GWP
Reproduction and Genetics
WP2-7

Presenter: Neil Duncan, IRTA

1st year project meeting
4-6 November 2014
Bari, Italy

BOTTLENECKS

Increasing importance of reproduction

Pikeperch (*Sander lucioperca*)

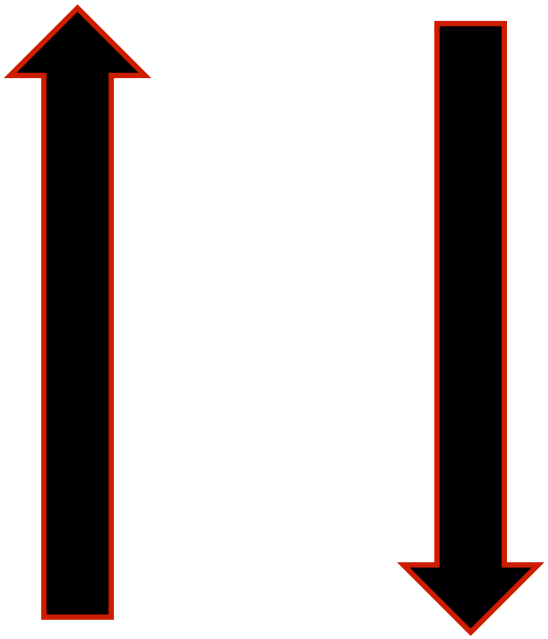
Meagre (*Argyrosomus regius*)

Atlantic halibut (*Hippoglossus hippoglossus*)

Greater amberjack (*Seriola dumerili*)

Wreckfish, (*Polyprion americanus*)

Grey mullet (*Mugil cephalus*)



Increasing importance of genetics

WP4 Pikeperch (*Sander lucioperca*

Bottlenecks

- **Lack of knowledge of the genetic variability of current broodstocks and variable or unpredictable growth rate during grow-out.**

Pikeperch (*Sander lucioperca*)

Objectives

- **1. Evaluate the genetic variability of captive broodstock in commercial RAS farms in Europe,**
- **2. Compare this variability with the variability of wild individuals and define how a future genetic breeding program should be established for sustainable optimal performances through domestication of pikeperch**

HCMR, UL

WP4 PM5 20,000€

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De
Task 4.1																				
Task 4.2																				

Pikeperch (*Sander lucioperca*)

Over 1000 samples collected so far

	Collection	Wild / Domesticated	Sample Size
1	Szabolsi, Halaszati Kft, Hungary	domesticated	50
2	Aquapri A/S, Danemark (VanMecklen, Holland)	domesticated	55
3	Aquapri A/S, Danemark (Czech Rep.)	domesticated	40
4	Aquapri A/S, Danemark (Excellence fish, Holland)	domesticated	14
5	Aquapri A/S, Danemark (Hungary)	domesticated	150
6	Aquapri A/S, Danemark (Mosso)	domesticated	20
7	IfB, Potsdam, Germany	domesticated	50
8	FGFRI Kainuu fisheries research station, Finland	domesticated	32
9	FGFRI Laukaa Fish Farm, Finland	domesticated	20
10	INAGRO, Belgium (>100 fin-clips of German Origin)	domesticated	100
11	INAGRO, Belgium (>100 fin-clips of Dutch Origin)	domesticated	100
12	ASIALOR, France	domesticated	65
13	URAFPA-DAC, Czech Rep.	wild	70
14	Domaine de Lindre, France	wild	51
15	Sarag Lake, Poland	wild	14
16	Wymoj Lake, Poland	wild	9
17	Gyori Elore, HTSZ, Hungary	wild	26
18	Gyori Elore, HTSZ, Hungary	wild	27
19	Lake Oulujärvi, Finland	wild	32
20	Lake Hiidenvesi, Finland	wild	31
21	INSTM, Tunisia	wild	59

Pikeperch (*Sander lucioperca*)

Microsatellite Cross-species amplifications two multiplexes being used

MULTIPLEX 1 with 4-plex

Locus	Acc.Number	Repeat Sequence	Range	Na	Ho	He	Forward	Reverse
PflaL3	AF211828	(TG)18	101–119	8	0.34	0.29	GCCGAATGTGATTGAATG	CGCTAAAGCCAACTTAATG
SviL8	AF144741	(TG)22	107–145	8	0.34	0.20	GCTTATACGTCGTTCTTATG	ATGGAGAAGCAAGTTGAG
Za138	HM622317	(AC)8	135-148	5	0.27	0.43	TTCTTTATACAAGAGGAATAGTTGCAG	TTTTTGTGATTGTGCTATTTTAAAGG
Za199	HM622334	(TCT)13	201-234	7	0.67	0.74	CCTTCCCCTCAAAAGCATGT	AGGAAATGGAAAGGGAATGC

MULTIPLEX 2 with 7 -plex

Locus	Acc.Number	Repeat Sequence	Range	Na	Ht	Hs	Forward	Reverse
Za038	HM622298	(AC)11	107-130	6	0.80	0.77	TGAATCGCTGCTCTTTCTCA	TATGCAATTACATCGGAGCG
Svi4	G36961	(AC)16	120–166	15	0.70	0.65	ACAAATGCGGGCTGCTGTTC	GATCGCGGCACAGATGTATTG
Za024	HM622294	(AC)7	127–139	4	0.47	0.43	TGAACCTCCCTATCCCCTCT	TCTTTTCCACAGCAGGAAGC
PflaL9	AF211834	(TG)24	182–214	4	0.65	0.52	GTTAGTGTGAAAGAAGCATCTGC	TGGGAAATGTGGTCAGCGGC
Za237	HM622342	(CA)10	171-178	5	0.57	0.54	ATCTCAAGTCATGGGGCATC	GGTCCTCTGGTGCAGCTATAA
Za144	HM622319	(AC)8	199-228	8	0.70	0.80	GCCCACAATAGCACCGTAAT	TTTGTGAATGTGAGTGAGAGTCAG
Za207	HM622337	(GT)13	222-237	5	0.67	0.64	GGATTCCAGAAGCAAAAGAGG	TGGGACAAGGCTTTAACCAC

Pikeperch (*Sander lucioperca*)

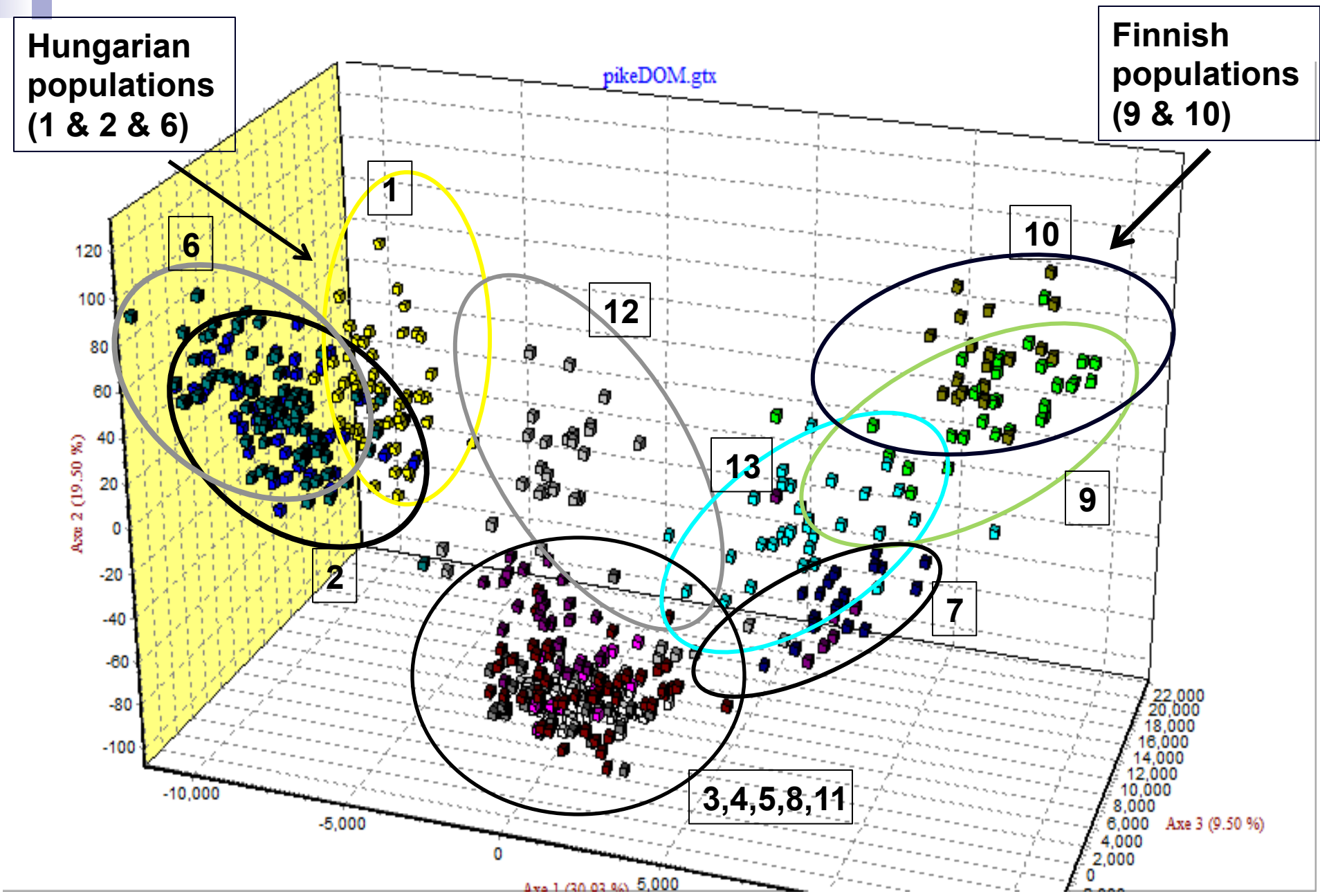
DNA extractions and PCR amplifications

- DNA extractions ~80% completed
- PCR amplifications
  - currently focused on domesticated samples

Pikeperch (*Sander lucioperca*)

Data analysis

- Domesticated stocks, quantify the loss of genetic variability
- Ensure sufficient genetic variation
- Basic population genetics parameters
  - allelic richness,
  - heterozygosity indices,
  - inbreeding coefficients
- Compare stocks to improve management
  - genetic selection
  - traceability of products


Hungarian populations (1 & 2 & 6)
Finnish populations (9 & 10)
pikeDOM.gtx
1
6
12
10
13
9
2
7
3,4,5,8,11
Axe 2 (19.50 %)
Axe 3 (9.50 %)
Axe 1 (30.93 %)

WP2 Meagre (*Argyrosomus regius*)

Bottlenecks

- **Limited genetic variation of current broodstocks**
- **Variable growth rate in pre-growing phase and grow-out in cages.**

Meagre (*Argyrosomus regius*)

Objectives

- **1. Evaluate the genetic variation in the available captive broodstocks of meagre,**
- **2. Genetic characterization of fast and slow growers,**
- **3. Development of tools that facilitate the implementation of genetic selection programs,**
  - **a. Develop protocols for the paired crossing of breeders with spontaneous spawning,**
  - **b. Describe sperm quality and cryopreservation techniques,**
  - **c. Develop in vitro fertilization protocols to provide planned genetic crosses,**
  - **d. Develop a set of SNP markers for genetic selection and stock characterisation.**

Meagre (*Argyrosomus regius*)

WP2

IRTA, HCMR, FCPCT, IFREMER

PM40.65 – 320,656€

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	44	48	51	54	57	60
Task 2.1																				
Task 2.2																				
Task 2.3																				
Task 2.4																				
Task 2.5																				

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.1 Evaluation of the genetic variation in captive meagre broodstocks (led by FCPCT, Juan Manuel Afonso).

