

REPRODUCTION OF WRECKFISH IN CAPTIVITY AND INDUCTION OF SPAWNING WITH GnRH α

**WRECKFISH WORKSHOP
19TH JULY**

**INSTITUTO ESPAÑOL DE
OCEANOGRAFÍA
Centro oceanográfico de Vigo**



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Blanca Álvarez-Blázquez.

Instituto Español de Oceanografía
Centro Oceanográfico de Vigo

Spain

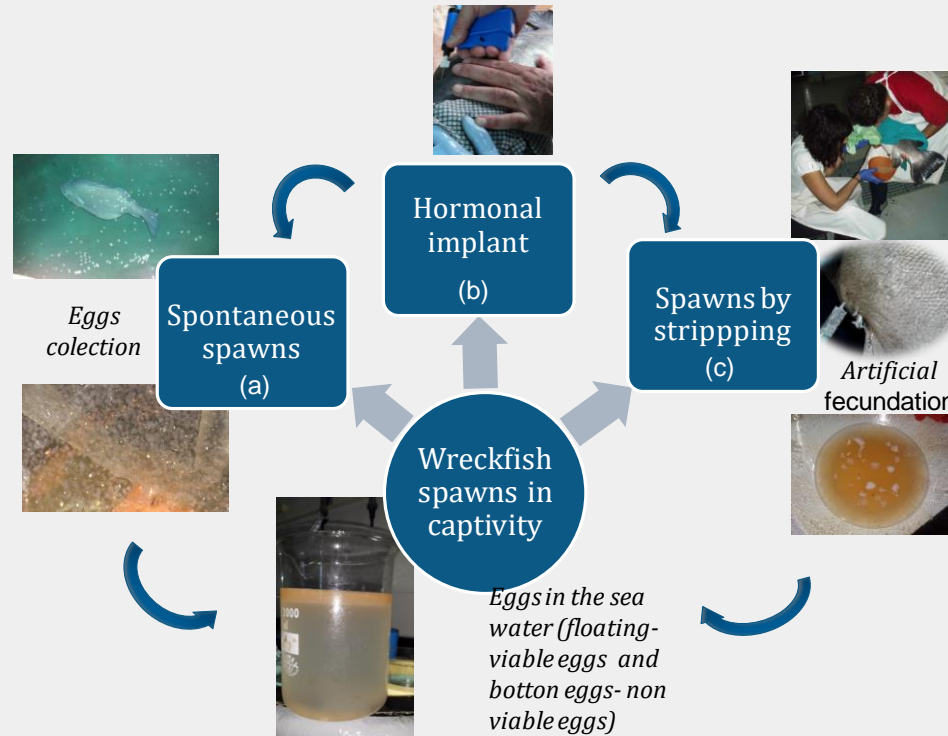
Procedures to obtain wreckfish spawns in captivity

b) Spawning induction with exogenous hormones (GnRHa)

- In large tanks (>40 m³) under controlled photothermal conditions, allowing the fish to spawn spontaneously, and fecund by the male.
- Or in smaller tanks (< 15 m³) by stripping will be conducted and fish will monitored for ovulation.

a) Natural and spontaneous spawns:

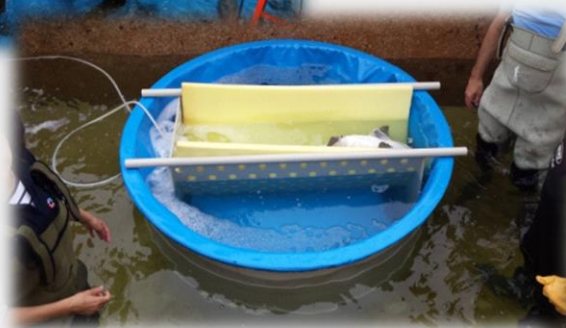
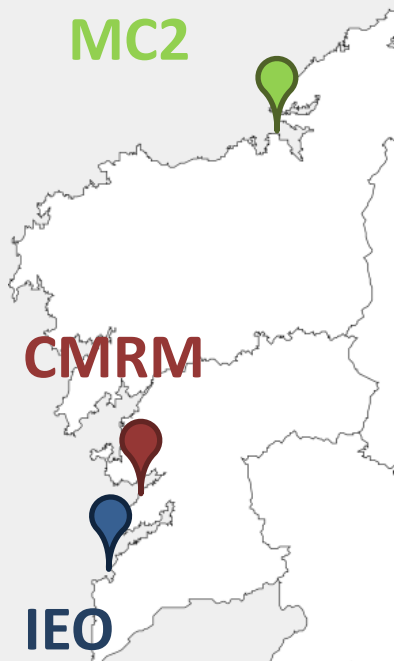
- Eggs were fertilized by the male in the sea water and were collected by floating in the tank
- In large tanks (>40 m³)



