biology Bergen, University of Bergen

11:00 AM on Monday, April 16, 2018
Abstract Code: K3

Audrey J. Geffen is a fish biologist, primarily concerned with the effects of environmental variables on the growth of larval and juvenile fish. Much of her work has involved theoretical and applied research on the growth, formation, and composition of fish otoliths. She has been part of the development of many of the otolith-based approaches, especially pushing for more communication between physiology, ecology, biomineralization, and geochemistry. She is currently working to develop standardized methods for using fish otoliths as recorders of environmental change, particularly for environmental impact and monitoring studies.

Dr. Benjamin Walther
Department of Life Sciences, Texas A&M University - Corpus Christi, USA

08:50 AM on Tuesday, April 17, 2018
Abstract Code: K4

Benjamin Walther conducts research on a variety of topics related to fish migration, habitat use, population connectivity and environmental histories. This work generally focuses on using the “natural tag” properties of hard parts in marine and diadromous fishes to examine individually-variable patterns of movement and life history dynamics of species with mobile phases. His research has primarily involved otolith chemistry, but has recently been adapted for use in alternative structures such as fish scales and fin rays. In addition, he is currently investigating chemical indicators of lifetime hypoxia exposure with redox-sensitive markers, and he is coupling these markers with isotopic indicators of altered food web interactions. Together, this work is aimed at providing essential information about ecological consequences of variable life histories and environmental exposures for important mobile species.
Dr. Christopher Izzo
Fisheries Research and Development Corporation (FRDC), Adelaide, Australia

08:50 AM on Thursday, April 19, 2018
Abstract Code: K5

I have a broad research background, having worked across a range of fields and taxonomic groups. However, a near constant in my research has been the examination and use of calcified structures to address biological and ecological questions. More recently my research has focused on investigating environmental and anthropogenic stressors affecting freshwater and estuarine ecosystems and the fish communities within them – namely, using element and growth chronologies to reconstruct changes in environmental conditions and biological processes.

Dr. Henk-Jan Hoving
GEOMAR, Helmholtz Centre for Ocean Research, Kiel, Germany

08:50 AM on Friday, April 20, 2018
Abstract Code: K6

Henk-Jan Hoving investigates the biology and ecology of deep-sea and oceanic pelagic fauna. One important part of his research program is the study of how the pace of life of deep-sea organisms differs from shallow water relatives. Towards this end he studies life history strategies (growth, longevity and reproductive strategies) of oceanic invertebrates. So far most of his work focused on cephalopods. These typically semelparous (single reproductive cycle) molluscs are very abundant in all marine ecosystems in particular in the open ocean and the deep sea. Via quantification of increments in hard body structures (statoliths, beaks) his group determines age at size, growth and longevity of cephalopods. Additionally, the quantitative assessment of reproductive systems reveals information on spawning strategies, and with that on longevity. Finally, Hoving applies observational technology in the deep sea to study the behavioral ecology of deep-sea organisms in their natural environment. This combined approach has resulted in novel insights in the life cycles of deep-sea cephalopods.
Workshops

Workshop I: A beginner’s (and/or refresher) course in otolith ageing and imaging

Organizer
Steven Campana (University of Iceland, Iceland)
Bronwyn Gillanders (University of Adelaide, Australia)

Description
Otoliths are the primary source of age data (yearly) for fish around the world. Otolith preparation techniques vary with the species, but the basics of otolith interpretation are common to most species. In this introductory and informal, hands-on workshop, participants will start by learning a few of the main methods for otolith preparation. The majority of the workshop will be spent in learning modern imaging and image enhancement techniques, followed by interpretation to identify and count the annual growth bands. Participants are welcome to bring their own otoliths, otolith sections or images of prepared otoliths to work on, although there will be insufficient time to section any otoliths if they are not already embedded in epoxy. Participants are also encouraged to bring their own laptops to carry out image enhancements; copies of Photoshop will be available for loan to Windows users.

Goal and aims
The specific objectives of the workshop are to:
• Observe the epoxy embedding and sectioning procedure to prepare an otolith thin section
• Use an Isomet saw to prepare a thin section of an epoxy-embedded otolith provided by the participant
• Learn the “crack and burn” method for ageing long-lived species like Sebastes
• Learn how to image and enhance burnt or sectioned otoliths; enhancement will be with Photoshop
• Learn how to interpret otolith annuli, either in the samples provided or in images/sections provided by the participant
• Learn the do’s and don’ts of otolith storage

This workshop is intended to provide an introduction to ageing for those with little or no prior experience in the interpretation of otoliths for annual age determination, as well as to provide a refresher for those with more experience. The image enhancement techniques, in particular, may be new to experienced otolith readers.
Workshop II: Otolith shape analysis with R

Organizer
Lisa Anne Libungan (Marine & Freshwater Research Institute, Iceland)

Description
Morphometric analysis of otoliths is a well-established method to delineate fish stocks, characterize population movements and to detect the natal origin of fish. For otolith shape analysis, two main morphometric methods are used: landmark analysis and outline analysis. With outline analysis it is possible to quantify boundary shapes so that patterns of shape variation within and among groups can be evaluated based on a large number of independent variables. The advantage of using such methods in population identification is that they are cost effective and only require otolith images from which outlines can be extracted and analyzed with statistical software.

Goal and aims
This workshop will focus on fish population discrimination using otolith shape analysis. It will cover all aspects of otolith shape analysis, from start to end. In the workshop, you will learn:

- how to take images of otoliths and prepare them for import into R
- the folder structure needed for the analysis
- how to use the R package ShapeR
- how to statistically compare fish populations with other available R packages

During the demonstrations and interactive practical sessions in this workshop, we will extract, visualize and generate otolith shape data with a small number of easy-to-use functions. In ShapeR there are built-in functions which allow users to perform automatic processes such as extract the otolith outlines from images, visualize the mean shape, smooth the outline by eliminating pixel noise and transform the outlines into independent coefficients using either Normalized Elliptic Fourier or Discrete Wavelet, which can be entered into a wide range of statistical packages in R.

* We suggest participants bringing their own laptops and pre-installing R and the package.

Figure 1. Otolith shape analysis based on Wavelet reconstruction using the R package ShapeR for three discrete fish populations from Iceland (IC), Norway (NO) and Scotland (SC) (Libungan and Pálsson 2015).
Workshop III: Can otolith chemistry be used for age estimation of fish?

Organizer
Karin Hüssy (Technical University of Denmark, Denmark)
Karin Limburg (State University of New York, USA)
Audrey Geffen (New York, USA Bergen, Norway)

Description
Some of the most basic data inputs to stock assessment are based on information relying on accurate age determination. For many fish species and stocks, such data are readily available through traditional age estimation based on counting annual growth zones in the otoliths. For some stocks (e.g. European hake, Baltic cod, anadromous alosine herrings), traditional age estimation has proven subject to extensive inaccuracy and bias. New methods to obtain age data therefore need to be explored. Analysis of otolith chemical composition has long been a useful tool for fisheries scientists for deriving stock identification, connectivity between spawning and nursery areas, migration patterns, and much more. In recent years, research into mechanisms regulating incorporation of elements and different isotopes thereof has gained increasing interest. In particular the possibility of some chemical constituents having chronometric properties – like a "chemical calendar" - may prove highly interesting for age determination in species that are difficult to age using traditional methods.

Analysis of otolith chemical composition has long been a useful tool for fisheries scientists for deriving stock identification, connectivity between spawning and nursery areas, migration patterns, and much more. In recent years, research into mechanisms regulating incorporation of elements and different isotopes thereof has gained increasing interest. In particular the possibility of some chemical constituents having chronometric properties – like a "chemical calendar" - may prove highly interesting for age determination in species that are difficult to age using traditional methods.

An example of Mg/Ca dynamics in a flounder otolith

Mg/Ca peaks in summer, has minima at annulus formation (winter) – a most excellent "chemical calendar"

(Limburg et al., in prep.)

Figure 1. Another example of a chemical calendar: Magnesium:calcium ratios in Baltic flounder otoliths. Again, validated by examining daily growth increments and annulus formations.

In salmoniform fishes, Zn/Ca ratios correlate with growth rate(*) - so we can distinguish summer from winter.

Figure 2. Example of a chemical calendar: zinc in salmoniforms increases in summer and decreases in winter. Thus in the example above, we can use Zn/Ca ratios to estimate the timing of a migration event from a river (low Sr, pale colors) to a lake with elevated Sr (darker reds). Here this Atlantic Salmon switched habitats at the end of its third summer growing season.

Goal and aims
This workshop aims to improve our understanding of the "chemical calendar" in order to promote the development of microchemistry analyses as age estimation tool. We encourage contributions focusing on:

• Methodological approaches to resolve seasonal signals on spatially and temporally relevant scales
• Statistical methodology for identifying seasonal signals in longitudinal or spatial microchemistry data
• Approaches aimed at validating seasonal microchemistry signals
• Identification of species and stocks where microchemistry based age determination might be applicable
• Identification of phylogenetic groups wherein different "chemical calendars" are found

We would like to invite oral and poster presentations on all above topics. The workshop will consist partly of presentations of relevant studies and of a time slot for discussions of ideas, collaborations and hypotheses for testing.
Workshop IV: Chemical archives in fishes beyond otoliths: Advancements in microchemical analyses of alternative fish body parts to advance the conservation and management of fishes

Organizer
Patricia Lastra (AZTI-Tecnalia Marine Science Institute, Spain)
Malte Willmes (University of California Davis, USA)
Orian Tzadik (University of South Florida, USA)

Description
Microchemical analyses of fish otoliths has revolutionized fishery sciences as it allows for the investigation of pressing issues such as habitat use, migration, stock structure, and even dietary patterns. Alternative hard tissues such as scales, spines, and fin rays may also provide valuable chemical information and are particularly useful as a non-lethal alternative to otolith in endangered or protected species.

In addition, otoliths do not contain high enough concentrations of organic matter to allow for the reconstruction of food-web relationships via multiple stable isotopes. Finally, some fish species exhibit vateritic otoliths that are difficult to analyze using conventional microchemical techniques. Currently, we have limited knowledge of the feasibility of using other body parts such as fin spines, rays, eye lenses or scales as alternatives or complements to otolith analyses for inferring life and environmental histories in fishes.

Goal and aims
The goal of this workshop is to explore the use of chemical archives in anatomical tissues beyond otoliths promoting its applications in fish ecology, fisheries management, and conservation issues. Over the last 40 years, the rapid development of microchemical analytical techniques, particularly trace element and stable isotope analyses (TEA and SIA respectively), has greatly expanded our abilities to address pressing issues in the field of fish ecology. As such, this aims to update participants on the more specialized tools available and the recent advances in technology that allows for more precise analyses of chemical properties and isotopic ratios in alternatives anatomical tissues. The workshop will also examine examples of different microchemical analysis protocols, and compare the techniques used for different species, providing an accessible summary of the methodological and statistical treatments. In addition, the workshop seeks to promote alternative analyses that potentially document movement, diet, physiological events, or environmental conditions, as well as new areas of application of microchemical analyses in tissues in an attempt to gain knowledge of fish life traits and environmental histories, highlighting the need of further validation studies using controlled laboratory setting and to develop a network of researchers with expertise in the fields of bio-chronology, chemistry and fish population dynamics research.

The content is targeted at researchers with backgrounds in microchemistry of anatomical tissues, with particular interest on those with wide experience in the use of non-carbonate hard structures for chemistry studies. The workshop will feature introductory and overview talks followed by informal discussions and a workshop with the aim of producing a short document that outlines the potentials and pitfalls of using alternative chronological recorders in fisheries research. Workshop presentations will inform participants of the theoretical and practical bases for these techniques and their application as natural tags for reconstructing habitat use, migrations, and other fundamental aspects of fish life histories that are beyond the spatial or temporal reach of most other approaches.
Program

Sunday 15th April 2018

Optional Tour
North Coast and Yehliu (Juming Museum), 08:00-16:30, one-day tour: NT$2600

15:00 PM Registration
Room: Lobby of Museum Dormitory

17:00 PM Welcome Reception
Room: Lounge Room of Museum Dormitory

Monday 16th April 2018

08:20 AM Opening Ceremony
Room: IMAX Theater

Keynote Session
Chair: Peter Grønkjær

09:10 OL-I01 Field metabolic rate recorded in otoliths: Geochemical techniques used to study fish physiological ecology
Ming-Tsung Chung, Department of Bioscience, Aarhus University, Denmark

Chair: Beatriz Morales-Nin

09:50 OL-I02 The organic flight recorder: Lifetime connectivity and habitat use reconstructions with fish otolith amino acids.
Kim Vane

10:30 AM Coffee Break

11:40 AM Group Photo

11:50 AM Lunch

Room 1
Theme: Life History and Fisheries
Chair: Allen H Andrews

13:30 OL-I03 The evolution of Otolith Science
Tanja Schulz-Mirbach, Ludwig-Maximilians-University Munich, Germany

OM-I01 Bias in fish length back-calculation models derived from otolith shape ontogeny. Allan Souza

13:50 OL-I04 Laser ablation AMS reveals complete bomb 14C signal in an otolith with confirmation of 60-year longevity for red snapper (Lutjanus campechanus).
Allen H Andrews

OM-I03 Variability in otolith shape shows evidence of stock structure in Patagonian toothfish (Dissostichus eleginoides) in the South-west Atlantic. Brendon Lee

14:10 OL-I05 Element histories from archival-tagged fish.
Ewan Hunter

OM-I05 Separating fish stocks in a mixing area by using genetics and ShapeR based otolith shape analysis: a case study on Baltic Sea cod (Gadus morhua L.). Franziska Maria Schade

14:50 OL-I06 Inferring life history of a long-lived deepwater grouper based on otolith radiocarbon analysis.
Beverly Barnett

OM-I07 Spatial distribution, total length frequencies and otolith morphometry as tools to analyze the effects of a flash flood on populations of roach (Rutilus rutilus). Fabien Morat

15:10 PM Coffee Break

15:40 OL-I07 Distinguishing brown trout (Salmo trutta) parr from different natal streams and hatcheries based on otolith elemental fingerprints – with some insights on adult sea trout natal origin.
Lagle Matetski

OM-I06 New services in AFORO (Shape analysis of otoliths): From data-base to data-bank. Antoni Lombarte

16:00 OL-I08 Comparative morphology of the otoliths of clupeid fishes from Japan and application for species identification.
Shota Mitsui

OM-I05 New services in AFORO (Shape analysis of otoliths): From data-base to data-bank. Antoni Lombarte

11:40 AM Group Photo

11:50 AM Lunch

Room 2
Theme: Morphology and Physiology
Chair: Tanja Schulz-Mirbach

13:30 OL-I09 Inferring life history of a long-lived deepwater grouper based on otolith radiocarbon analysis.
Beverly Barnett

OM-I05 Spatial distribution, total length frequencies and otolith morphometry as tools to analyze the effects of a flash flood on populations of roach (Rutilus rutilus). Fabien Morat

13:50 OL-I07 Distinguishing brown trout (Salmo trutta) parr from different natal streams and hatcheries based on otolith elemental fingerprints – with some insights on adult sea trout natal origin.
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Lagle Matetski

OM-I07 Comparative morphology of the otoliths of clupeid fishes from Japan and application for species identification. Shota Mitsui
Tusday 17th April 2018

Room: Room 1

Chair: Karin Limburg

08:50
K4
The Art of Otolith Chemistry: interpreting patterns by integrating perspectives.
Benjamin Walther, Texas A&M University - Corpus Christi, USA

Room 1: Chemistry and Composition
Chair: Benjamin Walther

09:30
OC-II01
Is otolith Mg adding nuance to the hypoxia story? Karin Limburg

09:50
OC-II02
An innovative approach to dating fish otoliths with near infra-red spectroscopy (NIRS). Irina Benson

10:10
OC-II03
Pathways of trace elemental incorporation into bivalve shells. Liqiang Zhao

10:30 AM Coffee Break

11:00
OS-II01
Beyond otoliths – considering the complexities of the ocean environment in order to understand age data for deep-sea corals. Dianne Tracey

11:20
OS-II02
Age and growth of the Saunsio undosquamis in the water off western Taiwan using otolith ring marks. Yuan-Tian chou

11:40
OS-II03
SmartDots: A new age reading software tool for the analysis of calcified structures of marine species. Karen Bekker

12:00
OC-II07
Stable carbon isotopes as metabolic biomarkers. Jasmin Martino

12:20 AM Lunch

13:30
OC-II08
Applicability of otolith delta13C and delta15N isotope analysis for addressing ecological questions. Karin Hüssy

13:50
OC-II09
Temperature-dependent trophic fractionation of stable nitrogen and carbon isotope in otolith organic matter. Jane Aanestad Godiksen

14:10
OC-II10
Discriminating wild and hatchery red snapper by using stable C and N isotopic signals on fish scales. Chian-Yu Lu

14:30
OC-II11
Isotope tools to track floodplain rearing of native fishes. Rachel Johnson

14:50
OC-II12
Natural abundance nitrogen isotopic composition of the organic matrix of fish otoliths across diverse taxa. Jessica Lueders-Dumont
**Wednesday 18th April 2018**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td><strong>Workshop I</strong>: A beginner's (and/or refresher) course in otolith ageing and imaging</td>
</tr>
<tr>
<td>Room 1</td>
<td><strong>Workshop II</strong>: Otolith shape analysis with R</td>
</tr>
<tr>
<td>Room 2</td>
<td><strong>Workshop III</strong>: Can otolith chemistry be used for age estimation of fish?</td>
</tr>
<tr>
<td>Room 3</td>
<td><strong>Workshop IV</strong>: Chemical archives in fishes beyond otoliths: Advances in microchemical analyses of alternative fish body parts to advance the conservation and management of fishes (see p.43)</td>
</tr>
</tbody>
</table>

**13:00 Optional Tour**
1. Northeast Coast Gold Mine and Old Street Tour, 13:00-18:00, half-day tour: NT$1900
2. Baoduzi fishing village guide, 13:00-17:00, half-day tour: NT$600

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**Thursday 19th April 2018**

**Keynote Session**
Room: Room 1
Chair: Bronwyn Gillanders

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:50</td>
<td><strong>KS</strong> A Hitchhiker's Guide to the Otolith Galaxy</td>
</tr>
<tr>
<td></td>
<td>Christopher Izzo, Fisheries Research and Development Corporation (FRDC), Adelaide, Australia</td>
</tr>
</tbody>
</table>

**Poster Session & SmartDots demo**
Room: Poster Exhibition Hall

**Committee Meeting**

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**Chair: Ming-Tsung Chung**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:20</td>
<td><strong>5-minutes Talk Session 1</strong> (F-201 - F-210), see p.41</td>
</tr>
<tr>
<td>Room 1</td>
<td><strong>Workshop I</strong></td>
</tr>
<tr>
<td>Room 2</td>
<td><strong>Workshop II</strong></td>
</tr>
<tr>
<td>Room 3</td>
<td><strong>Workshop III</strong></td>
</tr>
<tr>
<td>Room 4</td>
<td><strong>Workshop IV</strong></td>
</tr>
</tbody>
</table>

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**Chair: Ming-Tsung Chung**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:30</td>
<td><strong>OL-IV01</strong> Natal Origin and Connectivity of Pacific Bluefin Tuna.</td>
</tr>
<tr>
<td></td>
<td>David Wells</td>
</tr>
<tr>
<td>09:50</td>
<td><strong>OL-IV02</strong> Estimation of age composition and migration pattern of Pacific bluefin tuna (Thunnus orientalis) in the main spawning ground in the southwestern North Pacific Ocean. Taki Ishihara</td>
</tr>
<tr>
<td>10:10</td>
<td><strong>OL-IV03</strong> Investigating the origin of yellowfin tuna (Thunnus albacares) in the western Indian Ocean using an otolith chemistry approach. Iraide Artetxe Arrate</td>
</tr>
<tr>
<td>10:30</td>
<td><strong>OL-IV04</strong> Do fin spines preserve stable isotopes signatures suitable for reconstructing Atlantic bluefin tuna migration patterns? Patricia L. Luque</td>
</tr>
<tr>
<td>11:00</td>
<td><strong>OL-IV05</strong> Age and growth estimation of striped marlin in the eastern North Pacific using otolith micro-increments and fin spine sections. Tamaki Shimose</td>
</tr>
<tr>
<td>11:20</td>
<td><strong>OL-IV06</strong> Early life history traits of some small pelagic fishes in the Humboldt Current System. Guido Plaza</td>
</tr>
<tr>
<td>11:40</td>
<td><strong>OM-IV01</strong> Convergent evolution in vestibular systems of fish and cephalopods. Alexander Arkhipkin</td>
</tr>
<tr>
<td>11:40</td>
<td><strong>OM-IV02</strong> Functional effect of vaterite on escape trajectory of Salmo trutta. Matthias Vignon</td>
</tr>
<tr>
<td>11:40</td>
<td><strong>OM-IV03</strong> Large lapillus found in two tribes of Sciaenidae: The American Stelliferini and Indo-West Pacific Johnini. Ning Chao</td>
</tr>
<tr>
<td>11:40</td>
<td><strong>OM-IV04</strong> Do environmental conditions (temperature and food composition) affect otolith shape during fish early-life stages? An experimental approach applied to European seabass (Dicentrarchus labrax). Keilig Mahe</td>
</tr>
<tr>
<td>11:40</td>
<td><strong>OM-IV05</strong> Composite variations in genetic structure, life-history traits, and statolith morphology of Sepioteuthis lessoniana populations around Nagasaki Prefecture, Japan. Tzu-Yun Ching</td>
</tr>
<tr>
<td>11:40</td>
<td><strong>OM-IV06</strong> Abundant otoliths in fossil and modern coral reefs provide Caribbean reef fish community baselines. Chien-Hsiang Lin</td>
</tr>
</tbody>
</table>
Friday 20th April 2018

Keynote Session
Room 1: Alexander Arkhipkin

08:50 K6
The pace of life in deep living cephalopods.
Henk-Jan Hoving, GEOMAR, Helmholtz Centre for Ocean Research, Kiel, Germany

Room 1

09:30 OS-V01
Evaluating regional synchrony in multi species otolith growth chronologies: insights on population plasticity, resilience and ecosystem productivity in a changing ocean. Susanne Tanner

Room 2

09:30 OS-V01
Otoliths provide new insights into the importance of life history diversity in regulated systems.
Anna Sturrock

09:50 OL-V02
Demography and growth during early stages of Sproatelloides delicatulus in different marine coastal habitats of Con Dao, the oldest MPA of Vietnam. Hung Manh Pham

09:50 OL-V02
Is there a global signature of biological change in marine hotspots? John Morrioniello

12:00 OL-IV07
Fine-scale temporal changes in otolith microstructure and chemistry expose the early life history of a coastal southern Australian finfish. Troy Rogers

12:20 PM Lunch & SmartDots demo at Room 2

13:30 OC-IV08
Elements in otoliths as environmental proxies: a meta-analysis. Bronwyn Gillanders

14:10 OC-IV10
Validation of species specific otolith chemistry and salinity relationships. T. Reid Nelson

14:30 OC-IV09
Reconstruction of the salinity history associated with movements of mangrove fishes using otolith oxygen isotope analysis. Kotaro Shirai

15:40 OL-IV13
Connectivity of lemon sole Microstomus kitt in the northern North Sea as determined from a combination of otolith characteristics and particle tracking. Richard D. M. Nash

16:00 OL-IV14
Interannual variability in early life history of European Flounder, Platichthys flesus and common sole, Solea solea. Ana Vaz

16:20 OL-IV15
Marine dispersion of two related anomalous European shads along French Atlantic coast before 2000s population's crash. David José Nachón

16:40 OL-IV16
Changes in environmental salinity during the life of Pagosomus brevifili in the Mekong Delta (Vietnam) revealed by otolith Sr:Ca ratios. Trong Ngan Tran

17:10 PM 5-minutes Talk Session 2 (F-401 - F-410), see p.42
Room: Room 1
Chair: Benjamin Walther

18:30 PM Banquet
Room: The Historical Site (located by Poster Exhibition Hall)
10:10  | OS-V03  | Otolith tools in the ecotoxicology toolbox: Unraveling sources and pathways of Se exposure in wild Sacramento spliail with spinal deformities.  
Frederick Feyrer

10:30 AM  | Coffee Break
Chair: Audrey Geffen

11:00  | OS-V04  | Sclerochronological approach for the identification of herring growth drivers in the Baltic Sea.  
Szymon Smoliński

11:20  | OS-V05  | Interannual variation in population growth for Japanese Spanish mackerel (Scomberomorus niphonius) and differing respond to environmental change.  
Chi Zhang

11:40  | OS-V06  | Northern Benguela Merluccius paradoxus chronologies and annual change in length from age-length-keys used for age validation; and as indicators of response to longterm changes.  
Margit Wilhelm

12:00  | OS-V07  | Food deprivation records in the otoliths of juvenile sockeye salmon (Oncorhynchus nerka).  
Yuliya Kuzmenko

12:20 PM  | Lunch & SmartDots demo at Room 2

Theme: Chemistry and Composition
Chair: Kotaro Shirai

| 13:30  | OC-V08  | Otolith microchemistry approach to determine population structure and movements of European anchovy (Engraulis encrasicolus) along the Atlantic Coast of Iberian Peninsula.  
Carmen Hernandez

13:50  | OC-V09  | Reproducing migration history of Japanese sardine using otolith 8°O and a data assimilation model.  
Tatsuya Sakamoto

14:10  | OC-V10  | Discerning population connectivity and natal origins of Pacific herring (Clupea pallasii): inferences on population structure from otolith chemistry.  
Alexandra Bagarinao

14:30  | OC-V11  | Fish stock identification: otolith chemistry versus truss morphometry and microsatellite DNA.  
Mohammad Afzal Khan

14:50  | OC-V12  | Lactitudinal variation of life history traits in an amphi-dromous fish Plecoglossus altivelis altivelis: how does growth before upstream migration affect the performance in rivers?  
Iki Murase

15:10 PM  | Coffee Break

Theme: Life History and Fisheries
Chair: Patrick Reis-Santos, Francoise Daverat

| 15:40  | OL-V10  | Eel otoliths of known age-a comparison between readers.  
Hakan Wickstrom

| 16:00  | OL-V11  | Variation in age, growth and otolith kernel size among and within populations of marbled flounder in Japan.  
Peiqi Hong

| 16:20  | OL-V12  | Pelagic larval duration, growth rate and population genetic structure of the tidepool snake moray Uropterygius micropterus around southern Ryukyu islands, Taiwan and central Philippines.  
Wen-Chien Huang

17:00 PM  | Closing Ceremony
Room: Room 1
**Tusday 17th April 2018**

**5-minutes Talk Session 1**

**Room: Room 1**

**Chair: Ming-Tsung Chung**

<table>
<thead>
<tr>
<th>Time</th>
<th>Talk Number</th>
<th>Title</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:20</td>
<td>F-201</td>
<td>Effect of metabolic rate on time-lags change in otolith microchemistry: An experimental approach using <em>Salmo trutta</em></td>
<td>Matthias Vignon</td>
</tr>
<tr>
<td>15:25</td>
<td>F-202</td>
<td>Tracking hypoxia in Baltic flounder: do spawning ecotypes differ in hypoxia exposure?</td>
<td>Melvin Samson</td>
</tr>
<tr>
<td>15:30</td>
<td>F-203</td>
<td>Early growth history differences of the three cryptic grey mullet (<em>Mugil cephalus</em>) species in Taiwan.</td>
<td>Yu-Ling Nien</td>
</tr>
<tr>
<td>15:35</td>
<td>F-204</td>
<td>Age and growth of Atlantic Chub Mackerel (<em>Scomber colias</em>) in the Northwest Atlantic.</td>
<td>Taylor Daley</td>
</tr>
<tr>
<td>15:40</td>
<td>F-205</td>
<td>Can otolith stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) of Red Ear Emperor <em>Lethrurus lentjan</em> be used as proxies to reconstruct its historical habitat experiences in tropical coastal water?</td>
<td>Dung Le</td>
</tr>
<tr>
<td>15:45</td>
<td>F-206</td>
<td>Using otoliths to relate wave action to daily growth of an intertidal/shallow subtidal fish.</td>
<td>Becky Focht</td>
</tr>
<tr>
<td>15:50</td>
<td>F-207</td>
<td>Age validation of western Baltic cod (<em>Gadus morhua</em>).</td>
<td>Kate McQueen</td>
</tr>
<tr>
<td>15:55</td>
<td>F-208</td>
<td>Growth and validation of the age estimation of chub mackerel (<em>Scomber colias</em>) in the North and Northwest of the Iberian Peninsula.</td>
<td>Maria Rosario Navarro</td>
</tr>
<tr>
<td>16:00</td>
<td>F-209</td>
<td>Evaluating estuarine nursery use and life history patterns of <em>Pomatomus saltatrix</em> in eastern Australia.</td>
<td>Hayden Schilling</td>
</tr>
<tr>
<td>16:05</td>
<td>F-210</td>
<td>Tracking invasive lionfish population dynamics and demographies in the northern Gulf of Mexico via analysis of otolith microstructure.</td>
<td>Kristen Dahl</td>
</tr>
</tbody>
</table>

**Thurday 19th April 2018**

**5-minutes Talk Session 2**

**Room: Room 1**

**Chair: Benjamin Walther**

<table>
<thead>
<tr>
<th>Time</th>
<th>Talk Number</th>
<th>Title</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>17:10</td>
<td>F-401</td>
<td>Migratory behaviour as coping mechanism to resource availability: riverine <em>Galaxias maculatus</em> in Chile, <em>Konrad Górski</em></td>
<td></td>
</tr>
<tr>
<td>17:15</td>
<td>F-402</td>
<td>Can we reconstruct the growth history of the Pacific halibut (<em>Hippoglossus stenolepis</em>) population using otolith increment analysis?</td>
<td>Dana Rudy</td>
</tr>
<tr>
<td>17:20</td>
<td>F-403</td>
<td>Reconstructing growth chronologies of catadromous eels in New Zealand from historic and contemporary otolith collections.</td>
<td>Eimear Egan</td>
</tr>
<tr>
<td>17:25</td>
<td>F-404</td>
<td>Hatch dates of young-of-the-year Pacific cod tell us the place of their birth.</td>
<td>Masayuki Chimura</td>
</tr>
<tr>
<td>17:30</td>
<td>F-405</td>
<td>Pelagic larval growth and recruitment success of <em>Platichthys flesus</em> in the Mondego estuary.</td>
<td>Ana Primo</td>
</tr>
<tr>
<td>17:35</td>
<td>F-406</td>
<td>Millennia of fishing along the Pacific coast of Panama and the potential impacts on populations and life histories of <em>Cynoscion</em>.</td>
<td>Abhy Verdurmen</td>
</tr>
<tr>
<td>17:40</td>
<td>F-407</td>
<td>Using otolith growth autocorrelation to detect critical periods during early life of fish.</td>
<td>Dominique Robert</td>
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<tr>
<td>17:45</td>
<td>F-408</td>
<td>The diversity of fish otoliths from the sea bottoms: on their taphonomic significance.</td>
<td>Chien-Hsiang Lin</td>
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<tr>
<td>17:50</td>
<td>F-409</td>
<td>Otolith shape analysis - a tool for separation of stock in anchovies from Indian waters.</td>
<td>Shardul Gangan</td>
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<tr>
<td>17:55</td>
<td>F-410</td>
<td>Growth estimation of the European hake (<em>Merluccius merluccius</em>) from the Eastern coasts of Algeria, based on length-frequency and otolith analyses.</td>
<td>Nadjette Boursalah</td>
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</tbody>
</table>
Tuesday 18th April 2018

Workshop IV program

NOTE: Participants should each bring a laptop.

Session 1 [09:00-11:00]

09:00 Opening remarks
09:05 Introduction to chemical archives in fishes beyond otoliths: A review on the use of other body parts as chronological recorders of chemical constituent. Orian Tzadik, Malte Willmes, Patricia L. Luque
09:20 Elements and elasmobranchs – using elemental chemistry of calcified structures for conservation and management of sharks. Bronwyn M. Gillanders
09:50 Exploring the elemental and isotopic proxies in dorsal fin spines: an alternative chronological recorder that complements otoliths analyses in Bluefin tuna. Patricia L. Luque
10:00 Fin ray microchemistry as a tool to reconstruct White Sturgeon life history. Malte Willmes
10:10 Fish Eyes, Fish Ears: Revealing the Secret Lives of Fishes with Eye Lens and Otolith Chemistry. Hadis Miraly
10:20 Application of enriched 113Ba tracer to mark juvenile Persian sturgeon (Acipenser persicus). Hadis Miraly
10:30 Radiocarbon and Nd isotopic compositions in fish skeletal hard parts as new proxy for habitat use and migration ecology. Kotaro Shirai
10:40 Fish and stable isotopes - examples from Finnish studies. Sami Vesala
10:50 Otolith microchemistry research in the Baltic Sea region and beyond. Mehis Rohtla
11:00 Otolith microstructure at early life history stages. Troy Rogers
11:10 Filling in the blanks – where and how can chemical archives add value to archival tagging programmes for marine fish & shellfish? Ewan Hunter
11:20 COFFEE BREAK OR REFRESHMENT

Session 2 [11:30-13:00]

General discussion and concluding remarks

Drafting a summary document with the information gathered at the workshop and outline a path forward to be presented at the AF5 Symposium, Atlanta 2018.

Discussion points:
- Analytes: new analytes that potentially document movement, diet, physiological events, or environmental conditions
- Tissue types: soft, hard or a multiproxy approach
- Instrument and lab techniques: i) recent advances in microchemical analytical techniques (Microsampling, Mass spectrometry techniques, SIMS, X-Ray, XPS), ii) the need of validation studies using a controlled lab setting.

Poster

Theme: Chemistry and Composition

PC-01 Hatching cohort discrimination in stotolith microstructure and chemical signatures of swordsp squid (Loligo ridulph edulis) off northeastern Taiwan. Zhi-Zheng Hsu
PC-02 Application of enriched 113Ba tracer to mark juvenile Persian sturgeon (Acipenser persicus). Hadis Miraly
PC-03 Fish Eyes, Fish Ears: revealing the secret lives of fis hes with eye lens and otolith chemistry. Hadis Miraly
PC-04 Sr/Ca and Ba/Ca ratios in otoliths - tracing the migratory behavior of grey mullet in the estuary. Sung-Hung Yang
PC-05 Proteomics study of otolith matrix proteins. Marta Kaika
PC-06 Using the otolith 87Sr/86Sr ratio to reveal the differences in habitat salinity among three sympatric ninespine sticklebacks (genus Pungitius). Konomi Fudamoto
PC-07 Uptake and fractionation of nitrogen stable isotopes in otolith organic materials. Jen-Chieh Shiao
PC-08 Synchrotron µ-X-ray fluorescence analysis of Baltic cod otoliths reveals high resolution patterns in Sr concentrations. Kate McQueen
PC-09 Comparison of analytical methods assessing otolith chemistry. Viktor Finnäs
PC-10 Eye lenses as an alternative to otoliths for reconstructing habitat use of juvenile salmon. Rachel Johnson
PC-11 Exploring sub-population structure of Atlantic bluefin tuna (Thunnus thynnus) within the Mediterranean Sea. Patricia Lastra
PC-12 The effect of laser spot size on data averaging and accuracy while using LA-ICPMS. Karin Limburg
PC-13 29 years on, your PhD otoliths may still surprise you. Karin Limburg
PC-14 Procedures for LA-ICPMS: the use of R software and Bayesian analyses for trace element quantification. Silvia Pérez-Mayol
PC-15 Retracing the habitat use and movement patterns of sockeye salmon (Onchorhynchus nerka) in the North Pacific Ocean. Wade Smith
PC-16 Change in otolith density as age progressed using CT scan. Satoshi Katayama
PC-17 Stock composition analysis and connectivity of Prochilodus lineatus from Plata Basin (South America) using otolith fingerprints: temporal variation of a dominant cohort. Alejandra Volpeado
PC-18 Spatial segregation and connectivity in young and adult stages of Megaleporinus obtusidens inferred by using otolith signatures and management implication. Alejandra Volpeado
PC-19 Otolith shape and stable isotopes analysis as stock discrimination tools for Yellowtail snapper, Ocyurus chrysurus, in northeastern Brazilian coast. Beatrice Ferreira
PC-20 Where does it go? Provenance and stock structure of capelin in Greenland using microchemistry. Peter Fink-Jensen
PC-21 Effect of temperature on calcium carbonate structure δ18O and δ13C of cuillefish. Ching-Yi Chen
PC-23 Horning natal and resident behavior in a giant Amazonian catfish. Karin Limburg
Theme: Life History and Fisheries

PL-01 The application of SIMS δ¹⁸O analysis for estimating spawning temperature of Pacific bluefin tuna using otolith. Yulina Hane

PL-02 Using digital imaging of otolith dissection in ageing of Baltic herring (Clupea harengus membras). Sami Vesala

PL-03 Remarkable homing ability of a pelagic cruncarp "Carassius auratus grandulus" endemic to Lake Biwa: Evidence from otolith Sr stable isotope. Yoshiyuki Uehara

PL-04 Unraveling the life cycle of three small gobies: age validation and determination. Beatriz Morales-Nin

PL-05 Between reader differences in age estimation of Baltic cod larvae and early juveniles. Katarzyna Spich

PL-06 Historical variation in age structure of parental stock of the southern hake (Merluccius australis), hoki (Macrurus magellanicus) and southern blue whiting (Micromesistius australis) fishery resources in southern Chile. Vilma Ojeda

PL-07 Natai origin and feeding grounds of adult Pacific bluefin tuna (Thunnus orientalis) before the spawning. Ching-Chun Cheng

PL-08 Spatial gradients and temporal variations in size at age of Northeast Arctic cod (Gadus morhua) off the coast of Norway during the last two decades. Côme Denechaud

PL-09 Movement patterns of brown trout (Salmo trutta) in a large New Zealand river system. Roland Svigdsden

PL-10 Combining otolith analysis and particle drift modeling to identify and locate the spawning areas of the gilthead sea bream Sparus aurata in the Gulf of Lions (NW Mediterranean). Frank Ferraton

PL-11 Otolith Shape Indices as a tool for discriminating the stocks of Milkfish (Chanos chanos) in Indian waters. Sri Hari Murugesan

PL-12 Lunar timing of migration patterns differ between migrant and non-migrant types of a partially migratory fish. Heidi Heim-Ballew

PL-13 Determining habitat use in juvenile lionfish: addressing the nursery hypothesis. Derek Hogan

PL-14 Age and growth of tailor (Pomatomus saltatrix) in eastern Australia. Hayden Schilling

PL-15 Determination of daily age of pikeperch fry in a deep temperate reservoir. Petr Blabolil

PL-16 Investigating connectivity and temporal changes in nursery habitat use for two over-exploited local stocks of the southern hake (Merluccius australis) in Northwestern Patagonia. Maylis Labonne

PL-17 Determining life history of Blueback Herring in the Mohawk River using otolith microchemistry. Cara Ewell Hodkin

PL-18 Otolith increment formation reveals growth patterns in early life history of tropical clownfish Amphiprion ocellaris. Thanh Nguyen Thi Hai

PL-19 Discrimination by the otolith of the landlocked Japanese Grenadier Anchovy Coilia nasus. Tomoyuki Jouachi

PL-20 First estimates of age and growth of four-spot megrim (Lepidorhombus bosci) on the Porcupine Bank (west of Ireland). Carmen Hernández

PL-21 Age-Based Growth Variation in Populations of Green-Blotched Parrotfish, Scarus quoyi in Southern Philippine Seas. Merlene Elumba

PL-22 Application of isotopic analysis to reconstruct habitat use of Japanese sea bass (Lateolabrax japonicas) and discrimination from reared fish. Yu Hsieh

PL-23 Otolith chemical composition of Solea solea and Merluccius merluccius along the Northeast Atlantic and the Mediterranean Sea. Audrey Geffen

PL-24 Spatial variance in otolith chemistry of juvenile Pacific halibut (Hippoglossus stenolepis): the importance of scale-dependence and trending for correctly inferring nursery origins. Timothy Loher

PL-25 Tracking natinal habitat contributions in a freshwater migratory fish, Clear Lake hitch (Lavinia exilicauda chi) using otolith strontium isopes. George Whitman

PL-26 Evaluation of ²³⁰Th mass-marking technique in the eary-life-history stages of Sepioteuthis lessoniana. Chun-I Chiang

PL-27 Preliminary study on age and growth of Apolemichtys arcaus and Holocanthus africanus (Pomacanthidae). Chen-Yi Lee

PL-28 Otolith microchemistry reveals the decline of wild fall-run salmon on the Feather River, California. Malte Willmes

PL-29 Distribution of naturally recruited Japanese eels in rivers as inferred from otolith stable isotope ratios. Hikaru Itakura

PL-30 Estimation of the age of jack mackerel (Trachurus murphyi) using otolith weight, daily growth rings and annual rings, do it easy! Lilian Cisterna

PL-31 Using daily growth increments in otoliths to age fourfinger threadfin (Eleutheronema rhdinum) in waters off western Taiwan. Yi-Sin Lu

PL-32 Migratory dynamics of narrow-barred Spanish mackerel (Scomberomorus commerson) in the water off Taiwan. Jen-Chieh Shiao

PL-33 Studies on age and growth of Sparus latus in the waters off Taiwan. Shao-Jhu Chiu

PL-34 Age, growth and reproductive study of the mangrove snapper (Lutjanus argentimaculatus) in the waters off Taiwan. Yun-Ting Yeh

PL-35 Using otolith microchemistry to discriminate wild and released Red snapper (Lutjanus argentimaculatus). Yu-Ren Huang

PL-36 Study on Age Growth of Acanthopagrus schlegelii in Kinmen area. Guan-Yi Li

PL-37 Biological tags in fin rays and otoliths for identifying "released" fourfinger threadfin (Eleutheronema tetradactylum) in coastal waters off western Taiwan. Nan-Jay Su

PL-38 Age and growth of eleven fish species from the western Arabian Gulf. Yu-Jia Lin

PL-39 Young-of-the-year Yellowmouth barracuda Sphyraena viridensis (Cuvier, 1829) growth in Eastern Algeria based on otolith microstructure analysis. Boureihail Nadjette

PL-40 Preliminary Result of Age and Growth Research of Pacific Bluefin Tuna Caught by Taiwanese Longline Vessels. Hsin-Ming Yeh

PL-41 Migration of giant catfish from the Orinoco River basin revealed by otolith microchemistry. Karin Limburg

PL-42 Early life-history of the fork-tail siganid Siganus argenteus inferred from otolith microstructure. Víctor Soliman

PL-43 Annual variability in growth and maturation of Uroteuthis edulis in the Yilan Bay. Chih-Shin Chen

PL-44 Age and Growth of the Acoupa weakfish, Cynoscion acoupa (Lacepéde, 1801), on the north coast of Brazil. Francisco Marcarante Santana
Theme: Morphology and Physiology

PM-01 Microphotogrammetry for the obtention of 3d otolith images. Francisco Cerna
PM-02 Morphometric analysis of sagitta otoliths of Polydactylus virginiensis, Menticirrhus littoralis, and Canodon nobilis in Sergipe, Brazil. Kátia de Meirelles Felizola Freire
PM-03 Ontogenetic and intraspecific changes in otolith shape of anchoveta (Engraulis ringens) as indicator of stock structure in the Pacific southeast off Chile. Francisco Cerna
PM-04 Understanding the Population Structure of the European Anchovy (Engraulis encrasiciulus) in the Black Sea, Mediterranean Sea and Northeast Atlantic Ocean by Using Otolith Shape Analysis. Kélig Mahe
PM-05 Comparison of otolith morphology and growth rate of Central Baltic herring populations. Szymon Smoliński
PM-06 Inter- and intraspecific relationships in the genus Antimora (Gadiformes: Moridae) based on the comparison of the otolith shape. Alexander Arkhipkin
PM-07 Otolith shape development in young-of-the-year chub (Cyprinidae, Squalius cephalus). Pierre Gibert
PM-08 Otolith age estimation by Mojette Transform descriptors and machine learning. Kélig Mahe
PM-09 Evolutionary trends on the sagitta otolith shape of recent marine bony fishes. Antoni Lombarte
PM-10 Otolith morphometry and fish length relation of Amblycephalodon moio (Hamilton,1822) caught from middle Ganga region (India). Neetu Nimesh
PM-11 What makes the otolith magnesium chemical calendar-clock tick? Plausible mechanism and empirical evidence. Karin Limburg
PM-12 Validation of the periodicity of growth increment formation in sprat (Sprattus sprattus) in the eastern North Sea. Olof Lövgren
PM-13 Otolith Shape Analysis and Dimensions of an endemic species (Alburnus tarichi (Güldenstädt, 1814)) inhabiting Lake Van (Turkey). Nazmi Polat
PM-14 Age and Morphometric Variations in two Terapon jarbua (Forskål 1773) populations in Mindanao, Philippines. Maybelle Fortaleza
PM-15 Fish Otoliths from a Tidal Flat Ecosystem of Brazil (Western Atlantic Ocean). Carolina Siliprandi
PM-16 Morphology of otoliths and other characteristics of the Gadidae genera Eleginus, Gadus and Micragadus, and mutual relationships of these genera. Pieter Gaemers
PM-17 Atlas of fish otoliths of the Argentina. Alejandra Volpedo
PM-18 New contour descriptors with invariance to rotation, to scale, and to position. Pere Martí-Puig
PM-19 Morphological variation in the Sagitta of Menticirrhus americanus (Linnaeus, 1758) in a subtropical environment. Alejandra Volpedo

Theme: Sclerochronology and Environment

PE-01 Application of otolith analysis to study the occurrence of temperature-dependent sex determination in wild cobaltcap silverside. Kaho Miyoshi
PE-02 Losing track of time: Use of otolith chemistry to solve the problem of age determination in Baltic Sea cod. Yvette Heimbrand
PE-03 Extraction of high resolution juvenile life history information from adult otoliths of diadromous fishes. Tymofiy Sposivy
PE-04 Evaluation of age reading methods for the invasive round goby, Neogobius melanostomus. Martina Blass
PE-05 Use of AFORO otoliths database in recent reconstruction of deep-fish communities and their relationship with recent climate changes. Antoni Lombarte
PE-06 Daily growth patterns of three species of young-of-the-year of the Eastern coasts of Algeria fishes. Bourhadj Nadjet
PE-07 Tracing of escaped meagre (Argyrosomus regius) through otolith microchemistry. Silvia Pérez-Mayol
PE-08 Daily increments of juvenile fish under environmental change – a case study with Baltic Sea perch. Rickard Yngwe
PE-09 Modelling the growth of juvenile Dolphinfish: Relating daily environmental factors and otolith microstructure. Beatriz Morales-Nin
PE-10 Estimating hypoxia exposure rates and consequences using redox-sensitive chemical markers in otoliths. Benjamin Walther
PE-11 Age and growth of Scyllorhinus canicula (L., 1758) in the Strait of Sicily from vertebrae readings. Flavia Berlinghieri
PE-12 Otolith growth and chemical chronologies in dusky grouper: the effect of ENSO. Patrick Reis-Santos
PE-13 Validating annual age of roach (Rutilus rutilus) from harsh environments using daily increments. Malin Hållbom
PE-14 The temperature-growth relationship in two co-existing populations of juvenile Atlantic cod (Gadus morhua). Peter Groenkaer
PE-15 Otolith opacity tracks annual cycles of the bioenergetic status of adult Atlantic cod (Gadus morhua). Peter Groenkaer
### Theme: Statistics and Modelling

**PS-01**  The effect of ageing errors on Von Bertalanffy parameters estimation using a Bayesian sensitivity analysis approach. Matthias Vigon

**PS-02**  Evaluation of the effects otolith sampling strategies and ageing error on the precisions of age composition and growth curve: a case study for the Pacific bluefin tuna. Jhen Hsu

**PS-03**  Exploring 2-d otolith multi-element microchemistry maps with cluster analysis software. Dennis Swaney

### Theme: Others

**PO-01**  Diadromous Fishes in the Lower Mekong Basin: a review. An Vu

**PO-02**  Ageing of fish in Swedish waters by SLU Fish Ageing Network. Marie Leiditz

**PO-03**  SmartDots : a flexible open source software tool for fish age reading based on otoliths. Kevin De Coster

**PO-04**  An otolith interdisciplinary network birth in Latin America: origins and challenges. Alejandra Volpedo

**PO-05**  Preliminary study on the Plio-Pleistocene otoliths of Taiwan. Chien-Hsiang Lin

**PO-06**  Age and growth of the finless porpoises (*Neophocaena*) in the Taiwan Strait. Kuan-Tzu Huang

**PO-07**  Establishing relationship of fossil otoliths through geometric morphometrics: a case study of sciaenid otoliths from the Eocene gulf coast. Chien-Hsiang Lin

**PO-08**  Using otolith microchemistry to determine migratory patterns of Indonesia freshwaters fishes: A prospects review. Vipen Adiansyah

**PO-09**  Otolith collection of Panamanian Caribbean Fishes. Brigida De Gracia

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### Participant List

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Field metabolic rate recorded in otoliths: Geochemical techniques used to study fish physiological ecology

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2 Ocean and Earth Science, University of Southampton, UK
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Metabolic rate is a fundamental property setting daily energy requirements for individuals and knowledge of metabolic costs associated with maintenance, foraging, growth and reproduction under natural conditions are important for understanding the energetic consequences of climate change at population, community and ecosystem levels. If we want to understand how animals perform in the wild and operate within a complex environment, time-integrated individual-level field metabolic rate is the ecologically and evolutionarily relevant trait to study, and it can be investigated through natural stable carbon isotope tracers in otoliths. The isotopic composition of carbon in fish otoliths is linked to oxygen consumption through metabolic oxidation of dietary carbon. The proportion of metabolically derived carbon can be estimated with given knowledge of δ¹³C values of dissolved inorganic carbon in the water, and inorganic and organic otolith carbon, assuming that the δ¹³C values of the organic otolith material mirrors that of the dietary carbon. We describe the mechanistic relationship between otolith inorganic δ¹³C values and oxygen consumption under laboratory conditions, and the proportion of metabolically derived carbon demonstrates an increasing exponent decay relative to oxygen consumption. The laboratory-determined relationship is applied to free ranging wild fishes, showing that reconstructed field metabolic rates are 3.5 to 1.5 times higher than standard metabolic rates from the larval to adult stages. This developed methodology offers a possibility to evaluate individual energy budgets and specific body-mass scaling to metabolism, and to compare behavioural responses to environmental changes among species, populations and geographic distributions. The information contained in the carbon isotopic proxy can be utilised in ecological models to obtain more accurate and precise predictions of population dynamics exposed to environmental changes, especially temperature increases.

Keywords: Stable carbon isotope, Otolith organic materials, Atlantic cod, Climate change
Fish otoliths: Unravelling the secrets of shape diversity and function

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In the fish ear, otoliths play an important role for the senses of balance and hearing. Otoliths also serve as useful tools in fisheries sciences. Considering the tremendous shape diversity in fossil and extant otoliths, two main questions arise. Why did solid carbonatic otoliths with species-specific shapes evolve in teleost fishes whereas most other (aquatic and terrestrial) vertebrates possess numerous tiny otoconia? How do differences in otolith shape and mass affect ear function such as hearing? Until now, the selective forces and/or constraints driving the evolution of solid otoliths and otolith shape diversity in teleosts are largely unknown. I will briefly present a hypothetical framework proposing that the advent of otoliths may have initially been a selectively neutral “by-product” of other key innovations during teleost evolution and subsequently otolith shape diversity evolved while auditory abilities in teleosts improved. From this evolutionary viewpoint, I will focus on functional aspects which have to be scrutinized for a better understanding of otolith evolution as well as effects of e.g. global climate change along with ocean acidification on fish hearing. To elucidate otolith function, we need integrative and comparative approaches, that 1) yield quantitative data characterizing the structural relationship of otolith, otolithic membrane, and the underlying sensory epithelium in 3D in different teleost groups, 2) implement these 3D data in advanced mathematical modelling of otolith motion, and 3) include new experimental set-ups enabling us to precisely characterize the relative motion between otolith and sensory epithelium. I will present and discuss outcomes from my current projects investigating fish ear morphology in 3D and visualizing the in-situ motion of otoliths in combination with advanced modelling. In these projects, imaging techniques such as high-resolution contrast enhanced microCT, neutron imaging, and hard X-ray phase contrast imaging provide important tools for more detailed structural and functional insights.

