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Report of the Workshop on Sexual Maturity Staging of Mackerel and Horse Mackerel (WKMSMAC)

26–29 November 2007

Lisbon, Portugal

ICES International Council for the Exploration of the Sea

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Executive summary

- Under the EU Data Collection Regulation, maturity samples are collected for mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*). These data are used for fishery stock assessment and also for different types of studies regarding the maturation cycles of these species.
- Several different maturity scales are currently used by different institutes. Most of these scales are used for very specific purposes, and the data produced are difficult to compare or jointly analyse. The Walsh scale, in particular, has been used for 17 years, and is used as routine or for special purposes by most of the observers present at the workshop.
- Taking into consideration the generic standard scale proposed in a previous workshop (WKMAT), and the characteristics of the mackerel and horse mackerel reproductive cycles, the workshop has proposed a standard scale for these species. The Walsh scale was fitted into this standard scale, which was used in the calibration exercises along with the Walsh scale.
- Ideally a calibration should be carried out with gonads of a 'known' stage. For practical reasons such an exercise was not possible. Therefore, the objective of this workshop was restricted to compare and improve the agreement between observers, assuming that the "correct stage" was the one given to each gonad by the majority of the observers.
- The agreement between observers, regarding the determination of sex, was high for both species. However, the gonads of very small fish are difficult to classify, both in terms of sex and maturity stage, especially for less experienced observers. Agreement in horse mackerel is slightly lower compared to mackerel suggesting horse mackerel is a difficult species to attribute sex to.
- For mackerel, the highest agreements in maturity staging were obtained with the Walsh scale and in general there was a decrease in agreement when using the standard scale. The standard scale agreement for immature fish was in particular much lower than the one obtained with the Walsh scale.
- For horse mackerel, the level of agreement was higher for the standard than for the Walsh scale, however, this was also not the case for fish in immature stage, which had a lower agreement with the standard than with the Walsh scale. Almost one third of the immature fish were classified as being in a maturing stage when using the standard scale, which may indicate a low precision in the calculation of maturity ogives.
- The standard scale is proposed by the workshop as a good description of the mackerel and horse mackerel reproductive cycle, with an adequate level of detail for most purposes, being especially useful for exchanging and comparing data between observers or institutes. However, many observers are still very used to the Walsh scale, and the fitting of the Walsh scale into the standard one must be better assimilated.
- WKMSMAC recommends that a similar workshop should be repeated every three years, in the year prior to the Atlantic mackerel and horse mackerel egg survey. These should be used to train and calibrate

observers, and also to address several questions on the reproduction of these species.

1 Introduction

At the 93rd Statutory Meeting of ICES (2006) it was decided that a Workshop on Sexual Maturity Staging of Mackerel and Horse Mackerel [WKMSMAC] (Chair: Alberto Murta, Portugal) should be held. This workshop took place in Lisbon, Portugal, from 26 to 29 November 2007 with the following terms of reference:

- Compare the macroscopic maturity scales for Mackerel and Horse Mackerel used in the different laboratories.
- Compare and calibrate the criteria, followed by the scientists/technicians involved in maturity stage sampling, to classify each maturity stage for males and females.
- Standardise the criteria to classify each maturity stage.
- Propose a common maturity scale, with common classification criteria, to be used by all laboratories.

WKMSMAC reports for the attention of ACFM, RMC and PGCCDBS.

Under the Data Collection Regulation DCR (Council Regulation 1543/2000 and Commission Regulations 1639/2001, 1581/2004), maturity samples are collected for a wide range of species, including mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*). These data are used for fishery stock assessment, e.g. for calculation of SSB, for the egg production methods, and also for different types of studies regarding the maturation cycles of these species. Therefore, the Planning Group on Commercial Catch, Discards and Biological Sampling PGCCDBS (ICES, 2006) proposed a workshop to calibrate the criteria for the classification of sexual maturity stages of mackerel and horse mackerel. The structure of this report reflects roughly the sequence of the workplan followed during the Workshop:

Section 2 provides the agenda followed during the workshop.

Section 3 gives an overview of the sexual maturity scales currently used for mackerel and horse mackerel, and describes how the Walsh scale (Walsh et al., 1990) is applied in each lab/institute. This scale is the most frequently used, and has been slightly adapted within each lab. This means that even the labs that use the Walsh scale are not using exactly the same criteria to classify maturity stages.

Section 4 proposes a new sexual maturity scale, which was obtained by fitting the original Walsh scale into the scale proposed by WKMAT (ICES, 2007). This scale, called here the "standard scale", is proposed to be used by all labs either for all purposes, or at least for data exchange, in the case the Walsh scale continues to be used (given that the translation from the Walsh to the standard scale is straightforward).

Section 5 describes the procedure followed for the exercises of calibration between observers, and also presents the results of the analyses made to the data produced during the calibration.

Section 6 summarises the main conclusions of the Workshop and presents an overview of the recommendations.

Section 7 has the references quoted in the text, and finally

Section 8 has an Annex with the list of participants, and several tables with the raw data obtained during the calibration exercises.

2 Agenda and participation

2.1 Agenda

Monday, 26th	Morning	Welcome and introduction. A short description of the work carried out in each lab involving the maturity staging of mackerel and horse mackerel.
	Afternoon	Attribution of a secret number to each participant, so that the participants can be kept anonymous in the analysis of the workshop results. Review of the existing maturity scales. Analysis of photos of mackerel and horse mackerel, and discussion of the application of criteria to each photo.
Tuesday, 27th	Morning	Analysis of mackerel and horse mackerel gonads at the lab. Classification of maturity stages by each participant (anonymous).
	Afternoon	Analysis of the results of the exercise carried out in the morning.
Wednesday, 28th	Morning	Repetition of Tuesday's exercise.
	Afternoon	Analysis of the results of the exercise carried out in the morning.
Thursday, 29th	Morning	Discussion and evaluation of the workshop results. Drafting the report. Farewell.
	Afternoon	

2.2 Participants

This workshop was attended by 24 people, from 8 countries. Table A.1, in Annex 1, presents a list of names and addresses of all participants.

3 Overview of sexual maturity scales currently used

3.1 Maturity scales

Several different maturity scales are currently used by different institutes. Most of these scales are used for very specific purposes, e.g. some institutes may use a maturity scale for market sampling, another one during egg surveys and still another for the bottom-trawl surveys. This diversity of maturity scales has lead to a situation in which data from different sources (surveys or market samples) are difficult to compare or jointly analyse. A detailed overview of maturity scales in the ICES area, including some for mackerel and horse mackerel, was made by WKMAT (ICES, 2007). However that overview did not cover a few scales currently used for mackerel and horse mackerel, which are described below in Tables 3.1, 3.2, 3.3 and 3.4.

Table 3.1 Mackerel and horse mackerel maturity scale used at IMARES (Netherlands) for market sampling.

STAGE	DESCRIPTION
Immature	No signs of development.
Maturing	Developing or resting after Spent and recovering.
Running	Roe or eggs run with slight pressure, translucent eggs in ovary/cavity.
Spent	Testes and ovaries empty, a few eggs remain. Solid, no signs of development.

Table 3.2 Mackerel and horse mackerel maturity scale proposed by Nikolsky (1963) used at HCMR (Greece).

STAGE	SEX	DESCRIPTION
1. Virgin	Male	Transparent, very thin gonad. Difficult recognizable.
	Female	Very small and transparent gonad. Difficult recognizable.
2. Developing Virgin/ Inactive	Male	Thin, whitish/ transparent gonads with flat shape slightly increased in width.
	Female	Transparent, pinky/orange, more rounded gonad. Gonads occupying 1/4 to 1/3 of the abodominal cavity. Oocytes not visible to naked eye.
3. Developing	Male	Larger, whitish/creamy gonads.
	Female	Larger, salmon-pinky/ orange gonad. Gonads occupying 1/3 –1/2 of body cavity. Oocytes are visible to naked eye. Some vascularisation is visible.
4. Mature	Male	Larger gonad occupying significant part of the abdominal cavity. White/ creamy colour. Sperm duct is visible. Sperm flows when it is cut.
	Female	Compact ovaries cover the 2/3 of the abdominal cavity. Ovaries orange-red.
5. Ripe	Male	The whole abdominal cavity covered. Testis structure is not so connected. Sperm easily flowing on pressure.
	Female	The whole abdominal cavity covered. Many hydrated oocytes are visible. Well vascularized gonads.
6. Spent	Male	Testes flaccid, yellowish/ creamy. A little residual milt may exist in the lumen.
	Female	Gonads occupying <1/3 of abdominal cavity. Ovaries flaccid with dark red/ dark brown colour. A few residual oocytes are visible as dark areas. Vascularization is dark or not visible.

Table 3.3 Mackerel and horse mackerel maturity scale used at IMR (Norway).

STAGE	SEX	DESCRIPTION
Blank	Undecided	Not checked
1. Immature a	Male	Juvenile phase. Gonads thread-like, thin and completely transparent and colourless. Difficult to determine sex.
	Female	Juvenile phase. Gonads thread-like, thin and completely transparent and colourless. Difficult to determine sex.
2. Immature b	Male	Gonads are somewhat larger in volume, sex is easier to determine. The gonads continue to be transparent and coulorless with a hint of coulor.
	Female	Gonads are somewhat larger in volume, sex is easier to determine. The gonads continue to be transparent and coulorless with a hint of coulor.
3. Maturing a	Male	Gonades opaque but developing in volume. Distinct veins. Testes white or with white spots. Firm consistency.
	Female	Gonades opaque but developed in volume. Distinct veins. Ovaries have yellow / white eggs in lamellae and can occupy half of the body cavity or more.
4. Maturing b	Male	Gonads larger in volume. Distinct veins. Testes light grey or white, milt thick and slow-flowing.
	Female	Gonads larger in volume. Distinct veins. Ovaries yellowish or white, can occupy 2/3 or more of the body cavity depending on the condition of the fish. The eggs can be seen distinctly and feel like grain. The eggs in the front part of the gonad are beginning to become transparent.
5. Maturing c	Male	Testes are grey or white. The milt runs easily. Gonads are not yet running, however, a light pressure on the abdomen causes the milt to run.
	Female	Ovaries fill the entire body cavity. Most of the eggs are transparent.
6. Spawning	Male	Running gonads. A light pressure on the abdomen causes the milt to run.
	Female	Running gonads. A light pressure on the abdomen causes the eggs to run.
7. Spent	Male	Gonads loose, contain remaining milt.
	Female	Gonads loose, contain remaining eggs.
8. Resting	Male	Gonads are small. Milt are not visible. Difficult to distinguish from stages 2/3.
	Female	Gonads are small. Eggs are not visible. Difficult to distinguish from stages 2/3.

Table 3.4 Mackerel and horse mackerel maturity scale used at DIFRES (Denmark).

STAGE	SEX	DESCRIPTION
1. Immature	Male	Testes very thin like a translucent ribbon lying along an unbranched blood vessel. No signs of development.
	Female	Ovaries small, elongated, whitish, translucent. No signs of development.
2. Maturing	Male	Development has obviously started, colour is progressing towards creamy white and the testes are filling more and more of the body cavity but sperm cannot be extruded with only moderate pressure.
	Female	Development has obviously started, eggs are becoming larger and the ovaries are filling more and more of the body cavity but eggs cannot be extruded with only moderate pressure.
3. Spawning	Male	Will extrude sperm under moderate pressure to advanced stage of extruding sperm freely with some sperm still in the gonad.
	Female	Will extrude eggs under moderate pressure to advanced stage of extruding eggs freely with some eggs still in the gonad.
4. Spent	Male	Testes shrunken with little sperm in the gonads but often some in the gonoducts which can be extruded under light pressure. 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.

