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REPORT OF THE WORKSHOP ON SEXUAL MATURITY STAGING OF COD, WHITING, HADDOCK AND SAITHE (WKMSCWHS)

13–16 NOVEMBER 2007

COPENHAGEN, DENMARK

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CIEM Conseil International pour l'Exploration de la Mer

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1 Introduction

1.1 Participants

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Annex 1 provides addresses and contact information for all participants.

1.2 Term of Reference

2006/2/ACFM33

A Workshop on Sexual Maturity Staging of Cod, Whiting, Haddock and Saithe [WKMSCWHS] (Co-Chairs: Jørgen Dalskov and Jonna Tomkiewicz) was established and took place in Copenhagen, Denmark, from 13–16 November 2007 to:

a) Compare applied maturity scales and main criteria followed by the scientists/technicians involved in the national sampling, to classify each maturity stage for males and females.

b) Validate macroscopic maturity determination with histological analysis.

c) Standardise the criteria to classify each maturity stage.

d) Propose a common scale, with common classification criteria, to be used by all laboratories.

e) Identify the optimal sampling time to estimate maturity ogives.

1.3 Agenda

Agenda for the ICES workshop on Sexual Maturity staging of Cod, Whiting, Haddock and Saithe (WKMSCWHS): Charlottenlund Castle, Denmark, November 13th to 16th.

Tuesday 13th

10.00:	Welcome by Jørgen Dalskov and Jonna Tomkiewicz
10.30:	Presentation of collected material and histological results by Rikke Hagstrøm Bucholtz
11.00:	Coffee break
11.30:	Introduction to female maturity stage determination using histology and visual documentation by Jonna Tomkiewicz and Rikke Hagstrøm Bucholz
12.30:	Lunch
13.30:	Teamwork on female cod to discuss, group and describe stages macroscopically for practical use in maturity determinations based on collected material.
15.00:	Coffee break
15.30:	Teamwork continued
17.00:	End of Day 1

Wednesday 14th

09.00:	Presentation of teamwork and discussion of the stage determination of cod
10:30:	Coffee
11:00:	Teamwork on female saithe, haddock and whiting to discuss, group and describe stages macroscopically for practical use in maturity determinations based on collected material
12.30:	Lunch
13:30	Teamwork continued
16.30:	Presentation of teamwork results on stage determination and formulation of a useful female maturity scales
17.30:	End of Day 2
19:00:	Social event

Thursday 15th

09.00:	Introduction to male maturity stage determination using histology and visual documentation by Gavin Power, Rikke Hagstrøm Bucholtz and Jonna Tomkiewicz
10:00	Teamwork on male cod, saithe, haddock and whiting to discuss, group and describe stages macroscopically for practical use in maturity determinations based on collected material
11:00:	Coffee break
11:30	Teamwork continued
12.30:	Lunch
13:30	Teamwork continued

15.00: Coffee

15.30: Teamwork continued

16.30: Presentation of teamwork results on stage determination and formulation of a useful female maturity scales

17.30: End of Day 3

Friday 16th

09.00: Presentation of present sampling and data collection by participants for each country / laboratory

10.00: Coffee

10.30 Discussion of improvement of methods and quality assurance: maturity scales, timing of sampling, frequency of sampling, etc., reporting and application in assessment-moderator Jørgen Dalskov

12.30: Report of workshop

13.00: End of workshop

1.4 Background

For stocks assessed by ICES data on maturity by age is needed as the advice given on the stock status is based on spawning stock biomasses. Therefore, it is of importance that these data are accurate. Data on sexual maturity are collected onboard research vessels during surveys, by observers participating on commercial fishing trips or at ports when carrying out marked sampling.

Furthermore, according to the EU data collection regulation (DCR) Council Reg. 1543/2000, Commission Reg. 1630/2001 and Commission Reg. 1581/2004 data on sexual maturity has to be collected for a number of species. Precision levels on data collected have to be estimated in order to be able to calculate precision of the stock estimate. Calculated precision on collected data does not necessarily give a true picture of the quality of the data. As these data is based on a subjective estimate of the sexual maturity, the accuracy of the maturity determination is essential.

For some species comprehensive sexual maturity manual exists while for other species there are only limited descriptions in e.g. survey manuals. For the International Bottom Trawl Survey (IBTS) a 4-stage scale exists for gadoids and data are provided to the ICES databases in this format. For the Baltic, a similar 5-stage scale exists for the Baltic International Trawl Survey (BITS), which also is the reporting format to DATRAS.

In some cases, maturity is determined according to these scales while in other cases, national scales are used to grade maturity and the data are then subsequently converted to ICES scales and reported. As data on sexual maturity for the same stock are collection by a number of countries and within a country by a number of different scientists/technicians it is of outmost importance that the data collection is carried according to a uniform standard.

Analyses on historical sexual maturity data have shown some discrepancies in the collected data. It was therefore decided at the ICES, PGCCDBS meeting in 2007 to establish a workshop on sexual maturity for cod, haddock, whiting and saithe (ICES, 2007).

1.5 Preparation and outline of the workshop

The procedure was to obtain photos of the fresh gonads, records of national staging and preserved gonad samples for histological analysis for subsequent maturity evaluation of all four species. Sampling procedures were elaborated at DTU Aqua and sent to collaborating institutes in all countries participating in the IBTS. Photographs, records and samples were after each national cruise sent to DTU Aqua, where gonad samples were selected for histological processing to validate the maturity stage of both females and males. The histological sections were photographed and the gonadal developmental stage was determined. The histological characteristics of ovaries was graded on a scale from 1 to 10 (Tomkiewicz *et al*., 2003), and a similar scale was developed for testes.

The ovaries and testes of each species were categorised according the histological staging. Photographs of the fresh gonads and matching histological sections were used as basis for discussions during the workshop. The histological characteristics were compared with the original stage determination and used to elaborate a common scale, with revised macroscopic and histological classification criteria.

The reproductive cycle and strategy of each species was described. Photographs of gonads and tissue were selected as basis for draft manuals. The best sampling time to estimate maturity ogives in relation to existing IBTS cruises was judged for all species based on the timely occurrence of different stages and the accuracy of the stage determination.

The workshop has thus had a practical focus with the aim to discuss and elaborate draft manuals. The literature has been consulted but a comprehensive literature review was not part of the ToR for the present workshop. The elaboration of illustrated and histologically documented and maturity manuals follows the concept developed for Baltic cod (Tomkiewicz *et. al*., 2002, 2003) and Baltic herring (Bucholtz *et al*., 2007).

2 Sampling and histological analyses

2.1 Sampling

The sampling was conducted in cooperation between the participating countries during the IBTS 1Q and IBTS 3Q 2008. Institutes from Denmark, France, Germany, Holland, Norway, Scotland and Sweden participated in the sampling during the IBTS 1Q in January to March. Denmark, England, Germany, Norway, Scotland and Sweden participated during the IBTS 3Q in August to September. A few specimens from Greenland sampled during May and June were also included.

During each national cruise a sub-sample of 5 individuals per 10 cm length group per sex was collected by sampling randomly from the catch. The sampled fish were stored on ice until processing. As it is not likely that all length groups are represented in one haul, the preferred sampling strategy was to commence the sampling by random selection of fish, and as length groups were completed, sampling focused on length groups not yet covered. With respect to cod which presently are scarce either all specimens in the catch were sampled or, if the most cod in the catches were of similar size, maximum 10 specimens per 10 cm length group per sex were sampled. It was attempted to spread the sampling on as many locations as possible, but at the same time considering that as many length groups as possible were filled.

Preferably the sampling procedure should have been executed 4 times during a year to follow the reproductive cycle and development of the gonads. However, cruises are not conducted each quarter and limitations in sampling capacity were also recognised. The sampling procedure was therefore restricted to the existing IBTS 1Q and IBTS 3Q cruises, during which the maturity sampling at present is conducted and data subsequently reported to ICES. The total numbers sampled per species and sex during Q1 and Q43 surveys are given Table 2.1.1 and 2.1.2, respectively.

Table 2.1.1. Samples from IBTS 1Q

SPECIES/SEX	♀	♂	?	TOTAL
Cod	252	271	10	533
Whiting	174	167	4	345
Haddock	146	140	1	287
Saithe	60	73	1	134
Total	632	651	16	1299

Table 2.1.2. Samples from IBTS 3Q

SPECIES/SEX	♀	♂	?	TOTAL
Cod	96	75	10	181
Whiting	76	55	0	131
Haddock	80	66	0	146
Saithe	47	45	0	92
Total	299	241	10	550

For the data collection and histology samples, each specimen was given an identification number including the following information: Country, station, date and fish number, e.g. DK01-010307/1 (Denmark, Station 1, 1st March 2007, fish number 1). For each specimen the following information was recorded:

1) Total length (L_T)
2) Total weight (M_T)
3) Sex
4) Maturity stage (according to the maturity scale normally used and the ICES IBTS 4 scale, see Annex 2)
5) Gonad weight (M_{GO})
6) Liver weight (M_{LI})
7) Gutted weight (M_{GU})

A series of photographs of the fish and gonad including the identification number were taken during the process according to a predefined sampling program (See examples for each of the set-ups in Annex 3):

1) Fish with gonad was photographed (Example 1)
2) Fish with gonad lying next to it was photographed (Example 2)
3) Close-up photo(-es) of gonad was taken (Example 3)

The gonad or sub-samples of the gonad tissue were preserved after the photographs were taken. If small (immature) the entire gonad was sampled. For larger the gonads, 3 transverse slices app. 2 cm wide were selected from the anterior, middle and posterior part respectively (Example 4) of one of the gonad lobes. The sampled tissue was preserved in separate containers with a 4% formaldehyde solution buffered by NaH_2PO_4-H_2O and Na_2HPO_4-$2H_2O$ for histological processing. Slices from the right or the left lobe were randomly chosen for preservation. It was recorded from which lobe the slice was taken (right/left) and whether it was from the anterior, middle or posterior part.

For all samples, cruise ID, station, date, latitude, longitude, the initials of the persons who collected the fish and the species and stock was recorded. The samples were sent to the Technical University of Denmark, National Institute of Aquatic Resources (DTU Aqua) (previously name Danish Institute for Fisheries Research) for further examination as well as copies of the data, photographs and applied maturity scales.

The numbers per length group, quarter and sex for cod, whiting, haddock and saithe are given in Annex 4. The numbers per maturity stage based on the original staging and according to IBTS 4-scale is given in Annex 4 per species, sex and quarter.

2.2 Histological analysis

A variety of stages were represented in the samples from IBTS first quarter survey as this coincided with the spawning period for all 4 species (Annex 5). Consequently, most maturity stages were covered during the 1Q IBTS. In contradiction, only few stages were represented during the 3 quarter survey. In order to optimise the histological processing and analysis of samples which is time requiring, a subset of samples were selected from the total amount collected from the IBTS 1Q for the purpose of the workshop. The samples were selected using the following criteria for each of the four species:

- Within each stage and sex: one with high, intermediate and low GSI
- Optimally with at least one fish per length group

Cod was used as a model for the histological analysis and around 10 fish per country were processed. The samples from IBTS 3Q was used to supplement the samples from IBTS 1Q in order to cover all maturity stages histologically as well as by photographs for each country if possible. For the other species the number of samples processed was lower, but the sample selection followed the same procedure.

A transverse section of about 5 mm from the middle part of the preserved lobe was sampled for histological analysis. The tissue was dehydrated and embedded in paraffin using standard procedures. The paraffin embedded tissue was sectioned on a microtome. Three consecutive sections were taken from the paraffin block. The sections were stained using haematoxylin and eosin (H & E) and mounted.

Table 2.2.1 shows the total number of gonad samples processed histologically for each species and each sex. Tables showing the number of processed gonad samples according to length groups are provided in Annex 4.

Table 2.2.1. Histologically processed samples from IBTS quarter 1 and 3.

SPECIES/SEX	♀	♂	NOT SEXED	TOTAL
Cod	71	73	4	148
Whiting	54	32	1	87
Haddock	52	37	1	90
Saithe	33	29	1	63
Total	210	171	7	388

3 Determination of gonadal maturity of gadoids

3.1 Introduction

The maturity scales and main criteria applied by the scientists and technicians involved in the national sampling to classify maturity stages differed among the institutes involved in the IBTS (Annex 2) as well as among areas including the Baltic Sea and Kattegat. Most countries participating in the IBTS survey uses the IBTS 4-stage maturity scale but with some exceptions. Norway uses a 6-key scale–where stages 1–4 are similar to IBTS, while "blank" in undecided/undetermined and 5 stage "Uncertain" is used when immature and spent/resting can not be distinguished. England uses a 5-level scale including I (immature), M (maturing), H (Hyaline), R (running) and S (spent). Sweden uses Maier's 8-stage scale for cod. The national scales are translated into the IBTS 4-stage scale before reporting to ICES.

