

Female tuna reproductive cycle - Protocol for histology analysis

BFT (*Thunnus thynnus*), Atlantic and Mediterranean

BET (*Thunnus obesus*), Tropic and subtropic areas

YFT (*Thunnus albacares*), Tropic and subtropic areas

ALB (*Thunnus alalunga*), Temperate and tropic areas

SKJ (*Katsuwonus pelamis*), Tropic and warm-temperate areas

FEMALE REPRODUCTIVE CHARACTERISTICS

- Iteroparity
- Gonochorism
- Ovuliparity
- Asynchronous oocyte development
- Indeterminate fecundity
- Batch fecundity

GONAD DEHYDRATION

Gonad cutting (1 cm)

Tissue processor

PARAFFIN		RESIN	
Baths	Immersion time	Baths	Immersion time
Alcohol 70%	1 h	Alcohol 70%	32 h
Alcohol 90%	1 h	Alcohol 90%	16 h
2 x Alcohol 96%	2 x 2 h 45'	Alcohol 96%	8 h
2 x Xylene	2 x 2 h 15'	Resin + Ethanol	48 h
2 x Paraffin	2 x 2 h 30'	Resin	~60 h

HISTOBLOCK PREPARATION AND SLICING

Microtome

PARAFFIN		RESIN	
Baths	Immersion time	Baths	Immersion time
Absolute alcohol	2 min	Haematoxylin	5 min
Absolute alcohol	2 min	Running tap water	5 min
Tap water	5 tips		
Haematoxyline	2 min		
Running tap water	2 min		
Acid alcohol	5 tips		
Running tap water	2 min	Eosin	5 min
Eosin	1 min		
Absolute alcohol	3 min		
Absolute alcohol	3 min	Running tap water	5 min
Xylene	5 min		
Xylene	5 min	Alcohol absolute	1 tip

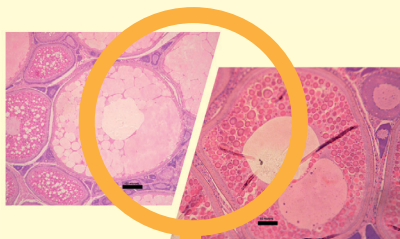
SLIDES STAINING

READING FOR MICRO IDENTIFICATION

ANNUAL FECUNDITY ESTIMATION - INDETERMINATE FECUNDITY

Multiplying the batch fecundity, spawning fraction (i.e. % of females spawning per day), and duration of spawning season

SAMPLE SELECTION



- Ovaries with oocytes in GVM / Hydration stages
- No presence of new Postovulatory Follicles (POF) (i.e., residual follicle layer remaining in ovary after ova releasing)
- Ovaries with current batch formed

METHODOLOGY

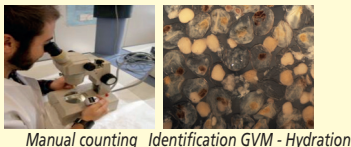
Sample weight **0.1 g**
+ 1-2 drops Glycerin



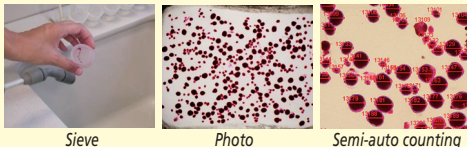
Sample weight **0.04 g**

+ Rose Bengal (min 24h)
2 g Rose Bengal powder / Litre 4% buffered formaldehyde

GRAVIMETRIC - MANUAL METHOD



GRAVIMETRIC - IMAGE ANALYSIS METHOD



RESULTS

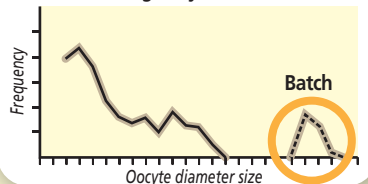
Counting translucent oocytes (O_i)

Formula:

$$BF = \frac{[\sum_i (O_i / W_i)]}{n} \times W_o$$

where O_i is the number of oocytes in a weighed sample (W_i).
 W_o is the total mass (g) of the ovary
 n is the number of subsamples required to reach CV < 10% of the n° of oocytes per unit weight.

Counting oocyte of the batch



Authors

- Iker Zudaire (IRD) iker.zudaire-balardi@ird.fr (IKERBASQUE) current position
- Emmanuel Chassot (IRD) Emmanuel.Chassot@ird.fr
- Constance Diaha (CRO) constance.diaha@cro-ci.org
- Maria Cedras (SFA) mcedras@sfa.sc
- Hilario Murua (AZTI) hmurua@azti.es
- Nathalie Bodin (IRD) nathalie.bodin@ird.fr

References

- Ganias, K., et al. 2014. Handbook of Applied Fisheries Reproductive Biology for Stock Assessment and Management: 1-109.
- Hunter, J.R., et al. 1985. Batch fecundity in multiple spawning fishes, in: Lasker, R. (Eds.), An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. NOAA Tech. Rep. NMFS 36, 67-78
- Murua, H., Saborido-Rey, F. 2003. Female reproductive strategies of commercially important fish species in the North Atlantic. J. Northwest Atl. Fish. Sci. 33, 23-32.
- Schaefer, K.M. 2001. Reproductive biology of tunas. In: Block, B. A., Stevens, E. D., Hoar, W. S., Randall, D. J., Farrel, A. P. (eds), Tuna: Physiology, ecology, and evolution, pp 225-270. Academic Press, San Diego, California, USA.
- Zudaire, I., et al. 2013a. Fecundity regulation strategy of the yellowfin tuna (*Thunnus albacares*) in the Western Indian Ocean. Fish. Res. 138, 80-88.