Keywords: Otolith motion, Otolith evolution, Fish hearing, X-ray imaging
The evolution of Otolith Science (?)

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Each symposium has been a platform to showcase a growing number of techniques and approaches centred on information contained in otoliths. The original Age and Growth of Fish conferences were concerned with validation and age estimation, and the revolution in larval fish studies triggered by the discovery of microincrements. Over time, a critical mass of researchers developed, eager to discover new applications for otolith analysis, and the occasion conference about otolith research and application evolved into an International Otolith Symposium series. Looking back through the previous symposium volumes, especially through the themes highlighted by the convenors, it is clear that the evolution can be represented as a rapid adaptive radiation. The diversity of themes has increased to the point where we can ask whether there is, in fact, a field of “Otolith Science”. Taking a phylogenetic approach to the IOS themes, we can examine the relationships between techniques and research questions – and speculate on future developments.
Laser ablation AMS reveals complete bomb $^{14}$C signal in an otolith with confirmation of 60-year longevity for red snapper (*Lutjanus campechanus*)

Allen H Andrews$^1$*, Christiane Yeman$^2$, Caroline Welte$^2$, Marcus Christl$^2$, Lukas Wacker$^2$, Bodo Hattendorf$^3$

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Red snapper (*Lutjanus campechanus*) is an important fishery species in the Gulf of Mexico. Fishery sustainability is supported by knowledge of valid life history parameters, like longevity. Maximum age for fishes was often underestimated because the age-reading method was not validated—the maximum estimated age for red snapper ranges from ~35 year to more than 50 years by counting growth zones in otolith (fish ear stone) cross sections. The age of some fish species has been validated with bomb $^{14}$C dating—using the rise in marine $^{14}$C due to atmospheric testing of thermonuclear devices in the 1950s and 1960s—by extracting calcium carbonate from the otolith core (earliest growth) with a micromilling machine. This core material is analyzed for $^{14}$C and an alignment with a coral bomb $^{14}$C reference to determine age. The method is typically limited to a single sample because the temporal specificity of micromilling becomes difficult as the otolith grows in successive layers that become thinner with increasing age. Hence, a fish with a pre-bomb birth year can be aged to only a minimum birth year of ~1958 because the coral $^{14}$C reference levels plateau prior to this date. To provide a basis for age validation beyond the normal limits of bomb $^{14}$C dating, red snapper otoliths were analyzed for $^{14}$C using a novel laser ablation accelerator mass spectrometry (LA-AMS) technique to provide a nearly continuous record of bomb $^{14}$C uptake. As a result, the maximum validated age for a red snapper is now approaching 60 years and the method show promise as a more efficient way to validate the age of fish from otoliths.

**Keywords:** Radiocarbon, Gulf of Mexico, Lutjanidae, Age validation
The organic flight recorder: Lifetime connectivity and habitat use reconstructions with fish otolith amino acids

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With increasing disturbances to fish habitats, it is crucial to understand which and how habitats are utilized during a fish’s lifetime to predict the impact on the population. The otoliths proteinaceous matrix can give us a unique insight into such habitat use by elucidating resource utilization, trophic position (TP), and migration. With the analysis of δ¹³C and δ¹⁵N values in otolith amino acids we describe how these parameters illustrate diverse life histories among individual Cynoscion acoupa.

In the Amazon region of Brazil, the carnivorous C. acoupa is known to occur in mangrove estuaries as larvae and juveniles, and exclusively on the coastal shelf as adults. To track resource utilization across different size classes of C. acoupa, we inferred amino acid δ¹³C fingerprints (δ¹³CEAA) from otolith cores and edges. While the δ¹³CEAA fingerprints generally follow the expectation that early life stages tend to be supported by estuarine algal resources and adults by coastal shelf resources, this resource utilization pattern inclined to be interchangeable between these life stages. Our δ¹⁵N analysis of AAs results support that adults tend to have different feeding strategies: C. acoupa adults exploit TPs between 3 and 4, but during juvenile stages all individuals displayed a consistent TP of ~2.8 in habitats that were distinct from the adult stage. We hypothesise that low TP and estuarine resource use can indicate that some adults in the Amazon occupy habitats closer to the coast, potentially predating on fish that make migrations out of the estuaries. Our study shows that the C. acoupa population is quite versatile on an individual level where estuarine resources can be important at all life stages. Thus, this relatively new approach of utilizing the otoliths organic matrix has great potential to aid in identifying habitat use and connectivity.

Keywords: Amino acids, Trophic position, Resource utilization, Migration
Impact of fishing activities on Faroe marine food web investigated using δ¹³C and δ¹⁵N of archived otolith (1950-2014)

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It is widely accepted that fishing activities can alter the composition of fish communities, the life history traits of the populations and the structure of food webs. But conversely, the changes in trophic functions, i.e. prey-predator interactions, created by these perturbations remain largely unexplored, although crucial for understanding marine population dynamics. So far, this lack of knowledge was primarily due to the complexity of assessing trophic relationships over time in a natural habitat, but a method developed recently allows the use δ¹³C and δ¹⁵N from otolith organic matter to reconstruct historical diet characteristics. In this study, we used this method to infer the effects of fishing activities on a marine food web structure and function. To this aim and to understand the influence of the food compartments on these potential disturbances, we used an archived collection of otoliths from four co-occurring gadoids experiencing different degree of fishing pressure and belonging to different trophic niches, namely the pelagic saithe, the benthic-pelagic cod, the demersal haddock and the blue ling. For each species, we assessed the annual δ¹³C and δ¹⁵N of otoliths from individuals caught in Faroe Islands between 1955-2015 (1084 otoliths in total: 331 for haddock, 359 for saith, 341 for cod and 53 for blue ling), these signatures were corrected for baseline variations of the Faroe region using the isotopic signatures of A. islandica shell protein. Beside the degree of fishing pressure and the trophic compartment, our model also included environmental parameters (e.g. SST, pH) and climate indices (e.g. NAO, AMO) in order to disentangle the effect of climate change from that of the fishing activities. The results of this study, the first investigating the long term effects of fishing activities on marine trophic functioning, will contribute to a better understanding of fishing impact on harvested ecosystems.

Keywords: Impacts of fishing activities, Food web, Otolith, δ¹³C and δ¹⁵N
Eye lens core $\delta^{14}C$ validates otolith-based age estimation in Western Atlantic gray triggerfish, *Balistes capriscus*

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Gray triggerfish, *Balistes capriscus*, support economically important fisheries in U.S. waters of the western Atlantic and Gulf of Mexico, where stock status in each region is estimated with age-structured integrated assessment models. Traditionally, gray triggerfish ages have been estimated via translucent zone counts in dorsal spine sections. However, a significant difference between counts of dorsal spine (translucent) and otolith (opaque) growth zones suggests considerable bias exists in age estimates derived from at least one of these structures. The bomb radiocarbon chronometer has been successfully employed to validate otolith-based age estimation in other western Atlantic and Gulf of Mexico reef fishes, but gray triggerfish otoliths are too small to effectively core and spines may be subjected to physiological reworking. Therefore, eye lens cores, which are composed of the protein crystallin and not resorbed once formed, were extracted and analyzed for $\delta^{14}C$ with accelerator mass spectrometry. Resulting data were plotted against otolith-derived and spine-based birth year estimates which were superimposed on a function fit to a regional coral and known-age red snapper, *Lutjanus campechanus*, otolith $\delta^{14}C$ time series. Data based on otolith-derived ages were within the 95% prediction intervals of the coral-red snapper function but diverged considerably from expectation for spine-based birth year estimates. Therefore, it appears that while otolith opaque zone counts provide accurate estimates of gray triggerfish age, dorsal spines are not reliable ageing structures for this species. On average, mean sex-specific size at age was approximately 15% smaller for otolith- versus spine-based age estimates, and estimated longevity nearly doubled with otolith ageing. These results have important implications for natural mortality and growth rate estimates, as well as for stock assessment performance.

**Keywords:** Eye lens, Radiocarbon, Age validation
Inferring life history of a long-lived deepwater grouper based on otolith radiocarbon analysis

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3 Riverside Technology, Inc. under contract to National Marine Fisheries Service, SEFSC, Panama City Laboratory

Warsaw grouper, Hyporthodus nigritus, is a western Atlantic deepwater species that is listed as a species of concern by the U.S. NMFS and as critically endangered by the IUCN. However, little information exists on its life history in the Gulf of Mexico (GOM) and its stock status in that region is currently unknown. The goal of this study was to investigate Warsaw grouper life history in the GOM based on otolith microstructure and radiocarbon analysis. Age was estimated via opaque zone counts in otolith thin sections. Traditional methods used to verify or validate annual opaque formation are problematic for this species given year-round samples are not available, and chemically marking otoliths was impractical given the outer shelf and upper slope depths adults occupy. Therefore, we employed the bomb $^{14}\text{C}$ chronometer to validate ageing in Warsaw grouper. Otolith cores ($n = 14$) were analyzed with accelerator mass spectrometry and resulting $\Delta^{14}\text{C}$ values were plotted against a regional coral $\Delta^{14}\text{C}$ time series. Results from an analysis of residuals between predicted $\Delta^{14}\text{C}$ values from a Loess regression fit to the coral time series and the measured values in Warsaw grouper otolith cores indicate there is no significant difference in the two data series, thus validating otolith-based ageing and enabling growth and longevity estimation for Warsaw grouper. Comparison of otolith $\Delta^{14}\text{C}$ values versus a deepwater coral $\Delta^{14}\text{C}$ time series from the upper slope of the nGOM (depth ~310 m) also suggests Warsaw grouper are more likely to spend their early life on the shelf before moving to deeper (to 600 m) waters later in life. Overall, results of this study provide critical life history information for Warsaw grouper, and also suggest otolith radiocarbon analysis of other poorly studied deepwater reef fishes in the western Atlantic may be illuminating.

Keywords: Otoliths, Age validation, Warsaw grouper, Deepwater species
Elemental histories from archival-tagged fish

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A key objective for investigations wishing to use otolith chemistry to provide a natural marker of location is to demonstrate that variations in otolith chemistry caused by intrinsic effects are smaller than those reflecting changes in ambient environmental conditions. To gain a fuller picture of the relative importance of intrinsic and extrinsic influences on otolith chemistry in wild plaice, Pleuronectes platessa, a sample of male and female fish from the western (WNS), central (CNS) and eastern North Sea (ENS) with “known” movements and environmental histories based on archival tag records were studied. Daily geolocations were estimated by matching archival tag-recorded pressure (depth) records with a tidal database when an individual spent a full tidal cycle on the seabed. Temperature records, hidden Markov models and ‘behavioural switching’ between high and low activity states were used to refine geolocation estimates and provide a ‘most probable track’ for each fish. Otoliths from the same individuals were analysed across the experimental period (202-560 days) using SIMS (δ¹⁸O, Sr, Ba, Mg, K, Li) and high-resolution LA-ICPMS (Sr, Ba, Mn, Zn, Cu). For each element, spatial and temporal trends were examined and linear mixed effects models used to explore whether concentrations were best explained by extrinsic (population subunit, migratory behaviour, temperature, salinity) or intrinsic (sex, age, size, growth rate, condition) factors. With a specific focus on the elements Li, Mn, Sr and Ba, the potential for each element to serve as a natural geolocator is considered.

Keywords: Pleuronectes platessa, SIMS, LA-ICPMS, Population structure
Distinguishing brown trout (*Salmo trutta*) parr from different natal streams and hatcheries based on otolith elemental fingerprints – with some insights on adult sea trout natal origin

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Sea trout (anadromous form of brown trout (*Salmo trutta*)) is ecologically, recreationally and commercially important fish species in coastal waters and rivers around the world. Currently, many sea trout stocks in the Baltic Sea are considered to be in sub-optimal state and therefore different management and conservation actions require ecologically valid information for implementation. Present study used brown trout parr otolith elemental signatures combined with water chemistry data to: (i) explore the relationship between otolith and water chemistry (ii) examine if and to what extent brown trout parr from different spawning rivers and hatcheries can be distinguished from one another and (iii) give some insights on natal origin of adult sea trout caught from the coastal waters of Estonia. Juvenile brown trout (n=592) were collected from sea trout spawning streams and hatcheries in Estonia (n=28 streams; one hatchery), Finland (n=3; three hatcheries), Latvia (n=8; four hatcheries) and Sweden (n=1). Water samples were collected from 63 Estonian and 19 Finnish streams. Adult sea trout (n=312) were collected from several areas in the Estonian coastal sea. Sr:Ca, Ba:Ca and 87Sr:86Sr were the most useful markers in distinguishing parr from different rivers and streams. For Estonian and Finnish fish the mean reclassification accuracy for parr was 84.4%. Preliminary data indicated that adults can mix thoroughly during sea residency and in some regions almost half of the sampled adult fish were immigrants from other countries. For example, adults caught from the Estonian northern coast were a mixture of Estonian and Finnish origin fish. Latvian fish were common around Saaremaa (West Estonian Archipelago). Most notably, hatchery reared fish were well represented in all sampled areas. The results indicate that although sea trout recruitment in Estonian coastal sea is mainly driven by local fish, immigrants from foreign streams and hatcheries are substantial in catches.

**Keywords:** Otolith microchemistry, Brown trout, Baltic sea, Management and conservation
Habitat use, thermal history, and growth of the endangered winter-run Chinook salmon during a drought period

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The resilience of species to climate change depends greatly on their physiological plasticity and to some extent on their capacity to shift their distribution. However, climate change poses an additional challenge to endangered species since it might put them on the brink of extinction. This is the case of the Sacramento River winter-run Chinook salmon (winter-run Chinook hereafter) in the California Central Valley, United States. Winter-run Chinook is listed as an endangered species, and much of its imperiled status owes to the destruction of their natural spawning habitats, like many other anadromous fish species. Winter-run Chinook historically spawned exclusively in the headwaters of the Sacramento River basin, but now their spawning habitat occurs in a highly modified habitat along a stretch of few kilometers downstream a dam. Winter-run Chinook spawning season occurs during summer, and cold water is essential for the survival of eggs and juveniles. During the 2012-2015 drought in California, the amount of cold water released from dams into the Sacramento River was insufficient for winter-run Chinook to maximize reproduction success. The first few survivors of the 2012-2015 drought returned in 2017 to spawn. Thus, this work aims at disclosing the life and thermal histories of the first winter-run Chinook survivors of the 2012-2015 drought before their ocean-exit, and compare it with those of individuals reared in non-drought conditions. We hypothesize that adjacent habitats to the Sacramento River main stem continued to be vital for winter-run Chinook, as observed during non-drought conditions. However, early growth must have been lower due to heat stress. Life histories and drought impacts were assessed by combining strontium and oxygen isotopes analyses to identify rearing habitats and thermal histories with sclerochronology measurements of sagitta otoliths. As droughts will become more frequent and severe, this work will disclose essential information for winter-run Chinook management plans.

Keywords: Climate change, Drought, Endangered species, Otolith chemistry
Reconstructing life history and thermal resilience of a small endangered fish in California from strontium and oxygen isotope ratios

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The Delta Smelt (Hypomesus transpacificus) is a small pelagic fish and rapidly approaching extinction. It is endemic to the Sacramento-San Joaquin Bay-Delta which is an important link in California’s water supply. This estuary is managed for human use as well as for several species of threatened and endangered fish and the Delta Smelt is at the center of conflict between human and environmental uses of the limited water resources. Extensive monitoring surveys have shown that Delta Smelt exhibit a diverse life history with both resident and migratory phenotypes within a genetically homogenous population, however the details of this life history remain unclear. Here we used strontium (87Sr/86Sr) and oxygen (δ18O) isotope tracers from archived Delta Smelt otoliths to reconstruct life history and thermal resilience at fine temporal scales (<1 week). 87Sr/86Sr ratios from otoliths can be used to reconstruct salinity habitats, while δ18O reflects the water temperature a fish has experienced. δ18O is also influenced by the ambient water conditions and species dependent isotope fractionation. As a first step, we calibrated the δ18O temperature-dependent fractionation and validate the reconstruction of ambient water temperature from otolith δ18O using Delta Smelt from lab experiments at UC Davis. This new method will then be applied to wild-caught Delta Smelt for which detailed salinity habitat reconstructions based on 87Sr/86Sr have already been established. The combination of these two isotopic tracers will allow us to investigate the relationship between Delta Smelt abundance, freshwater outflow and water temperature. Understanding this relationship can give new insights into resilience and habitat utilization of Delta Smelt in the face of warming water temperatures during prolonged drought periods and long-term climate change.

Keywords: Strontium, Oxygen, Endangered, Resilience
Bias in fish length back-calculation models derived from otolith shape ontogeny

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Otoliths have been widely used in many fishery and ecology studies to access growth, structure and dynamics of fish populations. Through age rings it is possible to back-calculate the size of fish through time using mathematical models. However the models available in the literature do not account for shape dissimilarities between individuals and populations. The shapes of the European perch (*Perca fluviatilis*) otoliths were compared among fish of various ages and populations. Age readings were performed on whole otoliths of more than 700 *P. fluviatilis* individuals from 12 freshwater reservoirs of Czech Republic and The Netherlands. Photos of *P. fluviatilis* otoliths were taken from a fixed distance, aspect, magnification and camera to avoid bias due to different image processing procedures. Images were then imported to R software where the outlines of individual otoliths were obtained. Otolith outlines were posteriorly decomposed using Fourier transformation and then analyzed using Multivariate analysis of variance (MANOVA). The standard body length of *P. fluviatilis* were back-calculated using 5 widely used models (Biological intercept, Dahl-Lea, Fraser-Lee, Monastyrsky and Modified-Fry) and a Three-parameters logistic model (3PL). Otolith shape varied among reservoirs and ontogenetic variation were also recorded. The ratio between otolith length and width was lower in small and big individuals (ovate shape) compared to intermediary sizes (lanceolate shape). The comparisons of back-calculation models revealed that 3PL model outperformed other models in all reservoirs, being particularly noticeable in reservoirs where the ontogenetic variation in otolith shape was stronger. Our study highlighted that the otolith ontogeny is an important issue for body length back-calculations and the 3PL elegantly cope with this issue, providing more accurate estimative of fish length through the entire life cycle should be chosen in detriment of models that does not cope with otolith ontogeny.

**Keywords:** *Perca fluviatilis*, Back-calculation, Growth
Variability in otolith shape shows evidence of stock structure in Patagonian toothfish (*Dissostichus eleginoides*) in the Southwest Atlantic

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Otolith shape analysis is a useful tool for discriminating between fish stocks. While retaining its unique taxonomic characteristics, otolith shape can vary in relation to disparate environmental and genetic factors, or differences in body condition or growth. In the southwest Atlantic, life-history traits of a deep-sea notothenioid, Patagonian toothfish (*Dissostichus eleginoides*), suggest a potential for high levels of connectivity through advection by currents during a protracted egg and larval stage of development or infrequent long-distance migrations of adults. While toothfish populations found on the Patagonian Shelf are genetically distinct from South Georgia, limited genetic structuring has been observed within each of those locations. Oceanographic modeling and otolith microchemistry studies suggest complex localized population structures. However, the complexity of subdivisions and connectivity between regional and within localised population groupings of this species remains unclear. Here we show how variability in otolith shape can be used to reflect isolation and connectivity within and among stocks of Patagonian toothfish across the Patagonian Shelf, and South Georgia and the South Sandwich Islands (SGSSI). Variation in otolith shape indicated clear differentiation between fish from the Patagonian Shelf and SGSSI confirming population isolation between these two geographic regions. Furthermore, significant differences in the otolith shape of fish caught within these two regional groupings suggests that despite some degree of connectivity, large-scale mixing of adult toothfish across the Patagonian Shelf and SGSSI are limited. These results have provided some alternative insights into stock structure of Patagonian toothfish across southern South America, South Atlantic and SGSSI. We anticipate these findings to provide a baseline for future research further investigating the variables explaining the dynamics of Patagonian toothfish stock structure on a range of temporal and spatial scales.

**Keywords:** Stock structure, Otolith shape, Patagonian toothfish, Southwest Atlantic
Genetic factors have a major effect on otolith shape in Atlantic herring (*Clupea harengus*)

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Atlantic herring, *Clupea harengus*, have complex population structures and different populations can be found in fully marine as well as nearly freshwater conditions. Mixing of populations is known, but the extent of connectivity is still unclear. Ripe spring spawning herring were collected in marine (35 psu, Atlantic) and brackish water (6 psu, Baltic Sea) conditions. One Atlantic herring female was crossed with one Atlantic and one Baltic male generating an F1-generation consisting of Atlantic purebreds and Atlantic/Baltic hybrids which were incubated and co-reared at two different salinities, 16 and 35 psu, for three years. The F1-generation was repeatedly sampled and otoliths were extracted for shape analysis. Atlantic purebreds grew better than Atlantic/Baltic hybrids at 35 psu, but not at 16 psu. Otolith aspect ratio were higher for Atlantic purebreds than Atlantic/Baltic hybrids, consistent with the parental groups. There were no differences in otolith aspect ratio between herring with the same genotype but raised in different salinities. A Canonical Analysis of Principal Coordinates was applied to analyze the variation in wavelet coefficients that described otolith shape. The first discriminating axis identified the differences between Atlantic purebreds and Atlantic/Baltic hybrids, while the second axis represented salinity differences. These results demonstrate that otolith shape have a significant genetic component and are potentially useful in studies of population dynamics and connectivity.

Keywords: Otolith shape, Atlantic herring, Hybrids
Separating fish stocks in a mixing area by using genetics and ShapeR based otolith shape analysis: a case study on Baltic Sea cod (Gadus morhua L.)

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For sustainable fisheries management, fish stocks in a mixing area need to be separated according to their stock affiliation. When tissue samples are available, genetics is the approved tool to assign fish with unknown origin to its fish stock. In some cases and particularly for historical samples, tissue is often not available, but archived otoliths. The combination of otolith-based shape analysis and genetics enable the determination of both present and past mixing proportions. Here, we show for the first time how genetic information and the newly developed R package ShapeR can be combined for assigning unknown fish otoliths to their original stock, using the two Baltic stocks of cod Gadus morhua L. as a case study. Stock specific genetic markers, i.e. single nucleotide polymorphisms (SNPs) were developed from whole genome data to genotype individual cod from the mixing area (Arkona Sea), as well as from reference areas (Belts, Sounds and Bornholm Basin). Their otolith shapes were analysed using normalized elliptic Fourier descriptors from the ShapeR package. Subsequently, the otolith shape baseline, derived from genetically validated cod reference samples, was used to assign otolith shapes with unknown origin either to the western or eastern Baltic cod stock. By combining these two stock separation techniques, we were able to determine the mixing proportions of Baltic cod stocks from the last 40 years with a classification success of 85%, which in turn could be validated by additional genetic analyses of tissue attached to historical otoliths. The ShapeR package is a convenient and time saving approach to analyse large amounts of otolith shapes for developing time series of mixing proportions in transitional areas of fish stocks. This is of great importance for establishing meaningful reference points for mixed stocks with historically fluctuating stock levels.

Keywords: Otolith shape analysis, SNPs, Baltic Sea, Cod
Spatial distribution, total length frequencies and otolith morphometry as tools to analyze the effects of a flash flood on populations of roach (*Rutilus rutilus*)

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Floods are known to be the major source of natural variability and disturbance in stream ecosystems. However, the management and channelization of large rivers have impacted the fluvial geomorphology and disconnected the main channel and floodplains used as nurseries by many species of fish. This study examines the influence of a first autumnal flood event of the Ardèche River on the roach population, *Rutilus rutilus* (Linnaeus, 1758), in the Caderousse reach on the Rhône River (South of France). Fish were sampled in three contrasted sections of the reach before and after the first autumnal flood event. This influence was evaluated through an analysis of the spatial distribution for the abundance and size of juvenile roach and an analysis of otolith shape by both Fourier and shape indices. Juvenile roach from the three sections of the reach formed well-defined local populations before and after the flood event. Juvenile roach were always more abundant in the less modified section (BPS) of the river than in the other sections (RES and TAIL) for the two year studied. While the original primary river channel was directly impacted by the flash flood, no significant difference in otolith shape was observed before or after the flash flood. Our results suggest that this part of the reach provides refuge habitats used by fish during high flow events.

**Keywords:** Cyprinidae, Otolith morphometry, Lower Rhône River, Flood event
New services in AFORO (Shape analysis of otoliths): From data-base to data-bank

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The AFORO system (http://aforo.cmima.csic.es) is a public access web site to an otolith guide. It was conceived as a database cataloguing fish otolith images and since then, the system has evolved by incorporating many other services to interactively exploit the database information. Thus, in 2006, AFORO incorporated an expert system dealing with automatic taxon identification based on wavelets (WT) applied to otolith contours. Further improvements have been made since then with the inclusion of a 3D representation of otoliths, and the connection to a database of complete morphometric information (measures and morphological indexes) considering, among others, Elliptic Fourier (EFA) and wavelet (WT) descriptors. Since 2014, specific software for automatic computation of morphometric parameters from scaled images is available at this site. Recently, two new services have been implemented: 1) a remote system access to obtain WT (at 9 levels) from the contour of an object; and 2) a database of parameters of the potential function allowing to compute the fish length based on the otolith length for roughly about 400 species. A remarkable aspect is that these functions are updated when new specimens are included in the database. These new changes improve the biometric database, making it especially appealing for research applications in Paleontology, Archeology and Trophic Ecology. Presently, the number of specimens uploaded has increased, reaching to 5707 images, and thereby giving a biodiversity representation corresponding to 1716 species (belonging to 234 families) as well as its geographical extension. Thanks to the invaluable collaboration of different authors, the database has been enriched with images (permission allowed) from already published specialized books on otoliths. Thus the AFORO system includes now species from North Atlantic (Campana; Leopolod et al), Persian Gulf (Sadighzadeh et al) and Taiwan waters (Lin & Chang) among others.

Keywords: Morphometry, Wavelets, Shape analysis, Automatic taxon identification
Comparative morphology of the otoliths of clupeid fishes from Japan and application for species identification

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Analysis of the stomach contents of marine animals is an important tool to elucidate the aquatic food web but digestion of the ingested materials often limits its usefulness. Fish otoliths are relatively resilient to digestion and may retain their original shape for a considerably length of time in the digestive tract of predators, and thus provide valuable insight on the diet of piscivorous species such as mammals, sea birds and fish. This study examined the amount of intra/interspecific variation of otolith shape in clupeid fishes, a dominant prey item in the diet of piscivorous predators in adjacent waters of Japan, in order to provide diagnostic features for prey species identification during stomach content analysis of these predators. Otoliths from twelve clupeid species (*Etrumeus micropus*, *Spratelloides gracilis*, *Ilisha elongata*, *Sardinops melanostictus*, *Sardinella zunasi*, *S. lemu*, *S. melanura*, *Herklotzichthys quadrimaculatus*, *Clupea pallasii*, *Konosirus punctatus*, *Nematalosa japonica* and *N. come*) were included in the interspecific morphological comparison. In the intraspecific comparison, the ontogenetic changes in otolith morphology of *S. melanostictus* and *E. micropus* were investigated. In the interspecific comparison, variation was detected in morphological features such as the outline shape, otolith length/height ratio (OL/OH), excisura depth, location of the crista superior and its crest-like ridge, and edge characteristics of the ventral margin. In the intraspecific comparison, the OL/OH of *S. melanostictus* increased with growth over the range of SL analyzed whereas that of *E. micropus* increased up to 100 mm SL and then levelled off. The outline shape became elongated in both species and in *S. melanostictus* a projection on the dorsal margin developed in fish above 100 mm SL. It is concluded that a combination of the above features, with due consideration to interspecific variation, provides suitable diagnostic tools for species identification based on otolith morphology.

**Keywords:** Otolith morphology, Clupeidae, Stomach contents, Species identification
Otolith shape: A population marker for Atlantic and Pacific herring

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Atlantic herring (Clupea harengus) is one of the most abundant fish species in the world and due to its commercial value, an understanding of its population dynamics, is needed for the sustainable management of the resource. The biogeography of herring is complex and populations are often defined on where and when they spawn. Extensive sampling of two herring species, Atlantic and Pacific herring (C. pallasii), was conducted throughout the North Atlantic, along the coast of Norway, Russia, and the Pacific. A large number of otoliths needed image processing and therefore the open source software package shapeR was created in the programming language R. Otolith shape variation was detected among herring populations compared and these differences could be traced to three morphological structures on the otolith which showed a correlation with the stocks spawning time. A classifier based on shape differences could discriminate with 94% accuracy between the Icelandic summer-spawners and Norwegian spring-spawners, which are known to mix at feeding grounds east of Iceland. In separate studies on local populations in Norway, variation in otolith shape was detected, and among local populations along the coast, a latitudinal gradient emerged where neighboring populations were more similar to each other than to those sampled at larger distances. These morphological differences are likely to reflect environmental differences but also indicate low dispersal among local herring populations. At the species level, a comparison was conducted between Atlantic and Pacific herring and among subspecies of Pacific herring which revealed similarity of herring occupying the Bering Sea in the NW-Pacific, Balsfjord in N-Norway and the SE-Barents Sea in Russia, results which are in accordance with former genetic studies. The results of these studies show that otolith shape can serve as a marker to identify herring populations, subspecies and species at small and large geographic scales.

Keywords: Otolith shape, Population discrimination, Population dynamics, Pelagic fish
A combined phylogenetic and otolith morphometric analyses of ecomorphological trends in Pomacanthidae

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Fishes of the Pomacanthidae, commonly as marine angelfishes, distribute widely in coral reefs in tropical oceans of the world. There are at least 8 genera and 82 species of pomacanthids in the world. A great variation in zoogeographic range, fish shape and size enables pomacanthids an adequate model for ecomorphological analysis. In this study, four molecular markers (COI, 16s, Rhodopsin, and ETS2) and three morphological methods (outline morphometric parameters, elliptic Fourier analysis, and geometric morphological analysis) were examined for otolith sagittae in Pomacanthidae in a global scale. The ecomorphological trend and phylogenetic inertia will be discussed.

**Keywords:** Pomacanthidae, Otolith morphology, Phylogeny, Ecomorphology
The rapid acquisition of high-resolution chemical profiles across otolith increments has led to an explosion of data from diverse taxa in coastal, marine and freshwater systems worldwide. Advances in instrumentation have widened the periodic table of elements that can be reliably assayed across growth increments, and two-dimensional mapping of chemical patterns has further illuminated spatial heterogeneity across these complex structures. The primary goal for most otolith chemistry studies is typically interpretation of single or multi-elemental signatures to identify putative origins, nursery habitats or lifetime environmental exposure histories. Yet, although the environmental drivers of observed elemental signatures in otoliths are often assumed to be paramount, multiple intrinsic and extrinsic biotic and abiotic factors can modify or even disrupt the ability of certain elements to provide a straightforward reflection of a single environmental parameter alone. Further, our understanding of the unique responses of specific elements to these factors allows a greater appreciation for the diversity of processes that may be recorded in multi-element profiles. Insights into phylogenetic constraints, physiological status, ion transport across membranes, dietary contributions, and calcification dynamics are all necessary to continue developing the field of otolith chemistry so that it can provide defensible interpretations of fish life histories. Although the importance of these factors were appreciated in the earliest days of otolith chemistry analyses, gaps in our understanding remain that must be filled for further progress. This talk will discuss advances and challenges in understanding environmental drivers, physiological regulation, and calcification dynamics that affect our ability to effectively interpret elemental patterns in otoliths, and suggest future avenues of experimentation and collaboration to drive the field forward.

Keywords: Chemistry, Environment, Physiology, Migration
Is otolith Mg adding nuance to the hypoxia story?

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Hypoxia is a growing problem worldwide, contributing to habitat degradation and loss, with adverse consequences for many fish species and fisheries. Recently, otolith Mn/Ca ratios were demonstrated to correlate strongly with fish dwelling in hypoxic vs. normoxic habitats (Limburg et al. 2015). We have been monitoring other trace elements as well, including the physiologically sensitive element magnesium, another divalent cation. In a separate poster, we provide evidence here that Mg/Ca ratios reflect metabolism, and propose a two-stage mechanism for the uptake and incorporation of Mg into otoliths. Here, we present evidence that Mg/Ca can reflect the metabolic state of a fish living under hypoxia, particularly seasonal hypoxia. This takes the form of a “decoupling” of Mg/Ca from Mn/Ca, in situations where hypoxia is particularly severe, and demonstrate that Mg/Ca in those cases is correlated with poor condition factor (Fulton’s K). Thus, Mg/Ca has potential to bring nuance to our understanding of hypoxia impacts on individual fish, such that we may proxy both exposure (through otolith Mn/Ca) and response (otolith Mg/Ca) lifetime histories.

Keywords: Mg/Ca ratios, Hypoxia, Fish condition
An innovative approach to ageing fish otoliths with near infra-red spectroscopy (NIRS)

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Fish ages are one of the most fundamental data elements of integrated stock assessments because they provide critical information on recruitment, growth, maturity, mortality and production which inform models and management advice. Despite the increase in demand for age data, the capacity to support the need has not increased. As such, we investigate an innovative technology to derive machine-based ages from near Infra-red spectroscopy (NIRS), which is a method of measuring light absorbance signatures in organic substances. After the initial calibration-validation procedure, scan and age estimation of ageing otoliths takes 30 seconds for walleye Pollock compared to approximately 5 minutes per specimen by a trained ageing expert, so efficiency can be increased 500% to 1000%. Based on a pilot study of EBS walleye Pollock from otoliths collected during the 2016-2017 survey years, we successfully replicated the ageing process using FT-NIR with equal or better precision than the traditional ageing process. While these results are extremely promising additional research is needed to evaluate the spatial and temporal variation in calibration models from different species (i.e., Pollock vs. Pacific cod), to evaluate quality control procedures (statistical metrics) in an operationalized ageing process, and quantify the cost-benefit of moving away from a traditional to machine-based ageing approach.

Keywords: Near infra-red spectroscopy, Otolths, aging, Walleye Pollock
Pathways of trace elemental incorporation into bivalve shells

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The formation of bivalve shells relies on calcium ions as mineral substrate. During growth other element impurities potentially record physicochemical changes of the surrounding environment. Of particular interest are divalent cations that can substitute Ca\(^{2+}\) in the crystal lattice. However, the underlying mechanisms responsible for their incorporation into shells have not yet been fully elucidated. It is commonly assumed that Ca\(^{2+}\) and other divalent ions may share the same transport mechanisms because of similar ionic radii and electrochemical properties. So far, little effort has been devoted to clarifying this interpretation. To disentangle the role of Ca\(^{2+}\) in shell formation and elemental incorporation, the Asian clams (Corbicula fluminea) were reared for four weeks in three sets of experiments: (1) different aqueous Ca\(^{2+}\) levels (3, 4, 5 and 6 mM); (2) the presence of Ca\(^{2+}\)-channel blockers (lanthanum: 1 and 10 mM; verapamil: 0.1 and 1 mM), and (3) the presence of Ca\(^{2+}\)ATPase inhibitor (ruthenium red: 0.1 and 1 mM). Our findings demonstrate that altering water Ca\(^{2+}\) level did not affect the rate of shell growth. Likewise, two Ca\(^{2+}\)-channel blockers had little effect on shell production, but exposure to 1 mM ruthenium red led to ca. 32% reduction. On the other hand, with increasing water Ca\(^{2+}\) level, the amounts of Mn, Cu and Sr incorporated into the shells decreased, whereas the Zn, Ba and Pb contents remained constant. Evidently, blocking Ca\(^{2+}\)-channel significantly reduced the incorporation of Mn, Cu, Zn and Pb into the shells and of these elements, Mn/Cashell and Cu/Cashell decreased simultaneously when inhibiting Ca\(^{2+}\)-ATPase. Nevertheless, neither Sr/Cashell nor Ba/Cashell were changed implying intracellular Ca\(^{2+}\) transport mechanisms are not responsible for their incorporation into the shells. Findings of the present study may pave the way for disentangling the driving mechanisms responsible for the element partitioning between the ambient water and the shells.

Keywords: Bivalve shells, Trace elements, Incorporation pathways
Trace element-protein interactions in endolymph from the inner ear of fish: implications for environmental reconstructions using otolith chemistry

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Otoliths, the biomineralised hearing “ear stones” from the inner ear of fish, grow throughout the lifespan of an individual, with deposition of alternating calciferous and proteinaceous bands occurring daily. Profiles of trace element:calcium ratios measured by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) are often used in to reconstruct environmental histories. There is, however, considerable uncertainty as to which elements are interacting with either the proteinaceous or calciferous zones of the otolith, and thus their utility as indicators of exogenous (i.e., environmental) or endogenous change. To answer this, we used size exclusion chromatography-inductively coupled plasma mass spectrometry (SEC-ICP-MS) of endolymph, the otolith growth medium, to determine the binding interactions for a range of elements. In addition, we used solution ICP-MS to quantify element concentrations in paired otolith and endolymph samples to estimate relative enrichment factors. We found 12 elements that are present only in the proteinaceous fraction, 6 that are present only in the salt fraction, and 4 that are present in both. These findings have important implications for the reconstruction of environmental histories based on changes in otolith elemental composition: (1) elements occurring only in the salt fraction are most likely to reflect changes in the physico-chemical environment experienced during life; (2) elements occurring only in the proteinaceous fraction are more likely to reflect physiological rather than environmental events; and (3) elements occurring in both the salt and proteinaceous fractions are likely to be informative about both endogenous and exogenous processes, potentially reducing their utility for reconstructing environmental or life-history events.

Keywords: Otolith microchemistry, Physiological regulation, Organic matrix, Element partitioning
The protein landscape of the fish inner ear: an integrated omics approach

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Otolith growth is mediated by a range of organic macromolecules. Research to date has identified less than a dozen potential otolith matrix proteins. In addition to these, a handful of accessory proteins and other macromolecules thought to be implicated in biomineralization have been identified in the endolymph as well as in the cells of the kinocilia and saccular epithelium. Our understanding of the structure and function of these macromolecules as well as their probable metal cofactors is still developing. Otolith chemical profiles have typically been used to reconstruct environmental histories of fish. However, there is considerable uncertainty as to whether variation in trace element concentrations reflect environmental change or endogenous processes. We previously employed size exclusion chromatography-ICP-MS to determine the range of protein-element interactions present in endolymph of black bream (Acanthopagrus butcheri). To further elucidate this issue and identify potential metalloproteins, we employed an integrated multi-omics workflow. First, we sequenced and assembled the transcriptome of black bream. This was annotated through basic local alignment techniques and used to create a species-specific search database. We then extracted and digested the organic phase of otoliths and endolymph from wild adults. These were fractionated through reversed-phase high performance liquid chromatography (RP-HPLC) and used to generate proteomic data through nanoscale liquid chromatography-tandem mass spectrometry (Nano LCMS/MS). Finally, mass spectra were then searched against our transcriptome database, resulting in the discovery of many previously unknown inner ear proteins and metalloproteins. Our work both increases the current understanding of otolith biomineralization, as well as raises further questions regarding the interpretation of chemical chronologies in otoliths.

Keywords: Biomineralization, Proteomics, Transcriptomics, Metalloproteins
“Lend me your ears”: macroecological study of the field metabolic rate of fishes

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Field metabolic rate (FMR) is the total (time averaged) metabolic cost experienced by a wild organism operating in its natural environment. There has been increasing interest in the macroecological study of FMR particularly as metabolism defines many aspects of species’ responses to climate change, and FMR is a more ecologically relevant measure of metabolism than standard or maximum metabolic rate. Despite this interest, there are relatively studies of FMR in ectotherms, particularly fishes - largely due to a lack of suitable measures of FMR. It is well known that carbon released to the body from diet (metabolic carbon) is depleted in 13C compared to dissolved inorganic carbon derived from ambient waters. $\delta^{13}C$ values in otoliths therefore vary with the relative rate of metabolic oxidation of carbon (i.e. oxygen consumption and metabolic rate. Consequently, it is possible to use otolith $\delta^{13}C$ as a proxy for FMR. The availability and ease of sampling otoliths available makes this technique ideal for the study of FMR at a macroecological scale. We aim to investigate macroecological drivers of FMR in teleost fishes by examining how FMR varies among phylogenies, climate zones, habitats and functional groups. We will achieve this by collecting otoliths from fish taxa spanning broad ranges in phylogenies, lifestyle characteristics, for example migratory behaviour and depth range and climate zones. Here we present preliminary analyses showing ecological differences in FMR across a wide range of North Atlantic fish species. We are in the process of expanding our analyses by establishing collaborations with other otolith scientists.

**Keywords:** Field metabolic rate, Macroecology, Stable isotope
Stable carbon isotopes as metabolic biomarkers

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Understanding past physiological trends in vital in anticipating future population responses. A geochemical tracer of fish metabolic rate would allow development of long-term histories which would unearth a wide-range of historical biological and ecological trends. This study assessed the relationship between metabolic rate and carbon isotopes ($\delta^{13}$C) in otoliths and investigated carbon source contributions to otolith composition. Juvenile snapper (*Chrysophrys auratus*), a commercially, culturally and recreationally important fish species in Australasia, were raised in varied temperatures for up to two months and intermittent-flow respirometry was used to measure oxygen uptake rates and calculate metabolic rates (Standard, Maximum and Absolute Aerobic Scope). Otolith $\delta^{13}$C, diet $\delta^{13}$C and tank dissolved inorganic carbon (DIC) was then analysed using isotope ratio mass spectrometry (IRMS). We determined proportional contributions from DIC and diet to otolith $\delta^{13}$C with temperature and metabolic rates driving this relationship. Metabolic rates and $\delta^{13}$C both reflected thermal performance curves. These findings refine the use of an isotopic biomarker in otoliths and increases its efficacy in biological reconstructions, with methods developed being applicable to other fish species.

**Keywords:** Stable carbon isotopes, Carbon uptake, Metabolic rate, Geochemical tracer
Applicability of otolith delta13C and delta15N isotope analysis for addressing ecological questions

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Analysis of delta13C and delta15N isotopes has gained increasing interest to study trophic interactions in fish. We will demonstrate the applicability of otolith isotopes for addressing feeding ecology of Atlantic cod (Gadus morhua) in the North Sea by testing whether 1) the opportunistic feeding behaviour of cod reflects a generalist strategy of the individuals or individual diet specialization on a number of different prey types, 2) specialization persists across years, and 3) growth rates differ between feeding groups, by linking growth patterns with stomach content composition, environmental temperature and delta13C and delta15N isotopes. Four feeding groups were identified where one prey type dominated the stomach contents: ‘Sandeel’, ‘Herring’, ‘Norway pout’ and ‘Crustaceans’. Growth patterns were estimated from measures of otolith growth increments and delta13C and delta15N isotope composition was analysed separately for the year of capture and the year before that. Cod as a species are opportunistic predators while individuals show a strong preference for a specific prey. Prey type had a significant effect on growth, while temperature had no effect. Slowest growth was observed in the ‘Sandeel’ group, the ‘Norway pout’ group the fastest. No significant difference occurred between the ‘Crustaceans’ and ‘Herring’ groups. delta13C and delta15N signals in the year of capture differed significantly between feeding groups, suggesting that cod had a preference for specific prey throughout the feeding season. However, isotope patterns within feeding groups did not overlap between years in the ‘Norway pout’, ‘Sandeel’ and ‘Herring’ groups suggesting that individual cod’s prey selection is not consistent across years. An exception was the ‘Crustaceans’ group where the overlapping isotope signals suggest a strong selection for crustaceans across years. Combined information from otolith microstructure and chemical composition thus provides a useful tool for studying patterns of prey selection and resulting growth patterns in wild fish.

Keywords: Nitrogen and carbon isotopes, Prey selection, Growth, Atlantic cod
Temperature-dependent trophic fractionation of stable nitrogen and carbon isotopes in otolith organic matter

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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. However, ambient temperature may influence the trophic enrichment of stable isotopes from diet to predator tissue. To test this, a 10-month rearing experiment with juvenile cod held at four different temperatures (4, 7, 10 and 14°C) was conducted. In total otoliths from 97 fish and heart and muscle tissue from 117 fish were used for the analysis. There was a decrease in otolith δ¹⁵N with increasing temperature of -0.041 ‰ per °C (F₁,₈₂ = 6.3, P = 0.014) and a decrease otolith δ¹³C with increasing temperature of -0.11 ‰ per °C (F₁,₈₂ = 23.2, P < 0.001). The absolute growth of the otoliths during the experiment did not have a significant effect on the whole otolith isotope values (ANCOVA, P > 0.25). Only the δ¹⁵N of muscle tissue showed a similar response to temperature decreasing 0.022 ‰ per °C (F₁,₁₀₃ = 16.3, P < 0.001), whereas there was no effect on heart tissue and muscle δ¹³C. Our results indicate that the trophic signals recorded will reflect changes in diet isotope values with a minor, albeit significant, effect of the temperature experienced by the fish. The results from this study will be used when testing archived cod otoliths for diet changes over the past century.

Keywords: Trophic position, Nitrogen isotope, Temperature experiment, Northeast Arctic cod
Discriminating wild and hatchery red snapper by using stable C and N isotopic signals on fish scales

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The aquaculture of red snapper *Lutjanus argentimaculatus* has earned a huge concern throughout the world and is one of the important species in fry releasing program for stock enhancement in Taiwan. To assess the impact of hatchery groups on wild populations, identifying fish origin is a critical issue. In this study, wild and hatchery fish were collected and three culture experiments were conducted, including group A and B fish fed by different artificial feeds in the indoor tank and group C fish kept in semi-open seawater to feed natural bait. Stable isotopes of scales were analyzed for discriminating wild and hatchery red snapper. The isotopic signals of the hatchery fish enriched ontogenetically. The $\delta^{15}$N in the scale core of group C and B were the same, but different in the edge. The two groups of fry were from the same hatchery but later fed on different foods, with consistency to the stable isotope pattern. The $\delta^{13}$C of hatchery samples were $-17.2 \pm 0.9 \%$ that were significantly lighter and more uniformed than the wild population at $-13.4 \pm 2.2 \%$. Results of cluster analysis and random forest model analysis were found that 94.7 % of fish were correctly classified. The study demonstrated that the chemical signals in scales is an sensitive recorder for non-lethally fish origin determining method and can be applied to trace releasing and restocking program for further ecological evaluation.

**Keywords:** *Lutjanus argentimaculatus*, Natural tag, Restocking program
Isotope tools to track floodplain rearing of native fishes

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Previous research shows that juvenile salmon and other native fishes that gain access to floodplains grow faster than those that remain in the river. This is due to a relatively productive food web created by a longer residence time of water, lower water velocities, and the decomposition of plant matter compared to the extremely channelized Sacramento River, California, USA. We observed fish from the Yolo Bypass floodplain having isotopically light and distinct sulfur isotopes (\(^{34}S/^{32}S; \delta^{34}S\)) in their prey content and muscle tissue. Juvenile salmon on the floodplain were depleted in \(\delta^{34}S\) in muscles \((\delta^{34}S = -1.7\pm2.9, 1999; \delta^{34}S = -1.67\pm1.1, 2016)\) relative to salmon collected in the Sacramento River \((\delta^{34}S = 4.3\pm4.7, 2012; \delta^{34}S = 7.2\pm1.04, 2016)\), consistent with the trend in particulate organic matter between the two adjacent habitats. Microbial decomposition of plants (e.g., rice) on floodplains fractionates sulfur isotopes which are incorporated into the invertebrate prey items of salmon, their muscle tissue, and permanently recorded in their eye lenses and otoliths. Here, we investigate the feasibility of using sulfur isotopes in juvenile salmon eye lenses and/or otoliths to link the extent to which floodplains have population-level benefits to multiple salmon populations and other native fishes.

**Keywords:** Sulfur, Salmon, Habitat, Migration
Natural abundance nitrogen isotopic composition of the organic matrix of fish otoliths across diverse taxa

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Otoliths are composed of calcium carbonate, as the mineral aragonite, and a small amount of organic matter called the organic matrix (OM). Natural abundance nitrogen isotopic content (δ¹⁵N) of fish tissues is a useful tool for investigating diet and migration in fishes; however, the δ¹⁵N of the nitrogenous components in otolith OM is still an underutilized source of information in part due to lack of validation across many species. Here, we compared δ¹⁵N of white muscle tissue (δ¹⁵N_{wmt}) and otolith OM (δ¹⁵N_{oto}) for twenty-four teleost fish species across seven orders. We also hydrolyzed otolith OM to measure the variations in amino acid concentrations (AAs) across fish taxa as a coarse assay for differences in organic matter composition among species. Lastly, we developed a simple model incorporating the differences in time averaging between otolith and white muscle to understand the implications of whole-life-history averaging provided by whole-otolith δ¹⁵N analyses. We report that δ¹⁵N_{wmt} and δ¹⁵N_{oto} are highly correlated across and within species for wild and farmed fish including freshwater, estuarine, and marine fishes. For most species, the difference between δ¹⁵N_{oto} and δ¹⁵N_{wmt} from the same fish individuals was close to 0. With the exception of otoliths from species in the Gadiforme order (Family: Gadidae), amino acid concentration profiles did not appear to explain interspecies variation in the differences between δ¹⁵N_{oto} and δ¹⁵N_{wmt}. Results from the otolith model suggest that differences in the otolith-to-muscle δ¹⁵N offset are unlikely to derive exclusively from lifetime variations in dietary δ¹⁵N and differences in time averaging between otolith and white muscle. Even with these small interspecies variations in the offset, the high degree of correlation across and within diverse taxa suggests that the factors affecting fish δ¹⁵N_{wmt}—diet, baseline, and metabolism—also control δ¹⁵N_{oto}.

Keywords: Otolith matrix, Nitrogen isotopes, Amino acids
Beyond otoliths – considering the complexities of the ocean environment in order to understand age data for deep-sea corals

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Radiometric methods such as radiocarbon \(^{14}C\), Uranium-Thorium, and lead-210 \(^{210}\text{Pb}-^{226}\text{Ra}\) dating are increasingly being used to obtain colony age of habitat forming deep-sea coral fauna. These methods are particularly important to apply when assumed annual growth zones are not visible in the branch cross-section of the skeleton, or when growth is unable to be observed \textit{in situ}. By applying these methods, various studies on branching scleractinian corals and tree-like gorgonian octocorals are able to determine growth as linear extension rates or radial thickening respectively. For radiocarbon dating, interpreting the resultant \(^{14}C\) age data requires knowledge of regional ocean circulation and the radiocarbon age of the water i.e., the “marine reservoir age”, as well as how errors in the reservoir age can modify age estimates. Altered levels of \(\Delta^{14}C\) can also occur due to localised upwelling events and productivity blooms. All these factors need to be considered when ageing these marine calcifiers and it is in this space that the biologists and oceanographers meet, to help solve these conundrums. Here we describe how we have interpreted recent age data for these important ecosystem taxa.

\textbf{Keywords:} Corals, Ageing methods, Ocean environment
Age and growth of the *Saurida undosquamis* in the water off western Taiwan using otolith ring marks

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*Saurida undosquamis* (Richardson, 1848) is one of most abundant component of trawl fishery captures in the southwestern waters of Taiwan, but they have since decreased dramatically in recent year. Assessment of lizardfish stocks and establishment of fishery management guidelines are urgently needed. A total of 1237 individuals of the *Saurida undosquamis*, collected in the waters of southwestern Taiwan from March 2011 to June 2012, were using for fisheries biology study. A total of 804 individuals with fork lengths (FL) ranging from 85.92 to 416mm were examined using polished thin sections of otolith (sagittae). Monthly changes in the marginal increments, indicate that ring marks form once a year, from February to March. Male and Female specimens both had 1–8 ring marks. The estimated von Bertalanffy growth curves were male as \( FL = 386.251 \times (1- e^{-0.15333 \times (t-2.06)}) \) and that of female as \( FL = 420.158 \times 0.16354 (1- e^{-0.45 \times (t-1.53)}) \). Our results suggest that females grow faster and grow bigger than males. The Growth performance index (Φ) estimated from von Bertalanffy growth equation \((L_\infty, k)\) were male as 4.36 and that female as 4.46. We applied simultaneous confidence region (SCR) approach to the set of nearly Taiwan region. The results consider our Φ is in the credible range of growth index. Monthly variations in the gonadosmatic index and hepatosomatic index indicated that the *Saurida undosquamis* spawned from December to February. Two recruitments per year were found in January to February and April to June.