In the Baltic Sea, an ICES 5-level scale exists that compares to the IBTS 4-stage scale, but with a stage to identify resting specimens and skip spawning. In many countries, data are sampled according to national scales and translated into the standard scale before reporting to ICES.

An important problem is that it is difficult to distinguish late immature (part of stage 1) and resting (part of stage 4) outside the maturation and spawning period, which the Norwegian scale also recognises. Similarly it can also be very difficult to distinguish early maturing from late maturing specimens and late immature/resting in the early ripening period. This may severely impact estimates of the spawning stock.

Another problem is that female gadoids like cod are batch spawners, which implies that the females in spawning stage will be in running condition only occasionally. The IBTS scale does not consider this and the females will switch between maturing and spawning during their entire spawning period, while males will remain spawning. This does not affect estimation of maturity ogives but mapping of spawning areas and peak time e.g. will be imprecise.

In the present study, the samples were processed histologically and examined under microscope to enhance maturity determination. The histological stage of each of the samples was evaluated by identification of specific characteristics. The microscopic criteria applied in the division of the specimens into specific stages are widely accepted developmental changes in the oocytes of the ovary such as formation of cortical alveoli and yolk vesicles/granules, as well as changes in the order of position between the cellular composites such as nuclear migration for females. Similarly for the males such criteria as the presence and relative abundance of spermatozoa and their precursors were applied. The exact criteria are specified in Section 3.3. The specimens were assigned stages both according to a 10 stage scale (Tomkiewicz *et al*., 2003) and graded into immature, maturing, spawning, spent and other (e.g. diseased).

Prior to the workshop the corresponding macroscopic taken during the cruises and histological photographs were aligned for each species and each sex and sorted in order of maturity judged by the microscopic characteristics of the specific specimen.

During the workshop the participants used the same technique aligning the photographs, and from these discussed the originally assigned maturity stages with the histological validation. The participants were grouped to represent different institutes in order to exchange views, perception and experience. The discussions

were used as basis for the determination of reproductive strategy, elaboration of a new common maturity scale including macroscopic and microscopic criteria.

3.2 Reproductive strategies

The most common reproductive traits and strategies of commercially important fish species of the North Atlantic based on oocyte development, ovary organization, recruitment of oocytes and spawning pattern of females are summarised in Table 3.2.1 with examples. Most species are interoparous, i.e. they can participate in spawning more times in life, and only few are semelparous i.e. they have only one spawning season in life and die subsequently. Gadoids are interoparous.

The fecundity can be either determinate or indeterminate. In the determinate fecundity type the numbers of eggs to recruited and developed for the coming spawning season is determined at relatively early stage of maturation, and no more eggs are recruited during the spawning period. In species, the individual fecundity and egg production can therefore be estimated prior to the spawning period from sampled ovaries. The individual fecundity is often increasing proportionally to fish weight, and this is the reason for using the Spawning Stock Biomass (SSB) as an index of the egg production.

Species with indeterminate fecundity continue to recruit new cohorts of oocytes throughout the spawning period and the amounts of eggs produced by the individual relates to e.g. water temperature, food availability and stored energy level as well as total body weight or length. In these species, which tend to be opportunists the length of the spawning season and amounts of eggs produced per female or unit of SSB tend to differ significantly among years and the spawning stock biomass therefore tend not to be a useful index of the potential egg production and the reproductive potential.

The oocyte development in species with determinate fecundity may be either synchronous or asynchronous. In species with synchronous development, all oocytes recruited for the coming spawning season develop in a fairly synchronous way during the vitellogenesis. In some species, all oocytes go through final maturation and hydration over a short period of time and are spawned in a continuous event and over a short period. Species with this spawning pattern is called total spawners, e.g. herring (*Clupea herengus).* In other species, the oocytes are arrested at the late vitellogenic stage, and batches are recruited for final maturation, hydration and ovulation separately. These are called batch spawners and they may spawn over a long period of time. Cod (*Gadus morhua)* is a determinate, group synchronous batch spawner (Kjesbu and Kryvi, 1989; Morrison, 1990). In other species, a number of batches are developing asynchronously in cohorts which are then spawned in subsequent batches.

Table 3.2.1. Common reproductive traits of female teleosts in the Northwest Atlantic. Modified from Murua and Saborido Rey, 2003.

Reproductive strategies of female teleost fishes				
Breeding opportunities	Fecundity type	Oocyte development	Spawning pattern	Examples
Semelparous	Determinate	Synchronous	Total spawner	Pacific salmon, Lamprey
		Asynchronous	Batch spawning	Eel
Iteroparous	Determinate	Group synchronous	Total spawner	Herring, sea trout, redfish
			Batch spawner	Cod, saithe, plaice
		Asynchronous	Batch spawner	Mackerel, sole
	Indeterminate	Asynchronous	Batch spawner	Anchovy, sprat, tuna

Species with indeterminate fecundity have asynchronous development with a number of subsequent cohorts being present in the ovary simultaneously. In contrast to the determinate spawners with asynchronous development, where the cohorts gradually phase out, the indeterminate species recruits new cohorts continuously and spawning session is characterised by arrested development and the break down of cohorts at different development stages. In these species, the egg production can be estimated form the batch fecundity, the spawning frequency and duration of the spawning period. Or the same parameters can be applied to egg production estimates from ichthyplankton surveys to derive the female spawning stock biomass (the egg production method).

In species with synchronous or group-synchronous development the fecundity can be estimated prior to spawning, in species with a synchronous development only the batch fecundity can be correctly estimated using traditional fecundity estimation methods.

The male reproductive strategy is less investigated but some of the traits correspond to the female characteristics, e.g. number of breeding opportunities. Males of species with synchronous and group-synchronous development in females also seem to recruit and develop the spermatozoa over a relatively short period, while males of species with asynchronous female development tend to continue to recruit and develop spermatozoa over a long period thus matching the female spawning period. This pronounced asynchronous development in males often is related to a gradient in the development in the testes tissue.

3.3 Histological criteria

3.3.1 Histological criteria for determination of female maturity

The oogenesis tends to follow a general pattern in teleosts and specific characteristics can be used to divide the oogenesis into different oocyte growth phases. The morphological development of the oocytes accompanied by increased oocyte size cause in combination with an increase of stroma is changing the appearance of the ovary. Similarly, during and after the spawning where resorption of postovulatory follicles, atretic cells etc. takes place and the ovary regenerates. The following description is based on cod (Kjesbiu and Kryvi, 1989; Morrison, 1990).

The reproductive tissue of the ovary is formed by several ovigerous folds extending from the wall to the centre of the ovary. Within these folds oogonia are formed by mitosis from the primordial germ cells. At this premature stage, oogonia are always present although not visible by the naked eye. In the juvenile fish, the oogonia develop into oocytes with densely staining cytoplasm and a large central nucleus with few, large peripheral nucleoli, the so-called peri-nuclear stage (PN). During their first growth phase the oocytes increase slightly in size, both the nucleus and the entire cell, and by the end of the first growth phase the cytoplasm has expanded and in cod e.g. ring formed structure has appeared i.e. circumnuclear ring (CNR) stage.

The second growth phase is under influence of sex hormones and initiates the oocyte maturation. The first clear sign of the second growth phase is the appearance of spherical and transparent vesicles (cortical alveoli stage, CA) in the periphery of the cytoplasm. During this stage, granules of yolk intensely stained (vitellogenic oocytes, VT), initially appear peripherally, but as they increase in number and size, they fill and expands the cytoplasm.

As the oocyte approaches final maturation, the shape of the nucleus becomes irregular. The final maturation is marked by the migration of the nucleus towards the micropyle and the hydration process. Before the ovulation, the nuclear wall disintegrates and yolk granules coalesce forming large irregular spheres (FM oocytes). The subsequent hydrolysation of the yolk protein results in hydrated eggs (HYD). The hydrated egg is transparent and the cell content appears completely homogeneous. HYD tend to loose their round shape during the fixation procedure and often fall out when the tissue is sectioned.

At the ovulation, oocytes are released into the lumen, while the ruptured follicles (post-ovulatory follicles, POF) remain in the ovary. The POFs are resorbed over relatively short time while vitellogenic oocytes that do not complete the maturation become atretic (AT and are resorbed.) Encapsulation of non-spawned hydrated egg can also occur and may cause disturbance of the tissue if they are numerous.

The first appearance of oocytes showing the different specific characteristics identifies the maturity stage i.e. in general the most developed oocytes are used as stage indicators. The combination of different stages of oocytes present at same time in the ovary characterises the reproductive strategy, e.g. synchronous vs. asynchronous and determinate versus indeterminate (Murua and Saborido-Rey, 2003).

3.3.2 Histological criteria for determination of male maturity

The early divisions of the germ cells in the male reproductive tissue regulate the fecundity in male teleosts. Histologically, development is associated with spatial heterogeneity in the tissue development observed between distal and proximal tissue regions of the testis. In cod, the germ cells are concentrated mainly in the distal part or 'frill region' of the testes and a branching system of efferent ducts passes into the proximal part of the testes (Morrison, 1990). These efferent ducts fuse to form the sperm duct which has a highly folded wall. The following outline of characteristic microscopic development stages is after Gokhale, 1957 and Morrison, 1990. Due to the differential development in the proximal and distal parts of the testes and frills, it is very important to dissect the testes transversely and keep the orientation of the sample.

Early in the male development there is a characteristic presence of 'germ cells' or spermatogonia which may be migratory, with an elongate appearance and a lightly staining cytoplasm. Some germ cells may be located proximally near to or within

inter-lobular walls but most are located distally. Singular germ cells eventually lodge within a 'cyst' and while undergoing transformation become rounded, increase in size and the nucleolus becomes more prominent. In the reproductive tissue of juvenile males, such cells can be seen dividing mitotically giving rise to groups or 'cysts' of germ cells.

Primary spermatocytes are the result of mitotic division of germ cells or spermatogonia. These cells are stained more deeply than spermatogonia or germ cells, display a smaller nucleus and the distinct nucleolus is lost. As division progresses, generations of cells are retained within the original cyst wall. Later as spermatogenesis progresses, cysts expand and gametes are retained between the inter-lobule walls of the distal tissue.

Primary spermatocytes divide by mitosis to form smaller secondary spermatocytes. Characteristically the chromatin material of secondary spermatocytes is unevenly dispersed making the nucleus appear mottled in appearance. Secondary spermatocytes now undergo a further meiotic division to produce smaller haploid spermatocytes. A further mitotic division now takes place forming haploid spermatids which have a characteristic elliptical shaped nucleus.

The spermatids develop flagella and become flagellate spermatozoa. The number of spermatozoa increases, particularly in the proximally part of the frills. Cyst and lobule walls disappear so that long tubules of spermatozoa are formed proximally with tubules will contain masses of spermatozoa. Mature spermatozoa become aligned so that their flagella lie alongside each other and the heads face the interstitial tissue between the tubules. Few migrating germ cells are now visible except at the extreme distal edges and no mitotic division is observed in these cells. The sperm duct and proximal efferent duct system contains ripe spermatozoa. Distal cysts may still contain earlier products of spermatogenesis that will develop and be spawned in later depending on the reproductive strategy of the species.

In the tissue of spent testes, the interlobular walls and the stroma of the testis increase in thickness. Towards the distal end of the tissue, thick septa of connective tissue can be seen as well as remaining germ cells. Atretic spermatozoa may be present in the ducts. These atretic spermatozoa loose the characteristic flagella and stain quite darkly. The tissue contains many blood vessels.

In resting or skip of spawning tissue the tissue appears quite dense and a re-organisation of the tissue appears to be in progress. New cysts are being formed and lobule walls contain many migrating germ cells or spermatogonia. Resting cysts of spermatogonia or primary spermatocytes may also be visible. Relict atretic spermatozoa contained in lobules or tubules are reabsorbed by larger phagocytes which stain a lighter colour. The numbers of spermatogonia increase progressively in resting or skip of spawning tissue until the process of spermatogenesis begins again.

Abnormal testicular tissue may contain histological irregularities in the developmental process of spermatogenesis. Mass atresia of maturing or ripe structures may be visible in some or all tissue regions. Spatial heterogeneity may be evident in the maturation of abnormal tissues with maturation evident in some areas and not in others. Tissues may be irregularly composed of dense stroma and connective tissue. Normal zonation patterns in spermatogenesis may not be visible. Inter-sex may be apparent in some specimens, histologically both oogonia and spermatogonia may be visible in tissue sections as well as later development stages of both male and female reproductive tissue.

