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## Contents

Executive summary ................................................................................................................ 2

1背景 .................................................................................................................................. 3

2 实现目标 .......................................................................................................................... 4

2.1 目标1。审查从硬质部位（鱼体、壳类和鳞片）生长线宽度开发的年轮的应用

2.2 目标2。审查年轮开发的基本原理

2.3 目标3。学习用于统计检查生长线年代学精度的软件

2.4 目标4。讨论年轮开发的假设和限制，以及适用于这些方法的物种和收藏的特性

2.5 目标5。在图像分析软件中测量鱼体生长线宽度

2.6 目标6。生成生长线年轮并与其气候指数相关

2.7 目标7。发起合作项目或训练演习

附录1。参与者名单 ...................................................................................................... 8

附录2。WKGIC2条款和条件 .......................................................................................... 10

附录3。议程 ...................................................................................................................... 13
WKGIC2 group picture, IMEDEA, Esporles, Spain, 21 April 2016
Executive summary

Over the past several decades, thousands of otoliths, bivalve shells, and scales have been collected for the purposes of age determination and remain archived in European and North American fisheries laboratories. Advances in digital imaging and computer software combined with techniques developed by tree-ring scientists provide a means by which to extract additional levels of information in these calcified structures and generate annually resolved (one value per year), multidecadal time-series of population-level growth anomalies. Chemical and isotopic properties may also be extracted to provide additional information regarding the environmental conditions these organisms experienced. Given that they are exactly placed in time, chronologies can be directly compared to instrumental climate records, chronologies from other regions or species, or time-series of other biological phenomena. In this way, chronologies may be used to reconstruct historical ranges of environmental variability, identify climatic drivers of growth, establish linkages within and among species, and generate ecosystem-level indicators.

Following the first workshop in Hamburg, Germany, in December 2014, the second workshop on Growth-increment Chronologies in Marine Fish: climate-ecosystem interactions in the North Atlantic (WKGIC2) met at the Mediterranean Institute for Advanced Studies headquarters in Esporles, Spain, on 18–22 April 2016, chaired by Bryan Black (USA) and Christoph Stransky (Germany). Thirty-six participants from fifteen different countries attended. Objectives were to i) review the applications of chronologies developed from growth-increment widths in the hard parts (otoliths, shells, scales) of marine fish and bivalve species ii) review the fundamentals of crossdating and chronology development, iii) discuss assumptions and limitations of these approaches, iv) measure otolith growth-increment widths in image analysis software, v) learn software to statistically check increment dating accuracy, vi) generate a growth-increment chronology and relate it to climate indices, and vii) initiate cooperative projects or training exercises to commence after the workshop.

The workshop began with an overview of tree-ring techniques of chronology development, including a hands-on exercise in crossdating. Next, we discussed the applications of fish and bivalve biochronologies and the range of issues that could be addressed. We then reviewed key assumptions and limitations, especially those associated with short-lived species for which there are numerous and extensive otolith archives in European fisheries labs. Next, participants were provided with images of European plaice otoliths from the North Sea and taught to measure increment widths in image analysis software. Upon completion of measurements, techniques of chronology development were discussed and contrasted to those that have been applied for long-lived species. Plaice growth time-series were then related to environmental variability using the KNMI Climate Explorer. Finally, potential future collaborations and funding opportunities were discussed, and there was a clear desire to meet again to compare various statistical techniques for chronology development using a range existing fish, bivalve, and tree growth-increment datasets. Overall, we hope to increase the use of these techniques, and over the long term, develop networks of biochronologies for integrative analyses of ecosystem functioning and relationships to long-term climate variability and fishing pressure.
1 Background

Over the past decade, a growing network of chronologies in the North Pacific has been developed from annual growth-increment widths in marine fish and bivalves using tree-ring techniques. These chronologies have been integrated across species, marine regions, and other biological time-series to reconstruct growth and environmental variability and identify climate drivers of productivity and functioning at the ecosystem level. For example, chronologies of rockfish (*Sebastes* spp.) and salmon (*Oncorhynchus* spp.) have been combined with indices of seabird reproductive success to demonstrate that winter upwelling is critical to ecosystem functioning in the California Current. This winter upwelling pattern is driven by broad-scale atmospheric pressure systems that facilitate or block onshore flows of precipitation. Due to their drought sensitivity, tree-ring chronologies can be used to hind-cast this biologically important winter pattern over the past six centuries, documenting that variance in the system has risen to unusually high levels over the past 100 years that has been driven by a series of winters with anomalously low upwelling. Moreover, these California Current chronologies have been compared to those developed in the Gulf of Alaska, showing that the two ocean domains co-vary out of phase. Robust growth in the north is associated with poor growth in the south and *vice versa*, a pattern largely driven by winter *El Niño* Southern Oscillation activity. Beyond the Pacific, such approaches have resulted in fish chronologies off New Zealand and along the Australia west coast.