**Keywords:** *Saurida undosquamis*, Ototlith
SmartDots: A new age reading software tool for the analysis of calcified structures of marine species

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Accurate age determination of fish is essential in fisheries biology for a sustainable management of fish stocks. To be able to perform correct and quality controlled stock assessments, it is imperative to develop reliable age determination methods. To facilitate age readings based on otolith images, a new software tool called SmartDots was initially developed at ILVO (Institute for Agricultural and Fisheries Research) in Belgium for internal use. Subsequently, the SmartDots software was integrated into a complete software platform aiming at managing the data for international age reading workshops and exchanges and automatically reporting the results in a standardized way. The platform (http://smartdots.ices.dk) was developed in collaboration with ICES (International Council for the Exploration of the Sea) and DTU-Aqua (Danish National Institute of Aquatic Resources). The SmartDots age readings platform is an open source solution offering different functionalities. To determine the age of the fish, end-users make annotations on otolith images. Each annotation contains a line and one or multiple dots plus a quality indication and a comment field. The number of dots is equal to the age. Automatic scale detection is included in the software so that distances between dots can be calculated and marginal increment analysis or microstructure analysis can easily be performed. Other features included are: an interactive line-chart which visualises brightness and growth data of the annotation, different dot shapes and colours which can help in annotating the image. All registered data are available in the connected reporting environment. This tool offers many advantages. It facilitates the carrying out and reporting of events related to age reading, making it a user friendly process with an improved management system for otoliths metadata. Moreover, as annotations can easily be compared after analysis the tool facilitates quality control procedures such as testing agreement between readers and calibration exercises.

Keywords: SmartDots software, Age reading, Otoliths, Quality control
Performances of otolith weight as a single predictor of age and a covariate in continuous age-length key in nine fish stocks from western Arabian Gulf

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The performance of using otolith weight to estimate age as a sole predictor and a covariate to construct age-length key was tested using nine fish stocks from five family (Scombridae, Serranidae, Sparidae, Lethrinidae, Siganidae, and Carangidae). The models being tested including generalized linear model with Gaussian, Poisson, and negative binomial for using otolith weight to predict age, and multinomial logistic regression model for the construction of the continuous age-length key. For prediction of the age by the otolith weight alone, the Gaussian model outperformed Poisson and negative binomial model in all fish stocks with smaller average relative prediction error (14 ~ 34 %, 59 to 65 %, and 59 to 65 %, respectively). Adding otolith weight improves the fit of multinomial logistic regression model when constructing a continuous age-length key as indicated by having the lowest Akaike Information criterion in eight out of nine fish stocks. When otolith weight is incorporated, the average relative prediction error have an improvement of 0.7 to 6.6 %. The exception occurred in Kingsolider bream (Argyrops spinifer) that the incorporation of otolith weight information did not improve the construction of continuous age-length key, and the prediction error had an increase of 2.5 %. The ratio of otolith weight and total weight, and the coefficient of determination in the relationship between age and otolith weight did not correlate significantly with the average relative prediction errors, while maximum age was positively correlated to prediction errors of continuous age-length key. The performance of otolith weight as a single predictor of age is probably not affected by fish taxon. When the fish is long-lived, it might be necessary to pool some oldest years to increase sample size in these categories to obtain a reliable age-length key with low prediction error.

Keywords: Otolith weight, Age, Generalized linear model, Age length key
Population structure and recruitment in a forage fish: it’s all in the otolith

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The sandeel (*Ammodytes marinus*) is a key forage fish in the North Sea and there are concerns about the role of climate change and fishing pressure on the state of stocks. Dispersal in this species is limited to the larval phase as sandeels have a close association with coarse sands following settlement. In this study a combination of otolith microchemistry and microstructure was used to gain insights into the scale of population processes and the causes of variation in year-class strength. A combination of supervised and unsupervised Random Forest approaches was used to assess: (1) the potential for variation in otolith chemical signature to discriminate between sub-populations and (2) the number and contribution of larval sources to these sub-populations. Spatial variation in natal otolith element signatures reflected the scale of hydrographical isolation of the sandeel larvae, as inferred from biophysical models, supporting earlier evidence of North Sea sub-populations. Yearclass variation was examined within one such sub-populations based on weekly larval samples and a hatch date analysis of survivors. Comparison between the timing of sandeel hatching and the timing of their copepod prey production revealed that the degree of mismatch was a strong determinant of recruitment.

**Keywords:** Sandeel recruitment, Population structure, Otolith microstructure, Otolith microchemistry
Tell me where you come from and I’ll tell you what you’ll become: importance of carry-over effects for population fitness in the sequential hermaphrodite *Sparus aurata* in the Mediterranean

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Carry-over effects (COE) are thought to be important drivers of fitness variation among individuals of the same population, especially in species who typically inhabit multiple habitats during their lifespan, thereby experiencing several different environments. Difficulty in linking life-history traits across life stages however makes specific examples of COE’s importance for the maintenance of animal populations quite rare. Here, we took advantage of the physiological and environmental information stored in fish otoliths to investigate whether using alternative nursery habitats could explain local intra-specific variation in life history in the sea bream *Sparus aurata*, a sequential hermaphrodite species of high commercial value in the Gulf of Lions (NW Mediterranean). For this, nursery origin was determined for 200 adult sea bream, by matching elemental signatures in the juvenile portion of their otoliths to a multi-annual database of the same signatures gathered from juveniles captured in contrasted local nursery habitats (four coastal lagoons with different depths and salinities and the sea). Then, this information was matched with the size, weight, body condition and sexual status at age of the same fish. Fish lifetime annual growth rates were also assessed (from otolith structure measurements) to investigate the potential influence of a juvenile life in each habitat on later individual survival, size-at-age and reproductive history (COE). The consequences of nursery habitat selection in terms of inter-individual variation in probable fitness (body size, timing of first sexual maturity and of male-to-female transition) are discussed in the light of recent evidence of polygenic adaptation to variable lagoon environments in this species.

**Keywords:** Juvenile habitat quality, Lifetime growth, Sexual history, Fitness
Multiannual life history traits of young-of-the-year chub, *Squalius cephalus* (Linnaeus, 1758) in different reaches of the lower Rhône River: implications from otolith analyses

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Fish growth is inherently plastic in response to environmental conditions such as temperature or flow constraints in lotic ecosystems. At the end of the first growing season, 0-group total length distributions exhibit high variability. Linking early life fish growth variability to abiotic factors requires an accurate and precise ageing tool. We used otolith analysis to assess the spatial and temporal variability of their shape and growth rate in young-of-the-year chub (Cyprinidae: *Squalius cephalus*) in the regulated Lower Rhone River (South of France). We compared results from two reproductive seasons (2013 and 2015) obtained in three contrasting sections of a hydraulic reach to investigate the effects of water temperature regime, streamflow patterns, and hatching date on daily growth rates.

Firstly, the otolith shape was studied to evaluate if fish moved between the three contrasting sections. The high discrimination observed between sections and between the two studied years suggested that fish was resident in sections and that the pattern was conserved in time, but the interannual variability influences highly the otolith shape. The otolith daily growth rates were higher in 2015 than in 2013 but showed similar profiles with a higher growth rate in the tailrace than in the by-pass section and the reservoir. As otolith growth rates increased coinciding with increasing temperature, the environmental conditions seemed to have a significant influence on both otolith shape and growth. The age estimation was used to back-calculate the hatching dates of all fish studied. Several cohorts were identified in both years, confirming the strategy of multiple spawning of the chub. In 2015, the spawning season ranged from April to June was narrower than in 2013 (April to early August). Differences observed were associated to contrasting hydrological and thermal conditions between the two years.

**Keywords:** Regulated river, Otoliths, Shape analysis, Daily growth rate
Lake trout abundance, growth rate and yield across 480,000 lakes in the Canadian Arctic

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Lake trout (Salvelinus namaycush) are one of the most abundant cold temperate fish species in the world, and are a valued target for recreational fishers. Although lake trout biomass is often high in unfished lakes, lake trout populations are easily overfished and slow to recover. Lake trout age, growth and longevity were measured using bomb-validated otolith sections in more than 4600 fish across a range of 55 fished and unfished calibration lakes in the Canadian Arctic. Geospatial and digital elevation data were then coupled with the calibration lake population data to characterize the size, depth and water temperature fields of more than 480,000 lakes in the Canadian Arctic, along with the abundance, growth rate and sustainable long-term yield of their resident lake trout populations. A Google Earth overlay was developed to allow point-and-click access to the lake morphometry, water temperature data and lake trout yield estimates of all 480,000 lakes.

Keywords: Growth, Productivity, Climate, Large-scale
The use of otolith microstructure to infer early life history stages and verify important assumptions in determining the stock structure of Blue-eye Trevalla, *Hyperoglyphe antarctica*

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Blue-eye Trevalla (*Hyperoglyphe antarctica*) is an important high value commercial and recreational target species occurring on the outer continental shelf and upper slope waters, and also offshore seamounts around the southern half of Australia and New Zealand. Little is known about the population structure of *H. antarctica* and even less about their early life history. This is in part because fishery-derived data are sparse, but also because the species has an unusual and elusive early lifehistory. The apparent knowledge gap for this species lead to the need for a wider stock discrimination study to be conducted. This study utilised a different array of methods including otolith chemistry life history profiles and stable isotope analysis, ecological dispersal modelling and the spatial analysis of age and growth across their Australian range. All of these methods rely, in part, on various assumptions made around early life history stages such long their apparent long larval phase, potential habitat use and relatively fast initial growth in the first year of life. In an attempt to verify these assumptions, the micro-increment structure from several Blue-eye Trevalla otoliths was examined to provide an alternate means of obtaining this information and to verify time periods in the otolith which may correspond to the larval period and potential habitat shift. This study also presents the first description of otolith microstructure available for this species and indicates the potential for daily growth zones to provide valuable information on the early life history of species that would be largely unattainable by other traditional means (i.e. catch and release, pre-recruit sampling etc). We also provide further evidence that Blue-eye Trevalla are extremely fast growing initially and that the large length attained at age 1 (40 cmTL) and 2 (50 cmTL), as estimated by annual reading is not unreasonable.

**Keywords:** Blue-eye Trevalla, Early life history, Stock structure, Micro-increments
Use of sclerochronology to obtain life history traits of deep-water fishes

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The use of otoliths to obtain information on fish age and growth, for the purposes of fisheries stock assessments and subsequent management, has been standard practice in this field. However, this assumes that otolith increment formation is annually formed. Unfortunately, not all species have been validated, and this is especially the case for deep-water fishes, where little research has been done and standard validation techniques are of limited use. We investigate the application of sclerochronology methods for age validation of a deep-water snapper, *Pristipomoides zonatus*. This is possible because of crossdating, a dendrochronology method that assumes climate-driven growth patterns that are synchronous and time-specific. It is a rigorous statistical tool that enables multiple individuals to be cross-matched. When individuals display a high degree of precision (an indication of low ageing errors) and there are strong correlations between fishes and climate records, this indicates that the growth increments have been correctly identified and accurately assigned a calendar year, hence the age estimates are correct. The use of these climate-induced natural markers has been successfully shown for long-lived fishes, and here, we show its applicability to deep-water fishes, which are not only data poor, but also tend to be long-lived, making it a suitable candidate for this approach. The successful application of this method would be relevant to other long-lived and deepwater fishes that have not had their otolith increments validated. This method would be a faster and cheaper alternative to traditional age validation methods, and particularly for fisheries agencies in developing countries.

**Keywords**: Deep-water snapper, Life history traits, Fisheries, Sclerochronology
Determining the response of NZ migratory fish to sites of productivity across environmental gradients

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Island systems often have freshwater fishes that exhibit a migration strategy known as amphidromy, where individuals hatch in freshwater and quickly migrate seaward, returning as juveniles to freshwater systems to complete the life cycle. However, partial migration is common among species and populations; partial migration, where only some individuals within a population make a migration, has been documented in several amphidromous New Zealand freshwater fishes. The mechanisms that underscore partial migration are not well understood but it is believed environmental factors are influential. Climate variability can affect the productivity of freshwater and marine systems influencing the success of aquatic migrants between those systems (i.e., diadromy). Migratory success can fluctuate in response to variable environmental conditions such as stream flow or productivity. The primary hypothesis of this study is that alteration in productivity, via natural processes (i.e., El Niño), and distance to a productive site is influential to the proportion of individuals that migrate and successfully recruit to adult populations. Amphidromous species, Gobiomorphus cotidianus was sampled along productivity gradients and longitudinally from oceanic and freshwater productive sites on the South Island of New Zealand. Otolith microchemistry was used to determine the proportion of migrants within adult freshwater populations to assess how distance to productivity sites (lentic vs. oceanic) influences demography of freshwater adult populations across environmental gradients.

Keywords: Amphidromy, Otolith Chemistry, Partial Migration
Age and growth in distinct male morphotypes of a loliginid squid (*Doryteuthis gahi*) in Falkland Islands waters

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Understanding patterns in migration and the degree of connectivity within a population is necessary for stock assessment and management. The Patagonian long-finned squid *Doryteuthis gahi* is a commercially important species within Falkland Islands waters. The population structure consists of two temporally distinct spawning cohorts. Mixing between cohorts maintains genetic homogeneity within Falkland Islands waters, yet the mechanisms connecting these cohorts are yet to be fully resolved. A recent study has suggested that two distinct morphotypes of male squid occur in this species. The elongated mantles and large fins of the rare ‘super large’ males were suggested to be a morphological adaption to facilitate extended migrations and connect geographically distinct subpopulations. Very few age data are available on these larger individuals. Thus, to test the hypothesis that this ‘super large’ morphotype provides temporal connectivity in addition to geographical connectivity, statoliths were used to estimate the age of mature males belonging to both morphotypes and to determine the age structure of the population. To investigate when these ‘super males’ occur and how temperature affects size, hatching dates were back calculated and monthly temperature histories were reconstructed using CTD data collected within the designated fishing zone. Results indicate that ‘super large’ morphotypes are a similar age to their smaller counterparts and are not responsible for providing temporal connectivity, but hatching occurs year-round for the rest of the population, resulting in overlap between cohorts.

**Keywords:** Cephalopods, Statolith, Ageing, Connectivity
Microchemical analyses of fish otoliths have revolutionized fisheries science. Molecules deposited within otoliths may originate from either ambient water or diet, with molecular concentrations being subject to subsequent physiological alteration after exposure. Analyses of otolith microstructure and incorporation of inorganic elements have led to major advances in stock assessment and fisheries ecology. However, the use of otoliths for microchemical analyses has drawbacks. Specifically, otolith removal from live specimens requires specimen sacrifice, which may be forbidden in the case of protected species. In addition, otoliths rarely contain sufficient concentrations of organic matter to allow reconstruction of food-web relationships via multiple stable isotopes, and otolith microstructure can be difficult to interpret in some species. Here, we review alternatives to otoliths that can provide microchemical analytes for life-history studies in fishes. Our focus is to describe advantages and disadvantages to the use of each alternative structure, with particular attention paid to trace-element analysis for inorganic matrices and stable-isotope analysis for organic ones. In general, the chronological analysis of elemental and isotopic values within each structure depends on the inert nature (or lack of molecular turnover) of the tissue. Structures with high turnover rates or those that are metabolically active will not effectively record elemental or isotopic compositions over time. Here we provide an assessment of the use of bony endoskeleton, fin spines, fin rays, scales, and eye lenses as alternatives or complements to fish otolith analysis.
Elements and elasmobranchs: using elemental chemistry of calcified structures for conservation and management of sharks

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Quantifying the elemental composition of elasmobranch calcified cartilage (hard parts) has the potential to answer a range of ecological and biological questions, that can address conservation and management issues. Increasingly, researchers are starting to analyse shark vertebrae, but there is potential to also use spines and teeth for elemental analyses. This presentation will provide an overview of some of the applications of elemental and isotopic analysis in elasmobranchs to date and look at how some of the assumptions around use of calcified structures for population structure and environmental reconstructions are being addressed in elasmobranchs. Gaps in knowledge and future research needs will also be addressed.
Investigating chemical patterns in scales as non-lethal alternatives: a multi-proxy approach

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The use of scales as a non-lethal alternative to otoliths for reconstructing migration and dietary histories of mobile fishes is gaining popularity across the world and in numerous taxa. However, the complexity of scale growth geometry is an important determinant for the potential utility of sequential analyses of both elemental (e.g. strontium and barium concentrations) and isotopic (e.g. carbon and nitrogen isotope ratios) proxies. We present methods and results investigating both types of proxies in scales of a highly migratory euryhaline predator, the Atlantic tarpon, *Megalops atlanticus*. Scales were cross-sectioned for analysis to avoid issues with rugosity and thickness variation inherent in top-down lasering, as well as to target the calcified upper layer exclusively. Paired subsamples from comparable growth increments were also removed for isotope analyses dominated by the poorly-calcified underplated portions of the scales. Consistency in both elemental and isotopic proxies among multiple non-regenerated scales from the same individual indicated these proxies provide reliable information about movements across salinity gradients and associated trophic shifts across different life history stages. Our findings highlight novel opportunities to use scales as non-lethal alternative to monitor fish migrations across chemical gradients in species where sequential sub-sampling is made possible by scale architecture.
Exploring the elemental and isotopic proxies in dorsal fin spines: an alternative biogenic structure that complements otoliths analyses in Atlantic bluefin tuna

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Trace elements and isotopes analysis (TEA and SIA, respectively) on otoliths has been effectively used to address ecological pressing issues such as natal origin, habitat use, and more recently migration patterns in Atlantic bluefin tuna (*Thynnus thunnus*). However, the removal of otolith has also drawbacks, particularly for commercially valuable species like bluefin tuna as it requires manipulating the fish that greatly affects its appearance, diminishing their market value. Alternative hard tissues such as fin spines may provide valuable micro-chemical information that complements otoliths analyses and are particularly useful as a non-lethal otolith’s alternative in endangered or protected species. However, currently we have limited knowledge of the ability of this biogenic structure in preserving elemental and isotopic signatures suitable for inferring life and environmental histories in tuna species. In this context, here I will present preliminary results investigating both elemental and isotopic proxies in dorsal fin spines of a highly migratory marine predator, bringing up where and how fin spine microchemistry would add value to otolith research.
Fin ray microchemistry as a tool to reconstruct White Sturgeon life history

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White sturgeon (Acipenser transmontanus) are a species of special concern in California. They inhabit the San Francisco Estuary and much of their historical freshwater habitat has been severely altered and disconnected due to impassible barriers, insufficient freshwater flows, agricultural diversions, elevated water temperatures, and environmental contaminants. Currently, recovery efforts for sturgeon are complicated by a lack of basic life history information. Previous research has focused primarily on monitoring efforts and tagging studies which have gathered information for only a small portion of the sturgeon life cycle. Analyzing fin ray strontium isotope ratios (\(^{87}\text{Sr}/^{86}\text{Sr}\)) via laser ablation MC-ICP-MS may provide a non-lethal method to resolve fine-scale movement patterns in sturgeons. However, unlike otoliths that are a well-established tracker of life history changes, fin rays are complicated by analytical interferences, and uncertainty regarding their formation timing and resistance to chemical overprint. Using experimental validation studies, we developed new analytical protocols to investigate and overcome these constraints. After validating the method, we examined pectoral fin ray sections from wild adult White Sturgeon collected from the San Joaquin River and the San Francisco Estuary. We found that White Sturgeon exhibited a complex life history, with high variability both across individuals and through time. This new analytical tool will help resource managers refine flow management and habitat restoration strategies for this imperiled species.
Fish Eyes, Fish Ears: Revealing the Secret Lives of Fishes with Eye Lens and Otolith Chemistry

Hadis Miraly, Karin E. Limburg & Roxanne Razavi

For decades, chemical analysis of calcified structures such as otoliths, scales and spiny fin-rays have been used to the study of life history of fishes, from age determination to fish movements. More recently, other “chronometric structures” are increasingly being studied in this way. Among these structures, the eye lens is an interesting candidate for providing chemical information complementary to otoliths. Like otoliths, eye lenses grow throughout the life of a fish; yet unlike the aragonitic (CaCO$_3$) otolith, eye lenses are made entirely of protein covered by an external, living layer of epithelial cells. Further, eye lenses have been found to take up mercury, a known environmental contaminant, preferentially. The aim of this study is to compare the chemical composition of eye lenses with corresponding otoliths of Yellow Perch (Perca flavescens) and Rainbow Smelt (Osmerus mordax) in Lake Erie and Herring (Clupea harengus) from the eastern and central part of Baltic sea to (1) develop a concordance of chronologies between these two structures, and (2) gain better understanding of mercury (Hg) exposure histories. We will test; the uptake trend of mercury (Hg) and Selenium (Se) in otoliths compare with the eye lenses through the fish lifetime, and if Hg interferes with the natural Ca distribution in outer layer of retina. This research will provide useful micro-chemical information which help to better understanding of life history of fishes.
Application of enriched $^{137}\text{Ba}$ tracer to mark juvenile Persian sturgeon ($\textit{Acipenser persicus}$)

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The present study evaluates the variations of $^{137}\text{Ba}$ abundance in pectoral fin spine of 1-month-old juvenile Persian sturgeon ($\textit{Acipenser persicus}$) upon marking using the stable isotope approach. The marking of the fish was achieved by incorporation of $^{137}\text{Ba}^{2+}$ in the calcified lattice of the pectoral fin spine through substitution with structural Ca$^{2+}$. This process was carried out by rearing juveniles in treatment tanks containing elevated concentrations of $^{137}\text{Ba}$ for 1, 3 and 5 days. The marked fish were then retained in natural abundance fresh and brackish waters, to evaluate the trend of exchange of $^{137}\text{Ba}$ from the fin spines. The abundance of $^{137}\text{Ba}$ in fin spines during marking and post-marking experiments were detected by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that a significant isotope mark can be obtained with no mortality and 100% marking rate on the first day of exposure to the isotope. The marked juveniles maintained their isotopic signature for at least 25 days. Statistical analysis of the obtained $^{138}\text{Ba}/^{137}\text{Ba}$ ratios demonstrated that the successful incorporation of $^{137}\text{Ba}^{2+}$ in pectoral fin spines provides an effective marking method for Persian sturgeon restocking programs.
Radiocarbon and Nd isotopic compositions in fish skeletal hard parts as new proxy for habitat use and migration ecology

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One of the challenging topic using fish otoliths is that an identification of habitat use and migration area of the pelagic zone. Amano et al. (2015) proposed radiocarbon and neodymium isotopic composition in fish otoliths are a potential proxy for this purpose. The distribution of radiocarbon in oceanic DIC shows a decreasing trend in the vertical direction and characteristic horizontal distribution patterns of radiocarbon composition ($\Delta^{14}C$) in the pelagic zone. Neodymium isotopes also show a characteristic value depending on water masses. However, the most serious difficulty in using them as the proxy is that relatively large amount of the material is required for the analysis. Our research group is now trying to establish a method for using these compositions as a proxy. As a first step, we applied the analytical technique to fish vertebrae, because it is much easier to obtain more materials than from otoliths. In the presentation, I will present a brief background of these geochemical compositions and recent progress of technical development in our research group.
Stable isotope analysis (SIA) has become more widely used in a large variety of ecosystem studies in Finland within the last decade. In this presentation, I give two examples of projects where fish stable isotope composition has been an important component. The first study was a whole lake experiment to investigate the role of terrestrial dissolved organic matter as a carbon source in the lake ecosystem. We were able to manipulate the $^{13}$C content of zooplankton and macroinvertebrates and subsequently of fishes in a small boreal lake by adding cane sugar with a distinct carbon stable isotope value. The results confirmed that external dissolved organic carbon passed through the lake food web to the fish top consumers. The other study is an ongoing investigation of terrestrial support of lake food webs and the factors affecting it such as lake size, structure of the food web within the lake, land use etc. In this study, isotopic composition of carbon, nitrogen, and hydrogen is being determined from terrestrial sources to all levels of the food web in a sample of 50 Finnish lakes. The results presented are preliminary as the sampling is continuing through 2018.
**A Hitchhikers Guide to the Otolith Galaxy**

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This is certainly not a ‘guide’ guide, but more of a map through 10+ years of research with otoliths (and other calcified structures). The chemical and growth properties of otoliths provide excellent environmental and biological loggers of fish. From an inauspicious start, cracking and burning whiting otoliths (to a singe!?) as an under-graduate; I have been fortunate to exploit the properties of otoliths to examine species life histories and address multiple issues in fisheries science. My key focus has been assessing stock structure and habitat use as well as the age and growth of fish.

This talk will discuss the use of otoliths in fisheries science, with an emphasis on the different forms of spatial and temporal information that otoliths provide, and how they can be integrated with other approaches towards developing a holistic view of stock structure. Given the prominence placed on the use of multiple approaches to assessing stock structure in fisheries science, there is an increasing need to better integrate and interpret these (sometimes) complementary data sets.

In addition, I will highlight synergies between the uses of otoliths to inform fisheries management planning with an improved understanding of the life histories and habitat use of fish. This has increased the scope of the types of research and management questions that may be addressed. Furthermore, the increased integration of otolith derived information in decision making aids in aligning management planning with the life history and biology of species.

**Keywords:** Stock structure, Growth, Life history, Holistic
Natal Origin and Connectivity of Pacific Bluefin Tuna

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Uncertainties about stock structure continue to complicate fisheries management, particularly for highly migratory species such as Pacific Bluefin Tuna (PBF, *Thunnus orientalis*). The PBF stock assessment is structured under the assumption of a single stock in the Pacific Ocean with two discrete spawning grounds in the western Pacific Ocean (WPO). One located around the Philippines north to the Ryukyu Islands and one in the Sea of Japan. Age-0 fish remain in waters around Japan, but at 1-2 years old, an unknown portion of fish migrate to the eastern Pacific Ocean (EPO) where they remain for several years before returning to the WPO. The dynamics and timing of these migrations are poorly understood and questions remain about how many fish migrate to the EPO and from which spawning ground they originate. The purpose of this study was to identify unique chemical signatures in otoliths from young-of-the-year (YOY, age-0) PBF from both spawning grounds over multiple year classes to validate our ability to identify spawning grounds or natal origin. A total of 100 YOY PBF <40 cm fork length were collected over a five year period (2011-2015) from the two known spawning grounds (n=10/spawning ground/year) in the WPO. Several trace elements and stable isotopes of \(\delta^{18}O\) and \(\delta^{13}C\) were measured and quantified in core areas of YOY PBF otoliths as a proxy for nursery signatures. Results indicate correct classification success to spawning ground ranged from 70-93% each year, which validated the ability to distinguish YOY PBF nursery grounds. Age class matched sub-adults are currently being analyzed in order to determine relative contribution rates of both spawning grounds to PBT in the EPO. The ability to use natural tracers to describe population dynamics of PBT will allow us better understand movement patterns and connectivity of PBT throughout the North Pacific.

**Keywords:** Pacific bluefin tuna, Migration, Nursery, Connectivity
Estimation of age composition and migration pattern of Pacific bluefin tuna (*Thunnus orientalis*) in the main spawning ground in the southwestern North Pacific Ocean

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Knowledge about age composition of spawning fish and staying duration in spawning ground is important to estimate the reproductive potential of the fish stock. We provided the new information about that of Pacific bluefin tuna (*Thunnus orientalis*, PBF) in the southwestern North Pacific spawning ground (SWNP) through the analysis of catch at size data and otolith aging results collected in the SWNP from 1995 to 2016. Matured PBF migrate to the spawning ground in the SWNP from the North Pacific during April to July. Relationships between age, body length and catch locations showed that larger individuals migrate to more southern part of the SWNP. The relationships between age and body length also indicated that the smaller and younger individuals appeared in the early spawning season and the larger and older individuals appeared in the mid to late spawning season, and staying duration was considered to be about one month. The age compositions of PBF caught in the SWNP suggested that the main age groups of spawning stock were 7 to 20 years old. After 2013, the main spawning cohorts in the SWNP shifted from the late 1990’s cohorts to the late 2000’s cohorts.

**Keywords:** Otolith ageing, Age composition, Spawning migration, Pacific bluefin tuna
Investigating the origin of yellowfin tuna (*Thunnus albacares*) in the western Indian Ocean using an otolith chemistry approach

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Stable isotope (δ¹³C and δ¹⁸O) and trace element (Mg, Fe, Sr and Ba) ratios were measured in otoliths of age-0 and age-1 yellowfin tuna (*Thunnus albacares*) collected from two different regions in the western Indian Ocean, namely Mozambique Channel (13-16°S) and northwest Indian Ocean (10°S-10°N). Stable isotopes in otolith core, and trace elements in otolith core, near-core and edge, together with transects along the ventral arm of the otolith, were used to determine whether yellowfin tuna from these two regions have different nursery habitat and life history characteristics. Otolith edge chemistry proved that the two areas could be discriminated, Mozambique Channel showing more depleted values of Ba:Ca and Sr:Ca compared to northwest Indian Ocean. We found no difference in otolith core isotopic and elemental composition, but the two regions were differentiated based on otolith near-core Mg:Ca and Ba:Ca ratios (Yuen t test<0.05). This suggests distinct nursery origins for both groups. Differences in Ba:Ca ratios along life history transects were also detected, likely reflecting different residence times in nutrient rich-like waters. The percentage of fish correctly assigned to their respective nursery areas ranged from 62% with linear discriminant analysis to 92% with random forest analysis, proving the importance of correct classification method choice for habitat discrimination. Mixed-stock analysis indicated that all age-1 yellowfin tuna collected were originated from the northwest Indian Ocean nursery, highlighting the production importance of the area. The results from this study support the use of otolith chemistry as a tool to analyse yellowfin stock structure in the Indian Ocean.

**Keywords:** Yellowfin tuna, Otolith microchemistry, Stock structure, Indian Ocean
Do fin spines preserve stable isotopes signatures suitable for reconstructing Atlantic bluefin tuna migration patterns?

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The use of stable isotopes (SI) as powerful "natural tags" for reconstructing fishes’ environmental and life history, has been largely restricted to ear bones or otoliths. Alternative hard tissues such as fin spines may also provide valuable chemical information and are particularly useful as a non-lethal alternative to otoliths in endangered or protected species. But currently, we have very limited knowledge of the feasibility of using other body parts such as fin spines as alternatives or complements to otoliths stable isotopes analyses. The Marie Curie project SIFINS aims to investigate whether tuna fin spines preserve SI signatures into their cellular matrix suitable for reconstructing habitat use, migrations, and other fundamental aspects of tuna life histories that are beyond the spatial or temporal scale of most other approaches. Firstly, we analyzed $\delta^{18}$O and $\delta^{13}$C values in fin spine sections (across annual growth bands) of 65 bluefin tuna collected offshore the Bay of Biscay (Northeast Atlantic Ocean) and the Mediterranean Sea (including western, central, and eastern regions). Secondly, we analyzed whether SI profiles among locations i) are distinct enough to accurately classify individuals to their capture locations, and ii) vary according to temperature in a predictable manner as it has been hypothesized in otoliths. Results of this study will provide a better understanding of tuna movement pattern seasonal, spawning migration, and ontogenetic habitat shifts over time, with important implications in fish ecology, fisheries management, and conservation issues.

Keywords: Atlantic bluefin tuna, Dorsal fin spine, Stable isotopes, Migration
Age and growth estimation of striped marlin in the eastern North Pacific using otolith micro-increments and fin spine sections

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Age of billfishes (families Istiophoridae and Xiphiidae) have been estimated using fin spine (or ray) sections for decades. Recent studies also use otolith micro-increments to estimate earlier growth and to estimate the position of the first-year annual growth band in the sectioned fin spine. This study tried to estimate age and growth of striped marlin Kajikia audax, which is an important commercial and recreational fisheries target billfish species, using otolith micro-increments and fin spine sections. Total of 29 otolith and 175 fin spine samples were collected during the longline research cruise by R.V. Shoyo-Maru in the tropical areas of the eastern North Pacific Ocean (5–18°N, 103–134°W) between September and November 2004. Daily ages of small striped marlin (87–123 cm lower jaw-fork length) were estimated to be from ca. 90 to 180 days by counting micro-increments in the sectioned otolith. Back calculated hatch dates were estimated to be from April to July which is earlier than the known spawning season in slightly northern area. Some of these 0-year old striped marlin had one or two distinct growth bands in sectioned spine which were thought to be false annual growth bands. Assumed annual growth bands in the sectioned spine of 175 individuals (87–228 cm in Lower Jaw Fork Length) were counted avoiding false annual growth bands. Ages were estimated to be from 0 to 5 years old, and dominated by 0 to 3 years old (>90%). Striped marlin in the area grew to 102 cm (4.2 kg body weight) within 4 months in average.

Keywords: Eastern North Pacific, Fin spine sections, Otolith micro-increments, Striped marlin
Early life history traits of some small pelagic fishes in the Humboldt Current System

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Small pelagic fishes, such as anchovies and sardines, play key ecological and economic roles in nearly all the world’s oceans where they inhabit; however, many aspects of its early life history are still poorly understood. To contribute to fill this gap a line of research was initiated in the anchoveta (Engraulis ringens) in Chile, based in monitoring of otolith micro-structure analysis, whose main findings are summarised in this presentation: (a) primary growth-increments are formed on a daily basis, validated through chemical otolith marking of young reared adults and newly-hatched larvae reared in a quasi-natural environment and subsequently examined and measured by international otolith experts in a laboratory-based workshop; (b) a very fast growth and early age at recruitment of anchoveta, based on the analysis of daily age and growth patterns for juveniles caught in northern Chile, suggesting most fish are removed by the fisheries at very early ages; (c) when analysing growth patterns for wild larvae, pre-recruits, recruits and young adults, four allometric patterns, which were not fully described for any traditional curvilinear growth models; (d) existence of spatial segregation of stock units along the Chilean coast showing very distinctive early growth patterns, particularly the occurrence of reduced growth rates in higher latitudes, a feature also shared with other small pelagic fishes, such as the sardine (Strangomera bentincki) and the Chilean sprat (Sprattus fuegensis). The results support the hypothesis of rapid growth/young age through the juvenile stage for some anchovy species in other highly productive ecosystems recently reported, but also suggest a high plasticity of this species along the Humboldt Current System.

Keywords: Speckle, Otolith, Fish
Fine-scale temporal changes in otolith microstructure and chemistry expose the early life history of a coastal southern Australian finfish

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Establishing connectivity between spawning and nursery areas for fish is difficult but necessary for understanding movement pathways, population dynamics and stock structure. The microstructure and elemental chemistry of otoliths provide unique tools for retrospectively reconstructing early life history processes and investigating connectivity at fine temporal and spatial scales. This study explored the early life history of King George whiting (Sillaginodes punctatus), the mostly highly-sought, inshore finfish of southern Australia. Post-larvae were sampled on nine occasions fortnightly throughout the settlement season of July to November 2016, at a significant nursery area in Gulf St. Vincent, South Australia. For the smallest twenty fish from each sample, i.e. the most recent settlers, daily increments were counted and measured to estimate pre-settlement duration and growth trajectories throughout the larval period. Considerable variation was identified in these early life history characteristics with average pre-settlement duration increasing (108 to 164 days) and average growth rate (0.16 to 0.11 mm.day⁻¹) progressively declining throughout the settlement season. The long pre-settlement duration and slow growth relate to the late autumn and winter spawning season and subsequent larval advection during winter. Trace element chemistry was used to consider the natal origins of the same fish and identified two different multi-elemental signatures that related to fish spawned up to April, and those spawned thereafter. The fine-scale temporal change in otolith chemistry describing the natal origin reflected higher ⁸⁸Sr and lower ⁷Li later in the spawning season and suggested two possible scenarios: either a change in environmental conditions that influenced otolith chemistry; or there were two different spatial natal origins. Considering otolith microstructure and chemistry concurrently has provided a greater understanding of early life history processes.

Keywords: Otolith microstructure, Trace element chemistry, Settlement, LA-ICPMS
Elements in otoliths as environmental proxies: A meta-analysis

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Use of otolith chemistry as an environmental proxy has increased greatly since inductively coupled plasma-mass spectrometers (ICP-MS) were introduced in the 1980s. Yet, the suitability of individual elements as reliable and accurate environmental indicators may vary depending on a range of factors reflecting species variation, life history characteristics and climatic conditions. Furthermore, research has shown complex physiological influences on element uptake may decouple otolith composition from ambient environmental conditions. We used a systematic review to identify records in Web of Science related to search terms incorporating otolith(s) and *chem* (where * incorporates all words with chem in the middle, e.g. microchemistry, or beginning of words and variations of chemistry, chemical etc at the end of the word) in combination with six different environmental indicator terms (upwelling, pollution, salinity, temperature, hypoxia or pH). Approximately 1700 papers were compiled and then screened against predefined criteria to ensure relevance and where possible used in both a qualitative and quantitative synthesis (meta-analysis). A wide range of elements have been used as environmental indicators in past studies, although most studies have focused on strontium and barium as indicators of either temperature or salinity. Our meta-analyses and treemap plots suggest that some elements reflect environmental conditions better than others, but that study-level covariates (e.g. species, niche) also influence elemental incorporation. Systematic reviews such as the one performed here are important for elucidating the links between otolith chemistry and extrinsic and intrinsic factors. Ultimately, disentangling the influence of multiple factors on otolith chemistry will aid in evaluating the extent at which intrinsic factors may counter or exacerbate environmental signals. Establishing a predictable link between environmental conditions and otolith chemistry is essential for the continued use of otoliths as environmental proxies.

Keywords: Meta-analysis, Review, Environmental proxy, Environmental indicator
Reconstruction of the salinity history associated with movements of mangrove fishes using otolith oxygen isotopic analysis

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Recent degradation and loss of tropical estuaries due to human impact have made necessary the protection of essential habitats for estuarine fishes. A better understanding of estuarine use patterns contributes greatly in identifying essential habitat conditions. This study presents quantitative reconstruction of the salinity history experienced by individual estuarine fishes using an otolith oxygen isotope. \( \delta^{18}O \) otolith was determined from the kuhliid *Kuhlia rupestris* and pomacentrid *Chrysiptera cyanea* for use as freshwater and marine end-member references, respectively. The salinity history of three species of estuarine mangrove fishes, often dominant in tropical East Asian estuaries, viz. the apogonid *Fibramia amboinensis*, pomacentrid *Neopomacentrus taeniurus* and terapontid *Terapon jarbua*, were examined. The \( \delta^{18}O \) otolith ranges of \(-2.3\) to \(-5.4\)‰ for *F. amboinensis* and \(-2.3\) to \(-3.7\)‰ for *N. taeniurus* corresponded to 6 to 30 psu and 19 to 30 psu, respectively, when converted to salinity, based on the two salinity extreme values of the reference fishes. *T. jarbua*, on the other hand, exhibited \( \delta^{18}O \) otolith variation range \((-1.8\) to \(-3.0\)‰) corresponding to 25 to 34 psu. Field observations in other studies have indicated that *F. amboinensis* and *N. taeniurus* may be resident species, hovering near mangrove roots and tolerating a variable salinity environment, whereas *T. jarbua* may be a vagile species which can move to areas of preferred higher salinity conditions. Such behavioral ecologies are largely consistent with the reconstructed salinity histories from \( \delta^{18}O \) otolith. These results suggested that *F. amboinensis* and *N. taeniurus* may be estuarine resident fishes, whereas *T. jarbua* may be a marine migrant.

**Keywords:** Salinity, Oxygen isotope, Mangrove fishes, Estuarine
Validation of species specific otolith chemistry and salinity relationships

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The ratios of strontium (Sr) and barium (Ba) to calcium (Ca) in otoliths are used to reconstruct salinity use of fishes, given that generally Sr:Ca exhibits a positive and Ba:Ca exhibits a negative relationship with salinity. However, these relationships are non-linear, confounded by surrounding water chemistries, and vary among species. To determine if reconstructions of past salinity use are possible in northern Gulf of Mexico estuaries (GOM), the objectives of this study were to investigate the relationships of Sr:Ca and Ba:Ca with salinity in both ambient water and otoliths of juvenile Red Drum (*Sciaenops ocellatus*) and Gulf Killifish (*Fundulus grandis*). Fishes were held in low (< 1 psu), medium (10 psu), and high (30 psu) salinity recirculating lab systems for 8 weeks. These systems were filled with field collected water, and water trace metal samples were taken weekly. Element:Ca edge concentrations of otoliths were quantified using LA-ICPMS and water element:Ca concentrations were determined on the same instrument. Sr:Ca was positively related to salinity in otoliths and water, and Killifish Sr:Ca was higher than Drum at all sites. Ba:Ca was negatively related to salinity in both water and otoliths. The low treatment had the highest Ba:Ca for both species, but Killifish concentrations were 4 times higher than Drum at that site. The partition coefficients differed between species and treatments, Gulf Killifish ranged from 35 – 49% for Sr:Ca and 7 – 22% for Ba:Ca. Red Drum had Sr:Ca coefficients of roughly 20% across all treatments, and Ba:Ca ranged from 3 – 20% increasing with increasing salinity. These results demonstrate that it should be possible to distinguish oligohaline use from high salinity use using otolith element:Ca ratios in northern GOM estuaries. However, the differences between species and among treatments in partition coefficients and element:Ca ratios elucidate how important species specific validation is.

**Keywords:** Salinity, Sr:Ca, Ba:Ca, Estuaries
Linking daily growth chronologies and otolith chemistry of juvenile European flounder *Platichthys flesus* in estuarine nursery areas

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Otolith chemical composition has been extensively used as a natural tag to resolve stock structure, population connectivity and movement patterns of fish. The incorporation of elements in the otolith matrix is influenced by the environment and physiology. However, these processes remain poorly understood, particularly in early life history stages during which larval migrations, metamorphosis, and ontogenetic, behavioral or ecological shifts can further hamper our understanding. Unraveling these processes is especially complex in estuarine environments where environmental conditions vary markedly on small spatial and temporal scales. In this work, we combine otolith daily increment widths (as a proxy for individual growth) and otolith chemical composition of juvenile European flounder *Platichthys flesus*, from the Mondego estuary nursery (Portugal). We use increasingly complex mixed models to partition intrinsic (age and early life stage – pelagic, metamorphosis and benthic) and extrinsic (environment: temperature, river flow, juvenile density) factors that may influence growth and otolith element concentrations through time. We explore the importance of different environmental and physiological drivers to individual growth in different life stages and over five recruitment cohorts. Similarly, drivers of otolith chemical composition are assessed to evaluate the applicability of this natural tag to reconstruct estuarine nursery habitat use. Furthermore, we evaluate how otolith daily growth may influence elemental composition. Results contribute to disentangling life stage, environmental driven growth patterns and otolith chemistry, which ultimately can provide insights on juvenile fish habitat use and estuarine nursery role.

**Keywords:** Growth chronologies, Otolith chemistry, Estuarine habitat use, Life stage
Stable $\delta^{18}$O and $\delta^{13}$C isotope signatures in the otolith nucleus as a potential proxy for stock assignment of individual Baltic Sea cod (*Gadus morhua*)

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In the Baltic Sea a western and eastern stock have distinct spawning grounds in different basins but considerable mixing occurs in the adult stage hampering stock assessment. Juvenile cod are known to occur rather localized in their coastal habitats. Given the distinct isotopic characteristics of the water bodies between the basins, we aimed at assessing the usefulness of stable isotope composition in Baltic cod otolith nuclei as a potential indicator of spawning ground origin. Stable isotope oxygen ($\delta^{18}$O) and carbon ($\delta^{13}$C) were obtained from otolith nuclei of spawning Baltic Sea cod females of the known spawning grounds from Belt, Arkona and Bornholm Sea to establish spawning stock baselines. The baselines were validated by otolith shape analyses. Subsequently, the isotopic composition of the nuclei of juvenile cod otoliths sampled along a west-east axis was compared to the baselines to evaluate the mixing in the coastal habitats. The results suggest two baselines, one western (Belt Sea) and one eastern (Arkona and Bornholm Sea combined). Despite thorough selection of the baseline otoliths by spawning time and area, the shape analysis of validated otoliths detected assignment uncertainties of 12% (Belt Sea) and 13-20% (Arkona and Bornholm Sea). $\delta^{13}$C lacked any spatial pattern. Based on $\delta^{18}$O all juvenile cod otoliths could be assigned to either the western or eastern baseline suggesting that $\delta^{18}$O in the nucleus of the otolith can be used as proxy for stock assignment. The results also suggested a mixing of juvenile cod in the eastern Arkona and western Bornholm Sea. The $\delta^{18}$O signature of the 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 of origin, suggesting that validation by methods independent of the environmental influence such as genetic analysis are required.

**Keywords:** Otolith chemistry, Oxygen 18, Stable isotopes, Stock interactions
Connectivity of lemon sole *Microstomus kitt* in the northern North Sea as determined from a combination of otolith characteristics and particle tracking

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Lemon sole, *Microstomus kitt*, is a commercially valuable flatfish species that occurs in shelf waters from the White Sea and Iceland in the north to the Bay of Biscay in the south. Spawning in the North Sea is generally assumed to peak between May and August with spawning being completed in October. Lemon sole larvae were caught in November and December 2016 in the northern North Sea and also in similar areas during January and February 2017. In both cases the larvae were caught using ICES standard 2m Midwater Ring Trawls, MIK, during the IMR standard transect sampling and the ICES IBTS MIK survey. All larvae were measured and sagittal otoliths removed. The primary increments were counted using standard techniques using a light microscopy. The larvae caught in November and December ranged in nominal age from 4-45 days post-hatch which suggests spawning continuing into late October and November. However, the presence of small larvae in February suggested spawning may continue in to late December/early January. Using projected trajectories and the modelled thermal regimes along with estimated otolith growth rates, indicated the most likely sources of the larvae i.e. their spawning grounds.

**Keywords:** Larval drift, Overwintering, Microstructure, Flatfish
Interannual variability in early life history of European flounder, *Platichthys flesus* and common sole, *Solea solea*

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Recruitment variability is one of the most important subjects in fisheries biology, especially considering commercially important species that use estuaries and inshore areas as nursery grounds. Since early life history plays a determinant part in defining such variability, we focused on the interannual variability in hatch dates, pelagic larval duration (PLD) and benthic settlement after metamorphosis of the European flounder, *Platichthys flesus* and common sole, *Solea solea*. Our dataset was obtained from monthly fieldwork in the Mondego estuary, Portugal, covering the period between 2010 and 2015. Early life history events were determined through otolith microstructure analysis, by which we determined age and duration of the pelagic and metamorphosis stages, as well as hatch dates, determined from age and date at capture. Hatching was estimated to start in late winter for these species, and interannual differences in hatch dates, PLD, metamorphosis and benthic settlement were discussed in the scope of high environmental instability during the period of 2010-2015 (sea surface temperature, NAO index, rainfall, chlorophyll a, upwelling), which are known to influence early life history of marine fish and migrations towards estuarine nurseries.

**Keywords:** Otolith microstructure, Migrations, Life stage, Environmental change
Marine dispersion of two related anadromous European shads along French Atlantic coast before 2000s population’s crash

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Allis shad, Alosa alosa (Linnaeus, 1758) and twaite shad, Alosa fallax (Lacépède, 1803), are a complex of two sister species of anadromous Clupeidae species, with A. alosa populations facing a great decline in the 2000s. Whereas the freshwater part of the biological cycle of these species was widely addressed (mainly for A. alosa), information about coastal and oceanic habitat use is scarce and mostly relying on accidental captures. Available data showed that both species inhabit shallow waters (<150 m) and aggregate in coastal regions near the river mouths of most important spawning grounds. However, stock-specific migratory behaviour of both species at sea are largely missing. A metapopulation functioning with a strong isolation-by-distance was determined for A. alosa using otolith microchemistry and a Bayesian model. On the other hand, genetic studies also pointed an isolation-by-distance for A. fallax. These results suggest that exchanges of individuals primarily occur between neighbouring rivers. However, whether straying occurs during the early stage of sea nursery phase or when the growth is accomplished at sea remains unclear. Straying would take place between the coastal feeding grounds corresponding to different watershed origins. Thus, discrimination of the stocks origins of fish at sea could help us to unravelling this question. In the present study, we aimed (i) at understanding the source and mixture of populations of both species throughout the French continental shelf area of the Atlantic Ocean (48°-45° N) from a pre-crash period (1986-1989) of supposed population’s stability, and (ii) to compare population connectivity of these two closely species by the use of natal chemical tags recorded in their otoliths. Using the Bayesian assignation model described in a previous work of our research group, implemented with new water samples and news juvenile signatures, 214 Alosa alosa and 170 Alosa fallax were assigned to a natal origin.

Keywords: Anadromous species, Marine connectivity, Natal origins, Bayesian model
Changes in environmental salinity during the life of *Pangasius krempfi* in the Mekong Delta (Viet Nam) revealed by otolith Sr:Ca ratios

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The knowledge of biology of the catfish *Pangasius krempfi* is still incomplete in the Mekong River but it is supposed to undertake migrations in the delta. To verify hypotheses on the migration patterns and the habitat at birth, strontium:calcium ratios in otoliths were measured to infer the salinities crossed by individuals during their life. From March to June 2017, 33 otoliths of *P. krempfi* were collected in the brackish waters of the lower Mekong Delta, embed in epoxy resin, and thin slices through the core were obtained after transverse sectioning and polishing. Strontium:calcium ratios were measured from the core to the otolith edge using laser ablation-ICPMS. Sr:Ca ratios were also measured from water samplings along the Mekong Delta using SB-ICPMS. The results showed that all fishes have similar Sr:Ca ratio in the core, probably meaning that they were all born in the same environmental salinity level. Changes in the Sr:Ca ratio was observed for many individuals along their life meaning that they faced different environmental salinities, and that they maybe undertake movements in the Mekong Delta waters. We hypothesized that all individuals come from a single natal origin. During their early life, *P. krempfi* individuals showed a same pattern: after hatching in freshwater, they reached higher salinity on early stage, and then they were mainly resident in saltiest waters during their life, and some of them are coming back in the original waters at older stages. This could have implications for the fisheries management of this high valuable species, and migration pattern should also be confirmed by using otolith strontium isotopes.

**Keywords:** *Pangasius krempfi*, Otolith, Salinity, Mekong Delta
Convergent evolution in vestibular systems of fish and cephalopods

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Fish and cephalopods have been coexisting for about 500 million years since late Cambrian and competing to dominate the world oceans. Very early in their evolution, both groups left their benthic habitats to live in the water column. Despite completely different origins and systematic positions, body plans and physiology (molluscs: protostomia and vertebrates: deuterostomia), both groups convergently evolved paired equilibrium organs located at the posterior part of their brain to monitor position of their body in three dimensional space and compensate eye movements. Each equilibrium organ (called the statocyst in cephalopods and inner ear in fish) has two main parts, namely the gravity receptor system and angular acceleration receptor system. In each group, the gravity receptor system consists of three compartments or sections containing calcium carbonate granules (otoconia=statoconia and otolith=statolith). Interestingly, primitive fishes have loose otoconia embedded in a gelatinous matrix, similar to primitive cephalopods (such as nautilus and cirrate octopods). The most advanced fish (teleosts) and cephalopods (decapods) have their CaCO$_3$ crystals fused into a solid structure (otolith and statolith, respectively). Apart from gravity detection, fish otoconia and otoliths are involved in hearing, whereas cephalopod statoconia and especially statoliths play their role in monitoring jet propulsive movement. Different evolutionary pathways that lead to convergent formation of solid calcareous structures in the vestibular organs of fish and cephalopods are discussed, with an emphasis on taxonomically specific shapes of otoliths and statoliths.

