Macroscopic visual criteria for the identification of the sex and maturity of billfish gonads, used in Reunion Island, following the ICES WKASMSF 2018 scale.

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Abstract:

Swordfish (*Xiphias gladius*) is the primary target species for large pelagic longliners operating around Réunion Island. Blue marlin (*Makaira nigricans*) is a significant bycatch for this fleet and a key target species for coastal fishers too. Other billfish species such as striped marlin (*Kajikia audax)*, black marlin (*Istiompax indica*), and sailfish (*Istiophorus platypterus*), while being less known species, have raised attention in recent years and were thus included in the National Biological Monitoring Plan under the Data Collection Framework (DCF). However, the maturity scales currently in use in Reunion Islands were actually designed for fish from mainland France, which differ considerably from the tropical species caught in the French overseas departments and territories—particularly the large pelagics and billfish monitored by the IOTC.

The purpose of this presentation is to discuss the technical fact sheets we designed to assist field observers in determining the sex and maturity of billfish in Reunion Island fisheries using macroscopic visual criteria of gonads. These facts sheets are based on the ICES WKASMSF 2018 scale which serves as the reference for European biological monitoring. The work on these species in the western Indian Ocean is still incomplete, particularly in terms of histological validation. However, this initiative could encourage collaborative efforts to develop standardized criteria based on various studies and regions, thereby contributing to a better understanding of these species.

1 . ICES WKASMSF 2018 scale presentation :

Adopting a single standardized scale across Europe for defining fish maturity as part of the EU's Data Collection Framework (DCF) was decided during the 2018 Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF), organized by the International Council for the Exploration of the Sea (ICES) in Copenhagen. This aims to improve data accuracy and enable the conversion of existing datasets into a unified format, which could be highly beneficial for ongoing monitoring in the Western Indian Ocean.

You can find the complete guide in Annex 1 . This guide takes into account all maturity phases and sub-phases alongside histological criteria, as well as the translation of the old 2012 scale for data harmonization (ICES WKASMSF 2018 REPORT). As a result of this working group, an atlas of illustrative sheets of the different maturity stages has been created for the Mediterranean species, Follesa and Carbonara (2019). This is the atlas we'd like to propose for the Indian ocean

Here, we will focus only on macroscopic criteria and won't use the sub-phase as it needs to be determined with histological criteria. We can resume our proposed guide like this :

Figure 1 : schematic representation of the cycle

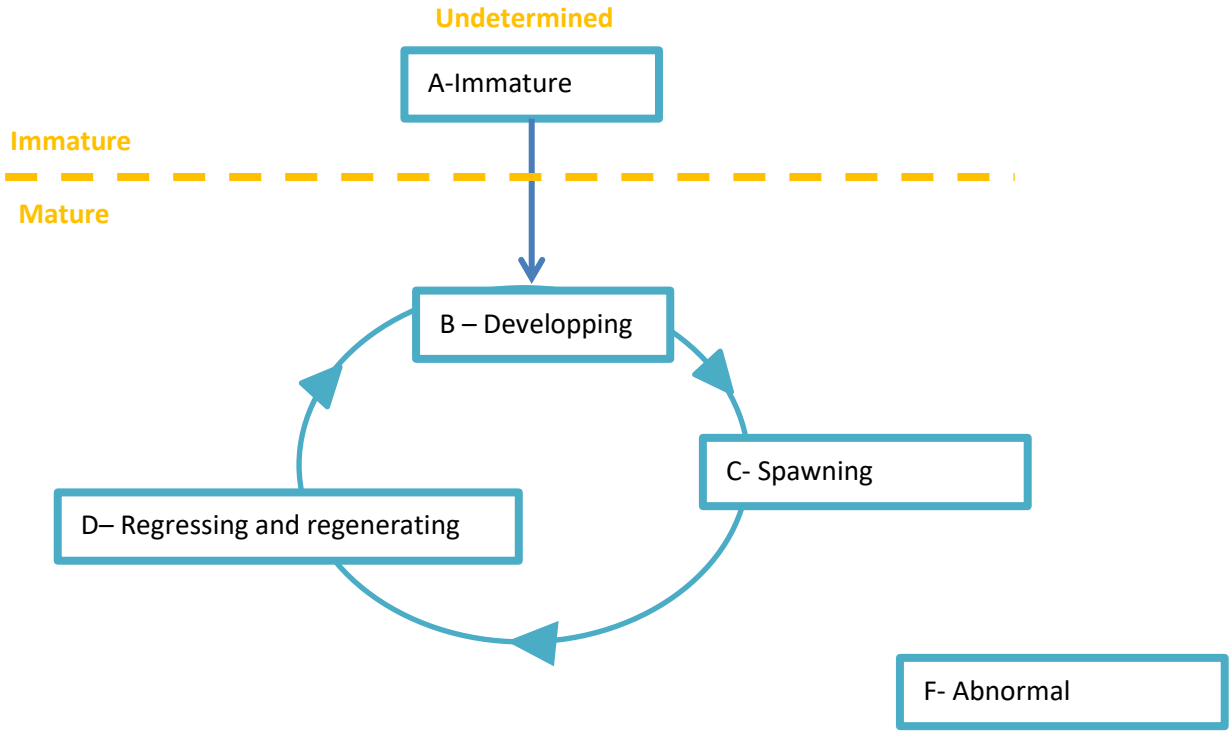


Figure 2 : Part of the ICES WKASMSF 2018 scale that describes the macroscopic criteria

