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REPORT OF THE WORKSHOP ON SEXUAL MATURITY STAGING OF HAKE AND MONK (WKMSHM)

21–24 NOVEMBER 2007

LISBON, PORTUGAL

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

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

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Contents

Executive summary

The Data Collection Regulation (DCR) programme covers extensive sampling of maturity data (Reg EC No1639/2001). Maturity stage is an important biological parameter that is used in the calculation of maturity ogives (and therefore of spawning stock biomass), for the definition of the spawning season of a species, for the monitoring of long-term changes in the spawning cycle, and for many other research needs regarding the biology of fish.

The terms of reference of this workshop were: a) compare the macroscopic maturity scales for Hake and Monkfish used in the different laboratories; b) compare and calibrate the criteria, followed by the scientists/technicians involved in maturity sampling, to classify each maturity stage for males and females; c) validate macroscopic maturity scales with histological analysis; d) standardise the criteria to classify each maturity stage; e) propose a common scale, with common classification criteria; f) evaluate alternative methods to identify immature and mature fish, namely GSI and HIS; g) identify the period best suited to estimate maturity ogives.

This report presents the results of a Workshop on Sexual Maturity Staging of Hake and Monkfish, held in Lisbon (Portugal) in 21st–24th November 2007.

Laboratories involved in the collection of Hake and Monkfish maturity data use different macroscopic maturity stage keys for the same species. Even for those that were using the same maturity stage key it was detected that they use different criteria to classify the maturity stages that are more prone to a subjective interpretation. The misinterpretation between institutes was based on maturity data collected with photographic registration and gonads collection for histology analysis. A calibration exercise with fresh hake specimens was carried out among all participants. Correspondence between each institute maturity stage key was provided.

The standardization of maturity stage classification is fundamental when stock assessment is based on several institutes' data. In order to estimate new maturity ogives all institutes involved in stock assessment should use the same criteria to distinguish immature and mature specimens. One of the main goals of this workshop was to present a standard maturity key for each species. The proposed macroscopic maturity stage key is in agreement with the histological information of the species and is based on knowledge of the reproductive cycle. The maturity stage keys of all participant institutes were analysed and the proposed one was the consensual that allow the minimization of macroscopic misclassification. Photo observation helped to establish macroscopic characteristics that define each stage and to identify the major sources of classification uncertainty.

Macroscopically it is not possible to distinguish immature from resting females. The use of GSI and HIS to differentiate these two stages was investigated but does not give an accurate answer. Only histology can allow the correct classification of resting females. Taking to account that the proportion of resting females during the peak of the spawning season is lower than the rest of the year, maturity ogives should only be based on data collected during the peak of the spawning season. In case of hake it is recommended to collect immature/resting female gonads for histology purposes in a length class basis to estimate a correction factor that could be applicable to the macroscopic data. If any doubts in the macroscopic maturity stage classification arise it is recommended to collect the gonad for histological analysis.

Few histology studies focusing on males have been produced. Considering that all the hake and monkfish stocks used sex combined maturity ogives, more histology work should be done and the same importance should be given on both sexes. Histology is the only tool to produce validated maturity data.

In case of monkfish, it was easier to achieve a consensus on a new scale, comparatively with hake, because of the similarity of the scales used by each institute. However, it should be noted that the histological knowledge of this species is less.

The detection of different stage interpretation between institutes were only possible with this workshop, where scientists from different institutes classified the same gonad with the same maturity key and explained which macroscopic characteristics gave rise to a given classification. Also the presence of experts in reproductive biology, namely in histology, is an essential key to support the correct macroscopic identification and to link them with the reproductive cycle. It is recommended that this kind of workshop should be carried out intra and inter-institutes on a routine basis. At the very least, maturity exchanges with macroscopic and microscopic photos should be carried out to calibrate maturity identifications between institutes.

Usually, maturity sampling is performed by a large number of people in each institute. It was not possible to evaluate maturity data quality of each country based on one or few workshop participants. It is recommended that in the near future a similar workshop should be carried out inside each institute to assess discrepancies and also to present the conclusions of this workshop and to convert each institute maturity data in the new standard maturity stage key.

This kind of workshop should be carried on during the main spawning season of the respective species, to ensure fresh sample availability and the maximum range of maturity stages. Even inside the spawning season, the closer to the beginning of the year will be preferable, as fresh specimens become unavailable when quotas are reached. A calibration exercise should always be conducted with fresh samples.

A standard tool should be developed to analyse observer's discrepancies as has been developed for otoliths exchanges and workshop (Eltink *et al*., 2000). This analysis should weight the differences between immature and mature and not only stages. Also considering the reproductive cycle circular statistic analysis should be applied.

1 Introduction

1.1 Participation and agenda

A complete list of participants is presented in Annex 1 of this report. Also the Workshop agenda is available in Annex 2. Several Working Documents were present at the Workshop. In Annex 3 are present the abstract of the Working Documents.

1.2 Terms of Reference

a) Compare the macroscopic maturity scales for Hake and Monkfish used in the different laboratories.

b) Compare and calibrate the criteria, followed by the scientists/technicians involved in stage sampling, to classify each maturity stage for males and females.

c) Validate macroscopic maturity scales with histological analysis.

d) Standardise the criteria to classify each maturity stage.

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

f) Evaluate alternative methods to identify immature and mature fish–GSI and HIS.

g) Identify the period best suited to estimated maturity ogives.

1.3 Scientific justification and aims

The maturity stage is an important biological parameter used in the calculation of maturity ogives (and therefore of spawning stock biomass), for the definition of the spawning season of a species, for the monitoring of long-term changes in the spawning cycle, and for many other research needs regarding the biology of fish.

Laboratories involved in the collection of ICES WGHMM maturity data use different macroscopic maturity scales for the same species. Even those that use the same scale may be using slightly different criteria to classify the maturity stages that are more prone to a subjective interpretation. This may lead to bias in the data that may be going to be used, for example, in fisheries stock assessment models, or in any other kind of analysis. Therefore, this workshop has the objective of reaching an agreement on a common stage key to be used, but also to define objective criteria to classify each stage of that key.

One of the more important objectives of this Workshop is to produce a comparative description of the stage keys in use in different laboratories and if possible a correspondence between maturity stages of those different scales. Based on fresh samples and on photos it is also important to measure to what extent the criteria to classify maturity stages is coherent between technicians, and to identify where the major sources of disagreement are.

Another important goal of this Workshop is to produce a standard maturity stage key to allow the easy exchange of data between laboratories. The proposed standard maturity stage key should take into account the reproductive cycle of the species as well as histology information.

Other aspects such as, the use of alternative methods to assess the maturity stages identification (GSI and HIS) should be analysed.

Considering the macroscopic similarities between resting and immature females the spawning period should be correctly defined in order to minimize the effect of this misclassification in the maturity ogives estimation.

1.4 Data collection before the workshop

The workshop was appointed in the end of the year to allow data collection to be analysed at the workshop, namely macroscopic photos of gonads; GSI and HIS information and histology.

Before the Workshop each institute collected during a one year cycle gonads of each species according to the following indication:

For both species, the sampling parameters were: total length; gonad visual inspection-maturity stage by a standard maturity scale and the usual maturity scale used by the institute; total weight; gonad weight; liver weight; gutted weight; gonad photo; age; histological maturity stage; microscopic preparation photo. In Annex 4 the protocols are sent to participants and also the standard proposed maturity stage key for Hake and Monkfish.

2 Hake

2.1 Introduction

European hake (*Merluccius merluccius* Linnaeus) is a gadoid species widely distributed throughout the Northeast Atlantic from Norway to the Guinea Gulf, being more abundant from the British Isles to the South of Spain (Casey and Pereiro, 1995). Hake is also an important resource in the Mediterranean waters (Orsi-Relini *et al*., 2002; Maynou *et al*., 2003; Oliver, 1991).

European hake is a partial/multiple spawner (Pérez and Piñeiro, 1958; Sarano, 1986) with indeterminate fecundity (Murua *et al*., 1998). High asynchrony is observed both at the individual and population level, i.e., all oocyte developmental stages are observed in the mature ovary at the same time and females in all ovary maturity stages are recorded all year around (Domínguez-Petit, 2007, WD1; Murua and Motos, 2006).

Several studies on hake reproduction are being carried out (BIOSDEF, 1998; Dominguez-Petit, 2006; Murua, 2006). Most of the reproductive hake studies refer to the protracted hake spawning season. These species have a main spawning season but the time of the year and the duration depends on the region (Murua *et al*., 2006). The text table below shows different works with spawning season information:

Table 2.1. Hake spawning season.

			Month											
ICES area	Non - ICES area	Author	1	2	3	4	5	6	7	8	9	10	11	12
II, IV, VI, VII and VIIIa, b, d		ICESa, 2007												
VIIIc and IX a		Alcázar *et al*., 1983; Pérez and Pereiro, 1985; Piñeiro and Sainza, 2003												
IX a – Golf of Cádiz		Silva *et al*., 2007 (WD 10)												
	Greek waters	Mytilineou and Vassilopoulou, 1988												

main peak of the spawning season
peak of the spawning season

2.2 Maturity stage keys used by Institute

Several institutes that have attended this Workshop use different hake maturity stage keys, with a variable numbers of stages. Also a four stage maturity key was proposed as a standard before the workshop, that is referred in this report as “protocol” (Annex 4). The “protocol” key is a consensual maturity stage key proposed by three institutes (AZTI, IEO and IPIMAR) and results from a large reproductive study (BIOSDEF, 1998). Table 2.2 presents the different maturity stage keys used by each institute, the correspondences among them, the protocol and the general maturity stage key proposed by WKMAT (ICESb, 2007). All Hake maturity stages key used by different institutes are presented in Annex 5. (WD2; WD 3; WD4; WD5; WD6; WD7; WD8; WD12; WD13).

In general it is possible to establish correspondence between the different maturity stage keys. The different nomenclature used in different maturity scales key highlights the importance of having a standard maturity stage key or a glossary. The main doubts were between resting and recovery and between running, spawning and spent.

Until now there is no evidence in the hake literature relating to the possibility of skipping a spawning season. That is the reason for the lack of correspondence between all the hake keys and the WKMAT key in the “omitted spawning”.

As for many other species, immature and resting females are not possible to distinguish macroscopically (Trippel *et al.*, 1997; Saborido-Rey and Junquera, 1998; Dominguez-Petit, 2007, WD1; Gonçalves and Morgado, 2007, WD8; Gonçalves, 2007, WD9). In both stages the oocytes are not visible. It is only possible to accurately identify each stage by the means of histology. This misclassification has an impact on the estimation of the mature proportion of the stock because resting females have already contributed to the spawning biomass of that year and are macroscopically considered as immature. In the peak of the spawning season mature females are active and number of resting females should be minimal. In order to overcome the differentiation between immature/resting females it is recommended that these gonads are collected in a length-stratified sampling scheme for histology analysis to obtain a correction factor applicable to macroscopic data.

During the workshop the meaning of spawning was debated, *i.e.*, if it is related to the entire spawning season or a single batch event. In case of the IPIMAR hake maturity stage key, the spawning season were considered to be a batch. For that reason the post-spawning stage for this institute means a partial spawning, while for all the other institutes this stage means the end of the spawning season. All the other institutes take into account the cycle inside the spawning (multiple batches) and so the hydrated (running) and between batches stages are considered as the spawning stage.

Another discrepancy that was detected between institutes was that IEO classified the partial spawning (females between batches) in the developing stage, since the macroscopic description of this stage considered the presence of opaque oocytes and the absence of hydrated oocytes, even if they are flaccid (Appendix D, Table D.4). For that type of gonads all the institutes classified as spawning, except for IPIMAR that classified as post-spawning. It should be noticed that IPIMAR and IEO are applying the maturity stage key resulting from BIOSDEF project (BIOSDEF, 1998), but as explained above with different stages interpretations. An example of this discrepancy could be seen on Figures 2.1where IPIMAR classified as post-spawning, IEO as developing and the others laboratories as spawning.

Table 2.2. Hake Maturity Stage Key used by different Institutes, protocol maturity stage key and maturity stage key proposed by WKMAT.

MATURITY STAGE KEY	AZTI[(1)]	CEFAS[(2)]	HCMR[(3)]	IEO[(4)]	IFREMER[(5)]	IPIMAR[(6)]	MARLAB[(7)]	PROTOCOL[(8)]	WKMAT[(9)]
Immature	immature beging maturing	immature	Virgin developing virgen /inactive	immature	immature - virgin	immature	virgin	immature	virgin
Mature	maturing	mature	developing Mature	developing	virgin - developing recovering maturing	developing	maturing	developing	maturing
	pre-spawning spawning	hyaline running	Ripe	spawning	mature/spawner	spawning	spawning	spawning	spawning
	post-spawning	spent	Spent	pos-spawning	spent	post-spawning	spent and resting	post-spawning	spent/recovery
	resting				resting				
Immature									omitted spawning

[(1)] Annex 5 - Hake maturity Stage key from AZTI; [(2)] Annex 5 - Hake maturity Stage key from CEFAS; [(3)] Annex 5 - Hake maturity Stage key from HCMR; [(4)] Annex 5 - Hake maturity Stage key from IEO; [(5)] Annex 5 - Hake maturity Stage key from IFREMER; [(6)] Annex 5 - Hake maturity Stage key from IPIMAR; [(7)] Annex 5 - Hake maturity Stage key from MARLAB; [(8)] Annex 4 – Protocol Hake maturity Stage key; [(9)] Annex 5 – WKMAT Maturity Stage key.

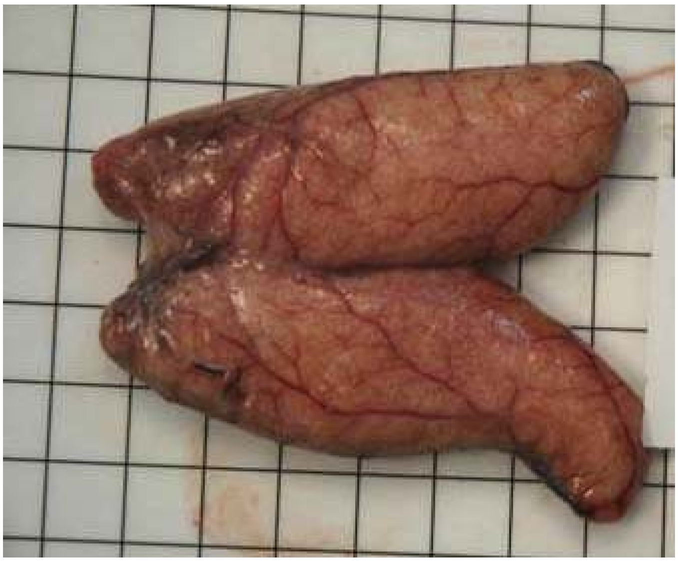

Figure 2.1. Hake female gonad, between batch stages. This gonad is characterized by the presence of many opaque oocytes; absence of hydrated oocytes and is flaccid. IPIMAR classified as post-spawning, IEO as developing and the other institutes as spawning.

2.3 Maturity stage calibration exercise

A calibration exercise was carried out with fresh hake specimens. After a plenary discussion on the protocol maturity stage key all the participants performed an independent maturity stage classification of 50 hake, with the protocol maturity stage key.

2.3.1 Material and methods

The length of the 50 specimens ranged from 28 to 52 cm. All specimens had an abdominal cavity incision to allow gonads inspection.

Each participant classified independently the gonad development according to the protocol maturity stage key (four stages key). After the independent gonad classification, the specimens with discrepancies were analysed and discussed in order to get a consensual maturity stage.

2.3.2 Results and discussion

The calibration exercise results are presented in Annex 6. Participants that as a routine are using scales with more than four stages showed more discrepancies.

The absence of larger individuals and the time of the year (outside the main spawning season) didn't allow spawning and post-spawning females. Most of the individuals were immature. In the case of females there were no difficulties in immature/mature differentiation. In the case of males, the boundary between virgin and the start of developing was difficult to establish, and that differentiation has been postponed for the discussion with histology.

The maturity stages classification were analysed using the Eltink *et al.* (2000) spreadsheet, developed for otoliths comparisons. This tool was just used to calculate the CV, the percentage of agreement relatively to the agreed stage and the bias to the agreed stage. Considering that the majority of the samples were immature females the discrepancies between observers were not evident, mainly between those who were used to the protocol stage key.

2.3.3 Comments

This calibration exercise was very important, mainly for those participants with less experience in macroscopically classified hake gonads.

The plenary discussion to get a consensual macroscopic maturity stage was very useful in order to establish macroscopic characteristics that defined each stage.

In general the majority of the participants have good agreement in macroscopic maturity stage classification.

A standardize tool should be developed to analysed observers discrepancies as it has already be developed for otoliths exchanges and workshops (Eltink *et al*, 2000). This analysis should weight the differences between immature and mature and not only stages. Also considering the reproductive cycle circular statistic analysis should be applied.

2.4 Standard maturity stage key

Based on the calibration exercise results, on the different maturity stages keys available and the discrepancies detected in the macroscopic gonads classification using the same maturity stage key a new standard hake maturity stage key was proposed. This key considers the reproductive cycle of this species and has also a correspondence to the histological characteristic (see Section 2.5).