However, all institutes in the ICES area, except DIFRES (Denmark), use the Walsh scale (Walsh *et al*., 1990), either for all purposes, or at least in certain occasions, e.g. when sampling for the mackerel and horse mackerel egg surveys (WGMEGS). The Walsh scale (Table 3.5) also seems an adaptation of the Nikolsky scale, used at the HCMR (Greece). Therefore, it was decided, regarding TOR b (calibration of criteria), to use the Walsh scale for the calibration during the workshop.

The Walsh scale has been used for 17 years, and the original criteria have been slightly adapted, which was obvious during the plenary discussions of photos of gonads. In fact, most participants clearly admitted, during these discussions, that the exact criteria stated in the original description of the Walsh scale were not strictly followed. For this reason, a description of the different ways that the Walsh scale is being applied, across and within institutes, is given in the next section.

Table 3.5 Sexual maturity scale proposed by Walsh *et al.* (1990) for mackerel, also used for horse mackerel.

Stage	State	External appearance
1	Immature	Gonads small. Ovaries wine red and clear, torpedo shaped. Males pale, flattened and transparent.
2	Early ripening	Gonads occupying 1/4 to 3/4 body cavity. Opaque eggs visible in ovaries giving pale pink to yellowish colouration, largest eggs without oil globule. Testes off-white, milt not running.
3	Late ripening / Partly spent (early)	Gonads occupying 3/4 to almost filing body cavity. Ovaries yellow to orange. Largest eggs may have oil globules. Testes creamy white.
4	Ripe	Testes filling body cavity, milt freely running. Ovaries characterized by externally visible hyaline eggs no matter how few or how early the stage of hydration. Ovaries with hyaline eggs only in the lumen are not included. Ovary size variable from full to 1/4.
5	Partly spent (late)	Gonads occupying 3/4 to < 1/4 body cavity. Ovaries slacker than in stage 3 and often bloodshot. Testes with free running milt and shrivelled at anus end.
6	Spent / Recovering spent	Gonads occupying 1/4 or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs. Testes opaque with brownish tint and no trace of milt.

3.2 Application of the Walsh scale across institutes

In this section a description is given on the practical application of the Walsh scale in each institute, or in different labs within institutes. While the criteria to follow are mostly the same as in the original description of the scale, the relative importance of each criterion is very variable across institutes.

AZTI (Spain)

AZTI uses the Walsh scale to assign the maturity stage for mackerel and horse mackerel. The table below summarises the description of the character applied in AZTI and the order of the criteria during the staging. The description is practically similar to those of Walsh (1990) except for phase 4 where AZTI does not consider the aspect "Ovaries with hyaline eggs only in the lumen are not included".

Table 3.6 Criteria followed in AZTI to apply the Walsh maturity scale.

PHASE	STAGE	DESCRIPTION (AZTI WALSH SCALE)	MAIN CRITERIA
1	Inmature	Gonads Small. Ovaries wine red and clear, torpedo shaped. Testes pale, flattened and transparent.	Gonad size; No presence of eggs; Testes size and colour (transparent or not).
2	Early ripening	Gonads occupying 1/4 to 3/4 body cavity. Opaque eggs visible in ovaries giving pale pink to yellowish colouration, largest eggs without oil globule. Testes off-white, milt not running.	Gonad size; Colour; Presence of eggs; Testes size and colour.
3	Late ripening/ partly spent (early)	Gonads occupying 3/4 to almost filing body cavity. Ovaries yellow to orange. Largest eggs may have oil globules. Testes creamy white.	Gonad Size; Colour; Testes size and colour.
4	Ripe	Ovaries characterized by externally visible hyaline eggs no matter how few or how early the stage of hydration. Ovary size variable from full to 1/4 of body cavity. Testes filling body cavity, milt freely running.	Presence of hyaline eggs; Eggs running; Gonad size and aspect; Colour; Testes size and presence of sperm.
5	Partly spent (late)	Gonads occupying 3/4 to < 1/4 body cavity. Ovaries slacker than in stage 3 and often bloodshot. Testes with free running milt and shrivelled at anus end.	Colour and aspect; Gonad Size; Presence of hyaline eggs; Testes size; Presence of running milt; Testes aspect; Testes colour.
6	Spent / Recovering spent	Gonads occupying 1/4 or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs. Testes opaque with brownish tint and no trace of milt.	Gonad size; Gonad colour; Presence of eggs; Testes size and colour.

BFA (Germany)

A German translation of the original Walsh Scale is used for the macroscopic determination of the maturity of mackerel and horse mackerel during the mackerel and horse mackerel egg survey. For all other samples, from commercial cruises and from the IBT-survey, a modified Walsh-Scale is used where scale 2 and 3 as well as scale 4 and 5 are merged. Main characteristics for the determination are the size and consistency of the gonads.

DIFRES (Denmark)

Denmark has no maturity sampling from Mackerel and Horse mackerel, in the market sampling programme. However, Denmark is running several cruises in the North Sea during the year, where Mackerel and Horse mackerel are usually part of the catches; an acoustic survey in the summer (July), and a participation in the IBTS in the 1st and 3rd quarters. In the acoustic survey there is no sampling of maturity data from Mackerel or Horse mackerel. At the IBTS cruises in the North Sea, maturity data on Mackerel and Horse mackerel are sampled from 2007, using the four stages scale as recommended in the IBTS manual.

FRS (Scotland)

Stage 1, **Females**: Transparent very thin sometimes. Sex sometimes indistinguishable. Occasionally a small tubular reddish transparent structure

can be seen to tell it is a female. First prognostic at this stage is the size of the fish. If <=23cm then it would be expected to be a stage one. **Males**: As with females. Transparent and even smaller than the females. Often just a very small strip of material can be seen.

Stage 2, **Females**: Firstly, size of gonads ¼ to ¾ of body cavity. Then presence of small opaque eggs that can just be seen with the naked eye. Some vascularisation but not a large portion of the surface is covered. Colour yellow or light orange. Gonads feel firm with no slackness. **Males:** Size as with females. Then colour which can be off-white with some red parts. Gonad also firm.

Stage 3, **Females:** Gonads are ¾ or more of the body cavity. There is an increase in vascularisation. Colour can be bright yellow or orange. Eggs are larger than in stage 2. **Males:** Size is larger than stage 2. Testes whiter but still no extrusion of sperm when pressure is applied.

Stage 4, **Females:** Gonads show a smattering of hydrated eggs. They occupy the whole cavity and some eggs can be extruded if pressure is applied. **Males:** Firstly if some sperm can be extruded upon pressure. Gonads whiter and larger than in stage 3.

Stage 5 **Females:** Presence of hyaline eggs but some shrivelling seen especially towards the anus end of the gonad. Gonad heavily vascularised especially towards the anus end. Slackness. Extrusion of eggs if pressure is applied. **Males:** Gonads look slack and become off-white in some patches. We look for shrivelling towards the lumen. Differentiation between this stage and a stage 3 will be judged on the apparent "wear and tear" of the gonad, its slackness and its colour. In early stage 5 it can still be possible to see some residual milt but in later stage 5 none can be seen.

Stage 6 **Females**: Colour red to light pink and murky in appearance with some possible opaque eggs still remaining.Gonad slack and baggy, possibly some striations visible. **Male**: Colour from brown to dark orange. Gonads flat and in some cases stretched in appearance.

IEO (Spain)

The scale that is used in all the IEO laboratories is the Walsh scale with a few little interpretation points. Within IEO there are small differences when applying the scale. Table 3.7 describes the criteria used at the IEO lab in A Coruña, while Table 3.8 describes the criteria used in the Vigo and Santander labs.

Table 3.7 Criteria followed in the IEO (A Coruña lab) to apply the Walsh maturity scale.

PHASE	STAGE	CRITERIA USED IN THE CORUÑA LAB.
1	Inmature	Gonad very small, in males almost invisible, in females little whith no colour. The size of the fish is very important to assign the stage
2	Early ripening	Size criteria is similar to Walsh. In females sometimes there are some specimen with colouration yellowish without any eggs, or in other cases with little eggs but very numerous giving colouration. Males similar to Walsh. No idea about oil globules.
3	Late ripening/ partly spent (early)	Same as Walsh, females gonad very compact, eggs with similar size, and not bloodshoot.
4	Ripe	This stage is given when there is at least one visible hydrated egg, and the rest is similar to Walsh. Is the immediately before and after the running. Spawning. In males it is necessary that the milt runs freely when pressed. If not, the gonad is considered in stage five.
5	Partly spent (late)	Similar to Walsh. In females the ovaries are slacker and bloodshot around the anus. The appearance must be redish and is more important than the size to distinguish this stage from stage 3. In males it is important that there is no freely running milt and the presence of light purple areas. Size still large.
6	Spent/ Recovering spent	Same as Walsh . In females it is very common to find ovaries with only a few eggs but well visible. For us thís is the difference between late 6 and early 2. Males very narrow, the purple areas become brownish. Sometimes the gonads are hard to see, but they always have a dark, brown or redish colour. If the gonads have no colour or are lightly white the fish is considered immature (stage 1). The size of the fish is an important criterion to distinguish between stages 6 and 1.

Table 3.8 Criteria followed in the IEO (Vigo and Santander labs) to apply the Walsh maturity scale.

PHASE	STAGE	CRITERIA
1	Inmature	Gonads Small. Practically invisible. Ovaries wine red and clear, torpedo shaped. Males pale, flattened and transparent
2	Beginning of the development	Gonads occupying about 1/4 of body cavity, with pale pink to yellowish colouration, largest eggs without oil globule. Testes off-white, milt not running.
3	Ripe or end of development.	Gonads occupying 3/4 to almost filing body cavity. Ovaries yellow to orange. With visible opaques eggs. Testes creamy white.
4	Spawning	Ovaries and testes size variable from ¾ to full. Testes filling body cavity, milt freely running. The colour is creamy white. Ovaries characterized by externally visible hyaline eggs no matter how few or how early the stage of hydration. Eggs freely running.
5	Post-spawning	Gonads occupying 3/4 to 1/4 body cavity. Ovaries slacker than in stage 3 and often bloodshot. With residual eggs (opaques and hyalines). Testes with free running milt and shrivelled at anus end.
6	Post-spawning / Recovery	Gonads occupying 1/4 or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs. Testes opaque with brownish tint and no trace of milt.

IMARES (The Netherlands)

At IMARES during the mackerel and horse mackerel egg survey we use the Walsh scale as described in the Portuguese macroscopic scale of females and males of horse

mackerel, Atlantic mackerel and chub mackerel (Table 3.9). The size and firmness or the slackness of the gonads are the main characteristics used for the staging. If oocytes are visible and the ovary is firm this is considered Stage 3, if the oocytes are visible but the ovary is slack and bloody this is considered Stage 5. If hydrated eggs are visible this is considered Stage 4.

IMR (Norway)

The Norwegian maturity scale (Table 3.3) is used for regular sampling for both market and survey sampling. The Walsh scale is used only for the Mackerel and Horse Mackerel Egg Survey. This has given the Norwegian observers little experience in using the Walsh scale. Especially working with first time spawners, distinguishing between immature (1) and early ripening (2), is at times difficult when using the Walsh scale. The Walsh scale has been used as described in Table 3.5.

IPIMAR (Portugal)

The Walsh scale is used in the Lisbon lab of IPIMAR, but some criteria have been slightly changed. For females, the first characteristic to look at is the presence/absence of oocytes and whether they are hyaline or not. Other characteristics are the colouration and consistency of the gonads and the proportion of the body cavity occupied by the gonad. For males, the main characters to look at are the colour of the testes (whether they are white or greyish), the presence/absence of milt and finally the proportion of the body cavity filled by the gonad. The criteria used are summarised in Table 3.9.