State description	Phase		New terminology		Macroscopic criteria	
SI : Sexually immature, (without gonad development)	Immature	Possible sub-phase	A	Possible sub-phase	Small pinkish and translucent (often clear) ovaries shoter than 1/3 of body cavity. Indistinct blood vessels and no visible eggs	Possible sub-phase
SM : Sexually mature (with gonad development)	Developping	Developing but functionally immature (first-time developer)	B	Ba	Enlarging ovaries, blood vessels become more distinct	Small pinkish/reddish ovaries shorter than 1/2 of the body cavity. Eggs not visible to the naked eye
		Developing but functionally mature		Bb		Pinkish-reddish/reddish-orange and translucent ovaries that have a length of about 1/2 of the body cavity. Visible blood vessels, no visible eggs
	Spawning	Actively spawning	C	Ca	Large ovaries, blood vessels prominent, individual oocytes visible macroscopically	Pinkish-yellow ovaries with a granular appearance and a length of about 2/3 of the body cavity. Eggs are visible to the naked eye through the *ovaric tunica* which is not yet translucent. No eggs are expelled under light pressure
		Spawning capable		Cb		Orange-pink ovaries with conspicuous superficial blodd vessels and a length from 2/3 to full length of the body cavity. Large, transparent, ripe eggs are clearly visible and can be expelled under light pressure, or escape freely
	Regression / Regeneration	Regression	D	Da		Reddish ovaries of about 1/2 of the body cavity length. Flaccid ovarie walls, prominent blood vessels, and possible remnants of disintegrating opaque and/or translucent eggs
		Regeneration		Db		Small pinkish and translucent ovaries that have a length of aboiut 1/3 of the body cavity, with reduced but present blood vessels. No visible eggs
	Omitted spawning		E			
	Abnormal		F		Problems in the gonad development (necrosis, sclerosis, intersex, majority of the gonad that looks unhealthy)	

We use this scale for all fishes, including large pelagic and demersal species. We have clear guides for tuna, swordfish, and certain species of demersal fish because they occur and are fished at Reunion island all year round,resulting in having all seasons and all phases present in our guides. The problem with other species of billfish is that they are seasonal and we do not have all the phases of the cycle, which hinders the comparison with established guides and the validation of maturity stages, particularly with macroscopic criteria.

2 . Presentation of the swordfish visual guides used as a model

For the swordfish, we use the Poisson and Taquet (2001) study, which involved 1727 pairs of gonads (1,107 females/620 males) around Réunion Island using macroscopic criteria and histology. With this, they developed a precise scale of 6 stages for females and 4 for males. We have then translated and illustrated in our proposed new scale format shown in Annex 2 showing precise macroscopic criteria. This visual guide is therefore the model we aim to develop for other species.

Figure 3 : criteria for determining swordfish gender

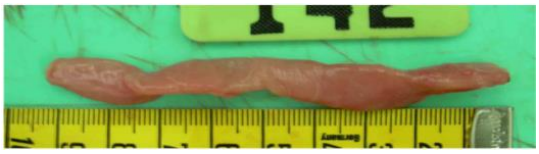
	FEMALE	MALE
General aspect	The **ovaries** have a smooth, round external appearance and can weigh up to several kilograms	The **testicles** are elongated, flattened, and have numerous nodules on the outer part. They usually weigh less than 300g
		
cross-section	The cross-section is usually cylindrical ,often has a lumen (empty space inside) and the internal material is rough. Mature females have oocytes that are visible to the naked eye	The cross-section is ovoid, has no lumen and has a very smooth appearance. Sperm is easily observed on mature individuals
		

Figure 4 : macroscopic criteria of female swordfish's (*Xiphias gladius)* maturity phase

A Immature

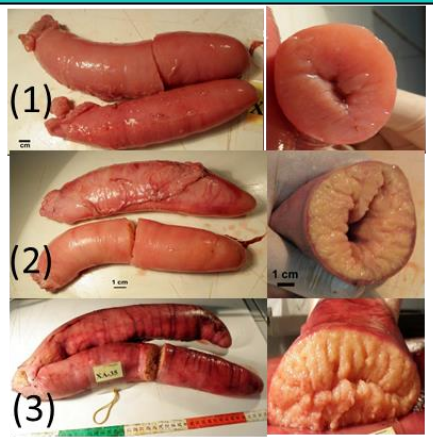
- Round and cylindrical section
- Length< 7 cm

B Gonads in Development: From the beginning to the end of maturation

Beginning of maturation (1):
- Length> 7 cm / no visible oocytes / thin and non-vascularized wall / usually presence of lumen / Weight< 500g

Maturation (2):
- The oocytes are visible to the naked eye /granular appearance /weight < 1 kg

End of maturation (3):
- Opaque cream-coloured individualised oocytes / thin and highly vascularised wall / weight >1kg



C Spawning or spawning capable

- Turgid gonads / weight >1kg / oocytes visible through the wall / transparent oocyte called "hydrated"

D Gonads in regression / regeneration

Regression(1):
- Flaccid gonads and thick wall/ Bloody/stunted appearance/ The cross-section looks like brown leaves/Some hydrated eggs may still be present

Regeneration (2):
- Be careful at this stage the lumen may not be visible /The gonad is more /the wall are really thick/ / The cross-section have mush-like consistency

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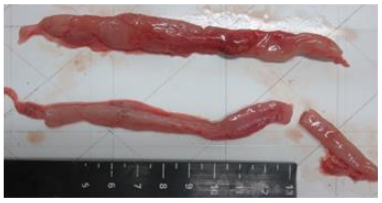
Figure 5 : macroscopic criteria of male swordfish's (*Xiphias gladius)* maturity phase