A number of exactly dated chronologies have also been developed for the extremely long-lived bivalve species *Arctica islandica* and *Glycymeris glycymeris* in the North Sea and North Atlantic for the purposes of reconstructing ocean circulation and climate. However, the “tree-ring” approach for chronology development has not yet been applied to fish or to address ecological or management issues in the North Atlantic and adjacent seas. The first workshop on Growth-increment Chronologies in Marine Fish: climate-ecosystem interactions in the North Atlantic (WKGIC) met at the Johann Heinrich von Thünen Institute in Hamburg, Germany, on 2–3 December 2014, chaired by Bryan Black (USA) and Christoph Stransky (Germany). During this meeting, we reviewed several pilot studies that have generated strong preliminary chronologies for Atlantic cod, plaice, and the greater Argentine. However, the greatest impediment to expanding this work remains a lack of knowledge as to suitable species and collections available for chronology development in the Atlantic, North Sea, Mediterranean, and Baltic region. To this end, we proposed WKGIC2 as a training workshop in which participants could learn these techniques and foster new collaborations by developing an otolith biochronology. During WKGIC2, emphasis was placed on short-lived species for which there are numerous and extensive otolith archives in European fisheries labs.
2 Fulfilment of Objectives

2.1 Objective 1. Review the applications of chronologies developed from growth-increment widths in the hard parts (otoliths, shells, and scales) of marine fish and bivalve species

Bryan Black provided a talk on the possible applications of chronologies developed from annual growth increments in the hard parts of long-lived fish, mollusc, and coral species. Given that they are exactly placed in time and extend over multiple decades, chronologies can be used to quantify impacts of climate on growth and hind-cast climate prior to the start of instrumental records. Moreover, chronologies can be integrated with observational records of phenology, growth, community composition, and reproduction to yield ecosystem-level perspectives on climate impacts and generate ecosystem indicators. For example, a Pacific rockfish and salmon chronologies have been combined with records of seabird reproductive success and tree-ring chronologies to document the importance of winter climate in the California Current and adjacent terrestrial systems. On broader spatial scales, chronologies from the California Current inversely co-vary with chronologies from the southern Gulf of Alaska, highlighting an inverse productivity regime between these two systems and the potential to compare across ocean domains. Finally, crossdating can be used to improve accuracy of age estimates, better resolving episodic recruitment events than simple ring counts in the long-lived Pacific geoduck (*Panopea generosa*). A network of growth-increment chronologies in the northeast Pacific (20+) is rapidly expanding and represents a tool to identify key climatic variables and their effects within and among species, trophic levels, and ecosystems.

2.2 Objective 2. Review the fundamentals of chronology development.

Bryan Black provided a talk on basic chronology development technique with particular emphasis on the dendrochronology technique of crossdating, which is employed to ensure that all increments are assigned the correct calendar year of formation. Crossdating is based on the assumption that some aspect of the environment influences growth, and that as the environment fluctuates over time, it induces a synchronous growth pattern among individuals of a given site and location. For living samples, crossdating is the process of cross-matching this synchronous ‘bar code’ among all individuals, working backward through time from the partial increment formed at the known year of collection. If an increment has been missed or falsely identified, the growth pattern in that individual will be offset by a year, beginning where the error occurred. The error is then confirmed by re-examining the wood for a micro or false ring. Samples with unknown death dates may also be crossdated among each other or with live-collected samples, assuming there is sufficient temporal overlap. In this way, highly accurate recruitment histories and growth-increment chronologies may be generated. Crossdating has proven effective on a wide range of long-lived freshwater and marine bivalve and fish species.

