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Report of the Workshop on Age Validation Studies of Gadoids (WKAVSG)

6 - 10 May 2013

IMEDEA, Mallorca

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

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1 Participant list

1.1 Active Participants

Karin Hüssy	Denmark	kh@aqua.dtu.dk
Beatriz Morales-Nin	Spain	beatriz@imedea.uib-csic.es
Carmen Gloria Piñeiro Álvarez	Spain	Carmen.pineiro@vi.ieo.es
Javier Rey	Spain	javier.rey@ma.ieo.es
Sally Songer	United Kingdom	sally.warne@cefas.co.uk
Joanne Smith	United Kingdom	joanne.smith@cefas.co.uk
Kelig Mahe	France	Kelig.Mahe@ifremer.fr
Hélène de Pontual	France	Helene.De.Pontual@ifremer.fr
Uwe Krumme	Germany	uwe.krumme@vti.bund.de
Didzis Ustups	Latvia	Didzis.Ustups@bior.gov.lv
Yvonne Walther	Sweden	yvonne.walther@slu.se
Ian McCausland	Ireland	ian.mccausland@afbini.gov.uk
Stefania Vittori	Italy	svittori@unica.it
Christian Irgens	Norway	christian.irgens@imr.no

1.2 Passive participants

Eugenia Lefkaditou	Greece	teuthis@ath.hcmr.gr
Krzysztof Radtke	Poland	radtke@mir.gdynia.pl
Francesca Vitale	Sweden	francesca.vitale@slu.se
Fred Serchuk	USA	fred.serchuk@noaa.gov
Pietro Rizzo	Italy	pietro.rizzo@iamc.cnr.it
João Pereira	Portugal	jpereira@ipma.pt
Jane Godiksen	Norway	jane.godiksen@imr.no

2 TOR's

a) Review information on age estimations, otolith exchanges, workshops, and validation works done so far on the following species: European hake, cod, pollock, saithe, haddock, whiting and blue whiting;

b) Assemble and compare the results of different validation methods (i.e. marking and recapture, marking the calcified structure, marginal increment analysis, marginal analysis, modal progression analysis, length back-calculation, etc.);

c) Discuss and propose the most appropriate validation methods of age and growth pattern of calcified structures (CS), for each species and stock;

d) Propose the appropriate validation methods to recognise the growth check as well as the spawning ring, demersal ring, migration ring, etc.;

e) Propose an ICES Cooperative Research Report on: Age Validation Studies for ICES and GCFM Gadoid Stocks, to ICES PGCCDBS, using previous studies and the outcome this workshop;

f) Based on results, conclusions and recommendations from this workshop to initiate and design an international cooperation project on validation methods (such as on the validation of checks and spawning rings) to commence after the workshop

3 Introduction

This workshop was initiated by PGCCDBS 2012. PGCCDBS was approached by the ICES Publications and Communications Group (PUBCOM) with a request for a Co-operative Research Report (CCR) aiming at providing guidelines for age validation studies. The objectives of the CCR are:

1) to compile existing information on ageing protocols, workshops and otolith exchanges and the recommendations issuing from these initiatives

2) to review and compile existing knowledge of validation studies undertaken so far, compare methods and provide guidelines for good validation practice

The responsibility for organising and editing the CRR was placed in WKNARC. WKAVSG was thereafter initiated in order to compile available knowledge for gadoid species. The TOR's a)-e) largely reflect the different paragraphs intended for the CRR. Consequently, the text found in this workshop report is to a large extent identical to the text in the CRR.

The only TOR not represented in the CRR is Tor f) aiming at "initiating and designing an international cooperation project on validation methods". In the present report, a first draft of a call text is presented.

4 Review of Background Information

Addressing: TOR a) - Review information on age estimations, otolith exchanges, workshops, and validation works done so far on the following species: European hake, cod, pollock, saithe, haddock, whiting and blue whiting.

Status: Accomplished

In this paragraph a review of efforts carried out to reach a common agreement in interpretation of otolith growth zones, including an overview of exchange programs, workshops and summaries of problem identifications and recommendations is presented.

The precision of age estimates is improved by means of otolith exchange schemes and age reading workshops. For gadoids in ICES waters there are 5 reports available (Easy et al., 2005; ICES, 2008; ICES, 2010; Mahé, 2008; Worsøe Clausen *et al*., 2005). A summary of the results from these workshops can be found in Table 1.

Table 1. Summary of workshops and exchanges by species

Species	ICES Areas	Number	Ageing preparation	Number of readers	% agreement	CV	Workshops
SAITHE	VI	137	sectioning otolith	18	82.8	5.4	Exchange 2007-2008
SAITHE	IV	154	sectioning otolith	20	95.9	3.3	Exchange 2007-2008
WHITING	VIId, IVa, IVc and VI	120	sectioning otolith	17	80.7	10.3	Workshop 2009
HAKE	VIIIab, VIIIc, Ixa	104	sectioning otolith	16	46.3	41.2	Workshop 2009 *
COD	IV	118	sectioning otolith	21	74.0	39.8	Workshop 2008
BLUE WHITING	IVa	100	whole otolith	15	86.5	12.2	Workshop 2005

(*) WKAEH 2009: Ageing Method was invalid

5 Summary of general age estimation methods and problems

5.1 Saithe (Polliachius virens)

Difficulties of interpretation: Differences could be explained by the position of the first ring and identification of increments representing ages older than 8 years. However, in general this species is considered to be relatively easy to read.

Recommendations: It was recommended to compare the two methods of preparation (sectioning and breaking). It is still necessary to present a direct or indirect validation of the formation of the rings (1 ring per year).

5.2 Whiting (Merlangius merlangus)

Difficulties of interpretation: There was confusion in some fish over the first annual zone because of splits and the wide range of growth that can occur during the first year. Indecision over zone formation at the edge of the otolith could lead to differences of one year between reader ages. The wide difference in growth rates between fish caught in the same area also adds to the problem of interpreting the ring structure as does the limited parts of the otolith where the ring structure is suitable for ageing.

Recommendations: There was no significant difference in the results between the two ageing methods of broken otoliths or sections. Each method has its own advantages and disadvantages. The workshop concluded that both ageing method were acceptable for whiting. 'Humphries shadow' is a feature that is present on most otoliths although not in every year and as such has only limited use in the interpretation of the ring structure.

5.3 Hake (Merluccius merluccius)

Difficulties of interpretation: Otoliths are difficult to interpret due to the complexity of the macrostructure and growth variability that has been related among other reasons with the long spawning season. The internationally agreed ageing criteria are based on a concentric pattern of translucent and opaque rings/bands around the nucleus of otolith sections. The growth pattern presents several translucent rings per year that probably correspond to short environmental and/or physiological events, and the difficulty in interpreting such otoliths often increases with the size of the fish. The classification of the edge type tends to be complicated since translucent edges appear year-round indicating a high incidence of checks (> 60%), particularly in summer (Piñeiro and Saínza, 2003). Recently, blind interpretation of marked hake otoliths at the last workshop (WKAEH 2009), demonstrated with tagging material that the internationally agreed ageing criteria are neither accurate nor precise and provide overestimation of age. This raises concern about the use, for stock assessment, of ALKs that were inaccurate (ICES 2010). At this time, a replacement ageing method with sufficient precision and accuracy is not available. (De Pontual *et. al*, 2006; Piñeiro *et al.*, 2007).

