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## Report of the Workshop on Sexual Maturity Sampling (WKMAT)

15–19 January 2007

Lisbon, Portugal



International Council for the Exploration of the Sea  
Conseil International pour l'Exploration de la Mer

**International Council for the Exploration of the Sea  
Conseil International pour l'Exploration de la Mer**

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](http://www.ices.dk)  
[info@ices.dk](mailto:info@ices.dk)

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## Executive summary

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*The Data Collection Regulation (DCR) programme covers extensive sampling of maturity stages, but up-to-date results are rarely used in the assessment of the Spawning Stock Biomass SSB. In several cases, calculation of the proportion of mature fish is based on information collected far outside the spawning season, or on incomplete coverage of the stock distribution area. Market sampling, fishery independent stock surveys and observer programmes provide information on the timing of the spawning season. Samples shortly preceding, or in the early phase of the spawning season may achieve adequate spatial coverage. Improved sampling programmes, further analyses and consideration of the consequences of inter-annual variation in maturity on stock assessments, will further eliminate the current problems in the maturity sampling programmes.*

This report presents the results of a Workshop on Sexual Maturity Sampling, held in Lisbon (Portugal), 15–9 January 2007.

The Data Collection Regulation (DCR) programme covers extensive sampling of maturity stages for stocks within Community waters, mostly on a tri-annual basis. The current Regulation prescribes a pre-defined precision level (level 3, the highest: 5% precision), but sampling strategy and the actually achieved precision have hardly been addressed yet. ICES stock assessments are often based on time-invariant maturity ogives, derived from information collected outside the spawning season and/or covered the spatial distribution of the stocks incompletely. This Workshop was set up to develop sound approaches to maturity sampling for the wide range of species included in the Data Collection Regulation programme.

Appendix XVI of the DCR lists all stocks for which maturity data need to be collected. This includes over 150 stocks, each of which has its details and peculiarities. Rather than specifying maturity sampling protocols for each and every species/stock, the Workshop addressed species groups with similar life history traits and sampling requirements. Chapter 2 addresses the full list of species/stocks with their characteristics, and derives a pragmatic grouping. Defining characteristics relate to longevity, seasonality, pelagic/demersal, and spawning pattern. The remainder of this report draws examples from individual species/stocks, but presents recommendations for typical species/stock groups. Implementation of improved maturity sampling protocols necessarily requires detailed planning at the species/stock level, in the relevant Regional Coordination Meeting.

For individual stocks, maturity information is usually collected by several Member States and institutes. The proper identification of maturity stages for a small number of species/stocks will be the subject of a series of workshops organised later this year (WKMSHM, WKMSMAC, WKMSCWHS). In the current workshop, it was realised that the coding schemes in use (varying from a 4-grade scale to a 10-grade scale), and the interpretation of particular stages (in particular immature versus post-spawning or skipped-spawning), might give rise to misinterpretations, both with respect to the actual biological maturity stage, as to the assessment of the spawning stock biomass. In chapter 3, an improved 5-stage maturity scale is proposed, which accommodates for these problems, while allowing consistent mapping of the more detailed scales. It is recommended that this proposal is further considered by the species-specific workshops later this year.

The majority of species/stocks covered by the DCR spawn during a limited time interval of the year, nearly always in restricted spawning areas. Sampling protocols, therefore, should accommodate for temporal and spatial variation in maturity composition. Additionally, length selectivity of gears might influence the sampling. Market sampling, stock surveys and observer programmes constitute sources of information on the maturity status of stocks. Market sampling most easily provides full temporal coverage, but catches might be stripped before landing, or the fishery may target the spawning component of the stock

disproportionally. Stock surveys, primarily set up for year class abundance estimation, will not necessarily match the spawning season. Manpower on observer trips often does not allow for additional sampling for maturity. Section 4 presents case specific examples of sources of variance, and a check list for future maturity sampling programmes.

Sampling protocols on board research surveys, as well as market sampling procedures, differ between countries, between stocks, and even between different surveys for the same stock. Section 5 provides an overview of current procedures, and highlights limitations of the existing procedures. Evidently, problems occur with respect to the sampling protocols, as well as the raising of the data for usage in stock assessments. However, it is also apparent from this overview, that up-to-date information on maturity is often not used by assessment working groups. Consequently, this chapter highlights how and where improvements can be implemented.

The last Section, Section 6, summarises the main conclusions from the workshop, and recommends further steps to improve maturity sampling. This concerns: the practical implementation of the proposed 5-stage maturity scale; planning of improved maturity sampling programmes in the Regional Coordination Meetings, using the check-lists provided in this report for improving the sampling protocols; further analysis of available data sets, to quantify spatial and temporal variation patterns, and to establish the relation between sample sizes and achieved precision levels; and finally, the analysis of potential effects of inter-annual variation in maturity on assessed trends in spawning stock biomass.

## 1 Introduction

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At the 93<sup>rd</sup> Statutory Meeting of ICES (2006) it was decided that a Workshop on Sexual Maturity Sampling WKMAT (Chair: Willem Dekker, the Netherlands) would be established, which actually took place in Lisbon (Portugal) from 15 to 19 January 2007, to:

- 1 ) Develop standard operational procedures on maturity sampling for various life history trait species groupings (slow/fast growth, short/long spawning period, batch/fractional spawners), with particular emphasis on:
  - a ) Temporal synchronisation of maturation and the sampling programme (time of the year, or relative to peak spawning)
  - b ) Spatial coverage of the population, especially when differential distribution of spawners and non-spawners occurs
  - c ) Precision levels achieved, in relation to DCR precision requirements.
- 2 ) Review maturity at length/age requirements from appendix XVI of Commission Regulation 1639/2001; in particular the periodicity for which these parameters should be collected, with the aim at revising the Regulation
- 3 ) Review ongoing sampling programs (for instance for Northeast Arctic cod) for liver index sampling and evaluate to which extent such sampling programs could usefully be implemented for other stocks.

This workshop was attended by 26 people, from 13 countries; Annex 1 presents a list of names and addresses of all participants.

Under the Data Collection Regulation DCR (Council Regulation 1543/2000 and Commission Regulations 1639/2001, 1581/2004), maturity samples are collected for a wide range of species, mostly on a tri-annual basis. The strategy of the sampling programmes and the utilisation of aggregated data collected from different areas at potentially different times of the year have not been addressed yet. Moreover, the number of fish stocks, for which the assessment of the spawning stock biomass SSB is based on actual and up-to-date information on the maturity composition of the whole stock, is extremely limited. The Planning Group on Commercial Catch, Discards and Biological Sampling PGCCDBS (ICES, 2006a) therefore proposed to convene a workshop, to look into sampling design and aggregation of maturity data. Rather than focussing on individual species or stocks, this workshop looked at the possibility of stratifying species into a number of groups depending on life history traits, and considered whether different approaches to sampling could be applied to each group. On the basis of these groupings, sampling designs are specified to optimise the collection of material for species within each grouping. In addition, the Workshop addressed other methodological issues in relation to maturity sampling such as the optimisation of spatial coverage of sampling for widely distributed stocks and for stocks with differential distribution patterns between spawners and non-spawners, the optimisation of sampling at length, and the calculation of aggregated maturity-at-length-keys for species/stocks where maturity shows spatial variation.

The structure of this report does not strictly follow the order of the Terms of Reference for the meeting, since different aspects of subjects were covered under different headings and a rearrangement of the Sections by subject was considered preferable.

**Section 2** presents a description of biological characteristics of species and stocks, and derives an appropriate grouping of the species for the remainder of the discussions.

**Section 3** discusses classification schemes for maturity stages, presenting both an overview of the classification schemes in use, and a condensed scale for information exchange. Moreover, the use of additional information, such as liver weight, to determine the likelihood of maturation before the next spawning season is discussed.

**Section 4** discusses sources of variation in maturity data, encompassing spatial and temporal variation in the stocks, as well as sampling-related sources of variation, such as length-selectivity of fishing gears.

**Section 5** presents an overview of sampling protocols currently in use, covering market sampling, stock surveys and observer programmes, and derives recommended protocols for future sampling.

**Section 6**, finally, summarises the main conclusions, and presents an overview of the recommendations.

The Workshop focused on sampling programmes and raising procedures. Analysis of the biological processes involved in maturation has been considered only marginally. Moreover, fecundity related issues, although included in the DCR programme for a few stocks, were not addressed. Two papers on the effect of variation in maturity on stock assessments (Poulding 1997; Catchpole, 1999) were available, but this topic was not pursued any further.

The information presented is detailed by species and/or stock, where possible, using the information available to the Workshop. Obviously, the details for individual stocks might need to be improved by the assessment working groups concerned, and our recommendations reconsidered in detail by the appropriate Regional Coordination Meetings.

## **2 Species grouping**

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### **2.1 Introduction**

Grouping of species according to its life history traits is a first step in order to develop standard operational procedures for maturity sampling. In this sense, species of the appendix XVI of Commission Regulation 1639/2001 and 1581/2004 are grouping by spawning pattern (batch, total or terminal spawner). Additional biological characteristics as longevity (short or long) and spawning period by species and fishing area are also shown (Table 2.1).

### **2.2 General comments**

In spite of the fact that general biology of the main fishing species is supposed to be well known, it is not so easy to find basic information as this table has shown. For instance, spawning season or slow/fast growth vs. longevity are not a very well known issue and presents a high variability between areas. That is always a point for a discussion; in fact we decided to use longevity instead of slow/fast growth because it is easier to agree on.

### **2.3 Data sources**

The information was obtained through the participants of this workshop, web pages (<http://www.fishbase.org>) and bibliography (Murua and Saborido, 2003; Murua, 2006; Quincoces, 2002). The spawner pattern and spawning season for some species/area is incomplete but this table could be a useful tool for develop the ToR's of this meeting.

### **2.4 Recommendations on species grouping**

- To transmit this table by Internet in order to fill the gaps and improve it.
- To focus the sampling procedure as a function of spawning pattern, short/long spawning period and another variability one should consider is whether the stock is pelagic or demersal fish.
- To collect basic information on reproductive biology, priority on stocks for which information is currently incomplete.

- Improve the knowledge on reproductive strategy based on the histological analyses of the gonads.

**Table 2.1. Summary of the species of the appendix XVI grouping according to its life history traits. Longevity and spawning period are shown by species/area.**

[illegible]

**Tabel 2.1. Continued.**

[illegible]

[illegible]

**Tabel 2.1. Continued.**

[illegible]

**Tabel 2.1. Continued.**

Spawning pattern	Common name	Scientific name	Longevity**	Spawning season												Area	
				Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
ICES areas I, II				Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
Mediterranean (Western Mediterranean Sea only)				Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
Batch spawner	Anchovy	<i>Engraulis encrasicolus</i>	Short													all areas	
	Brown ray	<i>Raja miraletus</i>	Long													1.3, 2.1, 2.2, 3.1	
	Giant red shrimp	<i>Aristeomorpha foliacea</i>	Long													1.3, 2.2, 3.1	
	Gilthead sea bream	<i>Sparus aurata</i>	Long													1.2, 3.1	
	Hake	<i>Merluccius merluccius</i> (*)	Long													1.1, 1.2, 2.2, 3.1, 1.3, 2.1	
	Horse mackerel	<i>Trachurus trachurus</i>	Long													1.1, 1.3, 3.1	
	Mackerel	<i>Scomber spp.</i>	Long													1.3, 2.2, 3.1	
	Mediterranean horse mac	<i>Trachurus mediterraneus</i>	Long													1.1, 1.3, 3.1	
	Norway lobster	<i>Nephrops norvegicus</i>	Long													1.3, 2.1, 2.2, 3.1	
	Red mullet	<i>Mullus barbatus</i>	Long														all areas
	Red shrimp	<i>Aristeus antennatus</i>	Long														1.1, 1.3, 2.2, 3.1
	Sardine	<i>Sardina pilchardus</i>	Short														all areas
	Seabass	<i>Dicentrarchus labrax</i>	Long														1.2
	Sole	<i>Solea vulgaris</i>	Long														1.2, 2.1, 3.1
	Striped red mullet	<i>Mullus surmuletus</i>	Long														all areas
Terminal spawner	Thornback ray	<i>Raja clavata</i>	Long													1.3, 2.1, 2.2, 3.1	
	White shrimp	<i>Parapenaeus longirostris</i>	Short													1.1, 1.3, 2.2, 3.1	
	Common octopus	<i>Octopus vulgaris</i>	Short													all areas	
	Common squid	<i>Loligo vulgaris</i>	Short													1.3, 2.2, 3.1	
	Cuttlefish	<i>Sepia officinalis</i>	Short													1.3, 2.1, 3.1	
Total spawner	Squid	<i>Illex spp.</i> (*), <i>Todarodes spp.</i>	Short													1.3, 2.1, 2.2, 3.1	
	Horned octopus	<i>Eledone cirrhosa</i>	Short													1.1, 3.1, 1.3, 2.1, 2.2	
	Anglerfish	<i>Lophius piscatorius</i>	Long													1.1, 1.3, 2.2, 3.1	
?	Black-bellied angler	<i>Lophius budegassa</i>	Long													1.1, 1.3, 2.2, 3.1	
	Eel	<i>Anguilla anguilla</i>	Long													all areas	
	Albacore	<i>Thunnus alalunga</i>	Long													all areas	
	Atlantic bonito	<i>Sarda sarda</i>	Long													all areas	
	Billfish	<i>Istiophoridae</i>	Long													all areas	
	Bluefin tuna	<i>Thunnus thynnus</i>	Long													all areas	
	Bogue	<i>Boops boops</i>														1.3, 2.1, 2.2, 3.1	
	Caramote prawn	<i>Penaeus kerathurus</i>	Short													3.1	
	Clams	<i>Veneridae</i>														2.1, 2.2	
	Dolphinfish	<i>Coryphaena spp.</i>	Long													all areas	
	Grey gurnard	<i>Eutrigla gurnardus</i>	Long													1.3, 2.2, 3.1	
	Grey mullet	<i>Mugilidae</i>	Long													1.3, 2.1, 2.2, 3.1	
	Mantis shrimp	<i>Squilla mantis</i>														1.3, 2.1, 2.2	
	Musky octopus	<i>Eledone moschata</i>	Short													1.3, 2.1, 2.2, 3.1	
	Pandora	<i>Pagellus erythrinus</i>	Long													1.1, 3.1, 1.2, 2.1, 2.2	
	Picarels	<i>Spicara maris</i>														3.1	
	Picarels	<i>Spicara spp.</i>														1.3, 2.1, 2.2, 3.1	
	Sharks	<i>Shark-like Selachii</i> (*)	Long													all areas	
	Swordfish	<i>Xiphias gladius</i>	Long													all areas	
	Tub gurnard	<i>Trigla lucerna</i>	Long													1.3, 2.2, 3.1	

\* For baltic herring Annual variation

\*\* Long: 3 years plus

### 3 Maturity coding

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The objective of the section was to present an overview of the variety of maturity scales for different stock/areas and species and to what extent the scales were validated with histology. In order to do so, a compilation of all the maturity scales that were available to the Workshop (Annex 2) was collected and a table with different species, the number of maturity stages and if the maturity key is histologically validated or not is presented in Annex 2, finally the Workshop discussed the definitions of some of the stages in the macroscopic maturity scales.

From the discussion the need of keys with precise and clear descriptions of the main characteristics of gonads in different stages or even pictures (see Tomkiewicz *et al.*, 2002 as a good example) became evident in order to minimize mistakes in the assignment of the maturity stages.

An attempt to create a standardized maturity scale for which people could add images for the analyzed species and into which almost the majority of the currently used scales could be converted was made.

The IBTS 4 grade maturity scale was used as a template and the addition of a fifth stage was discussed since recent research has showed that in several species, a substantial part of mature individuals from the younger age classes can omit spawning if energy resources are scarce (Jørgensen *et al.*, 2006) and the current IBTS maturity key does not allow to classify and give an appropriate code to those individuals skipping spawn. This process has been reported amongst other in: Greenland halibut (*Reinhardtius hippoglossoides*; Fedorov, 1971; Walsh and Bowering, (1981) found reabsorption of oocytes among females attempting to mature for the first time, but no evidence for later skipping), sole (*Solea solea*; Ramsay and Witthames, 1996), long rough dab (*Hippoglossoides platessoides*; Bagenal, 1957), Norway pout (*Trisopterus esmarkii*; Gokhale, 1957), winter flounder (*Pleuronectes americanus*; Burton, 1991, 1994), carp (*Cyprinus carpio*; Ivanov, 1971), chub (*Leuciscus cephalus*; Fredrich *et al.*, 2003), perch (*Perca fluviatilis*; Holmgren, 2003), hake (*Merluccius merluccius*; Hickling, 1930), and herring (*Clupea harengus*; Engelhard and Heino, 2005). The terminology we use here (omit spawning) is considered synonymous to skip spawning and artresia (the histological observation), which are also used in literature.

The importance of recording such omitting spawners comes from the fact that if a substantial part of the mature population omit spawning this will have an effect on the spawning stock biomass (SSB) estimate. The SSB is commonly used in ICES management advice in the modelling of the S/R relationship used for projections of the stock and to classify the state of the stock in relation to its biological points of reference ( $B_{pa}$  and  $B_{lim}$ ).

The difficulties in judging some of the stages macroscopically remain, in particular for the ripening stage of the first spawners as well as when omitting spawning occur. Using histology would eliminate these problems but applying histology routinely would be a time-consuming and expensive task.

However, collecting gonad weight and liver weight would clarify uncertainties in judgement in visual staging. Vitale *et al.* (2006) has shown that visual inspection overestimates proportion spawners (Figure 3.1), particularly the first-spawners and using GSI and HSI can improve data considerably at reasonable expense. Quincoces (2002) showed that the analysis of the annual variation of the GSI could help to identify wrong assignments of maturity stages in some species. In Figure 3.2 it is shown that in the Bay of Biscay black Anglerfish females above  $L_{25}$  (mature) showed higher GSI values than the ones showed by the immature females ( $L < L_{25}$ ) for the whole year, especially in the spawning season (May to July).

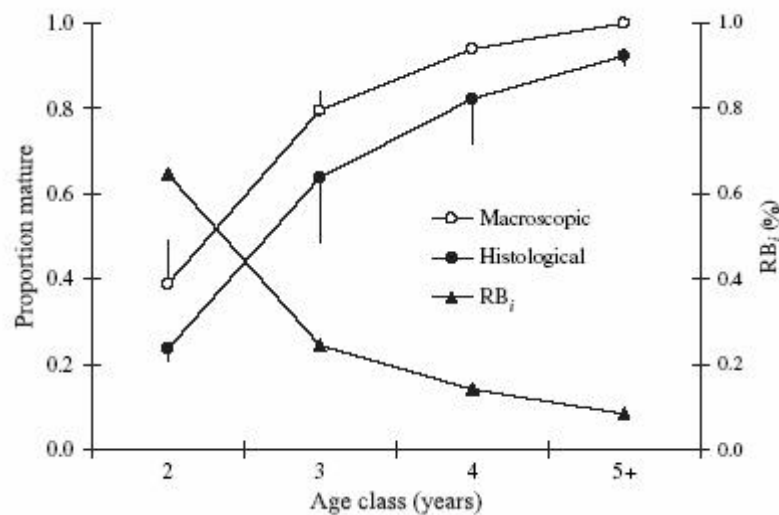


Figure 3.1. Comparison between histological and visual staging of the gonads. Bars represent standard errors. RB<sub>i</sub> is the relative bias per age class. (From Vitale *et al.*, 2006)

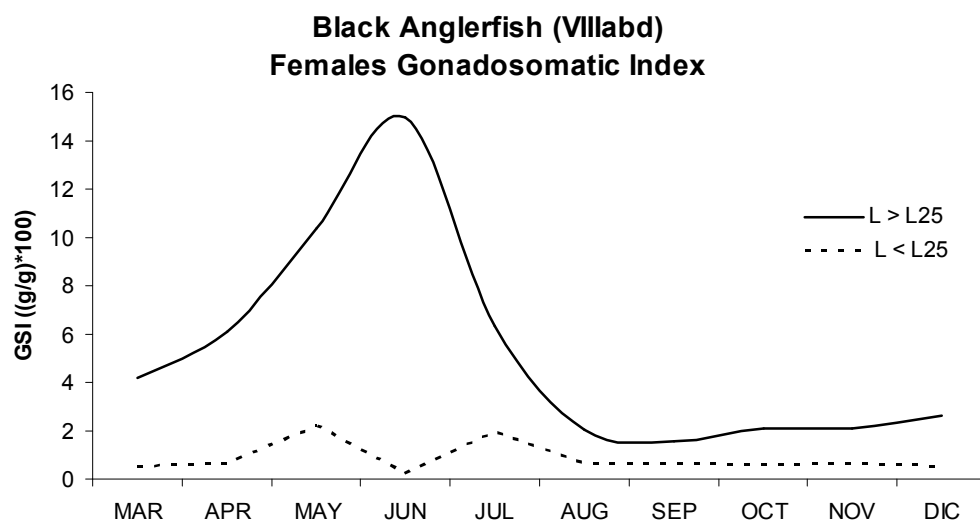


Figure 3.2. Black Anglerfish females Gonadosomatic Index (GSI) in the Bay of Biscay. Modified from Quincoces (2002).

From the collection of national maturity scales was evident that almost all the countries can convert their maturity scales into the one used in the IBTS, although as pointed before, the problems of the omitting spawners and the difficulties in the distinction between the Recovery and Virgin stages for hake led the Workshop to propose a new scale adapted from the one internationally agreed by the IBTS group. Table 3.1 presents the new proposal.

**Table 3.1. Proposed new Five-stage Maturity scale.**

FEMALES	STAGE	MALES	MATURES / IMMATURES
Ovaries translucent without visible oocytes.	IM Virgin	String-like and translucent testes.	Immature
Larger, opaque ovaries, individual opaque/yolk oocytes often visible.	MI Maturing	Larger and grey-whitish testes.	Mature
Even larger ovaries and with translucent/ hydrated oocytes (running).	MA Spawning	Larger white testes with sperm that can be extruded under pressure or visible in the ducts.	Mature
Ovaries slack with residual eggs or already in a recovering stage (lighter colours, smaller and with no oocytes visible).	SP/RE Spent /Recovery	Slack testes and blood stained or already in a recovering stage (no longer blooded, presents ribbon lying aspect).	Mature
Contracted and greyer ovaries.	OS Omitted spawning	Contracted and greyer testes.	Sexually mature individuals not contributing to the SSB in the current year.

In a substantial number of cases, either sex or maturity can not be determined from the observation. It is essential to record this as an unknown sex or maturity, rather than to leave the fish out of the sampling, since omission will bias population estimates in an unpredictable way. It is therefore recommended to augment the scales for sex and maturity with a code for unknowns, and to accommodate for these in the raising procedures.

