2 Gadoids

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2.1 Introduction

This chapter was written during the Workshop on Age Validation of Gadoids (WKAVGS 2013) which was held 6–10 May 2013 at Imedea in Esporles, Mallorca. The terms of reference for the workshop were to:

1. 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;
2. 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.);
3. Discuss and propose the most appropriate validation methods of age and growth pattern of calcified structures (CS), for each species and stock;
4. Propose the appropriate validation methods to recognize the growth check as well as the spawning ring, demersal ring, migration ring, etc.;
5. 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 of this workshop;
6. 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.

2.2 Age estimation methodologies in gadoids

A review of efforts carried out to reach common agreement on the interpretation of otolith growth zones, including an overview of exchange programmes, workshops, and summaries of problem identifications and recommendations, is presented.

The precision of age estimates by different national institutes is improved by means of otolith exchange schemes and age estimation workshops. Several reports on gadoids in ICES waters are available (Easey *et al*., 2005; Worsøe Clausen *et al*., 2005; ICES, 2008a, 2009a, 2013a, 2015a; Mahé, 2009; Piñeiro and Sainza, 2011; Mahé *et al*., 2014). A summary of the results from these workshops can be found in Table 2.1.

Table 2.1. Workshops and exchanges by species. Further information in "WK Ex SG History Master Table by Species 2018" in WGBIOP's Data quality assurance repository (ICES, 2018a, 2018b).

Species	ICES area	*n*	Preparation of the age estimation process	No. of readers	Agreement (%)	*CV*	Workshop/ Exchange
Saithe	Division 4.a Division 6.a	154 137	Sectioned otolith	20 18	95.9 82.8	3.3 5.4	Exchange 2007–2008 (Mahé, 2009)
	Division 2.a Subarea 4 Division 4.a	24 34 237	Sectioned otolith	13	85.9	6.2	Exchange 2013 (Mahé *et al.*, 2014)
	Division 2.a Division 6.a	50 10	Sectioned otolith	10	79.2 reflected light 82.3 transmitted light	3.7 4.6	WKARPV 2015 (ICES, 2015)
Whiting	Various areas around the British Isles	200	Broken otolith Sectioned otolith	11 19	72.6 80.9	16.3 13.7	Exchange 2004 (Easey *et al.*, 2005)
	Divisions 7.d, 4.a, 4.c, and Subarea 6	120	Sectioned otolith	17	80.7	10.3	Workshop 2009 (report not available)
	Division 3.a and subareas 4 and 7	134	Sectioned otolith	16	70	14	WKARWHG2 2016 (ICES, 2017a)
Hake	Divisions 8.a–b, 8.c, and 9.a	104	Sectioned otolith	16	46.3	41.2	WKAEH 2009* (ICES, 2009a)
	Divisions 8.c and 9.a	237	Sectioned otolith	12	62.3	33.1	Exchange 2011 (Piñeiro and Saínza, 2011)
Cod	Divisions 3.b–d		Sectioned otolith				Several workshops and exchanges (summarized by Hüssy *et al.*, 2016)
	Subarea 4	118	Sectioned otolith	21	74.0	39.8	WKARNSC 2008 (ICES, 2008a)
	Divisions 4.a and 4.b	120	Broken/ sectioned otolith	17	66	14.7	Exchange 2010 (ICES, 2011a)

Species	ICES area	*n*	Preparation of the age estimation process	No. of readers	Agreement (%)	*CV*	Workshop/ Exchange
Haddock	Subareas 4 and 6	NA	Sectioned otolith	12	84.2	18	Exchange 2009 (ICES, 2010a)
			Broken otolith	12	85	7.5	
Blue whiting	Division 4.a	100	Whole otolith	15	86.5	12.2	Workshop 2005 (Worsøe Clausen *et al.*, 2005)
	Divisions 4.a, 4.b, 2.a, and 5.a	189	Whole otolith	21	46.4	17.1	Exchange 2010–2011 (Mehl *et al.*, 2012)
	Subdivision 5.b.1	158	Whole otolith	19	57	13.4	WKARBLUE 2013 (ICES, 2013a)
	Mediterranean/ ICES divisions 9.a, 8.c, 7.j, 7.c, 7.b, 6.a, 4.a, 2.b, and 14.b / NAFO 1C	245	Whole otolith	29	68.7	44.2	WKARBLUE2 2017 (ICES, 2017b)

* The age estimation method was invalid.

2.3 General age estimation methods and problems

In this section, the general age estimation methods for gadoids are described by species. A series of images exemplifying the relevant otolith structures to analyse when estimating the age of a particular gadoid species are shown in Annex 2.

2.3.1 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 eight years. However, this species is generally 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 (one ring per year).

2.3.2 Whiting (*Merlangius merlangus*)

Difficulties of interpretation: For some fish there was confusion 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 fact that the ring structure is only suitable for age estimation on limited parts of the otolith.

Recommendations: There was no significant difference in the results between the two age estimation methods of broken otoliths or sections. Each method has its own advantages and disadvantages. The workshop concluded that both age estimation methods 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.

2.3.3 European hake (*Merluccius merluccius*)

In the Northeast Atlantic, northern and southern ICES hake stock assessments have been based on age structure from 1992 to 2010. To that effect, age data have been demanded routinely from different research institutes, with many attempts at improving the precision of otolith age estimations through successive age estimation calibration exercises, such as exchanges and workshops (for more details, see Table 2.1.1 in Piñeiro *et al.*, 2009). During the 1980s, when different preparation techniques were used, scientists undertook several exchanges and workshops to agree on standardized preparation techniques and age estimation 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 1990s, several workshops and calibration exercises resulted in common age estimation criteria suitable for 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, age estimation of hake still presented problems for older ages, which was a limiting factor for assessments. In 2004, an ICES otolith workshop focused on older fish in an attempt to deal with these problems. The results indicated that the precision of age estimations dropped from 0–5 to 0–3 years old. This was a consequence of the difficulty in using non-validated age estimation criteria in hake otolith reading, especially after the presentation of the first tagging results indicated that the age estimation criteria in use at that time were not accurate (de Pontual *et al.*, 2003, 2006). As a consequence of these results, another workshop was organized in 2009 using a reference collection of 104 OTC-marked otoliths. Eight research institutes (AZTI [Spain], IPIMAR [Portugal], Cefas [UK], MI [Ireland], Ifremer [France], IEO [Spain], AFBI NI [Northern Ireland], and VTI-DF [Germany]) participated in the evaluation of age estimation errors (accuracy and precision).