Following this introduction, the group completed a crossdating exercise using images of freshwater drum (*Aplodinotus grunniens*) otolith thin sections from samples collected in 2010 and 2009. Experienced crossdaters were paired with inexperienced participants and each group was charged with describing synchronous growth patterns among fish by listing those calendar years in which conspicuously narrow or wide increments occurred. In this way, participants were able to see the synchronous growth ‘bar code’ shared among fish, and that all groups arrived at the same dates and conclusions.
2.3 Objective 3. Learn software to statistically check increment dating accuracy

Bryan Black discussed approaches for statistically verifying crossdating accuracy with particular emphasis on the program COFECHA. In COFECHA, otolith measurement time-series are fit with a flexible cubic spline to track low-frequency variability, including age-related growth declines. Observed measurements are divided by those predicted by the spline, removing these low-frequency patterns and isolating only high-frequency (year-to-year) variability. These high-frequency patterns are then cross-correlated among samples. Should the correlation between any individual and the average of all other samples be unusually low (less than $p = 0.01$), then that individual should be visually re-inspected for possible measuring or dating errors. Edits are made only if an error is clearly identified in the visual re-inspection process.

Participants were provided with measurement time-series from the drum used in the visual crossdating exercise as well as measurement time-series from Pacific geoduck. All participants were then instructed on how to format data for entry into COFECHA, how to execute COFECHA, and how to interpret the output.

2.4 Objective 4. Discuss the assumptions and limitations of chronology development as well as the characteristics of species and collections for which these approaches would be ideally suited

Bryan Black led a discussion on the underlying assumptions and limitations of tree-ring approaches for developing biochronologies and ensuring that all increments are exactly placed in time. The first of these assumptions is that growth increments must be clearly visible and easily delineated. If growth-increment boundaries are unclear, then increment widths cannot be seen for crossdating or measuring. There must also be interannual (year-to-year) variability of increment width for crossdating to be possible. If increment width is too regular from year to year, then there is no pattern to use for crossdating. The second assumption is that individuals are sufficiently long-lived to crossdate (greater than 20 years). This is necessary to have the confidence that pattern matches among individuals are not spurious. Moreover, it is unlikely to find distinctive growth signatures in very short intervals of time, greatly limiting the use of crossdating.

Compared with tree-ring chronologies, there are additional considerations for otolith chronologies. The first of these is that fish are mobile, and caution must therefore be used when attempting to apply crossdating to migratory species. There are also issues of differences between sexes and diversion of energy from growth to reproduction, especially for females. Finally, juvenile and adult life stages must be considered as there may be changing ecology and climate-growth relationships in the first few years of life. There are no rules for identifying ideal species for crossdating. Testing whether crossdating applies to a collection of otoliths is the ultimate test for identifying whether synchronous growth patterns occur, and the spatial extents across which they are coherent. Finally, there is the assumption that increments are indeed annual. If a collection crossdates and the resulting chronology relates to annually resolved climate records in ways that make sense ecologically, then there is strong evidence of annual periodicity. Ideally, however, the annual periodicity should be confirmed independently with for example radiometric dating, bomb-carbon dating, mark recapture, or marginal increment analysis. Note that crossdating has not been attempted on subannual increments (e.g. daily rings), but this may be a possibility.
Objective 5. Measure otolith growth-increment widths in image analysis software.

Ultimately, the greatest potential for centennial-length chronologies may be for short-lived species with archival collections, especially in the North Atlantic and Mediterranean. For this reason, European plaice (*Pleuronectes platessa*) otoliths from the North Sea were used as a case study. Christoph Stransky provided images of more than 700 otoliths from the archives of the Johann Heinrich von Thünen Institute in Hamburg, Germany. Collections began in the mid-1980s and continued through the 2010s, providing an archive of otoliths spanning more than 30 years. Christoph Stransky also provided the group with an overview of plaice biology and details of the collection effort, which was focused in the south-eastern North Sea. In addition, the time-series on stock biomass, recruitment, weight-at-age and length-at-age available through ICES (assessment working group reports, DATRAS survey database) were presented.

Participants were divided into groups of three to five and each group was assigned a block of approximately 50 otoliths to measure. Image Pro Premier v. 9.1.4 or Image J/Object J were used for to measure growth-increment widths from the core of the otolith to the edge. Temporary licenses for Image Pro Premier v. 9.1.4 were provided by the manufacturer, Media Cybernetics, Silver Spring MD, USA. More than 1400 increment widths were measured from the otoliths provided.

