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Report of the Workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL)

11–15 October 2010

Valetta, Malta

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The European Union requires member states to collect maturity data under the Data Collection Framework (DCF) but there is no international agreement on the maturity scales to be applied to elasmobranchs. Therefore, this workshop was proposed by the Planning Group on Commercial Catches, Discards and Biological Sampling and the Planning group for the Mediterranean (PGCCDBS & PGMed 2009) as part of ongoing ICES and DCF work to improve data collection, methodology and quality assurance. The objectives were to agree on a common maturity scale for elasmobranchs (sharks, skates and rays), oviparous and viviparous species, across laboratories and compare existing scales and standardize maturity determination criteria. A system was developed to convert the current scales being used in various laboratories into the proposed maturity scales developed during this workshop. The proposed macroscopic maturity scale was validated by examining results from microscopic determinations. Guidelines were developed in order to propose an optimal sampling strategy to estimate accurately the maturity ogives for elasmobranchs.

Data

Participants from research institutes from Europe and North Africa provided macroscopic maturity scales and microscopic determinations for both oviparous and viviparous species. Digital photos on macro and microscopic maturity stages of reproductive systems were provided. These included a metric reference, identification of geographical area, date and biological characteristics (length, sex, etc) including the protocol used to gather this data. Sampling methods and estimators describing maturity were also provided.

Discussions and conclusions

The participants reviewed the currently employed maturity scales and produced two new scales of macroscopic maturity stages, one for oviparous and other for viviparous species. These scales were proposed to be adopted by all Institutes which are involved in elasmobranchs sampling both within the framework of EU data collection and by non EU countries. Common problems in both micro- and macroscopic stage determination were discussed and possible solutions were also provided. Results and comments are added to the present report. A reference photographic collection was built up thanks to the contribution of all the participants. Histological validations and stage descriptions were also illustrated and discussed. All participants felt that the aims of the workshop were attained and suggested a future meeting in three years time (2013) in order to test the proposed scales and improve the standardization with the consideration of more species, especially with those which have long life cycles and reproductive stages, such as large pelagic sharks.

Abbreviations and acronyms

AFRD	–	Agriculture and Fisheries Regulation Department
ARPAT	–	Environmental Protection Agency - Tuscany Regional
ATZI	–	Tecnalia Sukarrieta Txatxarramendi Ugartea z/g Spain
CAMPBIOL	–	CAMPionamento BIOLogico
CEFAS	–	Centre for Environment, Fisheries & Aquaculture Science
DCF	–	Data Collection Framework
ENSSMAL	–	Ecole Nationale Supérieure des Sciences de la Mer et de l'Amenagemet du Littoral
ETOH	–	Ethanol
EU	–	European Union
F	–	Female
FAO	–	Food and Agriculture Organization of the United Nation
FDBIG	–	Fisheries Department Balearic Islands Government
GFCM	–	General Fisheries Commission of the Mediterranean
GLM	–	General Linear Model
GRUND	–	GRUppo Nazionale Demersale
GSA	–	Geographical Sub Area
GSI	–	Gonadosomatic index
HCMR	–	Hellenic Centre for Marine Research
ICCAT	–	International Commission for the Conservation of Atlantic Tunas
ICES	–	International Council for the Exporation of the Sea
INRB – IPIMAR	–	Instituto Nacional de Recursos Biológicos-Instituto de Investigação das Pescas e do Mar
ISPRA	–	High Institute for Environmental Protection and Research
ISTM	–	Institute de sciences et technologies de la mer
M	–	Male
MCFS	–	Malta Centre for Fisheries Sciences
MEDITS	–	Medietrranean International Trawl Survey
MEDLEM	–	Mediterranean Large Elasmobranchs Monitoring
MiPAAF	–	Ministry of Agricultural, Food and Forestry Policies
NAFO	–	Northwest Atlantic Fisheries Organization
PAF	–	Parafolmaldehyde
PGCCDBS	–	Planning Group on Commercial Catches, Discards and Biological Sampling
PGMed	–	Planning Group for the Mediterranean
RCMs	–	Regional Co–ordination Meetings
SMALK	–	Sex Maturity Age Length Key
TL	–	Total Length
ToR	–	Terms of Reference
TW	–	Total Weight
USTHB	–	University os Sciences and Technology Houari Boumediene
VG	–	Van Gieson solution
WebGR	–	Web services for support of Growth and Reproduction studies
WGEF	–	Working Group on Elasmobranch Fishes
WKMAT	–	Workshop on Sexula Maturity Sampling
WKMOG	–	Workshop on Maturity Ogive Estimation for Stock Assessment
WKMSEL	–	Workshop on Sexual Maturity Staging of Elasmobranchs
WKMSSPDF	–	Workshop on Sexual Maturity Staging of sole, plaice, dab and flounder

1 Opening of the meeting

1.1 Opening of the meeting and adoption of the Agenda

The ICES "Workshop on Sexual Maturity Staging of Elasmobranchs" was held at the Waterfront Hotel in Sliema, Malta, from the 11 to 15 October 2010. The event was attended by 16 participants coming from: Algeria, Greece, Italy, Malta, Norway, Portugal, Spain and Tunisia. Representatives of United Kingdom participated through e-mail correspondence. List of the participants is attached as Annex 1.

The workshop was hosted by the capture fisheries research unit of the Agriculture and Fisheries Regulation Department. It was opened by Mark Dimech (co-chair and host) who welcomed the participants and recalled the importance of the workshop and the positive presence of people also from non EU countries. Elasmobranchs species present in both the Mediterranean and in the ICES area were used as examples for the proposal of a single maturity stage scale that it is foreseen to be adopted in both regions.

Fabrizio Serena (co-chair) presented a brief introduction on the background of the reproduction stage scales in use in the Mediterranean Sea, making a particular reference to the coordination meeting held in Kavala in 2006, where a standard maturity stage scale was adopted by the countries participating in the Mediterranean International Trawl Survey (MEDITS). Fabrizio also mentioned the relevance of the FAO MedSudMed project in supporting the participation in workshops that aim the study of the biology of Elasmobranch fishes and the production of technical documents. Acknowledgement was expressed to the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) by Fabrizio Serena and Cecilia Mancusi for supporting their participation.

Following the introduction of the participants, the co-chair Mark Dimech proposed the approval of the Agenda (attached as Annex 2), and appointed Cecilia Mancusi and Monica Barone as rapporteurs.

The workshop working documents and presentations are available on the ICES Share point web page: http://groupnet.ices.dk/WKMSEL2010/default.aspx

1.2 Scientific justification and aims

The development pattern of the gonads and determination of maturity stage are important biological aspects to be studied in fisheries science. They allow to discriminate life phases or "stanzas" (recruits, juveniles, adults, hermaphrodites, neoteny occurrence, etc), to characterise the follicle development and spawning modality (total or serial batch spawner), to set the spawning season of a species, monitoring of long term changes in the spawning cycle, and to estimate the achievement of sexual maturity (and the related computation of the Spawning Stock Biomass). Furthermore these studies are relevant for many other topics regarding the life cycle (for example, energy budget allocation and maturity-survival-longevity trade offs) of any exploitable or already exploited marine living resource.

For example, a comparison of the reproductive structure (as deduced by maturity stages), spawning occurrence, investment rate in the gonads (gonadosomatic index) and size at maturity, among different geographical areas and under heterogeneous level of exploitation, can be used to highlight the response of the investigated resources to both environmental and fishing variations.

Any accurate comparison of the reproductive pattern, however, would require a higher coherence and consistence among the different methods employed to gather the basic data. Indeed, logistic and administrative constraints, the accumulation of new knowledge or the personal feelings of the scientists in charge have determined the use of different macroscopic maturity scales, even for the same species, often in the same geographical area in successive times. Such difficulties are enhanced in case of elasmobranchs species, whose reproductive patterns and their links with the life cycle traits are less documented than with the more usual fish species.

The need of a common and standardized system for identification and macroscopic classification of maturity stages in the assessment of the fishery resources by the laboratories collecting maturity data has to be considered as an important priority to optimize the Data Collection Framework (DCF). Therefore, this workshop had the objectives of reviewing the previous methodologies and scales, defining objective criteria to classify the maturity stages both on micro and macro scale, reaching an agreement on the common scales to be used in the future, and figuring out conversion rules between the old and new scales.

A collateral but still relevant goal of this Workshop was to build a collection of micro and macro descriptions (mainly photos) provided by the different laboratories, to get an objective measure to what extent the criteria to classify maturity stages is coherent between technicians, and to pinpoint the major sources of disagreement.

Finally, it is worth pointing out the fact that if the new standard macro- and micro-maturity scales are accepted, this would improve considerably the standardisation and exchange of data between laboratories.

1.3 Terms of Reference

1) Agree on a common maturity scale for elasmobranchs (sharks, skates and rays), oviparous and viviparous species, across laboratories;

2) Compare existing scales and standardize maturity determination criteria;

3) Develop a system to convert the old and new maturity scales for the available time series;

4) Reduce sources of error on maturity determination by validating the macroscopic maturity stages;

5) Propose an optimal sampling strategy to estimate accurately the maturity ogives.

1.4 Data Collected before the Workshop

Each participant (or laboratory group) was invited to preliminary gather and present information, by species and whenever possible separately for females and males on:

1) Macroscopic maturity scale, old and in use, for both oviparous and viviparous species;

2) Microscopic maturity stages to validate macroscopic observations;

3) Digital photos on macroscopic maturity stages of reproductive systems. The photos have to be taken on fresh fish and have to include a metric reference and the sampling ID (for the identification of geographical area, date, biological data etc.);

4) Digital photos on microscopic maturity stages of the gonads and other reproductive organs. The photos have to include a metric reference and the sampling ID;

5) Staining protocol used for histological samples;

6) Methods and estimators describing maturity;

7) Collect reference material in order to share it among participants;

Before the workshop, each participant was invited to communicate the type of data (macroscopic and microscopic observations, photos, maturity estimation etc.) and the species on which they have collected information. Moreover participants were invited to communicate their intention to present a contribution during the workshop (e.g. powerpoint presentation) and if possible to provide a working document containing the information collected.

2 Summary of Presentations

2.1 Contribution to Guidelines for Age Determination of Chondrichthyes fish from the Mediterranean Sea (application to selected species).

Presented by Fabrizio Serena and Mark Dimech on behalf of the FAO project MEDSUDMED.

The main objective of the workshop was to provide an overview of techniques currently used in the MedSudMed Project area for preparation and reading of spines and vertebrae of a series of cartilaginous fish species. Direct observation of vertebrae and spines was conducted, along with tests of two methods for enhancing the appearance of the growth bands (red alizarin and cobalt nitrate). On the basis of the laboratory work and of the relevant bibliography available, trials were performed to identify the most relevant techniques for each species studied. The document details the work done during the workshop and provides general guidelines for cartilaginous fish age determination, as well as the limits of the methods currently used and improvements that could be made in the near future. It appeared that direct observation gave good results for all species except *Centroscymnus coelolepis*. Red alizarin and cobalt nitrate staining procedures also improved the appearance of growth patterns except for *Carcharhinus plumbeus*, *C. coelolepis* and *Scyliorhinus canicula* and *Galeus melastomus*. Good results were obtained for some species (*C. plumbeus*, and *G. melastomus*), according to the size of the observed individuals. Further investigations are needed; in particular, larger samples should be examined to confirm the methods used and to provide detailed guidelines to be used at the regional level.

2.2 Oviparous species *Galeus melastomus*: macroscopic maturity stages of females

Letizia Sion

Reproductive information are collected on *Galeus melastomus* oviparous species. The specimens were sampled during trawl surveys Italian "Gruppo Nazionale Demersali"(GRUND) and International Bottom Trawl Survey in the Mediterranean (MEDITS) carried out in the North-western Ionian Sea. Macroscopic analysis on females was carried out, five macroscopic maturity stages were individuated considering the detailed MEDITS scale (Kavala meeting, 2006).

2.3 Viviparous species: macroscopic maturity stages of females

Letizia Sion

Reproductive information are collected on viviparous species. The specimens were sampled during trawl surveys (GRUND and MEDITS) carried out in the North-western Ionian Sea. Macroscopic analysis on females of *Etmopterus spinax*, Dalatias licha, *Centrophorus granulosus* and *Torpedo torpedo* was carried out, seven macroscopic maturity stages were individuated according Stehmann scale (2002).

2.4 Maturity scale for the viviparous cartilaginous fishes for the Tuscany area, northwest Mediterranean (south Ligurian Sea–north Tyrrhenian Sea), GSA 9

Mancusi Cecilia., Barone Monica, Serena Fabrizio.

Different species of sharks, rays and skates were sampled during scientific bottom trawl survey (MEDITS) and occasionally during other project (European framework collection data CAMPBIOL, DISCARD; and Mediterranean Large Elasmobranchs Monitoring (MEDLEM) of accidental catches and strandings along Tuscany coast from 1985 to 2010. In particular the samples analysed for the attribution of the maturity stage were demersal species as *Centrophorus granulosus*, *Etmopterus spinax*, *Dalathias licha*, *Squalus blainvillei*, *Torpedo marmorata*, *Torpedo nobiliana*, *Torpedo torpedo* (mainly sampled thanks to the Medits project), pelagic species as *Alopias vulpinus*, *Carcharhinus obscurus*, *Carcharhinus plumbeus*, *Cetorhinus maximus*, *Hexanchus griseus* (data collected thanks to the monitoring of the MEDLEM project in Tuscany area) and other species like *Dasyatis centroura*, *Dasyatis pastinaca*, Myliobatis aquila, *Pteromylaeus bovinus*. For these species we have only performed a macroscopic analysis supported by photos but no microscopic validation was undertaken. The different maturity stage were attributed to males and females following the new Medits tables discussed in Kavala in 2006. For females we recognised 7 different stages (immature/virgin, maturing, mature, developing, differentiating, expecting, resting) and for males only 4 (immature/virgin, maturing, mature, mature/extruding active).

2.5 The research on the reproductive biology in the cartilaginous fishes at the Italian High Institute of Environmental Protection and Research (ISPRA)

Massimiliano Bottaro, Ivan Consalvo, Umberto Scacco

Since 2000 ISPRA (formerly Central Institute for Applied Marine Research-ICRAM) collected biological information on the cartilaginous fishes in the Italian waters (GSA 9, 11, 15, 17). About the reproductive features, we collected and analyzed samples since 2005 from the following species: *Cetorhinus maximus*, *Dalatias licha*, *Galeus melastomus*, *Mustelus mustelus*, *Prionace glauca*, *Squalus acanthias*, *Dipturus oxyrinchus*, *Raja asterias*, *Raja brachyura*, *Raja clavata*, *Raja polystigma*, *Torpedo marmorata*, *Torpedo torpedo* and *Chimaera monstrosa*. To define a common standard for our data we applied the Stehmann's proposal macroscopical scale (2002), but in order to clarify the real situation we histologically fixed and stored the most of the samples for compare the macroscopic information with the microscopic details. Our protocol to collect sample and data on the reproductive organs is based on the following steps: 1) taking a picture of each specimen with ID and metric reference; 2) providing total length (TL) and total weight (TW) of each specimen; 2) taking a picture of the gonads with ID and metric reference; 3) in males, measuring the length of the claspers and evaluating their consistence; 4) weighting the gonads to define the gonadosomatic index (GSI); 5) fixing the gonads in 4% Paraformaldehyde (PAF) or in Bouin liquid; 6) washing the gonads in posphate buffered saline (PBS) pH. 7,4 and storing them in ethanol (ETOH) 70%. Our analyses have allowed to evidence some interesting features about the species in the considered geographical area, such as maturity season and size segregation for *D. licha*, correlation between the first maturity length and difference sampling sites for *G. melastomus*, the reproductive patterns for *T. marmorata* and *T. torpedo*, and the first data on the maturity staging for *C. monstrosa*. The research is actively in progress and

more information will be collected in order to clarify all the details of the reproductive biology in the different species, both from a macroscopic and a microscopic point of view.

2.6 Macroscopic maturity scales and photos for oviparous and viviparous elasmobranch species used for the Maltese data collection system.

Francesca Gravino, Mark Dimech

The Agriculture and Fisheries Regulation Department of Malta collects data on biological parameters for cartilaginous species as part of the obligations from the EU Data Collection Framework (EC 93/2010).

Samples of cartilaginous fish among other species are collected from the Geographical Sub-Area (GSA) 15 and are collected through scientific surveys (e.g. MEDITS survey) and fisheries dependent surveys from on-board observations on commercial vessels (Cartilaginous fish are now listed as Group 1 species, i.e. species that have to be sampled whenever encountered) and market and port sampling

Malta determines maturity stages of elasmobranchs macroscopically and uses the MEDITS common maturity scale for elasmobranchs maturity stages. Up to 2008, the MEDITS 1994 scale (not detailed) was employed, but as from July 2009, the more detailed MEDITS scale (Kavala meeting, 2006) was used for both MEDITS and fishery dependent surveys. The MEDITS scale is for oviparous species but has been adapted also for viviparous species.

Malta presented macroscopic photos for oviparous species namely *Scyliorhinus stellaris*, *Raja clavata* and *Raja miraletus*, and also for viviparous species namely *Squalus blainvillei*, *Mustelus mustelus*, *Mustelus asterias* and *Mustelus punctulatus*. Most stages for both males and females were represented.

Malta also pointed some considerations, namely that some of the details (MEDITS scale) on the 'Gonad state' and 'Maturation state' differ between different species (e.g. the length of claspers) and that the scale does not include 'Spent' and 'Recovering' stages as in the MEDITS scale these are all included as 'Resting'.

2.7 Sexual Maturity Staging of the viviparous longnose spurdog *Squalus blainvillei* for the Sardinian waters (Central-western Mediterranean Sea)

C. Porcu, A. Mulas, S. Cabiddu, R. Cannas, M.C. Follesa, A. Cau

A maturity male and female scale for the aplacental viviparous *Squalus blainvillei* (Risso, 1826), based on that proposed by Stehmann in 2002, using both macroscopical and histological observations (digital photos and sampling ID are provided), is presented. For the drafting of the scale, fresh samples were collected from scientific surveys (GRUND and MEDITS) and commercial landings in the waters surrounding Sardinia (GSA11, Central-western Mediterranean Sea), between 2008 and 2010. According to Stehmann, four maturity stages for males are individuated. After the findings of some female specimens with apparent asynchronous uteri development, Stehmann's uterine stages 4 and 5 (developing and differentiating) are merged. According to the MEDITS scale (Relini *et al.*, 2008), all stages in which the individuals are mature, are indicated as 3, identifying two sub-stages (3a = mature, adult; 3b = mature, active) for males and three for females (3a = mature; 3b = develop-

ing/differentiating; 3c = expecting). Macroscopic stages are validated with the microscopic analysis.

2.8 Sexual Maturity Staging of the oviparous blackmouth catshark *Galeus melastomus* for the Sardinian waters (Central-western Mediterranean Sea)

C. Porcu, A. Mulas, S. Cabiddu, R. Cannas, M.C. Follesa, A. Cau

A maturity male and female scale for the oviparous *Galeus melastomus* Rafinesque, 1810, based on that proposed by Holden and Raitt (1974), Stehmann in 2002, and mainly the last MEDITS scale in 2008, using both macroscopical and histological observations (digital photos and sampling ID are provided), is presented. For the drafting of the scale, all fresh samples were collected from scientific surveys (GRUND, MEDITS and deep-water surveys) and commercial landings along Sardinian waters (GSA11, Central-western Mediterranean Sea) between 2008 and 2010. According to the MEDITS scale, five maturity stages for males and females are individuated, all confirmed by the histological observation. In general, the MEDITS scale is confirmed except for some descriptions as immature stage in females (description and appearance of the ovary) and maturing (length of claspers) and resting stages (description and appearance of the testis) in males. It is important to highlight, also, the presence of sperm in the seminal vesicle of some individuals with uncalcified claspers. As calcified clasper is required for copulation, the presence of sperm could be not always used as a reliable indicator of functional maturity.

2.9 Maturity scales used for oviparous elasmobranchs (Portugal, IPIMAR)

Bárbara Serra Pereira, Teresa Moura and Ivone Figueiredo

In Portugal, at IPIMAR, the assignment of the maturity stages in oviparous elasmobranchs, both males and females, have been based on the scales proposed by Stehmann (2002), but the proposal of a new scale by Serra-Pereira *et al*. (in press) is presented, due to the need to adapt the old scale to the species under study and to standardize the reproductive terms used among all fish studies. Maturity data is collected from mainly five skate species: thornback ray *Raja clavata*, blonde ray *Raja brachyura*, spotted ray *Raja montagui*, undulate ray *Raja undulata*, cuckoo ray *Leucoraja naevus*. The same scale is also applied to other oviparous elasmobranch species sampled in Portugal. Samples are collected under the National Data Collection Program: a) from three yearly IPIMAR's research demersal trawl surveys undergone along the mainland coast (30-950 m depth); and b) monthly from commercial landings of the artisanal fleet operating with trammel nets, gillnets, longline and trawl (most below 100 m depth) in the north (Matosinhos) and centre (Peniche) of Portugal mainland. The correspondence between the two maturity scales for both males and females was presented. In the case of males, the proposed scale includes a new stage - regressing -, to assign to males with hard, enlarged claspers with regressing gonads or those that appeared reproductively inactive. In the scale proposed for females there are two major differences between the two scales. One is the creation of a unique stage for females containing egg capsules (Actively spawning) instead of the three stages from the old scale. The second main difference is 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 (regenerating).

2.10 Maturity scales used for viviparous elasmobranchs (Portugal, IPIMAR)

Bárbara Serra Pereira, Teresa Moura and Ivone Figueiredo

In Portugal, at IPIMAR, the assignment of the maturity stages in viviparous elasmobranchs, both males and females, have been previously based on the scales proposed by Stehmann (2002), but currently the scale in use is the one from Figueiredo *et al.* (2008), which is an adaptation from the former. Maturity data is collected from mainly two deep-water shark species: leafscale gulper shark *Centrophorus squamosus* and Portuguese Dogfish *Centroscymnus coelolepis*. The same scale is also applied to other viviparous elasmobranch species sampled in Portugal. Samples are collected monthly under the National Data Collection Program from commercial landings of the artisanal fleet operating with longline (800-1600 m depth) in Sesimbra, in the centre of Portugal mainland. The correspondence between the two maturity scales for both males and females was presented. In the case of males, the two scales are equivalent. In the case of females, there were some inconsistencies and difficulties in assigning the old maturity stages. This was particularly evident in maturity stage 2 (Maturing), which included females maturing for the first time and females that had previously matured at least one time. Thus, the main difference between the two scales is the subdivision of maturing females into two groups: one composed by females maturing for the first time (2: maturing, 1st time) and, a second group, comprising maturing females that had already matured before, that are easily recognized after observation of the reproductive organs (8: regenerating).

2.11 Description of the macroscopic development of the reproductive system in skates as example of elasmobranchs oviparous species

Monica Barone, Cecilia Mancusi and Fabrizio Serena

In skates, the attainment of the sexual maturity requires different stages of development of the reproductive system in males and females. Females have paired ovaries and paired oviducts. Each oviduct is differentiated in ostium, anterior oviduct, oviducal gland, uterus, cervix and urogenital sinus, common to both tracts. Males have external paired claspers; the internal organs include the testes, genital ducts, Leydig gland. The genital ducts consist of efferent ductules, epididymis, ductus deferentes and seminal vesicle. In the females the ovary, at first dorso-ventrally flattened and milk-like white, is filled by follicles granulose and just visible. Then the ovary increase and follicles begin to differentiate in different sizes, but they are still white. At the onset of maturity the ovary walls become more transparent and the follicles are mostly large and yellow filling the whole ovary, which lost the flattened shape. At the same time, at oviduct level, the oviducal gland increase in dimension with the development of the ovary and reach the maximum size just before the ovulation, then its size regress or increase following the ovarian cycle. At the peak of the maturity cycle egg capsules are present in the oviducts, while in the specimens that have already laid the egg capsules the oviducts are still distended, vascularized and the urogenital sinus is very enlarged. During the sexual development of males the growth of claspers and the change of the consistency of their skeletal, becoming less flexible, are observed. At the end, the tip of claspers becomes nearly pointed. When testes are observed, the germinal region is not visible and it is located on the ventral surface, where the seminiferous ampullas develop and migrate in radial direction towards the dorsal region. At the beginning the testes is not entirely filled by the seminiferous

ampullas, encircled on the dorsal side by a white layer; when maturity is reached the testis is rose and almost completely filled by seminiferous ampullas. The ducti deferentes follow the development of testes, firstly not differentiated and narrow increase in thickness and at the onset of maturity they meander showing a whitish spiral-like formation representing the sperm flowing.

2.12 Data collection and sexual maturity staging for Rhinobatids in the Gulf of Gabès (southern Tunisia, central Mediterranean Sea) Macroscopic maturity scale

S. Enajjar, B. Saidi, M.N. Bradaï

Guitarfishes were collected between 2001 and 2005 from commercial bottom trawlers and a specific gill-netters from the Gulf of Gabe`s (14 GFCM geographical Sub-Areas). All specimens were sexed and sized. For males, measurements also took into consideration clasper length. The constancy of clasper was also described. Fully yolked oocytes, encapsuled eggs and embryos were weighed and then measured. The embryos were also sexed. The assignment of the maturity stages, both males and females, has been previously based on the scales proposed by Stehmann (2002). Four categories of males were distinguished: immature (stage A), maturing (stage B), mature (stage C) and mature active (stage C1) for females, sex stages were described: immature (stage A), maturing (stage B), mature (stage C), mature developing (stage C1), mature differentiating (C2) and mature resting (stage C3).

2.13 Length-weight relationships and length at maturity for skates (Rajidae) around the British Isles, and an overview of the collection of reproductive data for elasmobranchs in English groundfish surveys

During the workshop, this working document was sent by e-mail from Jim Ellis and Sophy McCully to the Co-chair Fabrizio Serena as contribution of the CEFAS laboratory, UK. In their e-mail, the authors expressed their concern about the differences between the "new standardized maturity scales" and those in use in their laboratory since 1990s. The working document was added as Annex of this report.

3 The standardised macroscopic maturity scale

The participants examined the available maturity scales, comparing the photos by stage with macroscopic and microscopic description in order to describe the reproduction cycle. The common analyses were done first for the oviparous species focussing on both males and females. A review of the examined materials by all participating institutes is reported in Annex 4, including the study areas and modes of reproduction of the species.

3.1 Oviparous maturity scales – macroscopic

The oviparous macroscopic scales were based on the results from skate (Rajidae) and shark (Scyliorhinidae) species studied by the participants of this workshop (see abstracts section 2). The descriptions were made as wide-ranging as possible, but it is important to highlight that in future applications of these scales to other oviparous elasmobranch species (e.g. the bullhead sharks of Heterodontiformes and the orectolobiform carpetsharks of Parascycilliidae, Hemiscyllidae and Stegostomatidae), differences can occur, and should be documented and discussed in a future WKMSEL meeting.

The terminology adopted for the scales is the one proposed by Brown-Peterson *et al.* (in press), as an effort to standardize the terms used to define the phases in the reproductive cycle of fish, including both teleosts and elasmobranchs. The scales include five maturity stages for males (1, 2, 3a, 3b and 4) and six for females (1, 2, 3a, 3b, 4a and 4b). When applying these scales in the field, it is important to retain that the subdivision of some of the stages (e.g. 3 and 4) cannot be ignored and considered as a single stage, i.e. they refer to distinct aspects of the cycle although sharing some similarities. It is also essential for a correct maturity stage assignment that the gonads are inspected in both sides (e.g. ventral and dorsal view in skates), because the visualization of the lobes in males and the follicles in females can be visible only in one of the sides (dorsal) during early maturation. These last two reminders are also applied to viviparous species.

Following is a short summary of the main aspects discussed in the meeting when building the maturity scales for males and females.

3.1.1 Males

In elasmobranch fishes the differentiation of testes usually anticipate the full development of claspers (asynchronous development), which rigidity is a condition necessary but not enough for the determination of the maturity stage. Moreover, in case the specimen is resting, the complete development of clasper is coupled with testes small and empty. Also in the last situation, not observing the testes status could lead to the wrong evaluation of the maturity stage

For example, when a male has flexible claspers but the internal organs already developed it is considered 2.Developing and not 3a.Spawning capable because physiologically it is not yet ready for copulation.

Differences in clasper development were discussed between skates and sharks. Generally in mature skates claspers reach a length largely greater than the pelvic fins, which is not observed in sharks that have generally very small claspers being sometimes only as long as the pelvic fins in mature males. So, it was established that when

the claspers are smaller than pelvic fins it is considered stage 1.Immature, and when claspers reach or surpass the length of pelvic fins it is considered stage 2.Developing.

In skates, the extension of testes surface occupied by the lobules cannot be used as a feature to define the stages. In general, only in stage 3.Spawning capable the lobules can occupy the whole testes surface. However, stage 2.Developing males were observed with lobes in the entire surface although less developed; and in 3.Spawning capable male testes with fully developed lobes not occupying the whole surface and with some epigonal organ visible in the posterior region were observed.

In the present scale a 4.Regressing stage for males was considered. This stage was based in few data, so that more results should be collected for the next WKMSEL meeting to validate its occurrence and add more accurate details to the description.

3.1.2 Females

One of the features used to differentiate between 1.immature and 2.developing females is the visualization of the oviducal gland in the latter. This feature was agreed to be true for all skate species, but when referring to oviparous sharks, the oviducal gland can be considered to be slightly visible in the end of the immature stage. So, for sharks the assignment of the 1.immature stage must rely on the absence of follicles in ovary and on the thread-like appearance of the uteri.

The use of the expansion of the cloaca after copulation as a distinctive feature between female's maturity stages was mentioned. It was reached the conclusion that this morphological feature cannot be used, since it is subjective information, depending on the degree of elasticity of the cloaca muscles of a female, which can also be variable with the species.

Based on the old scale proposed by Stehmann (2002) three distinct stages corresponding to the extrusion of the egg capsule were considered (D/4: active, E/5: advanced and F/6: extruding), depending on the stage of development of the egg capsule. To simplify, in the current scale only one stage was considered to describe the encapsulation of the eggs: 3b.Actively spawning; i.e. females beginning formation of the egg capsule (e.g. only the horns or tendrils visible) or containing fully formed egg capsules in the uterus are considered to be in the same stage.

The proposed scale introduced a new stage to differentiate between the adolescent females that are maturing for the first time (2.Developing), from those adult females that are beginning a new cycle and had already spawned at least once (4b.Regenerating).

To improve the accuracy of the descriptions of the stages 4a.Regressing and 4b.Regenerating it will be essential to collect macroscopic information, for the next WKMSEL meeting, about the occurrence of atretic follicles and postovulatory follicles in the ovaries of the females.

3.1.3 The proposal for a common macroscopic scale for Oviparous species

MALES

TITLE	OVIPAROUS ELASMOBRANCHS (SKATES AND SHARKS)			
SEX	PROPOSAL	MATURITY STAGE	STAGE	MATURITY
M	Claspers flexible and shorter than pelvic fins. Testes small (in skates, sometimes with visible lobules). Sperm ducts straight and thread-like.	**IMMATURE (Immature)**	**1**	**IMMATURE**
M	Claspers still flexible, and as long as or longer than pelvic fins. Testes enlarged (in skates, lobules clearly visible but not occupying the whole surface). Sperm ducts developing and beginning to coil (meander).	**DEVELOPING (Immature)**	**2**	**IMMATURE**
M	Claspers fully formed, skeleton hardened, rigid and generally longer than pelvic fins. Testes greatly enlarged (in skates, filled with developed lobules). Sperm ducts tightly coiled and filled with sperm.	**SPAWNING CAPABLE (mature)**	**3a**	**MATURE**
M	Description similar to stage 3a, however with clasper glands dilated, sometimes swollen and reddish. Sperm may be present in clasper groove or glans. On pressure sperm is observed flowing out of the cloaca or in the sperm ducts.	**ACTIVELY SPAWNING (mature)**	**3b**	**MATURE**
M	Claspers fully formed, similar to stage 3. Testes shrunken and flaccid, (in skates, with few visible lobules). On pressure sperm does not flow. Sperm ducts empty and flaccid	**REGRESSING (mature)**	**4a**	**MATURE**

FEMALES

SEX	**PROPOSAL**	**MATURITY STAGE**	**STAGE**	**MATURITY**
F	Ovaries barely visible or small, whitish; undistinguishable ovarian follicles. Oviducal (nidamental) gland not visible in skates and may be slightly visible in sharks. Uterus is thread-like and narrow.	**IMMATURE (Immature)**	**1**	**IMMATURE**
F	Ovaries enlarged with small follicles (oocytes) of different size. Some relatively larger yellow follicles may be present. Developing oviducal gland and uterus.	**DEVELOPING (Immature)**	**2**	**IMMATURE**
F	Large ovaries with enlarged yolk follicles of different sizes. Oviducal gland and uterus fully developed.	**SPAWNING CAPABLE (mature)**	**3a**	**MATURE**
F	Description similar to stage 3a, however with the presence of egg capsules.	**ACTIVELY SPAWNING (mature)**	**3b**	**MATURE**
F	Ovaries shrunken with few follicles of different sizes. The oviducal glands diameter may be reducing. Uterus appears much enlarged (relative to stage 2), collapsed, empty and reddish.	**REGRESSING (mature)**	**4a**	**MATURE**
F	Ovaries full of small follicles similar to stage 2, enlarged oviducal glands and uterus.	**REGENERATING (mature)**	**4b**	**MATURE**

3.2 Viviparous maturity scales – macroscopic

The group used the same template for the oviparous, and built up the one for viviparous starting from this. Most of the concepts and descriptions for maturity stages of viviparous species are the same as oviparous, especially for males. The major differences between oviparous and viviparous were the female's pregnancy stages.

Considering the multitude of reproductive strategies that are found among viviparous elasmobranchs, and to reinforce that the scales here presented are based on a limited number of species and consequently not all strategies are covered, the modes of reproduction in which these scales were based are summarize in Annex 4.

3.2.1 Males

The descriptions for the maturity stages in viviparous are very similar to oviparous species, so those presented in section 3.1.1 were further applied, with some notes described below.

Since the group examined a few number of species, there was no certainty if the all the specie have segmented testes or if some have testes filled with lobules (e.g. *Torpedo torpedo*).

In some species, the tip of the claspers can be more flexible in 3b.Actively spawning males. Also in this stage, in sporadic cases, the claspers can be opened (exploded) and more flexible on the tip.

The group did not have enough data to reach an agreement on the existence and on the corresponding description of 4a.Regressing males. However, in doubt, the group considered useful to add this stage to the maturity scale for future references. From experience, the group expects that in stage 4a.Regressing the testes and spermducts are shrunken and flaccid. It was agreed that will be essential that, until the next WKMSEL meeting, additional data should be collected for future discussion on the maintenance of this stage in the male's maturity scale.

3.2.2 Females

The separation of the embryos inside the uterus when observed from the outside is here referred as segmentation. It was discussed, and photo referenced, that, depending on the species, this segmentation can be observed earlier in the in 3c.Mid-pregnancy for some species, or latter just in the 3d.Late pregnancy stage.

The use of two homograph terms "candle case" and "candle-shape uterus phase" was discussed and defined.

The term "candle case" refers to the thin pliable transient egg candle case (yolk sac species), or thin pleated egg envelope (most placental species) produced around the fertilized egg and retained in the uterus until disappearing during the embryogenesis of viviparous species (Hamlett *et al.* 2005a).

Stages 4a.Regressing and 4b. Regenerating share similar features but refer to very different phases of the cycle. Typically, in viviparous species due to their determinate fecundity, 4a.Regressing females don't have follicle development which is a main difference from 4b. Regenerating, in which the ovaries start to produce the next generation of follicles, and several small yellow follicles are visible.

Stage 4b. Regenerating can also be mistaken with stage 2.Developing, but the former refers to those females that are maturing not for the first time, being distinguishable from the latter by their enlarged and flaccid uterus and well developed oviducal gland.

In the maternal stages, when the embryos are analysed, one additional feature that differentiates 3c.Mid pregnancy and 3c.Late pregnancy is the presence of external gill filaments in the embryos of the former and the absence of external gill filament in the embryos of the latter ones.

Note that, for some species, e.g. *M. mustelus*, *R. rhinobatos*, *S.blainvillei*, *C. granulosus*, vitellogenesis continues to occur simultaneously with embryogenesis, i.e. during stages 3b.Early pregnancy, 3c.Mid pregnancy and 3c.Late pregnancy, as reported and documented with photos by Samira for *R. rhinobatos* from Tunisian waters, and by Bárbara for *C. granulosus* from Portugal.

For maturity ogive estimation: females from stages 1.Immature and 2. Developing are considered immature; females from stages 3a.Capable to reproduce to stage 4b.Regenerating are considered as mature. For maternal ogive estimation, only females from stages 3b.Early pregnancy, 3c. Mid pregnancy and 3d.Late pregnancy are considered (maternal).

3.3 The proposal for a common macroscopic scale for Viviparous species

MALES

PROPOSAL	MATURATION STATE	STAGE	MATURITY
Claspers flexible and shorter than pelvic fins. Testes small (in rays, sometimes with visible lobules). Sperm ducts straight and thread-like.	**IMMATURE (Immature)**	**1**	**IMMATURE**
Claspers slightly more robust but still flexible. Claspers as long as or longer than pelvic fins. Testes enlarged; in sharks testes start to segment; in rays lobules clearly visible but do not occupy the whole surface. Sperm ducts developing and beginning to coil (meander).	**DEVELOPING (Immature)**	**2**	**IMMATURE**
Claspers fully formed, skeleton hardened, rigid and generally longer than pelvic fins. Testes greatly enlarged; in sharks testes are fully segmented; in rays filled with developed lobules. Sperm ducts tightly coiled and filled with sperm.	**SPAWNING CAPABLE (mature)**	**3a**	**MATURE**
Description similar to stage 3a, however with clasper glands dilated, often swollen and reddish (occasionally open). Sperm often present in clasper groove or glans. On pressure sperm is observed flowing out of the cloaca or in the sperm ducts.	**ACTIVELY SPAWNING (mature)**	**3b**	**MATURE**
Claspers fully formed, similar to stage 3. Testes and sperm ducts shrunken and flaccid.	**REGRESSING (mature)**	**4**	**MATURE**

FEMALES

PROPOSAL	MATURATION STATE	STAGE	MATURITY
Ovaries barely visible or small, whitish; undistinguishable ovarian follicles. Oviducal (nidamental) gland may be slightly visible. Uterus is thread-like and narrow.	**IMMATURE (Immature)**	**1**	**IMMATURE**
Ovaries enlarged with small follicles (oocytes) of different size. Some relatively larger yellow follicles may be present. Ovaries lack atretic follicles. Developing oviducal gland and uterus.	**DEVELOPING (Immature)**	**2***	**IMMATURE**
Large ovaries with enlarged yolk follicles all of about the same size so that they can be easily distinguished. Oviducal gland and uterus developed without yolky matter, embryos and not dilated.	**CAPABLE to REPRODUCE (mature)**	**3a**	**MATURE**
Uteri well filled and rounded with yolk content (usually candle shape). In general segments cannot be distinguished and embryos cannot be observed.	**Early pregnancy (maternal)**	**3b**	**MATERNAL**
Uteri well filled and rounded, often with visible segments. Embryos are always visible, small and with a relatively large yolk sac.	**Mid pregnancy (maternal)**	**3c**	**MATERNAL**
Embryos fully formed, yolk sacs reduced or absent. Embryos can be easily measured and sexed.	**Late pregnancy (maternal)**	**3d**	**MATERNAL**
Ovaries shrunken without follicle development and with atretic (degenerating) follicles. The oviducal glands diameter may be reducing. Uterus appears much enlarged, collapsed, empty and reddish.	**REGRESSING (mature)**	**4a**	**MATURE**
Ovary with small follicles in different stages of development with the presence of atretic ones. Uterus enlarged with flaccid walls. Oviducal gland distinguishable.	**REGENERATING (mature)**	**4b***	**MATURE**

*** Be careful, these stages can be easily confused**

3.4 Comparison and conversion of previous maturity scales with the new one WKSMEL 2010

The terminology commonly used to describe different reproductive phases in elasmobranchs is variable among authors. A broad comparison between the reproductive terminologies from several published works on oviparous elasmobranchs throughout the world can be consulted in Serra-Pereira *et al*. (in press). Following is a comparative summary of the terminologies from old and new scales used by WKMSEL participants and from literature. Any discrepancies and remarks were pointed out some suggestions for the new proposed scale, to be discussed with the whole group.

Oviparous Females

STAGE		Holden and Raitt (1974)	Relini *et al* (1999) "OLD MEDITS"	Stehmann (2002)	Relini *et al* (2008) "MEDITS Kavala"	Ungaro (2008) "ICES"	ICES (2009) "WGEF"	Serra-Pereira *et al*. (in press)
IMMATURE	**1**	1 Immature	1 Immature	A or 1 Immature, juvenile	1 Immature/ Virgin	F1 Immature, Juvenile	A Immature	1 Immature
DEVELOPING	**2**	2 Maturing	2 Maturing	2 or B Maturing, adolescent	2 Maturing	F2 Adolescent, Maturing	B Maturing	2 Developing
SPAWNING CAPABLE	**3a**	3 Mature	3 Mature	3 or C Mature, adult	3a Mature	F3 Adult, Mature	C Mature	3a Spawning capable
ACTIVELY SPAWNING	**3b**	3 Mature	3 Mature	D or 4 Active E or 5 Advanced F or 6 Extruding	3b Mature/ Extruding, Active	F4 Active, uterine stage; Advanced, uterine stage; Extruding, uterine stage	D Active	3b Actively spawning
REGRESSING	**4a**	No stage comparison	No stage comparison	No stage comparison	4a Resting	No stage comparison	No stage comparison	4 Regressing
REGENERA-TING	**4b**	No stage comparison	No stage comparison	No stage comparison	4 Resting	No stage comparison	No stage comparison	5 Regenerating

Oviparous Males

STAGE		Holden and Raitt (1974)	Relini *et al* (1999) "OLD MEDITS"	Stehmann (2002)	Relini *et al* (2008) "MEDITS Kavala"	Ungaro (2008) "ICES"	ICES (2009) "WGEF"	Serra-Pereira *et al*. (in press)
IMMATURE	**1**	1 Immature	1 Immature	A or 1 Immature, juvenile	1 Immature/ Virgin	M1 Juvenile	A Immature	1 Immature
DEVELOPING	**2**	2 Maturing	2 Maturing	2 or B Maturing, adolescent, sub-adult	2 Maturing	M2 Adolescent, Maturing	B Maturing	2 Developing
SPAWNING CAPABLE	**3a**	3 Mature	3 Mature	3 or C Mature, adult	3a Mature	M3 Adult, Mature	C Mature	3a Spawning capable
ACTIVELY SPAWNING	**3b**	3 Mature	3 Mature	4 or D Active	3b Mature/ Extruding, Active	M4 Active, copulating	D Active	3b Actively spawning
REGRESSING	**4a**	No stage comparison	No stage comparison	No stage comparison	4a Resting	No stage comparison	No stage comparison	4 Regressing

Viviparous Females

STAGE		Stehmann (2002)	Stehmann (2002) Adapted by Samira	Relini *et al* (2008) "MEDITS Kavala"	Ungaro (2008) "ICES"	ICES (2009) "WGEF"	Figueiredo *et al*. (2008) (Stehmann, 2002 adaptation)
IMMATURE	**1**	A or 1 Immature, juvenile	A Immature, juvenile	1 Immature/ Virgin	F1 Immature, Juvenile	A Immature	1 Juvenile
DEVELOPING	**2**	2 or B Maturing, adolescent	B Maturing, adolescent, sub-adult	2 Maturing	F2 Adolescent, Maturing	B Maturing	2 Maturing
CAPABLE to REPRODUCE	**3a**	3 or C Mature, adult	C Mature, adult	3a, 3b	F3 Adult, Mature	C Mature	3 Adult
EARLY PREGNANCY	**3b**	4 or D Developing	C1 Active	4a	F4	D Early gravid	4 Developing
MID PREGNANCY	**3c**	E or 5 Differentiating	C2	4b	F5	E Mid-term gravid	5 Differentiation
LATE PREGNANCY	**3d**	F or 6 Expecting	C2	5	F6	F Late gravid	6 Extrusion
REGRESSING	**4a**	G or 7 Post-natal, spent	C3	6	F7	G Post-partum	7 Resting
REGENERA-TING	**4b**	B or 2 (Maturing for the 2nd or more times)	No stage comparison	No stage comparison	No stage comparison	No stage comparison	8 Maturing (not for the first time)

Viviparous Males

STAGE		Stehmann (2002)	Stehmann (2002) Adapted by Samira	Relini *et al* (2008) "MEDITS Kavala"	Ungaro (2008) "ICES"	ICES (2009) "WGEF"	Figueiredo *et al*. (2008) (Stehmann, 2002 adaptation)
IMMATURE	**1**	A or 1 Immature, juvenile	A Immature, juvenile	1 Immature/ Virgin	M1 Juvenile	A Immature	1 Juvenile
DEVELOPING	**2**	2 or B Maturing, adolescent, sub-adult	B Maturing, adolescent, sub-adult	2 Maturing	M2 Adolescent, Maturing	B Maturing	2 Maturing
SPAWNING CAPABLE	**3a**	3 or C Mature, adult	C Mature, adult	3a Mature	M3 Adult, Mature	C Mature	3 Adult
ACTIVELY SPAWNING	**3 b**	4 or D Active	C1 Active	3b Mature/ Extruding, Active	M4 Active, copulating	D Active	4 Actve
REGRESSING*	**4a**	No stage comparison	No stage comparison	No stage comparison	No stage comparison	No stage comparison	No stage comparison

* There are no photos available for this stage.

4 Histological determination of microscopic maturity stages

4.1 Microscopic maturity scales

The microscopic scales for both males and females, that support the macroscale here presented, were based on results collected for oviparous (*Raja clavata* - ICES Area IXa and GSA 9; *Raja asterias* - GSA 9; *Scyliorhinus canicula* (GSA 9) *Galeus melastomus* - GSA 11) and viviparous (few data on *Squalus blainvillei*- GSA 11) elasmobranch species. The descriptions were made as wide-ranging as possible, but due to the limited data available, future work is recommended so that can be documented and discussed in the next WKMSEL meeting. Histological data should be collected for the different reproductive organs by maturity stage: ovaries, oviducal gland and uteri in females; and testes and sperm ducts in males.

It is worth noting that, in both male and female gonads, different stages of development of spermatocysts and follicles can be observed during the gametogenesis. Thus, when microscopy is associated to macroscopic description, the main microscopic observations should be described per each macroscopic stage. In addition, quantitative analyses giving the proportion of spermatocysts/follicles at different stages in sections of testes/ovaries are advisable. The following table describes the microscopic description of the macroscopic maturity scales for oviparous and viviparous species.