Table 3.9 Criteria followed in the IPIMAR (Lisbon lab) to apply the Walsh maturity scale.

PHASE	STAGE	EXTERNAL APPEARANCE
1	Immature	Gonads Small. Ovaries wine red and clear, torpedo shaped. Males pale, flattened and transparent. The main criterion is the size of the gonads when it is difficult to distinguish from stage 6.
2	Beginning of the development	Gonads occupying 1/4 to 3/4 body cavity. Opaque eggs visible in ovaries giving pale pink to yellowish colouration, largest eggs without oil globule. Testes off-white, milt not running. In females, a special attention is given to the presence of eggs because this stage in small females can be mistaken with stage 1. Also, the aspect of the tissue (if it is hard or soft) is important to distinguish between stages 2 and 6.
3	Late Ripening/partly spent (early)	Gonads occupying 3/4 to almost filing body cavity. Ovaries yellow to orange with the presence of opaque eggs. Largest eggs may have oil globules. Testes creamy white.
4	Ripe	Ovaries characterized by externally visible hyaline eggs no matter how few or how early the stage of hydration. Ovaries with hyaline eggs only in the lumen are not included. Ovary size variable from full to 1/4. Ovary size variable from full to 1/4. Testes filling body cavity, milt freely running.
5	Partly spent (late)	Gonads occupying 3/4 to 1/4 body cavity. Ovaries slacker than in stage 3 and often bloodshot. It may have residual eggs (opaque and a few hyalines in the gonad. Testes with free running milt and shrivelled at anus end.
6	Spent / Recovering spent	Gonads occupying 1/4 or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs. Testes opaque with brownish tint and no trace of milt.

A different adaptation of the Walsh scale is used at the Matosinhos lab of IPIMAR. For Stage 1, the size of the gonads is the most important criterion. The colour, shape and the absence/presence of opaque eggs are the most important characteristics observed on females. Male's gonads are thin, flattened and transparent. For Stage 2, the first characteristic to observe is the presence/absence of opaque eggs and eggs without oil globule. Testes are off-white to white and there is no milt running. In Stage 3, the size and colour are important. Ovaries are yellow and without blood vessels. Testes are creamy white and milt is not running. Stage 4 is characterised by the testes filling the body cavity and milt freely running, and ovaries with abundance of visible hyaline eggs. Orange ovaries, slacker, flaccid, with spaces and bloodshot, characterize Stage 5. In this stage, testes have a few running milt and are shriveled at anus end. There is no milt running in Stage 6 testes. These are usually opaque with brownish/wine tint and no trace of milt. Stage 6 ovaries are reddish and often murky in appearance, sometimes with a few opaque eggs. These characteristics are summarised in Table 3.10.

Table 3.10 Criteria followed in the IPIMAR (Matosinhos lab) to apply the Walsh maturity scale.

STAGE	STATE	EXTERNAL APPEARANCE
1	Immature	Gonads small. Ovaries wine red or pale pink and clear, torpedo shaped. Absence of opaque eggs. Male thin, flattened and transparent.
2	Early ripening	Presence of opaque eggs and eggs without oil globule in ovaries giving pale pink. Testes off-white to white, milt not running. Gonads occupying ¼ to ¾ body cavity (is not the first characteristic to check).
3	Late ripening	Gonads occupying ¾ to almost filing body cavity. Ovaries yellow, without blood vessels. Gonads without spaces (compact). Largest eggs may have oil globules. Testes creamy white, milt not running.
4	Ripe	Testes filing body cavity, milt freely running. Ovaries characterized by high quantity of visible hyaline eggs, independently of the stage of hydration. Ovary size variable from full to ¾ of body cavity.
5	Partly Spent	Gonads occupying ¾ to ¼ body cavity. Ovaries slacker, flacid, with spaces, orange and bloodshot. Testes with a few running milt and shriveled at anus end.
6	Recovering Spent	Gonads occupying ¼ or less of the body cavity. Ovaries redish and often murky in appearance, sometimes with a scattering or patch of few opaque eggs. Testes opaque with brownish/wine tint and no trace of milt.

4 Proposal of a new scale for sexual maturity stages

In January 2007, a workshop on sexual maturity sampling, WKMAT (ICES, 2007) took place in Lisbon. During that workshop, a standardized maturity scale was created into which the majority of the currently used scales could be converted. The IBTS 4 grade maturity scale was used as a template and the addition of a fifth stage, omitted spawning, was discussed. Recent research has shown that in several species, a substantial part of mature individuals from the younger age classes can omit spawning if energy resources are scarce (Jörgensen *et al*., 2006). Since the current IBTS maturity key does not allow classifying for those individuals that skip spawning, a fifth stage was added. This process of skipped spawning, also known as omitted spawning and massive atresia (the histological observation), has not been so far reported for mackerel or horse mackerel. Although there are no published descriptions of omitted spawning occurring in these species, some photos of mackerel gonads were shown to the WKMSMAC plenary, which looked macroscopically in a spent stage, but microscopically it could be seen that massive atresia was taking place in those individuals (Figures 4.1 and 4.2).

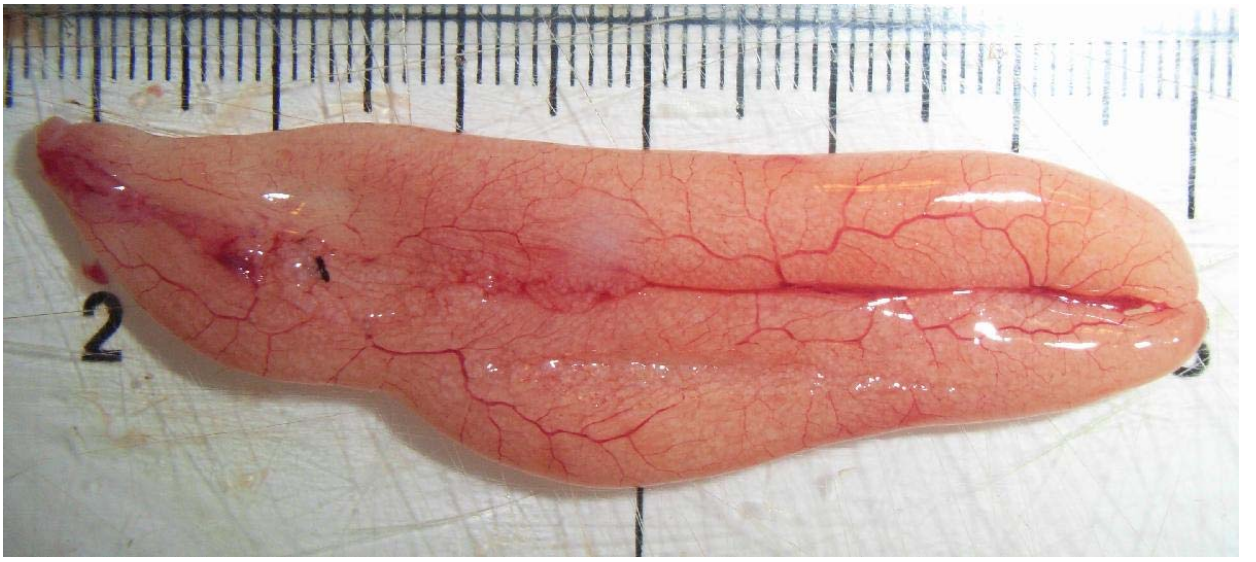

Figure 4.1 Ovary of a female mackerel, which could be considered as a post-spawning fish by macroscopic examination, but in fact is under a process of massive atresia (see also the histological slide in Figure 4.2). Copyright: Merete Fonn, IMR, Norway.

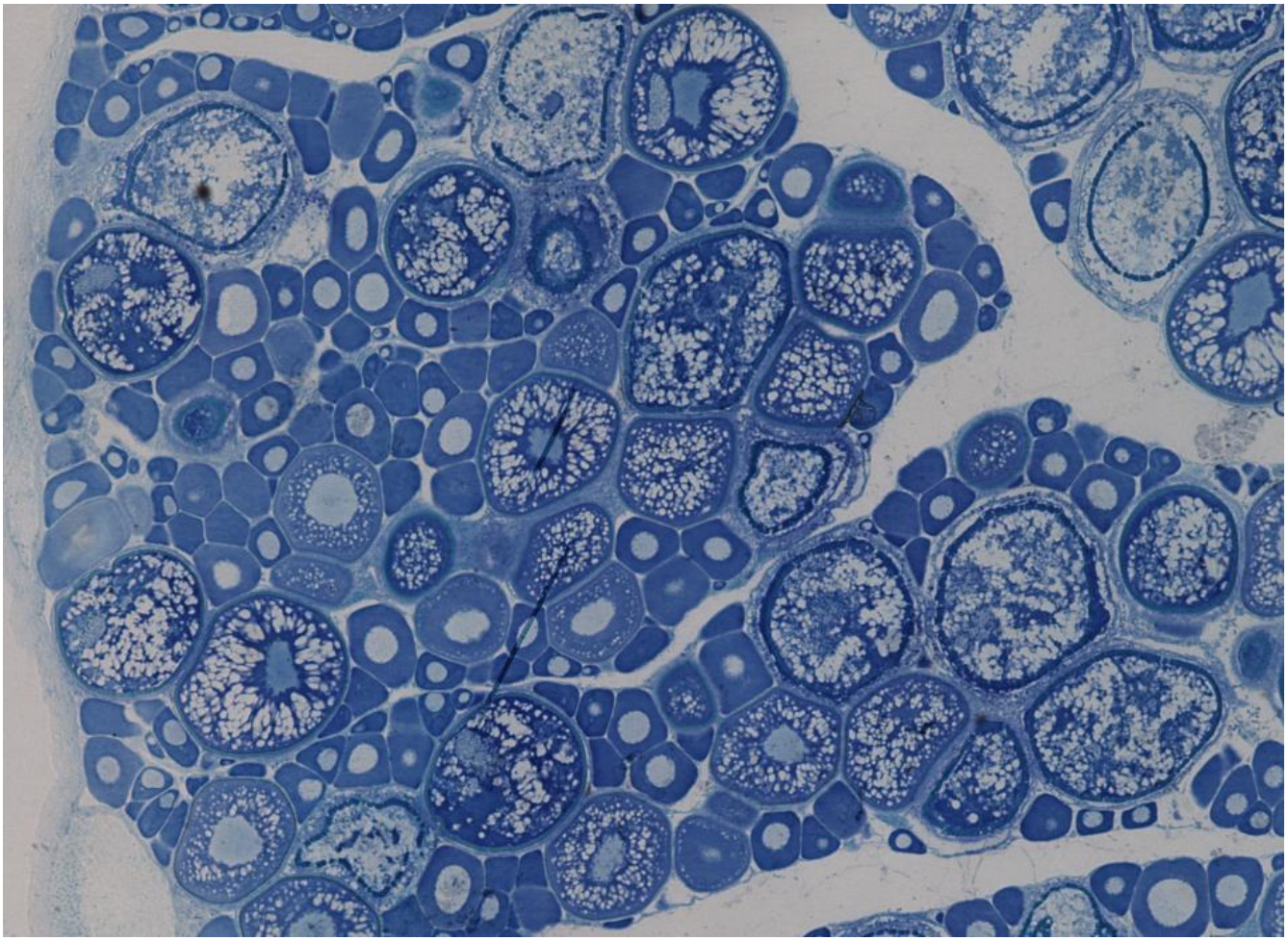

Figure 4.2 Histological slide of a gonad of a female mackerel showing the occurrence of massive atresia. Copyright: Merete Fonn, IMR, Norway.