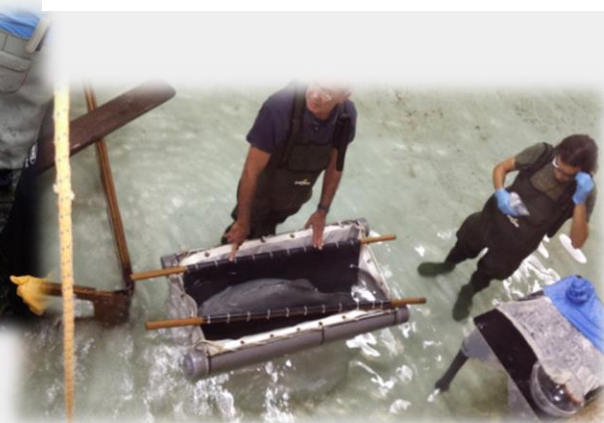
c) By stripping of the mature males and females followed by in vitro fertilization

- Maintained in smaller tanks (<15 m³)

OUR STOCKS



These fish were maintained in a variety of environmental conditions in regards to tank size and photothermal regime, including indoor (IEO, MC2) and outdoor tanks with natural photothermal conditions (IGAFA), and indoor tanks with simulated natural photothermal conditions (IEO, IGAFA)) or constant temperature (MC2).



	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



NATURAL AND SPONTANEOUS SPAWNS

The number of natural and spontaneous spawns was increased in all the broodstocks along the years. Females and males produced a large number of fertilized eggs and achieved satisfactory fertilization success.



- Spawning periodicity was 3-5 days in all stocks and the time of spawning was mainly between 05:00 and 08:00 h, with some exceptions that took place at mid day.
- Fertilization success was between 50 and 100% with better quality eggs towards the mid or end of the spawning season for each female. It has been found that a female is able to spawn an average of ten times per breeding season.

NATURAL AND SPONTANEOUS SPAWNS

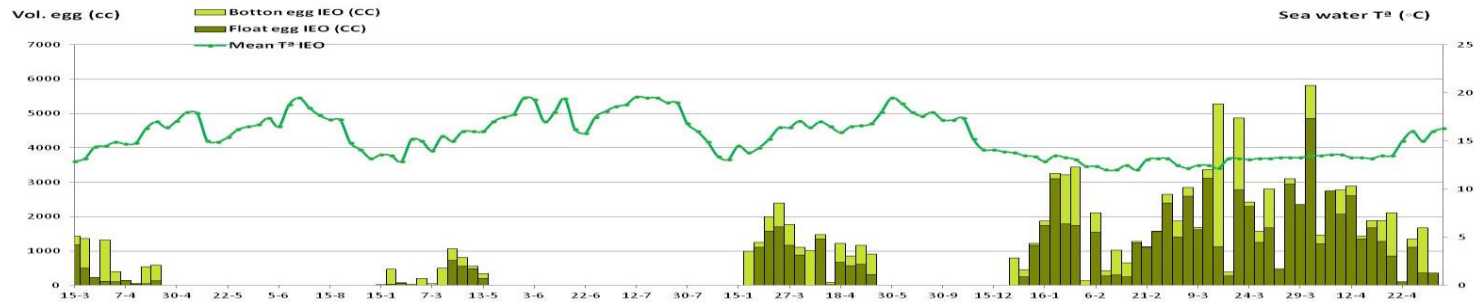
- Characterized by males chasing the females followed by the release of the gametes.



