WORKSHOP ON ELASMOBRANCHS MATURITY (WKSEL3; outputs from 2018 meeting)

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Contents

i Executive summary

The WKMSEL3 meeting aimed to update and validate the international maturity stages for elasmobranchs species (viviparous and oviparous) and prepare conversion tables for the maturity scales used by the institutes to the international scale. The update was carried out on the base of the results presented from the participants including hystological validation.

Some changes were made on the criteria and description of each maturity stages of the WKMSEL2 scale, considering the expertise and experiences from both Atlantic and Mediterranean species. Therefore particular attention was devoted to have clear elements useful to discern juveniles and adults stages. Indeed, this item has a high impact on the Stock Spawning Biomass quantification and, more in general, on the stock assessment for the elasmobranchs species.

For oviparous species, in the male maturity scale, the specification of thorns presence was introduced as a characteristic that can help to distinguish some maturity stages from each other (Stage 1 vs Stage 2; Stage 2 vs Stage 3a). Moreover, lobuli segmentation and seminal vesicles were deeply specified in the stage descriptions. Furthermore, the name of Stage 4 was changed from REGRESSING to SPENT. This term was considered as more appropriate to include all phases (post-spawning, rest and regenerating) described in this stage.

In oviparous, female maturity scale, the uteri appearance and oviducal glands in some maturity stages was taking into account to help the distinction of maturity stages from each other.

In the viviparous females, the description of the ovary and oviducal gland for the Stage 4a, 4b, 4c, 5 and 6 was added to maturity scale. Moreover it was inserted a Stage 7 (LATE REGENERATING) in which the atretic follicles are unlikely because they are developing and the Stage 6 was renamed (EARLY REGENERATING).

Two staging exercises were carried out, one using a shark viviparous species (*Etmopterus spinax*) and another one using a shark oviparous species (*Galeus melastomus*). Generally, participants felt that *G. melastomus* was easier to stage than *E. spinax*. Indeed, the viviparous scale is more complicated mostly for the females (9 stages). Moreover, in males and females, there was a confusion also in the sex determination for the juveniles of this species, since the claspers in the stage 1 are not easily visible determining a source of errors. Regarding the maturity stage for *G. melastomus*, the agreement was 71.8%, while for *E. spinax*, the agreement was 68.5%.

The macroscopic maturity stages were validated with the histological analysis in *G. melastomus* (oviparous) and *E. spinax* (viviparous) for both sexes. The microscopical validation was done taking into account the criteria fixed in WKMATHIS (ICES, 2018).

The picture references collection was improved from the previous workshop (WKMSEL2) by the photos of the participants discussed in plenary. The Atlas was amplified in term of case studies (*Galeus atlanticus*, *Prionace glauca*, *Urolophus* spp, *Squalus acanthias*, *S. blainville*) and macroscopic features suitable for the determination of each maturity stage.

For all scales, conversion tables are presented to the international agreed scale.

It was recommended that the next meeting should be in 3 years. It was also recommended that the national institutes should be strongly encouraged to put effort into making pictures, and should find time and money to do so. Successful maturity staging workshops cannot be carried out without these pictures.

Moreover, the establishment of a Working Group including also the ageing analysis was encouraged from all participants.

ii Expert group information

Expert group name	Workshop on Sexual Maturity Staging of Elasmobranchs (WKSEL3)
Expert group cycle	Annual
Year cycle started	2018
Reporting year in cycle	1/1
Chair(s)	Maria Cristina Follesa, Italy
	Pierluigi Carbonara, Italy
Meeting venue(s) and dates	19-22 February 2018, Cagliari, Italy (13 participants)

1 Opening of the meeting

WKMSEL3 met 19-22 February 2018 in Cagliari, Italy. Thirteen participants from 5 countries (representing 9 different institutes) joined the meeting. The participant list is in the Annex 1.

The meeting aimed to update and validate the WKMSEL2 maturity scales for elasmobranchs species (viviparous and oviparous) and prepare conversion tables for the maturity scales used by the institutes to the international scale. The update was carried out on the base of the results presented from the participants including histological validation.

Terms of Reference for the meeting where:

a) Update the international maturity scales based on macroscopic features both for oviparous and viviparous species
b) Validate both maturity scales based on macroscopic features through histological analysis
c) Update the conversion tables both for oviparous and viviparous species;
d) Compile an Atlas using both macroscopical and histological gonad pictures
e) Increase the number of case studies with particular attention for viviparous species

ToR A was discussed in sub-groups following to the plenary, ToR B was developed on base of histological analysis presented from the participants, ToR C was produced on plenary considering the results of the ToR A. ToR D and ToR E were done taking into consideration the pictures collections from the participant laboratories.

Each ToR was discussed in separate chapters.

2 Adoption of the agenda

The agenda, reported in the Annex 2, addressed all ToRs. The meeting started with the background presentations on the scales used on each lab. Moreover, some emblematic cases (*G. melastomus* and *E. spinax*) as well as *G. atlanticus*, *P. glauca*, *Urolophus* spp, *S. acanthias* and *S. blainville* were presented. On the base of the presentations and discussion, an update of WKMSEL2 scales was done in two sub-groups (oviparous and viviparous). Following, an exercise of staging oviparous (*G. melastomus*) and viviparous (*E. spinax*) species has been made.

2.1 Abbreviations and acronyms

DISVA – Dipartimento di Scienze della Vita e dell'Ambiente.

DW – Disc Width

GFCM – General Fisheries Commission of the Mediterranean

GSA – Geographical Sub Area

ICES – International Council for the Exploration of the Sea

IEO - Instituto Español de Oceanografía

MEDITS – Mediterranean International Trawl Survey

TL – Total Length

ToR – Terms of Reference

TW – Total Weight

WKMATCH – Report of the Workshop for maturity staging chairs

WKMSEL Workshop on Sexual Maturity Staging of Elasmobranchs

3 Update the international maturity scales based on macroscopic features both for oviparous and viviparous species (ToR A)

Some changes were made on the criteria and description of each maturity stages of the WKMSEL2 scales (ICES, 2012a), considering the expertise and experiences from both Atlantic and Mediterranean species following the recommendations of the WGBIOP (ICES, 2017). During the presentations about key-species and the discussions after the staging rounds, it was pointed out that further macroscopic features should be highlighted to allow a more complete description of each maturity stage. It was done an effort also to describe the borders between the stages as clearly as possible, since these are the most difficult to assess.

Expertise agreed that having two separated tables for each sex might be preferable and more user-friendly. Special concern was reserved to viviparous species, since males and female reproductive cycles do not coincide (e.g. stages 3a and 3b).

3.1 Abstracts of presentations

Abstracts of some presentations (see Annex 6) of participants are reported below:

3.1.1 Maturity scales used in HCMR for Elasmobranchs

Aikaterini Anastasopoulou (HCMR, Greece)

In Greece, at HCMR, for the period from 2010 to 2012 the maturity scale for Elasmobranchs proposed by the WKMSEL Workshop, which held in 2010 in Malta, has been applied. Since December of 2012 is in use the updated, by the WKMSEL2 Workshop, maturity scales for the oviparous and viviparous species. Samples of Elasmobranchs species are elaborated either on board or in the Laboratory. Some species are studied in standard basis within the framework of DCF project, whereas others are studied only within the framework of other scientific projects. At the present Workshop, macroscopic maturity stage images for the oviparous *Galeus melastomus* and the viviparous *Squalus blainville* were presented. The proposed macroscopic scale proved to be adequate when applied to female and male Elasmobranchs. However, some problems may be arise as the maturity stages may vary from species to species or by the fact that some maturity stages need a better definition because they can be confused with other maturity stages.

3.1.2 Maturity data of spurdog (*Squalus acanthias*) in Norwegian waters

Marlén K. Myrlund (IMR, Norway)

The Institute of Marine Research use a joint oviparous scale and a joint viviparous scale for both sexes. The IMR scale and the WKMSEL2 scale have the same number of stages, but the numbering and terminology of each stage are to some extent different. However , the description of the stages match up nicely and a simple transition should be possible. The IMR started a sampling program in October 2015 and the following results include the samples collected until January 2017. The spurdogs were collected from five different statistical areas along the Norwegian coast and from two sampling periods (May and September-December). A sample of 50 spurdogs was

collected from each of two catches *per* area and *per* sampling period. The large majority of males were mature and active. Females were found in all stages, except 7 and 8. Using all the data from females, L_{50} was estimated of 79 cm of TL. Females in early pregnancy were mainly found in May, while females with near-term pups in the uteri were mainly found in late autumn. Furthermore, larger mature, but not active females, were more often found in late autumn than in spring. Measurements of the oviducal gland shows an enlargement of the gland in stage 3 and 6. Excluding females with zero pups in one uterus or with a between-uteri-difference of 4 pups or more, the uterine fecundity were of 8.3 pups. The uterine fecundity and the length of near-term pups increased with maternal length.

3.1.3 Oviparous and viviparous Elasmobranch species: critical analysis of the macro (MEDITS *vs* WKMSEL2) maturity scales

Follesa Maria Cristina, Porcu Cristina, Cau Alessandro, Bellodi Andrea, Marongiu Martina Francesca, Mulas Antonello (UNICA, Italy)

In this work useful elements to clear discern the different maturity stages of oviparous and viviparous Elasmobranch species and improve the maturity scales were presented. In particular, for oviparous male species, claspers' length did not always represent a good maturity indicator. In fact, in some species, they never surpass the pelvic fins (e.g. genus *Scyliorhinus*). In addition, for batoid species, the presence of alar thorns could be useful to discern immature specimens (Stage 1) from developing ones (Stage 2). As regard to batoid females in Stage 2 (developing), ovarian follicles were not always clearly distinguishable at naked eye differently from sharks. Instead, the oviducal gland is one of the most important element in discerning macro stages (in particular Stage 1 vs Stage 2 and Stage 2 vs Stage 4b) because of its clear development through the different maturation phases. In viviparous species, it could be useful to differentiate species with "asynchronous" (e.g. *Etmopterus spinax*) and "synchronous" (e.g. *Squalus blainville*) ovaries, especially for pregnancy and post-partum stages because the first one presented a regressing ovary and a subsequent slow recovery, whereas the latter showed always a developed ovary with yolked follicles, differently from what described in the previous maturity scales. Moreover, it could be assumed that, in viviparous species, the oviducal gland is not an important element in discerning the different maturity stages because it less differentiated.

3.1.4 Discussion and comparison on MEDITS and WKMSEL2 maturity scales (oviparous and viviparous)

Donnaloia Marilena and Carbonara Pierluigi

The MEDITS program aims at conducting coordinated surveys from bottom trawling in the Mediterranean Sea and provides two official scales adopted by the countries participating to MEDITS surveys in order to determine sex and maturity stages for both oviparous and viviparous elasmobranchs. The present work aims to compare and discuss the MEDITS maturity scales in respect to the scales proposed at the last Workshop on Maturity (WKMSEL2, 2012).

Each maturity stage is analysed and discussed through images of different oviparous and viviparous Elasmobranch species collected during experimental surveys (MEDITS program) and commercial landings (CAMPBIOL program) within the European Data Collection Framework context. The samples come from three different Geographical Sub-Areas (GSAs): Southern Adriatic Sea (GSA18), Ionian Sea (GSA19) and Southern Central Thyrrenhian Sea (GSA10).

The key anatomical elements described in the MEDITS and WKMSEL2 scales were compared in order to highlight the crucial differentiation among the maturity stages along the sexual development. A first clarification regarded the attribution of the developing stage (Stage 2) to an immature or mature phase among the MEDITS and WKMSEL2 scales. This aspect is very crucial in order to perform a correct ogive calculation and its relative implication for the Spawning Stock Biomass estimation purpose. Therefore, in the MEDITS scale the "developing" stage is included in the adult mature fraction, while in the WKMSEL2 the same Stage is considered an immature phase considering that the specimen theoretically has not yet reached full reproductive capacity. In the Figure, the difference of the length at first maturity of *Raja clavata* when males of Stage 2 were considered in the mature portion (right) or the immature one (left) is presented. A difference of 6 cm in the length at first maturity can be detected. The same can be observed for females (Fig.3.1.4).

Another relevant point of discussion regarded the main difficulties laying in transitional stages mostly in relation to the female reproductive cycle. In this sense, a more accurate oocytes and uterus description proposed for the WKMSEL2 scales results more helpful compared to the MEDITS ones. Anyway, the need to improve the scales taking into account the variability existing in the elasmobranch reproductive strategies is highlighted. Some examples of the ovary specie-specific characterization among different maturity stages from viviparous (*Mustelus* and *Squalus blainville*) and oviparous species (*Raja clavata* and *Dipturus nidarosiensis*) are represented. The regenerating stage is the main critical stage difficult to distinguish because it could be confused with: 1) resting 2) capable to reproduce and 3) developing stages. Therefore, it is indicated that further biological data should be collected (e.g. reproductive organ weights and measures of meristic and anatomical reproductive elements) to improve the knowledge of the different patterns of reproduction. Moreover, it is underlined that such generic descriptions (e.g. clasper lengths in relation to the pelvic fins) do not fit for all species. Therefore, it needs to add indicative ad helpful elements for the more common species (i.e. in the Rajadae males the detection of vestigial thorns represents a further and useful element to discriminate macroscopically the immature specimens from the mature ones).

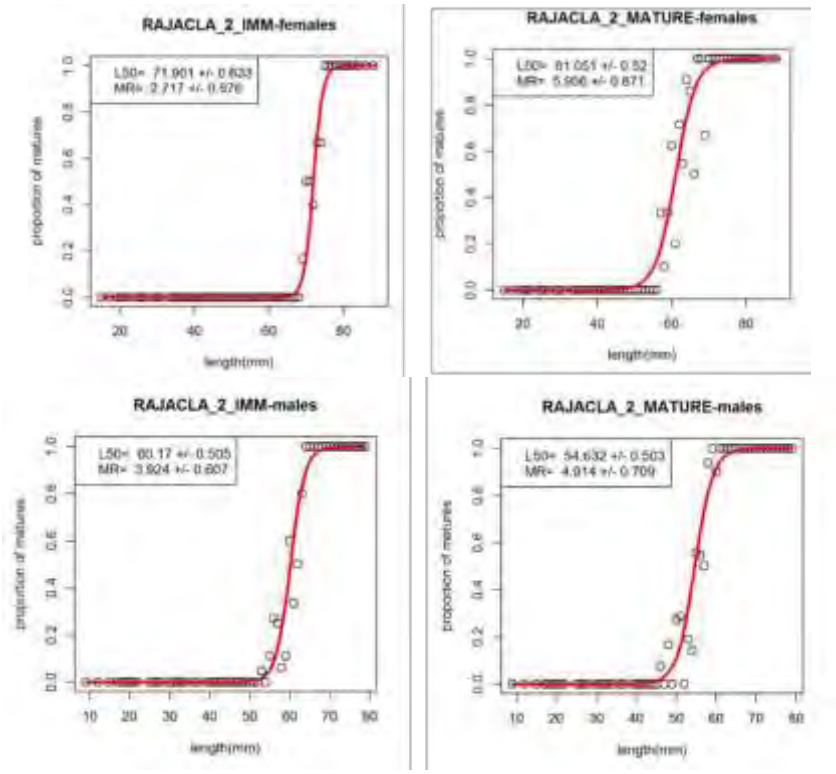


Fig. 3.1.4 Different application of the Stage 2 respectively in male and female ogive calculations. Implementing the Stage 2 as mature proportions, the results showed as the lengths at first maturity are lower of about 6 and 10 cm of respectively for male and female.

3.1.5 Maturity stages of Scyliorhinus canicula

Marina Sartini (APLYSIA Soc. Coop. r.l.)

The Elasmobranchs maturity stage determination in GSA9 (Ligurian Sea and Northern Tyrrhenian Sea) is made using only the MEDITS maturity scales. In this workshop macroscopic gonad pictures of the oviparous *Scyliorhinus canicula* were presented. The specimens analyzed were collected within the DCF program, during experimental surveys (MEDITS survey) and landing monitoring project (CAMPBIOL). The MEDITS maturity scale for oviparous species can be considered quite good for the stage determination of *S. canicula,* even if the macroscopic description of Stage 2 for males is not suitable for this species and the genus *Scyliorhinus* in general. As a matter of fact in *Scyliorhinus* spp. claspers don't extend to the posterior edge of the pelvic fins. From a general point of view, it could be useful to have a better macroscopic description for some stages that can be confused (e.g. Stage 2: maturing with Stage 4b: regenerating).

3.1.6 Viviparous species of the Northern Ionian Sea: comments on the use of macroscale

Letizia Sion, Antonella Indennidate (UNIBA, Italy)

In this work the authors presented useful elements to highlight some uncertainties on maturity stages of Elasmobranch viviparous species in order to improve the assignment of each maturity stage.

Different species of sharks, rays and skates were sampled during experimental bottom trawl and long line surveys (MEDITS, CoralFish) carried out in the Western Ionian Sea, Geographical Sub-Area 19. In particular in the present work the samples analysed for the attribution of the maturity stage were demersal viviparous Elasmobranch species (from 2006 to 2016) as *Centrophorus granulosus*, *Dalatias licha*, *Etmopterus spinax*, *Torpedo nobiliana*, *Torpedo* (mainly sampled thanks to the Medits project) and pelagic viviparous Elasmobranch species as *Pteromyleus bovinus*, *Pteroplatytrygon violacea,* (data collected, from 2010 to 2012, thanks to CoralFish project). The samples were analysed in the laboratory and the maturity stage classification was based on the scales proposed by WKMSEL Workshop, held in Malta in 2010. For the aforementioned species we have only performed a macroscopic analysis supported by photos but no microscopic validation was undertaken.

Problems concerning particular stages were discussed as the differentiation between the adolescent females that are maturing for the first time (developing stage) from those adult females that are just ending to reproduce (regressing) or just beginning a new cycle (re-generating).

Moreover the following studies were presented and the slides of them are reported in the Annex 6:

Medits macroscopic maturity scale of oviparous species : The case of *Galeus atlanticus* in Alboran Sea (Mediterranean waters)
Francisca Salmerón (IEO, Spain)

Obligate or facultative diapause in batoids
Fabian Trinnie, Terence Walker, Paul Jones, Laurie Laurenson)

3.2 Oviparous macroscopic maturity scales

The oviparous macroscopic scales (females and males) were updated on the base of the results coming from skates (mainly Rajidae), catsharks (Scyliorhinidae and Pentanchidae) and the holocephalan *Chimaera monstrosa* description, the main taxa studied by the participants of this workshop. Stages' descriptions were made considering a geographic area as wide-ranging as possible.

The general structure of WKMSEL2 maturity scales was maintained with 6 female maturity stages (1. Immature, 2. Developing, 3a. Capable to reproduce; 3b. Egg-laying; 4a. Post-laying, 4b. Regenerating) and 5 male maturity ones (1. Immature, 2. Developing, 3a. Capable to reproduce; 3b. Active; 4. Regressing).

3.2.1 Female maturity scale

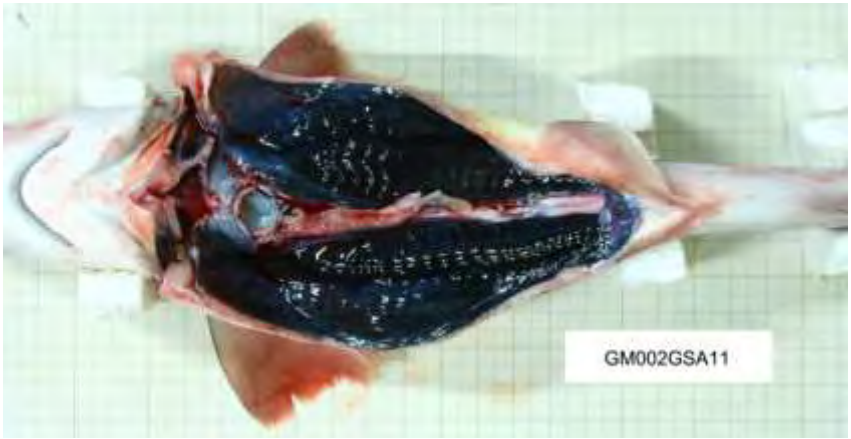
The nomenclature of the maturity stages was changed. In particular, the name of the Stage 3b has been changed in ACTIVE (the old name was EGG-LAYING).