The standard maturity stage key proposed has four stages for both sexes (see Annex 7). Although, in case of females, the stage that corresponds to spawning has two sub-stages. One stage is related to the presence of hydrated oocytes and the other is related to the period between batches where gonad flaccidity is an important macroscopic aspect. In both cases, the presence of opaque oocytes is imperative. The reason to have the sub-stages and not a different one is to keep the correspondence between females and males stages keys.

In Annex 8 is presented some macroscopic hake gonads classified according to the standard hake maturity.

I–IMMATURE/RESTING

Females: The absence of oocytes (opaque and hyaline); small, transparent or pink/grey gonads characterize this stage. Gonads are firm and not vascularized. The absence of oocytes is the determinant characteristic. These gonads are easy to identify. This is the only immature stage; however it is important to remember that outside the spawning season, resting females are macroscopically similar to virgin. Taking this into account, for maturity ogive estimation proposes it is recommended to use that data from the main spawning season, when the number of resting females is at a minimum. If an accurate differentiation between virgin and resting is mandatory, only histology can solve this problem.

Males: The absence of sperm and small, transparent or whitish gonads with a thin ribbon shape characterize this stage. These gonads do not present signs of developing, with no curling or like a thin curling band. Very few male histology studies have been conducted, and the differentiation between virgin and developing is difficult (Gonçalves, 2007, WD15).

II–DEVELOPING/MATURING

Females: The presence of opaque oocytes and absence of hydrated ones characterize this stage. Gonads classified as developing should be medium or large sizes and no signs of flaccidity. This stage is the first one to be considered as mature.

Males: Whitish gonads, with medium size, showing signs of developing (curling band) characterize this stage. Compared with stage I, these gonads are more curling and not so thin.

III–SPAWNING

Females: This stage comprises all the macroscopic aspects that female gonads present during the spawning season, *i.e.*, during the hydrated period (IIIA) and the inter-batch period (IIIB). All gonads should present opaque oocytes, large size and a pink to reddish orange colour. IIIA–Hydrated. Gonads are characterized by the presence of hydrate (hyaline) oocytes and firm gonads and IIIB – Partial spawning. Gonads are characterized by the absence of hydrate oocytes and flaccidity. This stage includes gonads with different macroscopic description (A and B) however both sub-stages occur inside the spawning period since hake is a multiple batch spawner. This fact and the need to have correspondence between males and females maturity stages keys were the reasons to consider sub-stages and not different stages. Stage IIIB can be misclassified when gonads are not fresh since the flaccidity is the main characteristic that differentiates it from stage II. However IIIB gonads have spaces next to the oviduct and are more vascularized than gonads from stage II.

Males: The gonads at this stage are a large white band with sperm that flow with pressure on the abdomen.

IV–POST-SPAWNING

Females: Gonads classified at this stage are flaccid and small or medium size. The colour is between a dark pink and purple. The oocytes are absent or residual. The colour of this stage is the main characteristic that allows a better differentiation with stage I. The absence of oocytes is the main characteristic that differentiates between this stage and stage II and IIIB.

Males: Gonads at this stage should present an empty and deformed band. They should be large gonads with residual or without sperm. Near the spermduct a large and thin space is evident.

2.5 Validation of macroscopic maturity stages

Histology is the only tool that allows an accurate identification of the maturity stage. This method is enough to solve identification of immature and resting females, because in the first one, all the oocytes in the ovary are in primary growth stage and well packaged while in the second one, ovary is without mature oocytes, with wide ovary wall, but lamellae are not so compact as in immature ovaries and blood vessels could be more abundant. Post-spawning females (inactive) are characterized by a high level of atresia, old postovulatory follicles, disorganization of ovary structures, numerous blood vessels, thick ovary wall and absence of yolked oocytes groups (excepting some atretic yolked oocytes).

In general fish histological studies are conducted on females and very few studies on males are available (Trippel, 2003) and hake is not an exception. IPIMAR has conducted some male gonads histology according to the workshop protocol (Gonçalves, 2007, WD15), mainly with the immature and developing males.

A correspondence between the proposed standard macroscopic maturity stage and the histological characteristic was established during the workshop.

2.5.1 Females

The female microscopic maturity stage key is defined as:

> **Stage I**: This stage includes two stages that are not macroscopically distinguishable - immature and resting. In the case of immature females, all the oocytes in the ovary were in primary growth stage and the oocytes are well packaged. The resting ovary doesn't present mature oocytes, has a wide ovary wall, lamellae are not as compact as in immature ovaries, and blood vessels may be more abundant.
>
> **Stage II**: This stage is characterized by the occurrence of cortical alveoli and/or vitellogenic oocytes, but post-ovulatory follicles are not present and no signs of

advanced spawning processes, such as a like thick ovary wall, high vascularization of gonad and/or disorganization of lamellae.

Stage IIIa: Presence of high percentage of hydrated oocytes or at the beginning of the hydration process.

Stage IIIb: Post-ovulatory follicles are observed throughout the ovary together with vitellogenic oocytes in different stages. There are no signs of advanced spawning process could be detected such as high number of blood vessels, swelling ovary wall, atresia, disorganization of ovary structures, etc.

Stage IV: Females at this stage will no longer produce more oocytes to be released during the current breeding season. They are characterized by a high level of atresia, old post-ovulatory follicles, disorganization of ovary structures, numerous blood vessels, thick ovary wall and absence of yolked oocytes groups (excepting some atretic yolked oocytes).

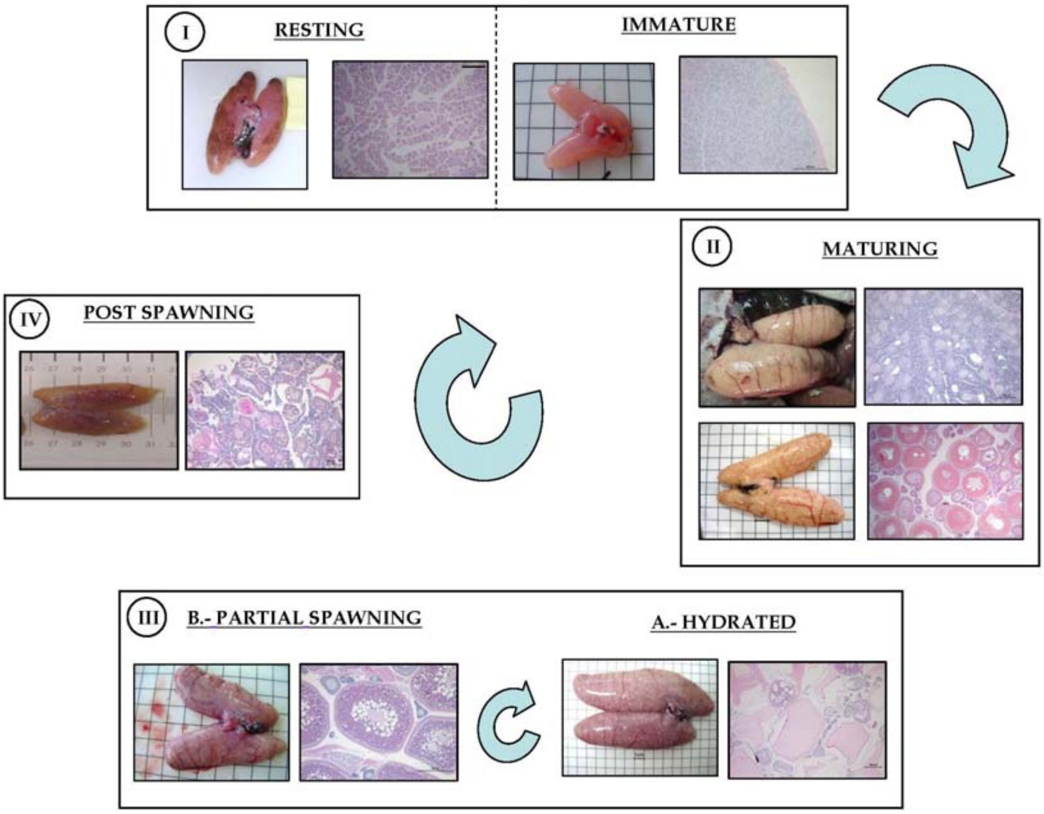

Figure 2.2. Hake reproductive cycle for females. Macroscopic and microscopic maturity stages.

2.5.2 Males

Male's microscopic maturity stage key is defined as:

Stage I: There are only spermatogonia and primary spermatocytes.

Stage II: The presence of secondary spermatocytes and spermatids is the main characteristic.

Stage III: Seminiferous tubules are thick and full of spermatozoa.

Stage IV: Seminiferous tubules are empty, with some residuals spermatozoa. Spermatogonia in the testes cortex.

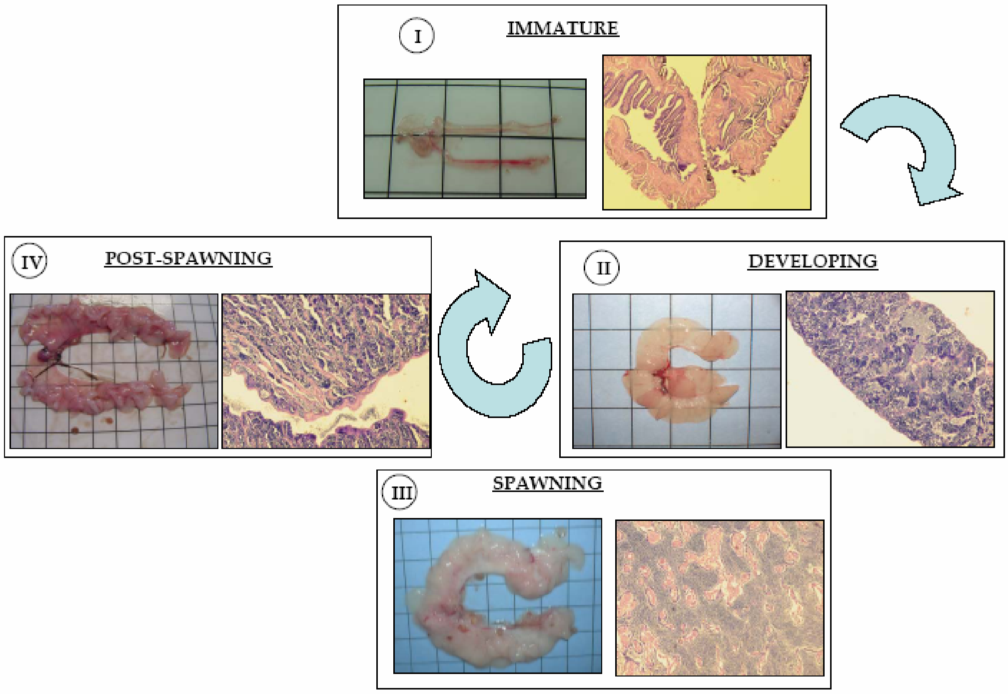

Figure 2.3. Hake reproductive cycle for males. Macroscopic and microscopic maturity stages.

2.6 Alternative methods to identify mature/immature

Several studies with condition factor, gonadosomatic index (GSI) and hepatosomatic index (HIS) were presented at the Workshop (Dominguez-Petit, 2007, WD 1; Morgado, 2007; WD11; Silva *et al.*, 2007, WD10). All these indices that are related with the used of energy reserves for growth or reproduction, do not allow an effective identification of the maturity stage. However those values can be used as indicative of the period of the spawning season (BIOSDEFF, 1998).

3 Monkfish (Lophius budegassa and L. piscatorius)

3.1 Introduction

Black anglerfish (*Lophius budegassa* Spinola, 1807) and white anglerfish (*Lophius piscatorius* Linnaeus, 1758) are important species in European fisheries. They are very similar, being distinguished by the peritoneum colour (*L. budegassa* black and *L. piscatorius* white) or by the number of rays in the second dorsal fin (*L. budegassa* 9–10 and *L. piscatorius* 11–12) (Caruso, 1986). Both black and white anglerfish are bottom living species, the former having a depth range between 70–800 m and the latter extending to depths >1000 m (Dardignac, 1988; Azevedo and Pereda, 1994). Black anglerfish has a more southern distribution (Mediterranean and Eastern North Atlantic from British Isles to Senegal) compared to white anglerfish (Mediterranean, Black Sea and Eastern North Atlantic from Barents Sea to the Strait of Gibraltar), but there is considerable overlap.

The studies conducted in the last years by Afonso-Dias and Hislop (1996), Duarte *et al.* (2001) and Quincoces (2002) have showed several aspects of the reproduction on anglerfish in different areas, as the general morphology and histology of the gonads, the maturation process, the spawning seasons, the maturity ogives, and the lengths and ages at first maturity. Different studies with information on the spawning season are showed in Table 3.1.

Anglerfish is a determinate spawner (Afonso-Dias and Hislop, 1996). The process of oocytes maturation is similar to that in other teleosts, although the morphology of ovaries differs markedly from that of most other teleosts. A gelatinous matrix is produced inside the ovaries, the oocytes are arranged in clusters and, within each oocytes cluster, there is a gradation in the size of oocytes. As the ovaries develop, one group of oocytes becomes clearly demarcated from the others (Afonso-Dias and Hislop, 1996). The eggs seem to be shed in a single batch, because no modes can be seen in the size distribution of the advanced eggs, and the egg ribbons are far too long and wide to be a result of only one of several batches (Afonso-Dias and Hislop, 1996).

Table 3.1 Anglerfish spawning periods: a) *L. budegassa,* b) *L. piscatorius.*

a)			Month											
ICES area	Area	Author	1	2	3	4	5	6	7	8	9	10	11	12
VIIIabd	Northern Bay of Biscay	Quincoces *et al*., 2002												
VIIIc-IXa	Iberian Peninsula	Azevedo, 1996a												
VIIIc-IXa	Iberian Peninsula	Duarte *et al*., 2001												
VIIIc	North of Spain	Landa *et al*., 2007												
	Greek waters	Maravelias and Papaconstantinou, 2003												

b)			Month											
ICES area	Area	Author	1	2	3	4	5	6	7	8	9	10	11	12
IVa	Shetland Islands	Laurenson, 2003												
IV	East of Scotland	Fulton, 1898												
IV	North Sea	Bowman, 1919												
VIa	North of Scotland	Afonso-Dias and Hislop, 1996												
VIIIabd	Northern Bay of Biscay	Quincoces *et al*., 2002												
VIIIc-IXa	Iberian Peninsula	Duarte *et al*. 2001												

3.2 Maturity stage key used by Institute

A variety of keys are currently in use throughout ICES (Table 3.2), consisting of 4, 5 and 6 maturity stages (Annex 9). Four-stage keys are used by the IBTS and by IFREMER (France) and the descriptions of the developmental stages are quite similar.

CEFAS (England and Wales), IPIMAR (Portugal), FRS (Scotland), AZTI (Spain) and IEO (Spain) all use five-stage keys. Although the developmental stages are generally similar between all institutes, differences are seen for males and females at the second stage. For males in the CEFAS key, no sperm are present at stage, whereas for the other institutes, some sperm may be present. For females in the FRS and IPIMAR keys, there are no visible oocytes present. In contrast, in the AZTI, IEO and CEFAS keys, the presence of visible opaque oocytes is given in stage 2.

HMRC (Greece) is the only institute to use a 6-stage key. The first three stages of this key equate to the first two stages of the 5-stage keys, by the addition of a developing virgin/inactive stage. In the females, this additional stage differentiates between ovaries with and without opaque oocytes.

The proposed WKMAT key (Table 3.2.) is similar to the IBTS key, with the addition of an omitted spawning stage.

Comparing the maturity stages of the institutes concerned (Table 3.2.), it can be seen that there is already good consensus between the developmental stages. This would probably make it easier for each institute to move towards a standardised key, than would be the case with hake. For each stage, a few, well chosen words have been used, rather than long descriptions of the state of the gonad. It is hoped that this will make assigning gonads to the correct stage easier for technicians. For the proposed key, Stage 2 is immature, but it also contains resting fish. However, if only data from the spawning season are used, then this problem will be minimised (as these fish will be in stages 3, 4 or 5).

Table 3.2. Monkfish Maturity Stage Key used by different Institutes, protocol maturity stage key and maturity stage key proposed by WKMAT.

<table>
<tr><td>WKSMSHM</td><td colspan="2">I - Immature</td><td>II – Developing/ resting</td><td>III – Pre-spawning</td><td>IV - Spawning</td><td>V – Post-spawning</td></tr>
<tr><td>IBTS</td><td colspan="2">1 – Virgin</td><td colspan="2">2 - Maturing</td><td>3 - Spawning</td><td>4 - Spent</td></tr>
<tr><td>Cefas</td><td colspan="2">1 - Immature</td><td>2 - Maturing</td><td>3 - Mature</td><td>4 - Spawning</td><td>5 - Spent</td></tr>
<tr><td>IFREMER</td><td colspan="2">I - Immature</td><td>II - Development</td><td colspan="2">III - Maturity</td><td>IV – Post-spawning</td></tr>
<tr><td>HCMR</td><td>I - Virgin</td><td>II – Dev. virgin/ inactive</td><td>III - Developing</td><td>IV - Mature</td><td>V - Ripe</td><td>VI – Spent</td></tr>
<tr><td>IPIMAR</td><td colspan="2">I - Immature</td><td>II - Maturing</td><td>III – Mature or pre-spawning</td><td>IV - Spawning</td><td>V – Post spawning</td></tr>
<tr><td>FRS</td><td colspan="2">I - Virgin</td><td>II Developing/ recovering/ resting</td><td>III - Maturing</td><td>IV - Ripe</td><td>V - Spent</td></tr>
<tr><td>AZTI</td><td colspan="2">1 - Immature</td><td>2 - Maturing</td><td>III – Maturing or pre-spawning</td><td>IV - Spawning</td><td>V – Post-spawning</td></tr>
<tr><td>IEO</td><td colspan="2">I – Immature or virgin</td><td>II - Maturing</td><td>III – Mature or pre-spawning</td><td>IV - Spawning</td><td>V – Post spawning</td></tr>
</table>

3.3 Standard maturity stage key

Based on a plenary discussion on the macroscopic characteristics that defined each maturity stage a standard maturity key with five stages was proposed (see Annex 10). This key considers the reproductive cycle of both *Lophius* species and has also a correspondence to the histological characteristic (see Section 3.4).