We recommend using this scale primarily for bony fishes since the workshop is aware of the differences in reproductive strategies among the other major commercial groups of species (elasmobranches, crustaceans and cephalopods). The workshop recommends to the IBTS planning group to reconsider the presently used 4-grade scale and replace it by the proposed 5-grade one.

For species with a clear difference between recovering females and the virgin ones there is no problem with the use of the data for a maturity ogive. On the other hand, in species which are difficult to classify macroscopically (i.e. not clear distinction between recovering and virgin females or identification of the omitting spawning individuals) the workshop recommends the use of GSI, HSI or histological analysis in order to check the accuracy of data before using it with maturity ogive calculation purposes.

Species specific workshops later this year (WKMSHM, WKMSMAC, WKMSCWHS) should also consider the 5-grade scale proposed here.

**Table 3.2. List of fish species (Teleosts and Elasmobranchs) sampled by the commercial fleet and in the surveys for each country member of the WG. MATUR. KEY: number of stages included in the maturity scale for a given species and a given country, HISTOL. VALID.: if the maturity scale has been validated (y) or not (n) with histology, ADDITIONAL DATA: if gonad and liver weight data are routinely used to help the identification of matured individuals (y), occasionally (occas.) or not at all (n).**

SPECIES	COMMON NAME	COUNTRY	STOCK/AREA	MATUR. KEY	HIST. VALID.	ADDITIONAL DATA	
						GONAD	LIVER
BONY FISH							
<i>Aphanopus carbo</i>	Black Sccabardfish	PT	IXa	5	y	n	n
		PT	X (Azores)	5	y	y	y
<i>Argentina sphyraena</i>	Argentine	GER	V, VI	4	n	n	n
<i>Beryx spp.</i>	Alfonsinos	PT	X (Azores)	6	y	y	y
<i>Clupea harengus</i>	Herring	SWE	IIIId, IIIa	8	n	n	n
		IRL	VI, VII	8	n	n	n
		SCO	IV, VI, II	8	n	n	n
		FIN	IIIId, 31, 30, 25 to 29, 32	5	n	n	n
		UK	IV, VIId, VIa, VIIabcj	8	na	n	n
		EST	25 to 29, 32, Gulf of Riga	5	n	n	n
		LAT	25 to 29, 32, Gulf of Riga	6	n	n	n
		LTU	26	6	n	n	n
		NED	IV	4	na	na	na
		DK	IIIaS, IIIb,c, IIIa, IIIb,d	8	y	na	na
		GER	IVa,b,c, VIId	4	n	n	n
		GER	IIIb,c,d	8	n	na	na
<i>Conger conger</i>	European conger	PT	X (Azores)	6	n	y	y
<i>Dicentrarchus labrax</i>	European seabass	UK	All Excluding IX	5	na	n	n
<i>Engraulis encrasicolus</i>	Anchovy	SP	VIII, IXaS	6	y	n	n
<i>Gadus morhua</i>	Cod	IRL	VI, VII	7	y	n	n
		SWE	IIIa, IIIId	4, 5, 8	y	occas.	n
		SCO	IV, VI, Rockall	4	na	na	n
		EST	25 to 32	5	n	n	n
		UK	IV, VIIabedj, VIa,b, VIIb-k, VIII, XII, XIV	5	na	n	n
		GER	IVa,b	4	n	n	n
		GER	IIIb,c,d	8	n	y	y
		LAT	Gulf of Riga, 26	6	n	n	till 1991
		LTU	26	6	n	n	n
		DK	IIIaS, IIIb,c	10	y	y	y
<i>Helicolenus dactylopterus</i>	Blackbelly rosefish	PT	X (Azores)	6	y	y	y
<i>Lepidopus caudatus</i>	Silver scabbardfish	PT	X (Azores)	6	y	y	y
<i>Lepidorhombus boscii</i>	Four-spotted megrim	PT	IXa	5	n	n	n
		SP	VIIIc, IXa	4	n	occas.	?
		SCO	IV, VI, Rockall	4	n	n	n

						ADDITIONAL DATA	
<i>Lepidorhombus whiffiagonis</i>	Megrim	IRL	VI, VII	7	y	n	n
		UK	IV, VII, VIIIabd	5	na	n	n
		PT	IXa	5	n	n	n
		SP	VIIIa,b,c,d, IXa, VI, VIIbk	4	n	occas.	n
		SCO	IV, VI, Rockall	4	n	n	n
<i>Lophius budegassa</i>	Black-bellied angler	IRL	VI, VII	7	n	n	n
		SP	NS (VIIa,b,d, VIIbk), SS	5	y	occas.	n
		UK	IV, Vb, VI, XII, XIV, VII, VIIIabde	5	na	n	n
		PT	IXa	5	y	n	n
		SCO	IV, VI, Rockall	4	n	n	n
<i>Lophius piscatorius</i>	Anglerfish (white)	IRL	VI, VII	7	n	n	n
	Monkfish	SP	NS (VIIa,b,d, VIIbk), SS	5	y	occas.	n
		UK	IV, Vb, VI, XII, XIV, VII, VIIIabde	5	na	n	n
		GER	IVa,b	4	n	n	n
		PT	IXa	5	y	n	n
		SCO	IV, VI, Rockall	4	n	n	n
<i>Melanogrammus aeglefinus</i>	Haddock	IRL	VI, VII	7	y	n	n
		SCO	IV, VI, Rockall	4	na	na	n
		NED	IV	4	na	na	na
		UK	IV, VIa, VIb, VIIa,d, VII, VIII, XII, XIV	5	na	n	n
		DK	IIIaS	4	na	na	na
		SWE	IIIa	4, 8	n	n	n
		GER	IVa,b	4	n	n	n
<i>Merlangius merlangius</i>	Whiting	IRL	VI, VII	7	y	n	n
		SCO	IV, VI	4	na	na	n
		UK	IV, Vb, VI, XII, XIV, VIIa, VIIb-k, VIII	5	na	n	n
		DK	IIIaS	4	na	na	na
		NED	IV	4	na	na	na
		GER	IVa,b	4	n	n	n
<i>Merluccius merluccius</i>	Hake	SP	NS, SS (VIIc, IXa)	4	y	occas.	n
		IRL	VI, VII	7	y	n	n
		UK	IIIa, IV, VI, VII, VIIIab, VIIIc, Ixa	5	na	n	n
		PT	IXa	4	y	y	n
		SCO	IV, VI	4	n	n	n
<i>Micromesistius poutassou</i>	Blue-whiting	IRL	VI, VII	7	n	n	n
		PT	IXa	5	y	n	n
		SP	IXa, VIIIb,c	6	n	?	n
		SCO	IV, VI	4	n	n	n
		GER	IVa and Vb, VI,	4	n	n	n

						ADDITIONAL DATA	
			VII, XII, XIV				
<i>Microstomus kitt</i>	Lemon sole	UK	IV, VIId	5	na	n	n
		GER	IVa,b	4	n	n	n
<i>Molva dypterygia</i>	Blue ling	PT	X (Azores)	6	n (in progr.)	y	y
<i>Molva molva</i>	Ling	UK	All Areas (Western)	5	na	n	n
<i>Pagellus acarne</i>	Axillary seabream	PT	X (Azores)	6	y	y	y
<i>Pagellus bogaraveo</i>	Blackspot seabream	SP	IXa, VIIIc	5	n	n	n
		PT	X (Azores)	6	y	y	y
<i>Phycis phycis</i>		PT	X (Azores)	6	y	y	y
<i>Pleuronectes flesus</i>	Flounder	EST	32	5	n	n	n
<i>(Platichthys flesus)</i>		GER	IIIb,c,d	4	n	n	n
<i>Pleuronectes platessa</i>	Plaice	IRL	VII	7	y	n	n
		SWE	IIIa	4, 8	n	n	n
		UK	VIIa,d, e, f, g	5	na	n	n
		BEL	IV, VIId, VIIa, VIIf,g	7	n	y	n
		NED	IV	4	na	na	na
		DK	IIIaS	4	na	na	na
		SCO	IV, VI	4	n	n	n
		GER	IVa,b	4	n	n	n
<i>Pollachius virens</i>	Saithe	IRL	VI, VII	7	n	n	n
		SWE	IIIa	4, 8	n	n	n
		UK	Vb, VI, XII, XIV, IV, VIId	5	na	n	n
		DK	IIIaS	4	na	na	na
		SCO	IV, VI, Rockall	4	n	n	n
		GER	IVa,b	4	n	n	n
<i>Polyprion americanus</i>	Atlantic wreckfish	PT	X (Azores)	6	n (in progr.)	y	y
<i>Psetta maxima</i>	Turbot	GER	IVa,b	4	n	n	n
<i>Sardina pilchardus</i>	Sardine	SP	VIIIc, IXa	6	y	n	n
		PT	IXa	6	y	y	occas.
		GER	VII, VIII	4	n	n	n
<i>Scomber japonicus</i>	Spanish mackerel	PT	IXa	6	n	y	n
		PT	X (Azores)	6	y	y	y
<i>Scomber scombrus</i>	Mackerel	IRL	VI, VII	6	y	n	n
		UK	II, IIIa, IV, V, VI, VII, VIII, IX (exc. VIIIc, IXa)	6	na	n	n
		PT	IXa	6	n	y	n
		NED	na	4	na	na	na
		DK	IIIaS	4	na	na	na
		SP	IXa, VIII	6	y	n	n
		SCO	IV, VI	6	n	n	n
		GER	IVa,b and IVa, VI, VIII	4/6 (MEGS)	y	y	y
<i>Scophthalmus rhombus</i>	Brill	GER	IVa,b	4	n	n	n
<i>Sebastes spp.</i>	Redfish	GER	V and XIVb	6	n	n	n
<i>Solea senegalensis</i>	Senegalese sole	PT	IXa	5	n	y	n
<i>Solea solea</i>	Sole	IRL	VI, VII	7	y	n	n

						ADDITIONAL DATA	
		PT	IXa	5	y	y	y
		UK	VIIId, VIIa, fg, e, hjk	5	na	n	n
		BEL	IV, VIIId, VIIa, VIIIf,g, VIIla,b	7	n	y	n
		NED	IV	4	na	na	na
		GER	IVb,c	4	n	n	n
<i>Sprattus sprattus</i>	Sprat	EST	22 to 32	5	n	n	n
		LAT	25 to 29, 32	6	n	n	n
		LTU	26	6	n	n	n
		DK	IIIaS, IIIb,c, IIIa, IIIb,d	4	y	na	na
		FIN	IIIId	5	n	n	n
		SWE	IIIa	8	n	n	n
		GER	IVb,c	4	n	n	n
		GER	IIIb,c,d	8	n	n	n
<i>Trachurus picturatus</i>	Blue jack-mackerel	PT	X (Azores)	10	y	y	y
<i>Trachurus trachurus</i>	Horse-mackerel	IRL	VI, VIII	9	y	n	n
		SP	IXa, VIII	6	y	n	n
		UK	IV, VIIId	6	na	n	n
		PT	IXa	6	Y	Y	N
		NED	na	4	na	na	na
		SCO	IV, VI	4	n	n	n
		GER	IVa,b and VI, VII, VIII	4/6 (MEGS)	y	y	y
<i>Trisopterus esmarkii</i>	Norway Pout	SCO	IV, VI	4	n	n	n
		GER	IVa,b	4	n	n	n
		UK	IV	5	na	n	n
		DK	IIIaS	4	na	na	na
		SWE	IIIa	4	n	n	n
<i>Trisopterus luscus</i>	Pouting, Bib	SP	VIIIc, IXa	4	n	n	n
		PT	IXa	5	n (in progr.)	y	na
ELASMOBRANCHS							
<i>Leucoraja naevus</i>	Cuckoo Ray	IRL	VI, VII	6F/4M	n	n	n
		PT	IXa	6F/4M	y	n	n
<i>Raja brachyura</i>	Blonde Ray	IRL	VI, VII	6F/4M	n	n	n
		PT	IXa	6F/4M	y	n	n
<i>Raja clavata</i>	Thornback Ray	IRL	VI, VII	6F/4M	n	n	n
		PT	IXa	6F/4M	y	n	n
<i>Raja montagui</i>	Spotted Ray	IRL	VI, VII	6F/4M	n	n	n
		PT	IXa	6F/4M	y	n	n
<i>Raja undulata</i>	Undulate Ray	PT	IXa	6F/4M	y	n	n
Rajidae	Rays (various)	UK	all areas	4	na	n	n
Rajidae	Skates (various)	SCO	IV, VI, Rockall	4	n	n	n
Selachimorpha	Sharks (various)	PT	IXa	8F/4M	y	n	n

**Table 3.3. List of the maturity keys used by the different countries and for each species or group of species (Teleosts and Elasmobranchs).**

COUNTRY	CODE	SPECIES	NATIONAL MATURITY SCALES	REFERENCE
Belgium	BEL	Plaice, Sole	7-stage	Annex 2, Figure A2.1
Denmark	DK	Herring	8-stage	Annex 2, Figure A2.2
		Cod	10-stage	Annex 2, Figure A2.3
		Other pelagic and demersal fish	4-stage	IBTS Annex 2, Figure A2.4
Estónia	EST	All pelagic and demersal fish	5-stage	BITS Annex 2, Figure A2.5
Finland	FIN	Herring, Sprat	5-stage	BITS Annex 2, Figure A2.5
Germany	GER	Herring, Sprat	4-stage (North Sea, NEA)	IBTS Annex 2, Figure A2.4
		Herring, Sprat	8-stage (Baltic Sea)	Modif. from Heincke 1898 (BITS Manual)
		Cod	4-stage (North Sea, NEA)	IBTS Annex 2, Figure A2.4
		Cod	8-stage (Baltic Sea)	Modif. from Maier (1908) (BITS Manual)
		Redfish	6-stage	Annex 2, Figure A2.6
		Mackerel, Horse-mackerel	6-stage (surveys), 4-stage (market)	Walsh et al. 1990 (surveys), IBTS Annex 2, Figure A2.4 (market)
		Other pelagic and demersal fish	4-stage	IBTS Annex 2, Figure A2.4
Ireland	IRL	Herring	8-stage	Annex 2, Figure A2.7
		Gadoids	7-stage	Annex 2, Figure A2.8
		Flatfish	7-stage	Annex 2, Figure A2.9
		Horse-mackerel	9-stage	Annex 2, Figure A2.10
		Mackerel	6-stage	Annex 2, Figure A2.11
		Anglerfish	7-stage	Annex 2, Figure A2.12
		Rays and skates	6-stage	Annex 2, Figure A2.13
Latvia	LAT	Herring, Cod, Sprat	6-stage	Annex 2, Figure A2.14
			converted to 5-stage	BITS Annex 2, Figure A2.5
Lituânia	LTU	Herring, Cod, Sprat	6-stage	Annex 2, Figure A2.14
			converted to 5-stage	BITS Annex 2, Figure A2.5
Netherlands	NED	All pelagic and demersal fish	4-stage (before 7-, 8-, and 9-stage)	IBTS Annex 2, Figure A2.4
Portugal	PT	Megrim	5-stage	Annex 2, Figure A2.15
		Anglerfish	5-stage	Annex 2, Figure A2.16
		Hake	4-stage	Annex 2, Figure A2.17
		Blue-whiting	5-stage	Annex 2, Figure A2.18
		Sardine	6-stage	Annex 2, Figure A2.19
		Horse-mackerel, Mackerel, Spanish Mackerel	6-stage	Annex 2, Figure A2.20
		Blue jack-mackerel (Azores)	10-stage	Adapted from Annex 2, Figure A2.21
		Flatfish	5-stage	Annex 2, Figure A2.22
		Pouting	5-stage	Annex 2, Figure A2.23
		Black Scabbardfish	5-stage	Annex 2, Figure A2.24
		Deep-water sharks	8-stage (♀), 4-stage (♂)	Annex 2, Figure A2.25

COUNTRY	CODE	SPECIES	NATIONAL MATURITY SCALES	REFERENCE
		Rays	6-stage (♀), 4-stage (♂)	Annex 2, Figure A2.26
		Other fish sampled in the Azores	6-stage	Annex 2, Figure A2.27
Spain	SP	Pelagic fish	6-stage	Annex 2, Figure A2.28
		Demersal fish	4-stage	Annex 2, Figure A2.29
		Anglerfish	5-stage	Annex 2, Figure A2.30
		Hake (AZTI)	4-stage	Annex 2, Figure A2.31
		Teleosts	8-stage (Mediterranean surveys)	Annex 2, Figure A2.32
		Eslamobranch fish	5-stage	Annex 2, Figure A2.33
Sweden	SWE	Herring, Sprat	8-stage	Annex 2, Figure A2.34
		Cod	4-stage	Annex 2, Figure A2.35
			5-stage	Annex 2, Figure A2.35
			8-stage	Annex 2, Figure A2.35
		Other Gadoids and Flatfish	4-stage	Annex 2, Figure A2.35
			8-stage	Annex 2, Figure A2.35
		Norway Pout	4-stage	IBTS Annex 2, Figure A2.4
United Kingdom	UK	Herring	8-stage	Annex 2, Figure A2.36
		Gadoids	5-stage	Annex 2, Figure A2.37
		Anglerfish	5-stage	Annex 2, Figure A2.38
		Other demersal species	5-stage	Annex 2, Figure A2.37
		Horse-mackerel	6-stage	Annex 2, Figure A2.39
		Mackerel	6-stage	Annex 2, Figure A2.40
		Flatfish	5-stage	Annex 2, Figure A2.37
		Rays	4-stage	Annex 2, Figure A2.41
UK - Scotland	SCO	Herring	8-stage	Annex 2, Figure A2.2
		Mackerel	6-stage	Walsh et al. 1990
		All demersal fish	4-stage	IBTS Annex 2, Figure A2.4
		Elasmobranch	4-stage	Not available

## 4 Sources of Variation

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### 4.1 Introduction

There are a large number of considerations regarding the design of maturity sampling programmes. This chapter introduces the main problems, providing real examples where possible.

A consistent problem with maturity-at-age data is that even datasets that are quite large tend to become sparse after disaggregating by length, age, sex, maturity, area and/or fleet. Therefore, it is essential that data from different countries are combined before maturity data can be analysed, in order to obtain suitable sample sizes. It is preferable to produce one maturity ogive from combined data than to combine a number of maturity ogives from various countries.

Presently, maturity data are not collected in a coordinated way. Differences between the stratifications used by various institutes will result in problems with raising the combined data. Data collection will need to be coordinated and there is a need to assign some sort of 'stock coordinator' to collect and combine data from various sources in advance of any workshops, as it is not possible to perform this task during a workshop due to time constraints.

In this chapter, the suitability of various data sources are investigated and sources of variation described, citing practical examples or existing literature, where available, leading to a checklist of the main considerations concerning sampling design.

### 4.2 Data requirements

For species-specific workshop or other bodies to set up co-ordinated sampling programs for maturity data, it is essential to know the requirements from the assessment process. Therefore the workshop compiled a checklist (below) to be filled in by stock co-ordinators which can be included in a stock annex.

Questions A to C are general whereas questions 1 to 9 require a specific answer. The clarification is hoped to be as extensive as possible to avoid confusion about the essence of the requirements.

#### **Question 1: Sex specific or combined**

The Workshop examined the stock annexes and found that most maturity ogives were sex-combined. This can be due to lack of data or due to the particularities of the assessment process. Therefore the workshop asks what essential information is required to better incorporate maturity data.

#### **Question 2: Maturity at length or age**

Does the maturity ogive have to be compiled by age or length? Currently most maturity data are collected during sampling for age-length keys. Getting accurate maturity ogives at age is not straightforward but essential if it is required for the assessment process.

#### **Question 3: Sensible age/length range**

When landing practices allow obtaining maturity information, market sampling is a cost effective way of data collection. Minimum landing sizes often make it difficult to collect data for the younger ages/lengths where the maturity ogive has the highest variation. Is the maturity data essential for all the ages or can there be a smaller age/length range because of the particularities of the assessment process. At some age/length, the proportion mature levels

off. Defining this top range can avoid collecting excessive non-informative data for all-mature age/length classes, e.g. plus-group.

#### **Question 4: Different maturity keys per sub-area**

Is there evidence in the compiled data sets of the co-ordinator that different maturity ogives per sub-area are essential in the assessment process .

#### **Question 5: Data to reflect the landings or the population.**

Surveys are an expensive way to collect data reflecting the population rather than the landings, but does the assessment process use the full potential of that information, e.g. only as a tuning fleet.

#### **Question 6: fleets**

If "landings" is preferred, it is needed to specify if the information has to be extensive by fleet and what fleets are of the essence.

#### **Question 7: sensible minimum update frequency**

The workshop found that many ogives are fixed, knife-edge, etc and that for many stocks they have not been updated recently. Therefore a pragmatic and sensible update frequency can avoid non-essential efforts.

#### **Question 8: Minimum length of the time series**

Can the maturity data be incorporated immediately or does a time series have to be built and for how many years.

#### **Question 9: Raising procedures**

Raising procedures prove to be often problematic not only for a single dataset but also when combining datasets from different sampling schemes. The stock co-ordinators are asked to describe the current raising procedures but also to give guidance on future possibilities/modifications which are to be avoided or favoured when better incorporating maturity in the assessment process. The bodies setting up co-ordinated programs should also pay extreme attention with regards to the raising procedures inherent to their proposed sampling scheme.

### **4.3 Data Sources**

#### **4.3.1 Market sampling**

In order to be suitable for market sampling, species need to be landed with gonads intact and landings need to include a size range that has both mature and immature fish. Some flatfish species meet these criteria, small haddock and whiting are sometimes landed round which might also make them suitable. Species like sea bass or tuna are also generally landed round, but the cost of obtaining samples might be prohibitive. Many other species are landed gutted, making them unsuitable for maturity determination. Additionally, the size range of the landings often consist mainly of mature fish, making maturity analysis impossible.

Advantages of market sampling include the availability of large numbers of samples and the timing of sampling is easy to control.

Disadvantages include the lack of detailed spatial information. As the proportion of mature fish can vary with location, lack of knowledge on the catch locations can result in biased estimates (see also section 4.4.3. Spatial variation)

### 4.3.2 Surveys

Species that are caught in large enough numbers by on surveys might be suitable for maturity analysis. Combining data from different counties/years might result in useful data for species that are caught in low numbers.

Advantages of surveys include the fact that sampling parameters are controlled and the availability of detailed spatial information which can be used to avoid bias if the stock is not uniformly distributed. Additionally it is possible to collect more detailed biological information on surveys (liver weight, histological samples etc).