From the collection of maturity scales that were reviewed during WKMAT, it was evident that almost all could be converted into the one used in the IBTS, although as pointed before, the problems of the omitting spawners and the difficulties in the distinction between the Recovery and Virgin stages for hake lead WKMAT to propose a new scale adapted from the one internationally agreed by the IBTS group. This proposed standard scale is described in Table 4.1. WKMAT further recommended that species specific workshops, such as WKMSMAC, should also consider the 5 grade standard scale proposed.

Table 4.1 Standard maturity stage scale proposed by WKMAT (ICES, 2007).

FEMALES	STAGE	MALES	MATURE/IMMATURE
Ovaries translucent without visible oocytes.	IM Virgin	Stringlike 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 do not contribute to the SSB in the current year.

That recommendation was taken into account by WKMSMAC. In fact, in one of the institutes present at the workshop (DIFRES, Denmark) the old IBTS scale is currently used, which facilitates the adoption of the new standard scale. However, most of the institutes use the Walsh scale, therefore a way to convert the maturity stages as described in the Walsh scale into those of the standard scale was discussed during WKMSMAC. Although mackerel gonads have been found with ongoing massive atresia, this evidence was considered still scarce in order to consider including an omitted spawning stage. Therefore, a 4 maturity stage standard scale, based on the one suggested by WKMAT was considered by WKMSMAC.

The reproductive cycle of mackerel and horse mackerel, as described by the Walsh scale, follows the scheme in Figure 4.3. Once the sex of a young fish can be visually identified, that fish is considered immature, until it starts the reproductive cycle for the first time. Then, it passes maturity Stages 2 and 3 till it spawns (reaching Stage 4) for the first time. Both species are batch spawners, meaning that the eggs spawned during a spawning season are matured and released in separate batches; the fish goes to maturity Stage 5 after releasing a batch, but returns to Stage 4 as soon as another batch starts to be spawned. This cycle is repeated the number of times that a batch is released. After all batches in the current season are spawned, the fish goes from Stage 5 to Stage 6 and starts a new reproductive cycle from Stage 2 (having spawned once, it will never return to Stage 1).

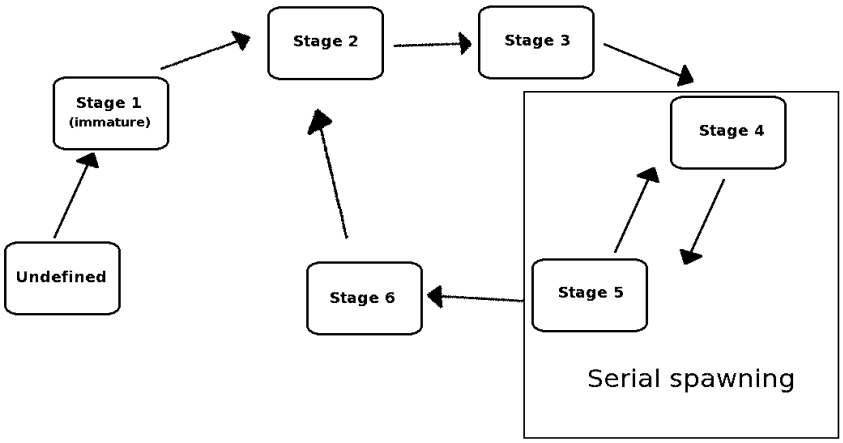

Figure 4.3 Reproductive cycle of mackerel and horse mackerel as described by the Walsh scale.

Taking into consideration the standard scale proposed by WKMAT and the description of the reproductive cycle given by the Walsh scale, the Workshop has proposed that the Walsh scale could be fitted into the standard scale in the way described in Table 4.2. This scale was also used for calibration between observers (TOR b) along with the Walsh scale.

Table 4.2 Proposed combination of the Walsh maturity scale into the standard scale.

STANDARD	WALSH	MATURE/ IMMATURE	STATE	FEMALE	MALE
1	1	Immature	Immature	Gonads small. Ovaries wine red and clear, torpedo shaped.	Gonads small. Males pale, flattened and transparent.
2	2	Mature	Maturing	Gonads occupying 1/4 to 3/4 body cavity. Opaque eggs visible in ovaries giving pale pink to yellowish colouration, largest eggs without oil globule.	Gonads occupying 1/4 to 3/4 body cavity. Testes off-white, milt not running.
	3	Mature	Maturing	Gonads occupying 3/4 to almost filing body cavity. Ovaries yellow to orange. Largest eggs may have oil globules.	Gonads occupying 3/4 to almost filing body cavity. Testes creamy white.
3	4	Mature	Spawning	Ovaries characterized by externally visible hyaline eggs no matter how few or how early the stage of hydration. Ovary size variable from full to 1/4.	Testes filling body cavity, milt freely running.
	5	Mature	Spawning	Gonads occupying 3/4 to < 1/4 body cavity. Ovaries slacker than in stage 3 and often bloodshot.	Gonads occupying 3/4 to < 1/4 body cavity. Testes with free running milt and shrivelled at anus end.
4	6	Mature	Spent/ Recovery	Gonads occupying 1/4 or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs.	Gonads occupying 1/4 or less of body cavity. Testes opaque with brownish tint and no trace of milt.

Because of the loop between Stages 4 and 5 (Figure 4.3) the Workshop proposes to include these into Stage 3 (spawning) of the standard scale, following the rationale that the alternation of those stages corresponds to a single spawning season. Some participants were of the opinion that the loop could, in certain cases, include also the Stage 3 of the Walsh scale, which would include those gonads in the Spawning state of the standard scale. This is especially relevant for the horse mackerel females, given that, being an indeterminate spawner (Abaunza *et al*., 2003), new oocytes are produced during the spawning season and there is the possibility that in a given batch, the number of oocytes to be released is so high that the gonad takes the aspect corresponding to stage 3 of the Walsh scale. This would not be the case with mackerel which is considered, for practical purposes, a determinate spawner in Northeastern Atlantic waters (Greer Walker *et al.*, 1994). However, in the Northwestern Atlantic mackerel is considered an indeterminate spawner (Auteuil and Grégoire, 1993). If there is not a production of new oocytes once spawning has begun, it is unlikely that the ovaries may have, in the middle of the spawning season, the size and aspect corresponding to the Stage 3 of the Walsh scale. The Workshop agreed that in both species, the majority, if not all, of the gonads in Stage 3 (Walsh scale) belong to fish that did not spawn yet in the current spawning season, and that including Stage 3 of the Walsh scale in the Maturing state of the standard scale would minimize the misclassification rates.

5 Calibration experiments

5.1 Procedure

Before the Workshop several samples of mackerel and horse mackerel were collected during surveys, both within and out of the spawning season, in order to have a wide range of maturity stages in the calibration. The fish were kept frozen and thawed just prior to the calibration exercise, in order to avoid deterioration of the gonads. Each individual was identified with a number, and carefully opened to keep the gonads intact. The gonads were observed together with the whole fish to ensure the conditions under which the staging took place resembled the field situation as much as possible and giving the opportunity to use the important staging criteria, such as volume of the gonad in relation to the body cavity.

Ideally a calibration should be carried out with gonads of a 'known' stage. This 'known' stage can only be assessed through microscopic determination of histological sections of the gonad. The results of such a calibration exercise could show the deviation of the observers from the "real" maturity stage. And at the same time check if the descriptions of the different stages of the maturity scale describe the reproductive cycle of each species. For practical reasons such an exercise was not possible. The gonads were frozen (destroying the cell structure) and the duration of the workshop was too short to carry out detailed histological analysis and discussion. Therefore, the objective of this workshop was restricted to compare and improve the agreement between readers.

Before the first calibration experiment, each observer was randomly assigned a number. This number was used to identify the observer in the experiments carried out during the Workshop. No personal information was recorded concerning the classification of the maturity stages. Therefore, the calibration results will be analysed on an individual basis, given that it is not possible to analyse those results by pooling observers from the same lab or institute.

The calibration was carried out twice. On the first day (Nov. 27th morning) the observers were asked to classify the maturity stages of the fish using three different scales:

- the one they currently use (Walsh scale adapted by each lab)
- the original Walsh scale (following strictly the criteria described there)
- the standard scale proposed by the Workshop.

The (anonymous) results of this calibration were discussed in a plenary session during the afternoon, while looking at photographs from the gonads analysed in the morning. This experiment was mainly meant to compare agreement in staging and sexing between observers, using the different scales. The morning of the second day a new calibration exercise was carried out. Using only the new proposed standard scale, to asses the increase in agreement between observers using this scale, these results were again discussed and reviewed in the afternoon plenary session.

All observers were trained in the classification of maturity stages of one or both species, although levels of experience differed (e.g. some observers stage fish through the year while others only do the staging while on survey). Hence, it was assumed that the maturity stage attributed to a given gonad by the majority of the observers (the modal stage) is the "correct" one. Results of the calibrations were analysed by

comparing the maturity stage attributed by each observer to the modal stage of the gonad.

5.2 Results

Tables A.2 to A.13 in the Annex contain the raw data obtained from both calibration exercises. Some observers preferred not to participate in the experiments for one of the species (mackerel or horse mackerel) because they usually do not sample that species. Those observations are therefore missing from the tables. All observers are identified by their number, which was the same in both exercises.

5.2.1 Sex identification

Tables A.2 to A.5 in the Annex show the sex of each fish, as attributed by each observer. Tables 5.1 and 5.2, for mackerel and horse mackerel respectively, show the agreement of each observer with the modal sex. Agreement is high for both species and increases on the second exercise. In the first calibration gonads of very small fish were included. These are difficult to classify, both in terms of sex and maturity stage, especially for less experienced observers. Also, the overall condition of the gonads was better in the second exercise because of a faster thawing process. This, together with the discussion of the gonads after the first exercise, explains the lower agreement for some observers on the first day. Agreement in horse mackerel is slightly lower compared to mackerel suggesting horse mackerel is a difficult species to attribute sex to.

Table 5.1 Agreement with the modal sex attributed to mackerel by each observer.

OBSERVER	SEX 1ST EXERCISE	SEX 2ND EXERCISE
1	0.9	
2		1
3	0.62	0.83
4	0.84	1
5	0.95	1
6	0.9	1
7	0.95	1
8		
9	0.88	1
10		
11	0.81	1
12		0.92
13	0.95	1
14	0.86	1
15	0.9	1
16	0.57	1
17		0.92
18	0.9	1
19	0.86	1
20	0.95	1
21	1	1
22	1	1
23	0.83	0.75
24	0.89	1
Mean	0.87	0.97

Table 5.2 Agreement with the modal sex attributed to horse mackerels by each observer.

OBSERVER	SEX 1ST DAY	SEX 2ND DAY
1	0.96	
2		0.95
3	0.72	0.9
4	0.91	0.95
5	0.88	1
6	0.92	1
7	0.92	0.95
8	0.92	1
9	0.96	1
10	1	1
11	0.77	0.95
12	0.92	1
13	0.92	0.95
14	0.92	1
15	0.92	0.95
16	0.86	0.9
17		0.86
18	0.96	1
19	0.84	0.95
20	0.95	0.67
21	0.92	0.95
22	0.88	0.95
23	0.91	0.95
24	0.88	0.95
Mean	0.9	0.95

5.2.2 Classification of sexual maturity stages of mackerel

Figure 5.1 shows the proportion of observers, which attributed the modal maturity stage to each mackerel. The highest agreements (around 0.9) were obtained with the Walsh scales (the modified and the original one). The worst agreement was also obtained with these scales (around 0.4). While the standard scale had lower maximum agreement (around 0.8), the worst agreements in these scales (around 0.5) were higher compared to the Walsh scales. There is a slight overall improvement in agreement in the standard scale from the first to the second exercise.