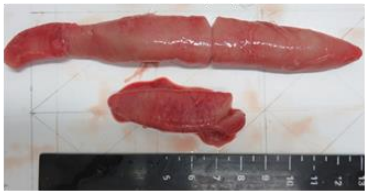
A Immature

- Thin, tapered testicles
- Cross-sectional width< 1 cm



B Gonads in Development: From the beginning to the end of maturation

- Cross-sectional width> 1 cm
- After section and slight pressure, "non-opaque whitish" sperm may appear



C Spawning or spawning capable

- Fully developed testicles
- Flow of white and opaque semen to the cut without pressure

D Gonads in regression / regeneration

- Highly vascularized and bloody-looking flaccid gonads
- Semen residue may be observed

3 . Presentation of the billfish visual guides

All the following sheets are unfortunately incomplete (at least one phase photo missing). The purpose of presenting them here is to exchange ideas and advice on your practices, your experiences, and your studies, so these can be either completed or corrected resulting in a simple and precise criteria.

Figure 6 : criteria for determining billfish gender

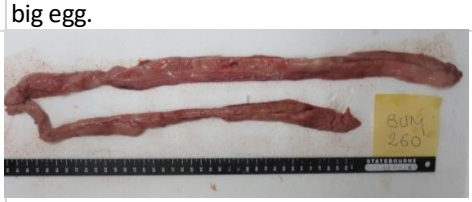
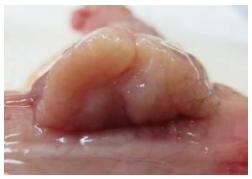
	FEMALE	MALE
General aspect	The **ovaries** have a smooth, round external appearance and can weigh up to several kilograms.	The **testicles** are elongated, flattened, and have numerous nodules on the outer part. be careful when it's mature it can look like big egg.
		
cross-section	The cross-section is less cylindrical than swordfish and often has a lumen (empty space inside). Mature females have oocytes that are visible to the naked eye	The cross-section is also in several leaflet nodules and has no lumen. Sperm is easily observed on mature individuals
		

Figure 7 : macroscopic criteria of female blue marlin (*Makaira nigricans)* maturity phase

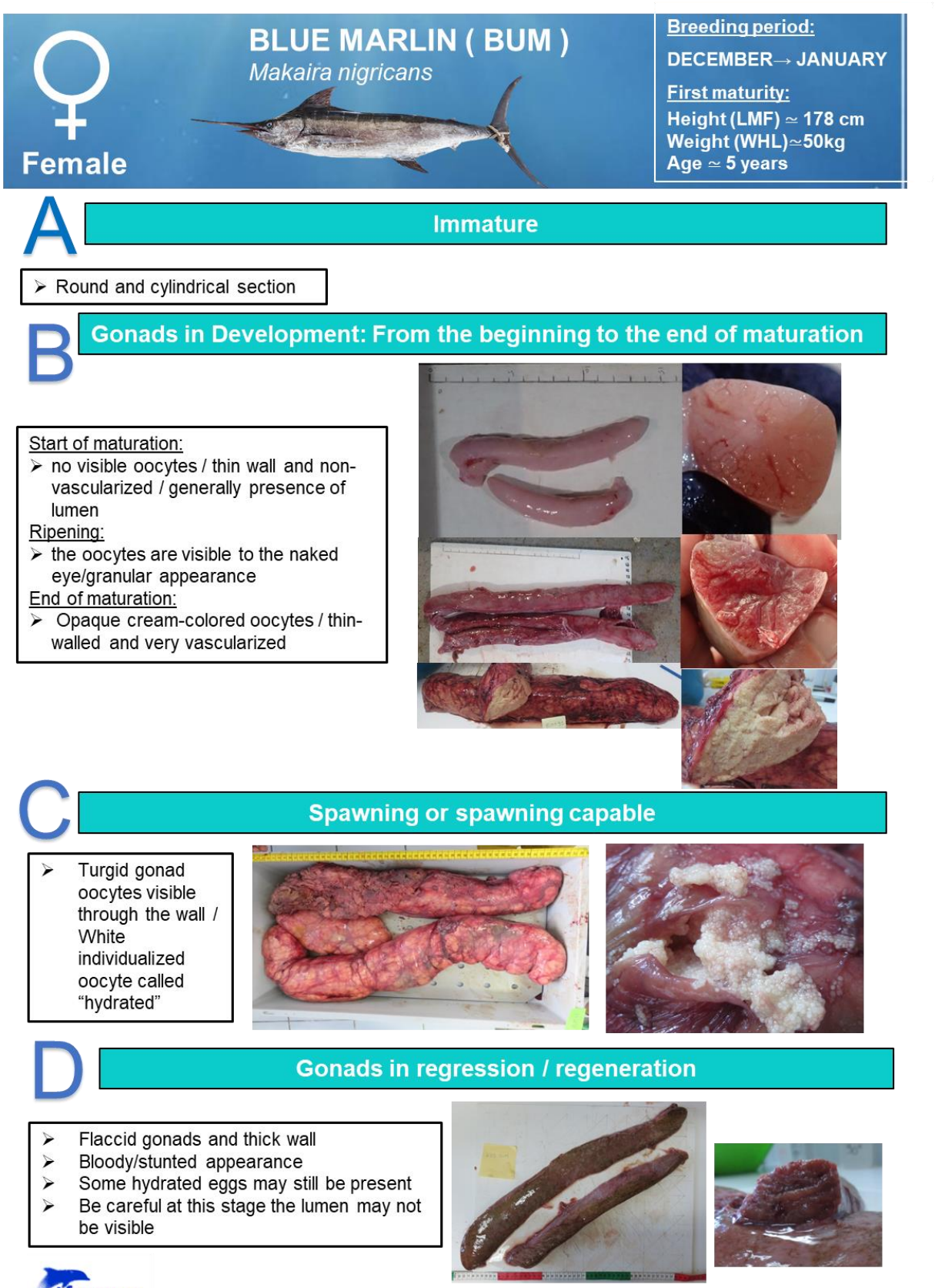


Figure 8: macroscopic criteria of male blue marlin (*Makaira nigricans)* maturity phase