Disadvantages include the high cost of surveys, and low sample numbers for some species. Surveys also tend to be targeted at a number of species at the same time, which makes it difficult to optimise the sampling strategy. Existing surveys often take place during a time of year that is not suitable for maturity sampling.

### 4.3.3 Observers

Observer trips offer good potential for maturity data collection. The observer has access to both the landed and discard component of the catch and information on the catch locations. While the workload on most observer trips does not allow for additional sampling, dedicated maturity sampling trips might be a cost-effective alternative to surveys. Presently, most countries are not using this potential source of maturity data.

### 4.3.4 Self-sampling

Self-sampling is unlikely to be viable option due to legal problems with landing under-sized fish. However, it can be useful to request fish to be landed without being gutted for analysis in the port or lab if enough immature fish exceed the minimum landing size. It should be noted that the quality of the gonads deteriorates quickly; it is more difficult to stage fish that are not fresh

## 4.4 Factors influencing accuracy and precision

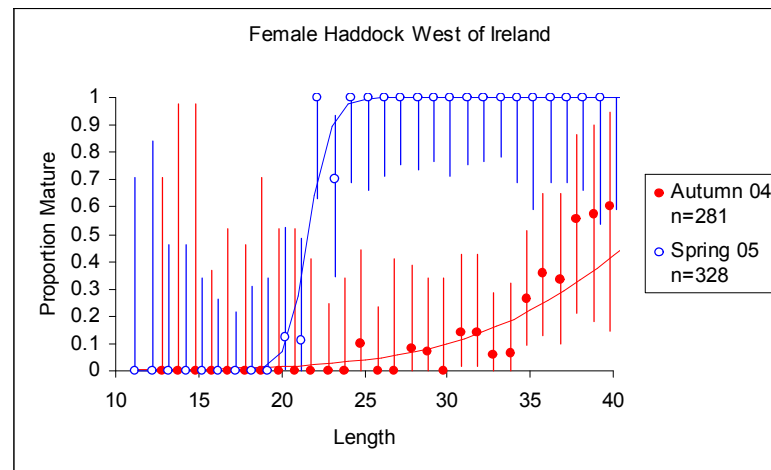
### 4.4.1 Time of year

When gonads are recovered after spawning, they are often indistinguishable from virgin gonads, even by histological analysis. Therefore the timing of sampling needs to take into account when it is possible to distinguish fish that will spawn or have recently spawned from fish that are virgins and will not spawn in the current season. The highest agreement between macro- and microscopical analysis of gonads tends to be just before the peak of spawning (Tomkiewicz *et al.*, 2003; Vitale *et al.*, 2006). However, the gonads of some species take a long time to develop so it is possible to determine well before the spawning season whether they are likely to take part in spawning. Similarly, some species might show signs of previous spawning activity after the spawning season has ended.

When sampling outside the spawning season is possible, it has to be considered which question is being addressed: is the fish likely to spawn in the next season, or: is the fish likely to have spawned in the previous season. For example: a two-year old fish, sampled in autumn, which looks like it will spawn in the next spring should count towards the proportion of mature three-year-olds. However, if this fish shows signs of previous spawning, it should count towards the proportion of mature two-year-olds.

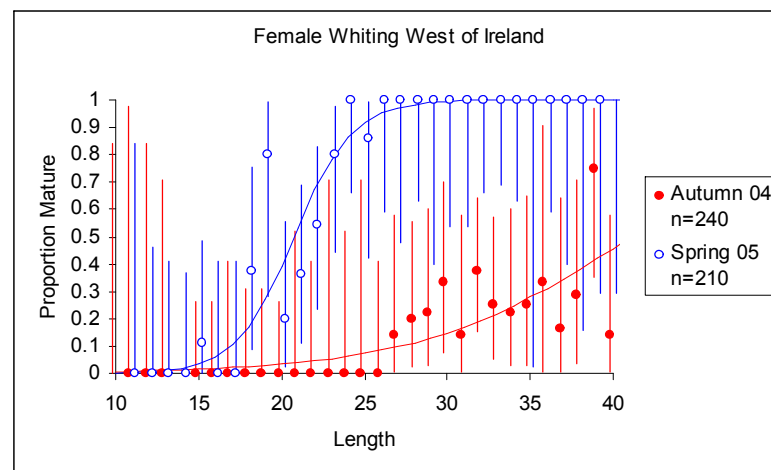
In order to illustrate the importance of the timing of sampling, survey data will be used that was collected in the same region but in different seasons. In the autumn of 2004, maturity data were collected on the Irish Groundfish Survey (IBTS) in the area to the west of Ireland (VIIb and VIIj). In spring of the next year, the same area was sampled again on the Irish Biological

Sampling Survey. As growth is limited during winter, this dataset offers the opportunity to compare maturity ogives collected outside the spawning season with ogives collected during the spawning season. If it is possible to accurately determine whether fish are likely to spawn in the following season during the autumn, the maturity ogives from the two surveys should be similar.



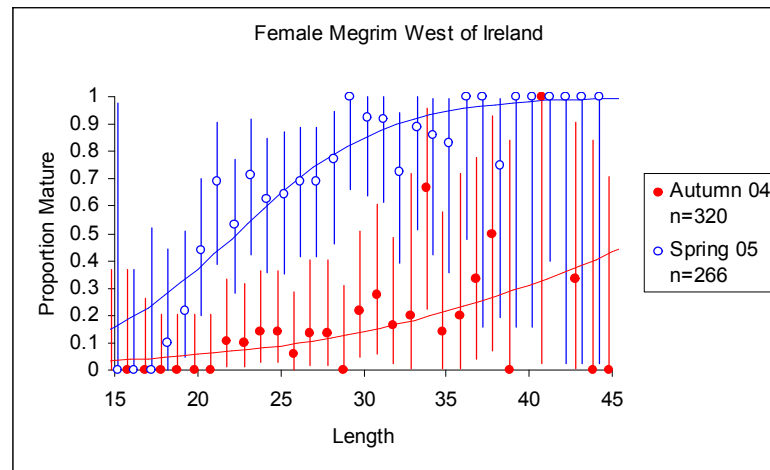
**Figure 4.1. Sampling for maturity in different seasons for haddock.**

It is clear from Figure 4.1 that it was not possible to determine maturity of haddock in autumn. Only the largest fish were assigned as being mature in autumn, smaller, mature fish were indistinguishable from virgin fish. The error bars represent 95% confidence intervals for the proportions mature.



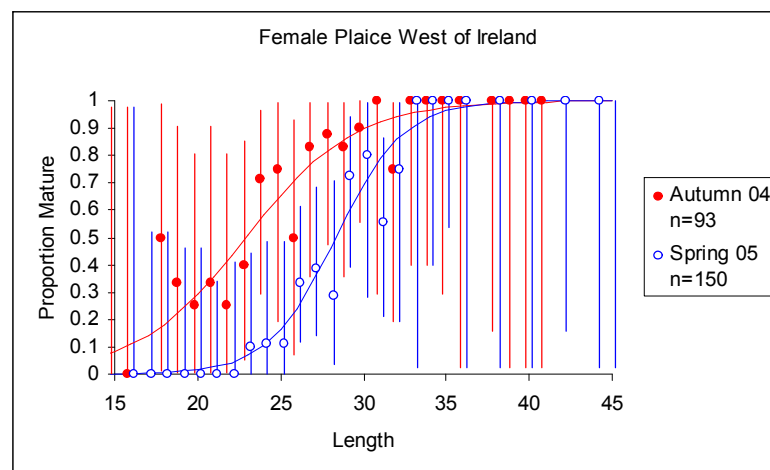
**Figure 4.2. Sampling for maturity in different seasons for whiting.**

*Whiting (figure 4.2) show a similar pattern to haddock.*



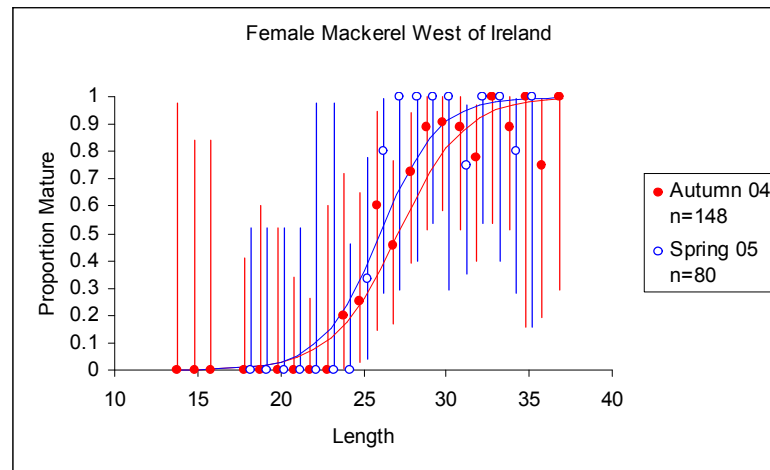
**Figure 4.3. Sampling for maturity in different seasons for megrim.**

*The maturity stage of megrim (Figure 4.3) cannot be determined accurately in autumn either.*



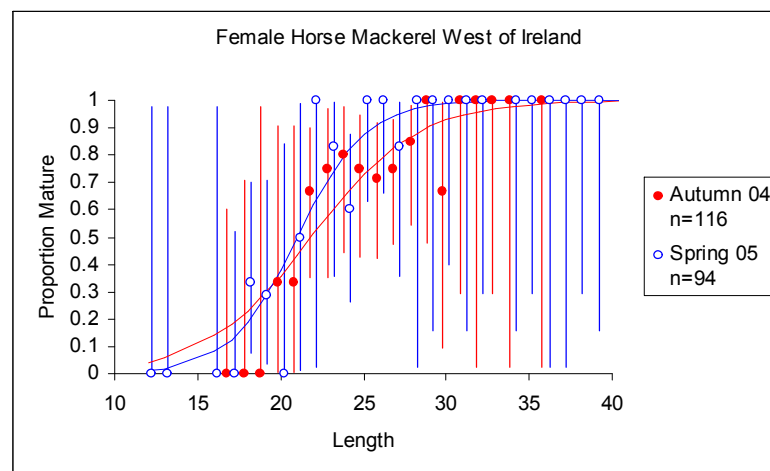
**Figure 4.4. Sampling for maturity in different seasons for plaice.**

*It is possible that information on maturity of plaice (Figure 4.4) from the autumn survey can be useful. However, the difference of around 5cm between the maturity ogives is unlikely to be explained by growth between autumn and spring. It is more likely that the smaller fish start showing signs of maturation later in the season.*



**Figure 4.5. Sampling for maturity in different seasons for mackerel.**

*The maturity ogives of mackerel (Figure 4.5) are very similar between the two surveys, suggesting that maturity might be determined in autumn.*



**Figure 4.6. Sampling for maturity in different seasons for horse mackerel.**

*The maturity ogives of horse mackerel (Figure 4.6) are also very similar between the two surveys, suggesting that maturity of horse mackerel might also be determined in autumn. More detailed analysis is necessary to confirm or reject these findings.*

#### **4.4.2 Inter-annual variation**

Inter-annual variation is known to exist, e.g. in the Bothnian Sea herring: the proportion mature seems to be inversely correlated with recruitment (unpublished data, Finn. Game & Fish. Res. Inst.)

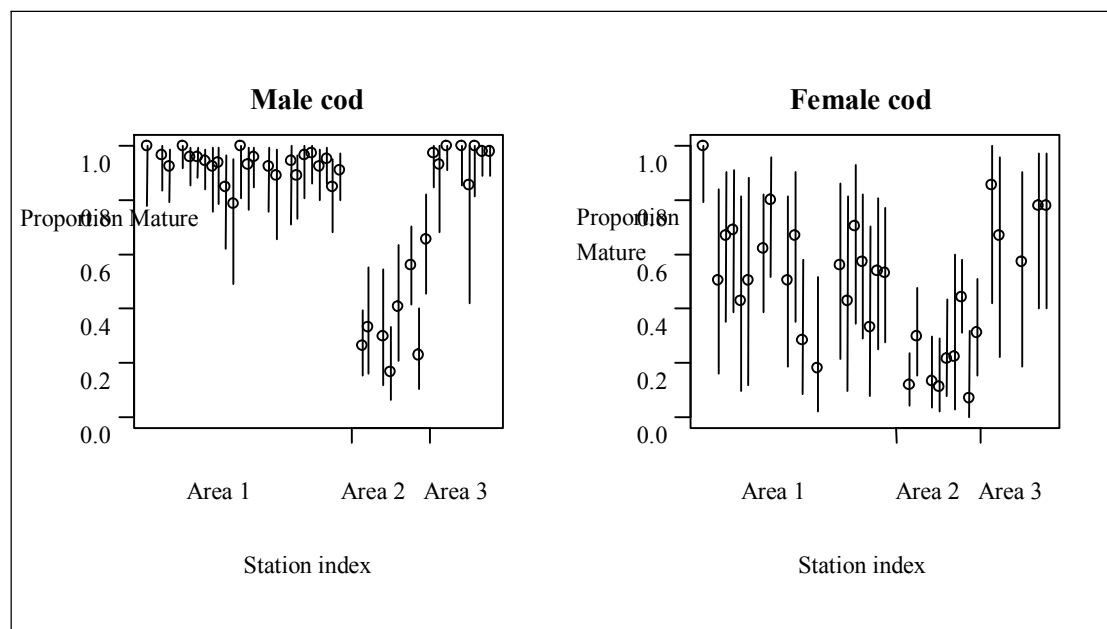
Trends in maturation have been widely reported in the literature (e.g. Oosthuizen and Daan, 1974; Hubold, 1978; Beacham, 1982; Ponomarenko and Yaragina, 1995; Trippel *et al.*, 1997; Cardinale and Modin, 1999; Rochet, 2000; Gerritsen *et al.*, 2003; Armstrong *et al.*, 2004a, 2004b; Olsen *et al.*, 2005).

Because trends in maturity are known to exist, the inter-annual variability for each stock would need to be evaluated in order to determine whether a three-yearly cycle of updating maturity information is appropriate. It needs to be noted that variation between years can be a consequence of sampling variability or real changes in the population. Therefore, frequent

updates of maturity ogives might improve the accuracy but deteriorate the precision of sss estimates.

#### 4.4.3 Spatial variation

In many cases, only mature fish migrate to spawning grounds while immature fish of the same age/length remain on the feeding grounds (e.g. Armstrong *et al.*, 2004b). This can result in biased sampling if spatial coverage is incomplete. If spatial patterns exist, it is essential to sample both the spawning and non-spawning areas. Use of landings data might not be appropriate in these cases if detailed spatial information is not available.



**Figure 4.7. Sampling for maturity in different areas.**

Figure 4.7 illustrates the proportion of mature two-year-old cod in three areas of the Irish Sea over the years 1992–2002. Area 2 is a non-spawning area, while areas 1 and 3 are spawning areas. The proportion of mature two-year olds was higher in the spawning areas, particularly for males (Based on Armstrong *et al.*, 2004b)

#### 4.4.4 Stratification

Stratification by fleets/gear types; area; time of year; sex, age, length etc. tends to result in datasets with low numbers in each cell. In certain cases it can be shown that it is not necessary to maintain all levels of stratification:

- Whiting in the Irish Sea appear to spawn throughout the area and no spatial patterns in maturity are apparent (Gerritsen *et al.*, 2003). In this case it is not necessary to provide complete spatial coverage to obtain unbiased data.
- In some cases, the main factor determining maturity is length and age does not play a significant role after length has been taken into account. In these cases, it is not necessary to reduce the dataset to a Maturity-Age-Length-Key, instead, a length-ogive can be applied directly to estimated length distributions-at-age, reducing the requirements for high sample sizes. However, in most cases, within each length class, the age of individuals will influence their likelihood of being mature (see also example in Section 5).
- Length stratified sampling is common for biological parameters; however in certain cases random sampling might circumvent many problems with raising the data.

Problems with raising data from different sources arise if stratification varies between institutes. Data need to be weighted correctly to take account of total numbers in population in relation to their distribution. E.g. large number of samples might be from a small area that might not be representative (presentations given during the workshop indicated that biological sampling is often concentrated in specific areas, while other areas are ignored).

#### **4.4.5 Sample size**

Sampling strategies and targets on surveys and in the ports are often determined by the requirements for age sampling. Maturity information is often simply collected for the fish that are aged. However, the sampling levels that are set for age samples are often not appropriate for maturity analysis. As maturity data is of limited value if the fish are not aged, the sampling levels for otolith samples might need to be increased during the maturity sampling period. This will have unavoidable cost implications.

#### **4.4.6 Number of samples**

Fish that are caught together tend to be more similar to each other than to fish in the population (Pennington *et al.*, 2002). The variability between hauls can make up a considerable amount of the total variability. Precision levels are therefore determined by both the number of samples (number of hauls or sampling locations) and the number of individuals sampled (sample size). Increases in the sample size, therefore do not necessarily result in significant improvements in precision if the number of sampling locations does not increase.

#### **4.4.7 Gear selectivity**

In some cases, certain gear types might select for fish in certain maturity stages. Gillnets, for example, select fish by their girth, which is larger for ripe fish. Certain gear types (or discard practices) might also select fish in sizes that are all mature or all immature.

#### **4.4.8 Differences between the sexes**

Differences in length and age at first maturity between the sexes are common, however most assessments are carried out for the combined sexes, even if significant differences in growth, mortality and maturity are known to exist (e.g. megrim, ICES, 2006b). Maturity data need to be weighted by the sex ratio if they are to be used in a single-sex assessment. In many cases, however, a female-only SSB estimate might be a more accurate measure of reproductive potential (Marshall *et al.*, 2003; Marshall *et al.*, 2006). As maturity data are always available by sex, they can be supplied to working groups as such, if desired.

#### **4.4.9 Ageing error**

Ageing errors will have obvious consequences for maturity-at-age estimates. Recently, there have been a number of ageing workshops addressing ageing errors (Anon., 2002; Egan *et al.*, 2004; Duarte *et al.*, 2005; Easey *et al.*, 2005; Worsøe Clausen *et al.*, 2005).

#### **4.4.10 Variability in assigning maturity stages**

Experiments investigating the variability in assignment of maturity stages using fresh material, indicated that the mature and immature fish were generally distinguished consistently, however individual maturity stages were not assigned consistently, even when the one person assigned the same fish twice (Gerritsen and McGrath, 2006). These results do not invalidate the need for maturity scales with more than two stages, as there is much information to be gleaned from scales that distinguish e.g. ripening, ripe, running and spent fish, even if these categories are subject to variability.

#### 4.5 Checklist for maturity sampling

The main considerations for sampling maturity data are listed below:

- Does the maturity ogive vary significantly between years? If so annual sampling / updating might be necessary.
- Is the timing of the sampling suitable for distinguishing mature fish from immature fish?
- Does the sampling method select an appropriate size range of fish: including both mature and immature fish? If the landings only contain very small numbers of immature fish, maturity data are meaningless. On the other hand, if the assessment does not include the youngest age classes, it might not be necessary to sample these.
- Is it possible to obtain suitable sample numbers? Surveys might not provide large enough sample numbers for certain species, while market samples might be too costly for certain species like tuna or sea bass.
- Does the fleet or gear type select for a certain stage in the life history? Gillnets select fish by their girth, which might be related their maturity stage. Different fleets might target different components of the stock.
- Is the stock uniformly distributed in space? If spawning takes place in localised areas, it is necessary to sample both on and off the spawning grounds. Information on the sampling locations is then essential and commercial data might be unsuitable.
- Are there differences between the sexes? If so, maturity ogives need to be weighted by the sex ratio, or presented separately by sex.
- Is it necessary to sample in a length-stratified way? Random sampling avoids many problems with raising the data, but might require higher sample sizes than length-stratified sampling.

#### 4.6 Conclusions on sources of variation in maturity sampling

- The main considerations when sampling for maturity are:
  - sampling period
  - sampling locations
  - sampling levels

For many stocks, commercial data are not appropriate and existing surveys are in the wrong period, in those cases the workshop suggests setting up specific maturity sampling schemes if maturity data are required (new surveys or dedicated observer trips). This will have financial implications.

- The workshop suggests that coordination of maturity data collection should take the form of targeting specific stocks in certain years that are sampled by all countries concerned at the same time in the same way. This approach has worked well for species like *Nephrops* and mackerel/horse mackerel.
- It is important to receive input from the end-users of the maturity data. Future workshops need a detailed description of the requirements from stock assessment working groups. The workshop suggests that in the stock annex of the assessment working groups Table 4.1 is included to illustrate the requirements (if any) on maturity data:

**Table 4.1 Checklist data requirements for stock assessment coordinators.**

	STOCK		
		requirements	clarification
a	is maturity data needed		
b	is maturity data sufficient at present		
c	will improved maturity data improve the assessment model		
1	sex specific or combined		
2	at length or at age		
3	sensible age/length range (min-max)		
4	different maturity keys per subarea		
5	data to reflect the landings (fleets) or the population		
6	Fleets		
7	sensible minimum update frequency		
8	minimum length of the data series		
9	raising procedures		

## 5 Overview of current collection and usage of maturity information

Available data on maturity sampling, raising and the use in the assessment were compiled and analysed during the workshop.

### 5.1 Maturity sampling from stock surveys

Table 5.1 (*Survey timing and maturity sampling.xls*) provides an overview of maturity sampling activities in the different countries. Listed are the stocks and countries (ex. Herring in SD 25-29, 32). Table 5.1 (sampling on surveys) lists if there is a survey conducted during the spawning season, the name of the surveys and the sampling procedures used, samples collected in a simple random way or length stratified.

Some countries provide data for maturity ogives from surveys, both within and outside the spawning season. Data collected outside spawning season can be biased due to difficulties in distinguishing between immature fish and potential spawners. This can bias the SSB estimation.

Maturity data obtained outside the spawning season may be useful for additional biological information.

It was the opinion of the Workshop that the collection of maturity data for assessment purposes for a given stock, in most instances, was best undertaken either pre or during the spawning period.

Using the data available to the Workshop, each cruise was looked at in relation to cruise timing and spawning time (where known) for those species listed in Appendix XVI of the current DCR. Expertise at the Workshop limited this exercise to surveys in the following areas:

ICES AREA III inc. Baltic

NORTH SEA & EASTERN CHANNEL & Area II

NE ATLANTIC AREA & WESTERN CHANNEL

Expertise in relation to cruises in the Mediterranean was restricted to the North West of the area and was insufficient for the purposes of the exercise.

If a species detailed in Appendix XVI was given as a target species for a cruise it was thought that, given collaboration internationally, it should be possible to provide precise estimates of stock maturity for assessment purposes. Cruise timing was then compared to the spawning periods for other species to indicate where the possibility to collect maturity data during the appropriate period existed. The number of individuals these samples may provide for maturity sampling is not fully available for quantification but for some stocks, turbot and rays for example, will still be too low to provide precise maturity data without input from other sources such as market and on-board sampling.