Keywords: Convergent evolution, Cephalopods, Fish, Otolith organs
Functional effect of vaterite on escape trajectory of *Salmo trutta*

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Sagittal otoliths are normally composed of aragonite. However, the inclusions of vaterite in abnormal, or “crystallized”, sagittal otoliths has been reported in a number of marine and freshwater species from different environments. In particular, several authors have hypothesized that exposure to acute or chronic stress (e.g. starvation, high density, temperature stress, diseases) can promote the development of aberrant otoliths. Due to stressful conditions encountered in hatcheries, large numbers of vateritic otoliths are therefore frequently observed in farmed fish, compared to wild populations. In this context, investigating the functional effect of vateritic otoliths is of primary concern for restocking programs based on captive-bred fish. More generally, since otoliths play an important role in sensory and balance functions, the presence of vaterite may have implication for perception of congeners, preys, and predators, as well as in the perception of acceleration and the maintenance of postural equilibrium while swimming. There is currently strong theoretical and empirical (i.e. auditory brainstem response) evidences that vaterite deposition has a negative impact on auditory sensitivity in fishes. However, to date, the integrated response (i.e. behavioral) of fishes remains uninvestigated. Here, using juvenile (0+ years) *Salmo trutta* originating from the wild in experimental conditions, we report for the first time that escape trajectories from vateritic individuals significantly differ from aragonitic individuals and exhibit a much more stereotyped response (i.e. more predictable). We also report that natural behavior from vateritic individuals is largely compensated by lateral line (as revealed by using streptomycin in an attempt to chemically ablate canal and superficial neuromasts). We ultimately emphasize that the presence of an alternative crystalline structure in otolith could alter fish’s behavior in ways that decrease their chances of survival in experimental predation test.

**Keywords:** Vaterite, Behavior, Morphology, Predation
Large lapillus found in two tribes of Sciaenidae: The American Stelliferini and Indo-West Pacific Johniini

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Among 300 species of sciaenid fishes, two independently evolved tribes, Stelleferini from tropical Americas, and Johniini from Indo-West Pacific have much large lapillus otoliths. Stelliferini includes 5 genera and 45 species, while Johniini consists a monotypical genus with 33 species. Genus Stellifer, include Ophioscion, has an oval-shaped lapillus the same size of the sagitta, other genera Bairdiella, Corvula, Ellatarchus and Odontoscion have lapillus size graduate reduced to 1/2 of the triangular sagitta. Johnius has a proportional smaller lapillus about 1/4 of sagitta, which is unique by having a bent anterior portion and a deep crater-like caudal of the sulcus. Corresponding to an enlarged lapillus, Johnius has an expanded anterior portion of the gas bladder, with appendages extended anteriorly to cranium and laterally to cleithrum, externally visible under a thin skin. In Stellierini, the gas bladder has a separated yoke-shaped anterior chamber, with its tips attach to cranial case. Two taxa have evolved independently and endemic to Americas and Indo-West Pacific respectively. Inner ear of fishes, as a sensory organ for equilibrium and sound reception, what can be the functional advantage of two large pairs of otoliths? Is it evolutionarily selective for certain habitats? Stelliferini and Johniini mostly inhabit in turbid shallow coastal and estuarine waters, which is also typical for many sciaenids and coastal fishes. Benthic fishes often have larger otoliths than pelagic fishes. Among Sciaenidae, there are species with thick or out cropped sagitta (e.g. Micropogonias, a bottom fish), or long sagitta (e.g. Cynoscion virencens, a mid-water predator); their cranial cases also expanded accordingly. Is an additional large pair of otoliths to enhance sound reception or equilibrium? Species richness of these two taxa also indicates that they can quickly evolve and adapt to changing habitats, such as a warmer ocean. Is it just a convergence of natural selection?

Keywords: Sciaenidae, Lapillus, Otolith morphology, Phylogeny
Do environmental conditions (temperature and food composition) affect otolith shape during fish early-life stages? An experimental approach applied to European seabass (Dicentrarchus labrax)

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Otolith shape is an efficient tool for stock discrimination. Current global climate change generates a global increase in ocean temperature that also negatively impacts the production of polyunsaturated fatty acids (EPA and DHA) by phytoplankton and thus their availability in marine trophic webs. The goal of this study was to assess experimentally the combined influences of warming temperature and polyunsaturated fatty acids content of food on European seabass (Dicentrarchus labrax) otolith morphogenesis during early-life stages. 300 individuals were reared from 94 to 200 days post-fertilization (dpf) at 15 or 20ºC and fed only fish oil (1.85% EPA+DHA) or mixed fish and vegetable oils (0.85% EPA+DHA) according to a full factorial design, with 3 replicates (tanks) for each experimental condition (i.e. temperature-food combination). To follow the development of mean otolith shape during the experiment, twelve individuals per condition and replicate were sampled three times. Shape analysis was carried out on the two sagittae of each sampled fish. Firstly, otolith morphometric measurements, shape factors and normalized Elliptical Fourier coefficients describing outline shape were extracted using image analysis. Then, the effects of temperature and food EPADHA content on otolith shape variation with respect to time, were estimated using linear mixed effect models accounting for variation across replicates. Whatever the model, temperature has a significant effect on otolith morphogenesis as well as on fish growth in length. Food EPA-DHA content effect was significant for all otolith morphometric measurements and for normalized Elliptical Fourier descriptors whereas regarding shape factors only circularity was significantly affected. In conclusion, this study shows that otolith shape and its ontogenetic trajectories could be affected by climate change through both global warming and the resulting reduction of polyunsaturated fatty acids production. Such effects could have implications for the use of otolith shape as an indicator of fish stocks.

Keywords: Otolith shape analysis, Global warming, PUFA, Temperature change
Composite variations in genetic structure, life-history traits, and statolith morphology of *Sepioteuthis lessoniana* populations around Nagasaki Prefecture, Japan

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The life-history traits, statolith morphology and molecular structure are analysed and revealed to understand the population structure of *Sepioteuthis lessoniana*. Squid samples were collected at six locations (Tameshi, Western Nomozaki, Eastern Nomozaki, Sasebo, Goto Islands, and Omura Bay) around Nagasaki Prefecture from June, 2016 to September, 2017. In total, we collected 142 individuals for biology measurement and statolith morphology analysis, aged 134 samples by reading daily rings in statolith and investigated the genetic structure based on mitochondrial COI gene. The results demonstrated that two lineages (B, and C) of *Sepioteuthis lessoniana* were found in Nagasaki prefecture, and this study revealed two subpopulations of lineage C (C1 and C2) which are related to different season cohorts. Life-history parameters are different between sexes, between locations, and between seasons. The morphology of statoliths also differs between the left and right side, and between stocks and cohorts subpopulations of *S. lessoniana*. We considered the subpopulation in different locations as stocks and different seasons as cohorts, and revealed the boundary between lineage B and C. The life-history parameters, statolith shape and DNA markers are useful methods to contribute to the fishery management units.

**Keywords:** *Sepioteuthis lessoniana*, Genetic structure, Statolith shape, Life-history traits
Abundant otoliths in fossil and modern coral reefs provide Caribbean reef fish community baselines

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Contrary to conventional thinking we discover abundant and well-preserved otoliths in Holocene and modern Caribbean carbonate reef sediments from Panama and the Dominican Republic. Here we explore the utility of these otolith assemblages to observe changes in the ecology of coral reef fishes over the last 7000 years. We find that otolith assemblages from modern reef sediments faithfully (with some important caveats) capture the driving ecological signal of the living reef fish community. Excavation of >160 kg of carbonate reef matrix has yielded more than 8000 fossil otoliths representing 38 fish families. Analysis of these assemblages will provide a pre-human baseline of reef fish communities and help reveal how the taxonomic and functional structure of Caribbean reef fish communities have changed over the last 7000 years. We discuss the limitations of this approach, and its potential application to the conservation efforts of coral reef ecosystems.

Keywords: Paleoeconomy, Caribbean, Fossil, Coral reefs
Morphology and morphometric traits of sagittae for different fish species in the eastern coast of LIBYA

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Sparus aurata, Oblada melanura, Mugile cephalus, Oreochromis niloticus, Dicentrarchus labrax and Sciaena umbra fish used in the present study were collected from the artisanal catch of eastern Libya Mediterranean Sea during April to July 2017. The length weight relationship of S. aurata and O. melanura has b values of 2.91 and 3.012 consecutively, indicating isometric growth. M. cephalus and O. niloticus have negative allometric growth (b: 2.487 and 2.770). Morphological features of sagitta of each species were described on bases of sagittal Length, Height, Weight, Dorsal and Ventral margins, Sulcus acusticus, Ostium, Cauda, Rostrum and Antirostrum, and Posterior and Anterior ends. Morphometrics of sagitta (sagitta length, height and weight) of Sparus aurata, Oblada melanura, Mugile cephalus and Oreochromis niloticus, were related to fish morphometrics (length and weight) by linear equations. Numbers of Dicentrarchus labrax and Sciaena umbra were too few to establish relations

Keywords: Sparus aurata, Oblast melanura, Mugler cephalus, Saittal
Losing track of time: Dubious age determination of Baltic cod, probable causes and promising solution

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Cod (\textit{Gadus morhua}) ranges across the North Atlantic, where it is an economically and ecologically key species. In the Baltic Sea, increasing eutrophication and expanded areas with oxygen deficiency have resulted in the loss of spawning and feeding areas. The worsened environmental conditions, together with high fishing pressure and a growing seal population that has infected the Baltic cod with parasites, have had dire consequences for the fish. The stocks have declined and body size and condition have decreased. Age estimation of Baltic cod for stock assessment and fisheries advice has never been straightforward; however, lately the annual growth zones in the otoliths have become so unclear that no reliable age data are available. Subsequently, analytical assessment and forecasts have not been possible, resulting in suspension of sustainable seafood certification for this species. An alternative way to age estimate Baltic cod by searching for seasonal patterns in otolith chemistry is now under development. Trace elements from the surrounding water are incorporated into the otolith during the fish’s life. Micro-chemical analyses assay the lifetime elemental variations in the otolith, which can reflect migration behavior, chemical changes in the environment as well as physiological and metabolic processes in the fish. Results from North Sea cod otoliths with clear seasonal growth zones reveal that chemistry displays a strong correlation with the visual pattern of the otolith. Several elements in ratio to calcium can be assessed for interpreting seasonality. Manganese represents exposure to seasonal hypoxia in the Baltic Sea during summer. Strontium is salinity and temperature dependent. Higher strontium concentrations during winter may be a result of kinetics but also migration. Magnesium levels rise during summer, likely coupled to increased metabolic activity. This multivariate chemical method shows promising potential for determining ages of Baltic cod and other species with age estimation issues.

Keywords: Age validation, Otolith chemistry, Seasonal patterns, Cod (\textit{Gadus morhua})
You can’t swim from the past! Using otolith micro-chemistry to infer ontogenetic movements of maturing gilthead seabream

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Estuaries act as important nursery areas for a number of valuable fish species. Greater understanding of the drivers of larval mortality and recruitment success, and the levels of connectivity between adjacent environments is key for successful management of these systems and associated fish stocks. The gilthead seabream Sparus aurata is an important fish, targeted by both commercial and recreational fishers. Predominantly found in the Mediterranean and off the Atlantic coast of Portugal and Spain, the rising sea temperature is thought to be responsible for an apparent range expansion into the English Channel and Celtic Sea. In the Mediterranean, wild S. aurata populations spawn during winter at sea, and their pelagic larvae swim for approximately 3 months before settling in coastal lagoons. Relatively little is known about the UK population at the northern edge of range expansion, where adults are being caught more frequently in spring and summer months and juveniles have been observed in recent years. Here we used sagittal otoliths from northern S. aurata to characterise the ontogenetic variation in trace element:Ca ratios (Li, B, Na, Mg, Cr, Mn, Sr, Ba, and Co) using laser ablation inductively coupled mass spectrometry (LA ICP-MS). Coupled with aging, this allowed us to determine the timing of juvenile settlement into nursery areas, and subsequent seasonal movements between estuarine/lagoon and marine waterbodies. As well as providing new information on the dynamics of climate driven colonisation in a range expanding fish species, our data have the potential to inform sustainable management plans that would benefit fishers and the coastal tourism sector.

Keywords: Otolith micro-chemistry, Climate change, Range-expansion, Fisheries management
Otolith microstructure and chemistry validate age and subsequently provide insights into early life history and behaviour in southern blue whiting (*Micromesistius australis australis*, Norman 1937)

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Accurate age determination for commercial fishes is essential for effective fisheries management however validation of the ageing methodology is frequently overlooked. The microstructure of Patagonian southern blue whiting (*Micromesistius australis australis*) otoliths reveals check marks within the otolith microstructure that have potentially led to overestimations of age. This study aims to use otolith microstructure and trace elemental analyses to correctly interpret the otolith microstructure and produce the first validated ageing methodology for *M. a. australis* found in Falkland Islands waters. Firstly, daily increment formation was confirmed by conducting daily increment analyses on otoliths from individuals of a single cohort over the course of 1 year. Daily increment counts were used to determine the location of the first winter check and marginal increment analysis was further conducted on otoliths from 1-5 year old individuals to validate the annual formation of subsequent winter checks. Otolith microstructure was further investigated and revealed accessory growth centres and a subsequent check mark. Trace elemental analyses (LA-ICP-MS) was conducted to further investigate these structures. Results revealed significant changes in otolith chemistry, confirming a marked change in physiology and/or a change from a pelagic to a demersal habitat. This study confirms the location of the first winter check through daily increment counts and trace elemental analyses, and provides a validated ageing methodology which will benefit the accuracy of age determination and subsequent age-based stock assessments and growth models for *M. a. australis* found in Falkland Islands waters.

**Keywords:** *Micromesistius australis australis*, Otoliths, LA-ICP-MS, Life-history
Elemental patterns in shark vertebrae reflect age and upwelling histories

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Characterizing long-distance migrations of large apex predators typically involves conventional or electronic tagging that requires capturing and handling individuals, high expense, and low reporting rates. Natural chemical tags such as trace elements contained in chronologically formed band pairs of shark vertebrae, may increase our understanding of individual life histories, migration patterns and population connectivity. In this study, trace element profiles were quantified by laser ablation ICP MS in vertebrae of common thresher (n=10), shortfin mako (n=9), and blue sharks (n=7) with known tag and recapture locations between the United States and Mexico between 2004-2009. These sharks were all injected with oxytetracycline (OTC) when they were initially captured and released allowing for identification of the vertebral band associated with their initial tagging location and validation of the number of band pairs deposited between tagging and recapture. Known movements included both latitudinal and longitudinal patterns that ranged from <10 km to > 600 km and individuals exhibited time at liberty from 215 to 1385 days. Manganese (Mn) peaks were related to the number of validated band pairs in vertebrae, suggesting Mn could be used to age these species. Barium (Ba) concentrations in the outer edges of vertebrae, reflecting the previous month of life, were correlated with coastal upwelling indices that varied among year of capture. These results indicate that shark vertebral chemistry has the potential to drastically advance our knowledge of shark life history including age and migration patterns.

Keywords: Elasmobranchs, Vertebral chemistry, Upwelling, Age-validation
Nursery origin of bull sharks in the northwestern Gulf of Mexico: Inferences from vertebral chemistry

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Bull sharks possess a unique ability among elasmobranchs to move freely across the freshwater-marine continuum, and are heavily reliant upon estuarine systems as nursery habitat. However, there is evidence that not all estuaries are of equal value to bull shark populations, and effective delineation of specific nurseries as essential habitat requires a better understanding of which areas might make the greatest relative contributions to population growth and adult biomass. The chemistry of shark vertebrae may serve as an effective natural tracer of nursery origin for bull sharks inhabiting estuarine habitats along a broad physicochemical gradient. In this study, we assess the utility of vertebral chemistry to discriminate young-of-the-year bull sharks from putative nurseries along the Texas coast. Here we quantify trace element (\(^{7}\)Li, \(^{24}\)Mg, \(^{43}\)Ca, \(^{88}\)Sr, \(^{55}\)Mn, \(^{59}\)Co, \(^{65}\)Cu, \(^{66}\)Zn, \(^{137}\)Ba) concentrations and stable isotope values (\(\delta^{13}\)C and \(\delta^{18}\)O) of vertebrae from 100 juvenile bull sharks collected from five distinct estuarine systems along the Texas coast from 2013 to 2016. We present the results of these analyses and discuss future lines of research and alternative approaches in characterizing the use of bull shark nursery habitat along the Texas coast.

**Keywords:** Elasmobranchs, Vertebral chemistry, Stable isotopes, Trace metals
Cod growth, climate, and stable isotopes in Godthaabsfjord, West-Greenland

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Changes in climate and exploitation have caused large fluctuations in the productivity of many North Atlantic cod populations, including the Greenlandic Godthaabsfjord population. To what extent these changes are driven by or augmented by changes in the foodweb is largely unknown, primarily due to the lack of historical data on ecosystem structure. To study the link between cod growth, environmental change and ecosystem trophic structure, we therefore developed an 82-year (1927-2009) chronology of cod growth, and a corresponding chronology of stable carbon and nitrogen isotopes derived from otolith protein. The time-series showed significant variability in growth with low frequency variation correlated to the Atlantic Multidecadal Oscillation (AMO) and high frequency variation with a period of 5-8 years. Similar patterns were seen in baseline uncorrected $\delta^{15}N$ & $\delta^{13}C$ values, which were significantly correlated to AMO. Regime shift analysis showed significant changes in growth around 1970 and in the mid 1990’ies, while abrupt shifts in $\delta^{15}N$ were seen in 1970 and 1997 and for $\delta^{13}C$ in 1970. These two time-points appear to mark profound changes in the Godthaabsfjord ecosystem and in the climatic and tropho-dynamic factors that control the system. The transitions in 1970 and mid 1990’ies suggest a major shift in the trophic position of cod. Interestingly the strong correlation between AMO and $\delta^{15}N$ & $\delta^{13}C$ breaks down in 1997 with all subsequent isotope ratios grouped far from the expected values. This suggests that the effect of a climatic factor such as AMO is dependent on the state of other e.g. climatic or population dynamic factors, and that the factors that control of ecosystem productivity may vary over time.

Keywords: Stable isotopes, Food web, Growth, Climate
Isotopic records of Pacific halibut otoliths in detection of recent climate-related regime shifts in the Washington coast

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The use of stable oxygen and carbon isotope ratios ($^{18}$O/$^{16}$O or $d^{18}$O, and $^{13}$C/$^{12}$C or $d^{13}$C) in otoliths has received growing attention in recent years in fisheries management, particularly in examining climate-related regime shifts (from $d^{18}$O) and the effects of ocean acidification (from $d^{13}$C). Otoliths are likely formed in oxygen isotopic equilibrium with the ambient seawater, while carbon isotopes may reach isotopic equilibrium with dissolved inorganic carbon (DIC). In this study we report stable isotopic records of otoliths of Pacific halibut (\textit{Hippoglossus stenolepis}) and their connection with climate regime shifts from Makah’s usual and accustomed (U&A) fishing areas. The long-term otolith collections (four intervals from 1991, 2002, 2012, and 2017 fishing season), which cover the past 48 years in the same fishing area along the Washington coast, provide valuable information on climate regime shifts. The $d^{18}$O values of Pacific halibut otoliths of this study ranged from -0.74 to +3.21 ‰, whereas the $d^{13}$C values of the same samples ranged from -3.64 to +0.26 ‰. As previously reported, the isotopic composition of mature halibut (ages 8-12) suggest that the 1977 regime shift was a result of warming; while the 1990 regime shift may reflect a bottom seawater temperature decrease of about 2 °C. More regime shift events are being determined in this dataset. The connection between stable isotope variation in otoliths and the climate regime shifts is thus potentially useful in studying the population dynamics of Pacific halibut stocks, and decadal-scale (e.g., the last 40-50 years) ocean environmental changes.

\textbf{Keywords:} Pacific halibut otoliths, Stable isotopes, Climate regime shift, Washington coast
Oxygen stable isotopes in otoliths used to reveal fractionation curves, fish condition, and fish age validation

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Stable isotopes found in otoliths can be used to address a variety of environmental and fisheries management questions. This is especially useful in studying climate change, climate related effects on fish growth and health, and paleotemperature. In this study we used stable oxygen isotopes, in the form of $\delta^{18}O$, as an indicator of water temperature experienced by individual fish. First, we developed empirical fractionation curves of otolith $\delta^{18}O$ in relation to water temperature using laboratory based studies on four North Pacific Gadid species: Artic cod (*Boreogadus saida*), saffron cod (*Eleginus gracilis*), walleye pollock (*Gadus chalcogrammus*), and Pacific cod (*Gadus macrocephalus*). We found statistically significant relationships, with up to $r^2 = 0.94$. We analyzed lifehistory seasonal cycles of microsampled otolith $\delta^{18}O$ to validate the accuracy of ages estimated by counting otolith growth zones from wild caught Artic cod. Lastly, we used early-life-stage otolith $\delta^{18}O$, and its relationship to temperature, to consider late-life-stage fish condition in terms of body mass, fish length, and otolith mass.

**Keywords:** Otolith stable isotope $^{18}O$, Age validation, Temperature, Fish condition
Otolith oxygen isotope analysis and experienced temperature in early stage of chub mackerels *Scomber japonicas*

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The growth of chub mackerel is influenced by marine environment such as water temperature. It has been reported that the growth rate of the juvenile has a positive correlation between the sea surface temperature. However, the water temperature that chub mackerel juvenile actually experiences is not directly observed, and the growth and the experienced environment are not compared. In this study, oxygen stable isotope ($\delta^{18}O$) of chub mackerel otolith in early stage were analyzed and the experienced temperature was estimated. Larvae and juveniles of chub mackerel *Scomber japonicus* were collected in the western North Pacific. After measuring fork length, radius of otolith, number of daily rings and otolith daily growth rate, $\delta^{18}O$ were analyzed with isotope ratio mass spectrometer for whole of otolith samples from 2004 to 2015 (10-20 samples per year, excluding 2006). For the juveniles, the $\delta^{18}O$ value was positively correlated with the radius of otolith ($R=0.73$, $n=130$). It shows a high $\delta^{18}O$ value as the otolith size increases. On the other hand, $\delta^{18}O$ decreases with the growth of otolith during the larval stage ($R=-0.54$, $n=31$). The yearly trend was small and measured values were widely distributed. From the cluster analysis for the initial growth rate, the six clusters (CL1 - CL6) resulted. CL5, 6 (showing high growth) showed higher otoliths $\delta^{18}O$ than CL1, 2 (showing low growth) and it was suggested that CL5, 6 experienced the lower water temperature range. When converting the difference of otolith $\delta^{18}O$ to the water temperature, CL5, 6 experienced low water temperature as about 1.1 °C. Therefore, it was shown that a positive spiral, in which individuals with high initial growth proactively entered to the low water temperature area and obtained high nutritious diet.

**Keywords:** Oxygen isotope, Chub mackerel, Cluster analysis, Experienced temperature
The pace of life in deep living cephalopods

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A growing body of literature shows that life for deep-sea organisms (living in waters >200 m) proceeds at a slower pace than that of their shallow water relatives. This trend has been found for example in bivalves, fishes and sharks. Cephalopods are among the fastest growing invertebrates in the ocean and these typically opportunistic, carnivorous, marine molluscs only have a single reproductive cycle (semelparity). Ongoing themes in GEOMAR’s deep-sea biology lab is to investigate: 1) what feeding strategies cephalopods have evolved to fuel the semelparous life history strategy, and 2) how the lifecycle length and growth rates of deep-sea cephalopods compare to that of coastal and epipelagic species. In this seminar I will present an overview of the current state of knowledge of life history strategies in deep-sea cephalopods. Also, I will discuss the direct and indirect methods that are employed to study age and growth in deep-sea cephalopods. These include in situ observations of animals in their natural habitat, the quantification of increments in hard body structures (statoliths, stylets, beaks), and the estimation of life cycle length via modelling using life history parameters like environmental temperature, egg size and size at maturity. Finally, I will discuss the future of the study of life history strategies in deep-sea cephalopods, a field that could benefit from the ongoing advancement of deep-sea technology in combination with traditional established techniques.
Evaluating regional synchrony in multi species otolith growth chronologies: insights on population plasticity, resilience and ecosystem productivity in a changing ocean

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Fish growth is an ideal proxy to measure organisms’ response to environmental change as it is sensitive to a range of drivers including temperature effects on physiology, changes in food webs and fishing intensity as well as playing a fundamental role in determining fitness. Growth rate information naturally archived within otoliths of a set of species (pelagic, demersal and deep water, from short- to long-lived) is used to develop chronologies documenting growth variations over the last 50 years in a wide marine region (Iberian Atlantic coast and oceanic islands of Azores and Madeira). Firstly, species- and population-specific mixed effects models capturing both intrinsic and extrinsic sources of variation in growth are used to assess the relative importance of drivers as well as the temporal and spatial patterns in population productivity. Secondly, these models are used to investigate individual- and population-level plasticity of growth rates through time in relation with extrinsic drivers and may provide insight on species resilience and adaptive potential to climate change. Thirdly, the chronologies are used to investigate regional synchrony in growth patterns among the different species and uniformity in response to environmental change aiming at identifying environmental drivers of system productivity. The integrated assessment of regional productivity across species, habitats and life history traits over wide temporal and spatial extents, offers a means to evaluate environmental and climate driven effects in contrasting ecosystems (coastal upwelling and deep sea) which may be differently affected by climate change.

Keywords: Individual growth, Multi species, Northeast Atlantic, Regional synchrony
Is there a global signature of biological change in marine hotspots?

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The world’s oceans are changing at an unprecedented rate due to climate change. In turn, a growing number of studies have identified the signature of oceanic warming in marine life through shifts in species distributions, abundances, and phenology. But not all the world’s oceans are warming at the same rate: it is likely that the degree of biological sensitivity to environmental change is dependent on the rate of change itself. A recent study by Hobday and Pecl (2014) identified ~24 areas globally where the rate of warming is 90% faster than the rest of the oceans. Termed ‘global marine hotspots’, it is expected that there will be an intensification of biological response (either positive or negative) within these areas compared to neighbouring waters. Here, we present a global analysis, centred on the Pacific Ocean, of long-term trends in biological response to ocean warming inside and outside marine hotspots. We focus on the rich, annually-resolved data available in the long-term biochronologies developed from marine organism hard parts (e.g. otoliths, shells, coral). Analysis of hard part structures allows for the development of biological time series that extend long before other monitoring or observation took place, allowing for unparalleled insight into how marine organisms have responded to past environmental change. Using our database of 60+ annually resolved growth times series (20-100 years in length), we show that 77% of time series inside hotspots show significant directional change, compared to just 49% outside hotspots. Interestingly, we did not detect any difference in the rate of change between areas. Our results suggest that rapid warming inside hotspots does indeed induce growth rate change in more exposed species, but that once initiated this change is not necessarily more extreme than observed in areas experiencing ‘normal’ warming.

Keywords: Climate change, Marine hotspots, Fisheries
Otolith tools in the ecotoxicology toolbox: Unraveling sources and pathways of Se exposure in wild Sacramento splittail with spinal deformities

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Selenium (Se) is an essential nutrient required for oxidative and enzymatic processes, but at elevated levels it can disrupt protein synthesis resulting in deformities in developing offspring of fish and birds. Individuals with deformities consistent with Se toxicity (e.g., S-shaped spines) have been observed in Sacramento splittail (splittail) \textit{Pogonichthys macrolepidotus}, a cyprinid endemic to the upper San Francisco Estuary. Juvenile splittail can be exposed to elevated Se through direct ingestion of prey or through maternally-derived yolk. Here, we use scanning X-ray fluorescence microscopy (SXFM) at Cornell’s High Energy Synchrotron Source to detect Se and quantify the chronology of Se exposure in wild-caught juvenile splittail otoliths that display spinal deformities. We evaluate the spatial-temporal distribution of Se in the otoliths and compare the core (maternal) and edge (environmental) to test the pathway of Se exposure. Results of this study implicate different anthropogenic sources of Se that influence the foodwebs in the San Francisco Estuary or freshwater habitat as the proximate source of toxicity in these individuals.

Keywords: Synchrotron, Contaminants, Selenium, X-ray fluorescence
Sclerochronological approach for the identification of herring growth drivers in the Baltic Sea

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Functioning and structure of the entire ecosystem of the Baltic Sea, one of the biggest brackish water basins, is considered to be highly vulnerable to global climate change. Both the fish species and the fisheries are strongly affected by climate variations, but multidecadal indicators by which to assess these effects are still scarce. Sclerochronological studies based on hard structures of marine organisms may help in the evaluation of climatic impacts on the fish resources. The objective of this project was to identify the factors that influence the inter-annual variations in the somatic growth rate of the herring (*Clupea harengus*) in the Baltic Sea. This work covers otolith samples collected from commercial catches and during scientific surveys from 1954 to 2017 in the Polish zone of the southern part of the Baltic. Otolith increments widths, which represent changes in fish growth, were analyzed with linear mixed models to investigate different intrinsic and extrinsic sources of fish growth variation. Sea surface temperature, Baltic Sea Index, stock biomass of pelagic stocks (herring and sprat) were considered potential predictors of herring growth. It was hypothesized that fish are responding to climatic factors, as well as to intraspecific and interspecific competition. Analysis was conducted taking into account life history traits, focusing on juvenile and adult phase of individuals growth. Developed multidecadal biochronology showed response of the commercially important Baltic herring to the environmental variability. These results may be used for the prediction how fish will respond in the future under different climatic scenarios and support ecosystem-based management of stock.

**Keywords:** Herring, Biochronology, Growth, Otolith
Interannual variation in population growth for Japanese Spanish mackerel (*Scomberomorus niphonius*) and differing respond to environmental change

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Understand how individual growth of key species varies and respond to environmental changes is essential for effective and responsive fisheries management. In this study, otolith growth chronology was developed to reconstruct the growth history of a commercially and ecologically important species, Japanese Spanish mackerel (*Scomberomorus niphonius*). Using a mixed modeling method, a functional relationship between inter-annual and spatial growth variance and a range of intrinsic and extrinsic predictor variables were analyzed. The population growth demonstrated annual fluctuation but in a relatively small range. According to spatial growth difference observed, three stocks were identified and provided further evidence for the change in population structure. Two stocks showed markedly different growth effecting factors and dynamics responding to these factors. The result indicated a potential of local decline and highlight the importance of future study evaluating fishing induced changes.

**Keywords:** *Scomberomorus niphonius*, Growth variation, Environmental change, Mixed effect model
Northern Benguela *Merluccius paradoxus* chronologies and annual change in length from age-length-keys used for age validation; and as indicators of response to longterm changes

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Age and growth of *Merluccius capensis* (Cape hake) in the northern Benguela upwelling system off Namibia have been extensively studied showing fast growth and previous overestimates of ages. However, age validation of *M. paradoxus* (deep-water hake) has not been done because of a lack of juvenile growth data and few monthly otolith samples. In this paper we attempt to fill this gap. Mean annual growth calculated from chronologies of 141 sliced *M. paradoxus* otoliths were compared with annual growth rates determined from whole otoliths routine age-length-keys (ALKs) in Namibia (change in mean length at age 3 to mean length at age 4 from one year to the next). Annual growth rates calculated from the two methods (1999-2013) were strongly correlated ($\rho = 0.66$, $n = 15$, $p < 0.005$). Annual *M. paradoxus* growth was significantly negatively correlated with Sept-Oct (austral spring) sea surface temperatures ($\rho = -0.45$, $n = 26$, $p < 0.025$) and positively correlated with spring meridional wind stress (upwelling-favourable winds) ($\rho = 0.46$, $n = 15$, $p < 0.1$). Growth variability between years thus responds to local forcing. This species (occurring at > 300 m bottom depth and as a top demersal predator) is thus a good indicator species for long-term changes in the northern Benguela upwelling system. Growth rates determined from ALKs have increased significantly (1977-2016) at 0.07 cm.year⁻¹ ($t = 2.37$, $df = 60$, $p < 0.025$), indicating fishery-induced evolutionary changes. Additionally, upwelling favourable winds in the northern Benguela have decreased since the late 1990s, and appear to have increased again since 2006, with *M. paradoxus* growth also responding to these changes.

**Keywords:** Interannual growth variability, Fishery-induced changes, Deepwater hake, Upwelling
Food deprivation records in the otoliths of juvenile sockeye salmon (*Oncorhynchus nerka*)

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Growth rate is food and temperature dependent, contributing significantly to juvenile salmon condition and ultimate survival. Periods of food deprivation are common during the early life history of many fish and may be a leading cause of their mortality (Tzeng, 1992). Restricted access to food can affect physiology, growth and behavior which may cause increased risk of death. Occurrence of food deprivation during the early marine stage of sockeye salmon is difficult to quantify in the field. However, otolith records are a proven tool for the study of individual fish growth dynamics, with growth being recorded in otolith daily increments (Molony, 1996). We used juvenile sockeye salmon of outmigration age (age-1) to test the effects of food deprivation on the width of daily otolith increments during the transition to salt water. Three treatment groups were fed 1.5%, 0.5% and 0.25% of body weight (BW)/day respectively for two weeks, followed by a recovery period of 2 weeks. Growth and recovery in response to treatments were reflected in the width of the otolith daily increments. Daily growth increments in the 0.25% treatment were significantly smaller than in the 1.5% treatment. The response in otolith increment growth to diet treatment was delayed by ~ 5 days and this needs to be accounted for during life history reconstructions. The 0.25% treatment group maintained low growth rates for 10 days after normal feeding (1.5% BW) was resumed. Our results show that otolith daily increments can be used to determine periods of low food availability in wild juvenile sockeye, but there is a time lag between the onset of food deprivation and the growth response. In addition, food deprivation may have a prolonged effect on juvenile salmon growth even after normal feeding has resumed, and this has significant implications for survival during wild fish outmigration.

**Keywords:** Salmon, Otolith, Starvation, Daily increments
Otolith microchemistry approach to determine population structure and movements of European anchovy (*Engraulis encrasicolus*) along the Atlantic Coast of Iberian Peninsula

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The anchovy (*Engraulis encrasicolus*) present in the European Atlantic waters is separated in two distinct stock management units, one distributed in the Bay of Biscay (ICES Sub-Area 8) and the other distributed in ICES Division 9a (Portuguese coast and Spanish waters of the South of Galicia and Gulf of Cadiz). Traditionally, the distribution of anchovy in the ICES Division 9a has been concentrated in the Subdivision 9a South (Gulf of Cadiz). Outside the main nucleus of the Gulf of Cadiz, resilient anchovy populations seem to be abundant only when suitable environmental conditions occur. The objective of this work is to analyze the phenomenon of sporadic increases in the availability of anchovy in the northern part of the ICES Division 9a, testing the following hypotheses: 1) The increase in anchovy availability in the northern part of Division 9a is due to an exceptional increase of local residual populations, 2) The increase in anchovy availability in the northern part of Division 9a is a consequence of the population productivity increase in one of the two management units. To answer these hypotheses, we evaluate the feasibility of using otolith chemical composition. We analyze the spatial variation of trace elements in the anchovy otoliths in Division 9a and Subarea 8 to investigate natal origins and population movements of European anchovy in the Atlantic waters of the Iberian Peninsula. The elemental composition of anchovy otoliths was analyzed at the core and otolith edge. Li, Na, Mg, K, Ca, Mn, Sr and Ba were measured using LA-ICPMS on the sagitta otoliths of anchovy sampled in 6 different areas. Samples (collected 2015) were classified by area and age group (1 and 2 years old). The chemical composition was compared statistically between areas within age groups and between age groups within areas.

**Keywords:** *Engraulis encrasicolus*, Otolith microchemistry, Natal origin, Atlantic Coast of Iberian Peninsula
Reproducing migration history of Japanese sardine using otolith $\delta^{18}$O and a data assimilation model

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Although tracking the movement and understanding the migration of fish is a key for fisheries science, it has been technically difficult especially for small sized fish that cannot be tagged. In this study, a new scheme was developed to estimate migration history of a captured fish using high resolution otolith $\delta^{18}$O analysis and a data assimilated ocean model. $\delta^{18}$O in otolith is affected by both $\delta^{18}$O and temperature of ambient water. $\delta^{18}$O in seawater is correlated with salinity. Using salinity and temperature distribution of an ocean model, therefore, it is possible to estimate the area that the fish located from otolith $\delta^{18}$O profile. The estimated areas can further be narrowed by filtering out areas that are impossible to reach by passive transport and active swimming. This new scheme was tested for immature Japanese sardine. High precision micro-milling system Geomill 326 and ultramicro-volume carbonate analyzing system MICAL3c were used to extract otolith $\delta^{18}$O profile, which resulted in temporal resolution of 10-30 days through whole life. The relationship between seawater $\delta^{18}$O and salinity in the Kuroshio-Oyashio system was updated through numbers of water sampling: $\delta$seawater = 0.56*S – 19.06 ($r^2$ = 0.86, p < 0.01). Because the estimated migration histories expressed northward migration, consistently with previous sampling surveys, and closely approached the actual captured location in the end, we concluded that the scheme successfully worked for the Japanese sardine. The scheme can be applied to other species without large modification and would be helpful to understand its migration.

Keywords: Oxygen stable isotope, Data assimilation model, Japanese sardine, Western North Pacific
Discerning population connectivity and natal origins of Pacific herring (*Clupea pallasi*): inferences on population structure from otolith chemistry

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Pacific herring, *Clupea pallasi*, undertake annual migrations between feeding and spawning grounds that link life stages, habitats, populations, communities, and ecosystems. The extent and direction of herring movements has a significant influence on food web dynamics and also affects the diversity and stability of herring populations. However, movement patterns of these highly mobile fish are poorly understood. Declines in Pacific herring abundance and slow population recoveries in the absence of fishing pressure have elevated concerns over the status of this ecologically, economically, and culturally important species. Pacific herring spawn on substrate in nearshore habitats where eggs and larvae develop for approximately two weeks before hatching. Early development within discrete spawning grounds could facilitate the incorporation of distinctive chemical signatures within otoliths that could be used as intrinsic markers to trace movements and mixing among groups or regions. Identifying the direction and strength of connectivity among groups can reveal source populations and promote the development of population- and ecosystem-based management strategies that reflect ecologically relevant spatial scales. We applied otolith microchemistry data to: 1) test the utility of elemental signatures to distinguish the natal origins of larval herring; 2) evaluate inter-annual variation in natal signatures within spawning sites; and 3) assess the similarity of edge and natal signatures of adult herring within and among spawning sites. In 2015 and 2016, we sampled actively spawning adult herring and their offspring across British Columbia, Canada. Otoliths were extracted, aged, and their elemental composition analyzed using laser ablation inductively coupled plasma mass spectrometry. Cohort-specific analyses were applied to assess consistency among elemental signatures and broader, age-specific movement patterns. Our analyses show that otolith elemental signatures of Pacific herring can provide insight into complex population structure at scales of 10s – 1000 kms to inform and enhance spatially-explicit approaches to conservation and management.

**Keywords:** Population connectivity, Natal origins, Herring, Migration
Fish stock identification: otolith chemistry versus truss morphometry and microsatellite DNA

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Otolith science has contributed immensely to the understanding of fish populations and their effective management. Owing to otoliths’ unique characteristic of being metabolically inert and getting the elements accreted onto its surface mainly from surrounding water and food, the variability in elemental profile of otoliths has often been used to decipher the stocks of a target fish species. However, in many cases the results are not in agreement to the stock structure deciphered by other popular methods such as truss network system, biochemical markers, microsatellite DNA etc. For example, in case of threatened stinging catfish *Heteropneustes fossilis* and *Clarias batrachus* from the Ganga River and its tributaries; the truss network analysis distinguished separate stocks of fish in the three rivers however, the elemental profile of otoliths further discriminated the stocks at the two sampling stations within the river Ganga. In another example, the microsatellite markers and truss network analysis identified three stocks of *Sperata aor* within the river Ganga whereas the otolith chemistry revealed four different stocks of the selected fish species from the same river. Otolith chemistry provides information on the extent of mixing between populations at finer scale. It may provide early indication about a group in a fish population developing as a separate stock. Freshwater ecosystems experience strong anthropogenic pressure that leads to greater spatio-temporal variability in the habitat conditions of the fish. In such a case, otolith chemistry can be a useful tool to detect any spatio-temporal variability at intraspecific level. Stock identification by any approach should consider the spatio-temporal dynamics of populations and their interaction with physical and biological components. Generally, no single method will unambiguously determine fish stock structure. Thus, an integrated approach to fish stock identification is highly desirable to minimize the prevailing inconsistencies between the scales of population structure.

**Keywords:** Stock structure, Otolith chemistry, Freshwater
Latitudinal variation of life history traits in an amphidromous fish Plecoglossus altivelis altivelis: how does growth before upstream migration affect the performance in rivers?

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The latitudinal cline in growth-related traits often reflects the latitudinal gradient of environmental factors. The latitudinal patterns can be different between marine waters within a species, but such a perspective has been generally overlooked so far. The ayu Plecoglossus altivelis altivelis is an amphidromous species migrating between coastal waters and rivers within an annual life span. In the first step, we aim to examine how the latitudinal variation in growth-related traits differs between the two sides of the Japanese Archipelago (i.e., the Pacific Ocean and the Sea of Japan). We analyzed the otoliths of 33 young individuals collected in 2001 to compare growth-related traits at the marine stage such as body size at upstream migration, length of the growing period and mean growth rates among river populations. The body size at upstream migration decreases with latitudinally decreasing mean growth rates in the Pacific, contrasting to the lack of latitudinal cline in body size on the Sea-of-Japan side. In the next step, we test the working hypothesis that the smaller body size at upstream migration may induce smaller body size at maturation with the assumption that low-latitude individuals maintain low growth rates in rivers. Reproduction begins later in low latitude and this trend allows low-latitude individuals to spent longer period in rivers than highlatitude individuals. In this context, the length of the growing period in rivers and the latitudinal variation in growth rates may play an important role to determine the body size at maturation and subsequent size-dependent reproductive success. We analyze the otoliths of adult individuals collected in 2017 from three Pacific rivers located in low (31.3°N), middle (34.8°N), and high (39.3°N) latitudes to estimate the growth rates before and after upstream migration, and discuss how growthrelated traits at the marine stage affect the performance in each river.

Keywords: Latitudinal variation, Amphidromous species, Growth-related traits
Reconstructing life history migrations across freshwater-estuarine-marine ecosystems: Filling the gaps of mark recapture information with an otolith chemistry approach

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Variability in migratory behaviour may confer increased survival and species resilience. Although mark-recapture studies (using non-archival artificial tags) can provide key observations on fish movement, these represent snap-shot events and cannot provide movement information prior to tagging or during the time between mark and recapture. Here, to complement a mark-recapture study of European flounder *Platichthys flesus* in freshwater, estuarine and coastal areas over multiple years, we used otolith chemical profiles of Sr:Ca and Ba:Ca to retrospectively reconstruct the environmental life-histories of recaptured fish. Otolith chemical composition varied throughout fish life history and revealed age-specific movements and different migratory behaviours among individuals, which suggest life history plasticity regarding spawning migrations, homing or site fidelity. The otolith chemistry data added more detailed information on movement patterns to the evidence obtained from mark-recapture, whereas the mark-recapture data allowed inferences drawn from otolith chemistry data to be validated. The integration of both approaches allows environmental thresholds in Sr:Ca and Ba:Ca elemental signatures for different habitats (marine, estuarine, freshwater) to be determined, with otolith chemical profiles indicating age at first seaward migration, movement frequency and site fidelity. Complementary studies using otolith chemistry and artificial tags can contribute to resolving individual movements at fine spatial and temporal scales over entire life histories, which ultimately are essential for developing effective management strategies.

**Keywords:** Otolith chemistry, Spawning migration, Life history plasticity, Estuarine habitat use
**Meta population dynamics of Alosa alosa, towards fluxes of strayers between rivers**

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Similar to many diadromous fish species, dramatic declines were observed for Allis shad *Alosa alosa*, an anadromous Clupeidae, since the beginning of the 2000s. The knowledge of population and metapopulation dynamics is a key issue for the management of migratory species. Although homing behaviour is dominant in Allis shad, straying causes exchanges between populations of each river catchment. Currently, the management of Allis shad is applied at the population scale, without accounting for a potential metapopulation structure. Herein we propose a method to estimate the exchanges flux between rivers and a method to identify source and sink rivers. We used otolith microchemistry within a Bayesian model of reallocation coupled with abundance estimates of spawners per watershed. Otoliths natal origin fingerprints were obtained from a previous study and more than 220 individuals were implemented in the data base. Assignation to natal origin was obtained with a Bayesian model based on water composition and juvenile otolith composition. Abundance estimates were obtained using every available data (fishery, fishways video counts, and bull counts) and an estimation of rivers carrying capacity, the river surface excluding the estuary. In order to handle homogeneous data, likely abundances were obtained by extrapolating abundance from data rich rivers as a function of watershed surface for data poor rivers. Results showed a metapopulation dynamic with several rivers acting as sources and other as sinks. However, the lack of precision and homogeneity in abundance data resulted in large credibility intervals, which calls for a better standardization in the acquisition of abundance data. Nevertheless, this method should provide an overview of the metapopulation dynamics of other anadromous species with management concerns.

**Keywords:** *Alosa*, Straying, Homing, Metapopulation
Migratory contingents of Striped Bass in the St. Lawrence River (Canada): why moving downstream?

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Migratory contingents are groups of individuals belonging to the same population that adopt different migratory patterns. They have been identified in numerous Striped Bass (Morone saxatilis) populations along the North American east coast. In the St. Lawrence River (Canada), the Striped Bass population was extirpated by the mid-1960s, due to cumulative effects of habitat destruction and overfishing. A re-introduction program started in 2002 and it is now recognized that the population self-reproduced during the last decade. Recently, three migratory contingents have been identified at the juvenile stage: freshwater residents, oligohaline migrants, and mesohaline migrants. However, little is known about factors promoting freshwater residency vs. downstream movements. In this study, we combined otolith microchemistry and otolith microstructure measurements to link migratory patterns to growth histories during Striped Bass early life. We hypothesized that downstream migrations were realized by small individuals that exhibited slow growth in their native freshwater habitat. Migratory patterns were inferred using multivariate analysis of four trace elements (Sr, Ba, Mn, and Mg) measured with a LA-ICP-MS along a transect on juveniles Striped Bass otoliths. Growth trajectories were reconstructed using otolith microstructure and size-at-age were compared between migratory contingents. This study provided results on Striped Bass early life stages that will contribute to the management of this re-established population in the St. Lawrence River.

Keywords: Migratory contingents, Growth trajectories, Otolith microchemistry, Striped bass
Otoliths provide new insights into the importance of life history diversity in regulated systems

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Juvenile salmonids exhibit large variation in the timing that they migrate through freshwater and their use of natal and non-natal rearing habitats. The expectation is that this phenotypic diversity increases population resilience, buffering over space (e.g. multiple habitats reducing competition and offering varying growth opportunities) and time (e.g. a broader outmigration window resulting in a greater probability of meeting favorable ocean conditions). The California Central Valley (USA) contains the southernmost extant populations of native Chinook salmon (*Oncorhynchus tshawytscha*) and some of the most regulated and fragmented rivers in the world. Juvenile chinook migrate to the ocean in January to June, with a large fraction dispersing downstream as small fry coincident with winter storm events. It is commonly assumed that these early migrants experience high mortality and do not contribute meaningfully to the adult population. We used otolith strontium isotopes to reconstruct natal origin and juvenile habitat use of more than 2500 post-spawned (i.e. successful) adults from the American, Yuba, Stanislaus and Merced Rivers across years and flow regimes. The extent of non-natal rearing (frequency and duration) varied between years and populations. Surprisingly, fry migrants consistently contributed to the adult returns, often representing more than 20% of the reproductive population. However, for populations experiencing greater flow regulation and habitat degradation, we observed clear truncation of life history diversity, with downstream processes selecting against both early and late outmigrants. Our ability to reconstruct the movements and rearing patterns of newly emerged fry was only made possible through the use of otoliths, as these individuals are too small and fragile to be externally marked using traditional tagging methods. These data provide new insights into how juvenile salmon may respond to changes in flow and habitat in an increasingly unpredictable climate.

Keywords: Salmon life history diversity, Juvenile rearing, Strontium isotopes, Outmigration
Demography and growth during early stages of *Spratelloides delicatulus* in different marine coastal habitats of Con Dao, the oldest MPA of Vietnam

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To limit the biodiversity loss, marine protected areas (MPA) had spread over but their design is rarely based on strict scientific considerations. Ideally to be effective, a MPA should protect all key habitats necessary for the achievement of marine species life cycle. Unfortunatly, identification of these keys habitats remains complex especially during early life stages of marine fishes. However, knowledge of life history traits of early life stages are crucial since marine fish population dynamic is intimately link to these stages. In this context, the spatial and temporal distributions of larvae and juveniles of the most aboundant species *Spratelloides delicatulus* were investigated in a mosaic of marine habitats in the Con Dao archipelago, the oldest MPA in Vietnam. The sampling was conducted monthly during one year (June 2016 to May 2017) using light traps in three main habitats (seagrass beds, coral reefs and harbor). The species was identified using both morphology and DNA barcoding, while age and growth parameters were estimated using otoliths. A total of 3536 larvae and juveniles was sampled all the year round, and daily growth increments were analyzed in a subsample of 216 larvae and juvenile otoliths (7 to 26 mm SL). Ages of larvae ranged from 5 to 43 days. *Spratelloides delicatulus* larvae were not present from December 2016 to March 2017, and this species recruited from April to October with sizes ranging from 7 to 26 mm SL (5 to 43 days old). Ages ranged from 5 to 35 days old in coral and harbor sites, and from 5 to 43 days old in seagrass beds. The species seems to colonize all habitats at early stages, but progressely concentrate on seagrass beds that could be nurseries for older specimens. Protection of all habitats seems then to be relevant in the Con Dao MPA.

**Keywords:** *Spratelloides delicatulus*, Larvae, Marine, Con Dao MPA
Age-at-maturity of *Sardinella lemuru* in two fishing grounds in the Philippines: discrepancy between age- and length-based growth model

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Sardines make up the bulk of total marine capture fisheries production of the Philippines. *Sardinella lemuru* is the most widespread, dominating 3 out of 6 fishing grounds where sardines make up a substantial part of total fisheries landings. Most fishing grounds in the Philippines are already overfished and many of the local sardine stocks show significant signs of overfishing such as reduced fecundity and attaining maturity at a smaller size/younger age, thereby decreasing its reproductive potential. A total of 87 sagittal otoliths were examined to determine the ages of mature *Sardinella lemuru* (11.5-16.2cm SL) in two fishing grounds in the Philippines, the Zamboanga Peninsula and Ticao Pass/San Bernardino strait. Otolith-based age of sardines show that they mature in less than a year contrary to what is known that they mature after a year. In Ticao Pass/San Bernardino strait, mature individuals range from 137-267 days with a mean age of 175.43±35.2. Much younger mature sardines are being caught off the Zamboanga which ages range from 110-188 days with an overall mean of 144.03±17.65 days. If the length-based model for this stock is used (K=1.0 and L L(=21.21), the equivalent age of fish this size (median=14.0) is over 3-4x older (~20mos.). Clearly, the results presented above highlight a rather large discrepancy between age-based and length-based growth model. Length-at-age (otolith) model show much faster growth than length-based VBGM. This large discrepancy may be due to the latter model depicting slow growth or may be due to underestimation of ages, particularly of the larger/adult specimens. It is imperative to provide correct and updated estimate of population parameters, especially for those that are directly influenced by fishing intensity, so that the status of stocks can be properly assessed, and harvest control measures can be designed for sustainability.