Recommendations: To stop the age estimations for the WGHMM until new validated/accurate criteria is available. Considering the age reading results obtained in the last workshop (ICES 2010 b) and the recent advances on hake age validation (tagging and recapture experiments, daily growth) (de Pontual et al., 2006; Piñeiro et al., 2007; 2008), it was concluded that there are currently no reliable ageing criteria. These overall findings led to substantial changes in the assessment conducted by ICES (2010a) that is now carried out using a length-based models instead of the age-based model XSA previously used (WKROUND, 2010). A better understanding of the complex otolith growth pattern of this species might be achieved through a better knowledge of fish behaviour (migrations, feeding activity etc.) and differences in individual life histories. Approaches coupling validation methods (eg. otolith structures and DST tagging, otolith microchemistry, otolith modelling) should be promoted.

5.4 Cod(Gadus morhua)

Difficulties of interpretation: The interpretation of the first annuli can be confused with a first translucent band most likely deposited in the time the juvenile cod settles to the bottom. This confusion can be avoided by considering that the first annulus is wider than the first translucent band, approximately two and one millimetre in diameter, respectively. Another difficulty was the interpretation of age 1 cod captured during quarter one, which have a quite wide opaque edge growth. Some readers aged these fish as two years old since they assumed the translucent band was deposited after the new year, and the opaque edge represented a summer growth period. The agreed interpretation is that the translucent band is deposited in the period autumn (new year), and the opaque edge growth is deposited during the winter months in quarter one. A third difficulty of interpretation is the occurrence of split rings. Some of the translucent annulus can consist of several thinner translucent bands that can be misinterpreted as true annulus which leads to overestimation of fish age. These bands can be identified as being thinner than true annulus and with less distance between them.

Recommendations: The workshop concluded that sectioning the otolith is the preferred method to use (versus the broken method). The various life history traits for the North Sea cod may differ within the North Sea and knowledge of this is highly important for the age readers. In addition, all age readers would benefit from more information on the formation of otolith structures in North Sea cod, especially the formation of split rings. Thus, the group recommended the inclusion of studies on otolith formation in general and the North Sea cod physiology/growth/behaviour in relation to this as a part of the training and updating of all North Sea cod age readers.

5.5 Blue Whiting (Micromesistius poutassou)

Difficulties of interpretation: The difficulty of interpretation was the position of the first ring where the Bower zone is clear. This is often seen in the younger individuals as the otolith is thinner and thus the structures more clear. The second difficulty of interpretation arose where some readers choose to leave out specific rings identified as splits while other readers identified the same rings as true annuli. This was more problematic after the second year of growth.

Recommendations: Inclusion of studies on otolith formation in general and blue whiting physiology/growth/behaviour in relation to this.

5.5.1 Ageing methods and problems of specific gadoid stocks

In the following the age reading problems of specific stocks where agreement on interpretation has not been achieved are addressed in detail.

5.5.2 Baltic Cod

Difficulties of interpretation: There are two Baltic cod stocks (SD 22-24, SD 25-32) that mix extensively. The interaction of various factors (e.g. different hydrographic conditions on the vertical and horizontal scale, successive onset of spawning from West to East) result in unclear growth ring formation. Otoliths of Baltic cod are at present aged on broken (Denmark, Sweden), broken and burned (Estonia, Latvia, Poland, Lithuania, Russia), or sectioned (Germany) otoliths. The key problems with age reading are: 1) identification of the first winter ring, 2) the timing of the winter ring formation, 3) interpretation of the edge. The interpretation of growth zones varies widely between countries, institutes and even between readers within institutes. To improve the precision of age estimation a reference collection of otoliths was compiled in 1995-1996. The purpose with the reference collection was to have a set of otoliths as reference material for calibration and training of new and established readers and to reach consensus of the interpretation of the characteristics of the otoliths. As the quality of the otoliths deteriorates with frequent handling, images of each otolith were digitized and the image collection distributed among all countries. Details of the ageing methods, problem descriptions and the reference collection can be found in the "Manual for Baltic cod age reading" (http://www.ices.dk/community/Documents/PGCCDBS/SGBCAR_2000.pdf). Despite 30 years of efforts to standardize preparation techniques, interpretation of growth zones with numerous workshops and otolith exchanges, precision in age estimates is still very low. As a result, the age distributions of catches alarmingly vary by country (WGBFAS report: http://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2012/WGBFAS/WGBFAS%20Report%202012.pdf; specifically section 2.4, Figure 2.4.1a-d) and may negatively influence the quality of the assessment (Reeves, 2003).

Recommendations: Due to the poor readability of the otoliths, there is still no consensus on the interpretation of growth zones of these two cod stocks. Consequently neither the ageing manual nor the reference collection has been updated in recent years. A Baltic Sea-wide tag-recapture study is urgently required to generate material for validation of fish age and assessing movement and exchanges processes within and between stocks.

5.6 European Hake

Difficulties of interpretation: Hake otoliths are difficult to interpret due to the complexity of their macrostructure pattern. In the North East Atlantic, Northern and Southern ICES hake stock assessments have been based on age structure from 1992 to 2010. To that effect, age data has been demanded routinely from different research institutes and many efforts have been carried out to improve the precision of otolith age estimations, through successive age reading calibration exercises, such as exchanges and workshops. (For more details see ICES Co-operative Research Report No. 294, table 2.1.1). During the 1980's, where different preparation techniques were used, scientists undertook several exchanges and workshops to agree on standardised preparation techniques and ageing methods. The main outcome of this decade was the adoption of a common preparation technique (transversal sections of otolith) and the identification of the main sources of discrepancies among readers i.e. the location of the first annulus, difficulty in discerning differences between annulus and other checks and the interpretation of otolith edge type. During the 1990's several workshops and calibration exercises were conducted and resulted in a common ageing criteria suitable for use on fish up to age 5, according to the accepted slow growth model at that time. These criteria were internationally adopted and applied by all readers from institutions involved in hake stock assessments. However, ageing hake still presented problems for older ages which was a limiting factor for assessments. In 2004, an ICES otolith workshop (was carried out focusing on older fish, in an attempt to deal with these problems. The results indicated that the precision of age readings dropped from 0-5 to 0-3 years old. This was a consequence of the difficulty of hake otolith reading using non-validated age determination criteria, especially after the presentation of the first tagging results which indicated that the ageing criteria in use at that time were not accurate. (de Pontual *et al.*, 2003, 2006). As a consequence of these results another workshop was organised in 2009 using a reference collection of 104 OTC marked otoliths. Eight research institutes (AZTI, IPIMAR, CEFAS, IM, IFREMER, IEO, AFBI NI, and VTI-DF) participated to evaluate age estimation errors (accuracy and precision).

Recommendations: The main results (ICES, 2010a) demonstrated that the ageing method was not only imprecise, but also inaccurate and lead to an overestimation of age (by a factor of two). The age estimation of European hake remains complex and further work is needed for both age related assessment and ecological studies.

6 Review of Validation Studies

Addressing: TOR b) - Assemble and compare the results of different validation methods (i.e. marking and recapture, marking the calcified structure, marginal increment analysis, marginal analysis, modal progression analysis, length back-calculation, etc.)

Status: Accomplished

According to Beamish *et al*. (1983) age validation is a process of establishing the accuracy of an age estimation method. Validation of an ageing procedure indicates that the method is sound and based on facts (Kalish, 1995). The term validation has two meanings; in a narrower sense the term is used to determine the temporal meaning of the growth increment used in ageing; in a wider sense, the term is used to prove that the whole age determination procedure is accurate.