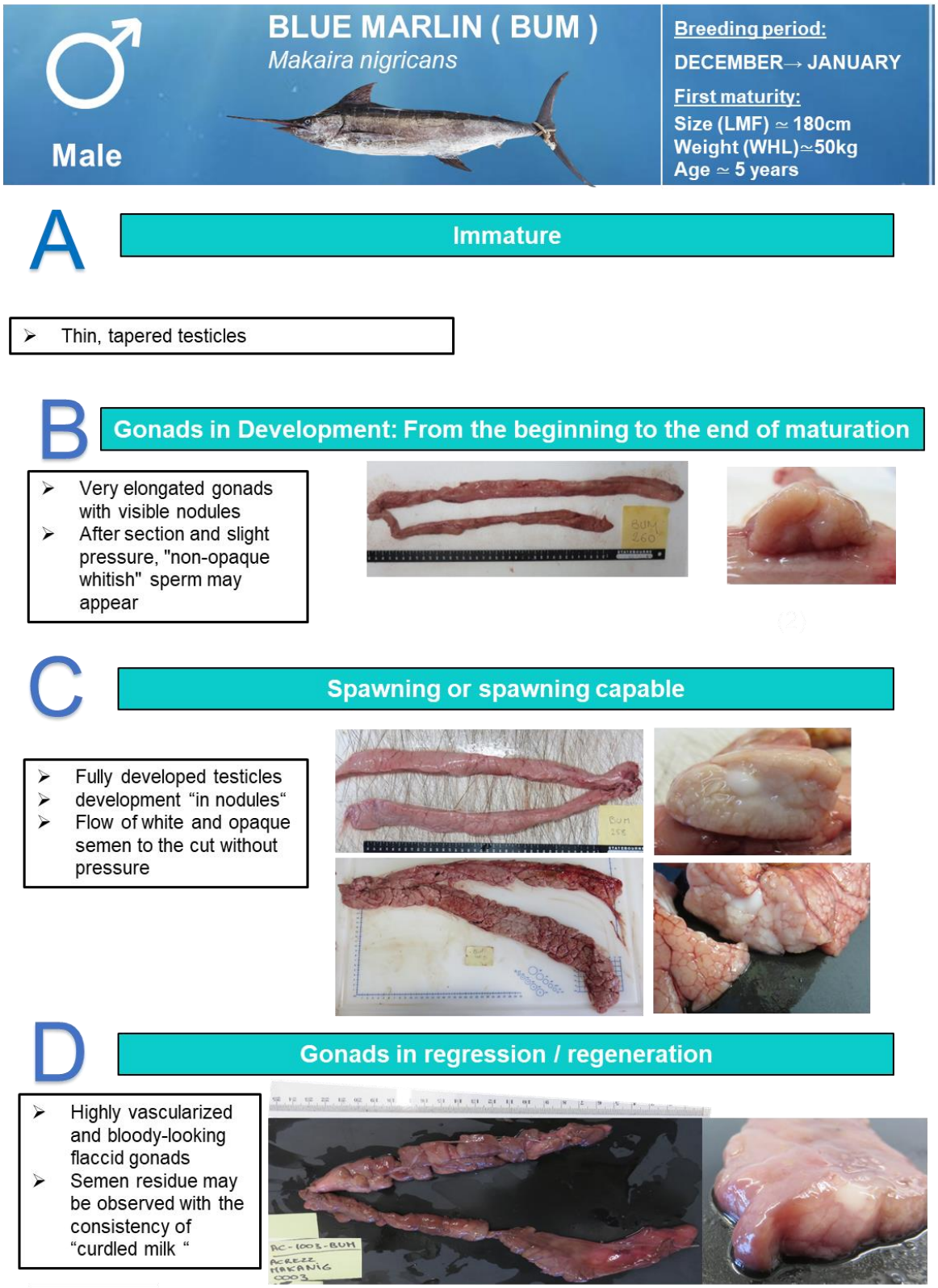


Figure 9 : macroscopic criteria of female Indo Pacific Sailfish (*Istiophorus platypterus)* maturity phase