3.4 Development of maturity scale for gadoids

Since 1991 the IBTS maturity data collections of gadoids has been reported to ICES as 4-grade scale (Annex 2.) The four stages are classified as

1 Immature, 2 Maturing, 3 Spawning, 4 Spent

In practice, these stages have been converted to a binomial scale separating juvenile fish from the spawning stock for assessment purposes and maturity ogive has been established as the proportion sexually mature = (n stage 2–4)/(n stage 1–4). In addition the data could also be used for estimating size at maturation and changes in L50 and A50.

However, definitions describing the stages have been vague and confounding, leading to misinterpretation of the gonadal status and resulting in possible erroneous estimation of the above. The issue was addressed already at WKMAT held in Lisbon in January 2007 and the workshop suggested an addition of a fifth stage comprising mature fish not contributing to the spawning biomass, so called "skippers". Recent research has shown that in several species, a substantial part of mature individuals from the younger age classes can omit spawning if energy resources are scarce (Jörgensen *et al*., 2006) and the current IBTS maturity key does not allow classifying and giving an appropriate code to those individuals.

It is also relevant to point out that the stage 3 which is named as spawning fish has been defined as running fish only and, by all countries present, used only for classifying running fish. It is unfortunate that the present definition in stage three suggests that spawning equals running since catching a fish that is running is quite random. As gadoids are batch spawner they will release eggs several times over a period of time and will have hydrated eggs during the entire spawning period. Therefore the stage where the fish has hydrated eggs and the stage when the fish has recently spawned ought to be considered spawners and should be included in stage 3. At present, the stage with hydrated eggs is included in the maturing fish which is irrelevant in regards to estimating SSB but counterproductive if you are working on temporal or spatial issues. How to deal with historical data in this respect was not discussed.

During WKMSCWHS the participants complied with the 5-stage scale proposed by WKMAT but all agreed on adding a 6th stage. This stage contains fish with abnormal gonadal development such as intersex and petrified roe, and they seem to exist in all species. Whereas specimens in resting stage may constitute a significant proportion of the adult fishes, fishes in stage 6 are considered rare. However, both stages seem useful as ecosystem state indicators. An increase in the proportion skipping spawning may indicate an unbalance, as well as a significant increase in abnormal specimens, e.g. the significant increase in intersex observed in eelpout males in some areas.

During the workshop, cod was used as a model for elaborating a common maturity scale and it was after wards tested on saithe, haddock and whiting. The proposed common scale thus includes 6 stages (Figure 3.4.1):

1 Juvenile/Immature, 2 Maturing, 3 Spawning, 4 Spent, 5 Resting/Skip of spawning and 6 abnormal.

Common classification criteria for females and males are given below. The dashed line around Stage 1 and 5 in Figure 3.4.1 illustrates that larger immature specimens and resting specimens often are difficult distinguish outside the spawning season

both macroscopically and histologically, because the tissue regenerates after spawning and resample late stage I. The small circle illustrates resting specimens that skip spawning. Abnormal fishes in Stage VI in general show irreversible signs of degeneration of the gonad tissue and are thus perceived to leave the reproductive cycle.

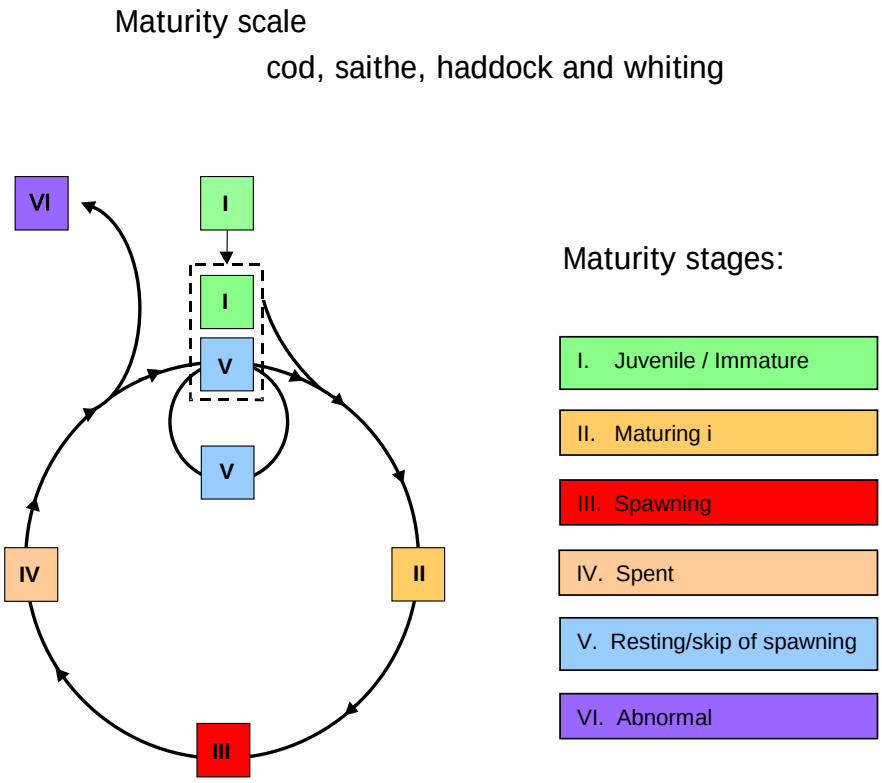

Figure 3.4.1. Proposed 6-stage maturity scale for cod, saithe, haddock and whiting.

The suggested scale facilitates the implementation of using spawning probability rather than a maturity ogive taking into account that all mature fish are not part of the spawning stock. Using only the proportion of fish that will spawn as basis for the assessment improves the accuracy of the SSB estimate as an index of the egg production for determinates spawners.

The estimation of the spawning probability (pS) should be pS = (n stage 2–3) / (n stage 1–6) in the prespawning period and pS = (n stage 2–4) / (n stage 1–6) during the spawning period.

Specimens below 15 cm should not be sexed as sex determination of males below this size is uncertain, but their maturity should be interpreted as Stage 1.

3.4.1 Female and male maturity scales

The macroscopic and histological criteria for the common maturity scale for females and males are given in Tables 3.4.2 and 3.4.2. The macroscopic descriptions were revised using the photo documentation and supplemented by histological criteria. Characteristic photographs for each species were selected for an illustration of the development of each species (Appendix 1–4).

Table 3.4.1. Suggested common maturity scale for female cod, saite, haddock and whiting including macroscopic and histological characteristics of the 6 stages. PN: perinuclear oocytes, CNR: circumnuclear oocytes, CA: cortical alveoli stage, VT: vitellogenic oocytes, FM: final maturation stage, HYD: hydrated eggs, POF: Post ovulatory follicles.

STAGE	DESCRIPTION OF APPEARENCE OVARIES	HISTOLOGY
1	Juvenile/Immature	
	No sex determination: juvenile below 15 cm, risk of mistaking gonads for bladder.	Oogonia / PN
	Sex determination: Juvenile-transparent ovaries.	PN
	Immature-translucent ovaries, coloration is pinkish to light orange, cast thin and clear. Blood vessels hardly discernable.	PN/CNR
2	Maturing: Firm, coloration ranges from reddish orange to creamy orange with granulated/oocytes clearly visible in issue. Blood vessels larger and diversified.	CA/T
3	Spawning: Distended, few to many hydrated eggs visible in tissue among vitelogenic oocytes or in lumen, occasionally running.	FM/HYD/POF
4	Spent: Slack with greyish cast, rich in blood vessels.	POF, perhaps atretia, PN, CNR
5	Resting/Skip of spawning*: No visible development-similar to Immature but simetimes with a greyish cast.	PN, CNR, perhaps atresia
6	Abnormal*: Hard parts (connective tissue), only one lope developed, intersex, or similar-fecundity at least partly reduced.	Variable

Ecosystem state indicators*

Table 3.4.2. Suggested common maturity scale for male cod, saite, haddock and whiting including macroscopic and histological characteristics of the 6 stages. SG: Sspermatogonia, SC1: Primary spermatocytes, SC2: secondary spermatocytes, ST: spermatides, SZ: spermatozoa.

STAGE	DESCRIPTION	HISTOLOGY
I	Juvenile/Immature.	
	No sex determination: juvenile below 15 cm, gonads difficult to identify.	Germ cells/SG
	Sex determination: Juvenile-transparent testes.	Germ cells/SG
	Immature-testes with developing frills, coloration is reddish to white, vascularisation is limited.	SG/SC1
II	Maturing: Whitish to almost opaque reddish-white, blood vessels more prominent, empty transparent spermatoducts.	SC1/SC2/ST, spermatids/non-motile flagellate SZ
III	Spawning: Opaque creamy white colour to reddish late in stage, semen visible in spermatoduct, milt often flows at ligth pressure.	Aligned ripe SZ proximally and in sperm duct, cyst, no lobule walls.
IV	Spent: Contracted, empty and flabby lobules, colour deep pink to reddish-purple, bloodshot, potentially with greyish cast.	Migrating germ cells/SG, interlobular walls thickens, atretic spermatozoa
V	Resting/Skip of spawning*: No visible development, spermatoducts often with a greyish cast, similar to immature, early maturing.	Migrating germ cells/SG, resting cysts of SG and SC1.
VI	Abnormal*: Adipose tissue, only one lobe developed, intersex, or similar.	Variable

Ecosystem state indicators*

The histological characteristics refer to the most advanced oocytes of traits in the tissue for females and characteristics of the advancing spermatogenesis and redistribution the mature spermatocytes into the spermatoducts. The histological characteristics are specified below.

The maturity stage 1. Juvenile/Immature, has been divided into 3 sub-stages: below 15 cm, juvenile and immature. Stages 5 and 6 will be applicable as ecosystem indicators. The appearance of Stage 6 will vary among specimens and also often among different part of the gonad. Some parts may show normal development.

3.4.1.1 Stage description for females

Stage 1-Juvenile/Immature

In this stage, the ovaries are small, transparent to translucent; the colour is pinkish to light orange and their wall thin and clear.

Oogonia are present in the tissue, and during development small oocytes with densely staining cytoplasm and a central nucleus with few, large peripheral nucleoli (PN) appear. A portion of the oocytes may have started the primary growth, characterized by a slight increase in size, both of the nucleus and of the entire cell, and by the presence of a light stained area around the nucleus, the so called circumnuclear ring (CNR). This ring indicates that cytoplasmatic changes occur and sexual maturation is approaching. Prior to vitellogenesis (i.e. formation of yolk), the circumnuclear ring moves towards the outer part of the cell.

Stage II-Maturing

The maturing ovaries become firm, coloration ranges from reddish orange to creamy orange with granulated/oocytes clearly visible in issue. Blood vessels become larger and diversified.

The circumnuclear ring gradually disintegrates, while spherical and transparent vesicles (cortical alveoli, CA) appear in the peripheral part of the cytoplasm. During this stage, granules of yolk intensely stained (vitellogenic oocytes, VT), initially appear peripherally, but as they increase in number and size, they distribute throughout the cytoplasm and finally expands the cell. Towards the end of the vitellogenesis the shape of the nucleus becomes irregular, but the nucleus is still centrally located.

The occurrence of cortical alveoli and yolk granules show that the maturation process is in progress, and under normal conditions, the individual will develop within the current spawning season.

Stage III-Spawning

The ovaries have become distended; few to many hydrated eggs visible in tissue among vitellogenic oocytes, or hydrated eggs are present in lumen and are occasionally running at light pressure at the abdomen. In spawning specimens the ovary is often filled with viscous fluid.

Histologically, the final maturation is marked by the nuclear migration by the hydration process. The nucleus moves from the centre towards the micropyle and eventually breaks down when reaching it, before the ovulation, the yolk granules coalesce forming large irregular spheres (FM oocytes), yolk protein is hydrolysed and hydrated eggs (HYD) are formed. The hydrated egg is transparent and the cell content appears completely homogeneous. HYD tend to loose their round shape

during the fixation procedure and often fall out when the tissue is sectioned. At the ovulation, oocytes are released into the lumen, while the ruptured follicles (post-ovulatory follicles, POF) remain in the ovary. Therefore at this step, 3 different developmental stages, oocytes in final maturation, hydrated eggs and/or POFs are all visible.

Stage IV-Spent

As spawning cease the ovary retracts and becomes slack with greyish cast, but is still rich in blood vessels.

The ovaries are dominated by post-ovulatory follicles (POF), marking the occurred ovulation, are abundant among perinuclear or circumnuclear stage oocytes. The development of vitellogenic oocytes sometimes fail and their maturation is not completed. These oocytes under intra-ovarian resorption are called "atresia".