Objective 6. Generate a growth-increment chronology and relate it to climate indices

Once growth-increment measurements were completed, data were compiled and assessed for synchronous growth patterns from calendar year to calendar year. An initial assessment of the data indicated that there was very little, however, the reason may be that the environment-growth relationships change as the fish age. Steve Campana introduced the group to the theory behind non-linear mixed effects models, which could be an effective tool for assessing effects of sex, age, and differences in growth from calendar year to calendar year. In a series of analyses, it became apparent that environment-growth relationships do change as fish age. Most notably, increments formed when the fish were > 4 year in age showed a distinct decline in width over time. Spawning-stock biomass has risen dramatically in recent decades, and this decline in growth-increment width could be in response to increasing competition.

Bryan Black led the group in an exercise using the KNMI Climate Explorer as a tool to investigate relationships between growth-increment chronologies and climate. The plaice chronology was investigated, as was a chronology for splitnose rockfish from the California Current. Participants learned how to correlate chronologies against global fields of gridded sea surface temperatures, sea level pressure, and other climate data.

Objective 7. Initiate cooperative projects or training exercises to commence after the workshop.

The group agreed that the next major direction for research is to compare the various statistical techniques currently used to generate chronologies from growth-increment data. Ideally, a workshop or working group would be formed that would compare outcomes of these different statistical approaches using a variety of existing datasets, including fish bivalves, and trees. Strengths and weaknesses could be assessed, as
could the ideal circumstances under which each should be applied. The European Union COST program was identified as a possible source of funding for such a project. There may be other funding sources available with which to fund biochronology research, including the European Commission’s Horizon 2020 programme. The specific goals of the research would be guided at least in part by the requirements in the call for proposals.
## Annex 1. List of participants

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Annex 2. WKGIC2 terms of reference

WKGIC2 - Workshop on Growth-Increment Chronologies in Marine Fish: climate-ecosystem interactions in the North Atlantic

2015/2/SSGIEOM12

A Workshop on Growth-increment Chronologies in Marine Fish: climate-ecosystem interactions in the North Atlantic (WKGIC2) chaired by Bryan Black, USA, Christoph Stransky, Germany and Beatriz Morales-Nin, Spain, will meet in Palma de Mallorca, Spain in 18–22 April 2016.

This will be a hands-on training exercise in which participants will work as a group to develop an otolith growth-increment chronology, including all phases of data collection, analysis, and interpretation. The chronology will be developed from one of the North Atlantic collections identified during WKGIC in December 2014. The 2016 workshop will involve learning:

a) Fundamental dendrochronology (tree-ring analysis) technique, with particular emphasis on visual cross dating followed by statistical verification using programs such as COFECHA.

b) How to prepare and photograph otolith samples, then measure growth-increment widths using image analysis software (i.e. Image Pro Premier).

c) Statistical techniques for generating biochronologies from growth-increment width measurements. Topics will include the removal of age effects, issues of minimum sample size, and maximizing signal-to-noise ratio. Special consideration will be given to datasets for chronologies developed using archival collections of short-lived individuals.

d) Correlation and regression techniques for relating the biochronology to instrumental climate records, principally through the use of the KNMI Climate Explorer.

A new otolith chronology based on candidate species and collections and their relationships to climate will be established over the course of the workshop.

WKGIC2 will report by 1 June 2016 for the attention of WGBIOP, SCICOM, ACOM and SSGEPD.

Supporting Information

Priority: Over the past several decades, thousands of otoliths, bivalve shells, and scales have been collected for the purposes of age determination and remain archived in European and North American fisheries laboratories. Advances in digital imaging and computer software combined with techniques developed by tree-ring scientists provide a mean to extract additional levels of information in these calcified structures and generate annually resolved (one value per year), multidecadal time-series of population-level growth anomalies. Given that they are exactly placed in time, chronologies can be directly compared to instrumental climate records, chronologies from other regions or species, or time-series of other biological phenomena. In this way, chronologies may be used to reconstruct historical ranges of environmental variability, identify climatic drivers of growth, establish linkages within and among species, and generate ecosystem-level indicators.
The first workshop on Growth-increment Chronologies in Marine Fish: climate-ecosystem interactions in the North Atlantic (WKGIC) met in 2014. WKGIC identified that the greatest limitation to developing biochronologies in the North Atlantic is lack of training in the specialized crossdating and statistical approaches involved. WKGIC2 will be a longer training workshop in which participants will learn these techniques (i.e. Crossdating and detrending, including common dendrochronology programs ARSTAN and COFECHA,) by developing a biochronology using otoliths from the North Atlantic region.