The definition of accuracy is a matter of degree, which measures how close an estimated age is likely to be to the true age (Francis, 1995). This wider sense certainly should be preferred, because each age reader will achieve a different level of accuracy and especially because an individual age reader will reach different levels of accuracy for each age group (older specimen are more difficult to age). Therefore, for each age group the accuracy should be measured from age readings of individual age readers by estimating how close the estimated ages are to the true ages.

Three levels of validation are possible (Francis, 1995):

1) The first increments are annual, but there are insufficient data to determine the periodicity of the latter increments and to make a quantitative estimate of the accuracy

2) All the increments are effectively annual but there are insufficient data to make a quantitative estimate of the accuracy

3) The increments are effectively annual and quantitative estimate of the accuracy is provided.

A comprehensive review of validation methods, including advantages and limits was presented by Campana (2001), while the status of age validation in Europe was reviewed by Appelberg *et al.* (2005). In gadoids, only a limited number of these methods have been applied. A summary thereof is given in Table 2, which is a modification of Campana's table with a column showing what gadoid species have been studied and which technique was used.

Table 2: Summary of age validation methodolgies, modified from Campane (2001) with methods used for gadoids

Method	Annual/DGI	Age	Advantages	Limits	Gadoids in which this validation techniques has been employed
Released marked fish	A+D	All	Validate absolute age and periodicity	Source of known age fish, recaptures old fish null	N/A
Mark recapture chemically tagged fish	A+D	All	Validate periodicity post release	low recaptures, some markers may affect survival	Hake
Captive rearing from batch	A+D		Validate absolute age and periodicity	Differences with wild fish	N/A
Microstructure	A	1 yr	Validation of 1st year	Daily periodicity assumed	Hake, cod
MLA	A+D	0-5 yr	Validation of ages 1-2	No overlapping length modes, No length based migrations	N/A
Marginal increment analysis	A	All	Validate periodicity	Not so straightforward in slow growing/older individuals. Need adequate sample size by month.	Cod, Saithe, Whiting, Haddock, Hake
Radiochemical dating	A	Plus 5yr old	Validate absolute age old fishes	Can only distinguish between divergent estimates	N/A
Bomb radiocarbon	A	All	Validate absolute age and periodicity	Very old fish needed	N/A

In the following, the validation studies carried out on gadoids referred to in Table 2 will be described in detail. The methods are reported according to whether they are indirect and direct validation methods.

6.1 Indirect validation methods

6.1.1 Length-based analyses

The length-based methods (LFA) analyse the length composition to identify the different age groups present, based on the assumption that each age group has a normally distributed length distribution and a differential modal length. Petersen (1891) was the first to identify that the modes of the length composition correspond to age groups. The age class 0 correspond to the smallest mode present in the sample obtained after the spawning period, with subsequent modes corresponding to age 1 and so on. The modal lengths corresponding to age classes can be identified through different methods and then compared to individual length at age observed from the calcified structures (Morales-Nin & Panfili 2002). This method has been applied to European hake (Aldebert & Morales-Nin, 1997).

Among the gadoid species discussed in WKAVSG (Table 3), length frequencies analysis has been used successfully on hake in the western Mediterranean Sea, obtaining an indirect validation of the first 3 age classes and to confirm the first winter ring of Baltic Sea whiting (Ross & Hüssy, 2012).

In the case of European hake (*Merluccius merluccius* Linnaeus, 1758) in the Mediterranean sea the formation of the translucent zone corresponds generally to winter months (Colloca *et al.*, 2003) and the frequency distribution of the ring distances from the nucleus show two principal peaks for each annual ring (Figure 1), in agreement with the presence of two spawning periods (spring-summer and fall-winter) (Arneri and Morales-Nin, 2000; Belcari et al., 2006). Consequently, in this case, at the time of the first hyaline ring formation, two spawning groups of individual should be recognizable, those who hatched in summer (age 0+ group) and those who were born the previous winter (age 1 group). This pattern would appear also for the following age groups.

Table 3. Summary of species where length frequency analysis has been applied

Species	Method	Area	Age/Size Range
WHITING	Mode progression + daily increments	Western Baltic	0-1 years
HAKE	LFA	Tyrrhenian Sea	0-3.5 years
Hake	LFA+otolith daily increments	Gulf of Lyons	7.5-30 cm

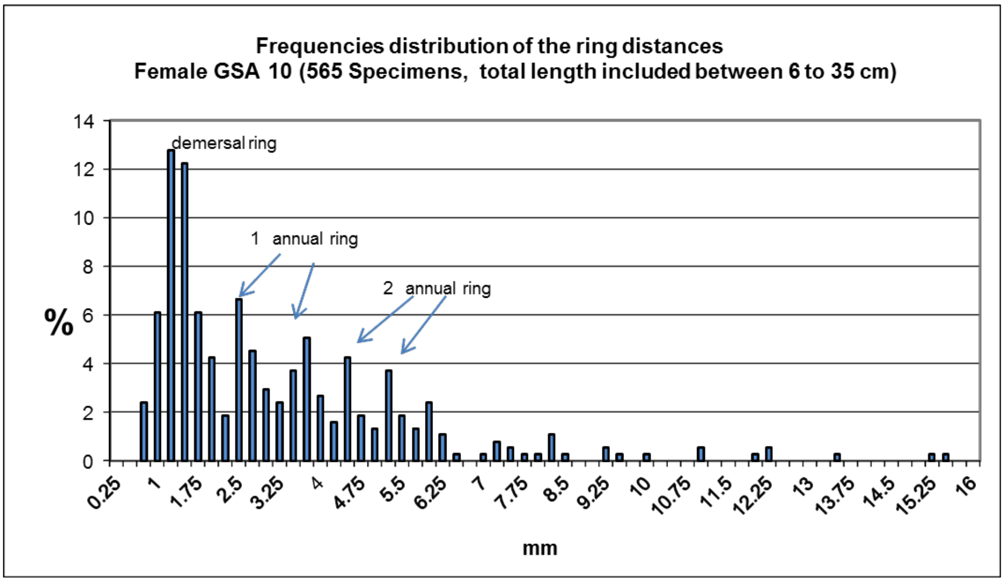

Figure 1. Frequency distribution of the distance between the nucleus and specific growth zones in European hake (Peaks representing different age classes indicated with arrows).

6.2 Conclusions on Technique

This technique verifies the growth rates associated with each age class by comparing them with another independent method of age estimation. This method is easily applicable because it is based on data which are routinely gathered in fishery studies (length frequencies). However, this procedure doesn't allow the validation of the periodicity in the deposition of hyaline rings and it is difficult to apply when an overlap between modes is present. For this reason, if used for a species with a relatively long spawning period like European hake, it provides reliable growth rate verification only for the first few age classes.

6.2.1 Marginal increment analysis

The evolution over time of the otolith marginal structure shows the period of increment formation. Marginal increment analyses (MIA) validates increment formation. If a growth increment is formed on a yearly cycle, the average state of completion of the outermost increment should display a yearly sinusoidal cycle when plotted against month (Figure 2).