Locus	Fluorochrome	Redesigned forward primer sequence (5'→3')	Redesigned reverse primer sequence (5'→3')	C (µM)	Original Reference
Meagre-STRI					
Cacmic14	5' 6-FAM	TGTCCTCACTCCTCTTTTTCTTTC	GTTTAAGGCGCATCTCCAGTCTC	0.02	1*
UBA054	5' 6-FAM	CCTTGTGAGAACATTAATTTGGATG	GTTTCCAAACCCTGATAGATGGATAGTT	0.02	2*
UBA050	5' 6-FAM	GCACAACTGCATCCCTTAGAT	GTTTAGAAGTGAAGACTGCGGACTG	0.05	2*
UBA053	5' VIC	TACTTCCTTCTACCCCTAAGTCTGG	GACTTTCCAGTGTAGCTGTCGTTT	0.05	2*
Soc431	5' VIC	GTGGTAGATGAAAACGTATAAAAG GAG	GTTTCATATATATAGTGTACAGCTCCAGCTTC	0.06	2*
UBA042	5' NED	TTTCTGCCTGACTAGATGTTCTTTC	GATTGTTGCTGGTTTTTCCAAT	0.05	2*
UBA853	5' NED	CAATGCTCAAGTTACAGGAAACC	GTTTGCACTCGTTCACCCTCAC	0.02	2*
UBA005	5' NED	CATCAGGATTGGCAACTAGC	GTTTCCTCCAGGTTTATTCTTCATTGAC	0.03	2*
Soc405	5' PET	AGCCTTTTGTTTAGTTTCCCTCAT	GGGGTGTAGCAGAACCACAC	0.03	2*
UBA006	5' PET	AGCACACGTAATCACACACAGAT	GTTTCCACTAGTGCAAAACGGTGGT	0.03	2*

Locus	Fluorochrome	Redesigned forward primer sequence (5'→3')	Redesigned reverse primer sequence (5'→3')	C (µM)	Original Reference
Meagre-STRS					
GCT15	5' 6-FAM	ATCCGGGCGTTACTACAGTC	GTTTCTCCACACAGTGCTTTTCAGA	0.02	3*
GA16	5' 6-FAM	CTACACAGTCTCTCTCACTCACTCG	GTTTCTGAAACAGCGCAGCATTG	0.02	3*
GA17	5' 6-FAM	CTAGAGAAATTCATCCAGGGAAGT G	GTTTAGAGCAGAGAGTTAGCGGTTGTT	0.015	3*
CA13	5' VIC	TTTTCCTTTTTCAGTAGTCTCCTTG	GTTTATAAGGAGGACGTGAGTTTGGTAG	0.035	3*
GA6	5' NED	GTCTGATGGCGACAGACAGG	GTTTCAGCCCGCTACTTTACCTACAAC	0.02	3*
CA3	5' NED	AAGTGGAGGCTCTTACATGAAAAC	GTGACAAATTGCCTTCTGTTTCTAC	0.03	3*
CA14	5' NED	ACTGAGAGTGAAGGTGGGAAACT	GTGAGTGTCTTTGTTTTTACCAACC	0.03	3*
GA2B	5' PET	AAGTGTGGCGTCATTTCCTCT	GTATTGATGGATAGCAAGTGTCAGA	0.05	3*

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.1 Evaluation of the genetic variation in captive meagre broodstocks (led by FCPCT, Juan Manuel Afonso).

- 906 samples received
  - DNA extractions 100% completed
  - PCR amplifications
    - ~80% completed
  - PCR readings
    - ~65% completed

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.1 Evaluation of the genetic variation in captive meagre broodstocks (led by FCPCT, Juan Manuel Afonso).

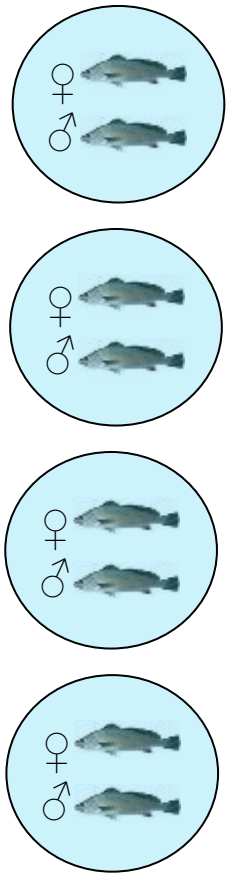
- Quantify the loss of genetic variability
- Ensure sufficient genetic variation
- Basic population genetics parameters
  - allelic richness,
  - heterozygosity indices,
  - inbreeding coefficients
- Compare stocks to improve management
  - genetic selection
  - traceability of products

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.2 Development of protocols for paired crossing in spontaneous spawning

Study in HCMR led by Constantinos (Dinos) Mylonas.



17 weeks, 17 induced spawnings

Females = 15µg/kg GnRHa, oocyte > 0,55 mm
Male = GnRHa implant dose 43–57 µg/Kg

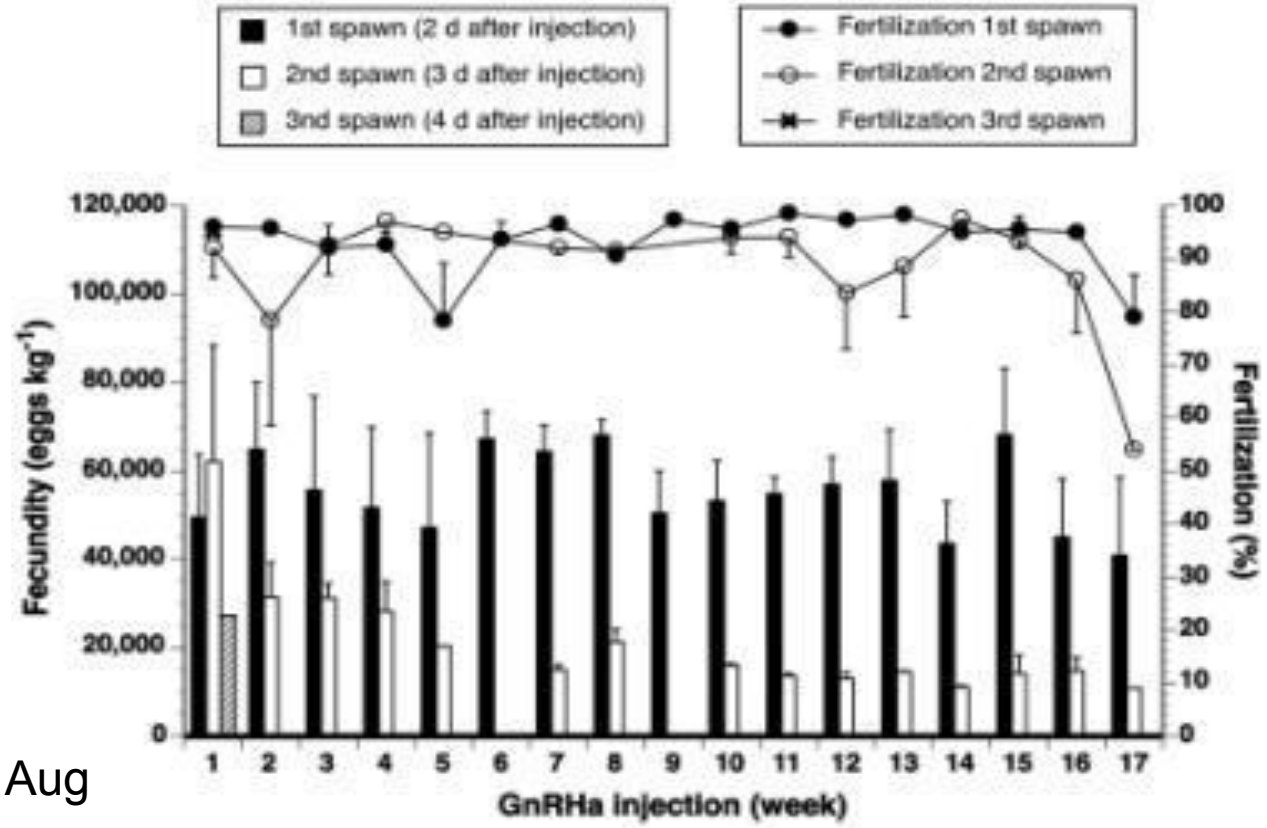
Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.2 Development of protocols for paired crossing in spontaneous spawning

Study in HCMR led by Constantinos (Dinos) Mylonas.

ANOVA Table (P values)	Fecundity	Fertilization
GnRHa injection (week)	0.834	0.159
Spawn No after injection	0.001	0.215
Interaction	0.737	0.564



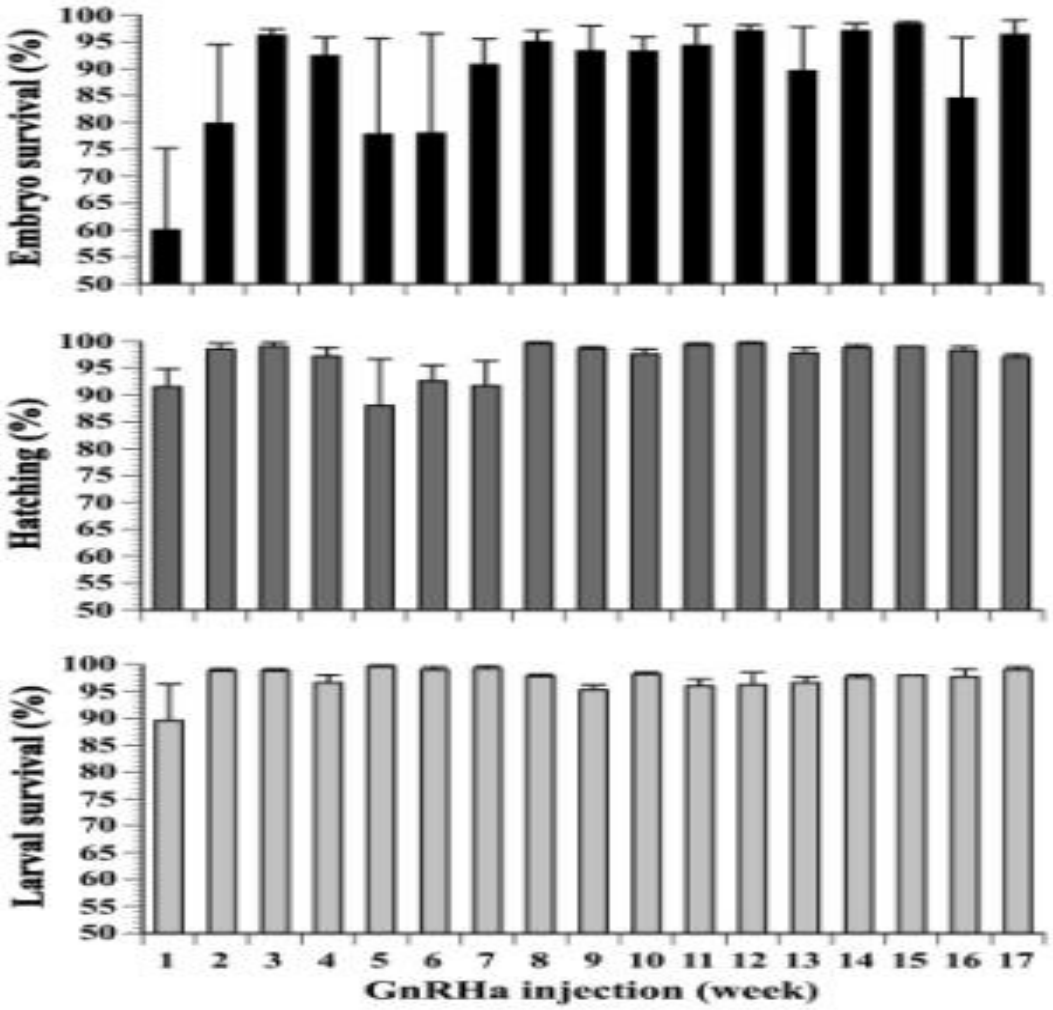
7 May to 28 Aug

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.2 Development of protocols for paired crossing in spontaneous spawning

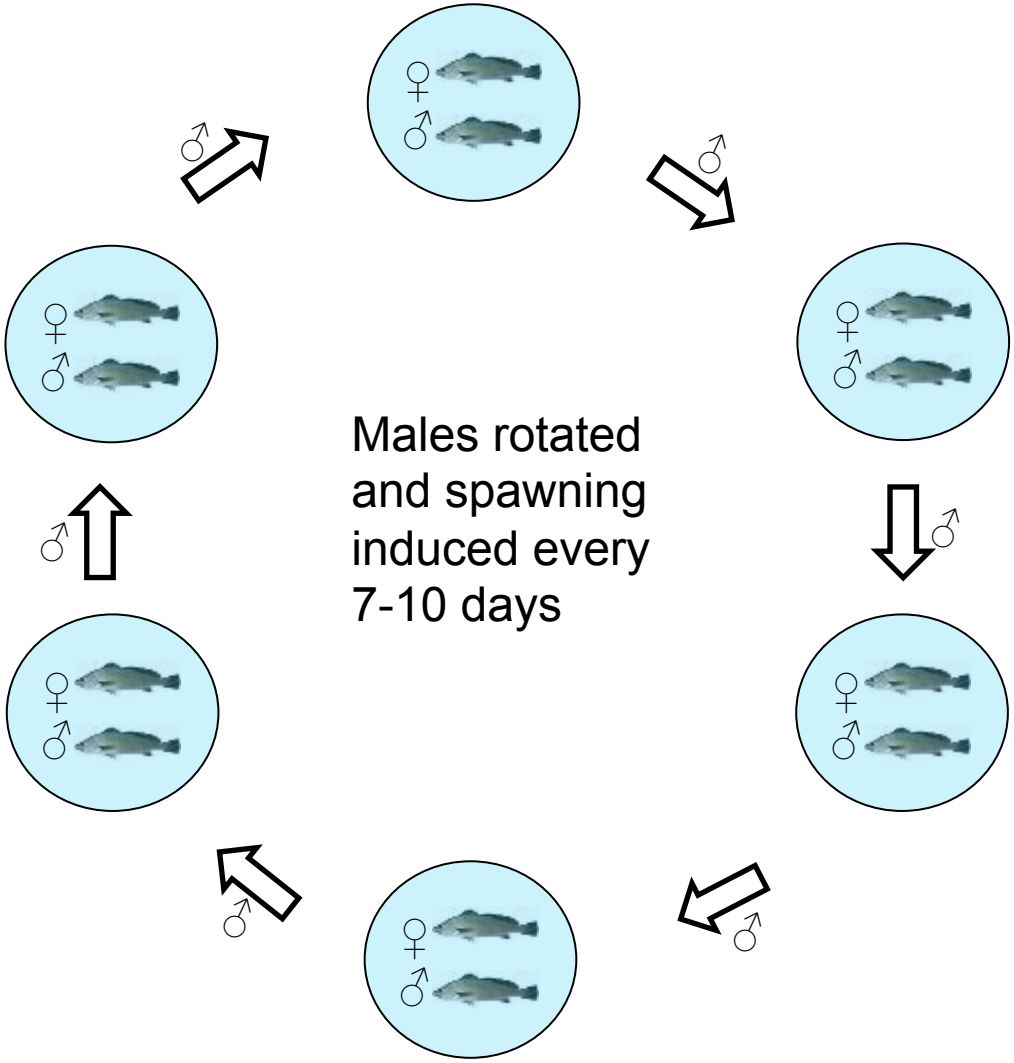
Study in HCMR led by Constantinos (Dinos) Mylonas.



Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.2 Development of protocols for paired crossing in spontaneous spawning (led by IRTA, Neil Duncan).

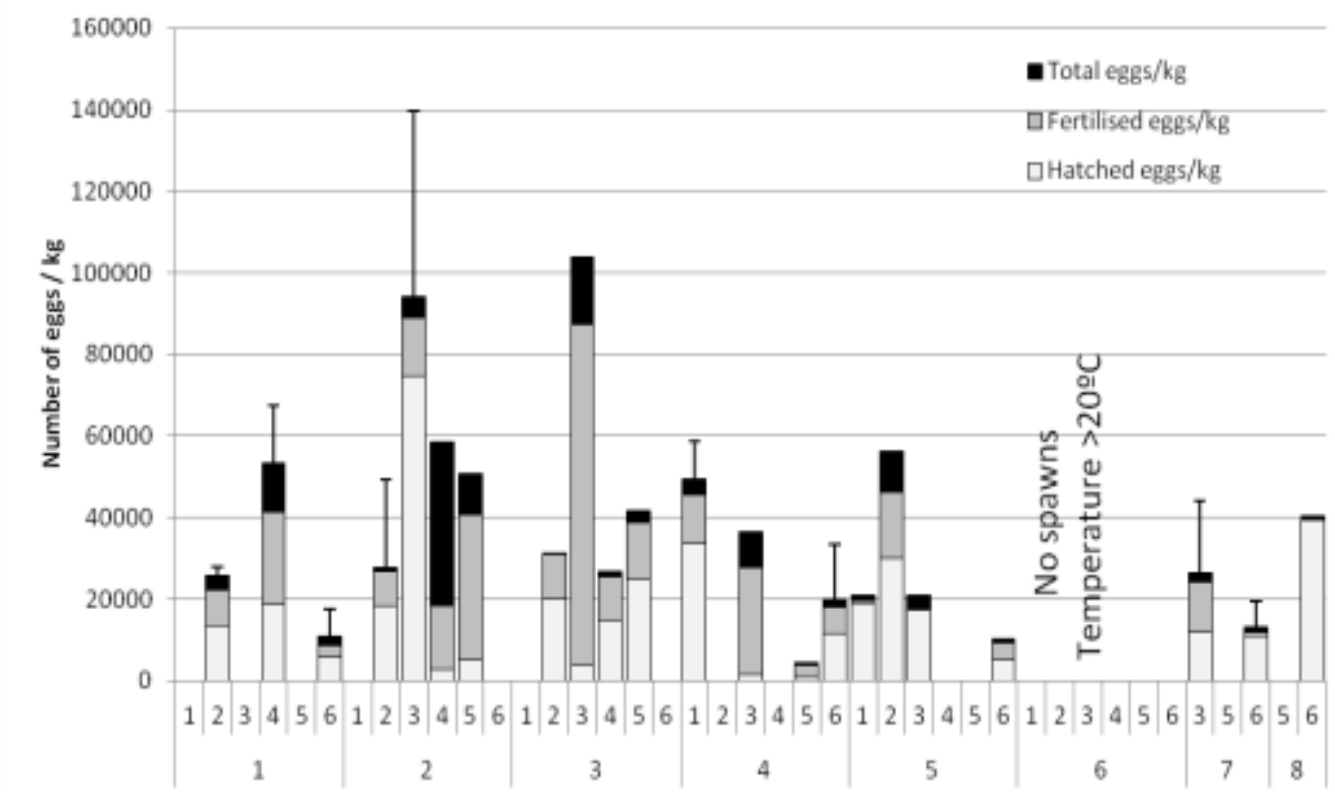


8 inductions
Females = 15µg/kg GnRHa, oocyte > 0,55 mm
Male = 7,5µg/kg GnRHa,

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.2 Development of protocols for paired crossing in spontaneous spawning (led by IRTA, Neil Duncan).



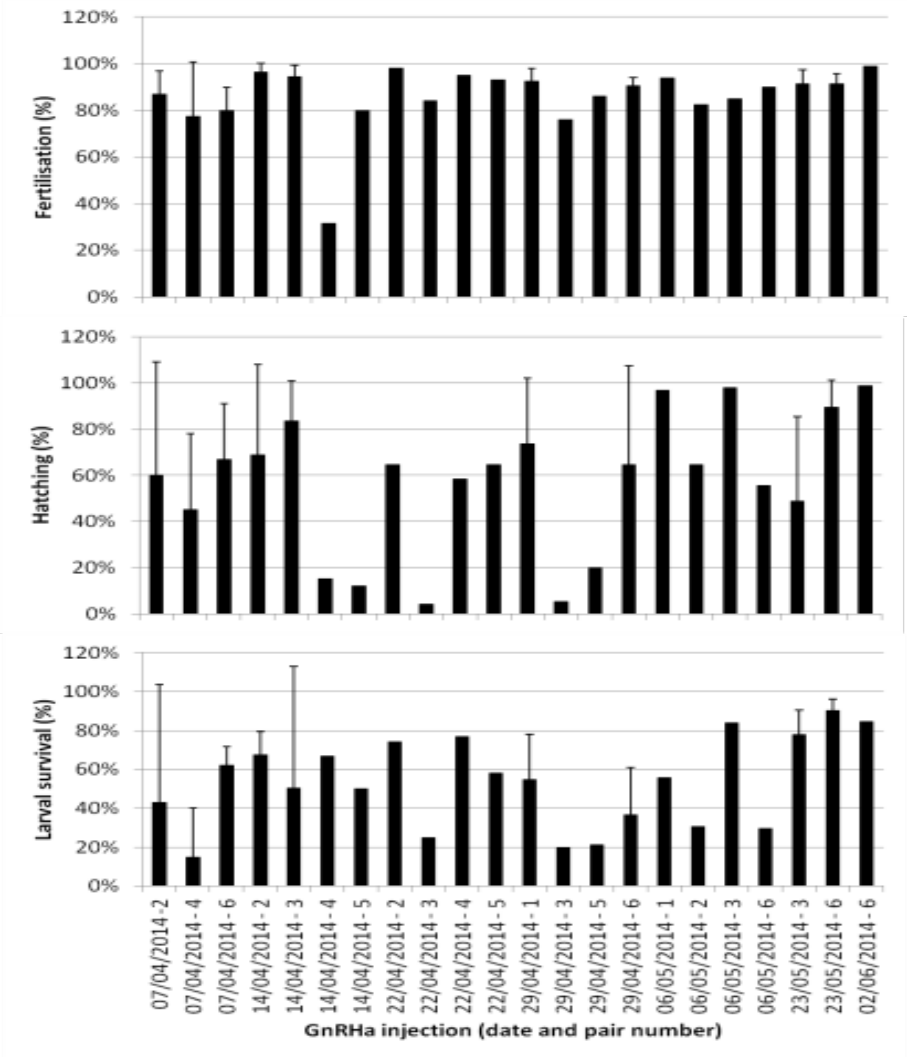
7 April to 2 June

22 (53%) pairs spawned out of a total of 41 pairs that were induced

Meagre (*Argyrosomus regius*)

2014 outputs:

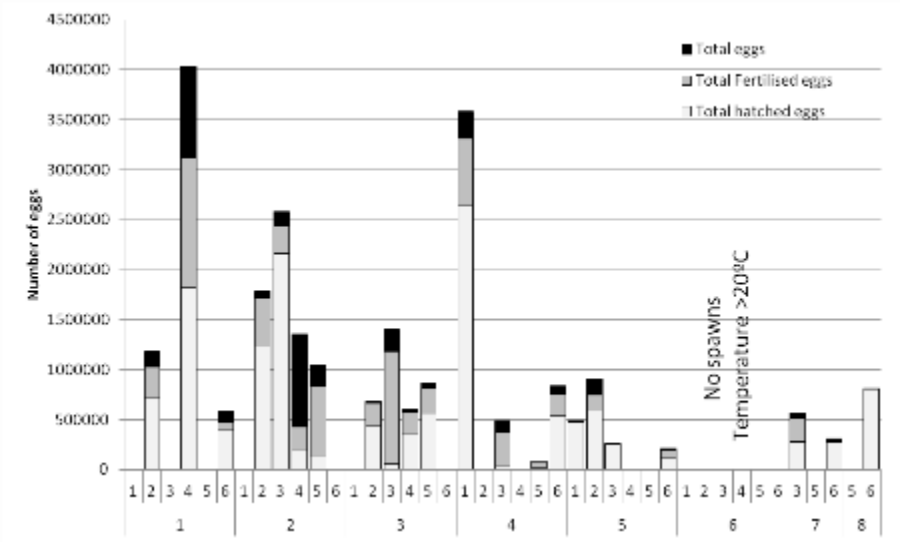
Task 2.2 Development of protocols for paired crossing in spontaneous spawning (led by IRTA, Neil Duncan).



Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.2 Development of protocols for paired crossing in spontaneous spawning (led by IRTA, Neil Duncan).



- No correlation amongst maturity stage and egg quality
- Weak correlation (R=0,5) between spawning efficiency and temperature
- Individual variation, fish that did not spawn and fish with significantly higher egg quality and quantity

Table

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.5 Development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers (led by HCMR, Costas Tsigenopoulos).

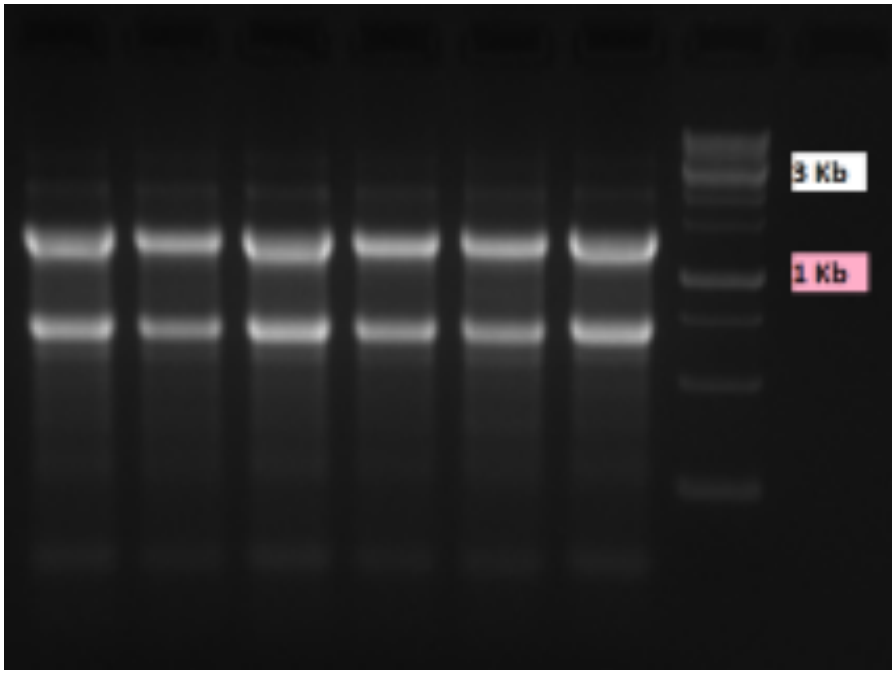
Family	Related half-sib family	Spawning Date (Tank)	Female	Male
1	3	24/04/2014 (V8-1)	5-wild	19-wild
3	1	01/05/2014 (V8-2)	1-wild	19-wild
4	-	24/04/2014 (C2)	16-cultured	21-wild
5	-	01/05/2014 (C1)	2-wild	22-wild
6	-	01/05/2014 (V6)	13-cultured	17-wild

- Parents that contributed to each family or half-sib family and spawning date.
- Liver and muscle samples taken from 16 individuals from different families and with different growth rates

Meagre (*Argyrosomus regius*)

2014 outputs:

Task 2.5 Development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers (led by HCMR, Costas Tsigenopoulos).



- Total RNA extraction profile from meagre liver tissues. The size marker on the right side of the gel is the 1Kb DNA ladder RTU from Nippon Genetics GmbH.