## 5.2 Maturity sampling from commercial fisheries (market and onboard)

Table 5.2 (*maturity from market sampling.xls*) includes information on maturity samples collected by market samplings as well as to samples onboard by observers, sampling frequency, the spatial coverage, and if market samples have been collected on a fleet basis and the stratification used in the collection.

Temporal and spatial pattern is differs by country. This ranges from monthly sampling by sub division to annual sampling by stock. If samples are collected only from fisheries in the peak spawning season when stocks tend to aggregate a bias to a high proportion of mature fish will be obtained. Because all countries do not cover all fleets, sampling could lead to a bias due to gear selectivity. Only a few countries are carrying out onboard samplings which may cover a broader length range.

The maturity data collected through market sampling was generally too sparse when disaggregated spatially and temporally at age to undertake a robust analysis for any stock. These data were for the period 2003–2005. The data were collected in order to produce Age Length Keys, usually at the quarterly level, and the numbers required for this are low in relation to those required for providing precise estimates of maturity at age.

The available data were investigated to see whether it could be used to give an indication as to the period of the year when spawning was occurring. Samples containing female fish in stages showing hyaline eggs (pre-spawning) or spawning were used to indicate a period where market samples could be used as a source of maturity data for use in assessments. This approach may also help identify any differences in the onset of spawning between different stocks of the same species or different parts of the same stock by area given sufficient sampling levels. It may also help identify the spatial distribution of spawning aggregations of the stock. The raw data provided in Table xxx is only indicative of what is possible using this method and should not be interpreted as being definitive.

## 5.3 Validation of maturity data

During the Workshop, an overview of samplings and validation procedures was compiled, but unfortunately, participants interpreted the question for validation differently. A positive validation could mean that:

- a ) The maturity ogive is based only on histological data (Southern horse mackerel),
- b ) A macroscopic maturity ogive is validated by histological studies (Baltic cod),
- c ) The maturity data are validated by a standard manual with pictures of macroscopic observations,
- d ) There have been consistency checks between maturity readers,

where a and b are referring to validation, c and d are referring to the quality control. For many stocks none of these options have been applied.

## 5.4 Use of maturity data in assessments

Table 5.3 (maturity data within the context of assessment, *sampling (ALL COUNTRIES).xls*) indicates if the maturity data are validated by histological investigations or just macroscopically. Furthermore, the table is referring to the use of the maturity data in the given ICES assessment groups, indicating the last year when the maturity ogive was updated and whether the collected data are used in the assessment. The following columns refer to the maturity ogive calculation, the ALK used to obtain maturity proportion at age (ALK/SMALK), if the data are weighed by the length distribution, and if the data is used in calculating the maturity ogive derived during spawning season only. Further, information is given on the ogives calculated on females only or sexes combined, and if sexes are combined, information on the use of a sex-ratio.

For most of the stocks assessed by ICES working groups, maturity ogives have not been updated on an annual basis. Some are using a fixed maturity ogive since the seventies or even earlier. For a number of stocks information on the last update of maturity ogive, the origin of the data and whether sex ratio has been applied is not available neither in the working group reports nor in the stock quality handbooks.

Even when annual data on maturity are available, these may currently not be in use in the assessment. For some species maturity data are collected according to the DCR requirements although these species are not assessed.

All countries are sampling on length basis but are using different methods to estimate the maturity ogive, namely a Sexual-Mature-Age-Length Key or just an Age-Length-Key. When samples are collected with a fixed number on a length stratified basis, some countries have not weighted their data by the overall length distribution. Due to lack of maturity data in the spawning season some stocks were updated with an ogive compiled of non validated off-season data.

For a few stocks a maturity ogive based on females only is used. In some cases sex ratios are not applied to the data.

## 5.5 Sampling and raising issues

Most ongoing surveys have been designed to obtain recruitment indices and as a result are not always conducted in the proper time of the year to obtain maturity proportions of the spawning stock. Also the spatial coverage of the survey may not match that of a particular stock, if the spawning part of the stock has aggregated in areas the survey does not cover. An example would be the acoustic spring survey in the Baltic targeting sprat and herring, but a large part of the herring stock is spawning along the coast at this time where the survey is not able to catch them.

- Only surveys conducted in the spawning time and with an adequate coverage can be used for a maturity index for the spawning stock. If maturity data are not available from spawning season, data from outside spawning season can only be used if there are validated by histological studies.

Many species are landed gutted and market maturity data are therefore not available. If data are available, there will still not be information on discarded fish – which could give an overestimation of maturity in the younger age classes. For a number of species commercial fishery will target spawning aggregations which may result in an overestimation of the proportion of spawning fish. If market samples are collected outside the spawning season, data will be easily misinterpreted and will not be accurate for maturity estimation.

If samples need to be bought from fishermen, high prices for large fish can bias the market samples towards smaller, cheaper priced fish. Also, on some occasions fishermen are not

willing to sell samples for scientific research. As fish auctions can start very early this could give logistic sampling problems for some institutes.

- Maturity data should be collected during the entire spawning season on a metier based sampling programme, and cover the entire stock distribution area.

On board (Catch) sampling is a better alternative than market sampling as a measure of discarded fish will also be obtained. On the other hand a good temporal and spatial coverage can be problematic as the possibility to get observers onboard is entirely dependent on vessel owners. Also, if samples are not analysed on board but are frozen and brought back interpretation of the maturity stage can be difficult due to decomposition in the freezing process.

- As with market samples it should be collected on a metier base to avoid gear and fleet selectivity and in the correct time frame compared to spawning.
- If possible maturity staging should be done on board.

For a number of stocks the maturity index is not validated, no proper manual exists and persons can interpret the scale differently.

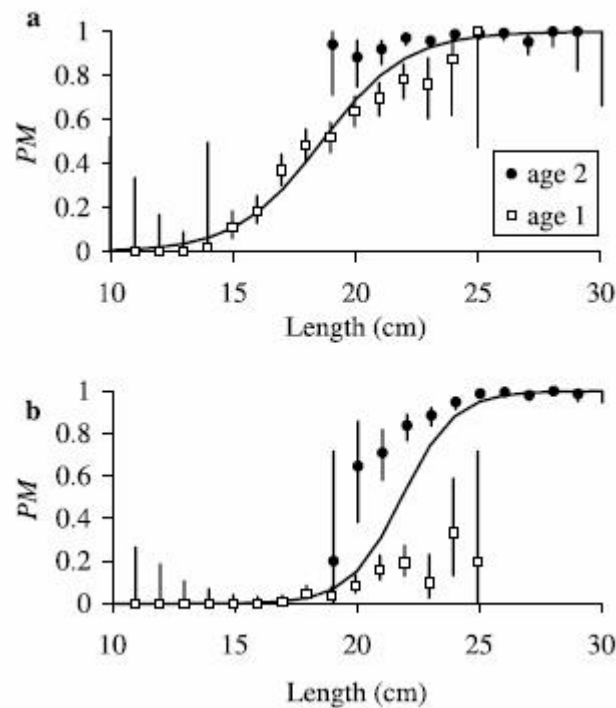
- A comprehensive illustrated manual should be available for all stocks where maturity observations are recommended (Tomkiewicz *et al.*, 2002).
- All macroscopic maturity scales used should be validated (see Section 5.1).

Much of the research sampling for age composition is stratified on a length basis, with a fixed number of samples taken from each length class. For most fish species, a given age can straddle several length classes. Further, the probability of being mature at a given age is influenced by length at that age. Often, the proportion mature at a given age is estimated from the observed fish at that age without taking into account the size distribution from which the age groups are drawn. This can result in biases in the estimation of proportion mature-at-age (Morgan and Hoening, 1997 and ICES, 2004).

- Maturity data should be weighed on an appropriate way (e.g. survey station, sub-division or stock level).
- If samples are collected on a random scheme or the stock is assessed on a length basis, no weighting according to the length distribution is required.

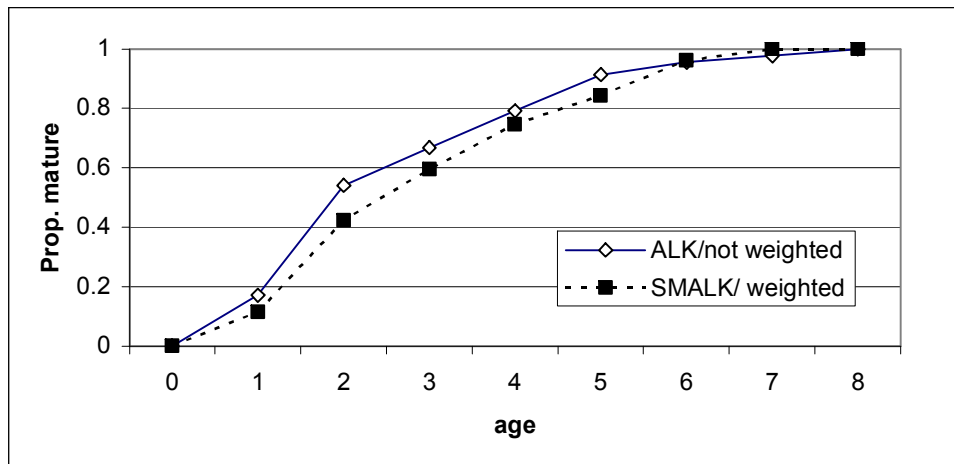
Maturity is generally dependent on age and/or sex as well as length. If maturity data is pooled by length with no age information and/or sexual segregation, the age maturity ogive do not comprise the age and/or sexual dependency.

Figure 5.1 shows the proportion of mature by length for the sexes in whiting. Male fish are maturing at a lower length and at a younger age than the females. Furthermore, splitting the maturity data by age for the same length range (20 to 25 cm) shows that the maturity proportion of age 1 is very low in comparison to age 2.



**Figure 5.1. Proportion mature (PM) at length for 1-year old and 2-year old whiting: (a) males and (b) females. Error bars are 95% confidence limits. The curve is the maturity ogive fitted to the combined data from fish of all age-classes (from Gerritsen *et al.*, 2003)**

Figure 5.2 shows the different results when a) a maturity ogive is calculated based on proportion of mature by length with no raising and then converted to age with and ALK; b) a maturity ogive is estimated with a SMALK weighted by length distribution. The two approaches were calculated using the same survey data. Not considering the length distribution of age 2 and age 3 fish, which have a peak in smaller length range, leads to an overestimation of the maturity proportion (mature to immature) at these age groups. The SMALK/raised procedure takes this into account in the maturity ogive estimation. This gives a more realistic picture of the maturity at age.



**Figure 5.2: Maturity proportion by age calculated using two different approaches: ALK/not raised) a maturity ogive is calculated based on proportion of mature by length with no raising and then converted to age with an ALK; SMALK/raised) a maturity ogive is estimated with a SMALK weighted by length distribution. (Data derived from IPIMAR groundfish survey)**

- If fish maturation process is dependent on age and/or of sex as well as of length Sexual Maturity-Age-Length-Key (SMALK) should be used. However, age reading precision is also very important in this context.

Recruitment is dependent on female egg production. For demersal fish the stock can decrease in a non length proportional way due to high fishing pressure on larger fish. This will reduce the most productive part of the stock, namely large females. In these instances a female maturity ogive may improve the stock recruitment relationship (ICES, 2004). Therefore it is recommended that if the stock shows a sexual difference in maturity to use maturity ogive for the females, or that the effect of combining the maturity data for both sexes is examined in detail.

## 5.6 Summarized guidelines for obtaining maturity data

- 1) Only surveys conducted in the right time compared to the spawning time and with an adequate coverage should be used for a maturity index for the spawning stock.

If survey data are not available at the right time maturity data obtained outside spawning season with a proper validation or market samples can be used.

- 2) Maturity data from market samples should be collected during the whole spawning season on a metier based sampling programme, and cover the whole stock distribution area.
- 3) As with market samples onboard samples should be collected on a metier base to avoid gear and fleet selectivity effect and in the correct time and spatial frame compared to spawning.
- 4) If possible maturity staging should be done onboard.
- 5) A comprehensive illustrated manual should be available for all stocks where maturity observations are needed.
- 6) All macroscopic maturity scales used should be validated either histologically or in another appropriate way.
- 7) Length stratified maturity data should be weighed with the length distribution. If samples are collected on a random scheme or the stock is assessed on a length basis, no weighting according to the length distribution is required.
- 8) If fish maturation process is dependent on age and/or of sex as well as of length a Sexual-Maturity-Age-Length-Key (SMALK) should be used. However, age reading precision is also very important in this context.

- 9) If the stock shows a sexual difference in maturity a female maturity ogive should be used, or the effect of combining both sexes considered in detail.

## 6 Conclusions from the Workshop

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The Data Collection Regulation DCR (Council Regulation 1543/2000 and Commission Regulations 1639/2001, 1581/2004) obliges Member States to collect information on, amongst others, the maturity composition of fish stocks. This information is needed to evaluate the situation of the fishery resources, as assessed by ICES. The sampling intensity is not specified in the DCR, and ICES stock assessment working groups have not indicated their information needs concerning maturity. The DCR, however, stipulates that maturity data are collected, achieving a level 3 precision (the highest level: 5% precision in parameter estimates at  $\alpha=0.95$ ). In practice, however, there is doubt on the soundness of the maturity information available, amongst others because of doubts on the operational procedures, the spatial coverage of the data in relation to the distribution of mature and immature fish, and a temporal mismatch between the spawning season and the data collection during recruitment surveys. Moreover, most assessments are based on time-invariant maturity ogives, for which the data source is obscure, or far outdated. There are only few examples of stocks, for which the precision of the maturity information has been assessed (far below required precision), and in these examples, the analysis has not led to an intensification of the sampling.

Based on an overview of stocks and existing sampling programmes in this report, the workshop recommends to improve and adjust maturity sampling along the following lines:

**Directly implementable improvements.** The different coding scales for maturity stages currently in use may give rise to inconsistencies and/or misunderstanding. Section 3 presents a detailed proposal for an improved coding scale, considered applicable for a wide range of fish species, while accommodating consistent mapping from the more detailed species- or country-specific coding scales, and facilitating the correct estimation of spawning stock biomasses in assessments. This recommended coding scale is addressed to the coming species-specific workshops on maturity, to stock coordinators exchanging information between labs, and to stock assessment working groups interpreting the maturity data.

**Checklists for pragmatic planning of sampling programmes.** The temporal and spatial mismatched, and potentially biased maturity sampling programmes currently executed must be improved by detailed consideration of stock- and area-specific options of existing or new sampling activities, in the appropriate Regional Coordination Meetings. To facilitate this process, Sections 4 and 5 present checklists for improvement of the maturity sampling.

**Further analysis of available data.** For a few stocks, statistical analyses of (spatial and temporal) patterns in maturation data have been presented, and achieved precision levels have been established. Although not all available data sets easily allow further analyses, some of the more extended data sets definitely provide opportunities. Further analyses will be required, augmenting the number of stocks for which (temporal and spatial) patterns are quantified, assessing the relation between sampling intensity and precision, and exploring options for maturity estimation based on multiple, complementary sources of information.

**Consequences of maturity variation on stock assessments.** For a few stocks, consequences of inter-annual variation in maturity data have been considered, indicating restricted effects for some, but not for all stock assessments. Given the extremely low number of stocks for which this has been investigated, these results need not be universally applicable. For those stocks for which inter-annual variation in maturity has been adequately quantified, stock assessment working groups should consider the potential effects on estimated trends in spawning stock biomass.

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## Annex 1: WKMAT List of participants

NAME	ADDRESS	PHONE/FAX	EMAIL
Guntars Strods	Latvian Fish Resources Agency Daugavgrivas str.8 LV-1048 Riga Latvia	Tel: +371 7613775	<a href="mailto:guntars.strods@latzra.lv">guntars.strods@latzra.lv</a>
Barbara Bland	Swedish Board of Fisheries Institute of Marine Research P.O. Box 4 SE-453 21 Lysekil Sweden	Tel: +46 523 187 20 Fax: +46 523 13977	<a href="mailto:Barbara.Bland@fiskeriverket.se">Barbara.Bland@fiskeriverket.se</a>
Rikke Hagstrøm Bucholtz	Difres Kavalergaarden 6 2920 Charlottenlund Denmark	Tel: +45 33 96 34 23 Fax: +45 33 96 33 33	<a href="mailto:rhb@difres.dk">rhb@difres.dk</a>
Ana Maria Costa	IPIMAR Avenida de Brasilia PT-1449-006 Lisbon Portugal	Tel: +351 21 302 7000 Fax: +351 21 301 5948	<a href="mailto:amcosta@ipimar.pt">amcosta@ipimar.pt</a>
Willem Dekker Chair	IMARES Institute for Marine Research and Ecosystem Studies Haringkade 1 P.O. Box 68 NL-1970 AB IJmuiden Netherlands	Tel: +31 255 564 646 Fax: +31 255 564 644	<a href="mailto:Willem.Dekker@WUR.NL">Willem.Dekker@WUR.NL</a>
Elena Fedotova	Fisheries research laboratory (FRL) LT-91001 Klaipeda PO Box 108 Lithuania	Tel: +370 46 391122 Fax: +370 46 391104	<a href="mailto:statrom@gmail.com">statrom@gmail.com</a> <a href="mailto:elena.fedotova@gmail.com">elena.fedotova@gmail.com</a>
Hans Gerritsen	Marine Institute Rinville Oranmore Co. Galway Ireland	Tel: +353 91 387297 Fax: +353 91 387201	<a href="mailto:hans.gerritsen@marine.ie">hans.gerritsen@marine.ie</a>
Patrícia Gonçalves	IPIMAR Avenida de Brasilia PT-1449-006 Lisbon Portugal	Tel: +351 21 302 7000 Fax: +351 21 301 5948	<a href="mailto:patricia@ipimar.pt">patricia@ipimar.pt</a>
Holger Haslob	Leibniz Institut für Meereswissenschaften Düsternbrooker Weg 20 D-24105 Kiel Germany		<a href="mailto:hhaslob@ifm-geomar.de">hhaslob@ifm-geomar.de</a>
Bart Maertens	ILVO Fisheries Ankerstraat 1 B-8400 Oostende Belgium	Tel: +32 (0) 59 56 98 34 Fax: +32 (0) 59 33 06 29	<a href="mailto:bart.maertens@dvz.be">bart.maertens@dvz.be</a>
David Maxwell	Cefas Lowestoft Laboratory Lowestoft Suffolk NR33 0HT United Kingdom	Tel: +44 1502 524 328	<a href="mailto:david.maxwell@cefas.co.uk">david.maxwell@cefas.co.uk</a>

Ana Moreira	IPIMAR Avenida de Brasilia PT-1449-006 Lisbon Portugal	Tel: +351 21 302 7000 Fax: +351 21 301 5948	<a href="mailto:amoreira@ipimar.pt">amoreira@ipimar.pt</a>
Cristina Morgado	IPIMAR Avenida de Brasilia PT-1449-006 Lisbon Portugal		<a href="mailto:cmorgado@ipimar.pt">cmorgado@ipimar.pt</a>
Alberto Murta	IPIMAR Avenida de Brasilia PT-1449-006 Lisbon Portugal	Tel: +351 21 302 7000 Fax: +351 21 301 5948	<a href="mailto:amurta@ipimar.pt">amurta@ipimar.pt</a>
Cristina Nunes	IPIMAR Avenida de Brasilia PT-1449-006 Lisbon Portugal	Tel: +351 213027000	<a href="mailto:cnunes@ipimar.pt">cnunes@ipimar.pt</a>
Jukka Pönni	FGFRI Kotka Unit Sapokankatu 2 FIN-48100-Kotka, Finland	Tel: +358 205 751 894 Fax: +358 205 751 891	<a href="mailto:ukka.ponni@rktl.fi">ukka.ponni@rktl.fi</a>
Iñaki Quincoces	AZTI Txatxarramendi Irla 48395 Sukarrieta Spain	Tel: +34 94 602 94 00 Fax: +34 94 687 00 06	<a href="mailto:iquincoces@suk.azti.es">iquincoces@suk.azti.es</a>
Tiit Raid	Estonian Marine Institute, University of Tartu Mäealuse 10A Tallinn EE- 12618 Estonia	Tel. +372 6718953 Fax +372 6718900	<a href="mailto:tiit.raid@gmail.com">tiit.raid@gmail.com</a> <a href="mailto:tiit.raid@ut.ee">tiit.raid@ut.ee</a>
Jari Raitaniemi	FGFRI Turku Game and Fisheries Research Itäinen Pitkätatu 3 FIN-20520 Turku, Finland	Tel: +358 205 751 685 Fax: +358 205 751 689	<a href="mailto:jari.raitanieni@rktl.fi">jari.raitanieni@rktl.fi</a>
Maria Sainza	Inst. Español de Oceanografía Centro Oceanográfico de Vigo Cabo Estay - Canido Apdo 1552 E-36280 Vigo Spain		<a href="mailto:maria.sainza@vi.ieo.es">maria.sainza@vi.ieo.es</a>
Romas Statkus	Fisheries research laboratory (FRL) LT-91001 Klaipeda PO Box 108 Lithuania	Tel: +370 46 391122 Fax: +370 46 391104	<a href="mailto:statrom@gmail.com">statrom@gmail.com</a> <a href="mailto:elena.fedotova@gmail.com">elena.fedotova@gmail.com</a>
Marie Storr-Paulsen	DIFRES Charlottenlund Slot 2920 Charlottenlund Denmark		<a href="mailto:msp@difres.dk">msp@difres.dk</a>
Jens Ulleweit	Institut für Seefischerei Palmaille 9 22757 Hamburg Germany		<a href="mailto:Jens.ulleweit@ish.bfa-fisch.de">Jens.ulleweit@ish.bfa-fisch.de</a>

Pedro Torres	Instituto Español de Oceanografía Centro Oceanográfico de Málaga Puerto Pesquero s/n, Apdo 285 29640 Fuengirola Spain		<a href="mailto:pedro.torres@ma.ieo.es">pedro.torres@ma.ieo.es</a>
Yolanda Vila	Instituto Español de Oceanografía Unidad de Cádiz CACYTMAR C/ República Saharaui s/n Polígono del río San Pedro Puerto Real Cádiz SPAIN		<a href="mailto:yolanda.vila@cd.ieo.es">yolanda.vila@cd.ieo.es</a>
Steve Wames	Cefas Lowestoft Laboratory Lowestoft Suffolk NR33 0HT United Kingdom	Tel: +44 1502 524550	<a href="mailto:Steve.Warnes@cefas.co.uk">Steve.Warnes@cefas.co.uk</a>

## Annex 2: Detailed overview of maturity stage coding keys currently in use

This annex presents an overview of the maturity coding keys currently in use. As far as possible, the original keys used in different countries are shown.