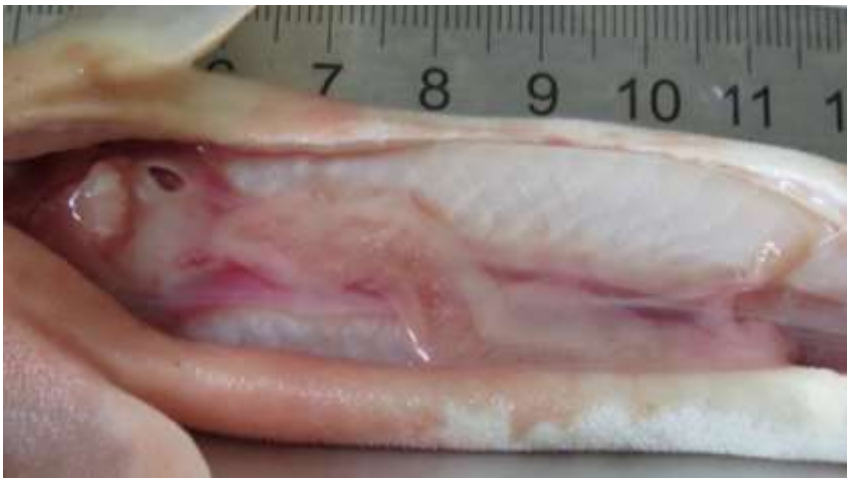
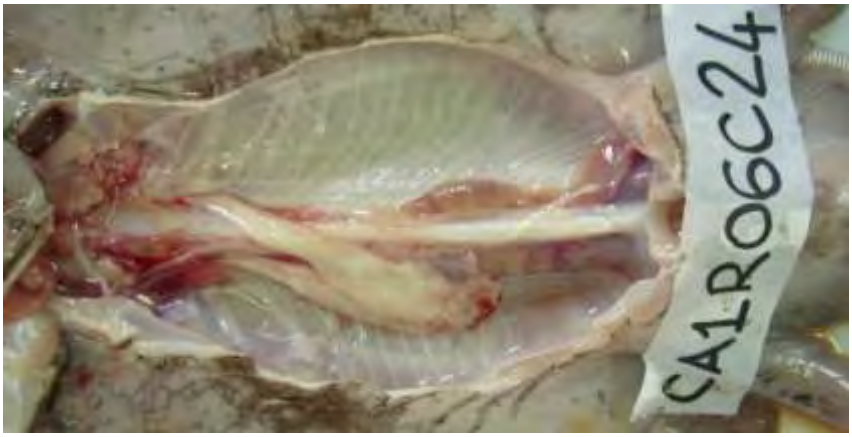
Below, a summary of the main features considered and the changes performed stage-by-stage was shown. Generally, in Notes the reason of the changes adopted is reported.

The changes derived from detailed observations of photographic material and discussion on it among the participants.

Stage 1. Immature: pictures of *Galeus melastomus*, *Scyliorhinus canicula* and *Dipturus oxyrinchuus* were observed and discussed in detail (Tab. 3.2.1.1).

Table 3.2.1.1. Pictures of female oviparous species in Stage 1.

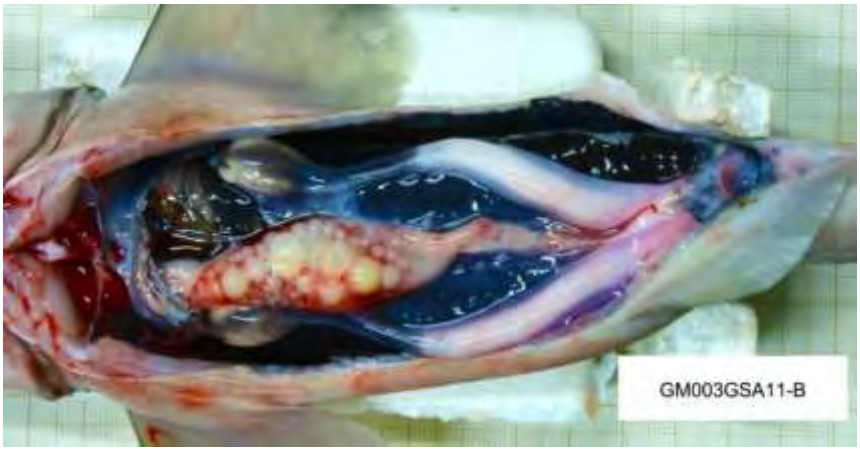
Stage 1 IMMATURE	NOTES
	Ovaries: in some sharks very small follicles are visible.
Galeus melastomus TL: 37.6 cm; TW: 133 g; GSA11	

Stage 1 IMMATURE	NOTES
	Ovaries: in some sharks very small follicles are visible.
Scyliorhinus canicula TL: 29 cm; TW: 75 g; GSA9	
	
Dipturus oxyrinchus TL: 57.2 cm; TW: 654 g; GSA11	
	
Galeus melastomus TL: 38.5 cm; TW:147 g; GSA20	

After the pictures observation (Tab.3.2.2.1) and discussion on the features present , it was decided that the sentence of "very small ovarian follicles" has to be added to the description of the immature ovary in WKMSEL2, as observed in some shark species as *Scyliorhinus canicula* and *Galeus melastomus*.

Stage 2. Developing: pictures of *Galeus melastomus* and *Dipturus oxyrinchuus* were observed and discussed in detail (Tab. 3.2.1.2).

Table 3.2.1.2. Pictures of female oviparous species in Stage 2.

Stage 2 DEVELOPING	NOTES
	Unyolked follicles: the group adopted the term unyolked follicles to distinguish them from the yolked ones according to development degree that could be observed in this stage.
Galeus melastomus TL: 41.4 cm; TW: 209 g; GSA20	
	Some small and medium yolked may be present:
Galeus melastomus TL: 41.5 cm TW: 193 g GSA11	
	Oviducal gland: distinguishable and developing mostly in skates.
Dipturus oxyrinchus TL: 96 cm; TW: 3849 g; GSA11	

Finally, after a detailed observation of the photos (Tab.3.2.2.1), the group decided to adopt and add the term "unyolked follicles" in the ovary description of the Stage 2 in order to distinguish them from the yolked ones according to the development degree observed at this stage. In addition, the developing oviducal gland, a feature common mainly in skates (e.g. *Dipturus oxyrinchus*), should be taken into consideration.

Stage 3a. Capable to reproduce: pictures of *Galeus melastomus*, *Dipturus oxyrinchus, Scyliorhinus canicula* and *Chimaera monstrosa* were observed and discussed in detail (Tab. 3.2.1.3).

Table 3.2.1.3. Pictures of female oviparous species in Stage 3a.

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Uteri: turgid differently from the Stage 4a.
Galeus melastomus TL: 46.3 cm; TW: 321 g; GSA11	
	Uteri: turgid differently from the Stage 4a.
Scyliorhinus canicula TL: 48 cm; TW: 246 g; GSA9	

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Uteri: turgid differently from the Stage 4a.
Raja miraletus TL: 39.2 cm; TW: 301 g; GSA11	

From the picture observation (see Tab. 3.2.1.3), the group decided to leave the same description of the ovary and oviducal gland reported in WKMSEL2. Only in the uterus description, the adjective "turgid" was inserted to better differentiate this stage from the 4a one.

Stage 3b. Active: pictures of *Galeus melastomus, Raja miraletus* and *Scyliorhinus canicula* were observed and discussed in detail (Tab. 3.2.1.4).

Table. 3.2.1.4. Pictures of female oviparous species in Stage 3b.

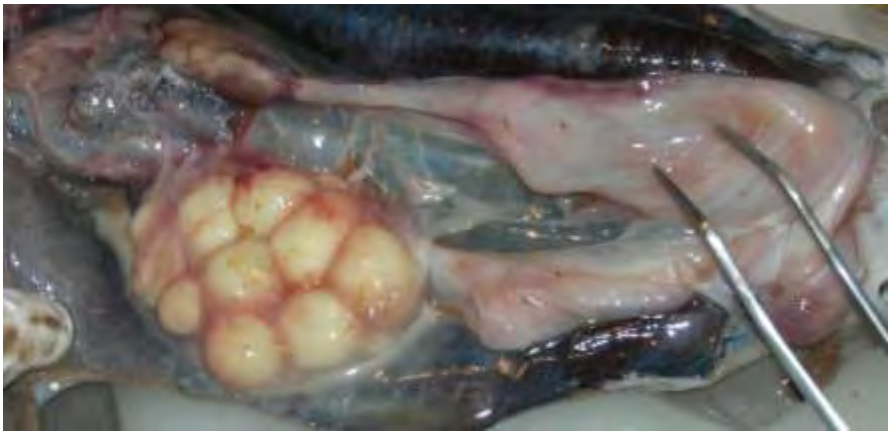
Stage 3b ACTIVE	NOTES
	
Galeus melastomus TL: 50 cm; TW: 363 g; GSA20	

Stage 3b ACTIVE	NOTES
	
Scyliorhinus canicula TL: 41.4 cm; TW: 241 g; GSA9	
	
Dipturus oxyrinchus TL: 101 cm; TW: 4177 g; GSA11	
	
Chimaera monstrosa GSA1	

The name of the stage has been changed in ACTIVE, in order to standardize the stage name with males. The group considered also necessary to insert the description of the ovaries and ovical glands, missing in the WKMSEL2 scale, even if similar to those observed in Stage 3a. For this reason, "**Ovaries and Oviducal glands**: similar to stage 3a" it was added to the WKMSEL2 stage description.

Stage 4a. Post-laying: pictures of *Galeus melastomus* and *Dipturus oxyrinchus* were observed and discussed in detail (Tab. 3.2.1.5).

Table 3.2.1.5. Pictures of female oviparous species in Stage 4a.

Stage 4a POST-LAYING	NOTES
	Uteri: the uteri appearance could be also vascularized.
Galeus melastomus TL: 50.6 cm; TW: 438 g; GSA20	
	Ovaries: flaccid appearance with unyolked and yolked follicles of different sizes, according to species.
Galeus melastomus TL: 51 cm; TW: 316 g; GSA9	

Stage 4a POST-LAYING	NOTES
	Oviducal glands: in some skate species could be reduced. Uteri: the uteri appearance could be also vascularized.
Dipturus oxyrinchus TL: 104 cm; TW: 5026 g; GSA11	

At the end of the discussion, it was decided that the development of the ovarian follicles (unyolked and yolked follicles) should be considered, according to the species photos observed (e.g. *Galeus melastomus*) (Tab. 3.2.1.5). The team highlighted that the oviducal gland could be reduced in some skate species as *Dipturus oxyrinchus*. In addition, the term "vascularized" was added to the WKMSEL2 uterus description.

Stage 4b. Regenerating: pictures of *Raja polystigma* and *Galeus melastomus* were observed and discussed in detail (Tab. 3.2.1.6).

Table 3.2.1.6. Pictures of female oviparous species in Stage 4b.

Stage 4b REGENERATING	NOTES
	Oviducal glands: in some skates species could be reduced. Uteri: in stage 4b uteri are not flaccid.
Raja polystigma TL: 45.8 cm; TW: 568 g; GSA11	

Stage 4b REGENERATING	NOTES
	Uteri: in stage 4b uteri are not flaccid.
Galeus melastomus TL: 51 cm; TW: 316 g; GSA9	

From the pictures above (Tab. 3.2.1.6), it was decided to leave the description of oviducal glands of the Stage 4a. The uterus texture was taking into account and "not flaccid" was added to distinguish it from Stage 4a.

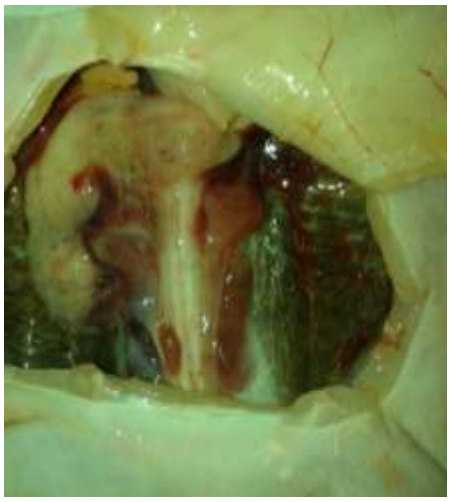
3.2.1.1 Easily confused stages

Stages that could be easily confused are the Stages 2 and 4b. In order to overpass this problem, the main features to be taken into consideration are the size and aspect of oviducal glands, higher and more developed (mainly in skates) in Stage 4b than in Stage 2, as we can see in the Tab. 3.2.1.1.1.

Table 3.2.1.1.1. Differences between Stage 2 and Stage 4b in female Rajidae and Pentanchidae species.

Stage 2	Stage 4b
	
Dipturus oxyrinchus TL: 95.9 cm; TW:3849 g; GSA11	*Dipturus oxyrinchus* TL: 111.6 cm; TW: 4670 g; GSA11

Stage 2	Stage 4b
	
Galeus melastomus TL: 42 cm; TW: 206 g; GSA11	*Galeus melastomus* TL: 46.5 cm; TW: 243 g; GSA11
	
Raja asterias TL: 56.2 cm; GSA18	*Raja asterias* TL: 52 cm; TW: 792 g; GSA11
	
Raja clavata TL: 78 cm; TW: 2769 g; GSA11	*Raja clavata* DW: 44.5 cm; TW: 1789 g; GSA11

Stage 2	Stage 4b
	
Raja miraletus TL:34 cm TW:197 g; GSA11	*Raja miraletus* TL:40 cm; TW: 298 g; GSA11
	
Raja polystigma TL: 40 cm; TW: 323 g; GSA11	*Raja polystigma* TL: 52.5 cm; TW: 983 g; GSA11

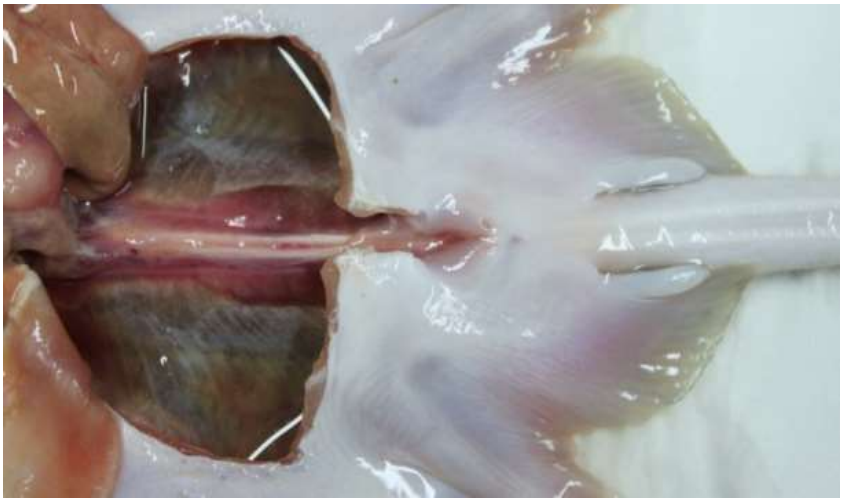
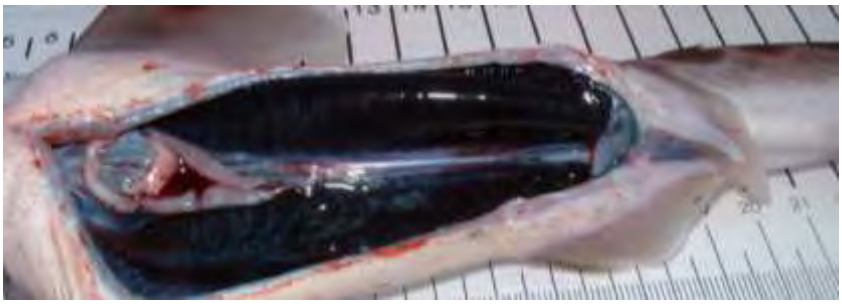
3.2.2 Male maturity scale

The nomenclature of the male maturity stages was the same of the WKMSEL2 scale except for the Stage 4 here reported as SPENT instead of REGRESSING, in order to better specify the last phase of the maturity cycle. In general, the specification of thorns presence was introduced as a characteristic helping the observers in the discrimination of some maturity stages from each other (Stage 1 vs Stage 2; Stage 2 vs Stage 3a). Moreover, the seminal vescicle appearance was specified in the maturity stage description.

Below, a summary of the main features considered and the changes stage-by-stage performed were shown. Generally, in Notes the reason of the changes adopted is reported.

Stage 1. Immature: pictures of *Raja clavata, R. asterias, Leucoraja circularis*, *Galeus melastomus,* and *Diptururs oxyrinchus* were observed and discussed in detail (Tab. 3.2.2.1).

Table 3.2.2.1. Pictures of male oviparous species in Stage 1.

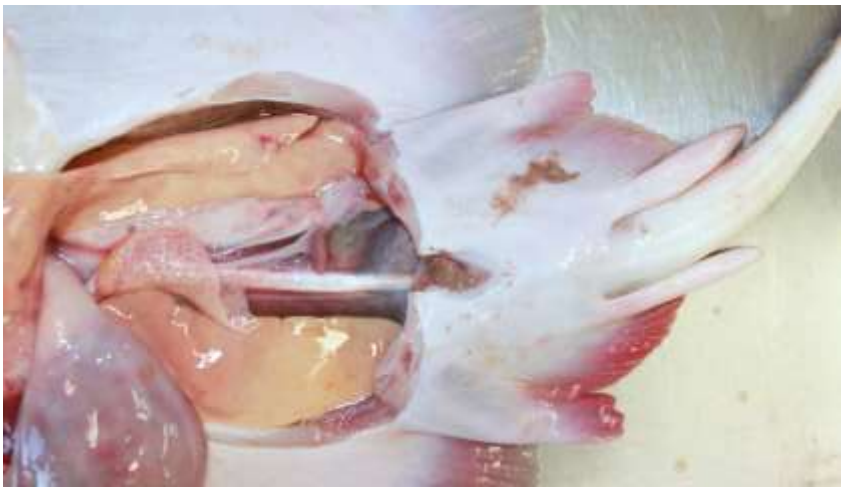
Stage 1 IMMATURE	NOTES
	None
Raja clavata TL 36.5 cm; TW 244.82 g; GSA19	
	None
Raja asterias TL 30.5 cm; TW 168.62 g; GSA10	
	
Galeus melastomus	

Stage 1 IMMATURE	NOTES
	None
Dipturus oxyrinchus TL: 55.7 cm; TW: 579.7 g; Clasper length: 27.9 mm; GSA 11	

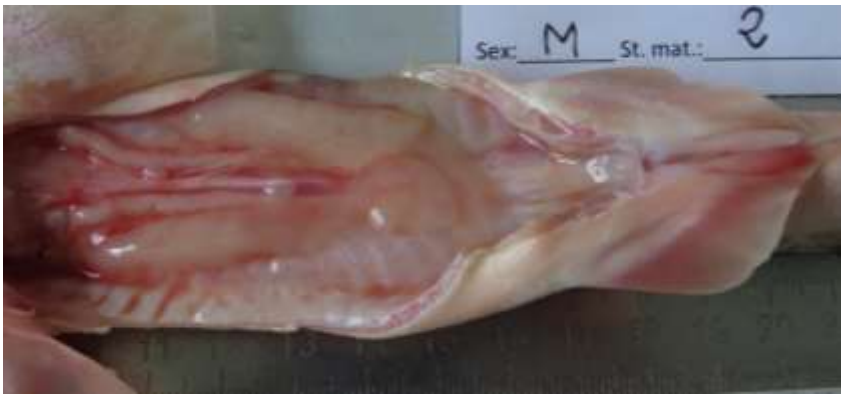
From the observation of the pictures, the description of testes, ducts and claspers was maintained exactly the same of WKMSEL2 scale.

Stage 2. Developing: pictures of *Raja clavata, R. asterias, Leucoraja circularis, Galeus melastomus, Scyliorhinus canicula* and *Diptururs oxyrinchus* were observed and discussed in detail (Tab. 3.2.2.2).

Table 3.2.2.2. Pictures of male oviparous species in Stage 2.