**Keywords:** sardines, age-at-maturity, otolith, length-based
Review of the fish otolith research in Taiwan since the 1980s

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Fish otolith research began in Taiwan in the 1980s. We first used the light microscope to examine the microstructure of daily growth increments (DGI) in otoliths of the milk fish, publishing the first paper in 1988. In the 1990s, techniques were developed for using the scanning electronic microscope to examine the DGIs of glass eel to study the larval transportation and recruitment dynamics of Japanese eel in the western Pacific, American eel and European eel in the Atlantic, and the short-finned eel in the eastern Australia and New Zealand. In 1994, we developed Electron Probe MicroAnalyzer (EPMA) techniques to measure otolith Sr/Ca ratios and published the first paper about salinity and ontogenetic effects on the Sr/Ca ratios in otoliths of Japanese glass eel during its migration from the spawning area to the estuaries of Taiwan. Otolith Sr/Ca ratios were then used to study the migratory environmental history of the Japanese eel in Taiwan, and for international collaboration on American eel in Canada, European eels in Baltic Sea and Mediterranean Sea countries, and the African long finned eel Anguilla mossambica from the Maningory River, Madagascar. In the past 10 years, we have also used inductively coupled plasma-mass spectrometry (ICP-MS) to measure trace elements in otoliths of southern blue-fin tuna in the Indian Ocean to study its migratory environmental history in relation to oceanic upwelling and to measure trace elements in otoliths of Japanese glass eel in the estuary of Taiwan to monitor industry heavy metal pollution. The achievements of otolith researches in Taiwan will be presented at the 6th International Otolith Symposium. The newly published book “Fish otoliths–discovering the mysterious life history of fishes” will also be introduced.

Keywords: Tzeng, Otolith daily growth increment, Otolith microchemistry, Eel
Otolith stable isotope ratios revealed decline of wild Japanese eel stock; a case study in Okayama, Japan

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Fisheries catches of Japanese eel, Anguilla japonica, have consistently decreased since the 1970s and are now in a historically critical situation, with the species listed as endangered (EN) in the IUCN Red List of Threatened Species. The only one stock assessment based on Japanese CPUE data, however, assumed that Japanese eel stock has been increasing from the 1990s. Because farmed individuals have been intensively released into natural rivers and lakes in Japan, we employed a novel method to discriminate wild eels from farmed-released individuals based on otolith oxygen and carbon stable isotope ratios in order to understand the dynamics of wild stock of this species. Freshwater areas of all the large rivers in Okayama prefecture, Japan, an area for a case study, were occupied by farmed-released eels (92.5% of 186 individuals). On the other hand, wild eels occupied brackish water areas (86.4% of 59 individuals). Annual eel catches of individual fishermen in brackish water areas of Okayama prefecture have been decreased more than 90% from 2003 to 2016, based on linear regression analysis. These results indicate that wild Japanese eel stock in this prefecture has been decreasing in these years. Existence of farmed-released eels can affect stock assessment of Japanese eel because their recruiting mechanism should be largely different from that of wild ones. Discrimination method used in this study might be essential to understand the stock dynamics of this species.

Keywords: Stable isotope, Anguilla japonica, Stocking, Discrimination model
Comparisons of otolith microstructure and microchemistry among anguillid eels during their early life stages

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The otolith microstructure and Sr:Ca patterns of 12 species of anguillid leptocephali and 9 species of glass eels have now been reexamined to understand the otolith characteristics during their early life stage of these species. Based on the scanning electron microscope observations, the otolith increments between the hatching check and first feeding check ranged from 8-16 days with widths of 0.43-0.68 µm. The successive otolith increments showed first peaks in increment widths at 20-70 days (0.74-1.36 µm) and second peaks at 80-360 days (0.73-2.73 µm). The number of days to reach the second peaks and the peak increment widths of the tropical eel species were shorter and higher (105±165 days, 2.25-2.73 µm) than those of temperate eels (185-305 days, 0.73-1.18 µm). The Sr:Ca ratios of glass eel otoliths analyzed by EPMA increased with the peaks (19×10⁻³-23×10⁻³) at 50-121 µm distances from the core and then dropped down at the start of metamorphosis. Ages and body sizes of both leptocephali and glass eels had linear relationships with otolith sizes for each species. The otolith daily increments corresponding to the leptocephalus durations and the total oceanic migration periods differed widely among species (91.0±15.6 to 280.0±31.3 days, 108.5±14.8 to 351.2±52.0 days, respectively). These wide variations of otolith features reflect the diversity of early life histories and environmental conditions of the eel species living in different regions of the world.

Keywords: Microstructure, Sr:Ca ratios, Eels, Early life history
Effect of ENSO events on larval duration and transportation process of the Japanese eel (Anguilla japonica)

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Japanese eel (Anguilla japonica) has been categorized as “endangered” by the International Union for Conservation of Nature Red List in 2014. Eel spawning ground is located near the West Mariana Ridge seamount. The species travels through the North Equatorial Current (NEC) and then enters the Kuroshio during its shoreward migration toward East Asia. Therefore, El Niño–Southern Oscillation (ENSO) events serve as the most obvious drivers of interannual variability across the equatorial Pacific. Because the NEC bifurcation and salinity profiles are related to ENSO events, we investigated the influence of locations of the NEC bifurcation and salinity front on drifting time, recruitment size, and success of transport of the larval Japanese eel by analyzing glass eel otoliths, quantifying variations in eel length (size), and numerically modeling particle transport in ocean currents. Circulation and hydrography used for particle tracking were obtained from the results of the Model for Interdisciplinary Research on Climate (MIROC) high-resolution forecasting experiment. Our results demonstrated that during El Niño years, (1) the southward movement of the salinity front might cause the larvae to experience slower currents and (2) the southward movement of the salinity front and northward movement of the NEC bifurcation might broaden the separation between their spawning ground and NEC bifurcation, thus prolonging the time needed for the larvae to enter the Kuroshio from their spawning ground, because of which the duration of entrainment in the water column and body size increase when eels reach estuarine waters. On the other hand, the northward movement of the NEC bifurcation might cause more water to flow into the Mindanao Current, leading to a decline in the rate at which larvae get entrained into the Kuroshio.

Keywords: Anguilla japonica, ENSO events, Otolith, Larval migration
Extreme variability of European eel growth revealed by extended mark-recapture experiment in Camargue Mediterranean habitats (France)

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The European eel panmictic population is endangered since few decades due to its peculiar life history cycle, to fishing pressures and to difficulties in population management at a global scale. To better understand the population dynamics of the species, an extended mark-recapture experiment has been conducted in Camargue (France) over a period of 8 years. Around 1300 silver eels and 2000 yellow eels have been marked with pit-tags in 2007 in the Vigueirat water swamps, 2.5 kg of elvers have been released each year from 2008 to 2012 in this habitat, and captures using gill nets were operated yearly in June and October for ten consecutive days between 2007 and 2015. After captures and recaptures, measures of body parameters, sex and maturity stage, parasites were recorded, and the otoliths were extracted. Age was estimated observing the whole right otolith under reflected light and a dark background. In 2015 at the end of the experiment, 1322 otoliths of silver eels from individuals between 352 and 875 mm (ages from 17 to 185 months), and 2038 otoliths of yellow eels from individuals between 170 and 868 mm (ages from 12 to 123 months) were interpreted. Age estimations were validated using mark-recaptures but showed an underestimation of the age in 16% of the cases. Corrections could be done but more than 5% of otoliths did not give an accurate age. The variability of the growth for the European eel is extreme (e.g. at 48 months old, sizes ranged between 243 and 774 mm, meaning that some individuals had sizes two times bigger than others at the same age. The growth variability started from the beginning of growth in continental waters with the same magnitude during the lifespan. Results emphasize the difficulty of management of the eel populations at least in the Mediterranean area.

Keywords: European eel, Otoliths, Age estimation, Validation
Proportion of restocked individuals and fresh water use among European eels (*Anguilla anguilla*) inhabiting the Baltic Sea

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Restocking of glass and pre-grown European eels (*Anguilla anguilla*) into inland waters to enhance the plummeted wild stock and aid the fisheries is a common practice throughout the range, but the success of these actions in terms of escapement is seldom addressed. In this study, 121 eels of TL >60 cm were collected from the Estonian coastal sea and analysed for 24Mg, 43Ca, 55Mn, 66Zn, 88Sr and 137Ba in otoliths to investigate (i) provenance (wild or restocked) and (ii) migratory patterns between brackish and fresh waters. Eels were also collected from five Estonian inland lakes into which eels are restocked, two lakes into which restocked eels are known to migrate, and from an eel farm to determine representative otolith element-to-calcium values for restocked individuals originating from those sources. 73% of analysed eels were of wild origin and 27% were restocked. 61% of the restocked eels had descended into the Baltic Sea within 0-2 years after restocking. None of the coastally collected restocked eels possessed post-restock otolith Sr:Ca values matching the corresponding values from eels sampled in those Estonian lakes which are restocked with eels. However, seven individuals from one coastal site (Käsmu Bay; n=17) possessed similar post-restock otolith Sr:Ca values present also in Estonian eel farm individuals. Sr:Ca profiles of those individuals indicated that, after the restocking event, most of them emigrated immediately to Lake Peipsi (i.e. did not stay in the original restocking lake) and from there shortly to the sea. Only 24% of all the wild and restocked (i.e. after descent to the sea) individuals had used fresh waters for rearing, meaning that brackish water was the preferred biome. For the Estonian eel management plan it is suggested that eels should be also restocked to brackish bays and freshwater bodies with a more direct connection to the sea.

Keywords: European eel, Otolith microchemistry, Baltic Sea, Conservation
Eel otoliths of known age - a comparison between readers

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Age and growth rates are essential parameters used for management of the European eels (Anguilla anguilla (L.)). Ageing is nowadays often done using their sagitta otoliths, after polishing and dyeing with e.g. Neutral Red. However, due to frequent supernumerary zones and very varying growth rates and patterns, ageing of eels are normally quite complicated low accuracies and precision. Recent comparisons within SLU Aqua have shown an alarmingly high variation, both between readers as well as between two ageing units. That study could not determine the accuracy as there were no known age otoliths at hand. This paper is based on a follow up study using known age otoliths from eels with diverse growth and that from habitats in both Sweden and Finland. Otoliths arrive from both marked and tagged eels recaptured after a number of years in nature. We present data on both the variation between readers and age reading teams in two countries as well as the accuracy in relation to the known true ages. The discrepancies are considered as background for an indispensable co-ordination in methodology and interpretations at SLU Aqua in Sweden and LUKE in Finland. The consequences of deviations from true ages are also discussed from a stock management aspect.

Keywords: Eel, Otolith, Known age
Variation in age, growth and otolith kernel size among and within populations of marbled flounder in Japan

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Marbled flounder (Pseudopleuronectes yokohamae) is a kind of demersal fish living on coastal sand and mud bottoms, as one of the most important economic species of flatfish, it well supports the commercial fishery. In order to contribute to the fish resource management, we compared the age composition and growth trajectory of marbled flounder by their otolith among various coastal waters around Japan, and examined variation in the otolith annual structures, otoliths extracted from the marbled flounders were collected in Kagawa Prefecture, Yamaguchi Prefecture, Chiba Prefecture, Tokyo Bay, Sendai Bay and Hokkaido with commercial fishing gear. The majority of samples are aged 2 and 3, and female individuals show the larger total length (TL) and growth rate than those of male. Growth curves for these samples were shown to be significantly different among different coastal waters, and for each area, it also showed a significant difference between males and females that females displayed a larger theoretical maximum total length (TL), longer life span and faster growth rate than males. For the annual structure, wide variation of the kernel size was found among sea areas and within the population. The kernel size from Hokkaido was significantly larger than other areas. Comparison of the kernel size within the populations showed no significant liner relationship with body length of sequence ages of 1 and 2 years old. These suggest that the benthic water temperature and hatching date were deemed to influence the wide variations in the otolith kernel size, rather than the early growth.

Keywords: Marbled flounder, Age and growth, Otolith kernel size
Pelagic larval duration, growth rate and population genetic structure of the tidepool snake moray *Uropterygius micropterus* around southern Ryukyu islands, Taiwan and central Philippines

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The relationship between pelagic larval duration (PLD) and geographic distribution patterns or population genetic structures of fishes remain obscure and highly variable among species. To clarify the effects of PLD on the population structure of the tidepool snake moray *Uropterygius micropterus* in the study area, the otolith microstructure and population genetics based on concatenated mtDNA sequence (cytochrome b and cytochrome c oxidase I, 1,336 bp) were analyzed for 195 specimens collected from eight locations around southern Ryukyu islands, Taiwan and central Philippines. Otolith microstructure shows that the longer PLD and slower otolith growth rate were observed at higher latitudes with lower water temperature (54.6 ± 7.7 days and 1.28 ± 0.16 μm day⁻¹ in Ishigaki island vs. 43.9 ± 4.9 days and 1.60 ± 0.19 μm day⁻¹ in Badian), suggesting that leptocephali grow faster and shorten the pelagic periods in warmer temperature. Meanwhile, PLD was longer in southwestern Taiwan (57.9 ± 10.5 days) which associated with the more complex ocean current conditions compared to the east (52.6 ± 8.0 days), but both sides showed similar otolith growth rates (1.33 ± 0.19 μm day⁻¹ vs. 1.36 ± 0.16 μm day⁻¹). Despite the inter-population variation in PLD, genetic analysis revealed fluent gene flows of the eels among study region, implying the poor influence of intra-species PLD variation on genetic structure. The leptocephalus stage might ensure the widespread gene flows among the study area, however, distinct environments that the larvae experienced can still be revealed by their distinct early life characteristics.

**Keywords:** Muraenidae, Otolith microstructure, Pelagic larval duration, Population structure
Effect of metabolic rate on time-lags change in otolith microchemistry - An experimental approach using *Salmo trutta*

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During the last decades, ecologists have paid a great deal of attention to the proper techniques to track fish movement in the wild. Otolith material incorporates chemical elements from the surrounding water in a layered manner that preserves the timing of deposition. Elemental composition along otolith center-edge transects thus represent a permanent record of the growing habitats experienced by the fish throughout its entire lifetime. In this context, the ability to reconstruct the environmental history of fish from otolith chemistry largely relies on the concentration of elements in otoliths changing in predictable manners with environmental variables such as temperature, salinity, and ambient elemental concentration. Recent research has however highlighted that physiological factors can complicate this relationship by controlling metal transport and biokinetics. An important consideration in reconstructing migratory history based on otolith fingerprint is the period of time required for the chemical composition of the otolith to reflect a change in water composition. Experimental designs have indeed demonstrated that there can be significant lags (i.e. weeks to months) between changes in water chemistry and subsequent change in otolith composition. In particular, the rate at which elements are incorporated into otoliths is hypothesized to depend on fish physiology. However, to date, time-lags have mostly been quantified at a population scale and potential determinants of its individual kinetics remain poorly investigated. Here, we report result from controlled experiments (artificially enriched environment) on individual trace element uptake rates in *Salmo trutta* (Salmonidae) and investigated whether inter-individual variability in time-lags change in otolith microchemistry is related to metabolic rate. Future models that aim at extracting environmental histories (i.e. migratory patterns) from otolith should be conducted at appropriate temporal scales that reflect an adequate period of time required for environmental variables to influence otolith chemistry, based on a priori information on fish physiology.

Keywords: Time-lags, Metabolic rate, Individual kinetics, Experimental
Tracking hypoxia in Baltic flounder: do spawning ecotypes differ in hypoxia exposure?

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The occurrence of low-oxygen and oxygen-free zones in coastal and oceanic regions around the world has been increasing worldwide since the 1960s and low-oxygen events are observed with increasing frequencies and severities. The Baltic Sea is considered as the largest hypoxic zone in the world with a total area of over 60,000 km$^2$ in recent years. The restricted intrusion of oxygen-rich, saline waters combined with a permanent density stratification limits oxygen exchange between bottom and surface waters, especially in the central basins. One of the few fish species that has adapted to the brackish conditions in the Baltic Sea is the European flounder (Platichthys flesus), locally called Baltic flounder. Baltic flounder generally mix to feed in the shallow waters during summer-autumn and to overwinter in the deep central basins, but migrate either to the coast or to the deep waters in spring for spawning. These different spawning strategies have resulted in the evolution of two distinct ecotypes that differ in their egg characteristics and spawning habitats. Individuals of the two spawning ecotypes may be differentially exposed to hypoxia as the deep waters in the central basins have a much higher prevalence for low oxygen conditions than the coastal waters. Previous studies have indicated that otolith microchemistry analyses using the redox-sensitive element manganese (Mn) can provide individual exposure histories of Baltic flounder to hypoxia. We compare differences between the two spawning ecotypes in the duration and frequency of hypoxia exposure using otolith chemistry.

**Keywords:** Microchemistry, Hypoxia, Ecotypes, Baltic Sea
Early growth history differences of the three cryptic grey mullet (*Mugil cephalus*) species in Taiwan

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The study focused on the early life history of 3 cryptic species (NWP1-3) of *Mugil cephalus* juvenile in the estuaries around Taiwan. Monthly juvenile samples were collected during November 2013 to March 2014 from varied estuaries, and genotyping for species discrimination were applied to understand spatial-temporal distribution for each cryptic species. Otolith daily age and microchemistry were then analyzed to investigate their early life histories. The most abundant species is NWP2 (81%), and exists in most estuaries over spawning season. NWP1 accounts of 17% overall, and increases with month in Yilan. Only 2% (all in Checheng) of mullet juvenile is NWP3. Secondly, otolith microstructure was analyzed to investigate the early life stage in mullet juveniles. Both daily age (50.4 - 11.1 days) and duration before settle down (31.5 - 6.2 days) of NWP2 are smaller than NWP1. The linear relationship between daily rings and total length demonstrates that growth rate of NWP2 is higher than NWP1. Otolith increment widths of NWP2 in Checheng estuary are wider than other estuaries, which indicate the growth rate is different between species and various estuaries. According to daily age and sampling date, hatching dates of 3 species mullets were back-calculated. Hatching months of NWP1 are from December 2013 to February 2014, and peak is January. NWP2 hatching months are from September 2013 to January 2014, and peak is December. October 2013 is the only hatching month of NWP3. Otolith microstructure and microchemistry analyses early life history of the mullet juveniles can be divided into three stages, including drifting, transition and stable growth stage. Both drifting and transition stage of NWP1 generally longer than NWP2. This study shows the habitat change of mullet at different life stage through detailed information recorded in otolith.

**Keywords:** Mullet, Otolith, Early life stage
Age and growth of Atlantic Chub Mackerel (*Scomber colias*) in the Northwest Atlantic

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Recent efforts to manage Atlantic Chub Mackerel (*Scomber colias*) in the Northwest Atlantic have necessitated description of the stock’s life-history characteristics, specifically the length-at-age relationship. Atlantic Chub Mackerel (n = 316) were collected in the Northwest Atlantic using fishery independent and fishery-dependent sampling from July 2016 to October 2017. Total lengths (TL) ranged from 17.7 to 39.7 cm. All ages (assuming a hatch date of January 1st and adjusted by date of capture) ranged from 0.58 to 7.58 years. In order to anchor the growth curve, juvenile fish (n = 60, TL = 2.1 to 7.7 cm) were collected in January from SEAMAP plankton surveys and were assigned an age of zero. Four non-linear candidate growth models were fit to length measurements and otolith derived age estimates and the best candidate model was selected using DIC. Models were fit within a Bayesian framework using a combination of informed and uninformed priors. The three-parameter von Bertalanffy growth function was selected as the best candidate model, as it provided the lowest DIC score. The resulting median parameter estimates were 33.58 (95% credible interval 33.19 to 33.98) TL cm, 1.45 (1.33 to 1.59) y⁻¹, and -0.07 (-0.09 to -0.06) y for, k, and to respectively. These results will directly inform management of *S. colias* in the Northwest Atlantic.

**Keywords:** *Scomber*, Length-at-age relationship, Bayesian, Growth parameters
Can otolith stable isotopes (δ¹³C and δ¹⁸O) of Red Ear Emperor *Lethrinus lentjan* be used as proxies to reconstruct its historical habitat experiences in tropical coastal water?

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*Lethrinus lentjan* is among vital commercial reef fishes for marine fisheries in Malaysia. As a reef-associated fish, they are supposed to inhabit in mangroves and seagrasses as nursery habitats before ontogenetically migrating to coral reefs as adults. However, such movement and habitat connection between juvenile and adult habitats are scarce for this species. To understand the ecological connection, the juvenile and adult emperors were collected in Setiu mangrove lagoon and coral reefs around Redang Island in Terengganu coastal water, respectively. Otoliths stable isotopes (δ¹³C and δ¹⁸O) in both juvenile and adult fish were analyzed. For adult fish, the otolith stable isotopes (OSI) were measured in juvenile and adult portions on cross-sectioned otolith surfaces. Additionally, the OSI of two adult fish species, freshwater (*Trichogaster pectoralis*) and marine (*Caesio cuning*) fishes, were measured as end-member references. The δ¹⁸O otolith values were ranges of $-7.3$ to $-7.0\%$ for *T. pectoralis* and $-2.2$ to $-2.1\%$ for *C. cuning* corresponded to 0 psu and 32.3 psu in waters, respectively. δ¹⁸O otolith values of juvenile portions differed from those of adult portions in adult emperor fish. Similar δ¹⁸O otolith values of otolith edges were found between adult *L. lentjan* and *C. cuning*. Interestingly, δ¹⁸O otolith values of juvenile portions in adult fish conformed to those of juveniles. Thus adult *L. lentjan* were likely to experience in the brackish water during their juvenile periods, suggesting that the δ¹⁸O otolith could be used as a reliable proxy for the salinities experienced by fishes. The wide range of δ¹³C otolith values among fish species might be prone to otolith carbon cumulation derived from diet sources and dissolved inorganic carbon in their habitats rather than relative to the variation of ambient salinity or temperature. Thus, δ¹³C otolith seems not be suitable to use as the biomarker for construction of environmental parameters where the fish inhabited

**Keywords:** Reef-associated fish, Stable isotopes, Historical migration, Otolith
Using otoliths to relate wave action to daily growth of an intertidal/shallow subtidal fish

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Species inhabiting intertidal/shallow subtidal marine habitats are exposed to wave action varying in intensity. While intertidal fish are adapted to their frequently changing environment, the fitness consequences of wave action are poorly known, particularly in species where larger fish have a competitive advantage. We reconstructed the growth histories of adult and juvenile common triplefin (*Forsterygion lapillum*), using otoliths, across a gradient of wave exposed sites in Wellington, New Zealand. Sagittal otoliths were extracted and otolith microstructure (age and growth increments) were characterised. Growth rates using the daily increment widths of juveniles and adults were compared with in situ water acceleration data (measured with a nearby HOBO logger) to determine differences in growth within and among sites with varying wave exposure. At both exposed and sheltered sites, juvenile growth rates were significantly lower than adults. However, early growth rates of adults were significantly correlated with growth rates in later-life only in fish from exposed sites. This suggests early growth rates may persist into adulthood at exposed sites, influencing an individual’s ability to attain a competitive advantage as an adult. Larval carry over effects have been documented in *F. lapillum* juveniles, but their impacts on adult growth and relationships with environmental factors are less clear. With the intensity of storm events due to increase with climate change, we highlight the need to link growth and environmental variables to better understand the compensatory potential of fish to perturbations in intertidal/shallow subtidal habitats.

**Keywords:** Wave exposure, Triplefin adults, Otolith
Age validation of western Baltic cod (Gadus morhua)

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The contrasting zones within otoliths are commonly used to infer age of temperate fish, assuming that one opaque and one translucent zone form per year. However, even in commercial fish species the periodicity of zone formation is often unvalidated and susceptible to errors and uncertainties, which propagate into age-structured calculations. The western Baltic cod (WBC; Gadus morhua) stock is currently evaluated through an age-based assessment, using the unvalidated assumption that translucent zones form in autumn/winter. The periodicity of zone formation was evaluated through a mark-recapture experiment on 0, 1 and 2 year old WBC, captured in a shallow nursery area. Cod were tagged externally and injected internally with tetracycline-hydrochloride to induce a permanent fluorescent mark on their otoliths. Between 2014 and 2016, 6600 cod were tagged and released into the wild. Of 69 cod recaptured by 2017, 30 were at liberty for more than 3 months. Timing of translucent zone formation was reconstructed through examination of their otoliths. Recaptured cod consistently formed one translucent zone per year, between summer and autumn, contrary to the previous assumption. Even with relatively low recapture rates, recaptures from a three year period yielded sufficient data to validate the timing of the 2nd and 3rd translucent zones. High-resolution length distribution data collected from the same sampling site confirmed that two age classes of WBC occupy this nursery area each year. The length frequency data was combined with otolith edge analysis to track the development of translucent zones in the otoliths of age 0 and age 1 cod, and to indirectly validate the timing of 1st and 2nd translucent zone formation. Timing of translucent zone formation estimated using this method agreed with the mark-recapture results. The results highlight the importance of conducting age-validation experiments on commercially important species, and will facilitate improved age estimates of WBC.

Keywords: Otolith growth, Chemical marking, Tagging, Age reading
Growth and validation of the age estimation of chub mackerel (*Scomber colias*) in the North and Northwest of the Iberian Peninsula

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The Atlantic chub mackerel (*Scomber colias*), a pelagic fish distributed in warm and temperate Atlantic waters and in the Mediterranean Sea, may be considered the southern congener of Atlantic mackerel (*S. scombrus*), both species overlapping in Iberian waters. Chub mackerel has shifted in distribution further north in the last decade, possibly associated with climatic variation, what has led to an abundance increase in northern Iberian Atlantic waters (ICES Div. 8c and 9a), with a great impact on pelagic fisheries and foreseeably on the ecosystem. Despite this population is not assessed, a fishery advice could be soon required. Age and growth are essential features for analytical stock assessment, although the age estimation of this population has to be validated or corroborated before using it for any type of model. This work presents the growth pattern and parameters of chub mackerel in southern Bay of Biscay and Galician waters (ICES Div. 8.c and 9.a-north) based on otolith age estimates, length and weight variation and length-weight relationship of individuals sampled from a seven years time-series (2011 to 2017). Besides, a semi-direct validation study of the age estimation of this species in both areas is performed, based on the otolith marginal increment analyses (both, absolute and relative) and the edge nature analysis, based on samples from two consecutive years. In addition, a length-frequency analysis of the time-series is carried out with the purpose of corroborating the growth pattern. A back-calculation analysis is also performed and the consistency of the age interpretation is tested by the regularity of the increments formation and the comparison of the growth obtained from direct age estimation and back-calculation. Finally, the results obtained are compared with those from other areas.

**Keywords:** *Scomber colias*, Growth, Validation, North-Northwest Iberian Peninsula
Evaluating estuarine nursery use and life history patterns of *Pomatomus saltatrix* in eastern Australia

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Estuaries provide important nursery habitats for juvenile fish, but many species move between estuarine and coastal habitats throughout their life. We used otolith chemistry to evaluate the use of estuaries by juvenile *Pomatomus saltatrix* in eastern Australia. Our study also sought to assess variation in otolith elemental profiles as a means of tracking movement between estuarine and coastal marine environments. Otolith chemical signatures of juveniles from 12 estuaries, spanning 10° latitude, were characterised using laser ablation-inductively coupled plasma-mass spectrometry. Otolith elemental signatures of fish collected in most estuaries could not be successfully discriminated and this was attributed to the influence of marine water on otolith elemental composition. Using a reduced number of estuarine groups, the juvenile otolith elemental signatures of 52% of adult *P. saltatrix* were most similar to the signature from juveniles from the marine dominated estuary (as opposed to all other estuaries). Elemental profiles across adult (Age-1) otoliths highlighted a variety of life history patterns, not all consistent with a juvenile estuarine phase. Furthermore the presence of age-0 juveniles in coastal waters was confirmed from historical length frequency data from coastal trawls. Combining multiple lines of evidence suggests juvenile life history plasticity and that *P. saltatrix* in eastern Australia utilises both estuarine and coastal nurseries. Ultimately, knowledge of juvenile life history is important for the management of coastal species of commercial and recreational importance.

**Keywords:** Bluefish, Juvenile habitats, Global life history strategy
Tracking invasive lionfish population dynamics and demographics in the northern Gulf of Mexico via analysis of otolith microstructure

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Invasive Red Lionfish (*Pterois volitans*) have become well-established within reef ecosystems across the western Atlantic where they pose substantial threats to native fish communities. Red lionfish otoliths were sampled across 5 years in the northern Gulf of Mexico (nGOM) to examine growth and year class progression in the expanding population of this invasive species on natural and artificial reefs in the system. Samples (n = 3,270) were collected in 2013-17, and fish were aged by counting opaque zones in otolith thin sections. Examination of percent opaque margins indicates a single opaque zone is formed in lionfish otoliths in late spring. Preliminary analysis estimated ages ranging from 1 to 7, with birth years between 2008 and 2017. Lionfish were first observed in the nGOM in 2010, yet age distributions reported here indicate they were likely present in the system before then. There was a significant difference in size at age between sexes and between natural and artificial reefs. Red lionfish are sexually dimorphic with males attaining larger size at age than females. The significant habitat effect is likely due to lionfish density being two orders of magnitude higher on artificial reefs, but also may result from lower diversity and abundance of small demersal reef fishes in that habitat type. Fish condition was also significantly lower at artificial reefs, and we earlier reported density-dependent cannibalism in this species. Results of cohort analysis based on annual age distributions will also be presented to further elucidate population dynamics of this invasive species.

**Keywords:** Lionfish, Invasive species, Gulf of Mexico
Migratory behaviour as coping mechanism to resource availability: riverine *Galaxias maculatus* in Chile

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Many species of Southern Hemisphere galaxiids were shown to display highly flexible migratory life histories, but the mechanisms driving evolution of different strategies remain unclear. Our objective was to assess mechanisms driving the selection of migratory strategies of *Galaxias maculatus* from rivers across a gradient of latitude. Fifteen fish from three size classes were collected from upper, middle and lower sections of 10 river systems in Chile between the latitudes of 36°-47°S. Otoliths of captured fish were used to estimate growth rates of early life stages, and to assess their migratory life histories (using Laser Ablation Inductively Coupled Plasma Mass Spectrometry). Otolith elemental composition was analysed through depth profiling. We assessed the effect of latitude on migratory behaviour (i.e., a binomial response) using generalised linear mixed models. Subsequently, we compared growth rates between migratory and non-migratory individuals using linear regressions. We report a strong effect of the latitudinal gradient on the probability of migratory behaviour regardless of the reach of capture. Fish were more likely to display migratory behaviour in more southern rivers. Furthermore, presence of large headwater lake interacts with this trend. Populations in lower river reaches migrated to marine habitats at higher latitudes in systems with these lakes present, compared to systems with lakes absent. This might be an effect of more predictable flow regimes controlled by these lakes, with floodplain inundation and associated nursery habitats. Interestingly, also there were no differences in size at 50 days between migratory and non-migratory fish, regardless of their reach of capture. We conclude that migratory strategies may confer a selective advantage on fish in less productive river systems at higher latitudes with shorter growing seasons. It is not clear whether migratory behaviour in *G. maculatus* has a genetic basis, or if it represents an environmentally determined plastic trait.

**Keywords:** Galaxiids, Depth-profiling, Life-history strategies, Latitudinal gradient
Can we reconstruct the growth history of the Pacific halibut (Hippoglossus stenolepis) population using otolith increment analysis?

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The Pacific halibut (Hippoglossus stenolepis) is one of the largest and longest-lived flatfish in the world, reaching up to 200 kg in body weight and 2.4 m in length and with the oldest aged individual being 55 years old. In this species females attain much larger sizes than males, and the average size at age for both males and females significantly decreased during the last 25 years, particularly in the Gulf of Alaska, leading to a decrease in the fisheries yield and stock biomass. Different factors, including environmental, biological, and fisheries-related effects, could be contributing to the observed decrease in the size at age of this species. Using the International Pacific Halibut Commission (IPHC) long-term, coast-wide otolith collection, we investigated whether otolith growth corresponds with somatic growth in Pacific halibut. Specifically, we examined otoliths from the 1977, 1987, 1992, and 2002 cohorts from three different regions of biological significance within the Pacific halibut’s distribution range. Despite the significant decline in Pacific halibut size at age, we did not find a similar decline in otolith growth during the examined time period. For example, among 15 year-old females sampled in the Gulf of Alaska from the 1977 and 1992 cohorts, there was a 2.45% increase in mean otolith radius during that time period, despite a 14.97% decrease in mean body length. Additionally, we found that otolith accretion in male and female Pacific halibut does not reflect their large dimorphic size differences. Although factors regulating otolith growth in Pacific halibut are not well understood, otolith growth appears to be decoupled from somatic growth in this important species.

Keywords: Pacific halibut, Otolith growth, Somatic growth, Fisheries
Reconstructing growth chronologies of catadromous eels in New Zealand from historic and contemporary otolith collections

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The endemic longfin (Anguilla dieffenbachii) and native shortfin (A. australis) eels in New Zealand are the basis of important cultural, recreational and commercial fisheries. The viability and sustainability of the fishery is under increasing scrutiny, particularly for longfin eels which are declining. Freshwater habitat degradation, disease and migration barriers are widely known to impact eels. However, the effects of environmental variability associated with climate change on longfin and shortfin eel growth rates and life history schedules are unknown. New Zealand is experiencing more frequent droughts and increased temperature variation associated with El Niño/ La Niña events, indicating climate change is likely a key stressor on New Zealand’s eels. Their longevity and slow growth further suggest longfin and shortfin eels may be particularly susceptible to the effects of climate change. Here, we reconstruct otolith-derived growth histories of longfin and shortfin eels from lakes and rivers in New Zealand to investigate climate effects on eel growth rates. Archived and contemporary otolith collections were used to generate growth chronologies spanning at least 25 years for each species. Mixed effects modelling was used to partition annual growth rates into intrinsic (e.g. sex, ontogeny) and extrinsic (e.g. temperature, precipitation) components, to better understand drivers of annual growth rate variation. We discuss the application of this bio-chronological approach to understand multi-decadal patterns in eel growth rates and implications for their life histories.

Keywords: Growth chronology, Eels, Environmental variation, Mixed effects models
Hatch dates of young-of-the-year Pacific cod tell us the place of their birth

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Pacific cod *Gadus macrocephalus* is widely distributed in the coastal area of the North Pacific Ocean and is one of the most important fishery resources in northern Japan. The large variation in the year-class strength of cod has been reported for several local populations in Japan. The year-class strength of cod may be determined during early life, however, there is little understanding of population structure, spawning locations and hatch period of cod in the waters around Hokkaido, northernmost Island of Japan. In this study, the hatch dates of young-of-the-year (YOY) cod, caught by bottom trawls from western and eastern Pacific coast of Hokkaido between late June and early July in 2017, were estimated by counting daily growth increments in lapilli of them. The standard length of YOY cod ranged from 6 to 9 cm and the majority of them have hatched between late January and early February. The back-calculated spawned dates of YOY cod were concentrated in January. From the temporal change in the maturity stage of adults, spawning season of cod was estimated to be from late December to January on western Pacific coast of Hokkaido (Hattori et al. 1992) and from February to March on eastern Pacific coast of Hokkaido (Hamatsu 1996). In January 2017, sea surface temperatures in the coastal area of western Pacific coast of Hokkaido were 5-8°C (Japan Meteorological Agency) and would be suitable for egg survival (Bian et al. 2014). YOY cod caught from eastern coast may have hatched on western coast and moved to eastern coast like YOY walleye pollock *Gadus chalcogrammus* (Honda et al. 2004).

Keywords: Hatch date, Otolith microstructure analysis, Pacific cod *Gadus macrocephalus*, Pacific coast of Hokkaido
Pelagic larval growth and recruitment success of *Platichthys flesus* in the Mondego estuary

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Understanding the causes of variation in recruitment of marine fishes has been a central goal over the last century. The relationship between recruitment variability and larval growth and survival is poorly known for most species. In this study, we examined the effects of the variation in the early life characteristics (pelagic larval growth, pelagic larval duration, size-at-hatching and size-at-settlement) on post-settlement survival of European flounder (*Platichthys flesus*) in the Mondego estuary over 5 years. Otolith analysis was used to back-calculate daily growth rates and other larval life traits. Results were related with the density of early juveniles (<70mm) in the Mondego estuary from 2011-2015. Larval growth presented interannual differences during the period analysed. Growth from larval age 16 to 20 d was the best predictor of recruitment. This study highlights the larval life traits as tool for predict recruitment, thus its importance for the management of resources.

**Keywords:** Larval growth, Recruitment, *Platichthys flesus*, Otolith
Millennia of fishing along the Pacific coast of Panama and the potential impacts on populations and life histories of *Cynoscion*

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*Cynoscion* species have been an important food resource since pre-Columbian periods across Tropical America and remain a highly valued and vital component of modern commercial fisheries. Despite their cultural and economic importance, however, there is a lack of basic life history information and stock assessments. Here we use *Cynoscion* otoliths from archaeological middens (2500-520 BP) and modern fishes from the Pacific coast of Panama to assess size at capture and changes in life histories over time. We focussed our study on the three species for which we had sufficient archaeological material – *C. albus*, *C. praedatorius* and *C. squamipinnis*. Fishes from mark-gets were used to determine the relationship between body and otolith length, and both modern and archeological otoliths were sectioned for growth band analysis. Results show that while *C. praedatorius* was an important constituent of the pre-Columbian diet it is no longer present in fish captures and may be locally extirpated. Growth-ring analysis shows that pre-Columbian fisheries targeted juveniles of *C. albus* on a seasonal basis, and the adults of *C. praedatorius* and *C. squamipinnis*. We observe that the rate of growth of *C. albus* is significantly faster today than in pre-Columbian periods. Increased food availability could be the cause, yet the same pattern is not apparent in *C. squamipinnis* suggesting a species-specific response. We propose that persistent harvesting of juveniles in *C. albus* may have been an agent of selection for the fish to mature faster today, although further research is required to better understand life histories of this important resource. Several aspects on pre-Columbian fishing and the implication of these findings for modern fisheries management are discussed.

**Keywords:** *Cynoscion*, Otolith, Panama
Using otolith growth autocorrelation to detect critical periods during early life of fish

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The Growth-Survival Paradigm stipulates that recruitment strength is positively linked to growth rate during the larval stage of fish. Fast-growing individuals would benefit a reduced period of high vulnerability to predation, a narrower range of effective predators, and increased capacity to escape predator attacks. In a previous study, we demonstrated over a wide species range that serial correlation in otolith growth during the early ontogeny was always positive but highly variable among species. Serial correlation was generally stronger in fast-growing species that displayed a strong association between feeding success and growth rate. The timing of rapid increase of growth autocorrelation is indicative of periods during the early ontogeny where larvae that have achieved relatively fast or low growth respectively remain on a fast or slow growth trajectory. Given the strong link between survival and growth, we argue that the Growth-Survival Paradigm might apply differently among fish species and that measuring the rate of change in the level of growth autocorrelation during the early otolith growth trajectory may be a promising avenue for detecting the timing of potential critical periods for survival, as well as estimating the nature of mortality that affects the early larval stage in a given population.

Keywords: Growth rate, Growth autocorrelation, Survival, Critical periods
The diversity of fish otoliths from the sea bottoms: on their taphonomic significance

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Otoliths represent a significant biogenic carbonate component in marine sediments, the diversity of sea bottom otolith assemblages, however, remains largely unexplored. Otolith assemblages from the bottom sediments (late Quaternary to Recent) of the North-eastern Atlantic (NE Atlantic), the central Mediterranean, and the Red Sea were investigated for their taxonomic composition. Samples were compared and diversity among them was estimated. The otoliths documented the full range of preservation textures reflecting the degree of early taphonomic processes. The assemblages are distinct according to their sampling areas, because they are structured by a unique combination of mesopelagic taxa that are specific to each area, though those of the central Mediterranean and middle-latitude NE Atlantic share many taxa and a certain biogeographical continuity was evident. We find that different from modern fish communities, the richness of otolith taxa peak at mid-water depths and decrease at depths >2000 m. The lower diversity at shallow water is interpreted as environment not favourable for otolith preservation. Further, it is critical to recognize the distinct ecological properties between the pelagic and benthic-benthopelagic components that are both spatial-averaged vertically in the otolith thanatocoenoses.

Keywords: Fish community, Diversity estimator, Paleoecology, Biogeography
Growth estimation of the European hake *Merluccius merluccius* from the Eastern coasts of Algeria, based on length-frequency and otolith analyses

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Age and growth estimates are crucial in formulating fisheries management policies mainly because of their influence in developing stock assessment models. The quality of the estimation for these parameters directly impacts on species stock assessment. The present study estimates age and growth parameters of the European hake *Merluccius merluccius* (Linnaeus, 1758) from the eastern coasts of Algeria, based on otolith analysis. Hake is well known as being a species in which otolith interpretation is difficult. A total of 125 otoliths sections were examined, covering a length range of 122 - 717 mm, sampled from commercial catches. Transverse sections of sagittal otoliths showed well defined structural increments consisting of alternating translucent and opaque bands which were validated as annuli. Age-length keys for combined sexes were obtained; the maximum age was 6 years. An indirect validation based on length-frequency analysis was performed using the Bhattacharya method. The estimated Von Bertalanffy growth parameters were $L_\infty = 109.5$ cm, $k = 0.2$ year$^{-1}$ and $t = -0.5$ year. These values are similar to those reported by other authors for the same species. Length-weight relationship exhibited majorates allometric growth.

**Keywords:** Age, Growth, *Merluccius merluccius*, Otoliths
Hatching cohort discrimination in statolith microstructure and chemical signatures of swordtip squid (*Uroteuthis edulis*) off northeastern Taiwan

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Statolith microstructure and chemical signature were analysed to investigate monthly early life growth variation of swordtip squid (*Uroteuthis edulis*) off northeastern Taiwan, and to discriminate different hatching cohorts. Samples were collected between July 2011 and December 2012 from northeastern Taiwan. Radius of natal ring (NRR) and incremental widths (IW) in statolith were measured and correlated to with sea surface temperature (SST). Natal ring radius of statoliths was significantly lower in the individuals hatched in July and August, but no significant difference among the other months. The mean first 30 incremental width was significantly narrower in the individuals hatched in January and February but significantly wider in May. The mean first 30 days statolith incremental width was positive corresponded to sea surface temperature (SST), apart from the warmest June and July. A total of 65 statoliths elemental composition of *U. edulis* at paralarval stage were qualified and it can discriminate the individuals from spring cohort (January to August) to autumn cohort (September to December). Overall, 87.7% individuals can correctly be classified between spring and autumn cohorts by using discriminant function analysis.

**Keywords:** Statolith, Microstructure, Trace element, *Uroteuthis edulis*
Application of enriched 137Ba tracer to mark juvenile Persian sturgeon (*Acipenser persicus*)

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The present study evaluates the variations of 137Ba abundance in pectoral fin spine of 1-monthold juvenile Persian sturgeon (*Acipenser persicus*) upon marking using the stable isotope approach. The marking of the fish was achieved by incorporation of 137Ba²⁺ in the calcified lattice of the pectoral fin spine through substitution with structural Ca²⁺. This process was carried out by rearing juveniles in treatment tanks containing elevated concentrations of 137Ba for 1, 3 and 5 days. The marked fish were then retained in natural abundance fresh and brackish waters, to evaluate the trend of exchange of 137Ba from the fin spines. The abundance of 137Ba in fin spines during marking and post-marking experiments were detected by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that a significant isotope mark can be obtained with no mortality and 100% marking rate on the first day of exposure to the isotope. The marked juveniles maintained their isotopic signature for at least 25 days. Statistical analysis of the obtained 138Ba/137Ba ratios demonstrated that the successful incorporation of 137Ba²⁺ in pectoral fin spines provides an effective marking method for Persian sturgeon restocking programs.

**Keywords:** Fish marking, Pectoral fin spine, Persian sturgeon, ICP-MS
Fish Eyes, Fish Ears: revealing the secret lives of fishes with eye lens and otolith chemistry

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For decades, chemical analysis of calcified structures such as otoliths, scales and spiny fin-rays have been used to the study of life history of fishes, from age determination to fish movements. More recently, other “chronometric structures” are increasingly being studied in this way. Among these structures, the eye lens is an interesting candidate for providing chemical information complementary to otoliths. Like otoliths, eye lenses grow throughout the life of a fish; yet unlike the aragonitic (CaCO$_3$) otolith, eye lenses are made entirely of protein. Further, eye lenses have been found to take up mercury, a known environmental contaminant, preferentially. The aim of this study is to compare the chemical composition of eye lenses with corresponding otoliths of Yellow Perch (Perca flavescens) and Rainbow Smelt (Osmerus mordax) in Lake Erie and Flounder (Platichthys flesus) in of the Baltic Sea to (1) develop a concordance of chronologies between these two structures, and (2) gain better understanding of mercury (Hg) exposure histories. We will test the uptake trend of mercury (Hg) and Selenium (Se) in otoliths compare with the eye lenses through the fish lifetime, and if Hg interferes with the natural Ca distribution in outer layer of retina. This research will provide useful microchemical information which help to better understanding of life history of fishes.

Keywords: Eye lenses, Otoliths, Micro-chemical information
Sr/Ca and Ba/Ca ratios in otoliths - tracing the migratory behavior of grey mullet in the estuary

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Although otolith microchemistry has been validated as a powerful tool for studying the migratory environmental history of fish, the application of otolith microchemistry for estuarine fishes has not been well-studied. The migratory behavior of grey mullet Mugil cephalus in the Tanshui River estuary of Taiwan was examined via the temporal changes in otolith Sr/Ca and Ba/Ca ratios, which were measured respectively by EPMA and LA-ICPMS, and compared with the spatial changes of the elements Ba, Sr and Ca concentration in fresh, estuarine, and sea waters. The results indicated that (1) the contents of Sr and Ca in sea water were positively correlated with salinity ($R^2 = 0.82$~$0.95$ for Sr and $0.93$~$0.98$ for Ca). In contrast, Ba levels originating from fresh water were negatively correlated with salinity but the correlation was low ($R^2 = 0.01$~$0.07$). This is because the solubility of Ba is highest at a salinity of approximately 18 psu. The Ba concentration peaked in tidal mixing areas of the river where turbidity was maximal. The behaviors of elements in the estuary enabled study of the migratory behavior of estuarine fishes such as mullet. (2) The temporal changes in otolith Sr/Ca and Ba/Ca ratios indicated that the mullet recruited to the Tanshui River in the larval stage and grew there until the spawning migration. The peak otolith Ba/Ca ratios implied that grey mullet preferred to stay in the tidal mixing and turbidity maximum area for feeding where organic debris abundantly accumulated. In conclusion, otolith Sr/Ca and Ba/Ca ratios are reliable tracers to reconstruct the estuarine migratory behavior of grey mullet.

Keywords: Grey mullet, Estuary, Otolith microchemistry, Migratory behavior
Proteomics study of otolith matrix proteins

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Otoliths, which are ear stones of teleost fish, consist predominantly of a mineral phase which is found within an organic matrix. A complex network of macromolecules, such as proteins and proteoglycans creates this organic matrix. Special role in regulation of mineral phase and crystal morphology is played by these proteins. Due to their highly acidic nature and structural properties, they promote Ca²⁺ binding which allows them to act as a specific regulatory factor. Proteomics is a large-scale comprehensive study of a specific proteome, including information of protein content, their variations and modifications, along with their interacting partners and networks, in order to understand cellular processes. Mass spectrometry is a highly sensitive technique, which does not require large sample sizes, and thus is ideally suited for the otolith matrix proteins studies. Our project was divided into several sections. The first part was focused on otolith extraction and purification, the second on matrix protein extraction. A protocol for protein extraction included decalcification in EDTA and protein precipitation was developed. After digestion peptides were analyzed with LC-MS/MS. Our studies allowed to identify proteins matrix from several fish species, such as zebrafish, rainbow trout or carp. Post-translation modifications in proteins as phosphorylation were also shown. Therefore, proteins compositions of aragonite and vaterite zebrafish otoliths were compared. Otolith matrix proteins control the biomineralization process, that is why the obtained results are of significant importance for understanding the mechanism of otolith formation. This work was supported by National Science Centre grant (UMO-2015/19/B/ST10/02148) and partly by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the faculty of Chemistry of Wroclaw University of Science and Technology. Travel costs were covered by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018.

Keywords: Otolith matrix proteins, Proteomic, Protein identification
Using the otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio to reveal the differences in habitat salinity among three sympatric ninespine sticklebacks (genus *Pungitius*)

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A difference in habitat salinity can be an important isolating barrier among closely related species of aquatic organisms. To reveal the role of salinity in speciation of fishes, a method to estimate the habitat salinity of individual fish through their life history is needed. In this study, we used the otolith strontium isotope ratio ($^{87}\text{Sr}/^{86}\text{Sr}$) as a proxy for habitat salinity of each individual fish to reveal the habitat differences between the three sympatric ninespine sticklebacks, the brackish-water type (*Pungitius pungitius*), the freshwater type (*Pungitius* sp.), and *Pungitius tymensis*, in the Shiomi River of eastern Hokkaido, Japan. *Pungitius tymensis* was identified by external morphology and the morphologically cryptic brackish-water type and freshwater type were distinguished by microsatellite marker analysis. The relationship between $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and salinity of the water sampled at several sites in Shiomi River was explained by a two-component (river water and seawater) mixing curve, indicating that $^{87}\text{Sr}/^{86}\text{Sr}$ ratio can be a good proxy for habitat salinity in this river system. The otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio was different among the three sympatric ninespine sticklebacks, showing the differences in habitat salinity among the three species.

**Keywords:** Strontium isotope ratio, Ninespine stickleback, Habitat salinity
This study elucidates the nitrogen isotopic fractionation between diets and otolith organic materials via two kinds of feeding experiments. First, larval stage tilapias (*Oreochromis mossambicus*) were fed diets having different isotopic compositions for up to one and a half years. Then the otoliths were converted to N\textsubscript{2}O gas by a peroxodisulphate oxidation-bacterial method and nitrogen isotopic compositions (δ\textsuperscript{15}N\textsubscript{oto}) were measured by an isotopic ratio mass spectrometer (IRMS). This highly sensitive method reduced the minimal mass of otolith required for δ\textsuperscript{15}N\textsubscript{oto} analysis to as low as 2 mg compared to conventional methods, which required approximately 8-155 mg of otolith. The tilapia otolith δ\textsuperscript{15}N\textsubscript{oto} compositions did not significantly differ from the δ\textsuperscript{15}N values of each diet. This indicates that the δ\textsuperscript{15}N\textsubscript{oto} might be randomly derived from dietary amino acids without any biochemical transamination. For the second feeding experiment, the unicellular green algae (*Tetraselmis chui*) were incubated with \textsuperscript{15}N labeled potassium nitrate and fed to the tilapia juveniles for 18 days. The otoliths were extracted and measured for δ\textsuperscript{15}N\textsubscript{oto} values deposited before and after the feeding experiments by the NanoSIMS. The δ\textsuperscript{15}N\textsubscript{oto} values showed abrupt surge from the natural abundant level to 1500-2000‰ after the fish ate the spiked algae with δ\textsuperscript{15}N values of 2200‰, suggesting that the otolith organic nitrogen is derived from the food not the metabolic tissues of fish. Therefore, δ\textsuperscript{15}N\textsubscript{oto} can be used as a proxy for nitrogen in the food sources of the fish. Analysis of δ\textsuperscript{15}N\textsubscript{oto} especially by a manner of high temporal resolution may have new applications in ecological studies such as the detection of diet shift or migration at certain life stages, which are difficult to detect in the metabolic tissues due to the slow turnover rate of their isotopic compositions.