IRD Institut de recherche pour le développement

emotion Estimation of effects on the sustainability of large pelagic populations

azti tecnalia

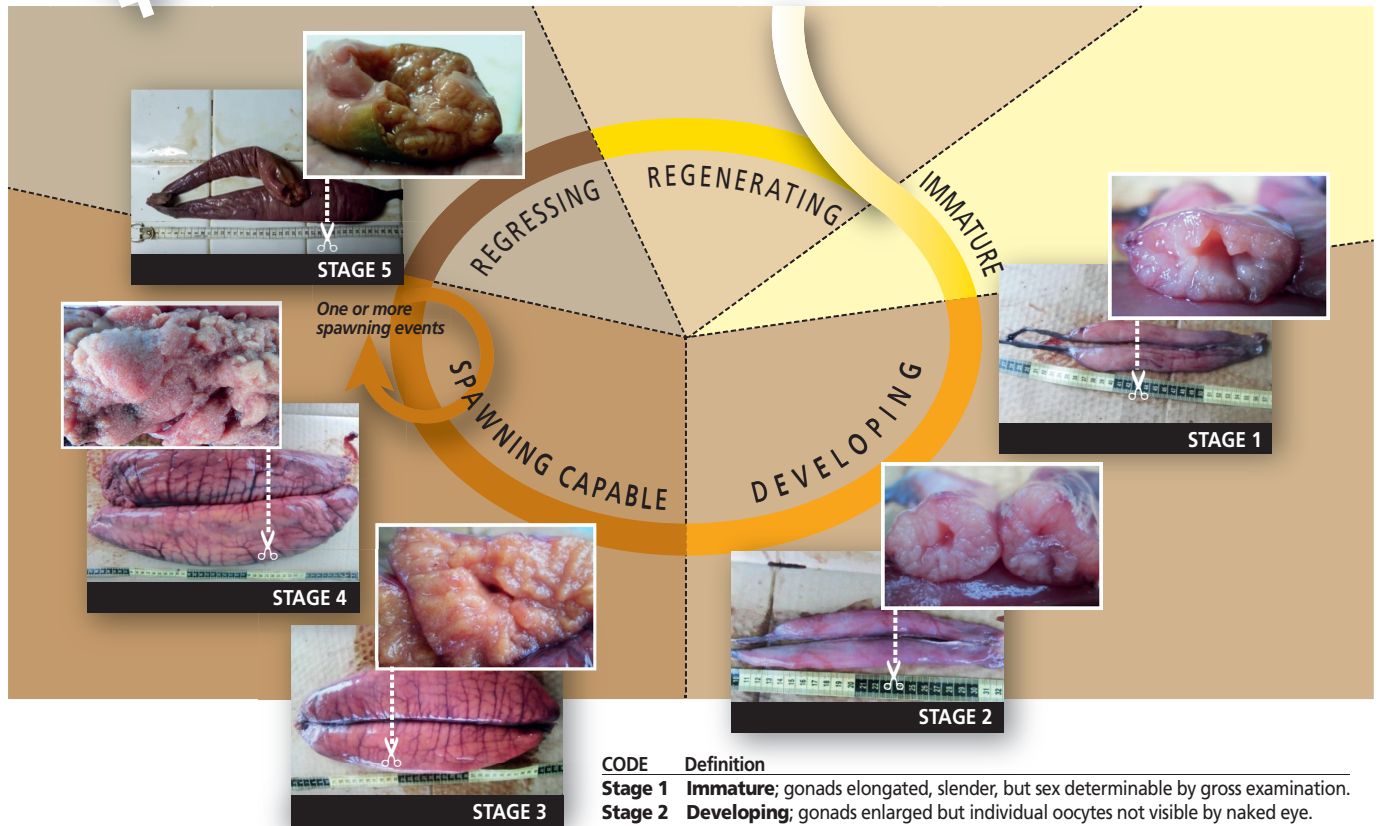
SECTEUR PÊCHE AUTHORITY

GPO CENTRE DE RECHERCHES Océanologiques

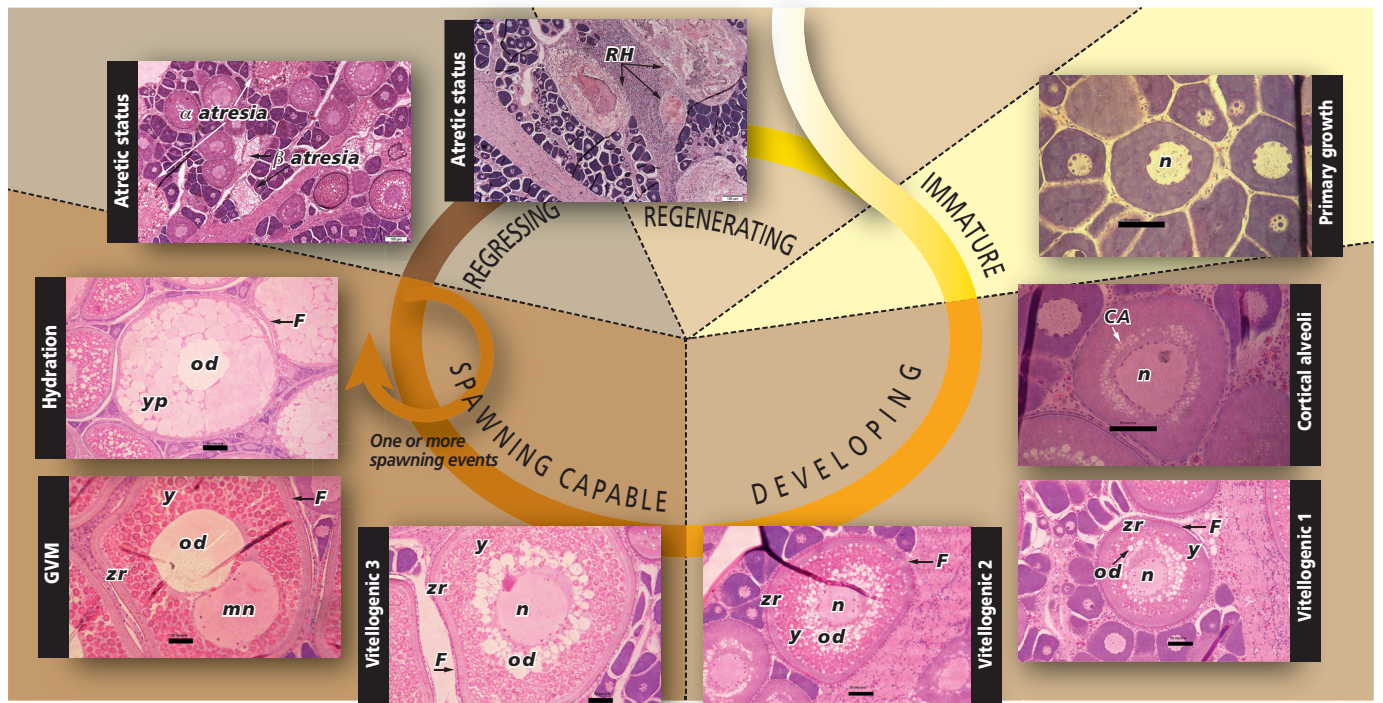
Female tuna reproductive cycle - Batch fecundity protocol



Female tuna reproductive cycle - macroscopic maturity scale



CODE	Definition
Stage 1	Immature ; gonads elongated, slender, but sex determinable by gross examination.
Stage 2	Developing ; gonads enlarged but individual oocytes not visible by naked eye.
Stage 3	Late developing ; gonads enlarged, individual oocytes visible by naked eye.
Stage 4	Spawning capable ; ovary greatly enlarged, oocytes translucent, easily dislodged from follicles or loose in lumen of ovary.
Stage 5	Regressing-Regenerating ; includes recently spawned and regressing fish, mature oocytes remnants in various stages of resorption (i.e., atresia), and mature oocytes remnants about 1.0 mm in diameter.



Phase	Oocyte development stage	Characteristic