<u>Difficulties of interpretation</u>: Otoliths are difficult to interpret due to the complexity of the macrostructure and growth variability that has been related, among other reasons, to the long spawning season. The internationally agreed age estimation 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 fish size. 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 (ICES, 2009a) demonstrated with tagging material that the internationally agreed age estimation criteria are neither accurate nor precise and provide overestimation of age. This raises concern about the use, for stock assessment, of age–length keys that were inaccurate (ICES, 2010b). At this time, a replacement age estimation method with sufficient precision and accuracy is not available (de Pontual *et al.*, 2006; Piñeiro *et al.*, 2007).

<u>Recommendations</u>: The main results (ICES, 2010b) demonstrated that the age estimation method was not only imprecise, but also inaccurate and led 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. Therefore, the age estimations used as input for the ICES Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk and Megrim (WGHMM) should be suspended until new validated/accurate criteria are available. Considering the age estimation results obtained in the last workshop (ICES, 2009a) 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 age estimation criteria. These overall findings led to substantial changes in the assessment conducted by ICES (2010b), that is now carried out using length-based models instead of the age-based model XSA previously used. 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 (e.g. otolith structures and DST tagging, otolith microchemistry, otolith modelling) should be promoted.

2.3.4 Cod (*Gadus morhua*)

Difficulties of interpretation: The interpretation of the first annulus can be confused with a first translucent band most likely deposited at the time the juvenile cod moves from the pelagic to the bottom zone. This confusion can be avoided by considering that the first annulus is wider than the first translucent band, ca. 2 and 1 mm in diameter, respectively. Another difficulty is the interpretation of age 1 cod captured during quarter one, when otoliths have a rather wide opaque-edge growth. Some readers estimated these fish at 2 years old because they assumed the translucent band was deposited after the New Year (1 January), and the opaque edge represented a summer growth period. The agreed interpretation is that the translucent band is deposited in autumn (New Year), and the opaque-edge growth is deposited during winter in quarter one. A third difficulty of interpretation is the occurrence of split rings. Some of the translucent annuli can consist of several thinner translucent bands that can be misinterpreted as true annuli, which leads to overestimation of fish age. These bands can be identified as being thinner than true annuli and with less distance between them.

Contrary to most other cod stocks, the eastern Baltic cod stock (subdivisions 25–32) is subject to extensive age estimation problems. 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. Age estimation of Baltic cod is presently performed with broken (Denmark, Sweden), broken and burnt (Estonia, Latvia, Poland, Lithuania, Russia), or sectioned (Germany) otoliths. The key problems with age estimation are: (i) identification of the first winter ring, (ii) timing of the winter ring formation, and (iii) interpretation of the edge. The interpretation of growth zones varies widely among countries and institutes, and even among readers within a given institute. To improve the precision of age estimation, a reference collection of otoliths was compiled in 1995–1996. The purpose of 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 on the interpretation of the otolith characteristics. 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 age estimation methods, problem descriptions, and the reference collection can be found in the revised "Manual for Baltic Cod Age Reading" (ICES, 2000). Despite 30 years of effort to standardize preparation techniques and 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 vary alarmingly by country (ICES, 2012a; specifically section 2.4, Figure 2.4.1a–d), which may negatively influence the quality of the assessment (Reeves, 2003). Since 2013, the traditional age-based stock assessment has been abandoned because of the extensive age estimation problems.

Recommendations: The workshop concluded that sectioning of the otolith is the preferred method to use (vs. the broken method). The various life history traits of North Sea cod may differ within the North Sea, and knowledge of this is highly important for 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 general studies on otolith formation and, in relation to this, North Sea cod physiology, growth, and behaviour as part of the training and updating of all North Sea cod age readers. Owing to the poor readability of eastern Baltic cod otoliths, there is still no consensus on the interpretation of growth zones of these two cod stocks. Consequently, neither the age estimation manual nor the reference collection have been updated in recent years. A tag–recapture study for the entire Baltic Sea is urgently required to generate material for validation of fish age and to assess growth, movement, and exchange processes within and between stocks.

2.3.5 Blue whiting (*Micromesistius poutassou*)

Difficulties of interpretation: The first difficulty of interpretation is the position of the first ring where the Bowers zone is clear. This is often seen in younger individuals as the otolith is thinner and the structures therefore clearer. The second difficulty of interpretation arises when some readers choose to omit specific rings identified as splits, while other readers identify the same rings as true annuli. This becomes more problematic after the second year of growth.

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

2.4 Age validation case studies in gadoids

In gadoids, only a limited number of validation methods described in the literature (Campana, 2001; Appelberg *et al*., 2005) have been applied. A summary, therefore, is given in Table 2.2, which is a modification of Campana's table, with a column showing the gadoid species that were studied and which technique was used.

Table 2.2. Summary of age validation methodologies, modified from Campana (2001), highlighting the methods used for gadoids. DGI = daily growth increments. N/A = not available.

Method	Annual/DGI	Age	Advantages	Limitations	Gadoids in which this validation technique has been employed
Released marked fish	Annual and DGI	All	Validate absolute age and periodicity	Source of fish with known age, recaptures of old fish are null	N/A
Mark–recapture chemically tagged fish	Annual and DGI	All	Validate periodicity post release	Low recaptures, some markers may affect survival	Hake (de Pontual *et al.*, 2003; 2006; Piñeiro *et al.*, 2007; Mellon-Duval *et al.*, 2010; ICES, 2010b)
Captive rearing from batch	Annual and DGI		Validate absolute age and periodicity	Differences with wild fish	N/A
Microstructure	Annual	1 year	Validation of 1st year	Daily periodicity assumed	Hake, cod (Morales-Nin and Aldebert, 1997; Arneri and Morales-Nin, 2000; Morales-Nin and Moranta, 2004; Belcari *et al.*, 2006; Hidalgo *et al.*, 2009; Hüssy, 2010; Pattoura *et al.*, 2011)
Most likely age (MLA)	Annual and DGI	0–5 years	Validation of ages 1–2	No overlapping length modes, no length-based migrations	N/A
Marginal increment analysis	Annual	All	Validate periodicity	Not so straightforward in slow-growing/older individuals. Needs adequate sample size by month.	Cod, saithe, whiting, haddock, hake (Mahé *et al.*, 2016)
Radiochemical dating	Annual	Plus 5-year-olds	Validate absolute age of old fish	Can only distinguish between divergent estimates	N/A
Bomb radiocarbon	Annual	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 2.2 will be described in detail. The methods are reported according to whether they are indirect or direct validation methods.

2.5 Application of indirect validation methods

2.5.1 Length-based analyses

Length frequency analysis has been applied successfully on some gadoid species (Table 2.3), especially European hake (Aldebert and Morales-Nin, 1992) and hake in the western Mediterranean Sea, obtaining an indirect validation of the first three age classes and confirming the first winter ring of Baltic Sea whiting (Ross and Hüssy, 2013).