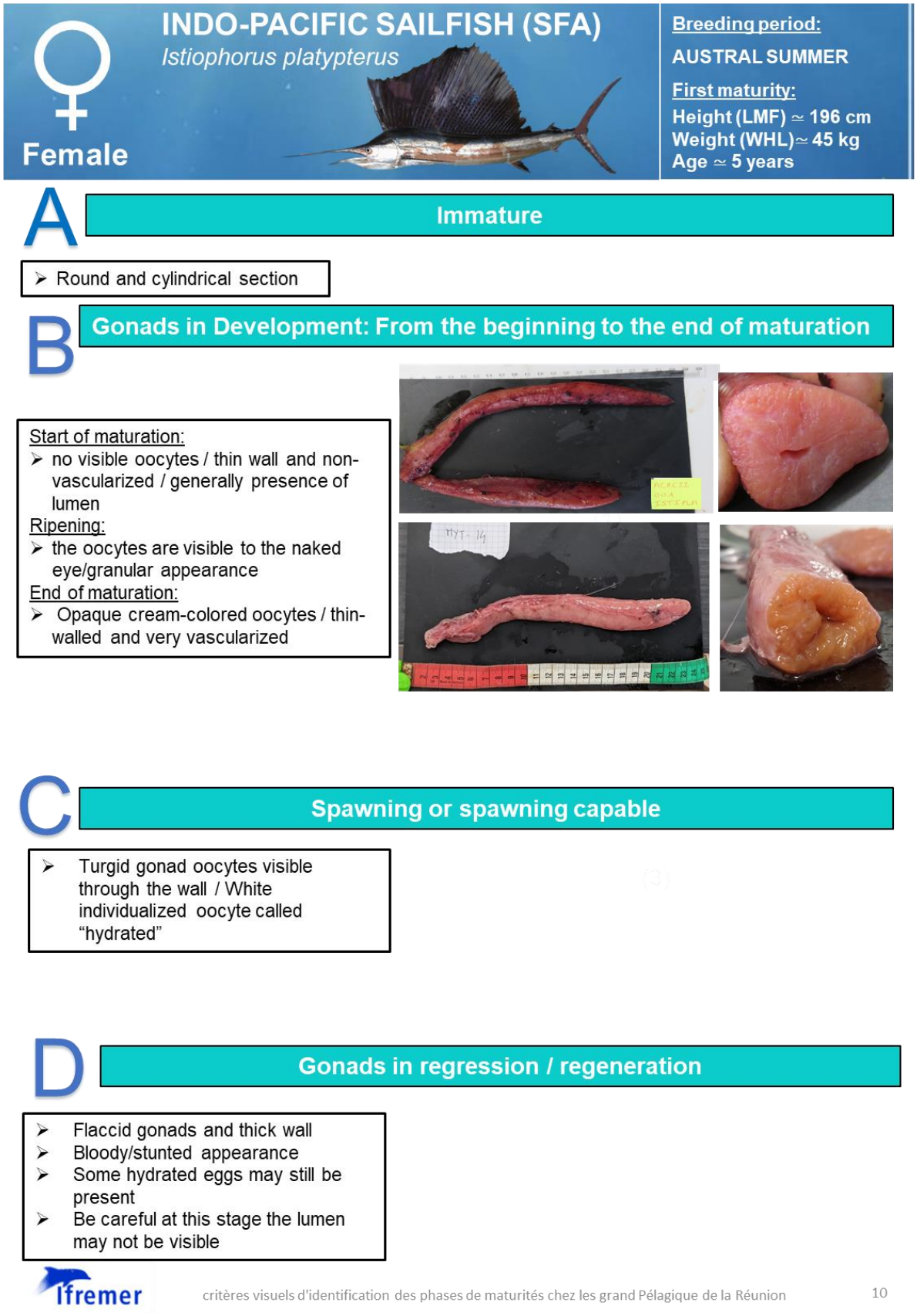


Figure 10 : macroscopic criteria of male Indo Pacific Sailfish (*Istiophorus platypterus)* maturity phase

A — **Immature**

- Thin, tapered testicles

B — **Gonads in Development: From the beginning to the end of maturation**

- Very elongated gonads with visible nodules
- After section and **slight pressure**, "non-opaque whitish" sperm may appear

C — **Spawning or spawning capable**

- Fully developed testicles
- development "in nodules"
- Flow of white/ivory and opaque semen to the cut **without pressure**

D — **Gonads in regression / regeneration**

- Highly vascularized and bloody-looking flaccid gonads
- Semen residue may be observed with the consistency of "curdled milk "

Figure 11 : macroscopic criteria of female stiped marlin (*Kajikia audax)* maturity phase