MALES

STAGE	MICROSCOPIC – OVIPAROUS AND VIVIPAROUS
1	Testes containing gonocytes, spermatogonia (more abundant), primary and secondary spermatocytes. Rarely more advanced stages of spermatocysts are observed (spermatids and spermatozoa).
2	Testes containing spematocysts in all spermatogenesis stages (generally, secondary spermatocytes are the most abundant). Spermatids and spermatozoa are more abundant than in immature males. Sperm ducts start to differentiate villosities. No spermatozoa is observed inside the ducts.
3a	Testes containing spematocysts in all stages. Spermatids and spermatozoa are more abundant than in developing males. Sperm ducts full with seminal liquid, more dense in the ducts deferens; spermatozoa bundles observed in the lumen.
3b	Description similar to Spawning Capable males. Spermatozoa is the most abundant stage observed.
4a	No observations

FEMALES

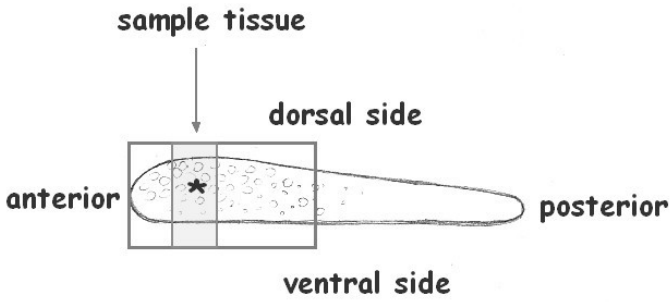
STAGE	MICROSCOPIC – OVIPAROUS	MICROSCOPIC – VIVIPAROUS
1	Ovary containing only early pre-vitellogenic follicles, connected to the germinal epithelium and tunica albuginea. Uterus composed mainly by connective tissue, covered by simple columnar epithelium with some invaginations.	Ovary containing only early pre-vitellogenic follicles, connected to the germinal epithelium and tunica albuginea.
2	Ovary filled with pre-vitellogenic follicles of various sizes and some vitellogenic follicles. Oviducal gland in the beginning of gland tubules formation or completely formed, with differentiation of the secreting zones, depending on the advance in maturation. Beginning of secretions production in the oviducal gland and uterus. Uterus more invaginated and vascularized than in immature females.	Ovary filled with pre-vitellogenic follicles of various sizes and some vitellogenic follicles.
3a	Ovary in all stages of development, being vitellogenic follicles the most abundant. Oviducal gland filled with secretions in the gland tubules. Uterus highly invaginated, with longitudinal folds producing secretions to the lumen.	no observations
3b	Postovulatory follicles can be present in the ovary. Oviducal gland tubules full of secretion materials. Secretions also present in the gland lumen.	no observations
4a	Follicles in all stages can be observed in the ovary. Postovulatory and atretic follicles, in which the basal lamina appears collapsed and invade the central lumen, are found. Oviducal gland completely formed but without secretions in the lumen. Uterus completely formed and with production of secretions.	no observations
4b	no observations	no observations

4.2 Recommendation for histological analyses

Following the ICES Guidelines for collecting maturity data and histological analyses for maturity workshops, the WKMSEL participants added some advises coming from their experiences on elasmobranchs.

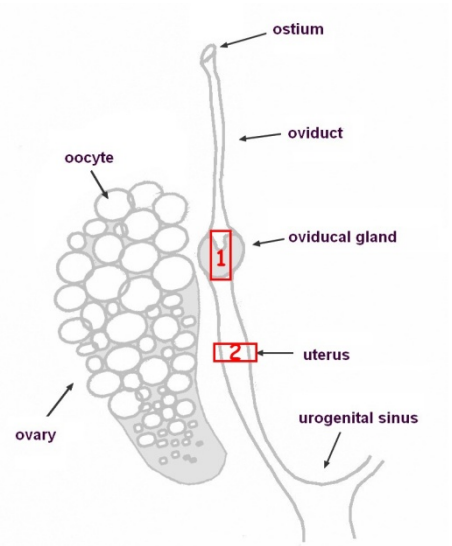
4.2.1 Extraction

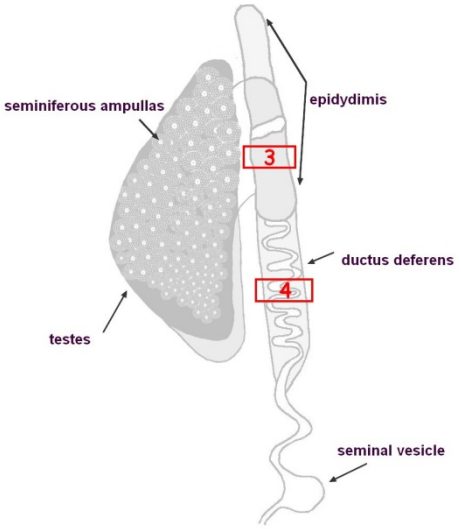
The gonad or sub-samples of the gonad tissue has to be extracted on fresh individuals. If only pieces of gonads are collected, these should be representative of the entire gonad. The participants recommend extracting the second quarter of the anterior half gonad as illustrated in the following scheme.



* second quarter of the anterior half gonad

Hereunder the section of other structures of the reproductive systems of females and males that can be extracted is illustrated. (These drawings are based on skates but can be considered valid also for sharks species).





1. Sagittal section of the middle part of the oviducal gland
2. Transversal section of the uterus
3. Sagittal section of epididymis
4. Sagittal section of ductus deferens

4.2.2 Fixation

The gonad or sub-samples of the gonad tissue has to be preserved immediately after collection in 10% formaldehyde 0.1 M phosphate buffered. The samples can be stored in formaldehyde or in ethanol 70°. Extreme dehydration was observed when some structures (e.g. oviducal glands) were stored in ethanol.

4.2.3 Embedding and sectioning

Pieces of tissue should be embedded in paraffin or resin. Thickness of histological section is not critical but should not exceed 5 microns (2-3.5 micron in resin, 3-5 micron in paraffin).

4.2.4 Staining

Haematoxylin-Eosin is a standard for paraffin and sometimes for resin; the toluidine blue and methylene blue is a standard for resin. For more details and characterization of ovaries histological structures (identification of the zona pellucida and yolk droplets) the PAS is suggested. For the identification of the nature of secretions being produced by the oviducal gland, combined Alcian blue and PAS staining is suggested.

5 Photo reference for the maturity scale proposed for oviparous and viviparous elasmobranch species

The participants of WKMSEL presented macroscopic descriptions of the different maturity stages of females and males of some oviparous and viviparous species, relying on the development of the different reproductive organs: ovaries, oviducal gland and uteri in females; testes and sperm ducts in males (see following table). The photos of macroscopic aspects for species and used by each institute are presented in Annexes 10 and 11.

The oviparous and viviparous macroscopic scales were based on the results from sharks and rays studied by the participants of this workshop. The descriptions were based on all the available photos, but it is important to highlight that in future applications of these scales to other oviparous and viviparous elasmobranch species (e.g. the bullhead sharks of Heterodontiformes and the orectolobiform carpetsharks of Parascycilliidae, Hemiscyllidae, Stegostomatidae, etc.), differences can occur.

Oviparous	Species	Institute	Area
Skates	*Raja clavata, Raja brachyura, Raja asterias, Raja montagui, Raja naevus, Rostroraja alba*	ARPAT, IPIMAR, USTHB	GSA9, ICES IXa, GSA4
Sharks	*Galeus melastomus, Scyliorhinus canicula*	ARPAT, Univ. Cagliari, Univ. Bari	GSA9, GSA11, GSA18, GSA19
Viviparous	**Species**	**Institute**	**Area**
Batoids	*Torpedo mormorata, Pteromilaeus bovinus, Miliobatis aquila, Torpedo torpedo, Rhinobatos rhinobatos, Gimnura altavela*	ARPAT, INSTM	GSA9, GSA13, GSA14
Sharks	*Etmopterus spinax, Squalus blainvillei, Centrophorus squamosus, Squalus acanthias, Centroscymnus coelolepis, Mustelus asterias, Dalatias licha, Mustelus punctulatus, Mustelus mustelus, Carcharhinus plumbeus, Alopias vulpinus, Centrophorus granulosus*	ARPAT, MCFS, IPIMAR, ISPRA, Univ. Cagliari, Univ. Bari, INSTM	GSA9, ICES IXa, GSA11, GSA13, GSA14, GSA18, GSA19

6 Guidelines on optimal sampling strategy for the determination of maturity stages in elasmobranchs

In response to the TOR to propose an optimal sampling strategy to estimate accurately the maturity ogives, based on two ICES documents, the Guidelines for collecting maturity data and histological analyses for maturity workshops developed by the Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) meeting 2010 (Annex 8) and the recommendations of the Report of the Workshop on Maturity Ogive Estimation for Stock Assessment ICES WKMOG REPORT 2008. From the experience gathered by the participating countries the following guidelines to collect data for maturity estimation of elasmobranch fishes was developed:

1) Sampling has to be conducted by cooperation between the participating laboratories.

2) The number of samples by length range, sex and location has to be clearly defined considering number of countries involved, timing, and spatial overlap of sampling.

3) Preferably, the sampling procedure should be executed several times during a year to follow the reproductive cycle and development of the gonads. At least 4 times at year, or more frequent depending on species.

4) However, cruises are normally not conducted each quarter or several times at year at the same location and hence limitations in sampling capacity are recognised. Commercial fleet samples (e.g., from observers onboard) can be used to complete sampling if gonads are properly preserved and observers properly trained for maturity staging.

5) For data collection and histology samples, each specimen should be given a fish ID including the following information: Country, station, date and fish number etc. as suggested by PGCCDBS 2010.

6) Staging should be performed on fresh samples when possible. Staging from pictures is more difficult than staging from fresh materials and hence this should be avoided.

7) For survey data to be used in a maturity index of the spawning stock, the survey must be conducted at the right time compared to the spawning period and have adequate coverage. If survey data are not available at the right time then histologically validated maturity data obtained outside spawning season can be used, although this should be confirmed on a stock by stock basis.

8) Where valid maturity data are available from market samples they can be used to estimate maturity. This is mainly the case for species with a protracted spawning season where survey data do not cover the whole spawning season or stock area. Also if survey and market data do not show systematic differences they can be used together.

9) Maturity data from market samples should be collected during the whole pre-spawning (for determinate species) or spawning (for indeterminate species) season on a métier based sampling programme, and cover the whole stock distribution area.

10) As with market samples, onboard samples should be collected on a métier-base to avoid gear and fleet selectivity effect and in the correct time and spatial frame compared to spawning.

11) If possible, maturity staging should be done onboard the survey vessel.

12) A comprehensive illustrated manual should be available for all stocks requiring maturity observations.

13) Macroscopic maturity scales used should be validated, either histologically or by another appropriate way.

14) Plot and map the data collected to assess differences by source, strata, location and time.

15) Length stratified maturity data should be weighted by the length distribution. If samples are collected on a random scheme or the stock is assessed on a length basis, no weighting according to the length distribution is required.

16) If the fish maturation process is dependent on age and/or sex as well as length then a Sex Maturity Age Length Key (SMALK) should be used. Age reading precision is important in this context.

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

18) Estimation of maturity oogive should take into account precision and bias in both the sampling and estimation process as described in the ICES WKMOG REPORT 2008.

7 Conclusion and recommendations

On the basis of the published literature, knowledge and experiences gained by the research groups attending the workshop, new maturity scales were proposed for oviparous and viviparous species. A comparison and conversion between the existing maturity scales and the WKMSEL 2010 maturity scale was also undertaken in order to provide a common tool for exchanging data and scientific information.

The most important change to the previously adopted scales was related to the development of two maturity scales (oviparous and viviparous) and the introduction of the pregnancy phases in viviparous species. Another important addition was the recovering stages. According to the experience and knowledge of the participants sometimes it is very difficult to distinguish between developing and recovering stages, however the participants deemed important to include the recovery stage and collect data on these stages for the next 2-3 years and analyse further data provided on this stage in a possible future workshop.

Participants recommended to:

1) Collect information on more species especially those which attain relatively large sizes, such as pelagic elasmobranchs.

2) Collect more information on all the different viviparous modes of reproduction (e.g. yolk-sac viviparity, limited histotrophyic, etc.)

3) Increase the geographical distribution of the data examined especially from Atlantic, North Sea, Baltic and the Eastern and Southern Mediterranean countries. Information from long distance fisheries (e.g. Pacific, Arctic etc.) exploited by European fleets would also be welcome.

4) Collect information on the 4a.Regressing and 4b.Regenerating stages of females and males both at the macro and micro scales and propose better descriptions to differentiate between those and the 2.Developing stages.

5) Perform histological analyses from different structures such as the uterus, ovaries and oviducal (nidamental) glands from females and sperm ducts and seminal vesicles for males.

6) More data should be collected on atretic follicles and post ovulatory follicles (POF) both at the macro and micro scale.

7) Collect information and photos on egg cases.

8) Compare colour of fresh and stored specimens.

Participants recommended to plan the next Workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL) in 2013 (Annex 3).

8 Glossary

When the maturity stage scales were produced, the use of appropriate and recent terminology was highlighted. Participants then suggested that a glossary should have been defined as complement of the table. Hereunder, the technical terms commonly used for the description of reproduction in elasmobranch fishes are defined. The glossary hereunder should be considered A "work in progress" and all participants are invited to revise it and/or propose new terms in view or the next WGMSEL.

Table 1. Technical terms commonly used in elasmobranch reproductive biology

TERM	DEFINITION	REFERENCES
Aplacental viviparity	This term includes different modes of elasmobranchs reproduction such as: yolk-sac viviparity, histotrophy (limited and lipid) and oophagy (Carcharinid or Lamnid).	Musick and Ellis 2005
Ductus deferens	Portion of the genital ducts immediately posterior to the epididym, and continuous with the seminal vesicle. The ductus deferens and seminal vesicle function as storage areas for seminal products.	Conrath, 2004
Efferent ducts	Fine tubules which cross the mesorchium at the anterior edge of the testis. Mature sperm are discharged from the testis through the ductus efferens, which joins the epididymis.	Conrath, 2004
Epigonal organ	Organ unique to the group, occurs in gonadal mesenteries, it is site of hemopoiesis.	Hamlett *et al.* 1999
Epididym	Portion of the genital ducts immediately posterior to the efferent duct, and continuous with the next section of the genital duct, the ductus deferens. The epididym can be seen along the vertebral column on either side of the dorsal aorta. In immature males the epididymis is a straight tube on the ventral surface of the kidneys; in sexually mature males the anterior portion is highly coiled, while the posterior becomes a septated thick-walled straight tube that enlarges to form the ductus deferens.	Carrier *et al*., 2004; Conrath, 2004
Follicle	Oocyte surrounded by granulose cells and delimited by a basal lamina.	Hamlett and Koob 1999
Follicular atresia	Degenerative process by which oocytes in various stages of their development and differentiation are resorpted from the ovary.	Guraya 1986
Genital ducts	include the efferent ducts, epididymides, ductus deferens and seminal vesicle	Conrath, 2004
Gestation	Period from fertilization to parturition.	Musick and Ellis 2005
Histotrophy	Matrotrophic mode of reproduction in which organically rich histotroph are produced and secreted by uterine villi called trohonemata, and ingested by the embryo.	W. C. Hamlett and T.J. Koob (in Hamlet *et al*. 1999)
Isthmus	A narrow portion of the oviduct that may occur between the oviducal gland and the uterus, and may function to isolate the contents of the uterus	Conrath, 2004
Lecithotrophy	Is a developmental pattern in which yolk, produced by the maternal liver and sequestered in the yolk sac, provides for embryonic nutrition.	Hamlett *et al*. 2005b
Lobule	Basic unit of the testis, signaling the beginning of the sexual cycle in male gonads.	Skinner and Griswold 2005
Maternity	A female is in maternal condition if it is in pregnant condition, determined by the presence of eggs or embryos in the uteri.	Musick and Ellis 2005

TERM	DEFINITION	REFERENCES
Matrotrophy	Is a developmental pattern in which the maternal organism supplements yolk from other sources such as uterine secretions called histrotroph (histotrophy), ova (ovatrophy), siblings (adelphotrophy) or placentral transfer (placentatrophy).	Hamlett *et al*. 2005b
Maturity	Capable of mating and producing viable offspring. Female are assumed to be mature when ovarian follicles in the ovary are in an advanced stage of developed.	Musick and Ellis 2005; King 1995
Multiple oviparity	(Same as retained oviparity) Species in which egg cases are retained in the oviduct during most of development before deposition and hatching on the seabed.	Musick and Ellis 2005
Oophagy	Matrotrophic mode of reproduction in which embryos develop primarily from material in the yolk sac and thereafter, development relies on the continued supply of yolk in the form of ovulated eggs.	W. C. Hamlett and T.J. Koob (in Hamlet *et al*. 1999)
Oogenesis	Development and growth of follicles, from oogonia to maturation.	Wallace and Selman 1981; Patiño and Sullivan 2002
Ostium	Anterior funnel-shaped opening of the oviduct which functions to collect the ovulated eggs. It is located in apposition to the ovary	Conrath, 2004
Oviducal gland	(Same as nidamental gland and shell gland). Specialized region of the anterior portion of the oviduct in cartilaginous fishes, responsible for the production of the components of the egg jelly that surrounds the fertilized egg and the tertiary egg envelope (including the rigid egg capsule of oviparous species, the thin transient egg candle case of yolk sac species and the thin egg envelope in most placental sharks).	Hamlett *et al*. 2005a
Oviduct	Paired, long tubular structures that run the length of the body cavity on both sides of the vertebral column. Each oviduct is differentiated into an ostium, the anterior oviduct, the oviducal gland, the isthmus and the uterus	Carrier *et al*., 2004
Oviparity	Reproductive mode in which elasmobranchs enclose eggs in an egg case and deposit them into the environment where embryos develop external to the body of the mother and with expense of yolk (lecithothrophy). May be divided into two types: single and multiple	Musick and Ellis 2005
Ovarian cycle	Period from completion of one ovulation to completion of the next.	Musick and Ellis 2005
Ovary	Paired or single structures located at the anterior end of the body cavity dorsal to the liver. They are attached to the body wall by a mesovarium.	Carrier *et al*., 2004 Wourms, 1977
Placental viviparity	The embryos receive additional nourishment from the mother following a phase of living off the egg prior to birth. The amount of food supplied by mother through the umbilical cord can vary.	Conrath 2005; Hamlett *et al*. 2005b
Primary oocyte	Oocyte undergoing the early stages of prophase and becoming arrested in the diplotene of the first meiotic division.	Janssen *et al*. 1995; Wallace and Selman 1981
Primordial follicle	Primary oocyte surrounded by a single layer of squamous cells and enlarged lipid-like cells.	Hamlett *et al*. 1999
Single oviparity	(Same as external oviparity). One egg is deposed at a time from each oviduct, usually in pairs. In this reproductive mode almost all of the embryonic development occurs within the egg case outside of the mother's body.	Compagno 1990; Musick and Ellis 2005;
Spermatogenesis	Morphological and physiological changes during development of male germ cells.	Nagahama 1983; Grier and Uribe-Aranzábal 2009

TERM	DEFINITION	REFERENCES
Testes	paired, symmetrical structures situated at the anterior end of the coelom, dorsal to the liver. Usually, each testis is suspended from the midorsal body wall by a mesorchium. In some species, the testes are embedded at the anterior end of the epigonal organ.	Carrier *et al*., 2004
Tertiary egg envelope	Includes the rigid egg capsule of oviparous species, the thin pliable transient egg candle case of yolk sac species and the thin pleated egg envelope in most placental sharks.	Hamlett *et al*. 1998
Uterus	Enlarged posterior part of the oviduct, where the embryos develop	Carrier *et al*., 2004
Vitellogenic oocyte	(Same as Secondary oocyte growth phase) Oocyte showing yolk droplets. Several sizes of vitellogenic oocytes can be found.	Wallace and Selman 1981
Viviparity	Reproductive mode in which the female retains developing eggs within reproductive tracts and gives birth to living offspring.	Hamlett *et al.* 2005
Yolk-sac viviparity	Retention of fertilized eggs throughout the development within the uterus with no additional maternal nutritional input beyond the yolk (lecithotrophic mode of reproduction).	Musick and Ellis 2005

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Annex 1: List of participants

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Annex 2: Agenda

Monday, 11 October 2010

9.00 – 10.30	Presentation of chairs and list of participants Adoption of the agenda and time-table Determine the list of species to be discussed during the workshop; Sharks and Rays / oviparous and viviparous species
11.00 – 13.00	Presentation by each country or laboratory of the protocol for data collection including maturity scales (5-10 min each)
14.00 – 15.30	Presentation of maturity photos, macroscopic and microscopic observations and staging by each country or laboratory (5-10 min each) and comparisons between different countries/laboratories, for the oviparous species to be discussed during the workshop. Maturity stage scale and definition of "maturity" to be determined.
16.00 – 17.30	Continue from previous session

Tuesday, 12 October 2010

9.00 – 10.30	Continue from previous session
11.00 – 13.00	Continue from previous session and summary of the conclusions achieved including the proposal of the new maturity scale.
14.00 – 15.30	Presentation of maturity photos, macroscopic and microscopic observations and staging by each country or laboratory (5-10 min each) and comparisons between different countries/laboratories, for the viviparous species to be discussed during the workshop. Maturity stage scale and definition of "maturity" and "maternity" to be determined.
16.00 – 17.30	Continue from previous session

Wednesday, 13 October 2010

9.00 – 10.30	Continue from previous session
11.00 – 13.00	Continue from previous session and summary of the conclusions achieved including the proposal of the new maturity scale

14.00 – 15.30	L/W relationship, sex ratio, length frequency distribution of oocytes, maturity oogive
16.00 – 17.30	Continue from previous session

Thursday, 14 October 2010

9.00 – 10.30	Comparison of the old and new maturity scales,	
	3 subgroups	- Setting up of photo reference material
		- Comparison of old maturity scales with the new one (oviparous)
		- Comparison of old maturity scales with the new one (viviparous)
11.00 – 13.00	Continue with sub-group sessions	
14.00 – 15.30	Continue with sub-group sessions	
16.00 – 17.30	Presentation of the sub-groups conclusions	

Friday, 15 October 2010

9.00 – 10.30	Update of the guidelines for collecting maturity data
11.00 – 13.00	Adoption of the Report
	List of recommendations and terms of reference for the next workshops, AOB

Annex 3: WKMSEL terms of reference for the next meeting 2013

The Workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL) (Co-Chairs: Fabrizio Serena, Italy and Mark Dimech, Malta) will meet in xxx in 2013 to:

a) review and include information on more species especially those which attain relatively large sizes such as pelagic elasmobranchs.

b) review and include information on different modes of viviparous species.

c) review information on the spent/resting stages of females and males both at the macro and micro scales and propose better descriptions to differentiate between the resting and the maturing stages.

d) review histological analyses from different structures such as the uterus, ovaries and oviducal (nidamental) glands from females and sperm ducts and seminal vesicles for males and update the histological descriptions of the macro maturity stages

e) validate and update the WKMSEL 2010 maturity scale based on the new information

Supporting information

PRIORITY:	**THE MATURITY STAGE IS AN IMPORTANT BIOLOGICAL PARAMETER TO BE USED IN THE CALCULATION OF MATURITY OGIVES (AND THEREFORE OF SPAWNING STOCK BIOMASS), FOR THE DEFINITION OF THE SPAWNING SEASON OF A SPECIES, FOR THE MONITORING OF LONG-TERM CHANGES IN THE SPAWNING CYCLE, AND FOR MANY OTHER RESEARCH NEEDS REGARDING THE BIOLOGY OF FISH.**
Scientific justification and relation to action plan:	One of the action of the European Action Plan for Sharks aims to promote the identification and reporting of species-specific biological and trade data, at least for the main species. The ICES working group on sharks (WGEF) is being developing the assessments of stocks status of the main species. The results of these assessments should be the basis for any future action on specific stocks. In this regard, the identification and macroscopic classification of maturity stages can play a key-role in the assessment fishery resources and there is an urgent need for reliable and up-to-date information on the maturity parameters for all assessed shark species to improve the quality of these estimates. The expectation of the TORs is that the Workshop produces a comparative description of the scales used in the different labs and set off standard operational procedures and methodologies to facilitate the validation and classification of the different maturity stages.
Resource requirements:	Before the Workshop the organising institute will setup a sampling plan for collecting samples for to be used during workshop. The sampling will be carried out during 2011/2012
Participants:	In view of its relevance to the DCR, the Workshop is expected to attract wide interest from both Mediterranean EU and ICES Member States.
Secretariat facilities:	
Financial:	None
Linkages to advisory committees:	There is a direct interest from several international (ICES, NAFO, GFCM, ICCAT) advisory committee for a common effort toward the standardization of assessing procedures. RCMs, PGMed and PGCCDBS.
Linkages to other committees or groups:	There are link with the EU DCR and ICES WGEF.

Annex 4: Species list with info on the study areas and mode of reproduction

GSA	Area
4	Algeria
5	Balearic Island
9	North Tyrrhenian Sea
11	Sardinia
14	Gulf of Gabes
15	Malta Islands
16	South of Sicily
17	North Adriatic
19	Ionian Sea
ICES	**Area**
IXa	Portugal

OVIPAROUS SPECIES				**GSA/ICES areas**		
Group	**Order**	**Family**	**Species**	**Macro**	**Micro**	**Mode of reproduction (Hamlet, 2005)**
Sharks	Carcharhiniformes	Scyliorhinidae	*Scyliorhinus canicula*	9, 19	9	Lecithotrophic - single oviparity
Sharks	Carcharhiniformes	Scyliorhinidae	*Scyliorhinus stellaris*	5, 15	5	Lecithotrophic - single oviparity
Skates	Carcharhiniformes	Scyliorhinidae	*Galeus melastomus**	9,11,19	11	Lecithotrophic - multiple oviparity
Skates	Rajiformes	Rajidae	*Dipturus oxyrinchus*	9		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Leucoraja circularis*	5, 9		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Leucoraja naevus*	5; IXa		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja asterias*	4,9	9	Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja brachyura*	5, 9; IXa		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja clavata**	5, 9, 15; IXa	9; IXa	Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja miraletus*	5, 9, 15, 19	9	Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja montagui*	IXa		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja polystigma*	5, 9		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja radula*	5		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Raja undulata*	IXa		Lecithotrophic - single oviparity
Skates	Rajiformes	Rajidae	*Rostroraja alba*	5, 9	5	Lecithotrophic - single oviparity

* Shark and skate species selected as main examples for the elaboration of the new maturity scale for oviparous elasmobranchs.

VIVIPAROUS SPECIES **GSA/ICES areas**

Group	Order	Family	Species	Macro	Micro	Mode of reproduction (Hamlet, 2005)
Sharks	Hexanchiformes	Hexanchidae	*Hexanchus griseus*	9		Lecithotrophic - yolk-sac viviparity
Sharks	Squaliformes	Centrophoridae	*Centrophorus granulosus*	ICES IXa; GSA 5, 9, 19		Lecithotrophic - yolk-sac viviparity
Sharks	Squaliformes	Centrophoridae	*Centrophorus squamosus*	ICES IXa		Lecithotrophic - yolk-sac viviparity
Sharks	Squaliformes	Somniosidae	*Centroscymnus coelolepis*	ICES IXa		Lecithotrophic - yolk-sac viviparity
Sharks	Squaliformes	Dalatidae	*Dalatias licha*	4, 5, 9, 19	9	Lecithotrophic - yolk-sac viviparity
Sharks	Squaliformes	Etmopteridae	*Etmopterus spinax*	9, 19		Lecithotrophic - yolk-sac viviparity
Sharks	Squaliformes	Squalidae	*Squalus acanthias*	17	17	Lecithotrophic - yolk-sac viviparity
Sharks	Squaliformes	Squalidae	*Squalus blainvillei*	11, 9, 15, 19	11	Lecithotrophic - yolk-sac viviparity
Sharks	Squatiniformes	Squatinidae	*Squatina squatina*	4		Lecithotrophic - yolk-sac viviparity
Sharks	Lamniformes	Alopidae	*Alopias vulpinus*	5, 9		Matrotrophic - Lamnid oophagy
Sharks	Lamniformes	Cetorhinidae	*Cetorhinus maximus*	9		Matrotrophic - Lamnid oophagy
Sharks	Lamniformes	Lamnidae	*Isurus oxyrinchus*	16	16	Matrotrophic - Lamnid oophagy
Sharks	Carcharhiniformes	Carcharhinidae	*Carcharhinus obscurus*	9		Matrotrophic - placental
Sharks	Carcharhiniformes	Carcharhinidae	*Carcharhinus plumbeus*	9, 16	17	Matrotrophic - placental
Sharks	Carcharhiniformes	Carcharhinidae	*Prionace glauca*	4,17	17	Matrotrophic - placental
Sharks	Carcharhiniformes	Triakidae	*Galeorhinus galeus*	4		Lecithotrophic - yolk-sac viviparity
Sharks	Carcharhiniformes	Triakidae	*Mustelus asterias*	15		Lecithotrophic - yolk-sac viviparity
Sharks	Carcharhiniformes	Triakidae	*Mustelus mustelus**	4, 5, 9, 15, 17	5, 17	Matrotrophic - placental
Sharks	Carcharhiniformes	Triakidae	*Mustelus punctulatus*	15		Little known. Presumably matrotrophic - placental
Rays	Rajformes	Rhinobatidae	*Rhinobatos rhinobatos*	14		Lecithotrophic - yolk-sac viviparity
Rays	Rajformes	Rhinobatidae	*Rhinobatos cemiculus*	14		Lecithotrophic - yolk-sac viviparity
Rays	Rajformes	Gymnuridae	*Gymnura altavela*	5		Lecithotrophic - yolk-sac viviparity
Rays	Rajformes	Myliobatidae	*Myliobatis aquila**	5, 9	5	Matrotrophic - lipid histotrophy
Rays	Rajformes	Myliobatidae	*Pteromylaeus bovinus*	9		Matrotrophic - lipid histotrophy
Rays	Rajformes	Dasyatidae	*Dasyatis centroura*	9		Matrotrophic - lipid histotrophy
Rays	Rajformes	Dasyatidae	*Dasyatis pastinaca*	5, 9		Matrotrophic - lipid histotrophy
Rays	Rajformes	Torpedinidae	*Torpedo marmorata*	9		Lecithotrophic - yolk-sac viviparity

VIVIPAROUS SPECIES **GSA/ICES areas**

Group	Order	Family	Species	Macro	Micro	Mode of reproduction (Hamlet, 2005)
Rays	Rajformes	Torpedinidae	*Torpedo nobiliana*	9		Lecithotrophic - yolk-sac viviparity
Rays	Rajformes	Torpedinidae	*Torpedo torpedo*	9, 19		Lecithotrophic - yolk-sac viviparity

* Shark and ray species selected as main examples for the elaboration of the new maturity scale for oviparous elasmobranchs

Annex 5: Length-weight relationships and length at maturity for skates (Rajidae) around the British Isles, and an overview of the collection of reproductive data for elasmobranchs in English groundfish surveys

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Summary

The relationships between total length and weight are provided for skates caught in groundfish surveys around the British Isles. Estimates of the length at first and 50% and maturity, and lengths of the largest immature skates for males and females of the main skate species encountered are given.

Introduction

Relationships between length and weight for skates (Rajiformes; Rajidae) in European waters are needed as basic parameters to aid the assessment and management of skate fisheries, as such conversion factors are required to estimate weights of fish measured in market sampling and on board commercial fishing vessels. Additionally, weight at size conversion factors can be used in recreational fisheries, to allow anglers to return specimen fish alive (Kohler *et al.*, 1995).

The length at maturity for skates is also an important parameter, given it is fundamental to the application of demographic and other assessment models, and that the examination of spatial and temporal differences in such life-history parameters can inform on issues such as stock identity and also potential fishing impacts.

Here we provide length-weight conversion factors for the main skate species occurring on the continental shelf of the British Isles, including starry ray *Amblyraja radiata* (Donovan), cuckoo ray *Leucoraja naevus* (Müller & Henle), blonde ray *Raja brachyura* (Lafont), thornback ray *Raja clavata* L., small-eyed ray *Raja microocellata* (Montagu), spotted ray *Raja montagui* (Fowler) and undulate ray *Raja undulata* (Lacepède).

The standard maturity stages for elasmobranchs used by Cefas during annual groundfish surveys are given, which are as indicated in ICES (2009), and sampling protocols are provided.

Materials and methods

Field studies and biological sampling

Skates were caught during groundfish surveys in the North Sea, English Channel, Irish Sea, Bristol Channel and Celtic Sea during bottom trawl surveys by *RV Corystes*, *RV Cirolana* and *RV Cefas Endeavour*. Additional data on length at maturity were also collected from commercial fishing vessels during a Fishery Science Partnership on thornback ray in the southern North Sea (Ellis *et al.*, 2008), and during on-going studies on skate discard survival. A summary of the surveys used for this study is given in Table 1.

Total lengths of skates were measured to the centimetre below and the total weight recorded to the nearest 1 g (juveniles) or 5 g (larger skates). All individuals were classified as immature (A), maturing (B), mature (C) or active (D), using the maturity key given in Table 2a. The comparative maturity scale used for viviparous elasmobranchs (e.g. spurdog) during Cefas surveys is also given (Table 2b).

Male maturity was usually assigned based on clasper state, as this saves time on surveys, and also allows some skates to be returned to the sea alive. In those instances where external observations on clasper state were felt to be inconclusive (e.g. for fish that may or may not have reached stage C), then those individuals were dissected and the internal reproductive organs examined to more accurately gauge the maturity.

Female maturity was assigned based on examination of internal reproductive organs. In recent years, however, females of either <40 cm (e.g. for spotted and cuckoo ray) and individuals <50 cm (e.g. blonde and thornback rays) are not usually examined and assumed to be immature (this was based on observations of the data that had been collected over a number of the initial years). Once again, this saves time during surveys and facilitates the live release of some fish. Although some other maturity scales for skates make distinctions regarding the stage of egg-case formation, this was not conducted, as only very low numbers of mature females were observed with egg-capsules (as most surveys are conducted either away from spawning grounds and/or at different times of the year).

Other data on the reproductive biology of skates are not routinely collected. Collecting quantitative data to validate the maturity stage information (e.g. clasper length for males, and nidamental gland width for females) is obviously a useful exercise in academic studies, but this is too time-consuming during annual groundfish surveys. Although estimates of fecundity are needed for many skate species, there is currently no resource to allow for the collection, preservation and subsequent laboratory examination of skate ovaries, and so this is not undertaken. Similarly, there is no resource for histological studies.

In terms of viviparous elasmobranchs, now that information is being collected for *Squalus acanthias*, data on total uterine fecundity will be collected where possible. Large numbers of female *S. acanthias* were examined for reproductive state in 2005, after a single large catch was made (Ellis & Keable, 2008).

Data analysis

The skate and ray records were separated according to ICES ecoregions and by sex. The total weights were then plotted against their total lengths and trend lines were fitted to obtain length-weight equations for each of the nine species by ecoregion and sex, where data allowed. Where sample sizes were limited, data were combined across ecoregions. Although data were most comprehensive for six species, we have included those data available for all species. The resultant length-weight plots are not shown, although a summary table of the equations, samples sizes and length ranges are given (Table 3).

The proportion of mature rays (maturity stages C and D), were plotted against length (cm) and a sigmoid function was fitted, with an asymptote set at near to 1. The equation used to fit a sigmoid curve was as follows:

$$y = 1 / 1+e^{((mid - x) / scale)}$$

where the mid and scale parameters were fitted to the data. Mid is the length at which 50% are mature, and the scale is the measure of 'steepness'.

The resultant plots for the seven species where sufficient data were available are shown in Figure 1, and the 50% maturities by sex are given in Table 3. Where data were limited, the function could not be fitted (e.g. *R. undulata* female), although some descriptive information is presented.

Results and Discussion

Length–weight relationships

The relationship of total weight to total length was strongly correlated (r^2 >0.9 for all species). The constants required for the conversion of these measurements are given in Table 3.

In terms of earlier studies, Holden (1977) gave length-weight relationships for *R. brachyura, R. clavata, R. montagui* and *L. naevus,* and Ryland & Ajayi (1984) gave length weight relationships for *R. clavata, R. microocellata* and *R. montagui*. More recently, Coull *et al*. (1989) provided length-weight information for *R. clavata, R. montagui* and *L. naevus*, although these data were limited by sample size and/or size range of fish examined. The present study provides the mos**t recent data for all species.** Other conversion factors (including gutted weight and wing weight relationships) are given by Bedford *et al.* (1986).

Length at maturity

Maturity data were examined for the seven most often caught, and descriptive notes are provided for two of the less frequent species (Table 3, Figure 1).

R. clavata: Females first matured at 47 cm and 50% maturity was obtained by 75.1 cm, with the largest immature female measuring 90 cm. First maturity in males was the same (47 cm) and 50% maturity was reached at 66.5 cm, and the largest immature specimen was 88 cm.

R. brachyura: This was one of the larger species routinely sampled in surveys, with specimens of up to 108 cm in length caught. Lengths at first maturity were 55 cm and 60 cm for males and females respectively, with 50% maturity reached at 78.2 cm (males) and 85.6 cm (females). The largest immature *R. brachyura* were 91 cm (male) and 93 cm (female).

R. microocellata: The smallest mature male and females observed were 66 cm and 73 cm respectively, with 50% mature at 69 cm (males) and 77.1 cm (females). The largest immature specimens were recorded at 74 and 73 cm.

R. undulata: Data were limited for this species, and so the following data should be viewed as preliminary. The lengths at first maturity were broadly similar in both species (80 cm and 79 cm in males and females respectively). The length at 50% maturity was estimated at 83 cm for males, although no estimation for the length at 50% maturity for females could be made, given the low sample size (n=45) and low numbers of larger females. Certainly, further dedicated studies on this species are required.

Two of the more frequently occurring rays, *R. montagui* and *L. naevus*, are smaller-bodied species than those described above and, in the present study, the maximum recorded lengths were 76 and 69cm respectively. However, both these species are of commercial importance.

R. montagui: The smallest mature males and females observed were 40 cm and 49cm respectively, and, whereas 50% of males were mature at 50.3 cm, 50% maturity was not reached until 64 cm in females. The largest immature males and females were 66 cm and 70 cm, respectively.

L. naevus: Males and females first matured at 48 cm and 50 cm, respectively with 50% maturity occurring at 56.3 cm and 59.4 cm. The largest immature males and females were 64cm and 65cm.

A. radiata: This was the smallest skate sampled in this study and is not of commercial importance, with the lengths at first maturity at just 30 cm and 32 cm for males and females, with 50% maturities only ca. 6cm larger (36.2 cm and 38.2 cm respectively). The largest immature fish were 44 cm (male) and 46 cm (female).

Data were more limited for other skate species, such as *L. fullonica* and *"Dipturus batis"* (soon to be *Dipturus intermediata* and *Dipturus flossada*). However, given the uncertainty in their general biology qualitative information on their maturity status is provided. There were 34 records of *L. fullonica* in the database, all but three of which were from the Celtic Seas ecoregion. There were 17 males (21–96 cm) and 17 females (24–70 cm). All 17 females were immature, indicating that their length at maturity is relatively large, perhaps in line with that of other larger rajids, while two of the males (75 and 96 cm) were mature (stage C), and the largest immature male was 82 cm.

There were 62 records of *"Dipturus batis"* in the database; all of these were from the Celtic Sea Ecoregion, and so are likely to be *D. flossada*. There were 32 females (length range 19–135 cm), of which only two were mature (a 125 cm specimen at maturity stage C, and a 135 cm specimen at stage D), and other specimens (up to 97 cm) were all immature. There were 30 males (length range 20–188 cm), again two of which were mature (both stage C, total lengths of 115 and 118 cm) and other specimens (up to 98 cm) were all immature.

The lengths at maturity for selected UK skates as observed in earlier studies are summarised in Table 4. It is often suggested that over-exploitation can reduce the length at maturity and potentially the maximum observed length. Indeed, the length at maturity for *R. clavata* may have declined since the studies of Steven (1934, 1936), as previously suggested by Nottage & Perkins (1983). There seems to be a slight indication of a slight reduction in the maximum length observed for *R. clavata*, with other published studies reporting maximum lengths of ca. 120 cm (Holt, 1910: estimated from disc width), 102 cm (Nottage & Perkins, 1983), 99 cm (Ryland & Ajayi, 1984), 101 cm (Fahy, 1989) and 98cm (present study). However, a recent discard observer trip has reported one individual of *R. clavata* at 130 cm (Fishing News, 12 June 2009). It is uncertain whether the occasional indication of a reduced length at maturity is due to differences in spatial differences in sampling location, maturing staging protocols or due to differences in sampling (e.g. gear selectivity) or a *bona fide* reduction in this parameter.

It should also be recognised that many of the earlier studies on skate maturity often do not identify clearly (a) the maturity scale used, and (b) what the length at maturity stated actually refers to (e.g. is it first or 50% maturity?). It is recommended that reports of skate maturity should consistently report the lengths at first and 50% maturity and the largest immature fish. Furthermore, although most recent studies on skate maturity provide information on the total sample size, this often includes disproportionate numbers of juvenile fish, and there is rarely an indication of the numbers of

mature fish observed, or an indication of the number of fish examined over the length range spanning first to 100% maturity. If published maturity data are to be used in assessments of skate stocks, there will need to be identify which estimates may be the most robust.

On-going fishery-independent surveys conducted by Cefas catch quite high numbers of several skate species, although those skates with patchy distributions may have lower sample sizes. Additionally, catch rates for larger individuals and species can also be low. In recent years, some skate species such as blonde, undulate and thornback ray have also been subject of more dedicated surveys. For example, in 2007 and 2008, a Fishery Science Partnership project used commercial inshore vessels (using fixed nets, longlines, and triple and twin rig otter trawl gears) to tag and release thornback ray *R. clavata* in the Greater Thames Estuary. In these years, length and maturity information were collected for 2,887 individuals. Similarly, recent and ongoing studies on the survival of discarded skates and rays in the south-west are providing useful information on blonde and undulate rays. This highlights the potential value of dedicated surveys for some of the larger-bodied and locally abundant elasmobranchs, as sample sizes of mature fish in commercial gears can be far higher than in groundfish surveys.

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Figure 1: Proportion of mature fish at length for male and female *Raja brachyura* (BLR), *R. clavata* (THR), *R. microocellata* (PTR), *R. montagui* (SDR), *L. naevus* (CUR), *A. radiata* (SYR) and *R. undulata* (UNR) (insufficient data for female *R. undulata*).

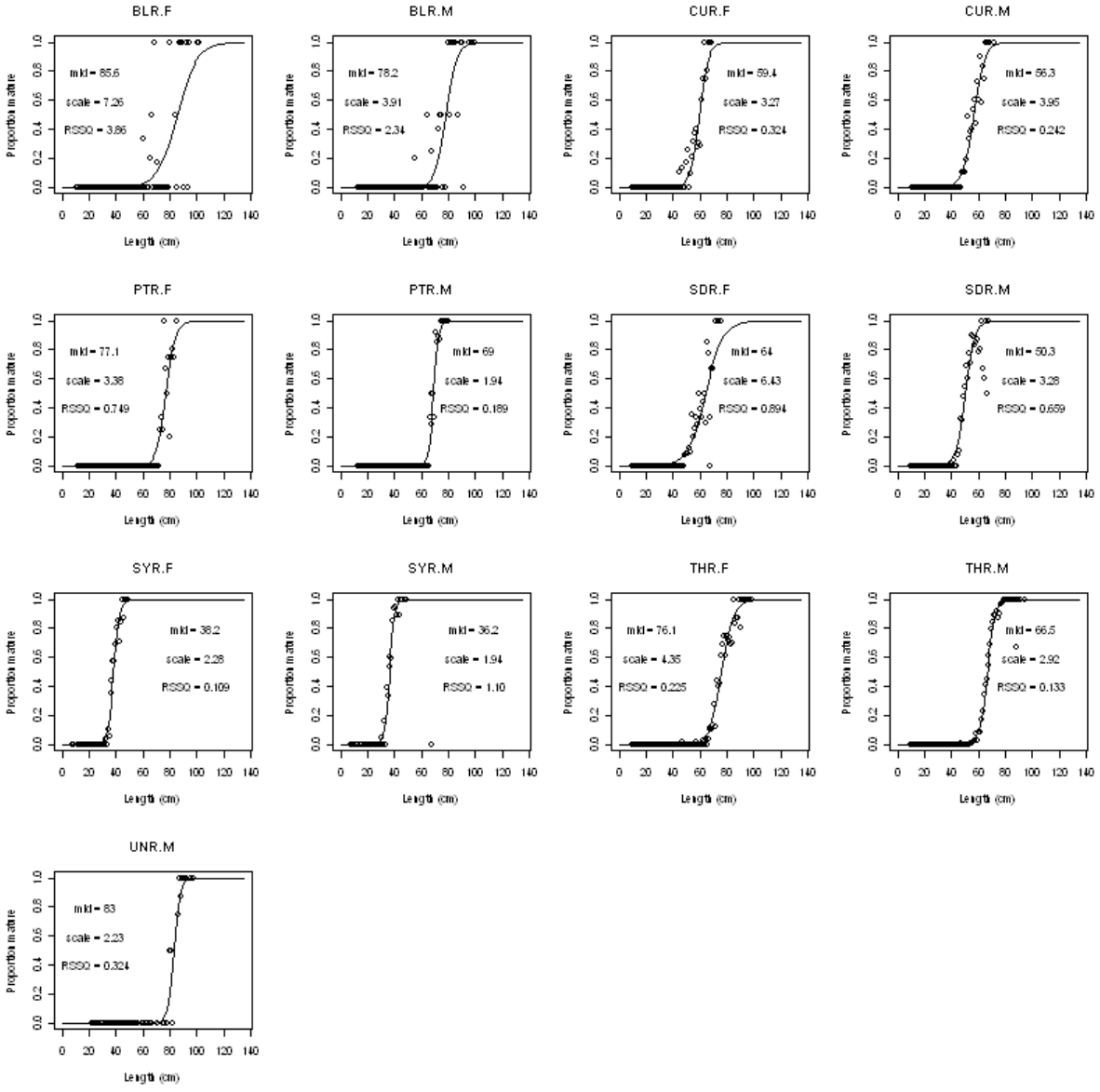

Table 1: Summary of groundfish surveys and research programmes that have been used in the present study

Survey name	Years used	Quarter	Fishing Gear used	Ecoregion	ICES Areas	N
Data Collection Framework Survey	2005–2010	1	PHH** trawl	Celtic Sea	Irish and Celtic Seas (VIIa, f, g, h, j)	1578
Q1 North Sea IBTS	2002–2004	1	GOV* trawl	North Sea and eastern Channel	North Sea (IVb and c)	214
Western Channel Q1SWBTS	2006–2010	1	Beam trawl	Celtic Sea	Western English Channel (VIIe)	560
West GFS (WCGFS)	1995 and 2004 only	1	PHH trawl	Celtic Sea	VIIe, f, g, h and j	161
Gear trials	2008	1 and 3	GOV with 3 ground gear bags attached	North Sea and eastern Channel	North Sea (IVb and c)	396
Thames thornback ray FSP	2007–2008	1-4	Longline and gillnet	North Sea and eastern Channel	Southern North Sea (IVc)	2887
Skate and Ray discard survival (M5202)	2010	2	Gillnet	North Sea and eastern Channel	English Channel (VIId-e)	118
Eastern English Channel Beam Trawl Survey	2002–2009	3	Beam trawl	North Sea and eastern Channel	Southern North Sea (IVc) and eastern English Channel (VIId)	1257
Q3 North Sea IBTS	2004–2009	3	GOV trawl	North Sea and eastern Channel	North Sea (IVa-c)	1047
Bristol Channel and Irish Sea BTS (NWGFS)	1992–2009	3	Beam trawl	Celtic Sea	Irish Sea (VIIa), Bristol Channel (VIIf) and parts of the Celtic Sea (VIIg)	7497
Irish Sea and Celtic Sea Q4SWIBTS	2003–2009	4	GOV trawl (rockhopper on harder ground)	Celtic Sea	Irish Sea (VIIa), Bristol Channel and Celtic Sea (VIIf-h) and western English Channel (VIIe)	2466

*Grand Ouverture Verticale Trawl

** Portuguese High Headline Trawl

Table 2a: Maturity scale used for skates and other oviparous elasmobranchs

MATURITY STAGE	MALES	FEMALES
A (Immature)	Claspers undeveloped, shorter than extreme tips of posterior margin of pelvic fin. Testes small and thread-shaped.	Ovaries small, gelatinous or granulated, but with no differentiated oocytes visible. Oviducts small and thread-shaped, width of shell gland not much greater than the width of the oviduct.
B (Maturing)	Claspers longer than posterior margin of pelvic fin, their tips more structured, but the claspers are soft and flexible and the cartilaginous elements are not hardened. Testes enlarged, sperm ducts beginning to meander.	Ovaries enlarged and with more transparent walls. Oocytes differentiated in various small sizes (<5mm). Oviducts small and thread-shaped, width of the shell gland greater than the width of the oviduct, but not hardened.
C (Mature)	Claspers longer than posterior margin of pelvic fin, cartilaginous elements hardened and claspers stiff. Testes enlarged, sperm ducts meandering and tightly filled with sperm.	Ovaries large with enlarged oocytes (>5mm), with some very large, yolk-filled oocytes (ca. 10mm) also present. Uteri enlarged and wide, shell gland fully formed and hard.
D (Active)	Clasper reddish and swollen, sperm present in clasper groove, or flows if pressure exerted on cloaca.	Egg capsules beginning to form in shell gland and partially visible in uteri, or egg capsules fully formed and hardened and in oviducts/uteri, or egg case being exuded from cloaca.