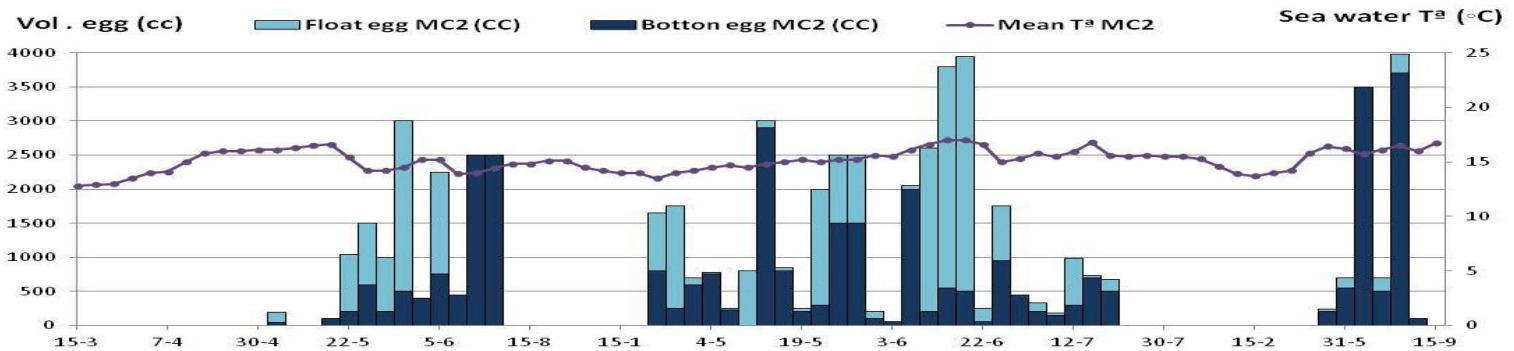
TOTAL NATURAL AND SPONTANEOUS SPAWNS



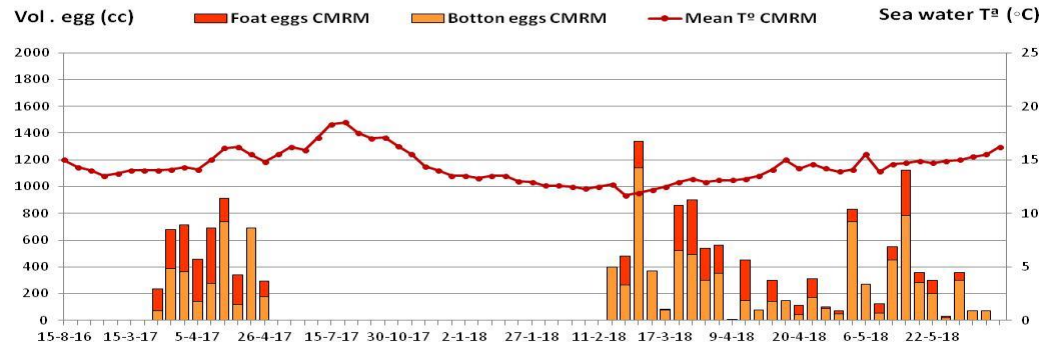
IEO



CM2



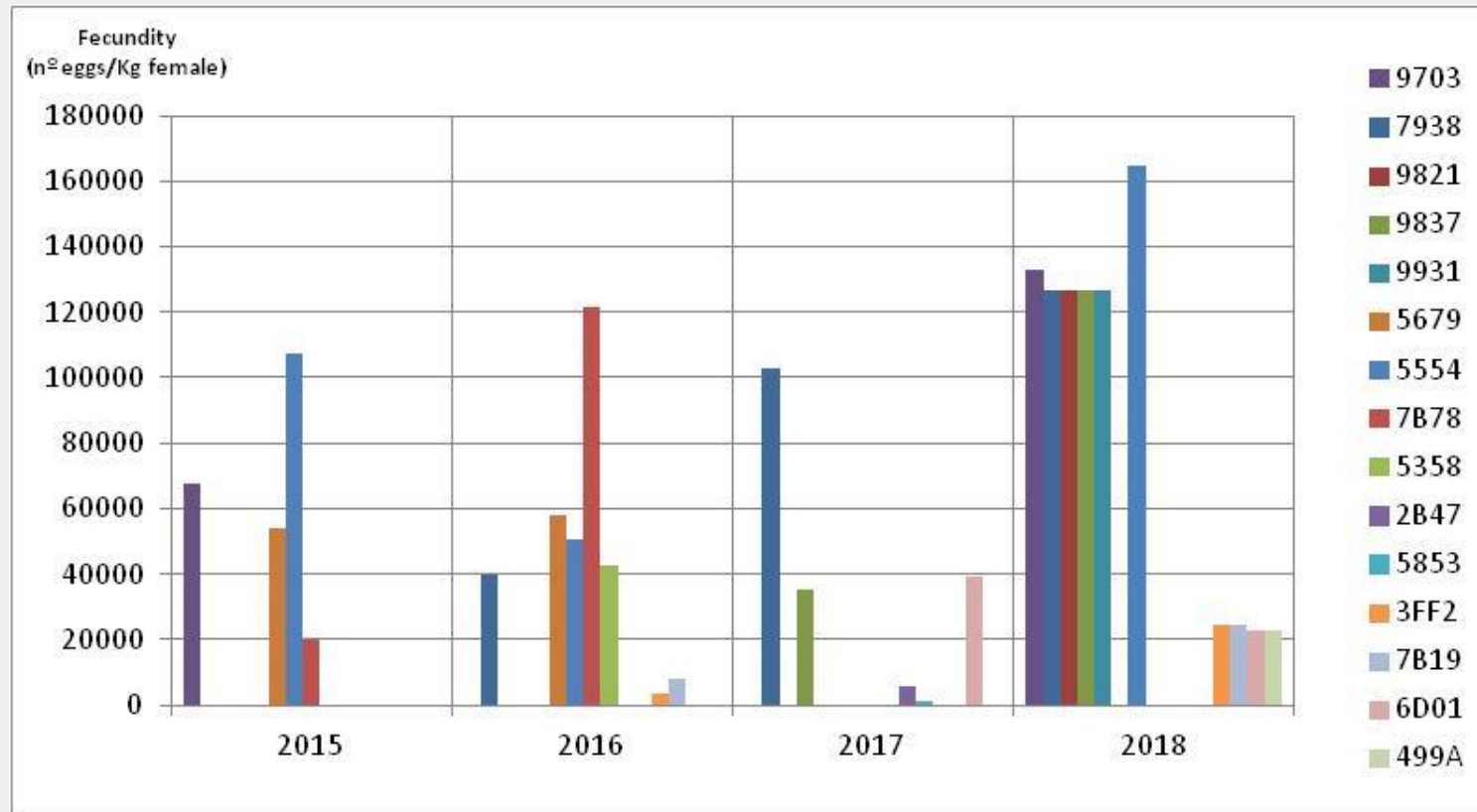
CMRM



Volume of viable floating and non-viable sinking eggs obtained from spawns at the IEO, CM2 and CMRM facilities between March 2015 and July 2018.

TOTAL NATURAL AND SPONTANEOUS SPAWNS

Respect to de females fecundity (nº eggs/kg de hembra), this was increased along the years, except in the MC2 for the death in 2017 of the best fertile females.