Meagre (*Argyrosomus regius*)

2014 outputs summary

- **Samples collected and analysis close to completion to characterize genetic diversity in current domesticated meagre broodstocks.**
- **Paired spontaneous tank spawning is possible and can provide a method to produce desired families.**
- **Samples collected and being analyzed to provide genetic markers for an assisted breeding program (SNP library).**

Atlantic halibut (*Hippoglossus hippoglossus*)

Bottlenecks

- **Irregular supply of fry particularly from F1/F2 broodstock; long production cycle**

Atlantic halibut (*Hippoglossus hippoglossus*)

Objectivos

- **1. Improve fecundity and gamete quality in F1/F2 broodstock.**

WP5

IMR, HCMR, NIFES, SWH

PM20.5 - 234,701€

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	44	48	51	54	57	60
Task 5.1																				
Task 5.2																				
Task 5.3																				

Atlantic halibut (*Hippoglossus hippoglossus*)

2014 outputs summary

- **Reproductive performance of wild and F1/F2 documentation.**
- **GnRHa induced spawning of F1/F2**

- **Presentation of results by Birgitta Norberg to follow → this talk…**

WP3 Greater amberjack (*Seriola dumerili*)

Bottlenecks

- **Lack of reliable reproduction and of egg availability**

Greater amberjack (*Seriola dumerili*)

Objectives

- **1. Describe the endocrine control of reproduction in captive broodstocks, and the nutritional status of fish during the reproductive season,**
- **2. Assess reproductive potential of wild vs. captive amberjack broodstocks and identify possible reproductive/metabolic dysfunctions during gametogenesis,**
- **3. Develop spawning induction methods for wild captive and F1 broodstocks of both the Mediterranean and Atlantic stocks,**
- **4.Apply the developed spawning induction methods for broodstocks maintained in cages, and examine the efficiency of an egg collector to obtain fertilized eggs,**
- **5. Develop a Computer Assisted Sperm Analysis method (CASA) for the evaluation of greater amberjack sperm during the reproductive season, and evaluate the possible effects of captivity.**

Greater amberjack (*Seriola dumerili*)

WP3

HCMR, UNIBA, FCPCT, IOLR, IEO, ULL, IFREMER, ARGO, ITICAL

PM148.6 – 751,471€

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	44	48	51	54	57	60
Task 3.1																				
Task 3.2																				
Task 3.3																				
Task 3.4																				
Task 3.5																				

Task 3.1 Description of the reproductive cycle of greater amberjack.

led by UNIBA
Aldo Corriero

First Annual Coordination Meeting
4-6 November 2014
Bari, Italy

WILD GREATER AMBERJACK SAMPLING (UNIBA)

SAMPLING AREA:

LAMPEDUSA

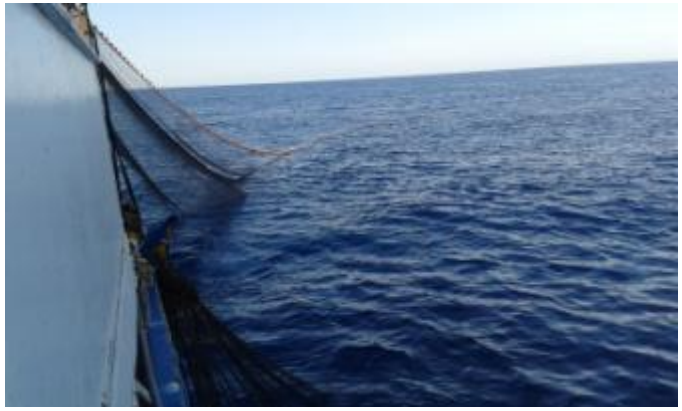
(Pelagic Islands, Sicily, Italy)

SAMPLING DATES:

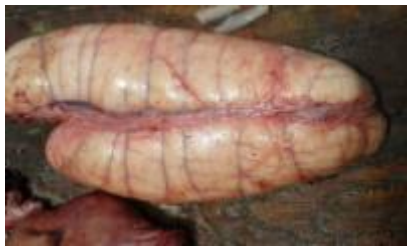
31/05/2014

30/06/2014

PURSE SEINE FOR GREATER AMBERJACK FISHERY IN LAMPEDUSA



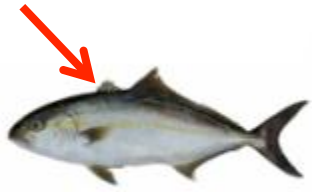
WILD GREATER AMBERJACK SAMPLES COLLECTED IN MAY AND JUNE 2014



OVARY

TESTIS

SPERM

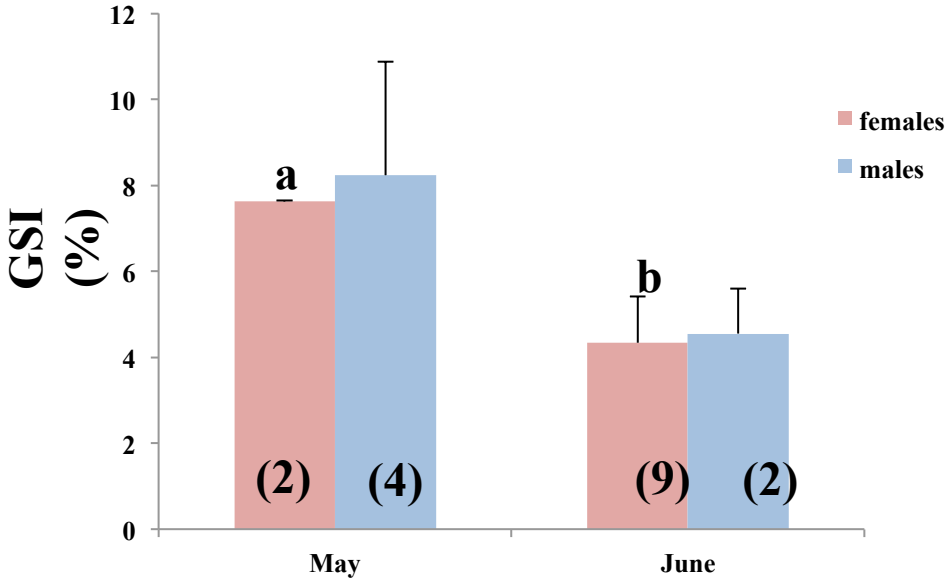


1st DORSAL SPINE

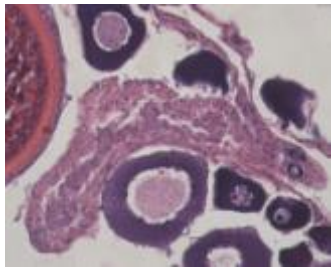
MEAN FORK LENGHT AND MEAN BODY MASS OF WILD GREATER AMBERJACK SAMPLED IN MAY AND JUNE 2014

Sampling Date	Sex	Mean Fork Lenght ± sd (FL; cm)	Total Body Mass ± sd (BM; kg)
31/05/2014	f	115.5 ±2.1	21.3±0.4
	m	110.0±11.6	17.3±4.3
30/06/2014	f	99.0±2.9	12.0±0.8
	m	99.5±0.7	10.7±0.3

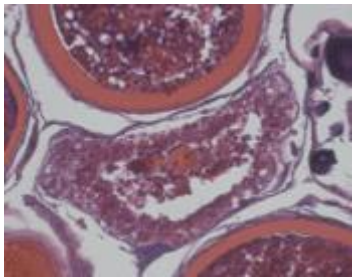
MEAN GONADO-SOMATIC INDEX OF WILD GREATER AMBERJACK SAMPLED IN MAY AND JUNE 2014 (ANOVA; P≤0.05)



Post- ovulatory follicles (POF)



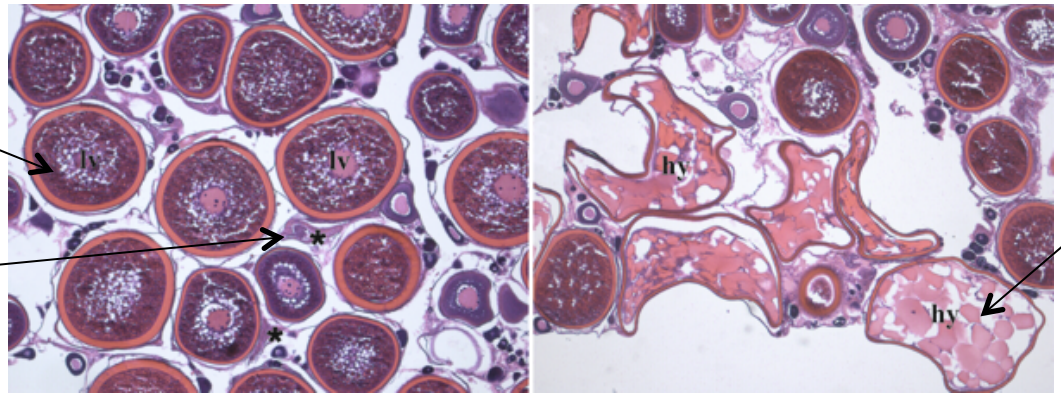
Atretic follicles



WILD FEMALE GREATER AMBERJACK REPRODUCTIVE STATE

late vitellogenic follicles

POFs



Hydrated oocytes

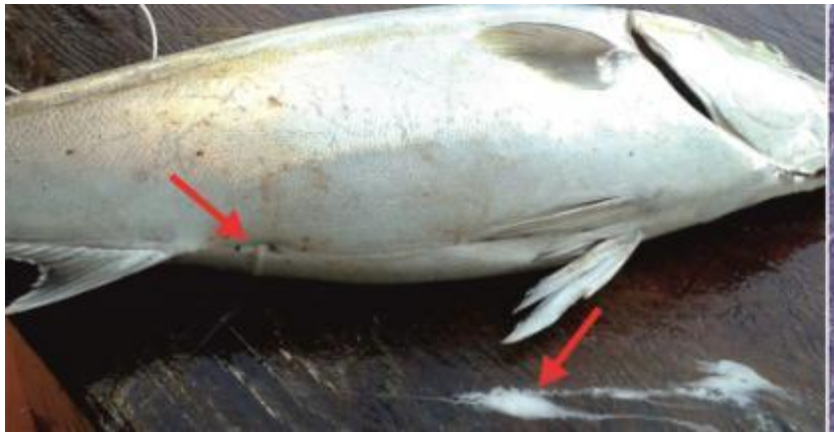
2 females in May and 7 in June:

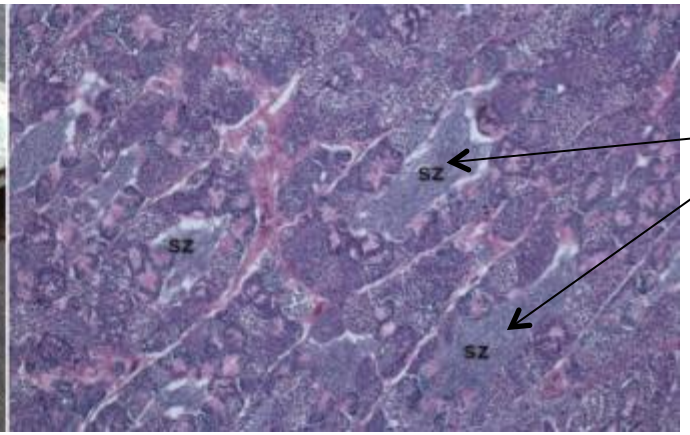
MATURE

2 females in June:

SPAWNING

WILD MALE GREATER AMBERJACK REPRODUCTIVE STATE





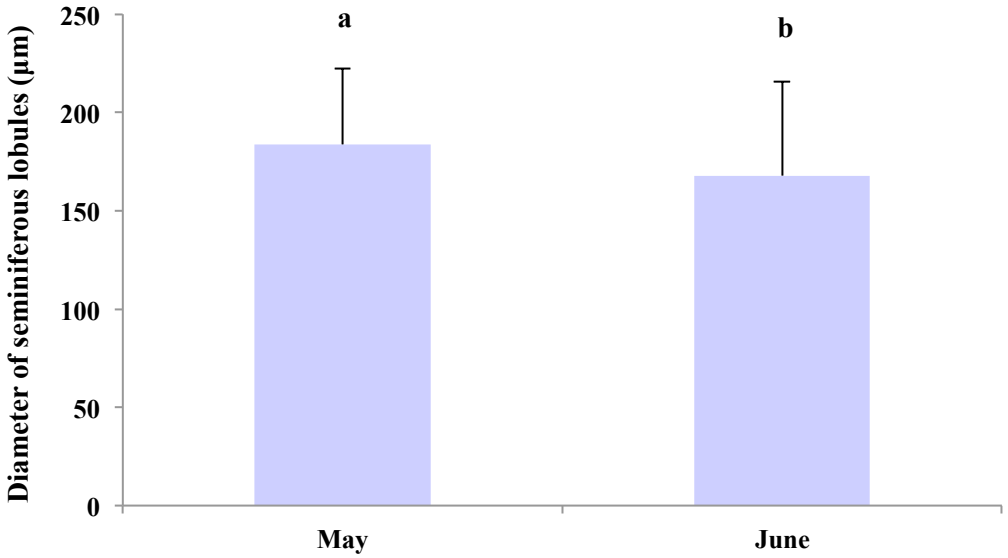
4 males in May :

MATURE, RELEASING SPERM

2 males in June:

MATURE, INTRA-TESTICULAR SPERM

MEAN SEMINIFEROUS LOBULE DIAMETER (ANOVA; P≤0.05)



PRELIMINARY CONCLUSION

- Wild greater amberjack is in spawning condition between the end of May and the end of June, when POF as well as hydrated oocytes were observed in the ovaries.