Table 2b: Maturity scale used for spurdog and other aplacental and placentally viviparous sharks

MATURITY STAGE	MALES	FEMALES
A	**Immature**: Claspers undeveloped, shorter than extreme tips of posterior margin of pelvic fin. Testes small and thread-shaped, sperm ducts straight	**Immature**: Ovaries small, gelatinous or granulated, but no differentiated oocytes visible. Oviducts small and thread-shaped, width of shell gland not much greater than the width of the oviduct.
B	**Maturing**: Claspers longer than posterior margin of pelvic fin, their tips more structured, but the claspers are soft and flexible and the cartilaginous elements are not hardened. Testes enlarged, sperm ducts beginning to meander.	**Maturing**: Ovaries enlarged and with more transparent walls. Oocytes differentiated in various small sizes (usually <5mm) and pale in colour. Oviducts small and thread-shaped, width of the shell gland greater than the width of the oviduct, but not hardened.
C	**Mature**: Claspers longer than posterior margin of pelvic fin, cartilaginous elements hardened and claspers stiff. Testes enlarged, sperm ducts meandering and tightly filled with sperm.	**Mature**: Ovaries large with very large, yolk-filled oocytes, (often 10–30 mm in diameter). Shell gland fully formed and hard. Uteri fully developed but without yolky matter (see Stage D) or embryos (see Stages E-F) and not dilated (see Stage G)
D	**Active**: Clasper reddish and swollen, sperm present in clasper groove, or flows if pressure exerted on cloaca.	**Early gravid**: Uteri filled with yolky matter, which may appear unsegmented, or if segmented, without visible embryos.
E		**Mid-term gravid**: Uteri filled with small developing embryos that have well developed yolk sacs. Uterine fecundity can be counted.
F		**Late gravid**: Uteri filled with well-developed term pups (for which the yolk sac has either been fullt absorbed or is very small). Uterine fecundity can be counted.
G		**Post partum**: Similar to stage C, but with a greater number of degenerating follicles and uteri dilated.

Table 3: Conversion factors for UK skates, giving the number of fish examined, their length range (by sex) and the relationships between total weight (W, g) and total length (L, cm) for nine species of ray, by ecoregion.

Species	Ecoregion	Number of fish (Length range)		Total weight and total length ($W = aL^b$)						No. of mature fish examined		First maturity		Largest immature		50% mature	
		Male	Female	Male			Female			Male	Female	Male	Female	Male	Female	Male	Female
				a	b	r^2	a	b	r^2								
R. brachyura	Combined	360	395	0.0027	3.2563	0.99	0.0026	3.2742	0.99	25	17	55	60	91	93	78.2	85.6
		(13-100)	(11-108)														
R. clavata	Combined	6002	3330	0.0046	3.0821	0.99	0.0038	3.1459	0.99	1123	208	47	47	88	90	66.5	75.1
		(10-94)	(10-98)														
	Celtic Sea	2448	2394	0.0042	3.1059	0.99	0.0036	3.1607	0.99								
		(10-89)	(10-98)														
	North Sea	3503	885	0.0061	3.0017	0.99	0.0046	3.0896	0.99								
		(11-94)	(12-94)														
R. microocellata	Combined	709	739	0.0032	3.1949	0.99	0.0030	3.1833	0.99	65	26	66	73	74	73	69	77.1
		(13-80)	(12-85)														
R. montagui	Combined	1947	1811	0.0042	3.1055	0.99	0.0032	3.1928	0.99	324	92	40	49	66	70	50.3	64
		(10-67)	(10-76)														
	Celtic Sea	1761	1677	0.0043	3.0942	0.99	0.0032	3.1859	0.99								
		(10-67)	(10-74)														
	North Sea	178	121	0.0034	3.1645	0.99	0.0028	3.2299	0.99								
		(14-67)	(17-76)														
L. naevus	Combined	988	986	0.0043	3.0866	0.96	0.0037	3.1309	0.98	138	87	48	50	64	65	56.3	59.4
		(11-72)	(10-69)														

Species	Ecoregion	Number of fish (Length range)		Total weight and total length ($W = aL^b$)						No. of mature fish examined		First maturity		Largest immature		50% mature	
		Male	Female	Male			Female			Male	Female	Male	Female	Male	Female	Male	Female
				a	b	r^2	a	b	r^2								
	Celtic Sea	841	827	0.0041	3.1052	0.99	0.0036	3.1450	0.99								
		(11-72)	(10-69)														
	North Sea	109	129	0.0032	3.1610	0.99	0.0030	3.1833	0.97								
		(17-63)	(15-62)														
A. radiata	North Sea	428	448	0.0083	3.0051	0.96	0.0114	2.9142	0.95	181	148	30	32	44	46	36.2	38.2
		(8-49)	(8-49)														
R. undulata	Combined	85	45	0.0035	3.1615	0.99	0.0034	3.1784	0.99	*28*	*2*	*(80)*	*(79)*	*(88)*	*(83)*	*(83)*	*na*
		(22-97)	(17-95)														
L. fullonica	Combined	17	17	0.0014	3.3173	0.99	0.0036	3.0751	0.98	*2*	*0*	*(75)*	*na*	*(82)*	*na*	*na*	*na*
		(21-96)	(24-70)														
"D. batis"	Combined	30	32	0.0041	3.1233	0.95	0.0026	3.2222	0.99	*2*	*2*	*(115)*	*(125)*	*(98)*	*(97)*	*na*	*na*
		(20-118)	(19-135)														

Table 4: Summary table for the length at maturity for UK skates, as recorded in the present study and earlier studies

Species	Area	Sex	N	Length range examined	Length at			
					First maturity	50% maturity	100% maturity	Source
R. brachyura	English Channel	Combined	100	17-105 cm	100 cm	-	-	Dorel (1986)
R. brachyura	Irish waters	Male	123		-	81.9	-	Gallagher *et al.* (2005)
R. brachyura	Irish waters	Female	61		-	83.6	-	Gallagher *et al.* (2005)
R. clavata	Plymouth	Female			94-100 cm (65-70 cm disc width)			Steven (1936)
R. clavata	Plymouth	Male			72.5 (50 cm disc width)			Steven (1936)
R. clavata	Irish waters	Male	165		-	65.7	-	Gallagher *et al.* (2005)
R. clavata	Irish waters	Female	90		-	71.8	-	Gallagher *et al.* (2005)
R. clavata	North Sea	Female			-	77.1	-	Walker (1999)
R. clavata	North Sea	Male			-	67.9	-	Walker (1999)
R. clavata	Bay of Biscay	Combined	23	11-98 cm	80 cm (Male) 95 cm (Female)	-	-	Dorel (1986)
R. clavata	English Channel	Combined	960	10-101 cm	80 cm (Male) 95 cm (Female)	-	-	Dorel (1986)
R. clavata	Bristol Channel	Female	1124	13-99.0	59.5	-	-	Ryland & Ajayi (1984)
R. clavata	Bristol Channel	Male	1019		60.5	-	-	Ryland & Ajayi (1984)
R. clavata	Mediterranean	Male				[75]		Capapé (1976)
R. clavata	Mediterranean	Female				[85]		Capapé (1976)
R. clavata	Irish waters	Male				55-62 cm (as 38-43 disc width)		Fitzmaurice (1974)
R. clavata	Irish waters	Female				66-73 cm (as 45.5-50.5 disc width)		Fitzmaurice (1974)

Species	Area	Sex	N	Length range examined	Length at			
					First maturity	50% maturity	100% maturity	Source
R. clavata	Solway Firth	Male		18.4-101.6	61.8			Nottage & Perkins (1983)
R. clavata	Solway Firth	Female		32.5-102.1	62.4			Nottage & Perkins (1983)
R. clavata	Adriatic	Male				[55-60]		Jardas (1973)
R. clavata	Adriatic	female				[80-85]		Jardas (1973)
R. microocellata	Bristol Channel	Female	1374	14-90.6	57.5	-	-	Ryland & Ajayi (1984)
R. microocellata	Bristol Channel	Male	1218		58.0	-	-	Ryland & Ajayi (1984)
R. microocellata	English Channel	Combined	97	15-87 cm	70 cm	-	-	Dorel (1986)
R. montagui	Irish waters	Male	274		-	53.7	-	Gallagher *et al.* (2005)
R. montagui	Irish waters	Female	175		-	57.4	-	Gallagher *et al.* (2005)
R. montagui	North Sea	Female			-	62.2	-	Walker (1999)
R. montagui	North Sea	Male			-	56.7	-	Walker (1999)
R.montagui	English Channel	Combined	81	12-102 cm	60 cm (Male) 65 cm (Female)	-	-	Dorel (1986)
R.montagui	Bristol Channel	Female	1019	12-72.9	57.3	-	-	Ryland & Ajayi (1984)
R.montagui	Bristol Channel	Male	986		56.2	-	-	Ryland & Ajayi (1984)
L. naevus	Irish waters	Male	353		-	56.9	-	Gallagher *et al.* (2005)
L. naevus	Irish waters	Female	191		-	56.2	-	Gallagher *et al.* (2005)
L. naevus	North Sea	Female			-	55.0	-	Walker (1999)
L. naevus	North Sea	Male			-	55.0	-	Walker (1999)
L. naevus	Celtic Sea	Combined	276	13-70 cm	60 cm	-	-	Dorel (1986)
L. naevus	Celtic Sea	Female				[59]		Du Buit (1976)
R. undulata	English Channel	Combined	439	13-101 cm	?	-	-	Dorel (1986)
A. radiata	North Sea	Female			-	39.5	-	Walker (1999)
A. radiata	North Sea	Male			-	39.6	-	Walker (1999)

Annex 6: WKMOG 2008 Guidelines

The following guidelines incorporate and extend those from WKMAT (ICES 2007b)

a) For survey data to be used in a maturity index of the spawning stock, the survey must be conducted at the right time compared to the spawning period and have adequate coverage. If survey data are not available at the right time then histologically validated maturity data obtained outside spawning season can be used, although this should be confirmed on a stock-by-stock basis.

b) Where valid (see 3) maturity data are available from market samples they can be used to estimate maturity. This is mainly the case for species with a protracted spawning season where survey data do not cover the whole spawning season or stock area. Also, if survey and market data do not show systematic differences they can be used together.

c) Maturity data from market samples should be collected during the whole prespawning (for determinate species) or spawning (for indeterminate ICES WKMOG REPORT 2008 | 39 species) season on a métier based sampling programme, and cover the whole stock distribution area.

d) As with market samples, on-board samples should be collected on a metier base to avoid gear and fleet selectivity effect and in the correct time and spatial frame compared to spawning.

e) If possible, maturity staging should be done on board the survey vessel.

f) A comprehensive illustrated manual should be available for all stocks requiring maturity observations.

g) Macroscopic maturity scales used should be validated, either histologically or by another appropriate way.

h) Plot and map the data collected to assess differences by source, strata, location and time.

i) Length stratified maturity data should be weighted by the length distribution. If samples are collected on a random scheme or the stock is assessed on a length basis, no weighting according to the length distribution is required.

j) If the fish maturation process is dependent on age and/or sex as well as length then a Sex Maturity Age Length Key (SMALK) should be used. Age reading precision is important in this context.

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

l) If the maturity data are modelled, a Binomial GLM with logic link is current standard practice. Alternative approaches should be compared against this baseline approach.

m) Check appropriate model diagnostics.

n) Report the number of maturity staged fish used to calculate the estimates. If length classes are used, report the width of length classes.

o) When maturity estimates (as proportions) are reported to DCR specifications, calculate the mean confidence interval width for the age and/or length range which correspond to a 20 % and 90% of mature fish. Convert this to a precision level using:

- if half confidence interval width is less than 0.05 then the precision level is 3
- if half confidence interval width is less than 0.25 then the precision level is 2
- if half confidence interval width is less than 0.4 then the precision level is 1

*This is based on likely new definitions: Level 3 making it possible to estimate a parameter with a precision of plus or minus 5% for a 95% confidence level, Level 2 as ±25% and Level 3 as ±40%.Optionally, report the range of precision levels achieved as well as the mean level.

Annex 7: WEBGR contact to upload photo reference library

WebGR is a European project that aims to develop Open Source software to support studies of fish growth and reproduction. In particular it promotes the use of online services to organise calibration workshops.

Scientists, who read otoliths to identify the ages of individual fish, have carried out calibration workshops for many years, to fine-tune their interpretation of the ages of fish within individual stocks or species. Calibration workshops have also recently been extended to cover the identification of fish gonad maturity stages. In general WebGR can be applied to all situations, where individual scientists need to discuss the interpretation of a protocol, for the identification of the status of biological material.

The consortium is constituted by: Laboratório Nacional de Recursos Biológicos – IPIMAR (Portugal) – Consortium leader, The Agri-Food & Biosciences Institute (UK), AZTI Tecnalia Foundation (Spain), Federal Agency for Agriculture and Food (Germany), Johann Heinrich von Thünen Institute (Germany), Hellenic Centre for Marine Research (Greece), Instituto Español de Oceanografia (Spain), Institut français de recherche pour l'exploitation de la mer (France), Institute for Marine Resources & Ecosystem Studies (The Netherlands), Institute of Marine Research (Norway), Swedish Board of Fisheries (Sweden), Italian Society for Marine Biology (Italy).

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For more information please visit http://webgr.berlios.de.

Annex 8: PGCCDBS Guidelines for Workshops on Maturity Staging

Introduction

The main objectives of a maturity staging workshop are: i) to agree on a common maturity scale for the species/stock of concern across laboratories, based on a comparison of existing scales and standardization of maturity determination criteria; ii) to establish correspondence between old and new scales so that time series of previous data can be converted; iii) to reduce sources of error in maturity determination by validating macroscopic staging, and iv) to propose an optimal sampling strategy to estimate accurate maturity ogives.

1) Topics to consider when preparing a Workshop

   a) Identify sources of data that, at present, are used to collect maturity data and their current sampling protocols.

   b) Gather information on the reproductive biology and ecology of the species / stock of concern with emphasis on the timing of the different stages of the reproductive cycle, particularly spawning time, delimitating clearly its duration.

   c) Studies are required on spawning synchronicity among individuals within a stock, as low synchronicity will mean there is temporal overlap of different stages (developing, spawning, spent and/or resting).

   d) The organization for the collection of the samples and the methods for histological analysis need to be decided amongst the experts but guidance can be found below (Guidelines for collecting maturity data).

   e) Maintain contact with participating countries to ensure adequate sample coverage is obtained prior to the workshop's analyses of samples. In this sense the following should be ensured:

   • Laboratories participating in stock assessment or data collection of the stock of concern should participate even if they do not collect routinely maturity data.

   • However, there are practical limits to the number of participants; in this case each laboratory will need to ensure that only the most suitable people attend.

   • Experts on histology, maturation process and the reproductive ecology/biology of the species of concern or at least a related species should participate in the workshop.

   f) Ideally, a fresh sample should be provided during the workshops. This needs to be taken into account when setting the timing of the meeting. The best time of year to do a workshop on maturity staging is when the diversity in maturity stages is high.

   g) Identify the metadata that are needed to accompany samples collected for analyses and specify it in the sampling protocols (see guidelines below).

   h) Provide detailed protocols on collecting images of the gonads sampled, including at least a precise description of the quality of images (set-up of camera and format) and image calibration. Additionally, in case of histologically images, agree on the histological protocol and microscope set-up (see guidelines for histological process below).

i) Use images as a tool for calibration prior to a workshop.

j) Gather information on how the data are, or could be used, in the assessment process.

k) Put in place arrangements for histological analyses of collected material taking into account that all participants may not have facilities or resources to meet this requirement. Arranging for centrally located analyses has proved effective in the past and has ensured that adequate samples are validated. Consider bi-lateral agreements to cover the cost of such work.

l) Each laboratory should carry out investigations into potential discrepancies in maturity staging between scientists within the laboratory. They should consider macroscopic staging and, if available, microscopic staging. If possible provide statistical analysis of precision and accuracy within the laboratory. Potential causes for lack of precision and accuracy should also be analyzed.

m) Prepare a full set of reference material covering both the spatial and temporal aspect of the species/stock of concern. These consist of pictures of all maturity stages together with their histology report.

n) The meeting should be held in an institute with suitable wet laboratory facilities and ideally with histological facilities. If not histological facilities are not available at least with sufficiently high quality research microscopes with attached high definition cameras.

2) Topics to consider during the Workshop

a) Provide information on participating laboratory procedures, including sampling procedures, macroscopic maturity determination process, maturity scale definitions and if applicable gonad preservation and histological methods, and protocols used to determine microscopic maturity.

b) Resolve interpretation differences between readers and laboratories both at macroscopic and microscopic scales. Differences may arise from:

i) Using different maturity scales

ii) Different interpretation of the same macroscopic stages (terminology and precise definition of stages are critical issues)

iii) Different sampling protocols, e.g. timing and/or gear selectivity or availability, see guidelines for collecting maturity data below.

iv) Different interpretation of gonad structures and gamete development in histological slides. This should not be an issue, so experts on gametogenesis should be involved in workshops.

c) Agree and create a single maturity scale. Consider the following aspects:

i) Keep the scale as simple and efficient as possible. Not everything can be extracted from a maturity scale and a complex maturity scale may introduce more errors than relevant information (See WKMAT report)

ii) Describe the stages precisely avoiding ambiguity and overly subjective description (like colour descriptions), for example, give measurements instead of saying “bigger”.

iii) If two stages are hard to distinguish macroscopically, they should normally be merged. This often occurs with resting and/or mature inactive stages that are confused with immature or developing (at early stages).

iv) In these cases, histology must be used to separate the merged maturity stage into the different real stages. It is necessary to define the minimum number of samples to be collected, the timing of the sampling, how they should be histologically processed, and what criteria should be used to distinguish between stages, and if possible define a reference lab (see below).

d) As a calibration exercise, each participant should classify the workshop sample collection using the agreed maturity scale. This will provide a test of the new scale and any discrepancies in interpretation should be identified and resolved.

e) Based on the experiences e.g. of the WKMSSPDF (22-26.02.2010) it is recommended to set the maximum fish to stage in one session to 120. However, the total numbers to stage should also take into account the species and any sample size requirements for statistical comparisons. This applies to fresh samples as well as pictures.

f) The results from the calibration exercise should be recorded to provide data for statistical analysis. If you want to measure improvements in agreement due to the workshop then ideally a different set of samples should be used, not the ones already staged earlier in the workshop.

g) Provide a statistical report comparing observed maturity stage with validated histological stage for the workshop participants to consider.

h) Differences in staging between laboratories should be statistically analyzed in terms of precision and accuracy; sources of discrepancies should also be analyzed.

i) Try to use standard terminology (Murua and Saborido-Rey, 2003; Brown-Peterson et al., 2007) during the workshop and in the report. Try to keep the recommended maturity scale as similar to the standard as possible.

j) When a new agreed maturity scale is proposed the impact on maturity historical series should be evaluated

k) Produce an agreed reference collection of preserved gonads, histological slides and images that should be stored in a reference lab and always available for the scientific community. Copies of histological slides can be made and distributed with referenced images of these slides.

l) A reference laboratory should be defined, for each species, with experience and equipments to define, with precision, maturity stages and to "solve problems".

m) The minimum output from species-specific workshops should be an illustrated manual.

n) Provide recommendations to stock assessment Working Groups and Benchmarks on relevant issues derived from maturity stage studies, such as timing of sampling, changes on maturity time series, spatial differences on maturity, differential sex maturation, etc.

Annex 9: Histological procedures in use for elasmobranchs and recommendations

A collection of the histological methodologies in use between participants is hereunder presented.

GSA 9 – *Raja asterias*, *R. clavata*, *R. miraletus* and *Scyliorhinus canicula*

Barone M. and Pirone A.

Sezione di anatomia – Dipartimento di produzioni animali, Universita` di Pisa, Pisa, Italy

1) Histological techniques requires the utilization of fresh samplings;
2) Gonads extracted in their entirely;
3) Fixed in 10% formaldehyde 0.1 M phosphate buffer, pH 7.4;
4) Small fragments of the anterior part of the gonad is cut (the second quarter of the anterior half gonad) (See Figure below);
5) The small fragments of each gonad were extensively washed in fresh water for one day;
6) Dehydrate in a graded ethanol series (30 min at 30%, 50%, 70%; 1 h at 80%; 2 h at 95%);
7) Embed in glycol methacrylate resin (JB-4, Polysciences Inc.);
8) Sections of 5 μm are collected on glass slides coated with 0.5% gelatine containing 0.05% chrome alum;
9) Sections were subsequently stained with toluidine blue and methylene blue
10) Cleared in xylene;
11) Mounted with DPX and examined with a light microscope (from x10 to x1000 magnification).

GSA 11 – *G. melastomus*, *S. blainvillei*

Porcu C., Mulas A., Cabiddu S., Cannas R., Follesa M.C. and Cau A.

Department of Animal Biology and Ecology, University of Cagliari, Italy.

1) A piece of tissue from the anterior region of the gonad is cut;
2) It is preserved in 5% formaldehyde 0.1 M phosphate buffer, pH 7.4 for 48 hours;
3) The tissues were stored in ETOH 70%;
4) Successively it is dehydrated;
5) It is infiltrated with resin (2-idrossiethylmetacrilate; glycol-methacrylate method; Technovit 7100).
6) The section is cut 3.5 μm thick;
7) Then they are stained with Harris's hematoxylin and eosin and cover slipped with a synthetic mounting media.

GSA 9 – *D. licha*, *S. acanthias*, *M. mus*telus, *P. glauca*

(Bottaro M.)

Istituto Superiore per la Protezione e la Ricerca Ambientale, Roma

1) The gonads is fixed in 4% Paraformaldehyde (PAF) or in Bouin liquid for a period ranging 8-18 hours, depending the size and volume of the samples;

2) It is washed washed 3 times (1 hours for each time) in posphate buffered saline (PBS) pH. 7,4 and we store samples in ethanol 70%.

3) Sub-samples are dehydrated in progressive series of ethanols (from 70% to 100%).

4) They are passed in xylene, then in xylene-paraffin and finally three 1-hour passage in paraffin before embedding.

5) 5µm sections by routinary microtome are stained;

6) The sections are stainedm with hematoxylin, after re-dehydration.

7) Then they are dehydrated again in progressive series of ethanols and xylene and the slides are closed with Eukitt.

ICES IXa - *C. squamosus*, *C. coelolepis*, *Raja clavata*, *R. undulata*, *R. brachyura*, *R. montagui*, *R. miraletus*, *R. microocellata*, *D. oxyrinchus*, *L. circularis*, *L. naevus*, *N. iberica*

(Serra-Pereira B.*, Moura T. and Figueiredo I.)

Marine Resources and Sustainability Unit (U-REMS), INRB, L-IPIMAR, I.P.

Avenida de Brasília, 1449-006 Lisboa

PORTUGAL

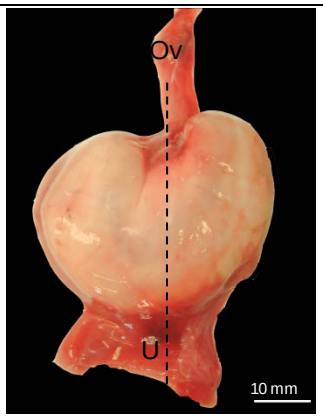
* contact: bpereira@ipimar.pt

1) The reproductive organs of both sexes, ovaries, oviducal glands (OG), uteri, testes and sperm ducts (both epididymis and vas deferens), are extracted and preserved in 10% buffered formaldehyde.

2) Transversal sections of about 3 mm thick are extracted from the middle of the anterior half of the ovaries and testes, from one of the gonads per specimen, covering all the maturity stages.

3) The modifications in the histological structure of the OG and the presence and nature of the secretions produced during the development process are analysed using a selection of one OG per specimen, covering all the maturity stages. Sagittal sections of about 3 mm thick are removed from the middle of the OGs (Fig. 1).

Figure 1. External anatomy of the OG at the actively spawning stage. Anteriorly, the OG communicates with the oviduct, while posteriorly it communicates with the uterus. The dotted line in the center indicates the position of sagittal sectioning (Adapted from Serra-Pereira *et al.* in press)



4) Transversal sections of about 3 mm thick are also removed from the middle of the uterus (anterior uterus) in females and Sgittal sections from both epididymis and vas deferens in males.

5) Sections are processed using an automated tissue processor (Leica TP1020, Germany) according to the standard protocol (Bancroft and Gamble 2002), i.e. 1) dehydration through a series of alcohols from 70% to absolute ethanol; 2) clearing with xylene; and 3) impregnation and embedding in paraffin wax.

6) Samples are embedded in paraffin wax blocks using a standard heated paraffin embedding system (Leica EG 1140H, Germany). The paraffin blocks are then sliced at 3-5 μm of thickness, using a rotary microtome (Leica RM2125RT, Germany).

7) Additional staining procedures are used to investigate the structure of the zona pellucida and stage of vitellogenesis in the ovaries and the chemical nature of the secretions produced by the OG and uterus: 1) periodic acid-Schiff (PAS), used to highlit the zona pellucida and yolk droplets in the ovaries and to detect neutral mucins (PAS+ structures stained pink) in the OG and uterus; 2) combined alcian blue and PAS to detect sulfated acid and neutral mucins (PAS/AB) (PAS+ structures stained pink, AB+ stained blue and PAS+AB+ stained in different intensities of purple); 3) Van Gieson stain (VG) to detect collagen, stained in red.

8) Histological staining protocols used by Bancroft and Gamble (2002) were followed, with some adaptations to improve the results. These included: a) in PAS and PAS/AB staining techniques, sections were covered with Schiff's solution, for about 8-10 minutes, instead of 15 minutes, since longer times caused background staining; b) in PAS/AB staining, after staining with AB, sections were covered with 1% Periodic acid, for 3 minutes, instead of 5 minutes, with the same results; and c) in VG staining, sections were covered in Van Gieson solution for 5 minutes instead of 3 minutes, for more intense staining .

9) Histological slides are observed with a stereo microscope (Olympus SZX9, USA) and an optic microscope (Carl Zeiss Axioplan 2 imaging, Germany). Images are obtained using a Sony DFW-SX910 camera and the imaging software TNPC 4.1 used with the stereo microscope and a Zeiss AxioCam MRc camera and the imagining software AxioVision 4.1 used with the optic microscope.

Annex 10: Histological photo reference for the maturity scale proposed for oviparous and viviparous elasmobranch species

Some participants of WKMSEL presented histological descriptions of the different maturity stages of females and males of oviparous *Raja clavata* (ICES Area IXa and GSA 9), *Raja asterias* (GSA 9), *Scyliorhinus canicula* (GSA 9), *Galeus melastomus* (GSA 11) and viviparous *Squalus blainvillei* (GSA 11), relying on the development of the different reproductive organs: ovaries (all studies), oviducal gland and uteri (Portugal) in females; and testes (all studies) and sperm ducts (Portugal, ARPAT, Italy) in males. The histological procedures used by each institute (IPIMAR, Portugal; ARPAT, Italy; and University of Cagliari, Italy) are presented in Annex 9

Reproductive development of *Raja clavata* from mainland Portugal

Bárbara Serra-Pereira, Teresa Moura and Ivone Figueiredo (IPIMAR, Lisbon, Portugal)

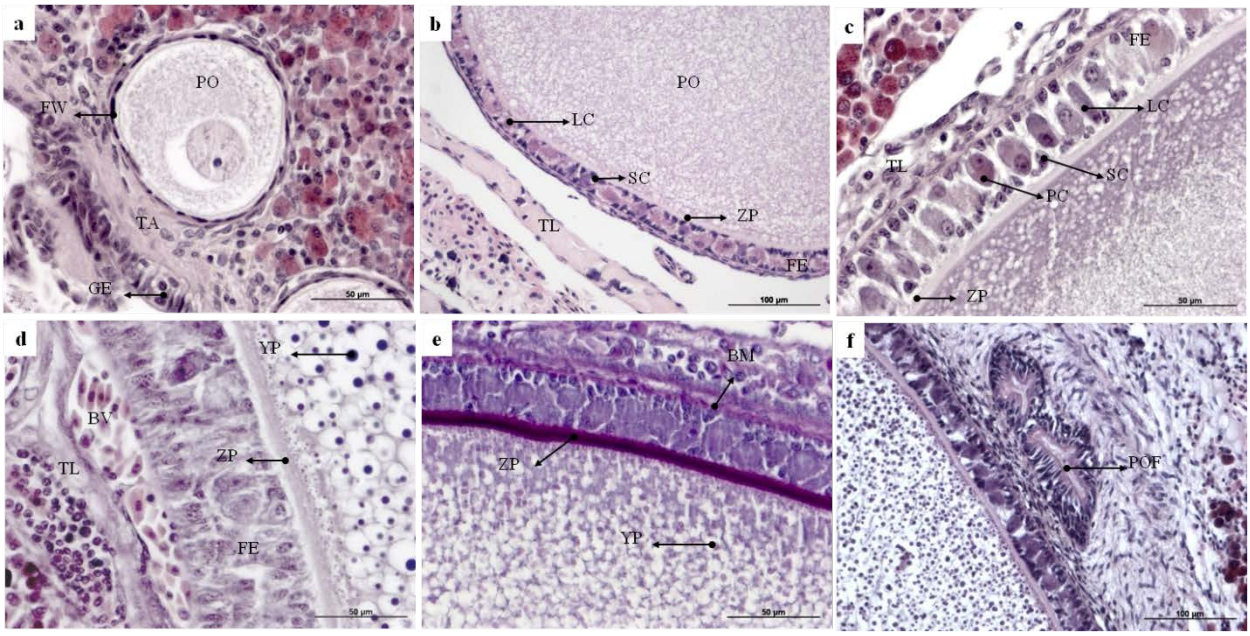


Figure 1. Oogenesis in *Raja clavata*. a) primordial follicle (105 μm in diameter). H&E; b) primary follicle (790 μm in diameter). H&E.; c) pre-vitellogenic follicle (1031 μm in diameter). H&E.; d) vitellogenic follicle (3480 μm diameter). H&E.; e) vitellogenic follicle (4100 μm diameter). PAS; f) post-ovulatory follicles (POF). H&E (PO: primary oocyte;FW: follicular wall (squamosus cells); GE: germinal epithelium; TA: tunica albuginea; ZP: zona pellucid; FE: follicular epithelium; SC: small cells; LC: large cells; TL: thecal layers; PC: pyriform cells; YP: yolk platelets; BV: blood vessel; BM: basement membrane; POF: post-ovulatory follicles). (Adapted from Serra-Pereira *et al*. in press a).

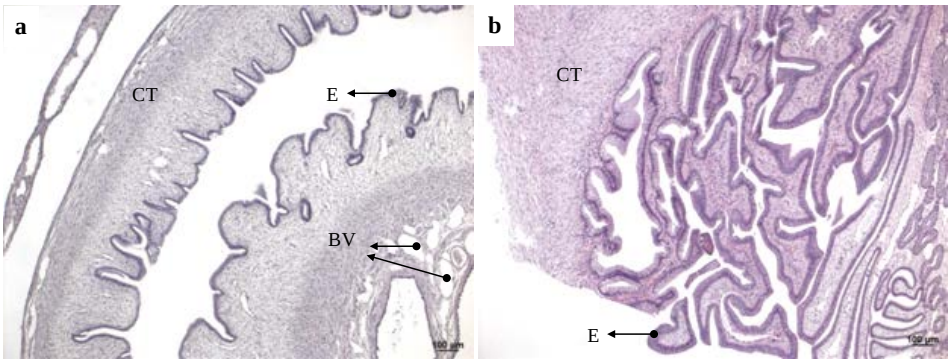


Figure 2. ***Raja clavata*** **tranversal section of the uterus in the immature stage (a) and spawning capable stage (b). H&E (CT: connective tissue; BV: blood vessel; E: epithelium). (Adapted from Serra-Pereira** ***et al*****. in press a).**

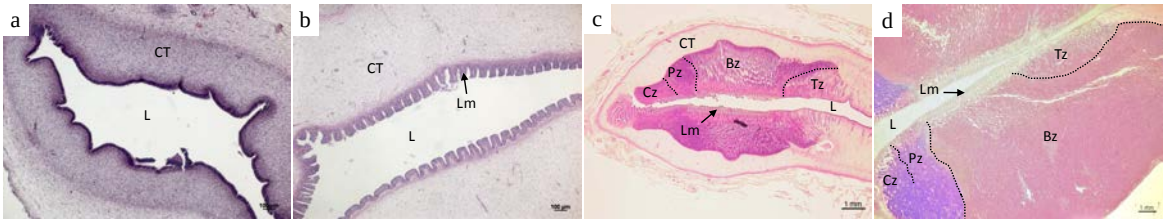


Figure 3. ***Raja clavata*** **sagittal sections of the oviducal gland (OG) in different maturity stages. a) Undifferentiated OG in an immature female; b) Beginning of the development of the OG in an early developing female; c) Formation of lamellae, gland tubules and differentiation of the four distinct zones in a developing female. d) Fully developed OG with full differentiation of the four secretory zones in a spawning capable female. H&E (CT: connective tissue; BV: blood vessel; E: epithelium; L: lumen; Lm: lamellae; Cz: club zone; Pz: papillary zone; Bz: baffle zone; Tz: terminal zone). (Adapted from Serra-Pereira** ***et al*****. in press b).**

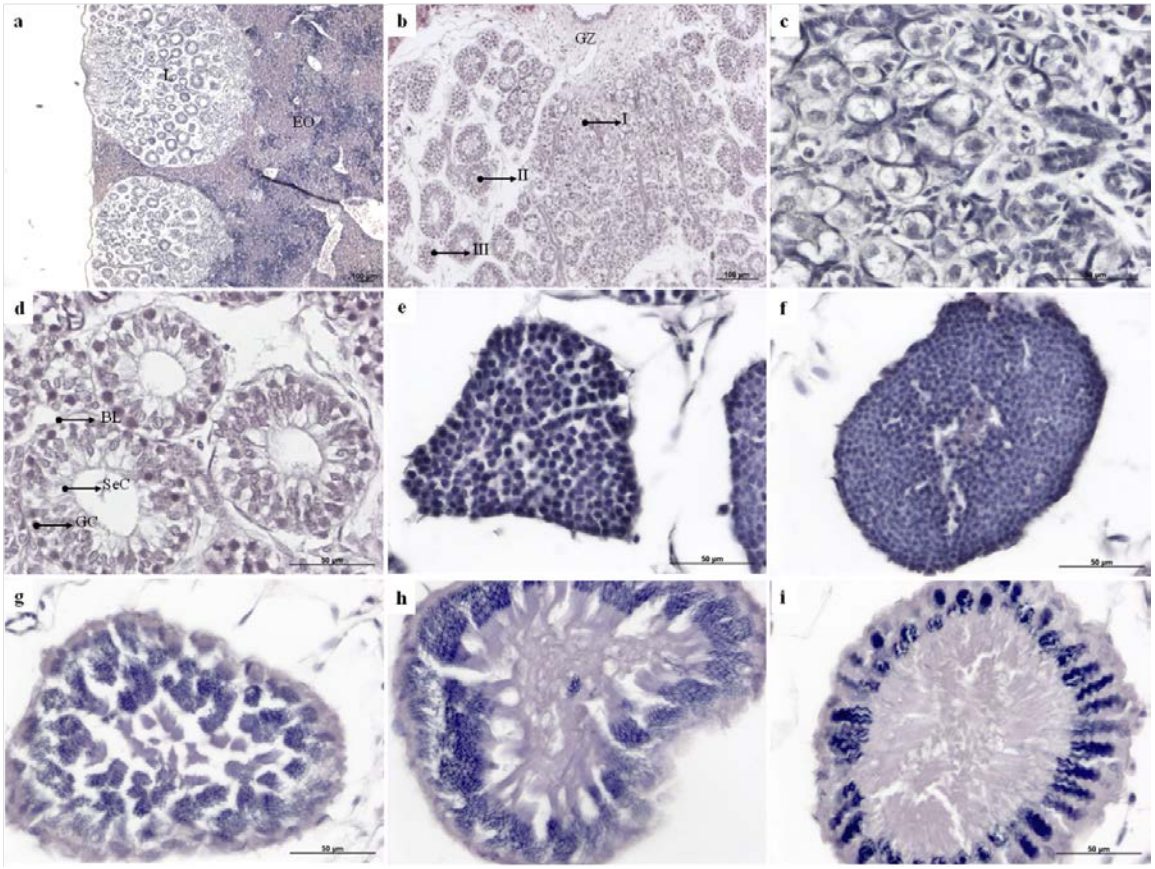


Figure 4. Spermatogenesis in *Raja clavata*. a) immature testis with small lobules starting to differentiate; b) first stages of the spermatogenensis in a lobe from an immature testis; c) stage I: gonocyte; d) stage II: spermatogonia; e) stage III: primary spermatocyte; f) stage IV: secondary spermatocyte; g) stage V: spermatid; h) stage VI: immature sperm; i) stage VII: mature sperm. H&E (L: lobules; EO: epigonal organ; GZ: germinal zone; I: gonocytes, II: spermatogonia, III: primary spermatocyte; GC: germ cells; SeC: Sertoli cells; BL: basal lamina). (Adapted from Serra-Pereira *et al.* in press a).

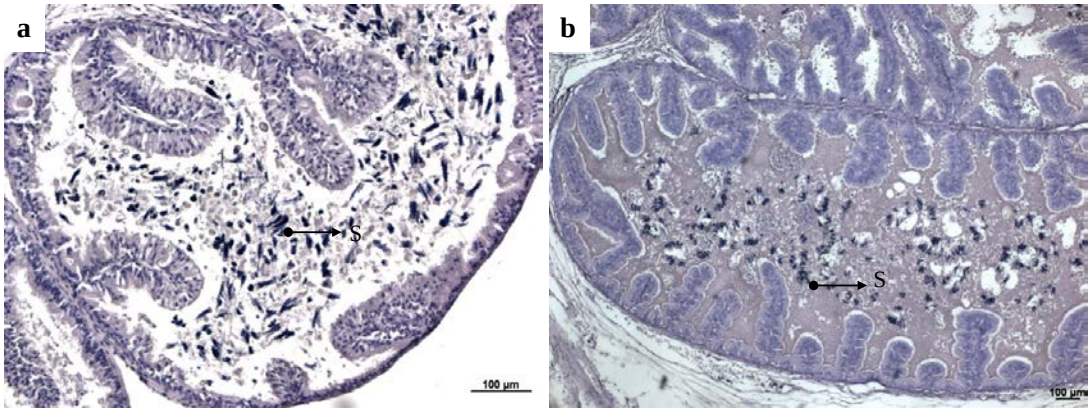
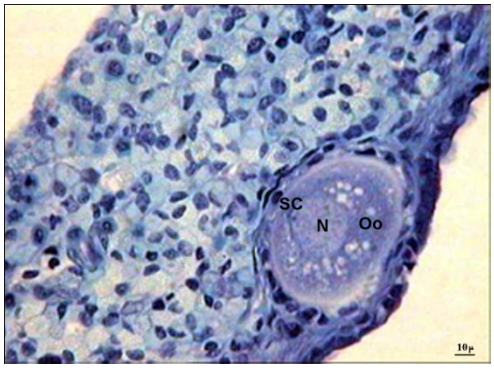


Figure 5. Tranverse sections of the Epididymis (a) and ducts deferens (b) in spawning capable (stage 3a) *Raja clavata* male. H&E (S: spermatozoa). (Adapted from Serra-Pereira *et al.* in press a).

Gametogenesis of Raja asterias, R. clavata, Scyliorhinus canicula from the northern Tyrrhenian Sea.

Monica Barone, Cecilia Mancusi and Fabrizio Serena (ARPAT, Livorno, Italy)

Raja asterias – oogenesis (adapted from Barone *et al.* 2007)



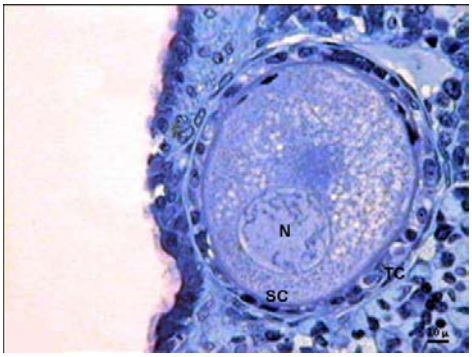


Figure 1 and 2 - Small previtellogenic follicles ~ 50 µm in diameter. The follicular epithelium is single layered and made up to small squamous cells (SC). TC theca cells; N, nucleus; Oo, oocyte.

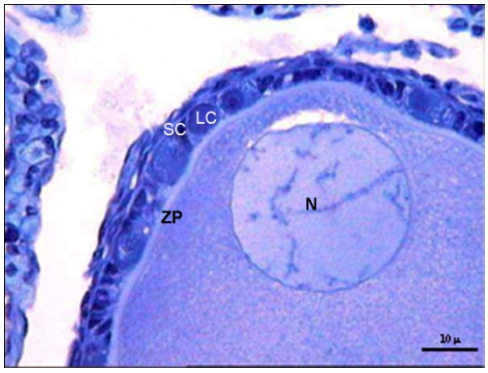


Figure 3 - Previtellogenic follicle ~ 100 µm in diameter. The follicular epithelium is double layered and made up to small (SC) and large (LC) cells. ZP zona pellucida.

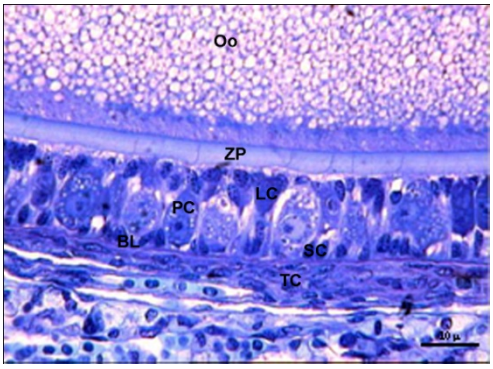


Figure 4 - Large previtellogenic follicles, up to 1500 µm in diameter. The follicular epithelium is multilayere and made up to small (SC), large (LC) and pyriform (PC) cells. Oo, oocyte; ZP, zona pellucida; BL, basal lamina.

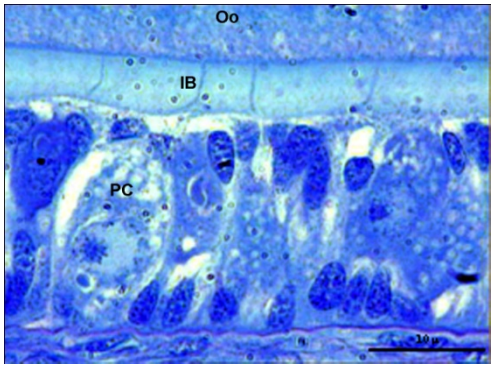


Figure 5 - Intercellular bridges (IB) link pyriform (PC) cells with oocyte (Oo).

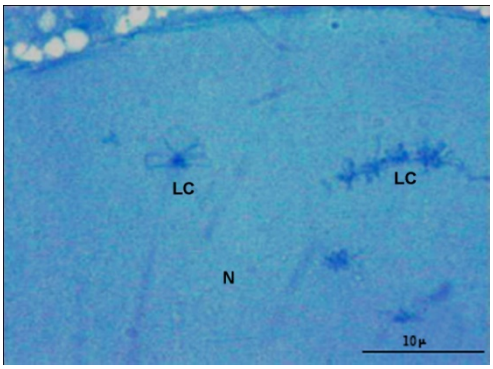
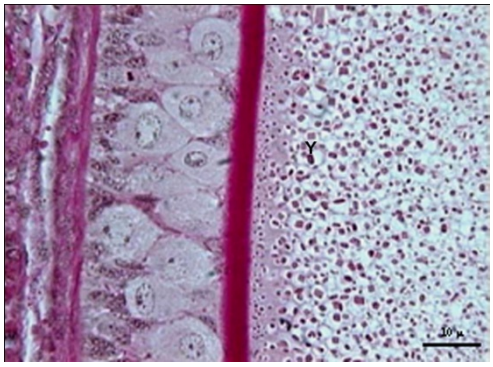


Figure 6 - Nucleus (N) of oocyte 1600 µm in diameter contained lampbrush chromosomes (LC).



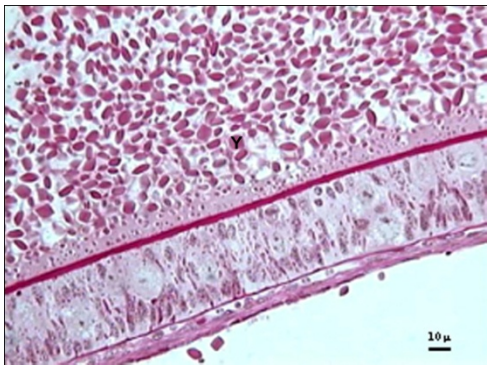


Figure 7 and 8 - Follicles up to 3000 μm filled with yolk platelets (Y).

Raja asterias – spermatogenesis (adapted from Barone *et al*. 2007)

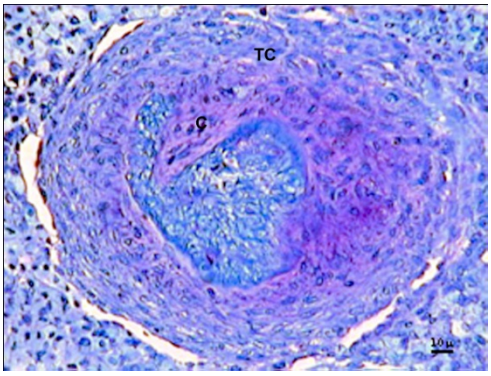


Figure 9 - Atretic previtellogenic follicle. Granulosa cells (C) and theca cells (TC) are hypertrophic.

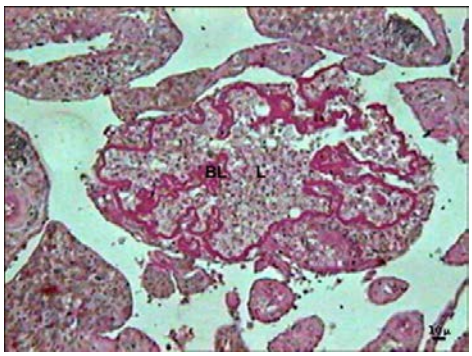


Figure 10 - Postovulatory follicle. The basal lamina (BL) appears collapsed and invades the central lumen (L).

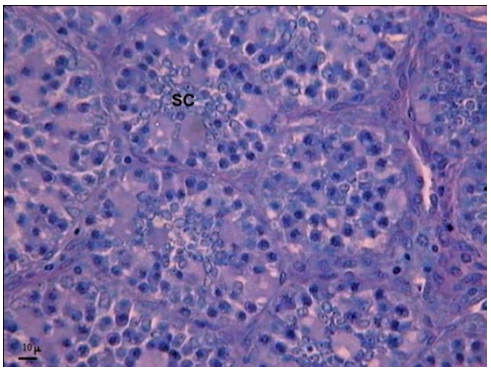


Figure 11 - Sertoli cell nuclei (Sc) migrating toward the periphery.