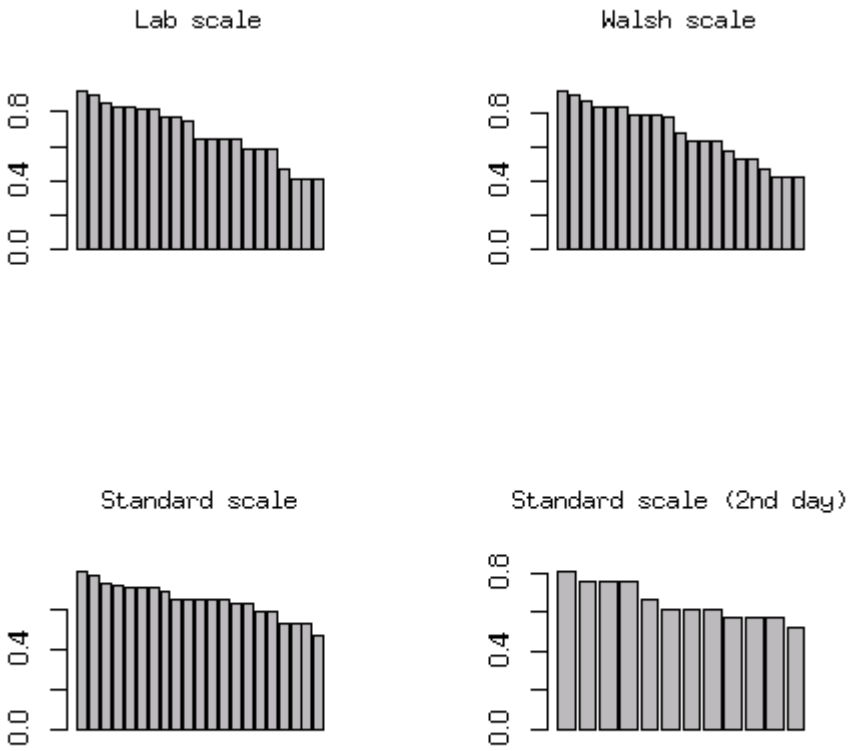

Figure 5.1 Proportion of observations that correspond to the modal stage, for each scale applied to mackerel. Each bar corresponds to a fish. Bars are ordered from highest to lowest agreement.

The Walsh scale adapted by each lab (Table 5.3) showed the highest agreement for modal Stage 1. While Stage 6 also had a high agreement (0.7), for the modal Stages 3 and 5 less than 50% of the observations corresponded to the modal stage. These last stages also shared the highest misclassifications: 38% of the observations from modal Stage 3 were classified as Stage 5, and 25% from Stage 5 were classified as Stage 3. Regarding the results obtained with the original Walsh scale (Table 5.4), these are very similar to those from the adapted scale, though the overall agreement increased in the adapted scale.

Table 5.3 The proportion each stage was attributed to mackerels in a given modal stage using the adapted Walsh scale.

		Attributed stage					
		1	2	3	4	5	6
Modal stage	1	0.84	0.06	0	0	0	0.1
	2						
	3	0	0.12	0.41	0.03	0.38	0.06
	4						
	5	0	0.12	0.25	0	0.49	0.14
	6	0.02	0.15	0	0.04	0.09	0.7

Table 5.4 The proportion each stage was attributed to mackerels in a given modal stage using the original Walsh scale.

Modal stage	Attributed stage 1	2	3	4	5	6
1	0.86	0.03	0		0	0.12
2						
3	0	0.16	0.45		0.34	0.05
4						
5	0	0.12	0.26		0.49	0.12
6	0.02	0.17	0.01		0.1	0.69

In the application of the standard scale in the first exercise (Table 5.5) some observers still used Stages 5 and 6 as in the Walsh scale. The agreement improved to all stages above 60%, higher compared to the Walsh scales. However, about 30% of the observations from gonads in modal Stage 1 (immature) were attributed to Stages 2 and 4 (both mature). This is a significant proportion, especially taking into account that an accurate distinction between immature and mature fish is very important for maturity ogives. Also the misclassification between pre-spawning (Stage 2) and resting (Stage 4) is about 30%. Given the high misclassification between Stages 3 and 5 of the Walsh scale (Tables 5.3 and 5.4), it is expected to find a higher misclassification, in the corresponding stages 2 and 3 of the standard scale.

Table 5.5 The proportion each stage was attributed to mackerels in a given modal stage using the standard scale proposed by the Workshop. The scale only has 4 stages, the classification into stage 5 and 6 were mistakes of the observers.

Modal stage	Attributed stage 1	2	3	4	5	6
1	0.86	0.03	0		0	0.12
2						
3	0	0.16	0.45		0.34	0.05
4						
5	0	0.12	0.26		0.49	0.12
6	0.02	0.17	0.01		0.1	0.69

In the second exercise (Table 5.6), after the discussion of the results from the previous day, agreement improved and the combination of the Walsh scale into the standard scale was followed by all observers. The misclassifications between Stages 2 and 3 were higher than those observed in the previous exercise, hence matching the misclassification between Stages 3 and 5 observed for the Walsh scale.

Table 5.6 The proportion each stage was attributed to mackerels in a given modal stage using the standard scale proposed by the Workshop.

Modal stage	Attributed stage 1	2	3	4
1				
2	0	0.52	0.43	0.05
3	0	0.34	0.65	0.01
4	0.02	0.18	0.1	0.7

The overall agreement with the modal sex by each observer (Table 5.7) shows for females the percentage of observations that contributed to the mode was around 75% throughout the calibration exercises. For males agreement decreased when switching from Walsh to the standard scales, and especially from the first to the second application of the standard scales.

Table 5.7 Overall agreement, by sex, between the modal stage and the stage attributed by each observer to each mackerel.

		Attributed stage			
		1	2	3	4
Modal stage	1				
	2	0	0.52	0.43	0.05
	3	0	0.34	0.65	0.01
	4	0.02	0.18	0.1	0.7

Some observers kept their misclassification rates stable during the whole exercise (e.g. observers 1 and 20, Table 5.8), while others increased the agreement with the modal stages from the Walsh to the standard scale and from one day to the other (e.g. observers 6, 18 and 5). Regarding the standard scale, eight of the observers improved the agreement from one day to the other, while nine observers showed the opposite trend.

Table 5.8 Agreement between maturity stages attributed to mackerels by each observer with the modal stage.

OBSERVER	WALSH MODIFIED	WALSH ORIGINAL	STANDARD 1ST EXERCISE	STANDARD 2ND EXERCISE
1	0.6	0.65	0.65	
2				0.58
3		0.48	0.57	0.33
4	0.81	0.81	0.88	0.83
5	0.67	0.67		0.75
6	0.33	0.33	0.43	0.67
7	0.86	0.86	0.81	0.58
8				
9		0.29	0.19	0.58
10				
11	0.7	0.75	0.75	0.83
12				0.83
13	0.76	0.76	0.81	0.67
14	0.75	0.75	0.75	0.58
15	0.76	0.76	0.86	0.83
16	0.24	0.62	0.81	0.5
17				0.75
18	0.33	0.33	0.43	0.67
19	0.71	0.71	0.52	0.33
20	0.57	0.57	0.57	0.58
21	0.72	0.72	0.89	0.83
22	0.9	0.9	0.6	0.83
23	0.89	0.89	0.44	0.58
24	0.89	0.89		0.58
Overall	0.68	0.67	0.64	0.65

5.2.3 Classification of sexual maturity stages of horse mackerel

The agreement to the modal maturity stage in horse mackerel (Figure 5.2) shows that for all scales, the highest agreement was close to 90% and the minimum around 40%. However, in the application of the standard scale in the second exercise, overall agreements (above 50%) were higher.

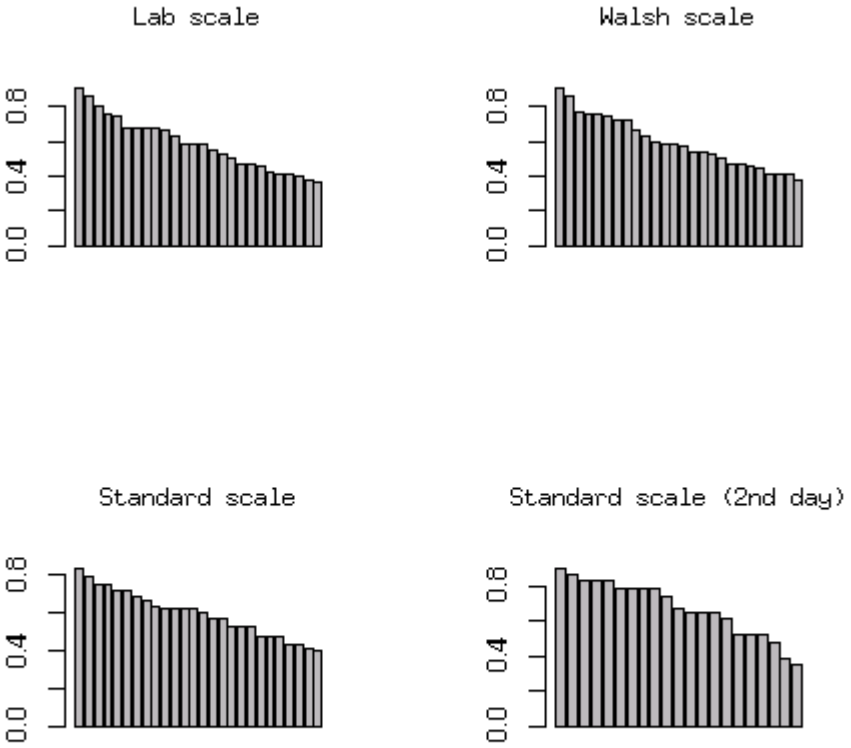

Figure 5.2 Proportion of observations that correspond to the modal stage, for each scale applied to horse mackerel. Each bar corresponds to a fish. Bars are ordered from the highest to the lowest agreement.

The application of the adapted Walsh scale (Table 5.9) showed agreement below 50% only for Stage 3. Most of the misclassified fish in modal Stage 3 were classified as Stage 5. The highest misclassifications were from Stage 3 into Stage 5 (33%), from Stage 1 into Stage 2 (29%), and from Stage 6 into Stage 5 (22%). The differences in results between the application of the adapted and the original Walsh scale (Table 5.10) were negligible.

Table 5.9 The proportion each stage was attributed to horse mackerels in a given modal stage using the adapted Walsh scale.

		Attributed stage					
		1	2	3	4	5	6
Modal stage	1	0.6	0.29	0	0	0	0.11
	2	0.03	0.65	0.08	0.01	0.14	0.09
	3	0.02	0.12	0.42	0.09	0.33	0.02
	4						
	5	0	0.12	0.2	0.06	0.58	0.04
	6	0.01	0.13	0.05	0.06	0.22	0.53

Table 5.10 The proportion each stage was attributed to mackerels in a given modal stage using the original Walsh scale.

Modal stage \ Attributed stage	1	2	3	4	5	6
1	0.6	0.29	0	0	0	0.11
2	0.03	0.65	0.08	0	0.14	0.1
3	0	0.14	0.44	0.11	0.29	0.03
4						
5	0	0.09	0.24	0.05	0.57	0.05
6	0.01	0.15	0.04	0.02	0.2	0.58

The results obtained with the standard scale in the first exercise (Table 5.11) were better compared to both the adapted and the original Walsh scales. In all modal stages agreement was above 50%. The highest misclassifications were between Stages 1 and 2 (28%) and between Stages 2 and 4 (26%). This latter is comparable with the misclassification between Stages 3 and 5 in the Walsh scale (which are respectively included in Stages 2 and 4 of the standard scale). In the second exercise the results obtained with the standard scale were generally improved (Table 5.12), although no fish in modal Stage 1 was present in this exercise. The highest misclassification was between Stages 2 and 4 (37%), while the agreement with the modal stage was well above 60% for all stages.