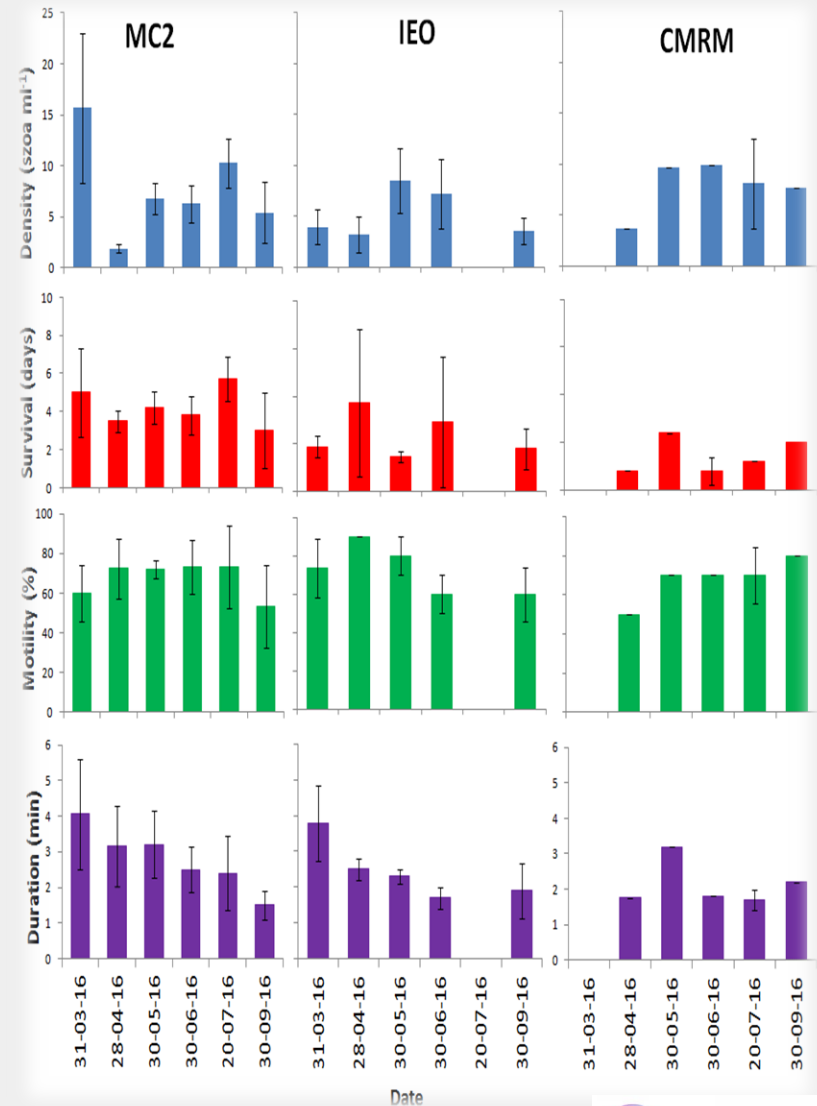
MALES CHARACTERISTICS

- The mean concentration of wreckfish sperm was 2.41×10^{10} (sd: 0.4×10^{10} , $n=9$) spermatozoa ml^{-1} in Galicia in January, while it remained around 1×10^{10} from April to September with no significant variation between sampling dates in Crete, Greece

- the mean survival time of sperm, conserved refrigerated at 4°C , is 4 days.