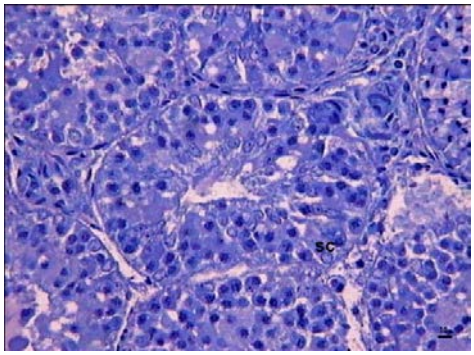


Figure 12 - Sertoli cells (Sc) in peripherical position just inside the basement membrane.

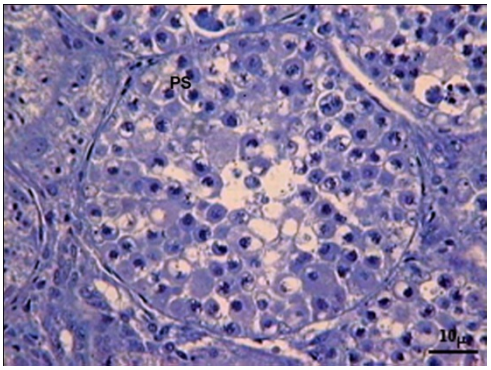


Figure 13 - Primary spermatocytes (Ps) showing large nuclei.

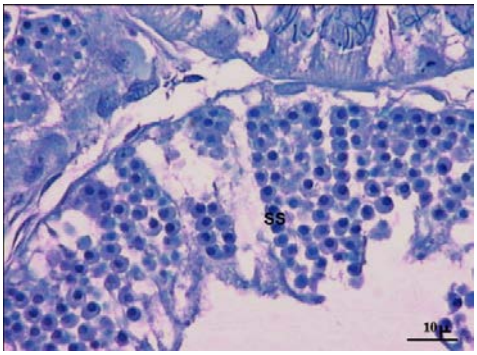


Figure 14 - Secondary spermatocytes (Ss) with nuclei containing condensed chromosomes.

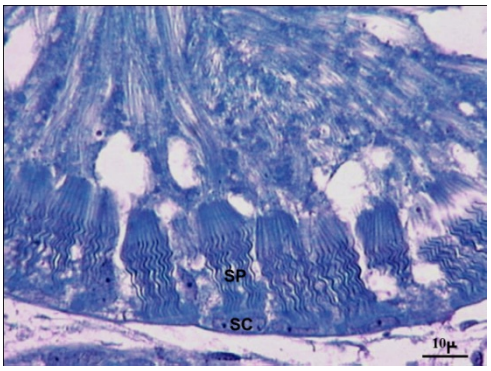


Figure 15 - Spermatozoa (sp) associated in linear arrays in the Sertoli cells (Sc).

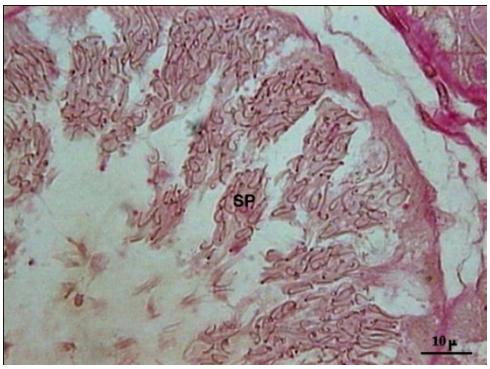
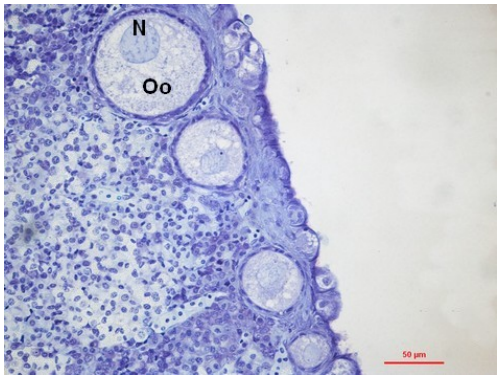
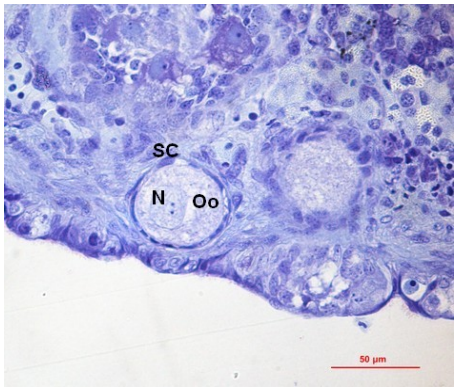


Figure 16 - Spermatozoa (sp) within the lumen.

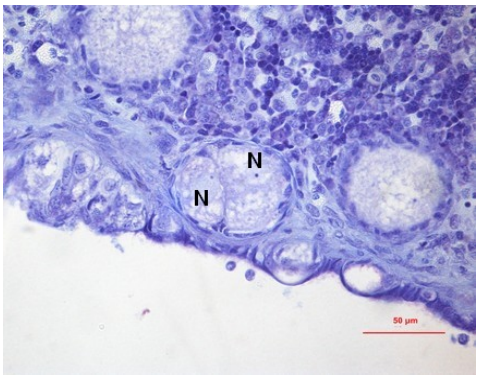
Raja clavata – Oogenesis (adapted from Barone 2009)



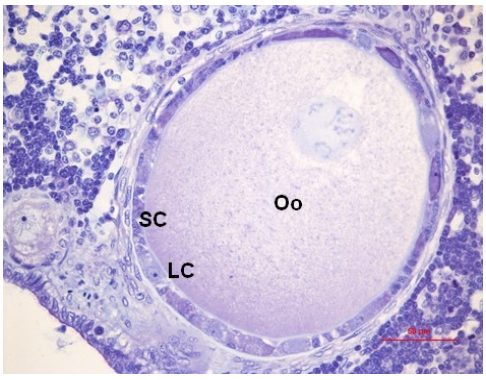
a - Primordial follicles and primary oocytes (Oo) ~50 µm diameter, under the tunica albuginea. N, nucleus.



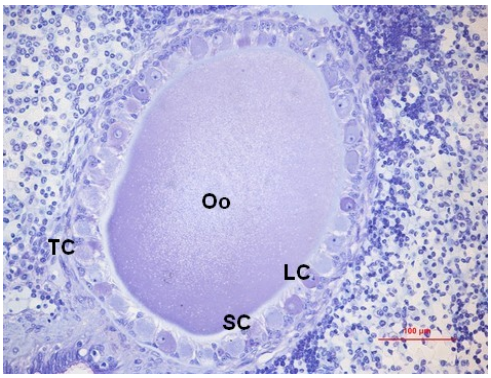
b - Primary oocytes (Oo) surrounded by a layer of small squamous follicle cells (SC). N, nucleus.



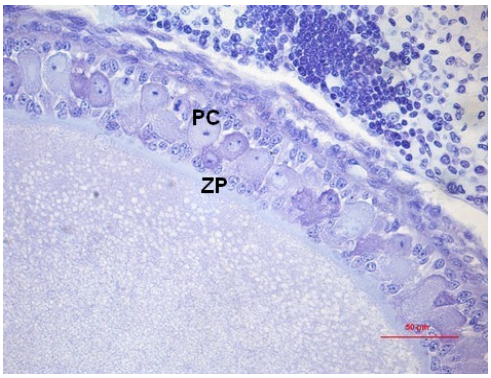
c - Couple of primary oocytes. N, nucleus.



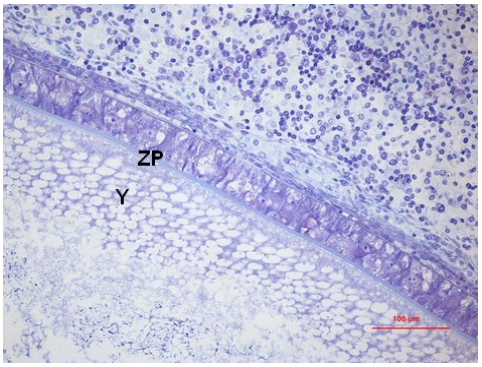
d - Previtellogenic follicle (Oo) ~150 µm in diameter. The follicular epithelium is made up to small (SC) and large (LC) cells.



e - Previtellogenic follicle (Oo) ~150 µm in diameter. The follicular epithelium is made up to small (SC) and large (LC) cells. ZP, zona pellucida; TC, teca cells.

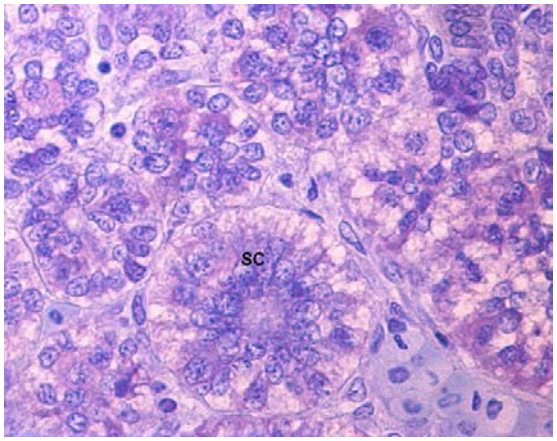


f - Large previtellogenic follicles, up to 1500 µm in diameter. The follicular epithelium is made up to small (SC), large (LC) and pyriform (PC) cells; ZP, zona pellucida.

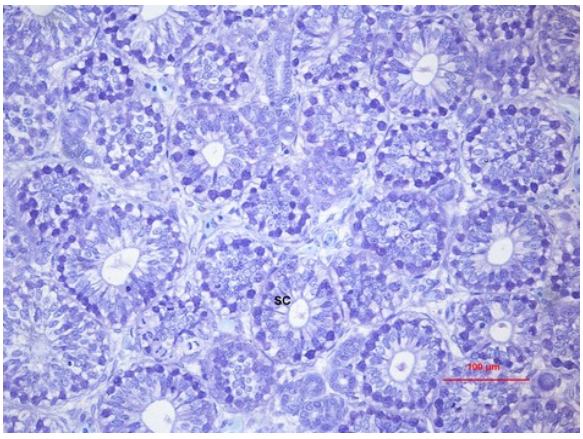


g - Follicles up to 3000 lm filled with yolk platelets (Y).

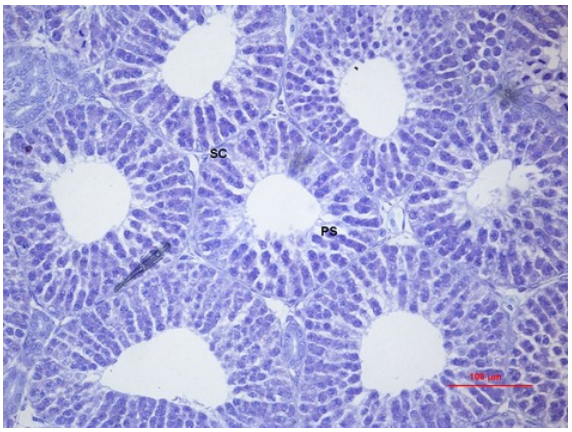
Raja clavata – Spermatogenesis (adapted from Barone 2009)



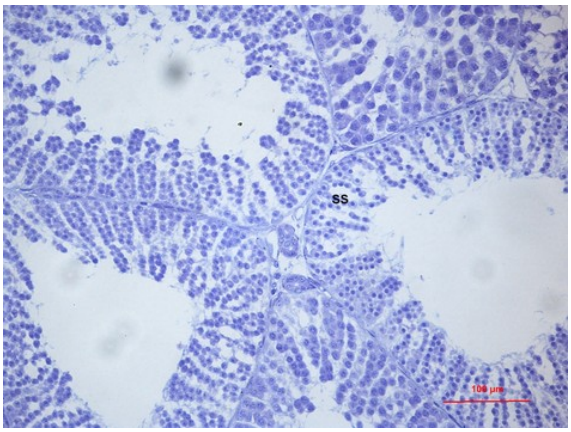
a - Sertoli cells (SC) occur at the lumen.



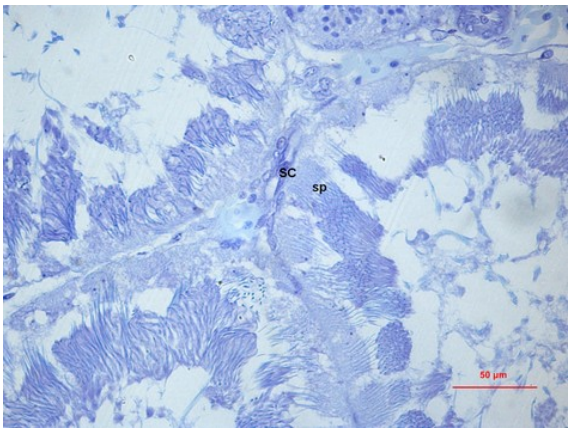
b - Sertoli cell nuclei (Sc) migrating toward the periphery.



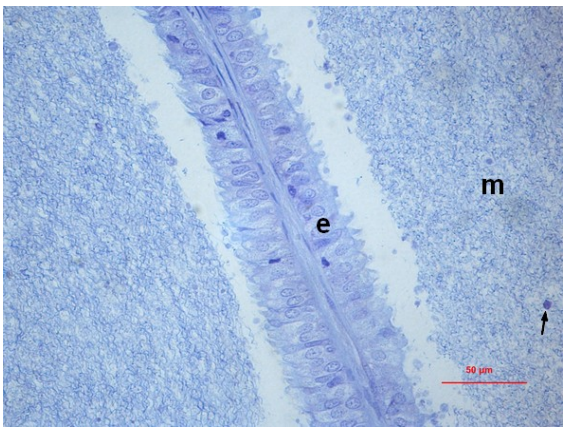
c - Nuclei of primary spermatocytes (PS).



d - Secondary spermatocytes (SS).

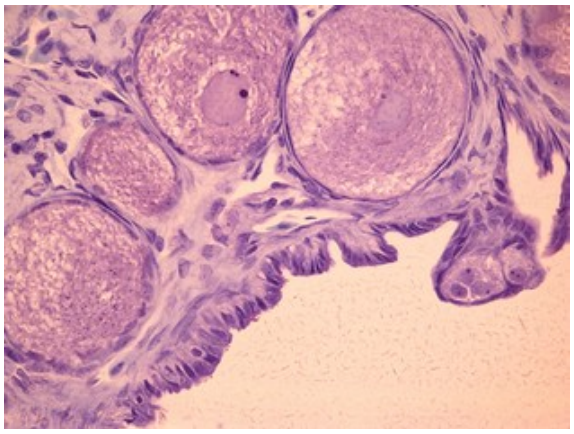


e - Spermatids (sp) are bundled together while being embedded on the Sertoli cell's apex (SC).



f – Columnar epithelium (e) of epididymis. Lumen is filled with matrix (m), vesicular bodies (arrow) and sperm

Scyliorhinus canicula – oogenesis



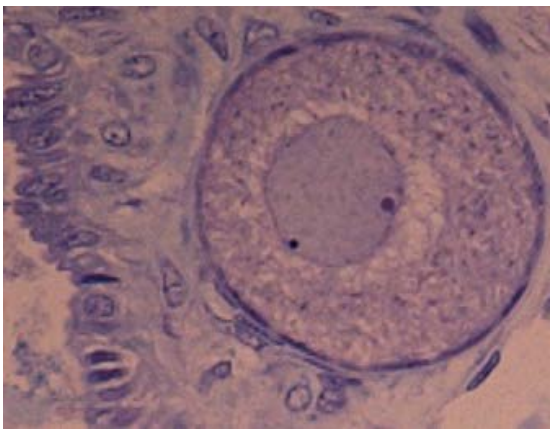
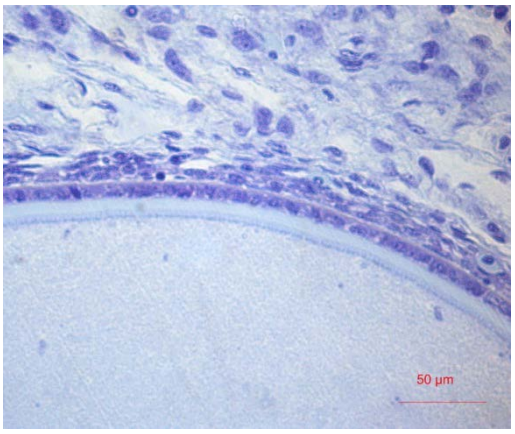


Fig 1 and 2 - Previtellogenic follicles (50-100 µm in diameter) lying below the ciliated epithelium



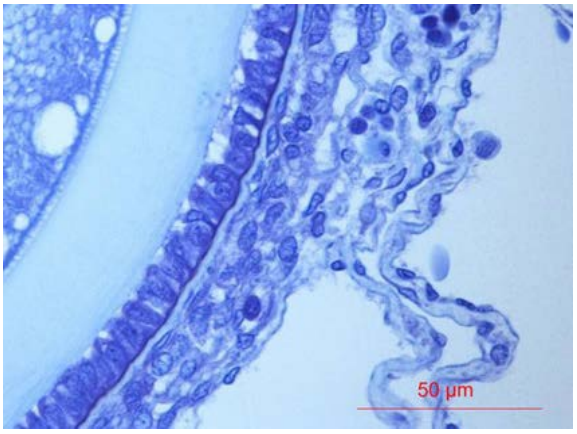
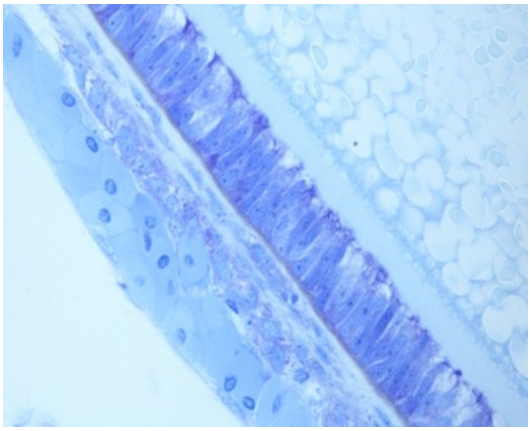


Fig 3 and 4 – Previtellogenic follicles (about 1000 µm in diameter)



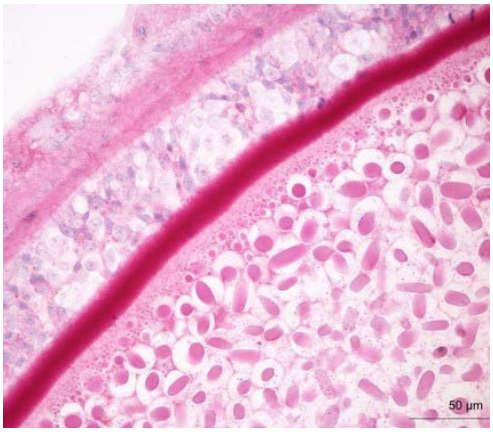


Fig 4 and 5 – Vitellogenetic follicles (about 3000 µm in diameter)

Scyliorhinus canicula – spermatogenesis

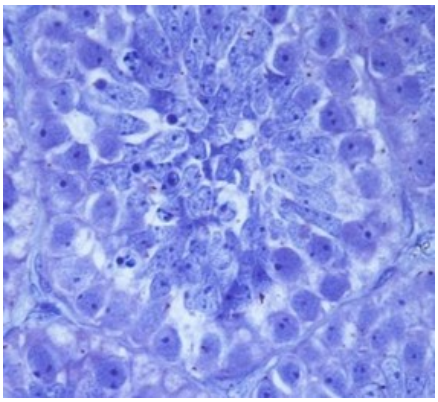


Fig - Sertoli cells occur at the lumen.

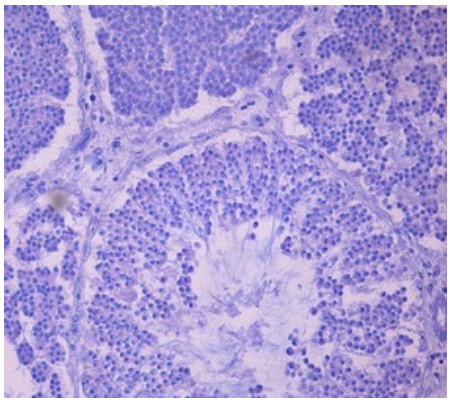


Fig - Secondary spermatocytes

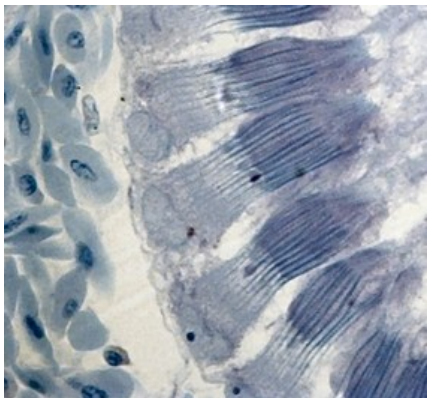


Fig – Spermatids bundled on the Sertoli cell's apex

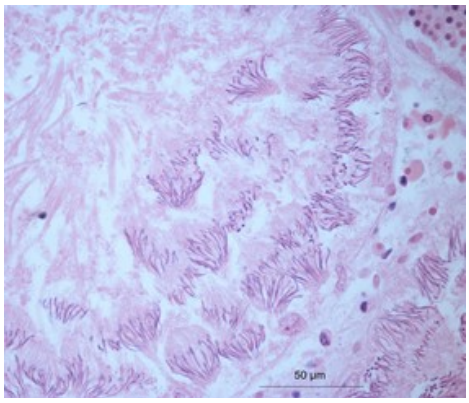


Fig - Spermatozoa within the lumen

Microscopic maturity stages of oviparous *Galeus melastomus*

Porcu C., Mulas A., Cabiddu S., Cannas R., Follesa M.C., Cau A. (University of Cagliari, Cagliari, Italy)

Oogenesis

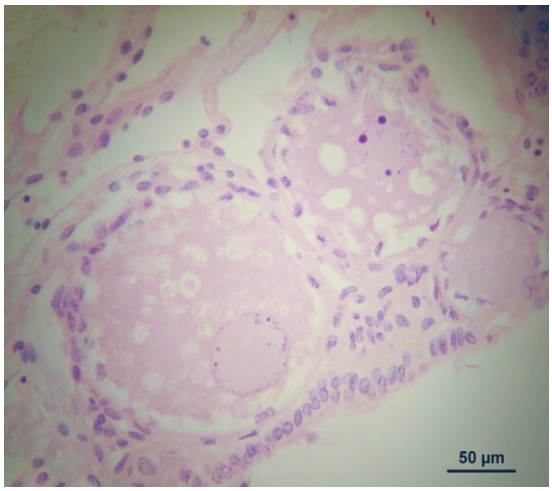


Figure 1 - Early previtellogenic follicles (50-100 µm). The follicular epithelium is single layered made up to squamous cells.

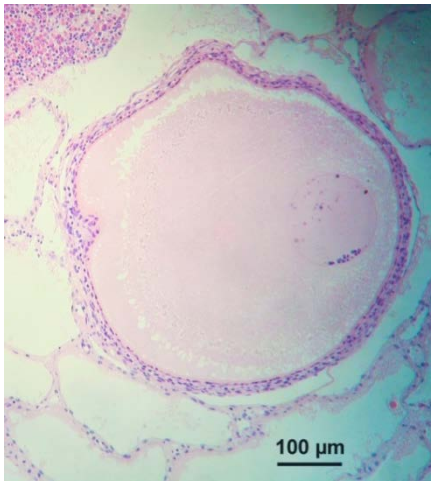


Figure 2 - Late previtellogenic follicle (~500 µm). The follicular epithelium is single layered. Lipid rich inclusions begin to appear (white arrow).

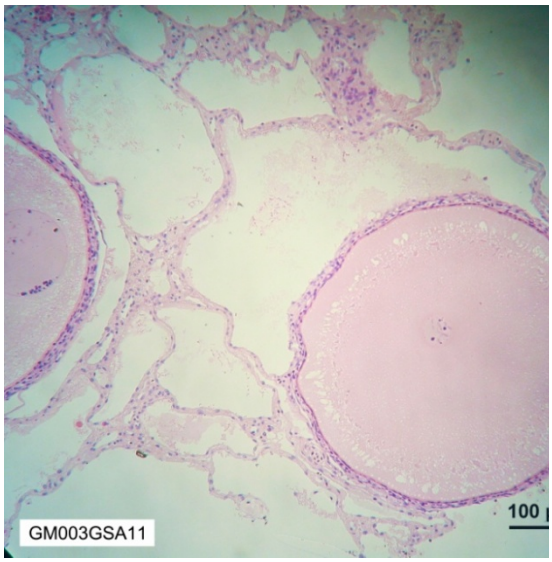


Figure 3 - Late previtellogenic follicle.

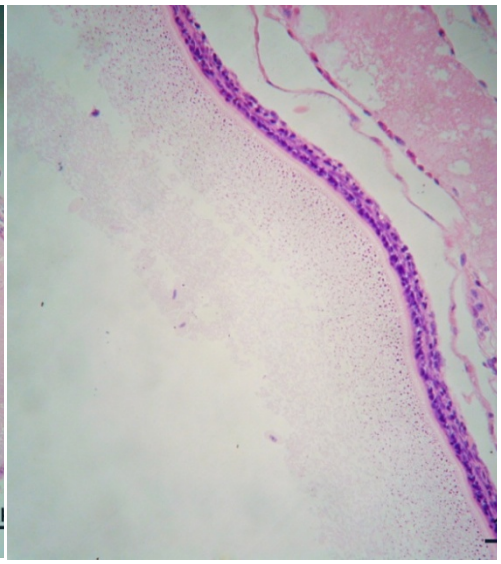


Figure 4 - Vitellogenic follicle. Yolk droplets appears on the ooplasm in follicle up 3000 µm.

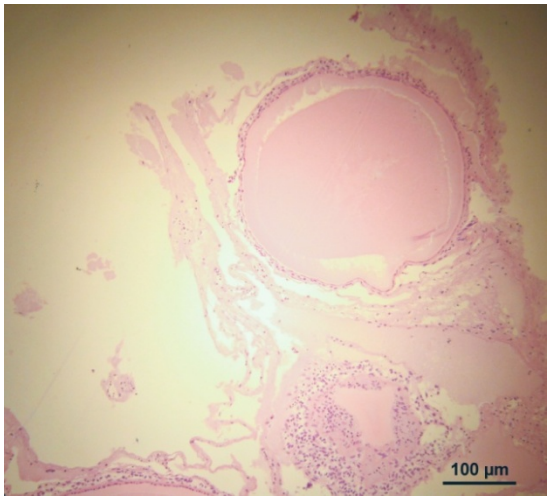


Figure 5 - Atretic follicle (white arrow) in which the basal lamina appeared collapsed and invaded the central lumen (resting stage).

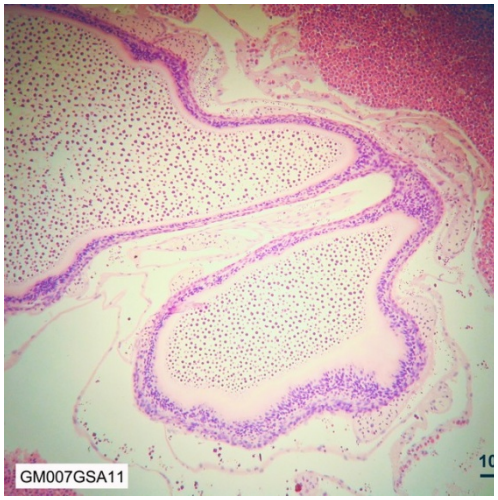


Figure 6 – Atretic follicle (yolk droplets are visible).

Smatogenesis

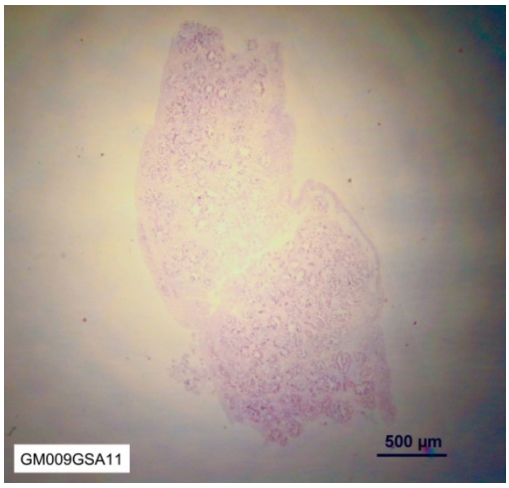


Figure 1 – Immature stage (cysts with only spermatogonia); virgin specimen

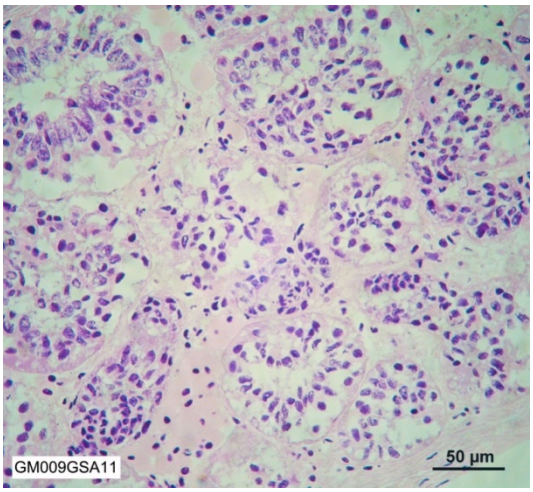


Figure 2 - Spermatogonia in immature gonad.

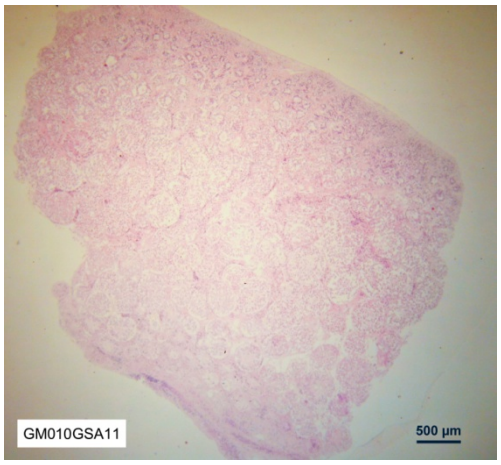


Figure 3 - Immature stage (adult specimen). All stages of spermatogenesis were distinguished, but the proportion of cysts with spermatozoa are very scanty.

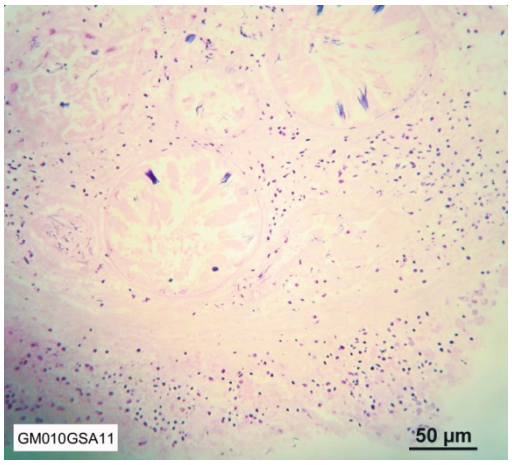


Figure 4 - Spermatocysts with spermatozoa in immature adult stage.

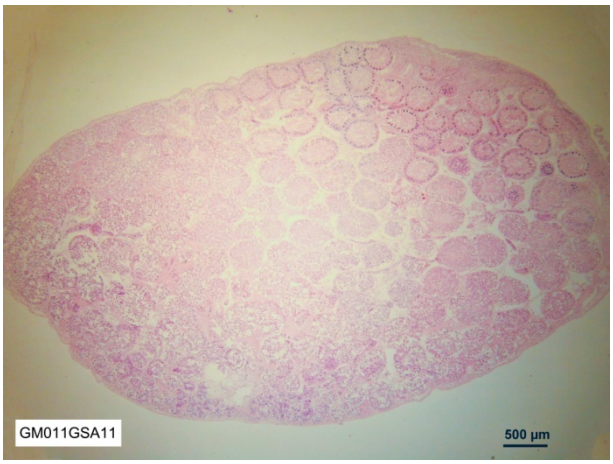


Figure 5 - Maturing stage. all stages of spermatogenesis were distinguished, but the proportion of cysts with spermatids and spermatozoa are greater than the previous stage.following the microscopic scale.

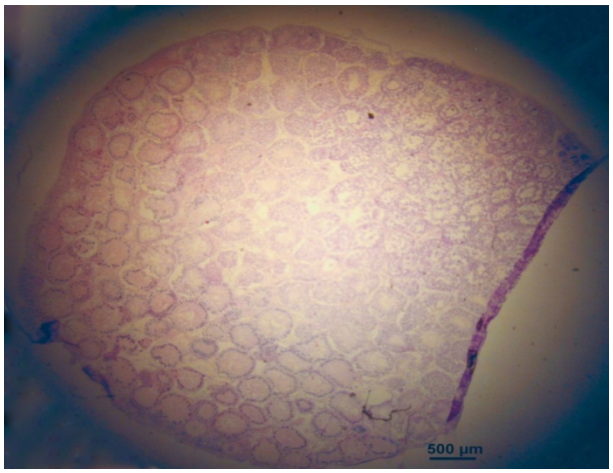


Figure 6 - Mature stage. The proportion of spermatozoa is very abundant.

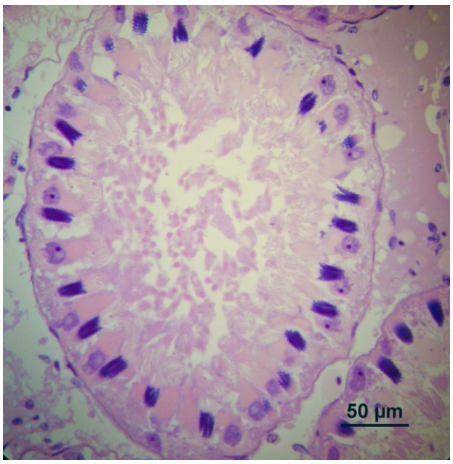


Figure 7 - Spermatocyst with mature spermatozoa. The Sertoli cells nuclei move and fix between the tight bundles of spermatozoa.

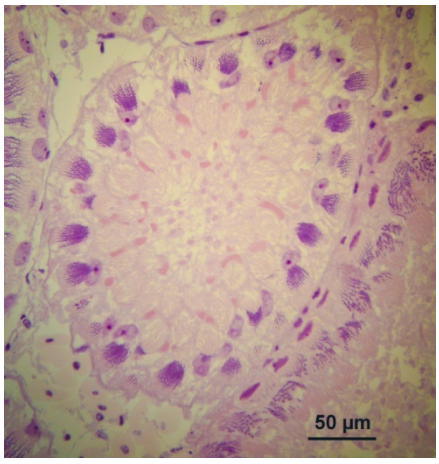


Figure 8 - Spermatocyst with mature spermatozoa.

Microscopic maturity stages of viviparous *Squalus blainvillei*

Porcu C., Mulas A., Cabiddu S., Cannas R., Follesa M.C., Cau A. (University of Cagliari, Cagliari, Italy)

Oogenesis

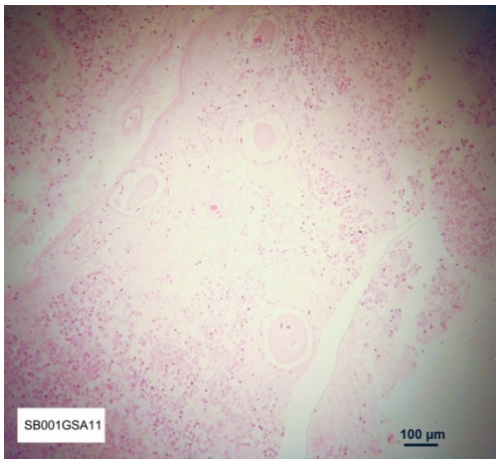


Figure 1 - Immature stage, early previtellogenic follicles.

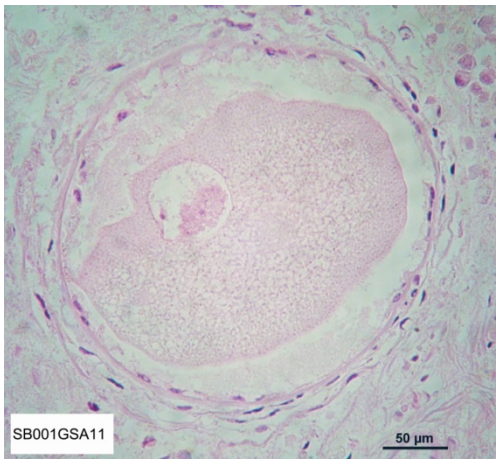


Figure 2 - Early previtellogenic follicle.

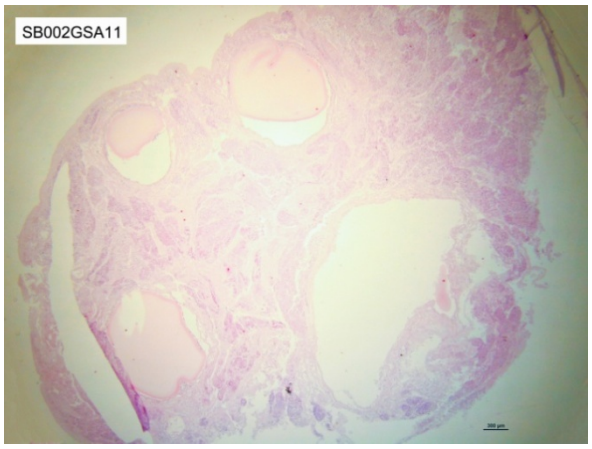


Figure 3 - Maturing stage, late previtellogenic follicles.

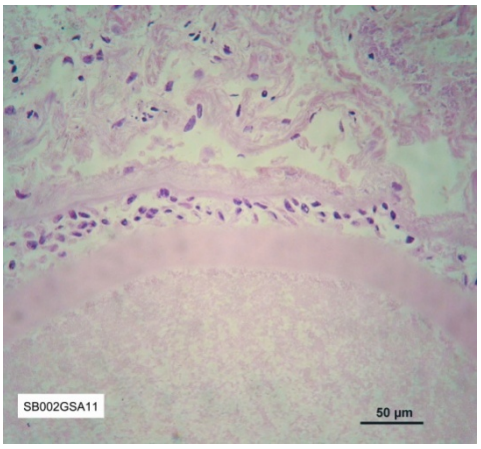


Figure 4 - Follicular epithelium of the late previtellogenic follicle

Spermatogenesis

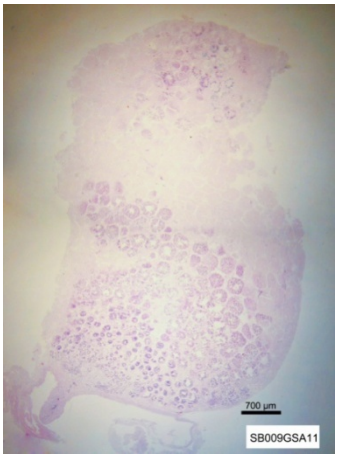


Figure 1 - Immature stage (adult specimen). All stages of spermatogenesis were distinguished, but the proportion of cysts with spermatozoa (spz) are very scanty.

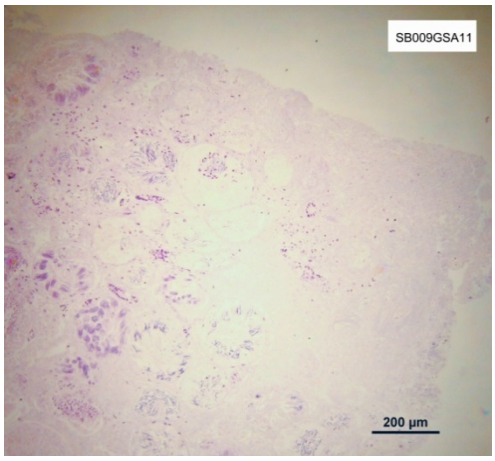


Figure 2 - Spermatocysts with spermatids and spermatozoa in immature adult stage.

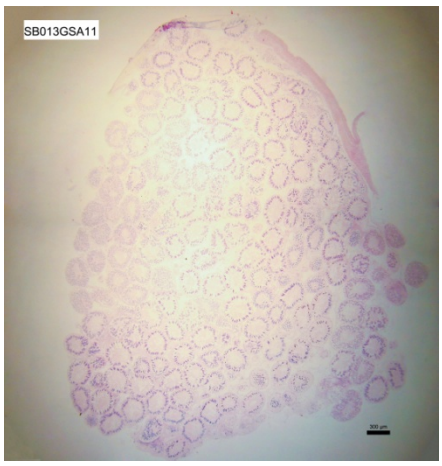


Figure 3 - Mature stage. The proportion of spermatozoa is very abundant.

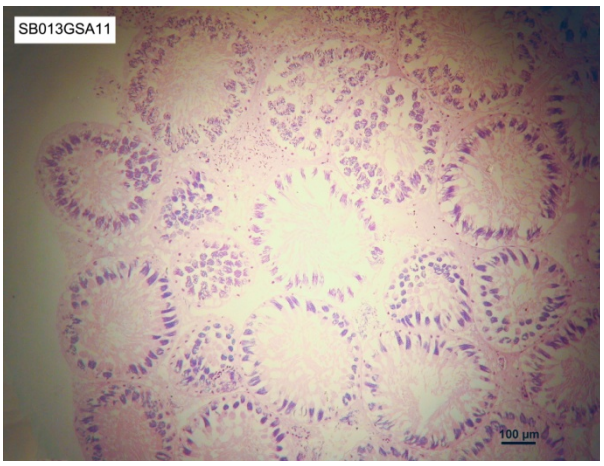
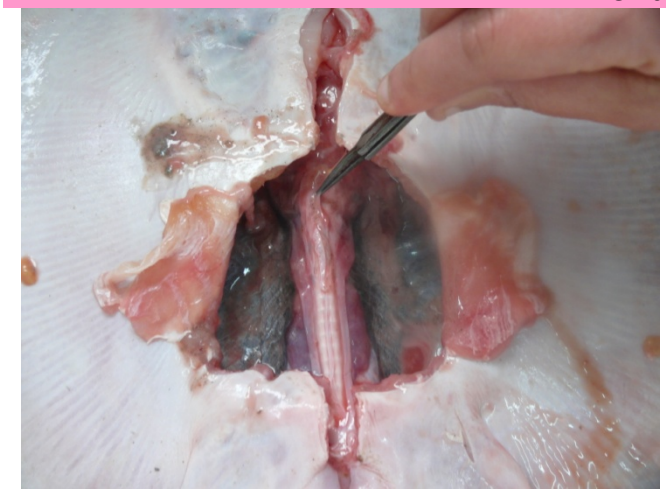


Figure 4 – Spermatocysts filled with spermatids and spermatozoa in mature stage.