Stage 2 DEVELOPING	NOTES
	None
Raja clavata TL 51.5 cm; TW 1385.04 g; GSA18	

Stage 2 DEVELOPING	NOTES
	
Leucoraja circularis TL 76 cm; TW 2276 g; GSA18	
	None
Raja asterias TL 45.8 cm; TW 624.37 g; GSA10	
	None
Galeus melastomus GSA20	

Stage 2 DEVELOPING	NOTES
	None
Dipturus oxyrinchus TL: 81.4 cm; TW: 2151 g; Clasper length: 70.8 mm; GSA 11	
	Claspers: in *Scyliorhinus* spp the claspers do not pass the pelvic fins, even when mature.
Scyliorhinus canicula TL: 16.6 cm; TW: 345 g; GSA 9	
 	Thorns: in several skates, primordial alar thorns are visible. The detection of the thorns could be useful to distinguish the Stage 2 from the Stage 1
Raja clavata TL 54.3 cm; TW 918.9 g; Clasper length: 93.3 mm; GSA11	

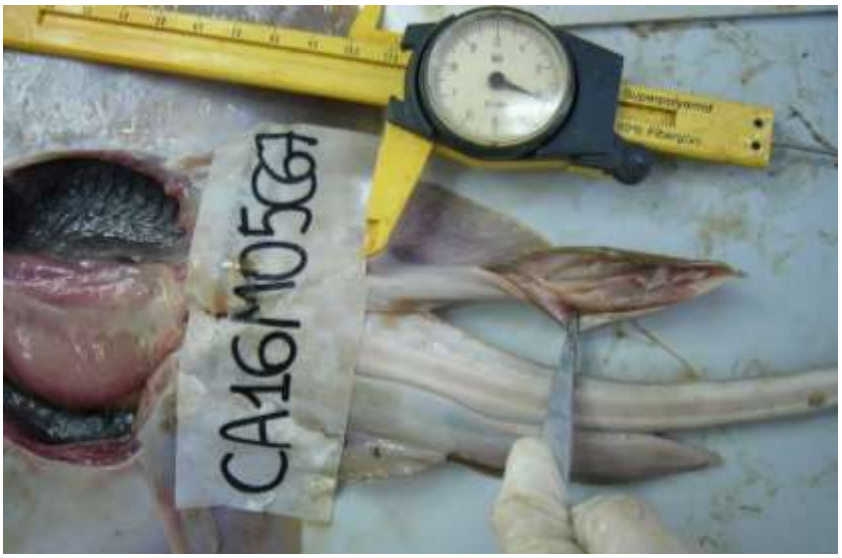
From the observation of the photos above (Tab. 3.2.2.2), the presence of alar thorns were reported as diagnostic feature for the developing stage in skates. In several skates, primordial alar thorns are clearly visible due to a sexual dimorphism. The detection of the thorns could be useful in males to distinguish the Stage 2 from the Stage 1 where alar thorns are totally absent. For this stage, it has been also specified that in some catsharks as those belonging to the *Scyliorhinus* genus (e.g. *S. canicula* and *S. stelleris*), claspers do not pass the pelvic fins, even when mature.

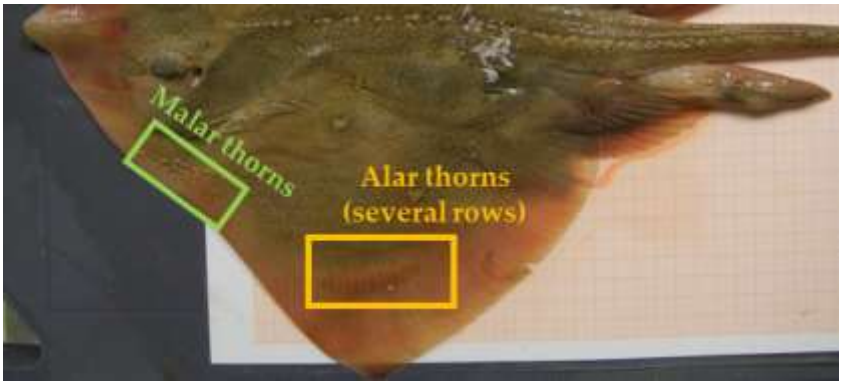
Stage 3a. Capable to reproduce: pictures of Raja clavata, R. brachyura, R. miraletus, R. asterias, R. polystigma, Galeus melastomus, Scyliorhinus canicula, Dipturus oxyrinchus, D. nidarosiensis and Chimaera monstrosa were observed and discussed in detail (Tab. 3.2.2.3).

Table 3.2.2.3. Pictures of male oviparous species in Stage 3a.

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Testes: they could appear fully developed with different structure (e.g. in skates with lobules)
Raja clavata TL 75.4 cm; TW 2108.8 g; GSA18	
	Testes: they could appear fully developed with different structure (e.g. in skates with lobules)
Dipturus nidarosiensis TL: 118 cm; TW: 6948 g Clasper length: 240 mm; GSA11	

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Testes: they could appear fully developed with different structure (e.g. in skates with lobules)
Dipturus oxyrinchus TL: 93.5 cm; TW: 3270 g; Clasper length: 190 mm; GSA11	
	Testes: they could appear fully developed with different structure (e.g. in skates with lobules)
Raja miraletus TL: 37.6 cm; TW: 219.6 g; Clasper length: 75.2 mm; GSA 11	
	Testes: they could appear fully developed with different structure (e.g. in skates with lobules)
Raja asterias GSA 1	

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Testes: they could appear fully developed with different structure (e.g. in skates with lobules)
Raja brachyura GSA 9	
	Testes: they could appear fully developed with different structure (e.g. in skates with lobules)
Raja polystigma TL: 47.2 cm; TW: 538 g Clasper length: 113 mm; GSA11	
	None
Chimaera monstrosa GSA1	

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Seminal vesicle: in some sharks as *Galeus melastomus* and *Scyliorhinus canicula* this organ is clearly evident.
Galeus melastomus GSA 20	
	Seminal vesicle: in some sharks as *Galeus melastomus* and *Scyliorhinus canicula* this organ is clearly evident.
Scyliorhinus canicula GSA 20	
	Thorns: in several skates alar and/or malar thorns , structured in several rows, are well visible. This detection could be useful to distinguish the Stage 3a from the Stage 2 (where are less evident).
Dipturus oxyrinchus TL: 95 cm; TW: 4183 g; Clasper length : 210.1 mm; GSA 11	
	Thorns: in several skates alar and/or malar thorns, structured in several rows, are well visible. This detection could be useful to distinguish the Stage 3a from the Stage 2 (where are less evident)
Raja asterias TL: 47.2 cm; TW: 538 g; Clasper length: 113 mm; GSA11	

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Thorns: in several skates alar and/or malar thorns , structured in several rows, are well visible. This detection could be useful to distinguish the Stage 3a from the Stage 2 (where are less evident)
Raja asterias TL: 47.2 cm; TW: 538 g; Clasper length: 113 mm; GSA11	

Finally, in this stage, a description of the seminal vesicle, clearly evident mainly in some sharks as *Galeus melastomus* and *Scyliorhinus canicula,* was added to the WKMSEL2 stage description. In addition, the observation of more structured alar/malar thorns organised in several rows in capable to reproduce males, should be considered to distinguish the Stage 3a from 2.

3b. Active: pictures of *Raja clavata, R. miraletus, R.brachyura, R. polystigma, R. naevus, Galeus atlanticus, G. melastomus, Scyliorhinus canicula* and *Diptururs oxyrinchus,* were observed and discussed in detail (Tab. 3.2.2.4).

Table 3.2.2.4. Pictures of male oviparous species in Stage 3b.

Stage 3b ACTIVE	NOTES
	
Raja naevus GSA1	

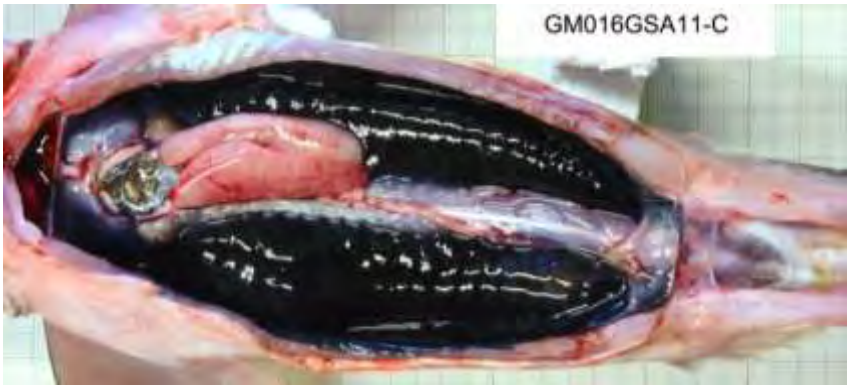
Stage 3b ACTIVE	NOTES

Raja brachyura TL: 88.7 cm; TW: 4783 g; Clasper length: 182 mm: GSA 11

Raja miraletus TL: 33.4 cm; TW: 160.4; GSA 11

Raja clavata TL 75.4 cm; TW 2108.8 g; GSA18

Stage 3b ACTIVE	NOTES
	
Raja polystigma TL: 45.6 cm; TW: 468.6 g; Clasper length: 117.7 mm; GSA 11	
	
Dipturus oxyrinchus TL: 98.2 cm; TW: 3692 g; Clasper length: 205 mm; GSA 11	
	Seminal vesicle: it is visible depending on the reproductive strategy.
Galeus atlanticus GSA 1	

Stage 3b ACTIVE	NOTES
 	Full seminal vesicle
G. melastomus GSA20	
	Empty seminal vesicle.
Galeus melastomus TL: 47 cm; TW: 297.7 g; Clasper length: 45.6 mm; GSA 11	
	Full seminal vesicle.
Scyliorhinus canicula GSA9	

Stage 3b ACTIVE	NOTES
 	Thorns: in several skates alar and/or malar thorns , structured in several rows are still visible.

Raja brachyura TL: 93.4 cm; TW: 5945 g; Clasper length: 174 mm; GSA 11

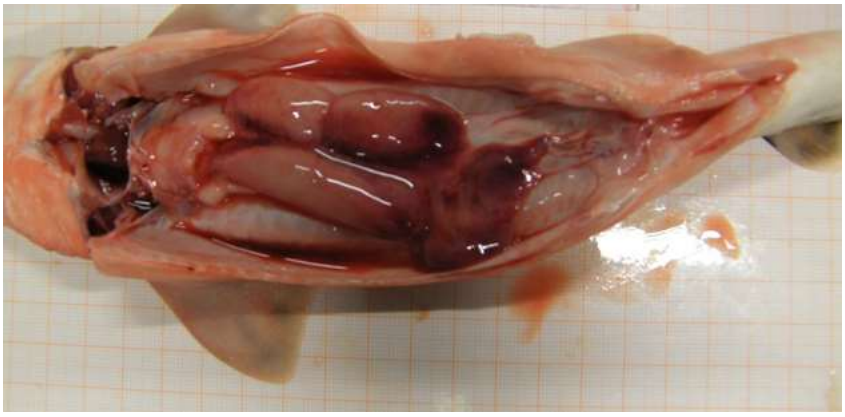
The description of testes and ducts was maintained the same of the WKMSEL2 scale. Moreover, seminal vesicle (that can be fully or empty depending on the reproductive strategy) and alar/malar thorns presence (organized in several rows) was taken into account.

Stage 4. Spent: pictures of *Raja clavata, R.brachyura, R. polystigma, Scyliorhinus canicula* and *Diptururs oxyrinchus,* were observed and discussed in detail (Tab. 3.2.2.5).

Table 3.2.2.5. Pictures of male oviparous species in Stage 4.

Stage 4 SPENT	NOTES
	

Stage 4 SPENT	NOTES
	
Dipturus oxyrinchus TL: 93.5 cm; TW: 3407 g; Clasper length: 190 mm; GSA 11	
	
Raja asterias TL: 46.9 cm; TW: 565 g; Clasper length: 122.5 mm; GSA 11	
	
Raja brachyura TL: 89.4 cm;TW: 5101 g; Clasper length: 287 mm; GSA 11	
	

Stage 4 SPENT	NOTES
Raja clavata TL: 67.5 cm; TW: 1722.5 g; Clasper length: 143.6 mm; GSA 11	
	
Scyliorhinus canicula TL: 40.3 cm; TW: 190.3 g; Clasper length: 29.4 mm; GSA 11	

According to the working group, the name of this stage has been changed in **SPENT** indicating a reproductive suspension (including regressing and regenerating phases). Seminal vesicle and thorns (alar and malar) description was inserted as features also in this stage. Indeed, the seminal vesicle was considered totally empty and the thorns similar to those observed in 3a and 3b Stages.

3.2.3 Female macroscopic maturity scale (with notes) proposed for oviparous Elasmobranchs

In Tab. 3.2.3.1, the female macroscopic maturity scale proposed for oviparous Elasmobranchs is reported. In red, changes made to WKMSEL2 maturity scale. A column "NOTES" has been added in order to better specify some features of the new scale.

Table 3.2.3.1 Female macroscopic maturity scale proposed for oviparous Elasmobranchs with notes (in red the modified sentence)

MATURITY	STAGE		DESCRIPTION	NOTES
IMMATURE	1	IMMATURE	**Ovaries**: small and whitish. Undistinguishable or very small ovarian follicles. **Oviducal gland**: often not visible. In some species a thickening of the uteri where the gland will develop may be visible. **Uteri**: thread-like and narrow.	**Ovaries:** in some sharks very small follicles are visible.
IMMATURE	2	DEVELOPING	**Ovaries**: Unyolked follicles and some small and medium yolked ones may be present. **Oviducal gland**: distinguishable and developing, mostly in skates.	**Unyolked follicles**: the researchers group adopted the term unyolked follicles to distinguish them from the yolked ones according

MATURITY	STAGE		DESCRIPTION	NOTES
			Uteri: enlarging	to the different development degree observed in this stage. **Oviducal gland**: distinguishable and developing mostly in skates.
MATURE	3a	CAPABLE TO REPRODUCE	**Ovaries**: presence of large yolked follicles ready to be ovulated. **Oviducal glands**: fully developed . **Uteri**: fully developed and turgid.	**Uteri:** are turgid differently from the Stage 4a.
MATURE	3b	ACTIVE	**Ovaries and Oviducal glands**: similar to Stage 3a **Uteri:** presence of egg capsules	
MATURE	4a	POST-LAYING	**Ovaries**: flaccid with follicles (unyolked and yolked) of different sizes. Post-ovulatory follicles and atretic follicles visible. **Oviducal glands**: fully developed but may be reduced in size, mostly in skates. **Uteri**: enlarged, flaccid and vascularized.	**Ovaries**: flaccid with unyolked and yolked follicles of different sizes, according to species. **Oviducal glands**: in some skates species could be reduced. **Uteri**: the uteri appearance could be also vascularized.
MATURE	4b	REGENERATING	**Ovaries**: large with small and medium sized yolked follicles. Pre-ovulatory follicles absent. **Oviducal glands**: fully developed but may be reduced in size, mostly in skates. **Uteri**: enlarged but not flaccid.	**Oviducal glands**: in some skates species could be reduced. [4b.2] **Uteri:** in Stage 4b uteri are not flaccid.

3.2.4 Male macroscopic maturity scale proposed for oviparous Elasmobranchs

In Tab. 3.2.4.1, the male macroscopic maturity scale proposed for oviparous Elasmobranchs is reported. In red, changes made to WKMSEL2 maturity scale.

Tab. 3.2.4.1 Male macroscopic maturity scale proposed for oviparous Elasmobranchs (in red the modified sentence).

MATURITY	STAGE		DESCRIPTION	NOTES
IMMATURE	1	IMMATURE	**Testes**: small and undeveloped (in skates, sometimes with visible lobules). **Ducts**: straight and thread-like. **Claspers**: flexible, non-calcified and shorter than pelvic fins.	No changes in respect to WKMSEL2 scale

MATURITY	STAGE		DESCRIPTION	NOTES
IMMATURE	2	DEVELOPING	**Testes**: developing (in skates, lobules clearly visible but not fully developed). **Ducts**: developing and beginning to coil. **Claspers**: flexible, partially calcified and usually or as long as or longer than pelvic fins. In some sharks they do not pass the pelvic fins. **Alar thorns**: could be present at primordial stage (single row) in some skate species.	**Claspers**: in *Scyliorhinus* spp the claspers don't pass the pelvic fins, even when mature. **Thorns**: in several skates, primordial alar thorns are visible due to a sexual dimorphism. The detection of the thorns could be useful to distinguish the Stage 2 from the Stage 1
MATURE	3a	CAPABLE TO REPRODUCE	**Testes**: fully developed according to species-specific characteristics. **Ducts**: tightly coiled and filled with sperm. **Seminal vesicle:** (when present) developed. Sperm does not flow on pressure. **Claspers**: rigid, fully calcified and longer than pelvic fins (in some sharks they may only be as long as the pelvic fins). **Alar and/or malar thorns:** could be present in some skate species.	**Testes:** they could appear fully developed with different structure (e.g. in skates with lobules)**.** **Seminal vesicle:** in some sharks as *Galeus melastomus* this organ is clearly evident. **Thorns:** in several skates, alar and/or malar thorns , structured in several rows are visible. Their detection could be useful to distinguish the Stage 3a from the Stage 2.
MATURE	3b	ACTIVE	**Testes**: similar to Stage 3a. **Ducts**: sperm observed flowing out of the cloaca on pressure. **Seminal vesicle** (when visible) can be full or empty **Claspers:** fully calcified sometimes with glans dilated, swollen and reddish. Sperm flows on pressure and it may be present in groove or glans. **Alar and/or malar thorns:** could be present in some skate species.	**Seminal vesicle**: depending on reproductive strategy. **Thorns**: in several skates, alar and/or malar thorns , structured in several rows are visible.
MATURE	4	**SPENT**	**Testes** shrunken and flaccid. On pressure sperm does not flow. **Sperm ducts** and **seminal vesicle** empty and flaccid. **Claspers**: fully formed. **Alar and/or malar thorns:** could be present in some skate species.	According to the working group, the term **SPENT** in males indicates the reproductive suspension (including regressing and regenerating phases) **Seminal vesicle and thorns**: similar to Stage 3b

3.2.5 Reproductive cycle of oviparous female and male Elasmobranchs

The working group prepared a schematic common reproductive cycle for female and male oviparous species (Fig. 3.2.5.1; 3.2.5.2). Immature phases (1-immature and 2 -developing stages) and mature phases (3a-capable to reproduce, 3b-active, 4a-post-laying and 4b-regenerating for females and 3a-capable to reproduce, 3b-active and 4-spent for males) were represented. In both sexes, when females and males are capable to reproduce (Stage 3a), they continue the cycle arriving to the regenerating/spent stage. Then, they return to the capable to reproduce stage.

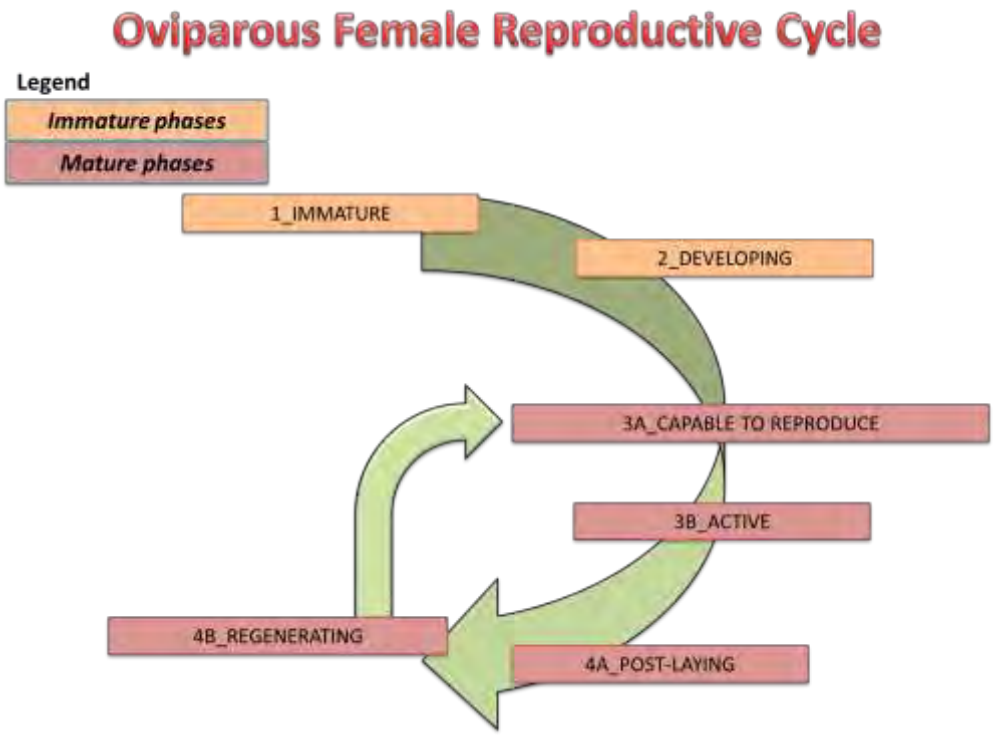


Fig. 3.2.5.1 Representation of a oviparous female reproductive cycle.