**Keywords:** Fish ecology, Nitrogen stable isotopes, Food source, Isotopic fractionation
Synchrotron µ-X-ray fluorescence analysis of Baltic cod otoliths reveals high resolution patterns in Sr concentrations

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Micro-chemical analysis of otoliths can reveal information about the environmental and physiological experience of a fish throughout its entire lifetime. The Baltic Sea is home to two commercially important cod populations. Despite their close proximity geographically, these populations experience different environmental conditions and have developed divergent life history strategies, both of which will potentially be reflected in the microchemistry of their otoliths. More than 40 cod (*Gadus morhua*) otoliths originating from both populations in the Baltic Sea, collected over the last 4 decades, were analysed using µ-X-ray fluorescence at the hard X-ray microprobe beamline at the synchrotron facility DESY. The high throughput raster scanning ability of the Maia detector system provided the opportunity to produce detailed 2D maps of otolith elemental distributions within a relatively short time period. The major elements which could be reliably detected using this configuration were Ca and Sr. Elemental maps produced for these elements indicated regular fluctuations in Sr over the life of some of the sampled Baltic cod, with more uniform distributions of otolith Sr apparent in others. The fluctuations in Sr may indicate movement between waters of different salinities and temperature in the Baltic Sea, though variations in individual growth rate are also likely to influence the signal. Chronological transects through the otolith growth axis were compared to fluctuations in otolith opacity to explore variation in growth and environmental experience over the life of each individual. The large amount of data produced by this analysis was also used to characterise the Sr profile of the entire otolith cross-section. Both approaches allowed a comparison of Sr incorporation into Baltic cod otoliths from different regions and decades, and highlighted the diversity of life history strategies between and within the two cod stocks in this stratified, brackish sea.

**Keywords:** Strontium, Otolith chemistry, Synchrotron µ-XRF, *Gadus morhua*
Comparison of analytical methods assessing otolith chemistry

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Almost 20 years has passed since Steven Campana wrote his frequently cited review paper on the chemistry and composition of fish otoliths. Since then, there has been a huge development of the instrumental methods achieving increased sensitive, precision and spatial resolution. The information on the chemistry and especially on the elemental distribution in the otoliths depends on the method of choice. Particle X-ray Emission and X-ray Fluorescence analysis are non-destructive methods well suited for measuring strontium and zinc distributions or multi-element mapping. The barium concentrations in otoliths are however often too low for these methods. Laser Ablation Inductively Coupled Plasma Mass Spectrometers are available in single- or multi-collector modes, the latter providing excellent information on isotopic ratios. These methods are sensitive enough to measure a wide variety of elements but the calibration is demanding. It is also possible to dissolve the whole otolith in acid and analyse the solution with Inductively Coupled Plasma Optical Emission Spectrometry. The information on the elemental concentrations becomes in this case more reliable and easier to assess statistically but at the same time information on the detailed life history of the fish is lost. In this paper we compare the information obtained by analysing whitefish otoliths with the different instrumental methods. The whitefish (n=30) were captured in the Baltic Sea close to their spawning ground. Among these whitefish there are seaspawners, river spawners and stocked fish.

Keywords: Otolith, Whitefish, Baltic Sea, Elemental analysis
Eye lenses as an alternative to otoliths for reconstructing habitat use of juvenile salmon

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Traditional methods used to track movement, habitat use, or dietary shifts in fish have relied on measuring carbon (δ¹³C), nitrogen (δ¹⁵N), and sulfur (δ³²S) isotopes in different tissues (gut content, blood, organs, muscle, bone, otoliths) with varying turnover rates (days, weeks, months, lifetime) to reconstruct diet over time. Fish eye lenses are a promising alternative for chronicling shifts in fish feeding ecology. Proteins (OMP-1 and Otolin-1) are small constituents in calcium-carbonate otoliths. In contrast, lenses, are made primarily of protein. Eye lenses are small, onion like spheres that lay within the eye of a fish. Each lens is comprised of layers that continue to accumulate throughout the life of a fish. Therefore, lenses represent an ideal tissue to measure light isotope ratios of C, N, and S, typically derived from diet sources which are in high concentrations in body proteins. Here, we 1) investigate the relationship between fish fork length and eye lens diameter using juvenile Chinook salmon (Oncorhynchus tshawytscha) of known rearing history and age, and 2) compare C, N, and S isotope ratios in fish eye lenses between fish rearing in floodplains compared to tributaries and mainstem rivers. We show that this method is a promising approach to reconstruct the proportion of salmon that use and benefited from growth on floodplains.

**Keywords:** Eye lens, Protein, Isotopes, Foodweb
Exploring sub-population structure of Atlantic bluefin tuna (*Thunnus thynnus*) within the Mediterranean Sea

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Atlantic bluefin tuna, *Thunnus thynnus*, is a large migratory fish with an extremely wide distribution over the North Atlantic Ocean and adjacent seas. In the Atlantic Ocean, two stocks are sustained by separate spawning populations: the western Atlantic population spawning in the Gulf of Mexico and eastern Atlantic population spawning in the Mediterranean Sea. Within the Mediterranean Sea, bluefin tuna spawning occurs in several sites, but principal spawning areas are in eastern (Balearic Islands), central (southern Tyrrenhenian Sea and western Ionian Sea), and western (Levantine Sea) regions. We used otolith trace element chemistry to assess the relative importance of each of the spawning areas. We analysed young-of-the-year bluefin tuna otoliths to investigate if these spawning areas can be discriminated by their trace element signature. If otolith core trace elements chemistry is distinct among the spawning areas, juvenile and adult bluefin tuna captured by fisheries both within and outside of the Mediterranean Sea could be assigned to one of the spawning areas.

**Keywords:** Bluefin tuna, Trace element, Subpopulation structure, Mediterranean Sea
The effect of laser spot size on data averaging and accuracy while using LA-ICPMS

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When conducting beam-based microchemical analyses of otoliths and other hard parts of fishes, decisions must be made on setting various parameters. In the case of using lasers to ablate material, the diameter of the laser beam (the spot size) is a parameter that many find difficult. On the one hand, a larger spot size will pass across more material, and thus average the signal. On the other hand, a smaller spot size collects less material, thereby affecting the signal:noise ratio adversely. Here I demonstrate the trade-off of larger vs. smaller spot sizes by analyzing the same cod otolith transect twice: first with a 35-micron spot, then with a 100-micron spot. The latter acquires approximately 8.2 times the amount of material as the former. Elements for which the ICP mass spectrometer has greater sensitivity (e.g. Ba, Mn), or which are relatively abundant (e.g. P), show good performance at either spot size; the slopes of 100-micron analyses regressed on 35-micron analyses are > 0.8, and R\textsuperscript{2} values exceed 0.75. Elements and isotopes in low abundance or with poor instrument sensitivity (e.g., 26Mg, 127I) have much lower signal:noise ratios at 35-micron spot size; both regression slopes and R2 values decline. This trade-off suggests that LA-ICPMS analyses should be carried out at the largest spot size possible for most trace elements and isotopes. Further, direct comparisons of analyses carried out at different spot sizes are not recommended; rather, it is better to keep the same spot sizes for inter-otolith comparisons.

Keywords: LA-ICPMS, Laser beam spot size, Signal:noise, Trade-offs in setting parameters
29 years on, your PhD otoliths may still surprise you

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I raised juvenile American shad (*Alosa sapidissima*) for bioenergetics experiments during my Ph.D. studies. These were reared at three different temperatures and three ration levels. At the end of the experiment, I extracted their otoliths, mounted and polished them, and used them primarily for an age validation study (*). A second use during my Ph.D. was to investigate whether otolith Sr/Ca was affected by a change in diet. Recently however, I had reason to re-analyze these to test hypotheses about physiologically sensitive trace elements. In the process, I discovered that not only was Sr/Ca affected by a switch from feeding on freshwater zooplankton to a marine fish-based diet, but so was Ba/Ca, even more strongly. Thus, holding onto research materials like otoliths, which do not degrade under most storage conditions, can enable you to ask questions in the future that never occurred to you at the time. (*) I presented that study at the first IOS in Hilton Head, South Carolina USA.

**Keywords:** The value of archiving one's otolith collections, Laboratory experiment, Thermal and feeding effects on otolith chemistry
Procedures for LA-ICPMS: The use of R software and Bayesian analyses for trace element quantification

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The use of Laser-Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) for elemental quantification of biological calcified structures is a generalized tool due to the possibility of high spatial resolution on the analysis. The technique has several constraints that need to be addressed with different approaches that involve several steps. The use of Certified Reference Materials (CRMs) of different concentrations and matrix compositions for the reduction of the raw signal into elemental concentrations allows having wider coverages for the possible scenarios. For instance machine drift is solved by analysing the different CRMs at the beginning and end of each session and in between a certain number of samples, and the use of an internal standard is essential when facing differences in yield. Here we propose a procedure developed at IMEDEA for data handling and discuss the use of Bayesian analyses for data reduction and trace element quantification. A Nd:YAG 213 nm NewWave Research LA system coupled to a Thermo-Finnigan Element-XR ICPMS was used to analyse otolith samples and 5 different CRMs (NIST612, NIST614, NIST616, FEBS-1 and NIES-22). Element profiles (time vs intensities) for each LA scan were visualized using the Glitter software (GEMOC, Macquarie Research LTD) in order to verify their proper quantification, select the plateau intervals for the background and signal, and obtain averaged counts after removing background. An R script developed by the Fish Ecology Group was used to standardize the data using Ca43 as internal standard and transform element intensities in element concentrations using the CRMs as standards and correcting for any instrumental drift. Element concentrations were estimated by fitting a hierarchical linear model, which was fitted using a Bayesian approach in order to properly propagate all the uncertainty sources involved.

Keywords: LA-ICPMS, Linear model, Data reduction, CRMs
Retracing the habitat use and movement patterns of sockeye salmon (*Oncorhynchus nerka*) in the North Pacific Ocean

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Pacific salmon (*Oncorhynchus spp.*) are renowned for their high mobility, complex population structure, and homing to natal spawning grounds. Following a year or more in freshwater and estuarine habitats, sockeye salmon (*O. nerka*) in British Columbia (Canada) are thought to migrate north and northwest along the coast before moving off the continental shelf in their first winter. Subsequently, they appear to move counterclockwise through the north Pacific, potentially occupying a vast high seas range between the Aleutian Islands and the Washington and Oregon coasts before returning to their home rivers and spawning grounds 2-3 years later. A growing number of studies have investigated the timing of out-migration, coastal residence time, and habitat use in juvenile sockeye salmon. However, much less is known about sockeye salmon habitat use and movements once they move offshore — a period that may comprise the majority of their lives. The lack of even basic information on high sea distribution limits our ability to predict the environmental conditions experienced by a fish and the potential impacts of regional shifts in ocean conditions. To address this knowledge gap, we explore the application of a combined otolith microstructure and microchemistry approach to compare and contrast growth and chemical profiles of sockeye salmon during their marine phase. Returning salmon were collected from Fraser River, Campbell River, and Rivers Inlet fisheries in 2015. Otoliths were imaged, measured, and aged prior to elemental analyses. Age-specific chemical profiles were constructed following laser ablation inductively coupled plasma mass spectrometry for trace elements and isotope ratio mass spectrometry for stable oxygen isotopes. We discuss the insights gained from the combination of age and chemical chronologies with respect to growth variation, movement patterns, and habitat use of sockeye salmon at large multi-regional scales.

**Keywords:** Migration, Profile analysis, Habitat use, Stable oxygen isotopes
Change in otolith density as age progressed using CT scan

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Growth and maturation are essential parameters to understand fish life cycle. Otolith is well known as a time recorder of the fish life profile. Through observation of annual structure, we can estimate the growth trajectory for individual fish. If additional information of maturation and reproduction should be obtained along annuli, life cycle characters of each fish can be revealed. I investigated the relationship between the otolith annuli and crystal density using the micro computed tomography (CT scan) for several fish species. Otolith in young period showed low density. The density changed drastically at one phase in their life, and is significantly high. In many case, this change in the density occurred around maturational age. These results suggested otolith density is corresponded to the growth and reproduction phases and provided new insight for understanding individual life history interpreting otolith characteristics.

**Keywords:** Otolith density, CT scan, Life history
Stock composition analysis and connectivity of *Prochilodus lineatus* from Plata Basin (South America) using otolith fingerprints: temporal variation of a dominant cohort

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*Prochilodus lineatus* is the most important fishery resource of the Plata Basin (South America). We evaluated the connectivity and contribution of the two main nursery areas to two fishing stocks (Uruguay and Paraná rivers), considering subadult (2 years) and adult (4 years) specimens. Estimates were made using two maximum likelihood methods. Chemical composition of young-of-year fish caught in nursery areas in 2010 was used as baseline of the models; while chemical composition of the core of subadult and adult otoliths (same cohort) was used as sample of unknown origin. Discriminant analysis based on the baseline showed high classification rate (98.9%). Results suggest that the subadult stock from Paraná was not mixed (contribution~100%), while the stock from Uruguay had a contribution from the Paraná nursery (1.5±1.2-17.9±3.96%). For the adults, the degree of mixing increased where the contribution from both nursery areas to the Paraná and Uruguay stocks varied between 14.8±4.18 and 85.2±4.18%. This paper shows the potential application of otolith fingerprints for determining the relative importance of recruitment sources of fish in the Plata Basin.

**Keywords:** Connectivity, LA-ICP-MS, Recruitment, Maximum likelihood
Spatial segregation and connectivity in young and adult stages of *Megaleporinus obtusidens* inferred by using otolith signatures and management implication

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*Boga* *Megaleporinus obtusidens* is a teleost fish of economical and sport importance from Río de la Plata Basin (South America). Otolith core and edge elemental ratios (Ba:Ca, Cu:Ca, Li:Ca, Mg:Ca, Mn:Ca, Pb:Ca, Rb:Ca and Sr:Ca) were compared between three sampling areas from the Río de la Plata Basin (Paraná and Uruguay Rivers and Río de la Plata Estuary) to evaluate the applicability of the fingerprint to study segregation and connectivity in young and adults stages. Several ratios were significantly different among sites for otolith core and edge (p<0.05). PERMANOVA (p<0.05) and quadratic discriminant function analysis (classification rates: 86.8% and 82.5 % for otolith core and edge, respectively) were found to be highly effective in detecting differences in otolith core and edge fingerprints between sampling sites suggesting the existence of spatial segregation in young and adult life stages, respectively. The presence of relatively isolated groups may require the need to manage the stocks separately.

**Keywords:** Connectivity, LA-IPCMS, Migration, Segregation
Otolith shape and stable isotopes analysis as stock discrimination tools for Yellowtail snapper, *Ocyurus chrysurus*, in northeastern Brazilian coast

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We investigated variations on otolith shape and oxygen and carbon stable isotopes rates for the yellowtail snapper *Ocyurus chrysurus* (Bloch, 1791) along the northeastern Brazilian coast (East Brazil Large Marine Ecosystem- LME). Two locations were chosen, off Ceará state, located on the zonally oriented coastline, and off Bahia state, at the meridionally oriented coast. A total of 415 otoliths were sampled and aged, 112 from Bahia and 303 from Ceará States between 1999 and 2000. Otoliths from individuals aged between 7 and 9 years were used for shape and stable isotopes analyses. A total of 58 left otoliths were photographed (36 from Bahia and 22 from Ceará) and submitted to elliptical Fourier Analysis. Whole otoliths (24 from Bahia and 22 from Ceará) were analyzed for \( \delta^{18}O \) and \( \delta^{13}C \) with Delta V Advantage isotope ratio mass spectrometer connected to the GasBench II. For shape analysis the PCA synthesized 80% of information in 11 principal components. The MANOVA was significant to distinguish between otoliths from the two regions. Discriminant analysis performed shared 61% individuals correctly. Although the other shape indices did not present a significant difference, otolith height indicated higher growth for Ceará State, and consequently, the otolith areas were different. The values for \( \delta^{18}O \) were also significantly different between regions, ranging from -0.46 to 0.11‰ for Bahia and from -1.34 to -0.37‰ for Ceará, likely responding to different seawater conditions of temperature and salinity. The values for \( \delta^{13}C \), known to be mainly influenced by fish metabolism and by dissolved carbon in water, was similar between regions: - 4.98 to -3.57‰ from Bahia, and -5.14 to -3.58‰ from Ceará. These results suggest population structuring of Yellowtail snapper across different regions of the northeastern Brazilian coast, and the importance of considering regional management units.

**Keywords:** Lutjanid, Brazil, Shape analysis, Stable isotopes
Where does it go? Provenance and stock structure of capelin in Greenland using microchemistry

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In this study, we investigate movement and structure of capelin (*Mallotus villosus*) stocks in Greenland by otolith microchemistry. Capelin is an essential species in many marine ecosystems, but little is known about its stock composition and migratory behavior. Such knowledge is crucial for proper management of the species, both in terms of sustainability and economy. During late spring and most of the summer, vast capelin schools arrive in the inner regions of Greenland’s fjords to spawn. We hypothesize that during this period capelin schools migrate from outer coastal regions (perhaps even offshore) to inner parts of the fjords and back out. Migration and stock mixing may even take place over much greater distances than previously assumed. Alternatively, capelin stocks stay in local fjords or offshore environments during autumn and winter, and as such only local stock mixing may occur. For this study, more than 50 spawning capelin were caught at each of 18 different localities along Greenland’s West-, South- and East coast. To discriminate between stocks at different localities, otolith microchemistry, as well as genetics, will be tested. Migration of individual fish will be tested by comparing microchemical patterns to otolith chronology, using parameters such as temperature, salinity and local geological discharge. Applied methods include in-situ LA-ICP-MS analysis and bulk compositional analysis, using element to calcium ratios of e.g. Mg, Mn, Cu, Sr and Ba, as well as isotopic relationships of O and Li. At certain localities, pollution of Pb, Zn and Ag from local mines may also be taken into consideration. The project is carried out in collaboration with the Greenland Institute of Natural Resources, industrial partners Polar Seafood & Royal Greenland and Geological Survey of Denmark and Greenland (GEUS). Preliminary results will be presented at IOS 2018.

**Keywords:** Microchemistry, Migration, Capelin, Greenland
**Effect of temperature on calcium carbonate structure $\delta^{18}$O and $\delta^{13}$C of cuttlefish**

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Stable oxygen and carbon isotopic values recorded in calcium carbonate structure of marine animals, such as otolith and corallum, can reflect thermal and metabolic histories. However, it is understudied on cephalopods calcium carbonate structures, such as statolith and cuttlebone. Therefore, hatchlings of pharaoh cuttlefish, *Sepia pharaonis*, were reared under a controlled temperature at 20, 25 and 30°C for one month and analyze $\delta^{18}$O and $\delta^{13}$C values in statolith, cuttlebone, water and food. Cuttlefish living at 25°C had higher survivorship and grew faster. Relation between temperature and $\delta^{18}$O values in statolith, cuttlebone, and water will be further analyzed and used for metabolic prediction.

**Keywords:** *Sepia pharaonis*, Cuttlebone, Statolith, Carbon and oxygen stable isotope
Could cooking change oxygen and carbon stable isotope compositions of fish otoliths?

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Stable oxygen (δ\(^{18}\)O) and carbon (δ\(^{13}\)C) isotope analysis of biological carbonates have been long used on archaeological research for paleo-environmental reconstruction and seasonality interpretation. However, it was still unclear whether the original otolith isotopic compositions remained the same after cooking. This research evaluated the effects of cooking methods i.e., steaming, pan-frying, and roasting on otolith isotopic compositions. The left sagittal otolith was extracted from javelin grunter (*Pomdasys kaakan*) before the treatments of different cooking methods to the right otolith in situ in the fish. Then the otoliths were cut to thin sections and powders were sequentially collected from the core to the edge by the micromill before the isotopic analysis. The isotopic variation profiles were very consistent between treated and untreated otoliths, indicating the relative variations of otolith isotopic profile can provided useful information of seasonal changes or migration across different saline waters. More samples will be analyzed on more samples to prove is isotopic composition completely the same between cooked and uncooked otoliths.

Keywords: Archaeology, Otolith, Stable isotope, Cooking
Homing natal and resident behavior in a giant Amazonian catfish

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Natal homing (philopatry) is the return of individuals to their area of origin to breed. Although this behavior is well known for diadromous species such as salmonids, it is still poorly documented for potamodromous species. Recently, otolith $^{87}$Sr/$^{86}$Sr analyses revealed a > 8000 km long migration with natal homing in the Amazonian giant catfish Brachyplatystoma rousseauxii. This extraordinary life cycle between the breeding area in the Andean piedmont and the nursery area in the lower Amazon-estuary area was demonstrated for fish caught and hatched in the upper Madeira basin. Here, otoliths with known Sr isotope profiles (LA-MC-ICPMS) were analyzed using scanning x-ray fluorescence microscopy (SXFM) in order to test whether Sr:Ca and Se:Ca could shed some light on migratory patterns of fish caught in the Upper Amazon mainstem. A 6.5-year-old individual showed elemental patterns consistent with natal homing behavior: low Sr:Ca and high Se:Ca in the core (corresponding to the Andean region), elevated Sr:Ca and low Se:Ca up to about 4 years (first 2 years in the estuarine environment followed by 2 years in the Central Amazon) and then low Sr:Ca / high Se:Ca, when it returned to the headwaters region in the Andes. Another young individual, about two years old, exhibited a different pattern with elevated Se:Ca in its early life (near the core) and intermediate bands that correspond to growth marks with low Sr:Ca throughout the otolith (indicating that it never migrated downstream to estuary). Our results indicate the existence of two distinct life-history strategies within this species: natal homing and resident behavior. Both LA-MCICPMS and SXFM, very different analyses, led to the same conclusions and help to unravel the migratory patterns of Amazonian catfish species.

Keywords: Potamodromy, Sr isotope, Trace elements, Brachyplatystoma rousseauxii
The application of SIMS δ\(^{18}\)O analysis for estimating spawning temperature of Pacific bluefin tuna using otolith

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Pacific bluefin tuna (PBT), *Thunnus orientalis*, is one of the most commercially valuable species in Japan. Their primary spawning grounds are located in the waters off eastern Taiwan and Ryukyu Islands in the south of Japan and in the Sea of Japan with spawning temperature to be approximately 26–27°C and 24°C, respectively. However, recent seawater temperature rise due to the climate change may cause their spawning grounds to be shifted northward, possibly leading to declining survival rates of larvae and juveniles. It is important to investigate changes in spawning mechanism driven by long-term climate variability for sustainable management. Otolith oxygen isotope ratio (δ\(^{18}\)O) is an effective proxy for estimating water temperature experienced by fish. Previous study has established an equation for estimating temperature using otolith δ\(^{18}\)O of bulk PBT larvae analyzed by isotope ratio mass spectrometry (IRMS). However, temperature experienced by individual larva has not been analyzed due to IRMS’s limited resolution. This study investigates spawning temperature of PBT caught off the waters around Japan by analyzing otolith core δ\(^{18}\)O by the SIMS (Secondary Ion Mass Spectrometry) techniques. The SIMS is a high sensitive surface analytical method with a spatial resolution of sub- to 10\(\mu\)m. It is crucial to establish a sample preparation protocol before SIMS measurement to assure data quality, as obtaining stable beam of δ\(^{18}\)O strongly depends on the surface condition and chemical composition of samples. This talk will introduce our recent efforts in developing the sample preparation protocol to obtain a mirror-surfaced thin section containing otolith core for SIMS analysis. In addition, the preliminary result on the first SIMS test analysis will be introduced.

**Keywords:** Pacific bluefin tuna, Otolith δ\(^{18}\)O, Spawning temperature, SIMS
Using digital imaging of otolith dissection in ageing of Baltic herring (*Clupea harengus membras*)

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Baltic herring is by far the most important species for commercial fisheries in Finland with 136 million kg catch while the total commercial catch was 157 million kg in 2016. Baltic herring stock assessments are conducted annually by the International Council for the Exploration of the Sea (ICES). To provide data for the stock assessments, Luke collects yearly about 5 000 individual herring samples in total, both from commercial catches and from research survey. The otoliths from sampled herring are moulded to epoxy and dissected with Stuers Accutom 100 precision saw to slices of 0.4 mm in thickness. The otoliths slices are then etched in mild acid dilution and histologically stained. Each individual sample is photographed from both sides of the slice with an optical microscope. Microscope is equipped with high resolution CCD camera with software able to produce pictures with multi-layer focal point technique. The images are uploaded to an FTP-server from where they are linked directly with corresponding herring individual in the fish sample database of Luke, SUOMU. In the database age reader has access to all the relevant data for ageing, including both individual data (weight, length, sex, maturity) and also the data of the fishing trip (date, location, etc.). The preliminary plan is to use SmartDots, application developed by ILVO (Flanders Research Institute for Agriculture, Fisheries and Food in Belgium) for age reading, since the ICES has chosen it to be the platform for age reading workshops and exchanges from 2018 onwards. Also possibilities to use or to develop automated ageing software will be studied.

**Keywords:** Baltic herring, Stock assesment, Digital image, Aging
Remarkable homing ability of a pelagic crucian carp "Carassius auratus grandculis" endemic to Lake Biwa: Evidence form otolith Sr stable isotope

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Lake Biwa is the biggest lake in Japan and the third oldest in the world. In this Lake, many endemic species evolved from their fluvial ancestors to adapt to the pelagic environment after the lake deepened through faulting ca. 0.4 million years ago. All of them migrate from pelagic to coastal area for reproduction. Crucian carp Carassius auratus grandculis is one of the endemic pelagic fish in the lake. During spring spawning season, it migrates to temporary waters, such as coastal reed areas and rice paddies. Using the otolith marking method with alizarin complexone (ALC), our previous research revealed that stocked fish can perform the spawning migration to the natal rice paddy after their growing period for 2-3 years. Considering that they perform long-term and wide-ranging feeding migration in the pelagic habitat, their homing ability with pinpoint accuracy is remarkable. However, it is still unclear if wild fish even show such a homing ability. Here we examine its homing ability for the wild population and trace its migration route, based on strontium isotope ratio (³⁷Sr/⁸⁶Sr) analysis for time-series micro-core samples of fish otolith (lapillus). We also measured the Sr isotope ratio for the otolith samples of the ALC marked fish which returned to the natal rice paddy for spawning. The Sr isotope analysis for the marked fish otolith showed that otolith cores which were formed during the nursery period had Sr isotope signatures similar to those of irrigated waters in the rice paddy where the fish were stocked, demonstrating that our isotope approach is a powerful tool to identify the natal sites. The otolith Sr isotope signatures of wild adult fish revealed that they returned to their natal area after migration from the pelagic habitat but show individual variation in the homing ability.

Keywords: Strontium stable isotope, Lapillus, Fish migration, Homing
Unraveling the life cycle of three small gobies: age validation and determination

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Three pelagic species of small gobies (<60 mm) *Aphia minuta* (Risso 1810), *Pseudaphia ferreri* (De Buen and Fage, 1908) and *Cristallogobius linearis* (von Düben, 1845) are the target species of a small-scale fishery which has developed particularly in the western and central Mediterranean. These progenetic species are poorly known, *A. minuta* age and growth have been determined, whilst there are no information on the other two species. Due to the short life span of less than one year, daily growth increments (DGI) should be used to determine their age. Therefore, the daily periodicity of the DGI in their sagittal otoliths was established. A direct validation method was applied. For validation purposes specimens of the three species were collected from the purse seine captures and transported to LIMIA facilities. There they were estabulated in eight experimental 90 l glass aquarium supplied with a continuous 1 µm filtered and UV-treated seawater (renewal rate 4lh\(^{-1}\)). Salinity was constant 38 g L\(^{-1}\) and temperature fluctuates between 15.4° to 22°C during the experience. Fishes were feed daily with *Artemia salina* nauplius and metanauplius. After an acclimataion period of seven days, the fishes were marked with an alizarine bath. After a period of 20 days *A. minuta* was marked for a second time using the same procedures. Mortality during the marking was 5%. The experiment ended after 3 months. Once the daily nature of the increments was validated age was determined in days and the increment width measured. All three species had maximum ages well below 10 months and back-calculated birthdates provided an indirect validation due to the agreement with the spawning season. Differences in growth rates and life traits between species were determined and will thus be available for assessing purposes.

**Keywords:** Direct validation, Marking, Gobies
Between reader differences in age estimation of Baltic cod larvae and early juveniles

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Cod Gadus morhua is a key species for the functioning of the Baltic Sea ecosystem and plays the role of top predator in the trophic food web in the Baltic Sea. It is also one of the most important commercial species for the Baltic Sea fishery. The condition of the Baltic Sea cod stocks has been deteriorating recently. When studying cod ecology, some of the analysis can be conducted to better understand processes occurring during the early, larval and juvenile, life stages of this species. Many studies on the early life history of fish, also cod, is based on the information about age of individuals - age structure, distribution of hatching dates, or assessment of the larvae growth rate. The determination of age is based on the analysis of otoliths microstructure by counting the number of daily growth increments, which is a very common method applied to different fish species. The accuracy of such readings may depend on the method applied and on the experience of the person reading the age. This is due to the difficulties in the interpretation of structures resembling daily growth increments, but not constituting them. Errors in age reading further affect the results of analyses in which they are applied, thus affecting total statistics and interpretation of data in terms of identifiable trends. In our study we focused on identification of differences in the precision and accuracy of age readings of Baltic cod larvae (4.1-33 mm SL) based on the daily increments analysis from both sagittae and lapilli. This was performed by reader experienced and inexperienced in age analysis of larvae. Significant differences between the two readers were found, which had a significant consequences in subsequent growth rate analysis.

Keywords: Early life stages, Gadus morhua, Daily increments, Otolith microstructure, Baltic Sea
Historical variation in age structure of parental stock of the southern hake (*Merluccius australis*), hoki (*Macruronus magellanicus*) and southern blue whiting (*Micromesistius australis*) fishery resources in southern Chile

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The southern demersal fishery of Chile, has resources of economic importance like a southern hake (*Merluccius australis*), hoki (*Macruronus magellanicus*) and southern blue whiting (*Micromesistius australis*), of which exploitation even when it has developed in recent years, based on the authorization of catch quotas, it has shown deterioration in the structure of parental stock. The State of Chile, represented by the fishing authority, in its mission to ensure the balanced exploitation of resources, finance each year a study programs in which it can obtain different rates that provide knowledge in explaining global behavior and characterizations by stratum of interest. The present study shows historical evidence of the internal stock structure over a period of sixteen years, its changes and the median age as a parameter that summarizes the variation over time. The southern hake, at the beginning of the studied series, the median age was found at age eleven and thirteen in males and females respectively, however in the present, the males registered the lowest historical value of median age (age six) and in the females have remained more stable (age thirteen). Hoki shows fall in this indicator, from median ages of seven and eight in males and females respectively, and to be recorded in the present median age three in males and four in females. In a similar way, southern blue whiting presents the median has declined from an age value of eleven for both sexes at the beginning of the series, until registering in the last year a median age of six years in males and eight in females.

**Keywords:** Age structure, Spawning stock, Historical series, Median age
Natal origin and feeding grounds of adult Pacific bluefin tuna (*Thunnus orientalis*) before the spawning

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Sagittal otoliths of 59 adult Pacific bluefin tuna (*Thunnus orientalis*, PBF) were analyzed for the $\delta^{18}O_{oto}$ compositions to determine their natal origin. Most PBF (88%, n = 52) caught in the spawning ground of northwest Pacific Ocean showed the first highest $\delta^{18}O_{oto}$ values (the first winter signal) between 800-1000 $\mu$m from the core while a small portion (12%, n = 7) showed the first highest $\delta^{18}O_{oto}$ values between 1300-1500 $\mu$m. The former was very likely to be hatched in the western Pacific Ocean in April to July and the later might be hatched in the Sea of Japan in July to August. Besides, 120 adult PBF were analyzed for muscle $\delta^{15}N$ composition to infer their feeding grounds before the spawning seasons. The $\delta^{15}N$ value suggested that most individuals (95%, n = 114) resided in the western Pacific Ocean with $\delta^{15}N$ values between 11.9-15.1‰ while few individuals (5%, n = 6) might newly migrate from the eastern Pacific Ocean to the spawning grounds near Taiwan because of relatively higher muscle $\delta^{15}N$ values of 15.2-16.1‰. These results suggested Pacific spawning grounds have greater contribution to the spawning stock biomass of the PBF. Since PBF with different natal origins can spawn at both spawning grounds, PBF shall be regarded as a signal stock for the fishery management due to sufficient gene flow.

**Keywords:** Pacific bluefin tuna, $\delta^{18}O_{oto}$, Muscle $\delta^{15}N$
Spatial gradients and temporal variations in size at age of Northeast Arctic cod (*Gadus morhua*) off the coast of Norway during the last two decades

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Fish growth and length at age can often be very variable depending on environmental, biological or genetic parameters. To some extent, models have successfully predicted these variations, but are often limited by a lack of data or predictive capabilities. Besides, natural spatial patterns in size at age often occur and allow to easily visualize large-scale gradients, but will sometimes be overlooked in favour of model predictions. Otoliths are a well-known and frequently used source of age and growth information for teleost fishes, and have been consistently used for ageing cod from Norwegian surveys. Our analysis of cod survey data already showed significant differences in the size at age for mature cod depending on the gear type and the area of sampling. Using survey data collected for immature and mature cod, we thus aim to investigate spatial gradients and potential long-term shifts in size at age in the last two decades spanning from 1996 to 2016. Large-scale patterns in size at age will be investigated throughout most of the survey area, including samples from the spawning ground in Lofoten and from fishing grounds in the Barents Sea. We expect it will not only give insight on spatial size at age variations for the northeast arctic cod, but will also potentially draw new conclusions on the decadal growth variability previously highlighted.

**Keywords:** Northeast arctic cod, Size at age, Spatial variations
Movement patterns of brown trout (*Salmo trutta*) in a large New Zealand river system

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Brown trout (*Salmo trutta*) were introduced to New Zealand’s South Island in the late 1860s. Brown trout are considered as highly valuable sport fish and are currently the most widely distributed non-indigenous fish species on the South Island. Although, it holds great importance as an angling fish, there are significant gaps in knowledge on brown trout migrations and recruitment patterns in New Zealand’s waterways. In this study we analysed movement patterns of adult brown trout in the Pomahaka and Waipahi rivers (tributaries to Clutha River). We used otolith Sr:Ca and 87Sr:86Sr values as markers for detecting shifts in habitat use. Additionally, to investigate some of the possible natal baseline values, we collected and analysed young-of-the-year (YOY) fish from four known spawning tributaries of Waipahi River. The results indicate that Pomahaka and Waipahi river systems are generally characterized by relatively high Sr:Ca (typically >1.5 mmol/mol) values and 87Sr:86Sr below marine value (<0.709). We detected several potential movement patterns in the studied river systems and it seems that there are some migration differences between fish sampled from Pomahaka and Waipahi rivers. Natal signatures of the adult fish indicate that there must be more spawning tributaries than those that were sampled in this study. Based on Sr isotopic signatures of adult trout, we did not identify any sea-run individuals. As a marker, 87Sr:86Sr produced more stable and more readily interpretable profiles compared to Sr:Ca (occasionally noticeably labile profiles). The findings of this study demonstrate that significant habitat shifts occur in brown trout among freshwater streams and number of possible spawning tributaries in Waipahi and Pomahaka river systems are greater than previously thought.

**Keywords:** Otolith microchemistry, Migration patterns, Brown trout, New Zealand
Combining otolith analysis and particle drift modeling to identify and locate the spawning areas of the gilthead sea bream *Sparus aurata* in the Gulf of Lions (NW Mediterranean)

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Sustainable management of marine fish resources requires a good knowledge of their local population structure and lifetime migrations. Yet this basic information is still lacking for many target species, especially with regards the number and location of the spawning grounds and their respective contribution to juvenile and adult sub-populations. This is the case for the gilthead sea bream (*Sparus aurata* L.) in the Gulf of Lions: little is known on the species’ local spawning areas and on the origin of the larvae that colonize each year the different lagoons spread along the coast, where most of the juveniles grow. In this work, we used the otoliths of early juveniles from the four largest lagoons of the region to evaluate the variability in hatching and recruitment dates among sites and model potential larval drift at sea for the corresponding periods, using three discrete zones in the Gulf where adults are known to aggregate during the winter spawning period as putative spawning sources. The potential migration routes obtained were then matched with concomitant environmental data (temperature, salinity, Chl a) for the entire area, and compared with the environmental (ratios in B, Ba, Ce, Cr, Mg, Mn, Pb, Sr, Zn and Y) and physiological (daily growth rate) information stored in the otoliths of the fish during their larval life. This allowed identifying their respective origin and characterizing the differences in larval life history among them. The results suggest that local sea bream juveniles originate mainly from only two of the three putative spawning areas, with different migration routes during larval drift from both sites according to fish spawning date. These later largely explain differences in spawning origin among lagoons, bringing new insights to our knowledge of the early life connectivity and population structure of *Sparus aurata* in this region.

**Keywords:** Larval drift modeling, Environmental histories, Daily growth, Multi-elemental signatures
Otolith Shape Indices as a tool for discriminating the stocks of Milkfish (Chanos chanos) in Indian waters

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Milkfish (Chanos chanos) is a sole member of the family Chanidae which is widely distributed in South and Southeast Asian region. Otolith shape indices were employed for the first time to discriminate the milkfish stocks. A total of 144 otoliths were extracted from the collected samples along both coasts of India from four locations namely Chilika Lake, Mandapam Lagoon, Cochin Backwaters and Madovi-Zuari estuary. In this study, otolith shape indices like circularity, ellipticity, rectangularity, roundness and form factor were used to analyze the stock structure of the species. The data were subjected to multivariate statistical procedures. The post hoc test indicated the significant difference between the sample means. Mean values significantly differed among sites for Circularity (F = 10.65, P < 0.00), Ellipticity (F=64.61, P< 0.00), Rectangularity (F= 4.71, P< 0.003), Form factor (F=13.81, P<0.00) and Roundness (F=33.41, P<0.00). Discriminant function analysis reveals the overall classification success of 59.6% for otolith shape indices. In conclusion, analysis of otolith shape indices revealed the existence of four different stocks along the Indian coast. The finding of this study will help in management and future breeding programmes of the species.

Keywords: Otolith, Milkfish, Discriminant analysis, Conservation
Lunar timing of migration patterns differ between migrant and non-migrant types of a partially migratory fish

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Biological timing associated with lunar rhythms can affect behavior such as migration in a wide variety of taxa. Aquatic organisms are specifically influenced by changes in illumination and hydrostatic variation driven by lunar phase progression. Lunar periodicity of spawning and recruitment is well documented for marine reef fishes, however, lunar mechanisms influencing freshwater migrations is not fully understood. Hawaiian freshwater fishes exhibit a migration strategy known as amphidromy, where individuals hatch in freshwater and quickly migrate seaward, returning as juveniles to freshwater systems to complete the life cycle. Not all species fully migrate; partial migration, where only some individuals within a population make a migration, has been documented in at least one amphidromous Hawaiian species Awaous stamineus. The mechanisms that underscore partial migration are not well understood but it is believed environmental factors are influential. A combination of otolith microstructure and microchemistry were used to determine if life-history timing was associated with specific lunar phases for both an obligate- (Sicyopterus stimpsoni), and a facultative-migratory species (A. stamineus). Surprisingly, A. stamineus non-migrants had lunar lifehistory timing like obligate migrant S. stimpsoni where more individuals hatched at the new moon and settled at the full moon. Significantly more A. stamineus migrant individuals hatched at the full moon and settled at the new moon. Divergent migration phenologies observed here can be explained by a balance of competing selective pressures of migration and predation resulting in differing lifehistory strategies between migrant and resident individuals in the same population. This study provides insight into endogenous and evolutionary mechanisms that maintain divergent migration phenologies.

Keywords: Otolith microstructure, Otolith microchemistry, Lunar rhythmicity, Amphidromy
Determining habitat use in juvenile lionfish: addressing the nursery hypothesis

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Pacific Red Lionfish have recently invaded the Western Atlantic, Caribbean and Gulf of Mexico. Several studies in the invaded range have shown that large lionfish adults are typically found on deep fore-reef habitats and juvenile individuals are more commonly observed in shallower, in-shore habitats such as sea grass beds and mangrove. This has lead researchers to assume that lionfish are using in-shore habitats as a nursery and at some point during ontogeny they migrate out to the forereef. The degree to which in-shore habitats act as a nursery for lionfish has not been empirically tested. We collected lionfish adults and juveniles from paired mangrove and reef habitats at Turneffe Atoll in Belize to determine what percentage of adult lionfish used mangrove habitat in their juvenile lifestage. We used otolith microchemistry to determine spatial differences in trace element chemistry from fishes collected in both mangrove and reef habitats. We used a maximum likelihood estimator to assign adults to either reef or mangrove habitats based on their juvenile otolith chemistry. We found that 36% of adult lionfish recruited to mangroves as juveniles, while 64% recruited to reef habitats. It is critical to determine how lionfish are using various reef-associated habitats in order to understand how to most effectively and efficiently manage invasive populations.

Keywords: Lionfish, Nursery hypothesis, Mangrove-reef connectivity, Invasive species management
Age and growth of tailor (*Pomatomus saltatrix*) in eastern Australia

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*Pomatomus saltatrix* is a globally distributed pelagic mesopredator with previously identified differences in life history patterns. Despite being a key recreational species and subject to high fishing pressure in eastern Australia, many characteristics of *P. saltatrix*’s life history remain undefined. Over 3 years, over 3,500 fish were sampled from recreational and commercial fishers across two jurisdictions. Annual formation of rings within otoliths was validated and whole otoliths were shown to be equivalent to sectioned otoliths. Growth was determined to be fast yet the oldest collected fish was 6 years old, suggesting possible age truncation of this stock as other populations of the same species are commonly caught at older ages.

**Keywords:** Age truncation, Whole otolith, Bluefish, Tailor
Determination of daily aging of pikeperch fry in a deep temperate reservoir

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Precise daily aging is important to determine the term of hatching and incubation period under natural conditions. Pikeperch (*Sander lucioperca*), a commercially and ecologically (top-predator) important species, was used as a study object because of disposable spawning and known variation in year-class strength. Sampling was conducted in spring and early summer 2007 and 2008 in deep Římov Reservoir, Czech Republic. Pelagic fix-frame trawl was applied mainly during night period in epipelagic layer and to prove deep fry migration during the day-time period in epilimnetic as well as in hypolimnetic layers too. Artificially bred larvae of known age marked by fluorescent dye (oxytetracycline hydrochloride) were stocked both years in the reservoir to correct the daily growth. Sagittal otoliths were extracted from 636 in 2007 and 2793 in 2008 pikeperch individuals to evaluate readability, timing of hatching and structure of fry cohorts with respect to temperature in the reservoir. This informations are helpful for managers to predict pikeperch recruitment based on temperature in critical periods.

**Keywords:** Daily growth, Pelagic fry, Control stocking, Hatching
Investigating connectivity and temporal changes in nursery habitat use for two over-exploited local stocks of the southern hake (*Merluccius australis*) in Northwestern Patagonia

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Understanding the population structure and lifetime migrations of marine fishes is essential for the sustainable management of their stocks. Yet this information is still lacking for many target species, especially with regards the connectivity among demographic units through dispersal at the larval and juvenile stages. In species like the southern hake (*M. australis*) who exhibit slow individual and population growth rates and late sexual maturity, identification of the juvenile habitats that sustain exploited adult stocks is even more crucial. In NW Patagonia, two distinct adult stocks are considered for management purposes, an oceanic stock exploited by the industrial fleet and an estuarine stock exploited by the artisanal fleet. Both have been intensely fished since the late 1970ies, which resulted in a drop in *M. australis* local landings in the 1990ies. The aim of this work was to identify the main juvenile habitats of each stock and to estimate the connectivity between them, as well as investigating changes in habitat use patterns over the last 30 years. For this, we analyzed lifetime otolith signatures in 7 elements (B, Ba, Na, Mg, Mn, P, Sr) for adult hakes from 3 cohorts (1996, 1999, 2004) and 5 distinct sampling zones (2 oceanic, 3 estuarine). Early juveniles were also captured after several months of residency within each sampling zone and their otoliths were analyzed to generate a multiannual database of otolith elemental signatures for all nursery sites. Matching the chemical fingerprints corresponding to the first year of life in the otoliths of the adults with those available in this database allowed identification of their juvenile habitats. This approach revealed important variation in nursery origin and lifetime migratory patterns for both stocks but the high level of connectivity found between them over the last decades do not support their treatment as separate demographic units.

**Keywords:** Elemental fingerprints, Stock connectivity, Lifetime migrations patterns, Nursery habitats
Determining life history of Blueback Herring in the Mohawk River using otolith microchemistry

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Hard parts in fishes have long since been deemed useful for use as natural life history trackers. Otoliths, as continuously growing structures, can be analyzed at the microchemical level for trace elements embedded in the carbonate matrix throughout an individual’s lifetime. This is particularly useful for anadromous fishes like Blueback Herring, which migrate annually from the Atlantic to spawn in freshwater habitats and illustrate corresponding patterns in microchemistry. This study aims to use trends in otolith and water microchemistry (particularly Sr:Ca, Ba:Ca, and Sr isotopes,) to determine the importance of the Mohawk River to the declining Blueback Herring population as part of the Hudson River system. The Erie Canal provided access to a novel ecosystem in the Mohawk River, and we question whether this nursery area serves as a source or sink for the population, as compared with historically used spawning areas in the Hudson and its other tributaries. Preliminary results indicate that Mohawk River Blueback Herring displayed differences in size at first egress to the ocean, as evidenced from 87/86 Sr signatures in the otolith. We plan to examine this further, along with thermal and salinity histories at sea, as these may indicate response to habitat quality and global climate change. Blueback Herring have a relatively narrow migration window, so early and late migrators may experience different environmental conditions at both the freshwater and oceanic ends of their pathway.

Keywords: Blueback Herring, Life History, Migration
Otolith increment formation reveals growth patterns in early life history of tropical clownfish *Amphiprion ocellaris*

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Planktonic duration is an important factor in the dispersal and recruitment of marine shore fishes. The analysis of otolith daily increments is a useful tool for studying larval dispersal and growth, and has been applied to several clownfish species. However, the early life history of *Amphiprion ocellaris*, the tropical clownfish, is not well studied. We used otolith increment analysis to investigate the growth in the period surrounding acclimation to anemones. We investigated increment deposition in laboratory – reared embryos and larvae stained with alizarin red S. Otoliths were marked at 3 days pre hatching and 8 days post hatching, and showed a conspicuous transition of width grow increments which seemly coincided with physiological and ecological changes that occur during fish larval stage, including first hatching, first food transition, and settlement. In addition, the microstructure of the false clownfish otoliths were examined, in term of the time for appearance and morphological parameters. These findings are expected to be applied to estimate post settlement growth rates, age determination, distribution and population structure of wild clownfishes and other damselfishes.

Keywords: Otolith, Alizarin, *Amphiprion ocellaris*, Clownfish
Discrimination by the otolith of the landlocked Japanese Grenadier Anchovy *Coilia nasus*

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Japanese Grenadier Anchovy *Coilia nasus* is one of important fisheries species in Ariake Sea of Japan and has been decreasing in recent years. However, the number of landlocked *C. nasus* was increasing in the Isahaya Bay regulating reservoir closed by 7 km long dike in 1997 and desalinated. These landlocked *C. nasus* were smaller than *C. nasus* in Ariake Sea by age assessment with the otolith. Otolith morphology, size and Sr / Ca ratio of *C. nasus* in the Isahaya Bay regulating reservoir were compared with *C. nasus* in the Ariake Sea, and the discrimination method was examined. These results suggest that it is difficult to discriminate by the otolith.

**Keywords:** *Coilia nasus*, Sr/Ca ratio, Landlocked fish
First estimates of age and growth of four-spot megrim (*Lepidorhombus boscii*) on the Porcupine Bank (west of Ireland)

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The state of the stock of four-spot megrim (*Lepidorhombus boscii*) in Celtic Seas and northern Bay of Biscay shelf (ICES Div. 7.b-k and 8.a,b,d) has not been assessed in ICES so far, and biological information for a forthcoming stock assessment process is required. Age and growth are key biological aspects for the age-structured assessment of exploited fish populations. The age of *L. boscii* is here determined by analysing sagittal otoliths following internationally standardized protocols, obtained from specimens caught in three annual groundfish surveys (2010–2012) carried out in Porcupine Bank. Yearly age-length-keys are built using the respective age estimates and applied to length distribution of each survey, thus obtaining mean lengths at age and year. To confirm the consistency of the age interpretation is essential for providing accurate age estimates, thus the consistency of the age estimation criterion and the growth pattern of *L. boscii* is demonstrated by analyzing the regularity of both the mean fish lengths at age class obtained along three years and the distances of the annuli to centre in the otoliths. The growth is also analyzed using back-calculation of otolith increments, and the growth patterns obtained from direct otolith age estimation and from back-calculation are also compared. The von Bertalanffy growth parameters obtained are also presented and the results are compared with those from other areas.

**Keywords:** Age estimation, Growth, *Lepidorhombus boscii*, Otolith
Age-based growth variation in populations of Green-Blotched Parrotfish, *Scarus quoyi* in Southern Philippine Seas

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Green-Blotched parrotfish or *Scarus quoyi* is one of the dominant scarine in most reefs in the Philippines. They are the ones that normally increase in density when an area is subjected to protection. Given the importance of reef fishes and its dearth of information in the tropics, this study was conducted to evaluate the variation in growth of this species across three different bays/gulf in Southern Philippine seas. These were Pujada Bay, Davao Gulf, and Sarangani Bay. Age structure of *S. quoyi* populations were determined through sagittal otolith increments. Results of von Bertalanffy growth parameters fitted to size-at-age plot revealed different growth curves for the three sites. Davao Gulf population had the largest asymptotic length ($L_\infty$) of 26.03 cm, followed by Sarangani Bay ($L_\infty=23.02$ cm) and Pujada Bay ($L_\infty=22.02$ cm). Curvature parameter $K$ also differed across populations where Pujada Bay had the highest growth rate ($K=0.1148$), followed by Sarangani Bay ($K=0.102$) and Davao Gulf ($K=0.032$). Otolith dimensions correlated with fish length and weight measurements showed significant difference across the three sites. Despite variations in length-weight relationships, all populations showed a negative allometric growth with $b$ values of 2.177 for Davao Gulf, 2.6036 for Sarangani Bay, and 2.657 for Pujada Bay. The variations could possibly suggest that growth rate differences are related to typology of indicators, environmental conditions and food availability among the populations examined even at small spatial scale.

**Keywords:** Age, Growth curve, Otolith, *Scarus quoyi*
Application of isotopic analysis to reconstruct habitat use of Japanese sea bass (*Lateolabrax japonicas*) and discrimination from reared fish

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Japanese sea bass (*Lateolabrax japonicas*) widely distributed from Japan to the South China Sea may show diadromous migration. However, the migratory behaviors of this species are rarely studied and still unclear. Japanese sea bass is a food fish providing from fish farming or fishery catch. Stable isotopic analysis of otoliths and muscles were conducted to clarify the habitat use of wild Japanese sea bass and to discriminate the wild population from cultured fish. Otolith $\delta^{18}$O values and water temperature during the corresponding period were used to predict the salinity experienced by the fish. The estimated salinity profiles suggested some Japanese sea bass entered the estuaries and rivers to forage during different life stage. However, some fish completely resided in the marine environment. The stable isotopic composition were more variable in wild fish ($\delta^{18}$O: -3.02 ± 1.3‰; $\delta^{13}$C: -4.92 ± 1.8‰, n=18) than in the reared fish ($\delta^{18}$O: -4.11 ± 0.3‰; $\delta^{13}$C: -9.08 ± 0.6‰, n=7). In addition, the stable isotope composition of otolith (K-W test, $\delta^{18}$O$_{oto}$: p = 0.025; $\delta^{13}$C$_{oto}$: p = 0.001) and muscle (K-W test, $\delta^{14}$N$_{mus}$: p = 0.036; $\delta^{13}$C$_{mus}$: p < 0.001) were significantly different between the hatchery and wild fish. These results suggested that Japanese sea bass can use diverse habitats from rivers, estuaries to oceans. The stable isotopic compositions of otoliths and muscles are useful tools to distinguish between wild fish and aquaculture.