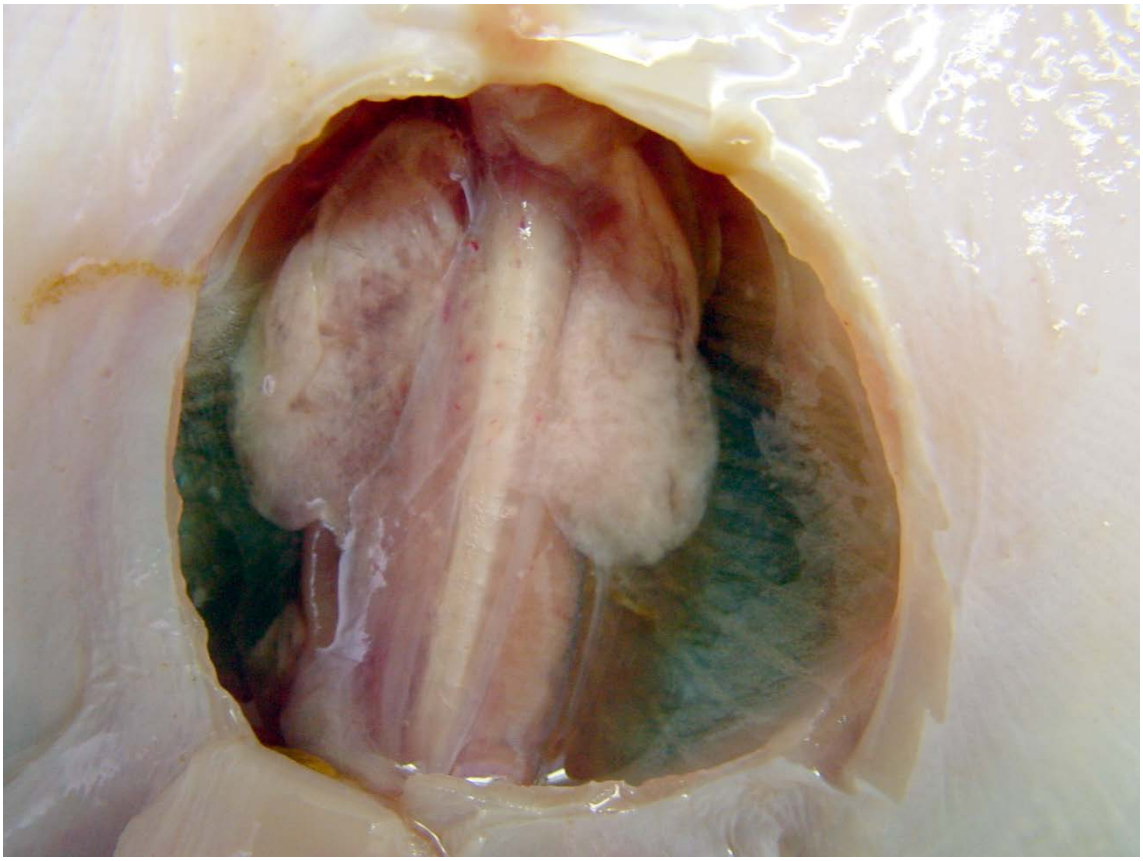
Annex 11: Macroscopic oviparous gonad reference photos

SKATES
Females 1



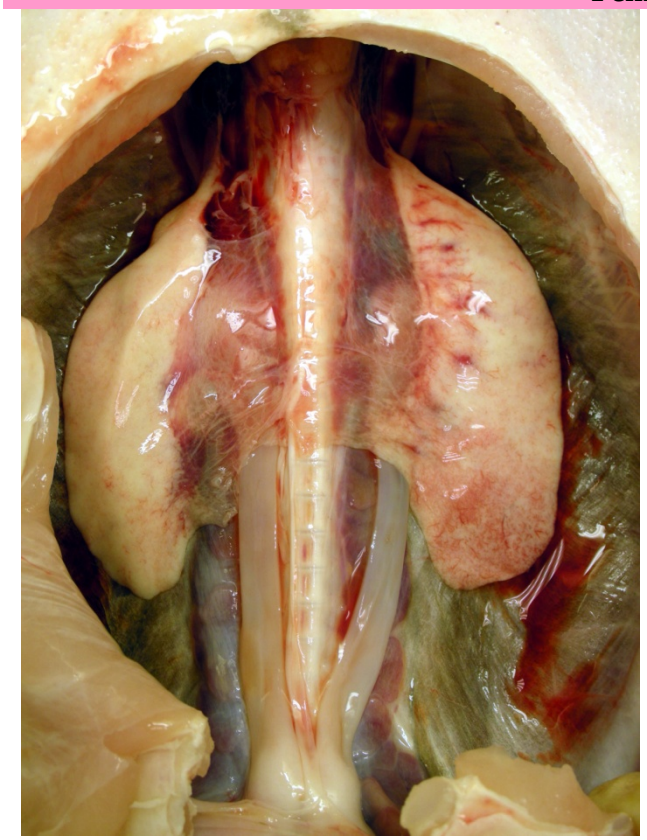
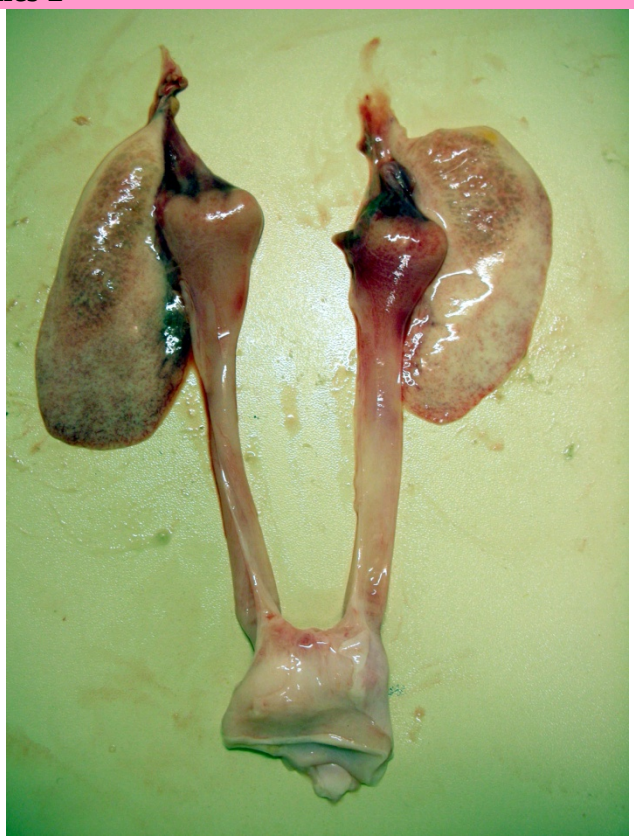
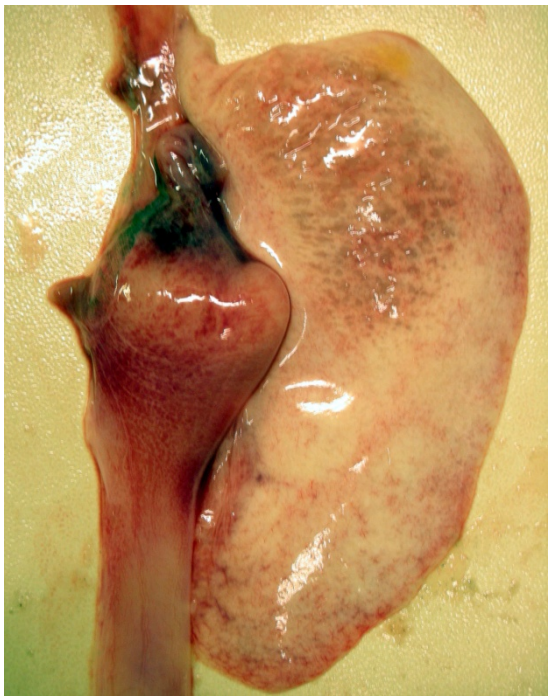
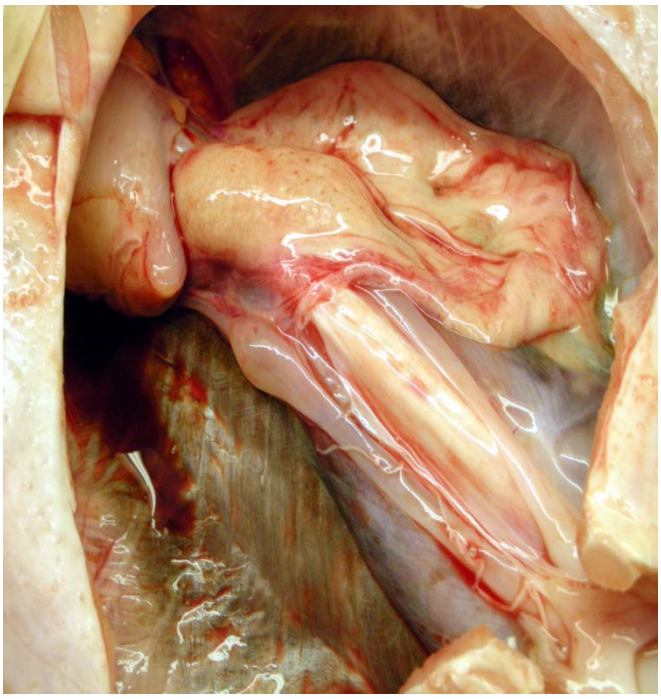
Raja asterias by USTHB