In the case of European hake 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 shows two principal peaks for each annual ring (Figure 2.1), in agreement with the presence of two spawning periods (spring–summer and autumn–winter; Arneri and Morales-Nin, 2000; Belcari *et al.*, 2006). Consequently, in this case, two spawning groups of individuals should be recognizable at the time of the first hyaline ring formation: those that hatched in summer (age 0+ group) and those that were born the previous winter (age 1 group). This pattern also appears for subsequent age groups.

Table 2.3. Summary of species where length frequency analysis (LFA) has been applied.

Species	Method	Area	Age/size range
Whiting (Ross and Hüssy, 2013)	Mode progression + daily increments	Western Baltic	0–1 years
Hake (Aldebert and Morales-Nin, 1992; Arneri and Morales-Nin, 2000; Belcari *et al.*, 2006)	LFA	Tyrrhenian Sea	0–3.5 years
Hake (Aldebert and Morales-Nin, 1992)	LFA + otolith daily growth increments	Gulf of Lion	7.5–30 cm

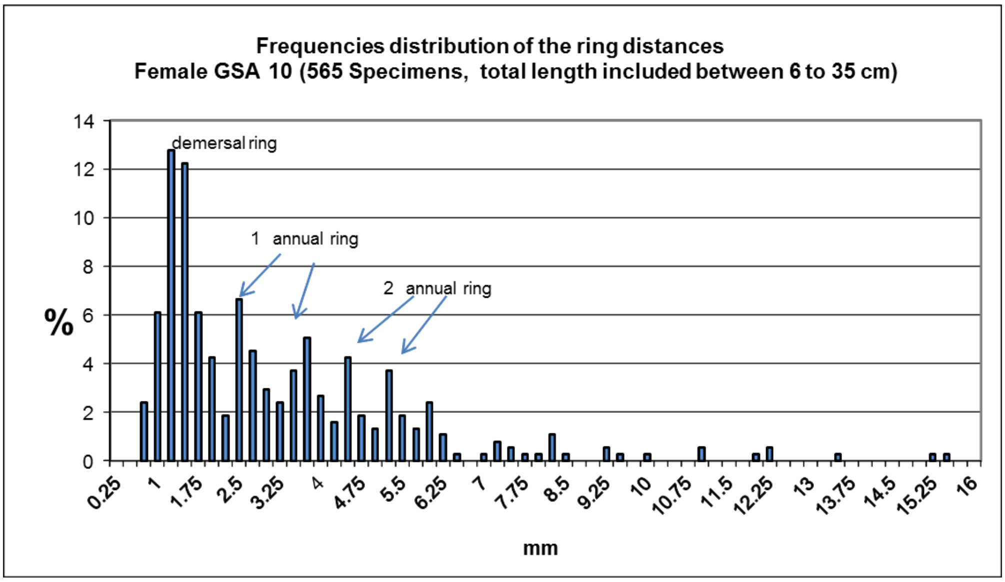

Figure 2.1. Frequency distribution of the distance between the nucleus and specific growth zones in European hake. The peaks represent different age classes, indicated with arrows.

In general, 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 gathered on a routine basis in fishery studies (i.e. length frequencies). However, this procedure does not allow 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 species with a relatively long spawning period (e.g. European hake), it provides reliable growth rate verification only for the first few age classes.

2.5.2 Marginal increment analysis

This method has been carried out on a number of gadoid species (Table 2.4).

Table 2.4. Summary of species where marginal increment analysis (MIA) has been applied. TL = total length (Mahé *et al.*, 2016).

Species	Method	Area	Time-series	Age/size range
Cod	Sectioned	Divisions 7.f–j and 8.a–b	Jan 2011–Jan 2012	2–9 years
Saithe	Sectioned	Division 6.a	Jan 2011–Jan 2012	2–14 years
Whiting	Sectioned	Subareas 4–6 and divisions 7.a–d	Jan 2011–Jan 2012	2–14 years
Whiting	Sectioned	Divisions 7.f–h	Jan 2011–Jan 2012	2–8 years
Haddock	Sectioned	Divisions 7.f–j and 8.a–b	Jan 2011–Jan 2012	2–8 years
Hake	Whole	Tyrrhenian Sea	Mar 1997–Feb 1998	2–16 years
Hake	Sectioned, burnt	Gulf of Lion	Jan 1989–Dec 1990	6–94 cm TL

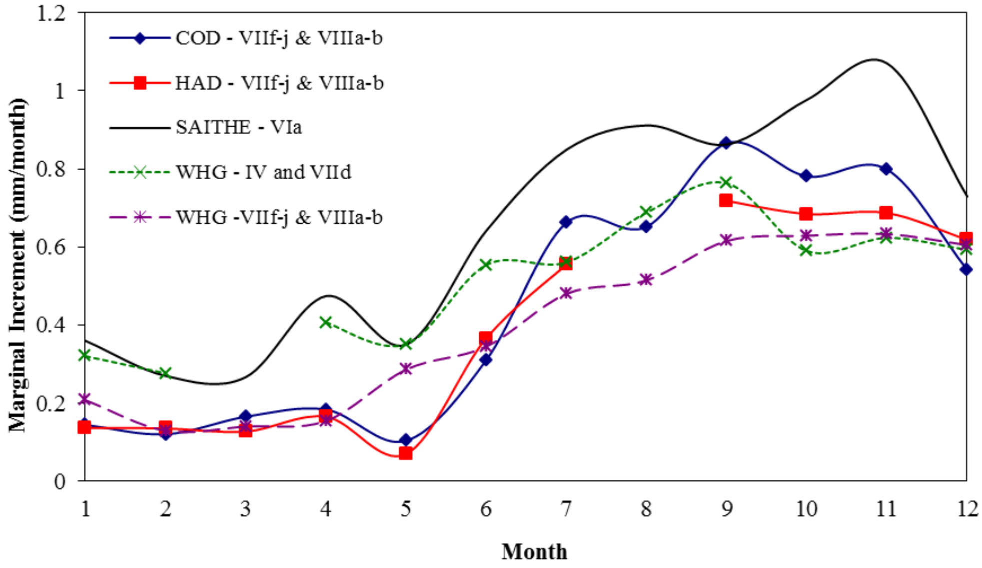

Figure 2.2. Marginal increment analysis of cod, haddock, saithe, and whiting from the North Sea to the Bay of Biscay (source: Mahé *et al.*, 2016).

For the gadoid species discussed at the Workshop on Age Validation Studies of Gadoids (WKAVSG) and listed in Table 2.4 above, the use of MIA proved to be a successful method to corroborate annual increment formation, using sectioned otoliths in fish across large age ranges. Figure 2.2 shows the application of MIA for a range of species.

2.5.3 Edge-zone analysis

See Section 1.2.5 on indirect validation methods.