This is the most commonly used validation method. It can determine the percentage of translucent and opaque increments in the edge with monthly periodicity and the marginal increment can be measured and some growth index can be estimated. As sexual dimorphism and multiplicity of ings avoiding the application of the method for Mediterranean hake has been reported (Morales-Nin et al. 1998) and differences depending on maturation could be found (Beckman & Wilson 1995), it is recommended to consider these factors when using this method. This method has been carried out on a number of Gadoid species (Table 4)

Table 4. Summary of species where marginal increment analysis was applied

Species	Method	Area	Time Series	Age/Size Range
COD	Sectioned	VIIf-j & VIIIa – b	Jan 2011 – Jan 2012	2-9 years
SAITHE	Sectioned	VIa	Jan 2011 – Jan 2012	2-14 years
WHITING	Sectioned	IV – VIId	Jan 2011 – Jan 2012	2- 14 years
WHITING	Sectioned	VIIf – h	Jan 2011 – Jan 2012	2-8 years
HADDOCK	Sectioned	VIIf-j & VIIIa – b	Jan 2011 – Jan 2012	2-8 years
HAKE	Whole	Tyrrhenian Sea	Mar 1997 – Feb 1998	2-16 years
HAKE	Transversal burnt section	Gulf of Lions	Jan 1989 - Dec 1990	6-94 cm TL

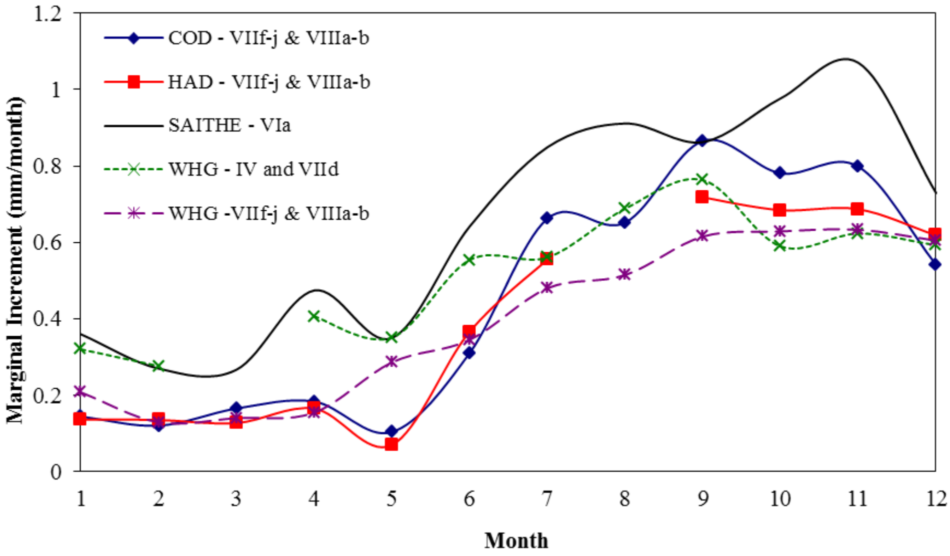

Figure 2. Marginal increment of Cod, Haddock, Saithe & Whiting in the North Sea (source: French data from 2010 & 2011)

6.3 Conclusions on Technique

Marginal increment analysis (MIA) should not be seen as a true validation technique. It is rather a corroboration that an annual growth structure is present. MIA is relatively cheap in terms of the time taken and the requirement to collect extra sample material. The measuring of the increments can however be a time consuming and repetitive task as many samples need to be processed to obtain a valid result. As samples are needed from every month throughout the time frame being investigated, insufficient sample size for some months may be problematic.

Despite being quite a straight forward technique to employ there are some limitations with MIA, mainly based around the difficulty in measuring small increments accurately. This problem is exacerbated in slow growing and older individual particularly at the edge of the otolith. Difficulties in interpreting opaque/translucent zones can arise, and lighting setup of the microscope may be a factor in this.

In the gadoid species discussed at WKAVSG, and listed in Table 4 above, the use of MIA proved a successful method to corroborate annual increment formation using sectioned otoliths in fish across large age ranges.

6.3.1 Edge zone analysis

In cases where there is low contrast between growth zones making Marginal Increment Analysis difficult, an edge zone analysis may be used to achieve similar, but less accurate results (Ross & Hüssy, 2013). This method is also based on the subjective interpretation of whether the edge zone under formation is opaque or translucent.

6.3.2 Daily increment analysis

Daily increment analysis is based on the close relationship between the widths of daily growth increments with the environmental temperature experienced by the fish (Mosegaard and Titus, 1987). The width of daily increments decreases with decreasing temperature and patterns of successively decreasing/increasing increment widths can therefore be used to identify macroscopic growth zones, providing that these occur during specific seasons (and thereby link with environmental temperature) (Hüssy et al., 2010). This technique is particularly useful for the verification of the first winter ring (Rehberg-Haas et al., 2012), as well as for corroborating subsequent structures (Hüssy et al., 2010, Hüssy, 2010).

Table 5. Summary of species where daily increment analysis has been applied

Species	Method	Area	Time Series	Age Range
COD	Sectioned	Baltic Sea, SD 22-24 Baltic Sea, SD 25	2009 2001	0-1 years < 3 years
WHITING	Ground in sagittal plane	Baltic Sea, SD 22-24	2009 – 2011	0-1 years
HAKE	Ground in sagittal plane	Ionian Sea	?	0 group
HAKE	Ground in frontal plane	Adriatic Sea	1992-1997	0-1 years
HAKE	Ground in frontal plane	Tyrrhenian Sea	2001	0-1 years
HAKE	Ground in sagittal plane	Gulf of Lions, Balearic Sea	1997, 2004	0-1 years

***Validation of the first winter ring*:** This analysis is based on the enumeration of daily increments from hatch to capture, where individuals are sampled repeatedly from the same cohort before, during and after the formation of the first winter ring. This approach was successfully used in European hake from the Ionian Sea (Pattaura et al., 2011), Adriatic Sea (Arneri and Morales-Nin, 2000), Tyrrhenian Sea (Belcari*et al.*, 2006), and the WMediterranean (Gulf of Lions and Balearic Islands (Morales-Nin & Aldebert, 1997; Morales-Nin & Moranta, 2004; Hidalgo et al., 2009)) and Western Baltic cod (Rehberg-Haas et al., 2012). Similarly, the enumeration of daily increments from hatch to capture can be used to identify the timing of growth zones, by linking the occurrence of translucent checks to the time of occurrence. In Western Baltic cod, this approach showed that fish hatched early in the year have a juvenile check which is visually separated from the winter ring, whereas the two structures are merged in fish hatched later in the year (Figure 3).

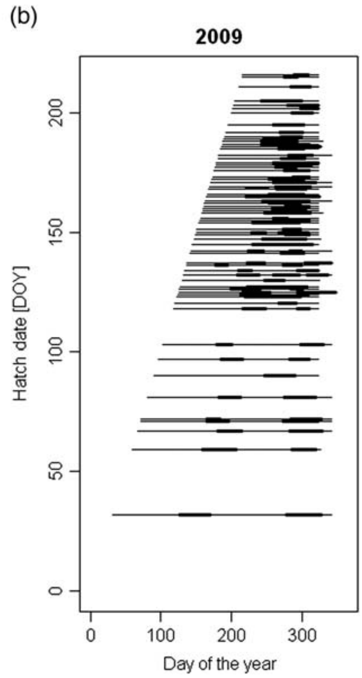

Figure 3. The temporal occurrence of translucent checks (bold lines) from hatch to death in juvenile 0-group cod hatched at different times of the year (from Rehberg-Haas et al., 2012)

Subsequent winter rings**:** Cyclical patterns with daily increments forming a bell-shaped pattern separated by periods without visible increments were linked with seasonal temperature cycles based on cod tagged with data storage tags and strontium chloride (Hüssy et al., 2009; Hüssy et al., 2010). Comparison of daily increment patterns revealed inconsistencies between winter zones identified by the lack of visible increments with formation of translucent zones (Figure 4) (Hüssy, 2010).