Raja clavata by IPIMAR



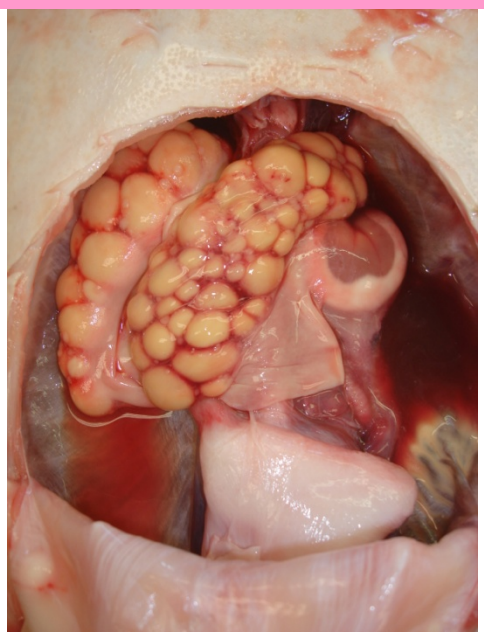
Raja clavata by ARPAT

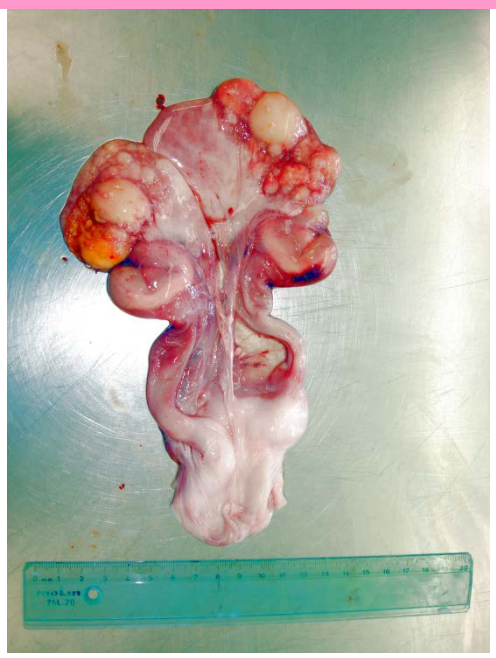
SKATES
Females 2

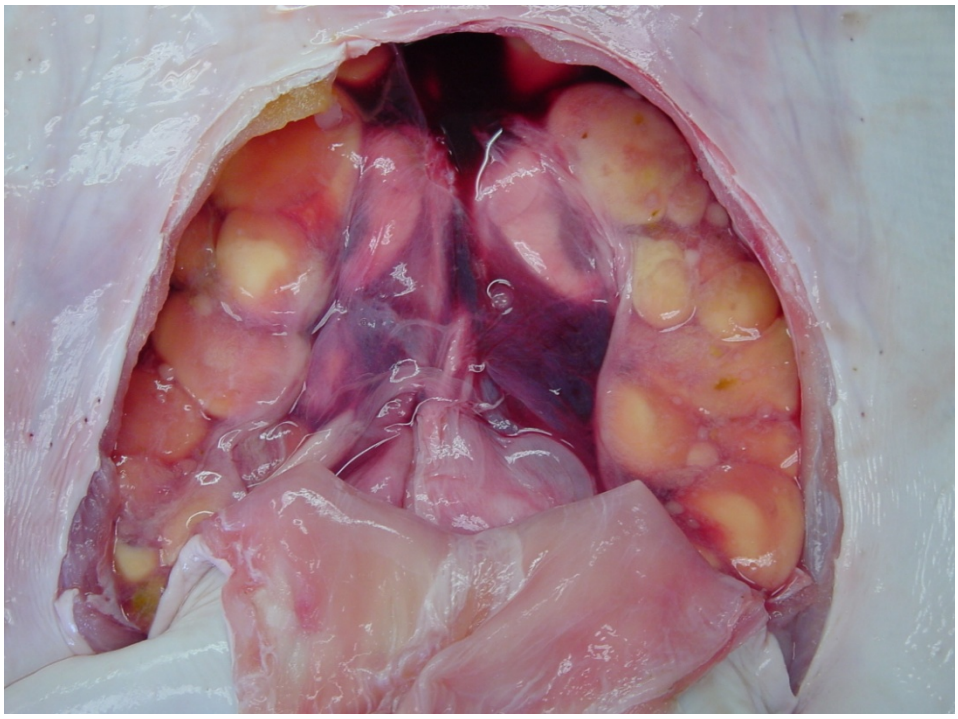
Raja clavata by ARPAT

SKATES
Female 3a



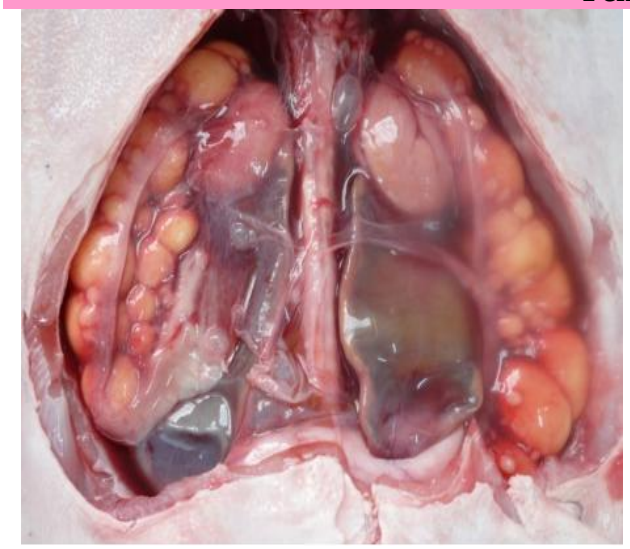


Raja clavata by IPIMAR

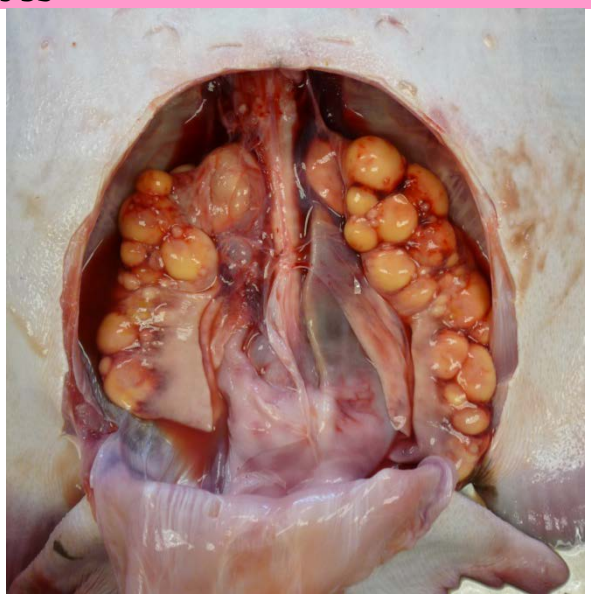


Raja montagui by IPIMAR

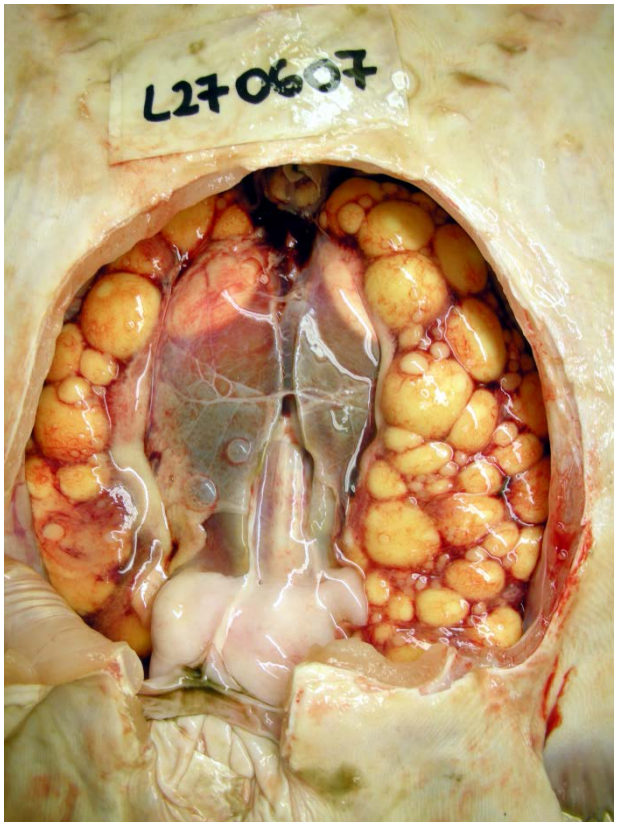
SKATES
Females 3b

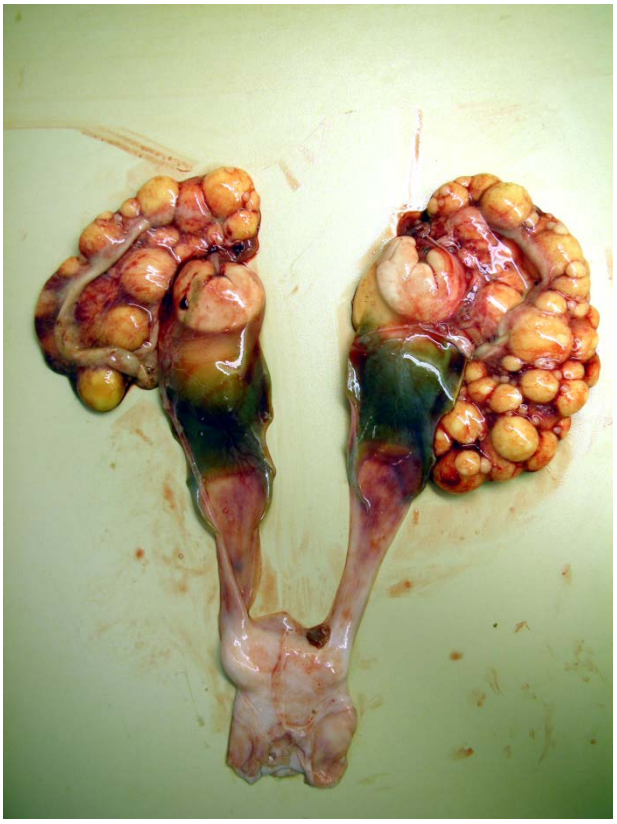


Raja asterias by USTHB



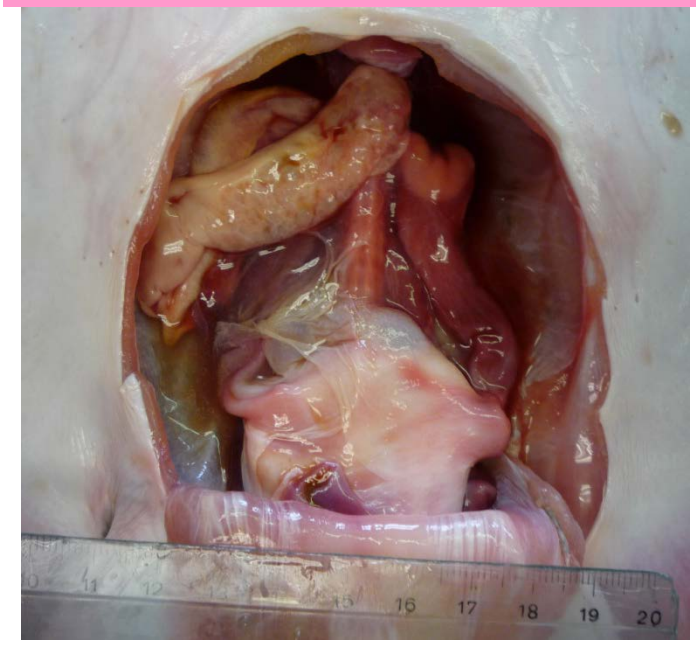
Raja clavata by IPIMAR



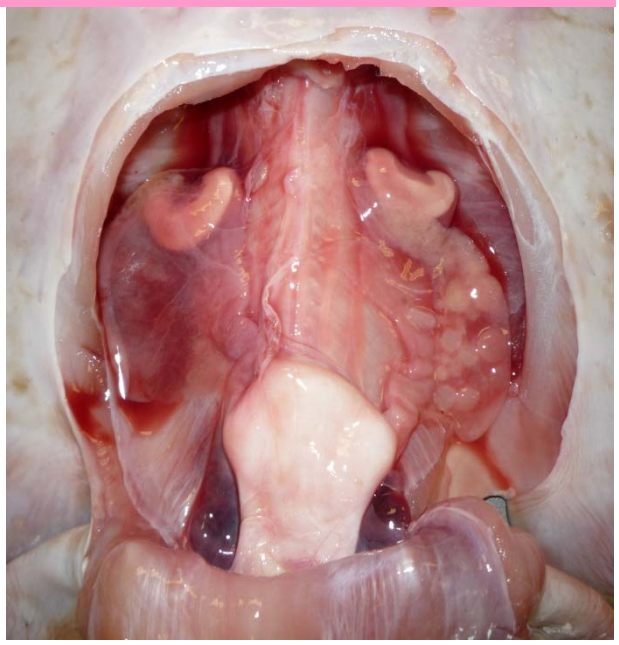


Raja clavata by ARPAT

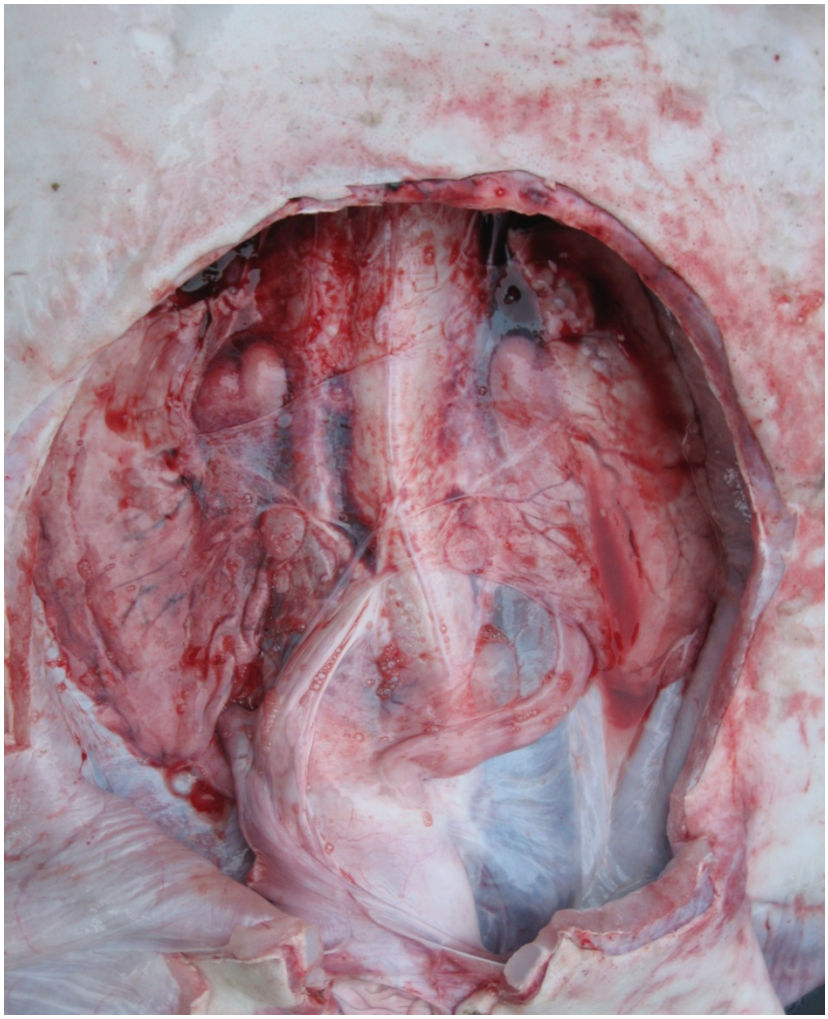
SKATES
Females 4a



Raja montagui by IPIMAR

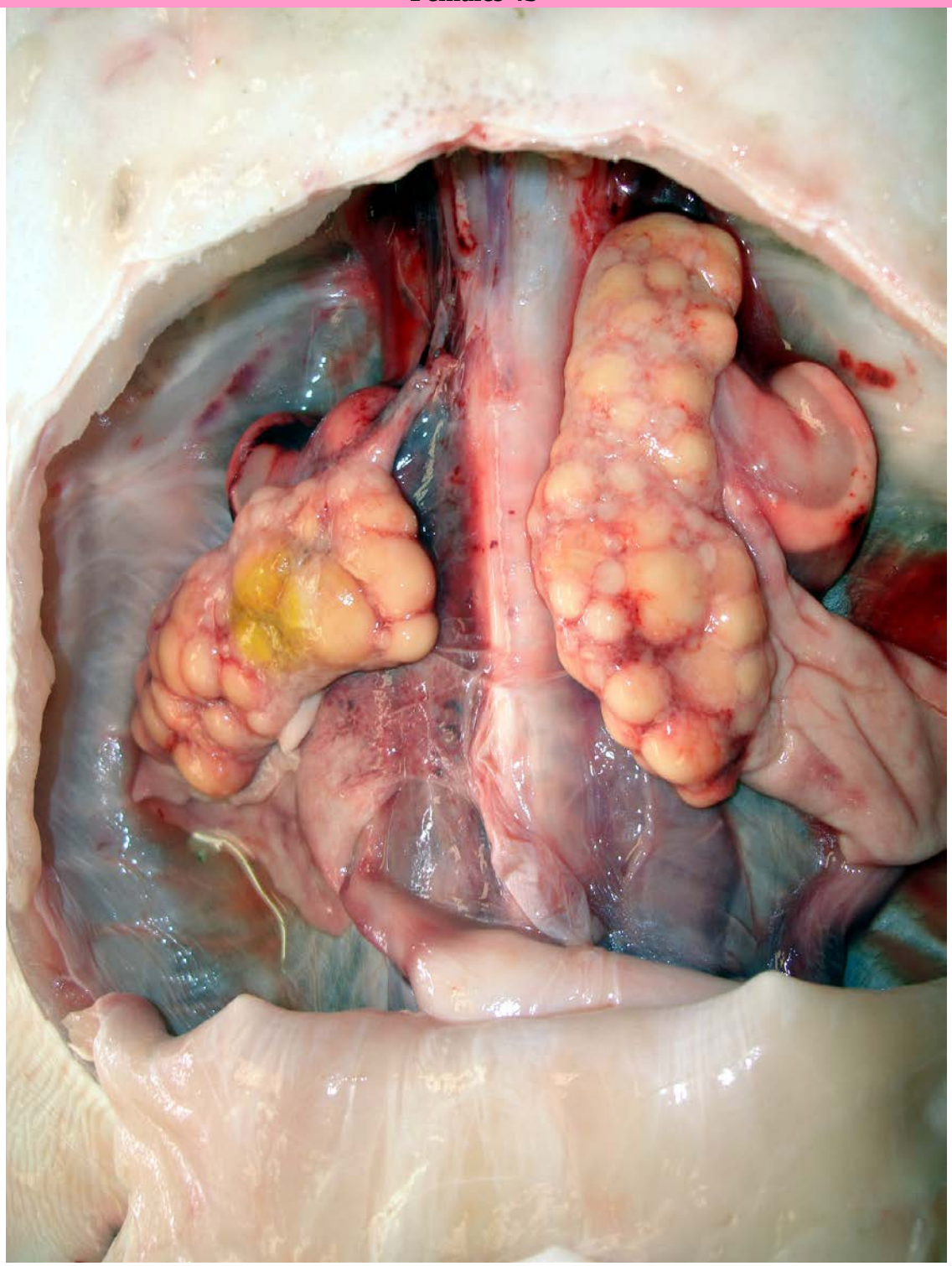


Raja naevus by IPIMAR



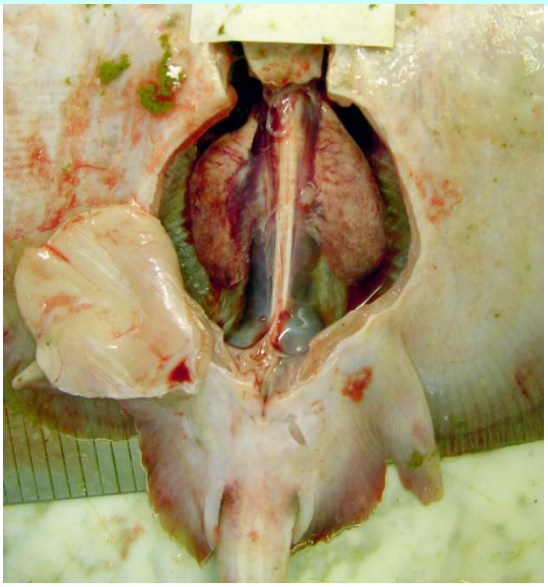
Rostroraja alba by ARPAT

SKATES
Females 4b

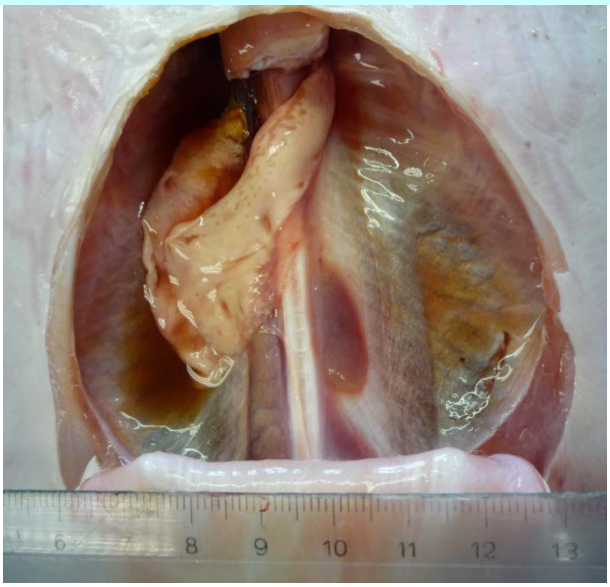


Raja clavata by IPIMAR

SKATES
Male 1

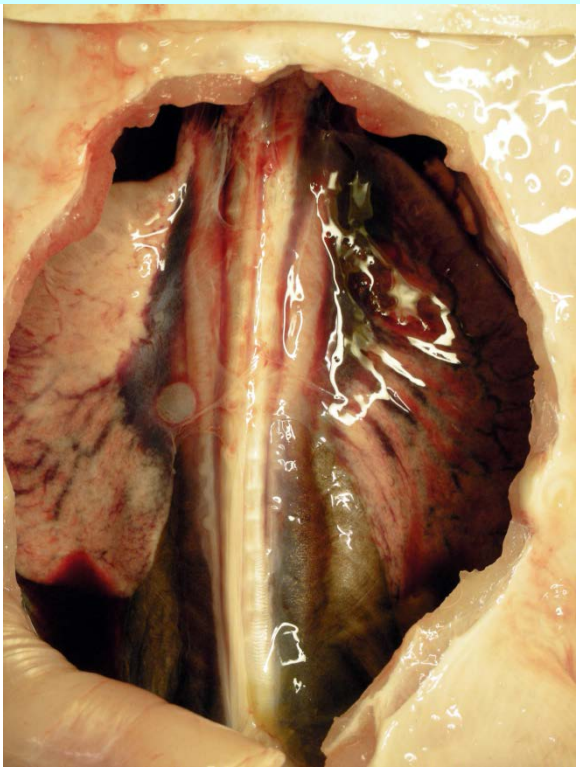


Raja clavata by ARPAT

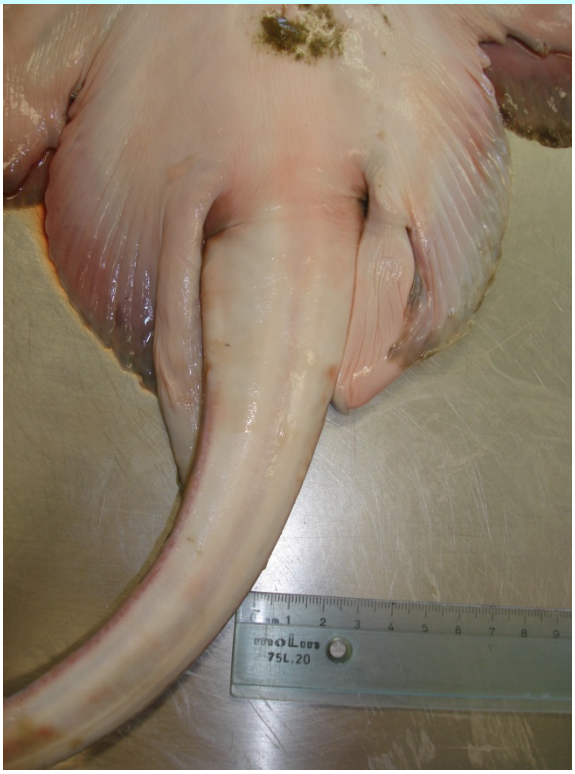


Raja clavata by IPIMAR

SKATES
Male 2

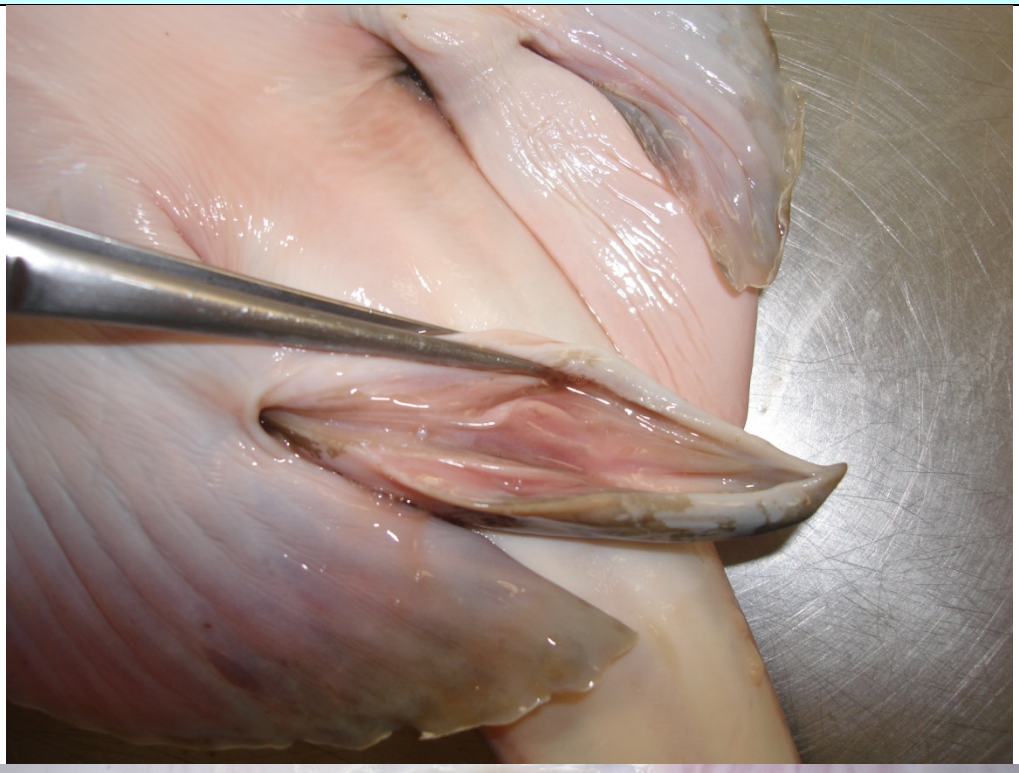


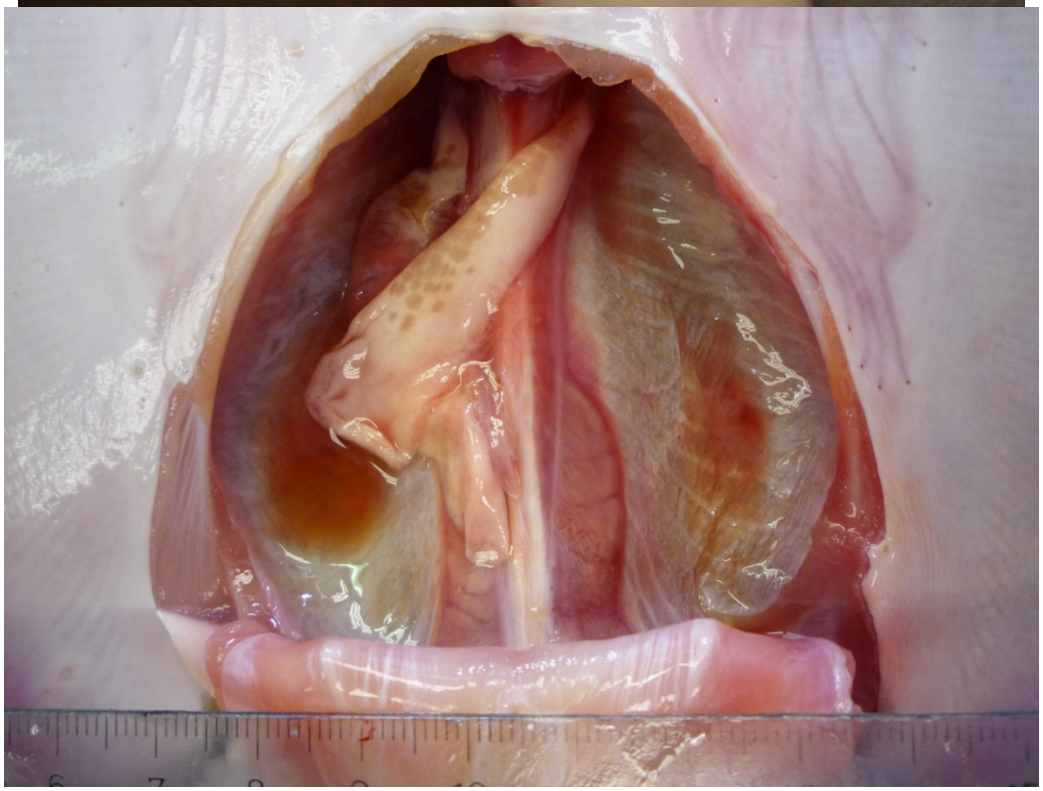
Raja clavata by ARPAT



Raja brachyura by IPIMAR

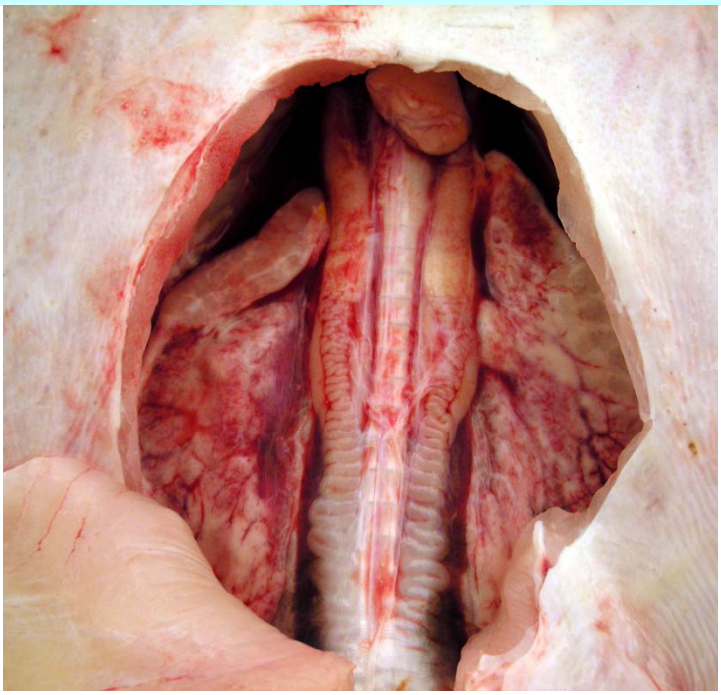
SKATES
Male 2

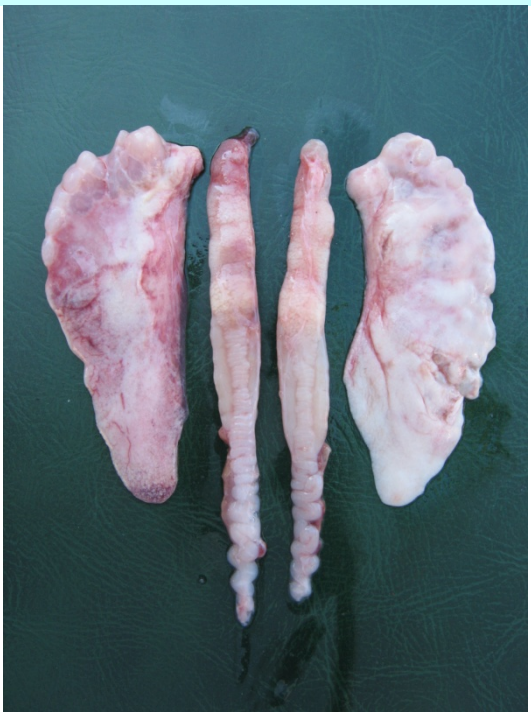




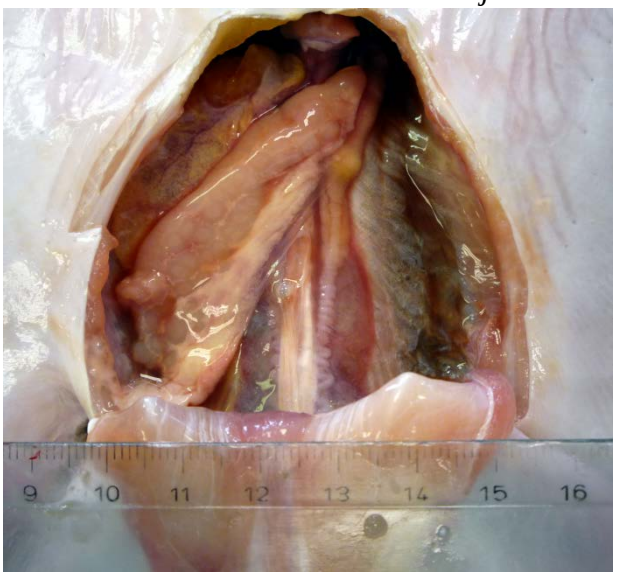
Raja brachyura by IPIMAR

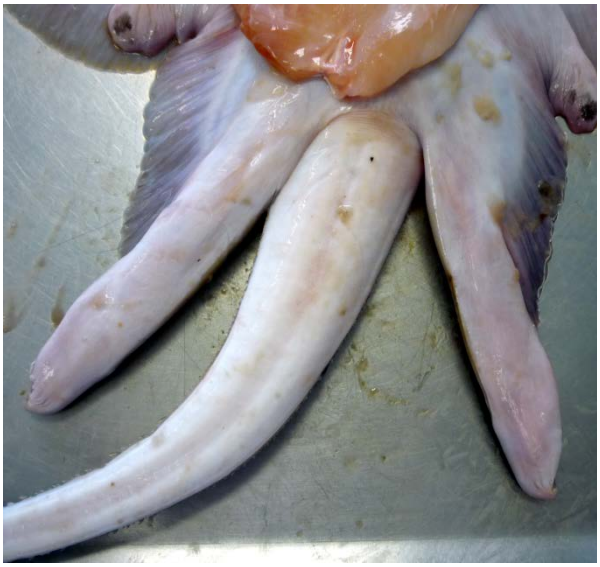
SKATES
Males 3a





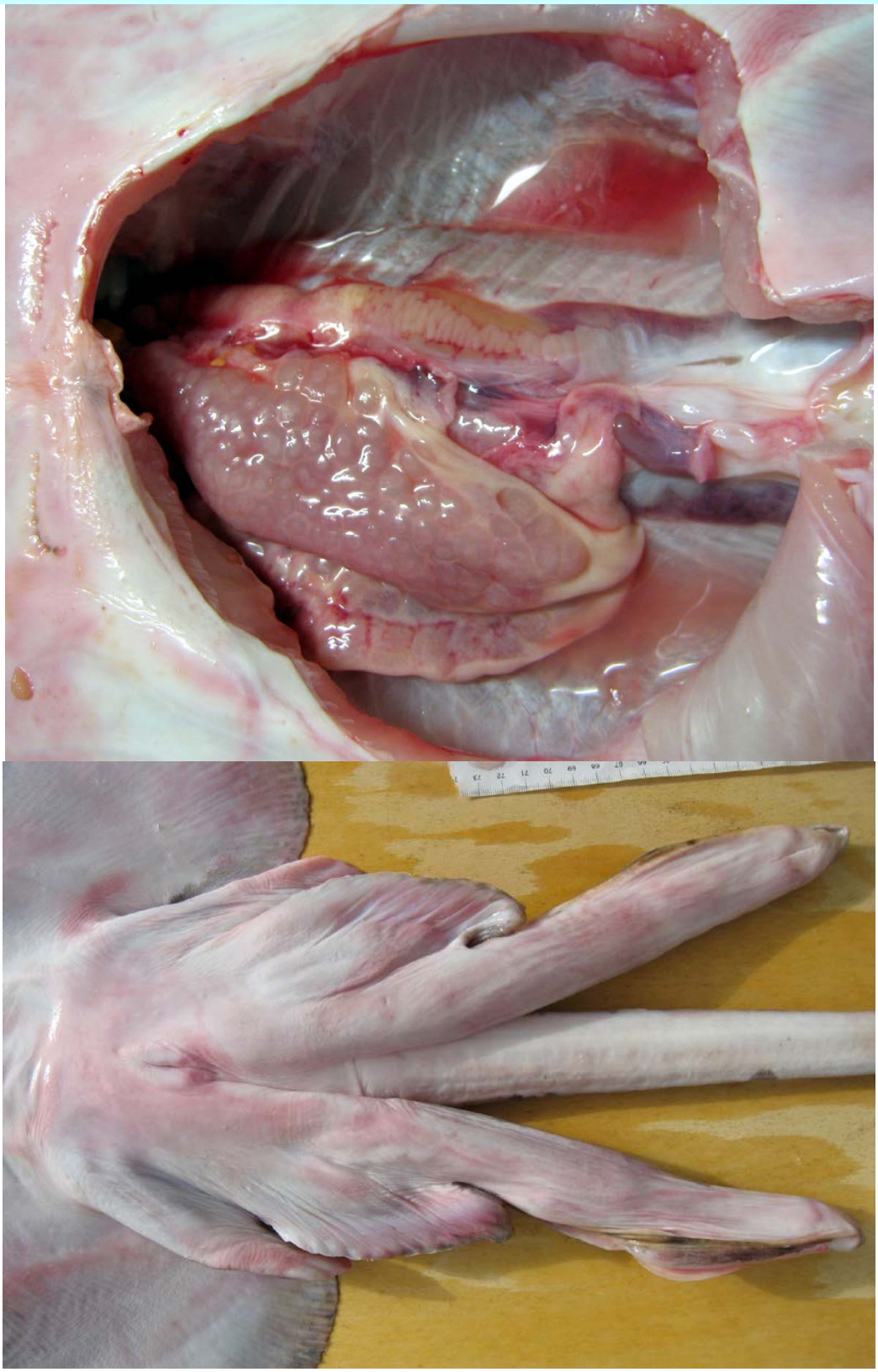
Raja clavata by ARPAT





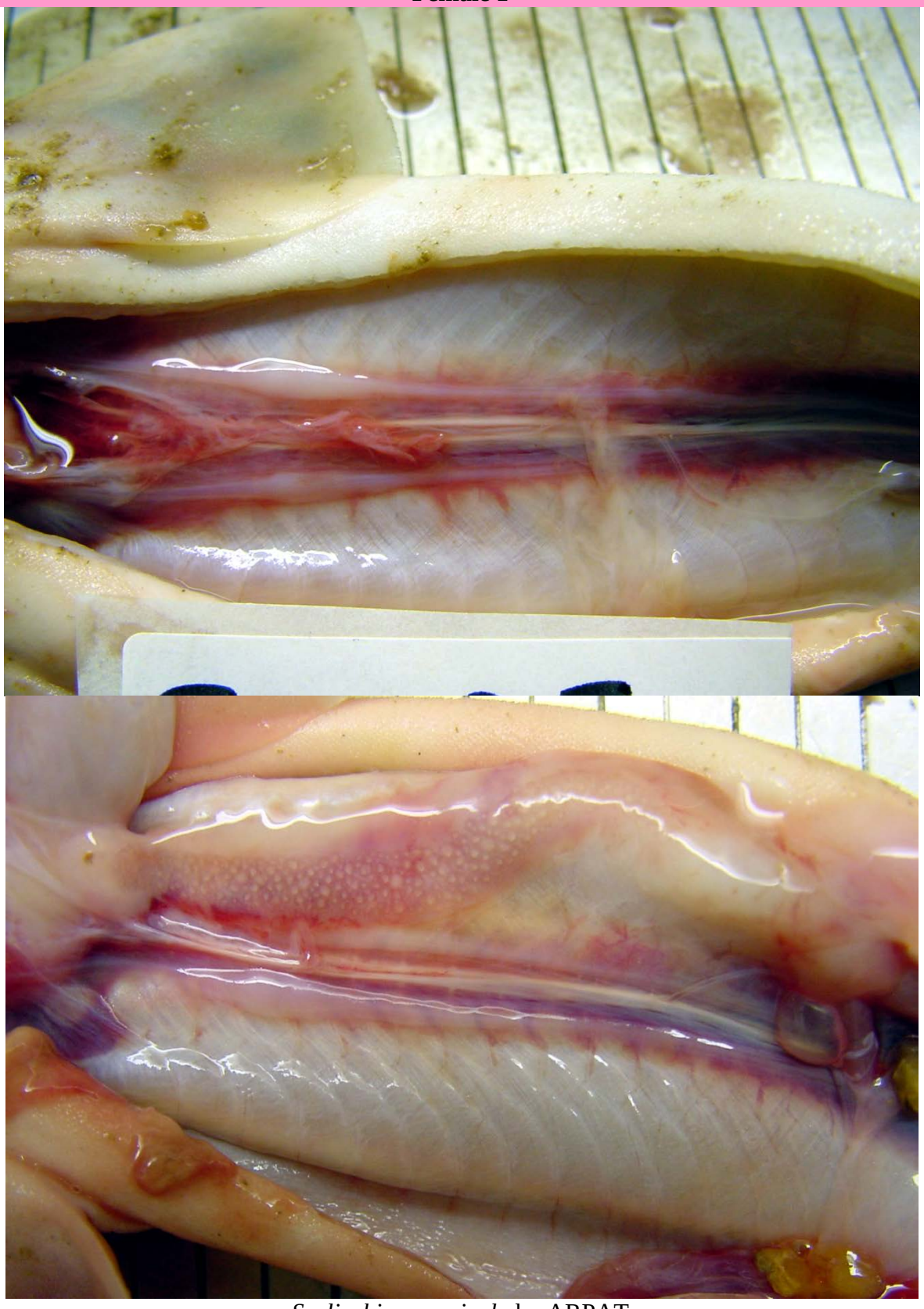
Raja naevus by IPIMAR

SKATES
Males 3b



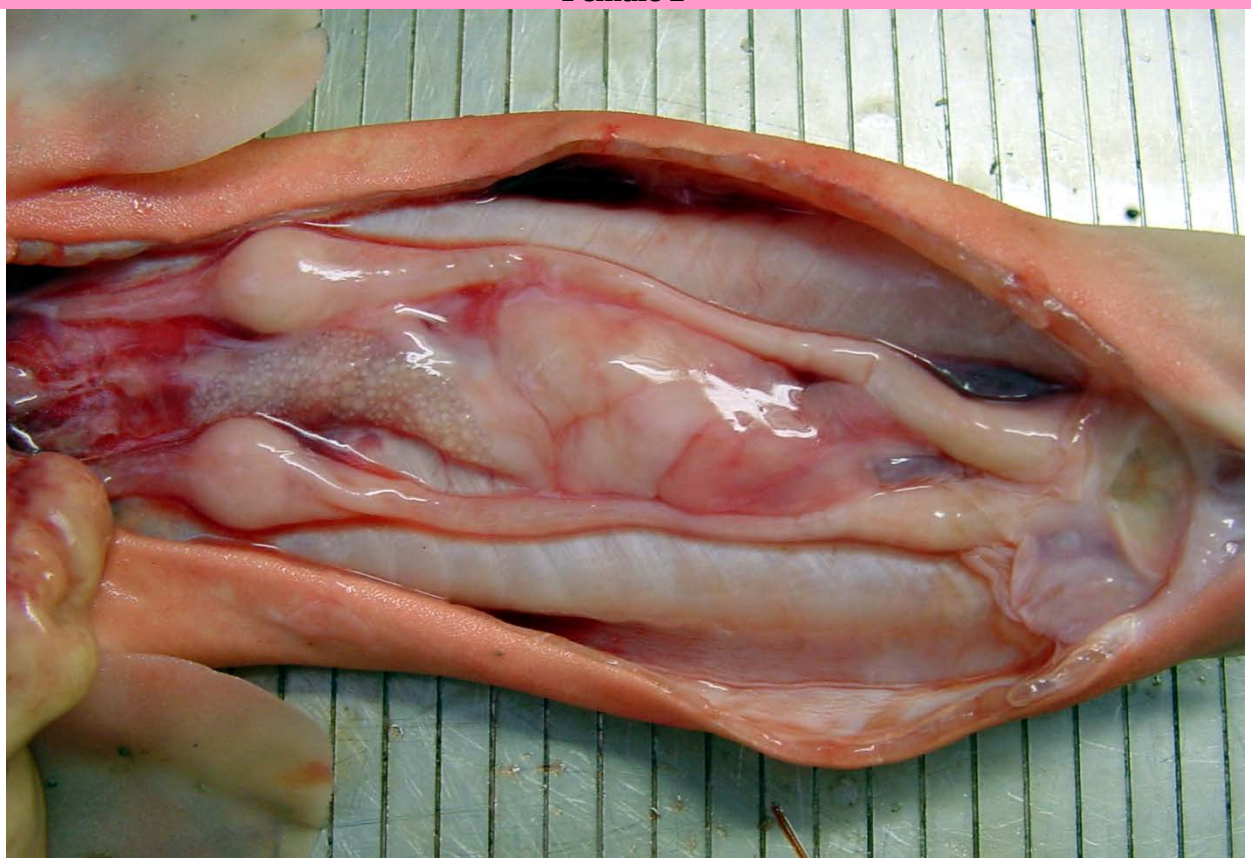
Raja brachyura by ARPAT

SHARKS
Female 1

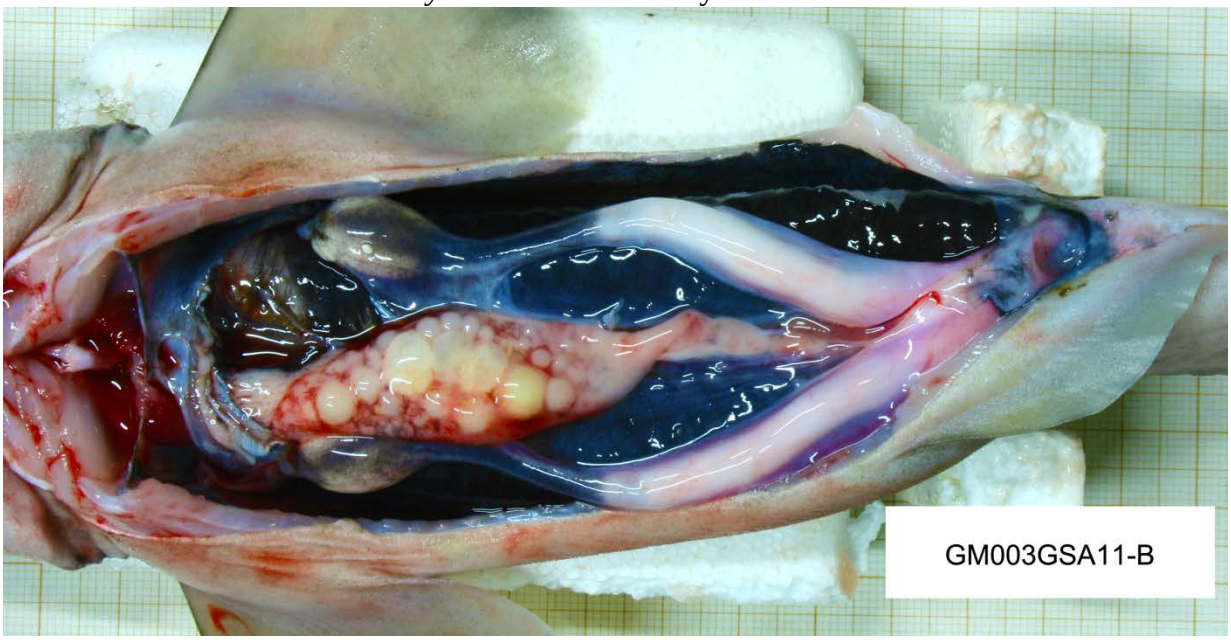


Scyliorhinus canicula by ARPAT

SHARKS
Female 2

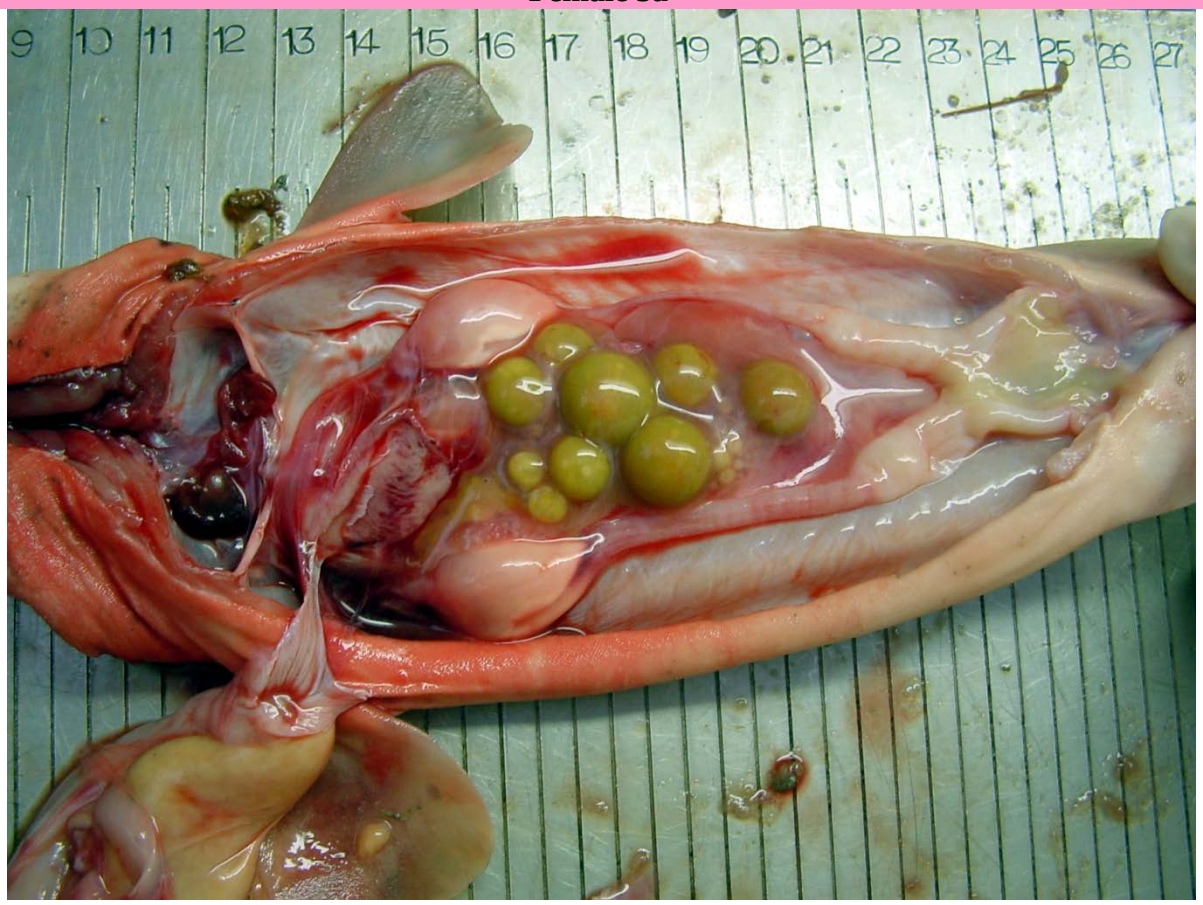


Scyliorhinus canicula by ARPAT



Galeus melastomus by Univ. Cagliari

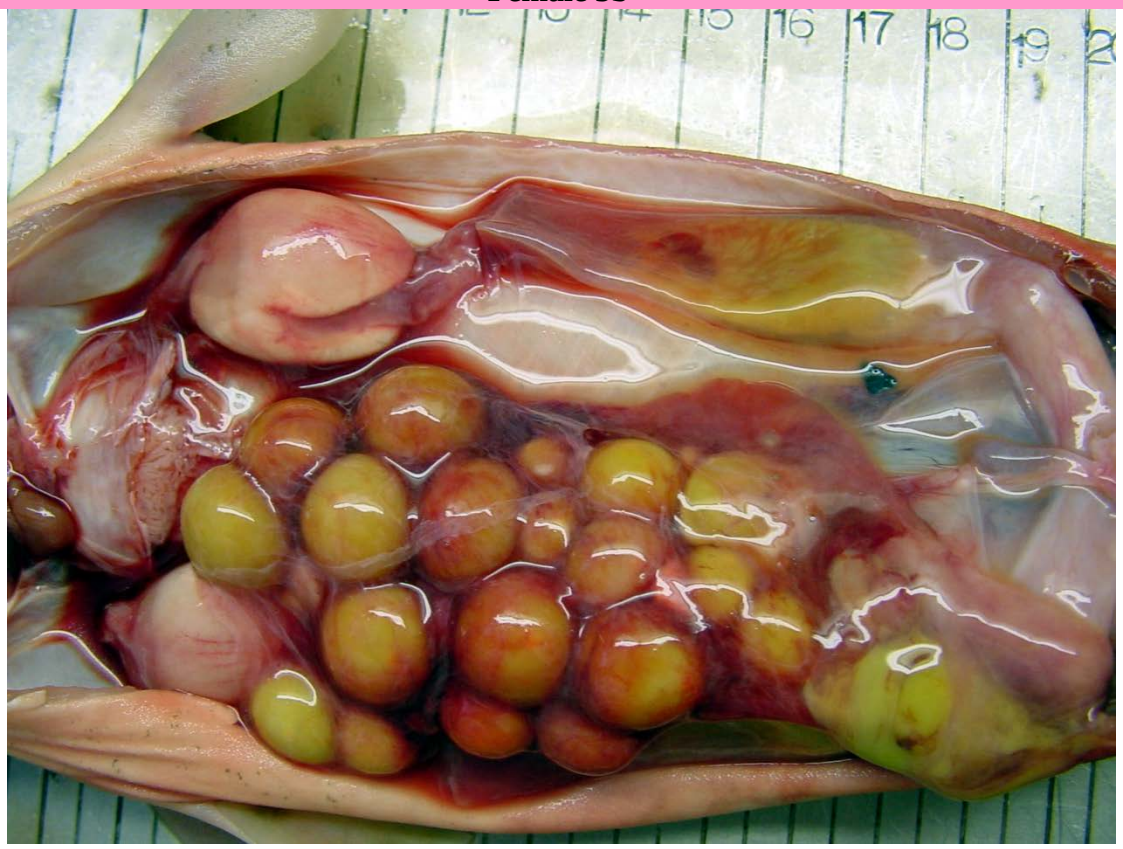
SHARKS
Female 3a



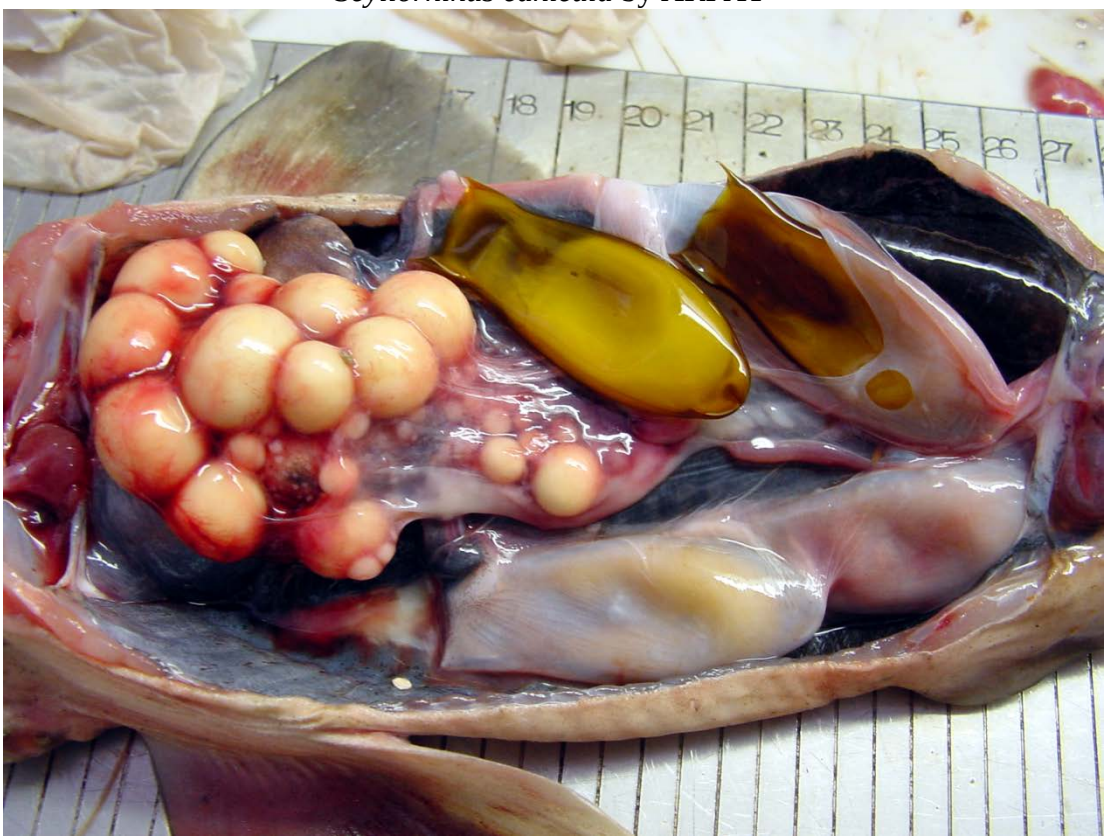
Scyliorhinus canicula by ARPAT

Galeus melastomus by Univ. Bari

SHARKS
Female 3b



Scyliorhinus canicula by ARPAT

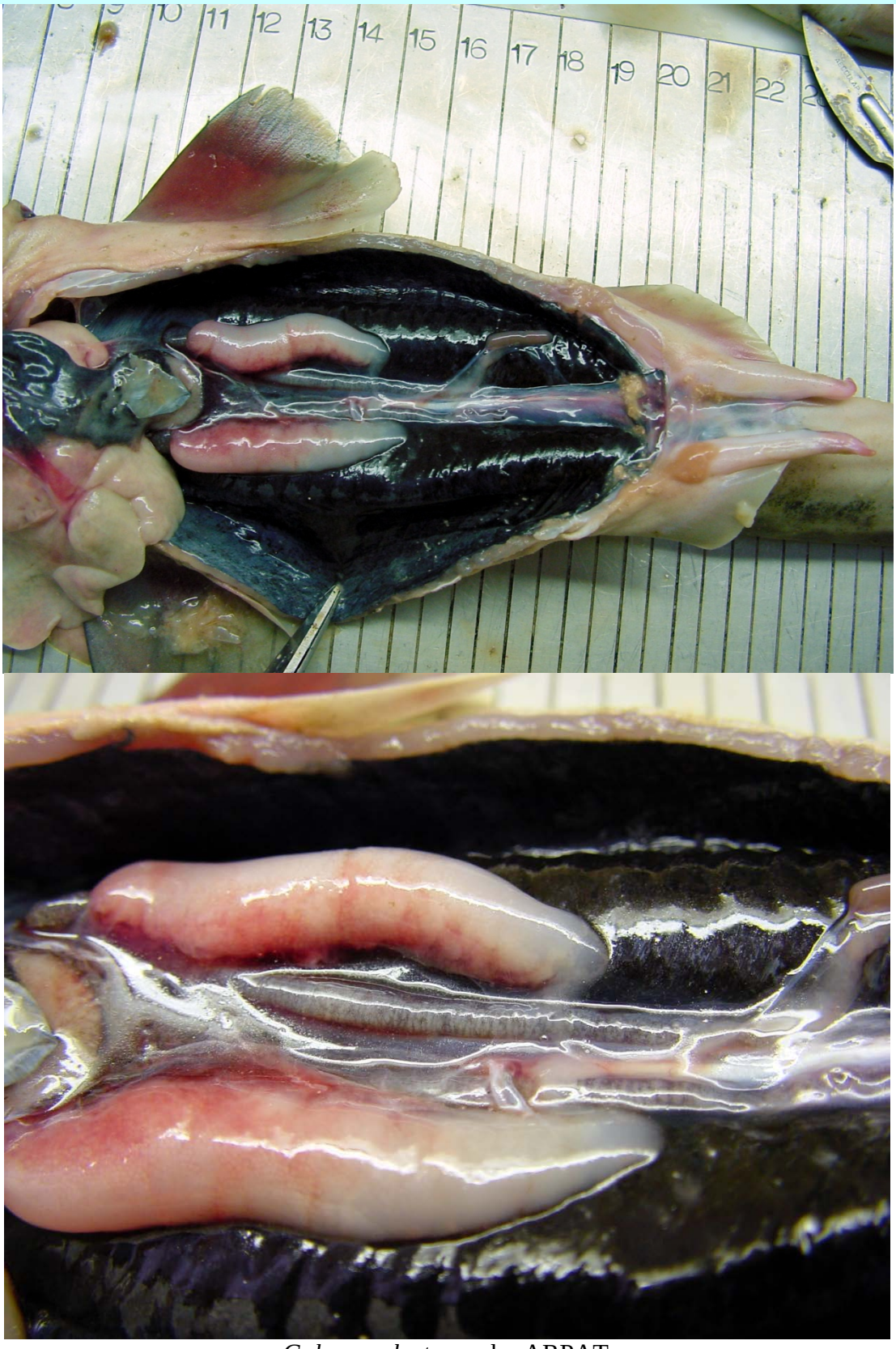


Galeus melastomus by ARPAT

SHARKS
Males 1

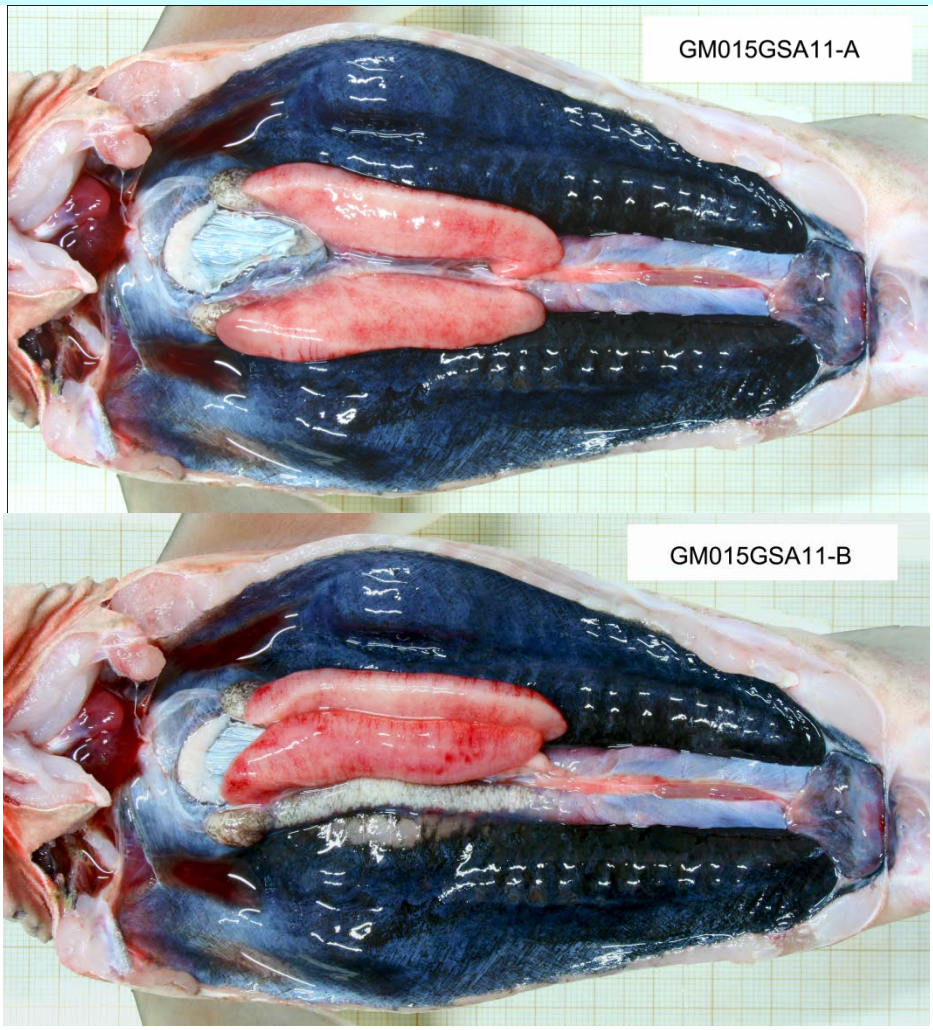
Galeus melastomus by Univ. Cagliari

SHARKS
Males 2

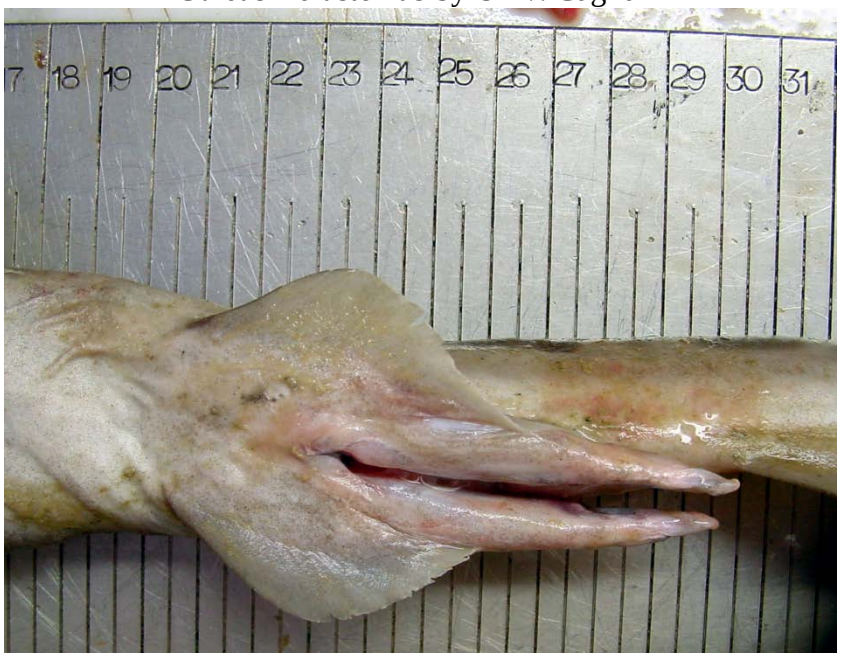


Galeus melastomus by ARPAT

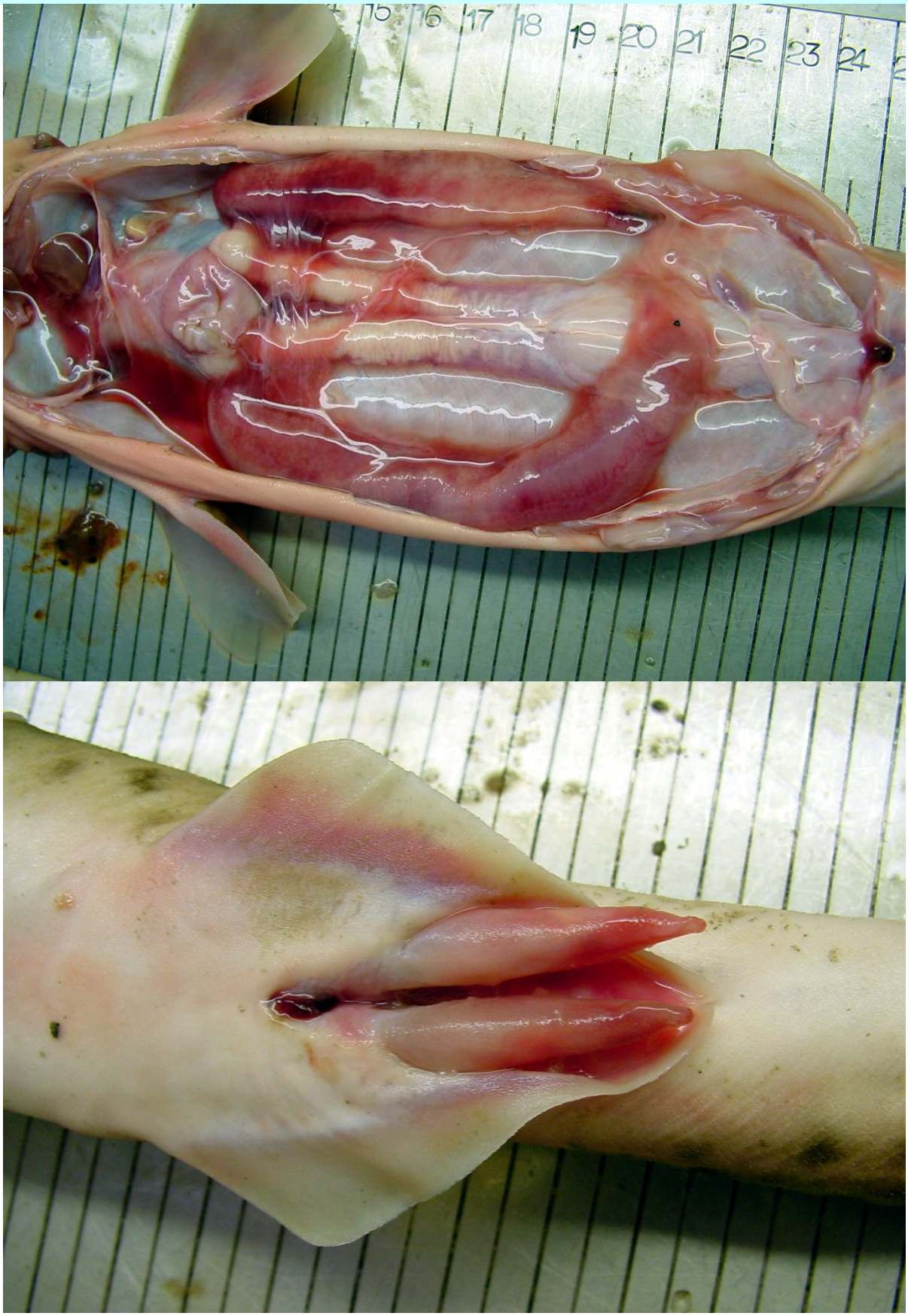
SHARKS
Males 3a



Galeus melastomus by Univ. Cagliari

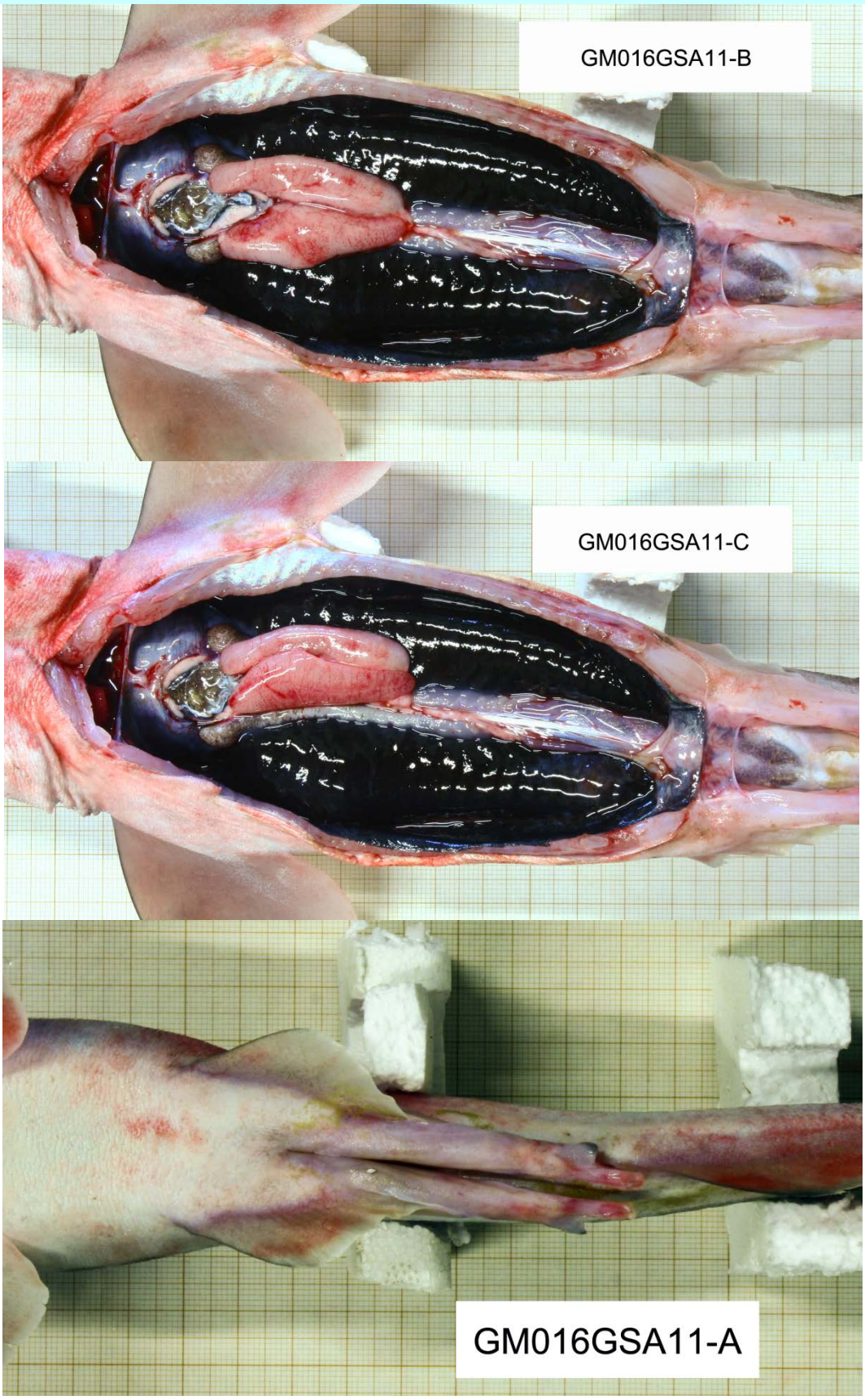


SHARKS
Males 3b



Scyliorhinus canicula by ARPAT

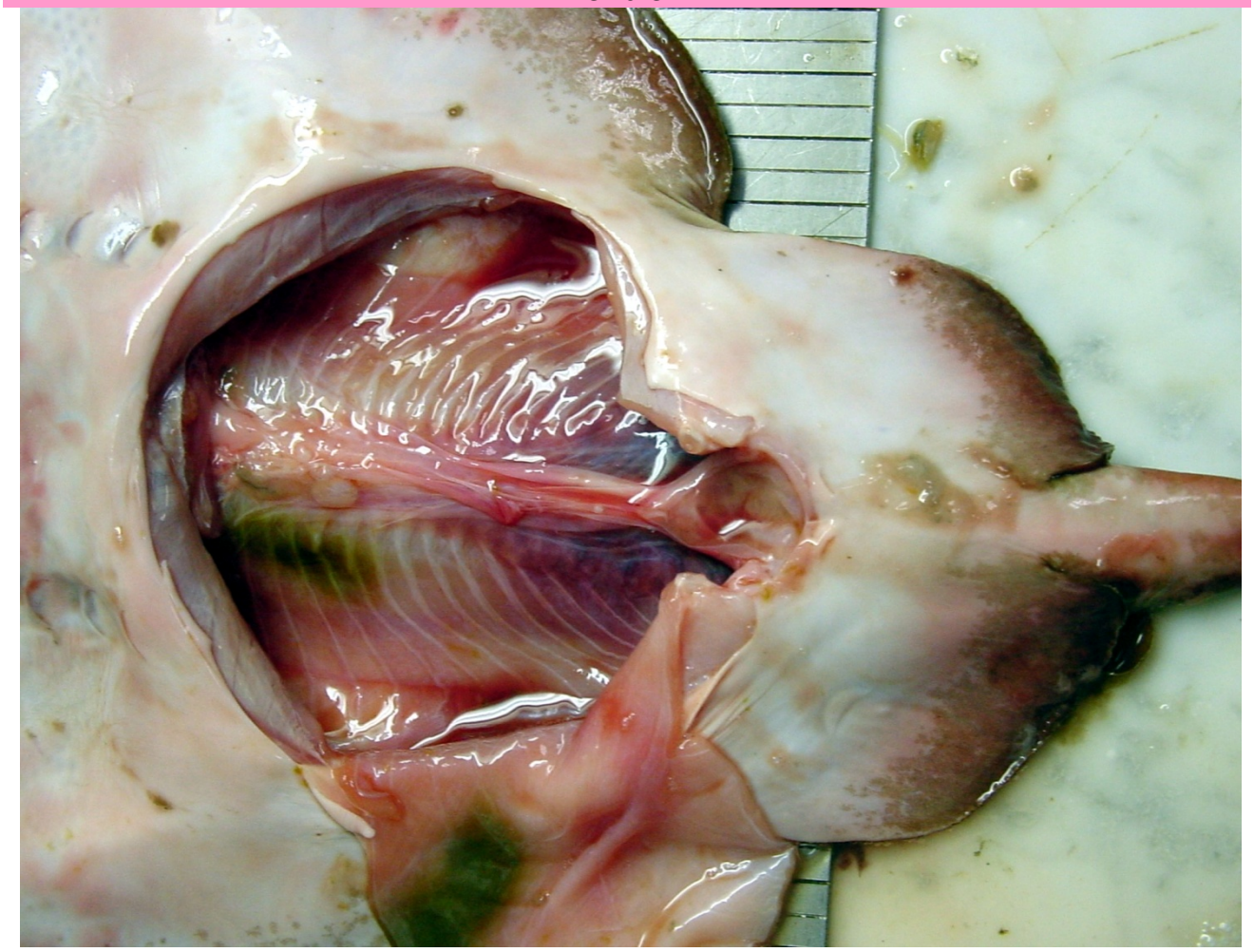
SHARKS
Males 4



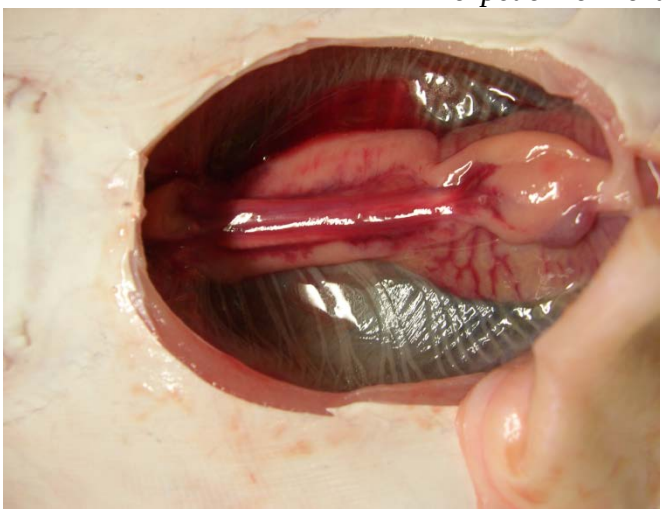
Galeus melastomus by Univ. Cagliari

Annex 12: Macroscopic viviparous gonad reference photos

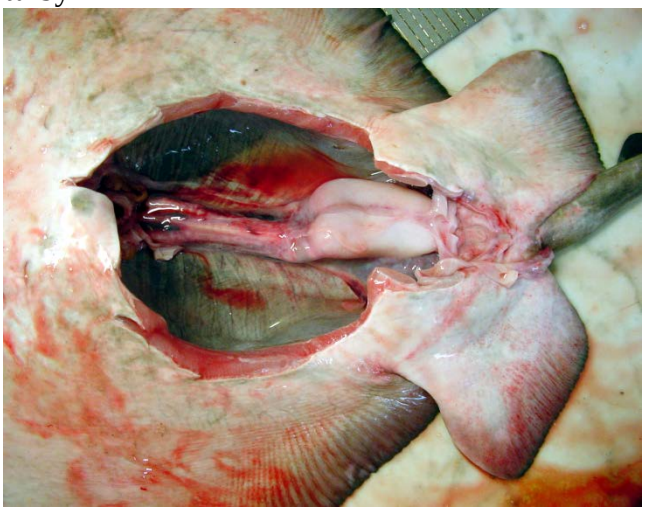
Batoids
Female 1



Torpedo mormorata by ARPAT

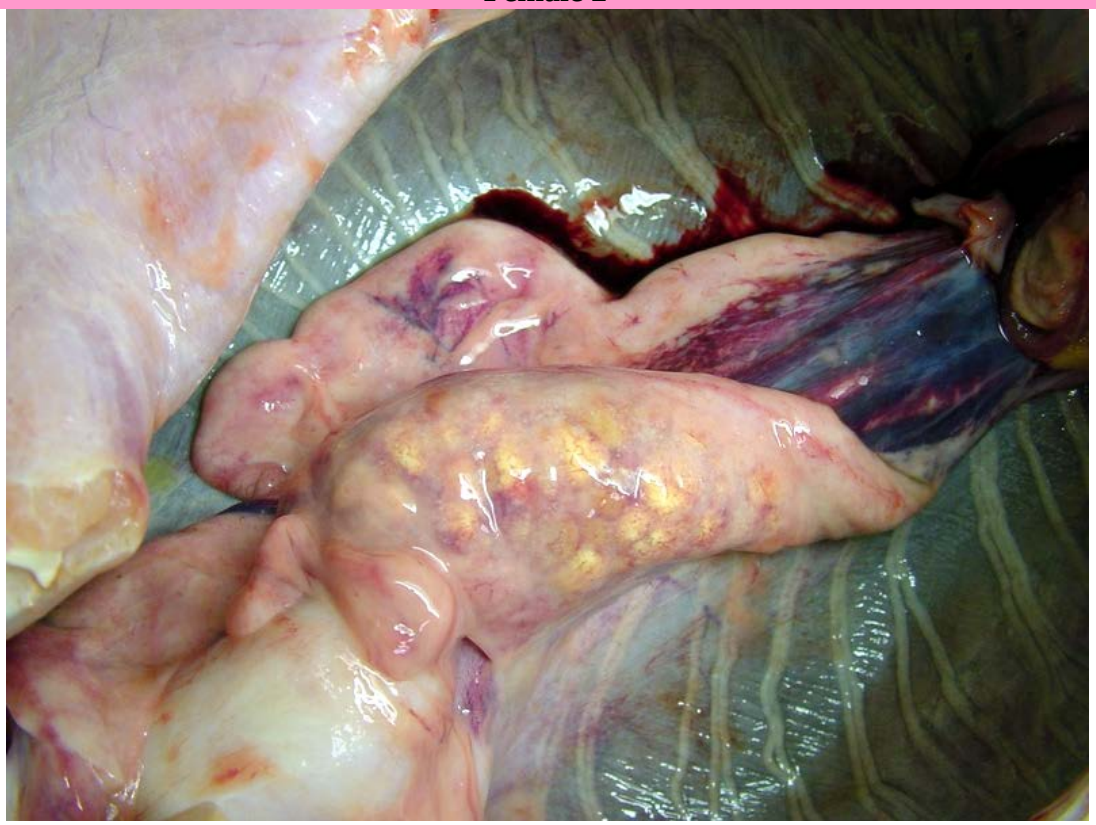


Pteromilaeus bovinus by ARPAT

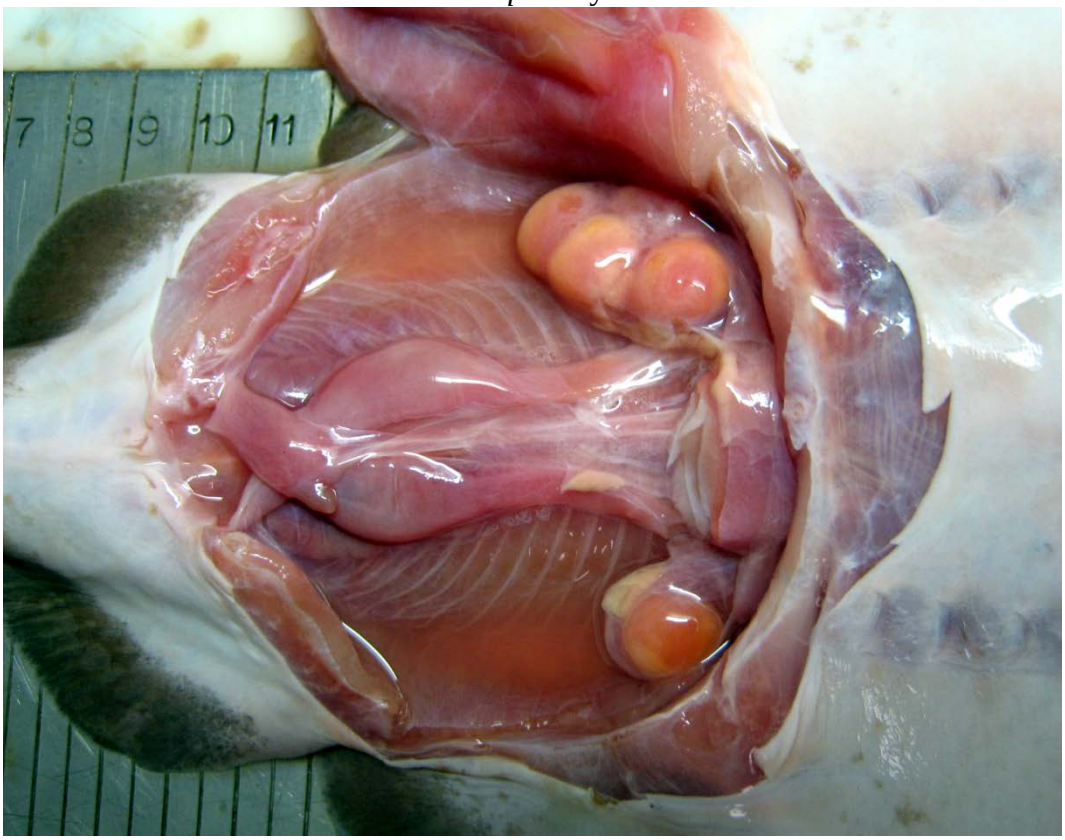


Miliobatis aquila by ARPAT

Batoids
Female 2

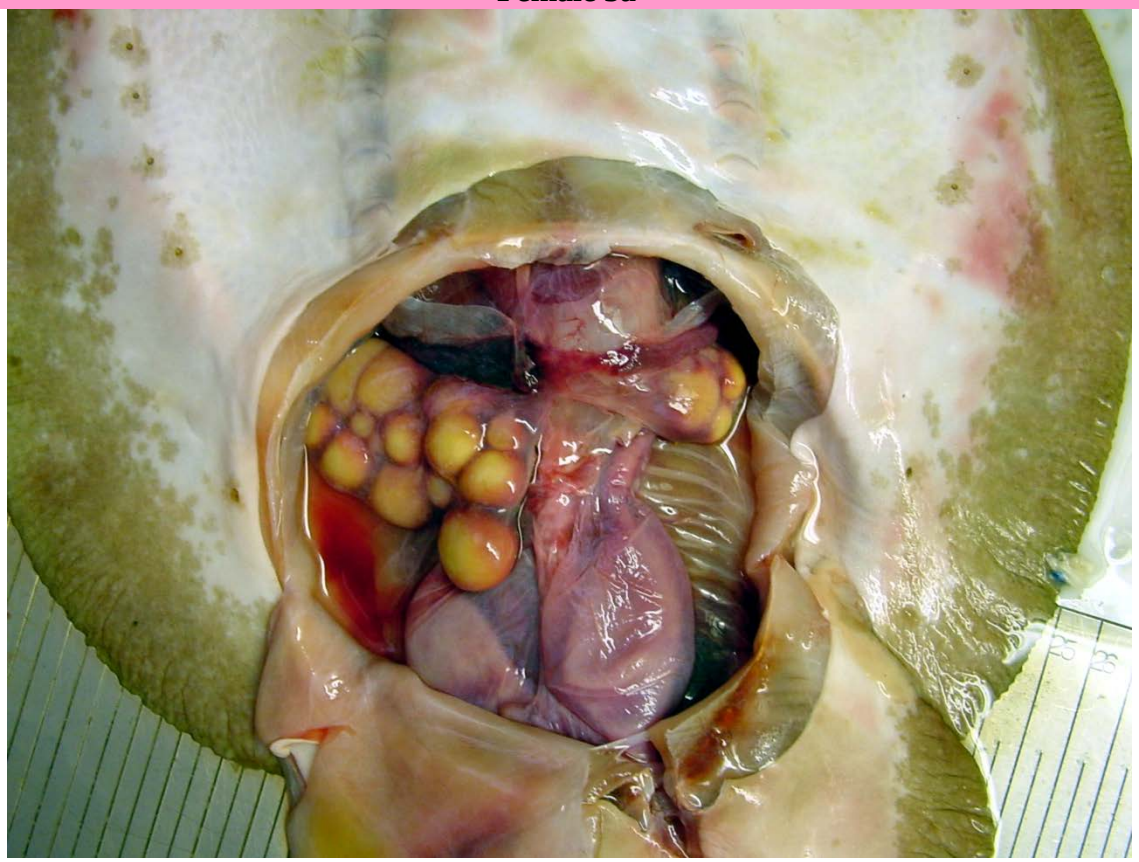


Miliobatis aquila by ARPAT

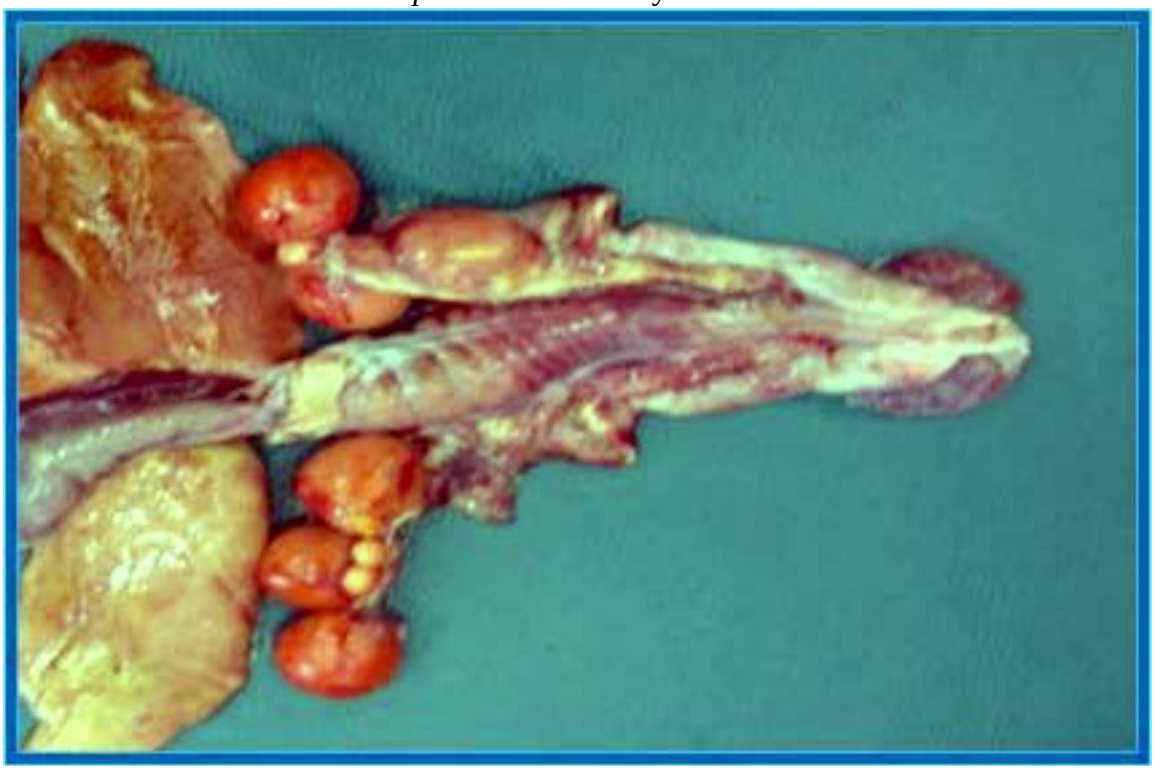


Torpedo torpedo by ARPAT

Batoids
Female 3a

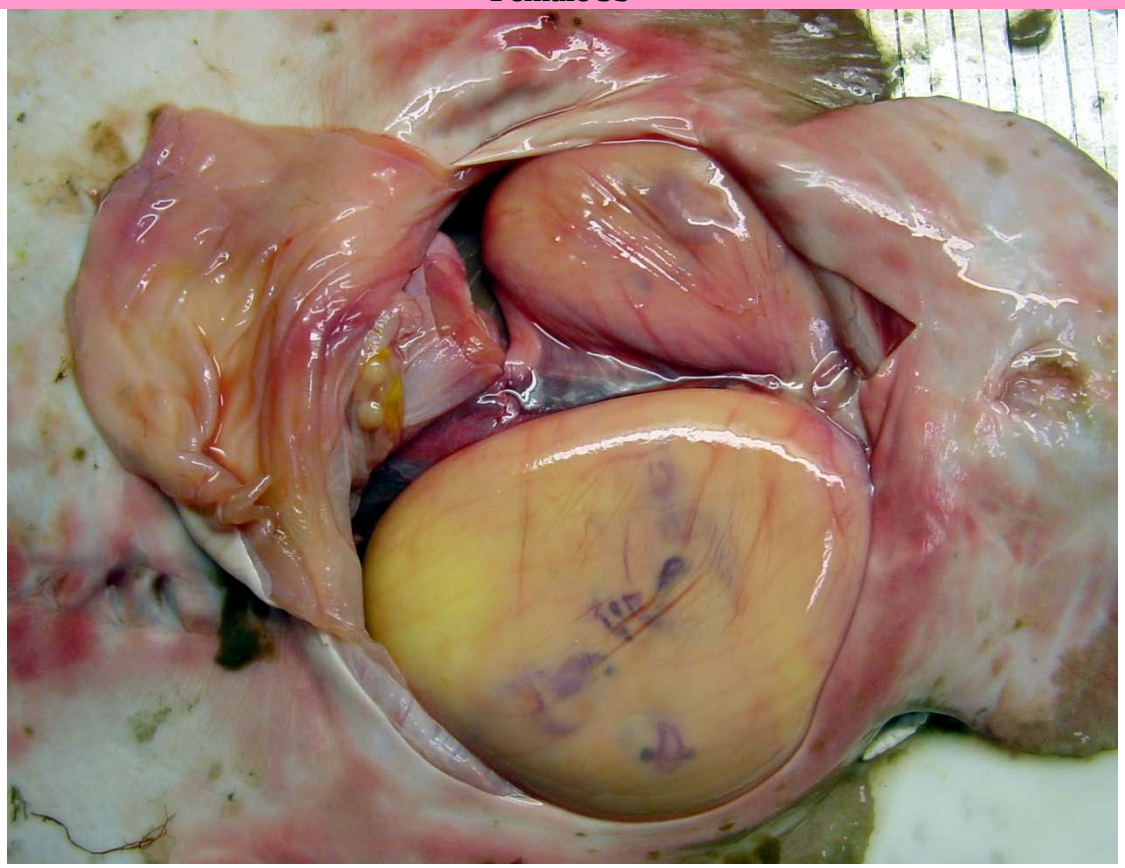


Torpedo mormorata by ARPAT

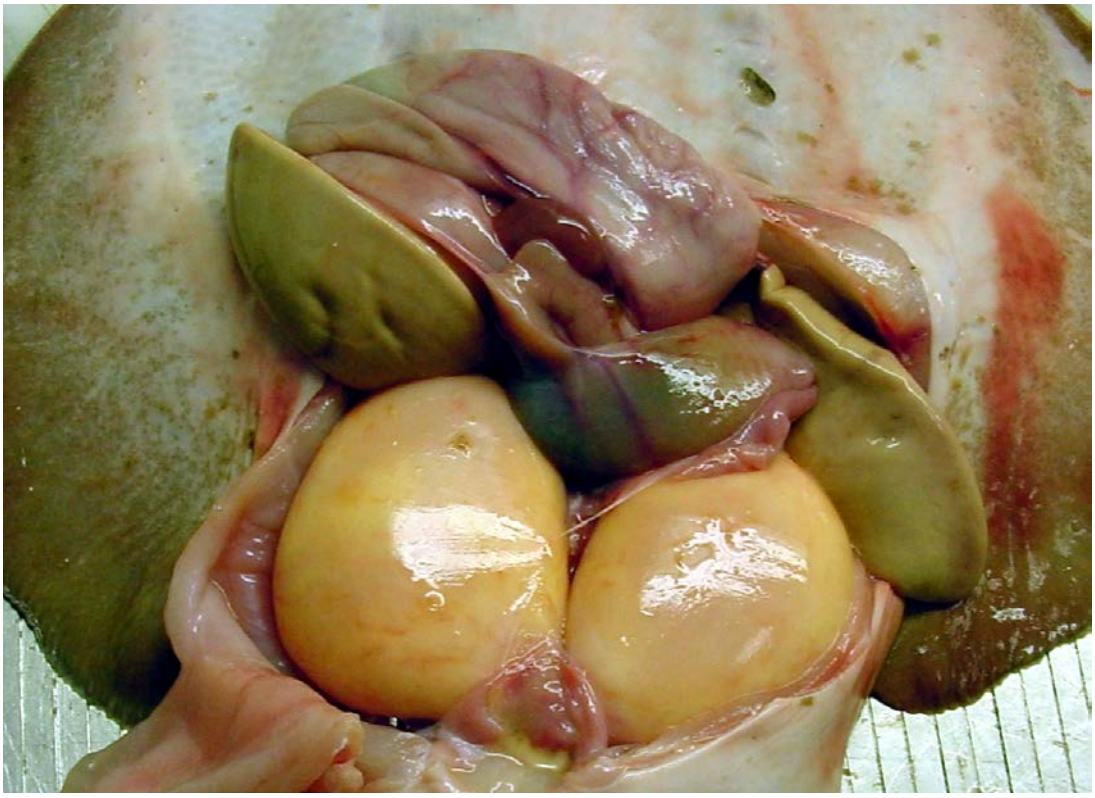


Rhinobatos rhinobatos By INSTM

Batoids
Female 3b



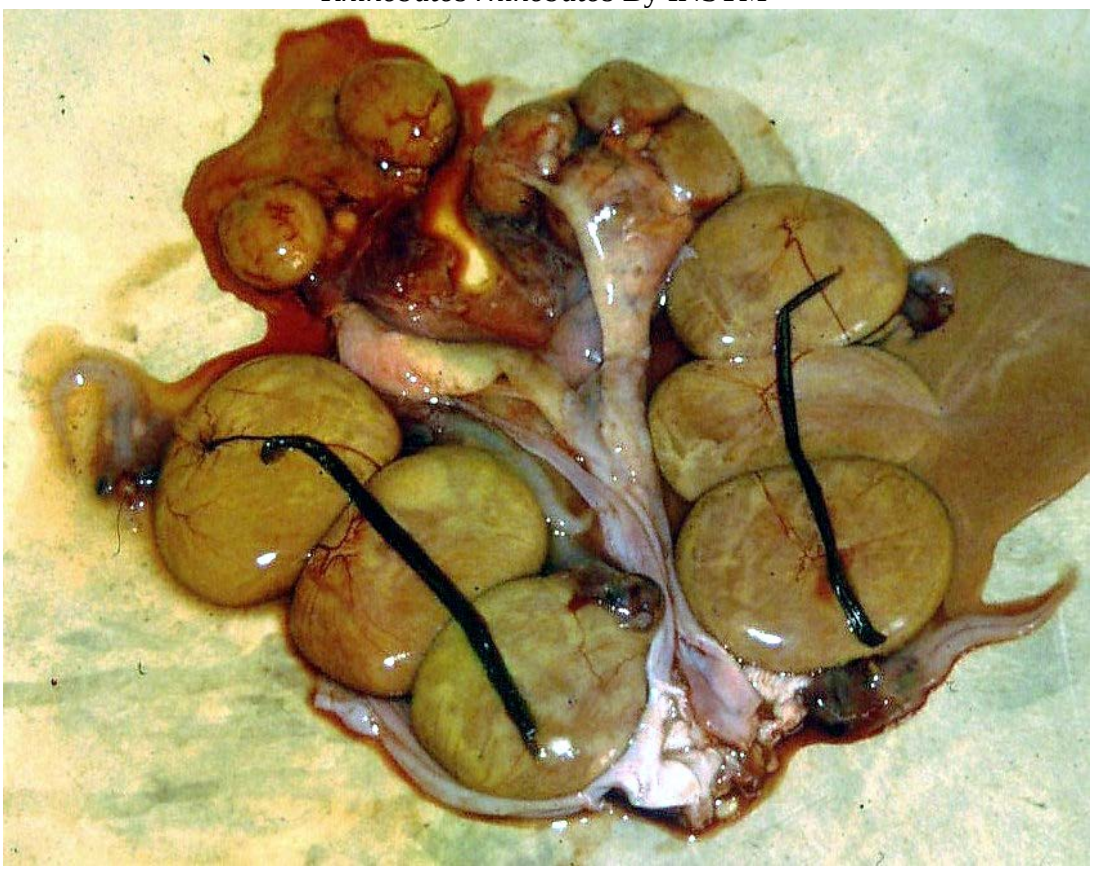
Torpedo mormorata by ARPAT



Torpedo torpedo by ARPAT

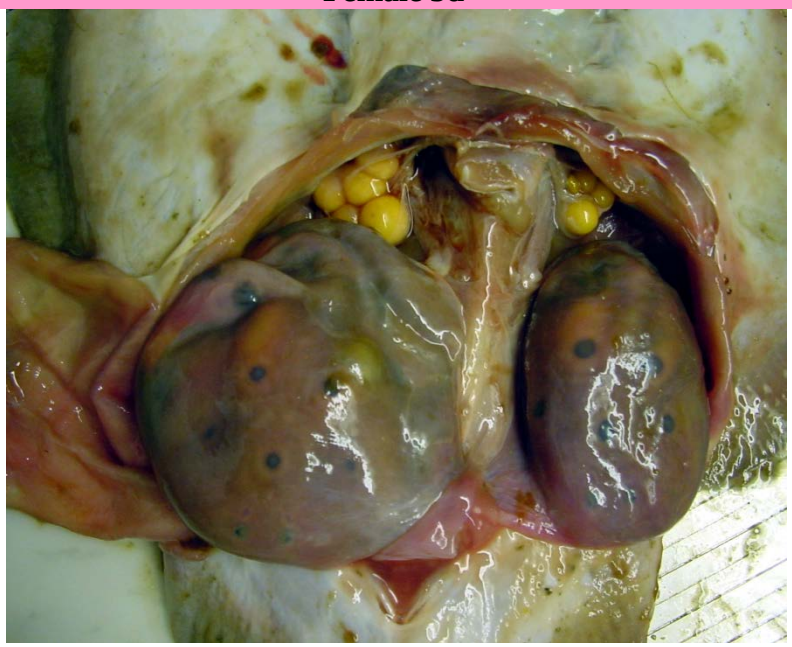
Batoids
Female 3c

Rhinobatos rhinobatos By INSTM

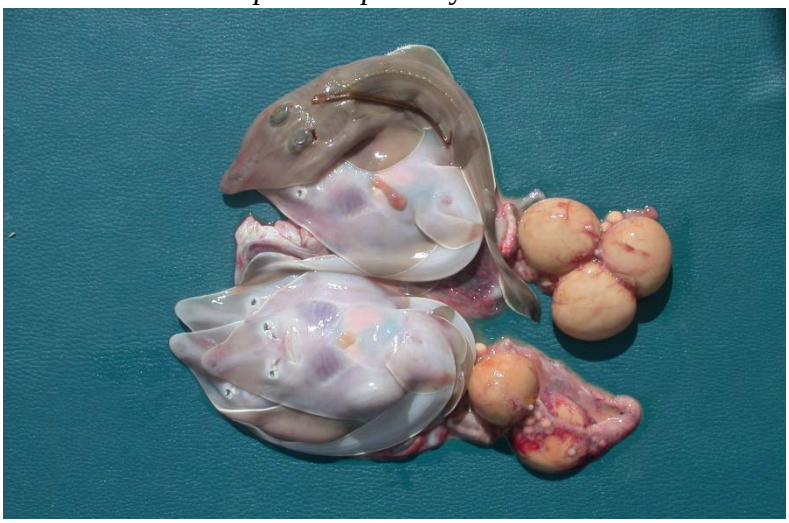


Rhinobatos rhinobatos By INSTM

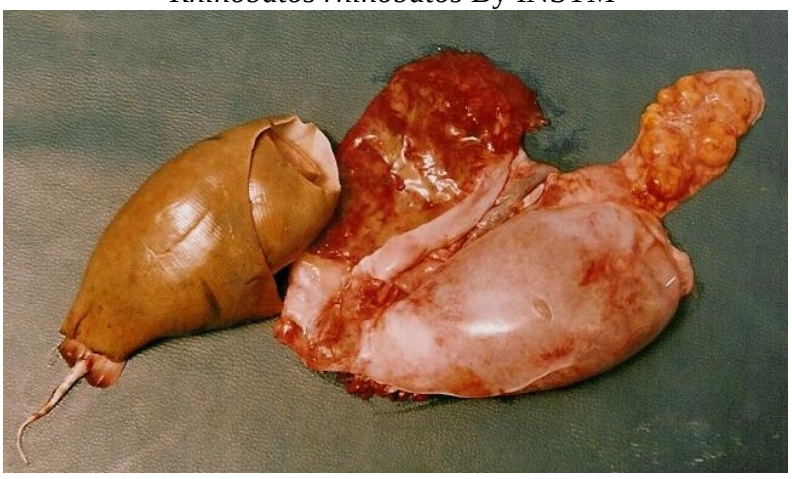
Batoids
Female 3d



Torpedo torpedo by ARPAT



Rhinobatos rhinobatos By INSTM

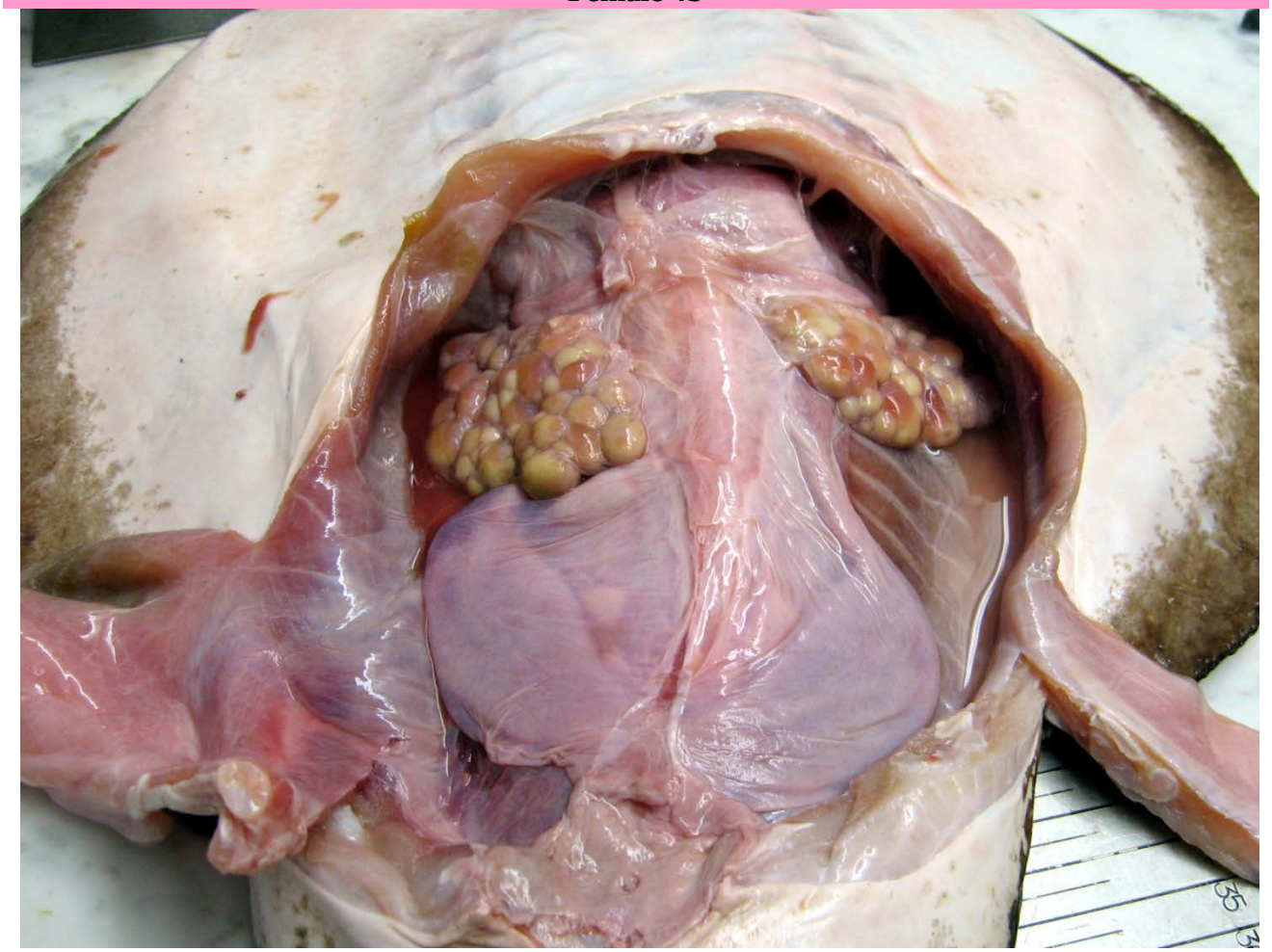


Gimnura altavela by INSTM

Batoids
Female 4a

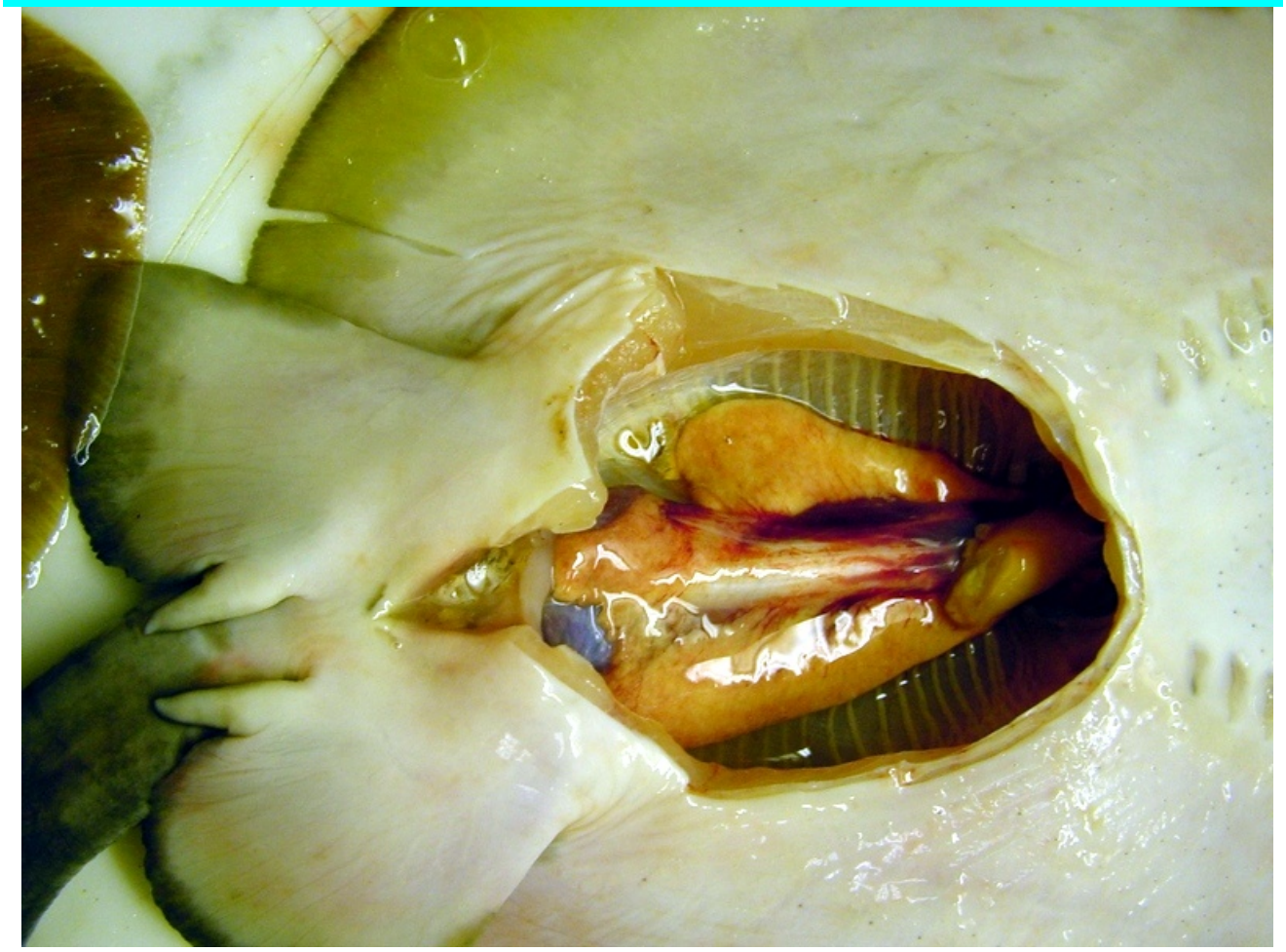
Image not Available

Batoids
Female 4b

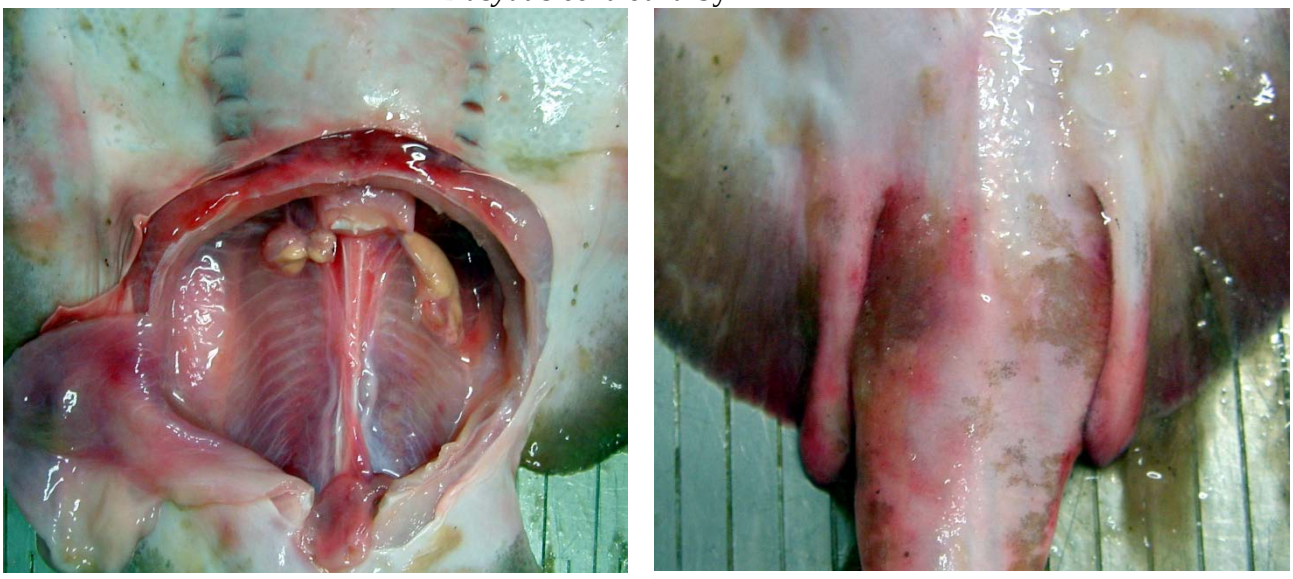


Torpedo mormorata by ARPAT

Batoids