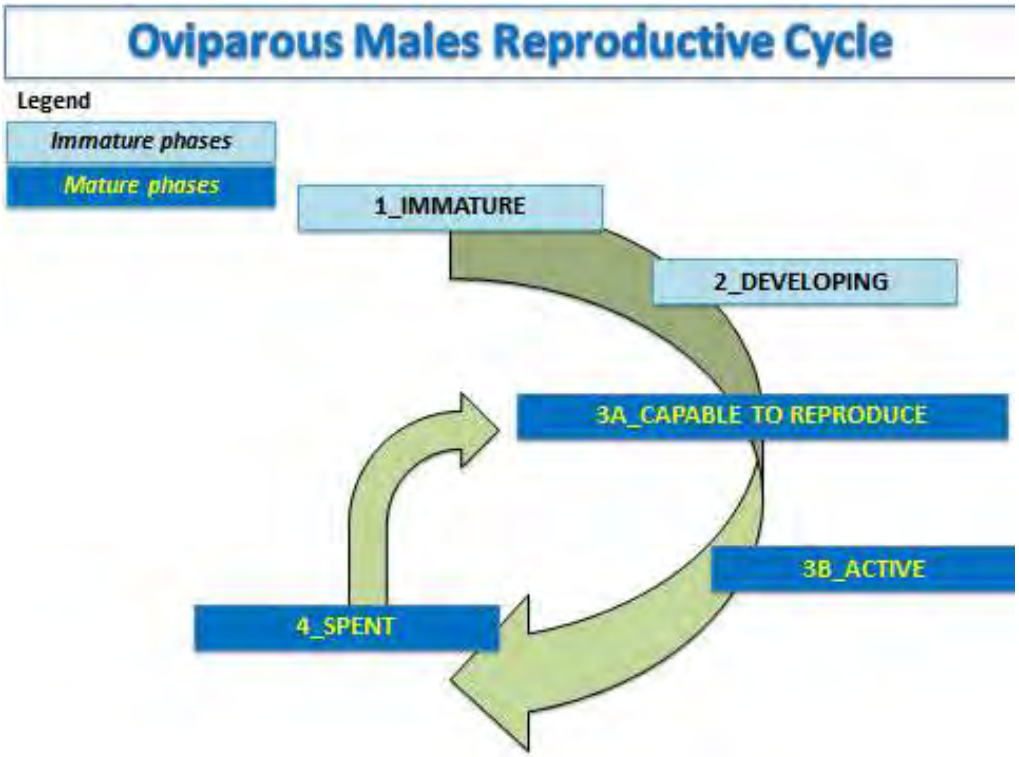


Fig. 3.2.5.2 Representation of a oviparous male reproductive cycle.

3.3 Viviparous macroscopic maturity scales

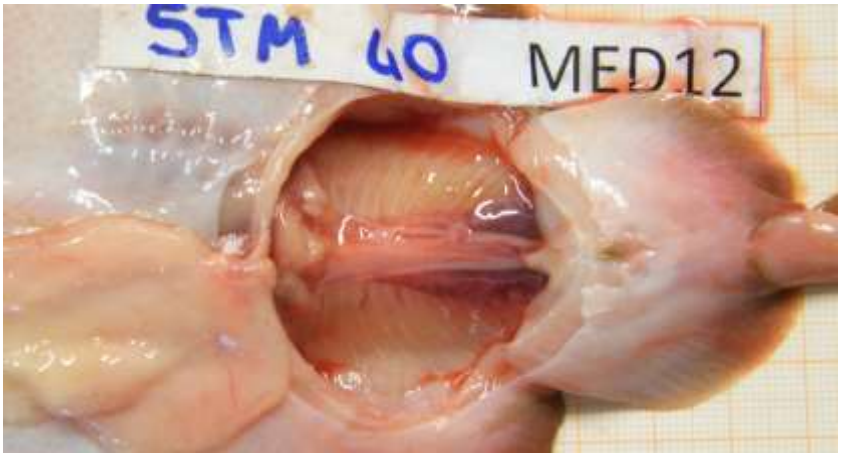
The viviparous macroscopic scales were based on the results from sharks and batoids (mainly Squaliformes and Torpediniformes), the taxa studied by the participants of this workshop. Stages' descriptions were made considering a geographic area as wide-ranging as possible. Changes in respect to the WKMSEL2 one were made only to the female macroscopic scale.

3.3.1 Female maturity scale

The general structure of WKMSEL2 female maturity scale was not maintained due to addition of a seventh maturity stage. The old Stage 6 REGENERATING has been split in two stages: 6. EARLY REGENERATION and 7. LATE REGENERATION. The group considered useful to make this division in order to distinguish the time of regeneration. Moreover, it was added a Stage 7 (LATE REGENERATION) in which the atretic follicles are unlikely because they are starting again the developing .

Stage 1. Immature: pictures of *Etmopterus spinax*, *Oxynotus centrina* and *Torpedo marmorata* were observed and discussed in detail (Tab. 3.3.1.1).

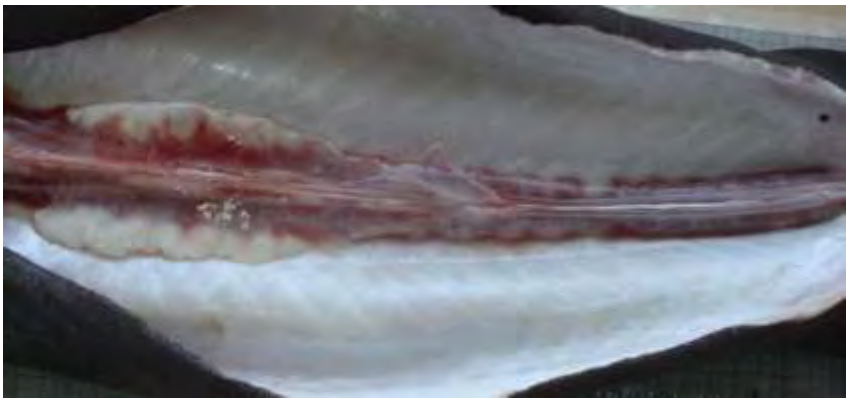
Table 3.3.1.1. Pictures of female oviparous species in Stage 1.

Stage 1 IMMATURE	NOTES
	
Etmopterus spinax TL: 28.3 cm; TW: 95 g; GSA11	
	
Oxynotus centrina TL: 27.4 cm; TW: 154 g; GSA11	
	
Torpedo marmorata TL: 16.2 cm; TW: 90 g; GSA11	

No changes has been made to Stage 1.

Stage 2. Developing: pictures of *Etmopterus spinax* and *Dalatias licha* were observed and discussed in detail (Tab. 3.3.1.2).

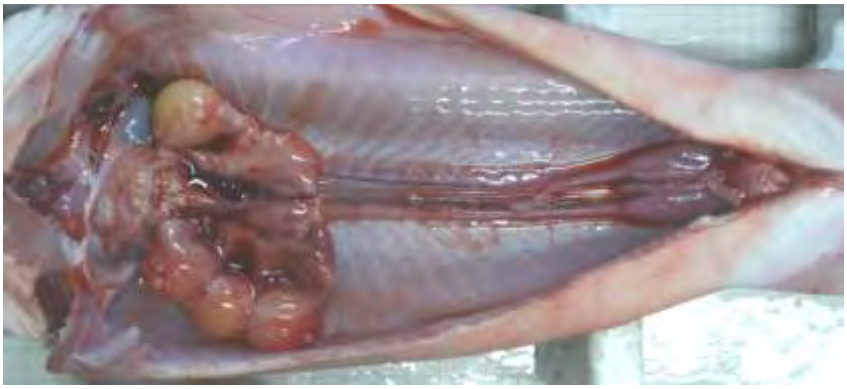
Table 3.3.1.2. Pictures of female oviparous species in Stage 2.

Stage 2 DEVELOPING	NOTES
	
Etmopterus spinax GSA11	
	
Dalatias licha GSA19	

No changes has been made to Stage 2.

Stage 3. Capable to reproduce: pictures of *Torpedo marmorata* and *Squalus blainville* were observed and discussed in detail (Tab. 3.3.1.3).

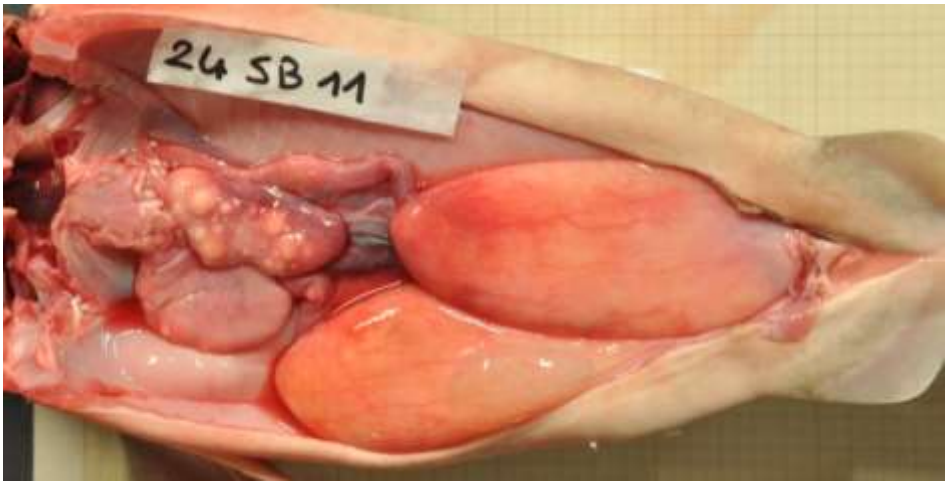
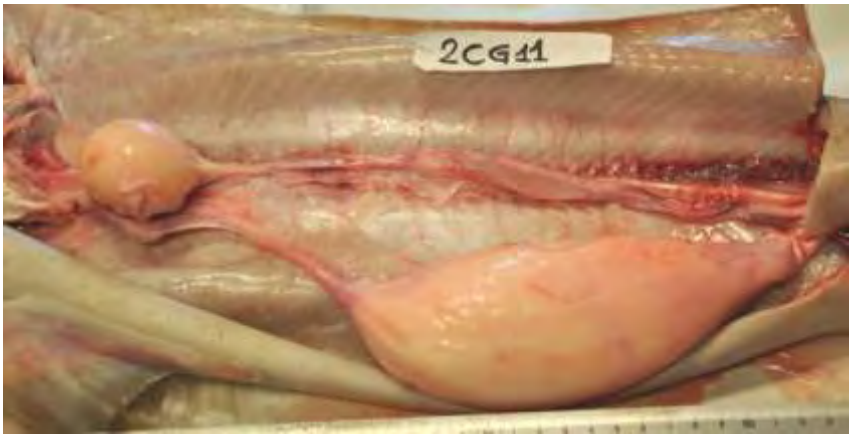
Table 3.3.1.3. Pictures of female oviparous species in Stage 3.

Stage 3 CAPABLE TO REPRODUCE	NOTES
	
Torpedo marmorata TL: 16.2 cm; TW: 90 g; GSA11	
	
Squalus blainville TL: 58 cm; TW: 1250 g; GSA11	

No changes has been made to Stage 3.

Stage 4a. Early pregnancy: pictures of *Squalus blainville* and *Centrophorus cf uyato* were observed and discussed in detail (Tab. 3.3.1.4).

Table 3.3.1.4. Pictures of female oviparous species in Stage 4a.

Stage 4a EARLY PREGNANCY	NOTES
	
Squalus blainville TL: 68.3 cm; TW: 1524 g; GSA11	
	
Centrophorus cf uyato TL: 103.8 cm; TW: 6542 g; GSA11	

From the observations, the researchers group decided to add ovary and oviducal gland description, differently from the WKMSEL2 scale where only the uteri description was present. Participants mentioned the different development of ovaries in sharks. Some of these (e.g. *Squalus blainville*) show active ovaries (yolked ovarian follicles always present) during the pregnant phases (synchronous development). Other Squaliformes (e.g. *Etmopterus spinax*) have inactive ovaries (unyolked and/or atretic follicles) during maternal stages (asynchronous development). The group also highlighted the possible presence of yolk in oviducts and/or oviducal gland (ovulation in process).

Stage 4b. Mid pregnancy: pictures of *Squalus blainville* and *Centrophorus cf uyato* are discussed (Tab. 3.3.1.5).

Table 3.3.1.5. Pictures of female oviparous species in Stage 4b.

Stage 4b MID PREGNANCY	NOTES
	Active ovaries.
Squalus blainville TL: 79.9 cm; TW: 2967 g; GSA11	
	
Centrophorus cf uyato TL: 103.8 cm; TW: 6542 g; GSA11	

The ovary and oviducal gland description has been added differently from the WKMSEL2 scale where only the uteri description was present. As mentioned in the previous stage, the ovary description reported the difference between active and inactive ovaries.

Stage 4c. Late pregnancy: pictures of *Squalus blainville, Etmopterus spinax* and *Dalatias licha* were observed and discussed in detail (Tab. 3.3.1.6).

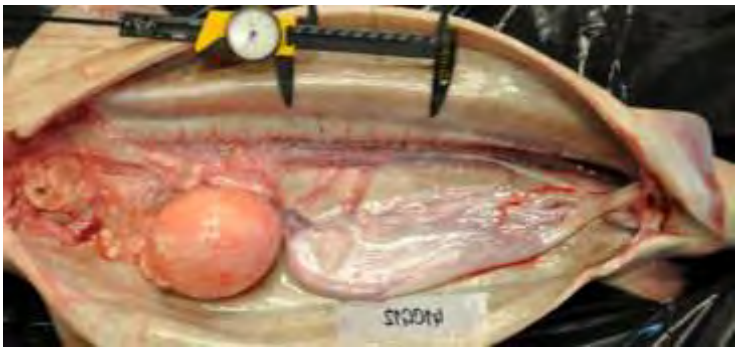
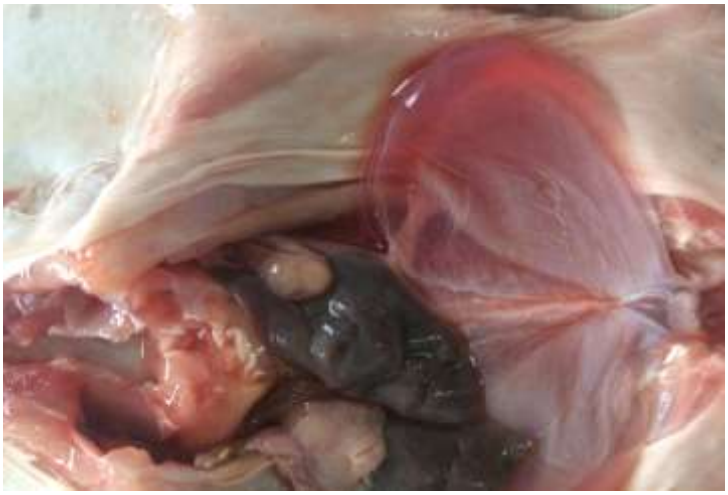
Table 3.3.1.6. Pictures of female oviparous species in Stage 4c.

Stage 4c LATE PREGNANCY	NOTES
	Inactive ovaries in which atretic follicles are visible. Embryos fully formed.
Etmopterus spinax TL: 38.2 cm; TW: 275 g; GSA11	
	Active ovaries with large yolked follicles. Embryos fully formed.
Squalus blainville TL: 77.2 cm; TW: 2300 g; GSA11	
	
Dalatias licha TL: 77.2 cm; TW: 6525 g; GSA11GSA11	

The ovary and oviducal gland description has been added differently from the WKMSEL2 scale where only the uteri description was present. As mentioned in the previous stage, the ovary description reported the difference between active and inactive ovaries.

5. Post-partum: pictures of *Torpedo marmorata, T. torpedo* and *Centrophorus cf uyato* were observed and discussed in detail (Tab. 3.3.1.7).

Table 3.3.1.7. Pictures of female oviparous species in Stage 5.

Stage 5 POST-PARTUM	NOTES
	
Torpedo marmorata TL: 53 cm; TW: 3204 g; GSA11	
	
Centrophorus cf uyato TL: 103.8 cm; TW: 7151 g; GSA11	
	
Torpedo torpedo GSA19	

The description of the stage reported in the WKMSEL2 scale has been modified only for the uterus. The participants agreed with the fact that the appearance of ovaries and oviducal glands was similar to what reported for Stage 4c.

6. Early regeneration: picture of *Etmopterus spinax* were observed and discussed in detail (Tab. 3.3.1.8).

Table 3.3.1.8. Pictures of female oviparous species in Stage 6.

Stage 6 EARLY REGENERATION	NOTES
	
Etmopterus spinax TL: 38.5 cm; TW: 253 g; GSA11	

The Stage 6 (REGENERATING) reported in WKMSEL2 scale has been changed in EARLY REGENERATION considering the time of regeneration in which females are. The group reported that the presence of small or medium yolked follicles in ovaries with atretic follicles can be observed.

7. Late regeneration: picture of *Squalus blainville* were observed and discussed in detail (Tab. 3.3.1.9).

Table 3.3.1.9. Pictures of female oviparous species in Stage 7.

Stage 7 LATE REGENERATION	NOTES
	
Squalus blainville GSA11	

The group proposed the creation of a new stage pointing out the different morphologies of reproductive organs during regeneration process. Indeed, differently from the Stage 6 (early regeneration), ovaries could have medium-large yolked ovarian follicles. Atretic follicles could be unlikely because ovaries are developing.

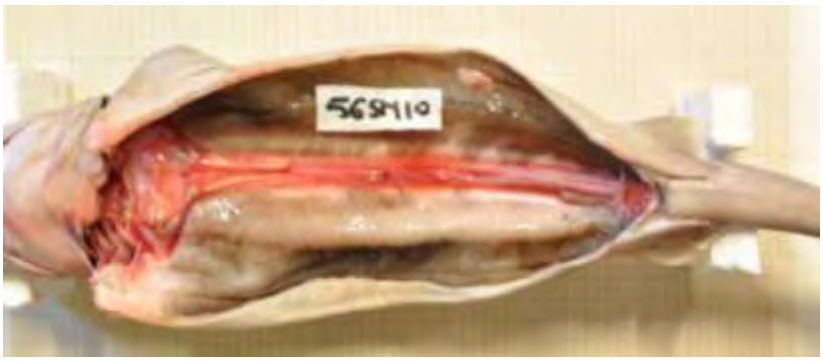
3.3.2 Male maturity scale

The nomenclature of the male maturity stages was the same of those of WKMSEL2 scale except for the Stage 4 here reported as SPENT instead of REGRESSING as in the macroscale of male oviparous species. In addition, the order of the organ description has been changed (testes, ducts and claspers instead claspers, testes and ducts).

Following, a summary of the main aspects discussed stage-by-stage during the revision of the males maturity scale is reported .

1. Immature: picture of *Centrophorus cf uyato* and *Squalus blainville* were observed and discussed in detail (Tab. 3.3.2.1).

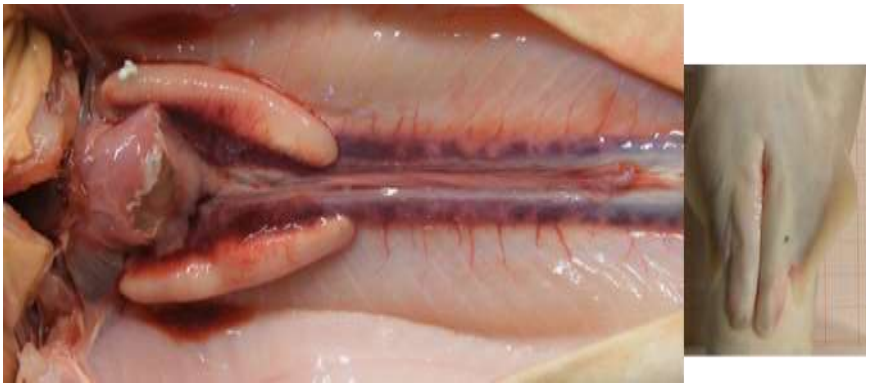
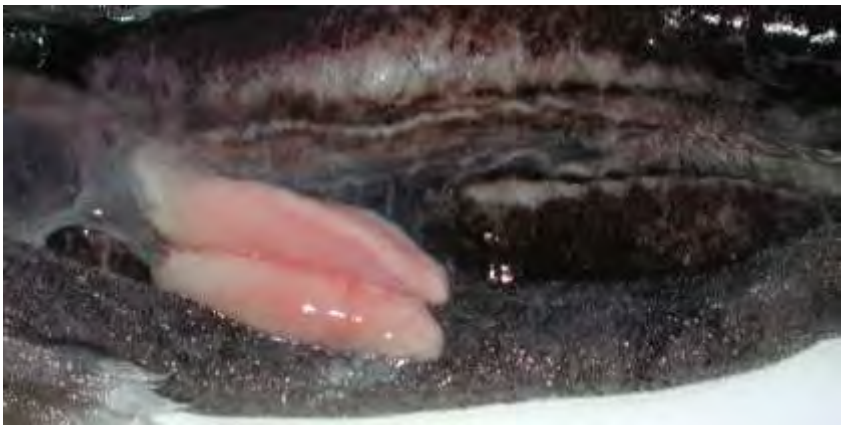
Table 3.3.2.1. Pictures of male viviparous species in Stage 1.

Stage 1 IMMATURE	NOTES
	
Centrophorus cf uyato TL: 58.1 cm; TW: 846 g; GSA11	
	
Squalus blainville TL: 44 cm; TW: 395 g; GSA11	

The description of immature stage was the same of WKMSEL2.

2. Developing: picture of *Etmopterus spinax* and *Squalus blainville* were observed and discussed in detail (Tab. 3.3.2.2).

Table 3.3.2.2. Pictures of male viviparous species in Stage 2.