Stage V-Resting / Skip of spawning

The ovaries show no visible development and look similar to ismmature but the fish may be fairly large and the ovary may have a greyish cast. It is often useful to cut such ovaries open to make sure that vitellogenesis has not started, because it can be difficult to judge behind the cast.

The ovary is characterized by oocytes in PN and CNR stages. Atretic oocytes might occur. This stage should be interpreted as resting if observed outside the spawning season and skip of spawning if observed during the spawning season.

Stage IV-Abnormal

The ovaries may possess dark and hard parts (connective tissue), only one lope developed or other abnormal traits that causes at least partly reduced fecundity.

Some part of the ovary may show normal development similar to above stages. The parts filled with connective tissue may contain encapsulated, hydrated eggs that have not been spawned. Instead of resorption they have become encapsulated in connective tissue.

3.4.2 Criteria for determination of male maturity

Stage I-Juvenile/Immature

In juveniles and immature specimens (above 15 cm), the testes are recognised as thin translucent strings which in late stage have developed small frills; coloration is reddish to whitish, vascularisation is limited.

This stage is characterised by the presence of 'germ cells' or spermatogonia (SG) which may be migratory, with elongate appearance and a lightly staining cytoplasm. Some germ cells may be located proximally near to or within inter-lobular walls but most are located distally. Singular germ cells eventually lodge within a 'cyst' and while undergoing transformation become rounded, increase in size and the nucleolus becomes more prominent. In stage 1 reproductive tissue, such cells can be seen dividing mitotically giving rise to groups or 'cysts' of germ cells. Immature tissues in preparation will contain primary spermatocytes (SC1) which are the result of mitotic division of germ cells or spermatogonia. As division progresses, generations of cells are retained within the original cyst wall. Later as spermatogenesis progresses, cysts expand and gametes are retained between the inter-lobule walls of the distal tissue.

Stage II-Maturing

During the maturing stage testes change from reddish-white to almost opaque white, blood vessels more prominent; spermatoducts remains empty and transparent.

In the early stage few remaining singular germ cells are present but groups or 'cysts' of germ cells have divided and form primary spermatocytes (GC1). During the stage primary spermatocytes divide by mitosis and form smaller secondary spermatocytes. The numbers of both primary and secondary spermatocytes increase considerably. Lobules elongate and widen so the testes enlarges. By the end of the stage secondary spermatocytes undergo a meiotic division to produce smaller haploid spermatocytes and a mitotic division to form haploid spermatids (ST) which have a characteristic elliptical shaped nucleus. Spermatids develop flagella and become flagellate spermatozoa or sperm within distended lobules. The presence of flagellate spermatozoa in maturing tissues is not uncommon especially in more proximal tissues which develop more rapidly. By the end of stage II, the numbers of spermatids and flagellate spermatozoa increases rapidly but no sperm is visible in the sperm duct. However it must be remembered that for asynchronous species, cysts containing all stages of spermatogenesis may be present in ripening fish.

Stage III-Spawning

Testes appear opaque creamy white to reddish late in the stage, semen visible in spermatoducts, in the early stage milt may appear as a viscous droplet, later in the stage milt flows at light pressure at vent.

The number of spermatozoa increases rapidly, particularly proximally, in the beginning of the spawning stage. Cyst and lobule walls disappear so that long tubules of spermatozoa are formed proximally with tubules will contain masses of spermatozoa. Mature spermatozoa become aligned so that their flagella lie alongside each other and the heads face the interstitial tissue between the tubules. Few migrating germ cells are now visible except at the extreme distal edges and no mitotic division is observed in these cells. The sperm duct and proximal efferent duct system contains ripe spermatozoa. Distal cysts may still contain earlier products of spermatogenesis that will develop and be spawned in later batches depending on the reproductive strategy of the species.

Stage VI-Spent

After spawning the testes contract and appear empty with flabby lobules, colour deep pink to reddish-purple, bloodshot, potentially with greyish cast.

The most noticeable histological change in spent tissue, apart from the great reduction of sperm, is that the interlobular walls and the stroma of the testis increase in thickness. Towards the distal end of the tissue, thick septa of connective tissue can be seen as well as remaining germ cells. Atretic spermatozoa can be seen contained inside the collapsing efferent ducts, proximal tubules and in the sperm duct. These atretic spermatozoa loose the characteristic flagella and stain quite darkly. Dilated blood vessels may still be visible throughout the tissue but are in the process of resumption to original size. Scattered blood cells may also be visible.

Stage VResting/Skipped Spawning

No visible development, spermatoducts often with a greyish cast, similar to immature, early maturing.

In resting or skip of spawning tissue the tissue appears quite dense and a re-organisation of the tissue appears to be in progress. New cysts are being formed and lobule walls contain many migrating germ cells or spermatogonia. Resting cysts of spermatogonia or primary spermatocytes may also be visible. Relict atretic spermatozoa contained in lobules or tubules are reabsorbed by larger phagocytes which stain a lighter colour. The numbers of spermatogonia increase progressively in resting or skip of spawning tissue until the process of spermatogenesis begins again.

VI-Abnormal

The reproductive tissue of testes may partly turn into adipose tissue giving the frills a dark yellow appearance, or only one lobe developed. Intersex occurs where part of the tissue contains oocytes or eggs.

Abnormal testicular tissue contains histological irregularities in the developmental process of spermatogenesis. Mass atresia of maturing or ripe structures may be visible in some or all tissue regions. Spatial heterogeneity may be evident in the maturation of abnormal tissues with maturation evident in some areas and not in others. Tissues may be irregularly composed of dense stroma and adipose cells. Normal zonation patterns in spermatogenesis may not be visible. Inter-sex may be apparent in some specimens, histologically both oogonia and spermatogonia may be visible in tissue sections as well as later development stages of both male and female reproductive tissue.

3.4.3 Reproductive strategy

The reproductive strategy of the four species was judged from the appearance of the different ovarian stages.

Cod and saithe were characterised by determinate fecundity, group synchronous development and batch spawning. During the maturation, oocytes gradually enter vitellogenesis and the oocyte development is fairly uniform. The maturation period is fairly long. Before final maturation the oocytes are arrested in the late vitellogenic stage and the eggs undergo final maturation and hydration in batches. The batches are spawned with intervals of several days and over a longer period. The potential fecundity and approximate number of eggs to be spawned during the entire spawning season can be judged in the late maturation period.

Whiting and haddock showed asynchronous oocyte development in females and as well as a gradient in development of testes from the efferent duct towards the periphery of the frills. During the maturation, oocytes are recruited in cohorts, which enter vitellogenesis. The development within the cohorts is fairy similar cohorts, while the cohorts can be clearly separated through their differential development. The eggs are hydrated and spawned in batches subsequently. There are thus different cohorts of developing cohorts at the same time in the ovary of a spawning female. In determinate fecundity types the number of batches recruited will be fixed, while for the indeterminate species the recruitment continues as long as the environmental conditions prey availability and energy stores allows. When spawning ends, all developing oocytes are resorbed. The “spent” stage is in this case is characterised by large numbers of atretic eggs.

Hardly any specimens were in spent stage and it was therefore not possible to determine with certainty whether they possess a fixed number of batches per female or they have indeterminate fecundity. If these species have indeterminate fecundity, the SSB is not a useful indicator of the egg production and there will be a need to

supplement with sampling methods applied for other species with similar reproductive pattern like horse mackerel.

Table 3.4.3. Reproductive strategies of cod, saithe, haddock and whiting. It was not possible to judge the exact fecundity type of haddock and whiting based on the available material.

BREEDING OPPORTUNITIES	FECUNDITY TYPE	OOCYTE DEVELOPMENT	SPAWNING PATTERN	EXAMPLES
Iteroparous	Determinate	Group synchronous	Batch spawner	Cod, saithe
		Asynchronous	Batch spawner	Hadock?
	Indeterminate	Asynchronous	Batch spawner	Whiting?

4 Maturity staging of cod and saithe

4.1 Cod

4.1.1 Existing information

Spawning peaks in the southern part of the North Sea from the last week of January to mid-February, whereas in the northern part the highest concentration of eggs may be found in April (Daan *et al*., 1978, Heesen and Rijnsdorp, 1989 and Raitt, 1967 in ICES FishMap). The cod recruits to the adult population at age 1 and some mature in their second year of life, but all are mature at the age of six. As for most fish, males mature slightly earlier than females, and in the southern North Sea there is a tendency for cod to mature at a younger age than in the northern part (Rijnsdorp *et al*., 1991 and Osthuizen and Daan, 1974 in ICES FishMap).

The oogenesis of cod (*Gadus morhua*) has been widely studied (Sivertsen, 1935; Sorokin, 1957; Woodhead and Woodhead, 1965; Kjesbu and Kryvi, 1989, Morrison, 1990; Kjesbu, 1991; Kjesbu *et al*., 1991, 1996 and Tomkiewicz *et al*., 2003). The spawning period can last several months as the eggs are being released in batches (Kjesbu, 1989; Worsøe *et al*., 2002), and the reproductive strategy of female cod has been categorized as an iteroparous, group-synchronous batch-spawner with determinate fecundity (Murua and Saborido-Rey, 2003). The present study agrees with this strategy for female cod. Two studies similar to the present have been conducted by Morrison, 1990 and Tomkiewicz *et al*., 2003, in which the gross appearance of the ovaries was correlated with histology in north-western Atlantic cod and in the Baltic Sea cod, respectively.

The histological development of male reproductive tissue has not been described as widely as for the female cod (Sivertsen, 1935; Sorokin, 1960; Vladykov *et al*., 1985). However, the study of north-western Atlantic cod conducted by Morrison, 1990 included testes histology and the gross appearance of the testes was correlated with histology.

For North Sea cod, no previous cross-institutional study to standardize and validate classification of female and male maturity stages has been conducted.

4.1.2 Data analysis

In total, the maturity of eighty one specimens was ascertained through histological examination of the gonadal tissue and the results compared with initial macroscopic assessments. Only specimens sampled from Q3 (Quarter three) were included in data analysis.

4.1.2.1 Females

Samples of ovaries were obtained from scientific cruises on IBTS Q1 and IBTS Q3 2007. Maturity was macroscopically assessed at sea using IBTS 4 stage scale, a modified 8-stage scale used on Dutch cruises and an 8-stage scale used on Swedish cruises, which can be translated directly into the IBTS 4 stage scale. Change to RIVO etc as for in males.

Overall, the maturity of over 36.8% of the females in the study group was miss-classified through the macroscopic examination (Table 4.1.2.1). The initial classification of non- spawners (Stages 1, 5 and 6) being classified as spawners (Stage 2–4) had an overall miss-classification rate of 47.1%. Within the group of specimens

classified histologically as spawners, 4.8% were initially staged as non-spawners. This means the SSB will be overestimated-in particular the misclassification of the Stage 1 specimens causes this discrepancy. Based on the existing guidelines the macroscopic criteria separating the two stages seem inadequate.

A proportion of females in spawning stage were incorrectly staged as being maturing. This is due to the previously mention problem that cod are batch spawners and the criteria that spawning females should be running condition fails to distinguish maturing from spawning. This has not effect on the estimation of SSB, but it affects studies of spawning habitats e.g. in relation to MPAs.

Table 4.1.2.1. Misclassification of North Sea cod based on histological characteristics. Stage 1 is in this case called undeveloped because it may include ovaries in resting condition which cannot be distinguished with certainty from immature histologically. The misclassification are related to the total histologically staged and the stages misclassified are given in brackets as numbers x stage.

MATURITY STAGE	COD	COD
I. Undeveloped (juvenile/immature/resting)	7/15 (5xII + 2xIV)	2/10 (1xII + 1xIV)
II. Maturing	2/11 (1xI + 1xIV)	4/5 (1xI + 1xIII + 2xIV)
III. Spawning	3/8 (2xII + 1xIV)	12/22 (1xI + 11xII)
IV. Spent	0/2	4/5 (1xI + 2xII + 1xIII)
V. Resting / Skip of spawning	0	0
VI. Abnormal	2 (1xI + 1xIV)	1 (1xIV)
Total missclassifications	14/38 (36.8%)	23/43 (53.5%)
Non-spawners classified as spawners initially	8/17 (47.1%)	3/11 (27.3%)
Spawners classified as non-spawners initially	1/21 (4.8%)	3/32 (9.4%)

4.1.2.2 Males

Samples of testes were obtained from scientific cruises on IBTS Q1 and IBTS Q3 2007. Maturity was macroscopically assessed at sea using IBTS 4 stage scale, a modified 8-stage scale used by RIVO and an 8-stage scale used on Swedish cruises. The latter two scales can both be translated directly into the IBTS 4 stage scale.