**Keywords:** Otolith, *Lateolabrax japonicas*, Estimate salinity profile, Stable isotope analysis
Otolith chemical composition of *Solea solea* and *Merluccius merluccius* along the Northeast Atlantic and the Mediterranean Sea

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Otolith chemistry has been used successfully to address issues related to stock identity and fish movements. Here we explore the applicability of otolith core and edge chemical signatures of two commercially important species, common sole (*Solea solea*) and European hake (*Merluccius merluccius*), to discriminate among multiple areas in a broad geographic region (Norway to eastern Mediterranean Sea). Otolith chemical composition was determined via laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), and the following elements were quantified: Na, Mg, Mn, Ca, Sr and Ba. Otolith core signatures are used to disentangle spawning groups and individual origin whilst otolith edge composition is used to evaluate traceability of individual fish to their capture locations. Low variability in otolith core composition was found with limited ability to identify individual origin. Geographically subsetting the datasets did not significantly improve discrimination of groups of origin. Moderate success in assigning fish to capture locations was observed with some regional patterns clearly evident (e.g. British Isles versus North Sea versus Kattegat/ Skagerak). Interspecific variations of otolith chemical composition were pronounced and exceeded geographical discrimination. Ultimately, identifying natural markers capable of discriminating populations and measuring connectivity throughout a species distribution range will be key for the safeguard of these species.

**Keywords:** Otolith chemistry, Stock identification, Inter-specific variation, Management
Spatial variance in otolith chemistry of juvenile Pacific halibut (*Hippoglossus stenolepis*): the importance of scale-dependence and trending for correctly inferring nursery origins

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Nursery grounds for Pacific halibut (*Hippoglossus stenolepis*) are located throughout the Gulf of Alaska, eastern Bering Sea, and Aleutian Islands. Following ~2 years of nursery residence, juveniles migrate to the south and east to arrive at feeding grounds where most individuals remain as sitefaithful residents. In addition to being harvested in commercial longline fisheries, they are encountered as bycatch in fisheries that are required to discard all Pacific halibut, thereby invoking varying rates of discard mortality. As a result, debate arises regarding the degree to which elements of the fleet – with respect to gear type and geographic region – impact the productivity of downstream harvest sectors. However, little is known about the distance that juveniles migrate or the degree to which any given geographic region receive recruits that originate from specific nurseries versus being composed of a mixture of individuals reared throughout the geographic range. While a long history of conventional tagging studies has provided insight regarding general movement patterns in juveniles, such studies do not allow for empirical mixture analyses due to artifacts associated with unknown rates of tag loss due to high natural mortality during early the juvenile phase, and the spatial distribution of fishery-based recaptures. In contrast, otolith microchemistry may allow unbiased analysis of nursery origin for all individuals. However, given the large geographic range over which these nurseries occur (~6000 km of coastline) and the extremely large number of nurseries likely to be contained within that range, otolith chemistry can only be useful if signatures are regionally-trended and do not require sampling of all nurseries. Here, we report the results of a study conducted over ~1000 km that was designed to examine variance in otolith chemistry at multiple spatial scales and compare that variance to the scales over which fishery management is implemented.

**Keywords:** Otolith chemistry, Nursery origin, Geographic scale
Tracking natal habitat contributions in a freshwater migratory fish, Clear Lake hitch (*Lavinia exilicauda* chi) using otolith strontium isotopes

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Clear Lake hitch are a cyprinid fish endemic to Clear Lake and its tributaries (California, USA). Once highly abundant, the population has seen a 100 fold decrease in size compared to historic levels prompting the designation of threatened under the California endangered species act in 2014 and a current petition for listing under the U.S. endangered species act. A major factor in their decline is the alteration and degradation of the tributary streams that serve as spawning grounds for adults and natal habitat for larvae. It is thought that historically the majority of Clear Lake’s tributaries played a role in supporting the vibrant Clear Lake fishery for many of the local tribal communities. Currently, little is known about which tributaries may be contributing to the persistence of the population. The diverse geology in the Clear Lake watershed suggests that variation in strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) may be a useful marker to quantify Clear Lake tributary usage. Here, we (1) demonstrate the among-tributary variation in $^{87}\text{Sr}/^{86}\text{Sr}$ in the water from potential natal tributaries that ranged from $^{87}\text{Sr}/^{86}\text{Sr}$ values of 0.70499 to 0.70679 and (2) test the feasibility of identifying the natal origins of adult hitch collected in the Lake using $^{87}\text{Sr}/^{86}\text{Sr}$ transects across the otoliths.

*Keywords:* Otolith, Hitch, Migration
Evaluation of $^{137}$Ba mass-marking technique in the early life-history stages of *Sepioteuthis lessoniana*

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*Sepioteuthis lessoniana* widely distributes in the offshore-inshore neritic waters of Indo-Pacific Ocean as an important commercial fishery species. Egg capsules were collected from northern Taiwan and assigned to one of the 3 $^{137}$Ba spiking experimental groups at 0.2, 0.5 and 1 ppm and 3 immersion durations (1-, 3- and 7-day). Significantly lower $^{138}$Ba/$^{137}$Ba ratios than control group are observed from statoliths of hatchlings that are immersed in higher $^{137}$Ba spiked concentrations or longer immersion. However, a statistical discrimination is achieved by treatments of 3-day immersion with more than 0.5 ppm or 7-day immersion with more than 0.2 ppm spike concentration. $^{138}$Ba/Ca, $^{137}$Ba/Ca, Pb/Ca and Sr/Ca offer the best classification (100% correction) of hatchlings of 7-day immersion by different spiked concentrations, and shows that the technique can be further used to identify recaptured squids from multiple markings or different releasing sites. In addition, Cu/Ca, Zn/Ca and Pb/Ca showed positive relationships to $^{138}$Ba/Ca, and indicate that the interactions among trace elements in statolith during biomineralization processes may be exist. This study established a $^{137}$Ba mass-marking technique to provide a potential approach for investigating the population dynamic of early life-history stage of cephalopods.

**Keywords:** *Sepioteuthis lessonina*, Barium isotope, Statolith, Early life-history stages
Preliminary study on age and growth of *Apolemichtys arcuatus* and *Holacanthus africanus* (Pomacanthidae)

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Banded angelfish *Apolemichtys arcuatus* from Hawaiian and Johnston islands in Eastern Central Pacific and Guinean angelfish *Holacanthus africanus* from Senegal to Democratic Republic of the Congo in Eastern Atlantic are both rare, high priced, and difficultly reared marine ornamental fish of Pomacanthidae. Otolith-based age and growth of the two angelfishes were preliminarily studied. Total lengths and ages of the banded angelfish (n=23) ranged 58.6-156.8 mm and 1-20 yr, with an asymptotic length of 136.3 mm and a growth coefficient of 0.736 that were fitted by the von Bertalanffy growth formula. Individuals at ca. 4 yr attended computed maximum size of the fish. Total lengths and ages of the Guinean angelfish (n=19) ranged 60.0-176.8 mm and 2-14 yr. Ages for larger sized individuals will be added to adequately compute growth parameters of the fish.

**Keywords:** Pomacanthidae, Otolith, Age, Growth
Otolith microchemistry reveals the decline of wild fall-run salmon on the Feather River, California

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Fall-run Chinook salmon (Oncorhynchus tshawytscha) from the Sacramento-San Joaquin River system form the backbone of California’s salmon fishery and are heavily supplemented through hatchery production. However, the spawning of hatchery origin Chinook with wild fish has been found to compromise the genetic integrity of the wild origin populations through processes such as outbreeding, genetic homogenization and reduction of life history diversity. Identifying temporal trends in the contribution of hatchery and wild origin fish to the overall in-river escapement is thus of vital importance for assessing the extinction risk and resiliency of fall-run Chinook in the Central Valley. Here we used otolith strontium isotope ($^{87}$Sr/$^{86}$Sr) ratios of otoliths collected during carcass surveys from 2002 to 2010 on the Feather River to reconstruct their life history patterns and natal origin. This timeframe is important because it spans the salmon stock collapse in 2007-2008 in California. Our results show that prior to the salmon stock collapse ~55-67% of in-river spawners were of hatchery origin; however, hatchery contributions increased drastically (89%) in 2010 following the collapse. Data from a recent hatchery marking program corroborate our results, showing that hatchery fish continued to dominate (~90%) in 2011-2012. Though the rebound in abundance of salmon in the Feather River suggests recovery of the stock post-collapse, our otolith chemistry data document a persistent decline of wild spawning salmon, likely leading to the erosion of locally-adapted populations. Central Valley salmon are at a critical juncture, with many populations close to extinction and hatcheries can play a key role in the recovery of wild stocks but only with careful and appropriate management.

Keywords: Strontium, Life history diversity, Hatchery management, Extinction risk
Distribution of naturally recruited Japanese eels in rivers as inferred from otolith stable isotope ratios

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Stocking of cultured Japanese eels (Anguilla japonica) has been widely conducted in rivers in Japan for the purpose of population restoration. Since these eels have been released outside the distribution range of naturally recruited eels (wild eels), the existence of cultured eels obscures the natural distribution range. A recent study has been successful in discriminating wild and cultured eels based on otolith oxygen and carbon stable isotope ratios. In order to determine the distribution range of wild eels in the river, we discriminated wild and cultured (stocked) Japanese eels that were collected from the lower to upper reaches in the Tone River system, a major river basin in Japan. We also investigated the distribution range of wild eels in Japan by discriminating wild and stock eels that were collected around the country. In the Tone River system, wild eels were found between the lower and middle reaches of the river basin, whereas none were found in the upper reaches of the system. Throughout Japan, wild eels were found in rivers in the south of Kyoto prefecture facing the Sea of Japan, and in rivers in the south of Iwate prefecture facing the Pacific Ocean.

Keywords: Japanese eel (Anguilla japonica), Natural distribution, Oxygen and carbon stable isotope ratio, Stocking
Estimation of the age of jack mackerel (*Trachurus murphyi*) using otolith weight, daily growth rings and annual rings, do it easy!

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Growth of jack mackerel (*Trachurus murphyi*) in Chile was estimated using daily growth rings, annual rings and otolith weight as an indicator of age. From the reading of daily increments, an adjustment was made between the increment density and otolith radius \((n = 4881)\). With the integration of the total radius of the otolith the individual age was estimated (in days) and then later it was transformed to years. Asymptotic length \(L_\infty = 75\) cm (fork length), growth coefficient \(K = 0.16 \text{ year}^{-1}\), theoretical length at which the length of the fish is zero, \(t_0 = -0.19\) years. The weight of the otolith was multiplied by the mass growth rate described for this species \((8.256 \text{ mg year}^{-1})\), the parameters in length of von Bertalanffy, Asymptotic length \(L_\infty = 75\) cm, growth coefficient \(K = 0.15 \text{ year}^{-1}\) and theoretical length at which the length of the fish is zero \((t_0) = -0.48\) years. In the present work the von Bertalanffy growth curves estimated with the daily increments and otolith weight use do not differ statistically.

**Keywords:** Age, Otolith weight, Daily growth, *Trachurus murphyi*
Using daily growth increments in otoliths to age fourfinger threadfin (*Eleutheronema rhadinum*) in waters off western Taiwan

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Daily growth increments in otoliths were used to age East Asian fourfinger threadfin, *Eleutheronema rhadinum*, in waters off western Taiwan. A total of 915 samples with fork length (FL) ranging from 11.5 to 80.0 cm and body weight from 29 to 8100 g were collected at fishing ports in Yunlin, Taichung, Miaoli and New Taipei during January 2015 to December 2016 in this study. There is no significant difference in the relationship of body weight and fork length between sexes. Daily periodicity of microincrement formation in otoliths was indirectly validated based on the backcalculated hatch date frequency distributions. Results indicated that daily increments could be used as age determination tools for fish smaller than 50 cm in FL. The East Asian fourfinger threadfin were aged from 87 to 751 days in this study, with a reading precision of 2.36% in average percent error (APE) and 3.16% in coefficients of variation (CV). The growth of the East Asian fourfinger threadfin can be best described using von Bertalanffy growth curve of length (FL) = 128.40 \times (1 – e^{-0.693(t-0.105)}). As a fast growing species, fourfinger threadfin can reach more than 50 cm in FL, with daily growth rates consistent among individuals. The relationship between daily age and otolith radius (OR): Daily age = 39.258 OR + 16.261 could be considered a convenient approach to age fourfinger threadfin in waters off western Taiwan as suggested from this study.

**Keywords:** Fourfinger threadfin, Age and growth, Otoliths, Daily growth increments
Migratory dynamics of narrow-barred Spanish mackerel (*Scomberomorus commerson*) in the water off Taiwan

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The migratory life history of narrow-barred Spanish mackerel is unclear and the recent decline of fishery catch suggest imperative management. Otolith stable oxygen isotope combined with seawater temperature and salinity data were used to investigate the migrations of this species in this study. The results suggested that the juvenile spanish mackerel were likely distributed near Taiwan Banks and western coasts of Taiwan, followed by the movement to East China Sea from spring to summer as the fish grew. Then the fish moved southward through Taiwan Strait to South China Sea in autumn to winter. The fish might return to Taiwan Banks again in the spring of following year. The migration routes of the Spanish mackerel was closely associated with the water temperature of 23 – 25 °C and salinity of 30-32, which might be influenced by the Kuroshio and China coastal current.

**Keywords:** Narrow-barred Spanish mackerel, Otolith stable oxygen isotope, Migration
Studies on age and growth of *Sparus latus* in the waters off Taiwan

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*Sparus latus* widely distributed in the global waters, and it lived in the shallow sea. In Taiwan, it is an important species in both aquaculture and fisheries, with high economic value. In recent years, the importance of coastal fisheries has risen daily. Therefore, the fishing pressure increased followed by the resources decreases. This study analyzed the age of *Sparus latus* to provide biological information and parameters for future fisheries management. Otolith was used to determine and validate the age of *Sparus latus*. The sampling duration was from January till August in 2017. A total of 204 specimens were sampled, including 122 females, 16 males and 66 unknown sex individuals. The periodicity of opaque ring will be validated by marginal increment ratio analyses. The oldest male and female were 2 and 6 years old, respectively. The Robertson growth model estimated for *Sparus latus* were $L_\infty = 35.28$ cm TL, $k = 0.465$ yr$^{-1}$ and $t_0 = -0.7206$ yr, and Gompertz growth model estimated for *Sparus latus* were $L_\infty = 36.11$ cm TL, $k = 0.365$ yr$^{-1}$ and $t_0 = -1.5597$ yr.

**Keywords:** *Sparus latus*, Age, growth, Otolith
Age, growth and reproductive study of the mangrove snapper (*Lutjanus argentimaculatus*) in the waters off Taiwan

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Mangrove snapper (*Lutjanus argentimaculatus*) is one of Taiwan's highly economic important species of fisheries, but also one of the selected species in the fry tagging and releasing program in recent years. In order to explore the reproductive biology and age and growth of mangrove snapper in the waters around Taiwan, a total of 152 samples ranging from 106-665 mm in TL, were collected from the surrounding waters off Taiwan every month between 2016 and 2017. Among them, 42 were females, 31 were males, and 79 were unknown-sex individuals. The gonadal development and the sex maturation are estimated according to the gonad index, morphology and tissue sections to estimate the reproductive cycle. The sectioned otoliths is used to interpret the age of the sample. Further research will be used to understand its age and length at maturity, and to estimate its growth equation.

**Keywords:** *Lutjanus argentimaculatus*, Otoliths, Reproductive biology
Using otolith microchemistry to discriminate wild and released red snapper 
(*Lutjanus argentimaculatus*)

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*Lutjanus argentimaculatus* is one of the restocking species in Blue Economic Program in Taiwan. At present, a mass external tagging work consume a lot of manpower. This study is aimed to establish an effective and sensitive natural discriminant method to replace. The composition of trace-element in fish otoliths will be affected by the compositions of water they grow, while the releasing juvenile fish come from aquaculture farms, which is different from the natural seawater of wild populations. We collected wild and cultured *Lutjanus argentimaculatus* to understand whether there is a difference in the otolith core elements. The composition of otoliths was analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Firstly, whether the otolith micro-sampling method between MicroMill and laser ablation has difference were tested. Then analyzed the composition in the otolith core to the edge, and compared the difference of elemental values in different time interval. The results showed that, there are significant difference between the sampling methods. It was suggested to use the same analysis method to facilitate comparison in the future. Wild and cultured fish otolith element values have a significant difference at the otolith distance of 600-700 μm from the core, indicating this interval can be used as a basis to distinguish fish sources. According to the composition of otolith, 100% can be correctly. This result showed its an effective discriminant tool to distinguish cultured and wild individuals.

**Keywords:** Tag-and-release, Trace element, Otolith
Study on age growth of *Acanthopagrus schlegelii* in Kinmen area

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*Acanthopagrus schlegelii* is one of the important economically species around Taiwan and has been captured in all areas. Due to its strong adaption to the environment and artificial propagation technology has matured, it has become an important target species in restocking. For increasing its coastal fishery resources, Kinmen Fisheries Research Institute has been restock *A. schlegelii* for many years. However, related research was lacking at Kinmen area. Therefore, this research focused on *A. schlegelii* at Kinmen area, and studies its age and growth by using otolith. The sampling was collected from March to September 2017 and continuously. The total length of samples ranged from 13.1 - 48.0 cm, and age ranged from 0 -10 yrs. Estimate the parameters of von Bertalanffy growth equation were 41.09 cm for the calculated asymptotic length ($L_\infty$), 0.2935 yr$^{-1}$ for the growth coefficient (k), and 1.8047 yr for the hypothetical age at which fish would have zero length ($t_0$). This study will be carried out continuously. In the future, we will compare the growth parameters of the releasing and wild populations for the reference of subsequent releasing.

**Keywords:** *Acanthopagrus schlegelii*, Age growth, Otolith, Kinmen
Biological tags in fin rays and otoliths for identifying "released" fourfinger threadfin (*Eleutheronema tetradoctylum*) in coastal waters off western Taiwan

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Fourfinger threadfin is the major species that are released regularly in coastal waters off western Taiwan. However, the information regarding to tagging and recapture is lack. Tagging technique for this species was studied to understand the benefits of stock enhancement, survival rates of released fish in natural environment, and impacts on the coastal fisheries. Results showed that external tagging was evaluated as a feasible approach to tag the animals. Water quality and soak time to dyeing the otoliths need to be controlled and improved by using this approach. A total of 3,613 individuals were marked by fin-clipping, which can be identified in their gill (83%), caudal fin (73%) and dorsal fin (50%). Other parts of the fish were evaluated unsuitable for clipping and mark. Otolith microchemistry analysis, including the time series of Sr/Ca and Ba/Ca ratios in otoliths, will be conducted in the future to understand their life history and potential stock structure, which are basic scientific data for conservation and assessment of this stock in coastal waters off western Taiwan.

**Keywords:** East Asian fourfinger threadfin, Mark-recapture, External tagging, Otoliths dyeing
Age and growth of eleven fish species from the western Arabian Gulf

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Age information is one of the most influential components in the population dynamics and key to development effective management strategies. To estimate the age, the otolith generally outperformed other hard structures in many teleost fishes for having relatively better precision, higher clarity of the increments and ease of sample preparation. With age information, growth model can be estimated, but a priori use of one model and ignorance of alternative models can lead to model selection bias which may contaminate following applications. To examine the age and growth the fish from the western Arabian Gulf, we collect the samples from eleven commercially important fish species *Lethrinus nebulosus*, *Lethrinus lentjan*, *Epinephelus coioides*, *Epinephelus areolatus*, *Cephalopholis hemistiktos*, *Argyrops spinifer*, *Rhabdosargus haffara*, *Scomberomorus commerson*, *Siganus canaliculatus*, *Nempterus japonicas* and *Carangoides bajad*. Three models, Schnute, von Bertalanffy, and Gompertz were fitted as the best model was chosen based on information theory. The transverse sections of otoliths show clear annular incremental patters, enabling the otolith-based ageing. According to the lifespan, the fish can be categorized into short- (< 5 years, *R. haffara* and *S. canaliculatus*), medium- (< 10 years *C. bajad*, *E. areolatus*, *E. coioides*, *Nempterus japonicas*, and *S. commerson*) and long-lived (> 10 years, *L. nebulosus*, *L. lentjan*, *Cephalopholis hemistiktos*, and *Argyrops spinifer*). Von Bertalanffy growth model was best supported by the lengths-at-age data derived from otoliths except for *R. haffara*, *S. commerson* and *S. canaliculatus*, in which Gompertz was selected. Sex-pooled growth models were supported in all fish species examined, suggesting an insignificant role of sex in affecting the growth curve.

**Keywords:** Age and growth, Arabian Gulf, Life history, Information theory
Young-of-the-year yellowmouth barracuda *Sphyraena viridensis* (Cuvier, 1829) growth in Eastern Algeria based on otolith microstructure analysis

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Daily age estimate of young-of-the-year (YOY) Barracuda *Sphyraena viridensis* Cuvier (1829), captured in the south-western Mediterranean Sea, was made on otoliths of 32 individuals. Increments were observed in sagittae sectioned in a transverse plane and viewed with light microscopy. Increment counts were made for age estimation. Estimated ages ranged from 27 to 299 days of fish ranging in size from 15.9 to 38.9 cm total length (TL). The observed mean growth rate is 3.487 mm.day$^{-1}$. Decreasing growth performance is evidenced according to age: 3.822 mm.day$^{-1}$ between 159 and 302 mm (27 - 139 days), 1.596 mm.day$^{-1}$ between 352 and 389 mm (172 - 299 days).

**Keywords:** Daily growth, Sagittae, *Sphyraena viridensis*, Eastern Algeria
Preliminary Result of Age and Growth Research of Pacific Bluefin Tuna Caught by Taiwanese Longline Vessels

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Pacific Bluefin Tuna (*Thunnus orientalis*) are caught by longline fishing vessels and landed in the Tongkang, Nanfangao and Chengkung fishing port. There were a total of 62,245 individual Pacific Bluefin Tuna (PBF) caught by Taiwanese small-scale longline fishing vessels (<100 gross registered tonnage) during 2001-2016. The annual catches in number of PBF ranged from 713 (in 2012) to 8,193 (in 2003). The PBF fishing seasons run from April to July, although 94.13% of PBF were landed in May and June. PBF is of great economic value and produce an average of NT$ 4.46 billion dollars annually during 2001-2016. The effective management of fish populations requires knowledge of the age and growth of the fish. In this study, sagittal otoliths of 304 individual Pacific bluefin tuna, caught in the waters off Northeast Taiwan and landed in Nanfangao fishing market in 2016, accompanied with fork length (FL) and whole body weight (BW) data were collected for age determination and length-weight relationship analysis. The FL ranged from 173 to 274 cm, and age ranged from 6 (3 samples) to 27 years (1 sample) of 294 PBF samples. The mean FL and age are 216.43 cm (±2.20 cm) and 11.44 (±0.46 years) years, respectively. The most common age distribution of PBF ranged from 7 to 13 years (83.33%) in 2016.

**Keywords:** Pacific Bluefin Tuna, Otolith, Age-Length key, Age structure
Migration of giant catfish from the Orinoco River basin revealed by otolith microchemistry

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The giant catfish *Brachyplatystoma rousseauxii* is an ecologically and economically important species in the Amazon River basin. Although much of the Amazonian fish fauna is poorly studied, the life history of this species is receiving increasing attention, particularly via otolith microchemistry. Recent studies have demonstrated that these fish spawn in Andean headwaters of the Amazon, and eggs and larvae drift downstream to nursery areas, often reaching the Amazon estuary. After growing 1–2 years in nursery areas, fish migrate back upstream, with some homing back to natal tributaries. The round trip is >8,000 km, one of the longest known fish migrations. However, populations living in the Orinoco River basin remain poorly studied; it is unknown if their life-history characteristics differ from conspecifics in the Amazon. Since the Orinoco is less than half the length of the Amazon, the life histories of these populations necessarily differ in at least this critical dimension. To elucidate their life histories, we measured otolith microchemistries of juveniles, subadults, and adults (n = 14 in total) from the Orinoco using scanning X-ray fluorescence microscopy, laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS), and laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS); we focused primarily on Sr:Ca, Se:Ca, Ba:Ca, and \(^{87}\text{Sr}:{^{86}}\text{Sr}\).

Although 3 individuals showed microchemistry patterns consistent with the long-distance migrations and possible estuary use reported previously, the remaining fish deviated from this; e.g., one fish appears to have been spawned in a headwater stream, then drifted downstream to and resided in a main tributary of the Orinoco, without ever entering the Orinoco main stem. Such a migratory life history would require a roundtrip 1/10th that reported for the Amazon. A larger-scale study may be needed to investigate the spatial variability of *B. rousseauxii* life histories in order to adequately manage this already-complex species.

**Keywords:** Amazon, Otolith microchemistry, Life history, Fish migration
Early life-history of the fork-tail siganid *Siganus argenteus* inferred from otolith microstructure

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In coral reef fishes, early life traits could directly relate to population dispersal. Longer pelagic larval duration suggests wider dispersal potential. *Siganus argenteus*, a widely distributed siganid species, exhibited short larval duration (24 days) and lowest proportion in multispecies settlement among congenerics. High mortality incurred in long travel by the fish toward the coast could be implicated for the low percentage in species composition among settlers. Otolith microstructure analysis revealed the core, hatch ring, first feeding rings and subsequent growth increments from polished sagittae. Coefficient of variation of increment counts was less than 3%. The short pelagic larval duration and first feeding, and restricted spawning period derived from otolith age indicate the urgency to settle. Highest settlement volume based on commercial-scale juvenile “runs” occurred on and 1-2 days about the new moon date. New moon could provide the advantage of darkness for better predator evasion and faster dispersal shoreward afforded by the wide tidal range. Spawning in the fish falls a few days before the first quarter moon from backcalculated otolith ages. The daily age structure of the fish sampled from the gut of skipjack tuna caught offshore and from off-reef and seaweed bed sites traced a consistent spatio-temporal path of its dispersal shoreward until settlement. But a coastal origin of larvae is unsubstantiated by the data.
**Annual variability in growth and maturation of *Uroteuthis edulis* in the Yilan Bay**

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Neritic squids are short-lived species whose life-history traits and distribution pattern are vulnerable to changes of environmental conditions. Annual variability in growth and maturation parameters of *Uroteuthis edulis* from a trawl in Yilan Bay was examined from January 2011 to December 2012. Mantle length (ML) of both sexes were larger in 2011 than those in 2012. The ML-body weight (BW) relationship was significant between sexes, and was significant between two years for the same sex. Hatching dates were back-calculated by statolith microstructure. Hatching month peaked in August, followed by December and March for 2011, while peaked in March-April, followed by September and January for 2012. Proportion of mature females were higher in January and May for 2011, while in March and May for 2012. Age-at-maturity for both sexes were similar between two years (<5 days). ML-at-maturity of both sexes were larger in 2011 than those in 2012. Annual variability in growth and maturation of *U. edulis* in Yilan Bay might be resulted from population movement, or influences of environmental condition changes.

**Keywords:** *Uroteuthis edulis*, Statolith, Growth, Maturation
Age and Growth of the Acoupa weakfish, *Cynoscion acoupa* (Lacepéde, 1801), on the north coast of Brazil

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Species of the Sciaenidae family are among the main fisheries resources in Brazil, constituting approximately 22% of the total production in tonnes from the country’s marine fisheries. Among the species of this family, we have the Acoupa weakfish, *Cynoscion acoupa*, one of the most exploited fishing resources in the north coast of Brazil. Although it is a species of high economic and social interest, studies on the biology of the species are still scarce, and data on age and growth are the basis for assessments of fish stocks, and for this reason, the objective present this study. Samples of *C. acoupa* were collected at artisanal fishery landings from three locations in São Marcos Bay, São Luís - MA, between 2007 and 2008, measured, weighed and removed the *sagittae* otoliths. A total of 208 otoliths of individuals between 13 and 107 cm in total length (without significant difference between the sexes) were collected and analyzed. Through the marginal increment analysis, it was possible to determine the formation of a ring per year in July, at the beginning of the rainy season in the region. The von Bertalanffy growth model was estimated for males and females, and did not present significant difference, resulting in a curve for both sexes with the following parameters: $L_\infty = 142.95$ cm; $k = 0.13$ and $t_0 = -0.45$ years. The ages varied from 1 to 10 years, with the age at maturity between 2.1 and 2.4 years. Young individuals are captured within the San Marcos Bay, while the older ones are found in the coastal marine region.

**Keywords:** Otoliths, Northern Brazil, Scianidae

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Age and growth of the endemic Xingu River stingray *Potamotrygon leopoldi* validated using fluorescent dyes

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The Xingu River stingray *Potamotrygon leopoldi* (Potamotrygonidae) is a valuable species in ornamental trade. Between 2003 and 2005, vertebrae of 151 individuals (75 males and 76 females) were analyzed to derive the first growth curve for this species. In the totality of the sample, there were significant differences in the size of both sexes with females measuring 149 to 700 mm disc width - *W₀* and males 109 to 500 mm *W₀*. The marginal increment ratio showed an increasing trend with the highest value in November, decreasing from December. Tetracycline (TET) specimens injected were held in captivity for 13 months displaying a fluorescent mark in vertebrae (two females of 410 and 503 mm *W₀*, and two males of 356 and 434 mm *W₀*, aged 3 to 7 years), confirming the periodicity of the band pair once a year. The Akaike Information Criterion (AIC) showed that, among the seven models considered, the best fit was obtained for the von Bertalanffy, modified with *W₀* for both sexes. Significant differences in growth by sexes were derived. Growth parameters for females were: *W₀* = 149 mm; *W∞* = 763.06 mm; *k* = 0.12 year⁻¹, whereas for males: *W₀* = 109 mm; *W∞* = 536.4 and *k* = 0.22 year⁻¹. The ages corresponded to males from 0+ and 7.2 years while in females were from 0+ to 14.3 years. Maturity is acquired in females between 5.0 and 5.7 years and in males, 3.5 - 4.2 years. Concerns on sustainability were raised due to the construction of the Belo Monte power plant for electricity (2015 and 2016) in the state of Pará causing changes to this species’ habitat which is endemic to the Xingu River and to two of its tributaries.

**Keywords:** Vertebrae, Potamotrygonidae, Amazonian region

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Using otolith and scales to investigate age and growth of wild blackhead seabream in the waters off western Taiwan

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In Taiwan’s surrounding waters, blackhead seabream (Acanthopagrus schlegelii) lives very close to regions of high human activity, and even though it’s a well-known resource but also vulnerable to anthropogenic influences. In view of this, the government selected blackhead seabream as an important stock enhancement species as a result its stock assessment is necessary. However, considering the convenience and cost of sampling, it is necessary to study the possibility of using scales as age determination material. Original scales determined age and body length data were used to establish VBGE, and to estimate the relationship between age and length of the fish. Finally, we compared the VBGE with that determined by otoliths, and also discussed the possible application of scale oriented data.

**Keywords:** Blackhead seabream (Acanthopagrus schlegelii), Otolith, Fish scale, age determination, VBGE
Simple predictions of growth under differing environmental pressures are not suitable for heavily fished species with complex ecology

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Populations with discrete distributions may experience varying environmental conditions, providing opportunities to explore the impact of environmental conditions on growth. Using analysis of size-at-age data from fisheries surveys, we examine sub-group, and wider North Sea, responses of European plaice (*Pleuronectes platessa*) to environmental variation and warming seas in the field. We show that female and male individuals undergoing a southern North Sea feeding migration are smaller at age than their northern counterparts until age 3, with southern males also remaining smaller from ages 4–7 but the females catching up with their northern counterparts. We suggest that historic fisheries induced evolution and current competition explain these variations in size between the areas and sexes. By considering sexually dimorphic growth differences between northern and southern feeding groups we suggest that higher temperatures do not limit growth of plaice up to age seven. Using a von Bertalanffy model to estimate maximum asymptotic size, we also show that responses to conditions in the North Sea are not clearly driven by a Pejus temperature limit. Using standardised survey data enabled this study to examine the relationship between fish length at age and whole otolith. The study did not provide consistent results between fish length measurements and otolith increment estimates of length at age, highlighting the need for very strong datasets to develop fish size to otolith size relationships in wild caught studies. Contrary to existing literature, we found no evidence that higher temperatures either limited recruitment to age 1, or reduced size in regularly sampled age groups of fish. Our study highlights how heavy fishing pressure and geographic separation may obscure environmental drivers of growth in commercially exploited species. The insight provided by the inclusion of known spatial separation in this species challenges the expected response of plaice to environmental variation.

**Keywords:** Temperature, *Pleuronectes platessa*, Fish growth, Otolith
Microphotogrammetry for the obtention of 3d otolith images

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Microphotogrammetry of otoliths is a technique that consists of obtaining 3D models from 2D images, which considerably broadens the possibilities of morphometric studies of otoliths in various fish resources with specific applications such as stock differentiation. It is possible through the sequential acquisition of images to obtain digital reconstructions in three dimensions. The objective of this work is to develop a working methodology for obtaining otoliths images 3d with materials and tools available in the age and growth laboratory of the Instituto de Fomento Pesquero and that can be replicated in other laboratories. Otoliths of some of the main species that make up the commercial fisheries of Chile were used, as well as an image analysis device with Image Pro Premier, Colmap, Agisoft, VSCD, Fiji softwares and an adjustable speed cutting machine. By means of this procedure, several measurements can be obtained in both two-dimensional and three-dimensional planes that can be used in various inter and intraspecific characterization studies. As a final result three-dimensional images of otoliths are obtained that offer possibilities for further studies, mainly of morphometric characterization and species differentiation.

Keywords: Microphotogrammetry, Otoliths, 2D, 3D
Morphometric analysis of sagitta otoliths of *Polydactylus virginicus*, *Menticirrhus littoralis*, and *Conodon nobilis* in Sergipe, Brazil

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*Polydactylus virginicus*, *Menticirrhus littoralis*, and *Conodon nobilis* are the main species caught in shore-based fishing tournaments in Sergipe. Specimens were sampled during events occurring from March 2014 to August 2015. Each specimen was measured (total length TL; cm) and weighed (FW; g) and their sagitta otoliths (right and left) were removed and stored separately in paper envelopes. Both otoliths were measured (length - OL and width - OWi) using stereomicroscope and micrometric ruler, and weighed (OW) with a scale (precision: 0.0001). A total of 229 *P. virginicus*, 183 *M. littoralis* and 77 *C. nobilis* were sampled. The relationships between TL and OL, and TL and OWi were estimated for each specimen and no significant difference between right and left otoliths was found for these three species. Variations in TL were better explained by OL than OWi and the relationships estimated for sex grouped were: TL = −1.9040 + 4.1898*OL, TL = −0.7153 + 3.1154*OL, and TL = −3.2945 + 2.2740*OL for each species, respectively. Relationships between fish and otolith weight estimated for the right and left otoliths were not statistically different, and a single relationship was estimated for sex grouped of each species: FW = 1.0285 + 9280.6*OW (*P. virginicus*), FW = −17.8891 + 3912.8*OW (*M. littoralis*), and FW = 4.4845 + 672.4*OW (*C. nobilis*). Such relationships allow for the estimation of fish length and weight in diet and fossil studies. Finally, the relationship between otolith weight and length were OW = 0.00006*OL^{2.9608}, OW = 0.00012*OL^{2.7474}, and OW = 0.00003*OL^{3.7362}, respectively, indicating isometry or different degrees of allometry (negative and positive).

**Keywords:** Morphometry, Polynemidae, Sciaenidae, Haemulidae
Ontogenetic and intraspecific changes in otolith shape of anchoveta (*Engraulis ringens*) as indicator of stock structure in the Pacific southeast off Chile

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The anchoveta, *Engraulis ringens*, support the most productive small pelagic fisheries of Humboldt current ecosystem that in Chilean coast are separated in three administrative stocks: zone I from Arica to Antofagasta (18.24°S 26.00°S), zone II from Caldera to Coquimbo (26.01°S 32.16°S), zone III from Valparaíso to Valdivia (32.17°S 41.77°S); however, population structure of this resource is unknown yet. The phenotypical variation in sagittal otolith, through Generalized Additive Model (GAM) of shape indices and canonical discriminant analysis of Elliptical Fourier harmonic were used to test for significant differences between the three zones. The deviance of shape indices GAM showed that form factor and Ellipticity indices varied significantly between the three zones, however roundness, circularity and rectangularity indices showed differences between zone I and II. The discriminant analysis of elliptical Fourier descriptors of juvenile and adult in 2016 and adult in 2013, showed a high discriminatory power of otolith outline to identify population units of anchoveta. The results showed significant differences between three the zones, with high classification percentages over 81%, suggesting that zona I (Arica-Antofagasta), II (Caldera-Coquimbo) y III (Valparaíso-Valdivia) corresponded to independent demographic units.

**Keywords:** Anchoveta, GAM Otolith shape indices, Elliptical Fourier analysis, Chile
Understanding the population structure of the European Anchovy (*Engraulis encrasicolus*) in the Black Sea, Mediterranean Sea and Northeast Atlantic Ocean by using otolith shape analysis

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European anchovy, *Engraulis encrasicolus*, is a small pelagic coastal marine fish largely spread from the North Sea to central Africa, including the entire Mediterranean and the Black and Azov Seas. The aim of the study is to understand the potential different population structure and their relationship between the Northeast Atlantic, Western, Eastern Mediterranean and the Black Sea anchovy population. The outlines of 2127 sagittal otoliths were collected from nineteen locations (English Channel, Atlantic, the Mediterranean and Black Sea), and added to existing information available from a previous study. Elliptical Fourier Analysis (EFA) was used to analyze the otolith shape variations among locations. Before examining the geographical differentiation by Linear discriminant (LDA) and Hierarchical Clustering Analysis, potentially confounding sources of variation (sex, fish length, otolith side and sampling year) were analyzed by partial RDA. Sex, sampling year and otolith side have no significant effect on the outline of otoliths. However, after taking into consideration the ontogenic effect, the geographical area has affects otolith shapes significantly. Three different groups of anchovies were identified: Atlantic-Southwestern Mediterranean, Northwestern-Eastern Mediterranean and Black Sea with a classification score of 80%. These results have implications for the stock management of anchovy populations from the North Sea to the Black Sea.

**Keywords:** Anchovy, Otolith Shape Analysis, Eastern Atlantic Ocean, Mediterranean Sea & Black Sea
Comparison of otolith morphology and growth rate of Central Baltic herring populations

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Identification of fish populations is essential for reliable resources management and is currently an integral part of modern fisheries stock assessment. Mixing of two spring-spawning components (northern and southern populations), and autumn-spawning herring are currently neglected in the stock assessment models for Baltic herring. In the presented work it is hypothesized, that morphology of otoliths can be driven both by environmental and genetic factors and show population-specific features, thus may be relevant marker of distinct fish groups. Otolith samples collected during the Baltic Acoustic Spring Survey conducted in May 2017 in the Polish areas of the southern Baltic Sea were used to investigate the applicability of elliptical Fourier analysis, shape indices of otolith outlines and variables correlated with otolith size in discrimination of Baltic herring populations. Presented paper aims also to compare growth rates of distinguished fish groups using von Bertalanffy growth functions. Results showed differences in otolith morphology of all herring groups, which may be used for discrimination of fish populations. The highest degree of dissimilarity of otolith shapes was found between spring and autumn spawners. However, significant differences were also found between spring spawners, which come from southern and northern areas of the Baltic Sea. According to the estimations of von Bertalanffy growth functions parameters, growth rate of all distinguished groups differ significantly. Spring spawning migrants from the northern parts of the Baltic show considerably lower growth rate than fast-growing herring of the southern coast. Findings of this study support the opinion of intensive migrations of the herring populations in the Baltic and highlighted the significance of that phenomenon for the proper management of fish resources.

Keywords: Herring, Population, Otolith, Discrimination
Genus *Antimora* (Moridae, Gadiformes) is represented by two species, Pacific flatnose *A. microlepis* Bean, 1890 and blue hake *A. rostrata* (Günther, 1878), differed by number and relative length of gill filaments only. *A. microlepis* inhabits the North Pacific while *A. rostrata* inhabits the rest of the world’s ocean. The main purpose of this work is to analyze inter- and interspecific relationships of two *Antimora* species based on comparison of the shape of their otoliths. More than 500 otoliths were used for the otolith shape analysis, including four samples of Pacific flatnose (Emperor Seamounts, SE Sakhalin, British Columbia, and US West Coasts) and three samples of blue hake (Greenland, Antarctic, and Falkland Islands). MANOVA results show very high levels of discrimination between samples. The results of the discriminant analysis show significant differences between Pacific flatnose and blue hake. Within blue hake cluster the Greenland and Antarctic groups of samples were significantly different from one to another. The Falkland Islands sample located separately from Greenland and Antarctic samples, but much closer to the Antarctic one, indicating some similarity of otolith shapes of blue hake from the Antarctic and Falkland Islands. No significant differences were found within the Pacific flatnose cluster, i.e. between US West Coast and British Columbia samples, SE Sakhalin and British Columbia samples, SE Sakhalin and Emperor Seamounts samples, indicating some uniformity in otolith shapes of Pacific flatnose in the North Pacific. Our observations corroborate rather well with recent genetic research based on polymorphism of CO1 gene. The differences found in otolith shapes in various areas might be attributed to different environmental conditions of deep-water areas where both species occur. This research was supported by the Russian Fund of Basic Research (grant 16-04-00516).

**Keywords:** *Antimora microlepis, Antimora rostrata*, Otolith shape, Intraspecific structure
Otolith shape development in young-of-the-year chub (Cyprinidae, *Squalius cephalus*)

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Only a very few studies have focused on the influence of age on the otolith morphometry of cyprinids. Chub *Squalius cephalus* (L.) is an abundant and widespread European rheophilic cyprinid. Otolith shape analysis was used in many studies to characterize diverse local populations. In the present study we have evaluated the influence of age on the shape of otoliths during the early life of Chub when reared for experiment. Features of the sagittae, asterisci, and lapilli of laboratory-hatched larvae of the chub, were investigated until day 180 after hatching. Our observations showed that the lapilli and the sagittae were both present at hatching, whereas the asterisci were formed only between 20 and 30 days post-hatching. From day 45, the shape of the lapilli and the sagittae became more complex. The lapilli took the shape of a bean whereas the sagittae became far too fragile and inappropriate for study due to its developing very thin anterior and posterior rostra. From day 45, Fourier analysis of the shape of lapilli showed significant differences dependent on age group. However, the shape of the lapilli did not differ significantly between individuals of the same age group, even if their total length was different. This result suggested that differences in individual growth rate do not greatly influence the shape of the otolith. For young-of-the-year chub, according to the link between age and otolith shape, we recommend taking this parameter into account in any study aiming to highlight a genetic or environmental regulation of the otolith form.

Keywords: *Squalius cephalus*, Otolith shape, Juveniles, Fourier analysis
Otolith age estimation by Mojette Transform descriptors and machine learning

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Age and growth are primordial essential data in stock assessment and management. However, contracting experts for age estimation using calcified pieces costs several million euros annually. Yet, alternative methods exist for fish ageing using the otolith shape (i.e., otolith shape descriptors or Elliptic Fourier Analysis). The goal of this study is to use a new descriptor of the otolith shape with Mojette Transform as an input of a Random Forest (RF) classifier. Mojette Transform is the exact discrete Radon transform used in tomographic reconstruction, image watermarking, or video compression. Its mathematical properties allow reducing the information and having enough redundancy to characterize the object/image according to a sufficient numbers of projections from the binarized image. Each projection is the sum of pixel luminance crossed with a specific angle. For otoliths, this projection well reflects the succession of the growth segments. Preliminary experiments were conducted on 334 plaice (*Pleuronectes platessa*) samples collected during the survey CGFS (October 2016) covering the Eastern English Channel. The calibrated image for the left sagittal otolith was realized for each fish. The image database was labeled by expert interpretation according to international rules. The recognition rate is based on the comparison with the RF labeling and the expert data. After rescaling (Gray transform centering) and resizing (from 50x50 pixels), RF obtained a 48% error rate and a 3-year average over/under estimation until 5-years bias for expert-identified ages greater than 6 years old. With the first 30 Mojette projections as inputs of the RF classifier, error rate was 46% with a smaller 2-year over/under estimation. These results could be optimized to realize automatic ageing.

**Keywords:** Otolith shape, Fish age, Random forest, Mojette Transform
Evolutionary trends on the sagittae otolith shape of recent marine bony fishes

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From 1990s it has been sequenced genes of fishes contributing to establish high phylogenetic relationships, which has transformed the fish classification used by ichthyologist. Here we show the first evolutionary approach on morphology of sagittae otoliths of recent marine bony fishes based in these phylogenetic studies. The otolith analysis was also performed considering the taxon family following the phylogenetic classification recently resolved. The otoliths selection such represented each family was based on considering three together criteria: (i) the abundance and amplitude of geographical distribution of species belonging it, (ii) the available of material in the databases and atlas, and (iii) the species used in the phylogenetic trees. The morphological analysis of sagittal otolith was performed using geometric morphological analysis. The otolith shape was digitalized from 12 landmarks defining the contour features, and others 12 landmarks drawing the sulcus acusticus. From these data, the otolith-shaped variability was analyzed from graphical methods and the morphological disparity. In addition, we provide the otolith features by taxon linked to phylogenetic differentiation.

Keywords: Otolith shape, Evolution, Phylogeny, Marine Fishes
Otolith morphometry and fish length relation of *Amblypharyngodon mola* (Hamilton, 1822) caught from middle Ganga region (India)

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The aim of this study was to determine the correlation between the length, widths and weights of otoliths and the length and weight of fish samples of *Amblypharyngodon mola* caught from middle Ganga region. Standard length (SL) measurements were made with a 1mm precision fish length measuring scale. Weights were measured with a 0.001 gm measurement precision scale. Then length and width of left and right otoliths were measured. The weights of otoliths were measured with a 0.0001gm precision microbalance. Image J software was used and T-test analysis carried out for statistical difference between otoliths. In this study deeply significant relationship between the otolith morphometry and standard length was specified.

**Keywords:** *Amblypharyngodon mola*, Rasborine fish, Otolith morphometry
What makes the otolith magnesium chemical calendar-clock tick? Plausible mechanism and empirical evidence

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The incorporation of a number of readily measured trace elements are considered to be under some sort of physiological control, but rarely are explicit mechanisms proposed. We have been studying the incorporation of the trace element magnesium, which in some taxa shows strong seasonal patterning, taking on the characteristics of a “chemical calendar-clock.” However, Mg/Ca and the isotopic ratio 26/24Mg are less “clock-like” in taxa that are less metabolically active. We hypothesize that Mg uptake and incorporation are related to metabolic activity. Further, we propose a two-step process of Mg incorporation: (1) limited entry into the otolith-bearing chamber through ion channels and (2) association with water-soluble proteins within the chamber. Supporting data from a range of taxa and life histories are provided; our aim is to stimulate discussion and encourage physiologists to test these and alternative mechanistic hypotheses.

Keywords: Mg/Ca and 26/24Mg incorporation in otoliths, Metabolism, Mechanistic hypothesis
Validation of the periodicity of growth increment formation in sprat (*Sprattus sprattus*) in the eastern North Sea

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Abstract Stock assessment procedures require an accurate and efficient determination of fish age for managing exploited fish populations. Routine techniques determine the age of an individual fish through the identification and count of periodic growth increments (annuli) on calcified structures such as otoliths. However, the interpretation of the annuli is often questioned and in need of validation. The present work attempts to validate the periodicity of formation of the growth increments on sprat otoliths (*Sprattus sprattus*) collected in the Skagerrak and Kattegat during 2002-2003, by the means of the widespread Marginal Increment Analysis. This is a novelty for this commercially important pelagic stock in the eastern North Sea. The results pointed out that the otolith hyaline and opaque zones were laid down once during the years analysed. The increment of the outermost ring increased slowly from February to May conforming to the slow growth of sprat during the winter period while the deposition of the new translucent ring was completed during the summer period (June-July). This sinusoidal annual pattern was common for both Skagerrak and Kattegat and for all age groups. The results validate the periodicity of growth increment formation in this exploited sprat stock.

**Keywords:** Otoliths, Growth zones, Seasonal pattern, Marginal Increment Analysis
Otolith Shape Analysis and Dimensions of an endemic species (*Alburnus tarichi* (Güldenstädt, 1814)) inhabiting Lake Van (Turkey)

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Introduction: Tarek is an important endemic species which live in only Van Basin. This species is assessed as NT according to IUCN. The aim of this study is to determine the shape index values and dimensions of tarek sampled from Lake Van. Material-Methods: The tarek samples were obtained from Lake Van (120 samples) in September 2016-August 2017. Lake Van, the largest lake in Turkey. Due to the highly salty-alkaline waters of the lake, it is known as a ‘soda lake’. All samples were obtained from fishermen. All captured fish were measured (± 0.1cm) for total length and weighted (± 0.01g). Utricular, lagenar and sagittal otoliths were removed by making left and right distinctions. All otoliths were photographed on distal side using Leica DFC295. Otolith breadth (OB), length (OL), area (A) and perimeter (P) (± 0.001mm) were determined by Leica Application Suit Ver.3.8 Imaging Software. Form Factor (FF), Circularity (C), Roundness (RD), Rectangularity (REC), Aspect Ratio (AR) and Ellipticity (EL) were used for otolith shape analyses. Paired t-test, Wilcoxon tests were used for analyses. Results-Discussion: There is no differences between right and left asteriscus in terms of otolith length. But otolith breadth of asteriscus are different from between right and left (P˂0.01). OL and OB are different in the lapillus between right and left otoliths (P˂0.01). Right and left sagittal otoliths are similar in terms of OL and OB (P>0.05). Otolith shape index are separately calculated for right and left three otolith pairs. FF, C, RD, REC, AR, EL for left asteriscus are 5.0542 ± 0.0404, 18.459 ± 0.166, 0.24733 ± 0.00163, 2.6522 ± 0.0388, 1.1295 ± 0.00560, 0.06005 ± 0.00250, for left lapillus are 4.0233 ± 0.0268, 14.901 ± 0.0391, 0.22613 ± 0.00141, 1.1725 ± 0.0193, 1.3194 ± 0.00733, 0.08883 ± 0.00175, for left sagittal otoliths are 1.8049 ± 0.0349, 52.170 ± 0.756, 0.041396 ± 0.000987, 0.2084 ± 0.0108, 5.3108 ± 0.0610, 0.67725 ± 0.00591, respectively. This is the first study for determining the shape index for lapillus, sagittal otoliths of tarek. Acknowledgements: This study was supported by TUBITAK(Project No:215Z148).

Keywords: Otolith shape indices, Lake Van, Stock separation, *Alburnus tarichi*
Age and morphometric variations in two *Terapon jarbua* (Forsskål 1773) populations in Mindanao, Philippines

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Metabolic factors and environmental gradients contribute to the plasticity of somatic and otolith growth in reef fishes. Mindanao is the second largest island in the Philippines with distinct monsoonal seasons where some coastal regions have upwelling areas. This study provides baseline information on the age structure and morphological variations of a euryhaline fish, *Terapon jarbua* from two different and distinct bodies of water, Davao Gulf and Iligan Bay in Mindanao, Philippines. Length-weight relationship of *T. jarbua* revealed negative allometric growth with a b value of 2.94 for Davao Gulf and b=2.54 for Iligan Bay. Growth parameters from von Bertalanffy growth equation generated an asymptotic length of $L_\infty=26.43$ for Davao Gulf and $L_\infty=22.02$ for Iligan Bay. Fitted age-at-length data resulted to a curvature parameter of $K=0.34$ ($R^2=0.98$) and $K=0.48$ ($R^2=0.89$) for Davao Gulf and Iligan Bay, respectively. Results of Principal Components Analysis (PCA) and Multivariate Analysis of Variance (MANOVA) with pairwise Hotelling’s Test (Bonferroni corrected) further suggested that the two *T. jarbua* populations are morphologically different. For Davao Gulf, there was an observed expansion of the dorsal and anal regions such that the mouth region is pointing more downward. Whereas in Iligan Bay, the expansion region was only observed between the pectoral and pelvic areas. This makes the mouth region to be pointing upwards. This suggests that the fish samples from Davao Gulf may seem to increase bottom feeding activity but not for the population from the Iligan Bay. The two populations may have targeting different food types that are abundant in the area.