- Sperm exhibits a high percentage of motile cells at activation and one of the highest initial speeds recorded for fish sperm. This high speed was associated with a long swimming duration compared to other marine fish. The long duration exhibited a double trajectory shape.

- However, in some cases it reaches the 18 days of survival after its recollection





MALES CHARACTERISTICS

- Sexual maturation covers the same period of females, reaching its peak in the months of April and June

The first trajectory of the spermatozoa was straight (associated with the search of target eggs) and then the trajectory began bending, which was interpreted as a phase of searching for the micropyle on the egg surface. Moreover, the results obtained by CASA are in agreement with field observations obtained by human inspection under the microscope and complement them by objective data that can be more easily statistically analyzed.

The spermatozoa concentration in wreckfish stripped semen was of the same order of magnitude as that of pelagic fish such as European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) or meagre (*Argyrosomus regius*) and it was higher than that of sole (*Solea solea*) and



The natural spawning behaviour. CONCLUSIONS

- males chasing the females followed by the release of the gametes.
- Spawning takes place during the night or very early in the morning.
- Spontaneous spawning in the IEO, MC2 and CMRM stocks produced a large number of fertilized eggs and achieved satisfactory fertilization success.
- Spawning periodicity was 3-5 days in all stocks
- the time of spawning was mainly between 05:00 and 08:00 a.m, with some exceptions that took place at mid day.
- Fertilization success was between 50 and 100% with better quality eggs towards the mid or end of the spawning season for each female.
- A female is be able to spawn an average of ten times per breeding season.
- The male is be able to fecundate oocytes of four females during 30-35 spawn in a spawning season (5 months, year 2018 IEO)

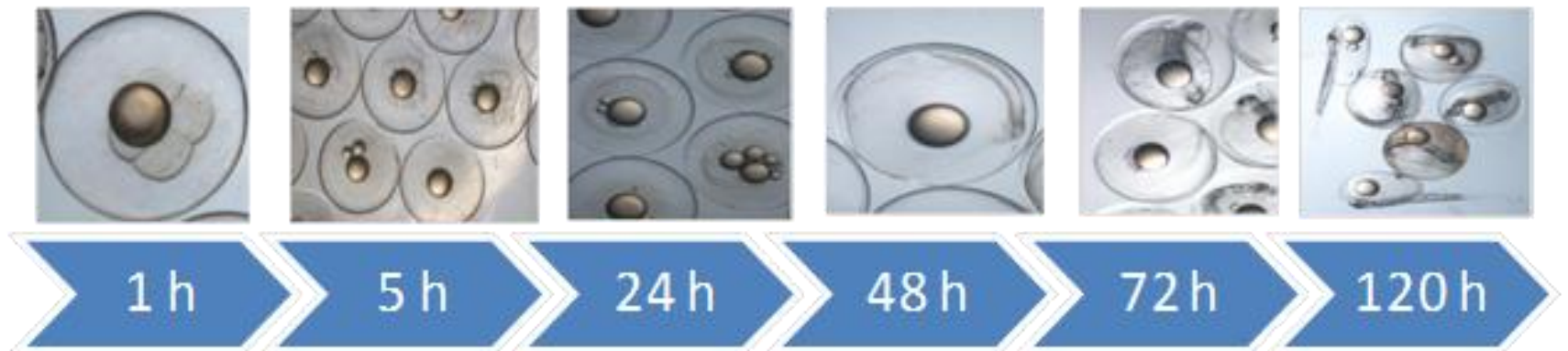


On the other hand, characteristics of wreckfish eggs and the embryogenesis were known.

The wreckfish viable eggs have high diameter (1.996 ± 0.034 mm), with a thick lipid droplet allowing to keep afloat.

Hatching take place after five days of incubation at $16 \pm 0.8^\circ\text{C}$ of water temperature.

Trials of incubation eggs are described in next chapter of larval husbandry.