In Annex 11 is presented some macroscopic monkfish gonads classified according to the standard hake maturity stage key.

I–IMMATURE

This stage only includes virgin fish showing no signs of gonadal development.

Females: Ovaries are very narrow (<2cm band width), thin and ribbon like. They are translucent with no oocyte clusters visible and minimal vascularisation, if any. Volume is negligible compared to that of other internal organs.

Males: Testes are long and narrow (<1cm broad). They are translucent with no significant vascularisation. Their volume is negligible compared to that of other internal organs.

II–DEVELOPING/RESTING

This stage for the most part includes virgin fish showing signs of early development although there is an acceptance that this stage also includes resting mature fish which are returning from the post spawning phase. Confusion in this stage can be minimised by only collecting maturity data from within the peak spawning period.

Females: Ovaries increase in length and width (>2cm band width) often undulating, especially at the margins. They become less translucent and the volume occupied is roughly the same as that occupied by the intestine. There are no visible oocyte clusters.

Males: Testes increase in length and width. They are no longer translucent but opaque in colour. Some vascularisation is visible around the seminiferous duct. No sperm is present at this stage, either in the seminiferous duct or in the lumen.

III–PRE-SPAWNING

Females: Ovaries increase considerably in width and particularly in length. They are highly vascularised and individual opaque oocyte clusters are visible embedded in a matrix. The colour also changes to yellow or orange.

Males: The testes are large and firm with a cream colouration. The seminiferous duct is now highly vascularised and moderate/ large amounts of milt are produced from the testes when cut and/or from the lumen/genital pore when moderate pressure is applied.

IV–SPAWNING

Females: The ovaries are extremely long and wide occupying most of the body cavity. The colour is typically bright orange and the ovaries are characterized by the presence of large hyaline oocyte clusters embedded within a gelatinous matrix.

Males: Milt is free running or freed from the genital pore with only light pressure to the abdomen. The testes are large and turgid with large amounts of sperm being produced upon dissection of the testes. Colouration is cream, sometimes with an olive green tint.

V–POST-SPAWNING

Females: The ovaries are flaccid and much reduced in size compared to that of stage IV. Their colour is dark pink or red often with longitudinal striations. No oocytes are present and the ovary is highly vascularised.

Males: Testes are reduced in size and are more flaccid than in stages III and IV. They exhibit pink/red bruised areas along the surface of the testes. At this stage sperm may still be visible in the seminiferous duct or when dissected though this is not always the case.

3.4 Validation of macroscopic maturity stages

Studies with monkfish histology are scarce and several doubts were raised in this workshop. More work should be conducted in the area to improve the macroscopic validation.

3.4.1 Females

Stage I: Only oogonias and oocytes in nucleolar chromatin.

Stage II: Resting: Oogonias, oocytes in nucleolar chromatin and perinucleolar stages; Developing: Oocytes in cortical alveoli are present.

Stage III: There are vitelogenic oocytes (early to advanced) and mucus matrix starts to develop.

Stage IV: Oocytes are in migratory nucleus stage or hydrated and mucus matrix is completely developed.

Stage V: Post-ovulatory follicles and atretic oocytes are observed with primary growth stages (nucleolar chromatin and perinucleolar).

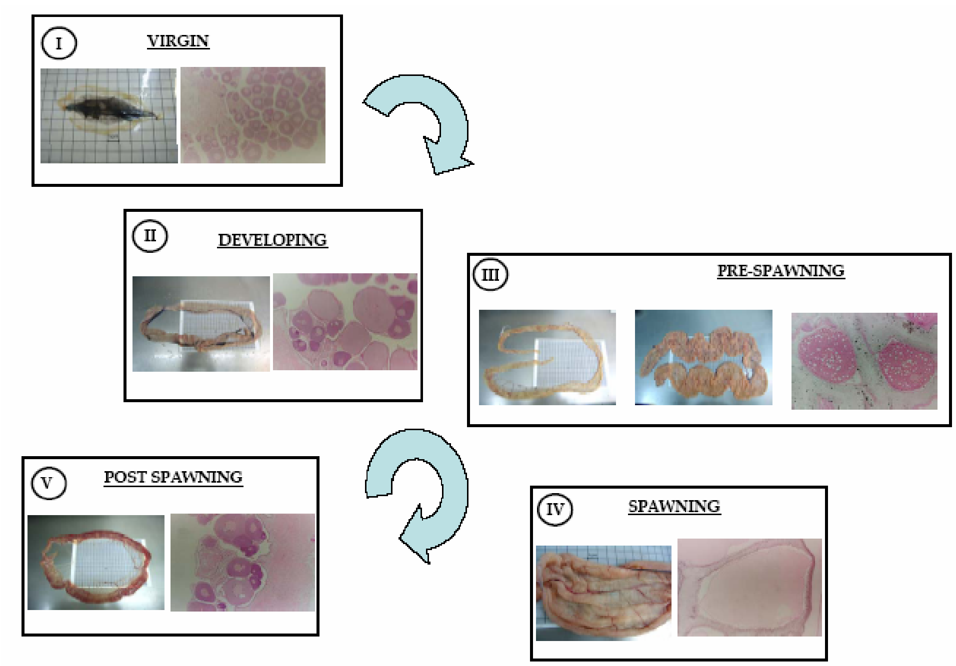

Figure 3.1. Monkfish reproductive cycle for females. Macroscopic and microscopic maturity stages.

3.4.2 Males

Stage I: There are only spermatogonia and primary spermatocytes.

Stage II: Spermatogonias, primary and secondary spermatocytes are predominant. Spermatids are scarce.

Stage III: Spermatids are predominant with a lot of spermatozoa in the seminiferous tubules. Spermatogonias and spermatocytes are only in testes cortex.

Stage IV: Seminiferous tubules are thick and full of spermatozoa.

Stage V: Seminiferous tubules are empty, with some residual spermatozoa. Spermatogonia in the testes cortex.

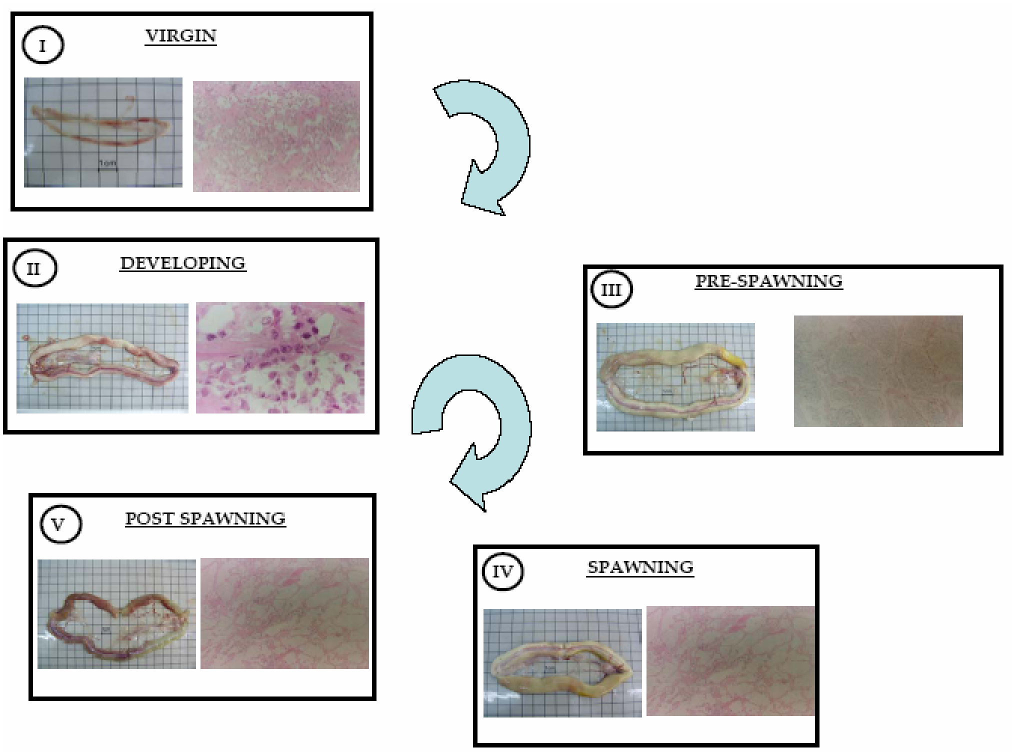

Figure 3.2. Monkfish reproductive cycle for males. Macroscopic and microscopic maturity stages.

3.5 Alternative methods to identify mature/immature

A study with condition factor (CF), gonadosomatic index (GSI) and hepatosomatic index (HIS) was presented at the workshop (Landa *et al.*, 2007, WD 12), analysed by month. This study was based on the specimens with lengths higher than $L_{50\%}$., assumed as adults. All these indices that are related to the use of energy reserves for growth or reproduction, do not allow an effective identification of the maturity stage. However, some results about the spawning period were obtained, mainly in males. The scarcity of mature females and lack of spawning females, mainly in white anglerfish, did not allow a precise spawning period for combined sexes to be estimated.

4 Stock assessment link

4.1 Hake

Hake is a partial/multiple spawner with a protracted spawning season. When maturity data is used for maturity ogive estimation two important issues should be taken into consideration: (i) the macroscopic misclassification of resting females as immature; (ii) the variability of the spawning season according to the large distribution of this species. As referred before, only histology allows an accurate classification between mature and immature, however if only maturity data from the spawning season is used the misclassification of resting females is minimized. Also it is recommended that as a sampling routine, female gonads that are macroscopically classified as immature should be collected in a length-stratified manner for histological analysis. With the histological results each institute can estimate a correction factor for the misclassification between mature and immature, and correct all macroscopic data.

When females' macro and microscopically estimated maturity ogives are compared, it is observed that $L_{50\%}$ is overestimated if macroscopic method is applied mostly because of the misclassification of immature and resting females (Dominguez-Petit, 2007, WD1).

Also for maturity ogives estimation purposes data should take into account the different times of the year of different geographic areas, as shown in Table 2.1.

It is recommended that routine $L_{50\%}$ estimation should be carried out. Reduction in $L_{50\%}$ may be an indicative of overexploitation of stock and/or a decrease in stock reproductive potential. This is because smaller females begin ovary development at later time and will probably be in spawning condition later (Kjesbu, 1994). These females will produce not only less but also smaller eggs (Kennedy *et al.*, 2007) during a shorter spawning season and a less intense reproductive activity (Alarcón *et al.*, 2004), which will result in smaller larvae with less probability to survive and be recruited to spawning stock biomass (Cardinale and Arrhenius, 2000). Increase or decrease of $L_{50\%}$ may affect the assessment results and, subsequently, management measures (Trippel, 1999; Marteinsdottir and Begg, 2002; Kraus, 2002; Lambert *et al.*, 2003; Marshall *et al.*, 2003; Tomkievicz *et al.*, 2003; Dominguez-Petit, 2007, WD1).

The Northern stock of Hake is assessed with the same maturity ogive in recent years based on Martin (1991) studies with Bay of Biscay's data. The Southern stock of hake is assessed with an annual maturity ogive. Both stocks use a sex combined maturity ogive. Based on the recommendation from the ICES WKMAT (ICESb, 2007) the Southern Hake maturity ogive applied in this year's assessment was weighted by the total length distribution.

4.2 Monkfish

Different methods are applied in the assessment of anglerfish within ICES. The Iberian Atlantic stocks (two species) are assessed with a stock production model and no maturity ogives are necessary. There are however maturity ogives available for both species and sexes, that are assumed constant along the years. The Anglerfish stocks in Divisions VIIb-k and VIIIa,b,d,e are assessed by an age structured model and maturity ogives are applied to estimate the annual spawning biomass. A constant maturity ogive is used along the overall period and changes in the age of first maturity can not be traced. The assumption of a constant maturity ogive is however a consequence of the low sampling levels of maturity stages in each year. Trends in the age of first maturity can cause bias in the spawning biomass estimates that affect the perception of the stock status. Misclassifications of maturity stages are probably less important in Anglerfish.

5 Conclusions

5.1 Hake

- Discrepancies between institutes were detected during the workshop maturity stages even when the same maturity scale was used. These discrepancies were identified and a standard maturity stage key was proposed to overcome theses problems;
- The detection of the referred discrepancies were only possible in a workshop like this, where scientists from different institutes classified the same gonad, with the same maturity key, and explained which of the macroscopic characteristics that conducted to a given classification. Also the presence of experts in reproduction biology, namely in histology, is an essential key to support the correct macroscopic identification and to link them with the reproductive cycle;
- The proposed standard maturity stage key takes into account the reproductive cycle which allow an easier interpretation of the stage classification;
- Maturity ogives should only be based on data collected during the peak of the spawning season considering geographical variation, since it is impossible to macroscopically distinguish immature from resting females. The proportion of resting females during the peak of the spawning season is lower than on the rest of the year;
- Monthly analysis of gonadasomatic index is an important tool for the spawning season identification. However, this index, the condition factor and the hepatosomatic index does not allow the identification of the maturity stage and the differentiation between immature and mature fishes;
- Gonads freshness is very important to the correct maturity stage identification. Frozen gonads can present flaccidity due to the freezing process and thus not being indicative of the post-spawning stage. Also frozen gonads are not appropriate to perform histology;
- The calibration exercise performed during this workshop was very useful, because the observation of fresh gonads, instead of photos, provides a better description of some macroscopic characteristics;
- More histology work should be done. The differentiation between immature and resting females is possible by means of histology. Also histology on males should be pursued to distinguish between stages I and II and on females to distinguish between stages II and IIIb, where the flaccidity is the only difference between these stages.

5.2 Monkfish

- Compared with the hake it was easy to achieve a consensus on a new scale, because of the similarity of the scales used by each institute;
- Males stages II, III and IV may present different macroscopic aspects depending on the samples freshness, mainly between fresh and commercial (retained) samples;
- GSI and HSI can help to define the main spawning season; however do not allow the maturity stage identification;
- Mature resting stage have been aggregated along with stage II (Immature) due to difficulties with discrimination. The correct identification of these stages is only possible by means of histology. However, samples in the spawning season should minimize this inaccuracy, when the proportion of resting females should be minimal.
- More histology work should be done. The differentiation between immature and resting females is possible by means of histology (stage II). Also histology on males should be pursued to distinguish between stages I and II.

6 Recommendations

- It is recommended that this type of workshops should be carried out on a routine basis. At the very least, there should be a maturity exchange with macroscopic and microscopic photos to calibrate the maturity identifications between institutes;
- It is also recommended that regular calibration exercises inside institutes, with fresh specimens (not only images) should be carried out;
- Maturity ogives should only be based on data collected during the peak of the spawning season considering geographical variation, since it is impossible to macroscopically distinguish immature from resting females. The proportion of resting females during the peak of the spawning season is lower than on the rest of the year;
- A standardized tool should be developed to analyse observer discrepancies as already has been developed for otolith exchanges and workshops (Eltink *et al*, 2000). This analysis should weight the differences between immature and mature and not only stages. Also considering the reproductive cycle circular statistic analysis should be applied;
- This kind of workshop should be carried out during the main spawning season of the respective species, to ensure fresh sample availability and the maximum range of maturity stages. That was the reason for lack of spawning specimens in the calibration exercise;
- Even inside the spawning season, the closer to the beginning of the year the better, in order to avoid reaching quotas and to ensure fresh specimens availability. That was the reason of the lack of a calibration exercise with monkfish;
- Histology was an important tool to achieve a consensus on maturity stage description/classification.

6.1 Hake

- Gonad histology should be undertaken to assess the differences between immature/resting stages, mainly during the spawning season (period used for the maturity ogive estimation). Immature/resting female’s gonads should be collected for histology purposes in a length class basis to estimate a correction factor applicable to the macroscopic data.
- Since the two hake stocks use sex combined maturity ogives, histology on males should be undertaken to validate the macroscopic classifications, mainly in the differentiation between immature and mature.
- In the case of doubts in the macroscopic classification of the maturity stage it is recommended to collect the gonad for histological analysis.

6.2 Monkfish

- More histology studies should be done to validate the macroscopic maturity key, for both species. Also, histology should be applied to quantify the inaccuracies due to misclassification between immature/resting, at least during the spawning season (period recommend for maturity ogives estimation).
- Maturity scales should include a reference catalogue of images to clarify identification (if possible validated by histology), since each stage can present a great variability in the macroscopic aspect.