2.6 Application of direct validation methods

2.6.1 Tag–recapture

Tag–recapture is one of the direct methods to validate the intrinsic age information of otoliths. Upon capture, individual fish are usually marked externally (e.g. with T-bar or spaghetti tags to ensure recovery) and internally (to produce a mark in the otolith at the time of capture). Different chemical marker substances can be used, e.g. fluorescent compounds such as oxytetracycline, that may fade over time but have also been shown to remain visible in cod otoliths 40 years after tagging (Krumme & Bingel, 2016). A chemical mark like strontium chloride is stable over time, but requires a scanning electron microscope to detect it in the otolith (Geffen, 1992). The combined use of injections with tetracyclin and strontium chloride may therefore provide a long-term solution (Stötera *et al.*, 2018).

Information on the suitability of alternative chemical markers for certain species is often poor, so the choice of which otolith marker to use and which concentration to apply in validation studies must often be made without evidence from robust experiments. The material and methods sections of published studies are often not sufficiently detailed to allow the experiments to be reproduced (e.g. it is unclear exactly which chemical substances were used, how they were prepared and how applied). Sometimes, it is not clear whether failure in marker experiments was caused by real biological or other reasons, or whether the chemical concentrations were wrong.

It is, therefore, necessary for large-scale tag–recapture studies, aimed at age validation of otoliths, to be preceded by robust experiments that allow determination of the suitability of different chemical agents at different marker concentrations. Such experiments should ascertain adverse effects on the tagged fish (increased mortality; Morales-Nin *et al*., 2011), perceptibility of the artificial time-mark in the otoliths at different marker concentrations, and visibility of the mark over time. The use of control groups is essential. Stötera *et al.* (2018) provide an example of a systematic approach to assessing the effect of different tetracycline concentrations on the fluorescent band quality of Baltic cod.

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 optimize 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 of ~2) could be clearly related to an overestimation of age (de Pontual *et al*., 2006; Figure 2.3).

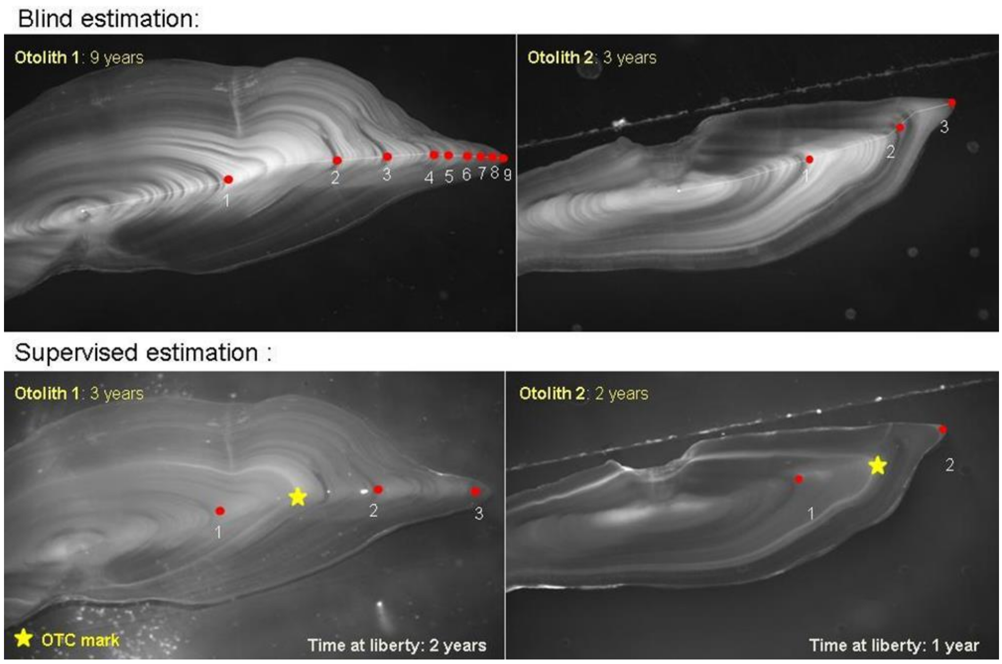

Figure 2.3. Comparison between blind and supervised interpretation of chemically marked (oxytetracycline [OTC]) European hake otoliths.

The approach was extended to the Iberian Peninsula (Piñeiro *et al*., 2007) and the Mediterranean Sea (Mellon-Duval *et al*., 2010), and sustained tagging effort was maintained in the Bay of Biscay during 2004–2007. In this region, 27 690 fish were tagged, chemically marked, and released. A total of 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 programmes carried out to date is given in Table 2.5.

The 104 marked otoliths resulting from this tagging programme 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 among hake readers, with substantial differences in ring positions). It was strongly recommended to stop producing annual age–length keys for use in ICES assessment of European hake.

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

Table 2.5. Summary of tagging programmes carried out on European hake.

Tagging experiment			Recapture results				
Location	**No. of fish released**	**TL range at release (cm)**	**Max time at liberty (days)**	**TL range at recapture (cm)**	**No. of tagged fish recovered**	**Recaptured (%)**	**Reference**
SW Ireland	78	28.9	255	40.6	1	1.3	Belloc (1935)
Southern Bay of Biscay	152	56	24	60	1	1.9	Lucio *et al.* (2000)
Bay of Biscay	1 307	21–40	1 066	24–67	36	3.1	de Pontual *et al.* (2006)
Bay of Biscay	27 690	9–84	1 555	19.2–78.9	1 199	4.33	de Pontual *et al.* (2013)
Mediterranean Gulf of Lion	4 277	15–40	717	16–57	280	6.5	Mellon-Duval *et al.* (2010)
NW Iberian Peninsula	527	29–36	466	31–56	6	1.3	Piñeiro *et al.* (2007)
NW Iberian Peninsula	2 725	28–46	466	31–56	27	1	C. Piñeiro (pers. comm.)
Balearic Islands	675	10–44	-	-	-	-	E. Massuti (pers. comm.)

The "tag–recapture" programme 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 age estimation criteria. A better understanding of the complex otolith growth pattern of this species might be achieved through better knowledge of fish behaviour (migrations, feeding activity, etc.) and differences in individual life histories. Approaches that couple validation methods (e.g. otolith structures and DST tagging, otolith microchemistry, otolith modelling; see Section 2.7 "Future perspectives") should be promoted.

2.6.2 Rearing in captivity

Rearing European hake in captivity from wild eggs has been done several times. In an experimental environment in Norway, European hake were reared from eggs up to 245 days 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 that start forming on day 8, probably related to the start of exogenous feeding. The lapillus showed a later increment formation.

2.6.3 Daily increment analysis

See Section 1.2.5 (Indirect validation methods). Table 2.6 summarizes studies where this technique has been applied specifically on gadoid species.

Table 2.6. Summary of species where daily increment analysis has been applied.

