



WRECKFISH REPRODUCTION. STATUS IN SPAIN



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OUR GOALS

WP 6. REPRODUCTION AND GENETICS

The research activities of DIVERSIFY regarding wreckfish reproduction are focused on five targets:

- I. Increase the availability of broodstocks.
- II. Describe the reproductive cycle in captivity.
- III. Develop spawning induction protocols for tank spawning, as well as artificial fertilization.
- IV. Develop protocols for Computer Assisted Sperm Analysis (CASA) and sperm cryopreservation.
- V. Determine the influence of broodstock feeds on fecundity and spawning quality (WP 12 –Nutrition wreckfish).

Location of the stocks

Isidro de la Cal
44 individuals 12-22 Kg



MC2



Natural temperature and simulated natural photoperiod

Exposition tank: 3500 m³
Auxiliary tank for breeders: 33 m³

PRIVATE COMPANIES

Aquarium O Grove.
6 individuals 10-14 Kg
CMRM



CMRM



Natural temperature and photoperiod

2 tanks 40 m³
1 tank 120 m³
1 tank 180m³

IEO



Natural temperature and photoperiod

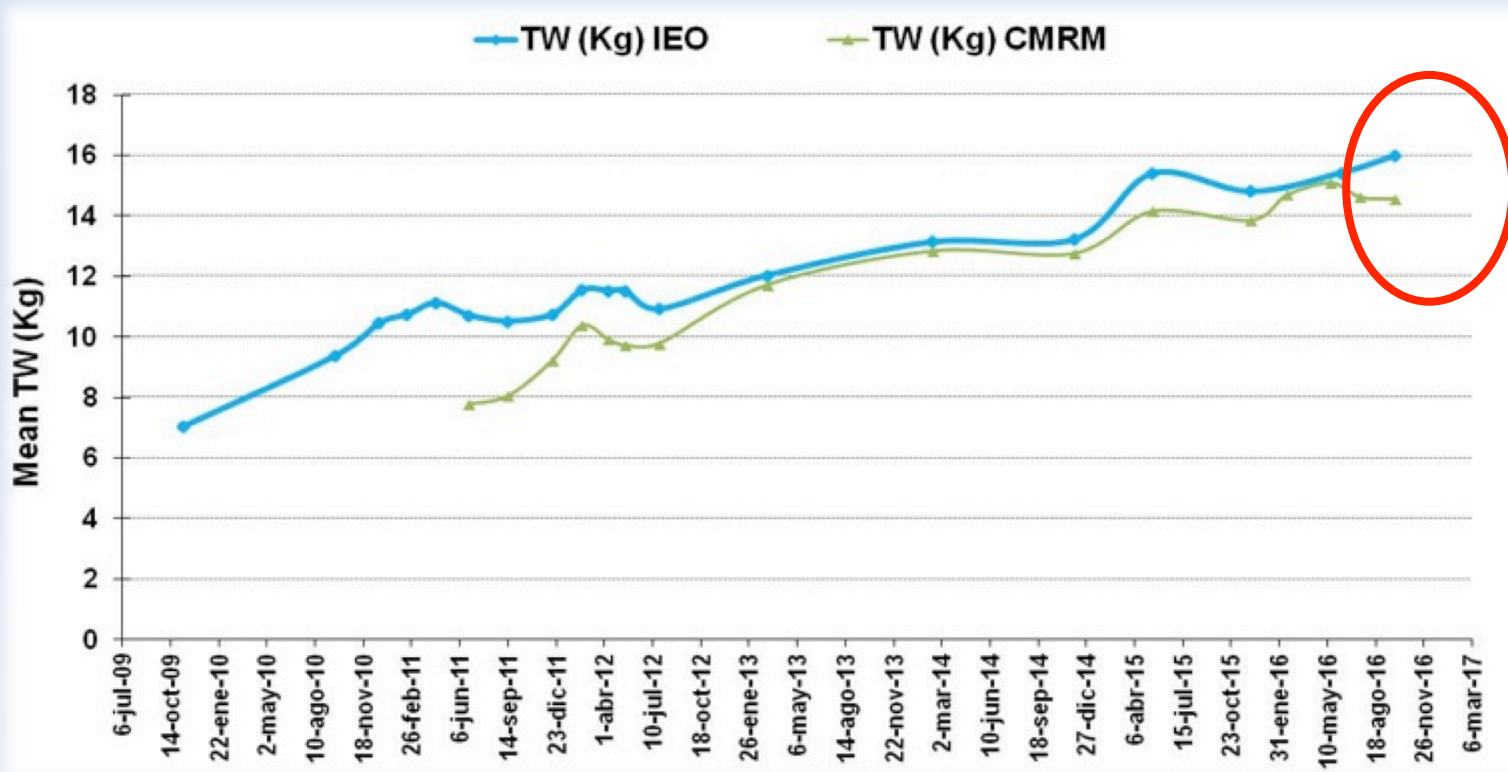
2 tanks 110 m³

During the years 2014, 2015 and 2016, broodstocks were overseen in order to describe the reproductive cycle in captivity. These fish were maintained in a variety of environmental conditions in regards to tank size and photothermal regime.

OUR STOCKS



	MC2 AQUARIUM FINISTERRAE	CMRM IGAFA/CIMA	IEO Centro Oceanográfico de Vigo	TOTAL
MALES	6	4	3	13
FEMALES	10	5	8	23
UNDETERMINED	3	2	3	8
TOTAL	19	11	14	44

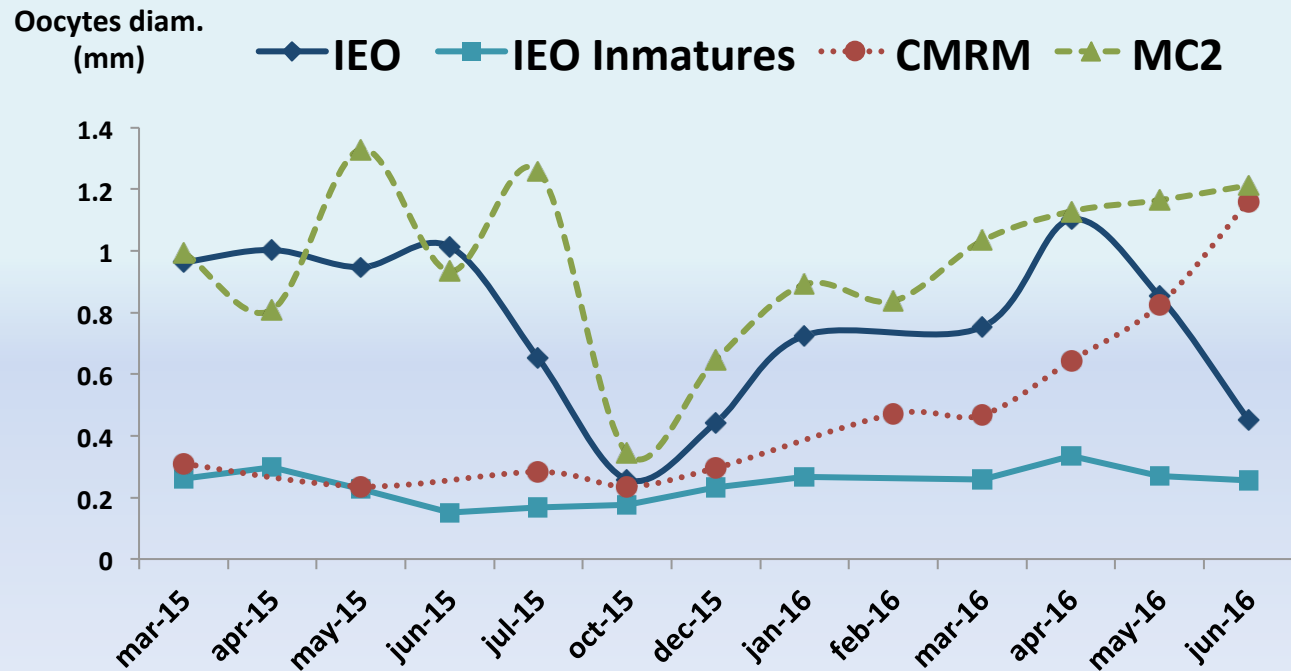


14-16 Kg.