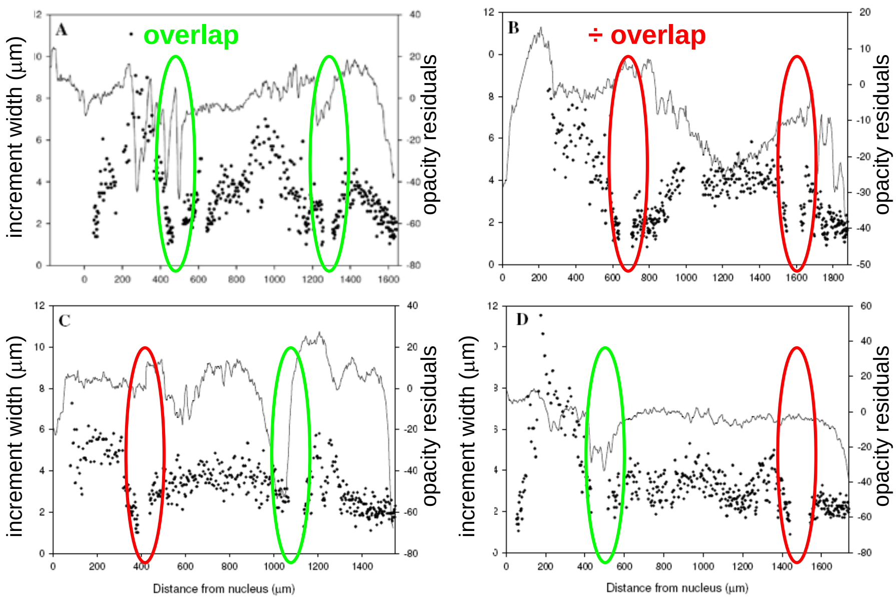

Figure 4. Four examples of 3-year old cod with different daily increment (dots) and opacity (lines) patterns over two consecutive years: (a) overlap in both years, (b) no overlap, (c) overlap in the second but not the first year, (d) overlap in the first but not the second year (modified from Hüssy, 2010).

6.4 Conclusions on Technique

Daily increment analysis cannot be considered a true validation technique, as it is based on the assumption that the growth increments used to validate a macroscopic structure are formed on a daily basis. Validation of the daily increments is necessary for this approach to be valid. Daily increment analysis is, however, a useful tool to help understand the mechanisms behind the observed structures in the otolith macrostructure and to corroborate that an annual growth structure is present. As the preparation of samples (grinding, polishing, image digitisation and analysis) is very time-consuming, this method is not suitable for routine validation.

6.4.1 Microchemistry

Three major assumptions underpin the application of otolith microchemistry to age validation:

1) Otolith composition reflects seasonal temperature variations
2) Temperature variations correspond to visible features
3) Variations in composition validate the seasonal frequency of visible features

***Elemental composition*:** The concentrations of the main micro-constituents of the otoliths (Ca, Sr, Na, K, Fe, Mn and Mg) can be measured using a Wavelength Dispersive Spectrometer (WDS or electron microprobe) to validate the seasonality of visible opaque and translucent (hyaline) bands used for age estimation. Elemental ratios in the otolith calcium carbonate, especially the Sr/Ca ratio, have been hypothesised to vary in response to seasonal changes in water temperature.

In a recent study in cod and hake (DGXIV Study Project 96-075) it was shown that the opaque and translucent zones were generally different in composition during the early stages of development although the variation declined toward the edge of the otolith. Sr/Ca ratios were generally higher in translucent zones than in opaque zones. Na/Ca ratios were inversely related to Sr/Ca ratios. The decreasing variations in Sr/Ca ratios between translucent and opaque bands towards the otolith edge could be a result of either the decreasing width of the bands or an ontogenetic effect. Because there was such a close coupling of the visual pattern of otolith zone formation and the chemical composition, it seemed unlikely that simple cyclic seasonal temperature fluctuations were responsible for all of the variation in the Sr/Ca signal. Therefore, it was not possible to use the Sr/Ca variations to validate which zones corresponded to annual otolith increments in many of the hake otoliths. There is increasing evidence in the literature that the Sr/Ca ratio in fish otoliths responds only indirectly to ambient temperatures. The elemental ratio may be more of a reflection of physiological processes, and these may simultaneously induce visual changes in the otolith. Thus, the Sr/Ca ratio is not independent of otolith growth and cannot be used as an independent validation tool.

***Isotopes*:** Isotopes have been used to validate the age estimates of fishes. They have mostly been used for long-lived species where the intention has been to 'validate' either the younger ages estimated from whole otoliths or older ages estimated from sectioned otoliths.

$^{210}Pb/^{226}Ra$ disequilibria in otoliths has been the most useful technique because it is suitable for ages of up to about 100 years. The $^{210}Pb/^{226}Ra$ disequilibria method has been the most widely used technique and there has been considerable debate over the validity of the results. An important assumption is that there are no losses or gains of

^{226}Ra or ^{210}Pb after uptake, other than by radioactive decay or in growth. One of the decay products is ^{222}Rn and it has been argued by Gauldie (1998) that this can leak from the otoliths and hence invalidate the results. Other authors argue that otoliths are similar to aragonitic corals where no losses of ^{222} Rn have been reported. Some recent publications relevant to the debate are Gauldie (1998), Gauldie and Cremer (1998), Gauldie et al. (1998) and Tracey and Horn (1999).

In a similar way to the Sr/Ca ratios, the stable oxygen isotope ($\delta\ ^{18}O$) signals show a seasonal variation coincident with the otolith's visible growth increments, generally supporting increment-count age estimates (Weidman & Millner 2000).

6.5 Conclusions on Technique

Microchemistry analysis is a method to corroborate the seasonal frequency of otolith macrostructure features through seasonally varying incorporation rates of different micro-constituents and stable isotopes. The elemental composition of otoliths seems to reflect physiological processes which simultaneously induce changes in the otolith macrostructure. This method is therefore not useful for independent age validation, but provides insight into the mechanisms behind observed macrostructure features.

7 Direct validation methods

7.1 Tag-recapture

A pilot tag recapture experiment was carried out for hake in the Bay of Biscay in 2002. A specific protocol was developed (including a modified gear designed to optimise fish survival). Recoveries showed much higher growth than previously expected (de Pontual *et al*., 2003)

Analysis of marked and recaptured fish and their otoliths showed that the previous underestimation of growth (by a factor ~2) could be clearly related to an overestimation of age (de Pontual *et al*., 2006) (Figure 5).