Stage 2 DEVELOPING	NOTES
	Testes with segments mainly found in sharks.
Squalus blainville TL: 48 cm; TW: 419 g; GSA11	
	Testes with segments mainly found in sharks.
Etmopterus spinax TL: 31.5 cm; TW: 129 g; GSA11	
	Testes with segments mainly found in sharks.
Etmopterus spinax GSA19	

The macroscopic description of testes has been changed pointing out that the gonad morphology is species-specific.

3a. Capable to Reproduce: picture of *Urolophus paucimaculatus*, *Tetronarce nobiliana*, *Torpedo marmorata*, *Myliobatis aquila* and *Squalus blainville* were observed and discussed in detail (Tab. 3.3.2.3).

Table 3.3.2.3. Pictures of male viviparous species in Stage 3a.

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Full seminal vesicle.
Squalus blainville GSA18	
	Testes with lobules mainly found in batoids.
Myliobatis aquila DW: 45.7 cm; TW: 1401.7 g; GSA11	

Stage 3a CAPABLE TO REPRODUCE	NOTES
 	Testes with lobules mainly found in batoids.
Tetronarce nobiliana GSA19	
	Testes with segments mainly found in sharks.
Squalus blainville TL: 60 cm; TW: 1018 g; GSA11	

Stage 3a CAPABLE TO REPRODUCE	NOTES
	Testes with lobules mainly found in batoids.
Urolophus paucimaculatus South-eastern Australia (Victoria)	
	Testes with lobules mainly found in batoids.
Torpedo marmorata TL: 34 cm; TW: 944 g; GSA11	

The changes made to the Stage 3a concerned the description of the testes and ducts. Segments (in some sharks) and lobules (in batoids) in testes and the description of seminal vesicle in ducts were taken into account.

3b. Active: pictures of *Centrophorus cf uyato* and *Squalus blainville* were observed and discussed in detail (Tab. 3.3.2.4).

Table 3.3.2.4. Pictures of male viviparous species in Stage 3b.

Stage 3b Active	NOTES
	Testes with segments mainly found in sharks. Full seminal vesicle.
Squalus blainville TL: 58.5 cm; TW: 857 g; GSA11	
	Testes with segments mainly found in sharks. Full seminal vesicle.
Centrophorus cf uyato TL: 82 cm; TW: 3089 g; GSA19	

As in Stage 3a, the seminal vesicle description was made. It may be full or empty depending on the reproductive strategy.

4. Spent: pictures of *Squalus blainville* and *Myliobatis aquila* were observed and discussed in detail (Tab. 3.3.2.5).

Table 3.3.2.5. Pictures of male viviparous species in Stage 4.

Stage 3b Active	NOTES
	Empty seminal vesicle.
Squalus blainville TL: 58.6 cm; TW: 896 g; GSA11	
	
Myliobatis aquila TL: 32 cm; TW: 2231 g; GSA19	

The name of the stage was changed in SPENT.

3.3.3 Viviparous female reproductive cycle

Short and long maternal cycle reflects the utilization of the stages and not the duration of the reproductive cycle.

Example of a long maternal cycle (generally asynchronous reproductive cycle) is referred to species where the follicles in the ovary are atretic or not developed at Stage 4c\5. The female then requires to go through Stage 6 (developing follicles) and then Stage 7 (follicles are fully developed) to give the opportunity to the gonad to start again its development (Fig. 3.4.1.1).

Example of a short maternal cycle (generally synchronous reproductive cycle) is referred to species where the follicles in the ovary are fully developed at Stage 4c\5. The female will then ovulate after a short Stage 5. This is seen in species with biannual or triennial reproduction.

Maternal cycles (intermediate between long and short maternal cycle) could be also detected. In this case, females, after the parturition at Stage 5, have the ovarian follicles still fully developed. The ovary after ovulation in Stage 5 does not occur directly to stages 6 and 7 (long maternal cycle)

but it needs to a longer time than female with short maternal cycle. For this reason, females skip Stage 6 and go straight to Stage 7.

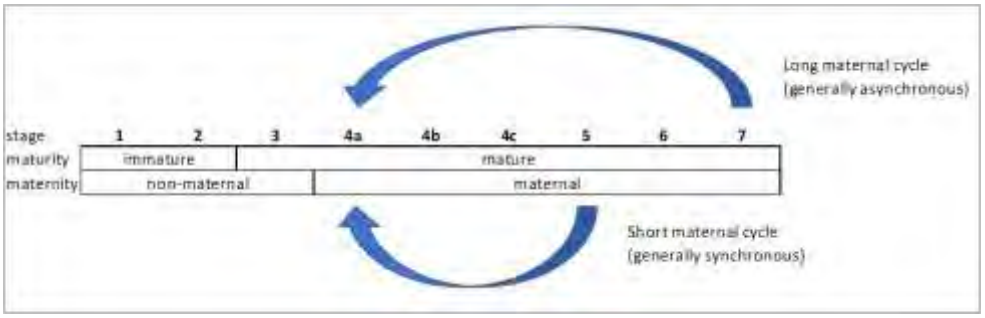


Figure 3.4.1.1 Representation of the female reproductive cycle of viviparous species.

3.4 Macroscopic maturity scale proposed for viviparous Elasmobranchs

3.4.1 Female macroscopic maturity scale proposed for viviparous Elasmobranchs

In Tab. 3.4.2.1, the female macroscopic maturity scale proposed for viviparous Elasmobranchs is reported. In red, changes made to WKMSEL2 maturity scale. A column "NOTES" has been added in order to better specify some features of the new scale.

Table 3.4.2.1. Female macroscopic maturity scale proposed for viviparous Elasmobranchs with notes (in red the modified sentence).

MATURITY/MATERNITY	STAGE		DESCRIPTION	NOTES
IMMATURE	1	IMMATURE	**Ovaries**: small and whitish; undistinguishable ovarian follicles. **Oviducal glands**: often not visible. In some species a thickening of the oviducts where the gland will develop may be visible **Uteri**: thread-like and narrow.	No changes in respect to WKMSEL2 scale
IMMATURE	2	DEVELOPING	**Ovaries**: follicles of different stages of development. Some small and medium sized yolked follicles may be present. **Oviducal glands**: distinguishable and developing **Uteri**: enlarging	No changes in respect to WKMSEL2 scale
MATURE	3	CAPABLE to REPRODUCE	**Ovaries**: presence of large yolked follicles ready to be ovulated. **Oviducal glands**: fully developed. **Uteri**: fully developed.	No changes in respect to WKMSEL2 scale
MATERNAL	4a	EARLY PREGNANCY	**Ovaries**: different sized follicles are present according to stages of ovulation. **Oviducal glands**: fully developed (it is possible to observe a starting regressing). **Uteri**: well filled and rounded with yolk content (usually candle shape). Embryos cannot be observed.	Yolk could be found in oviducts and/or oviducal gland (ovulation in process). Atretic follicles may be present in the ovaries (species specific).

MATURITY/MATERNITY	STAGE		DESCRIPTION	NOTES
MATERNAL	4b	MID PREGNANCY	**Ovaries**: small to medium, possibly yolked follicles (active ovaries) or small, unyolked and/or atretic follicles (inactive ovaries). **Oviducal glands**: fully developed (it is possible to observe a starting regressing). **Uteri**: well filled and rounded. Embryos are always visible, small and with a relatively large yolk sac.	In the different reproductive strategies follicles may begin to enlarge (synchronous) or ovaries may remain inactive (asynchronous).
MATERNAL	4c	LATE PREGNANCY	**Ovaries**: medium to large yolked follicles (active ovaries) or small, unyolked follicles and/or atretic follicles (inactive ovaries). **Oviducal glands**: fully developed (it is possible to observe a starting regressing). **Uteri**: embryos fully formed, yolk sacs reduced or absent.	In the different reproductive strategies follicles enlarged (synchronous) or ovaries may remain inactive (asynchronous).
MATERNAL	5	POST-PARTUM	**Ovaries**: Similar to stage 4c. **Oviducal glands**: Similar to stage 4c. **Uteri**: enlarged and flaccid (likely to have just given birth).	In the different reproductive strategies follicles enlarged (synchronous) or ovaries may remain inactive (asynchronous).
MATURE	6	EARLY REGENERATION	**Ovaries**: small or medium yolked follicles. **Oviducal glands**: fully developed (may be reduced in size). **Uteri**: enlarged post-maternal, but not so flaccid.	
MATURE	7	LATE REGENERATION	**Ovaries**: large yolked follicles of ovulatory size. **Oviducal glands**: fully developed (may be reduced in size). **Uteri**: enlarged post-maternal, but not flaccid.	Atretic follicles are unlikely because ovaries are developing.

3.4.2 Male macroscopic maturity scale proposed for viviparous Elasmobranchs

Some adjustments to the male maturity scale of WKMSEL2 has been made (in red) by the working group. In Tab. 3.4.3.1, the male macroscopic maturity scale proposed for viviparous Elasmobranchs by the participants is reported. In red, changes made to WKMSEL2 maturity scale. A column "NOTES" has been added in order to better specify some features of the new scale.

Table 3.4.3.1. Male macroscopic maturity scale proposed for viviparous Elasmobranchs with notes (in red the modified sentence).

MATURITY	STAGE		DESCRIPTION	NOTES
IMMATURE	1	IMMATURE	**Testes:** small and undeveloped. **Ducts**: straight and thread-like. **Claspers**: flexible, non-calcified and usually shorter than pelvic fins.	No changes in respect to WKMSEL2 scale
IMMATURE	2	DEVELOPING	**Testes:** developing according to species-specific characteristics (sometimes with segments in sharks or lobules in batoids) but not occupying the whole surface of the abdominal cavity. **Ducts**: developing and beginning to coil. **Claspers**: flexible, partially calcified and as long as or longer than pelvic fins.	Segments mainly found in sharks and lobules mainly in batoids.
MATURE	3a	CAPABLE TO REPRODUCE	**Testes:** fully developed according to species-specific characteristics (fully segmented in some sharks and lobed in some batoids). **Ducts**: tightly coiled and filled with sperm. Seminal vesicle (when visible) developed. **Claspers**: rigid, fully calcified and longer than pelvic fins.	Segments mainly found in sharks and lobules mainly in batoids.
MATURE	3b	ACTIVE	**Testes:** similar to stage 3a. **Ducts**: sperm flowing out of the cloaca on pressure. Seminal vesicles (when visible) can be full or empty. **Claspers**: fully formed. sometimes swollen and/or reddish. with clasper gland dilated, Sperm may be present in clasper groove or gland.	The fullness of the seminal vesicle Development depends on the reproductive strategy.
MATURE	4	**SPENT**	**Testes:** shrunken and flaccid. **Ducts**: empty and flaccid. Seminal vesicle (when visible) empty. Claspers: fully formed.	

4 Validate both maturity scales based on macroscopic features through histological analysis (ToR B)

The goal of ToR B is to validate the macroscopic staging keeping in mind that the histological features not always can be reflect macroscopically. For future references, it is recommended that if new information on maturity stages validation using histology are collected, the results should be documented and presented in the next WKMSEL meeting. In light of new information, the scales should be revised accordingly.

During the meeting, firstly the macroscopic pictures were shown and the maturity stage discussed. After that, the histological pictures were shown to validate the macroscopic determined stage.

Species with different reproductive modality were taken into account.

For oviparous species, *Galeus melastomus* and *Dipturus oxyrinchus* gonads were analysed macroscopically in plenary. Then, microscopic maturity features of each stage was presented. proposed for female oviparous elasmobranchs in WKMSEL2 has been followed.

A brief description of the microscopic features of each maturity stage previously defined macroscopically for females and males is presented below.

4.1 Female microscopic maturity stages

4.1.1 Oviparous species

Galeus melastomus and *Dipturus oxyrinchus*

Stage 1. Immature. Primary and early pre-vitellogenic ovarian follicles connected to the germinal epithelium and tunica albuginea, surrounded by the high vascularized epigonal organ (Fig. 4.1.1.1; 4.2.1.2).

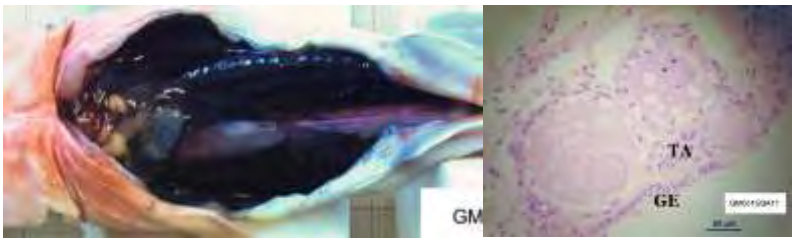


Figure 4.1.1.1. Macroscopic and microscopic pictures of *G. melastomus* in Stage 1.

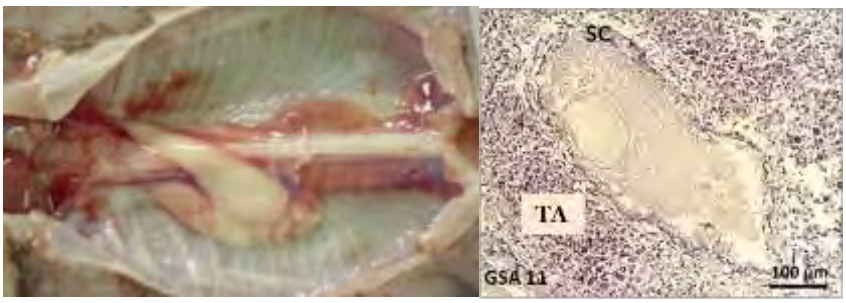


Figure 4.1.1.2. Macroscopic and microscopic pictures of *D. oxyrinchus* in Stage 1.

Stage 2. Developing: According maturation progress, the follicles increase in diameter, the zona pellucida thickens and the theca layers differentiate into an inner one, formed by eosinophilic squamous cells and an outer, comprising one or two layers of squamous basophilic cells. Lipid-rich inclusions begin to appear and enlarge in the cytoplasm. Besides the pre-vitellogenic follicles of various sizes, vitellogenic follicles may be present (Fig. 4.1.1.3).

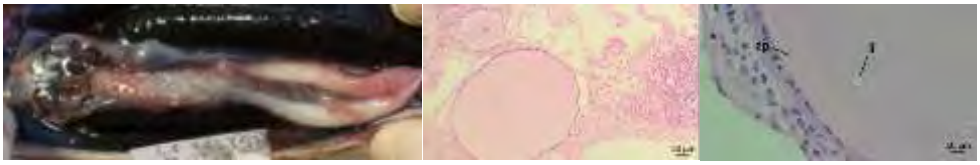


Figure 4.1.1.3. Macroscopic and microscopic pictures of *G. melastomus* in Stage 2.

Stage 3a. Capable to reproduce: vitellogenic follicles are the most abundant in the ovaries. Vitellogenesis consists on the formation of yolk platelets and pseudostratification of the follicular epithelium (Fig. 4.1.1.4; 4.1.1.5).

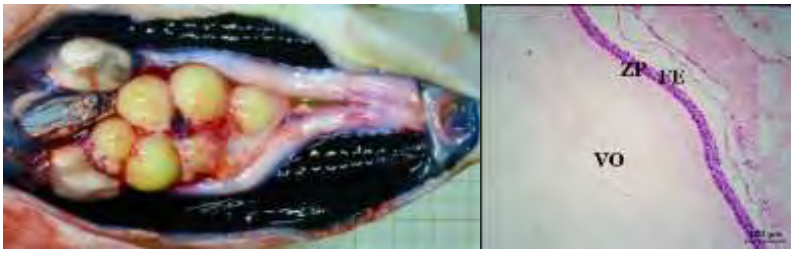


Figure 4.1.1.4. Macroscopic and microscopic pictures of *G. melastomus* in Stage 3a.

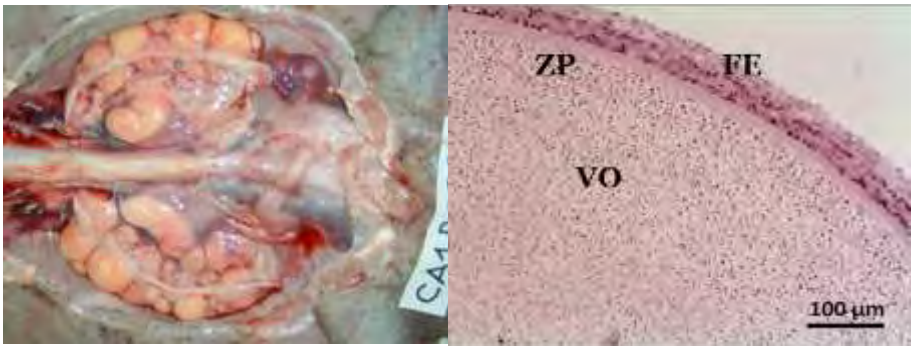


Figure 4.1.1.5. Macroscopic and microscopic pictures of *D. oxyrinchus* in Stage 3a.

Stage 3b. Egg-laying: the description was similar to Stage 3a. Post-ovulatory follicles was present.

4.1.2 Viviparous species

The histological description of female maturity stages of viviparous species was the same proposed for oviparous ones. However, the working group participants, in consideration of the differences in synchronous (e.g. *Squalus blainville*; Fig. 4.2.1.1) and asynchronous (e.g. *Etmopterus spinax;* Fig. 4.2.1.2) development of the ovaries in respect to the uteri, suggested to differentiate also the histological description for the two kind of development.

S. blainville shows active ovaries during pregnant phases (stages 4a,4b,4c,5) (Fig. 4.2.1.1). Ovarian histological sections at these stages highlighted the presence of vitellogenic ovarian follicles, differently from *E. spinax* that presents inactive ovaries with primary and previtellogenic ovarian follicles (Fig. 4.2.1.2).

Figure 4.2.1.1. Macroscopic an microscopic picture of a mid-pregnancy *S. blainville* (ovary). OV, ovaries; UT, uteri; VO, vitellogenic ovarian follicle.

Figure 4.2.1.2. Macroscopic an microscopic picture of a mid-pregnancy *E. spinax* (inactive ovary). OV, ovaries; UT, uteri; PO, primary ovarian follicle; PVO, previtellogenic ovarian follicl.

4.2 Staging Exercise

During the workshop, a staging exercise using the new scales for both viviparous and oviparous species was carried out. In particular 15 specimens belonging to *Etmopetrus spinax* (viviparous) and 15 specimens of *Galeus melastomus* (oviparous species) were analysed. The rendering data were analysed by Eltink sheet (Eltink, 2000).

Results of the staging exercise were discussed in plenary session in order to clarify the most controversial stages.

4.2.1 Etmopterus spinax

The overall agreement with modal stage was 68.5% (Table 4.2.1.1). The modal stage was calculated not stratified on the base of the participants' experience participants.

In the analysis, the stages with higher percentage of agreement and lower coefficient of variation were the stages 3 (Capable to Reproduce), 1 (Immature) and 6 (Early Regeneration) for females and stage 1 (Immature) for males (Table 4.2.1.2, Table 4.2.1.3 and Figure 4.2.1.1). Regarding the Stage 1, in some case, the dimension of the clasper (very small and short in this species) caused a misclassification among sexes.

In general, the results showed that females staged as POST-PARTUM (5) and EARLY REGENERATION (6) were confused each other. Moreover, for males, Stage 1 (Immature) was confused with Stage 2 (Developing). During the discussion of these results, it was attempted to clarify some of the controversial cases, especially when the gonads are located on the border between two stages.

Regarding the bias (Table 4.2.1.4), the results showed a positive bias (overestimation) for the stages 1 and 5 for the females and 1 and 2 for the males, while a negative bias (underestimation) for stages 6 (female) it was found.