Species	Method	Area	Time-series	Age range
Cod	Sectioned	Baltic Sea, subdivisions 22–24 Baltic Sea, Subdivision 25	2009 2001	0–1 years < 3 years
Whiting	Ground in sagittal plane	Baltic Sea, subdivisions 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 Lion, 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 (Pattoura *et al*., 2011), Adriatic Sea (Arneri and Morales-Nin, 2000), Tyrrhenian Sea (Belcari *et al*., 2006), Mediterranean, Gulf of Lion, and Balearic Islands (Morales-Nin and Aldebert, 1997; Morales-Nin and Moranta, 2004; Hidalgo *et al*., 2009). 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.

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, 2010). Comparison of daily increment patterns revealed inconsistencies between winter zones identified by the lack of visible increments with the formation of translucent zones (Figure 2.4; Hüssy, 2010).

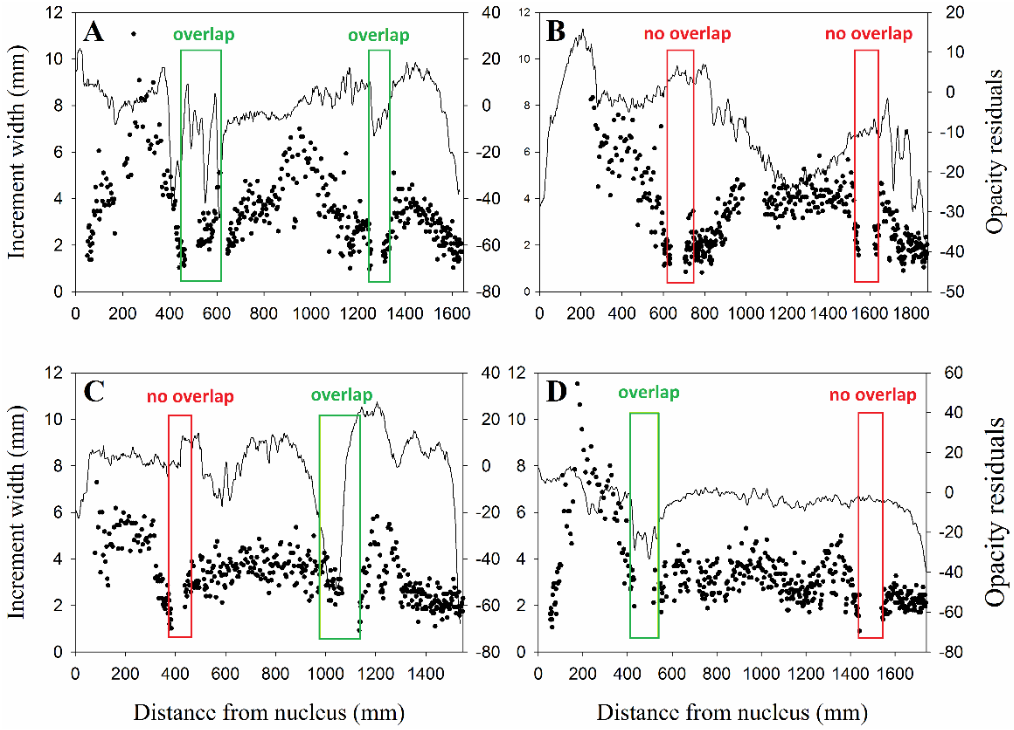

Figure 2.4. Four examples of 3-year old cod with different daily increment (dots) and opacity (lines) patterns over two consecutive years: (a) overlap in both years, (b) no overlap, (c) overlap in the second, but not the first year, (d) overlap in the first, but not the second year (modified from Hüssy, 2010).

2.7 Future perspectives in gadoids: available tools

2.7.1 Back-calculation of size at previous age and hatch date

Back-calculation is an important method of obtaining estimates of the length-at-age prior to capture of an individual fish (Panfili *et al.*, 2002). Specifically, the dimensions of one or more marks in some hard structures of the fish, together with its current body length, can be used to estimate a fish's length at the time of formation of each of the marks. The hard parts used are otoliths, opercular bones, vertebrae, fin rays, or spines (Francis, 1990). The marks are often annual rings associated with growth checks, but back-calculation has also been used in association with marks caused by the stress of liberation of hatchery fish (Davies and Sloane, 1986) and tetracycline injections in tagged fish (Panfili and Tomàs, 2000).

In order to carry out a back-calculation correctly, three main assumptions have to be met:

1. The size of the calcified structure (CS) mark remains unchanged from the time of formation (no resorption or degeneration).
2. The assumed time of formation is correct.
3. The back-calculation method accurately relates body size to CS size for each fish.

Back-calculation of previous size-at-age is an inexpensive tool, but cannot stand alone. This method should preferably be coupled to a length frequency distribution from a survey carried out in the period of the hyaline ring formation or size distributions of individual age classes.

2.7.2 Simulation tools for otolith macrostructure modelling

Improving the reliability of otolith-based individual and population data is critical to population dynamics and ecology. In this respect, the numerical model of otolith formation recently developed and validated by Fablet *et al*. (2011) deserves particular attention. Based on a general bioenergetic theory, it disentangles the complex interplay between metabolic and temperature effects on otolith growth and opacity patterns, resolves controversial issues, and explains poorly understood observations of otolith formation. It represents a unique simulation tool to improve otolith interpretation and applications. Scenario-based model simulations (where temperature and food series are forcing variables) are of primary interest to interpret and predict otolith characteristics in response to environmental features (e.g. Figure 2.5). Besides, they provide new means for the discrimination of seasonal vs. non-seasonal otolith structures, a crucial issue for the improvement of the accuracy of individual age estimation.