Total weight (mean) IEO and CMRM wreckfish stocks from 2009 to 2017

Our females

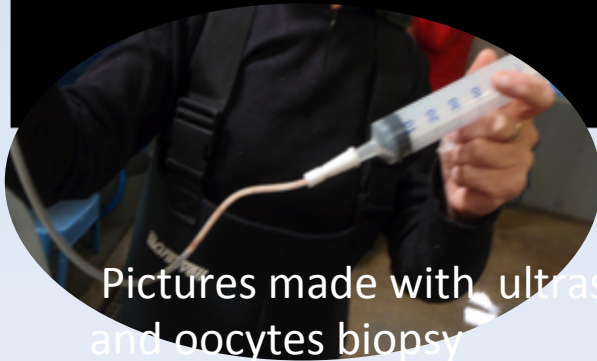
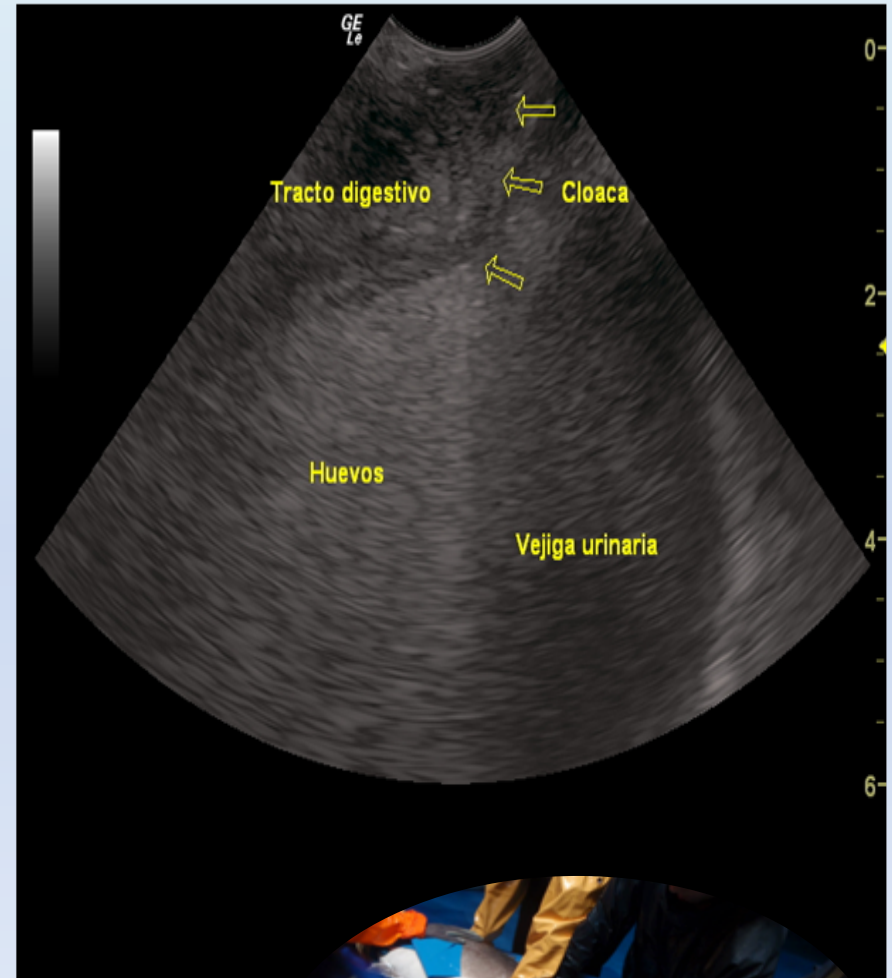
The degree of sexual maturation was studied in the three broodstocks. Each stock was sampled monthly during the period February-July (breeding season) and bimonthly during the period of resting August-January over the years 2015 and 2016.