### Maturity stages in sole

Stage		Female	Plate	Male	Plate
1	Immature.	Ovaries very small - not usually extending more than 6 cm down side of the body. Ovary wall thin and easily broken. Internally yellowish-orange variable in colour.	1	Gonads tight up against back of gut cavity and very small - not usually larger than about 2 mm.	9
1-3	Maturing for the first time.	Ovaries half full but as in stage I the ovary wall is still thin.	2		
3	Half full.	Ovaries filling with eggs.	3	Gonads filling - roughly half full all sperm from ducts resorbed.	10
4	Full.	Ovaries full and usually distending body. No sign of any hyaline eggs.	4	Gonads ripe, full, but will not run even with moderate pressure.	11
5	Full varying to part full depending on the progress through spawning.	Hyaline eggs present, may be just a few or many hyaline eggs visible but ovaries will not run even under heavy pressure. Red colour appears in nearly spent ovaries.	5		
6	Running.	Hyaline eggs can be extruded copiously under light pressure. During the spawning season ovaries cycle through stages 5 and 6 until spent.	6	A little sperm can be extruded under pressure.	12
7	Spent.	Few eggs in state of resorption (mainly opaque eggs) and much slime in ovaries	7	Gonads thin flabby and often red in places. Little sperm in gonads but very often some remaining in gonoducts which can be extruded under fairly light pressure.	13
2	Spent, Recovering.	All eggs resorbed little or no slime inside ovaries.	8	Gonads thin but recovering, back to normal colour i.e. redness has disappeared. Ducts may still contain a little sperm which can be extruded on moderate pressure.	14

Figure A2.1. Maturity Key for Flatfish (Belgium).

**Stage 1:**

Gonads thread-like, less than 4 mm wide. Ovaries torpedo-shaped. Testes knife-shaped.

(Juvenile)

♀: Oocytes 0.04-0.07 mm and winered-whitish

♂: Colour whitish-grey

**Stage 2:**

Gonads same shape as in adults

(Growth)

♀: Oocytes 0.08-0.2 mm, visible in , colour reddish

♂: Colour reddish-greyish

**Stage 3:**

Gonads wider, 8-15 mm.

(Small grains visible)

♀: Oocytes 0.2-0.5 mm, round, yolk is formed, colour reddish-yellow

♂: Greyish-greyishred

**Stage 4:**

Gonads almost filling the whole body cavity

(Red, bigger grains)

♀: Oocytes 0.5-0.8 mm, uneven outline

♂: Whitish

**Stage 5:**

Gonads filling the whole body cavity.

(Grains)

♀: Eggs 0.8-1.2 mm, round, some hydrated, yellowish colours

♂:

**Figure A2.2. Maturity key for Herring (modified from Maier, 1908) (Denmark).**

**Baltic cod maturity****Female scale**

STAGE	MACROSCOPIC CHARACTERS TO DETERMINE GONADAL MATURITY OF FEMALES
I	Juvenile Ovaries emerge as tiny, paired organs close to bladder; glassy transparent to orange-reddish translucent in larger specimens. $L_T$ rarely above 30 cm; $GSI < 1$ .
II	Preparation Ovaries small, but easily distinguishable posterior in body cavity; soft with even surface (flattens on a solid sheet); blurred translucent, reddish-orange. $L_T$ : 25-60cm; $GSI < 1.5$ .
III	<b>Ripening 1: Oocyte recruitment</b> Ovaries still small and restricted to posterior body cavity; firmer than II and roe shaped (keep form on a solid sheet), surface uneven; opaque orange-red to dark orange with greyish cast in large females. Tiny opaque oocytes emerge towards end of stage. $L_T$ rarely below 30 cm; $GSI$ : 1-7.5.
IV	<b>Ripening 2: Late vitellogenesis</b> Ovaries enlarged to mid body cavity; plump and firm with prominent blood vessels; opaque, orange to creamy yellow. Oocytes clearly visible and densely packed. $GSI$ : 3-14.
V	<b>Spawning 1: Initiation of spawning</b> Ovaries extending into anterior body cavity; distended and soft; opaque, orange to creamy yellow. Single glassy, hydrating oocytes among abundant opaque, vitellogenic oocytes (as in IV, but round and larger). Viscous fluid or hydrated eggs in lumen may occur. $GSI$ : 12-25.
VI	<b>Spawning 2: Main spawning period</b> Ovaries fill most of body cavity; very distended and soft; appear granulated orange- to reddish-grey from mixture of opaque and glassy oocytes. Lumen containing viscous fluid in excess or hydrated eggs. $GSI$ : 15-60.
VII	<b>Spawning 3: Cessation of spawning</b> Ovaries shrunk to posterior body cavity; flabby with prominent blood vessels; unclear reddish- grey. Hydrated oocytes present; opaque oocytes few or absent. Lumen with excess fluid and frequently hydrated eggs. $GSI$ : 3-8.
VIII	<b>Regeneration 1: Spent</b> Ovaries contracted; slack with greyish cast; rich in blood vessels; dim translucent reddish-grey. Vitellogenic oocytes absent, but single hydrated eggs or atretic oocytes (opaque, irregular granules) may occur. $GSI$ normally 2-3; with atresia up to 10.
IX	<b>Regeneration 2: Resting and spawning omission</b> Ovaries small as in II, but with signs of previous spawning; e.g. greyish cast and somewhat uneven walls; blurred translucent, reddish-grey, but more granulated and opaque than in II. $GSI$ : 1-3.
X	<b>Degeneration: Reduced fertility</b> A: Ovaries with fibrous tissue formation; affected areas compact and hard, brownish-yellow opaque; non-affected parts with normal development. Observed in females from 65 cm. B: Other abnormalities.

**Figure A2.3. Maturity key for Cod (Tomkiewicz et al., 2002) (Denmark).**

**Baltic cod maturity****Male scale**

STAGE	MACROSCOPIC CHARACTERS TO DETERMINE GONADAL MATURITY OF MALES
I	<b>Juvenile</b> Testes emerge as a pair of thin strings along air bladder. Lobules tiny, glassy transparent to reddish translucent in larger specimens. $L_T$ rarely above 30 cm; $GSI < 0.1$ .
II	<b>Preparation</b> Testes small, but distinguishable along air bladder. Lobules small, blurred translucent and reddish. $L_T$ : 20-50cm; $GSI$ : 0.1-0.5.
III	<b>Ripening 1: Early spermatogenesis</b> Testes still small, close to air bladder. Lobules plump and soft, rich in blood vessels, completely or partially opaque, reddish. $L_T$ rarely below 20 cm; $GSI$ : 0.5-6.
IV	<b>Ripening 2: Late spermatogenesis</b> Testes enlarged and prominent in body cavity; Lobules plump and brittle; reddish-white. Empty, transparent spermaducts with prominent blood vessels; no sperm release. $GSI$ : 1-18.
V	<b>Spawning 1: Initiation of spawning</b> Testes extending into ventral part of body cavity. Lobules distended and brittle, opaque creamy-white. Spermaducts filled with viscous semen and a viscous droplet may be released from vent. $GSI$ : 3-22.
VI	<b>Spawning 2: Main spawning period</b> Testes large and prominent in body cavity (as in V). Lobules still plump, but soft; completely opaque, whitish. Spermaducts filled with fluid, milky semen that easily flows from vent. $GSI$ : 3 to 25.
VII	<b>Spawning 3: Cessation of spawning</b> Testes shrunk to dorsal part of body cavity; soft and flabby. Lobules almost empty, opaque, reddish-white. Spermaducts still with fluid semen that easily flows from vent. $GSI$ : 0.5 to 4.
VIII	<b>Regeneration 1: Spent</b> Testes contracted, close to air bladder; rich in blood vessels. Lobules empty, flabby, reddish potentially with a greyish cast. Spermaducts with signs of previous distension, often with visible remains of semen. $GSI > 1.5$ .
IX	<b>Regeneration 2: Resting and spawning omission</b> Testes small (as in Stage II), but with signs of previous spawning; e.g. lobules slightly larger than in II; spermaducts often with greyish cast. $GSI < 1.5$ .
X	<b>Degeneration: Reduced fertility</b> A: Testes with adipose tissue formation; affected parts undeveloped, hard, yellowish; non-affected parts with normal development. Observed in males from 50 cm. B: Other abnormalities.

**Figure A2.3 (cont.). Maturity key for Cod (Tomkiewicz *et al.*, 2002) (Denmark).**

### Four stage IBTS standard maturity key.

Females	Stage	Males
Ovaries small, elongated, whitish, translucent. No sign of development.	IM Virgin	Testes very thin translucent ribbon lying along an un-branched blood vessel. No sign of development.
Development has obviously started, eggs are becoming larger and the ovaries are filling more and more of the body cavity but eggs cannot be extruded with only moderate pressure.	MI Maturing	Development has obviously started, colour is progressing towards creamy white and the testes are filling more and more of the body cavity but sperm cannot be extruded with only moderate pressure.
Will extrude eggs under moderate pressure to advanced stage of extruding eggs freely with some eggs still in the gonad.	MA Spawning	Will extrude sperm under moderate pressure to advanced stage of extruding sperm freely with some sperm still in the gonad.
Ovaries shrunken with few residual eggs and much slime. Resting condition, firm, not translucent, showing no development.	SP Spent	Testes shrunken with little sperm in the gonads but often some in the gonoducts which can be extruded under light pressure. Resting condition firm, not translucent, showing no development.

Figure A2.4. IBTS Standard Maturity Key.

**1. Virgin**

Male:	Testes very thin translucent ribbon lying along an unbranched blood vessel. No sign of development.
Female:	Ovaries small, elongated, whitish, translucent. No sign of development.

**2. Maturing**

Male:	Development has obviously started, colour is progressing towards creamy white and the testes are filling more and more of the body cavity but sperm cannot be extruded with only moderate pressure.
Female:	Development has obviously started, eggs are becoming larger and the ovaries are filling more and more of the body cavity but eggs cannot be extruded with only moderate pressure.

**3. Spawning**

Male:	Will extrude sperm under moderate pressure to advanced stage of extruding sperm freely with some sperm still in the gonad.
Female:	Will extrude eggs under moderate pressure to advanced stage of extruding eggs freely with some eggs still in the gonad.

**4. Spent**

Male:	Testes shrunken with little sperm in the gonads but often some in the gonoducts which can be extruded under light pressure.
Female:	Ovaries shrunken with few residual eggs and much slime.

**5. Resting (see remarks in ICES CM 1997/J:4, Section 2.5)**

Male:	Testes firm, not translucent, showing any development.
Female:	Ovaries firm, not translucent, showing any development.

**Figure A2.5. BITS 5-stage Standard Maturity Key (Baltic Sea) – BITS Manual (ICES, 2006c).**

**Females:**

Stage	Code	Ovaries description
Immature	1	Ovaries tubular, thin and small. Ovarian wall whitish and delicate. Without conspicuous blood vessels. If visible eggs occur, they are very small, whitish or pale yellowish. Pigmented eye larvae are never observed in the ovary.
Maturing/ Mature	2	The ovary has increased in size considerably and it is easy to distinguish in the body cavity. The ovary wall and eggs inside the ovary are clearly visible. Eggs are yellow and opaque.
Mature/ Fertilized	3	Ovaries are considerably bigger and occupy most of the body cavity. Colour is bright yellow. Many eggs are transparent (approx. 50%) because of yolk re-absorption the eye pigment of the larvae becomes visible.
Parturition	4	Ovary occupy practically the whole body cavity, it is delicate and the wall transparent and thin. The colour shift to a green-yellowish due to larval developing, the eyes are evident and there is little yolk. Larvae are easily released from the ovary when it is manipulated.
Post spawning	5	Ovary is flaccid, but still big. No visible larvae inside or just a remainder of them. The colour is purple or blackish, sometimes confused with the body cavity wall (peritoneum).
Recovery	6	Size is reduced to stage 3 or smaller, but no visible eggs, colour yellow to purple.

**Males:**





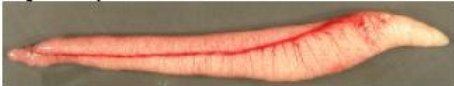




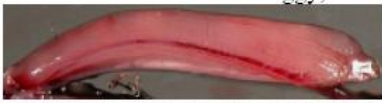


Stage	Code	Testes and genital papilla description
Immature	1	Testes are translucent, very thin and sometimes even difficult to detect, because it is confused with the mesentery. Width less than 1 mm. The penis is difficult to distinguish and easy to confuse with female genital papilla.
Maturing/ Mature	2	The Testes are more easily distinguishable because of increasing size. They are white. Width more than 1, 1–1,5 mm. There is no running sperm when the Testes are cut. Penis is visible, and it is easy to identify sex externally.
Mature/ Fertilized	3	Testes are bright white. The sperm is observed inside the Testes, but only when they are cut, i.e. sperm doesn't run out of the testes when they are pressed. Penis is thick, but no sperm is observed on it.
Parturition	4	Testes are big and with a cream colour. The sperm run out of the fish when belly is pressed. Penis is very conspicuous, with a purple tip and there are remains of sperm on it.
Post spawning	5	Testes are flaccid. The colour is still cream but with obvious dark (brown) patches. Practically no sperm inside the Testes.
Recovery	6	Size of the Testes has been reduced to stage 3, but the sperm is not visible. The colour is whitish.

Figure A2.6. Maturity Key for Redfish (Germany) – ICES, 2005.



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Foras na Mara

## Herring Maturity Scale

Females	Males
<b>1. Juvenile</b>	
Ovaries very small, transparent, no eggs visible to naked eye, up to 3mm wide.	Testes very small, transparent up to 3mm wide.
	
The sex is difficult to determine at this stage: ovaries have a pointed end and are shaped like a torpedo and testes have a rounded end and are shaped like a scalpel	
<b>2. Developing virgin</b>	
Ovaries somewhat larger in volume than stage I: 3-8mm wide. Eggs visible with magnifying glass. Still transparent.	Testes somewhat larger in volume than at stage I: 3-8mm wide. Still transparent
<b>3. Ripening 1</b>	
Ovaries more swollen and opaque, occupying about 1/2 of the ventral cavity. Yellow/white eggs in lamellae visible to naked eye.	Testes more swollen and opaque, occupying about 1/2 of the ventral cavity. Reddish grey or greyish.
	
<b>4. Ripening 2</b>	
Ovaries pale yellow, filling about 2/3 of the ventral cavity, eggs distinct and grainy not transparent yet.	Testes nearly filling 2/3 of the ventral cavity, milt whitish.
	
<b>5. Ripe</b>	
Ovaries filling ventral cavity. Some large transparent eggs present. Ovaries do not run under pressure	Testes filling ventral cavity, milt white, not yet running.
<b>6. Running</b>	
Ovaries run when light pressure is applied, eggs transparent	Testes run when light pressure is applied
	
<b>7. Recently spent</b>	
Ovaries slack with residual eggs	Testes baggy, bloodshot.
	
The sex is again difficult to determine at this stage, the shape of the gonads is more easily seen if they are spread out. Ovaries can be quite pointy while testes still have more or less scalpel shaped ends.	
<b>8. Spent-Recovering</b>	
Ovaries are firm and larger than stage 2. Walls striated vertically and blood vessels prominent	Testes are firm and larger than stage 2. Walls striated vertically and blood vessels prominent
	

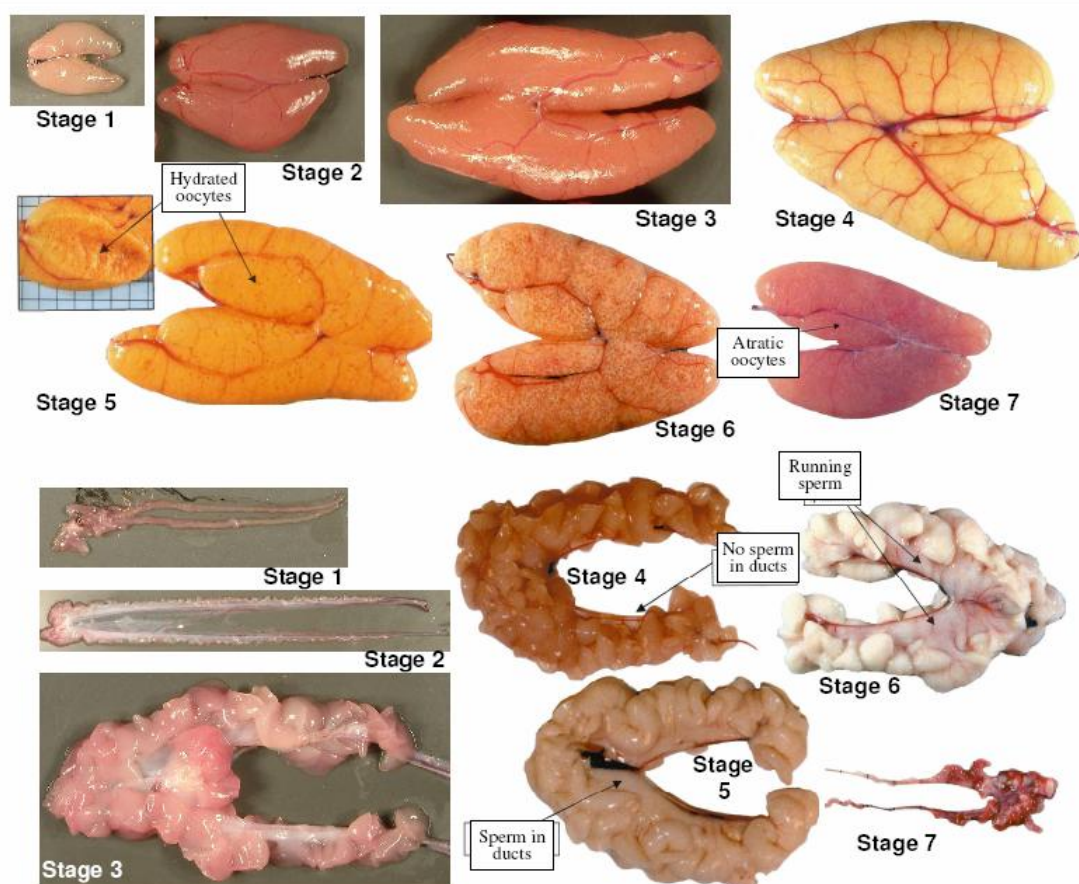
Based on: (Landry and McQuinn 1988; ICES 2003)

Figure A2.7. Herring Maturity Key (Ireland) – ICES, 2003.

### Gadoid Maturity Scale

(blue whiting, cod, haddock, hake, whiting, saithe)

Females	Males
<b>1. Juvenile</b>	
Translucent and very small, with thin walls.	Thin and translucent ribbons with tiny lobules
<b>2. Developing virgin / Resting spent</b>	
Blurred translucent, small.	Blurred translucent, small lobules.
<b>3. Ripening 1</b>	
Opaque. Yellow to orange colour visible when cut.	Completely or partially opaque, rich in blood vessels.
No individual oocytes visible.	
<b>4. Ripening 2</b>	
Individual oocytes visible. Plump and firm, colour yellow to orange.	Lobules plump. No sperm in spermaducts.
<b>5. Ripe</b>	
Hydrated eggs present. Does not run under moderate pressure. Distended and soft.	Some sperm visible when cut. Sperm in spermaducts. Not running under moderate pressure
<b>6. Running</b>	
Very distended and soft. Hydrated eggs can be extruded on slight pressure	Testes run on slight pressure
<b>7. Recently spent</b>	
Ovaries reduced in size and flaccid. Some residual eggs or slime still present.	Testes thin and flabby, some sperm remaining in spermaducts



Based on: (Tomkiewicz et al. 2002)

Figure A2.8. Maturity Key for Gadoids (Ireland) (Modified from Tomkiewicz *et al.*, 2002).

### Plaice Maturity Scale


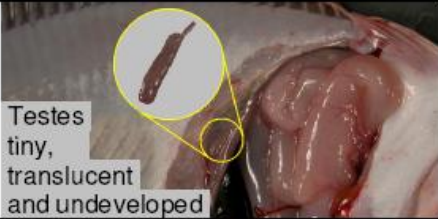






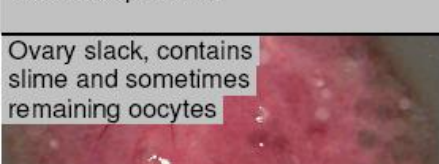
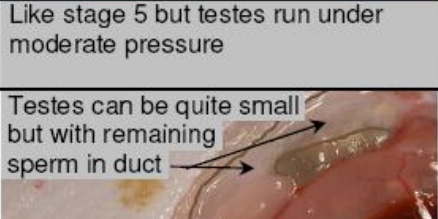


<p>Ovary small, contents translucent, nearly colourless</p> 	<p><b>1. virgin</b></p>	 <p>Testes tiny, translucent and undeveloped</p>
<p>Contents blurred translucent, pinkish, no oocytes visible</p> 	<p><b>2. early developing or resting spent</b></p>	 <p>Testes grey-white, opaque &amp; 10%-50% of maximum size</p>
no stage 3 in plaice		
<p>Ovary contains opaque oocytes that are clearly visible when ovary is cut</p> 	<p><b>4. preparing</b></p>	 <p>Testes nearly full size but no sperm visible when cut</p>
<p>Ovary contains hydrated cells but does not run</p> 	<p><b>5. ripe</b></p>	 <p>Sperm visible when cut but testes do not run</p>
<p>Like stage 5 but ovaries run under moderate pressure</p> 	<p><b>6. spawning</b></p>	<p>Like stage 5 but testes run under moderate pressure</p> 
<p>Ovary slack, contains slime and sometimes remaining oocytes</p> 	<p><b>7. recently spent</b></p>	 <p>Testes can be quite small but with remaining sperm in duct</p>

Figure A2.9. Maturity Key for Flatfish (Ireland).