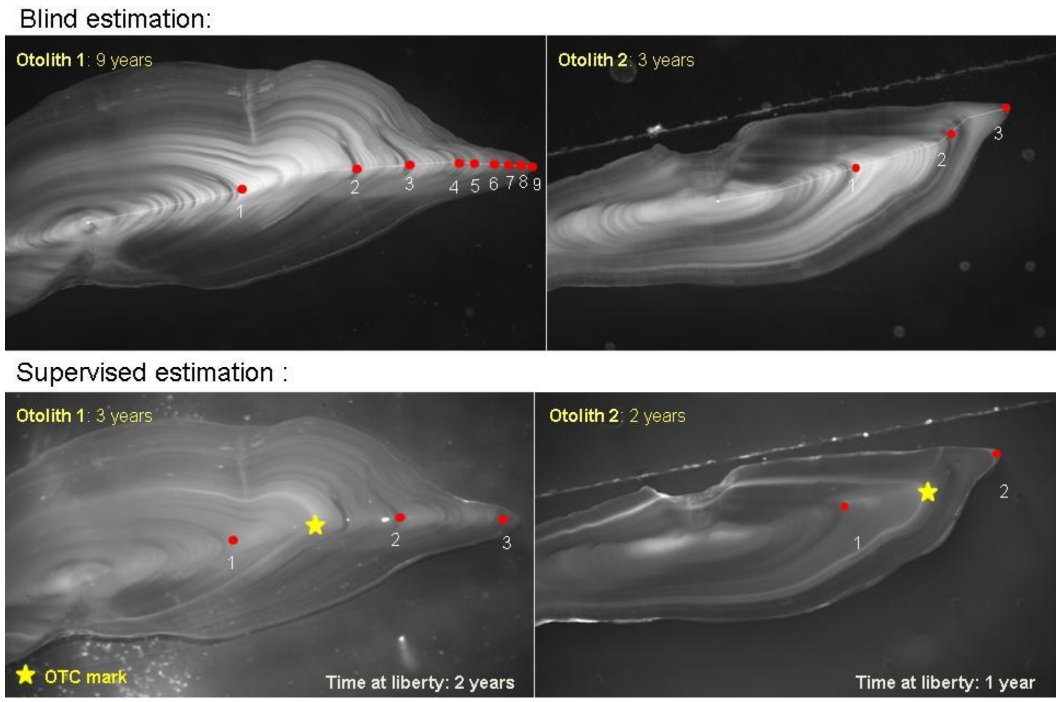

Figure 5. Comparison between blind and supervised interpretation of chemically marked (oxytetracycline) European hake otolith.

The approach was extended to the Iberian Peninsula (Pineiro et al, 2007 and Mediterranean Sea (Mellon et al., 2010) and sustained tagging effort was maintained in the Bay of Biscay from 2004-2007. In this region, 27690 fish were tagged, chemically marked and released. 1199 fish have been recovered to date leading to a new growth model being established as well as new insights on migrations and mortality. An overview of the tagging programs carried out to date is given in Table 6.

104 marked otoliths issued from this tagging program and were analysed in an international exchange and workshop (ICES 2010a). Supervised otolith interpretation (taking into account oxytetracycline (OTC) mark position and fish data) showed that the internationally agreed age estimation method was neither accurate (bias of a factor ~2) nor precise. Even the otolith supervised interpretation remained difficult (73.9 % agreement between hake readers with, moreover, substantial differences in ring positions). It was strongly recommended to stop producing annual ALKs to be used by the ICES assessment of European hake.

These overall findings led to substantial changes in the assessment conducted by ICES (2010a) that is now carried out using a length-based model (e.g. Stock synthesis 3, SS3) instead of the age-based model (XSA) previously used.

Table 6. Summary of tagging programs carried out on European hake

Tagging experiment			Recapture Results				
Location	Nº individuals released	TL range at release (cm)	Max Time at liberty (days)	TL range at recapture (cm)	N of recovered tag fish (nº)	% Recapture	Author
SW Ireland	78	28.9	255	40.6	1	1.3	Belloc, 1935
South Bay of Biscay	152	56	24	60	1	1.9	Lucio *et al.*, 2000
Bay of Biscay	1307	21-40	1066	24-67	36	3.1	de Pontual *et al.*, 2006
Bay of Biscay	27690	9-84	1555	19.2-78.9	1199	4.33	de Pontual *et al.*, in press
Mediterranean Gulf of Lions	4277	15-40	717	16-57	280	6.5	Mellon *et* al., 2010
NW Iberian Peninsula	527	29-36	466	31-56	6	1.3	Piñeiro *et al.*, 2007
NW Iberian Peninsula	2725	28-46	466	31-56	27	1	Piñeiro *et al. unpublished*
Balearic Island	675	10-44	-	-	-	-	Massuti *et al. unpubl.*

7.2 Conclusions on Technique

A "tag-recapture" program ended the controversy over the growth rate of European hake. Results invalidated the otolith-based age estimation method that was being used and it was concluded that there are currently no reliable ageing criteria. A better understanding of the complex otolith growth pattern of this species might be achieved through a better knowledge of fish behaviour (migrations, feeding activity etc.) and differences in individual life histories. Approaches coupling validation methods (eg. otolith structures and DST tagging, otolith microchemistry, otolith modelling see paragraph on "Future perspectives") should be promoted.

7.2.1 Rearing in captivity

Rearing European hake in captivity from wild eggs has been done several times. In an experimental setup in Norway, European hake were reared from eggs up to 245 days of age in temperature and salinity controlled stable conditions. The lapillus and sagitta of one of these fish were examined for microstructural features. The age derived from increment counts support the daily nature of the hake sagittal increments starting formation on day 8, probably related to the start of exogenous feeding. The lapillus showed a later increment formation.

7.2.2 Conclusions on Technique

Compared to all other techniques, rearing of fish in captivity from hatch under laboratory or mesocosm conditions provides a tool for the validation of a fish's true age. The drawbacks of this technique are that it is expensive and may not mirror conditions in the wild, resulting in otolith macrostructure features that do not correspond to those observed in wild fish.

8 Recommendations on use of Validation Methods

Addressing: TOR c) - Discuss and propose the most appropriate validation methods of age and growth pattern of calcified structures (CS), for each species and stock

Status: Accomplished

8.1 Specific conclusions for each method

- *Length based methods*: Estimates the growth rates and age of individual cohort from length frequency data; this has to be compared with other independent methods of age estimation. Does not allow the validation of the periodicity in the deposition of growth zones. Inexpensive and uses data routinely obtained.
- *Marginal Increment Analysis*: A successful method to corroborate annual increment formation across large age ranges, but hampered by the difficulty in measuring small increments accurately and the need for high contrast between growth zones. Similar alternative: Edge zone analysis. Inexpensive.
- *Daily increments*: A useful tool to 1) identify the first winter ring and 2) to help understand the mechanisms behind observed otolith macrostructure and to corroborate that an annual growth structure is present. Moderately expensive.
- *Microchemistry*: A tool to link otolith macrostructure features (though not necessarily seasonal structures) with environmental conditions through physiological processes affecting otolith accretion. As such not useful for age validation, but rather the understanding of otolith features. Expensive.
- *Tag-recapture*: The most direct validation method in use. A highly successful method that validates age directly and should be used if common agreement on age interpretation is not achieved. Very expensive. Risk of not getting back the released individuals.
- *Rearing in captivity*. The only method to validate the fish's true age. Expensive and may not mirror conditions in the wild resulting in otolith macrostructure features that do not correspond to those observed in wild fish.

8.2 General conclusions

- Otolith age readings and the ensuing ALK's should not be used indiscriminately by ICES stock assessment groups when there are clear indications of inaccurate age estimations of the species assessed (ICES 2010 a,b,c).
- Precision in age readings may be improved by workshops and otolith exchanges. But precision cannot replace the lack of accuracy, and validation should be carried out for all species.

- Age readings and their use in stock assessments should not be forced if:
    - o tag-recapture data indicate that otolith age estimations are inaccurate and therefore invalid.
    - o there is evidence that contradicts or is not in line with ageing results based on the accepted ageing criteria, such as:
        - Difficulties in following cohorts in the commercial catch or survey at age matrices.
        - Age estimation criteria demonstrate a low level of precision among expert readers.
        - There are studies whose results indicate somatic growth rates in disagreement with those derived from otolith aging, such as: studies of food consumption, marginal increment, modal progression analysis, daily growth rates, otolith typology, otolith chemical composition, etc.
- Attention should be given to every piece of information, including information that contradicts the accepted ageing paradigm, because each one of them provides an insight in what we still do not know.