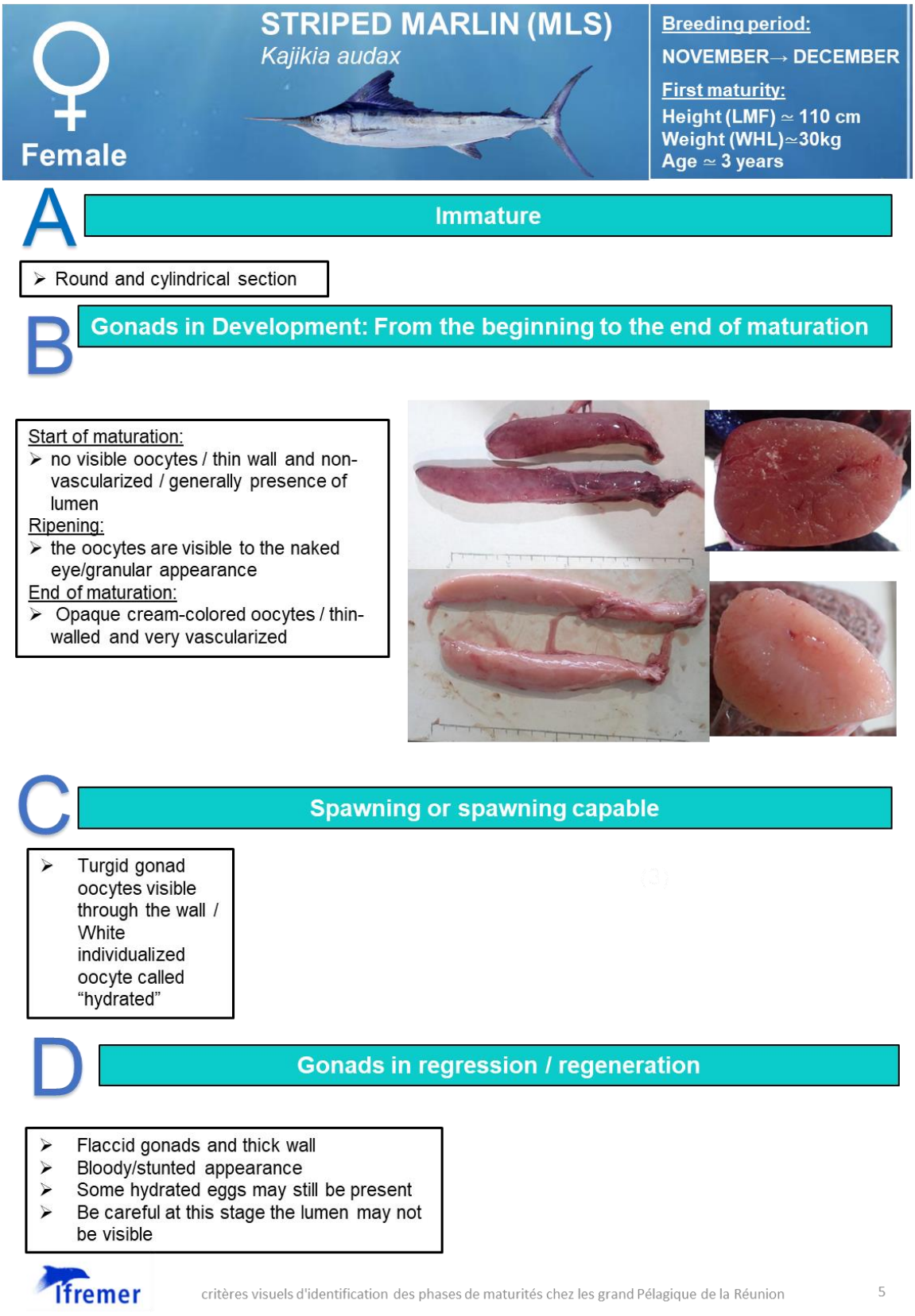


Figure 12 : macroscopic criteria of male stiped marlin (*Kajikia audax)* maturity phase

A — **Immature**

- Thin, tapered testicles

B — **Gonads in Development: From the beginning to the end of maturation**

- Very elongated gonads with visible nodules
- After section and slight pressure, "non-opaque whitish" sperm may appear

C — **Spawning or spawning capable**

- Fully developed testicles
- development “in nodules“
- Flow of white and opaque semen to the cut without pressure

D — **Gonads in regression / regeneration**

- Highly vascularized and bloody-looking flaccid gonads
- Semen residue may be observed with the consistency of “curdled milk “

Figure 13 : macroscopic criteria of female black marlin (*Istiompax indica)* maturity phase