## Sole Maturity Scale






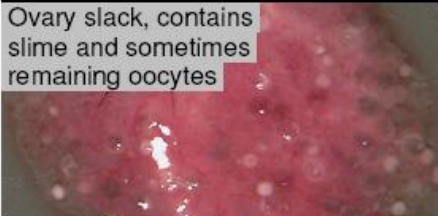
<p>Ovary small, contents translucent, pinkish</p> 	<p><b>1. virgin</b></p>	<p>Testes tiny, translucent and undeveloped</p>
<p>Contents blurred translucent, no oocytes visible. Colour varies from pink to orange to wine red</p> 	<p><b>2. early developing or resting spent</b></p>	<p>Testes up to half of their full size. Color varies</p> 
no stage 3 in sole		
<p>Ovary contains opaque oocytes that are clearly visible when ovary is cut</p> 	<p><b>4. preparing</b></p>	<p>Testes nearly full size but no sperm visible when cut</p>
<p>Ovary contains hydrated cells but does not run</p> 	<p><b>5. ripe</b></p>	<p>Sperm visible when cut but testes do not run</p>
<p>Like stage 5 but ovaries run under moderate pressure</p>	<p><b>6. spawning</b></p>	<p>Like stage 5 but testes run under moderate pressure</p>
<p>Ovary slack, contains slime and sometimes remaining oocytes</p> 	<p><b>7. recently spent</b></p>	<p>Testes can be quite small but with remaining sperm in duct</p>

Figure A2.9 (cont.). Maturity Key for Flatfish (Ireland).

### Megrim Maturity Scale




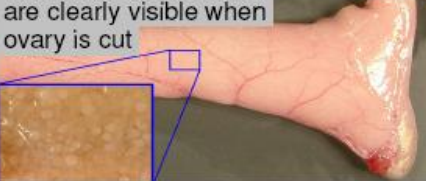

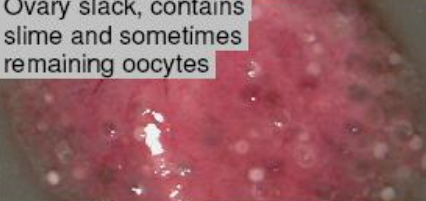
Ovary small, contents translucent, pinkish	<b>1. virgin</b>	Testes tiny, translucent and undeveloped 
Contents blurred translucent, no oocytes visible. 	<b>2. early developing or resting spent</b>	Testes up to half of full size. 
<b>no stage 3 in megrim</b>		
Ovary contains opaque oocytes that are clearly visible when ovary is cut 	<b>4. preparing</b>	Testes nearly full size but no sperm visible when cut
Ovary contains hydrated cells but does not run 	<b>5. ripe</b>	Sperm visible when cut but testes do not run
Like stage 5 but ovaries run under moderate pressure	<b>6. spawning</b>	Like stage 5 but testes run under moderate pressure
Ovary slack, contains slime and sometimes remaining oocytes 	<b>7. recently spent</b>	Testes can be quite small but with remaining sperm in duct

Figure A2.9 (cont.). Maturity Key for Flatfish (Ireland).

## SCAD maturity key

Maturity stage	Males	Females
1. Virgin	Flattened, <2mm broad, translucent; less than ¼ of length body cavity	Rounded, 1-2mm broad, translucent; less than ¼ of length body cavity; no oocytes visible
2. Developing virgin	Fattened, 2-4mm broad, about a ¼ of length body cavity	Rounded, 2-4mm broad, pink, about ¼ length of body cavity
3. Resting (mature)	Flattened, about 5mm broad, grey, about ½ of length body cavity	Rounded, about 5mm broad, pink, about ½ of length body cavity. No oocytes visible
4a. Developing (early)	Becoming flatter, off-white ½ – ½ of length body cavity	Yellow, between ⅓ and ½ of length body cavity; oocytes visible to naked eye
4b. Developing (late)	Firm, becoming whiter, ½ to whole length body cavity	Yellow, half to whole length of body cavity, large oocytes visible
5. Ripe	Almost fills body cavity, pure white, parts becoming soft.	Almost fills body cavity, hyaline eggs present, producing speckled appearance
6. Running	Milt runs from vent on slight pressure.	Oocytes run from vent on slight pressure
7. Partly spent	Smaller than stage 5; patches of white and grey give mottled appearance	Smaller and less firm than stage 5, patches of red and yellow give a mottled appearance, many oocytes still present
8. Spent	Flaccid, grey-brown; may be little residual milt	Flaccid, dark red, less than ½ of length body cavity, few large oocytes still visible
9. Recovering	About ⅓ of length body cavity or less; firmer than stage 8; dark patches visible through testis wall	Firmer than stage 8, mainly dark red, about ⅓ of length body cavity or less, few residual oocytes may be visible

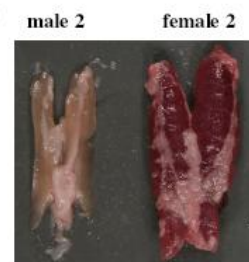


Figure A2.10. Horse-mackerel Maturity Key (Ireland) (From Edgar Mc Guinnes).

**Scomber scombrus**Proposed New Mackerel Maturity Key (Walsh scale)

Stage	State	External appearance
1.	<u>Immature</u>	Gonads small. Ovaries wine red and clear, torpedo shaped. Males pale, flattened and transparent.
2.	<u>Early ripening and Resting</u>	Gonads occupying $\frac{1}{4}$ to $\frac{3}{4}$ body cavity. Opaque eggs visible in ovaries giving pale pink to yellowish coloration. Testes off-white.
3.	<u>Late ripening</u>	Gonads occupying $>\frac{3}{4}$ body cavity. Ovaries yellow to orange. Largest oocytes with oil globules. Testes creamy white.
4.	<u>Ripe</u>	Gonads filling body cavity. Hyaline eggs present in ovaries, running milt in testes.
5.	<u>Partly spent</u>	Gonads occupying $\frac{3}{4}$ to $<\frac{1}{4}$ body cavity. Ovaries slacker than at stage 4 and often bloodshot. Freely running milt in testes, latter shrivelled at anus end.
6.	<u>Spent and Recovering spent</u>	Gonads occupying $\frac{1}{4}$ or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with scattering or patch of opaque eggs. Testes opaque with brownish tint and no trace of milt

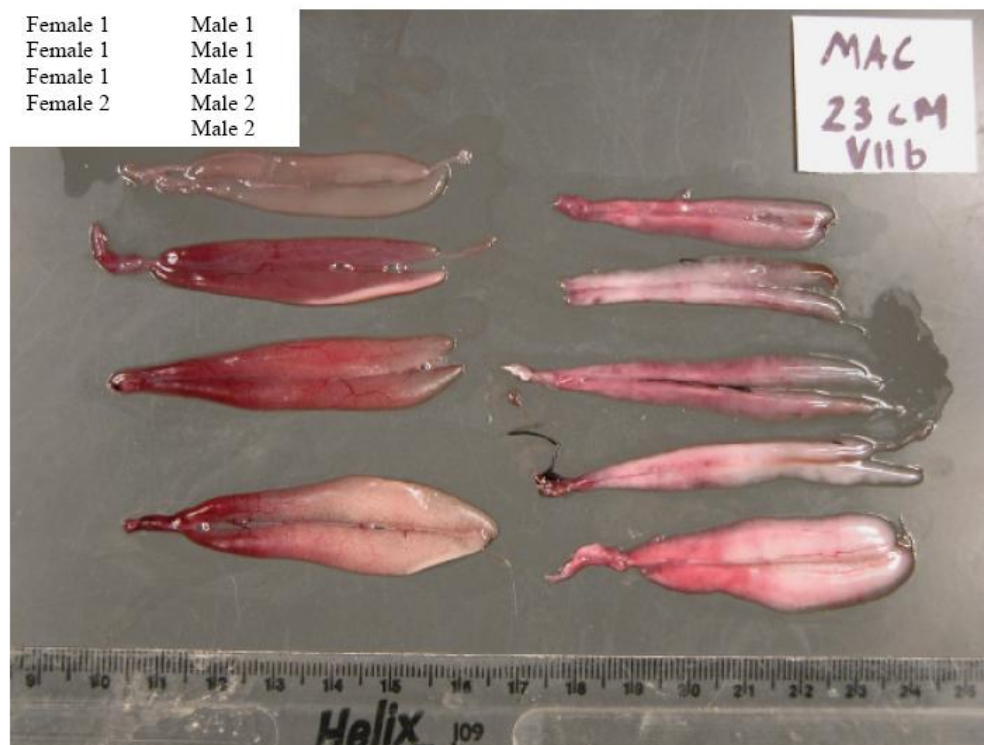


Figure A2.11. Maturity Key for Mackerel (Ireland) (From Walsh *et al.*, 1990).



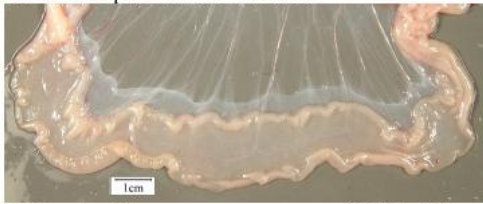


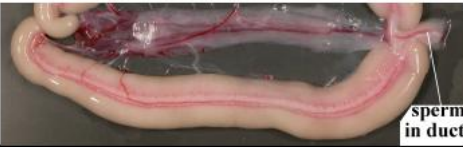
Monkfish Maturity Scale	
Females	Males
<b>1. Juvenile</b>	
Ovaries very narrow (<2cm), thin and ribbon-like. Translucent, no oocyte clusters can be seen. Their volume is negligible compared with other internal organs.	Testes long and narrow tube-like (<1cm), translucent. Volume is negligible compared with other internal organs.
	
At this stage the sexes can be difficult to distinguish: Ovaries are wider than testes in fish of similar length, the membrane connecting the ovary extends further out than that of the testes so that the gonad stretches further away from the vertebrae in females. Fish <20cm are very hard to sex, if in doubt record sex as 'U'.	
<b>2. Developing virgin / Resting spent</b>	
Ovaries wider (2-4cm) and less translucent, no visible oocyte clusters. Ovaries occupy roughly the same space as the intestine	Testes flattened tubular and wider than stage 1, creamy, opaque. Testes occupy roughly ½ the space occupied by the intestine
	
▲ Female: 61cm	► Male: 59cm
<b>3. Ripening</b>	
Ovaries much longer thickening but still ribbon-like, individual oocyte clusters visible embedded in gelatinous matrix. Ovaries occupy most of the abdominal cavity	Testes very firm texture, milt is produced when dissected, but testes do not run under pressure. Testes still occupy less space than the intestine
	
Stages 4 and 5 are not distinguished for monkfish	
<b>6. Ripe / Running</b>	
Ovaries extremely long (>6m) and wide (>30cm) Ovaries highly vascularized, oocytes hyaline, round and gelatinous with yellow-brown centre, look like frog spawn.	Milt runs on slight pressure. Large amounts of milt are produced when dissected.
<b>7. Spent</b>	
Ovaries flaccid and opaque, with longitudinal striations, still very wide (10-15cm) and highly vascularized. Some oocytes might still be present	Testes very flaccid, can have bruised appearance. Some milt often present in seminiferous duct. Testes still highly vascularized near seminiferous duct.
	
Based on Thangstad (2003)	

Figure A2.12. Maturity Key for Anglerfish (Ireland) (From Thangstad *et al.* 2003)

## Rays / Skates Maturity scale

### Females

- 1. Juvenile.** Ovaries small, No oocytes differentiated or all uniformly small. Oviducts narrow.
- 2. Maturing Virgin.** Oocytes differentiated to various small sizes up to around 1cm. Egg shell gland developing, creamy colour. Oviducts may become widened posteriorly.
- 3. Mature.** Some developing oocytes very large. Egg shell gland fully formed, uniformly white. Oviducts widened.
- 4. Active.** Large eggs present in fallopian tube (between ovary and egg shell gland) or passing through egg shell gland. Egg cases, if present, only partially extruded.
- 5. Laying.** Fully formed, hardened, egg capsules present in one of both oviducts.
- 6. Spent.** Oocytes in different stages of development like stage 2. Oviducts venous and stretched. Egg shell gland fully formed.

### Males

- 1. Juvenile.** Claspers undeveloped, flexible sticks. Do not extend beyond the tip of the pelvic fins.
- 2. Maturing.** Claspers extended beyond pelvic fins. Still flexible. Terminal stages of claspers (glans) becoming structured but still soft.
- 3. Mature.** Claspers full length and stiff. Cartilaginous components in glans are sharp and hard.
- 4. Active.** Glans clasper often dilated, its structures reddish and swollen. Sperm flowing on pressure from cloaca or present in clasper groove or glans.

From: Stehmann (2002) and Gallagher et al. (unpublished)

Female stages 2 and 6 are quite similar. The main differences are in the development of the egg shell gland and the width of the oviducts the judgement of which can be subjective. The size of the ray can be an indication: the total length at which the females mature is: THR: 70-80cm, SDR: 50-65cm, CUR: 50-60cm, BLR 75-85 cm.

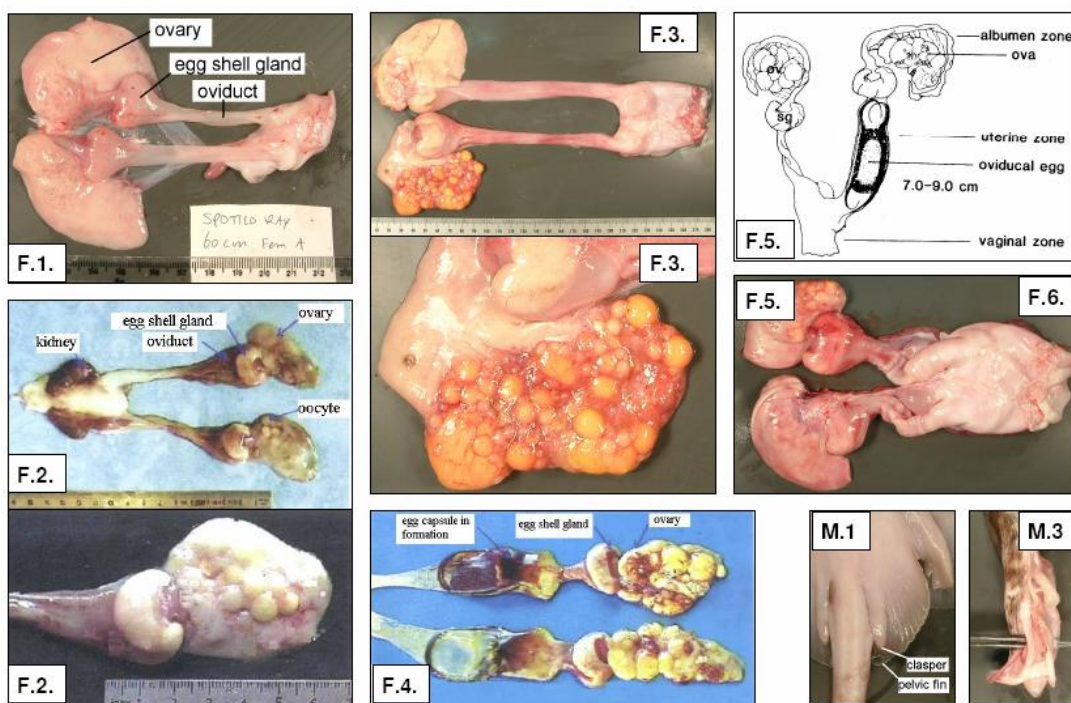


Figure A2.13. Maturity Key for Rays and Skates (Ireland) (From Stehmann, 2002).

State	Six-point maturity scale (Powles, 1958)	Eight-point maturity scale (Maier, 1908)	BITS ICES (1997)
Immature	II	I,II	1
Ripening	III	III	2
Ripe	IV	IV,V	2
Spawning	V	VI	3
Spent	VI	VII	4
Recovering	VI-II	VIII	5

**Figure A2.14. Maturity Key for Baltic cod (Latvia, Lithuania).**

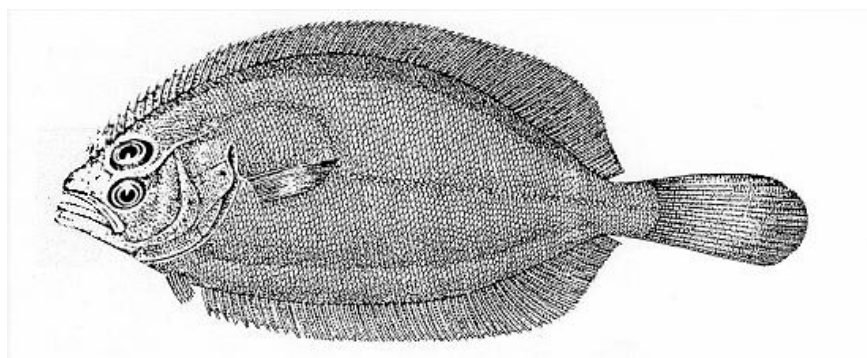
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I Immature	Tests smaller, flattened	Ovaries smaller, translucent, pink coloured.
II Development	Tests larger and longer	Ovaries larger, triangular shape, yellow, with visible opaque oocytes.
III Pre-spawning	Tests with sperm which flows under pressure.	Ovaries opaques, occupying all abdominal cavity with oocytes some of them are hydrated.
IV Spawning	Tests filling the abdominal cavity and extrude sperm easily.	Ovaries with almost all oocytes in the hydrated stage.
V Post-spawning	Tests total or parcial empty, brownish.	Ovaries total or parcial empty, red.

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**Figure A2.15. Maturity Key for Megrim (Portugal).**

Table 3  
Macroscopic gonad maturity stages by sex for both anglerfish species

Maturity stage	Males	Females
I Immature	Tube-shaped very small testicles, pink or transparent. Sperm not visible	Band-shaped ovaries, very transparent and without visible oocytes.
II Maturing	Testicles taking up a greater proportion of the visceral cavity. White coloured. Sperm not visible or just a little appearing in the lumen.	Ovaries occupying a little part of the visceral cavity, with a brown-orange colour. No vascularisation and no oocytes visible.
III Mature or pre-spawning	White coloured testicles with the lumen full of sperm.	Orange-coloured ovaries with accentuated vascularisation. Presence of some hyaline oocytes.
IV Spawning	Sperm is easily freed by applying pressure to the abdomen.	An enormous incolour gelatinous mass wraps the hyaline oocytes.
V Post-spawning	Testicles with red marks. There is no sperm, or a little residual.	Ovaries red due to the vascularisation. Residual oocytes present.

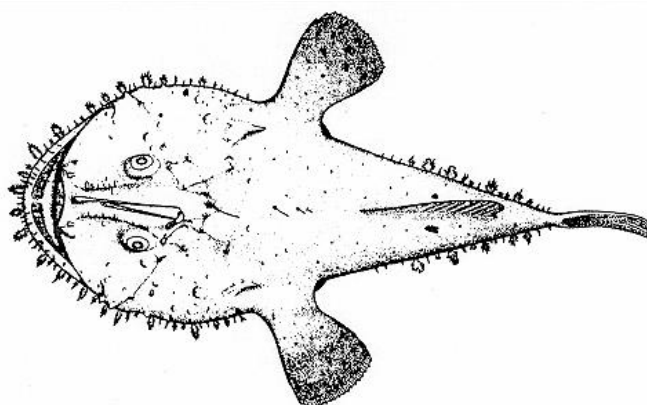


Figure A2.16. Maturity Key for Anglerfish (Portugal) (From Duarte *et al.* 2001).

## MALES

### I – IMMATURE/INACTIVE

**Size:** Small

**Shape:** Ribbon or small band

**Colour:** White/Transparent

**Sperm:** Absent



### II - DEVELOPMENT

**Size:** Medium

**Shape:** Large band

**Colour:** Pearl

**Sperm:** Flows when it is cut



### III - SPAWNING

**Size:** Large

**Shape:** Large band

**Colour:** Pearl

**Sperm:** Flows on applying pressure to the abdomen



### IV – POST-SPAWNING

**Size:** Large

**Shape:** Bands empty and deformed

**Colour:** White/pink

**Sperm:** Absent or a little residual



**Figure A2.17. Maturity Key for Hake (Portugal).**

## FEMALES

### I – IMMATURE/INACTIVE

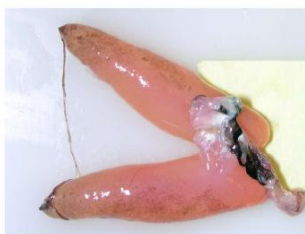
**Size:** Small

**Shape:** Ellongated

**Colour:** Pink/transparent

**Opaque oocytes:** Absents

**Hyaline oocytes:** Absents



### II - DEVELOPMENT

**Size:** Medium and Large

**Shape:** Cylindrical

**Colour:** Pink/ orange

**Opaque oocytes:** Present

**Hyaline oocytes:** Absents

**Macroscopic aspect:** Without stepped on areas or hurt areas



### III - SPAWNING

**Size:** Large

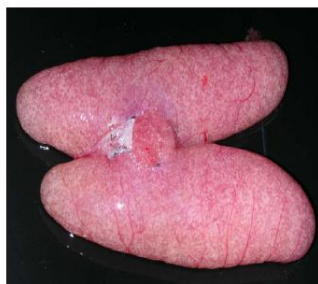
**Shape:** Cylindrical

**Colour:** Pink

**Opaque oocytes:** Present

**Hyaline oocytes:** Present

**Macroscopic aspect:** Involving membrane of the gonad is fine; hydrated oocytes may flow under some pressure in the abdomen.