Scientific justification and relation to action plan: A large and growing network of chronology datasets has been developed from annual growth-increment widths in marine fish and bivalves in the North Pacific. These chronologies have been integrated across species, marine regions, and other biological time-series to develop indicators and identify climate drivers of productivity and functioning at the ecosystem level. For example, chronologies of rockfish (Sebastes spp.) and salmon (Oncorhynchus spp.) have been integrated with indices of seabird reproductive success to demonstrate that winter upwelling is critical to ecosystem functioning in the California Current. This winter upwelling pattern is driven by broad-scale atmospheric pressure systems that facilitate or block onshore flows of precipitation. Due to their drought sensitivity, tree-ring chronologies can be used to hind-cast this biologically important winter pattern over the past six centuries, documenting that variance in the system has risen to unusually high levels over the past 100 years driven by a series of winters with anomalously low upwelling. Moreover, these California Current chronologies have been compared to those developed in the Gulf of Alaska, showing that the two ocean domains co-vary out of phase. Robust growth in the north is associated with poor growth in the south and vice versa, a pattern largely driven by winter El Niño Southern Oscillation activity. Such approaches have also been applied in fish chronologies off New Zealand and along the Australia west coast.

A number of exactly dated chronologies have also been developed for the extremely long-lived bivalve species Arctica islandica and Glycymeris glycymeris in the North Sea and North Atlantic for the purposes of reconstructing ocean circulation and climate. However, the “tree-ring” approach for chronology development has not yet been applied to fish or to address ecological or management issues. The first workshop on Growth-increment Chronologies in Marine Fish: climate-ecosystem interactions in the North Atlantic (WKGIC) met at the Johann Heinrich von Thünen Institute in Hamburg, Germany, from 2–3 December 2014, chaired by Bryan Black (USA) and Christoph Stransky (Germany). During this meeting, we identified several pilot studies have generated strong preliminary chronologies for Atlantic cod, plaice, and the greater Argentine. However, the greatest impediment to expanding this work remains a lack of knowledge as to suitable species and collections available for chronology development in the Atlantic, North Sea, and Baltic region. To this end, we propose a training workshop (WKGIC2) in which participants will learn these techniques and foster new collaborations by developing an otolith biochronology.

Resource requirements: All necessary samples, images, and meeting space will be provided by the chairs and other members.
<table>
<thead>
<tr>
<th>Participants:</th>
<th>We anticipate 10-20 participants from leading age labs and universities.</th>
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<td>Secretariat facilities:</td>
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<td>Financial:</td>
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Annex 3. Agenda

**Monday 18 April**
- 13:00  Introductions: participant backgrounds, organizations
- 13:15  Welcome to Esporles
- 13:20  Purpose of the meeting and objectives (TORs)
- 13:40  Introduction to crossdating
- 14:45  Break
- 15:00  Crossdating exercise
- 16:30  Applications and assumptions
- 17:00  Adjourn

**Tuesday 19 April**
- 9:00   Wrap-up of crossdating exercise
- 9:30   Introduction to Image Pro Premier
- 10:45  Break
- 11:00  Statistical verification of crossdating
- 12:30  Lunch
- 14:00  Hands-on use of COFECHA
- 14:45  Approaches to chronology development
- 16:00  Break
- 16:20  Review and update of available sample sets
- 17:00  Adjourn

**Wednesday 20 April**
- 9:00   Introduction to the case study: North Sea plaice
- 9:30   Measure samples
- 10:45 – 11:00 Break
- 12:30  Lunch
- 14:00  Continue measurements
- 15:00  Append measurements into final datasheet
- 15:30  Break
- 15:45  Chronology analysis and quality control
- 17:00  Adjourn
Thursday 21 April
9:00   KNMI Climate Explorer
10:30  Future directions: collaboration, funding, meetings
13:30  Group outing and banquet

Friday 22 April
9:00   Other case studies from the group members
12:00  Adjourn