Immature	Primary growth (PG)	The first phase of oocyte growth. Two stages are present Chromatin nucleolar and Perinucleolar. In 1st stage nucleus (n) is large and centrally located, containing a large and single nucleolus. In 2nd stage multiple nucleoli appear at the periphery nucleus. Presence of spherical vesicles (CA) at the periphery of the cytoplasm. Oil drops (od) begin to accumulate in the cytoplasm.
Developing	Cortical alveoli (CA)	Chorion and follicle (F) layers are apparent.
Spawning capable	Vitellogenic (Vtg)	Yolk vesicles (y) appear in the cytoplasm. Chorion separates in two layers: inner and outer zona radiate (zr). There are 3 Vtg stages: Oil droplets occupy more cytoplasmic area than yolk vesicles.
	Vtg 1	Oil droplets occupy similar cytoplasmic area than yolk vesicles.
	Vtg 2	Oil droplets occupy less cytoplasmic area than yolk vesicles.
Regression and regenerating	Oocyte Maturation	The nucleus migrates (mn) to the animal pole. The oil droplets fuse to coalescence into an unique oil globule. Yolk granules fuse in yolk plates (yp) forming a homogeneous mass. The nucleus has disintegrated.
	Atretic status	Regressing: $\geq 50\%$ α atresia, β atresia present in vitellogenic oocytes. Regenerating: late atresia (γ and δ), residual hydrated (RH) oocytes present.



Female tuna reproductive cycle - microscopic maturity scale