Embryonic development of wreckfish at $16 \pm 0.8^\circ\text{C}$



METHODS FOR HORMONAL INDUCTION

YEAR	STOCK	FISH	WEIGHT (KG)	DATE IMPLANT	OOCYTES SIZE (mm)	GnRH (µg)	Dosis (µg/kg)	SPAWNING DATE	Total eggs (ml)	FECUNDATION(%)	OBSERV.
2015	IEO	7938	16,5	09/06/2015	0,95	500	30,3				NO SPAWNS
	MC2	6015	27,35	11/06/2015	1,12	500	18,3				
		8438	33,15		1,09	500	15,1				
2016	MC2	5853	18,2	23/06/2016	1,20	1000	54,9	29/06, 3/07, 8/07, 12/07, 16/07 and 20/07	2200, 325, 180, 980, 730 and 670	86, 78, 49, 85, 75 and 60	ALL SPONTANEOUS. LARVAE ALIVE UNTIL 25 DPH
		5544	16		1,12	750	46,9				NO SPAWNS
	CMRM	3FF2	14,3	28/06/2016	1,39	750	52,4	11/07 y 12/07	100 y 150		SPONTANEOUS AND STRIPPING. NON VIABLE EGGS
		7B19	13,5	28/06/2016	1,26	750	55,6				NO SPAWNS
			13,5	12/07/2016	1,36	750	55,6	18/07 .	750	47	STRIPPING. NON VIABLE EGGS
			13,7	26/07/2016	2,23	750	54,7				NO SPAWNS
	2017	MC2	7B78	23	1st :07/06/2017	1,16	1750	76,1	20/06/2017	3500	0
2nd: 15/06/2017					1,16	2000	87,0				
2B47			18.5	1st: 11/05/2017	1,14	1000	54,0	27/05/2017	240	65,6	NON HATCHING
				2nd:	1,04	500	27	30/05	700	3,5	LARVAE ALIVE UNTIL 25 DPH
5853			21,3	1st: 30/06/2017	1,20	1750	82,2	16/07/2017	900	0	SPONTANEOUS. NON VIABLE EGGS
		2nd: 04/08/2017	1,80	1500	70,4	09/08/2017	3750	0	SPONTANEOUS. NON VIABLE EGGS		

GnRHa implant data of the different trials during 2015, 2016 and 2017 in some females of the three broodstocks : IEO, MC2 and CMRM.

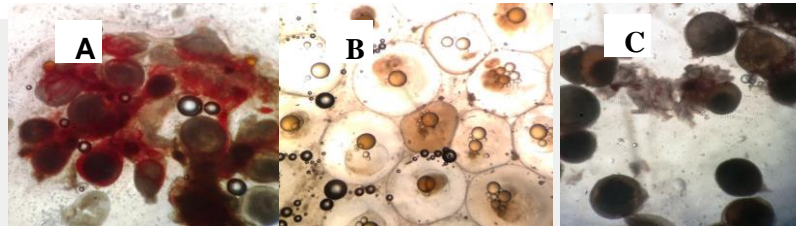
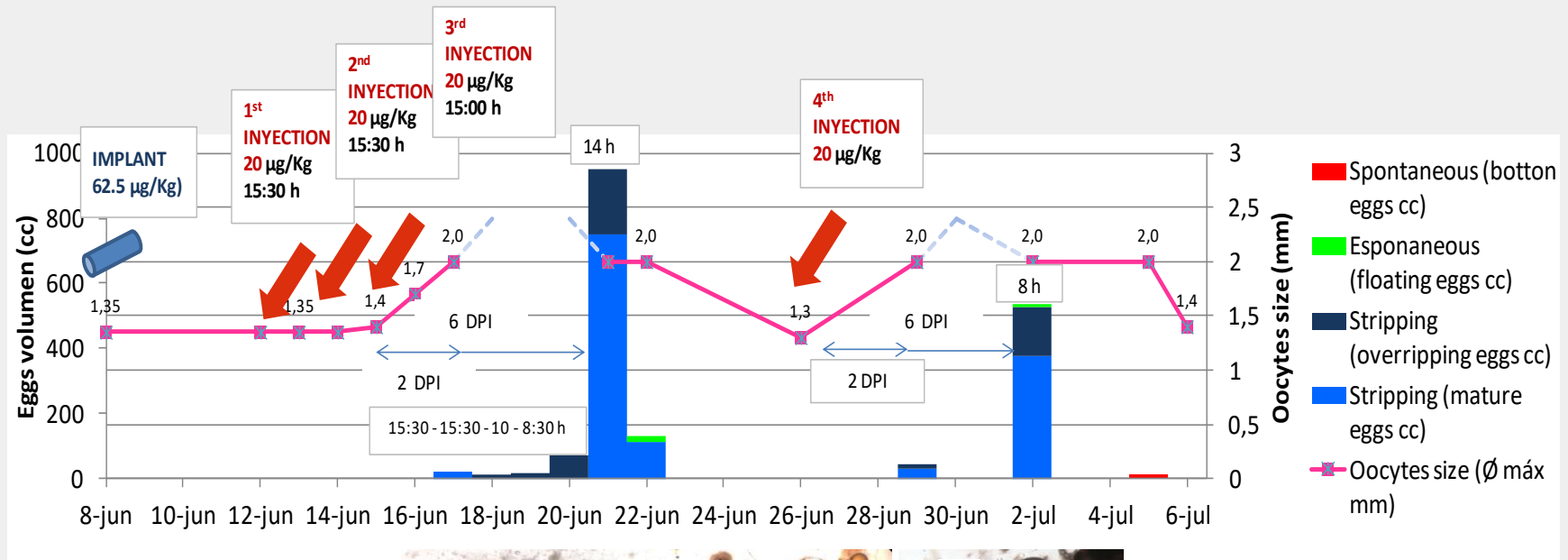
Females in three of the fourth stocks were implanted with 500 µg of GnRHa.
The response of the females were varied:

- No response in 2015.
- Spontaneous or stripping spawns with non-viable eggs in 2016, with only one spawn that was successfully cultured until 25-day post hatching in 2016.
- with good results in the fertilization, but not in hatching in 2017.
- With good results in the fertilization and quality of eggs in 2018, with a high dose of GnRHa hormone.