In total the maturity status of forty three male specimens was determined through histological analysis and the results compared with initial macroscopic assessments.

Overall, the maturity status of over 53.5% of the male specimens in the study was miss-classified via macroscopic assessment (Table 4.1.2.1). The initial classification of non- spawners (Stages 1, 5 and 6) being classified as spawners (Stage 2–4) had an overall miss-classification rate of 27.3%. Within the group of specimens classified histologically as spawners, 9.4% were initially staged as non-spawners. This means that also for males the SSB tends to be overestimated.

The most prominent misclassification of related to specimens judged as spawning while being maturing. Based on the existing guidelines, the macroscopic criteria separating the two stages are obviously inadequate. In this case, the new criteria that semen should be present in the spermatoducts can increase the accuracy.

4.1.3 Discussion and conclusions

If these high rates of misclassification are consistent it will without doubt bias the calculations of maturity ogives. Most errors associated with the macroscopic classification of male cod reproductive tissue were associated with assigning IBTS stage 2 (maturing) to that of stage 3 (spawning). Problems arose in the macroscopic identification of characteristic signs of ripeness in the testes. Specimen size and heterogeneous gonad size and appearance may have initially contributed to inaccurate assessments. The visible presence of spermatozoa in the sperm duct is the most distinguishing feature of ripe or spawning stage testicular tissue. This is often verified by the release of semen from the uro-genital pore, after pressure is applied. The gonads of male cod will progressively reduce over time. It is agreed that this may increase the probability of misclassification, as gonad size in relation to body cavity length is generally considered in maturity stage assessment. Furthermore, there seemed to be no consistency in the misclassifications of specimens in stage 1 and 4, the initial classifications given to these, were almost random among the four possible stages. All in all this means that there is a great confusion among institutions in the maturity staging of male cod.

The transition between the immature and maturing stages and the spent and maturing stages respectively, may in some cases only be possible to detect with certainty using histology. At present no quick method to detect signs of maturation aboard research vessels other than visual classification using macroscopic criteria is available. But the group agreed upon some species-specific macroscopic criteria which could facilitate the classification of reproductive tissue in cod. Furthermore, it was agreed that an illustrated manual describing these stages and incorporating such criteria, should be developed based upon the histologically verified macroscopic photographs taken during the IBTS surveys in 2007 and the proposed 6-stage scale. The manual is to be tested during the IBTS 1Q survey in 2008 and refined thereafter if deemed necessary. The draft manual is presented in Appendix 1.

As mentioned, the spawning period can last several days, as the eggs are being released in several batches (Kjesbu, 1989 in Worsøe *et al.*, 2002). Hydrated eggs may be macroscopically visible in between two spawning events and but slight pressure to the abdomen of the fish may not necessarily release eggs in such cases. The later criterion has been widely used to distinguish between maturing and spawning individuals, but may contribute to error in assessment. Therefore it is suggested to use the presence of hydrated eggs or viscous fluid in the lumen of the ovary as cutting edge criteria between the maturing and spawning stages.

For females it is possible to use light microscopy aboard the research vessels as a quick method to determine the presence or absence of maturing oocytes. The method has been developed by Olav Kjesbu (IMR, Bergen) in the 1980s. The definitive characteristic of female maturation stage development is the cortical alveoli, which appear as darker translucent vesicles in maturing oocytes. This diagnostic method can be used when training new staff in maturity classification as well as an ongoing quality assurance and testing of the maturity data. It was not possible to develop the method during the workshop, but would be a useful supplement.

For male cod, the transition between the immature and maturing stages, and the spent and maturing stages, is in some examples difficult to detect. In contrast for females it is possible to use light microscopy aboard the research vessels as a quick method to determine the presence or absence of maturing oocytes. For males the group agreed upon some more defined species-specific macroscopic criteria which

could facilitate the classification of male reproductive tissue in cod and reduce erroneous maturity assessment.

Furthermore, it was agreed that an illustrated manual describing these stages and incorporating such criteria should be developed based upon the histologically verified macroscopic photographs taken during the IBTS surveys in 2007 and the proposed 6-stage scale. The manual is to be tested during the IBTS 1Q survey in 2008 and refined if deemed necessary.

4.2 Saithe

4.2.1 Existing information

The reproductive strategy of female saithe has previously been categorized as iteroparous, with group-synchronous development, batch-spawning and determinate fecundity (Murua and Saborido-Rey, 2003). The present study confirmed the finding. Only limited information on saithe reproductive biology is available in the literature. The spawning period takes place from January in the southern part of the distribution area, and stretches until May further north (Reinsch, 1976). Juveniles recruit from their inshore habitats to the adult population occupying deeper waters over the shelf edge and beyond at the age of 2–3. Males and females have similar growth rates during the first 6 years of life, when the majority of the fish has reached maturity. Ultimately, the females become slightly larger than the males (ICES FishMap).

4.2.2 Data analysis

In total, the maturity of thirty three specimens was ascertained through histological examination of tissue and the results compared with initial macroscopic assessments. Only few specimens sampled from Q3 were included in the analysis. Data regarding misclassifications are given below and in Table 4.2.2.1.

4.2.2.1 Females

In total, the maturity status of eighteen female specimens was determined through histological analysis and the results compared with initial macroscopic assessments.

Table 4.2.2.1. Misclassification of North Sea saithe based on histological characteristics. Stage 1 is in this case called undeveloped because it may include ovaries in resting condition which cannot be distinguished with certainty from immature histologically. The misclassification are related to the total histologically staged and the stages misclassified are given in brackets as numbers x stage.

MATURITY STAGE	SAITHE ♀	SAITHE ♂
I. Undeveloped (juvenile/immature/resting)	2/9 (1xII + 1xIV)	2/5 (1xII + 1xIV)
II. Maturing	0/3	1/1 (1xIV)
III. Spawning	1/3 (1xII)	4/6 (3xII + 1xIV)
IV. Spent	1/3 (1xII)	1/2 (1xI)
V. Resting / Skip of spawning	0	1 (1xII)
VI. Abnormal	0	0
Total missclassifications	4/18 (22.2%)	9/15 (60%)
Non-spawners classified as spawners initially	2/9 (22.2%)	3/6 (50%)
Spawners classified as non-spawners initially	0/9 (0%)	1/9 (11.1%)

Overall, the maturity of over 22.2% of the females in the study group was miss-classified through the macroscopic examination (Table 4.2.2.1). The initial classification of non- spawners (Stages 1, 5 and 6) being classified as spawners (Stage 2–4) had an overall miss-classification rate of 22.2%. No spawners were classified histologically as non-spawners. As was the case for cod this indicates that the SSB will be overestimated the existing guidelines the macroscopic criteria separating the two stages seem inadequate. Relatively few female saithe were sampled and analysed for the workshop and further sampling and analysis would strengthen the analysis.

4.2.2.2 Males

In total the maturity status of fifteen male specimens was determined through histological analysis and the results compared with initial macroscopic assessments.

Overall, the maturity status of over 60% of the male specimens in the study was miss-classified via macroscopic assessment (Table 4.2.2.1). The initial classification of non-spawners (Stages 1, 5 and 6) being classified as spawners (Stage 2–4) had an overall miss-classification rate of 50%. Within the group of specimens classified histologically as spawners, 11.1% were initially staged as non-spawners. Relatively few saithe specimens were sampled and analysed for the workshop and further sampling and analysis would strengthen the analysis. Based on the existing guidelines, the macroscopic criteria are obviously inadequate.

4.2.3 Discussion and conclusions

Only very few samples were obtained during the IBTS 1Q and 3Q due to the poor overlap of the IBTS research area and the more northerly distribution of saithe.

The miss-classification of female saithe maturity with respect to non-spawners being spawners was lower than for cod the proportion of misclassified males was very high. This error has implications for the computation of maturity ogives and may bias results and causes an overestimation of SSB, if the data are applied as combined ogives are the normal bases of assessment. Data analysis indicated that for saithe most errors were associated in miss-classifying non-spawners as spawners.

The group identified that the appearance of a relatively thick tunica observed in immature saithe can mislead the macroscopic assessment by the resultant opaque appearance of ovarian tissue. The tunica of mature specimens was also observed to be quite opaque and display a grey cast-like appearance. The group agreed that such specimens were most commonly observed in samples for Quarter 3, outside the main spawning season.

In immature saithe, this appearance of the ovary wall may give the impression of ovarian maturation when in fact no maturation is evident. In such cases a diagnostic light microscopy analysis is to be favoured, as for female cod. This needs further investigation and elaboration of methods. Furthermore it was agreed that definitive species specific criteria should be included in the stage descriptions for saithe and that more detailed histology is required to compliment such an approach.

The group felt that similar problems were encountered as for male cod in identifying definitive criteria for testicular maturation. This was found to be particularly problematic in smaller specimens with reduced reproductive size. However the group acknowledged that few specimens were available for the analysis and this factor may have added to the heterogeneity in assessment.

There seems to be a hiatus in the literature regarding the available knowledge on saithe maturation. From the available material, the reproductive strategy of saithe appears similar to the one of cod, although the gross morphology and colour of the gonads differ slightly. It was agreed that the generalised IBTS stage descriptions for maturity classification is not well suited to saithe without adapted species specific criteria.

It was agreed that an illustrated manual with stage descriptions and incorporating such criteria, should be developed based upon the histologically verified macroscopic photographs taken during the IBTS surveys in 2007. It was recommended by the group that this manual should follow the proposed 6-stage IBTS scale. The manual is to be tested during the IBTS 1Q survey in 2008 and refined thereafter if deemed necessary. A draft manual based on the few examples available was elaborated and included in Appendix 2.

5 Maturity staging of haddock and whiting

5.1 Haddock

5.1.1 Existing information

Existing information on the maturity of haddock is scarce. The peak spawning period in the northern North Sea has been identified as March and April (Potts and Wotton, 1984). In the same study area, recruitment to the mature population is at three years of age, with males maturing at a younger age and smaller size than females (Potts and Wotton, 1984). The spawning strategy oh haddock has been described as iteroparous with group-synchronous development, batch-spawning and determinate fecundity (Murua *et. al*., 2003). However, this study identified an asynchronous development, but it could not be assigned whether the strategy is determinate or indeterminate from the existing samples.

5.1.2 Data analysis

Samples were obtained from scientific cruises on IBTS Q1 and IBTS Q3 2007. Maturity was assessed at sea using both the IBTS 4 stage scale and other scales. The maturity status of fifty-eight specimens was determined through histological analysis in total and the results compared with initial macroscopic assessments. No data from Q3 (Quarter three) were analysed histologically.

5.1.2.1 Females

The maturity status of thirty five female specimens was determined through histological analysis in total and the results compared with initial macroscopic assessments. Overall, the maturity of over 45.7% of the females in the study group was miss-classified through the macroscopic examination (Table 5.1.2.1). The initial classification of non- spawners (Stages 1, 5 and 6) being classified as spawners (Stages 2–4) had an overall miss-classification rate of 83.3%, while only 3.4% spawners were classified histologically as non-spawners. As was the case for cod and saithe this indicates that the SSB will be overestimated by the existing guidelines and macroscopic criteria used in separating stages. In addition a high proportion of the females in spawning condition were assigned as maturing.

The oogenesis of haddock appeared from the histology to be asynchronous and this might to be the cause that few in the maturing stage was sampled. This stage is considerably shorter in asynchronous batch spawners than in group synchronous as batches of oocytes are matured subsequently. Relatively few female haddock were sampled and analysed for the workshop and further sampling and analysis would strengthen the analysis.

Table 5.1.2.1. Misclassification of North Sea haddock based on histological characteristics. Stage 1 is in this case called undeveloped because it may include ovaries in resting condition which cannot be distinguished with certainty from immature histologically. The misclassification are related to the total histologically staged and the stages misclassified are given in brackets as numbers x stage.

MATURITY STAGE	HADDOCK ♀	HADDOCK ♂
I. Undeveloped (juvenile/immature/resting)	0/1	5/6 (5xII)
II. Maturing	0/1	1/9 (1xI)
III. Spawning	11/17 (9xII + 2xIV)	10/17 (10xII)
IV. Spent	0/4	0/3
V. Resting / Skip of spawning	0	0
VI. Abnormal	0	0
Total missclassifications	11/23 (47.8%)	16/35 (45.7%)
Non-spawners classified as spawners initially	0/1 (0%)	5/6 (83,3%)
Spawners classified as non-spawners initially	0/22 (0%)	1/29 (3.4%)

5.1.2.2 Males

In total the maturity status of twenty three male specimens was determined through histological analysis and the results compared with initial macroscopic assessments.