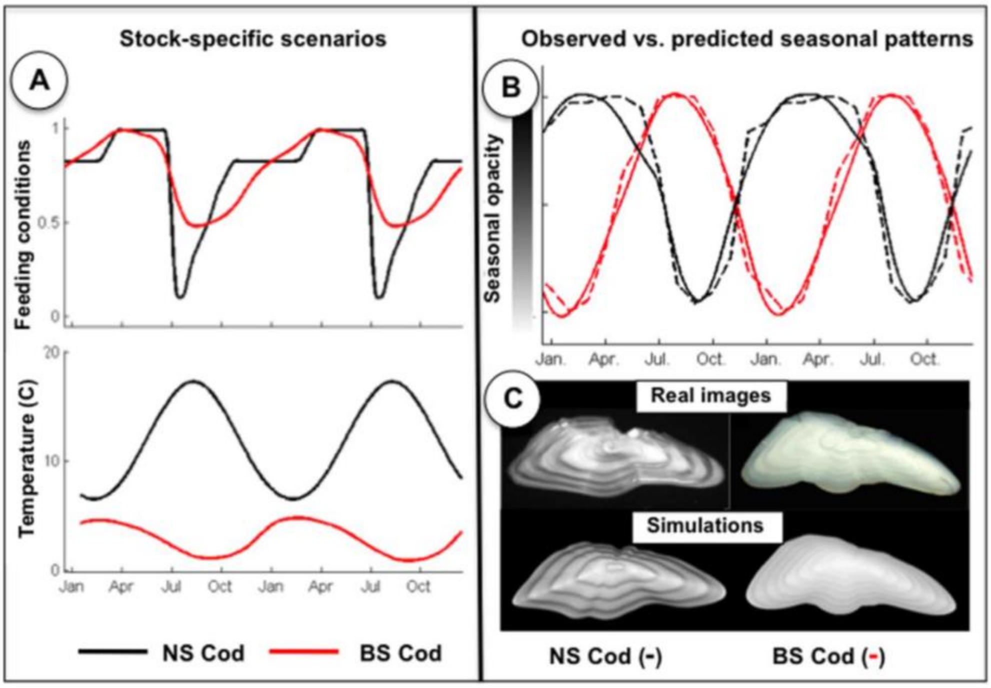

Figure 2.5. Resolving the non-synchronous seasonality of opacity patterns of Barents Sea (BS) and southern North Sea (NS) cod otoliths: feeding and temperature conditions (A) that explain otolith opacity patterns observed for southern North Sea (NS, black lines) and Barents Sea (BS, red lines) cod (B and C). Observed seasonal patterns (dashed lines), given as the relative proportions of opaque edges in the monthly sampled otolith sets (21), are compared to normalized simulated opacity patterns (solid lines). Model simulations reproduce both the opposite seasonal opacity patterns (B) and the remarkable differences in the contrast of the otolith images of the two populations (C). From Fablet *et al.* (2011).

2.7.3 Raman microspectrometry

Raman microspectrometry could be another useful tool to achieve a better understanding and validation of otolith growth patterns. This technique allows a quantitative characterization of the mineral and organic fractions of otolith structures (micro- and/or macro-structures). Recent studies (Jolivet *et al*., 2008, 2013) have provided new insights into understanding otolith biomineralization mechanisms, as well as for the interpretation and discrimination of otolith macrostructures.

2.7.4 Microchemistry as a supportive tool for age validation

Age estimation for most stocks is hampered by the fact that the process is based on counting annual growth increments (rings or annuli), whereas it is not certain that one ring is formed each year. The timing of the deposition of annuli is not verified and pseudoannuli, i.e. rings that can appear at any time during the year, induced possibly by stress, are a common problem. Using corroborative methods for validation of annual rings, such as elemental or isotopic cycles, could potentially support the counting of rings. This would be a correlative way of corroborating the age of the fish, primarily by confirming the identity of true annuli (Campana, 2001). It is necessary to be aware that the use of microchemistry as a supportive tool for age validation requires fundamental knowledge about the chemical environment of the species. While growth rate and metabolism can affect the deposition of chemical elements in the otolith, recent studies show that uptake directly from surrounding water is a main source of certain chemical elements found in otoliths (Walther and Thorrold, 2006).

Techniques commonly used for microchemistry analysis (elemental fingerprint) include:

- Laser ablation inductively coupled mass spectrometry (LA-ICPMS)
- Electron probe microanalysis (EPMA)
- Proton-induced X-ray emission (PIXE)
- Scanning X-ray fluorescence microscopy (SXFM)

Although microchemistry is not widely used for the purpose of validating age, the field has the advantage of being in rapid development (Campana, 2005). Methods have matured and are now more widely used for identifying natural tags. This also means that methods are becoming more affordable to use on a routine basis. The concept of elemental fingerprinting in otoliths is very attractive, telling a story about how to interpret the life history of fish and constructing environmental history. The use of elemental fingerprints is becoming more widely used for separation populations, migration, stock mixing, and map connectivity between habitats (Gillanders, 2005). The various uses of elemental finger printing implies that more effort will be invested in making elemental maps of otoliths, something that can also prove useful for age estimation purposes.

In a recent study of cod and hake (DGXIV Study Project 96-075), it was shown that 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 variations in the Sr/Ca signal. Therefore, it was not possible to use the Sr/Ca variations to validate which zones correspond 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.

Chemical elements suitable for corroboration of age will be highly variable, depending on species and even on stock level, and will reflect the environment. Investigations show that elemental fingerprints in cod otoliths based on the elements Li, Mg, Mn, Sr, and Ba are physically stable. The elemental ratio Sr:Ca is mainly connected with changes in salinity and is popular for tracking anadromous fishes (Walther and Limburg, 2012). Other elemental ratios that have shown variation in otoliths in offshore and coastal waters are Ba:Ca and Mg:Ca (Thorrold *et al.*, 1997). Additional ratios recently identified as promising in exhibiting seasonal patterns are Mg:Ca, Zn:Ca, Cu:Ca, and Rb:Ca (Hüssy *et al.*, 2015; Limburg et al., 2018).

Experiments were made with the Sr:Ca content in cod and hake; however, this elemental ratio proved not to be useful in the marine environment where the variation in concentration is connected to temperature and is not independent of otolith growth.

Another example is the findings from microchemistry analysis of cod in the Baltic Sea. An experiment hypothesized that the incorporation of Mn:Ca and Sr:Ca showed a potential of being related to seasonal events (Limburg *et al.*, 2011). Mn:Ca was found to have a strong correlation with hypoxic events (Itai *et al.*, 2012), which was also found in the experiment of Limburg *et al.* (2011).

Figure 2.6 maps variations in otoliths over a temporal scale of four historical periods of time. A seasonal pattern was detected when the ratio Mn:Ca was high in the summer months of young fish (1–2 years), which can be related to dwelling in shallow, relatively warmer nursery areas and with exposure to seasonal hypoxia. This pattern disappeared in later life when Sr:Ca was elevated (Figures 2.6 and 2.7). Sr:Ca is related both to temperature and salinity, and cod are known to migrate to deeper, more saline and colder water after their juvenile period to either feed or spawn.