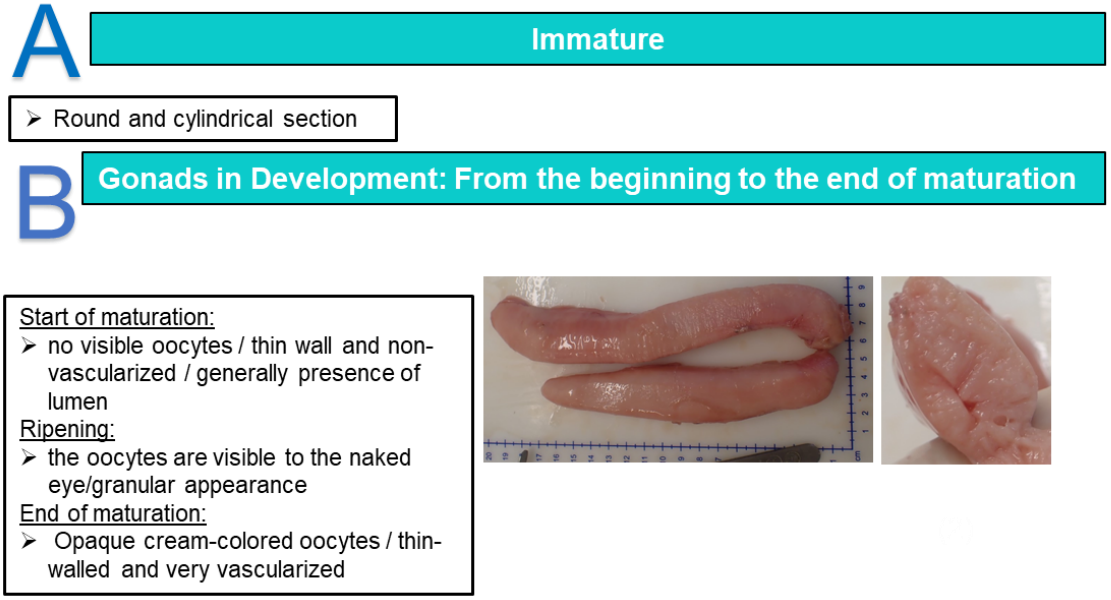
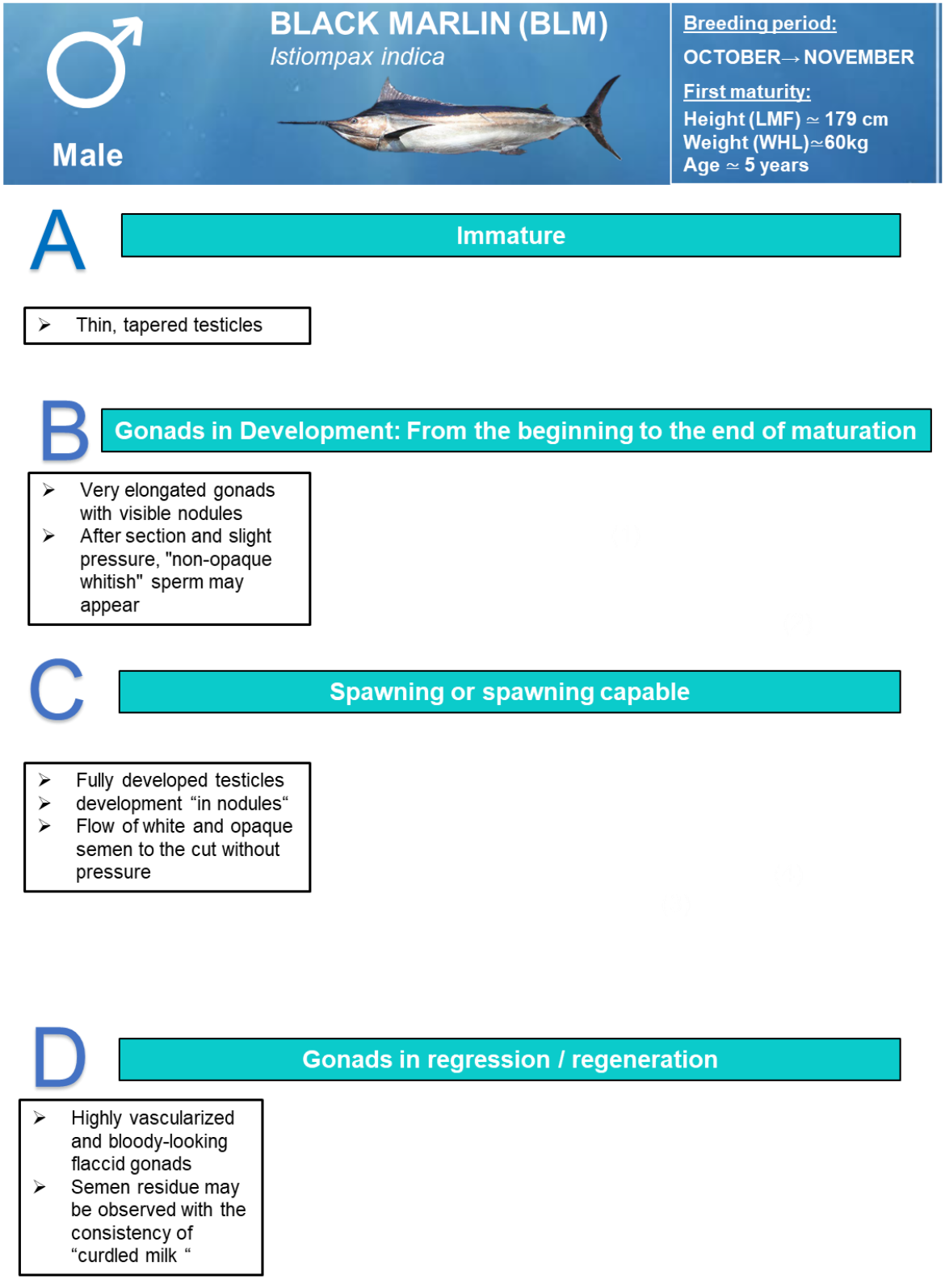


Figure 14 : macroscopic criteria of male black marlin (*Istiompax indica)* maturity phase



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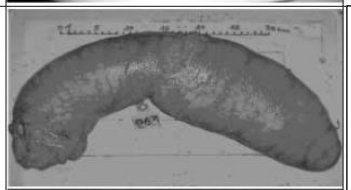
Follesa, M.C., Carbonara, P., eds. (2019). Atlas of the maturity stages of Mediterranean fishery resources. Studies and Reviews n. 99. Rome, FAO. 268 pp.