Overall, the maturity status of over 47.8% of the male specimens in the study was miss-classified via macroscopic assessment (Table 5.1.2.1). However, no non-spawners (Stages 1, 5 and 6) were misclassified as spawners (Stage 2–4) in the initial classification. Also within the group of specimens classified histologically as spawners alls specimens were initially staged correctly. All misclassified specimens were males in spawning condition that were assigned as maturing.

5.1.3 Discussion and conclusions

Errors associated with the macroscopic classification of female haddock reproductive tissue were associated with assigning IBTS stage I and II correctly, and maturing stage II and spawning stage III. Group discussions highlighted problems in macroscopically classifying tissue samples from smaller sized specimens. Observed errors associated with maturity stage I and II (IBTS 4 stage scale) female tissues can be attributed to a failure to macroscopically define between the end of the immature phase and the onset of the maturation process, particularly so for smaller specimens. This can be problematic for assessment as some specimens may show signs of development but may not eventually contribute to spawning during the current season. Under the existing IBTS four stage definitions, such tissues can be classed as stage II or maturing when in fact such individuals may not contribute to spawning during the current season. A refinement of stage I and II macroscopic definition, under the proposed IBTS six stage scale, will facilitate the assignment of such tissues to the more appropriate classification of stage I or immature/juvenile maturity stage. Lastly, the group agreed that more histological analysis is needed to verify the criteria for such defined stages definitions for female haddock.

The gross appearance of haddock ovaries, undergoing the process of asynchronous batch spawning, can prove problematic, as ovary size will progressively reduce over time. Furthermore, the characteristic visible presence of hyaline eggs in IBTS stage IV

spawning tissue (proposed new scale) can be temporally absent between batch phases, before the resumption of oocyte hydration. In such cases, other macroscopic characteristics should be considered in the assessment, such as a high degree of vascularisation and reduced size of gonads, or the presence of a viscous droplet containing hydrated oocytes when pressure is applied to the ovaries. The group agreed that, under the proposed IBTS 6-stage scale, a more defined spawning stage (IV) macroscopic definition is necessary and will include the visible presence of hydrated oocytes in the ovary. It is felt that this can be applied to the female maturity staging of all four species and will reduce the miss-classification of spawning stage (3) as maturing stage (2).

Errors associated with the macroscopic classification of male haddock reproductive tissue were primarily associated with assigning IBTS stage III (spawning) to that of stage II (maturing tissue). Problems arose in the macroscopic identification of characteristic signs of ripeness in the testes. Specimen size and heterogeneous gonad size and appearance may have initially contributed to inaccurate assessments. The visible presence of spermatozoa in the sperm duct is the most distinguishing feature of ripe or spawning stage testicular tissue. This is often verified by the release of semen from the uro-genital pore, after pressure is applied. However, the gross appearance of the gonads of asynchronous or group-synchronous batch spawning species may appear uncharacteristic during the batch spawning process. In male haddock this is due to the temporal maturation of successive batches of spermatozoa resulting in the transitory absence of ripe gametes in the sperm duct of ripe or spawning stage specimens. The gonads of batch spawning male haddock will progressively reduce over time. It is agreed that this may increase the probability of misclassification, as gonad size in relation to body cavity length is generally considered in maturity stage assessment.

The group agreed on the need for more histological analysis of the process of spermatogenesis in haddock. It is felt that, under the proposed IBTS 6-stage maturity scale, the use of histologically verified species-specific macroscopic criteria will facilitate the reduction in erroneous assessment of spawning stage tissue as maturing stage tissue in male haddock.

The group felt that more histological analysis was necessary on the process of spermatogenesis in this species. This is particularly necessary for both the onset of the maturation phase and the onset of the spawning phase. For this reason the group was unable to presently agree on the formulation and application of definitive macroscopic criteria for the maturity staging of this species. However, the group agreed on the need for more defined stage descriptions than is currently in use in the IBTS four stage maturity scale. Furthermore, the group agreed that existing knowledge on this species should be incorporated into a preliminary 'test' or draft manual and circulated to IBTS personnel during quarter one 2008.

The group concluded that more defined stage descriptions for maturity of haddock are necessary than those in use currently in the IBTS four stage maturity scale. Furthermore the group concluded that criteria for stage III or spawning tissue, in all four species, should include the presence of hydrated oocytes in the ovary. Methods used for other species with asynchronous development should be considered and there is a need for further investigation of whether the fecundity of haddock is determinate or indeterminate. This was difficult to assess based on the existing material as no specimens in the characteristic stage of general resorption of gametes was observed. To investigate this, the timing of the sampling needs to be extended. If

haddock is an indeterminate spawner, which is a spawning strategy known also from other gadoids, e.g. hake, the SSB will not be a useful indicator of the reproductive potential in stock-recruitment relationships.

The group generally concluded that we are unable at present to define specific stage criteria based on existing knowledge. The group recommends that more histological analysis be undertaken on the process of oogenesis in this species and supplementing methods investigated, but agrees that existing information be incorporated into a 'test' or draft manual and circulated to IBTS personnel during quarter one 2008. The photo-documentation and descriptions are included in Appendix 3.

5.2 Whiting

5.2.1 Existing information

In earlier maturity studies, Bowers, 1954 macroscopically outlined the seasonal maturation cycle of both sexes in the species. Bowers applied an arbitrary seven stage maturity scale to whiting gonad maturation which was adapted from a five stage scale proposed by Bull, 1928, which in turn was a modified version of a classification of cod maturity stages proposed by Graham, 1924.

There exists a large temporal-spatial range in the gonadal maturation of whiting (Bowers, 1954). The spawning period peaks in April but extends over at least five months of the year (February to June) throughout its distribution area in the north east Atlantic (Bowers, 1954; Potts and Wotton, 1984). Gokhale, 1957 investigated the seasonal histological changes in the gonads of the whiting using both macroscopic and microscopic comparisons and histologically verified the seven stage maturity scale adapted by Bowers, 1954.

Gokhale, 1957 also evaluated the gonadal development of the ovaries of whiting using macroscopic and histological comparisons. In this study Gokhale, 1957 outlined the process of oogenesis in the whiting ovary and correlated the characteristic development stages to the adapted seven stage maturity scale form Bowers, 1954. Results indicated that macroscopic difficulties arose in distinguishing between immature and spent-recovering females, particularly for smaller individuals. However, histological differences between these two tissue types were easily distinguished due to the relative absence of oogonia in spent-recovering ovarian tissues. Furthermore, spent recovering whiting ovaries were observed to contain many 'nests' of small oocytes, some degenerating oocytes, a thicker tunica albuginea and a visible re-organising 'inter-ovular' structure. Similarly, more advanced developmental stages of maturing, ripe and spawning females were found to be more easily identifiable.

In this study Gokhale, 1957 outlined the process of spermatogenesis in the testes and applied the characteristic development stages to the adapted seven stage maturity scale. Results indicated that difficulties arose, both macroscopically and microscopically, in distinguishing between immature and spent-recovering individuals, primarily due to similarities in germ cell organisational structure and appearance within both tissue types. However, more advanced developmental stages were found to be more easily identifiable.

Despite evidence of several more recent studies on the histological development of reproductive tissues in the whiting, no specific, histologically verified, maturity scale is in international circulation for this species. The current IBTS four stage scale used for the assessment of maturity in gadoids is understood to be a condensed version of

one or several earlier gadoid scale and may incorporate generalised gadoid macroscopic stage definitions.

The reproductive strategy of whiting has been described as iteroparous, group-synchronous, determinate batch-spawner (Murua, and Saborido Rey, 2003). However, the present study identified an asynchronous development, but it could not be assigned whether the strategy is determinate or indeterminate from the existing samples.

5.2.2 Data analysis

Samples of ovaries were obtained from scientific cruises on IBTS Q1 and IBTS Q3 2007. Maturity was assessed at sea using both the IBTS 4 stage scale and other scales. Samples were fixed fresh and later analysed in the laboratory. Maturity was validated histologically using the 10 stage histological cod maturity scale.

In total the maturity of forty five specimens was ascertained through histological examination of tissue and the results compared with initial macroscopic assessments. Specimens sampled from Q3 (quarter three) were included in data analysis.

5.2.2.1 Females

Two specimens from Q3 (Quarter three) were available for inclusion in the analysis. The maturity status of twenty eight specimens was determined through histological analysis in total and the results compared with initial macroscopic assessments.

Overall, the maturity of over 28.6% of the females was miss-classified through the macroscopic examination (Table 5.2.2.1). The initial classification of non-spawners (Stages 1, 5 and 6) being classified as spawners (Stage 2–4) had an overall miss-classification rate of 33.3%, while 13.6% of the spawners were classified histologically as non-spawners. As was the case for cod, saithe and haddock this indicates that the SSB will be overestimated by the existing guidelines and macroscopic criteria used in the separation of stage.

Table 5.2.2.1. Misclassification of North Sea whiting based on histological characteristics. Stage 1 is in this case called undeveloped because it may include ovaries in resting condition which cannot be distinguished with certainty from immature histologically. The misclassification are related to the total histologically staged and the stages misclassified are given in brackets as numbers x stage.

MATURITY STAGE	WHITING ♀	WHITING ♂
I. Undeveloped (juvenile/immature/resting)	0	2/6 (2xII)
II. Maturing	4/5 (2xI + 2xIV)	3/14 (3xI)
III. Spawning	7/9 (7xII)	3/8 (3xII)
IV. Spent	2/2 (1xII + 1xIII)	0
V. Resting / Skip of spawning	0	0
VI. Abnormal	0	0
Total missclassifications	13/16 (81.3%)	8/28 (28.6%)
Non-spawners classified as spawners initially	0/0 (0%)	2/6 (33.3%)
Spawners classified as non-spawners initially	2/16 (12.5%)	3/22 (13.6%)

The oogenesis of whiting appeared from the histology to be asynchronous and possibly fecundity is indeterminate. This might to be the cause why few in the spent and resting stages were sampled. This spawning period of asynchronous spawners with indeterminate fecundity is may extended as it the case of e.g. hake. Relatively few whiting were sampled and analysed for the workshop and further sampling and analysis would strengthen the analysis.

5.2.2.2 Males

In total the maturity status of sixteen male specimens was determined through histological analysis and the results compared with initial macroscopic assessments.

Overall, the maturity status of over 81.3% of the male specimens in the study was miss-classified via macroscopic assessment (Table 5.2.2.1). However, no non-spawners (Stages 1, 5 and 6) were misclassified as spawners (Stage 2–4) in the initial classification. Within the group of specimens classified histologically as spawners 12.5 specimens were initially staged as non spawners. Misclassified specimens attributed to males in maturing, spawning as well as spent males. In fact only 3 males out of 16 were classified correctly.

The difficulties likely relates to the asynchronous development, which also in the male gonads make them appear different from the general description of IBTS 4-stage scale.

5.2.3 Discussion and conclusions

As was found to be the case for haddock, erroneous classification of female maturity of whiting was observed to be smaller in magnitude than that for males. However, the general trend of assigning IBTS stage III (spawning) to that of stage II (maturing) accounted for most of the observed errors in the data. As discussed earlier, variables such as specimen size or the effects of the dynamics of the batch spawning process on the gross appearance may have compounded accurate assessment of maturity in some cases. Nevertheless, the group observed that the reproductive tissues of spawning females were frequently inaccurately assessed.

As for female haddock, the group agreed that more detailed descriptive macroscopic terminology and additional supplementing methods should be applied to the female maturity staging of whiting. The group discussed the application of species specific descriptive macroscopic terminology in concurrence with a proposed IBTS six stage scale and agreed that such an approach would compliment more accurate maturity assessment in the future.

The maturity of the majority of sample specimens was found to have been erroneously assessed for male whiting. Furthermore, most errors were again associated with assigning IBTS stage III (spawning tissue) to that of stage II (maturing tissue). In similarity to problems encountered with male haddock, specimens of smaller size and heterogeneous gonad size and appearance may have initially contributed to inaccurate assessment. The visible presence of spermatozoa in the proximal sperm duct and at the uro-genital pore is the defining characteristic between maturing and spawning male reproductive tissue. Consequently, problems may have arisen during sample collection in identifying this macroscopic characteristic. As previously outlined for haddock, this may be attributed to the macroscopic appearance of testes associated with the dynamics of the batch spawning process. Nevertheless, more detailed and refined criteria must now be

applied to macroscopic stage descriptions to improve the accuracy of maturity assessment for this species.