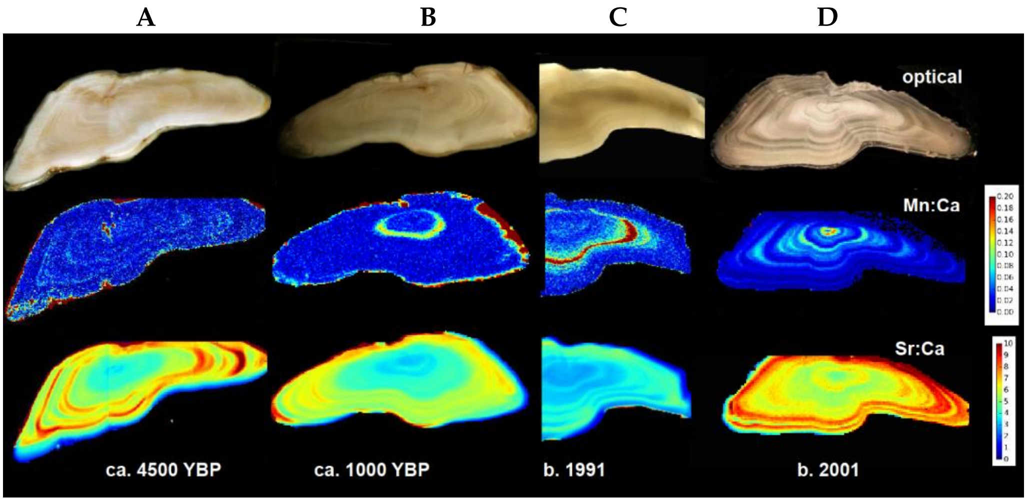

Figure 2.6. Transverse sections of eastern Baltic Sea cod otoliths from four different time periods: the Neolithic (A), late Iron Age/Middle Age (B), early 1990s when the areal extent of hypoxia was low (C), and 2001 when it was high (D). Regions in the core correspond to the juvenile stage. Note that Mn is high in the Neolithic and Iron Age otoliths in small cracks as well as along the edge, where it was in contact with soil pore water. The 1991 otolith is portrayed in a partial map. (Scale bar: 1 mm. Reproduced from Limburg *et al.*, 2011.)

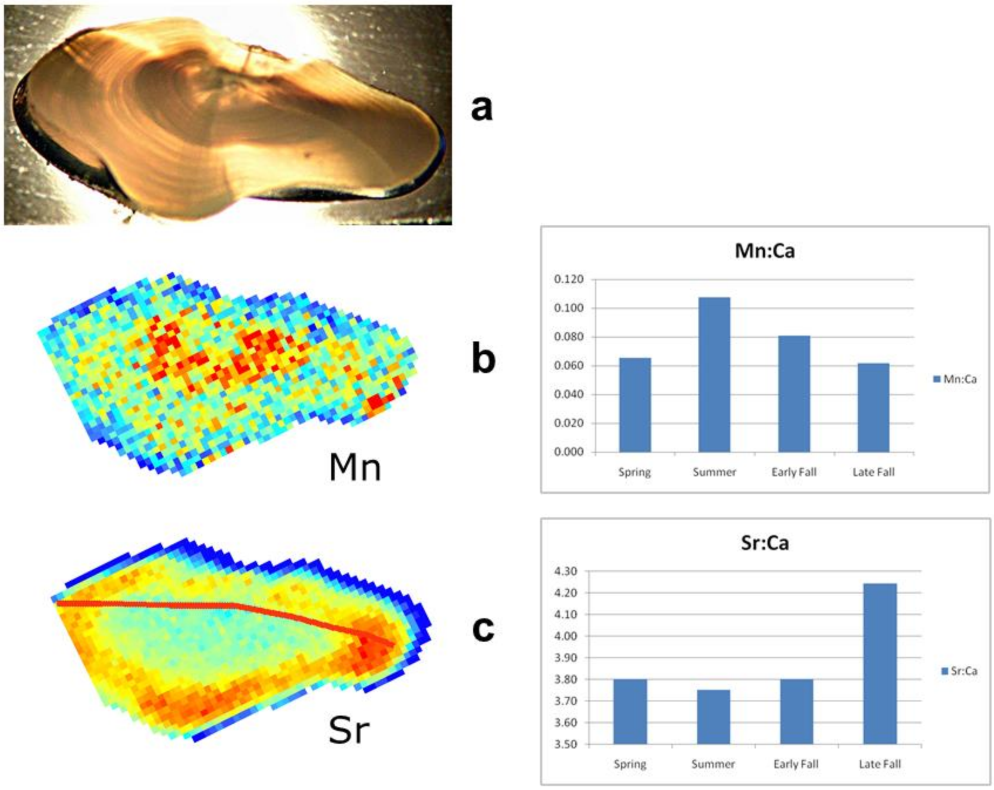

Figure 2.7. Otolith of a young-of-the-year eastern Baltic cod, length = 100 mm, captured 17 November 2008 in a special trawl survey. Scale bar = 1 mm. (a) Photo of a transverse section through the otolith. (b) Manganese elemental map (left) and Mn:Ca ratios (× 103, right) parsed out by season. (c) Strontium elemental map (left) and Sr:Ca ratios (× 103, right) parsed out by season. The red line represents transects of data that were extracted by GIS for preparing the graphs. Examination of daily increments (not visible) indicated the fish was born in May (Images: K. Limburg).

2.8 Ongoing and future work

For anglerfish (*Lophius piscatorius*) and hake (*Merluccius merluccius*), a European project (EASME/EMFF/2016/1.3.2.7/SI2.762036, Validating age-determination of anglerfish and hake) will investigate if calcified structures (otoliths and illicium) contain seasonal otolith microchemistry patterns that can be used for age estimation. A combination of analytical approaches (LA-ICPMS, IR-MS, SIMS) are employed to establish the most effective method for measuring age-related maxima and minima in elements and isotopes. The results will be used to develop the tools for otolith macrostructure modelling.

For Baltic cod (*Gadus morhua*), the seasonality of chemical element patterns occurring in otoliths is being validated through a large-scale tagging project "Tagging Baltic Cod" (TABACOD), funded by BalticSea2020. For further information visit the TABACOD home page at: http://www.tabacod.dtu.dk/

2.8.1 Validation of life history events – juvenile check

In some demersal species, several of the macrogrowth increments in the central part of the otolith confuse the attribution of the first seasonal increment. The growth of these increments during the early life phases may be related to one or several factors, such as changes in habitat (i.e. from pelagic to demersal settlement), feeding patterns (changes in diet), or metamorphosis (i.e. termination of cutaneous respiration and ossification). Any of these changes can result in changes in otolith formation, leading to changes in otolith shape and increment pattern. Changes in 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 2.8). In a study of European hake settlement (Arneri and Morales-Nin, 2000), the duration of the pelagic life phase was established at approximately 2 months, which was validated in other studies.