Table 5.11 The proportion each stage was attributed to mackerels in a given modal stage using the standard scale proposed by the Workshop. The scale only has 4 stages; the classification into stage 5 was a mistake of the observers.

Modal stage \ Attributed stage	1	2	3	4	5	6
1	0.53	0.28	0.03	0.12	0.03	0
2	0.03	0.62	0.08	0.26	0	0
3						
4	0.02	0.22	0.16	0.54	0.07	0

Table 5.12 The proportion each stage was attributed to mackerels in a given modal stage using the standard scale proposed by the Workshop.

Modal stage \ Attributed stage	1	2	3	4
1				
2	0.01	0.58	0.04	0.37
3	0	0.2	0.68	0.11
4	0	0.16	0.11	0.73

Overall agreement between the modal stage and the stages attributed by each observer (Table 5.13) shows, for both sexes, best agreement was obtained with the standard scales. Moreover, there is a clear increase in agreement from the first to the second exercise in the application of the standard scale.

Table 5.13 Overall agreement, by sex, between the modal stage and the stage attributed by each observer to each horse mackerel.

	Males	Females
Lab	0.62	0.59
Walsh	0.62	0.6
Standard day 1	0.65	0.61
Standard day 2	0.68	0.69

Table 5.14 Shows the agreement with the modal stages attained by each observer. The overall pattern is a similar agreement, for each observer, between the adapted and the original Walsh scale. The standard scale, showed a notorious improvement in the second exercise for the majority of the observers, though some showed a remarkable decrease. Agreement between maturity stages attributed to horse mackerels by each observer with the modal stage.

OBSERVER	WALSH MODIFIED	WALSH ORIGINAL	STANDARD 1ST EXERCISE	STANDARD 2ND EXERCISE
1	0.5	0.62	0.69	
2				0.71
3	0.42	0.54	0.73	0.19
4	0.65	0.65	0.35	0.85
5	0.69	0.69	0.73	0.86
6	0.42	0.42	0.62	0.85
7	0.62	0.62	0.58	0.67
8	0.54	0.5	0.42	0.81
9	0.54	0.54	0.69	0.43
10	0.84	0.84	0.68	0.81
11	0.42	0.42	0.54	0.48
12	0.65	0.65	0.72	0.9
13	0.52	0.52	0.61	0.6
14	0.58	0.58	0.69	0.57
15	0.67	0.67	0.42	0.38
16	0.57	0.57	0.53	0.57
17				0.57
18	0.5	0.5	0.65	0.85
19	0.52	0.52	0.68	0.67
20	0.36	0.36	0.27	0.67
21	0.62	0.62	0.58	0.85
22	0.84	0.84	0.52	0.67
23	0.61	0.61	0.39	0.67
24	0.68	0.68	1	0.86
Overall	0.58	0.59	0.6	0.67

6 Conclusions

The background of the participants in the Workshop was different, some have worked on the reproductive traits of mackerel or horse mackerel for several decades, while others did not have much (or none) experience with one of the species. Nevertheless, agreement in the identification of sex was very good. The biggest difficulties in sex identification had to do with the conjugation of several factors: the inexperience of some observers with one species or the other, the small size of some gonads and, in some cases, their lack of freshness.

The results of the maturity staging were different between mackerel and horse mackerel. For mackerel, highest agreements were obtained with the adapted Walsh scale and in general there was a decrease in agreement when using the standard scale. The Walsh scale provided a high agreement for Stage 1 (immature), which is important taking into consideration the use of maturity data for the calculation of maturity ogives. The standard scale had a more balanced agreement across stages than the Walsh scale. However, the agreement for Stage 1 was much lower (0.7) than the one obtained with the Walsh scale (0.86). This indicates that in some cases there is the possibility of substantial errors in the calculation of maturity ogives.

While most of the misclassifications using the Walsh scale on mackerel were between Stages 3 and 5, for the standard scale these were between Stage 2 and 4. If the fitting of the Walsh scale into the standard scale, as agreed during the Workshop, would have been strictly followed, highest misclassifications would be expected between Stages 2 and 3 of the standard scale. This incongruence is difficult to explain. Another unexpected result was the decrease in the agreement obtained with the standard scale from the first to the second exercise. This decrease seems to be due to the male fish present in the samples, given that the overall agreement for females was more or less constant during the whole calibration exercise.

For horse mackerel, the application of the standard scale resulted in more fish with agreement above 0.5 than with the Walsh scale. For this species there were no significant differences between males and females. Also, as expected, the level of agreement was higher for the standard than for the Walsh scale, and it increased from the first to the second exercise for most observers. However, this was not the case for Stage 1, which had a lower agreement with the standard than with the Walsh scale. Almost one third of the fish in modal Stage 1 were classified as being in Stage 2, which may indicate a low precision in the calculation of maturity ogives. The agreement for Stage 1 in the second exercise was not possible to determine since no immature fish were available.

The results of the application of the Walsh scale on horse mackerel showed that most of the misclassifications were between Stages 3 and 5. The corresponding misclassification on the standard scale would be between Stages 2 and 4, which is not evident in the data. In the application of the standard scale in the first day, no fish had Stage 3 as the mode, while several of those fish were mostly classified as being in the respective Stage 5 in the Walsh scale. This indicates that, also for horse mackerel, the agreed fitting of the Walsh scale into the newly proposed standard scale was not always strictly followed during the calibration exercise.

The standard scale is proposed by the workshop as a good description of the mackerel and horse mackerel reproductive cycle, with an adequate level of detail for most purposes, being especially useful for exchanging and comparing data between

observers or institutes. In some particular situations, a higher level of detail may be needed. For example, when sampling for the estimation of fecundity in the Daily Egg Production Method (currently applied to the southern horse mackerel), the objective is to obtain gonads with hyaline oocytes but without post-ovulatory follicles. This stage corresponds to Stage 4 of the Walsh scale, however in the standard scale the gonads in that stage are joined with gonads already spent, both corresponding to Stage 3 of the standard scale. Therefore, the use of more detailed scales is not prejudicial, as long as those can be fitted in a straightforward manner into the standard scale, and all observers using them show a good agreement between themselves. If these conditions are met, the translation of a more detailed scale into the standard scale should, in principle, increase the level of agreement between observers.

6.1 Recommendations

WKMSMAC recommends that the proposed standard scale for sexual maturity staging should be adopted for all sexual maturity sampling, as a minimum acceptable level of detail. This means that other scales currently used that are less detailed should be abandoned in favour of this standard scale. If more detailed scales, such as the Walsh scale, are being used, it should be ensured that those can be fitted into the standard scale and that there is a good agreement between observers using those scales.

WKMSMAC recommends that, when a maturity ogive based exclusively on histological data is not possible to obtain, data on the GSI, HSI, or from histological examination of part of the samples should be analysed, in order to check the accuracy of the resulting maturity ogive. This is based on the fact that it can be difficult to make a clear macroscopic distinction between recovering and virgin females (or identification of the omitting spawning individuals if they exist).

WKMSMAC recommends having a workshop on maturity of mackerel and horse mackerel every three years, in the year prior to the Atlantic mackerel and horse mackerel egg survey. Both technicians and scientist involved in the sampling of mackerel and horse mackerel should be involved in this workshop.

WKMSMAC recommends that, in case a future workshop will take place, all fish to be used should have the information on sampling date and area with it. These data are available when doing the real-time sampling and can be used to help to classify the maturity stages, especially in geographical areas where the spawning season is short and well defined. The circumstances at the workshop should be as much the same as possible as real-time sampling. If pictures are presented at the workshop, sampling date, area, length and weight should be included for each individual fish.

WKMSMAC recommends that, in a future workshop, the presentation of gonads should include pictures of histological sections as well as pictures of the whole gonad. The histological sections are the only means of resolving the differences in the macroscopic determination.

WKMSMAC recommends that the question whether or not skipped spawning occurs in mackerel and horse mackerel should be addressed in future workshops.

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Annex 2: Tables

Table A.2 Sex classification of mackerel (1st day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21
1	m	m	m	m	m	m	m	m		m	f	f	f	m	m	m	f	m	m	f	f
2																					
3	m	f	m	f	f	m	f	f						m	m	f	f	m	f	f	f
4	m	m	m		f	m		m	f	i	i	f	i	m	m	m	f	m	m	f	f
5	m	m	m	m	f	m	m	m	m	m	f	f	m	m	m	m	f	m	m	f	f
6	m	m	m	m	f	m	m	m	f	m	m	f	m	m	m	f	f	m	m	f	f
7	m	m	m	m	f	m	m	m	m	m	f	f	m	m	m	m	f	m	m	f	f
8																					
9	m	m	m	m	m	m	m	m			f			m	m	m	f	m	f	f	f
10																					
11	m	f	m	m	f	f	m	m	i	f	f	f	m	m	m	m	f	m	m	f	f
12																					
13	m	m	m	m	f	m	m	m	f	m	f	f	m	m	m	m	f	m	f	f	f
14	m	m	m	m	m	m	m	m	i	m	f	f	f	m	m	m	f	m	m	f	f
15	m	m	m	m	f	m	m	m	f	f	f	f	f	m	m	m	f	m	m	f	f
16	f	f	f	m	f	f	m	m	i	i	i	i	i	m	m	m	f	m	m	f	f
17																					
18	m	m	m	m	f	m	m	m		m	m	f	m	m	m	f	f	m	m	f	f
19	m	m	m	m	f	m	m	m	m	f	f	f	f	m	m	m	f	m	m	f	f
20	m	m	m	m	f	m	m	m	m	m	f	f	m	m	m	m	f	m	m	f	f
21	m	m	m	m	f	m	m	m	f		f	f		m	m	m	f	m	m	f	f
22	m	m	m	m	f	m	m	m		m	f	f	m	m	m	m	f	m	m	f	f
23	m	m	m	m	m	m	m	m			f	m		m	m	m	m	m	m	f	f
24	m	m	m	m	f	m	m	m		m	m	m		m	m	m	f	m	m	f	f
Mode	m	m	m	m	f	m	m	m	f	m	f	f	m	m	m	m	f	m	m	f	f

Table A.3 Sex classification of horse mackerel (1st day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21	Fish 22	Fish 23	Fish 24	Fish 25	Fish 26
1	f	m	f	m	m	f	f	m	f	f	m	m	f	f	f	f	m	m	m	f	f	f	m	m	f	f
2																										
3	f	m	f	m	f	f	m	f	f	m	f	m	m	f	f		m	m	m	f	f	f	m	m	f	f
4	f	m	f	m		f		m	f	f		m	f	f	f	f	i	i	m		f	f	m	m	f	f
5	f	m	f	m	m	f	f	m	f	f	f	m	f	f	f	f	f	f	m	m	f	f	m	m	f	f
6	f	m	f	m	m	f	f	m	f	m	m	m	f	f	f	f	m	m	m	m	f	f	m	f	f	f
7	f	m	f	m	m	f	f	m	f	m	m	m	f	f	f	f	m	m	m	m	f	f	m	f	f	f
8	f	m	f	m	m	f	f	m	f	f	m	m	f	f	f	f	f	f	m	m	f	f	m	m	f	f
9	f	m	f	m	m	f	f	m	f	f	m	m	f	f	f	f	m	f	m	m	f	f	m	m	f	f
10	f	m	f	m	m	f	f	m	f	f	m	m	f	f	f	f	m		m	m	f	f	m	m	f	f
11	f	m	f	m	f	f	m	f	f	f	m	m	f	f	f	f	i	i	m	f	f	f	m	m	f	f
12	f	m	f	m	m	f	f	m	f	f	m	m	f	f	f	f	f	f	m	m	f	f	m	m	f	f
13	f	m	f	m	m	f	f		f	f	f	m	f	f	f	f	m		m	m	f	m	m	m	f	f
14	f	m	f	m	m	f	f	m	f	f	m	m	f	f	f	f	f	m	m	f	f	f	m	m	f	f
15	f	m	f	m	m	f	m	f	f	f	m	m	f	f	f	f			m	m	f	f	m	m	f	f
16	f	m	f	m	m	f	m	f	f	m	m	m				f			m	m	f	f	m	m	f	f
17																										
18	f	m	f	m	m	f	f	m	f	m	m	m	f	f	f	f	m	m	m	m	f	f	m	m	f	f
19	f	m	f	m	m	f	f	f	f	f	f	m	f	f	f	f	f		m	f	f	f	m	m	f	f
20	f	m	f		m	f		f	f	f	m	m	f	f	f	f			m	m	f	f	m	m	f	f
21	f	m	f	m	m	f	m	f	f	f	m	m	f	f	f	f			m	m	f	f	m	m	f	f
22	f	m	f	m	m	f	m	f	f	f		m	f	f	f	f	m	f	m	m	f	f	m	m	f	f
23	f	m	f	m	m	f	m	f	f	f	m	m	f	f	f	f	m		m		f		m	m	f	f
24	f	m	f	m	m	f	m	f	f	f	m	m	f	f	f	f	m		m	m	f	m	m	m	f	f
Mode	f	m	f	m	m	f	f	m	f	f	m	m	f	f	f	f	m	m	m	m	f	f	m	m	f	f