Bowers, 1954 stated that no reliable macroscopic difference was found to distinguish maturing virgins from recovered spent specimens in male whiting. Similarly, this study observed the erroneous assignment of stage IV or spent tissue as stage II or maturing, in male whiting. The group agreed that the scope for error in such cases may be more prevalent in smaller sized individuals and that improved histology coupled with a more defined macroscopic criteria may help redress this issue. Furthermore, the group agreed that for species with protracted spawning seasons such as whiting, sampling for maturity outside the main spawning season will result in increased erroneous assessment of maturity.

In earlier maturity studies on Irish Sea whiting, the smallest individual in an advanced stage of maturity was found by Bowers, 1954 to measure 19 cm, and by both Nagabhushanam, 1964 and Gerritsen *et al*., 2003 to measure 15 cm. The group agreed that from the existing literature and the results of the present study, a cut off limit of 15 cm should be set for male and female whiting, below which sex and maturity will be taken as stage I immature (no sex). It was the consensus of the group that this category be incorporated into the proposed IBTS six stage scale.

For the maturity staging of male whiting the group recommends more defined stage descriptions and alternative methods than are currently in use in the IBTS four stage scale. However, as for haddock, based on available histological information, the group is at present unable to provide such definitive criteria for this species. More extended sampling during the early and late spawning period of whiting is necessary to cover the remaining stages and assess the fecundity type of whiting.

The group agreed that for whiting, maturity sampling for estimation of spawning proportion outside the main spawning season is unwise and that the maturity of all specimens of whiting below a length of 15 cm should be assessed as stage I immature (no sex). Lastly as for male haddock, the group agrees that existing information be incorporated into a test manual for testing on quarter one IBTS maturity surveys during 2008. However the manual should be perceived rather as assisting photo documentation than as a draft manual.

6 Improvement of sampling and quality assurance of maturity data

6.1 Present national sampling scheme

During the discussion on present sampling schemes it was realised that different national maturity scales were used. It was also shown that sampling takes place throughout the year. An overview over sampling schemes in use is given below.

Each member of the WS presented sampling schemes used at their national institute. The presentation of the national sampling schemes included information on: Maturity scale used, sampling freq. and spatial distribution, timing of sampling, validation-GSI, histology.

Present scheme in use in the North Sea, the Skagerrak and the Kattegat-IBTS survey

Sweden: From 1990 to 2006 the IBTS 4-grade scale was used routinely but in 2007 a national 8-grade scale was implemented. This scale is converted to the IBTS 4-grade scale prior to submitting data to DATRAS. In order to obtain a spatial cover of the surveyed area, one specimen per cm per haul is collected, except for gadoids > 70 cm where the aim is to collect all specimens caught. Sweden collects maturity data during the IBTS survey in Quarter 1 and 3 and no GSI or histology are performed on a regular basis. Maturity data are collected on cod, saithe and haddock.

Norway: IBTS 4 (5)-scale, 2 specimens per haul at the IBTS Q1 and Q3 surveys. No GSI or histology on regular basis. Maturity data are collected on cod, saithe, haddock and whiting.

Scotland: IBTS 4-scale, 1 specimen per cm per rectangle per haul at the IBTS Q1 and Q3 surveys. No GSI or histology on regular basis. Maturity data are collected on cod, saithe, haddock and whiting.

CEFAS: National 5-scale, 10 per cm in total or histology on regular basis at the Q3 IBTS. Maturity data are collected on cod, saithe, haddock and whiting.

Northern Ireland: National 7-scale, 1 specimen per cm per haul at the IBTS Q1 and Q3 surveys. No GSI or histology on regular basis. Maturity data are collected on cod, haddock and whiting (fecundity-haddock and cod recently).

Ireland: National 7-scale, 1 specimen per cm per rectangle per haul until 10 is reached at the IBTS Q3 survey. Maturity data are collected on cod, saithe, haddock and whiting. Germany: IBTS 4-scale, 2 specimens per cm per haul, 10 in total per cm and additional specimens to fill up, 10 per cm in total, no GSI or histology on regular basis at the IBTS Q1 and Q3 surveys. Maturity data are collected on cod, saithe, haddock and whiting.

Belgium: No participation in IBTS-no sampling of cod, saithe, haddock, whiting.

France: IBTS 4-scale, 1–2 specimens per cm per haul, 10–15 in total per cm and additional specimens to fill up per ground fish area. No GSI or histology on regular basis. Maturity data are collected on cod, haddock and whiting at the IBTS Q1 survey and at the Q3 English Canal, on cod and whiting.

Denmark: IBTS 4-scale, 8 in total per cm and additional specimens to fill up per ground fish area-spatial distribution is attempted. No GSI or histology on

regular basis. Maturity data are collected on cod, saithe, haddock and whiting at the IBTS Q1 and Q3 surveys.

Holland: No participation-contact persons sampling.

Those countries sampling maturity data on cod, haddock, whiting and saithe in the North Sea, the Skagerrak and the Kattegat were using the IBTS manual. If national maturity scaling differs from the IBTS scale the maturity data are converted into the IBTS 4 scale and reported to ICES DATRAS database.

Present scheme in the Baltic Sea-BITS

Denmark: National 10 scale-scale, 8 in total per cm and additional specimens to fill up per ground fish area-spatial distribution is attempted, GSI but not histology on regular basis. Maturity data are collected on cod at the BITS Q1 and Q4 surveys.

Sweden: In the Baltic several maturity scales have been in use over the years. Since XXXX the BITS 5-grade scale was used until 2007 when the previously used 8-grade scale was re-implemented. The 8-grade scale is converted to the BITS 5-grade scale prior to submission to DATRAS. Only maturity data on cod are collected in the Baltic. Five specimens per cm per SD are sampled except in SD 25 which is divided into 3 areas and therefore, when combining the data the number of fish collected amounts to 15 specimens per cm. Sweden collects maturity data during the BITS survey in Quarter 1 and 4, no GSI or histology are performed on a regular basis.

Germany: National 10-scale, 20 specimens per cm per SD, under 15 cm stages as immature no sex. GSI, individual egg size, but not histology on regular basis. Maturity data are collected on cod at the BITS Q1 and Q4 surveys.

Latvia: National 6-scale, more than 10 specimens per cm per sub area within SDs, Q1 and 4, no GSI or histology cod, data are also collected from commercial sampling.

If national maturity scaling differs from the BITS 5 scale the maturity data are converted into the IBTS 4 scale and reported to ICES DATRAS database.

6.2 Sampling time, numbers and spatial distribution

The micro- and macroscopical analyses have shown that sexual maturity data should not be collected in all season for the same species.

For the four species; cod, haddock, whiting and saithe, the following optimal sampling period and guide lines to estimate maturity ogives are recommended:

North Sea, the Skagerrak and the Kattegat:

- Cod: Sampling should only be carried out in the first quarter and the 1. quarter IBTS is recommended. Maturity staging should be carried out on all cod from 15 cm and above-below 15 no staging-no sex and maturity.
- Saithe: Sampling should only be carried out in the first quarter and the 1. quarter IBTS is recommended. Maturity staging should be carried out on all length class groups.
- Haddock: Sampling should only be carried out in the first quarter and the 1. quarter IBTS is recommended. Maturity staging should be carried out on all length class groups.

- Whiting: Sampling should only be carried out in the first quarter and the 1. quarter IBTS is recommended. Maturity staging should be carried out on all length class groups.

Baltic:

- Cod: Sampling should only be carried out in the first quarter and the 1. quarter BITS is recommended. Maturity staging should be carried out on all cod from 15 cm and above-below 15 no staging-no sex and maturity.

Furthermore, it is recommended that sampling is distributed spatially.

7 Final recommendations

7.1 General recommendations

1) **A common maturity scale including 6 stages is recommended for cod, saithe, whiting and haddock.** The maturity scale includes: 1. Immature, 2. Maturing, 3. Spawning, 4. Spent, 5. Resting/Skip of Spawning, and 6. Abnormal. The first 5 stages of the scale largely correspond to the 5-stage scale proposed by WKMAT. The 6th stage accounts for fishes with abnormal gonads, e.g. disease or intersex. Stages 5 and 6 will be applicable as ecosystem state indicators. Descriptions of stages were revised and supplemented by histological criteria. Specimens below 15 cm should be sexed as sex determination of males below this size is uncertain.

2) **Adaptation of DATRAS to include 6 maturity stages is recommended**. An extension of the number of maturity stages in DATRAS from 4 to 6 is recommended, if the revised maturity scale is agreed upon.

3) **It is recommended that the spawning proportion replaces the maturity ogive in the assessment of the spawning stock size.** Traditionally the SSB has been estimated using a maturity ogive that separates juvenile and adult fishes i.e. the proportion of sampled fish in stages 2–6/1–6. However, Stages 5 and 6 do not contribute to the spawning stock and viable egg production. As Stage 5 is difficult to distinguish from late immature in Stage 2, errors in the determination will not influence the estimated spawning proportion. The spawning proportion is estimated as the proportion of fish sampled in stages 2–4/1–6.

4) **It is recommended that sampling of maturity data for cod, saithe, whiting and haddock is only conducted during 1 quarter IBTS survey, but with increased intensity**. The first quarter surveys take part during the spawning period of all four species, while the third quarter survey takes place outside the spawning period. In the third quarter, most specimens were immature, early maturing or in resting condition, which are difficult to distinguish. The accuracy of sampling in the 3 quarter surveys was therefore low. A more efficient sampling will be obtained by focusing sampling efforts on the first quarter surveys. Broad coverage will be needed due to differences in habitats among spawning and non-spawning fishes.

5) **A follow-up Workshop is recommended in to be held in October 2009 considering cod, saithe, whiting and haddock.** The workshop distinguished between cod and saithe, both species are characterised by determinate fecundity, group synchronous development and batch spawning, and whiting and haddock that both has asynchronous development and sampling was too limited to assess whether they have determinate or indeterminate fecundity. Further work is needed to test and complete illustrated, histologically validated manuals on cod and saithe, and additional sampling is needed with respect to haddock and whiting. The species are described in detail in 7.2 and 7.3.

7.2 Cod and saithe

6) **It is recommended that the preliminary manuals for cod and saithe are tested on ITBS cruises in 1 quarter 2009 and that supplementing**

sampling is carried out to complete the manuals. Cod and saithe were characterised by determinate fecundity, group synchronous development and batch spawning. The general description of the spawning stages changed to match the batch spawning strategy of females i.e. including both female specimens with hydrated eggs and females in running condition. For the manuals (Appendices 1 and 2), the description of the specific maturity scales was adapted to the species and illustrated by selected photos of samples used during the workshop. In some cases, photos or stages are missing. Histological characteristics of stages were similarly described and included in the preliminary manuals. The manuals can be finalised with limited extra effort.

7.3 Haddock and whiting

7) In order to improve determination of reproduction pattern and routine sampling is recommended that additional sampling of haddock and whiting is conducted on ITBS cruises in 1 quarter 2009 and supplementing sampling is carried out by harbour sampling or during at-sea sampling to obtain missing stages. Whiting and haddock showed asynchronous oocyte development in females and as well as a gradient in development of testes from the efferent duct towards the periphery of the frills. Hardly any specimens were in spent stage and it was therefore not possible to determine with certainty whether they possess a fixed number of batches per female or they have indeterminate fecundity. If these species have indeterminate fecundity, the SSB is not a useful indicator of the egg production and there will be a need to supplement with sampling methods applied for other species with similar reproductive pattern like horse mackerel. Appendices 3 and 4 should therefore not be perceived as manuals at this stage but just as photo documentation. Prior to a follow up workshop an adequate sampling program should be developed.

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Annex 2: Maturity scale applied during IBTS cruises

A2.1 IBTS maturity scale

The 4-stage maturity scale that is included in the North Sea International Bottom Trawl Survey manual. This maturity scale is applied by Germany, Denmark, Holland, France, and Scotland.

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

2) MATURING:
   2.1) 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.
   2.2) 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:
   3.1) Male: Will extrude sperm under moderate pressure to advanced stage of extruding sperm freely with some sperm still in the gonad.
   3.2) Female: Will extrude eggs under moderate pressure to advanced stage of extruding eggs freely with some eggs still in the gonad.

4) SPENT:
   4.1) Male: 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.
   4.2) Female: Ovaries shrunken with few residual eggs and much slime. Resting condition, firm, not translucent, showing no development.

A2.2 National maturity scales

Examples of national maturity scales

Institute of Marine Research (IMR), NORWAY

Stage	Description
Blank	**Undecided / not checked.**
1	**Immature:** Gonads are small. No visible eggs or milt.
2	**Maturing:** Gonads are larger in volume. Eggs or milt are visible but not running.
3	**Spawning:** Running gonads. Light pressure on the abdomen will release eggs or milt.
4	**Spent/resting:** Gonad s small, loose and/or bloody.
5	**Uncertain:** Use only when difficult to distinguish stage 1 and 4.