Male 1

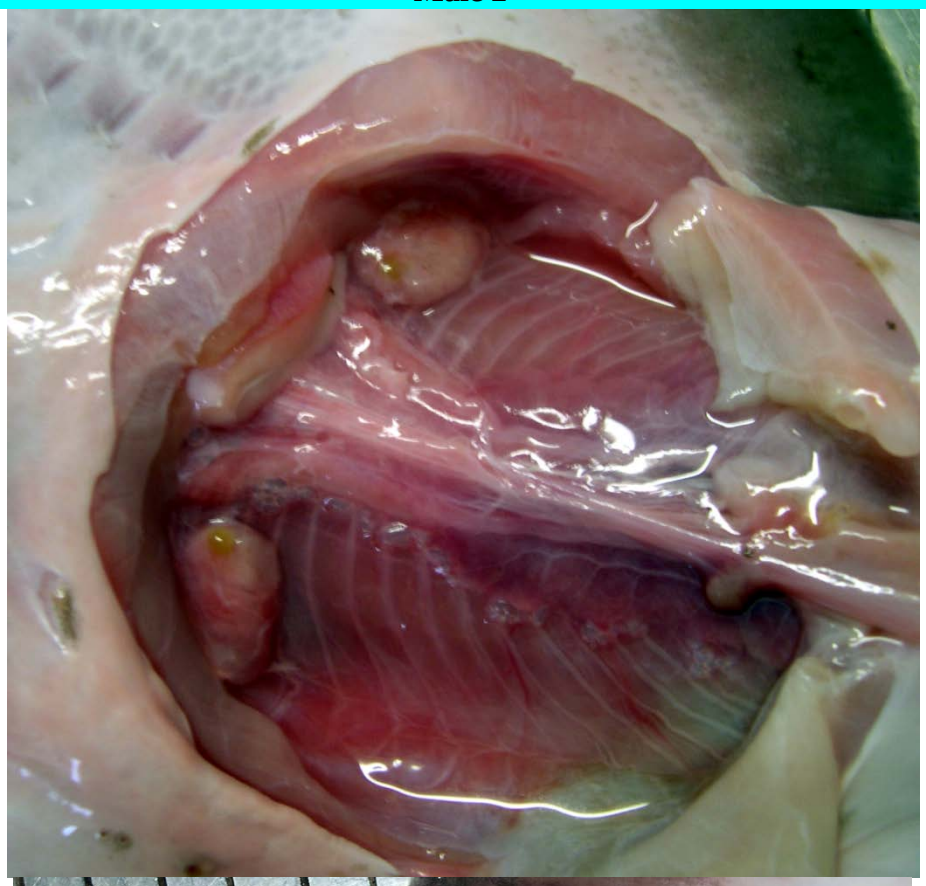


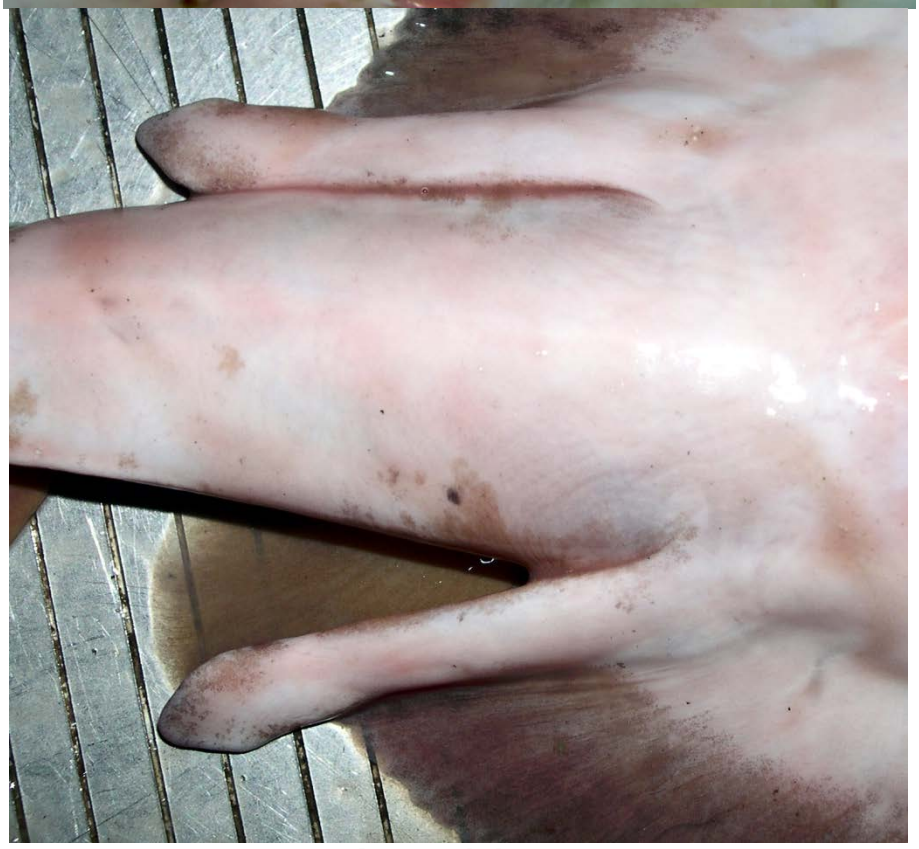
Dasyatis centroura by ARPAT



Torpedo torpedo by ARPAT

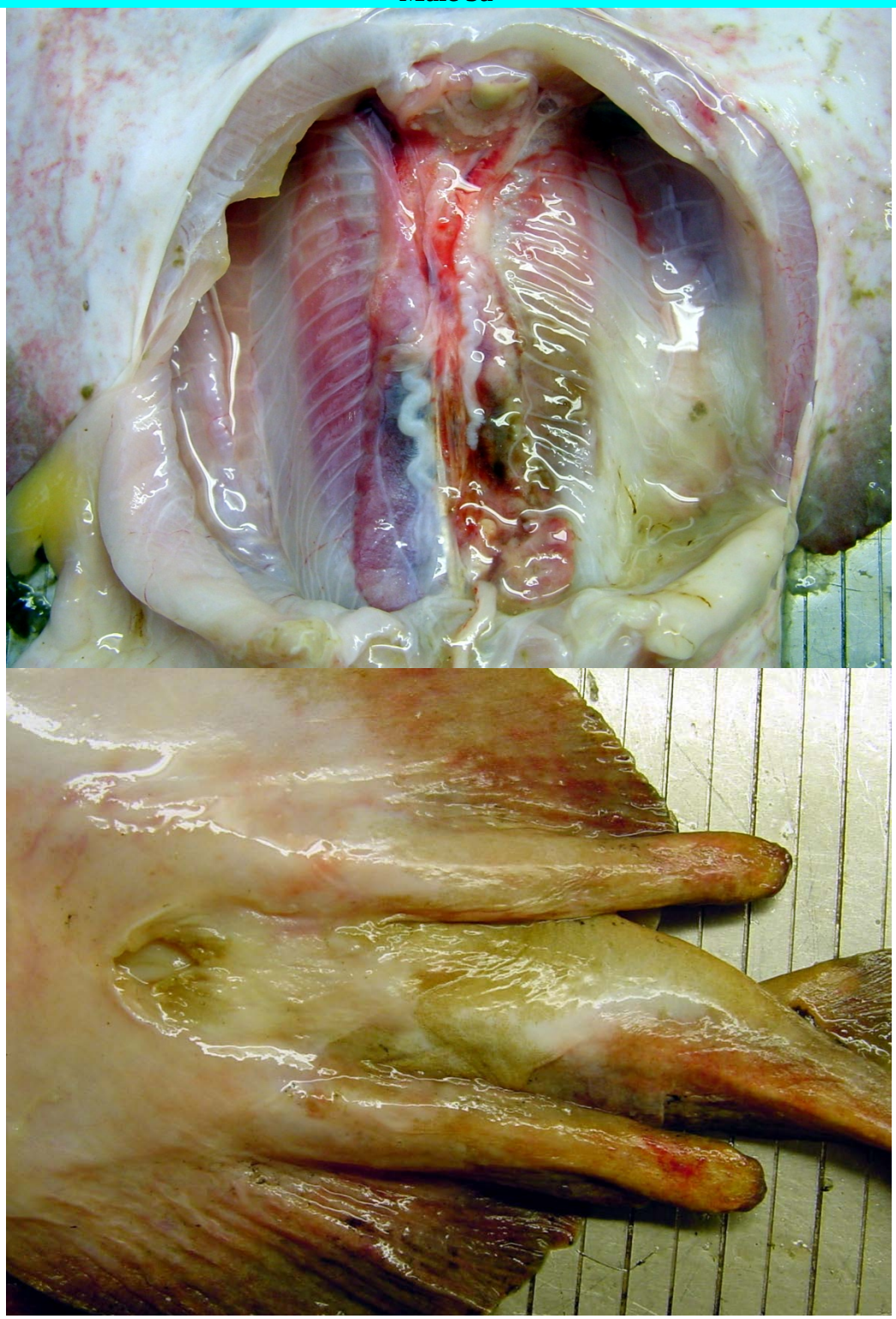
Batoids
Male 2





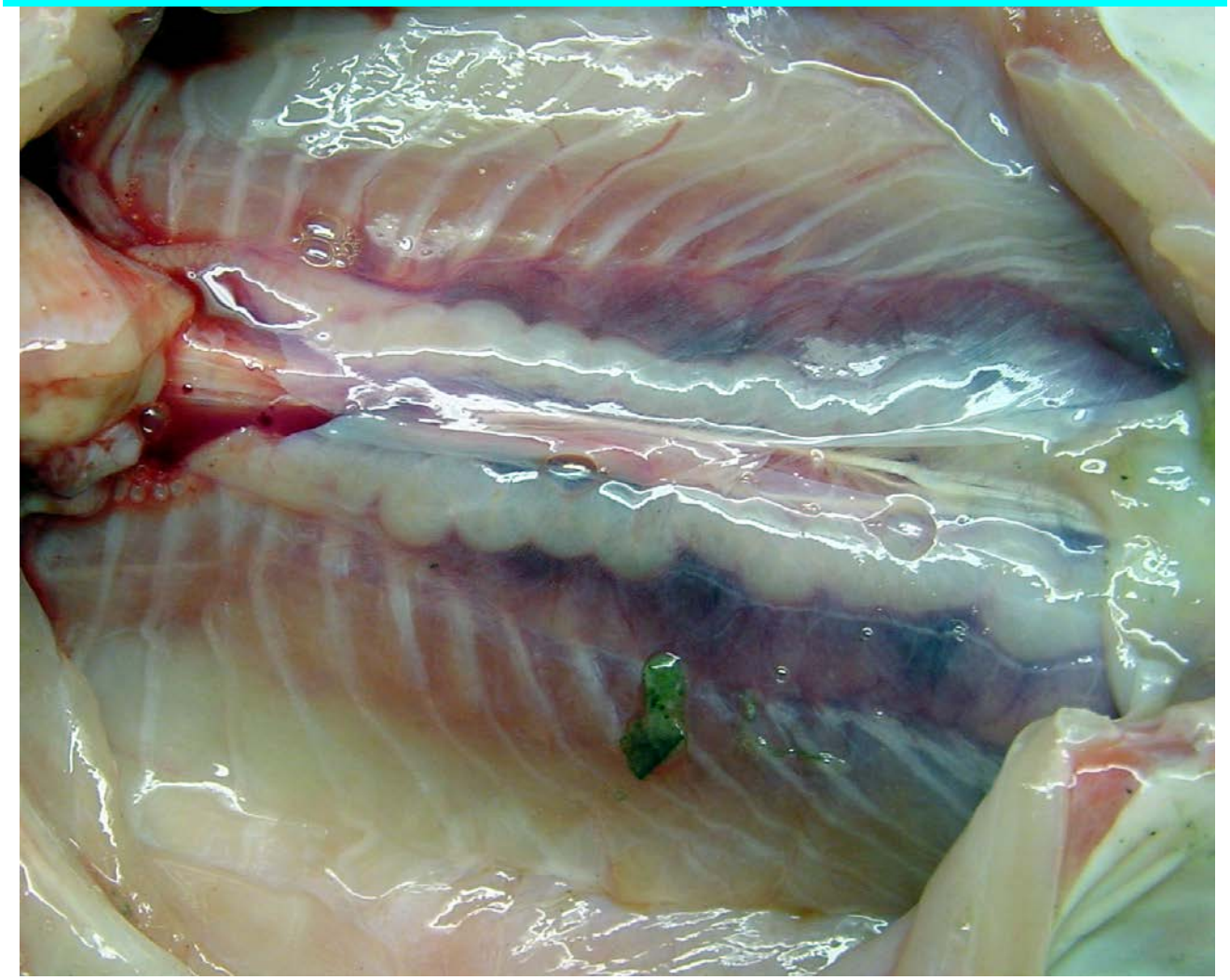
Torpedo torpedo by ARPAT

Batoids
Male 3a



Torpedo mormorata by ARPAT

Batoidi
Male 3b



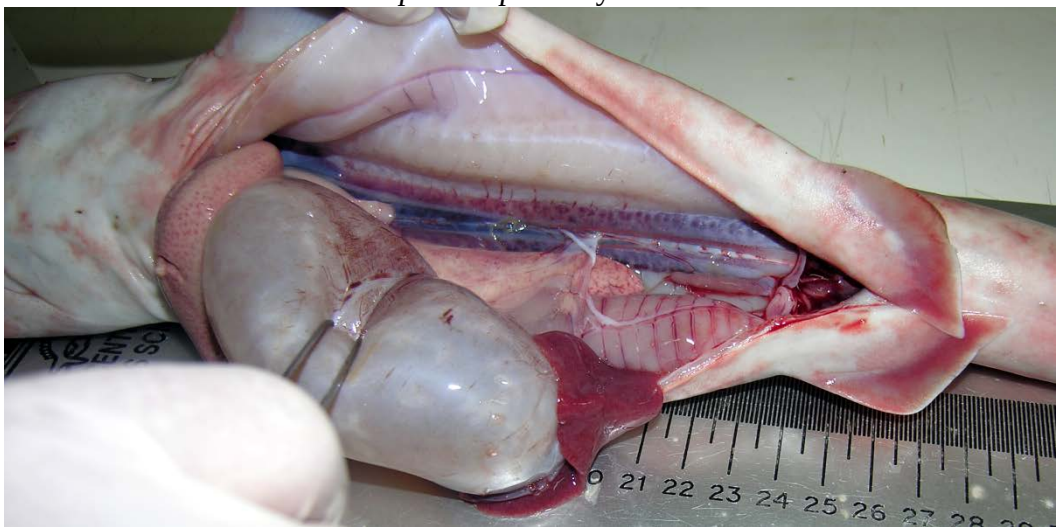
Torpedo torpedo by ARPAT

Batoidi
Male 4

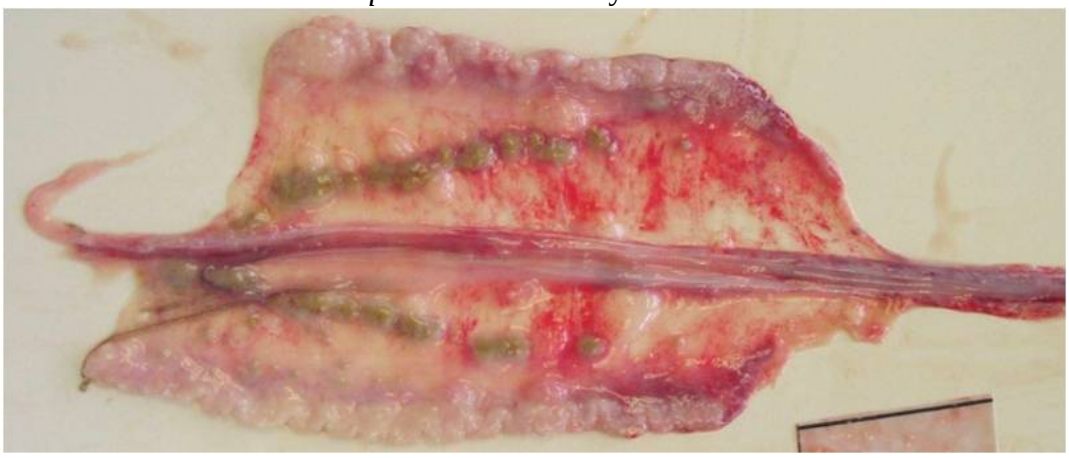
Image not available

Sharks
Female 1

Etmopterus spinax by ARPAT

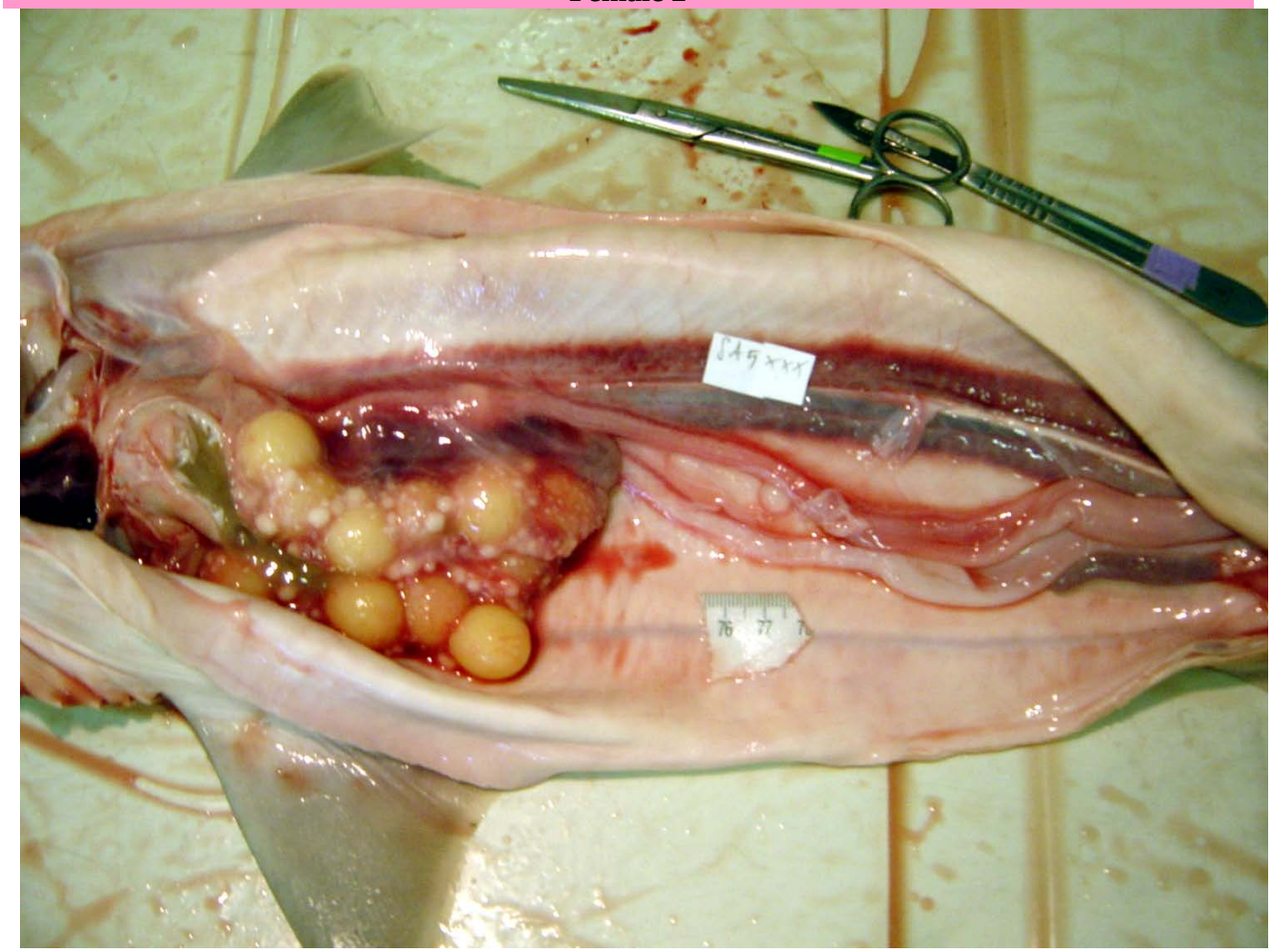


Squalus blainvillei by MCFS

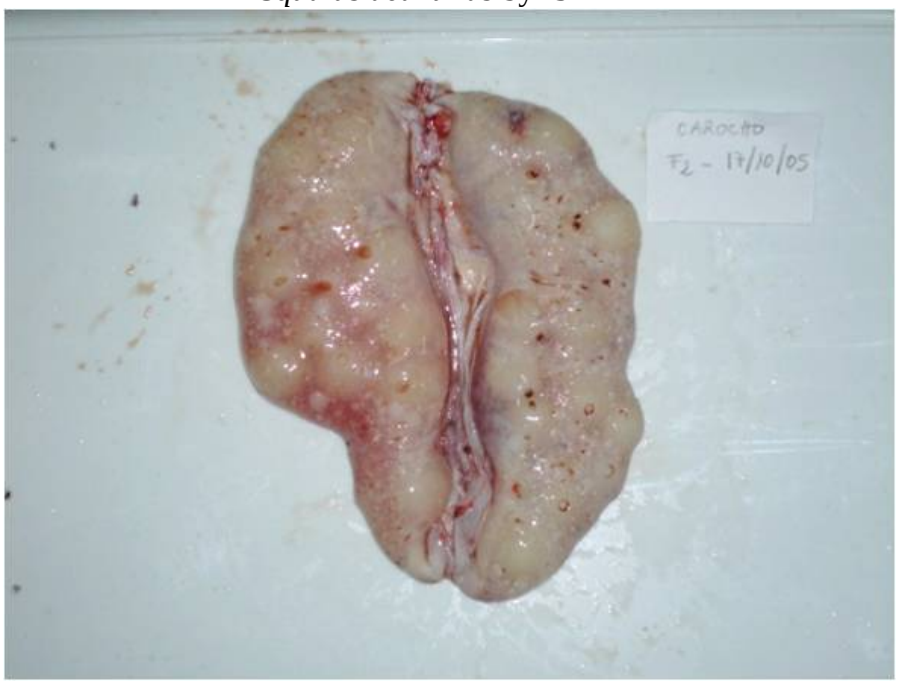


Centrophorus squamosus by IPIMAR

Sharks
Female 2



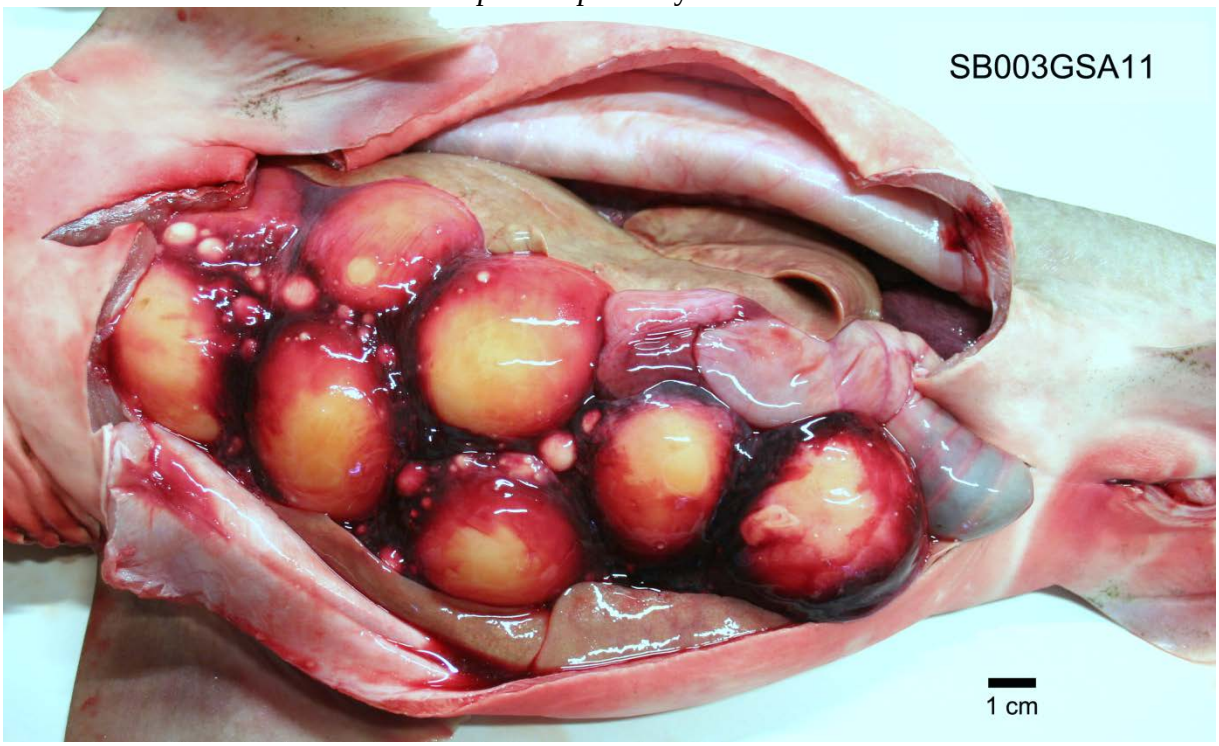
Squalus acanthias by ISPRA



Centroscymnus coelolepis by IPIMAR

Sharks
Female 3a

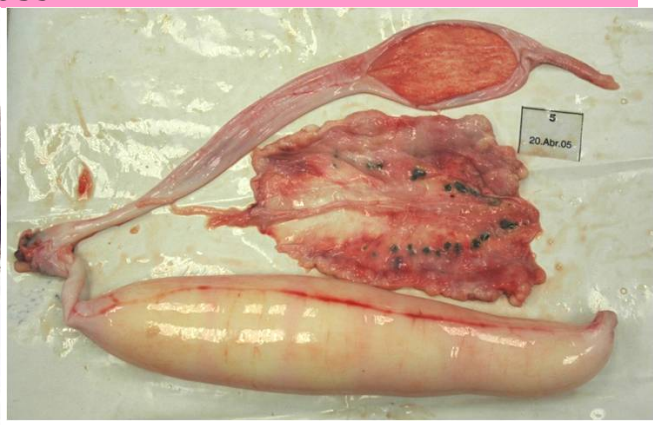
Etmopterus spinax by ARPAT



Squalus blainvillei by Univ. Cagliari

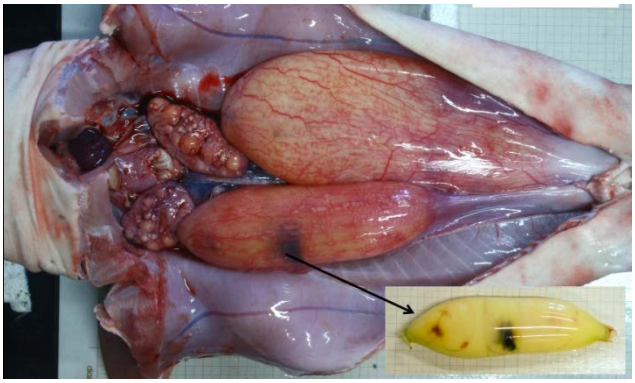
Sharks
Female 3b

Etmopterus spinax by Univ. Bari

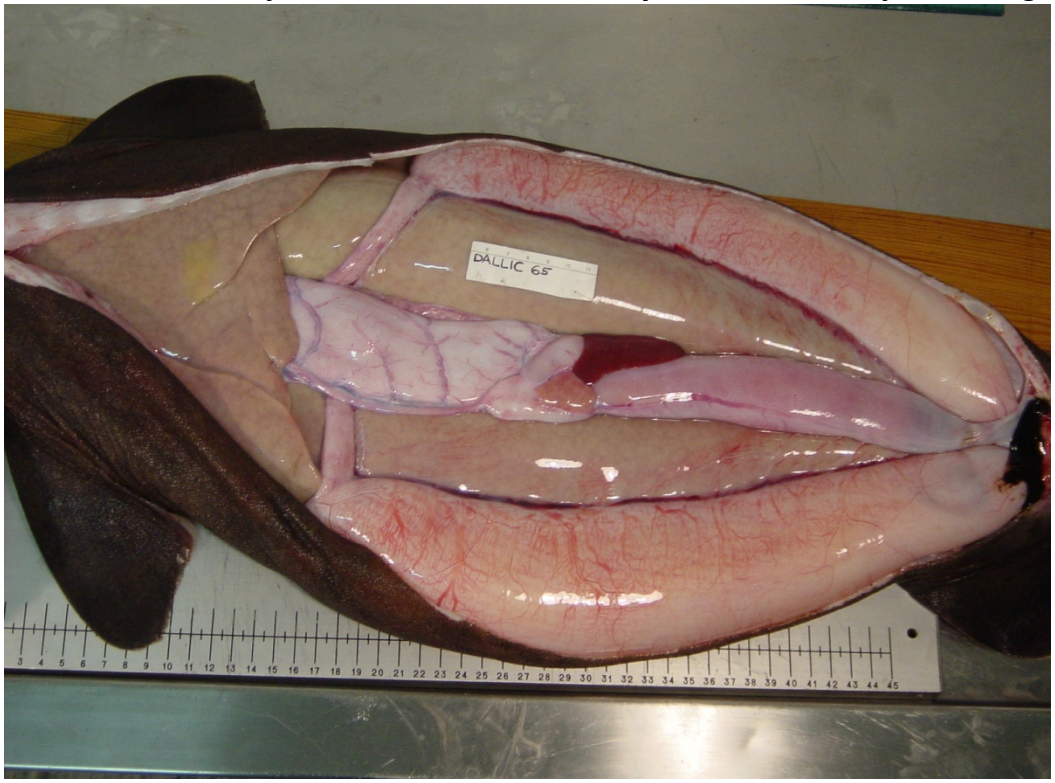


Centrophorus squamosus by IPIMAR

Mustelus asterias by MCFS

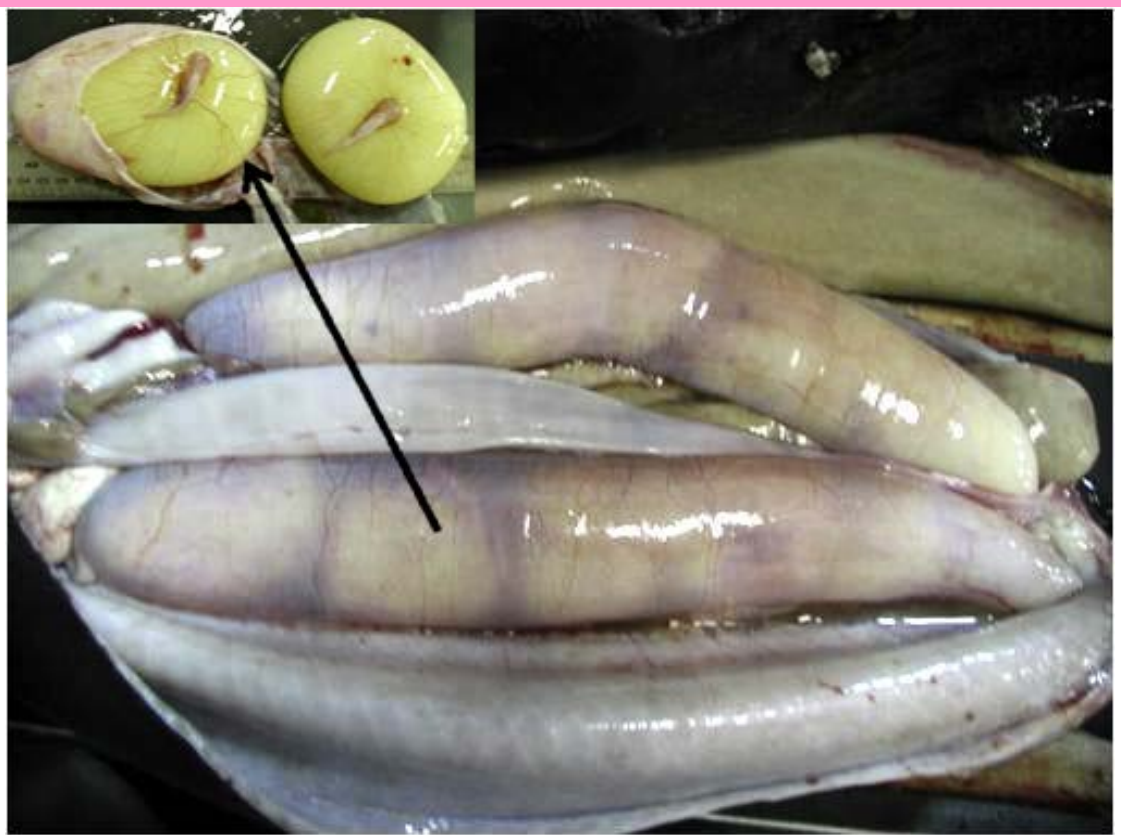


Squalus blainvillei by Univ. Cagliari

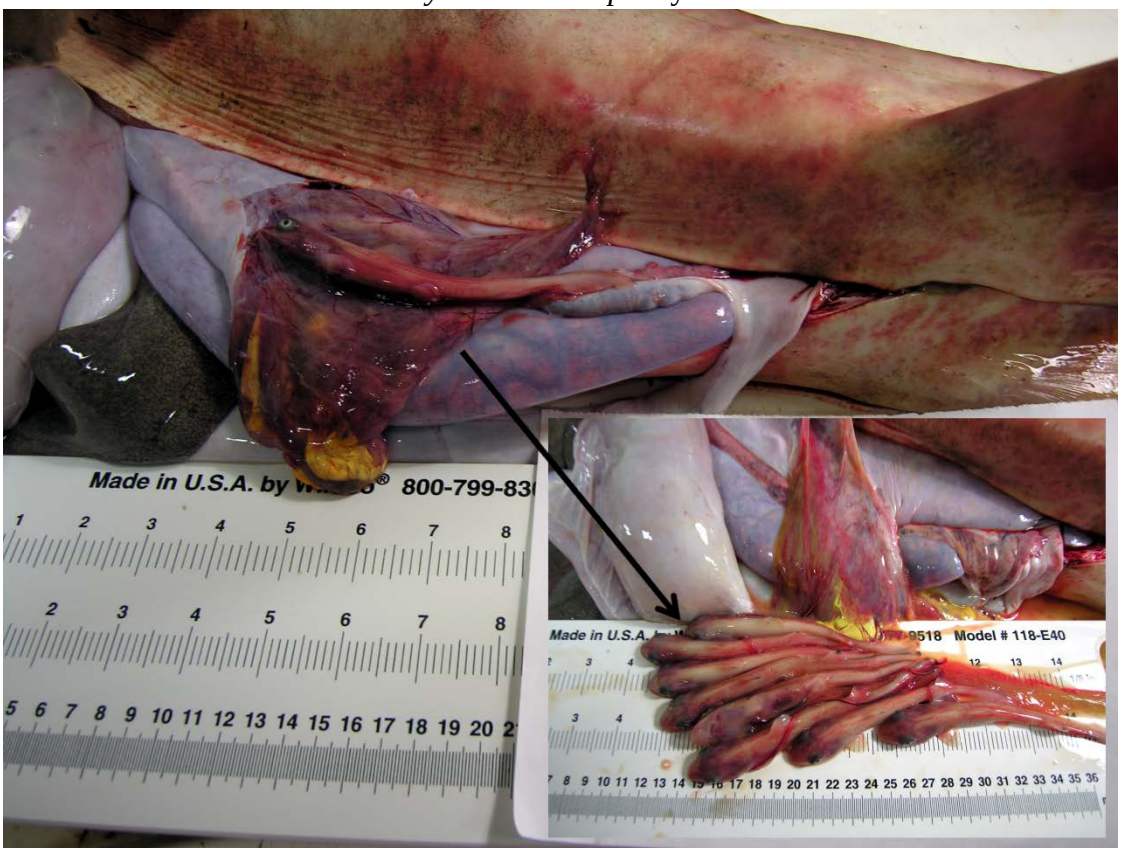


Dalatias licha by ISPRA

Sharks
Female 3c

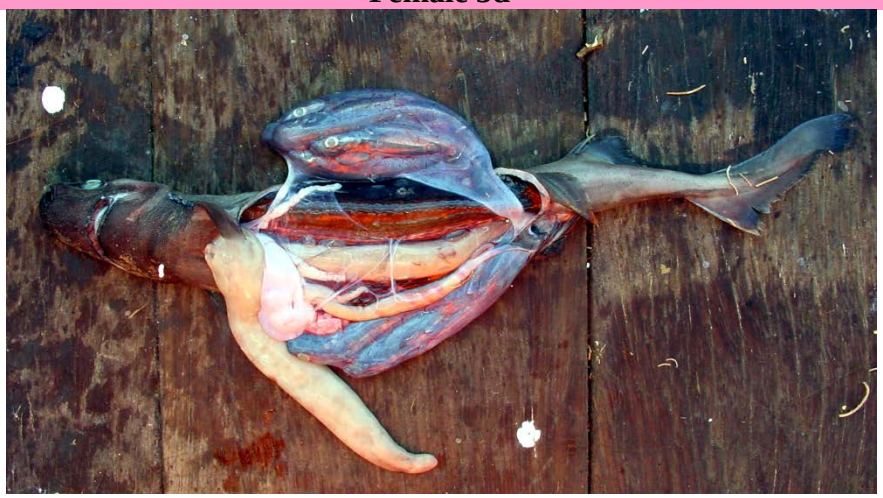


Centroscymnus coelolepis by IPIMAR



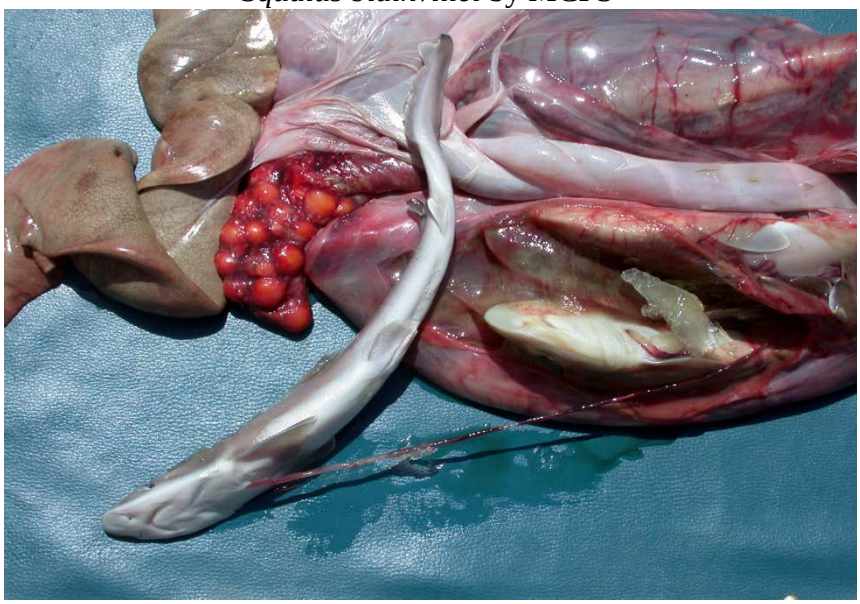
Mustelus punctulatus by MCFS

Sharks
Female 3d



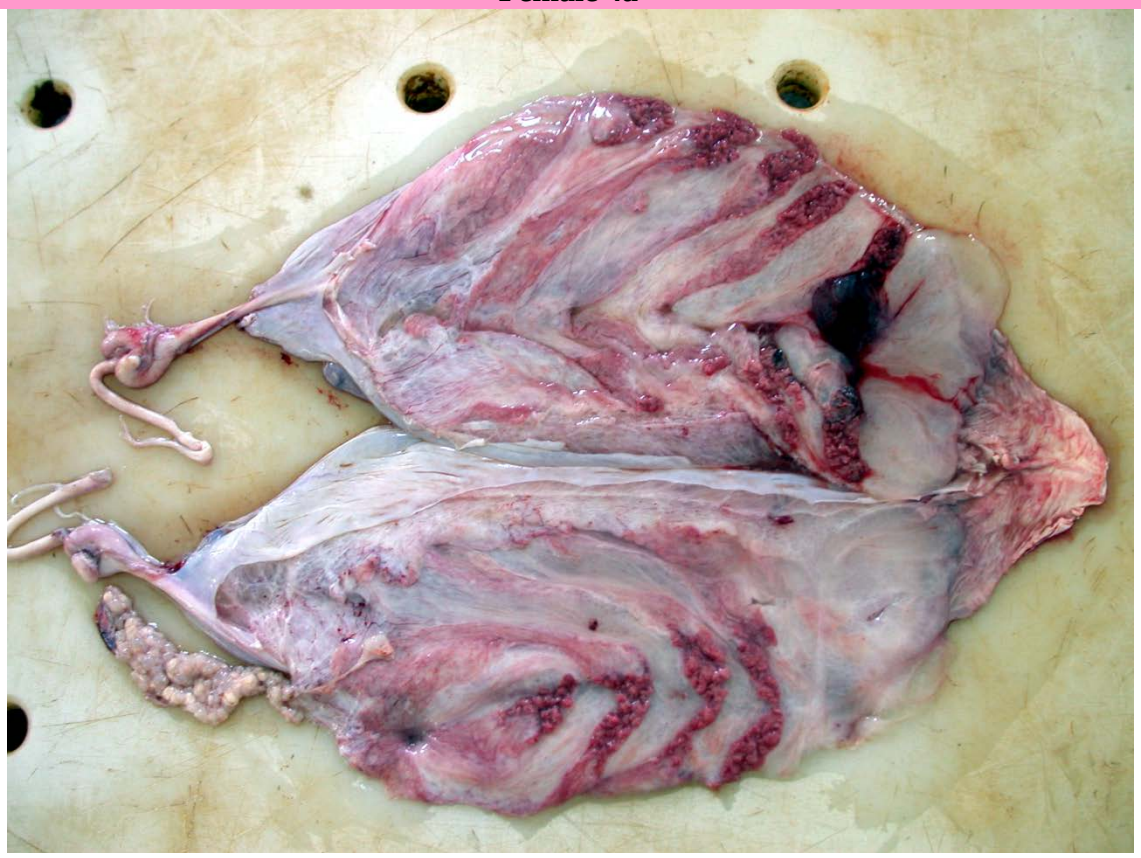
Etmopterus spinax by ARPAT

Squalus blainvillei by MCFS

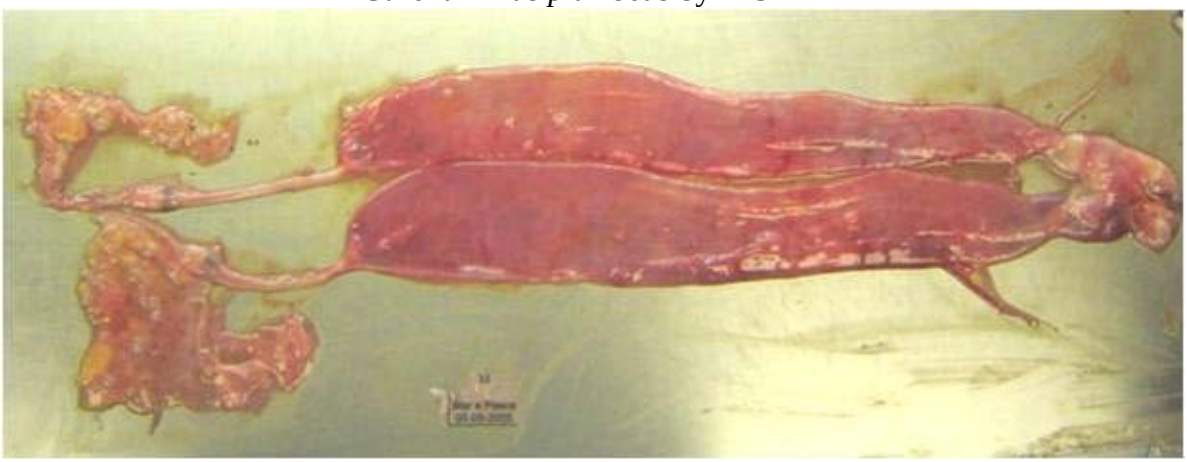


Mustelus mustelus by INSTM

Sharks
Female 4a

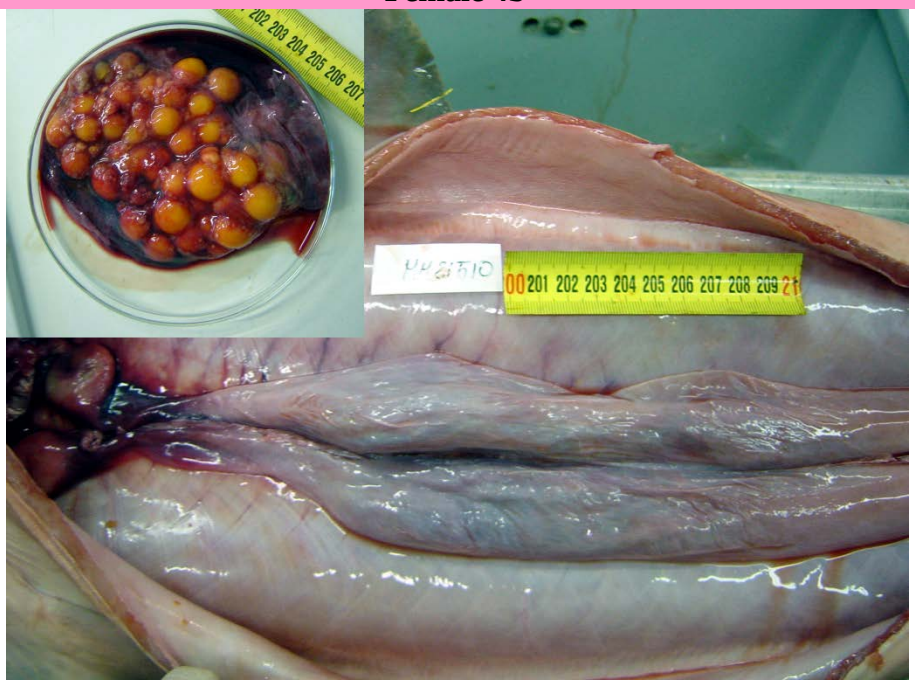


Carcharhinus plumbeus by INSTM



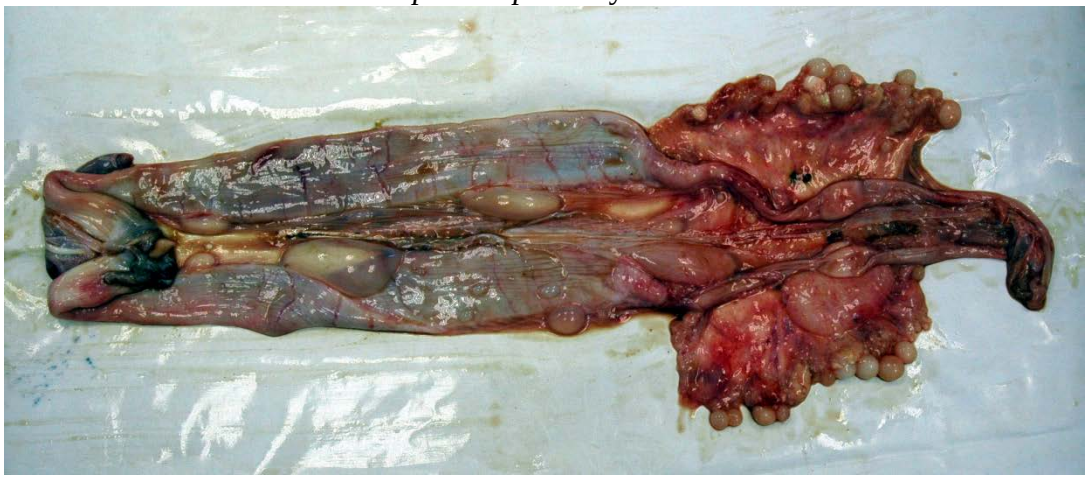
Centroscymnus coelolepis by IPIMAR

Sharks
Female 4b



Mustelus mustelus by ISPRA

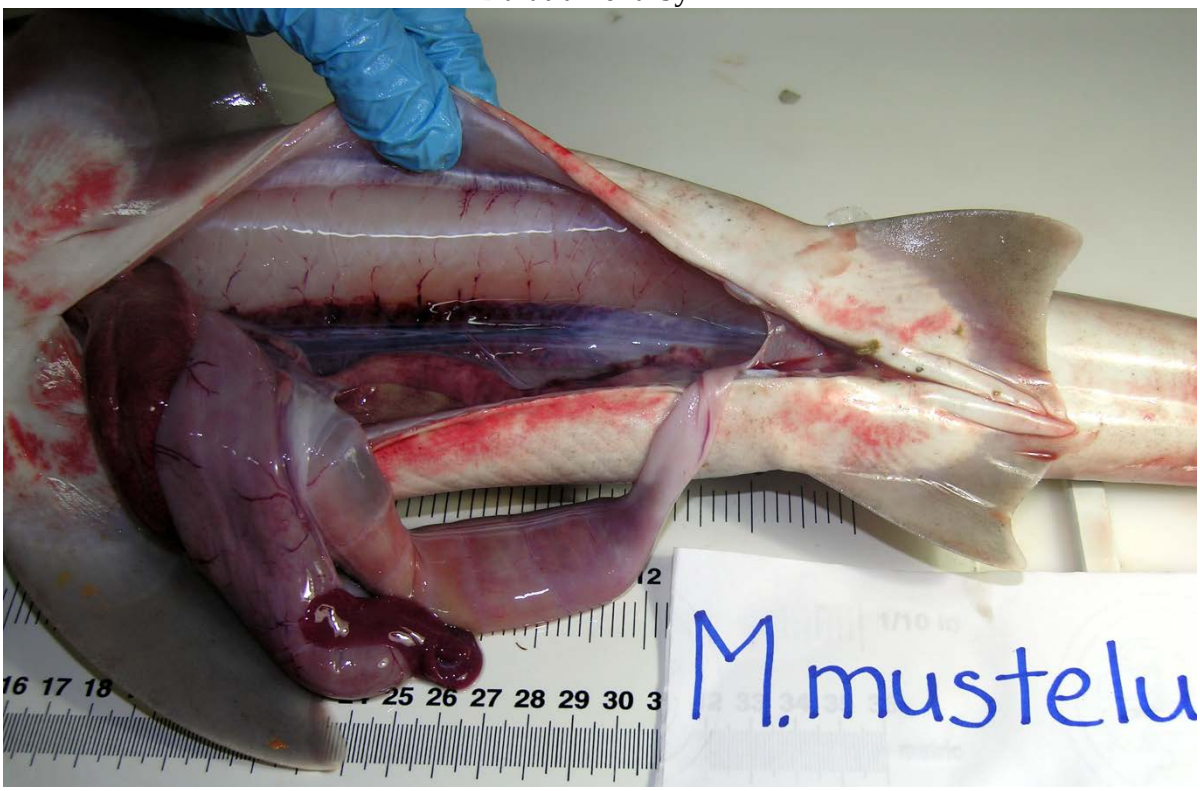
Etmopterus spinax by ARPAT



Centroscymnus coelolepis by IPIMAR

Sharks
Male 1

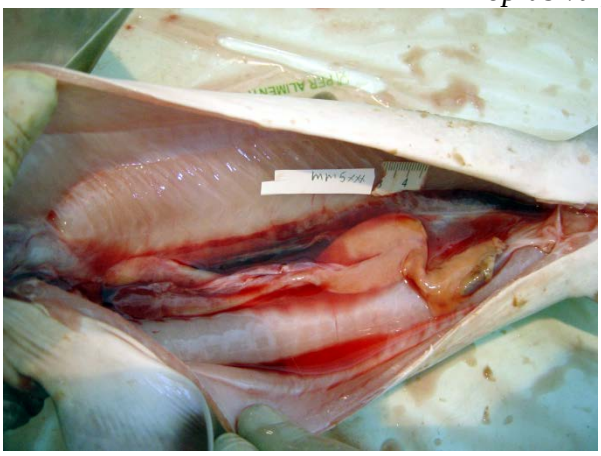
Dalatia licha by ARPAT



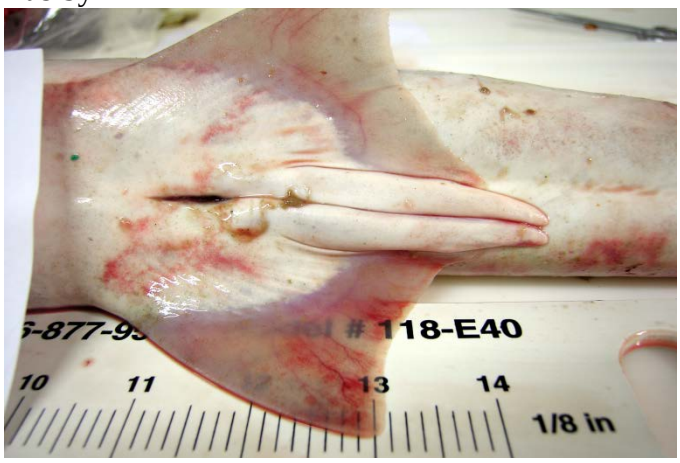
Mustelus mustelus by MCFS

Sharks
Male 2

Alopias vulpinus by ARPAT

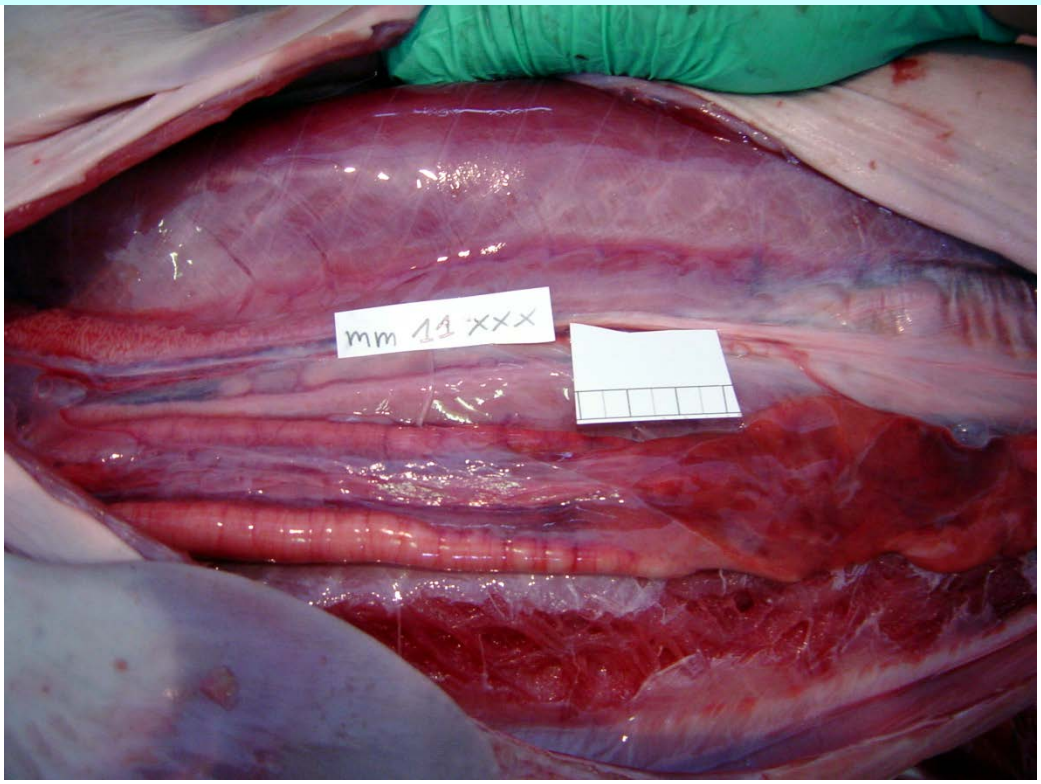


Mustelus mustelus by ISPRA



Mustelus mustelus by MCFS

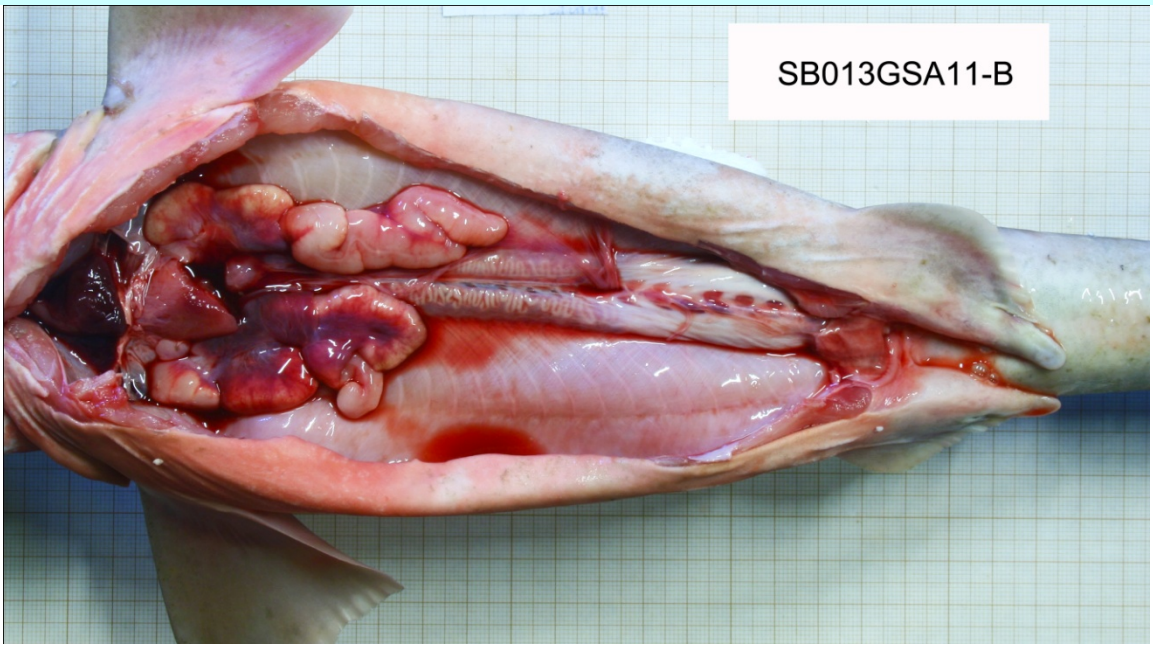
Sharks
Male 3a



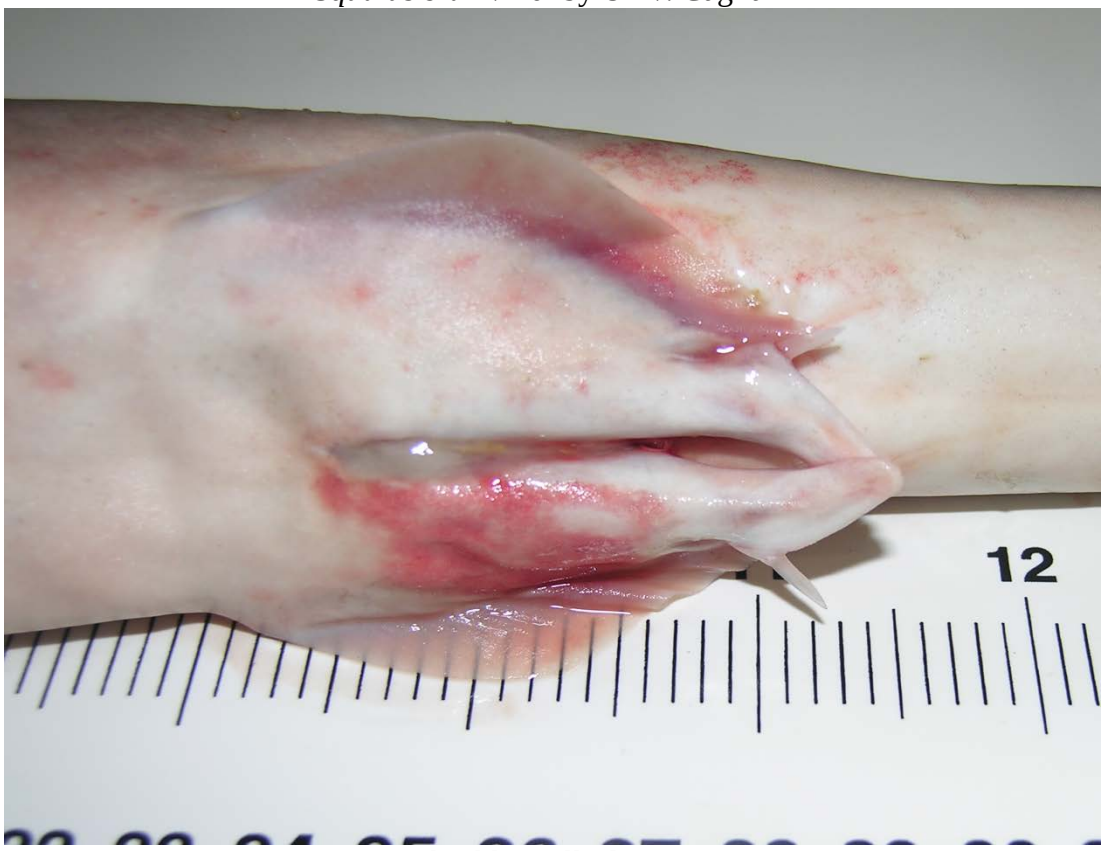
Mustelus mustelus by ISPRA

Mustelus mustelus by MCFS

Sharks
Male 3b



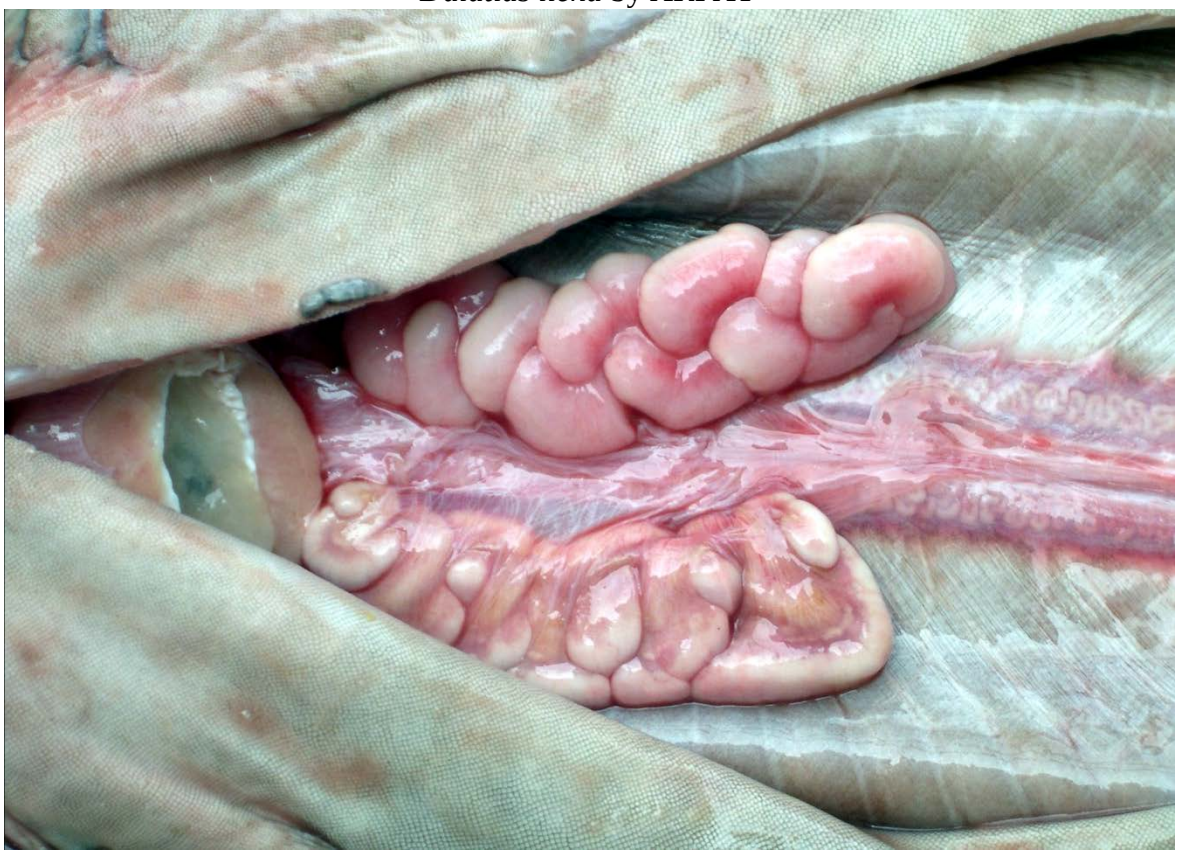
Squalus blainvillei by Univ. Cagliari



Squalus blainvillei by MCFS

Sharks
Male 3b

Dalatias licha by ARPAT



Centrophorus granulosus by ARPAT

Sharks
Male 4

Image not available

Annex 13: Proposed Final WKMSEL 2010 Macro maturity Scales

Oviparous elasmobranchs (skates and sharks)				
SEX	**GONAD ASPECT**	**MATURATION STATE**	**STAGE**	**MATURITY**
M	Claspers flexible and shorter than pelvic fins. Testes small (in skates, sometimes with visible lobules). Sperm ducts straight and thread-like.	**IMMATURE (Immature)**	**1**	**IMMATURE**
F	Ovaries barely visible or small, whitish; undistinguishable ovarian follicles. Oviducal (nidamental) gland not visible in skates and may be slightly visible in sharks. Uterus is thread-like and narrow.			
M	Claspers still flexible, and as long as or longer than pelvic fins. Testes enlarged (in skates, lobules clearly visible but not occupying the whole surface). Sperm ducts developing and beginning to coil (meander).	**DEVELOPING (Immature)**	**2***	**IMMATURE**
F	Ovaries enlarged with small follicles (oocytes) of different size. Some relatively larger yellow follicles may be present. Developing oviducal gland and uterus.			
M	Claspers fully formed, skeleton hardened, rigid and generally longer than pelvic fins. Testes greatly enlarged (in skates, filled with developed lobules). Sperm ducts tightly coiled and filled with sperm.	**SPAWNING CAPABLE (mature)**	**3a**	**MATURE**
F	Large ovaries with enlarged yolk follicles of different sizes. Oviducal gland and uterus fully developed.			