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Annex 1: List of participants

NAME	ADDRESS	TELEPHONE	FAX	E-MAIL
Cristina Morgado (Co-chair)	IPIMAR Avenida de Brasilia P-1449-006 Lisbon Portugal	+351 213 027 061	+351 213 025 948	cmorgado@ipimar.pt
Patrícia Gonçalves (Co-chair)	IPIMAR Avenida de Brasilia P-1449-006 Lisbon Portugal	+351 213 027 119	+351 213 025 948	patricia@ipimar.pt
Katerina Anastasopoulou	Athens Sounio, Mavro Lithari P.O. BOX 712, 19013 Anavissos Attica	+30 210 9856705	+30 210 9811713	kanast@ath.hcmr.gr
Susana Arengo	AZTI Txatxarramendi Irla 48935 Sukarrieta/Pedernales SPAIN	+34 94 6029455	+34 94 6870006	sarengo@suk.azti.es
Finlay Burns	Fisheries Research Services Marine Laboratory P.O. Box 101 Victoria road Aberdeen AB11 9DB United Kingdom	0044 (0)1224 295376 (Direct Dial) 0044 (0)1224 876544 (Switchboard)	0044 (0)1224 295511 (Fax)	f.burns@marlab.ac.uk
Begoña Castro	IEO C.O.A. Coruña Muelle de las Animas, s/n P.O. Box 130 E-15001 A Coruña	+34981205362	+34981229077	bego.castro@co.ieo.es
Ysabelle Cheret	IFREMER Lab.Ressources Halieutiques Bd Jean Monnet BP 171 34203 Sète Cedex	04 99 57 32 51	04 99 57 32 94	ysabelle.cheret@ifremer.fr
Joël Dimmet	IFREMER	02 97 87 38 15	02 97 87 38 36	Joel.Dimeet@ifremer.fr
Rosario Dominguez	IM (CSIC) C/ Eduardo Cabello, 6 36208, Vigo. SPAIN	+34 986 231 930		rosario@iim.csic.es

NAME	ADDRESS	TELEPHONE	FAX	E-MAIL
Sandra Dores	IPIMAR Avenida de Brasilia P-1449-006 Lisbon Portugal	+351 213 027 060	+351 213 025 948	sdores@ipimar.pt
Rafael Duarte	IPIMAR Avenida de Brasilia P-1449-006 Lisbon Portugal	+351 213 027 061	+351 213 025 948	rduarte@ipimar.pt
Robert Forster	CEFAS Boswednan Farm Tremethick Cross Penzance Cornwall TR20 8UA United Kingdom	+44 1736 350653 (office) +44 7899 690302 (mobile)	+44 1502 513865	robert.forster@cefas.co.uk
Maria Korta	AZTI Txatxarramendi Irla 48935 Sukarrieta/Pedernales SPAIN	+34 943 004800	+34 943 004801	mkorta@pas.azti.es
Jorge Landa	Instituto Español de Oceanografía Centro Ocenografico de Santander Promotorio de San Martin, s/n Apdo 240 E-39080 Santander Spain	+34 942 291060	+34 942 275072	jorge.landa@st.ieo.es
Maria Saínza	Inst. Español de Oceanografía Centro Oceanográfico de Vigo Cabo Estay - Canido Apdo 1552 E-36200 Vigo Spain	+34 986 492111	+34 986 498626	maria.sainza@vi.ieo.es
Carmo Silva	IPIMAR Avenida de Brasilia P-1449-006 Lisbon Portugal	+351 213027110	+351 213 025 948	mcarmo@ipimar.pt

NAME	ADDRESS	TELEPHONE	FAX	E-MAIL
Luis Silva	Instituto Español de Oceanografía Unidad de Cádiz Campus Rio San Pedro (CACYTMAR) P.O.Box 2606 E-11510 Puerto Real (Cádiz) Spain	+34956016290 / +34956016886	+34 956 263556	luis.silva@cd.ieo.es
Sarah Walmsley	CEFAS Lowestoft Laboratory Lowestoft Suffolk NR33 0HT United Kingdom	+441502527787	FAX: +44 1502 513865	sarah.walmsley@cefas.co.uk

Annex 2: Agenda

WKMSHM – AGENDA – 21 TO 24TH NOVEMBER

Wednesday, November 21

09:30–10:00 Opening of meeting (Co-chairs: C. Morgado and P. Gonçalves)

Welcome, local arrangements, incl. computer and network arrangements, and participant's presentation

10:00–10:30 Introduction; Adoption of Agenda; Brief overview of ToRs, discussion; report structure

10:30–10:45 Break

10:45–12:45 Brief presentations on maturity stage keys of each Institute (10 min each) and comments on the protocol maturity stage key – Hake

12:45–14:15 Lunch

14:15–14:30 Discussion on Hake protocol maturity stage key

14:30–16:00 Hake calibration exercise in maturity stages

16:00–16:15 Break

16:15–17:00 Hake calibration exercise in maturity stages (cont.)

17:00–17:30 Discussion on agreed maturity stages

Thursday, November 22

9:00–09:30 Hake calibration exercise results in maturity stages

09:30–10:30 Maturity studies presentation

- 9:30–9:50 Patricia Gonçalves
- 9:50–10:10 Rosario Domingues
- 10:10–10:30 Luis Silva

10:30–10:45 Break

10:45–11:05 Cristina Morgado

11:05–12:00 Brief presentations on maturity stage keys of each Institute (10 min each) and comments on the protocol maturity stage key-Monk

- 12:00–12:20 Joel Dimmet presentation

12:30–14:00 Lunch

14:00–14:30 Discussion on the monk protocol maturity stage key

14:30–15:00 Monk photos discussion

15:00–15:30 Hake photos discussion

15:30–16:00 Maturity data used in the assessment

16:00–16:15 Sub-groups tasks

16:15–16:30 Break

16:30–18:00 Sub-groups tasks

Friday, November 23

9:00–10:30 Sub-groups tasks

10:30–10:45 Break

10:45–12:30 Sub-groups tasks presentations

12:30–14:00 Lunch

14:00–16:15 Sub-groups tasks

16:15–16:30 Break

16:30–18:00 Sub-groups tasks and report

Saturday, November 24

9:00–10:30 Report

10:30–10:45 Break

10:45–12:00 Recommendations and conclusion

Annex 3: Working documents present to the Workshop

WD1 Domínguez Petit, R. 2007. Sexual maturity of European hake in the Galician Shelf: difficulty to work with an asynchronous species

European hake (*Merluccius merluccius*) is a partial/multiple spawner with indeterminate fecundity. In Galician waters high asynchrony is observed both at individual and population level, i.e. all oocyte developmental stages (primary growth, cortical alveoli, vitelogenic, migratory nucleus, etc.) are observed in mature ovary at the same time and females in all ovary maturity stages are recorded all year around.

One of the assessment tasks is identify different ovary maturity stages macroscopically. Two main handicaps have been observed in its consecution. On one hand, differentiate between immature and resting ovaries is not possible macroscopically and on the other hand, there are two concepts of post-spawning females, those ones that just has released a batch and those ones that has released all batches and their spawning season is completely finished until next year (inactive females).

Considering biological reality of reproductive cycle of partial/multiple spawner species is more correct considering post-spawning females as inactive females, i.e. those ones that are not going to contribute to offspring production in the current spawning season. When attention is focused on reproductive cycle, estimated based on spawning fraction and GSI, it is observed that, although there is a peak of spawning on Galician waters during February-March, females stock produces eggs through all the year. Then, it is not possible to use stock spawning season duration to determine which females are immature, resting or post-spawning.

In species with determinate fecundity, there is possibility to study fecundity variations (number of mature oocytes in the ovary) through the spawning season to estimate if one female is at the beginning, middle or the end of spawning season, because fecundity decreases along the spawning season. However, European hake has indeterminate fecundity and recruits new oocytes from primary growth to mature pool continuously; so, low fecundity is not a synonymous of advanced spawning season.

Consequently, it is necessary resort to histology. This method is enough to solve identification of immature and resting females, because in the first one, all the oocytes in the ovary are in primary growth stage and well packaged while in the second one, ovary is without mature oocytes, with wide ovary wall, but lamellae are not so compact as in immature ovaries and blood vessels could be more abundant. Post spawning females (inactive) are characterized by high level of atresia, disorganization of ovary structures, numerous blood vessels, thick ovary wall and absence of yolked oocytes groups (excepting some atretic yolked oocytes).

When females' macro and microscopically estimated maturity ogives are compared, it is observed that L50 is overestimated if macroscopic method is apply, because of misclassification of immature and resting females mainly. This error could be reduced sampling during the peak of spawning and calculating a correcting factor based on estimates of classification error between macro and microscopic maturity key from a random sample of confusing ovaries.

In addition, there is another issue that affects assessment results. Traditionally maturity ogives have been estimated based on both sexes combined which entails grave underestimation of L50, because males mature earlier than females and sex ratio varies when length increase. Reduction in L50 maybe an indicative of overexploitation of stock and/or a decrease in stock reproductive potential, because smaller females produce not only less eggs but also smaller, which will produce smaller larvae with less probability to survive and be recruited to spawning stock biomass. Increase or decrease of L50 may affect to assessment results and, subsequently, to management measures.

WD2: Sainza, M. and Piñeiro, C., 2007. Hake Maturity Sampling program at IEO(Northern and Southern stock)

IEO biological data is either based on market sampling or on scientific surveys and the individuals used are those also used for ageing proposes (Age Length Keys for Assessment). The sampling program happens along the year. The scheme is based on quarter. For Northern Stock of hake, we have not macroscopic maturity data due to fish for commercial fleet come gutted. For southern stock of hake, ogive period selected is from December to May.

We use four stages for the macroscopic maturity key (Inactive, Maturing, spawning and post-spawning). Our maturity scale is in agreement with standard maturity key proposed by WKMSHM.

WD3: Cherret, Y., 2007. IFREMER-Hake maturity stage key

Medits France Survey use for hake the same maturity stage key that for all the others bony fish. A four stages maturity key and more two corresponding to the undetermined stages when we could not recognize anything because the fish is damaged, any other reason, or when the gonads are so small that we could not determine if it’s male or female. We have difficulties to choice between stage 2 and stage 4. But for us the stage 3 is the easiest to classified, when the female is spawning and the male sperm is expelled.

WD4: Burns, F., 2007. FRS Marlab-Hake Sampling

Marlab has no routine sampling programme for hake however biological data is collected from its 5 bottom trawl surveys which occur during quarters 1, 3 and 4. This is a DCR commitment and although maturity data is collected it is recorded using the ‘generic’ IBTS 4 stage scale (devised primarily for gadoids) and is therefore not submitted for assessment purposes.

For WKMSHM, Marlab submitted data including gonad images from 12 hake which were staged using the 4 stage IBTS maturity key. Specimens were collected from the months of August and November. Ovaries and testes from 6 specimens were retained for histological validation.

WD5: Walmsley, S. A. and Foster, R., 2007. Hake and anglerfish sampling at CEFAS

This presentation gives the hake and anglerfish maturity keys used by Cefas. Also given are the sampling targets, the sampling protocols and the maturity sampling levels attained during research surveys in quarters 1, 2 and 3 in 2007. Maturity sampling for both species in the UK is problematic as fish are landed gutted (hake) or as tails (anglerfish).

WD6: Korta, M. and Arego, S., 2007. Maturity Stages of Hake and Anglerfish

In general, Sex and Maturity Stages of Hake and Anglerfish (Black and White) are collected routinely from market samples based on necessity of DCR minimum requirements for ageing estimations. Thus, Sex and Maturity Stages (de visu) are assigned to all samples analysed with ageing purposes.

Beside, there is a significant macro/micro Maturity data base on these species recorded through ad hoc biological studies within an on-board sampling program during the development of previous projects (BIOSDEFF, 1996–1997; DEMASSESS, 1998; ADULTOS DE MERLUZA, 2001–2002; RASER, 2002–2005; IMPRESS, 2004–2007; RASCOS, 2007–2008).

The intensive sampling of BIOSDEFF facilitated the elaboration of the Maturity Stage Keys for each species; 4 Stages were fixed for Hake-(I) Immature/Virgin, (II) Maturing, (III) Spawning, (IV) Post-spawning- and 5 for Anglerfish-(I) Immature-Resting, (II) Maturing, (III) Pre-spawning, (IV) Spawning, (V) Post-spawning.

These scales have been used for Maturity assignation in correspondence with AZTIs own Maturity Stage Keys, which displays 7 Stages, in the case of Hake, and takes into account the reproduction cycle within the spawning season-(1) Immature, (2) Beginning to mature, (3) Maturing, (4) Pre-spawning, (5) Spawning, (6) Post-spawning, (7) At Rest.

AZTIs Maturity Scale is easily to convert into the Maturity keys proposed by WKMSHM.

WD7: Anastasopoulou, A. and Mytilineou, Ch. 2007. Maturity scale used by HCMR for Hake

HCMR Hake samples come from market sampling all year round. All specimens collected for length composition are examined for sex and maturity. The Nicolsky's scale is used to classify the maturity stages, based on macroscopic criteria. According to this scale, there are six maturity stages; the descriptions of each one (and the correspondence photos) are given for males and females. The stages are: Stage I: Virgin, Stage II: Developing Virgin and Inactive (includes the resting gonads), Stage III: Developing, Stage IV: Mature, Stage V: Spawning and Stage VI: Spent. Also, some comments about the proposed scale are given. Histological analysis is also in progress. Anglerfish is a new studied species for our Institute and consequently we are trying to identify the maturity stages by macroscopic and microscopic criteria.

WD8: Gonçalves, P. and Morgado, C., 2007. Maturity stage key of hake (*Merluccius merluccius*)

A four stages macroscopic key for European hake has been presented. This scale has the following stages: I-immature, II-development, III-spawning and IV-post-spawning. Each stage corresponds to a phase of the spawning cycle. The main characteristics of each stage are described. Macroscopically is not possible distinguish between immature and resting females. By the means of histology we can distinguish these two stages (immature/resting). For this reason the females microscopic maturity stage key has composed by five stages, the stages one to four are the same of the macroscopic key and the fifth corresponds to resting. For males the microscopic key, at this moment, can not allow to distinguish between immature/resting. We don't know if macroscopically there is no misclassification between those stages or if we cannot identified due to the poor knowledge on males histology.

At IPIMAR hake samples are collected during: two annual research surveys (Autumn-since 1979; Winter-since 2005), monthly at the fishing harbour (Matosinhos, Póvoa do Varzim, Lisboa, Olhão) and by observers on board commercial vessels. The majority of samples from the fishing harbour are ungutted and composed by bigger length classes.

WD9: Gonçalves, P., 2007. Macroscopic maturity stage validation of hake

Samples from two bottom trawl surveys carried out during autumn 2006, winter 2007 and from fishing harbour (May, July of 2006 and February, March, April, August of 2007). A total of 215 individuals (124 females and 91 males) had been collected for macroscopic maturity classification and the gonads removed for histological maturity validation. Discrepancies between macroscopic and microscopic classification was observed in 38% of the total number of samples. The results reveal that the majority of discrepancies are: where the gonads are classified as development (macroscopically corresponds to stage II) and histological are in post-spawning (stage IV) (28.2%), or macroscopically as spawning (stage III) and histological as post-spawning (26.9%), or macroscopically as post-spawning being histological in development (16.6%). These percentages are given considering only the samples were the misclassification was been detected.

Being hake an indeterminate spawner and a multiple batch spawner, during the spawning season (inter-batches) hake gonadal maturity describes a continuous cycle, which explains the misclassification between development/post-spawning and post-spawning/development. The misclassification between spawning/post-spawning could be explained by the restriction on the macroscopic maturity stage where the stage III (spawning stage) on females, is the only where the hyaline oocytes are present. Due to the fact that hydration and ovulation is a continuous process we could observe remaining hyaline oocytes on post-spawners, or if we catch the female during the spawning event we observe post-ovulatory follicles and hydrated oocytes. In the case of males, being the main characteristic of stage III the flow of the sperm with pressure in the abdomen, in post-spawners we observe sperm near the spermoduct after the spawning event resulting in a wrong macroscopic classification.

In 12.6% of the samples females macroscopically classified as development, microscopically are spawners. Due to the fact that being the hydration is continuous process, sometimes the boundaries between development and spawning are difficult to achieve by visual inspection. But the microscopic observation can detect few hydrated oocytes or oocytes in the beginning of the process.

The classification in the immature stage the individuals that are at development or at resting (stage V-microscopic key) leads to an underestimation of SSB conducting to inadequate management options, and was been observed in 2.6 and 1.3% of the samples, respectively. In the males case it was been observed in 1.3% of the samples, misclassification between development and immature, what overestimates the SSB.

To minimize the impact of these wrong classifications on assessment, histology should be applied to quantified the percentage of misclassification between immature/mature. But in cases where histology cannot be applied, because of the time consuming and the fact that is an expensive technique, the gonads samples to estimate SSB should be collect during the main spawning season. Or according to Macewicz (2007), the data used to estimate SSB should be collect from the beginning to the peak of the main spawning season.