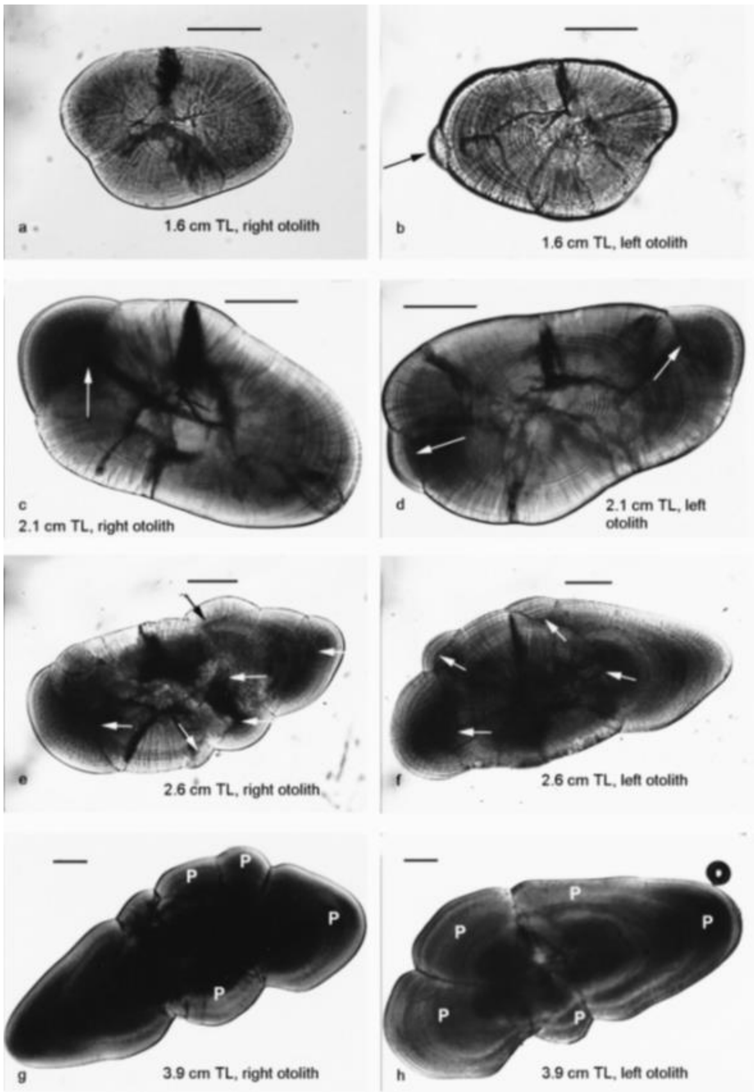

Figure 2.8. Juvenile hake otoliths, showing the changes in shape and increment structure (Arneri and Morales-Nin, 2000).

2.8.2 Validation of life history events – spawning check

Background

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 Rollefsen (1933, 1935), and were later used to estimate 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 types of analyses (ICES, 2001). At present, the Institute of Marine Research (IMR) in Norway uses otolith spawning checks for saithe north of 62°N (*Pollachius virens*) to determine the proportion of mature vs. immature saithe in assessments (ICES, 2005). The background here is that this survey cruise takes place in autumn, i.e. at a time of the year when gonad staging is not helpful in adequately separating maturing from immature individuals. In assessments of Northeast Arctic haddock (*Melanogrammus aeglefinus*), 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 of 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 assumed spawning indicators has only been brought to our attention in recent years. Previous work to validate "spawning zones" in cod otoliths began with the NFR (Research Council of Norway) project "Timing and determination of fecundity and skipped spawning" (2006–2009), followed by a second project to include haddock ("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 things, 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 projects 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 among individuals with regular or irregular annual reproductive patterns (skip spawners).

Future work

It is still unclear 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 spawning checks are recorded in the otoliths of individuals that skip spawning after one or several years of 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 hatching to age 3 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 regime will be switched to the other feeding regime. During the experiment, otoliths will be regularly stained by alizarin to determine the timing of spawning checks relative to time of spawning. The results of this experiment are not yet ready. Otolith growth and spawning checks will finally be analysed in transversal sections, using a fluorescence microscope to detect the alizarin stain marks. A disadvantage to such an experimental approach is the potential deviations that may occur 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 behaviour, migrations, and environment may vary among different gadoid fish stocks. Unfortunately, for some gadoid fish stocks, spawning checks may be difficult, if not impossible, to detect by conventional age estimation methods. Deciding which species are 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 estimation among involved age estimation institutions is a recommended prerequisite before undertaking further studies of spawning-zone validation in any specific gadoid stock. If individual age-at-maturation is not known, this type of spawning validation study has less value in the aspect of stock management.

The use of post-ovulatory follicles (POFs) is another good candidate for validating 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 ongoing study at IMR (Norway), otoliths and ovarian samples from Northeast Arctic saithe 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, results show good agreement between spawning zones and the presence of POFs among older individuals that 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, no spawning zones were found among individuals between two and four years old in 2012. Nevertheless, half of these ovarian samples showed evidence of 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.

3 Flatfish

Mark Etherton, Sally Songer, Joanne Smith, and Barbara Bland

3.1 Introduction

Flatfish form a large part of both the commercial fisheries and the stock assessment effort within ICES. The majority of category 1 stocks (defined as those stocks where a full analytical assessment and short-term predictions can be made) have age-based assessments. Accordingly, it is vital to obtain ages that are as accurate and precise as possible.

At least 18 flatfish species, comprising numerous stocks, have current age estimation programmes in marine institutes around Europe (Table 3.1). Many of these species are common in most sea areas within the Northeast Atlantic, Baltic, and Mediterranean, with a number of institutes doing age estimations. For others there is little age estimation effort; in some cases only a single laboratory has a flatfish age estimation programme. A list of the known flatfish species with age estimation programmes as covered by the EU Data Collection Framework is given in Table 3.2. A total of 22 institutes across Europe have flatfish age estimation programmes. Common sole (*Solea solea*) and European plaice (*Pleuronectes platessa*) are the most commonly read species and probably those that have the greatest commercial importance.

Table 3.1. Flatfish species with age programmes in European institutes and the number of institutes involved in each species.

Common name	Scientific name	No. of institutes
Common sole (Dover sole/Black sole)	*Solea solea*	10
European plaice	*Pleuronectes platessa*	10
Turbot	*Scophthalmus maximus*	9
Brill	*Scophthalmus rhombus*	7
Flounder	*Platichthys flesus*	7
Megrim	*Lepidorhombus whiffiagonis*	7
Dab	*Limanda limanda*	6
Lemon sole	*Microstomus kitt*	5
Witch (Witch flounder)	*Glyptocephalus cynoglossus*	2
Greenland halibut	*Reinhardtius hippoglossoides*	2
Long rough dab (American plaice)	*Hippoglossoides platessoides*	2
Scaldfish	*Arnoglossus laterna*	2
Four-spot megrim	*Lepidorhombus boscii*	1
Adriatic sole	*Pegusa impar*	1
Atlantic halibut	*Hippoglossus hippoglossus*	1
Sand sole	*Pegusa lascaris*	1
Solenette	*Buglossidium luteum*	1
Thickback sole	*Microchirus variegatus*	1

Within the framework of ICES, there have been a number of flatfish age calibration workshops and exchanges in recent years. Most have included analysis of interlaboratory comparisons of a set or sets of otoliths. Table 3.3 summarizes the available results from these workshops and exchanges.