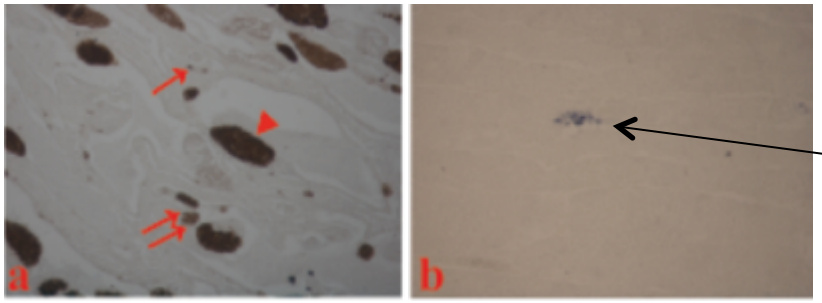
……TO BE DONE IN Y2

NEW SAMPLING CAMPAIGNS IN LATE APRIL-EARLY MAY AND, POSSIBLY, AFTER THE CESSATION OF THE SPAWNING SEASON

Male germ cell proliferation and apoptosis - Vitellogenesis

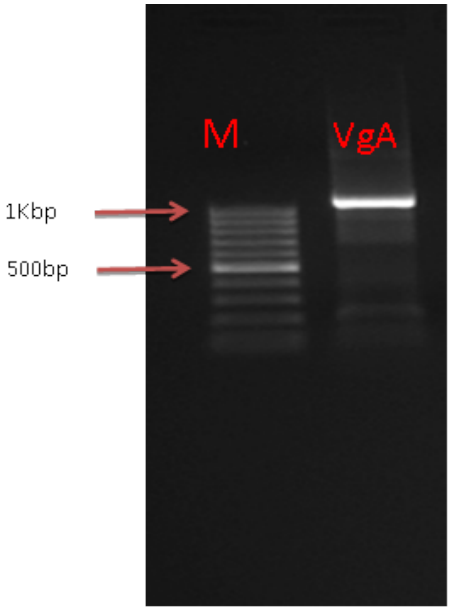
IDENTIFICATION OF PROLIFERATING (PCNA immunolocalization) AND APOPTOTIC (TUNEL method) GERM CELLS IN WILD MALES

PCNA POSITIVE GERM CELLS



TUNEL POSITIVE GERM CELLS

ESTABLISHMENT OF QUANTITATIVE PCR ESSAY TO MEASURE TRANSCRIPT LEVELS OF VITELLOGENIN



Vitellogenin A (Vg A) amplification

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.2. Development of an optimized spawning induction protocol for captive greater amberjack in the Mediterranean (led by HCMR Constantinos (Dinos) Mylonas).

- **GnRHa induced spawning studies**
- **Presentation of results by Constantinos (Dinos) Mylonas to follow → this talk…**

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

Twenty-two greater amberjack (average weight: females, 3.41 ± 1.12 kg; males, 2.37 ± 1.07 kg), originally captured in the South-western coast of Gran Canaria (Islas Canarias, España) in May 2011

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

May 2014, males average weight = 10.77 ± 2.33 kg; females = 10.72 ± 1.22 kg

40m^3 tanks

T A: 5♂; 2♀ (mean oocyte diameter = 837.1 ± 166.9 µm) – Natural spawn

T B: 4♂; 4♀ (mean oocyte diameter = 689.5 ± 99.8 µm) - Injected

T C: 3 ♂; 4 ♀ (mean oocyte diameter = 648.4 ± 58.5 µm) - Implanted

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

Injections: 20 µg/kg twice a week (Tuesday and Friday) alternating the broodstock couples (i.e., every 10-11 days). Oocyte > 0,69 mm

Implants: 500µg GnRHa implants / female (≈ 50 µg/kg); male 1/2 500µg implant (≈ 25 µg/kg) Re-administered when spawning stopped. Oocyte > 0,65 mm

Greater amberjack (*Seriola dumerili*)

2014 outputs

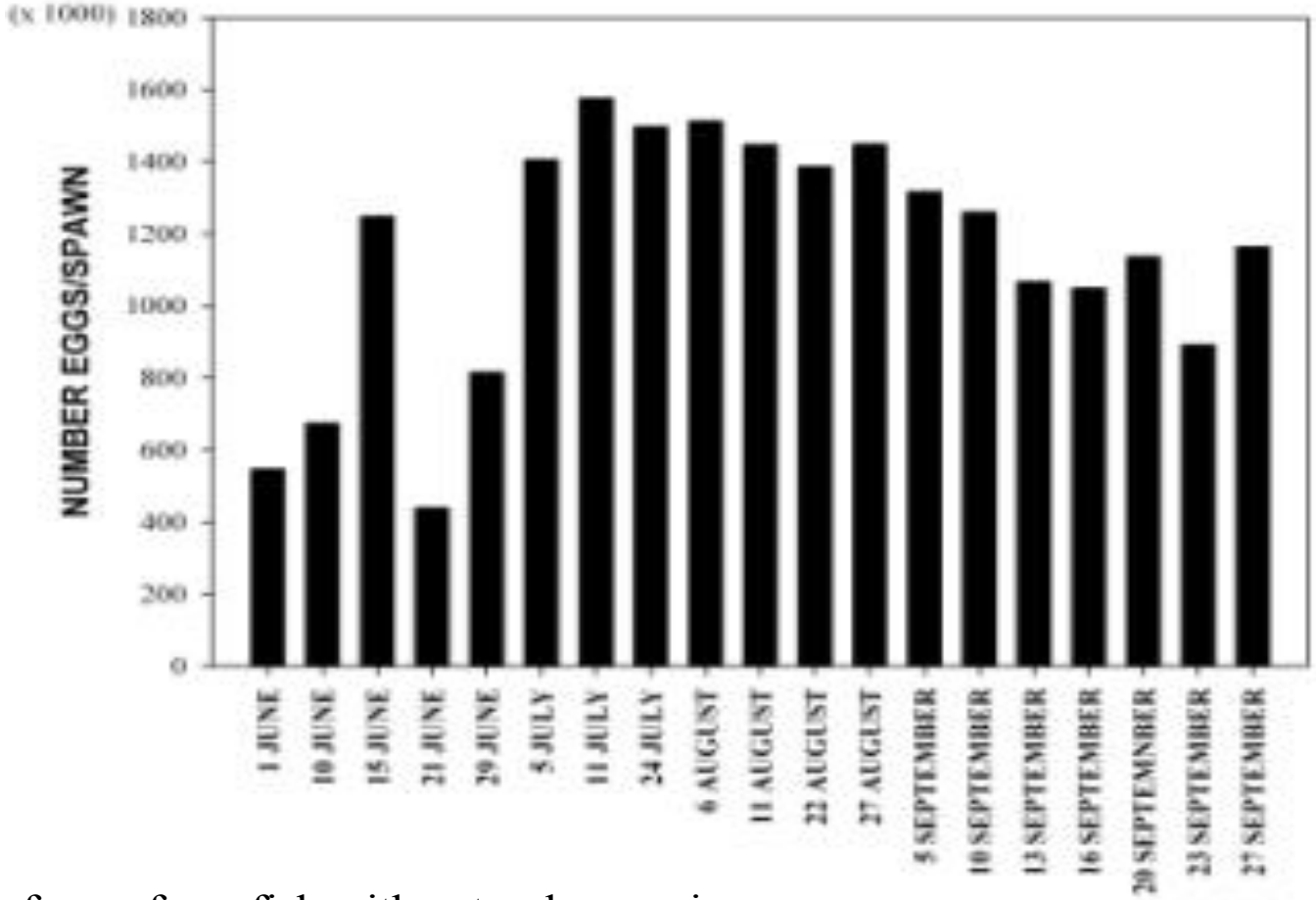
Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

Treatment	Nª females that spawned	Nº Inductions	Nº spawns	Spawns/ Induction $P < 0.01$	Spawn hour Latency period (h):	Nº eggs/spawn $P < 0.01$
Natural	-	-	19	-	5.38±1.65	1,151,610±339,375[a]
Injected	3	29	22	0.79±0.49[b]	43.14±2.36	448,483±265,552[b]
Implanted	3	12	36	3.0±1.65[a]	45.35±8.65	256,454±283,554[c]