METHODS FOR HORMONAL INDUCTION

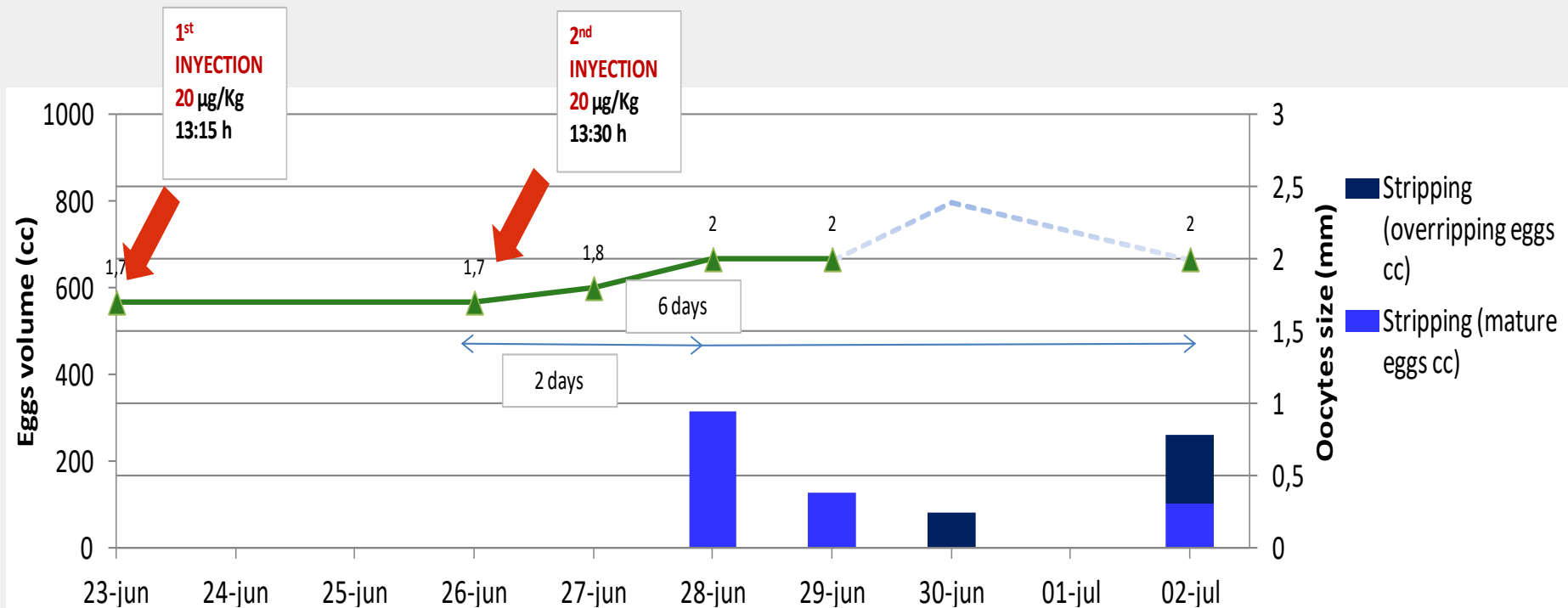
The necessity of more information on this subject required more females. Therefore, we used the stock from Isidro de la Cal Company, and worked with two of their females, based on an agreement made for that purpose. The experiments were carried out during the months of June and July of 2017.



Gonadal biopsies of the 16 Kg female, the 17th (A), 18th (B) and 19th (C) of June 2017.



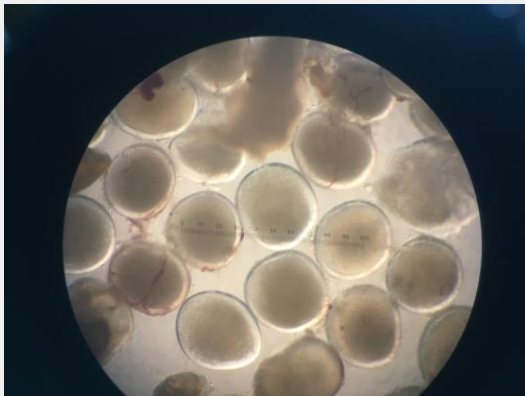
METHODS FOR HORMONAL INDUCTION



Another Isidro de la Cal's female was treated with a GnRHa injection with the same protocol. On the 23rd of June the first dose was administrated with oocytes size of 1.3 mm. Three days later, the second injection was given and one day after the female was very swollen and a plug was extracted. The next day, 315 ml of floating eggs were obtained and were fertilized. Every three days after the stripping, batches of overripped eggs were obtained.