WD10: Silva, L., Vila, Y., Acosta, J. J. and Tornero, J., 2007. Size at first maturity in European hake: Estimates based on different maturity criteria

During 2004 and 2007, two biological studies concerning European hake were carried out in the Gulf of Cádiz (ICES IXa-South) area. Sampling was made with bottom trawl and artisanal commercial fleet catches, as well as ARSA bottom trawl survey series samples. The key for maturity stage determination comprised four stages: I-Immature; II-Developing or maturing; III-Spawning; IV-Post-spawning. The spawning period in both years was determined by means of the analysis of the monthly evolution of the gonadosomatic index (GSI) and the percentages of different maturity stages. Three criteria were established to estimate size at first maturity: A. - Immature: stages I + II; Mature: stages III + IV; B.- Immature: stages I + II where GSI was lower than the lowest GSI value in stage III; Mature: stage II where GSI was higher than the lowest GSI value in stages III + IV; C.- Immature: stage I, Mature: stages II + III + IV. Maturity ogives were calculated for each criterion for both sampled periods and the results showed differences between sizes at first maturity, being the estimates for this parameter 52.2, 45.3 and 42.8 cm for A, B and C criteria respectively in 2004, and 55.6, 45.8 and 42.6 cm in 2007. In order to validate criterion B, histological samples of stage II female gonads with different GSI were studied, revealing the presence of cortical alveoli in all gonads. Therefore, we can conclude that all gonads in stage II must be considered as mature and consider the criterion C as the most suitable to estimate the size at first maturity in European hake.

WD11: Morgado, C. and Gonçalves, P., 2007.Condition factor, gonadossomatic and hepatossomatic index of Hake in the Portuguese continental waters

Biological data of 7 groundfish surveys were analysed. The surveys were carried out between June 2004 and March 2007. The condition factor, gonadossomatic and hepatossomatic index were calculated. These parameters vary according the year season and confirm that the main

Southern Hake stock spawning season is in February-March. However those parameters can not be used as a tool to estimate the maturity stage. Similar results are found when validated data are used.

WD12: Landa, J., Ámez, M.A. and Castro, B., 2007. Sexual maturity in white and black anglerfish (*L.piscatorius* and *L.budegassa*) in North of Spain based on the sampling at IEO

The macroscopic maturity scale of 5 stages used by IEO for white and black anglerfish, based on morphocromatic characteristics, is presented. Some problems have appeared in the distinction between some stages, mainly between the stages where the sperm or the oocytes are not showed or the stages where the quantification of the sperm is the most distinctive characteristic.

The fitting of the IEO scale to the WKMSHM protocol macroscopic maturity scale was not difficult due to the similarities between the stages in both scales.

The macroscopic gonadal stage was studied from 615 and 1332 black and white anglerfish, respectively, by month from 2003 to 2007, from commercial landings and bottom trawl surveys in North of Spain. The proportion of specimens by macroscopic stage, gonadosomatic index (GSI), hepatosomatic index (HIS) and condition factor (CF) were analysed by month based on the specimens supposed adults, those with lengths higher than L50. Some results about the spawning period were obtained, mainly in males. The scarcity of mature females and lack of spawning females, mainly in white anglerfish, did not allow estimate a precise spawning period for combined sexes. In general, the evidence presented agrees with that of Duarte *et al* (2001) in Iberian waters.

WD13: Burns, F., 2007. FRS-Marlab-Anglerfish Sampling

Marlab collects biological data for anglerfish on its 5 bottom trawl surveys which occur during quarters 1, 3 and 4. This is a DCR commitment and although maturity data is collected it is recorded using the ‘generic’ IBTS 4 stage scale (devised primarily for gadoids) and is not used in any analysis. In addition to the bottom trawl survey data, maturity data is also collected from the Anglerfish Abundance Project which started in November 2005. Those data are recorded using the 5 stage anglerfish maturity key.

For WKMSHM, Marlab submitted data including gonad images from 41 Lophius piscatorius and 14 Lophius budegassa which were staged using the 5 stage anglerfish maturity key. Specimens were collected from August, October, and November. Ovaries and testes from 10 specimens were retained for histological validation.

WD14: Duarte, R., 2007. Selection of gonads sampled on IPIMAR research surveys in 2006 and 2007

This presentation shows a selection of images of female and male anglerfish gonads (macroscopic aspect). The objective was to discuss the diagnostics and the general aspect that are used for each institute to classify the gonads.

WD15: Gonçalves, P., 2007. Males immature and development microscopic classification

On males maturity staging the most disagreement classification is between stage I (immature) and stage II (development). Due to the fact that some observers classified as development the gonads with a thin curling band and for others observers these tests are still immature. To define the boundaries between these stages we analyzed the histological slides. The microscopic classification for gonads without curling bands or with little curling bands was been presented. Being the maturation a continuous process, not always the beginning of the curling appearance indicates a development. Based on histological observation we decided to classify the beginning of curling as an characteristic of the development maturity stage (stage II). In order to minimize the errors on males SSB estimation using macroscopic maturity ogives.

WD16: Morgado, C., 2007. Hake and Monkfish maturity data in stock assessment

The main important of maturity data in stock assessment is to maturity ogives estimation. All stocks of Hake (*Merluccius merluccius*) and monkfish (*Lophius budegassa* and *Lophius piscatorius*), except the southern Hake stock (ICES div. VIIIc and IXa) used the same maturity ogive for all years. In the last 8 years assessment the maturity ogive didn't change. Only the Southern Hake stock used an annual maturity ogive.

WD17: Dimeet, J., 2007. Sampling data of *Lophius budegassa* and *Lophius piscatorius* from 2007 EVHOE Survey

Using the protocol defined before the Workshop, pictures of each maturity stage by sex are shown both for *L. piscatorius* and *L budegassa*, from individuals caught during the French EVHOE survey in autumn 2007.

Relevant data by species: total weight, total length, gonads weight, liver weight, gutted weight have been collected and recorded. Maps of sampling data in the area covered by the survey are also shown.

WD18: Silva, C. 2007. Maturity stage key for Monk and Anglerfish Microscopic Stages

Black and white Monkfish have unusual gonads. The testes are tubular and bean shaped in transverse section. The ovaries are confluent and form a single flatted tube and in transverse section it is possible to distinguish an ovigerous membrane and underlying connective tissue, and a non-ovigerous wall. A single layer of oocyte clusters projects from the ovigerous layer into the lumen. In males the maturation process is similar to the most other teleosts. For female as the maturation proceeds, the width and the length of the ovaries increases. In this stage key the ovaries were classified into five stages of maturity according Afonso-Dias and Hislop (1985) and based on the histological characteristics and development of the most avanced oocytes, presence of POF and atresia (Yamamoto and Yoneda *et al.*, 1998, Hunter and Macewicz,1985). The five stages are: I-Virgin; II-Resting/Developing; III-Pré-spawning; IV-Spawning and V-Pós-spawning. The five stages of the Microscopic key have correspondence with the stages of the macroscopic key.

Annex 4: Proposed maturity stage key for Hake

Maturity Stage Key for Hake Females

Characteristics	I - Inactiv/Iamture	II- Development	III- Spawning	IV- Post-Spawning
Size	Small	Medium/Large	Large	Large
Shape	Ellongated	Cylindrical	Cylindrical	Shrunken
Colour	Pink/transparent	Pink/orange	Pink	Pink dark
Opaque oocytes	Absents	Present	Present	Present
Hyaline oocytes	Absents	Absents	Present	Absents
Macroscopic aspect	-	Without stepped on areas or hurt areas	Gonad is fine; hydrated oocytes flow under pressure in the abdomen	Flaccid; spaces next to the hilo

Maturity Stage Key for Hake Males

Characteristics	I - Inactiv/Iamture	II- Development	III- Spawning	IV- Post-Spawning
Size	Small	Medium	Large	Large
Shape	Ribbon or small band	Large band	Large band	Bands empty and deformed
Colour	White/transparent	Pearl	Pearl	White/pink
Sperm	Absent	Flows when it is cut	Flows on applying pressure to the abdomen	Absent or a little residual

Maturity Stage Key for Anglerfish Females

Characteristics	I - Inactiv/Iamture	II- Development	III- Pre- Spawning	III- Spawning	IV- Post-Spawning
Size	Small	of the visceral cavity of the visceral cavity	Large	Large	Large
Shape	Band-shaped	Band longer	Longer	Longer	Retracted ovaries
Colour	Transparent	Creamy	Orange	Yellow	Pink dark
Opaque oocytes	Absents	Present	Present	Present	Present
Hyaline oocytes	Absents	Absents	Present	Present	Absents or possible residual ones (reabsorbed)
Macroscopic aspect	-	-	With vascularization	Enormous gelatinous mass wraps	High vascularization

Maturity Stage Key for Anglerfish Males

Characteristics	I - Inactiv/Iamture	II- Development	III- Pre- Spawning	III- Spawning	IV- Post-Spawning
Size	Small	Large	Large	Large	Large
Colour	Pink/transparent	Pearl	Pearl	Pearl	Red and stained
Sperm	Not visible	Some in the lumen	With the lumen full	Easily freed applying pressure to abdomen	Absent or a little residual
Macroscopic aspect	-	Testicles cover great proportion of the visceral cavity	-	-	-

Sampling proposed protocol for Hake and Monkfish

Before the Workshop each institute should collect during a one year cycle gonads of each species according to the following indications:

For both species, the sampling parameters are:

- total length (in centimetres);
- gonad visual inspection - maturity stage by a standard maturity scale
  - usual maturity scale used by the institute;
- total weight (in grams);
- gonad weight (in grams);
- liver weight (in grams);
- gutted weight (in grams);
- gonad photo
  - print and plasticize file photo_grid.ppt for photo grey background
  - at least one photo with the gonads inside the fish and another with the gonad alone (see examples);
  - save photos as jpg files
- histological maturity stage
  - anglerfish: males and females; hake: females
- microscopic photo of histological structures
  - anglerfish: males and females; hake: females.
  - photo with a 1 µm scale.

ID number should be as follow:

Species code (3 letters) **Institute code** (1 letter) **Year** (2 numbers) **Month** (2 numbers) **Day** (2 numbers) **Observation number** (2 numbers)

Species code	
Hake	HKE
Black anglerfish	ANK
White anglerfish	MON

Institute code	
AZTI	A
CEFAS	B
IEO	C
IFREMER	D
IPIMAR	E
MI	F
FRS	G

Gonads for histology:

Hake - 2 female per standard maturity stage per month *
Anglerfish – 2 males and 2 females per standard maturity stage per month *

* - note that on periods outside the spawning is difficult to sampling all the maturity stages

Annex 5: Hake maturity stage Keys

AZTI Hake maturity stage key

Female							
Maturity Stage	Immature		Mature				Immature
Reproductive State	Inactive		Active				Inactive
Developement	Immature	Beggining maturing	Maturing	Pre-spawning	Spawning	Post-spawning/ Full spent	At Rest
AZTI_Scale	1	2	3 (3´)	4 (4´)	5	6 (6´)	7
BIOSDEFF_Scale	I		II	III		IV	I
Macroscopic (*visu*)							
Size (*prop. abd. cav.*)	Small	~>1/3	Large, ~1/3 to 1/2	~>2/3	Whole	<1/2	<1/3
Aspect	Cylindrical		Blood capillaries			Flaccid, quite empty	Firm and contracted
Colour	Translucent	Pale white or rosy	Yellowish/orange			Purple. Bruised.	Translucent or rosy.
Opaque Oocytes	Absent	Absent	Present	Present	Present	Present	Absent
Hyaline Oocytes	Absent	Absent	Absent	Present	Present	Ocasionally, residual.	Absent
Batch evidence	Absent	Absent	Absent	Swollen ovaries	Flows under slight pressure	Absent	Absent
Microscopic (histology)	Chromatin nucleolus	Perinucleolus	Early vitellogenesis	Migratory nucleous and Hydrated oocytes		POFs and atresia	Chromatin nucleolus

Male							
Maturity	Immature		Mature				Immature
Reproductive State	Inactive		Active				Inactive
Developement	Immature	Beggining maturing	Maturing	Pre-spawning	Spawning	Post-spawning/ Full spent	At Rest
AZTI_Scale	1	2	3	4	5	6	7
BIOSDEFF_Scale	I		II	III		IV	I
Macroscopic (*visu*)							
Size (*prop. abd. cav.*)	Small (thread)	~>1/3	Large, ~1/3 to 1/2	~>2/3	Whole	Reduced, <1/2	<1/3
Aspect	flat		Festooned			Flaccid and empty; darkened capillaries.	Firm and contracted
Colour	Translucent		Pink/white	White	White cream	Reddened. Bruised.	Translucent
Sperm	Absent	Absent	On cutting	Flows if punture	Flows under slight pressure	Ocassionally, on cutting	Absent
Microscopic (histology)	Spermatocytes, not spermatozoids		Spermatocytes, few spermatozoids	Spermatozoids predominant		Empty ducts. Residual Spermatozoids	Spermatocytes, not spermatozoids

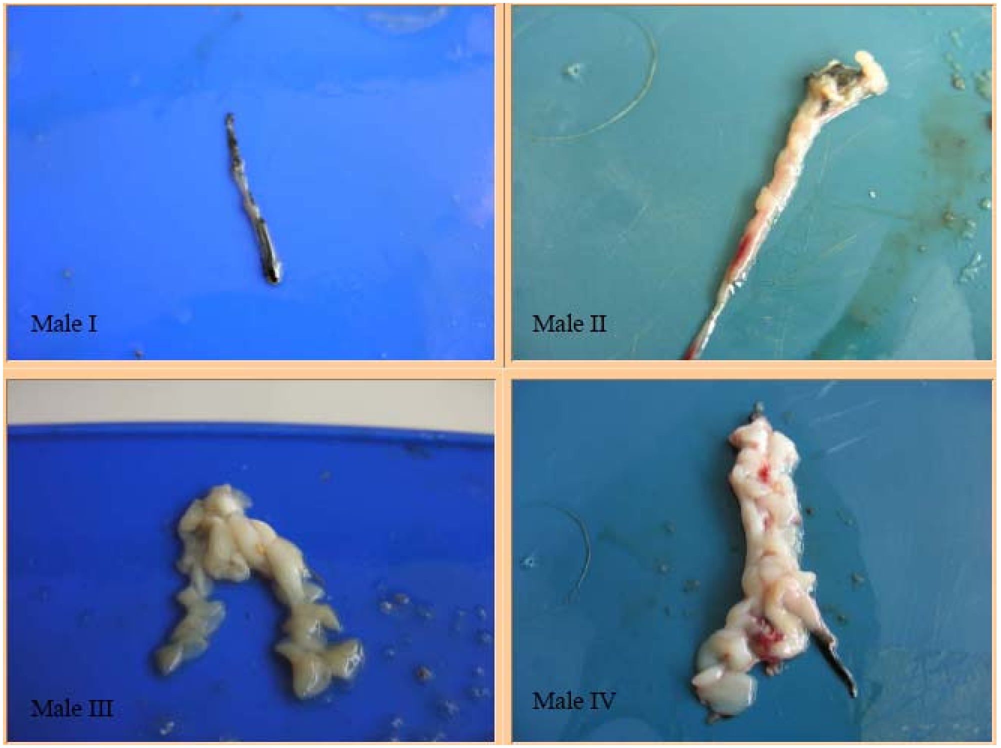
CEFAS Hake maturity stage key

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

HCMR Hake maturity stage key

Macroscopic maturity scale (Nikolsky)

Maturity Stage	Macroscopic description - Male
I Virgin	Transparent, ribbon gonad, flat in shape with slightly wavy edges. Difficult recognizable.
II Dev.Virgin / Inactive	Thin, whitish/transparent gonads with flat shape, slightly increased in width and wavy edges. Blood vessels slightly visible.
III Developing	Larger, frilled band, whitish/creamy in colour. Blood vessels are visible.
IV Mature	Larger gonad occupying significant part of the abdominal cavity. White/creamy colour. Sperm duct is visible. Sperm flows when it is cut.
V Ripe	The whole abdominal cavity covered. White genital pore. Testis structure is not so connected. Sperm easily flowing on pressure.
VI Spent	Flaccid, yellowish/creamy. A little residual milt may exist in the lumen.



Macroscopic maturity scale (Nikolsky)

Maturity Stage	Macroscopic description - Female
I Virgin	Very small, transparent/pinky gonad rounded in shape.
II Dev.Virgin / Inactive	Transparent, pinky/salmon, more rounded gonad, with blood vessels. Oocytes no visible to naked eye.
III Developing	Larger, salmon-pinky/orange gonad with cylindrical shape. Oocytes are visible to naked eye. Intensive blood vessels with wide dorsal vein full of junctions.
IV Mature	Compact ovaries cover the 2/3 of abdominal cavity. A few hydrated eggs may appear as transparent dots. Genital pore usually expand.
V Ripe	The whole abdominal cavity covered. Many hydrated oocytes are visible. Well vascularized gonads.
VI Spent	Flaccid gonad with dark red/dark yellow colour. A few residual oocytes are visible as dark areas. Vascualarization is dark or not visible.