Table A.4 Sex classification of mackerel (2nd day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12
1												
2	m	f	f	m	f	m	m	m	m	m	f	m
3	f	f	f	f	f	m	m	m	m	m	f	m
4	m	f	f	m	f	m	m	m	m	m	f	m
5	m	f	f	m	f	m	m	m	m	m	f	m
6	m	f	f	m	f	m	m	m	m	m	f	m
7	m	f	f	m	f	m	m	m	m	m	f	m
8												
9	m	f	f	m	f	m	m	m	m	m	f	m
10												
11	m	f	f	m	f	m	m	m	m	m	f	m
12	m	f	f	m	f	f	m	m	m	m	f	m
13	m	f	f	m	f	m	m	m	m	m	f	m
14	m	f	f	m	f	m	m	m	m	m	f	m
15	m	f	f	m	f	m	m	m	m	m	f	m
16	m	f	f	m	f	m	m	m	m	m	f	m
17	f	f	f	m	f	m	m	m	m	m	f	m
18	m	f	f	m	f	m	m	m	m	m	f	m
19	m	f	f	m	f	m	m	m	m	m	f	m
20	m	f	f	m	f	m	m	m	m	m	f	m
21	m	f	f	m	f	m	m	m	m	m	f	m
22	m	f	f	m	f	m	m	m	m	m	f	m
23	f	f	f	f	f	m	f	m	m	m	f	m
24	m	f	f	m	f	m	m	m	m	m	f	m
Mode	m	f	f	m	f	m	m	m	m	m	f	m

Table A.5 Sex classification of horse mackerel (2nd day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21
1																					
2	f	f	m	m	f	m	f	m	m	m	f	m	m	f	m	m	f	f	f	f	f
3	f	f	m	m	f	m	f	m	m	f	f	f	m	f	f	m	f	f	f	f	f
4	f	f	f	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
5	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
6	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
7	f	f	m	m	f	m	f	m	m	m	f	m	m	f	m	m	f	f	f	f	f
8	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
9	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
10	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
11	f	f	m	m	f	m	f	m	m	m	f	m	m	f	m	m	f	f	f	f	f
12	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
13	f	f	m	m	f	m	f	m	m	m	f	m	m	f	m	m	f	f	f	f	f
14	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
15	m	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
16	f	f	m	m	f	m	m	m	m	m	f	m	m	f	f	m	m	f	f	f	f
17	f	f	m	m	m	f	f	m	m	m	f	m	m	f	f	m	f	m	f	f	f
18	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
19	f	f	m	m	f	m	f	m	m	f	f	m	m	f	f	m	f	f	f	f	f
20	f	f	m	m	f	m	m	m	m	f	m	m	f	m	m	f	f	f	f	f	f
21	f	f	f	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f
22	f	f	m	m	f	m	f	m	m	m	f	m	m	f	m	m	f	f	f	f	f
23	f	f	m	m	f	m	m	m	m	m	f	m	m	f	f	m	f	f	f	f	f
24	f	f	m	m	f	m	f	m	m	m	m	m	m	f	f	m	f	f	f	f	f
Mode	f	f	m	m	f	m	f	m	m	m	f	m	m	f	f	m	f	f	f	f	f

Table A.6 Classification of mackerel maturity stages according to the Walsh scale with adaptations made by each laboratory.

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21
1	6	6	2	6	5	6	6	2		2	2	1	1	4	5	6	6	5	6	5	6
2																					
3																					
4	6	6	6		2	6		1	1			1		3	3	6	6	5	6	5	6
5	1	1	6	6	6	6	6	6	1	1	1	1	1	5	5	5	6	2	6	5	6
6	2	2	2	2	2	2	2	2	1	1	1	1	1	3	3	5	3	3	5	3	5
7	6	6	6	6	6	6	6	6	1	1	1	1	1	5	5	6	5	3	6	5	6
8																					
9																					
10																					
11	6	6	6	6	6	6	6	6		1	1	1	1	5	5	5	2	5	6	3	5
12																					
13	6	6	6	6	6	6	6	6	1	1	1	1	1	3	3	2	5	3	5	3	5
14	6	6	2	6	5	6	6	2		1	1	1	1	3	5	6	6	5	6	5	6
15	6	6	6	6	6	6	6	6	1	1	2	2	1	3	3	6	5	3	6	3	5
16	4	4	4	4	4	4	4	4	1	1	1	1	1	2	2	2	2	2	2	2	2
17																					
18	2	2	2	2	2	2	2	2	1	1	1	1	1	3	3	5	3	3	5	3	5
19	6	6	1	6	6	6	6	6	1	1	1	1	1	3	3	2	5	2	2	3	5
20	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6
21	6	6	6	6	6	6	6	6			6	6		5	3	6	6	5	5	5	6
22	6	6	6	6	6	6	6	6		1	1	1	1	5	5	6	5	5	6	5	6
23	6	6	6	6	6	6	6	6			1	1		2	2	6	5	5	6	5	6
24	6	6	6	6	6	6	6	6		1	1	1		5	5	6	5	5	6	5	6
Mode	6	6	6	6	6	6	6	6	1	1	1	1	1	3	3	6	5	5	6	5	6

Table A.7 Classification of mackerel maturity stages by strictly following the Walsh scale criteria.

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21
1	6	6	2	6	5	6	6	2		2	2	1	1	3	5	6	6	5	6	5	6
2																					
3	6	6	6	6	5	5	6	5	1	1	1	1	1	2	2	2	3	3	3	3	3
4	6	6	6		2	6		1	1			1		3	3	6	6	5	6	5	6
5	1	1	6	6	6	6	6	6	1	1	1	1	1	5	5	5	6	2	6	5	6
6	2	2	2	2	2	2	2	2	1	1	1	1	1	3	3	5	3	3	5	3	5
7	6	6	6	6	6	6	6	6	1	1	1	1	1	5	5	6	5	2	6	5	6
8																					
9	2	2	2	2	2	2	2	2			1			3	3	2	5	3	5	5	5
10																					
11	6	6	6	6	6	6	6	6		1	1	1	1	5	5	5	2	5	6	5	5
12																					
13	6	6	6	6	6	6	6	6	1	1	1	1	1	3	3	2	5	3	5	3	5
14	6	6	2	6	5	6	6	2		1	1	1	1	3	5	6	6	5	6	5	6
15	6	6	6	6	6	6	6	6	1	1	6	6	1	3	3	6	5	3	6	3	5
16	6	6	6	6	6	6	6	6	1	1	1	1	1	2	2	2	2	2	2	2	3
17																					
18	2	2	2	2	2	2	2	2	1	1	1	1	1	3	3	5	3	3	5	3	5
19	6	6	1	6	6	6	6	6	1	1	1	1	1	3	3	2	5	2	2	3	5
20	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6
21	6	6	6	6	6	6	6	6			6	6		5	3	6	6	5	5	5	6
22	6	6	6	6	6	6	6	6		1	1	1	1	5	5	6	5	5	6	5	6
23	6	6	6	6	6	6	6	6			1	1		2	2	6	5	5	6	5	6
24	6	6	6	6	6	6	6	6		1	1	1		5	5	6	5	5	6	5	6
Mode	6	6	6	6	6	6	6	6	1	1	1	1	1	3	3	6	5	5	6	5	6

Table A.8 Classification of mackerel maturity stages according to the standard scale (1st day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21
1	4	4	2	4	4	4	4	2		2	2	1	1	2	4	4	4	4	4	4	4
2																					
3	4	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2
4	4	4	4		4	4		4	1			1		2	2	4	4	4	4	4	4
5																					
6	2	2	2	2	2	2	2	2	1	1	1	1	1	2	2	3	2	2	3	2	3
7	4	4	4	4	4	4	4	4	1	1	1	1	1	4	4	4	4	4	4	4	4
8																					
9	2	2	2	2	2	2	2	2	4	4	1	4	4	2	2	2	3	2	3	3	3
10																					
11	4	4	4	4	4	4	4	4		1	1	1	1	4	4	4	2	4	4	4	4
12																					
13	4	4	4	4	6	4	4	4	1	1	1	1	1	3	2	2	3	2	4	2	4
14	4	4	2	4	4	4	4	2		1	1	1	1	2	4	4	4	4	4	4	4
15	4	4	4	4	4	4	4	4	1	1	4	4	1	2	2	4	4	2	5	2	4
16	4	4	4	4	4	4	4	4	1	1	1	1	1	2	2	2	2	2	2	2	2
17																					
18	2	2	2	2	2	2	2	2	1	1	1	1	1	2	2	3	2	2	3	2	3
19	6	5	1	5	5	5	5	5	1	1	1	1	1	2	2	2	4	2	2	2	4
20	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
21	4	4	4	4	4	4	4	4			4	4		2	2	4	4	2	4	2	4
22	2	2	2	2	4	2	2	2		1	1	1	1	2	2	2	4	2	4	2	4
23	2	2	2	2	2	2	2	2			1	1		2	2	2	4	2	2	2	4
24																					
Mode	4	4	4	4	4	4	4	4	1	1	1	1	1	2	2	4	4	2	4	2	4

Table A.9 Classification of mackerel maturity stages according to the standard scale (2nd day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12
1												
2	4	3	4	4	3	2	4	2	2	2	2	2
3	4	2	4	2	2	2	4	2	2	2	2	2
4	4	3	3	4	3	3	4	3	3	3	3	3
5	4	3	4	2	3	3	2	3	3	3	3	3
6	2	3	4	2	3	3	2	3	3	3	3	3
7	4	3	4	4	3	2	4	2	2	2	2	2
8												
9	4	3	4	4	3	2	4	2	2	2	2	2
10												
11	4	3	4	4	3	3	4	3	3	2	3	3
12	4	3	3	4	3	3	4	3	3	3	3	3
13	4	3	3	4	3	2	4	2	2	3	3	2
14	2	3	4	4	3	3	1	2	2	2	3	2
15	4	3	4	4	3	2	4	3	3	3	4	2
16	4	2	2	4	2	2	4	2	2	3	3	2
17	3	3	4	4	3	3	4	3	3	3	2	3
18	2	3	4	2	3	3	2	3	3	3	3	3
19	4	2	3	4	2	2	1	2	2	2	3	2
20	4	3	4	4	3	2	4	2	2	2	2	2
21	4	3	3	4	3	3	4	3	3	3	3	3
22	4	3	4	4	4	3	4	3	3	3	3	3
23	4	2	3	4	2	3	2	3	3	3	2	2
24	2	3	3	2	3	3	2	3	3	3	3	4
Mode	4	3	4	4	3	3	4	3	3	3	3	2

Table A.10 Classification of horse mackerel maturity stages according to the Walsh scale with adaptations made by each laboratory.