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

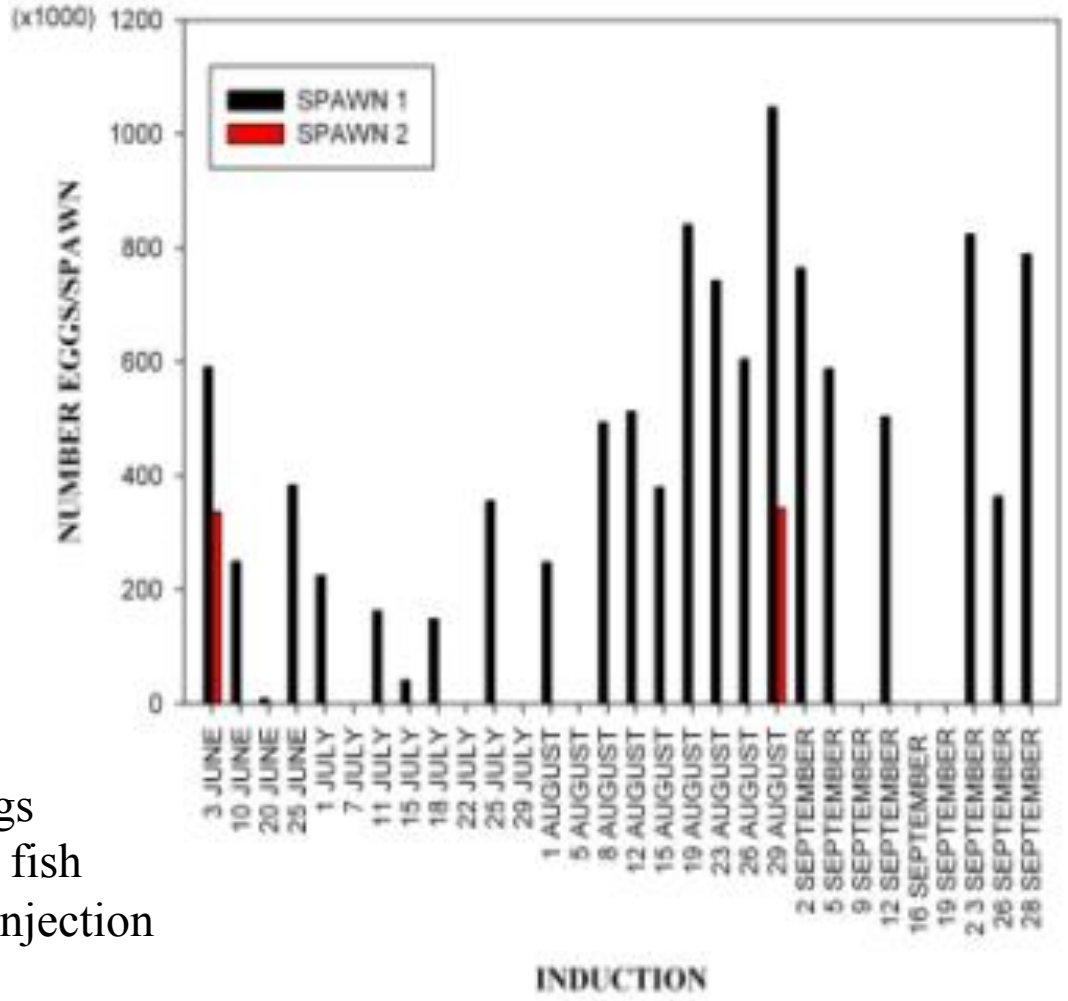


Number of eggs from fish with natural spawning

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

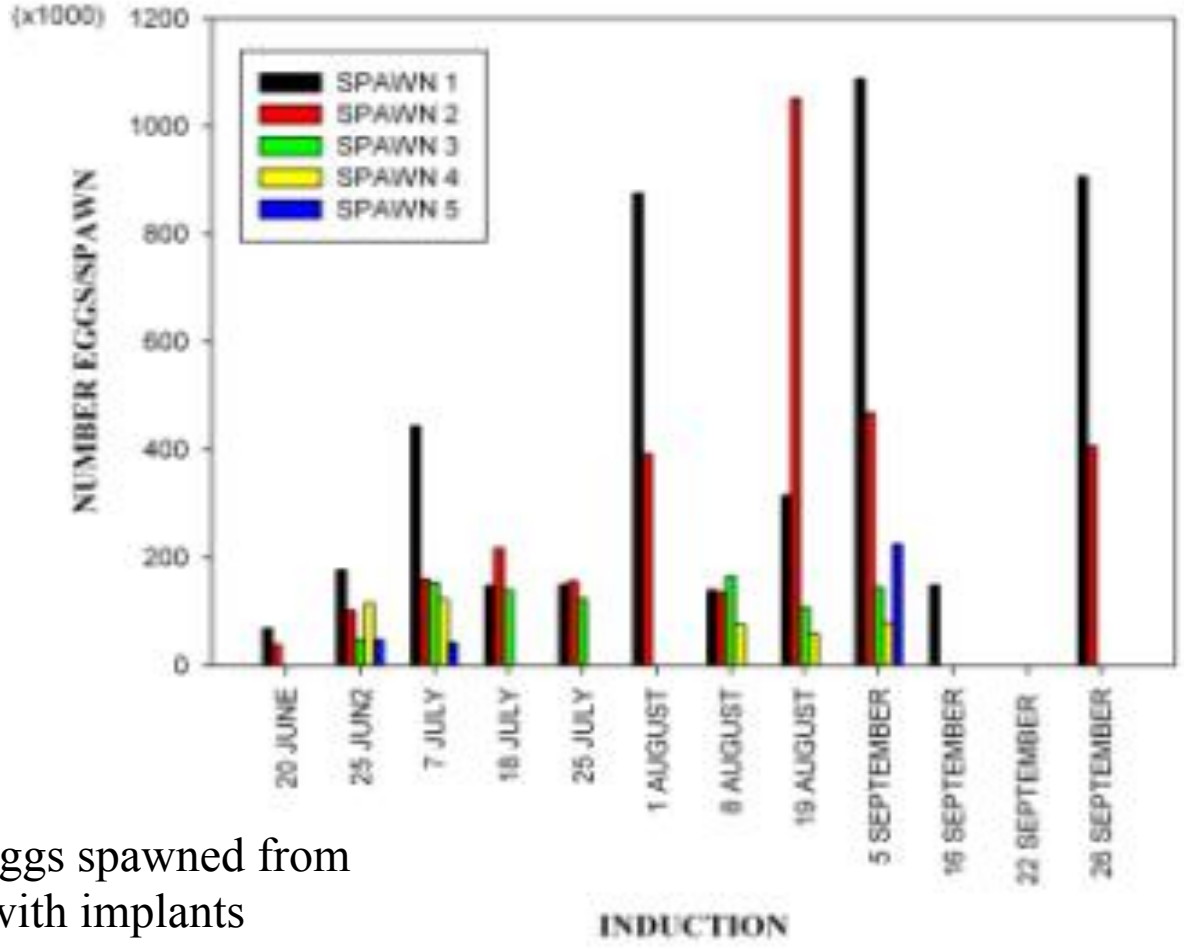


Number of eggs spawned from fish induced with injection

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

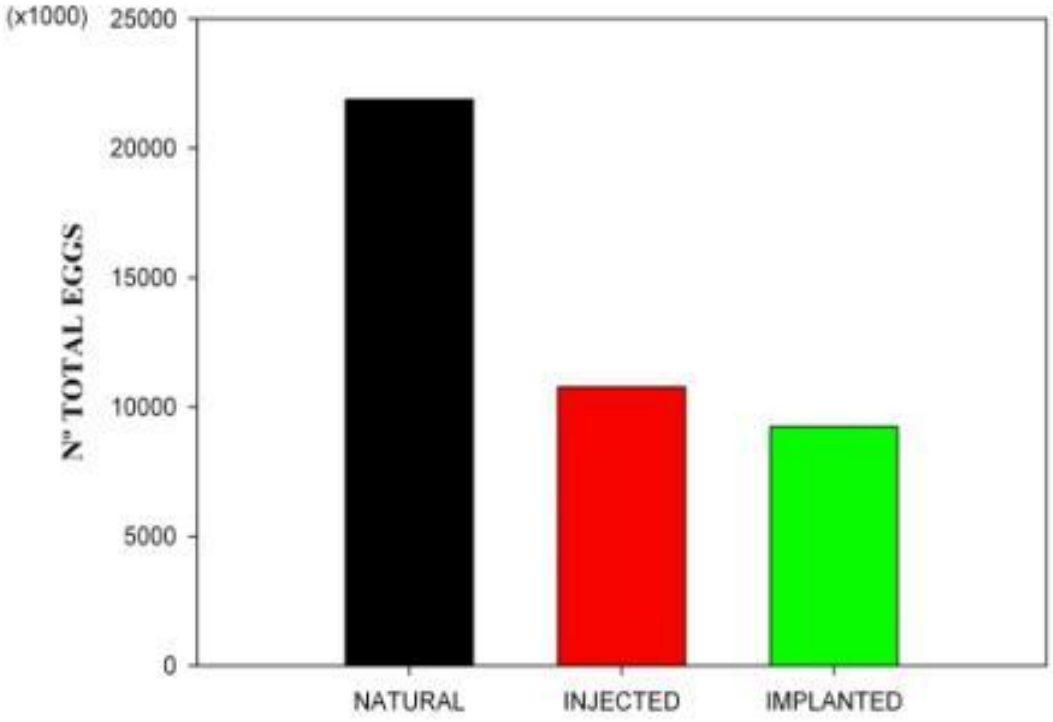


Number of eggs spawned from fish treated with implants

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).



Total number of eggs obtained in each treatment.

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.3 Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic (led by FCPCT, Hipílito (Pipo) Fernández-Palacios Barber).

Treatment	% Fertilization P < 0.01	% Viable 24 h P <0.01	% Hatching P < 0.01	% 4d Live P < 0.01	% 8d live P < 0.01
Natural	83.46±23.43^{a}	94.46±8.03^{a}	96.24±7.05^{a}	70.74±17.45^{a}	13.68±15.50^{a}
Injected	56.65±28.88^{b}	87.19±27.50^{ab}	89.45±28.10^{ab}	59.12±25.38^{ab}	7.13±7.40^{b}
Implanted	29.33±32.75^{c}	76.67±35.39^{b}	75.22±36.15^{b}	46,63±27.46^{b}	8.82±13.09^{ab}

Treatment	Egg diameter (n = 4500	Oil droplet diameter (n = 450)	Total length of larvae at day 0 (n = 450)	Total length of larvae at day 3 (n = 450)
Natural	1.13±0.03^{a}	0.30±80.02^{a}	2.59±0.09^{a}	3.85±0.13^{a}
Injected	1.10±0.02^{b}	0.27±0.02^{b}	2,58±0.13^{a}	3.82±0.13^{a}
Implanted	1.10±0.02^{b}	0.27±0.02^{b}	2.45±0.13^{b}	3.53±0.26^{b}

Task 3.4 Development of an optimized spawning induction protocols for F1 greater amberjack in the eastern Atlantic.

Led by IEO Canarias,
Salvador Jerez

First Annual Coordination Meeting
4-6 November 2014
Bari, Italy

Task 3.4 Development of an optimized spawning induction protocols for F1 greater amberjack in the eastern Atlantic

- **The broodstocks remaining were distributed in two groups (500 m3 and 50 m3 tanks volume).**

Group Tank 500 m^3			Group Tank 50 m^3		
ID	Weigth (kg)	Sex	ID	Weigth (kg)	Sex
584663	11.3	Female	**562180**	9.3	Male
566201	13.6	Male	**899854**	9.4	Male
883043	7.6	Male	**584537**	10.0	Female
594669	15.8	Male	**588159**	12.8	Unsexed
558222	15.9	Unsexed	**897974**	11.5	Male
560004	24.5	Unsexed	**885828**	11.0	Female
569463	11.3	Unsexed	**908613**	11.8	Male
904365	10.9	Male	**559560**	17.0	Unsexed
883043	6.6	Male			
592680	21.4	Female			

Task 3.4 Development of an optimized spawning induction protocols for F1 greater amberjack in the eastern Atlantic

No spawns obtained after treatment of fish in 50 m^3 tank

Results of induction treatment in the large tank (500 m^3)

- **Six eggs batches were collected (between 7/8/2014 and 2/9/2014)**
- **A total of 1.4 x 10^6 eggs and mean floating rate of 61.63 %**
- **Null fertilization rate although there were males in the group**

Date	Time (hour)	Nº eggs collected	Floating rate (%)
	9	501,384	65.11
	13	140,400	57.46
	18	101,268	46.05
07/08/2014		743,052	61.07
	9	157,140	57.08
	13	68,796	80.67
08/08/2014		225,936	64.27
	9	154,212	35.80
	18	74,376	74.62
09/08/2014		228,588	48.43
29/08/2014	9	158,100	88.25
01/09/2014	18	58,320	80.58
02/09/2014	9	44,160	5.43
Total-mean		1,458,156	61.63

Greater amberjack (*Seriola dumerili*)

2014 outputs

Task 3.5 Spawning induction of greater amberjack and egg collection in cages (led by HCMR Constantinos (Dinos) Mylonas).

- **GnRHa induced spawning studies**
- **Presentation of results by Constantinos (Dinos) Mylonas to follow → this talk…**

Greater amberjack (*Seriola dumerili*)

2014 outputs summary

- **Description of normal maturational development in wild stocks May and June.**
- **Natural spawning in captivity**
- **Initial development of hormonal spawning induction protocols.**
  - **In tanks in East Atlantic**
  - **In tanks in Mediterranean**
  - **In cages in Mediterranean**

WP6 Wreckfish, (*Polyprion americanus*)

Bottlenecks

- **Lack of reproduction control and of egg availability**

Wreckfish, (*Polyprion americanus*)

Objectives

- **1. Increase the availability of wreckfish broodstocks in captivity,**
- **2. Describe the reproductive cycle in captivity at the level of the pituitary and gonad,**
- **3. Develop spawning induction procedures for in vitro fertilization, as well as spontaneous tank spawning,**
- **4. Develop a CASA for evaluation of wreckfish sperm and establish cryopreservation protocols for use in in vitro fertilization applications.**

Wreckfish, (*Polyprion americanus*)

WP6

IEO, HCMR, IRTA, CMRM, MC2, ULL, IFREMER

PM28.96 - 260,932€

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	44	48	51	54	57	60
Task 3.1																				
Task 3.2																				
Task 3.3																				
Task 3.4																				
Task 3.5																				

WP6
Reproduction and Genetics species wreckfish

José Benito (Tito) Peleteiro IEO (P8)

First Annual Coordination Meeting
4-6 November 2014
Bari, Italy

Task 6.1 Collect wild fish to establish new broodstocks (led by CMRM).

▪ Fishing techniques: 3 animals were captured using a "**salabre**" since these animals are usually found below floating objects.

• Location: 5 miles West of Corrubedo Cape (La Coruña).

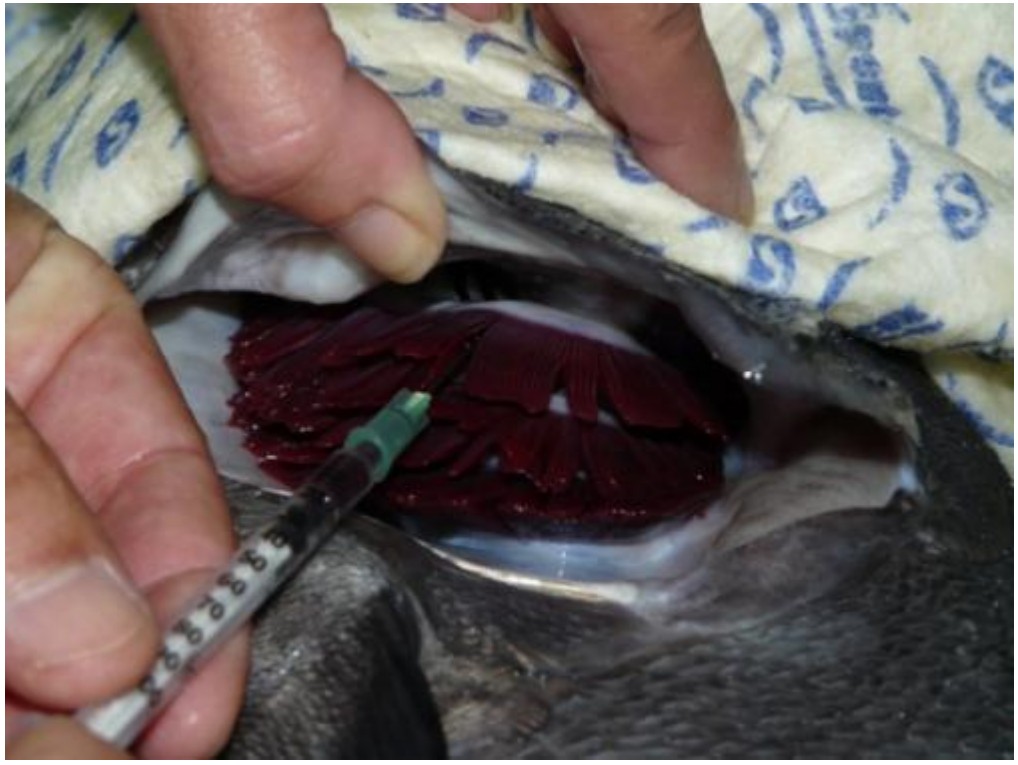
• Delivery date: 2 wreckfish of 2 kg each on the 12/06/2014 and 1 wreckfish weighing 1.5 kg on the 26/08/2014.

• Transportation: on a ship, by sea, in tanks with flow-through water , until the facilities from the "O Grove" Aquarium, where they were maintained in quarentene until weaning to inert food.

• Morphometric measurements were performed and a sample of the fin was taken, for future genetic identification.

Task 6.2 Describe reproductive cycle (led by IEO)

Blood samples from the CMRM (P19) stock (12 fishes), in order to determine evolution of sexual steroids between January and June 2014 (Samples are currently being processed).