IEO Hake maturity stage key

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

IFREMER Hake maturity stage key

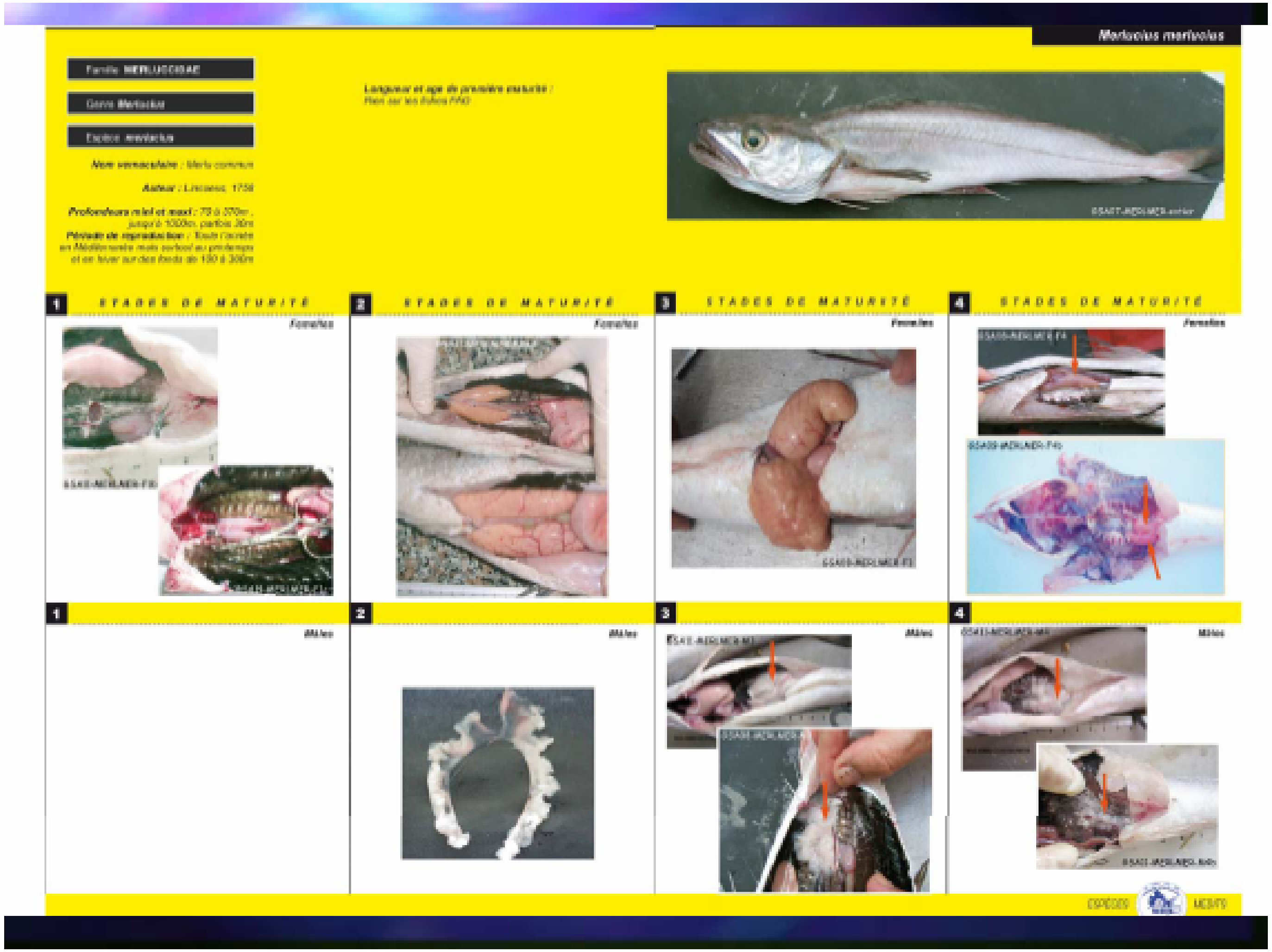
VII. Code of sexual maturity for fish

bony fish

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

Adult specimens

* : WARNING ! Be careful.These stages could be confused each other.


Merluccius merluccius
Famille MERLUCCIIDAE
Genre Merluccius
Espèce merluccius
Nom vernaculaire : Merlu commun
Auteur : Linnaeus, 1758
Longueur et age de première maturité :
STADES DE MATURITÉ
Femelles
Mâles
ESPÈCES
MEDITS

IPIMAR Hake maturity stage key

Macroscopic Hake Maturity Scale - Females

I - IMMATURE

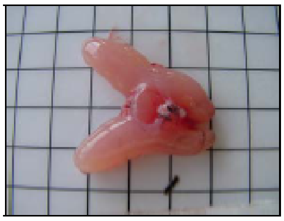
Size: Small

Shape: Cylindrical / Elongated

Colour: Transparent or pink

Opaque oocytes: Absents

Hyaline oocytes: Absents



II – DEVELOPMENT

Size: Medium or large

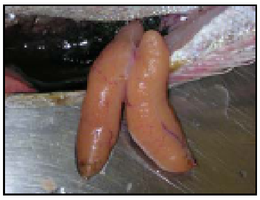
Shape: Cylindrical

Colour: Pink or yellow to orange

Opaque oocytes: Present

Opaque oocytes: Absents

Other characteristics: Without stepped on areas or hurt areas



III - SPAWNING

Size: Large

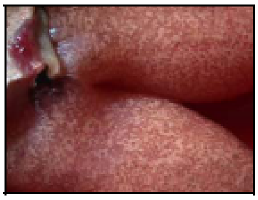
Shape: Cylindrical

Colour: Pink

Opaque oocytes: Present

Opaque oocytes: Present

Other characteristics: Involving membrane of the gonad is fine; hydrated oocytes may flow under some pressure in the abdomen



IV – POST-SPAWNING

Size: Large

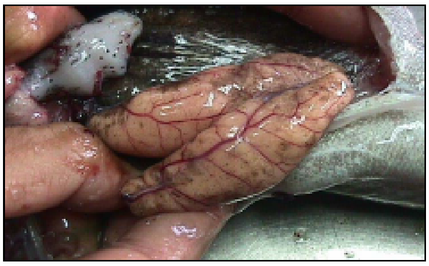
Shape: Slack cylindrical

Colour: Dark pink

Opaque oocytes: Present

Opaque oocytes: Absents

Other characteristics: Flaccid; with spaces next to the hilo



Macroscopic Hake Maturity Scale - Males

I - Immature

Size: Small

Shape: Ribbon or small band

Colour: Transparent or white

Sperm: Absent

II - Development

Size: Medium

Shape: Develop band

Colour: White

Sperm: Flows when it is cut

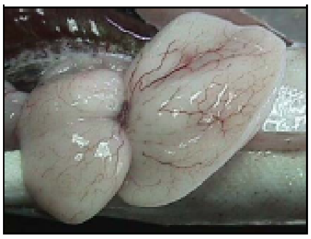
III - Spawning

Size: Large

Shape: Large band

Colour: White

Sperm: Flows with pressure in the abdomen



IV – Post-Spawning

Size: Large

Shape: Empty and deformed bands

Colour: White or light pink

Sperm: Absent or residual

MARLAB Hake maturity stage key

IBTS MATURITY KEY FOR ALL SPECIES SAMPLED

1. VIRGIN

Male - Testes very thin translucent ribbon lying along an unbranched blood vessel. No sign of development.

Female - Ovaries small, elongated, whitish, translucent. No sign of development.

2. MATURING

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

Female - Development has obviously started, eggs becoming larger and the ovaries are filling more and more of the body cavity but eggs cannot be extruded with only moderate pressure.

3. SPAWNING

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

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

4. SPENT and RESTING (returns to Stage 2)

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.

Female - Ovaries shrunken with few residual eggs and much slime. Resting condition firm, not translucent, showing no development.

WKMAT Hake maturity stage key

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

Annex 6: Hake maturity stage calibration exercise

Table 5.1. Maturity Stage Classification by observer.

Fish no	Fish length	Sex	CM Observ 1	PG Observ 2	SD Observ 3	RD Observ 4	MS Observ 5	LS Observ 6	BC Observ 7	MK Observ 8	SA Observ 9	RF Observ 10	SW Observ 11	FB Observ 12	YC Observ 13	KA Observ 14	JD Observ 15	JL Observ 16	Agreed stage	Percent agreement	Precision CV
1	42.0	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
2	37.8	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
3	37.4	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
4	39.7	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
5	38.0	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
6	42.2	f	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	88%	30%
7	49.5	f	2	2	2	2	2	2	2	2	2	3	2	2	2	3	2	2	2	88%	16%
8	43.1	f	1	1	1	1	1	2	1	1	1	1	1	4	2	2	1	1	1	75%	59%
9	50.6	f	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	94%	12%
10	52.2	f	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	94%	12%
11	32.3	f	1	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	94%	63%
12	30.7	f	1	1	1	1	1	1	1	1	1	1	1	1	4	2	1	1	1	88%	62%
13	30.4	f	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100%	0%
14	34.4	f	1	1	1	1	1	4	4	1	1	1	1	1	4	4	1	1	1	75%	77%
15	33.9	f	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	94%	63%
16	35.2	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
17	37.7	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	88%	30%
18	41.3	f	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	94%	12%
19	30.9	m	2	2	2	2	1	2	2	2	1	2	1	2	1	1	1	1	2	56%	33%
20	30.7	f	1	1	1	1	1	2	1	1	1	1	1	1	1	2	1	1	1	88%	30%
21	32.7	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
22	30.6	m	2	1	2	2	1	2	2	2	2	2	2	2	2	2	1	1	2	75%	26%
23	30.7	m	2	2	2	2	1	2	2	2	2	2	2	2	4	2	2	2	2	88%	28%
24	31.7	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
25	31.5	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
26	33.3	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
27	30.9	m	2	2	2	1	1	2	2	1	1	2	2	2	1	1	2	2	2	63%	31%
28	32.2	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
29	30.0	f	1	1	1	1	1	2	1	1	1	1	1	1	1	2	1	1	1	88%	30%
30	36.0	f	1	1	1	1	1	2	1	1	1	1	1	1	2	2	2	1	1	75%	36%
31	36.2	f	1	1	1	1	-	2	2	1	1	1	1	1	4	-	3	1	1	71%	63%
32	36.1	f	1	1	1	1	-	2	1	1	1	1	1	1	-	1	1	1	1	93%	25%
33	37.5	f	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	88%	30%
34	35.6	f	1	1	1	1	1	2	1	1	1	1	1	1	2	2	2	1	1	75%	36%
35	36.8	f	1	1	1	1	1	2	1	1	1	1	1	1	4	2	4	1	1	75%	69%
36	37.7	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
37	33.6	f	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	94%	13%
38	34.0	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	4	1	1	88%	62%
39	38.6	m	2	4	4	2	1	3	2	2	2	2	2	2	2	2	2	2	4	13%	34%
40	34.6	f	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	88%	30%
41	35.4	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
42	37.2	m	2	2	2	2	1	3	4	2	2	2	2	2	2	2	2	2	2	81%	29%
43	31.2	f	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	88%	30%
44	34.3	f	1	1	1	1	1	2	1	1	1	1	1	1	2	2	2	1	1	75%	36%
45	31.7	f	1	1	1	1	1	2	1	1	1	1	1	1	2	2	2	1	1	75%	36%
46	30.0	f	1	1	1	1	1	1	1	-	1	1	1	1	4	-	1	1	1	93%	66%
47	31.9	m	2	2	2	2	1	2	2	2	2	2	2	2	2	1	1	1	2	75%	26%
48	28.1	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
49	31.8	f	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100%	0%
50	31.3	f	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	94%	24%
	Total observ.		50	50	50	50	48	50	50	49	50	50	50	50	49	48	50	50		85.5%	31.8%
	Total NOT observ.		0	0	0	0	2	0	0	1	0	0	0	0	1	2	0	0			

Table 5.2. CV, percentage of agreement and bias by observer.

NUMBER OF STAGE OBSERVATIONS

	Agreed stage	CM Observ 1	PG Observ 2	SD Observ 3	RD Observ 4	MS Observ 5	LS Observ 6	BC Observ 7	MK Observ 8	SA Observ 9	RF Observ 10	SW Observ 11	FB Observ 12	YC Observ 13	KA Observ 14	JD Observ 15	JL Observ 16	TOTAL
	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	38	38	38	38	36	38	38	37	38	38	38	38	37	36	38	38	602
	2	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	176
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Total	0-15	50	50	50	50	48	50	50	49	50	50	50	50	49	48	50	50	794

COEFFICIENT OF VARIATION (CV)

	Agreed stage	CM Observ 1	PG Observ 2	SD Observ 3	RD Observ 4	MS Observ 5	LS Observ 6	BC Observ 7	MK Observ 8	SA Observ 9	RF Observ 10	SW Observ 11	FB Observ 12	YC Observ 13	KA Observ 14	JD Observ 15	JL Observ 16	ALL Readers
	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	0%	0%	0%	0%	0%	47%	46%	0%	0%	0%	0%	45%	63%	29%	58%	0%	34.6%
	2	0%	16%	0%	16%	37%	14%	28%	16%	22%	14%	16%	0%	39%	39%	33%	27%	21.5%
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	0.0%	3.5%	0.0%	3.5%	8.5%	38.7%	41.1%	3.5%	4.9%	3.2%	3.5%	34.3%	56.6%	30.8%	51.5%	5.9%	31.8%
	RANKING	1	4	1	4	10	13	14	7	8	3	4	12	16	11	15	9	

PERCENTAGE AGREEMENT

	Agreed stage	CM Observ 1	PG Observ 2	SD Observ 3	RD Observ 4	MS Observ 5	LS Observ 6	BC Observ 7	MK Observ 8	SA Observ 9	RF Observ 10	SW Observ 11	FB Observ 12	YC Observ 13	KA Observ 14	JD Observ 15	JL Observ 16	ALL
	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	100%	100%	100%	100%	100%	71%	95%	100%	100%	100%	100%	97%	59%	11%	79%	100%	89%
	2	100%	91%	100%	91%	36%	91%	91%	91%	82%	91%	91%	100%	73%	45%	64%	73%	82%
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	0%	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	13%
Weighted mean	0-15	98.0%	98.0%	100.0%	96.0%	83.3%	74.0%	92.0%	95.9%	94.0%	96.0%	96.0%	96.0%	61.2%	18.8%	74.0%	92.0%	85.5%
	RANKING	2	2	1	4	12	13	10	8	9	4	4	4	15	16	13	10	

ABSOLUTE BIAS

	Agreed stage	CM Observ 1	PG Observ 2	SD Observ 3	RD Observ 4	MS Observ 5	LS Observ 6	BC Observ 7	MK Observ 8	SA Observ 9	RF Observ 10	SW Observ 11	FB Observ 12	YC Observ 13	KA Observ 14	JD Observ 15	JL Observ 16	ALL
	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	0.00	0.00	0.00	0.00	0.00	0.34	0.11	0.00	0.00	0.00	0.00	0.08	0.73	1.00	0.34	0.00	0.16
	2	0.00	-0.09	0.00	-0.09	-0.64	0.09	0.18	-0.09	-0.18	0.09	-0.09	0.00	0.00	0.00	-0.18	-0.27	-0.08
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-2.00	0.00	0.00	-2.00	-3.00	-1.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-1.75
Weighted mean	0-15	-0.04	-0.02	0.00	-0.06	-0.21	0.26	0.08	-0.06	-0.08	-0.02	-0.06	0.02	0.51	0.71	0.18	-0.10	0.07
	RANKING	5	2	1	6	13	14	10	8	9	3	6	3	15	16	12	11	

Overall ranking

	CM Observ 1	PG Observ 2	SD Observ 3	RD Observ 4	MS Observ 5	LS Observ 6	BC Observ 7	MK Observ 8	SA Observ 9	RF Observ 10	SW Observ 11	FB Observ 12	YC Observ 13	KA Observ 14	JD Observ 15	JL Observ 16
Ranking Coefficient of Variation	1	4	1	4	10	13	14	7	8	3	4	12	16	11	15	9
Ranking Percentage Agreement	2	2	1	4	12	13	10	8	9	4	4	4	15	16	13	10
Ranking Absolute bias	5	2	1	6	13	14	10	8	9	3	6	3	15	16	12	11
OVERALL RANKING	2	2	1	5	12	13	11	8	9	4	5	7	16	15	13	10