**Keywords:** Age structure, Geometric morphometrics, Otolith, *Terapon jarbua*
Fish Otoliths from a Tidal Flat Ecosystem of Brazil (Western Atlantic Ocean)

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The morphology and morphometric relationships of the sagittae otoliths of 44 species of socialecologic interest inhabiting a tidal flat (Araçá Bay) of the southeastern coast of Brazil were analysed. Otolith morphology and morphometry, based on quantitative and qualitative features were analyzed to identify specific trends. The highlight of the study is that these species are common along the western Atlantic Ocean coast; hence our results could be of interest for other researchers of the area, in especial those studying trophic ecology and ecosystem fisheries models. Morphologically, the otoliths show a greater variability of shapes and features. The most common shapes are elliptic, oblong, fusiform and pentagonal; and the sulcus acusticus is mostly heterosulcoid, with median position. Morphometrically, five basic otolith types are identified: common, elongated, rectangular or squared shapes; a few of them present rounded and flattened patterns. The study reveals a smaller ratio between the otolith area and the sulcus acusticus area for pelagic fishes than to demersal or benthic ones. Also, the relationship between otolith length and weight provides very interesting specific information linked to specific life history of the species.

**Keywords:** Morphology, Morphometry, Sagittae, Tidal flat ecosystem
Morphology of otoliths and other characteristics of the Gadidae genera *Eleginus*, *Gadus* and *Microgadus*, and mutual relationships of these genera

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Otoliths of many families of bony fishes are very species specific and can also be used for recognizing genera and relationships between genera. The otoliths of the recent species *Eleginus gracilis* and *Microgadus proximus* show close relationship between these species, and those of *Gadus ogac* and *Microgadus tomcod* as well. This is supported by external characteristics, meristics and geographical distribution of these species, and genetic data. The taxonomic consequences will be discussed. Fossil otoliths of *Eleginus* or an ancestral genus of *Eleginus* have been found in Late Pliocene deposits from the Netherlands, proving that these fishes must have adapted themselves to much colder environments, from (warm)-temperate to arctic conditions, thus being opportunistic and entering the originally empty arctic environment that came into existence at the beginning of the ice ages. The fossil otoliths also show that *Eleginus* has a North-Atlantic origin.

**Keywords:** Gadidae, Taxonomy, Evolution, Adaptation
Atlas of fish otoliths of the Argentina

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The purpose of this Atlas is to provide a detailed description of morphological characteristics of otoliths (mainly sagitta, but also lapillus and asteriscus when possible) of 158 species, belonging to 64 families and 17 orders of marine and freshwater fishes from the Argentina. Five geographic areas are considered for the otoliths description: Paranaense forest, Paraná flooded savanna, La Plata Basin, Argentinean Sea and Austral Sea. Descriptions of sagittae are based on shape, contour, size, anteriorposterior parts, and sulcus acusticus features of otoliths. Characteristics of lapilli otoliths are made considering shape, margins, presence and structure of sulcus, gibbus maculae and line basalis maculae. Asteriscii otoliths are characterized by the lobus major, fossa acoustic, edge and structure of rostrum and antirrostrum. In addition to its description, each otolith is photographed and illustrated. The present Atlas provides information that complements the characterization of some fish species. In addition, it constitutes an important instrument for species identification using otoliths collected both in archaeological sites and in feeding remains of fish predators.

Keywords: Atlas, Otolith, Argentina
New contour descriptors with invariance to rotation, to scale, and to position

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One of the functionalities of AFORO system (http://aforo.cmima.csic.es) is the automatic classification of unknown otolith by a comparison with their previously classified records. The information used for classification is the otolith’s contour which is extracted from an image. In AFORO, otolith images are recorded following a strict positional normalization. Incoming images to be classified, however, do not always follow this normalization in such a way that they can appear rotated, inverted or with important variations of the scale. That normalization is critical for the classification accuracy. In this work, a new contour descriptor formulation that exhibits invariance to rotation, to scale, and to spatial displacement is presented. These descriptors operate in the transformed domain and are based on the Discrete Fourier Transform (DFT) and on some of its properties. The aim is to provide robustness way to feature shape contours, and in the AFORO database framework, to enhance the classification functionality of incoming otolith images regardless of how they have been taken.

Keywords: Contour descriptors, Automatic classification of otoliths, Invariance to rotation, AFORO system
Morphological variation in the Sagitta of *Menticirrhhus americanus* (Linnaeus, 1758) in a subtropical environment

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This study aimed to analyze morphological variations in the sagitta of *Menticirrhhus americanus* regarding sex and possible influences of the reproductive process in this structure. The specimens, caught in a Brazilian subtropical environment were measured for total length (TL), sexed and analyzed for gonadal development and the otoliths were extracted. The measurements of the right otoliths of each specimen were: length (OL greater longitudinal distance) and height (OH greater perpendicular distance) of the otolith, area and perimeter of the otolith (AO and PER, respectively) and sulcus acusticus area (AS). The applied shape indices were: Ratio Aspects OL/OT and OH/OL*100, Rectangularity [Rc = AO/(OL×OH)], Ellipticity [E = (OL-OH/OL+OH)], relative surface of the sulcus acusticus [SRS = AS/AO] and the wavelets for the otolith contour was also obtained. The results showed: adult specimens of similar size were selected (males meanTL = 24.6 ± 1.9 cm, n = 40 and females meanTL = 24.2 ± 1.7 cm, n = 50) to avoid the influence of ontogeny; the Wilcoxon MannWhitney test evidenced no significant differences for the shape indices between the sexes. The Kruskal-Wallis test of the 9 wavelets obtained showed only significant differences between the sexes in wavelet 4 in the ventral-posterior region of the otolith. It was also used young (meanTL = 17 ± 0.83 cm, n = 27) and adult (mean TL = 18.13 ± 0.98 cm, n = 29) females, with similar length; the otolith parameters was significant, except for rectangularity and SRS. The adult females and young females showed significant differences in wavelets 1 and 4 in the ventral-posterior region, in wavelet 5 in the ventral-posterior region and in the dorsal-posterior region and in wavelet 6 in the anterior end of the otolith. The results showed that the reproductive process influences the shape and the contour of otoliths.

**Keywords**: Morphological variation, Sagitta, *Menticirrhhus americanus*, Brazil
Otolith shape analysis of *Epinephelus marginatus* caught in the south of Brazil

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In the present work, we described the otolith morphometric changes during life of *Epinephelus marginatus* caught in the south of Brazil. 77 right sagittal otoliths were imaged with reflected light on a black background using digital camera. They were positioned with the sulcus acusticus faced to the camera, the rostral-postrostral axis aligned with horizontal axis of the image and the rostrum positioned to the left. All metrics and shape indexes were measured with the Software ImageJ. We measured the maximum distances from anterior to posterior regions (length) and from ventral to dorsal regions (height), total area, contour length (perimeter) and four shape indexes: aspect ratio, circularity, rectangularity and perimeter-area ratio, which is relative to otolith contour complexity. We evaluated how each of these indexes changes during the fish life using linear and non-linear regression models, considering immature and mature phases. All metrics, except area (b = 1.3), presented negative allometry (b < 1) in relation to fish length. The shape indexes presented different patterns between immature and mature fishes (ANCOVA, p-value < 0.05). In general, immature individuals presented more irregular otoliths.

**Keywords:** Dusky grouper, Ontogenetic variation, Sagittae, Brazil
Otolith atlas of fishes inhabiting the Ganges, India

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Otoliths (lapilli, sagittae and asterici) are paired structures present in the internal ear of fish. They are considered to assist the fish in hearing and balance. The unique characteristics of otoliths in being metabolically inert and not showing resorption even during starvation offers a variety of useful applications such as in deciphering precise age and growth pattern, movement pattern, nursing grounds, reconstructing the life history events, etc. The effectiveness and success of many fisheries management decisions depend directly or indirectly upon the outcome of the otolith science. Otoliths are also used to study the feeding habit of piscivores. The sagittal otoliths have been most commonly used for the purpose. Despite the fact that the freshwater ecosystem is considered fragile, there is paucity of information on the otoliths of freshwater fishes. Moreover, in India the otolith science is still in its infancy stage. Thus, the present study was undertaken with the aim to provide a photographic guide of the sagittae otoliths emphasizing on otolith length, otolith height and otolith weight of the commonly caught fish species from the Ganga River. This atlas consists of photographs of the sagittae otoliths of 50 species belonging to 19 families Schilbeidae, Cobitidae, Belonidae, Channidae, Claridae, Anabantidae, Cyprinidae, Sisoridae, Gobiidae, Clupeidae, Saccobranchidae, Mastacembelidae, Bagridae, Nandidae, Notopteridae, Centropomidae, Mugilidae, Siluridae, Sciaenidae distributed in seven orders.

**Keywords:** Otolith atlas, River Ganga, Sagittae
Sagittae as an efficient tool to diagnose three *Eucinostomus* species in a tidal flat of the southeastern Western Atlantic Ocean, Brazil

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In the tidal flat of Brazilian, fish species of genus *Eucinostomus* (Family Gerridae) have a high socio-economic value, but also are prey of other fishes and even birds. Considering that sagittae otolith is a specific species features, the present study analyzes the shape for three common species: *E. argenteus* \((n = 70)\), *E. melanopterus* \((n = 41)\) and *E. gula* \((n = 41)\). From morphological approach, the difference between species is focused in the ending of cauda of sulcus acusticus: slightly curved in *E. gula*, strongly curved in *E. argenteus* and markedly curved in *E. melanopterus*. To provide a robust analysis a Chi-square of cauda morphology revealed significant differences \((x^2; p < 0.05)\) within some length classes. From morphometric perspective, the ANOVA test (Tukey’s Multiple Comparison Test) showed intraspecific variability in the comparison of *E. argenteus* with *E. melanopterus* \((p < 0.05)\). The otolith weight in all analyzes is separated from the other features. The interspecific differences were related to the depth distribution, where deeper species (*E. gula* up to 55 meters depth) presented higher morphological variability.

**Keywords:** Otolith, Morphology, Morphometric, Depth distribution
Performances of otolith outline analysis in identifying fish species from western Arabian Gulf

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Otolith shape analysis is a well-established set of techniques that widely used to delineate fish stocks of the same species and distinguish several closely-related fish species. In this study, I examined the performance of otolith outline analysis as a cost-efficient tool to identify fish specimens across wider ranges of species and families. A total of 1104 fish individuals were collected comprising of twelve species and five families: Lethrinidae (*Lethrinus nebulosus* and *L. lentjan*), Scombridae (*Scomberomorus commerson* and *S. guttatus*), Serranidae (*Epinephelus coioides*, *E. areolatus* and *Cephalopholis hemistiktos*), Siganidae (*Siganus canaliculatus* and *S. javus*), and Sparidae (*Argyrops spinifer*, *Rhabdosargus haffara*, and *Sparidentex hasta*) from the western Arabian Gulf. Two outline analyses, discrete wavelet and Elliptic Fourier analysis, were applied on the 2-D projections of otoliths and linear discriminant analysis was applied to assign species or family based on normalized shape coefficients. Shape coefficient values (Wavelet levels and Fourier Harmonics) were significantly different between families and species (Multi Response Permutation Procedure of 9999 permutations, all ps < 0.001). However, two methods had poor performance in identifying the species (correction rates ranged from 0 to 67 %), and slightly better in predicting the families (23 to 73 %). Between-species distances were generally 79 to 189 % of within-species distances, and between families distances were 84 to 177 % of within-family distances. High within-species and family variability in otolith shape, which may arise from the occurrence of secondary structures in adults, masked the between-species and -families contrasts in terms of distances and consequently led to poor performances of otolith outline analysis in identifying the species.

**Keywords:** Outline analysis, Species identification, Arabian Gulf, Allometric growth
A first attempt to characterize three-dimensional speckle patterns in fish otoliths: A case study in the Southern blue whiting (*Micromesistius australis*)

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The speckle phenomena are observed when an optically rough surface is illuminated with light (e.g., a laser). The scattered light presents a particular intensity distribution, making the surface appear to be covered with a fine granular structure, which are then used to capture its roughness. In the current research three-dimensional speckle patterns (TDSP) were characterized in a first attempt to search for new otolith-based research properties in the Southern blue whiting (*Micromesistius australis*), used as a model species. The 3D speckle structure of otoliths was successfully revealed when a spot of 3 mm diameter were illuminated by collimated light \( S \), after which a set of speckle images were recorded with an 8-bit monochrome camera (CMOS of 4.4 x4.4 \( \mu \)m² pixel size), and then analyzed employing speckle contrast and speckle correlation techniques. In general, when the surface gets rougher, the specular component decreases as the diffuse component increases. The changes in this roughness property was analyzed in fish ranging from 52 to 56 cm in total length, from two spatially-separated spawning grounds [Atlantic (ATL) vs Pacific (PAC)]. The results showed the speckle contrast was similar in otoliths from ATL, while otoliths from PAC were not. Consequently, we have concluded for these samples that PAC otoliths has mayor diffuse component than ATL otoliths. Consequently, the results of the current research demonstrated TDSPs could be reliably characterized and quantified in otolith of adult fish, with promissory potential for developing further research in the field of fish stock identification or others phenotypic-based otolith research applications.

**Keywords**: Speckle, Otolith, Fish
Ecomorphological variation in the otolith shape of Siganids (Teleostei: Siganidae)

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Variability of otolith shape in juvenile *Siganus canaliculatus*, *Siganus virgatus*, *Siganus spinus*, and *Siganus argenteus* caught in Northeastern Philippines was investigated using shape analysis. Otolith extracted from 30 individuals per species were subjected to image analysis to extract otolith morphometrics (length, width, area, and perimeter) and to derive shape indices (form factor, circularity, ellipticity, and rectangularity). Otolith morphometrics and shape indices were statistically compared. Similarly, Elliptic Fourier Analysis (EFA), a technique for generating from poor outline a set of shape representative variables suitable for statistical comparison among samples, was performed. Normal distribution of fish sizes was ensured to elicit the confounding effects to otolith size and shapes. Except for rectangularity index, other shape indices among the four species showed that *S. argenteus* were significantly the most elliptical, but the least irregular, round and circular in shape than the other species. EFA shows that otolith shapes entailed high complexity in its form, thus higher number of harmonics were used to notably classify and define the otolith form of the four species. Finally, using non-metric multi-dimensional scaling ordination plot, four groups were successfully discriminated using the shape index as the variables. The results might suggest that otolith shape reflects the differential patterns that could be inherent to the life history and environmental conditions where the organism lives.

**Keywords:** Ecomorphological variation, Elliptic fourier analysis, Otolith morphometrics, Otolith shape indices, Siganids
Tracking life-history of fishes through otolith contour fluctuations

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It is well known that the chemistry, morphological and growth characteristics of fish otoliths have made the study of these calcified structures a very important tool to support management. Regarding the morphological aspects, the most common approach is the contour shape analysis frequently used to identify species or determine stocks of fishes. Very often based on Fourier or wavelets analyzes, the otolith contour is smoothed and represented by a coefficient matrix and, consequently, the small or even some large fluctuations of the contour are neglected. Here we show that multifractal analysis of the contour can uncover a straight relationship between otolith contours fluctuations and the fish metabolism. We have shown that changes in the otolith contour during the fish’s life caused by alteration of the metabolic rate is captured by the multifractal parameters. For all analyzed species, the changes were reflected in the α₀ parameter, meaning that roughness level of the sagittal otoliths follows the fish growth, while the general shape is kept unchanged. It was possible to identify at least one biological event during the fish life (e.g. length at first maturity, fish size of migration).

Keywords: Fish metabolism, Multifractal analysis, Contour decomposition, Life-history events
Diadromous fishes in the lower Mekong Basin: a review

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Fish migrations are an important characteristic of the Mekong River. Many fish species migrate between Mekong countries and several species need to connect with ocean as either adults or juveniles to complete their life cycle. Although Mekong River is ranked as the second fish species richest in the world, over one third of Mekong fish species is poorly known their migration status. Moreover, the persistence of these species is threatened by a series of dams planned across the Mekong Basin by blocking their migration routes. This is especially true for any diadromous fish that need to reach the ocean. Unfortunately, migration behaviors of diadromous species have very limited information and unlikely pay enough attention to this group, this can lead to poor management and extinction. Therefore, there is an urgent need to better-understand migration behavior of diadromous fishes in order to mitigate the environmental risks posed by dams. Currently, only one fish species is confirmed as diadromous in the Lower Mekong Basin, but considering the size of the system, and the large and complex delta region, it is thought that many more should exist. Over one thousand Mekong species were reviewed their diadromous status, distribution, and guild types in order to finalise a list of potential diadromous fish that need to be investigated further. Finally, otolith microchemistry approach will be discussed to reconstruct fish movements and migrations in the Mekong River.

Keywords: Diadromous fishes, Mekong River
Ageing of fish in Swedish waters by SLU Fish Ageing Network

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The majority of age determination of fish in Sweden are performed within the Department of Aquatic Resources at the Swedish University of Agricultural Sciences (SLU). To obtain high quality, development and continuous collaboration between all age readers at the department a network called SLU Fish Ageing Network (SFAN) has been established. About thirty species of fish, from lakes and rivers as well as from the offshore and coastal regions, are age analyzed here for assessment, monitoring and research projects. Mostly otoliths are used for age determination, examined as whole, broken and burned, grinded or sectioned and stained. Scales, operculum and bone structures are also used for ageing and growth measurements. In Skagerack and Kattegatt, a mixing area for autumn spring and winter spawning herring, we use otolith microstructures for spawning type determination by daily increments to separate the different stocks. Daily increments are also used for age validation and ageing of juvenile fish. Preparation of otoliths for chemical analyzes are performed to illustrate environmental impact and sclerochronology of fishes. To ensure a high quality level our work includes national and international inter calibrations, as well as training programs and reference archives. All methods and routines for preparation of otoliths and interpretation of annual growth zones for nearly thirty species has been documented in a manual that is available on our website.

Keywords: Swedish University of Agricultural sciences, SLU Fish Ageing Network, Otoliths, Age determination
**SmartDots** : a flexible open source software tool for fish age reading based on otoliths

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SmartDots is a software application which allows users to assess fish age readings through the visualization of otolith images. To determine the age of the fish, end-users make annotations on otolith images. Each annotation contains a line with one or more segments and a number of dots representing the annuli. SmartDots was initially developed at ILVO (Institute for Agricultural and Fisheries Research) in Belgium for internal use only. When other institutes showed interest, the SmartDots application was turned into a more flexible solution with two main modules: the SmartDots client and a Web API. With this setup the data and business logic were separated and the SmartDots solution became database independent. The SmartDots client is a Graphical User Interface which gets and sends annotation data to a Web API. The SmartDots client is an open source (GPL v3.0) Microsoft WPF application. The Web API, the interface between the SmartDots client and the database, contains all business logic. Consequently it decides how to process and where to store the data. Authentication and authorization is also handled here. The database can exist in any shape or form, and can be used for business intelligence or reporting. The SmartDots application is very flexible and can easily be adapted for internal needs. This solution results in an increased quality and efficiency for age readers.

**Keywords:** SmartDots, Software, Web API, Age reading
In the last four decades, otoliths have been recognised by scientists as powerful tools in fish ecology due to their ability to record vital environmental information throughout fish life history. Although its historical use on age determination analysis, from 90’s was recorded a sharp increase in studies using otolith with more than 200 papers published per year worldwide. Currently, among its many features, otolith chemical composition has emerged as a critical analysis in the discrimination of fish stocks, identification of key habitats and other correlated studies for environment conservation and stock management. Nevertheless, in Latin America, the development of otolith studies using microchemistry were still scarce (only 45 papers already published). The poor performance of such robust analysis in this region is somehow critical, compromising the conservation of the high Neotropical fish diversity in the continental and marine ecosystems. To change this scenario, researchers from 6 countries and 22 institutions joint in a workshop held in Alagoas, Brazil on November 2017. The workshop focused on the targets: 1. Discussion of methodological issues to identify main challenges and strategies to work in local and regional scale; 2. A methodological
intercalibration between the different research groups settled in Latin America and their international partners to defined standardized processes and protocols; 3. The current and expected application of otoliths microchemistry in biodiversity conservation and management of freshwater and marine Neotropical renewable resources. Apart of any other contribution expected from the audience after the presentation of the framework, the main result of this meeting was the formation of a network to boost the scientific production on marine and freshwater migratory species. Moreover, technicians and equipment mapping, consumption materials funding, and further determination of knowledge gaps in the region are part of the framework to trigger and consolidated key ecological studies.

**Keywords:** Otolith interdisciplinary network, Latin America
Fossil otolith assemblages have been reported from many regions of the world to interpret reginal paleoecology and to reconstruct paleobiogeography. The studies, however, are mainly concentrated in the European localities; those from the western Pacific region remain scarce, though several reports from Japan exist. Despite a few known Pliocene localities yielding otoliths, a systematic study of such fossil assemblages has never been carried out in Taiwan. The otoliths of the early Pliocene Kueichulin Formation in Shulin, Taipei County (northern Taiwan) and the late Pliocene to early Pleistocene Liuchungchi Formation in Chiayi County (southern Taiwan) were described for the first time. The recent otolith collection of Taiwan, deposited at the National Museum of Marine Biology and Aquarium, allows a well-comparable identification of fossil otoliths with great confidence. The otoliths of Sciaenidae and Apogonidae were the richest families in both localities. The sciaenids were, however, more diverse in the northern assemblage, while Pennahia was a dominant taxon in the southern one. Furthermore, the higher abundance of *Larimichthys polyactis* otoliths in the northern assemblage indicates a more pervasive distribution of the fish than it is today. The composition of the assemblages leads us to a conclusion that the paleoenvironment of both sites likely belongs to a near shore marine setting with sand to sandy mud bottom. Nevertheless, the occurrences of several myctophid otoliths in the southern assemblage suggest an environment much closer to an open ocean.

**Keywords:** Kueichulin Formation, Liuchungchi Formation, Taxonomy, *Larimichthys polyactis*
Age and growth of the finless porpoises (*Neophocaena*) in the Taiwan Strait

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Finless porpoises in the Indo-Pacific region include the Indo-Pacific (wide-ridged) finless porpoise (*Neophocaena phocaenoides*), narrow-ridged finless porpoise (*N. asiaeorientalis sunameri*) and freshwater inhabiting Yangtze finless porpoise (*N. asiaeorientalis asiaeorientalis*). Both the wide and narrow-ridged finless porpoises are commonly seen in the Taiwan Strait. One hundred and twenty-one specimens of finless porpoises stranded or obtained incidentally by fishing were recorded so far. In this study, ages of the two porpoise species were determined by growth-layer-group counting of thin-sectioned teeth and growth curves were fitted and compared. Finless porpoises stranded in western coast of Taiwan, Penghu, Kinmen, and Matsu from 2004 to 2017 were collected, which 33 specimens ranging 77-182 cm in body length were for the wide-ridged finless porpoise and 24 specimens with 85-156 cm for narrow-ridged ones. Ages of the 31 wide-ridged finless porpoise were determined between 0 yr and 10 yr for a length range of 77-168 cm, whereas those for the 21 narrow-ridged ones were 1-12 years and 85-146 cm. Age-length data were fitted by the Gompertz growth curve and the asymptotic length and growth rate were 172.82 cm and 0.34 for the wide-ridged finless porpoise, and 147.18 cm and 0.16 for the narrow-ridged one, that the wide ridged finless porpoise grew faster and reached larger size than the narrow-ridged ones.

Keywords: Finless porpoise, Growth-layer-group, Age, Growth
Establishing relationship of fossil otoliths through geometric morphometrics: a case study of sciaenid otoliths from the Eocene gulf coast

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The relationships of fossil otolith species rely heavily on the modern fish systematics. When tracing far back in the geological times, however, the increasing number of extinct otolith-based species that without comparable modern taxa usually impede establishing well-resolved relationships. One of the resulted problems might be the discrepancies of the interpretation on the first occurrences of the concerned taxa in the fossil record analyses. For example, the rich and abundant sciaenid otoliths from the Eocene gulf coast localities are remarkable compared to the contemporaneous European assemblages, but the otolith morphology of these fishes are not known to any of the recent relatives. Morphological analysis of the otoliths of seven Eocene sciaenid species revealed two main groupings according to the length of the cauda and the distance between ostium and posterior part of the bent cauda. Further resolution on the relationship of these species and their placement in the classification scheme is expected when other specimens from younger strata are added in the analysis. This exercise suggests that shape analysis such as geometric morphometrics might be a useful tool that objectively and quantitatively grouping otolith-based specie with similar shape and detect unnoticed lineages.

Keywords: Morphometrics, Systematics, Sciaenidae, Landmark
Using otolith microchemistry to determine migratory patterns of Indonesia freshwaters fishes: A prospects review

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Migratory behaviours are extremely common in fisheries, and understanding fish migrations is vital to sustainable management and conservation of fisheries resources in Indonesia. While diadromous species and populations have long been studied mainly in temperate region, potadromous migrations that are common in large tropical rivers, such in Indonesian Rivers are much less understood. Indonesia is one of the world’s most species-rich countries. Owing to their high diversity and endemism, freshwater fishes of Indonesia provide a prime example that reflects the complex geological and biogeographical history of the Indo-Australian Archipelago (IAA). To date, 1230 species belonging to 84 families have been reported from Indonesian freshwater including 1172 native species from 79 families among which 630 species are endemic of the country. Some fisheries are specialized on species with high economic and socio-cultural values such as Mahseer (Tor spp.). Most Tor spp. are rheophilic species know to migrate down streaming during the onset of the raining season for spawning and such have been largely affected by the development of dams. The dynamic of migration and life history traits are still poorly known in Indonesian population of Mahseers. The development of new tools for elucidating the migratory patterns of tropical rivers is urgently needed to improve the sustainability of the exploitation of the Indonesian freshwater fishes. A particularly useful method for studying the movement of fishes is otolith microchemistry analysis. Recent advances in otolith microchemistry methodology have enabled scientist to elucidate fish migrations, stock structure, and natal habitats.

Keywords: Otolith microchemistry, Fish migration, Indonesia Freshwater
Otolith collection of Panamanian Caribbean Fishes

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Systematic otolith collections for reference purpose are mainly established by paleontologists identifying fossil otoliths, and to a lesser extent, by ichthyologists studying the stomach contents of piscivores. The otolith reference collection in the Caribbean, however, has received little attention. Fish otoliths can be a tool in the fields of taxonomy, systematics, feeding ecology, zoogeography, paleoichthyology, archaeology and any study concerning fish identification using otolith morphology around the concern area. We present this reference collection of otoliths from the Panamanian Caribbean, which is an extract of the current Caribbean fish fauna. This reference collection is based on samples collected along a distance of ~1,287 km. The collection was made in different points of the Panamanian Caribbean coast, from the province of Bocas del Toro (STRI Point, Hospital Point) and Comarca Ngöbe Buglé (Bay of Kusapin) to the province of Colón (Rio Indio, Punta Galeta, Sherman, San Cristóbal) and Comarca Guna Yala (Archipiélago de las Mulatas). These samples work as indicators of main environments: shallow coral reefs, rocky bottom, soft bottom, mangroves, sea grasses, estuaries, islands and sandy areas with vegetation, shallow neritic areas, breakers. To obtain the otoliths, fishes were captured in an artisanal and various way such as rope, fishing rod, nylon monofilament, net, harpoon, otoliths were also obtained from the stomach contents of lionfish. The collection is represented by 45 families and 80 genera. The most abundant species is Halichoeres bivittatus. The collection is deposited at the Smithsonian Tropical Research Institute with a corresponding website (https://otolithspisciumpanama.jimdo.com). As enlarging our collection is still an ongoing process, we aim not only to increase the species number and the ontogenetic change within the common taxa, but also to anticipate on promoting regional otolith studies.

Keywords: Collection, Mangroves, Species, Taxonomy
Application of otolith analysis to study the occurrence of temperature-dependent sex determination in wild cobaltcap silverside

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Sex determination in fish can be influenced by water temperature during a critical period of development (Temperature-dependent sex determination; TSD), sometimes even overriding putative genotypic sex determinants. Extreme water temperatures caused by climate change may affect the reproduction of such species, for example, by inducing extremely unbalanced sex ratios. Species that present both TSD and a chromosomal sex marker may be useful indicators of the effects of climate change on fish reproduction, for example by monitoring mismatches between genotypic and phenotypic sex (sex-reversal). The occurrence of TSD can be demonstrated easily in rearing experiments but it is difficult to verify in wild populations. In this study, we applied otolith analysis to probe the occurrence of TSD in wild cobaltcap silverside, an Atheriniform from the Northwest Pacific Ocean in which we previously demonstrated both the presence of TSD and of a chromosomal sex marker. We screened a wild population in Tokyo Bay for the occurrence of sex-reversals from 2014 to 2016 and estimated the age in days and hatching period by otolith increment analysis. Daily water temperature data was then used to calculate the temperature experienced by each fish during the critical period of TSD. Sex-reversals were detected in 2014 (14.1% XX-males, 7.9% XY-females), 2015 (17.1% XX-males, 10.1% XY-females), and 2016 (42.9% XX-males, 0% XY-females). The average temperature of the estimated hatching period in 2014 (Jun.8~Aug.8), 2015 (Jul.7~Sep.8) and 2016 (Aug.7~Sep.24) were 23.1±1.5 °C, 25.4±1.7 °C and 26.2±0.9 °C, respectively. These data indicate that fish were born later and experienced higher temperatures during the first weeks of life in 2016 than in other years and suggest that exposure to relatively low and high water temperatures was behind the formation of sex reversed XX-males and XY-females, respectively. These results are consistent with laboratory results and suggest the occurrence of TSD in wild cobaltcap silversides.

Keywords: Sex determination, TSD, Temperature, Silverside
Losing track of time: Use of otolith chemistry to solve the problem of age determination in Baltic Sea cod

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Cod (Gadus morhua) ranges across the North Atlantic from Canada to the Baltic Sea. In the Baltic, cod has recently experienced marked declines in size and condition. This is due to a combination of worsening water quality conditions from hypoxia intensification, reduction in prey availability even as cod densities have risen, and increased occurrence of parasites and disease. A net result has been a decrease in formation of clear annual growth rings in otoliths of Baltic Cod. Otoliths are aragonitic structures that form part of the hearing and balance system in fishes, and grow incrementally as a fish grows. In seasonal environments, growth bands are laid down, much like tree rings. But in Eastern Baltic cod it has become increasingly difficult to "read" otoliths and determine ages. Here, we present 2-D otolith trace elemental maps that help us elucidate ages through seasonal variations in chemistry.

Keywords: Trace elements, Otolith microchemistry, Age estimation, Seasonal patterns
Extraction of high resolution juvenile life history information from adult otoliths of diadromous fishes

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Diadromous fish change their environments from fresh to the saltwater water, in either direction, at different stages of their lives. Changes of environment are often critical periods with increased mortality rates and are the focus of many contemporary studies. Shifts in the ratios of different trace elements, e.g., Ca, Sr, Ba, in otoliths have been used as indicators of movement between environments, especially helpful when visible transition marks in the otolith structure are faint or obscured. In this study, we developed an efficient, high precision method of processing sockeye salmon adult otoliths for extraction of daily growth information for the early marine stage. The method combines two stage laser-ablation inductively-coupled-plasma mass spectrometry (LAICPMS) (two different settings) for determination of the Sr/Ca ratio break point that identifies sea entry, and growth rate estimation from measurements of the daily increments growth with light microscopy. During the first LA-ICPMS stage, prepared, sectioned, polished and aged adult otoliths were ablated with a low resolution pass from the primordium to the edge of transverse section to identify the sea entry zone. First stage was aimed to determine rough location (±10 days) of the sea entrance zone. Next, otoliths were re-polished to enhance the visible daily increments which were photographed to preserve information on daily growth. A second high resolution ablation path was conducted across the previously estimated sea entry zone to determine more precise location (±2 days). Using this approach we acquired measurable daily growth increments (fish daily growth rates proxy) from the early marine stage of juvenile sockeye salmon together with sea entry date. Using the two step ablation process we reduced total laser time per otolith (5 mm in diameter) from ~ 1 hour to < 10 minutes. This method could be applied to any diadromous fish species.

Keywords: Otolith, Daily increments, Adult fish, Laser ablation
Evaluation of age reading methods for the invasive round goby, *Neogobius melanostomus*

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In the Baltic, the first observation of the round goby, *Neogobius melanostomus* was made in 1990. Within the past decade the species became invasive and spread rapidly throughout the Baltic Sea. Studies about the fishes potential impacts on resident species promote the need for an increasing knowledge of their basic stock structures such as growth rates, longevity and mortality, which all rely on accurate estimates of age. Former studies on the round goby have used several different age reading techniques. In this study, we compared three standard otolith preparation methods for ageing and present the best procedure for the invasive round goby. The results showed significant differences in age estimates of the same fish between the different preparation methods and between readers. The estimation of the first annulus, the first year, was the most problematic. The overall agreement was lowest when reading the whole otoliths while the best performance was achieved with sectioned and stained preparation method. Depending on method used the growth estimates also differed. The results question comparability between previous studies and highlight the importance of harmonised aging procedures for the round goby for obtaining correct estimates of population parameters such as growth rate, age at maturity, and longevity.

**Keywords:** Round goby, Method evaluation, Ageing, Otolith
Use of AFORO otoliths database in recent reconstruction of deep-fish communities and their relationship with recent climate changes

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Otoliths are one of the most commonly used fish structures for conducting a broad range of studies, from ichthyology to palaeontology, stratigraphy, archaeology and zoogeography. Their use as a diagnostic character in fish species determination has been enhanced by the publication of several otolith guides, digital catalogues and websites systems such as AFORO (Shape Analysis of Fish Otoliths, http://aforo.cmima.csic.es/). If well otolith determinations have also been widely applied to paleoreconstructions of communities from Jurassic to Holocene periods, but it is exceptional such analyses to include changes in fish assemblages of more recent decades obtained from marine sediments. In this study we present a reconstruction of recent (last few centuries) mud-bottom fish assemblages on the upper slope of the continental margin off Catalonia (western Mediterranean). Radiometric dating was determined using 210Pb 137Cs isotopes of marine sediments in one core taken at 398 m off the Ebre Delta gives us sediment geochronology. Using data base otoliths we can identify 247 otoliths from bellowing to 10 benthic and 24 meso-epipelagic species along the core. Mesopelagic fish are the most abundant remains and showed mid-time temporal oscillations. Abundance of some myctophid otoliths from Lampanyctus croccodilus and Benthosema glaciale was related with positive NAO periods. By contrast, periods of higher dominance of Ceratoscopelus maderensis could indicate opposite climatic and hydrographic conditions in some periods. Finally, AFORO provide public available information about otolith – fish length relationship based in the allometric power equation (y=axb). So, otolith size allow to reconstruct fish size structure of the commonest mesopelagic species by sediment strata, relating possible size changes with changes in the environment.

Keywords: Paleoecology, Climate changes, Morphometry, Fish communities
Daily growth patterns of three species of young-of-the-year of the Eastern coasts of Algeria fishes

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Otolith microstructure analysis was used to reveal daily growth patterns of young-of-the year (YOY) of three species of Sparides fishes, *Boops boops*, *Lithognathus mormyrus* and *Diplodus sargus* sargus. YOY collected in the Eastern coasts of Algeria from September 2016 to April 2017, ranged from 54 to 103 mm total length and from 46 to 337 days of age. In the three species, sagittal otoliths showed a slightly oval shape, symmetrical and laterally compressed. For all species, pairs of otoliths (sagitta & lapillus) were tested to identify the most easily interpretable otolith. Likewise, several methods of fine cutting and polishing have been carried out according to sagittal, frontal and transverse planes. The precision of age estimates was tested by applying both the average percent error (APE) and the coefficient of variation (CV).

**Keywords:** Sparides, Young-of-the-year, Sagittae, Eastern Algeria
Tracing of escaped meagre (*Argyrosomus regius*) through otolith microchemistry

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Escape incidents of farmed fish involve economic losses to fish farms, interactions with local fisheries and environmental impacts to coastal ecosystems. In case of escape incidents, it is therefore essential to gather any kind of information about the escapees for further management strategies. Based on the assumption that escaped fish experience different environmental conditions once they are outside the net-pen compared to their farmed conspecifics, otolith microchemistry might help us to trace fish individuals through their life-history. In this study, elements composition (i.e. Li, Na, Mg, Ca, Mn, Cu, Zn, Sr and Ba) was analyzed using laser-ablation ICP-MS along otoliths transects (from nucleus to border) on escaped and farmed meagre from the same coastal area in the W-Mediterranean Sea. Overall, results showed similar concentrations of analyzed elements between farmed and escaped meagre at the inner part of the otolith (next to the nucleus = early-stages, when both were in farmed conditions) but significant differences next to the border of the otolith. These results confirm that farmed and escaped fish shared the same origin (coastal farms) but inhabited in different conditions (farm vs. wild) during the last period of their life. Indeed, it might be possible to guess the exact moment (or period) when fish break out from the cage examining in detail explicit variations of element concentrations over fish life-time. Therefore, otolith microchemistry seems to be a crucial and practical tool to trace of escaped fish, and consequently, to help solving potential conflicts among coastal users and to improve management of potential negative socioeconomic and ecological impacts.

**Keywords:** Aquaculture, Escapes, Impacts, Microchemistry
**Daily increments of juvenile fish under environmental change – a case study with Baltic Sea perch**

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Daily increments provide knowledge on fish biology that is essential for understanding fish stock dynamics because they give detailed information on early life history. Although population dynamics of fish often is governed by events during their first year of life, early life history information is seldom used in fish stock assessment as methods for daily increments are lacking for many species. Growth of fish is highly temperature-dependent, especially early in life. Similarly, because growth is food-dependent, it may also depend on light conditions as these can affect feeding abilities through visibility. Daily increments may therefore vary greatly in different environments, and could be affected by warmer and darker waters induced by climate change. In our current study we develop a methodology for determination of daily increments of juvenile fish that is suitable for fish stocks in both coastal and freshwater habitats under varying environmental conditions. To obtain fish with known age, we hatched and reared European perch, *Perca fluviatilis*, one of the most common predatory fishes in Swedish lakes and along the Baltic Sea coast. By keeping the newly hatched larvae in different temperatures and light conditions in pelagic mesocosms situated in the Baltic Sea archipelago for 20 days we look at growth of the perch and the daily increments on the otolith. Our analyses reveal dramatically different growth patterns, despite a common origin and hatching date, depending on temperature and light conditions. Still, the method used for ageing could successfully identify hatching date across treatments. Thus, the method developed can provide robust and essential information on both daily age and growth across environments. Reliable methods for determination of daily increments are increasingly important, given more variable aquatic environments induced by climate change.

**Keywords:** Daily increments, *Perca fluviatilis*, Mesocosm, Climate change
Modelling the growth of juvenile Dolphinfish: Relating daily environmental factors and otolith microstructure

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The common dolphinfish (*Coryphaena hippurus*) is a large and highly migratory pelagic species distributed worldwide in tropical and subtropical regions. This species presents one of the fastest-growing rates registered for teleost fishes in the world. Due to its biological and physiological traits, it is likely that variations in environmental conditions quickly induce observable changes on its life-history parameters, such as growth rates. In the Mediterranean Sea, the dolphinfish represents a socioeconomically relevant resource. It reproduces during spring and early summer and the juveniles are fished in many coastal countries by a traditional and artisanal method using floating aggregating devices, representing an important income for local economies. In this work, an environmentally-dependent growth model for juvenile Mediterranean dolphinfish is performed based on individual daily increment measured on otoliths related with satellite-derived daily environmental history, using a wide array of Mediterranean locations, years and within-year birth dates in order to validate this model. Consequences of different thermal scenarios, derived from climate change effects on a warming Mediterranean Sea are explored.

**Keywords:** *Coryphaena hippurus*, Otolith microstructure, Individual daily growth, Environmentally-dependent growth model
Estimating hypoxia exposure rates and consequences using redox-sensitive chemical markers in otoliths

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Reconstructing patterns of hypoxia exposure in mobile fishes is essential to estimate population responses to this widespread environmental stressor in freshwater, estuarine and coastal habitats. Otolith chemistry offers a unique opportunity to identify sublethal hypoxia exposure using redox-sensitive chemical markers, such as manganese (Mn), that record lifetime exposure histories for individual fish. We applied this approach to quantify the proportion of Atlantic Croaker (Micropogonias undulatus) exposed to hypoxia in the northern Gulf of Mexico, which experiences widespread summertime hypoxia every year. We found the proportion of fish with sublethal hypoxia exposure during their first year of life was fairly consistent among sampled geographic regions (33-34%), indicating that exposure was common enough to have important consequences for reproductive sustainability. We observed only moderate effects of hypoxia exposure on growth and condition factor for Atlantic Croaker, suggesting some life history parameters in this species are relatively tolerant to hypoxia. Finally, we coupled tissue stable isotope measurements with otolith chemistry to assess whether hypoxia displaces demersal foragers to pelagic food webs, and found evidence for individually variable food web displacement. The effects of hypoxia are thus complex, and otolith chemistry offers a novel way to unravel the dynamics of this growing environmental stressor.

Keywords: Hypoxia, Manganese, Food web, Isotopes
Age and growth of *Scyliorhinus canicula* (L., 1758) in the Strait of Sicily from vertebrae readings

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A reference collection of 173 vertebrae (90 males and 83 females) was used for the estimation of age and growth of Lesser Spotted Dogfish (*Scyliorhinus canicula* L., 1758) by growth rings analysis of vertebrae. Vertebrae were prepared by the removal of adhering material with a solution of bleach, and were aged with the coloration of red Alizarin and read as a whole using a reflected light stereomicroscope at 10 magnification. A total of vertebrae of 77 specimens, ranging from 18.0 to 51.5 cm total length, were successfully read. Readings were taken twice and independently by three trained readers. Average Percent Age Error index (APE), coefficient of variation (CV), and percent agreement were computed to assess the precision. On the basis of counting of “annuli”, the estimated age ranged from 0 to 5 years with an overall high percent agreement and precision among the three readers. A preliminary set of von Bertalanffy growth parameters (sex combined) were estimated as: $L_\infty = 59,9798$, $K = 0,2716$, $t_0 = -1,1386$. This information represents a main prerequisite for assessment and management of Lesser Spotted Dogfish, a by-catch species regularly caught by trawlers targeted to Deep water rose shrimp in the Strait of Sicily.

**Keywords:** *Scyliorhinus canicula*, Vertebrae, Age, Growth, Mediterranean Sea
Otolith growth and chemical chronologies in dusky grouper: The effect of ENSO

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Dusky grouper Epinephelus marginatus is the only large Epinephelidae species commonly found along subtropical latitudes and is listed as ‘Endangered’ (IUCN Red List) due to a combination of overfishing and complex life history strategy (slow growth rate, late maturation, aggregative spawning behavior and sequential hermaphroditism). Here we aim to develop growth and otolith chemical chronologies for this species collected in southern Brazil in the coastal region adjacent to the Patos Lagoon. We use increasingly complex mixed models to partition intrinsic and extrinsic factors that may influence growth and otolith element concentrations through time. Extrinsic factors include SST, Salinity, chlorophyll a, multivariate ENSO index (MEI), prey availability. We explore the importance of different drivers to individual growth and otolith chemical composition over 30 years and extract climate- or environment-driven variations. Furthermore, we evaluate how growth may influence otolith elemental composition in this species. Ultimately, we aim to understand how local and regional environmental variability influence population productivity in this area.

Keywords: Biochronology, Otolith chemistry, Individual growth, South Atlantic
Validating annual age of roach (*Rutilus rutilus*) from harsh environments using daily increments

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Reliable information of the age of fish is at the center of fish stock assessments and advice for fisheries management, as well as of fish ecology. However, in temperate climates, strong seasonality can influence fish growth such that accurate determination of annual age becomes difficult. In Lake Jutsajaure in the north of Sweden the Common roach, *Rutilus rutilus*, hatch as late as the end of June. With a short, bright growth season, the roach have little time to grow to a size that allows them to survive the first winter. When applying traditional ageing methods to determine annual age, both the scales and the otoliths of such roach have a potential small annulus close to the core. The question is if it is a true annulus, or a false mark that occur during rapid growth? Using daily increments to validate the first annulus we examine this question, and also perform growth analysis to determine the size of the roach during their first winter. By using laboratory hatched and reared roach with known daily ages we could successfully validate the daily increment analysis and implement it on wild caught fish.

**Keywords:** Daily increments, *Rutilus rutilus*, Age validation
The temperature-growth relationship in two co-existing populations of juvenile Atlantic cod (*Gadus morhua*)

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In the southern Norwegian fjords, juveniles from the coastal Atlantic cod (*Gadus morhua*) population are mixed with cod from the offshore North Sea population. The offshore juveniles are on average larger than the coastal juveniles, - a pattern caused by earlier hatch and faster growth of the offshore population despite their common habitat. We hypothesize that different temperature-growth relationships between the two populations cause this difference in growth rates. To test this, we analysed sagittal otoliths of 26 juvenile cod from both populations sampled at Lillesand and Sandefjord on the Skagerrak coast of Norway. Daily growth rates were back-calculated from increments widths using the biological intercept method. A mean growth rate at a given date was calculated for each population and location and related to temperature using a polynomial of 4th degree. Daily temperatures at 5 m depth on the two locations were obtained from a hydrodynamical ocean model. Ages ranged between 144 and 224 days. Average growth rates varied between 0.46 to 0.86 mm day⁻¹. Peak growth rates were obtained between 11.8 and 13.2 °C and were 1.07 mm day⁻¹ in the offshore population at both locations, while it was 0.80 mm day⁻¹ in Lillesand and 0.98 mm day⁻¹ at Sandefjord for the coastal population. At temperatures above 7.5 °C offshore grew faster than coastal cod at Lillesand, whereas the two populations had similar growth rates until ca 11 °C at Sandefjord. The decrease in growth rates above the optimal temperature was fastest for offshore cod leading to similar growth at the maximum temperature (17 °C). We conclude that the larger size of offshore cod is at least partly due to their superior growth at higher temperatures, but we also find that location specific factors, such as food availability, may influence the growth-temperature relation and the absolute growth rates.

**Keywords:** Daily increments, Temperature, Growth, Atlantic cod
Otolith opacity tracks annual cycles of the bioenergetic status of adult Atlantic cod (Gadus morhua)

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We still lack a thorough understanding of what triggers the annual shift between translucent and opaque otolith growth zones, despite the fact that analysis and interpretation of these zones is the most widespread use of otoliths and a cornerstone of fisheries research and management. We tested the hypothesis that opaque increments are formed when standardized energy content (kJ/g standard weight) of the fish was above a certain threshold using a unique dataset of Atlantic cod from Kapisillit in the Godthåbsfjord, Greenland (64°25N, 50°20W). A total of 439 cod in the size range 45-55 cm were sampled approximately monthly throughout 13 months. Energy density (kJ/g wet weight) of muscle, gonad and liver was measured using bomb-calorimetry or proximate analysis in the case of the liver and multiplied with tissue weight to yield energy content. The marginal increment of each sagittal otolith was assigned as opaque or translucent by two independent readers. The monthly mean somatic energy density increased from ca 4.1 kJ/g in January-May 2010 to 4.7 kJ/g in August, after which it again decreased to ca 4.1kJ/g in November- January 2011. Concurrently, the proportion of opaque otoliths increased from <0.15 during January-May to >0.6 in June-July after which it again declined to <0.15 in November. The increase in energy density and proportion of opaque otoliths coincided with intense feeding on spawning capelin in May and with increasing temperatures. The subsequent decrease in the fraction of opaque otoliths occurred three months before the decrease in temperatures, but followed the decrease in the standardized energy content of the cod. This suggest that the formation of the opaque zones is linked to the improvement of standardized energy content during June-August whereas translucent zones are formed when standardized energy content returns to low values.

Keywords: Otolith opacity, Bioenergetics, Atlantic cod, Greenland
The effect of ageing errors on Von Bertalanffy parameters estimation using a Bayesian sensitivity analysis approach

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The analysis of age and growth information contained in the scales of fish has become a standard technique in fish population dynamics model. Commonly modeled using Von Bertalanffy growth function (VBGF), inferring growth for fish is dependent upon an accurate description of the age-length relationship, which may be undermined by ageing errors. Ageing errors frequently arise either from disagreements between scale readers or inability of scales to reflect true age (i.e. erosion, false growth ring). Here we use a Bayesian approach to infer *Salmo trutta* (L.) VBGF’s parameters from scales sample with ageing errors. Inferred from scale readings of 60 fish, age at capture (for all fish) and age at migration (for the anadromous fish) were extracted based on a hierarchical design with blind double reading using 4 scales per fish. We estimated VBGF’s parameters by sequentially integrating an increasing proportion of fish for which age determination is uncertain. By doing so, we can empirically determine the robustness of VBGF’s parameters at a population scale when using mixed-effects models as an alternative to fixed-effects models. This will result in more efficient analyses of growth patterns for stock assessment, life history trait evolution, and environmental monitoring.

**Keywords:** Ageing-errors, Von Bertalanffy parameters estimation, Bayesian sensitivity analysis
Evaluation of the effects otolith sampling strategies and ageing error on the precisions of age composition and growth curve: a case study for the Pacific bluefin tuna

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Age and growth information from otolith plays a fundamental role in fish stock assessment. The objective of this study is to explore minimum otolith samples required for estimating the catch-at-age composition and population growth curve under the limited resources and the presence of aging error. Using the Pacific Bluefin tuna as an example, we evaluated the efficiency of four otolith sampling methods: the random otolith sampling (ROS), the fixed otolith sampling (FOS), the proportional otolith sampling (POS), and the reweighting otolith sampling (REW) methods by using computer simulations in combination with various scenarios of sample sizes and multiple reader types. When the age reading had no bias, the estimation errors of catch-at-age composition and growth parameters reduced as the sample size increased to 400, and the POS performed better than other methods. However, when the reader consisted of the aging bias, none of the methods including changes in sample size increase, multiple readings, and sampling approaches could reduce the errors. This result can be regarded as a guideline for the future otolith sampling design.

Keywords: Pacific Bluefin tuna, Otolith sampling methods, Ageing error
Exploring 2-d otolith multi-element microchemistry maps with cluster analysis software

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We have begun to explore statistical techniques to analyze patterns of multi-element microchemistry of fish otoliths. Two software platforms are being used to examine relationships between elemental concentrations at varying distances from the otolith core: DISCO2 and ArcGIS spatial statistics toolbox. DISCO2 is a free, web-based data analysis and visualization tool built on the R language that allows users to upload data to a central site (hosted by Colby College), execute data exploration and analysis tools, and execute a variety of visualizations, including histograms and scatter plots, and obtain summary statistics about their data. Analysis tools currently implemented include Principal Components Analysis [PCA] and K-means clustering, which can be applied to both the original data and data projected onto the principal components provided by PCA. As the tool is built on the R language, adding new analysis techniques already written in R is possible and, once integrated into the system, can be executed by all users on their own data. It was originally developed with support from Land Ocean Interactions in the Coastal Zone (LOICZ) which is now Future EarthCoasts. ArcGIS is widely available commercial software developed by ESRI, used for a variety of environmental applications using spatial data. Its spatial statistics toolbox includes procedures for grouping, cluster, and "hot spot" analysis. Our aim is to use these tools to explore spatial patterns in concentrations of the above elements in fish otoliths, and to interpret environmentally and metabolically-driven variations occurring over entire lifetimes. We demonstrate this on Atlantic cod (Gadus morhua) from the Baltic and North seas. Two-dimensional maps of the spatial distribution of the elements Sr, Ca, Br, Mn across transverse sections of otoliths were generated using synchrotron-based X-ray fluorescence. These tools can be used on 2-D data sets generated by any means, including LA-ICP-MS, micro-PIXE, etc.

Keywords: Otolith microchemistry, Cluster analysis, Multi-element analysis, Spatial statistics