- Gonadal maturation in females begins in the autumn, but the main part of vitellogenesis takes place in winter (Dec-Feb). And oocyte maturation in captivity starts in March with peaks between April and June.
- Vitellogenesis continues until the oocytes reach a size of 1.2-1.400 mm in diameter, at which time oocyte maturation may take place.
- Biopsies from females that did not spawn had oocytes with similar size all year.

Our females

Gonadal tissue samples were obtained from females by cannulation through the ovarian duct



Pictures made with ultrasound with breeders of MC2 and CMRM for sex identification and oocytes biopsy

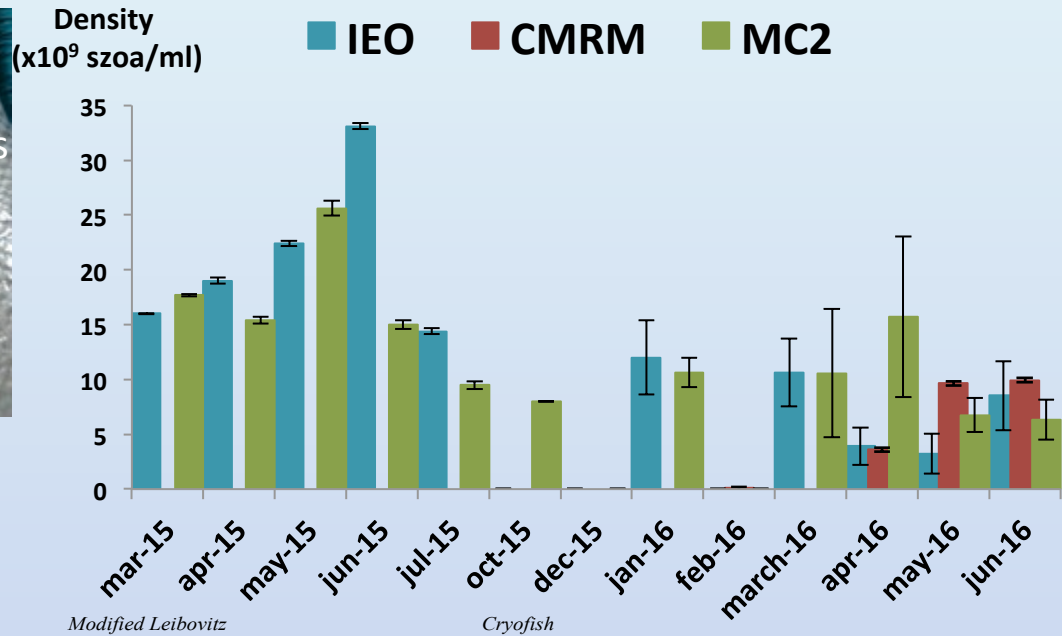
Our males



- Sexual maturation of males covers the same period of females, reaching its peak in the months of April and June, with peak concentrations of 25-35 x 10⁹ szoa/ml of sperm.

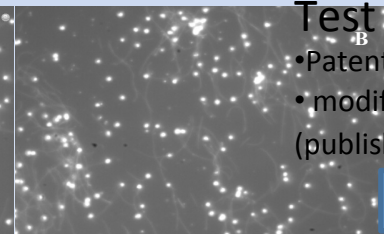
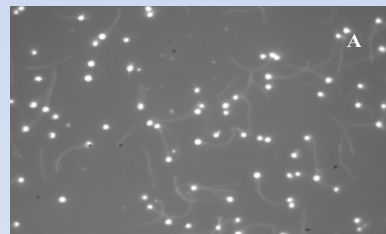
- The mobility ratio is high, with mean values between 2- 3 min. 30sec.

- The mean survival time of the sperm, conserved refrigerated at 4°C, is 4 days. However, in some cases it reaches the 18 days of survival after its recollection.