Task 6.2 Describe reproductive cycle (led by IEO)

Sampling in fish market

BIOMETRIC PARAMETER (58 WIDE WRECFISH)	MEDIA	STD
TOTAL LENGHT	76,09	6,788
ST LENGHT	66,38	7,629
PERÍMETER	55,68	5,986
WEIGHT (Kg)	7,52	2,169
EVIS. WEIGHT (Kg)	6,99	1,967
GONAD WEIGHT (g)	17,10	20,831
LIVER WEIGHT (g)	95,70	71,671
FAT PERIVIS. WEIGHT (g)	76,25	72,233
STOMACH WEIGHT (g)	125,90	56,183
INTESTINE LENGHT (cm)	94,53	15,555
INTESTINE WEIGHT (g)	99,27	62,688
GSI	0,20	0,161
SHI	1,21	0,497
VSI	10,31	17,233

Table 6.2.1. Biometric parameters and idexes (average) determined for the dead animals sampled.

Task 6.2 Describe reproductive cycle (led by IEO):

- From all animals sampled, besides “in visu” identification of female and male gonads, histological analysis were performed in order to confirm sex and study the possibility of hermafroditism in this specie.

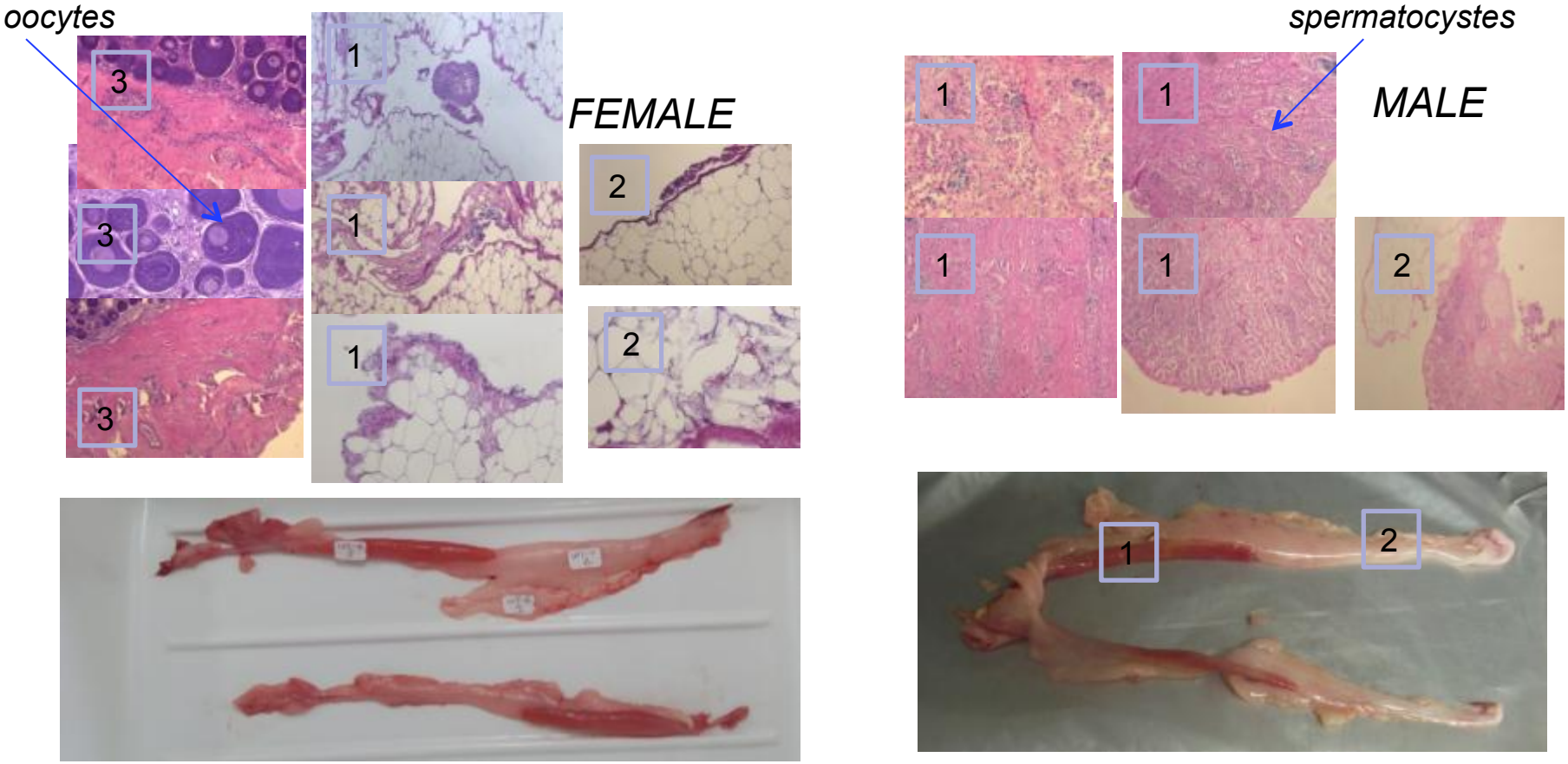


Fig. 6.2.3. Gonadal histology of females and males

Task 6.3 Development of spawning induction procedures (led by IEO) stock HCMR (P1)

- A stock of 3 wreckfish (two males of 7.4 and 11.2 kg, and female of 11.8 kg) 15-m^3 tanks, under simulated natural photoperiod and constant temperature (15°C). fed 3 times a week with raw fish (mackerel
- April 2014 female was undergoing vitellogenesis, oocytes of 1325
- 12 May 2014 contained vitellogenic oocytes (1250 μm) and oocyte maturation (1450 μm).
- Some eggs (25,000) were also released in the tank, but were not fertilized (Fig. 6.3.1).
- The female was given a GnRHa implant (500 μg) and males (400 μg GnRHa implant). Two spawns were obtained, but a very small number of eggs were fertilized <<1%.
- After that, the fish were biopsied and the female contained post-ovulated eggs and many vitellogenic oocytes, some in atresia/apoptosis (Fig. 6.3.1)

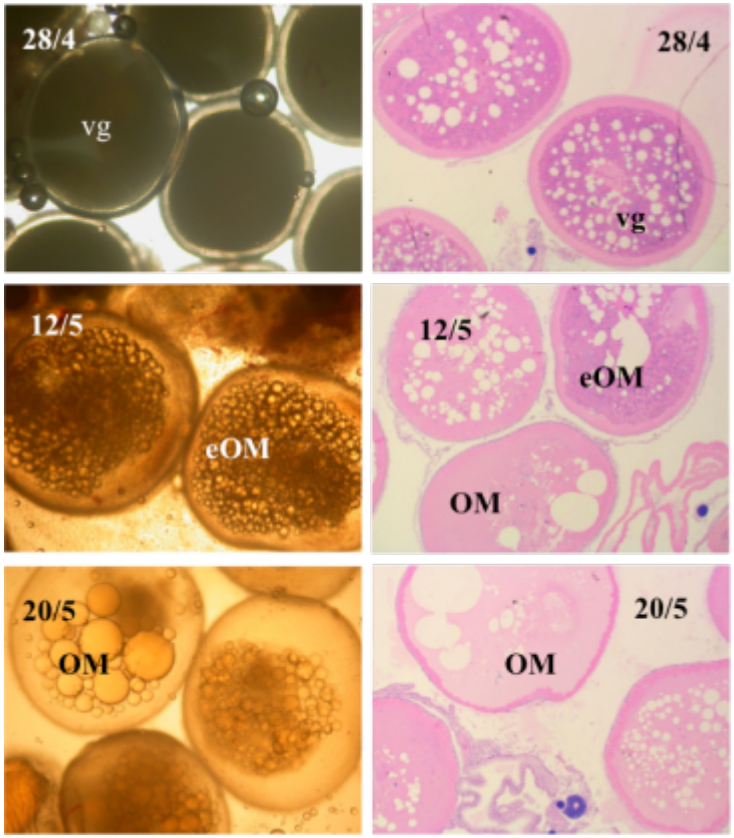


Fig. 6.3.1. Wet mount and histological sections of biopsies from wreckfish during the 2014 reproductive season (dates on each photo). eOM = early oocyte maturation, OM = oocyte maturation, Vg = vitellogenic

A final effort to induce spawning was undertaken, giving a higher dose of GnRHa (750 µg). At this time the female contained both post-ovulated eggs and vitellogenic oocytes, but with a high occurrence of atresia (Fig. 6.3.2)

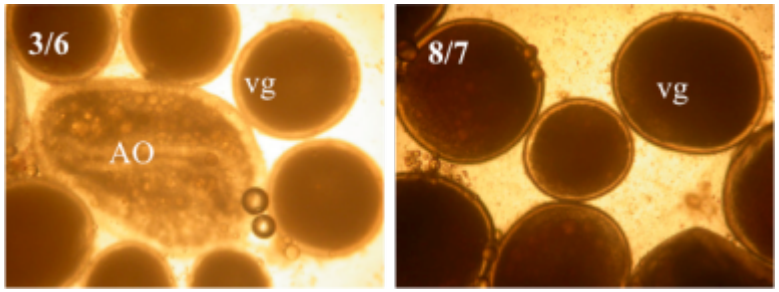


Fig. 6.3.2. Wet mount of biopsies from wreckfish during the 2014 reproductive season (dates on each photo). AO = apoptotic/atretic oocyte, Vg = vitellogenic.

• Sperm quality parameters were evaluated

Sperm quality was fairly high during the whole reproductive season

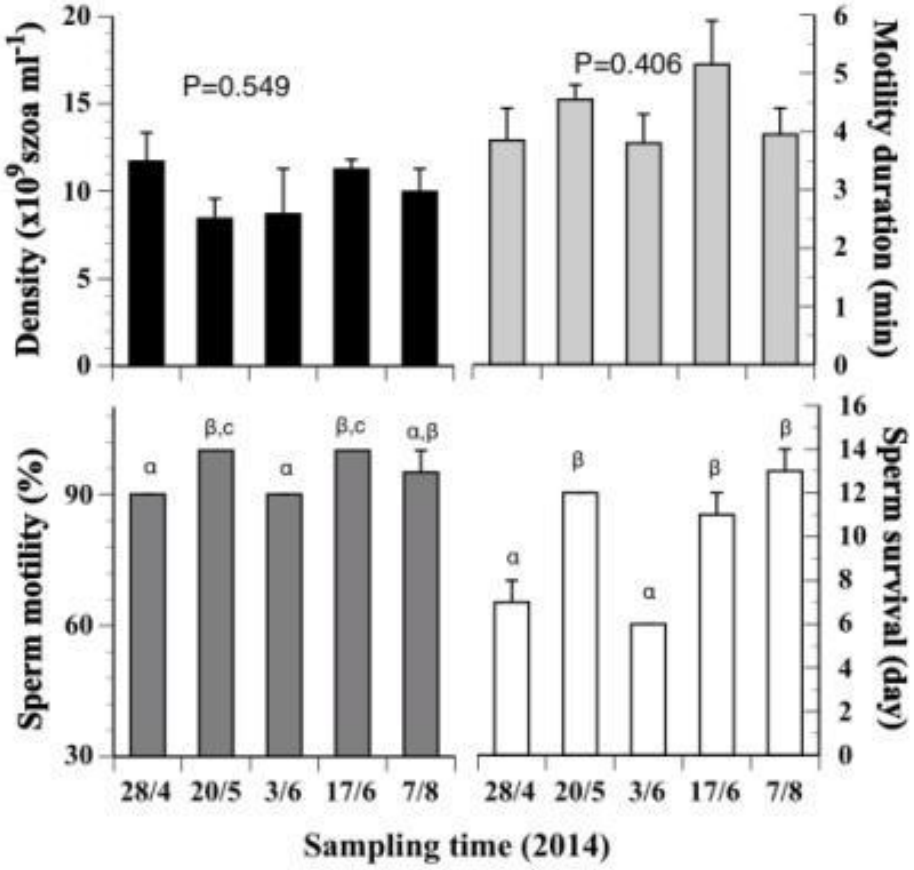


Fig. 6.3.3. Sperm quality parameters of wreckfish during the 2014 reproductive season. Different letter superscripts indicate the existence of significant differences (ANOVA, Duncan's New Multiple Range test, $P < 0.05$).

Task 6.3 Development of spawning induction procedures (led by IEO) stock CMRM (P19)

- 12 wreckfish 9.94 to 18.28 kg and sexually undifferentiated
- 80m^3 tank
- natural temperature and photoperiod.
- fed dry broodstock diets 3 times a week.
- This stock was sampled on a monthly basis (between January and August) to control sexual maturation. Blood samples were collected to determine sexual steroids. These samples were sent to the HCMR for future analysis.