Annex 1 : Full ICES WKASMSF 2018 scale

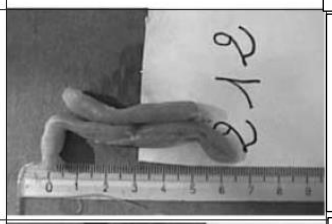
State description	Phase		Old terminology (ICES 2012)		New terminology		Macroscopic criteria		Histological features		Histological model
SI : Sexually immature, (without gonad development)	Immature	Possible sub-phase	I	Possible sub-phase	A	Possible sub-phase	Small pinkish and translucent (often clear) ovaries shoter than 1/3 of body cavity. Indistinct blood vessels and no visible eggs	Possible sub-phase	Only oogonia and PG oocytes present containing no oil droplets, rare atresia, no muscle bundles. Thin ovarian wall, scarce connective tissue around follicles and little space between oocytes	Possible sub-phase	Presence of either **ov**, **op1** or **op2** cells Absence of **oca**, **vit1**, **vit2**, **vit3**, **vit4**, **och**, **oh** and **POF**
SM : Sexually mature (with gonad development)	Developping	Developing but functionally immature (first-time developer)	II	IIa	B	Ba	Enlarging ovaries, blood vessels become more distinct	Small pinkish/reddish ovaries shorter than 1/2 of the body cavity. Eggs not visible to the naked eye	Marked increase in oocyte size, blood vessels become more distincts, PG, CA oocytes present. Vtg1 and Vtg2 oocytes can also be present. No POF. No Vtg3 oocytes. Some atresia may be present.		Presence of either **oca**, **vit1**, **vit2** or **vit3** cells Absence of **vit4**, **och**, **oh**, **oaB** and **POF** A percentage count of **oaA** of less than or equal to 50% of the total follicles quantified
		Developing but functionally mature		IIb		Bb		Pinkish-reddish/reddish-orange and translucent ovaries that have a length of about 1/2 of the body cavity. Visible blood vessels, no visible eggs			
	Spawning	Actively spawning	III	IIIa	C	Ca	Large ovaries, blood vessels prominent, individual oocytes visible macroscopically	Pinkish-yellow ovaries with a granular appearance and a length of about 2/3 of the body cavity. Eggs are visible to the naked eye through the *ovaric tunica* which is not yet translucent. No eggs are expelled under light pressure		Vtg3 oocytes are present. Early stage of OM can be present. No POF. Atresia of vitellogenic or hydrating oocytes may be present	Beginning of phase : Presence of either **vit4**, **och** or **oh** cells Absence of **POF** and **oaB** A percentage count of **oaA** of less than or equal to 50% of the total follicles quantified
		Spawning capable		IIIb		Cb		Orange-pink ovaries with conspicuous superficial blodd vessels and a length from 2/3 to full length of the body cavity. Large, transparent, ripe eggs are clearly visible and can be expelled under light pressure, or escape freely		Oocytes at the end of GVM, GVB, hydration or ovulation are present. Recently collapsed POF can be present	End of phase : Presence of either **vit4**, **och** or **oh** cells Absence of **oaB** A percentage count of **oaA** of less than or equal to 50% of the total follicles quantified A percentage of **POF** that does not exceed the number of **oh**
	Regression / Regeneration	Regression	IV	Ia	D	Da		Reddish ovaries of about 1/2 of the body cavity length. Flaccid ovarie walls, prominent blood vessels, and possible remnants of disintegrating opaque and/or translucent eggs		Atresia (any stage) and POF are present. Some healthy CA and/or Vtg1, Vtg2 oocytes present	
		Regeneration		IVb		Db		Small pinkish and translucent ovaries that have a length of aboiut 1/3 of the body cavity, with reduced but present blood vessels. No visible eggs		Only oogonia and PG oocytes present. Oil droplets can be seen in PG oocytes (species dependent). Muscle bundles, enlarged blood vessels, thick ovarian wall and/or atresia or old, degenerating POF may be present. There is more space and interstitial tissues around PG oocytes	
	Omitted spawning		V		E				No POF and at least 50% of the vitellogenic oocytes are atretic		Abscence of **POF** A percentage count of **oaA** of more than 50% of the total follicles quantified
	Abnormal		VI		F		Problems in the gonad development (necrosis, sclerosis, intersex, majority of the gonad that looks unhealthy)				

Annex 2 : Swordfish Maturity scales from F.Poisson, M.Taquet (2001)

Characterization of the different maturation stages identified for females.

Stages		Female Gonads
0	X	**Developing gonads**: the gonads are in the form of filaments in the form of filaments and cannot be identified by the naked eye
1		**Immature:** the sex can be identified with the naked eye.The ovaries are round and cylindrical, firm and pink in color
2		**Beginning of maturation:** the sex is identifiable.The ovaries are firm in consistency and whitish in color. The lumen is visible after sectioning, oocytes not visible
3		**Maturation**: the ovary is larger, more pronounced vascularization and visible oocytes
4		**End of maturation:** the gonad has grown significantly into the abdominal cavity, and is turgid, soft and highly vascularized. The well-individualized oocytes are cream-coloured.
5	 	**Ovulation:** the ovaries are highly vascularized and translucent.The hydrated oocyte fraction is clearly visible and their emission is imminent. Ova flow out after incision of the ovarian wall.
6		**Post-laying or atresia:** appearance depends on the time elapsed since the last laying and the time of year. Ovaries are flaccid and hollow, reddish to grayish in color. to grayish. The ovaries gradually return to a stage 3 appearance to begin a new maturation cycle or regress to sexual rest

Characterization of the different maturation stages identified for males.

Stages		Male Gonads
0	X	**Developing gonads:** the gonads appear as filaments in the form of filaments and cannot be identified by the naked eye
1		**Beginning of maturation:** the sex is identifiable, pinkish to red gonads are thin and tapered
2		**Maturation:** development increases in size, vascularization is more pronounced
3		**End of maturation:** the testicles are white to pink in color to pinkish in color, fully developed; after sectioning sperm is still flowing
4		**Post-maturation or atresia:** the testicles are highly vascularized, red to whitish in color

Translation to the new scale WKASMSF 2018

Female		**Male**	
F.Poisson, M.Taquet (2001) STAGES	WKASMSF (2018) STAGES	F.Poisson, M.Taquet (2001) STAGES	WKASMSF (2018) STAGES
0- Developing gonads	IND –Indeterminated	0- Developing gonads	IND –Indeterminated
1- Immature	A- Immature	1- Beginning of maturation	A- Immature
2- Beginning of maturation	B- developping	2- Maturation	B- Developping
3- Maturation		3- End of maturation	C- Spawning
4- End of maturation		4- Post-maturation or atresia	D- Regression /Regeneration
5- Ovulation	C-spawning		
6- Post-laying or atresia	D-regression /regeneration		