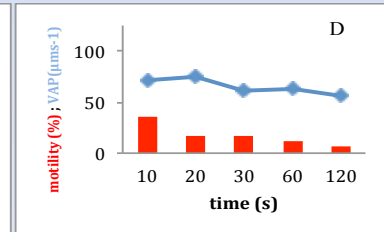
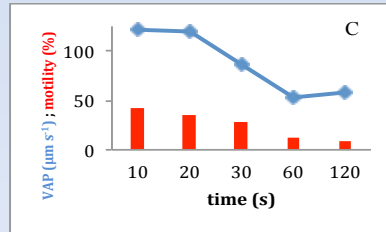
Modified Leibovitz

Cryofish



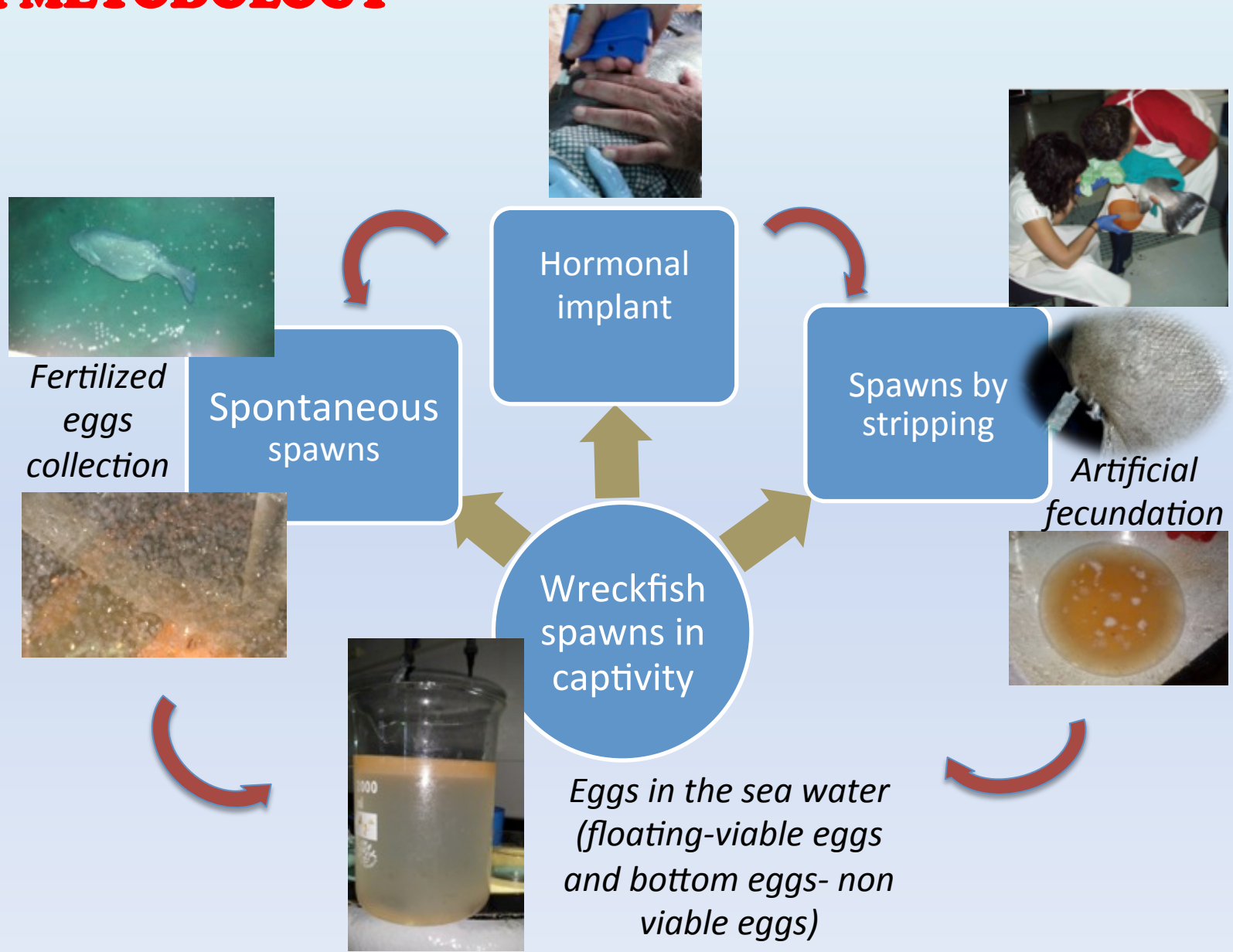
Test of 2 diluents

- Patented Cryofish
- modified Leibovitz L15 (published formula)