Task 6.3 Development of spawning induction procedures (led by IEO) stock IEO (P8)

- 9 wreckfish (4 females, 3 males and 2 undetermined), weighing between 9,50 and 18.86 kg,
- 130 m^3 tank
- natural temperature and photoperiod.
- fed 3 times a week with semi-moist broodstock diets.
- This stock was sampled twice a month during spawning season,
- No evidence of sexual maturation was observed on females.
- two males showed spermiation, and sperm quality was assessed

Task 6.3 Development of spawning induction procedures (led by IEO) stock MC2 (P32)

- 27 wreckfish (11 females, 12 males and 4 undifferentiated), weighing between 10.70 and 30.25 kg,
- 3500 m^3 exhibition tank (Nautilus transferred to a 50 m^3 tank for closer control during spawning season.
- During breeding season, when the first maturity signs were observed, the stock was sampled on a weekly basis to control the maturity stage evolution. Ovary biopsies were made at 9 females, to determine oocytes stages (Figure 6.3.4)

Plastic tagg	Tagg	06/03/2014	20/03/2014	29/04/2014	13/05/2014
	Nº 8 **00-0618-1E7D**	973,5 ± 37,150 µ	1040 ± 50,7093 µ	1160 ± 109,5445µ	1126± 66,619 µ
	Nº 9 **00-0618-16E6**		1143,3 ± 104,9943 µ	1155± 114593102 µ	1563± 352,04 µ
	Nº 11 **981023604036**	660 ± 118,300 µ	733,3± 143,5104 µ	925± 125,13151 µ	976± 74,527 µ
	Nº 12 **98102355554**		980 ± 64,9175 µ	1010± 96,7906042 µ	1074± 62,549 µ
	Nº 19 **98102357438**		846,7 ± 107,6812 µ	1060 ± 68,0557 µ	995± 71,10 µ
Wide mouth	Nº 20 **2356915**	403,5 ± 176,000 µ	870 ± 99,6422 µ	965± 122,581874 µ	940 ± 82,115 µ
	Nº 21 **00-0618-1779**		976,66 ± 67,7882 µ	1095± 114,593102 µ	2138 ± 135,6044 µ
	00-0643-7B78			940± 114,248114 µ	1016± 65,5526 µ
	00-061D-5679				1066± 82,346 µ

Fig. 6.3.4 Evolution in oocytes diameter from 9 females from the MC2 stock

Task 6.3 Development of spawning induction procedures (led by IEO) stock MC2 (P32)

- From the 5 females in isolation, three were submitted to abdominal massage for oocytes extraction. Sperm was obtained from males also. “In vitro” fertilization was performed, but spawn quality was poor, despite the oocytes were mature (2300µ in diameter).
- The remaining two females spawned naturally in the tank, from May until August (Fig 6.3.5) Eggs were collected and measured. Fecundity percentage was determined. In almost all cases egg quality was poor, except the one from June 4th, with a fertilization percentage of 70%. Nevertheless, this spawn was also of poor quality, since only 14% of these hatched.

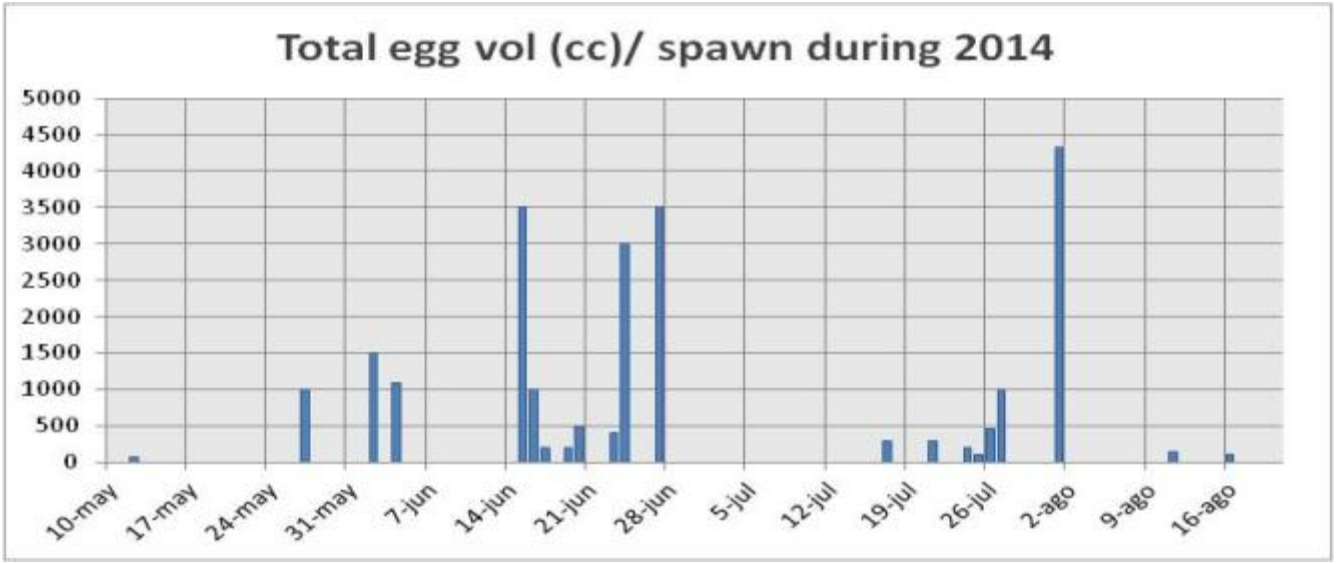
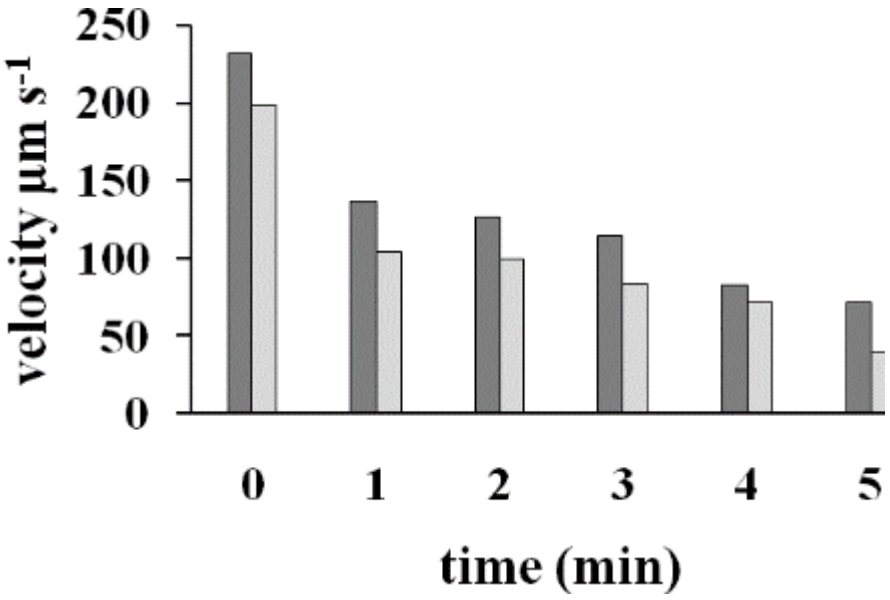


Fig. 6.3.5. Total collected eggs in Acuarium Finisterrae

Task 6.4 Evaluation of sperm characteristics and cryopreservation protocols (led by IFREMER).

- From April 8th to April 13th, sperm were collected from 10 males in la Coruña Aquarium , Luso Hispana Aquicultura (LHA) and IEO.
- sperm concentration was 2.41 10^{10} (sd :0.4 $10^{10,}$ n=9) spermatozoa per ml.



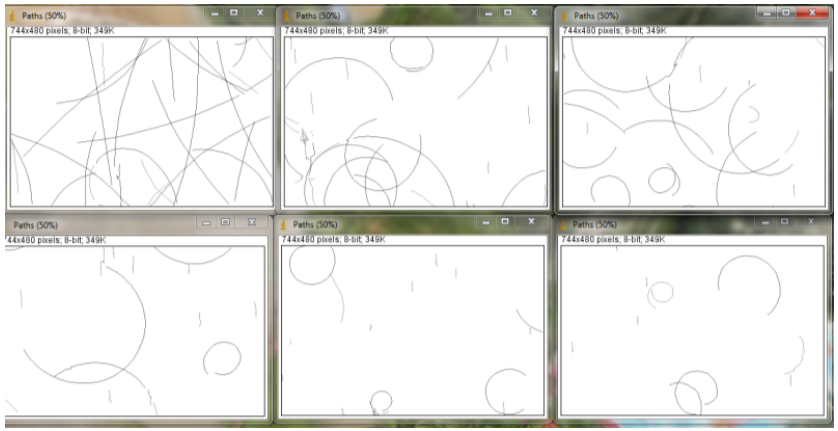


Fig. 6.4.1 *Variations with time of Average Path Velocity i.e along smoothed trajectory (dark grey) and Straight Line Velocity picturing the progressive movement (light grey)*

Wreckfish, (*Polyprion americanus*)

2014 outputs summary

- **Samples collected to describe the reproductive cycle of wreckfish.**
- **Natural spawning observed and data registered**
- **Preliminary work made to development hormonal spawning induction protocols.**
- **Initial data on sperm quality to develop in vitro fertilization procedures**

WP7 Grey mullet (*Mugil cephalus*)

Bottlenecks

- **Lack of control of the reproductive cycle; low and irregular egg quality**

Grey mullet (*Mugil cephalus*)

Objectives

- **1. Evaluate the effectiveness of hormone-based treatments on synchronizing gonadal development and improving gamete (eggs and sperm) quality in mature grey mullet,**
- **2. Develop hormone-based treatments for induced spawning of grey mullet,**
- **3. Optimize a scaled-up breeding of grey mullet in captivity under natural and manipulated photo-thermal regimes,**
- **4. Assess the effects of captivity on first sexual maturity and reproductive potential of captive-reared and hatchery-produced grey mullet broodstocks.**

Grey mullet (*Mugil cephalus*)

WP7

IOLR, HCMR, IRTA, UNIBA, ITICAL, DOR, ULL, IFREMER

PM24.3 - 149,981€

	Year 1 (2014)				Year 2 (2015)				Year 3 (2016)				Year 4 (2017)				Year 5 (2018)			
	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De	Ma	Ju	Se	De
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	44	48	51	54	57	60
Task 7.1																				
Task 7.2																				
Task 7.3																				
Task 7.4																				

Grey mullet (*Mugil cephalus*)

2014 outputs

Task 7.1 Evaluation of the effectiveness of hormone-based treatments on synchronizing gonadal development (led by IOLR, Hanna Rosenfeld).

- Recombinant FSH was produced using the *Pichia pastoris* yeast expression system
- Grey mullet breeders from IOLR-NCM hatchery F1 fish (n= 186; age: 5-year old); 4-m3 tanks; 40 ppt salinity natural photoperiod and temperature conditions (25°C in June).
- Methods to evaluate mullet sperm quality have been established.

Grey mullet (*Mugil cephalus*)

2014 outputs

Task 7.1 Evaluation of the effectiveness of hormone-based treatments on synchronizing gonadal development (led by IOLR, Hanna Rosenfeld).

- Hormonal acceleration of gonadal development
- In mid July 2014, both mullet females and males were administered recombinant FSH (7 µg/Kg BW) + dopamine antagonist: metoclopramide (15 mg/KgBW).
- The control fish were injected with saline solution.
- Early August 2014, males received 17alpha-methyltestosterone (MT) implants (5 mg/KgBW).

Relative abundance of fully mature females (oocyte >0,55mm) and spermiating males at early- and mid-spawning season (mid September and mid October, respectively) in control and hormonally treated fish.

	Control		Treatment	
	Mid September	Mid October	Mid September	Mid October
Fully mature females (%)	29	20	91	75
Spermiating males (%)	70	50	86	67

Grey mullet (*Mugil cephalus*)

2014 outputs

Task 7.2 Development of hormone-based treatments for inducing spawning (led by IOLR, Hanna Rosenfeld).

- Spawning trials are in progress.
  - Treatment: injections priming t=0: GnRHa 10 µg/kg; Met 15mg/kg and resolving t= 22,5h: GnRHa 20 µg/kg; Met 15mg/kg
  - female's failure to ovulate in 5 out of 12 spawning induction trials
  - fertilization rate ranging between 0 to 98%. (MT) implants

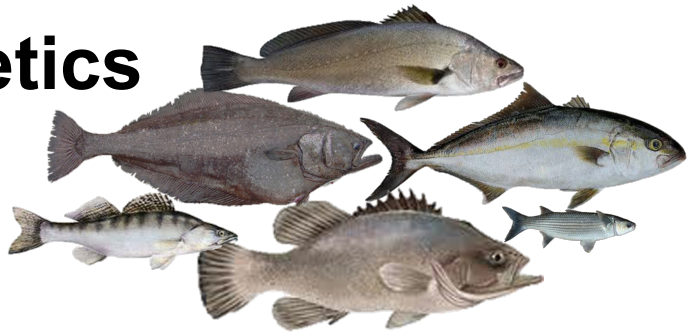
Date	Control-group				Treatment-group			
	No. of induction trials	Spawning Ratio (%)	Relative Fecundity ($x10^6$)	Fertilization rate (%)	No. of induction trials	Spawning Ratio (%)	Relative Fecundity ($x10^6$)	Fertilization rate (%)
10.9.14	2	0	0	0	2	0	0	0
29.9.14	1	0	0	0	3	100	2.6 ± 0.55	0-98
22.10.14					4	75	2.268±0.22	0-40

Grey mullet (*Mugil cephalus*)

2014 outputs summary

- **Recombinant FSH produced**
- **Methods for sperm evaluation established**
- **Percentage of breeders completing gametogenesis increased with treatments**
- **Spawning induction trails initiated**

GWP Reproduction and genetics 2014 outputs summary



- Pikeperch genetic evaluation in final stages
- Meagre paired spawning + genetic evaluation
- Greater amberjack natural and induced spawning + samples to describe gametogenesis
- Halibut F1 spawning increased
- Wreckfish natural spawning + samples to describe gametogenesis
- Mullet spawning trails initiated


Final expected result:
Massive productions of new aquaculture products

THE END

Thank for your attention