Table 4.2.1.1 – Results of the staging exercise on the viviparous species

Stratun	Sample year	Species	Fish no	Landing month	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	MODAL Stage	Percent agreemen	Precision CV
Italy	2018	E. spinax	es1	2	F1	F1	F1	M1	F1	M1	F1	F1	F1	M1	M1	**F1**	64%	106%
Italy	2018	E. spinax	es2	2	M1	M1	M1	M2	M1	M2	M1	M1	M1	M2	M2	**M1**	64%	5%
Italy	2018	E. spinax	es3	2	M3A	M2	M2	M3A	M2	M3B	M2	M1	M2	M3A	M3B	**M2**	45%	8%
Italy	2018	E. spinax	es4	2	F2	F1	F1	F1	F1	F1	F1	F1	F2	M1	M1	**F1**	64%	127%
Italy	2018	E. spinax	es5	2	M1	F1	F1	M1	F1	M1	F1	F1	M1	M1	M1	**M1**	55%	80%
Italy	2018	E. spinax	es6	2	M3B	M2	M3A	M3B	M2	M3B	M2	M1	M2	M3B	M3B	**M3B**	45%	10%
Italy	2018	E. spinax	es7	2	F2	F1	F1	F1	F1	F1	F1	F1	F1	F1	F1	**F1**	91%	28%
Italy	2018	E. spinax	es8	2	M1	M1	M1	M2	M1	M1	M1	M1	M1	M1	M1	**M1**	91%	3%
Italy	2018	E. spinax	es9	2	F1	F1	F1	F1	F1	F1	F1	F1	F1	F1	F2	**F1**	91%	28%
Italy	2018	E. spinax	es10	2	F6	F5	F5	F5	F5	F6	F5	F5	F6	F6	F6	**F5**	55%	7%
Italy	2018	E. spinax	es11	2	F6	F6	F6	F6	F6	F6	F6	F6	F6	F7	F7	**F6**	82%	5%
Italy	2018	E. spinax	es12	2	F7	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	**F3**	91%	51%
Italy	2018	E. spinax	es13	2	F3	F6	F5	F5	F5	F6	F5	F5	F6	F6	F6	**F6**	45%	20%
Italy	2018	E. spinax	es14	2	M2	M1	M2	M2	M1	M2	M1	M1	M2	M2	M2	**M2**	64%	5%
Italy	2018	E. spinax	es15	2	F6	F6	F6	F6	F5	F6	F6	F5	F6	F6	F6	**F6**	82%	5%
			Total read		15	15	15	15	15	15	15	15	15	15	15		**68.5%**	**32.4%**

Table 4.2.1.2 Results of agreement by reader and stage

PERCENTAGE AGREEMENT

MODAL Stage	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	ALL Readers
0	-	-	-	-	-	-	-	-	-	-	-	-
F1	50%	100%	100%	75%	100%	75%	100%	100%	75%	50%	25%	77%
F2	-	-	-	-	-	-	-	-	-	-	-	-
F3	0%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	91%
F4A	-	-	-	-	-	-	-	-	-	-	-	-
F4B	-	-	-	-	-	-	-	-	-	-	-	-
F4C	-	-	-	-	-	-	-	-	-	-	-	-
F5	0%	100%	100%	100%	100%	0%	100%	100%	0%	0%	0%	55%
F6	67%	100%	67%	67%	33%	100%	67%	33%	100%	67%	67%	70%
F7	-	-	-	-	-	-	-	-	-	-	-	-
M1	100%	67%	67%	33%	67%	67%	67%	67%	100%	67%	67%	70%
M2	50%	50%	100%	50%	50%	50%	50%	0%	100%	50%	50%	55%
M3A	-	-	-	-	-	-	-	-	-	-	-	-
M3B	100%	0%	0%	100%	0%	100%	0%	0%	0%	100%	100%	45%
M4	-	-	-	-	-	-	-	-	-	-	-	-
	60%	80%	80%	67%	67%	73%	73%	60%	80%	60%	53%	**68.48%**

Table 4.2.1.3 Results of Coefficient of Variation (CV).

COEFFICIENT OF VARIATION (CV)

MODAL Stage	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	ALL Readers
0	-	-	-	-	-	-	-	-	-	-	-	-
F1	38%	0%	0%	138%	0%	138%	0%	0%	40%	94%	86%	72%
F2	-	-	-	-	-	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-	-	-	-	-	-
F4A	-	-	-	-	-	-	-	-	-	-	-	-
F4B	-	-	-	-	-	-	-	-	-	-	-	-
F4C	-	-	-	-	-	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-	-	-	-	-	-
F6	46%	0%	8%	8%	8%	0%	8%	8%	0%	7%	7%	10%
F7	-	-	-	-	-	-	-	-	-	-	-	-
M1	0%	74%	74%	5%	74%	6%	74%	74%	0%	6%	6%	29%
M2	6%	7%	0%	6%	7%	12%	7%	0%	0%	6%	12%	6%
M3A	-	-	-	-	-	-	-	-	-	-	-	-
M3B	-	-	-	-	-	-	-	-	-	-	-	-
M4	-	-	-	-	-	-	-	-	-	-	-	-
	20.20%	15.74%	16.35%	40.33%	17.32%	39.61%	17.25%	16.42%	10.67%	28.52%	26.91%	**32.44%**

Table 4.2.1.4 Results of Relative Bias.

RELATIVE BIAS

MODAL Stage	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	ALL
0	-	-	-	-	-	-	-	-	-	-	-	-
F1	0.5	0.00	0.00	2.25	0.00	2.25	0.00	0.00	0.25	4.50	4.75	1.32
F2	-	-	-	-	-	-	-	-	-	-	-	-
F3	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54545
F4A	-	-	-	-	-	-	-	-	-	-	-	-
F4B	-	-	-	-	-	-	-	-	-	-	-	-
F4C	-	-	-	-	-	-	-	-	-	-	-	-
F5	1	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	1.00	0.45455
F6	-1.67	0.00	-0.33	-0.33	-0.67	0.00	-0.33	-0.67	0.00	0.33	0.33	-0.30
F7	-	-	-	-	-	-	-	-	-	-	-	-
M1	0	-3.00	-3.00	0.67	-3.00	0.33	-3.00	-3.00	0.00	0.33	0.33	-1.21
M2	0.5	-0.50	0.00	0.50	-0.50	1.00	-0.50	-1.00	0.00	0.50	1.00	0.09
M3A	-	-	-	-	-	-	-	-	-	-	-	-
M3B	0	-2.00	-1.00	0.00	-2.00	0.00	-2.00	-3.00	-2.00	0.00	0.00	-1.0909
M4	-	-	-	-	-	-	-	-	-	-	-	-
	0.33	-0.80	-0.73	0.73	-0.93	0.87	-0.87	-1.07	0.00	1.47	1.60	**0.05**

Figure 4.2.1.1 – Standard Deviation (STDEV), Coefficient of Variation (CV) and Agreement for each maturity stage analysed

Moreover, the difference between the stage interpretation of each readers with the "modal stage" and inter-reader bias was tested (Mann Whitney). The results showed that the most part of the readers showed possible significant differences between their staging analysis and the "modal stage". The analysis of the exercise results among each readers showed possible significant differences for the most part (42% of the comparison) of the case. The 31% showed the certainty presence of bias and in the 27% of the case no difference.

WKSEL3 exercise 2018

	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC
Reader MkM											
Reader AA	**										
Reader SS	*	–									
Reader MD	*	*	*								
Reader MS	**	–	–	*							
Reader AB	*	*	*	–	**						
Reader FS	**	–	–	*	–	*					
Reader LS	**	–	–	*	–	**	–				
Reader FT	*	–	*	*	*	*	*	*			
Reader MM	*	**	**	*	**	–	**	**	*		
Reader DC	**	**	**	*	**	–	**	**	*	–	
Modal Stage	*	–	–	*	*	–	–	*	–	*	*

–	= no sign of bias (p>0.05)
*	= possibility of bias (0.01<p<0.05)
**	= certainty of bias (p<0.01)

Figure 4.2.1.2 Inter-reader bias test and reader against modal age bias test of Etmopterus spinax gonad staging (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

4.2.2 Galeus melastomus

The overall agreement with modal stage was 71.8% (Table 4.2.2.1). The modal stage was calculated not stratified on base the experience of the participants.

In the analysis, the stages with higher percentage of agreement and lower coefficient of variation were stages 3b (Active), 1 (immature), 4a (Post-Laying) and 2 (Developing) for females and 3a (Capable to Reproduce) and 3b (Active) for males (Table 4.2.2.2, Table 4.2.2.3 and Figure 4.2.2.1).

In general, the results showed for females that stage POST-LAYING (4b) and REGENERATING (4a) were confused each other. Moreover, in males, stage 4 (Spent) has been confused with stage 3a (Capable to Reproduce). During the discussion of these results, it was attempted to clarify some of the controversial cases, especially when the gonads are located on the border between two stages.

Regarding the bias (Table 4.2.2.4), the results showed a positive bias (overestimation) for the stage 4a for females and 2 and 3a for males, while a negative bias (underestimation) for stages 4b and F2 in females and M3b and M4 in males it was found.

Table 4.2.2.1 Results of the staging exercise on the viviparous species.

Stratum	Sample year	Species	Fish no	Landing month	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	MODAL age	Percent agreemen	Precision CV
Italy	2018	G. melastomus	gm1	2	F3B	F3B	F3B	F3B	F3B	F3B	F3B	F3B	F3B	F3B	F3B	**F3B**	100%	0%
Italy	2018	G. melastomus	gm2	2	M3B	M3B	M3B	M2	M3B	M3B	M3B	M2	M4	M2	M2	**M3B**	45%	12%
Italy	2018	G. melastomus	gm3	2	F2	F2	F2	F1	F2	F2	F2	F1	F2	F1	F1	**F2**	64%	31%
Italy	2018	G. melastomus	gm4	2	M3A	M3A	M2	M2	M3A	M2	M2	M2	M4	M2	M2	**M2**	64%	11%
Italy	2018	G. melastomus	gm5	2	M4	M3A	M3A	M3A	M3A	M3A	M3A	M3A	M4	M2	M3A	**M3A**	73%	10%
Italy	2018	G. melastomus	gm6	2	M4	M3B	M3B	M3B	M3B	M3B	M3B	M3B	M4	M3B	M3B	**M3B**	82%	4%
Italy	2018	G. melastomus	gm7	2	F4A	F4B	-	F4B	F4B	F4B	F4B	F4A	F4A	F4B	F4B	**F4B**	70%	8%
Italy	2018	G. melastomus	gm8	2	F4A	F4A	-	F4B	F4A	F4A	F4A	F4B	F4A	F4A	F3A	**F4A**	70%	16%
Italy	2018	G. melastomus	gm9	2	M4	M3B	-	M3A	M3B	M3A	M3B	M3A	M4	M3A	M3A	**M3A**	50%	8%
Italy	2018	G. melastomus	gm10	2	M4	M3B	-	M3A	M3B	M4	M3B	M3A	M4	M3A	M3A	**M3A**	40%	9%
Italy	2018	G. melastomus	gm11	2	F4A	F2	-	F4B	F4B	F4B	F2	F4B	F4B	F4B	F4B	**F4B**	70%	33%
Italy	2018	G. melastomus	gm12	2	F4A	F3A	-	F4B	F4B	F4B	F3A	F4B	F4B	F4B	F4B	**F4B**	70%	24%
Italy	2018	G. melastomus	gm13	2	M3A	M3B	-	M3A	M3A	M3A	M3A	M3A	M3B	M3A	M3A	**M3A**	80%	5%
Italy	2018	G. melastomus	gm14	2	F3B	F3B	-	F3B	F3B	F3B	F3B	F3B	F3B	F3B	F3B	**F3B**	100%	0%
Italy	2018	G. melastomus	gm15	2	F3B	F3B	-	F3B	F3B	F3B	F3B	F3B	F3B	F3B	F3B	**F3B**	100%	0%
			Total read		15	15	6	15	15	15	15	15	15	15	15		**71.8%**	**11.4%**

Table 4.2.2.2 Results of agreement by reader and stage.

PERCENTAGE AGREEMENT

MODAL Stage	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	ALL Readers
0	-	-	-	-	-	-	-	-	-	-	-	-
F1	-	-	-	-	-	-	-	-	-	-	-	-
F2	100%	100%	100%	0%	100%	100%	100%	0%	100%	0%	0%	64%
F3A	-	-	-	-	-	-	-	-	-	-	-	-
F3B	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
F4A	100%	100%	-	0%	100%	100%	100%	0%	100%	100%	0%	70%
F4B	0%	33%	-	100%	100%	100%	33%	67%	67%	100%	100%	70%
M1	-	-	-	-	-	-	-	-	-	-	-	-
M2	0%	0%	100%	100%	0%	100%	100%	100%	0%	100%	100%	64%
M3A	25%	25%	100%	100%	50%	75%	50%	100%	0%	75%	100%	61%
M3B	0%	100%	100%	50%	100%	100%	100%	50%	0%	50%	50%	64%
M4	-	-	-	-	-	-	-	-	-	-	-	-
	40%	60%	100%	80%	80%	93%	73%	73%	47%	80%	80%	**71.79%**

Table 4.2.2.3 Results of Coefficient of Variation (CV).

COEFFICIENT OF VARIATION (CV)

MODAL Stage	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	ALL Readers
0	-	-	-	-	-	-	-	-	-	-	-	-
F1	-	-	-	-	-	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-	-	-	-	-	-
F3A	-	-	-	-	-	-	-	-	-	-	-	-
F3B	0%	0%	-	0%	0%	0%	0%	0%	0%	0%	0%	0%
F4A	-	-	-	-	-	-	-	-	-	-	-	-
F4B	0%	57%	-	0%	0%	0%	57%	10%	10%	0%	0%	23%
M1	-	-	-	-	-	-	-	-	-	-	-	-
M2	-	-	-	-	-	-	-	-	-	-	-	-
M3A	10%	5%	-	0%	6%	11%	6%	0%	5%	6%	0%	8%
M3B	14%	0%	0%	16%	0%	0%	0%	16%	0%	16%	16%	8%
M4	-	-	-	-	-	-	-	-	-	-	-	-
	4%	13%	0%	2%	2%	3%	13%	4%	3%	4%	2%	**11.36%**

Table 4.2.1.4 Results of Relative Bias.

RELATIVE BIAS

MODAL Stage	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC	ALL
0	-	-	-	-	-	-	-	-	-	-	-
F1	-	-	-	-	-	-	-	-	-	-	-
F2	0.00	0.00	0.00	-1.00	0.00	0.00	0.00	-1.00	0.00	-1.00	-0.30
F3A	-	-	-	-	-	-	-	-	-	-	-
F3B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
F4A	0.00	0.00	-	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.22
F4B	-1.00	-2.33	-	0.00	0.00	0.00	-2.33	-0.33	-0.33	0.00	-0.70
M1	-	-	-	-	-	-	-	-	-	-	-
M2	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	3.00	0.00	0.60
M3A	1.33	0.67	0.00	0.00	0.33	0.00	0.33	0.00	1.67	-0.33	0.43
M3B	0.00	0.00	0.00	-1.00	0.00	0.00	0.00	-1.00	1.00	-1.00	-0.20
M4	0.00	-1.00	-	-2.00	-1.00	0.00	-1.00	-2.00	0.00	-2.00	-1.00
	0.13	-0.33	0.00	-0.27	0.07	0.00	-0.47	-0.33	0.60	-0.40	**-0.11**

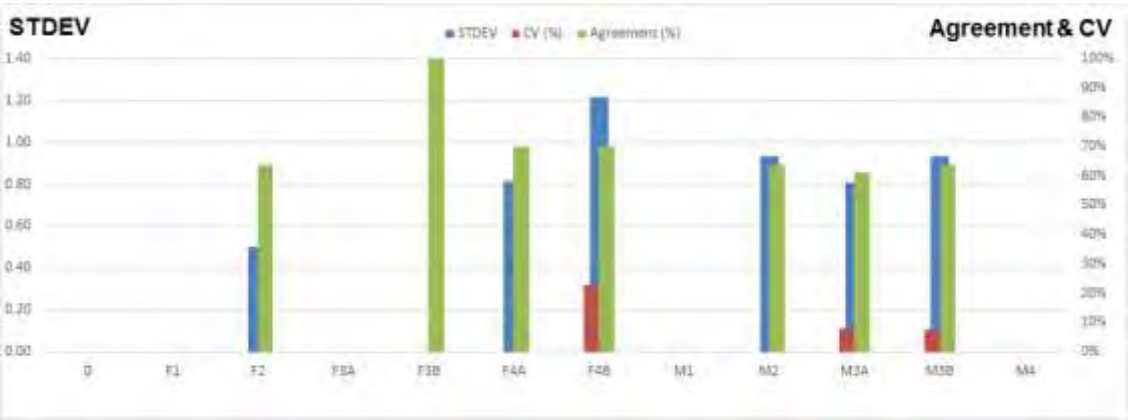


Figure 4.2.2.1 2 Standard Deviation (STDEV), Coefficient of Variation (CV) and Agreement for each maturity stage analysed

Moreover, the difference between the stage interpretation of each readers with the "modal stage" and inter-readers bias was tested (Mann Whitney). The results showed that for the most part of the readers was not recorded a significant difference between their staging analysis and the "modal stage". Moreover, the analysis of the results exercise among each readers did not showed significant differences for the most (42% of the comparison) of the case. The 27% showed the presence of possible bias and the 31% of the case recorded a certainty presence of bias.

WKSEL3 exercise 2018

	Norway MKM	Greece AA	Spain SS	Italy MD	Italy MS	Italy AB	Spain FS	Italy LS	Australia FT	Italy MM	Italy DC
Reader MKM											
Reader AA	**										
Reader SS	–	–									
Reader MD	**	**	–								
Reader MS	*	–	–	*							
Reader AB	*	*	–	–	–						
Reader FS	**	–	–	*	–	–					
Reader LS	**	**	–	–	*	*	*				
Reader FT	*	**	–	**	*	*	**	**			
Reader MM	**	**	–	–	*	–	*	–	**		
Reader DC	**	**	–	–	*	–	*	–	**	–	
Modal Stage	**	*	–	–	–	–	–	–	*	–	–

–	= no sign of bias (p>0.05)
*	= possibility of bias (0.01<p<0.05)
**	= certainty of bias (p<0.01)

Figure 4.2.2.2 Inter-reader bias test and reader against modal age bias test of Etmopterus spinax gonad staging (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

5 Update the conversion tables both for oviparous and viviparous species (ToR C)

The terminology commonly used to describe different reproductive phases in Elasmobranchs is variable among authors. Following, a comparative summary of the terminologies from the old and the new scales used by WKMSEL3 participants and from literature is reported (Tables 5.1 to 5.4).

Table 5.1. Conversion table among previous maturity scales and the WKMSEL 3 scale for Macroscopic scale proposed for Female oviparous Elasmobranchs.

WKMATCH (2012)		WKMSEL2 (2012)		MEDITS GFCM FAO (2016)		WKMSEL3 (2018)	
1	Immature	1	Immature	1	Immature Virgin	1	Immature
2a/2b	Developing but functionally immature / Developing and functionally mature	2	Developing	2	Maturing	2	Developing
3	Spawning	3a	Capable to Reproduce	3a	Mature	3a	Capable to Reproduce
3	Spawning	3b	Egg-laying	3b	Mature/ Extruding	3b	Active
4a	Regressing	4a	Post-laying	4a	Resting	4a	Post-laying
4b	Regenerating	4b	Regenerating	4b	Regenerating	4b	Regenerating
5	Omitted spawning	-	-	-	-	-	-
6	Abnormal	-	-	-	-	-	-

Table 5.2. Conversion table between previous maturity scales and the WKMSEL 3 scale for Macroscopic scale proposed for Female viviparous Elasmobranchs.

WKMATCH (2012)		WKMSEL2 (2012)		MEDITS GFCM FAO (2016)		WKMSEL3 (2018)	
1	Immature	1	Immature	1	Immature Virgin	1	Immature
2a/2b	Developing but functionally immature / Developing and functionally mature	2	Developing	2	Maturing	2	Developing
3	Spawning	3	Capable to Reproduce	3	Capable to Reproduce	3	Capable to Reproduce
3	Spawning	4a	Early pregnancy	4a	Early pregnancy	4a	Active
3	Spawning	4b	Mid pregnancy	4b	Mid pregnancy	4b	

WKMATCH (2012)		WKMSEL2 (2012)		MEDITS GFCM FAO (2016)		WKMSEL3 (2018)	
3	Spawning	4c	Late pregnancy	4c	Late pregnancy	4c	
4a	Regressing	5	Post-partum	5	Post-partum	5	Post-partum
4b	Regenerating	6	Regenerating	6	Regenerating	6	Early Regeneration
4b	Regenerating	6	Regenerating	6	Regenerating	7	Late Regeneration
5	Omitted spawning	-	-	-	-	-	-
6	Abnormal	-	-	-	-	-	-

Table 5.3. Conversion table between previous maturity scales and the WKMSEL 3 scale for Macroscopic scale proposed for Male oviparous Elasmobranchs.

WKMATCH (2012)		WKMSEL2 (2012)		MEDITS (2016)		WKMSEL3 (2018)	
1	Immature	1	Immature	1	Immature Virgin	1	Immature
2a/2b	Developing but functionally immature / Developing and functionally mature	2	Developing	2	Maturing	2	Developing
3	Spawning	3a	Capable to Reproduce	3a	Mature	3a	Capable to Reproduce
3	Spawning	3b	Active	3b	Mature/ Active	3b	Active
4a/4b	Regressing/ Regenerating	4	Regressing	4a/4b	Resting/ Regenerating	4	Spent
5	Omitted spawning	-	-	-	-	-	-
6	Abnormal	-	-	-	-	-	-

Table 5.4. Conversion table between previous maturity scales and the WKMSEL 3 scale for Macroscopic scale proposed for Male viviparous Elasmobranchs.