Centre for Environment, Fisheries & Aquaculture Science (CEFAS) ENGLAND

I=Immature maps to IBTS stage 1

M=Maturing maps to IBTS stage 2

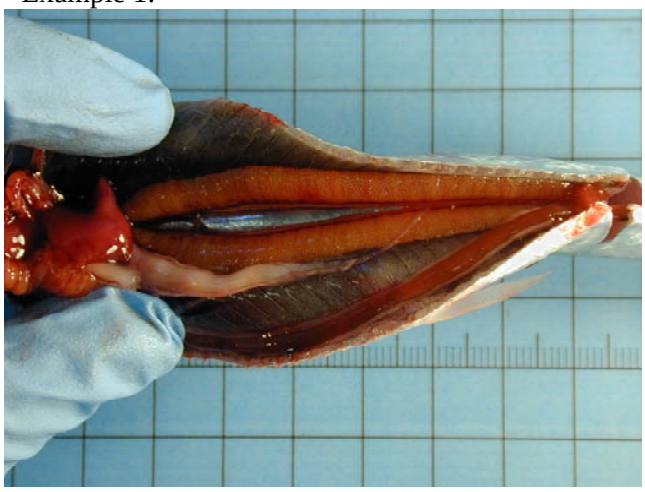
H=Hyaline maps to IBTS stage 2

R=Running maps to IBTS stage 3

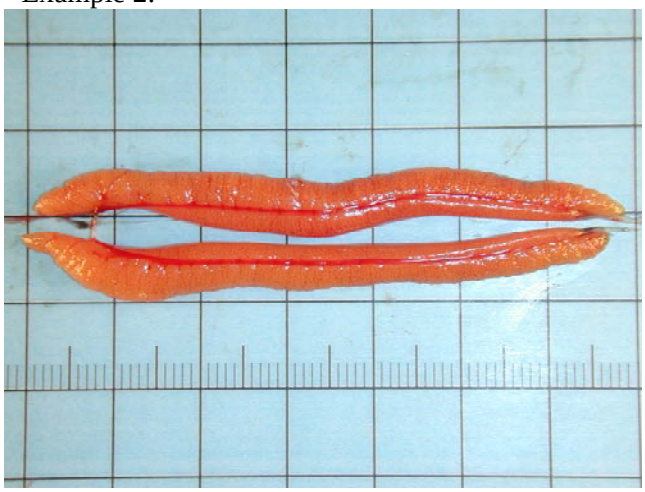
S=Spent maps to IBTS stage 4

Annex 3: Guidelines for photography

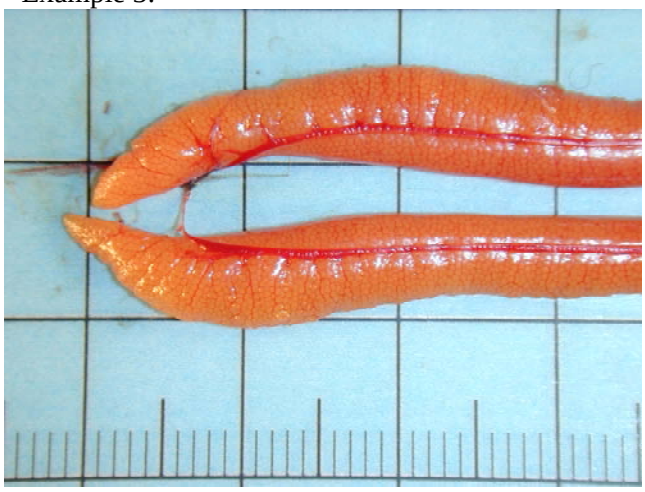
Example 1:



Example 2:



Example 3:



Guidelines for photography of samples: Example 1: Position and appearance in body cavity; Example 2: Entire gonad incl. measurement; Example 3: Close up illustrating characteristic features.

Annex 4: Samples collected and analysed by species and length

Samples collected, analysed and evaluated at the workshop by species (cod, saithe, whiting and haddock), sex and length per quarter.

Cod: *Gadus morhua* ♂ IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	19	4	3
20-29	45	3	3
30-39	63	7	6
40-49	45	7	5
50-59	26	7	5
60-69	28	9	6
70-79	25	8	5
> 80	20	6	6
Total	271	51	39

Cod: *Gadus morhua*-not sexed macroscopically IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	8	2	0
20-29	1	0	0
30-39	0	0	0
40-49	0	0	0
50-59	0	0	0
60-69	0	0	0
70-79	0	0	0
> 80	1	0	0
Total	10	2	0

Whiting: Merlangius merlangus ♀ IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 10	0	0	0
10-19	64	10	7
20-29	57	12	8
30-39	39	10	9
40-49	13	3	3
> 50	1	0	0
Total	174	35	27

Whiting: Merlangius merlangus ♂ IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 10	0	0	0
10-19	70	6	4
20-29	59	8	6
30-39	36	5	5
40-49	2	1	1
> 50	0	0	0
Total	167	20	16

Whiting: *Merlangius merlangus*-not sexed macroscopically IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 10	4	1	0
10-19	0	0	0
20-29	0	0	0
30-39	0	0	0
40-49	0	0	0
> 50	0	0	0
Total	4	0	0

Haddock: Melanogrammus aeglefinus ♀ IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	20	5	3
20-29	42	13	12
30-39	43	10	10
40-49	32	8	8
50-59	8	1	1
60-69	1	1	1
70-79	0	0	0
>80	0	0	0
Total	146	38	35

Haddock: Melanogrammus aeglefinus ♂ IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	18	1	1
20-29	47	6	4
30-39	40	10	9
40-49	28	5	5
50-59	7	1	1
60-69	0	0	0
70-79	0	0	0
>80	0	0	0
Total	140	23	20

Haddock: *Melanogrammus aeglefinus*-not sexed macroscopically IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	0	0	0
20-29	1	1	0
30-39	0	0	0
40-49	0	0	0
50-59	0	0	0
60-69	0	0	0
70-79	0	0	0
>80	0	0	0
Total	1	1	0

Saithe: *Pollachius virens* ♀ IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	0	0	0
20-29	3	1	1
30-39	7	1	1
40-49	27	11	7
50-59	15	5	4
60-69	5	2	2
70-79	1	1	1
> 80	2	1	1
Total	60	22	17

Saithe: *Pollachius virens* ♂ IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	0	0	o
20-29	4	1	1
30-39	7	1	1
40-49	28	7	3
50-59	21	7	5
60-69	8	2	2
70-79	1	0	0
> 80	4	2	2
Total	73	20	14

Saithe: *Pollachius virens*-not sexed macroscopically IBTS 1Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	0	0	0
20-29	0	0	0
30-39	1	1	0
40-49	0	0	0
50-59	0	0	0
60-69	0	0	0
70-79	0	0	0
> 80	0	0	0
Total	1	1	0

Cod: *Gadus morhua* ♀ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	1	1	0
20-29	14	5	1
30-39	14	4	0
40-49	23	6	0
50-59	17	2	0
60-69	8	3	0
70-79	7	0	0
> 80	12	5	2
Total	96	26	3

Cod: *Gadus morhua* ♂ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	0	0	0
20-29	12	4	0
30-39	13	3	1
40-49	19	5	0
50-59	10	3	1
60-69	7	3	0
70-79	8	1	2
> 80	6	3	1
Total	75	22	5

Cod: *Gadus morhua*-not sexed macroscopically IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	9	2	1
20-29	1	0	0
30-39	0	0	0
40-49	0	0	0
50-59	0	0	0
60-69	0	0	0
70-79	0	0	0
> 80	0	0	0
Total	10	2	1

Whiting: Merlangius merlangus ♀ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 10	0	0	0
10-19	11	2	0
20-29	23	8	2
30-39	22	4	0
40-49	19	4	1
> 50	1	1	0
Total	76	19	3

Whiting: Merlangius merlangus ♀ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 10	0	0	0
10-19	11	2	0
20-29	16	5	0
30-39	26	4	1
40-49	2	1	0
> 50	0	0	0
Total	55	12	1

Haddock: Melanogrammus aeglefinus ♀ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	5	2	0
20-29	19	4	0
30-39	25	3	0
40-49	22	3	0
50-59	6	1	0
60-69	3	1	0
70-79	0	0	0
>80	0	0	0
Total	80	14	0

Haddock: Melanogrammus aeglefinus ♂ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	6	3	0
20-29	19	4	1
30-39	25	3	2
40-49	13	3	0
50-59	3	1	0
60-69	0	0	0
70-79	0	0	0
>80	0	0	0
Total	66	14	3

Saithe: *Pollachius virens* ♀ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	0	0	0
20-29	1	1	0
30-39	6	1	0
40-49	12	4	0
50-59	12	2	1
60-69	12	2	0
70-79	0	0	0
> 80	4	1	0
Total	47	11	1

Saithe: *Pollachius virens* ♂ IBTS 3Q

Length groups	Total number of samples collected	Number of samples processed and screened histologically	Number of samples used for workshop
< 20	0	0	0
20-29	1	1	0
30-39	6	1	0
40-49	19	3	0
50-59	14	3	1
60-69	1	0	0
70-79	2	0	0
> 80	2	1	1
Total	45	9	2

Annex 5: Samples collected by species and IBTS maturity stage

Samples collected per maturity stage per species (cod, whiting, haddock and saithe), sex and quarter. The stage determination is the original made on broad the ships and given according to the IBTS 4-stage scale. The ICES IBTS 4-scale distinguishes I. Immature, II. Maturing, III. Spawning and IV Spent.

Cod: ***Gadus morhua*** **IBTS 1Q**

	Female 1Q		Male 1Q		No sex 1Q	
Stage	Number	%	Number	%	Number	%
1	100	39.7	127	46.9	9	90
2	129	51.2	71	26.2	0	0
3	7	2.8	54	19.9	0	0
4	13	5.2	17	6.3	0	0
No stage	3	1.2	2	0.7	1	10
Total	252	100.0	271	100.0	10	100

Cod: ***Gadus morhua*** **IBTS 3Q**

	Female 3Q		Male 3Q		No sex 3Q	
Stage	Number	%	Number	%	Number	%
1	18	23.1	25	40.3	10	100
2	19	24.4	2	3.2	0	
3	0	0.0	0	0.0	0	
4	41	52.6	35	56.5	0	
No stage	0	0.0	0	0.0	0	
Total	78	100.0	62	100	10	100

Whiting: ***Merlangius merlangus*** **IBTS 1Q and 3Q**

	Females 1Q		Female 3Q		Males 1Q		Males 3Q		No sex 1Q	
Stage	Number	%	Number	%	Number	%	Number	%	Number	%
1	37	21.3	14	24.6	14	8.5	16	32	4	100
2	121	69.5	11	19.3	134	81.2	8	16	0	0
3	12	6.9	3	5.3	8	4.8	0	0	0	0
4	1	0.6	29	50.9	3	1.8	26	52	0	0
No stage	3	1.7	0	0.0	6	3.6	0	0	0	0
Total	174	100.0	57	100.0	165	100.0	50	100	4	100

Haddock: ***Melanogrammus aeglefinus*** **IBTS 1Q and 3Q**

	Female 1Q		Female 3Q		Male 1Q		Male 3Q		No sex 1Q	
Stage	Number	%	Number	%	Number	%	Number	%	Number	%
1	25	17.1	7	11.9	15	10.7	22	36.7	1	100
2	103	70.5	16	27.1	86	61.4	1	1.7	0	0
3	9	6.2	0	0.0	26	18.6	0	0.0	0	0
4	9	6.2	36	61.0	13	9.3	37	61.7	0	0
Total	146	100.0	59	100.0	140	100.0	60	100	1	100

Saithe: *Pollachius virens* IBTS 1Q and 3Q

	Female 1Q		Female 3Q		Male 1Q		Male 3Q	
Stage	Number	%	Number	%	Number	%	Number	%
1	22	36.7	10	29.4	42	57.5	8	22.9
2	25	41.7	0	0.0	14	19.2	0	0.0
3	4	6.7	0	0.0	12	16.4	0	0.0
4	9	15.0	24	70.6	5	6.8	27	77.1
Total	60	100.0	34	100.0	73	100.0	35	100

WORKSHOP ON SEXUAL MATURITY STAGING OF COD, WHITING, HADDOCK AND SAITHE (WKMSCWHS)

Manuals on North Cod (*Gadus morhua* L), North Sea saithe (*Pollachius virens* L) North Sea haddock (*Melanogrammus aeglefinus* L) North Sea whiting (*Merlangius merlangus* L) can be found on the ICES Website at: http://www.ices.dk/reports/ACOM/2007/WKMSCWHS/directory.asp