Task 6.4. Evaluation of sperm characteristics (CASA) and cryopreservation protocols. D.6.1 and 6.2

OUR METODOLOGY



Our spawns

- During the spawning season of 2015, a total of 10 spawns were obtained from the IEO broodstock between March and June, and 14 from the MC2 broodstock. The majority of spawns were spontaneous, except one artificial stripping from the IEO and two from the Aquarium Finisterrae.
- During 2016, from April to July, 9 spontaneous spawns were obtained from the IEO, 22 spontaneous spawns from the MC2 and the first spawns from the IGAFa. The majority of spawns were spontaneous, except two by stripping (IEO), six by GnRh induction (MC2) and three, of the four from IGAFa., were by hormonal induction (GnRh).
- Spontaneous spawning in the IEO and Aquarium Finisterrae stocks produced a large number of fertilized eggs and achieved satisfactory fertilization success.

2015	FEMALE (TAG Nº)	WEIGHT (Kg)	SPAWNS				FECUNDITY		
			spont	stripp	induc+spont	induc+stripp	eggs(cc)	eggs(nº x10 ³)	(nºeggs/kg female)X10 ³
IEO	9703	14,5	9	1			6540	981	67,65
	5679	15,0	3	2			5390	8085	53,9
	5554	23,5	8				16825	2523,75	107,4
MC2	7B78	19,0	1				2500	275	19,7
	2016								
IEO	7938	15,6	9				4115	617,25	39,57
	5679	16,0	4	2			6180	927,00	57,94
MC2	5554	24,8	4				8350	1252,50	50,50
	7B78	19,0	7				15400	2310,00	121,58
	5358	18,0			6		5105	765,75	42,54
IGAFa	3FF2	14,2	1		1	1	350	52,50	3,70
	7B19	14,0				1	750	112,50	8,04