9 Recommendations for Validating other Structures

Addressing: TOR d) - Propose the appropriate validation methods to recognise the growth check as well as the spawning ring, demersal ring, migration ring, etc.

Status: Accomplished

9.1 Validation of life history events – juvenile check

In the central part of the otolith in some demersal species, there are several macro growth increments that confuse the attribution of the first seasonal increment. These increments laid down during the early life phases may be related to one or several factors like changes in habitat (i.e. from pelagic to demersal settlement); feeding patterns (changes in diet); or metamorphosis (i.e. termination of cutaneus respiration and ossification). Any of these changes can result in changes in the otolith formation leading to changes in the otolith shape and increment pattern. Changes in the optical density and spacing of daily growth increments may also accompany metamorphosis. The most frequent change in the otolith shape observed in gadoids is due to the formation of accessory growth centres (Figure 9). In a study of European hake settlement (Arneri and Morales-Nin 2000) the duration of the pelagic life phase was established to be around 2 months and this data was validated in other studies.

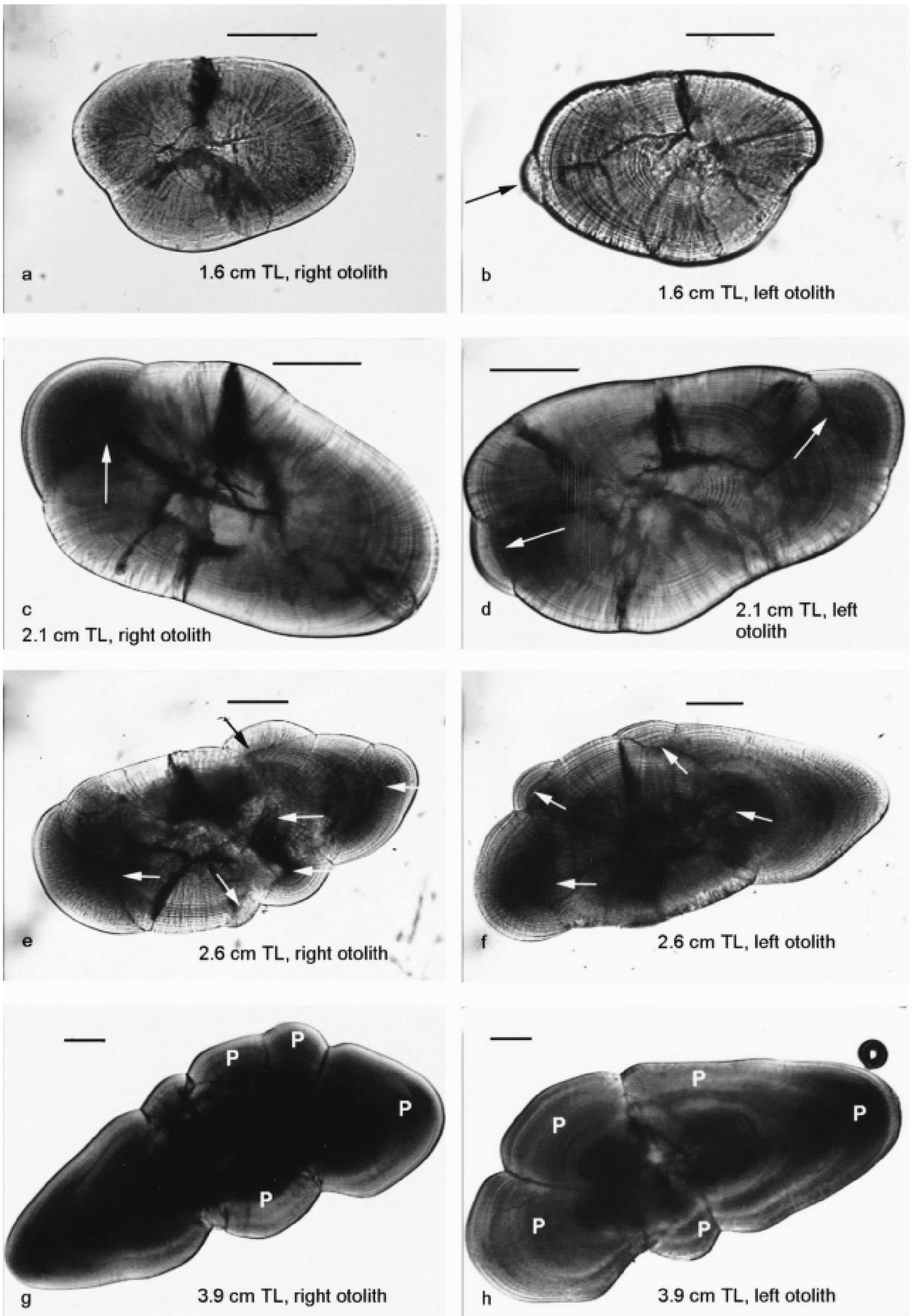

Figure 6. Juvenile hake otoliths showing the changes in shape and increment structure (Arneri & Morales-Nin, 2000).

9.2 Validation of life history events – spawning check

Background: Knowledge on age, size and growth at the onset of maturation are important stock assessment parameters (Godø and Haug, 1999). So-called spawning checks or "spawning zones" in otoliths may form an integral part of these otolith readings, especially in gadoids (Rollefsen, 1935). Spawning zones in Northeast Arctic cod were first described by Gunnar Rollefsen (1933, 1935), and has later been used to determine age at maturation and to construct maturity ogives for individual cohorts in the time period 1946-1981, although gonad staging is normally the main source of information in these type of analyses (ICES, 2001). At present, Institute of Marine Research in Norway (IMR) uses otolith spawning checks in saithe (North of 62 degrees) to determine proportion of mature vs. immature saithe in assessments (ICES, 2005). The background here is that this cruise takes place in the autumn, i.e. at a time in the year when gonad staging is little helpful in adequately separating maturing individuals from immature ones. In assessments of Northeast Arctic haddock, spawning checks are also currently being used but only to confirm maturation stages already determined by gonad inspection during the so-called 'winter survey'. .Moreover, otolith spawning checks may also be useful to studies on evolutionary changes in maturation-at-age related to commercial fishing – which may be in particular interest to institutions with large historical otolith archives.

Previous work: Despite many years with use of spawning checks in otoliths, direct validation of these assumedly spawning indicators has been brought to our attention only the recent years. Previous work to validate "spawning zones" in cod otoliths began with the NFR (The Research Council of Norway) project "Timing and determination of fecundity and skipped spawning" 2006-2009, followed by a second project to include haddock (NFR - The occurrence of skipped spawning and its importance for population dynamics in Northeast Arctic gadoids - 2009-2012). The first project included isotope and chemical comparative analyses to validate or corroborate visible "spawning zones", and to compare these in fish with and without gonad indicators of spawning (post-ovulatory follicles; POFs) (Witthames *et al.*, 2010, Skjæraasen *et al.*, 2012). The second fecundity project addressed among other aspects growth trajectories of fish before and after the "spawning zones" as a further method of validating these features.

The rationale for incorporating data from otolith composition analysis into the determination of fecundity project was to determine whether the physiological processes during gonad development would cause permanent, detectable features in the otoliths. There were two objectives:

1) to validate the so-called spawning checks by comparing these, in appropriate samples, with the presence of POFs, and
2) to use the isotope and element data to distinguish between individuals with regular or irregular, annual reproductive patterns (skip-spawners).