WKMATCH (2012)		WKMSEL2 (2012)		MEDITS (2016)		WKMSEL3 (2018)	
1	Immature	1	Immature	1	Immature Virgin	1	Immature
2a/2b	Developing but functionally immature / Developing and functionally mature	2	Developing	2	Maturing	2	Developing
3	Spawning	3a	Capable to Reproduce	3a	Mature	3a	Capable to Reproduce
3	Spawning	3b	Active	3b	Actively spawning	3b	Active

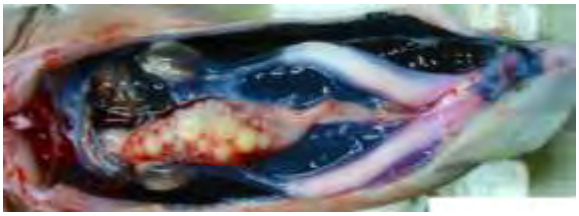
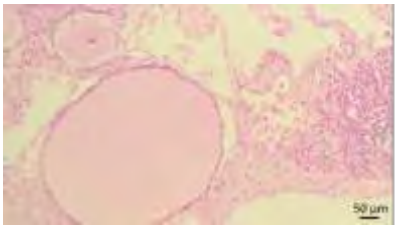
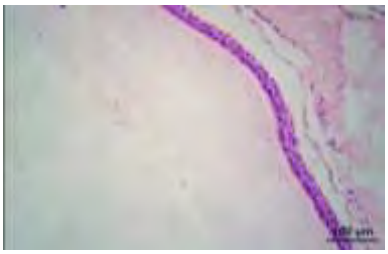
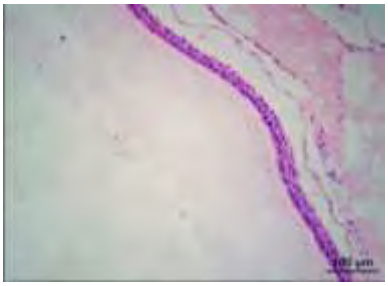
WKMATCH (2012)		WKMSEL2 (2012)		MEDITS (2016)		WKMSEL3 (2018)	
4a/4b	Regressing/ Regenerating	4	Regressing	4	Regressing	4	Spent
5	Omitted spawning	-	-	-	-	-	-
6	Abnormal	-	-	-	-	-	-

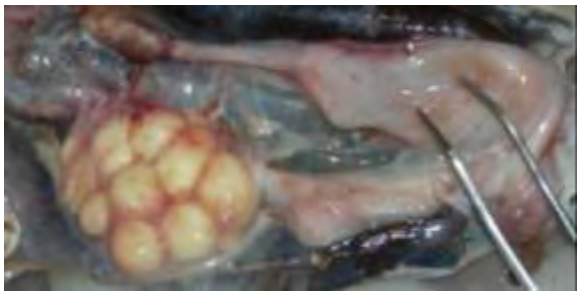
6 Compile an Atlas using both macroscopical and histological gonad pictures (ToR D)

An Atlas using male and female gonad pictures has been upgraded from the WKMSEL2 considering oviparous and viviparous species (mainly aplacental) adding when possible correspondent histological pictures.

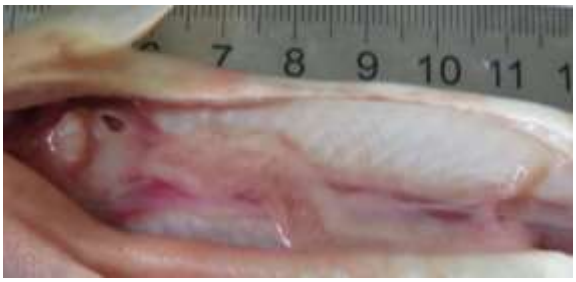
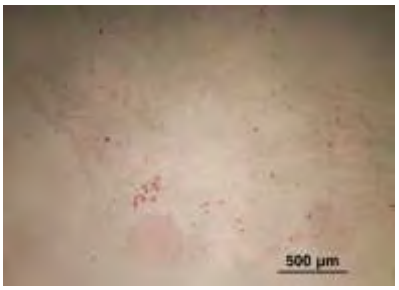
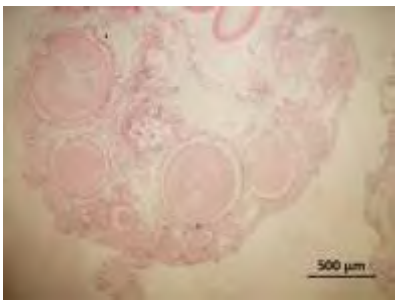
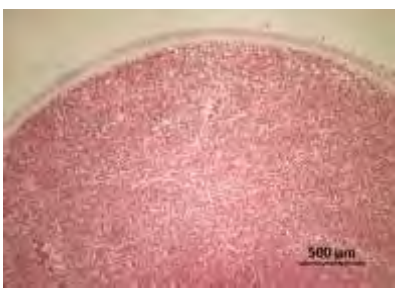
Females

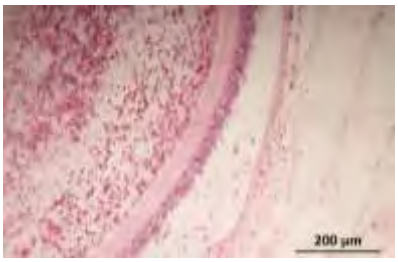
Oviparous species with multiple oviparity – *Galeus melastomus*

Stage		Macroscopic pictures	Microscopic pictures
1	Immature		
2	Developing	 	
3a	Capable to Reproduce		
3b	Active		

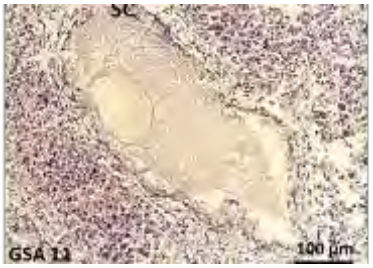
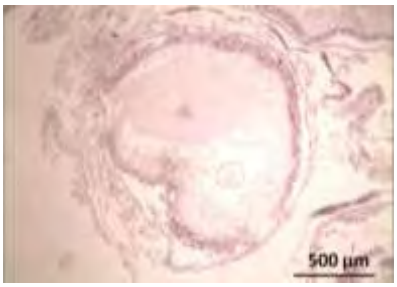
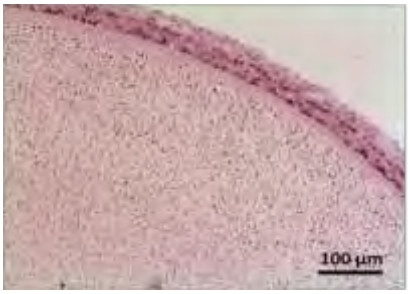
Stage		Macroscopic pictures	Microscopic pictures
4 a	Post-laying		
4 b	Regenerating		

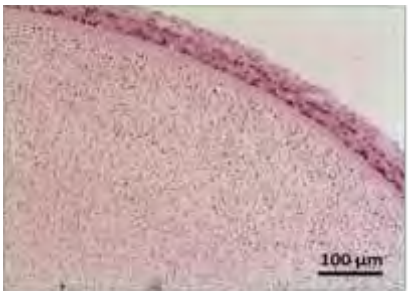
Oviparous species with single oviparity – *Scyliorhinus canicula*

Stage		Macroscopic pictures	Microscopic pictures
1	Immature		
2	Developing		
3a	Capable to Reproduce		

Stage		Macroscopic pictures	Microscopic pictures
3b	Active		
4a	Post-laying	NOT AVAILABLE	
4b	Regenerat-ing	NOT AVAILABLE	

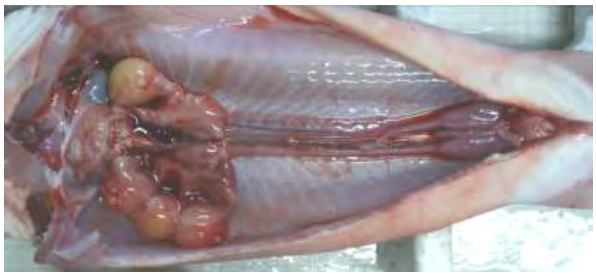
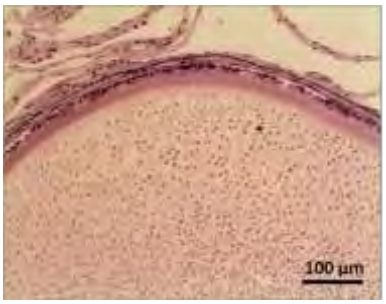
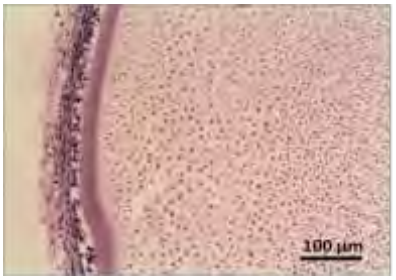
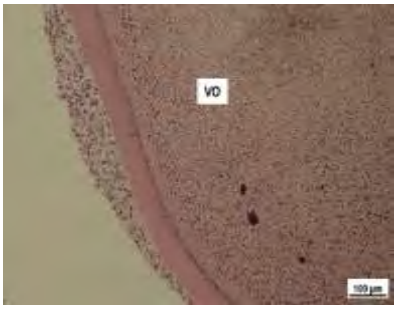
Oviparous species with single oviparity – *Dipturus oxyrinchus*

Stage		Macroscopic pictures	Microscopic pictures
1	Immature		
2	Developing		
3a	Capable to Reproduce		

Stage		Macroscopic pictures	Microscopic pictures
3 b	Active		
4a	Post-laying		
4 b	Regenerating		Not available

Lecitotrophic aplacental species: *Squalus blainville*

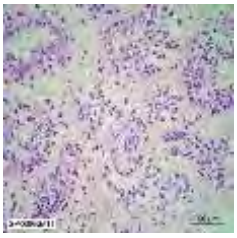
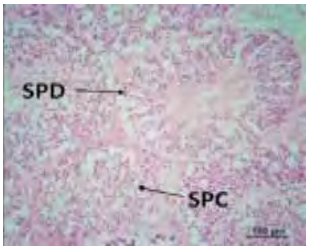
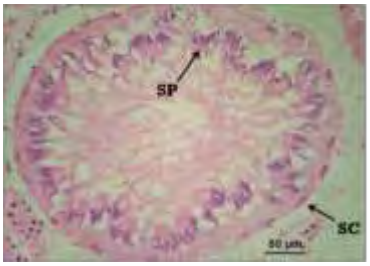
Stage		Macroscopic pictures	Microscopic pictures
1	Immature		

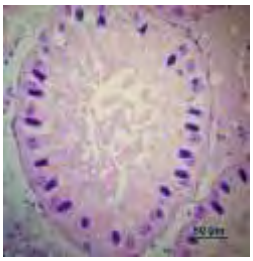
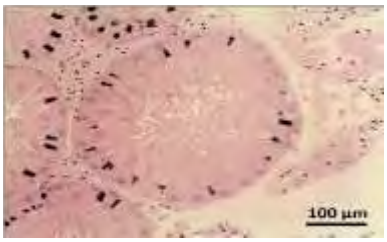
Stage		Macroscopic pictures	Microscopic pictures
2	Developing		
3	Capable to Reproduce		
4 a	Early Pregnancy		
4 b	Mid Pregnancy		
4c	Late Pregnancy		

Stage		Macroscopic pictures	Microscopic pictures
5	Post-partum		
6	Early regener-ation		
7	Late regener-ation		

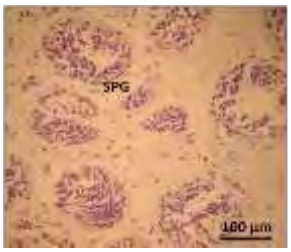
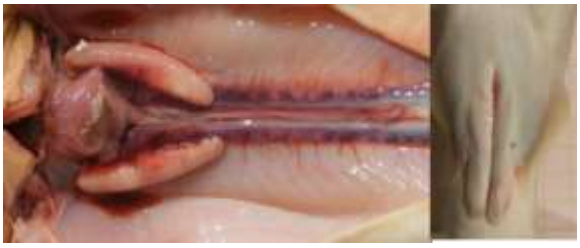
Males

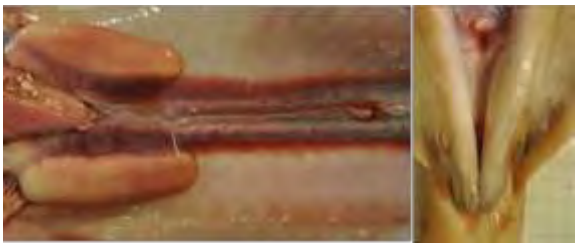
Oviparous species with multiple oviparity – *Galeus melastomus*

Stage		Macroscopic pictures	Microscopic pictures
1	Immature		
2	Developing		
3a	Capable to Reproduce		

Stage	Macroscopic pictures	Microscopic pictures
3b Active		
4 Spent		

Lecitotrophic aplacental species: *Squalus blainville*

Stage	Macroscopic pictures	Microscopic pictures
1 Immature		
2 Developing		
3a Capable to Reproduce		
3b Active		

Stage	Macroscopic pictures	Microscopic pictures
4 Spent		

7 Increase the number of case studies with particular attention for viviparous species (ToR D)

The participants highlighted the importance to increase the number of viviparous species to analyse. The following pictures from the viviparous species were discussed and staged in the plenary session on the base on the scale adopted during this workshop. In particular, pictures of lecitotrophic yolk-sac (Fig. 7.1, 7.2) and matrotrophic – placental viviparous species (Fig. 7.3-7.7) were analysed.

Figure 7.1. Early pregnant female of *Dalatias licha* (TL: 58 cm, GSA10).

Figure 7.2. Early pregnant female of *Centroscymnus coelolepis* (TL: 61.8 cm, GSA11).

Figure 7.3. Capable to reproduce female of *Mustelus* (TL: 117.5 cm; GSA18).

Figure 7.4. Early regeneration female of *Mustelus* (TL: 116.4 cm; GSA18).

Figure 7.5. Immature female of *Prionace glauca* (TL: 116 cm; GSA18).

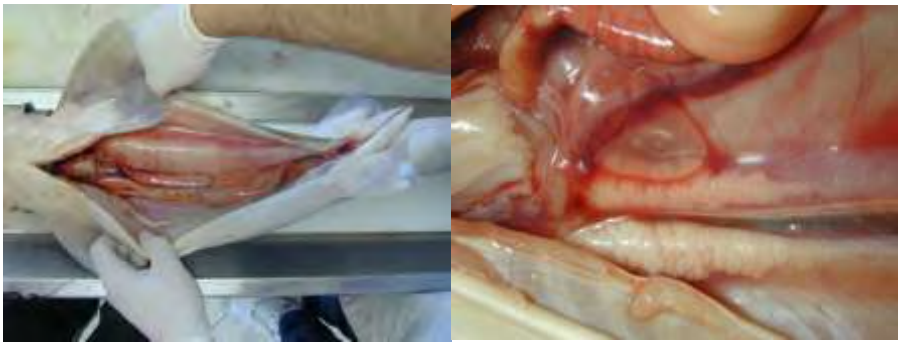


Figure 7.6. Active male of *Mustelus* (TL: 105.4 cm; GSA18).

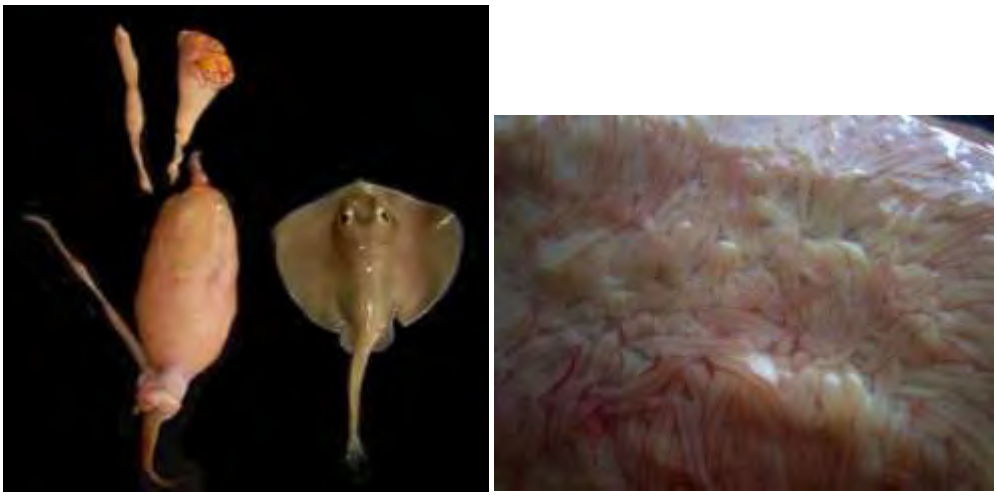


Figure 7.7 Early pregnant female of an Urolophidae. At right, a high magnification of uterine villi (uterine histotroph (milk)).

Figure 7.8. Developing male of *Pteromyleus bovinus*.

8 References

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ICES. 2017. Report of the Working Group on Biological Parameters (WGBIOP), 2–6 October 2017, Sardinia, Italy. ICES CM 2017/SSGIEOM:08. 129 pp.

MEDITS, 2016. International bottom trawl survey in the Mediterranean Instruction manual. MEDITS-Handbook. Version n. 7, 2016, MEDITS Working Group: 120 pp.

Annex 1: List of participants

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WKMSEL3 participants: From left to right standing: Aikaterini, Francisca, Letizia, Martina Francesca, Pierluigi. Sitting from left to right: Andrea, Samar, Marlén, Maria Cristina, Marilena, Marina and Fabien.

Annex 2: Agenda

ICES Workshop on Elasmobranchs maturity (WKMSEL3)

Agenda

The meeting will start at 14.00 of February 19, 2018 and will end on February 22 (~13.00)

Meeting place: Cagliari, ITALY,

Department of Life and Environmental Sciences

Via T.Fiorelli,1

Monday 19th February 2018

14.30-15.00

Welcome of the participants

Approval of the agenda

15.00-16.00 - Introduction round, general announcements

Explain setup of the workshop (presentation)

Approval of the Agenda

16.00-18.00 - Presentations from each lab/participant about the scale in use and common scale

16.00-16.30 – Follesa Maria Cristina et al. - OVIPAROUS and VIVIPAROUS ELASMOBRANCH species: Critical analysis of the macro maturity scales. (MEDITS VS WKMSEL2)

16.00-16.30 Coffee break

16.30-17.00 - Katerina Anastosopoulou - Maturity scale used by HCMR for Elasmobranche.

17.00-17.30 - Fabien Trinnie - Obligate or facultative diapause in batiods

Tuesday 20th February 2018

9.30 – 11.00 - Marilena Donnaiola - Discussion and comparison on MEDITS and WKMSEL2 maturity scale (oviparous and viviparous).

11.00-11.30 Coffee break

11.30- 12.00 – Pasqui Salmeron - Medits maturity scale of oviparous: the case of *Galeus melastomus*

12.00 – 12.15 - Samar Saber - "Macroscopic maturity of Blue shark Prionace glauca (Linnaeus 1758) caught by Spanish longline fleet".

12.15-12.30 – Marlen Knusten Maturity data of spurdog (*Squalus acanthias*) in Norwegian waters".

12.30-13.00 Letizia Sion - Viviparous speciesof the Northern Ionian Sea: comments on the use of macroscale.

13.00-13.30 - Discussion on the presentation's results

13.00-14.30 Lunch break

14.30 – 16.00 Working group on viviparous / Working group on oviparous

16.00-16.30 Coffee break

16.30-18.30 Working group on viviparous / Working group on oviparous

18.30-19.00 Common Discussion on the preliminary results

Wednesday 21th February 2018

9.30 – 11.00 Working group on viviparous / Working group on oviparous

11.30-12.00 Coffee break

12.00 – 13.00 Approvation on the plenary the updates of macroscopic scale iviparous and oviparous

13.00 -14.30 Lunch time

14.30 -16.00

Exercise staging oviparous species on specimens (*Galeus melastomus*)

Exercise staging viviparous species on specimens (*Etmopterus spinax*)

16.00 – 16.30 Coffe break

16.30-17.30

Discussion results on the exercise

Synthesis and conclusions from working groups

Ore 20.30

Social Dinner

Thursday 22th February 2018

9.30 – 11.00

Final Discussion on common scale

Synthesis and conclusions of the workshop

11.00-11.30 Coffee break

11.30-13.00

Planning of future work

Any Other Business and meeting closure

Annex 3: Terms of reference for the next meeting

WKSEL3 assumes it is the responsibility of WGBIOP to initiate a future workshop on maturity staging of Elasmobranchs' species. Recommendations for future workshops can be found Annex 4.