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21	Fish 22	Fish 23	Fish 24	Fish 25	Fish 26
1	3	3	5	2	2	6	2	6	5	2	2	3	2	2	6	3	1	2	2	6	6	2	4	2	5	5
2																										
3	3	3	5	3	6	6	6	5	5	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4	5	5	6	6		6		5	6	2		3	2	2	2	3			2		1	1	3	2	5	5
5	2	5	5	6	6	6	5	6	5	6	6	2	3	2	2	3	1	2	2	1	2	2	2	2	2	2
6	3	3	3	3	5	5	3	5	5	3	3	3	3	3	2	3	2	2	2	2	2	2	3	2	3	2
7	5	5	6	1	6	6	5	6	3	2	6	3	5	2	2	5	1	1	2	1	6	5	3	2	2	2
8	5	6	5	6	6	6	2	6	6	5	6	6	2	5	2	4	1	2	2	1	2	6	6	6	2	5
9	3	3	5	2	2	6	5	2	3	2	6	2	2	2	2	2	2	2	2	2	2	2	3	2	3	3
10	5	5	6	6	6	6	6	6	5	2	6	5	2	2	2	5	1		2	1	2	2	3	2	5	2
11	2	3	5	5	6	6	6	5	2	6	6	5	3	5	5	5			5	1	6	2	3	2	5	5
12	5	5	5	6	6	6	5	6	5	2	6	5	2	2	5	4	1	2	3	2	2	2	5	2	5	5
13	2	3	5	1	2	6	5		5	6	6	3	3	2	2	3	1		2		5	5	3	1	5	5
14	3	3	5	2	2	6	2	6	5	2	2	3	2	2	5	3	1	2	3	6	6	2	4	2	5	5
15	4	5	5	2	6	6	6	5	5	2	6	3	3	5	5	5			2	6	2	2	3	2	5	5
16	5	5	5	5	6	5	6	4	4	5	6	2				2			2	2	2	2	3	2	2	2
17																										
18	3	3	3	3	5	5	3	5	3	3	3	3	2	2	2	3	1	2	2	2	2	2	3	2	3	2
19	3	3	5	2	2	5	4	1	4	2	6	3	3	2	5	4	1		2	1	2	2	3	2	3	3
20	4	5	4		6	6		6	5	6	6	5	5	6	6	5			2	6	6	6	5	6	5	5
21	5	5	5	6	6	5	6	5	5	2	2	5	3	2	2	3			3	1	2	2	4	2	2	2
22	5	5	5	2	5	6	6	6	5	2		5	2	2	2	5	1	2	2	1	2	2	5	2	5	2
23	3	5	5	2	2	6	6	5	5	2	2	5	2	2	2	3	2		3		5		4	2	5	2
24	5	5	5	2	6	6	6	6	5	2	2	5	5	2	2	5	1		5	1	6	2	5	2	5	5
Mode	5	5	5	2	6	6	6	6	5	2	6	3	2	2	2	3	1	2	2	1	2	2	3	2	5	2

Table A.11 Classification of horse mackerel maturity stages by strictly following the Walsh scale criteria.

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21	Fish 22	Fish 23	Fish 24	Fish 25	Fish 26
1	3	3	5	2	2	6	2	6	5	2	2	3	2	2	6	3	1	2	2	6	6	2	4	2	5	5
2																										
3	3	3	5	3	6	6	6	5	5	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4	5	5	6	6		6		5	6	2		3	2	2	2	3			2		1	1	3	2	5	5
5	2	5	5	6	6	6	5	6	5	6	6	2	3	2	2	3	1	2	2	1	2	2	2	2	2	2
6	3	3	3	3	5	5	3	5	5	3	3	3	3	3	2	3	2	2	2	2	2	2	3	2	3	2
7	5	5	6	1	6	6	5	6	3	2	6	3	5	2	2	5	1	1	2	1	6	5	3	2	2	2
8	5	6	5	6	6	6	2	6	6	5	6	6	2	5	2	4	1	2	2	1	2	6	6	6	2	5
9	3	3	5	2	2	6	5	2	3	2	6	2	2	2	2	2	2	2	2	2	2	2	3	2	3	3
10	5	5	6	6	6	6	6	6	5	2	6	5	2	2	2	5	1		2	1	2	2	3	2	5	2
11	2	3	5	5	6	6	6	5	2	6	6	5	3	5	5	5			5	1	6	2	3	2	5	5
12	5	5	5	6	6	6	5	6	5	2	6	5	2	2	5	4	1	2	3	2	2	2	5	2	5	5
13	2	3	5	1	2	6	5		5	6	6	3	3	2	2	3	1		2		5	5	3	1	5	5
14	3	3	5	2	2	6	2	6	5	2	2	3	2	2	5	3	1	2	3	6	6	2	4	2	5	5
15	4	5	5	2	6	6	6	5	5	2	6	3	3	5	5	5			2	6	2	2	3	2	5	5
16	5	5	5	5	6	5	6	4	4	5	6	2				2			2	2	2	2	3	2	2	2
17																										
18	3	3	3	3	5	5	3	5	3	3	3	3	2	2	2	3	1	2	2	2	2	2	3	2	3	2
19	3	3	5	2	2	5	4	1	4	2	6	3	3	2	5	4	1		2	1	2	2	3	2	3	3
20	4	5	4		6	6		6	5	6	6	5	5	6	6	5			2	6	6	6	5	6	5	5
21	5	5	5	6	6	5	6	5	5	2	2	5	3	2	2	3			3	1	2	2	4	2	2	2
22	5	5	5	2	5	6	6	6	5	2		5	2	2	2	5	1	2	2	1	2	2	5	2	5	2
23	3	5	5	2	2	6	6	5	5	2	2	5	2	2	2	3	2		3		5		4	2	5	2
24	5	5	5	2	6	6	6	6	5	2	2	5	5	2	2	5	1		5	1	6	2	5	2	5	5
Mode	5	5	5	2	6	6	6	6	5	2	6	3	2	2	2	3	1	2	2	1	2	2	3	2	5	2

Table A.12 Classification of horse mackerel maturity stages according to the standard scale (1st day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21	Fish 22	Fish 23	Fish 24	Fish 25	Fish 26
1	2	2	4	2	2	4	2	4	4	2	2	2	2	2	4	2	1	2	2	4	4	2	3	2	4	4
2																										
3	2	2	2	2	4	4	4	4	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2
4	4	4	4	4		4		4	4	4		2	4	4	4	2			4		1	1	2	4	4	4
5	2	3	3	4	4	4	3	4	3	4	4	2	2	2	2	2	1	2	2	1	2	2	2	2	2	2
6	2	2	5	2	3	3	2	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
7	4	4	4	1	4	4	4	4	2	2	2	3	4	2	2	4	1	1	2	1	4	4	3	2	2	2
8	4	4	4	4	4	2	4	4	4	4	4	4	6	4	4	3	1	2	2	1	4	4	4	4	4	4
9	2	2	3	2	2	4	3	2	2	2	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
10	2	2	4	4	4	4	4	4	4	2	4	4	4	2	2	2	2	1		2	1	4	2	2	2	2
11	5	2	4	4	4	4	4	4	2	4	4	4	2	4	4	4			4	1	4	2	2	2	4	4
12	2	2	2	4	4	4	2	4	2	2	4	1	2	2	2	3		2	2	4	4	2	2	2	2	2
13	4	2	4	1	2	4	4		4	4	4	2	2	2	2	2	1		2		5	4	2	1	4	4
14	2	2	4	2	2	4	2	4	4	2	2	2	2	2	4	2	1	2	2	4	4	2	3	2	4	4
15	3	4	4	2	5	5	5	4	4	1	4	2	2	4	4	4			2	5	1	1	2	2	4	4
16	4	4	4	4	5	5	5	4	4	4	5	2				2							2	2	2	2
17																										
18	2	2	2	2	3	3	2	3	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2
19	2	2	4	2	2	4	3	1	3	2	5	2	2	2	4	3	1		2	1	2	2	2	2	2	2
20	4	4	3		4	5		4	4	4	4	4	4	4	4	4			2	4	4	4	4	4	4	4
21	3	3	3	4	4	4	4	3	3	2	2	3	2	2	2	2			2	1	2	2	3	2	2	2
22	4	3	4	3	3	4	3	4	4	2		3	2	2	2	4	1	2	3	1	2	2	3	3	4	2
23	2	3	4	3	3	3	3	3	3	2	3	3	2	2	2	2	3		3		4		3	3	4	2
24				2	4	4	4	4									1				4					
Mode	2	2	4	2	4	4	4	4	4	2	4	2	2	2	2	2	1	2	2	1	4	2	2	2	2	2

Table A.13 Classification of horse mackerel maturity stages according to the standard scale (2nd day).

Observer	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21
1																					
2	4	3	3	4	3	3	4	3	3	4	3	4	3	2	4	3	4	4	3	4	4
3	2	2	2	2	2	2	2	2	2	4	2	2	2	2	2	2	4	2	2	2	3
4	4	3	3	4	3		2	3	3	4	3	2	3	3	4	3	2	4	3	4	3
5	2	3	3	2	3	3	2	3	2	4	3	2	3	3	4	3	2	4	3	3	3
6	2	3	3	2	3	3	2	3	2		3	2	3	3	4	3	2	4	3	3	2
7	4	3	3	3	3	3	4	3	3	4	3	4	3	2	4	3	4	4	3	4	4
8	2	3	3	4	3	3	2	3	4	4	3	4	3	3	4	4	4	4	3	3	4
9	2	2	2	4	3	4	2	2	2	4	2	2	2	2	4	2	4	4	2	4	4
10	2	3	3	4	3	3	2	3	4	4	3	4	3	3	4	4	4	4	3	3	4
11	4	3	3	4	4	3	4	3	3	3	2	2	3	2	3	3	4	2	4	2	4
12	2	3	3	4	3	3	2	3	3	4	3	4	3	3	4	3	2	4	3	2	4
13	4	3	2	2	3	3	4	3	2		3	4	2	3	4	3	2	4	3	3	3
14	4	3	2	2	3	2	2	3	2	4	3	2	2	3	4	2	2	4	2	3	2
15	4	4	4	4	3	4	2	4	4	4	4	4	4	3	4	4	3	4	3	4	3
16	2	3	3	4	2	3	2	2	3	4	2	3	3	2	4	3	3	4	2	4	2
17	4	3	3	3	3	1	2	3	3	4	3	3	4	4	4	3	4	2	2	3	4
18	2	3	3	2	3	3	2	3	2		3	2	3	3	4	3	2	4	3	3	2
19	2	3	3	4	3	2	2	3	2	4	3	2	2	3	3	2	2	4	3	2	3
20	4	3	3	4	3	3	4	3	3	4	3	4	2	2	4	3	4	4	3	4	4
21	4	3	3	4	3		2	3	3	4	3	2	3	3	4	3	2	4	3	4	3
22	2	4	3	4	3	3	2	4	4	4	3	4	3	3	4	3	4	2	3	2	4
23	1	3	3	2	3	4	2	3	4	2	3	2	3	3	4	2	2	4	3	2	4
24	2	3	3	4	3	3	2	3	4	4	3	2	3	3	2	3	2	4	3	2	4
Mode	2	3	3	4	3	3	2	3	3	4	3	2	3	3	4	3	2	4	3	3	4