Future work: It still remains to fully understand when spawning checks are being recorded in gadoid otoliths (e.g. which months) relative to the actual time of spawning. It is also necessary to investigate whether or not spawning checks are recorded in the otoliths of individuals that skip spawning after one or several years with spawning. An experimental method of direct validation of spawning checks is now being conducted at Matre Research Station, Institute of Marine Research, Norway. Cod will be reared from hatch to age three, under two different feeding regimes (low and high), whilst individual growth, time of first maturation and spawning will be monitored. Half way through the experiment 50% of the fish from each feeding reime will be switched to the other feeding regime. During the experiment the otoliths will be regularly stained by alizarin. This will determine the timing of spawning checks relative to time of spawning. The experiment is expected to finish during 2014. Otolith growth and spawning checks will finally be analysed in transversal sections with the use of a fluorescence microscope to detect the Alizarin stain marks. A disadvantage with such an experimental approach is potential deviations in the otolith pattern from wild fish, although the husbandry protocols are designed to reflect the natural environment as far as possible.

Nevertheless, it should be expected that the appearance of spawning checks is species- and stock dependent, as spawning behavior, migrations and environment may vary between different gadoid fish stocks. Unfortunately for some gadoid fish stocks, spawning checks may be difficult, if not impossible, to detect by conventional age reading methods. Which species that is suitable for future validation studies, and the importance of such studies to the respective stock assessment, should therefore be discussed. Direct validation of age or at least high agreement in age reading among involved age reading institutions is a recommended prerequisite before undertaking further studies of spawning zone validation in any specific gadoid stock. Without known individual age at maturation, this type of spawning validation studies has less value in the aspect of stock management.

The use of post-ovulatory follicles (POFs) is another good candidate to validate spawning. POFs persist for about a year in gadoid ovaries post spawning, i.e. they can be used to justify the presences of an otolith spawning check produced within the last year. In an on-going study at IMR, otoliths and ovarian samples from Northeast Arctic saithe (*Pollachius virens*), captured along the Norwegian coast in October 2010, 2011 and 2012, are being analysed. Otolith transversal sections are being analysed for spawning checks, and gonad samples are being histologically processed (in resin) and stained with periodic acid-Schiff stain (PAS), before being analysed under a microscope for POF prevalence as well as ovarian morphology (method: see Witthames *et al*., 2010). So far the results show a good agreement between spawning zones and the presence of POFs among older individuals, which had spawned more than once. However, little correspondence was found between POFs present and spawning zones in the otoliths of younger individuals. As an example, in 2012 no spawning zones were found among individuals between 2 and 4 years old. Nevertheless, half of these ovarian samples evidently showed POFs, 40% had POFs defined as "almost certain" and only 10% had no POFs. Hence, our data suggest that Northeast Arctic saithe spawn at a younger age than expected from traditional otolith analyses.

10 ICES Cooperative Research Report

Addressing: TOR e) - Propose an ICES Cooperative Research Report on: Age Validation Studies for ICES and GCFM Gadoid Stocks, to ICES PGCCDBS, using previous studies and the outcome this workshop

Status: Accomplished

The previous chapters of this workshop report are to a great extent identical to the text found in the CRR chapter. For that reason, it is not repeated here. However, in order to get an overview over the CRR chapter for gadoids, the table of content is shown below.

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11 International Cooperation Project

Addressing: TOR f) - Based on results, conclusions and recommendations from this workshop to initiate and design an international cooperation project on validation methods (such as on the validation of checks and spawning rings) to commence after the workshop

Status: Accomplished

A first draft of an international cooperation project on validation methods was prepared following the PGCCDBS recommendations to promote a study under the EU "Call for Tender" program. This first draft was presented at WKNARC-2 held one week after WKAVSG. WKNARC-2 approved the ideas outlined and provided WKAVSG with comments for improving the call text. Below a draft of the call text is presented. The text follows the general layout of Call for Tender texts.

Important to note: This is not the final version, as the group wishes to clarify and improve the text further, before it is presented to PGCCDBS.

Proposed title: Improving accuracy in age estimation through understanding of the link between environmental conditions and physiological responses recorded in the otolith macrostructure.

Brief description of the study

The study aims at identifying the biological meaning of otoliths features such as annually recurring patterns, checks associated with spawning or other life stage events as well as periods of environmentally induced physiological stress. The timing of these features and the causal relationship between otolith feature and the fish's environment and behaviour can be validated by combining different validation techniques (micro and macrostructure analysis, microchemistry). Identification of the underlying processes affecting otolith macrostrucure should be based on species and stocks with an easily interpretable otolith structure. Results from these analyses will provide the necessary input data to calibrate a generic simulation tools that can link bioenergetic processes and environmental conditions with otolith visual appearance. The applicability of such an approach should subsequently be tested on stocks of the same species with highly complex otolith patterns and known otolith growth rates. This study will provide an evaluation of the applicability of this approach and should therefore focus on a limited number of species from different geographical locations/stocks where samples from tag-recapture programs are available.

The objective

With the objective of improving the accuracy of age data used in stock assessments, this study aims to validate different features within the calcified structure by combining well established validation techniques.

Background

Age estimates based on the interpretation of otolith macrostructure features has been used extensively in stock assessment for many years. For some stocks good precision in age estimation has been achieved, whilst in other stocks where otoliths are more difficult to interpret precision is lower. Even within the same species the otolith's visual appearance - and thus readability - may vary, presumably as a consequence of a combination of stock-specific environmental conditions and physiological responses. Validation of the biological significance of the structures used for age estimation is essential for improving both precision and accuracy of these estimates and, consequently, improving stock assessment. There are some well-established techniques available that can provide information on the timing of the formation of specific otolith features (micro structure analysis) and their relationship to physical and chemical properties of the environment experienced by the fish (micro-chemistry). Application of these methods simultaneously on known-age otoliths from tag-recapture programs will provide the key to understanding the biological meaning of otolith features.

Terms of reference

- References to ageing workshops, PGCCDBS, PGMED, WKNARC and WKAVSG
- Reference to projects TACADAR, EFAN, CODYSSEY, DECODE, AFISA, MARMER and French hake tagging
- Providing input to relevant ICES stock assessment working groups.

The main product would be to:

- Validation of features within otoliths.
- Accurate age data
- Greater understanding of different life histories of stocks within the same species.

The main tasks to be undertaken by the contractor are the following:

1) Compile available material for re analysis from existing otolith archives.
2) Perform comparative micro increment and micro chemical analysis on selected otoliths.
3) Re-evaluate age estimates in light of findings.
4) Present the recommendations to end users, to establish expertise and international cooperation for further work on other species.

Timetable and Final Report

The duration of the study shall not exceed 24 months from the signature of the contract. An interim report of the study should be made available after 12 months of the signature of the contract and a final report should be made available within one month of the termination of the project.

Budget

The maximum budget allocated for this study is € 1,500,000 covering all expenses, including personnel, preparation and analysis of samples, meetings, consumables

12 Recommendations for Future WKAVSG

The group considers that all TOR's have been fulfilled within the WKAVSG meeting 2013. All available information has been compiled and discussed. The conclusions are specified in a comprehensive chapter for age validation studies for the ICES CRR.

For that reason the group thinks that a continuation of WKAVSG beyond its current terms is not required at present. However the group recommends for another meeting to be held in approximately 3 years, depending on the progress made with respect to validation initiatives.

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