Annex 7: Proposed Hake maturity stage key

Macroscopic Hake Maturity Scale - Females

I – IMMATURE/RESTING

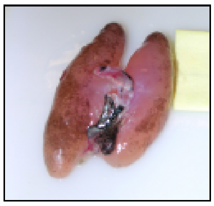
Oocytes (Opaque/Hyaline): Absents

Size: Small

Colour: Transparent or pink/grey

Vascularization: Minimal

Consistency: Firm



II – DEVELOPING/MATURING

Opaque oocytes: Present

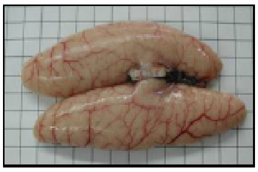
Hyaline oocytes: Absents

Size: Medium or large

Colour: Pink or yellow to orange

Vascularization: Variable, present and obvious

Consistency: Firm



III – SPAWNING

Opaque oocytes: Present

Size: Large

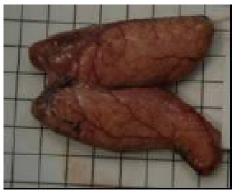
Colour: Pink or reddish orange

Vascularization: Variable, present

A: Hydrated

Hyaline oocytes: Present

Consistence: Firm



B: Partial spawning

Hyaline oocytes: Absent

Consistence: Flacid

IV – POST-SPAWNING

Consistency: Flaccid

Opaque oocytes: Absent or residual

Hyaline oocytes: Absents or residual

Size: Small / Medium

Colour: Dark pink/orange or purple

Macroscopic Hake Maturity Scale - Males

I - Immature

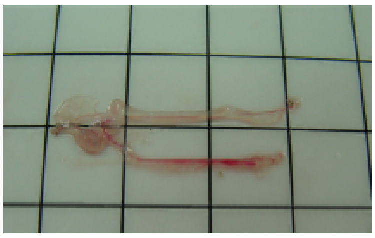
Sperm: Absent

Size: Small

Shape: thin Ribbon, no sign of developing, no curling or a thin curling band

Colour: Transparent or whitish

Test thickness: Very thin



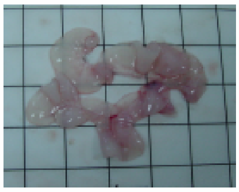
II - Developing

Shape: Develop and curling

Colour: Pink or Whitish

Size: Medium

Test thickness: Not thin



III - Spawning

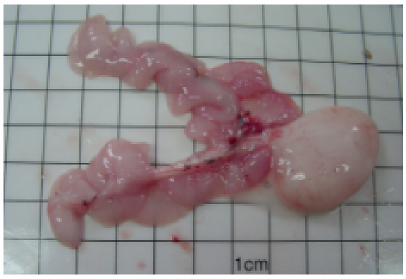
Size: Large

Shape: Large band

Colour: White

Sperm: Flows with pressure in the abdomen

Test thickness: Not thin



IV – Post-Spawning

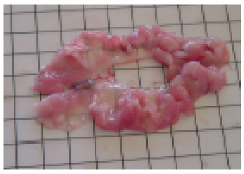
Shape: Empty and deformed bands

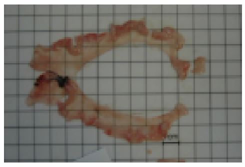
Size: Large

Colour: Reddish, to light pink

Sperm: Absent or residual

Test thickness: Very thin in the spermduct



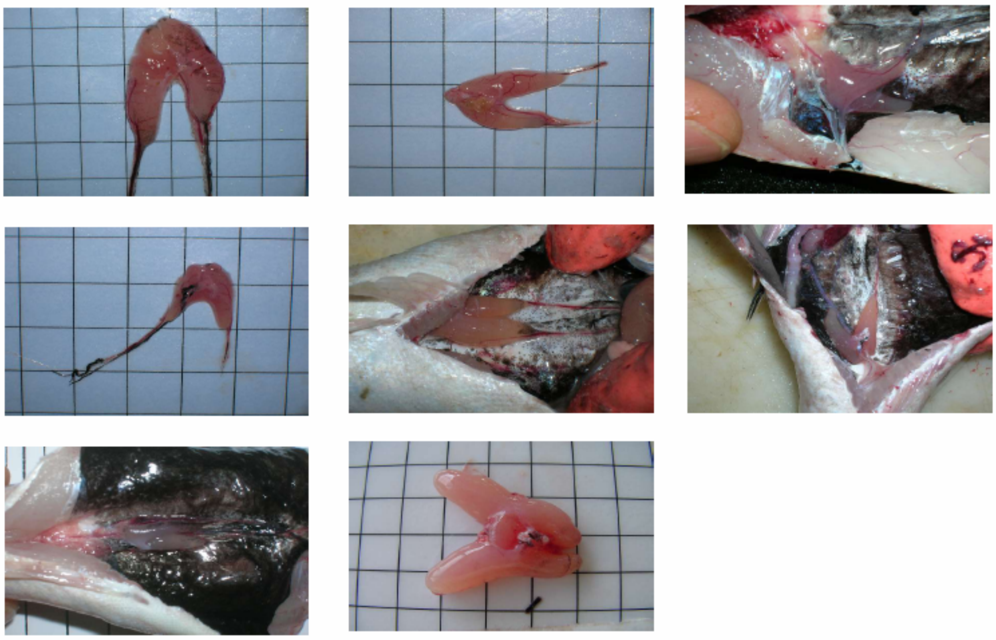


Annex 8: Hake gonads photos

Hake Maturity Stage Key

I - Immature/Resting

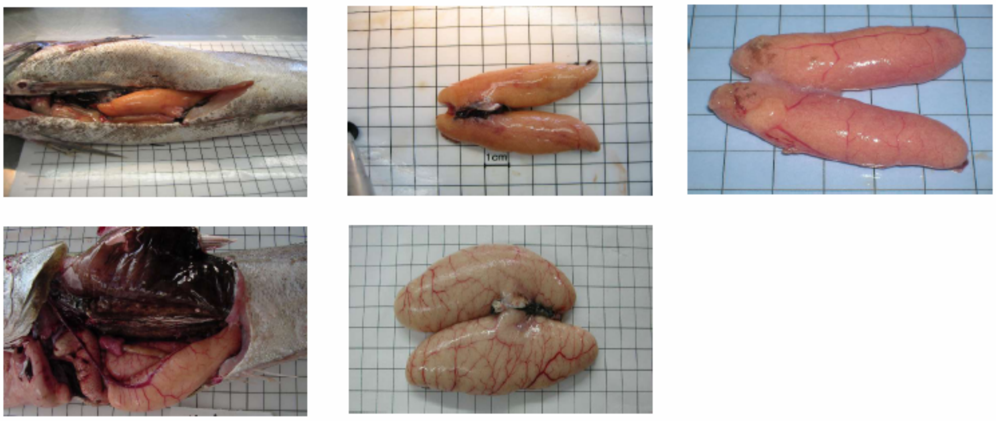
Oocytes (Opaque/Hyaline): Absents
Size:Small
Colour: Transparent or pinkgrey
Vascularization: Minimal
Consistence: Firm



Hake Maturity Stage Key

II - Developing/Maturing

<u>Opaque Oocytes:</u> Present
Hyaline Oocytes: Absents
Size: Medium or large
<u>Colour</u>: Pink or yellow to orange
Vascularization: Variable, present and obvious

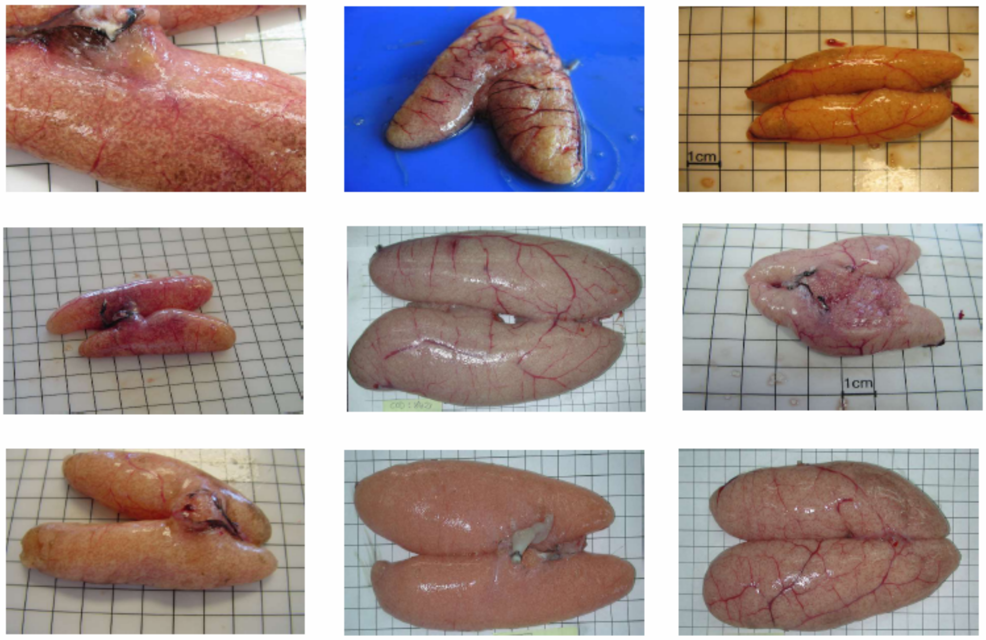


Hake Maturity Stage Key

III - Spawning

Opaque Oocytes: Present
Size: Large
Colour: Pink or reddish orange
Vascularization: Variable, present

A: Hydrated
Hyaline oocytes: Present
Consistence: Firm

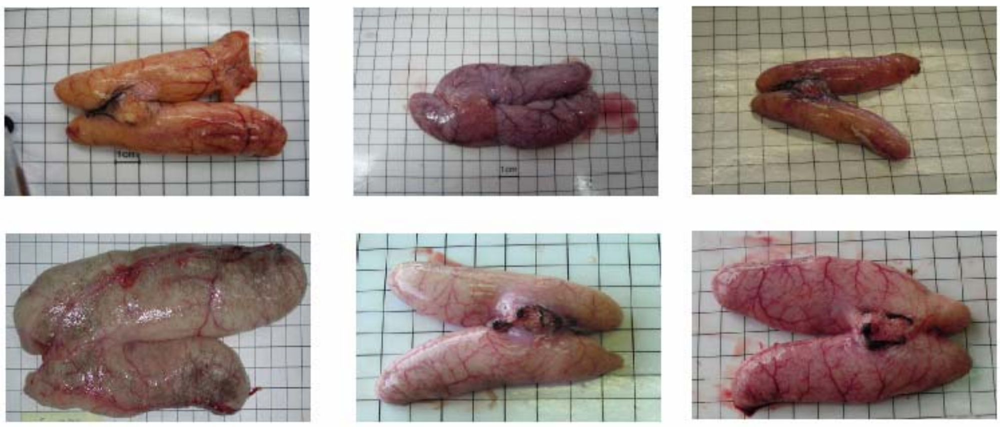


Hake Maturity Stage Key

III - Spawning

Opaque Oocytes: Present
Size: Large
Colour: Pink or reddish orange
Vascularization: Variable, present

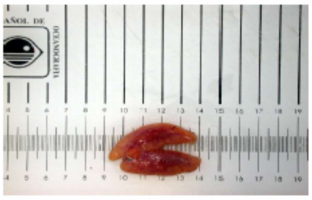
B: Partial Spawning
Hyaline oocytes: Absent
Consistence: Flaccid

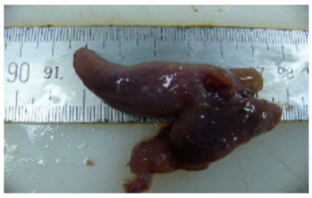


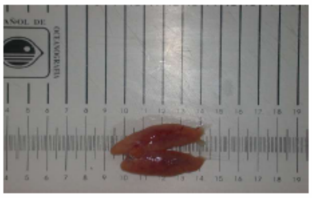
Hake Maturity Stage Key

IV - Post-Spawning

Consistence: Flaccid
Opaque oocytes: Absent or residual
Hyaline oocytes: Absent or residual
Size: Small/Medium
Colour: Dark pink/orange or purple



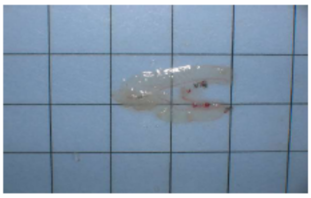
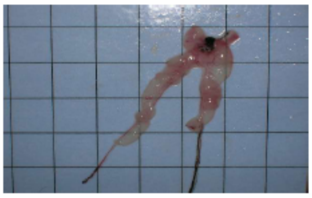
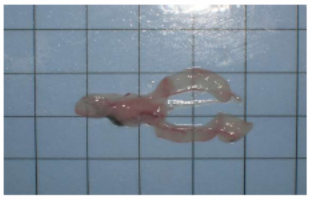




Hake Maturity Stage Key

I - Immature/Resting

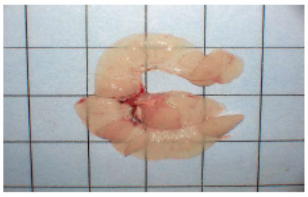
Sperm: Absent
Shape: Thin ribbon, no sign of developing or a thin curling band
Size: Small
Colour: Transparent or whitish
Testhickness: Very thin

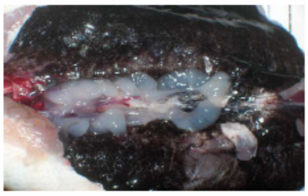





Hake Maturity Stage Key

II - Developing/Maturing

Shape: Develop and curling
Colour: Pink or whitish
Size: Medium
Testhickness: Not thin

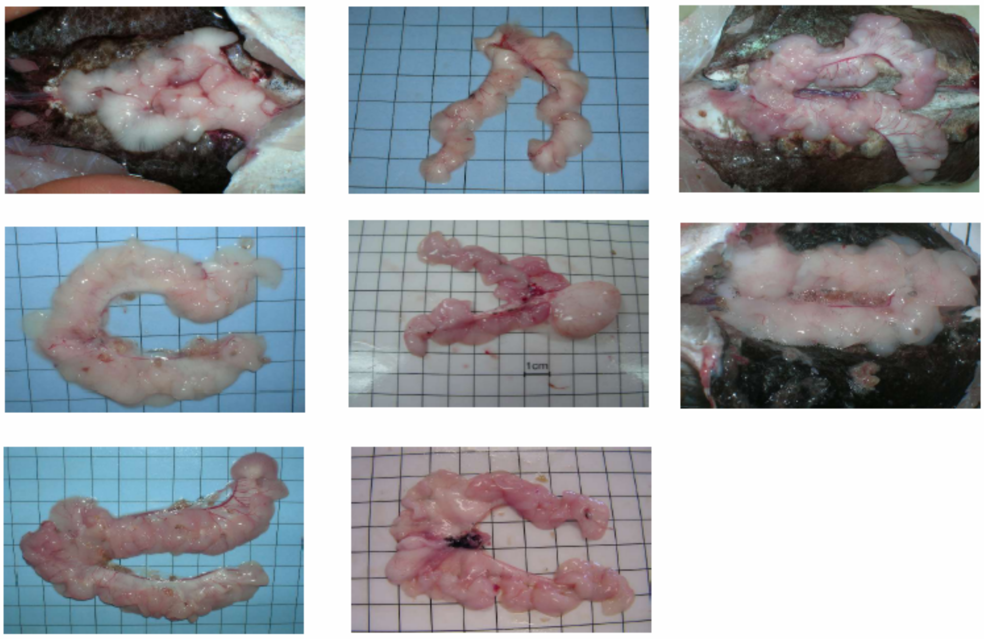




Hake Maturity Stage Key

III - Spawning

<u>Size:</u> Large
<u>Shape:</u> Large band
<u>Colour</u>: White
<u>Sperm:</u> Flows with pressure in the abdomen
<u>Testhickness:</u> Not thin



Hake Maturity Stage Key

IV - Post-Spawning

Shape: Empty and deformed bands
Size: Large
Colour: Reddish, to light or pink
Sperm: Absent or residual
Testhickness: Very thin the spermduct





Annex 9: Monkfish maturity stage keys

AZTI Monkfish maturity stage key

Female					
Maturity Stage	Virgin	Mature			
Reproductive State	Inactive	Active			
Developement	Immature	Maturing	Maturing or Pre-sapwning	Spawning	Post-spawning
BIOSDEFF_Scale	I	II	III	IV	V
Macroscopic (*visu*)					
Size (prop. Abd. cav.)	Small	Great proportion	Large		Retrated
Aspect	Band-shape	Longer band	Accentuated vasculariztion	Enormous gelatinous mass	Soft
Colour	Very transparent	Creamy	Orange	Yellow	
Opaque Oocytes	Not visible	Visible	Visible		Reabsorbed
Hyaline Oocytes	Not Visible	Not Visible	Visible	Visible	Residual
Microscopic (histology)	Chromatin nucleolus	Early vitellogenesis	Early migration. Mucus layer	Hydration. Great mucus layer	POFs and Atresia. Chromatin nucleolus

Male					
Maturity	Virgin	Mature			
Reproductive State	Inactive	Active			
Developement	Immature	Maturing	Maturing or Pre-sapwning	Spawning	Post-spawning
BIOSDEFF_Scale	I	II	III	IV	V
Macroscopic (*visu*)					
Size	Small	Great proportion			
Aspect	Tube-shape				
Colour	Pink or Transparent	Pearl-coloured	Pearl-coloured		Red and stained
Sperm	not visible	Some in lumen	full of the lumen	Flows applyinng preassure	Residual
Microscopic (histology)	Spermatocytes, not spermatozoids	Spermatocytes, few spermatozoids	Spermatozoids predom..; Spermatocytes in testis	Spermatozoids predominant	Empty seminiferal ducts. Residual spematozoids

CEFAS Monkfish maturity stage key

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

IEO Monkfish maturity stage key

Macroscopic maturity scale for anglerfish

MATURITY STAGES	MALES	FEMALES
I INMATURE OR VIRGIN	• Tube-shaped testicles covering a little proportion of the visceral cavity. • Pink or transparent. • Sperm not visible.	• Band-shaped and small ovaries. • Very transparent. • Without visible oocytes.
II MATURING	• Testicles cover greater proportion of the visceral cavity. • Pearl colour. • Some sperm in the lumen but it does not freed by applying pressure to the abdomen.	• Ovaries are a longer band covering a great proportion of the visceral cavity. • Creamy colour. • Visible oocytes (no hydrated).
III MATURE OR PRESPAWNING	• Testicles cover a great proportion of the visceral cavity. • Pearl colour. • Lumen full of sperm, but it does not freed by applying pressure to the abdomen.	• Ovaries are a big band covering the most of the visceral cavity. • Orange colour with accentuated vascularization. • Higher oocytes, being hyaline (hydrated) some of them.
IV SPAWNING	• Testicles cover a great proportion of the visceral cavity. • Dark-pearl colour. • Sperm is easily freed by applying pressure to the abdomen.	• Ovaries are an enormous gelatinous mass. • Yellow-orange colour. • All oocytes are hyaline (hydrated).
V POSTSPAWNING	• Testicles cover a great proportion of the visceral cavity. • Red and stained colour. • Sperm not visible, or a little residual	• Soft or retracted ovaries. • Haemorrhagic aspect. • Possible residual oocytes, reabsorbed.