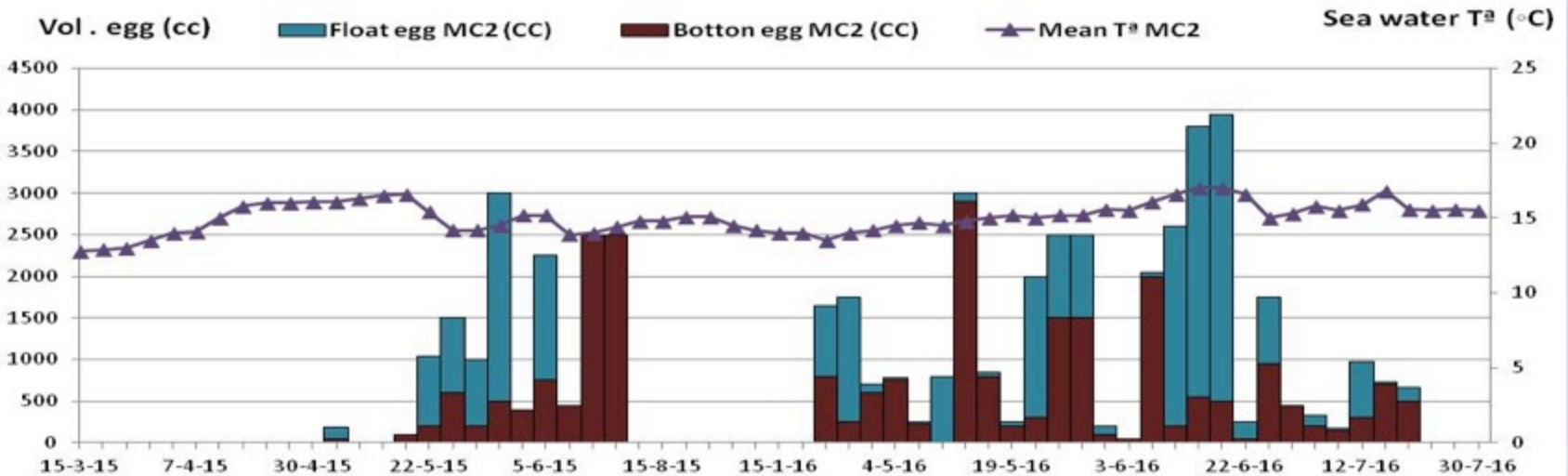
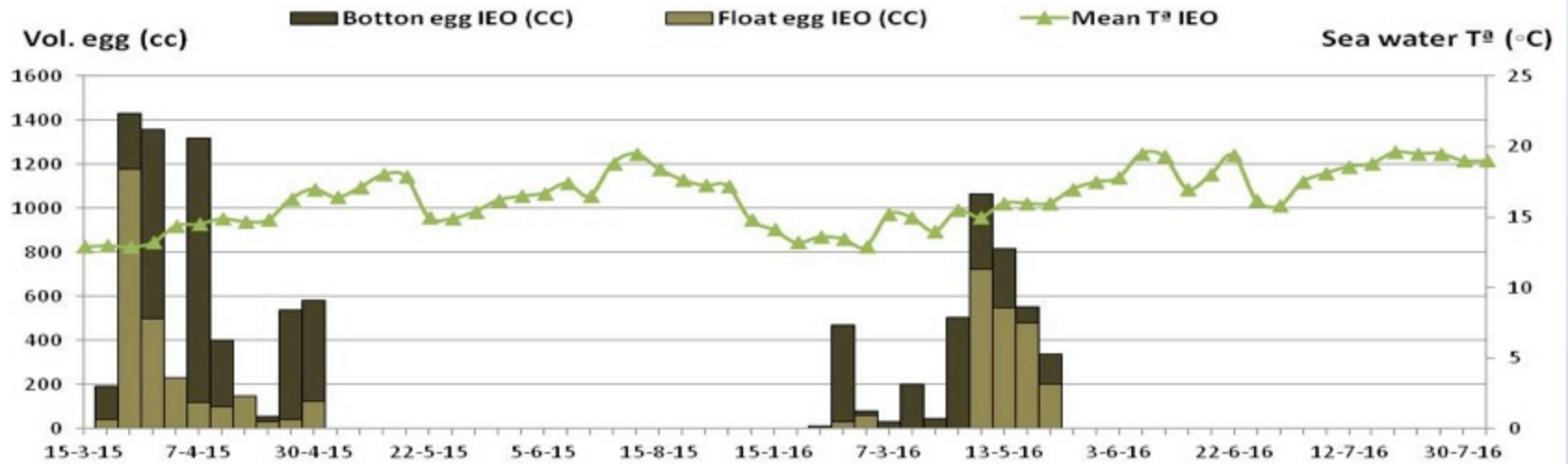
Total spawns

2015: 10 (IEO), 9 (MC2)

2016: 9 (IEO), 23 (MC2), 4 (MCMR)

Vitelogenesis
with FSH+LH
until 1.4 mm

Our spawns



Volume of viable floating and non-viable sinking eggs (cc) of wreckfish obtained from spawns at the IEO (upper) and Aquarium Finisterrae (lower) facilities between March 2015 and May 2016. The spawns were incubated and larvae were obtained.

Our spawns

In 2015

- Mature signals are present in 7 males in MC2, and in 2 males in IEO.

In 2016

- The same quantity of fluyent males are present in the MC2. However, 4 of them were present in the previous year as well.

- One male was moved from MC2 to CMRM. In both broodstocks the male behaved as fluyent.

- We have three mature males in the IEO. Two of them were fluyent as during the previous year.



FLUYENT MALES/ BROODSTOCK		
	2015	2016
MC2	5211	3484
	7705	5367
	8089	5830
	7705	
	6344	
	6582	
	5200	
CMRM	-----	7762
IEO		OCEE
	9902	
	9714	

Results

I. Biometric and biochemical data were obtained in 2014 from a large number of fish captured by the commercial fishery in the Azores Islands (Atlantic Ocean), and sold fresh at the market in Vigo, NW Spain.

A new dry food for wreckfish broodstock was formulated.

II. Success in spontaneous spawning in all of the three stocks..

III. The spawning season covers the months from March to July, and occurs sequentially in batches every 5 days for the females.