Annex 4: Recommendations

Recommendation	Addressed to
The group recommends to use the updated international maturity scale WKMSEL3 for both ICES (Atlantic) and GFCM (Mediterranean). The improvements are carried out on base of the experiences of the participants and the histological evidences for both oviparous and viviparous species	WGEF, WGBIOP
WKMSEL3 recommends to collect specie specific features of the male (e.g. testis, clasper characteristics, ducts) and female (e.g. ovaries, oviducts, glands) reproductive organs, due to the high variable characteristics of these reproductive organs	Maturity National Correspondence (WGBIOP)
WKMSEL3 recommends to organise an exchange using the new update scale (WKSEL3) in order to verify the agreement level among lab/country.	WGBIOP
WKMSEL3 recommends to improve the collection of the macro and micro images in order to amplify the reference collection.	Maturity National Correspondence (WGBIOP); WKMATHIS2
WKMSEL3 recommends to create a permanent group on the biological parameters (maturity, age), useful for the stock assessment	WGBIOP; EOSG; SCICOM\ACOM
WKMSEL3 recommends to adapt the SmartDOT platform also for the future maturity pictures exchanges and workshops.	WGBIOP

Annex 5: Macroscopic maturity scales proposed for oviparous and viviparous Elasmobranchs

Female macroscopic maturity scale proposed for oviparous Elasmobranchs			
MATURITY	STAGE		DESCRIPTION
IMMATURE	1	IMMATURE	**Ovaries**: small and whitish. Undistinguishable or very small ovarian follicles. **Oviducal gland**: often not visible. In some species a thickening of the uteri where the gland will develop may be visible. **Uteri**: thread-like and narrow.
IMMATURE	2	DEVELOPING	**Ovaries**: Unyolked follicles and some small and medium yolked ones may be present. **Oviducal gland**: distinguishable and developing mostly in skates. **Uteri**: enlarging
MATURE	3a	CAPABLE TO REPRODUCE	**Ovaries**: presence of large yolked follicles ready to be ovulated. **Oviducal glands**: fully developed. **Uteri**: fully developed and turgid.
MATURE	3b	ACTIVE	**Ovaries and Oviducal glands**: similar to stage 3a **Uteri:** presence of egg capsules
MATURE	4a	POST-LAYING	**Ovaries**: flaccid with follicles (unyolked and yolked) of different sizes. Post-ovulatory follicles and atretic follicles visible. **Oviducal glands**: fully developed but may be reduced in size mostly in skates. **Uteri**: enlarged, flaccid and vascularized.
MATURE	4b	REGENERATING	**Ovaries**: large with small and medium sized yolked follicles. Pre-ovulatory follicles absent. **Oviducal glands**: fully developed but may be reduced in size mostly in skates. **Uteri**: enlarged but not flaccid.

Male macroscopic maturity scale proposed for oviparous Elasmobranchs			
MATURITY	STAGE		DESCRIPTION
IMMATURE	1	IMMATURE	**Testes**: small and undeveloped (in skates, sometimes with visible lobules). **Ducts**: straight and thread-like. **Claspers**: flexible, non-calcified and shorter than pelvic fins.
IMMATURE	2	DEVELOPING	**Testes**: developing (in skates, lobules clearly visible but not fully developed). **Ducts**: developing and beginning to coil. **Claspers**: flexible, partially calcified and usually or as long as or longer than pelvic fins. In some sharks they do not pass the pelvic fins. **Alar thorns**: could be present at primordial stage (single row) in some skate species.
MATURE	3a	CAPABLE TO REPRODUCE	**Testes**: fully developed according to species-specific characteristics. **Ducts**: tightly coiled and filled with sperm. **Seminal vesicle:** (when present) developed. Sperm does not flow on pressure. **Claspers**: rigid, fully calcified and longer than pelvic fins (in some sharks they may only be as long as the pelvic fins). **Alar and/or malar thorns:** could be present in some skate species.
MATURE	3b	ACTIVE	**Testes**: similar to Stage 3a. **Ducts**: sperm observed flowing out of the cloaca on pressure. **Seminal vesicle** (when present) can be full or empty **Claspers:** fully calcified sometimes with glans dilated, swollen and reddish. Sperm flows on pressure and it may be present in groove or glans. **Alar and/or malar thorns:** could be present in some skate species.
MATURE	4	**SPENT**	**Testes** shrunken and flaccid. On pressure sperm does not flow. **Sperm ducts** and **seminal vesicle** empty and flaccid. **Claspers**: fully formed. **Alar and/or malar thorns:** could be present in some skate species.

Female macroscopic maturity scale proposed for viviparous Elasmobranchs			
MATURITY/MATERNITY	STAGE		DESCRIPTION
IMMATURE	1	IMMATURE	**Ovaries**: small and whitish; undistinguishable ovarian follicles. **Oviducal glands**: often not visible. In some species a thickening of the oviducts where the gland will develop may be visible **Uteri**: thread-like and narrow.

Female macroscopic maturity scale proposed for viviparous Elasmobranchs			
IMMATURE	2	DEVELOPING	**Ovaries**: follicles of different stages of development. Some small and medium sized yolked follicles may be present. **Oviducal glands**: distinguishable and developing. **Uteri**: enlarging.
MATURE	3	CAPABLE to REPRODUCE	**Ovaries**: presence of large yolked follicles ready to be ovulated. **Oviducal glands**: fully developed. **Uteri**: fully developed.
MATERNAL	4a	EARLY PREGNANCY	**Ovaries**: different sized follicles are present according to stages of ovulation. **Oviducal glands**: fully developed (it is possible to observe a starting regressing).**Uteri**: well filled and rounded with yolk content (usually candle shape). Embryos cannot be observed.
MATERNAL	4b	MID PREGNANCY	**Ovaries**: small to medium, possibly yolked follicles (active ovaries) or small, unyolked and/or atretic follicles (inactive ovaries). **Oviducal glands**: fully developed (it is possible to observe a starting regressing).**Uteri**: well filled and rounded. Embryos are always visible, small and with a relatively large yolk sac.
MATERNAL	4c	LATE PREGNANCY	**Ovaries**: medium to large yolked follicles (active ovaries) or small, unyolked follicles and/or atretic follicles (inactive ovaries). **Oviducal glands**: fully developed (it is possible to observe a starting regressing).. **Uteri**: embryos fully formed, yolk sacs reduced or absent.
MATERNAL	5	POST-PARTUM	**Ovaries**: Similar to Stage 4c. **Oviducal glands**: Similar to Stage 4c. **Uteri**: enlarged and flaccid (likely to have just given birth).
MATURE	6	EARLY REGENERATION	**Ovaries**: small or medium yolked follicles. **Oviducal glands**: fully developed (may be reduced in size). **Uteri**: enlarged post-maternal, but not so flaccid.
MATURE	7	LATE REGENERATION	**Ovaries**: large yolked follicles of ovulatory size. **Oviducal glands**: fully developed. **Uteri**: enlarged post-maternal, but not flaccid.

Male macroscopic maturity scale proposed for viviparous Elasmobranchs			
MATURITY	STAGE		DESCRIPTION
IMMATURE	1	IMMATURE	**Testes:** small and undeveloped. **Ducts**: straight and thread-like. **Claspers**: flexible, non-calcified and usually shorter than pelvic fins.
IMMATURE	2	DEVELOPING	**Testes:** developing according to species-specific characteristics (sometimes with developing segments in sharks or lobules in batoids) but not occupying the whole surface.of the abdominal cavity. **Ducts**: developing and beginning to coil. **Claspers**: flexible, partially calcified and as long as or longer than pelvic fins.
MATURE	3a	CAPABLE TO RE-PRODUCE	**Testes:** fully developed according to species-specific characteristics (fully segmented in some sharks and lobed in some batoids). **Ducts**: tightly coiled and filled with sperm. Seminal vesicle (when visible) developed. **Claspers**: rigid, fully calcified and longer than pelvic fins.
MATURE	3b	ACTIVE	**Testes:** similar to Stage 3a. **Ducts**: sperm flowing out of the cloaca on pressure. Seminal vesicles (when visible) can be full or empty. **Claspers**: fully formed, sometimes swollen and/or reddish; however with clasper gland dilated. Sperm may be present in clasper groove or gland.
MATURE	4	**SPENT**	**Testes:** shrunken and flaccid. **Ducts**: empty and flaccid. Seminal vesicle (when visible) empty. Claspers: fully formed.

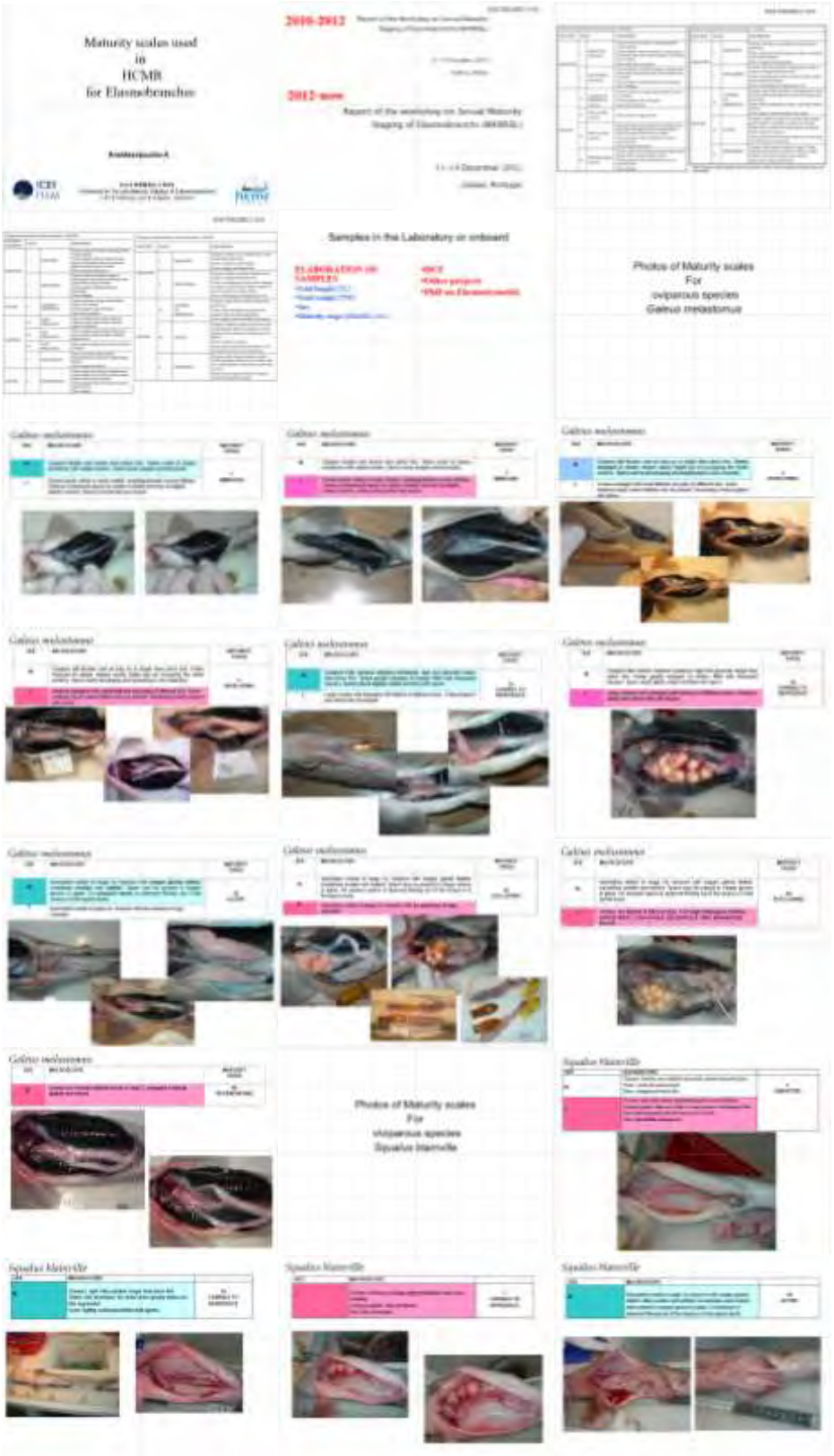
Annex 6: Workshop participants presentations


Oviparous FEMALES
List of the species presented in the photo


Oviparous FEMALES
Oviparous FEMALES
Oviparous FEMALES
Oviparous FEMALES
Viviparous FEMALES
Viviparous FEMALES
Viviparous FEMALES
Viviparous FEMALES
Viviparous FEMALES
Viviparous FEMALES

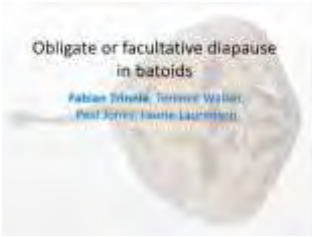

Viviparous FEMALES
Viviparous FEMALES
Viviparous FEMALES
• Stage 4b: Regenerating/Stage 4a: Resting
• Stage 4b: Regenerating/Stage 4a: Capable to reproduce
• Stage 4b: Regenerating/Stage 2: Developing
• Stage 4b: Regenerating/Stage 2: Developing
STAGE 2 DEVELOPING
STAGE 4B REGENERATING


ICES WKWKSEL3 2018
Workshop on Biomonitoring Maturity
? or
? or 3d
? or 4d
Thank you

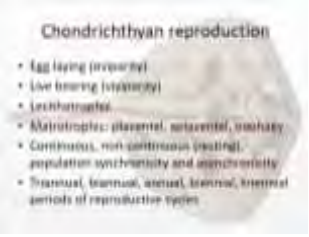

Maturity scales used
in
HCMB
for Elasmobranchs
2010-2012
2012 now
Samples in the Laboratory or onboard
Photos of Maturity scales
For
oviparous species
Galeus melastomus
Photos of Maturity scales
For
viviparous species
Squalus blainville

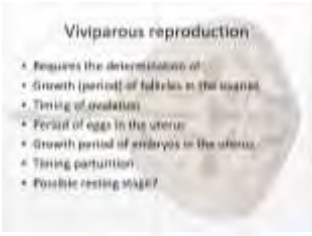

Thank you
for your attention


THANKS

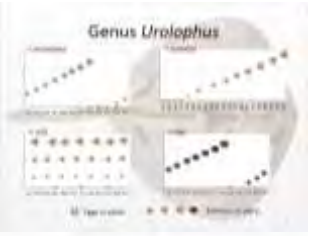

Obligate or facultative diapause in batoids

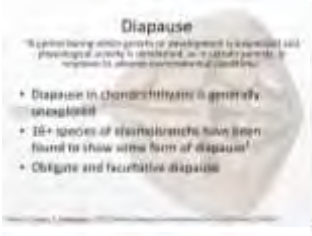

Fisheries management

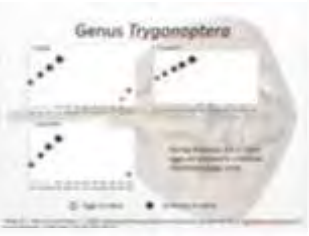

Chondrichthyan reproduction

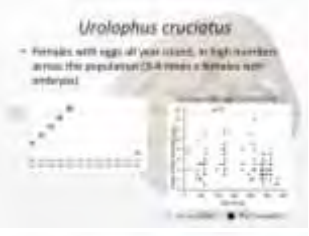

Viviparous reproduction


Urolophidae (stingaree) reproduction


Genus Urolophus


Diapause


Genus Trygonoptera


Urolophus cruciatus

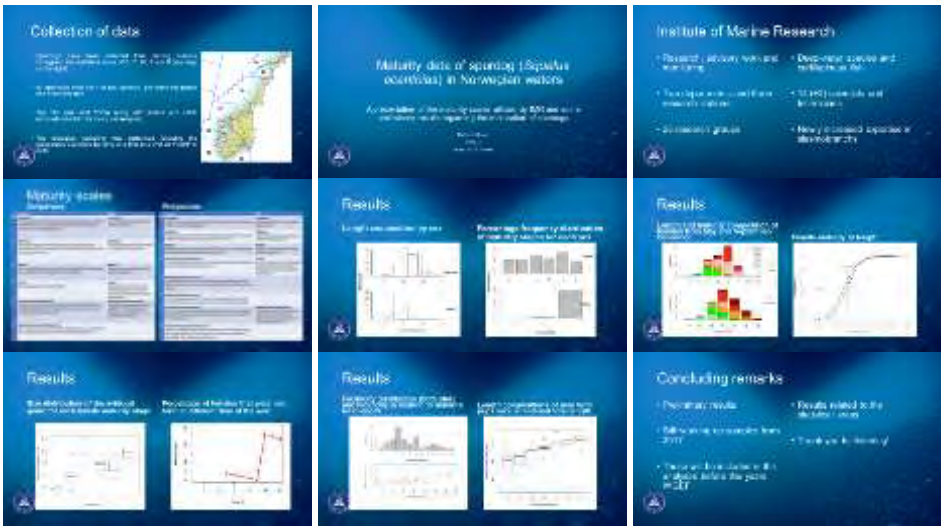

Obligate or facultative?

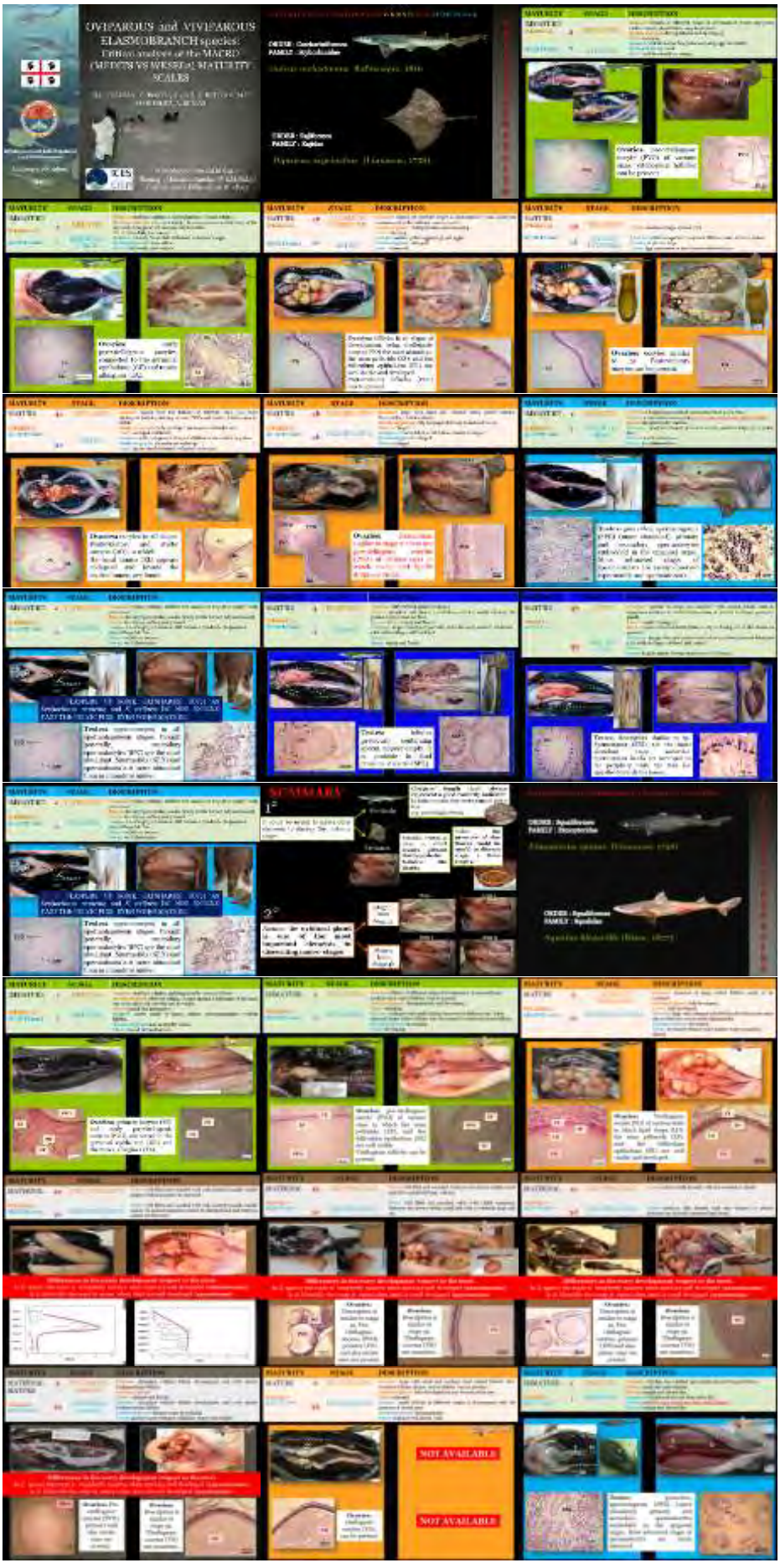

Diapause in other stingarees


Acknowledgements


Sampling Area


Maturity stages of Scyliorhinus canicula


Collection of data
Maturity data of spurdog (Squalus acanthias) in Norwegian waters
Institute of Marine Research
Maturity scales
Results
Results
Results
Results
Concluding remarks


OVIPAROUS and VIVIPAROUS ELASMOBRANCH species:
NOT AVAILABLE
NOT AVAILABLE


NOT AVAILABLE
NOT AVAILABLE