### IV – POST-SPAWNING

**Size:** Large

**Shape:** Shrunk

**Colour:** Pink dark

**Opaque oocytes:** Present

**Hyaline oocytes:** Absents

**Macroscopic aspect:** Flaccid; with spaces next to the hilo



**Figure 17 (cont.). Maturity Key for Hake (Portugal).**

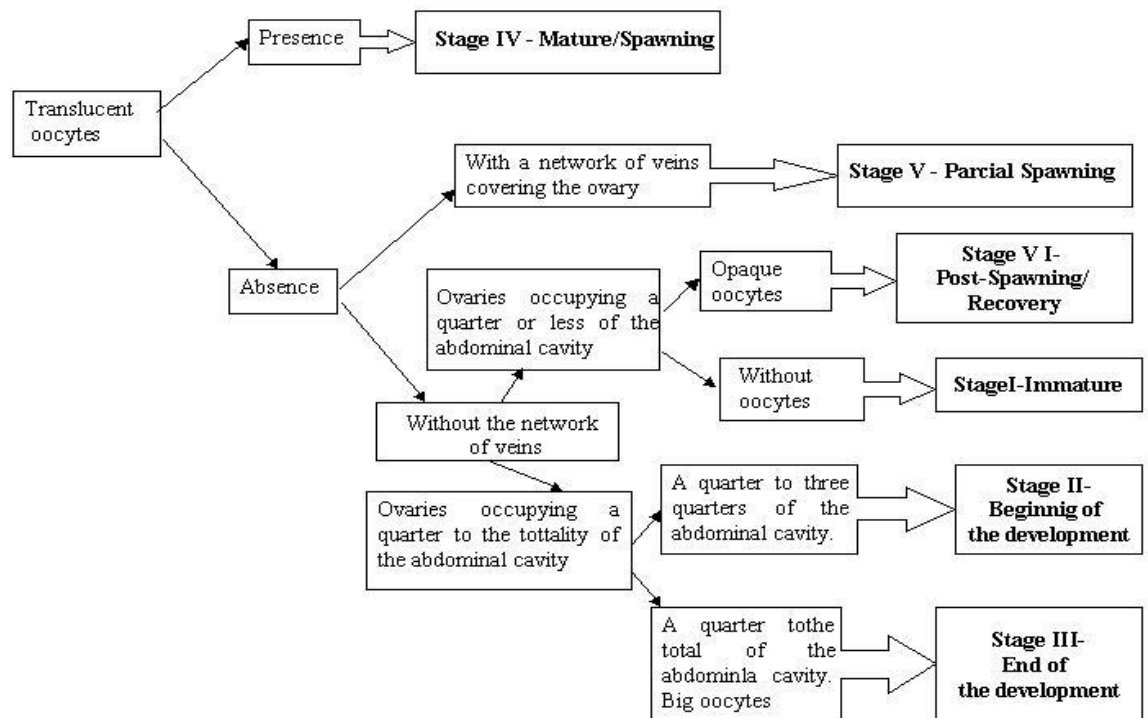
Maturity Stage		Characteristics
<b>1</b>	Resting Immature	Ovaries translucent, without visible oocytes, occupying 1/4 of the abdominal cavity  Testes thins, translucent, occupying 1/4 of the abdominal cavity
<b>2</b>	Beginning of development	Ovaries translucent, orange/red, occupying 1/3 of the abdominal cavity  Testes translucent, with/pink, occupying 1/2 of the abdominal cavity
<b>3</b>	End of development	Ovaries opaques, orange/pink, with visible oocytes, occupying ½ – 1/3 of the abdominal cavity  Testes opaques, with/pink, with blood vessels, occupying 3/4 of the abdominal cavity
<b>4</b>	Spawning	Ovaries orange/pink, with hydrated oocytes, occupying 3/4 of the abdominal cavity  Testes pure white colour, opaques, occupying all of the abdominal cavity
<b>5</b>	Post-spawning	Ovaries shrunken, pink/red, residual eggs, occupying 1/2 of the abdominal cavity  Testes yellow/brownish, avermelhados, occupying 1/2 of the abdominal cavity

**Figure A2.18. Maturity Key for Blue-whiting (Portugal).**

Ph	Macroscopic aspect of the ovaries	Diameter of larger oocytes observed fresh:		General aspect of dissected fresh ovaries
		Range ( m )	Mode ( m )	
I	Small translucent with almost no colour. Delicate but well defined blood vessels system.	210	75	The majority of the oocytes are translucent and polyhedral; however, some present very small cytoplasmic granulations.
II	Beginning of opacification. Through the ovarian membrane it is possible to observe several opaque oocytes. Coloration pink or yellow.	210-430	300	Oocytes very similar to those of the former phase; it is possible to observe oocytes beginning to develop cytoplasmic opacity (first group to mature).
III	Totally opacified. Oocytes clearly visible through the ovarian membrane and laid out regularly in parallel bands; no hyaline zones. Coloration pink or yellow.	430-680	500-680	The larger oocytes are totally opaque. It is also possible to see oocytes in early stages of development.
IV	Inflated and gelatinous. Oblique bands full with oocytes still opaque and transparent oocytes already ripe visible through the ovarian membrane.	800-1190	1000	The cytoplasm of ripe oocytes is hyaline and translucent; however there are still cytoplasmic opaque zones.
V(III)	Very similar to phase III, but the oocytes are laid out irregularly with scattered hyaline zones between them. Coloration red to salmon.	---	---	Similar to phase III, but with many very small transparent oocytes, formed during ovary recovery. Some residual ripe oocytes are wrinkled and in necrosis.
V	Very flaccid; hemorrhagic zones and, sometimes, whitish (blanchâtres) nodules (corresponding to residual oocytes reabsorbing). Blood coloration.	---	---	Oocytes of all stocks and sometimes of the residual ripe oocytes wrinkled. All opaque oocytes of other stocks are presented showing signs of necrosis.

Figure A2.19. Maturity Key for Sardine (Portugal) (From Pinto and Andreu, 1957).

## FEMALES



## MALES

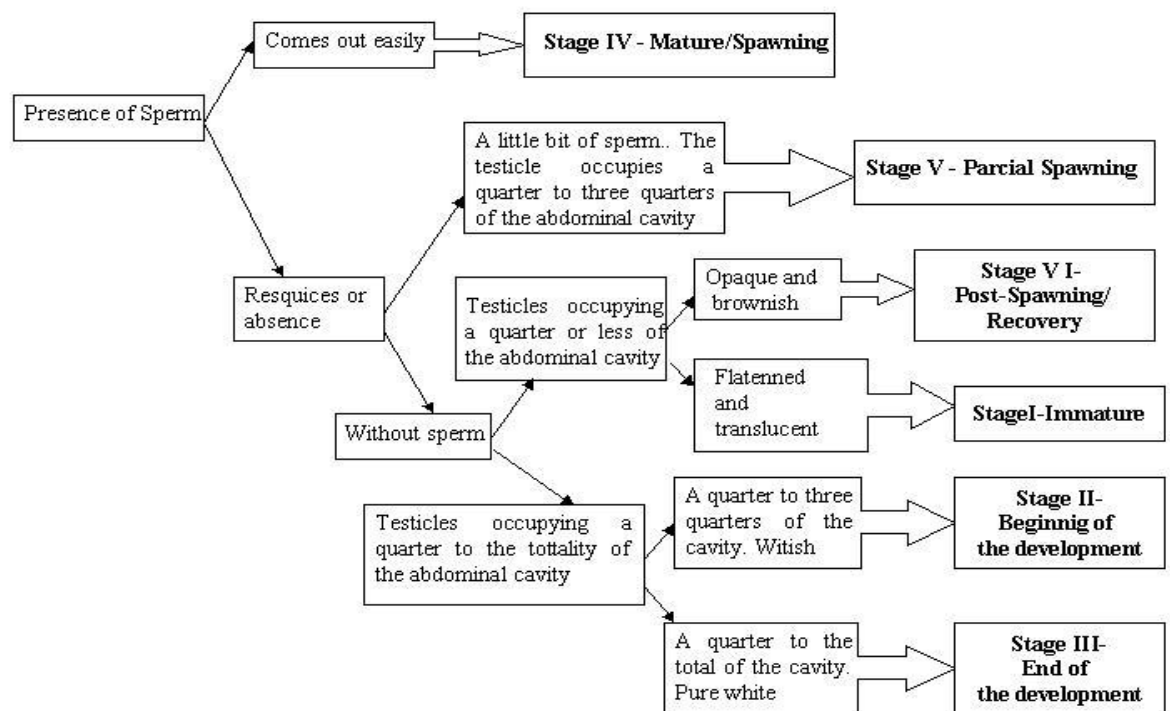


Figure A2.20. Maturity Key for Horse-mackerel, Mackerel, Spanish Mackerel (Modified from Walsh *et al.*, 1990) (Portugal).

Maturity stage	Testis External appearance	Ovary	
		External appearance	Histological appearance
1 Virgin	Flattened, 1–2 mm broad, translucent; less than a quarter of length of body cavity	Rounded, 1–2 mm broad, translucent; less than a quarter of length of body cavity; no oocytes visible	Few oocytes larger than 0.1 mm; oocytes have densely staining cytoplasm without vacuoles; ovary wall about 12 µm in thickness
1–3 Developing virgin	Flattened, 2–4 mm broad, grey; about a quarter of length of body cavity	Rounded, 2–4 mm broad, pink; about a quarter of length of body cavity	Many oocytes 0.1–0.25 mm present; larger oocytes have cytoplasmic vacuoles; ovary wall about 35 µm thick and not folded
2 Resting (mature fish)	Flattened, about 5 mm broad, grey; about a third of length of body cavity or less	Rounded, about 5 mm broad, pink; about a third of length of body cavity; no oocytes visible	Few oocytes larger than 0.1 mm; no cytoplasmic vacuoles; ovary wall 35 µm or more thick, may be folded
3 Developing (early)	Becoming fatter, off white; between a third and a half of length of body cavity	Yellow; between a third and a half of length of body cavity; oocytes visible to naked eye	Many oocytes 0.1–0.4 mm present; larger oocytes have cytoplasmic vacuoles and some may have small yolk droplets
4 Developing (later)	Firm, becoming whiter; half to whole length of body cavity	Yellow; half to whole length of body cavity; large oocytes visible	Many oocytes up to 0.6 mm present; cytoplasm of larger oocytes filled with densely-staining yolk granules
5 Ripe	Almost fills body cavity; pure white; parts becoming soft	Almost fills body cavity; hyaline oocytes present, producing speckled appearance	Hyaline oocytes present (usually collapsed by histological processing), as well as all stages of smaller oocytes
6 Running	Milt runs from vent on slight pressure	Oocytes run from vent on slight pressure	Hyaline oocytes present in lumen
7 Partly spent	Smaller than stage 5; patches of white and grey give a mottled appearance	Smaller and less firm than stage 5; patches of red and yellow give a mottled appearance; many oocytes still present	Conspicuous spaces present in septa; free follicular cells present in lumen
8 Spent	Flaccid, grey-brown; there may be a little residual milt	Flaccid, dark red; less than half of body length; a few large residual oocytes visible	Septa disorganized; residual oocytes undergoing resorption; lumen contains much 'debris', i.e. residual cells
9 Recovering	About a third of length of body cavity or less; firmer than stage 8; dark patches visible through testis wall	Firmer than stage 8, mainly dark red; about a third of length of body cavity or less; a few residual oocytes may be visible	An increase in the number of small oocytes is apparent, as is the organization of the septa; a few resorbing oocytes sometimes present

Figure A2.21. Maturity Key for Blue Jack-mackerel (Macer, 1974) (Azores, Portugal).

MATURITY STAGE		CHARACTERISTICS
1	Immature	Ovaries and testes translucent and of small size
2	Beginning of development	Ovaries opaque. Testes opaque but without visible sperm
3	Pre-spawning	Ovaries with oocytes visible. Testes firm and with sperm visible
4	Spawning	Ovaries with a large volume and with translucent oocytes. Testes with abundant sperm
5	Post-spawning	Ovaries and testes almost empty, slack and blood-stained

Note: a 6<sup>th</sup> stage can also be considered for females: stage 5-3 (Ovary partially spent)

**Figure A2.22. Maturity Key for other Flatfish (*Solea senegalensis*, *Solea solea*, *Dicologlossa cuneata*) (Portugal).**

MATURITY STAGE		CHARACTERISTICS
1	Immature/Resting	Very small gonads. Testes and ovaries translucent and/or rose-coloured
2	Beginning of development/Recovering	Gonads occupying a larger volume inside the abdominal cavity. Ovaries opaque with some oocytes visible. Testes opaque.
3	Advanced development	Gonads occupying an even larger volume inside the abdominal cavity and with blood vessels visible. Ovaries full of visible oocytes, some of them already translucent. Testes firm and opaque, with sperm stored in the lobes.
4	Spawning	Gonads filling the whole abdominal cavity. Ovaries with translucent oocytes. Testes white-coloured.
5	Post-spawning	Ovaries slack, yellow or red-coloured, with hematoma. Testes opaque, light green-coloured, with hematoma.

**Figure A2.23. Maturity Key for Pouting (Portugal).**

### **Females**

*I Immature/resting. Ovaries small, transparent or translucent. No oocytes can be seen with the naked eye.*

*II Developing. Ovaries larger and thicker, whitish or pinkish in colour. Small opaque oocytes can be seen with the naked eye.*

*III Pre-spawning. Ovaries thicker, occupying almost the whole body cavity. Large opaque oocytes fill the whole ovary.*

*IV Spawning. Ovaries occupy the whole body cavity. Hydrated oocytes dominate and will be easily extrude under a slight pressure on the abdomen.*

*V Post-spawning. Ovaries reduced in size and reddish in colour. Residual eggs can be seen with the naked eye.*

### **Males**

*I Immature/resting. Testes very small, firm and pinkish in colour.*

*II Developing. Testes white-pinkish in colour and larger than in previous stage.*

*III Pre-spawning. Testes white in colour and occupying a large part of the body cavity. Sperm can be extruded after a pressure on the abdomen.*

*IV Spawning. Testes white in colour. Sperm can be extruded very easily after a slight pressure on the abdomen.*

*V Post-spawning. Testes reddish in colour. Residual sperm can be observed especially in the sperm duct.*

**Figure A2.24. Maturity Key for Black and Silver Scabbardfish (Continental Portugal and Azores)**  
(from Gordo *et al.*, 2000)

**Males:**

<b>Maturity stage</b>		<b>Description</b>
Immature	Juvenile <b>1</b>	Testis thin, with a linear form. Flexible claspers shorter than the pelvic fin.
	Maturing <b>2</b>	Testis with a linear form, but well developed or starting the segmentation; Efferent ducts start to meandering. Claspers more robust but flexible, larger than the pelvic fin.
Mature	Adult <b>3</b>	Testis segmented. Rigid claspers.
	Active <b>4</b>	Testis segmented and highly vascularized. The dimensions may be smaller than in stage 3. Claspers more flexible than in stage 3, with open canals and red tip.

**Females:**

<b>Maturity stage</b>		<b>Description</b>
Immature	1      Juvenile	Ovary with small oocytes (diameter less than 1 cm), sometimes even not distinguished. Uterus narrow. Oviducal gland is not evident.
	2      Maturing	Ovary with vitellogenic oocytes with maximum diameter of 3 cm, and without atretic oocytes. Uterus narrow but larger than in stage 1 (interquartile ranges from 2.4-3.4 cm). Oviducal gland seldom distinguished.
Mature	3      Adult	Oocytes in both ovaries with large dimensions, attaining 8/9 cm. Uterus enlarged. Oviducal gland well developed in the anterior oviduct.
	4      Developing	Large eggs within the uterus. Embryos are not distinguished.
	5      Differentiation	Small embryos are distinguished, with external gill filaments and no pigmentation. Each embryo is linked by the yolk stalk to the yolk sac.
	6      Extrusion	Embryos are almost fully developed, pigmented and without external gills. The yolk sac is almost depleted or already absent. Inside the embryo, the internal yolk may be present.
	7      Resting	Ovaries present large sized atretic oocytes. The uterus is enlarged (its width may reach 8 cm) and is extremely vascularized.
	8      Maturing (not for the 1 <sup>st</sup> time)	Ovary with oocytes in many stages of development and high frequencies of atretic ones. Uterus enlarged, with chubby walls and with folds within the inner uterus wall. Oviducal gland distinguished.

**Figure A2.25. Maturity Key for Deep-water Sharks (Portugal).**

## MATURITY SCALE *ELASMO 1*

### *MALES*

- A or 1 = **immature, juvenile** (plate 3, figs 1-2; plate 6, fig. 2; plate 8, fig. 1)  
 Claspers undeveloped as small, flexible sticks being much shorter than extreme tips of posterior pelvic fin lobes. Gonads (testes) small, sperm ducts straight and thread-like.
- B or 2 = **maturing, adolescent, subadult** (plate 3, fig. 3; plate 8, fig. 2)  
 Claspers becoming extended, approaching tips of posterior pelvic lobes, as long as or a bit longer than posterior pelvic lobes, their terminal region (glans) becoming structured, but skeleton still soft and flexible. Gonads enlarged, sperm ducts eventually beginning to meander (coil).
- C or 3 = **mature, adult** (plate 4, fig. 1; plate 6, fig. 3; plate 7, fig. 2; plate 8, fig. 3)  
 Claspers full length, as long as or longer than posterior pelvic lobes, their external and internal glans structures fully formed, skeleton hardened so that claspers stiff and glans' free cartilaginous components sharp. Gonads greatly enlarged, sperm ducts meandering over almost their entire length and tightly filled with sperm.
- D or 4 = **active, copulating** (plate 4, figs 2 + 3; plate 8, fig. 4)  
 Glans clasper often dilated, its structures reddish and swollen. Sperm flowing on pressure from cloaca and/or present in clasper groove or glans. Sperm ducts largely as stage C/3 but may be less tightly filled, whereas seminal vesicle may be well filled. For oviparous sharks and chimaeras, this stage does not necessarily mean that the glans is spread open, but fleshy pads are obviously enlarged and sperm is present in clasper grooves.

### *FEMALES – ovarian stages*

- A or 1 = **immature, juvenile** (plate 1, fig. 1; plate 5, fig. 1; plate 9, fig. 1)  
 Ovaries small, their internal structure gelatinous or granulated. No oocytes differentiated or all uniformly small, granular. Oviducts (uteri) narrow, thread-like.
- B or 2 = **maturing, adolescent** (plate 1, fig. 2; plate 5, fig. 2; plate 9, fig. 2)  
 Ovaries somewhat enlarged, walls more transparent. Oocytes becoming differentiated to various small sizes. Uteri largely as stage A/1 but may become widened posteriorly.
- C or 3 = **mature, adult** (plate 1, fig. 3; plate 7, fig. 1)  
 Ovaries large and tight. Oocytes enlarged, with some being very large. Uteri enlarged and widening over nearly their entire length.

### *FEMALES - uterine stages*

- D or 4 = **active** (plate 2, fig. 1)  
 A distinctly large yolk-egg present in one or both Fallopian tubes. No egg capsule yet visible in shell gland, or beginning formation of egg capsule at most.
- E or 5 = **advanced** (plate 2, fig. 2; plate 6, fig. 1; plate 9, fig. 3)  
 Large yolk-eggs in Fallopian tubes, or already passing through into egg capsules. Egg capsules about fully formed in one or both oviducts but still soft at upper end and located very close to Fallopian tubes.
- F or 6 = **extruding** (plate 2, fig. 3; plate 7, fig. 3; plate 9, fig. 4)  
 Completed, hardened egg capsules in one or both oviducts, more or less separated from Fallopian tubes. Skate capsule surface mostly covered with dense silky fibres. Either no enlarged oocytes in Fallopian tubes, or one or two in position. If oviducts empty but still much enlarged and wide, capsules have probably just been extruded - this corresponds with either stage D/4 or E/5.

**Figure A2.26. Maturity Keys for Rays and Skates (from Stehmann, 2002) (Portugal).**

### FEMALES

Stages		Macroscopic description of the ovaries	Microscopic description of the ovaries
0	Immature	The gonads are small and translucent	Primary or previtelogenic oocytes only
II	Developing	The gonads are opaque, consistent, with some vascularization, the oocytes are scarcely visible with rose colour ( <i>H. dactylopterus</i> ); with yellow/orange colours ( <i>Beryx spp.</i> , <i>P. bogaraveo</i> )	Oocytes yolked undergoing through vitellogenesis, with yolk granules on the cytoplasm
III	Pre-spawning	The gonads are opaque, consistent, with a vascularization well developed, the oocytes are well visible, oocytes with orange, rose or white colours ( <i>H. dactylopterus</i> ); with yellow/orange colours ( <i>Beryx spp.</i> ); with orange/reddish color ( <i>P. bogaraveo</i> )	Oocytes in a final maturation usually with a migratory nucleus and coalescence of yolk material
IV	Spawning	The gonads are opaque, consistent, with a vascularization well developed, the hydrated oocytes are well visible, forming a gelatinous matrix well visible with orange, rose or white colours ( <i>H. dactylopterus</i> ); orange/reddish colours ( <i>Beryx spp.</i> , <i>P. bogaraveo</i> )	Hyaline oocytes or evidence of hyaline oocytes
V	Spent	The gonads are opaque, flaccid, with some vascularization, sometimes some residual oocytes visible, with a reddish colour ( <i>H. dactylopterus</i> , <i>Beryx spp.</i> , <i>P. bogaraveo</i> )	Atretic oocytes (the ovaries were only classified as atretic when a large percentage of yolked oocytes were atretic, or when atretic oocytes were accompanied only with unyolked oocytes)
I	Resting	The gonads are opaque, consistent, with some vascularization, the oocytes are not visible, with a reddish to rose colour ( <i>H. dactylopterus</i> , <i>P. bogaraveo</i> ); with yellow/orange colours ( <i>Beryx spp.</i> )	Primary and unyolked oocytes usually at the start of yolk vesicle accumulation

Figure A2.27. Maturity Key for the other fish sampled in the Azores (Portugal) (from Menezes *et al.*, 2001).

**MALES**

Stages		Macroscopic description of the TESTES	Microscopic description of the TESTES
0	Immature	The gonads are small and translucent	Small transverse section compared to resting male; spermatogonia and little or no spermatocyte development; sperm ducts and main spermatic duct not as evident as in resting stage
II	Developing	The gonads are large, consistent and white	Development of cysts containing spermatogonia in mitosis, spermatocytes and spermatids
III	Pré-spawning	The gonads are consistent, well developed, white and with visible sperm	Containing cysts in all stages of development, spermatozoa in sperm ducts and in main spermatic duct
IV	Spawning	The gonads are whitish in colour, with very visible sperm that run when abdomen is pressed	Predominance of spermatozoa in sperm ducts and in main spermatic duct, little or no occurrence of spermatogenesis
V	Spent	The gonads are opaque, flaccid, with "bloody aspect" and with some sperm	Some residual spermatozoa in sperm ducts and main spermatic duct, occurrence of spermatogonia and development of conjunctive tissues
I	Resting	The gonads are small, whitish or grey in colour, sometimes with residual sperm	Large transverse section compared with immature male, little or no spermatocyte development, beginning of spermatogonia activity, empty sperm ducts, sometimes residual spermatozoa is still visible

**Figure A2.27 (cont.).** Maturity Key for the other fish sampled in the Azores (Portugal) (from Menezes *et al.*, 2001)

### Macroscopic and microscopic maturity scale for female sardine

MATURITY STAGE	MACROSCOPIC DESCRIPTION	MICROSCOPIC DESCRIPTION	
I	VIRGIN AND RESTING	Invisible or very small cord-shaped ovaries; translucent with almost no colour.	Unyolked oocytes as the unique type of oocytes present in the gonad.
II	DEVELOPING	Wider ovaries occupying 1/4 to 3/4 of body cavity; opaque with pinkish or yellow colour . Visible oocytes are not present.	The most advanced batch of oocytes are partially-yolked ones.
III	PRE-SPAWNING	Bigger ovaries occupying 3/4 to almost fitting body cavity; opaque with yellow or orange colour . Small opaque oocytes are visible.	The most advanced batch of oocytes are yolked ones.
IV	SPAWNING	Large ovaries occupying the full body cavity; fully or partially translucent with gelatinous aspect. Hyaline oocytes are visible (some small opaque oocytes can be visible).	The most advanced batch of oocytes are hydrated ones.
V	PARTIAL POST-SPAWNING (recovering to Stage III)	Deflated and flacid ovaries occupying about 3/4 of body cavity; with some ruptured blood vessels that gives them a bloodshot aspect. Some small opaque oocytes are visible (some hyaline oocytes can be present).	Post-ovulatory Follicles may be present. Some ruptured blood vessels and free hemocytes present spread all over the ovary tissue (light hemocyte infiltration). Presence of all oocyte stages is possible.
VI	ULTIMATE POST-SPAWNING	Very deflated and flacid ovaries occupying from about 3/4 to 1/4 of body cavity; with many ruptured blood vessels that gives them a reddish color. Some small opaque oocytes can be visible (no hyaline oocytes are present)	Post-ovulatory Follicles may be present. Many ruptured blood vessels and free hemocytes invading the ovary tissue (generalised hemocyte infiltration). No yolked oocytes, or most of them in an atretic state.