IV. Males exhibit good sperm quality with large amounts of expressible sperm during an extended reproductive period (April-July), while a proportion of males were shown to be spermiating throughout the year.

V. Response to hormonal induction with GnRH

Conclusions



Sexual dimorphism was verified and the biochemical profile of the wild wreckfish was determined (WP12).

There is a clear relationship between the fatty acid profile of broodstock diet and the fatty acid profile of oocytes and larvae (WP12).

In captivity, easy adaptation and good handling, which is very encouraging for the future development of wreckfish aquaculture.

Period of sexual maturation coincides with those registered in the wild populations in the Atlantic.

Good maturation status of breeding stocks, with most specimens showing signs of gametogenesis in greater or lesser degree.

Sperm Cryopreservation-> efficient with acceptable impairment of motility parameters to be compensated by optimized sperm to egg ratio at fertilization (D6.1 and D6.2).

Advances in hormonal induction with GnRh, with induced spawns in two of the three broodstocks.

Challenges



- The biggest problem has been the capture of live wild wreckfish specimens in order to use them as future broodstock and study its growth, due to the shortage in the NW area of Spain on this last years. (Since 2014 only 5 fishes between 1-4 Kg body weight were captured).

(Delayed D6.4)

- The reason why there are specimens that made vitellogenesis, but do not reach the oocyte maturation, is still unknown and it should be considered in future studies.

What should be improved

Chilled sperm storage

Hormonal induction (FSH+LH). Delayed D6.3



EAS 2015

- **BIOMETRIC PARAMETERS OF WILD WRECKFISH (*Polyprion americanus*).**
B. Álvarez-Blázquez, F. Linares, R.M. Cal, J.L. Rodríguez, J.M. Martínez, M. Sánchez, N. Lluch, E. Pérez, P. Domingues and JB. Peleteiro
- **BIOCHEMICAL COMPOSITION OF WILD WRECKFISH (*POLYPRION AMERICANUS*).**
F. Linares, J. L. Rodríguez, J. B. Peleteiro, R. Cal, G.Pazos and B. Álvarez-Blázquez
- **DIVERSIFY: RESULTS FROM THE FIRST YEAR OF WRECKFISH (*POLYPRION AMERICANUS*) CULTURE.**
J.B. Peleteiro, F. Linares, A. Vilar, CH. Fauvel, N. Duncan, C. Rodríguez, M. Izquierdo & C. Mylonas

CNA 2015

- **MADURACIÓN SEXUAL DE TRES STOCKS DE CHERNA (*POLYPRION AMERICANUS*) EN GALICIA.**
Juan Manuel Martínez, Tito Peleteiro, Nuria Lluch, María Sánchez, Fátima Linares, José Luis Rodríguez, Antonio Vilar, Rosa Cal, Blanca Álvarez-Blázquez

EAS 2016

- **INFLUENCE OF BROODSTOCK NUTRITION OF WRECKFISH (*Polyprion americanus*) ON THE OOCYTES FATTY ACID COMPOSITION.**
F. Linares*, J. L. Rodríguez, J. B. Peleteiro, R. Cal, J.M. Martínez, G.Pazos, J.M. and B. Álvarez-Blázquez
- **DESCRIPTION OF THE WRECKFISH (*POLYPRION AMERICANUS*) REPRODUCTIVE CYCLE IN CAPTIVITY.**
J. M. Martínez-Vázquez*^a, J.B. Peleteiro^a, F. Linares^b, J. L. Rodríguez^c, A. Vilar^d, R. Cal^a, B.Álvarez-Blázquez^a

EAS PUBLICATION 2017

- **Advances in wreckfish (*Polyprion americanus*) research: the DIVERSIFY project**
Mylonas C.C., Papandroulakis, N., Fakriadis, I., Sigelaki, I. and Papadaki, M.
Peleteiro, J.B., Alvarez-Blázquez B., Pérez, E., Martínez, J.M., Lluch, N.
Vilar, A
Linares, F., Rodríguez, J.L., Pazos, G.
Fauvel, Christian



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