M	Description similar to stage 3a, however with clasper glands dilated, sometimes swollen and reddish. Sperm may be present in clasper groove or glans. On pressure sperm is observed flowing out of the cloaca or in the sperm ducts.	**ACTIVELY SPAWNING (mature)**	**3b**	**MATURE**
F	Description similar to stage 3a, however with the presence of egg capsules.			
M	Claspers fully formed, similar to stage 3. Testes shrunken and flaccid, (in skates, with few visible lobules). On pressure sperm does not flow. Spermducts empty and flaccid	**REGRESSING (mature)**	**4a**	**MATURE**
F	Ovaries shrunken with few follicles of different sizes. The oviducal glands diameter may be reducing. Uterus appears much enlarged (relative to stage 2), collapsed, empty and reddish.			
F	Ovaries full of small follicles similar to stage 2, enlarged oviducal glands and uterus.	**REGENERATING (mature)**	**4b***	**MATURE**
	* Be careful, these stages can be easily confused			

Viviparous elasmobranchs (rays and sharks)				
SEX	**PROPOSAL**	**MATURATION STATE**	**STAGE**	**MATURITY**
M	Claspers flexible and shorter than pelvic fins. Testes small (in rays, sometimes with visible lobules). Sperm ducts straight and thread-like.	**IMMATURE (Immature)**	**1**	**IMMATURE**
F	Ovaries barely visible or small, whitish; undistinguishable ovarian follicles. Oviducal (nidamental) gland may be slightly visible. Uterus is thread-like and narrow.			
M	Claspers slightly more robust but still flexible. Claspers as long as or longer than pelvic fins. Testes enlarged; in sharks testes start to segment; in rays lobules clearly visible but do not occupy the whole surface. Sperm ducts developing and beginning to coil (meander).	**DEVELOPING (Immature)**	**2***	**IMMATURE**
F	Ovaries enlarged with small follicles (oocytes) of different size. Some relatively larger yellow follicles may be present. Ovaries lack atretic follicles. Developing oviducal gland and uterus.			
M	Claspers fully formed, skeleton hardened, rigid and generally longer than pelvic fins. Testes greatly enlarged; in sharks testes are fully segmented; in rays filled with developed lobules. Sperm ducts tightly coiled and filled with sperm.	**SPAWNING CAPABLE (mature)**	**3a**	**MATURE**
F	Large ovaries with enlarged yolk follicles all of about the same size so that they can be easily distinguished. Oviducal gland and uterus developed without yolky matter, embryos and not dilated.	**CAPABLE to REPRODUCE (mature)**		
M	Description similar to stage 3a, however with clasper glands dilated, often swollen and reddish (occasionally open). Sperm often present in clasper groove or glans. On pressure sperm is observed flowing out of the cloaca or in the sperm ducts.	**ACTIVELY SPAWNING (mature)**	**3b**	**MATURE**
F	Uteri well filled and rounded with yolk content (usually candle shape). In general segments cannot be distinguished and embryos cannot be observed.	**EARLY PREGNANCY (maternal)**		**MATERNAL**
F	Uteri well filled and rounded, often with visible segments. Embryos are always visible, small and with a relatively large yolk sac.	**MID PREGNANCY (maternal)**	**3c**	**MATERNAL**
F	Embryos fully formed, yolk sacs reduced or absent. Embryos can be easily measured and sexed.	**LATE PREGNANCY (maternal)**	**3d**	**MATERNAL**
M	Claspers fully formed, similar to stage 3. Testes and spermducts shrunken and flaccid.	**REGRESSING (mature)**	**4**	**MATURE**
F	Ovaries shrunken withouth follicle development and with atretic (degenerating) follicles. The oviducal glands diameter may be reducing. Uterus appears much enlarged, collapsed, empty and reddish.	**REGRESSING (mature)**	**4a**	**MATURE**
F	Ovary with small follicles in different stages of development with the presence of atretic ones. Uterus enlarged with flaccid walls. Oviducal gland distinguishable.	**REGENERATING (mature)**	**4b***	**MATURE**
	* Be careful, these stages can be easily confused			