Figure A2.28. Maturity Key for pelagic species (Spain).

MATURITY STAGE	MALES	FEMALES
I (Inactive)	Small and flats gonads. Without sperm	Small cylindrical transparent ovaries. Without ovocytes.
II (Maturing)	Larger gonads with sperm on cutting. Pink/white colour.	Large ovaries with blood capillaries. Yellow/orange coloured. Visible opaque ovocytes without bruised areas.
III (Spawning)	Sperm flows on applying pressure to the abdomen	Translucent ovocytes which may flow or not on applying pressure.
IV (Post-Spawning)	Gonads reduced in size reddened. Occasionally with sperm on cutting. Bruised	Bruised ovary. Purple in colour. Flaccid. Occasionally with residual ovocytes

**Figure A2.29. Maturity Key for demersal species (Spain).**

Table 6.3.1. Maturity Stages for White Anglerfish assigned *de visu* (according to first Co-ordination Meeting of the BIOSDEF project (see Annex 1).

Maturity stage ( <i>de visu</i> )	Males	Females
I Immature or Virgin	Tube-shaped testicles, pink or transparent, cover a small proportion of the visceral cavity. Sperm not visible	Band-shaped ovaries, very transparent and without visible oocytes
II Maturing	Testicles cover a great proportion of the visceral cavity, pearl-coloured, some sperm in the lumen.	Ovaries cover a great proportion of the visceral cavity, with creamy colour. The band is longer and has visible oocytes.
III Mature or Prespawning	Pearl-coloured testicles and with the lumen full of sperm.	Orange-coloured oocytes with accentuated vascularization and the presence of some hyaline eggs (hydrated)
IV Spawning	Sperm is easily freed by applying pressure to the abdomen.	An enormous gelatinous yellow mass wraps the hyaline oocytes.
V Post-spawning	Testicles appear red and stained. There is no sperm, or a little residual.	Soft or retracted ovaries, possible residual oocytes, reabsorbed.

**Figure A2.30. Maturity Key for Anglerfish (Spain) (from BIOSDEF Report, 1998).**

Maturity stage ( <i>de visu</i> )	Males	Females
<b>I</b> <b>Inactive</b>	Small, flat gonads, <u>without sperm</u>	Small cylindrical transparent ovaries. <u>Without oocytes</u>
<b>II</b> <b>Maturing</b>	Large gonads, <u>with sperm on cutting</u> . Pink/white colour	Large ovaries with blood capillaries. Yellow/orange coloured. <u>Visible opaque oocytes</u> , without bruised areas.
<b>III</b> <b>Spawning</b>	<u>Sperm flows</u> on applying pressure to the abdomen	<u>Translucent oocytes</u> which may flow or not on applying pressure.
<b>IV</b> <b>Post-spawning</b>	Gonad reduced in size, reddened. Occasionally with sperm on cutting. <u>Bruised</u> .	<u>Bruised ovary</u> . Purple in colour. Flaccid. Occasionally with residual oocytes

**Table 4.2.1.** Hake maturity stages assigned *de visu*, as used in this study.

Maturity stage (microscopy)	Males	Females
<b>I</b> <b>Inactive</b>	Spermatogonia and Spermatocyte present, Spermatozooids absent	Oogonia, Chromatin nucleolus stage
<b>II</b> <b>Maturing</b>	Spermatogonia and Spermatocyte present, few Spermatozooids	Perinucleolus stage, early vitellogenesis
<b>III</b> <b>Spawning</b>	Spermatozooids predominant	Migratory nucleus stage and Oocyte hidration
<b>IV</b> <b>Post-spawning</b>	Empty seminiferal ducts, residual spermatozooids and few Spermatogonia	Post-ovulatory follicle and follicular atresias

**Table 4.2.2.** Hake maturity stages assigned by means of histology, used in this study.

**Figure A2.31.** Maturity Key used by AZTI (Bask Country, Spain) for Hake (from Lucio *et al.*, 1998, Lucio *et al.*, 2000).

SEX	GONAD ASPECT (TELEOSTEA)	MATURATION STATE	NEW STAGE	MEDITS STAGE
U	Sex not distinguished by naked eye. Gonads very small and translucent, almost transparent. Sex undetermined	UNDETERMINED	0	0
F	Small pinkish and translucent ovary shorter than 1/3 of the body cavity. Eggs not visible by naked eye.	VIRGIN	1	1
M	Thin and whitish Testes shorter than 1/3 of the body cavity.			
F	Pinkish/orange and translucent ovary long about 1/2 of the body cavity. Eggs not visible by naked eye.	IMMATURE- DEVELOPING *	2a	2
M	Whitish/pinkish Testes, more or less simmetrical, long about 1/2 of the body cavity.			
F	Pinkish/orange and translucent ovary long about 1/2 of the body cavity. Eggs not visible by naked eye.	SPENT-RECOVERING *	2b	
M	Whitish/pinkish Testes, more or less simmetrical, long about 1/2 of the body cavity.			
F	Ovary pinkish-yellow in colour with granular appearance, long about 2/3 of the body cavity. Eggs are visible by naked eye through the ovaric tunica, which is not yet translucent. Under light pressure, eggs are not expelled.	MATURING	2c	
M	Whitish to creamy Testes long about 2/3 of the body cavity. Under light pressure, sperm is not expelled.			
F	Ovary orange-pink in colour, with conspicuous superficial blood vessels, long from 2/3 to full length of the body cavity. Large transparent, ripe eggs are clearly visible and are expelled under light pressure. In more advanced conditions, eggs escape freely.	MATURE	3	3
M	Whitish-creamy soft Testes long from 2/3 to full length of the body cavity. Under light pressure, sperm is expelled. In more advanced conditions, sperm escapes freely.			
F	Reddish ovary shrunken to about 1/2 length of the body cavity. Flaccid ovaric walls; ovary may contain remnants of disintegrating opaque and/or translucent eggs.	POST-SPAWNING	4a	4
M	Bloodshot and flabby Testes shrunken to about 1/2 length of the body cavity.			
F	Small pinkish/reddish ovary long about 1/3 of the body cavity. Eggs not visible by naked eye.	SPENT **	4b	
M	Thin pinkish/whitish Testes long about 1/3 of the body cavity.			



#### Adult specimens

\* : WARNING ! These stages could be easily confused each other. The knowledge of species' maximum length and growth can help in discriminating the two stages.

\*\* : WARNING ! This stage could be easily confused with stage 1. The knowledge of species' maximum length and growth can help in discriminating the two stages.

**Figure A2.32: New Maturity Key for Teleost fish sampled in the Mediterranean coast of Spain.**

SEX	GONAD ASPECT (SELACHIA)	MATURATION STATE	NEW STAGE	MEDITS STAGE
U	Sex not distinguished by naked eye. Gonads very small and translucent, almost transparent. Sex undetermined	UNDETERMINED	0	0
F (Internal characteristics: dissection is necessary)	Ovary is barely discernible with small isodiametric eggs. Oviducts contain no eggs. Distal part of oviducts is thick-walled and whitish. The shell gland is very small	VIRGIN	1	1
M (External characteristics: dissection is NOT necessary)	Pterigopods are small and flaccid and do not reach the posterior edge of the pelvic fins			
F (Internal characteristics: dissection is necessary)	Whitish maturing eggs are visible in the ovary. The distal part of oviducts (uterus) is well developed but empty. The shell gland is small.	MATURING	2	2
M (External characteristics: dissection is NOT necessary)	Pterigopods are larger, but not ossified. They extend to the posterior edge of the pelvic fins.			
F (Internal characteristics: dissection is necessary)	Ovaries contain yellow eggs, except immediately after ovulation in viviparous species and at the end of spawning season in oviparous species. The shell gland is enlarged and oviducts are distended and, in viviparous species, thin-walled, flaccid and often highly vascularized.	MATURE	3a	3
M (External characteristics: dissection is NOT necessary)	Pterigopods extends well beyond the posterior edge of the pelvic fin and their internal structure is hard and ossified.			
F (Viviparous sharks; internal characteristics: dissection is necessary)	Ovaries at resting stages. It could be confused with stage 1 (or 2). The ovaries, although empty, are wider over their full length in contrast to stage 1 (or 2).	SPENT	3b	



Adult specimens

Figure A2.33. New Maturity Key for Eslamobranchs sampled in the Mediterranean coast of Spain.

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TABLE IV  
Maturity Scale Recommended by Herring Committee of I.C.E.S.  
*Explor. Rep 1962: 66-77*

Stage	Description
I	Virgin herring. Gonads very small, threadlike, 2-3 mm broad. Ovaries wine red. Testes whitish or grey-brown.
II	Virgin herring with small sexual organs. Height of ovaries and testes about 3-8 mm. Eggs not visible to naked eye but can be seen with a magnifying glass. Ovaries a bright red colour, testes a reddish grey colour.
III	Gonads occupying about half of ventral cavity. Breadth of sexual organs between 1 and 2 cm. Eggs small but can be distinguished with naked eye. Ovaries orange; testes reddish grey or greyish.
IV	Gonads almost as long as body cavity. Eggs larger, varying in size, opaque. Ovaries orange or pale yellow; testes whitish.
V	Gonads fill body cavity. Eggs large, round; some transparent. Ovaries yellowish, testes milk white. Eggs and sperm do not flow, but sperm can be extruded by pressure.
VI	Ripe gonads. Eggs transparent; testes white; eggs and sperm flow freely.
VII	Spent herring. Gonads baggy and bloodshot. Ovaries empty or containing only a few residual eggs. Testes may contain remains of sperm.
VIII	Recovering spents. Ovaries and testes firm and larger than virgin herring on Stage II. Eggs not visible to naked eye. Walls of gonads striated; blood vessels prominent. Gonads wine red colour. (This stage passes into Stage III.)

Figure A2.34. Maturity Key for Herring (Sweden) (from ICES 1962).

MALES	STAGE 1-8	STAGE 1-5	STAGE 1-4	FEMALES
Small rather translucent ribbons Immature	1	1	1	Yellow-reddish rather translucent gonads Immature
Slightly larger 1-3 cm long frill on gonad, colouration variable Immature	2	1	1	Grey-reddish translucent to opaque mediums sized gonads No visible oocytes Immature
Swelling grayish Mature	3	2	2	Yellow reddish gonads Small oocytes just visible Mature
Gonads fully developed in length but not entirely white or filled	4	2	2	Gonads fully developed in size, oocytes visible and opaque
Gonads filled and white	5	2	2	
	Maier 1908	BITS Manual	IBTS ICES	

Figure A2.35. Maturity Keys Correspondence for Cod, other gadoids and Flatfish (Sweden).

FEMALES	STAGE	MALES
Ovaries small, thread like, wine red in colour.	1  Virgin	Testes whitish or grey brown.
Ovaries bright red colour, eggs not visible to naked eye but can be seen under magnification.	2  Late virgin	Testes now 3-8 mm in breadth, reddish grey in colour.
Ovaries occupying ½ of ventral cavity, orange in colour and 1-2 cm in breadth. Eggs small and visible to the naked eye.	3  Early ripening	Testes occupying ½ of ventral cavity, reddish grey to greyish in colour.
Ovaries almost as long as body cavity, orange to pale yellow in colour.	4  Ripening	Testes almost as long as body cavity, whitish in colour.
Ovaries filling body cavity and are yellowish in colour. Eggs large round with some transparent.	5  Late ripening	Testes filling body cavity and are milk white in colour. Sperm can be extruded by pressure.
Eggs transparent and flow freely.	6  Ripe	Testes white and sperm flows freely.
Ovaries baggy and bloodshot, empty or containing only a few residual eggs.	7.1  Early spent	Testes baggy and bloodshot, may contain remains of sperm.
Ovaries colourless or wine red coloured. Firmer and slightly larger than virgin stage. No residual eggs.	7.2  Late spent	Testes reddish grey or greyish. Firmer and slightly larger than virgin stage. No milt present.
Ovaries firm and larger than stage 2. Eggs not visible to naked eye. Wine red colour. Walls of gonad striated, blood vessels prominent.	8  Recovering spent (Leads to stage 3)	Testes firm and larger than stage 2.

Figure A2.36. Herring Maturity Key (CEFAS, UK).

FEMALES	STAGE	MALES
<p>Roundfish - ovaries small, elongated, whitish, translucent.</p> <p>Flatfish – ovaries small, ovary wall thin and easily broken, internally yellowish-orange (although can be variable).</p> <p>No sign of development.</p>	<p><b>I</b></p> <p>Immature</p>	<p>Roundfish - Testes very thin ribbon lying along an unbranched blood vessel.</p> <p>Flatfish – Testes tight up against back of gut cavity and very small, not usually larger than 10mm x 2mm.</p> <p>No sign of development.</p>
<p>Eggs are beginning to develop.</p> <p>Roundfish - the ovaries are filling more and more of the body cavity.</p> <p>Flatfish – ovaries are extending down the side of the body of the fish.</p> <p>There are no signs of hyaline eggs.</p>	<p><b>M</b></p> <p>Maturing</p>	<p>Colour is progressing towards creamy white and the Testes are filling more and more of the body cavity.</p> <p>Roundfish – may be many lobed.</p> <p>Flatfish – at latest phase the Testes can become bulbous.</p>
<p>Hyaline eggs present, one or many. Ovaries will not run, even under moderate pressure.</p>	<p><b>H</b></p> <p>Hyaline</p>	<p>No equivalent stage.</p>
<p>Will extrude eggs under light pressure to advanced stage of extruding eggs freely with some eggs still in the gonad.</p>	<p><b>R</b></p> <p>Running</p>	<p>Will extrude sperm under light pressure to advanced stage of extruding sperm freely with some sperm still in the gonad.</p> <p>Flatfish – sperm evident in ducts.</p>
<p>Ovaries shrunk with few residual eggs and slime.</p> <p>Ovaries becoming tighter, no sign of egg development.</p>	<p><b>S</b></p> <p>Spent</p>	<p>Testes shrunk with little sperm in the gonads but often some in the gonoducts which can be extruded under light pressure. The gonad can shrink back to very small size.</p> <p>Flatfish gonad becomes knobbly.</p>

Figure A2.37. CEFAS Standard 5-stage Maturity Key (UK).

### ANGLERFISH MATURITY KEY

Note the growth rate for *L.budegassa* is slower than for *L.piscworious*, so mature *L.budegassa* will be found at a smaller size than mature *L.piscatorious*

STAGE	STATE	MALE	FEMALE
1	<b>Immature</b>	Difficult to distinguish sex in very small fish. Testes pink transparent, taking up an insignificant part of the body cavity. Slightly more rounded than the female, running almost parallel to the vertebral column. 1-2mm in width.	Ovaries flat & transparent, wider than and the testes in male fish of similar length. The membrane connecting the ovary extends further out than that in the testes, so the gonad stretches further away from the vertebrae than in the males. In larger fish the ovary looks frilly. No eggs.
2	<b>Maturing</b>	Taking up a larger proportion of the body cavity. Not transparent, firm, yellow creamy pink. No ripe milt visible in sperm ducts.	Taking up a larger proportion of the body cavity. The ovary is broader, flat, ribbon like and longer. Egg follicles are visible as white specks and are densely packed giving the ovary a creamy appearance.
3	<b>Mature</b>	Testes larger in diameter and comprise a significant proportion of the body cavity. Creamy and firm. Sperm ducts are more pronounced but no milt extrudes when pressure applied.	Thickening, becoming more orange but still flat and ribbon like. Eggs much larger and densely packed. Edges of the ovary beginning to curl and veins on membrane running to the ovary are more prominent. The ovary may be up to 9m in length.
4	<b>Spawning</b>	Milt is present in the sperm ducts of the testes or the testes contain running milt. The testes feel quite spongy and are a greeny cream colour when very ripe.	Eggs round and gelatinous with yellow brown centres and look similar to frog spawn. Eggs not in obvious ribbons as with the earlier stages, but appear free flowing within the ovary membrane and occupy most of the body cavity.
5	<b>Spent</b>	The testes look bruised, red and blotchy. They are not as firm as they were when maturing and there is no milt present in the sperm ducts.	The ovaries have shrunk back to the size they were when maturing and the membrane appears loose and flabby. The remaining oocytes are yellow red in colour, of different sizes and breaking down as they are reabsorbed.

Figure A2.38. Anglerfish Maturity Key (CEFAS, UK).

HORSE MACKEREL MATURITY STAGES.		
STAGE	FEMALE	MALE
1. Virgin (immature)	Ovary very small and ribbon-like. Edges rounded. No eggs visible.	Similar to female. Edges of Testes sharp.
1-3. Developing virgin	May not be distinguishable in scad.	As female
2. Resting (mature fish)	Ovary larger than above. Surface wrinkled. No eggs visible.	Testes larger than stage 1-3. Wrinkled.
3. Developing (A)	Ovary half-length of body cavity or less. Eggs visible. Red-orange in colour.	Testes fills half body cavity or less. Grey in colour.
	From half to whole length of body cavity. No hyaline eggs.	As female.
4. Developing (B)	Ovary almost fills body cavity. Hyaline eggs present. Blood vessels prominent.	Testes almost fills body cavity. White and firm.
5. Ripe	As above but eggs easily expressed by slight pressure on flanks.	As above but milt easily expressed by slight pressure on flanks.
	Ovary flaccid. Large free opaque eggs visible but many smaller ones still present.	As above but milt easily expressed by slight pressure on flanks.
6. Running.	Ovary a long, narrow sack. Wine-coloured. Some opaque eggs.	Some milt has been discharged but Testes obviously not empty.
7. Partly spent.	Ovary shrinking. Less than half-length of body cavity. Ovary wall thickening.	Testes a long, narrow sack. Brownish. Some residual milt present.
8. Spent		Testes less than half-length of body cavity. Dark clumps visible through wall, which is thickening.
9. Recovering.		

**Figure A2.39. Horse-mackerel Maturity Key (CEFAS, UK).**

FEMALES	STAGE	MALES
Ovaries small, wine red and clear. Torpedo shaped. No sign of development.	<b>1</b> <b>Virgin</b>	Testes small, pale, flattened and translucent. No sign of development.
Ovaries occupying $\frac{1}{4}$ to $\frac{3}{4}$ body cavity. Opaque eggs visible, giving pale pink to yellowish colouration. Largest eggs without oil globule.	<b>2</b> <b>Early ripening</b>	Testes occupying $\frac{1}{4}$ to $\frac{3}{4}$ body cavity, off-white, no milt running.
Ovaries occupying $\frac{3}{5}$ to almost filling body cavity. Yellow to orange in colour. Largest eggs may have oil globule.	<b>3</b> <b>Late ripening/partly spent (early)</b>	Testes occupying $\frac{3}{5}$ to almost filling body cavity. Creamy white in colour.
Ovaries size variable from a full to $\frac{1}{4}$ . Characterised by externally visible hyaline eggs, no matter how few or how early the stage of hydration. Ovaries with hyaline eggs only in the lumen are not included.	<b>4</b> <b>Ripe</b>	Testes filling body cavity. Milt freely running.
Ovaries occupying $\frac{3}{4}$ to $<\frac{1}{4}$ of body cavity. Slacker than stage 3 and often blood shot.	<b>5</b> <b>Partly spent (late)</b>	Testes occupying $\frac{3}{4}$ to $<\frac{1}{4}$ of body cavity, with free running milt and shrivelled at anal end.
Ovaries occupying $\frac{1}{4}$ or less of body cavity. Reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs.	<b>6</b> <b>Spent/Recovering spent</b>	Testes occupying $\frac{1}{4}$ or less of body cavity. Opaque with brownish tint and no trace of milt.

NB Ovaries in which hyaline eggs are present in the lumen should be marked with an asterisk next to the maturity stage ie spawning fish.

**Figure A2.40. Mackerel Maturity Key (CEFAS, UK) (from Walsh *et al.*, 1990).**

STAGE	STATE	MALE	FEMALE
<b>A</b>	Immature	<p>Claspers undeveloped, shorter than extreme tips of posterior margin of pelvic fin.</p> <p>Testes small and thread-shaped.</p>	<p>Ovaries small, their internal structure gelatinous or granulated and with no differentiated oocytes visible.</p> <p>Oviducts small and thread-shaped, width of shell gland not much greater than the width of the oviduct.</p>
<b>B</b>	Maturing	<p>Claspers longer than posterior margin of pelvic fin, their tips more structured, but cartilaginous elements are not hardened, and the claspers are soft and flexible.</p> <p>Testes enlarged, sperm ducts beginning to meander.</p>	<p>Ovaries enlarged and with more transparent walls. Oocytes differentiated in various small sizes (&lt;5 mm).</p> <p>Oviducts) small and thread-shaped, width of the shell gland much greater than the width of the oviduct, but not hardened.</p>
<b>C</b>	Fully mature	<p>Claspers longer than posterior margin of pelvic fin, cartilaginous elements hardened and claspers stiff.</p> <p>Testes enlarged, sperm ducts meandering and tightly filled with sperm.</p>	<p>Ovary/ovaries large and tight. Oocytes enlarged, with some very large, yolk-filled oocytes (&gt;5 mm).</p> <p>Uteri enlarged and widening, shell gland fully formed.</p>
<b>D</b>	Active	<p>Clasper reddish and swollen, sperm present in clasper groove, or flows if pressure exerted on cloaca.</p>	<p><u>Viviparous species</u> (e.g. Spurdog, tope and smoothhounds, sting/electric rays): Distinct yolk-filled eggs with developing embryos present in the oviducts.</p> <p><u>Oviparous species</u> (e.g. Lesser-spotted dogfish and skates (Rajidae): Egg capsules beginning to form in shell gland and partially visible in uteri, or egg capsules fully formed and hardened in oviducts/uteri.</p>

**Figure A2.41. Elasmobranch Maturity Key (CEFAS, UK)**