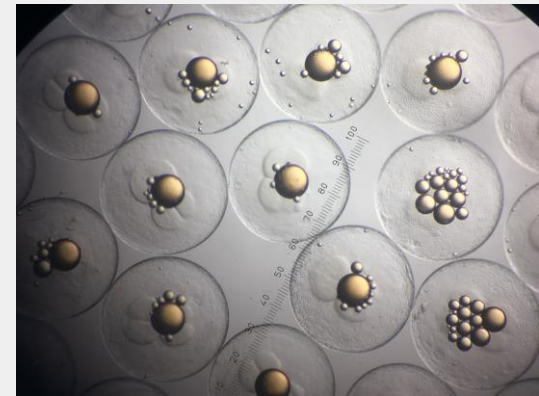
METHODS FOR HORMONAL INDUCTION

In 2018, another experiment with a female from MC2 with an induction with a higher dose of GnRHa implant was made, with good results and females spawned spontaneously with a good egg fertility (98 %), after 6 days from the hormonal induction. The same results were achieved with the injections trials a year before in Isidro de la Cal.



Oocytes with mean diameter 1,2 mm

Female implanted with **87 $\mu\text{g}/\text{kg}$ of GnRHa**



Fertilized eggs

METHODS FOR HORMONAL INDUCTION. Conclusions

More information on the ovulation time after induction with the GnRH α hormone

Results suggest that the correct oocyte size for implant should be $>1200\text{ }\mu\text{m}$, but it could be better at $>1300\text{-}1400\text{ }\mu\text{m}$.

Also, it was found that GnRH α injections are more effective than GnRH α implants and provide a faster response.

Results showed a time of response of about six days after the injection.

The risk of gonadal plugs, if the hormonal dose isn't adequate.

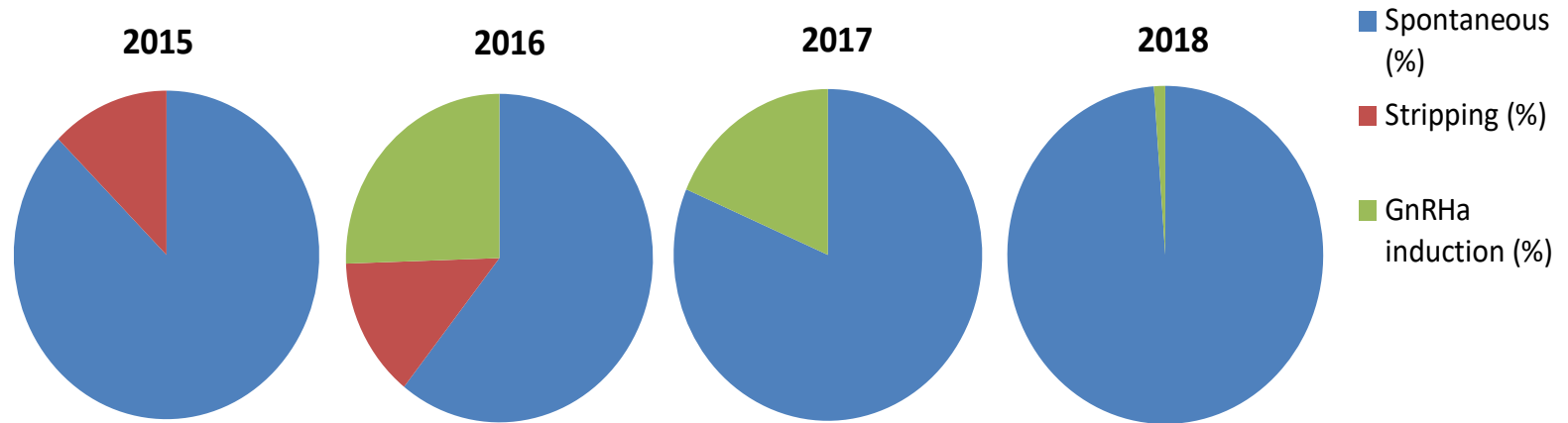


Gonadal biopsy



GnRH α implant

During these last years, the number of spontaneous spawns were increased, and the number of induced spawns was reduced. The reason is probably the better adaptation of the females to the captive conditions and the promotion of the natural maturation cycle, resulting in not only vitellogenesis, but also spontaneously oocyte maturation



THANKS!