IFREMER Monkfish maturity stage key

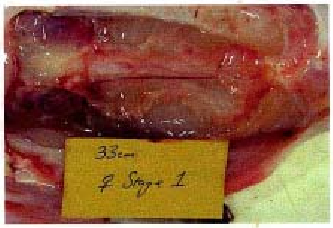
IPIMAR Monkfish maturity stage key

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

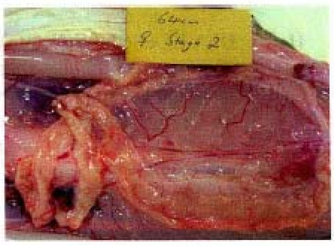
MARLAB Monkfish maturity stage key

Female Monkfish (***L piscatorius***) Maturity Key

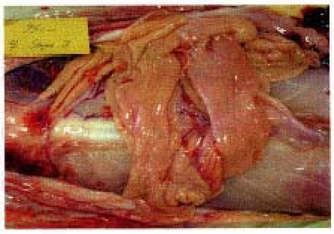
I. VIRGIN.
The ovaries are very narrow (<2cm broad), thin and ribbon-like. They are translucent, no individual oocyte clusters, or vascularisation can be seen. Their volume is negligible compared with that of the other internal organs.



II. DEVELOPING/RECOVERING/RESTING.
The ovaries increase in length and width (2-4 cm broad). They become less translucent and there is visible vascularisation. There are still no visible individual oocyte clusters. The volume occupied by the ovaries is roughly the same as that occupied by the intestine.



III. MATURING.
The ovaries increase in width (>4cm) and in length. They are highly vascularised and individual opaque oocyte clusters are visible embedded in a gelatinous matrix. At this stage the ovaries occupy most of the abdominal cavity.



IV. RIPE.
The ovaries are extremely long (>6m) and wide (30cm), and occupy most of the body cavity. The bright orange oocyte clusters (1-2mm) are easily visible and are embedded in a transparent gelatinous matrix. The ovaries are highly vascularised.

No image available

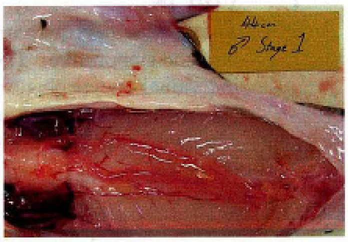
V. SPENT.
The ovaries are opaque and flaccid, with longitudinal striations. No oocyte clusters are visible with the naked eye. They are still very wide (10-15cm) and highly vascularized.

No image available

Male Monkfish (*L piscatorius*) Maturity Key

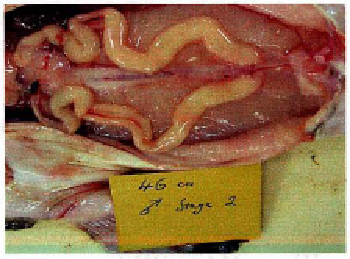
I. VIRGIN.

The testes are long, narrow (<1cm broad) and have a tubular-like structure. The medial seminiferous duct is distinct, being very pale with no visible vascularisation. Their volume is negligible compared with that of the other internal organs.



II. DEVELOPING/RECOVERING/RESTING.

The flattened tubular-shaped testes increase in length and, especially in width. Blood vessels become visible around the medial seminiferous duct. Their volume is roughly half that occupied by the intestine.



III. MATURING.

The testes have a very firm texture and moderate to large amounts of milt are produced when they are dissected. The seminiferous duct is now highly vascularised. Stage III testes still occupy less volume than the intestine.

No image available

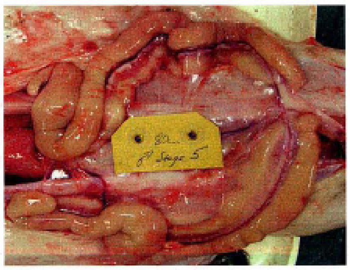
IV. RIPE.

Milt runs from the genital pore on slight pressure. The testes are extremely turgid and large amounts of milt are produced when they are dissected. Even at this stage, the testes still occupy less volume than the intestine (except in very large fish, where the volumes are similar)

No image available

V. SPENT.

The testes are very flaccid. Frequently there are roseate/salmon pink areas in the beige surface of the testis. Milt is often present in the seminiferous duct and also when dissected. The posterior edge is sometimes narrower than the anterior part of the gonad. At this stage, the testes are still highly vascularised in the vicinity of the seminiferous duct.



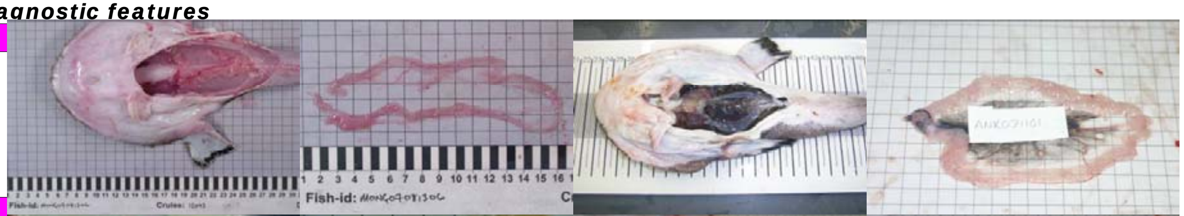
Annex 10: Proposed Monkfish maturity stage key

Macroscopic Anglerfish Maturity Scale – Females

Bold text indicates primary diagnostic features

I – IMMATURE

Oocytes (Opaque/Hyaline): Absent
Size: <2 cm band width
Colour: Transparent



II – DEVELOPING/RESTING

Opaque oocytes: Absent
Hyaline oocytes: Absent
Size: > 2 cm band width
Colour: Pink or creamy
Shape: Undulating margin

III – PRE-SPAWNING

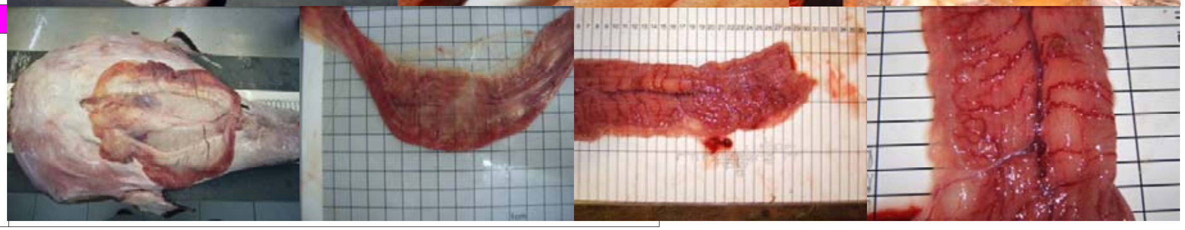
Opaque oocytes: Present
Hyaline oocytes: Absent
Size: Large
Colour: Yellow/orange
Macroscopic aspect: Vascularisation

IV – SPAWNING

Opaque oocytes: Absent
Hyaline oocytes: Present
Size: Large
Colour: Orange
Macroscopic aspect: Hyaline oocyte clusters embedded in a gelatinous matrix

IV – POST-SPAWNING

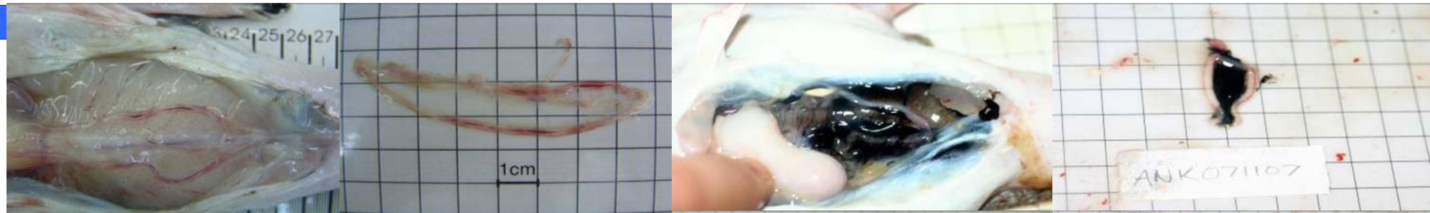
Opaque oocytes: Absent
Hyaline oocytes: Absent
Size: Large
Colour: Dark pink
Shape: Undulating margin
Macroscopic aspect: Highly vascularised



Macroscopic Anglerfish Maturity Scale - Males

I – Immature

Size: Small
Colour: Pink / transparent
Sperm: Absent



II – Developing / Resting

Size: Medium
Colour: Opaque*
Sperm: Absent

III – Pre-Spawning

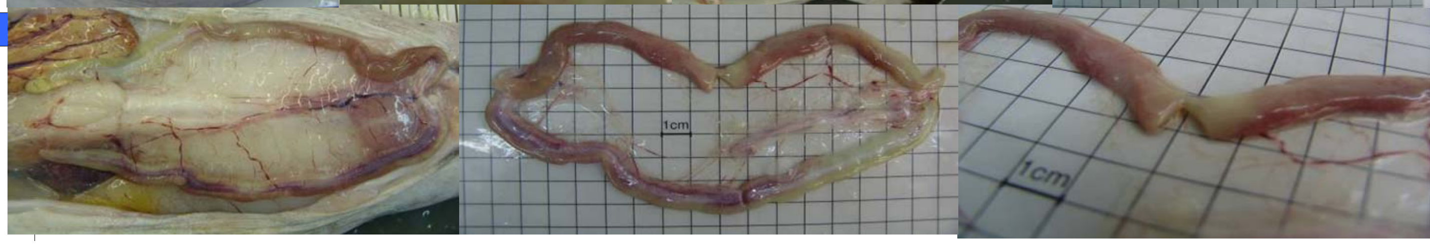
Size: Large
Colour: Cream*
Sperm: Present

IV – Spawning

Size: Large
Colour: Cream/olive*
Sperm: Free-running or freed with light pressure to abdomen

IV – Post-Spawning

Size: Large
Colour: Red / bruised
Sperm: Present



Bold text indicates primary diagnostic features

Annex 11: Monkfish gonads photos

Anglerfish Female Stage I

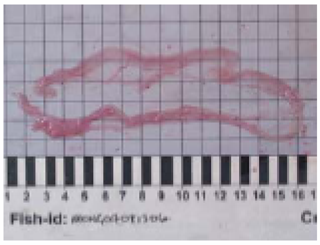
Early


gonad_2006_09_19_963a


gonad_2006_09_19_963c

Late

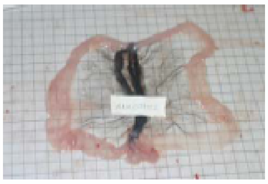

MONG07081306


MONG07081306-1

Anglerfish Female Stage II

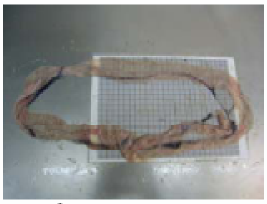
Early


ANKG07110302


ANKG07110302-1

Late


gonad_2007_03_19_223b


gonad_2007_03_19_223c

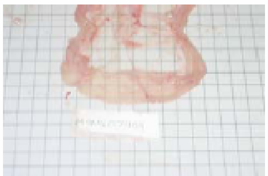

gonad_2007_03_19_223e


gonad_2006_05_25_445a


gonad_2006_05_25_445b


gonad_2006_05_25_445c

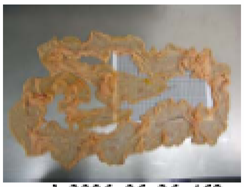

MONG07110309


MONG07110309-1

Anglerfish Female Stage III

Early


gonad_2006_06_26_469b


gonad_2006_06_26_469e

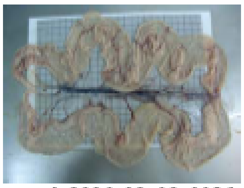

gonad_2006_06_26_469g


gonad_2006_12_19_1124 a


gonad_2006_12_19_1124 c


gonad_2006_12_19_1124 d


gonad_2006_12_19_1126 a


gonad_2006_12_19_1126 c


gonad_2006_12_19_1126 d


gonad_2007_03_19_222a


gonad_2007_03_19_222d


gonad_2007_03_19_222e


gonad_2007_05_03_260b


gonad_2007_05_03_260d


gonad_2007_05_03_260c

Anglerfish Female Stage III

<u>Early</u>

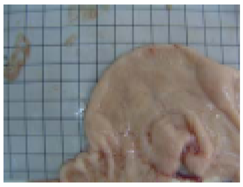

gonad_2006_10_10_1119 a


gonad_2006_10_10_1119 b


gonad_2006_10_10_1119 e


gonad_2006_11_14_1004 b.


gonad_2006_11_14_1004 c

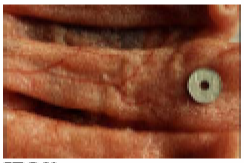

gonad_2006_11_14_1004 e


MONG07110201-1


MONG07110201

<u>Late</u>


IEO3a


IEO3b

Anglerfish Female Stage IV

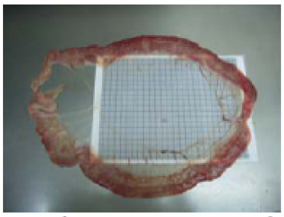

IEO4a


IEO4b

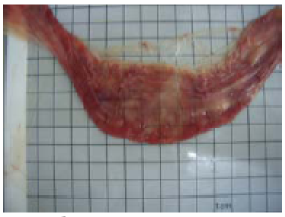

JA052_SWCOD060014

Anglerfish Female Stage V

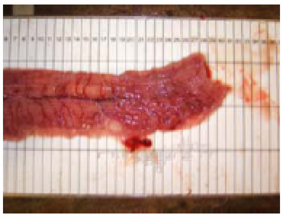
gonad_2006_09_19_ 972a.



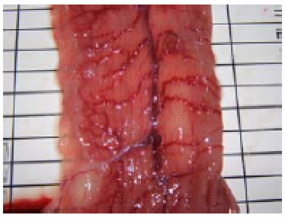
gonad_2006_09_19_ 972b



gonad_2006_09_19_ 972c.



JA050_SWCOD060004



JA051_SWCOD060009

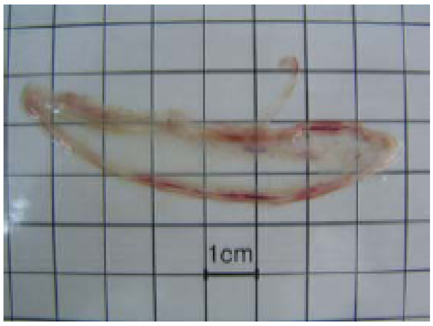
Anglerfish Male Stage I

<u>Early</u>


ANKG07110307

ANKG07110307-1

gonad_2006_09_26_814a



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MONG07081101-2

Anglerfish Male Stage II

Early

ANKG07110303

ANKG07110303-1

nov_bud2_m1_02

nov_bud2_m1_04

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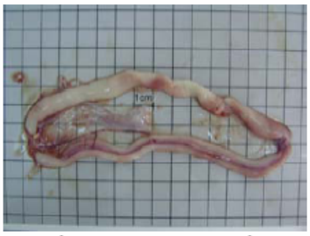

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Anglerfish Male Stage III

Early


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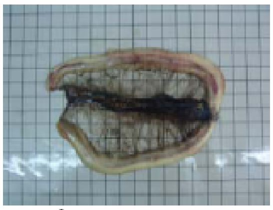
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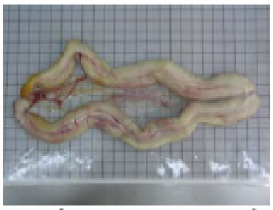
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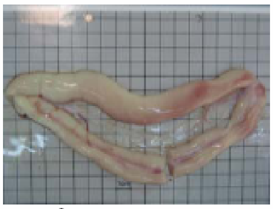
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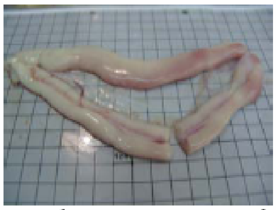
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MONG07102903-1


MONG07102903-2

Anglerfish Male Stage IV


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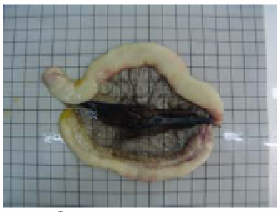
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gonad_2006_12_19_1130
c


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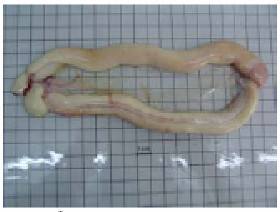
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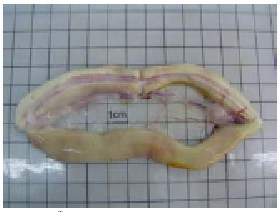
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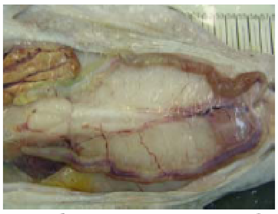
pis2_m3_06


pis2_m3_08


pis2_m3_05


DSC00051

Anglerfish Male Stage V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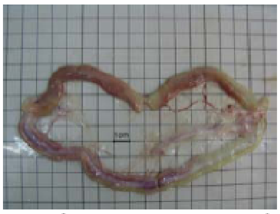
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