Spermatogenesis and sperm characteristics in captive greater amberjack

WP3_Reproduction and Genetics_
Greater amberjack

Third Annual Coordination Meeting
17-19 January 2017
Barcelona, Spain

WP3_Reproduction and Genetics_Greater amberjack

Task title	Deliverable title	Deliverable description	Delivery month
3.1_ Description of the reproductive cycle of greater amberjack (led by UNIBA)	D3.3_ Identification of possible reproductive dysfunction of gametogenesis of greater amberjack reared in captivity based on the comparative evaluation of fish sampled in the wild, in terms of proliferating and apoptotic germ cells, vitellogenin accumulation, yolk content in the oocytes and nutritional status	Comparative analyses between wild and captive amberjack: germ cell proliferation and apoptosis; liver Vg and VgR genes expression, oocyte yolk accumulation; key hormones and key nutrients related to fish nutritional status	24 DELIVERED (month 26)
	D3.4_ Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of greater amberjack sperm quality	Greater amberjack sperm motility and concentration	32 (DELIVERED)

- A decrease in sperm quality has been largely documented in captive fishes due to a variety of factors, including stress and rearing conditions (Rurangwa et al., 2004; Cabrita et al., 2009; Bobe and Labbé, 2010).

- An inadequate pituitary GtHs synthesis and/or release, and a consequent reduction of steroid secretion, has been considered as a major cause of reproductive dysfunctions in fish confined in captivity (Zohar and Mylonas, 2001; Mylonas et al., 2010; Berkovich et al., 2013).

Aim of this study was to identify possible dysfunctions of spermatogenesis in greater amberjack caught from the wild and reared in captivity, in terms of germ cell proliferation and apoptosis as well as sperm quality.

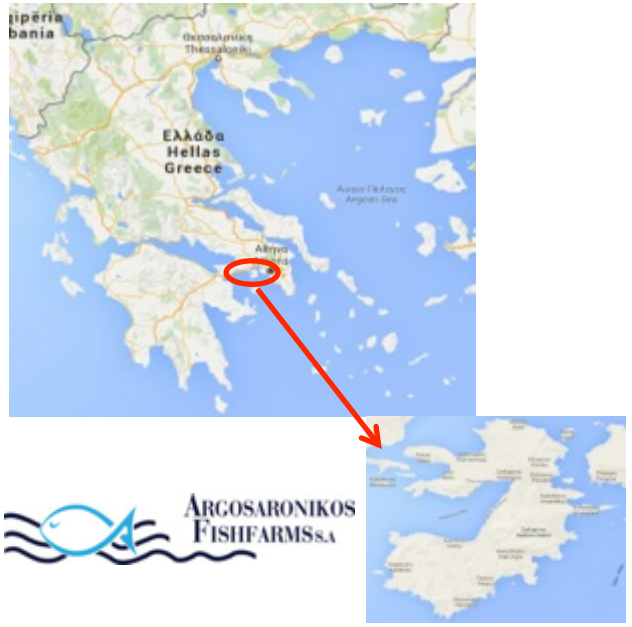
SAMPLING AREA: LAMPEDUSA

(Pelagic Islands, Sicily, Italy)

14 wild males

SAMPLING AREA: Argosaronikos Fish Farm

(ARGO), (Salamina Island, Greece)



12 captive-reared males

	Wild (2014-2015)	Captive-reared (2015)
Early gametogenesis (APR-MAY)	May: 1	April: 24
Advanced gametogenesis (MAY-JUN)	May: 31	June: 4
Spawning (JUN-JUL)	June: 29, 30	July: 2

May

Su	Mo	Tu	We	Th	Fr	Sa
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

April

Su	Mo	Tu	We	Th	Fr	Sa
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

May

Su	Mo	Tu	We	Th	Fr	Sa
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

June

Su	Mo	Tu	We	Th	Fr	Sa
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30				

June

Su	Mo	Tu	We	Th	Fr	Sa
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30				

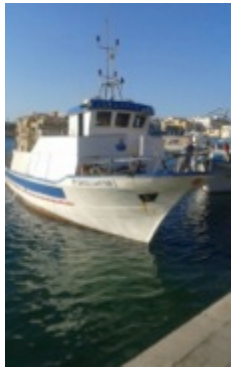
July

Su	Mo	Tu	We	Th	Fr	Sa
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

Sample collection

Biometric data: Fork Length (FL, cm); Body Mass (BM, kg); Testis Mass (TM, g)

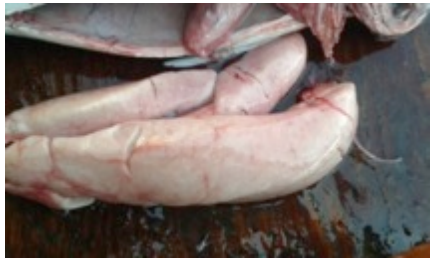
Sampling of wild fish



Sampling of captive-reared fish




Biological samples: blood; testis samples for histology; intratesticular sperm (only of captive-reared fish)

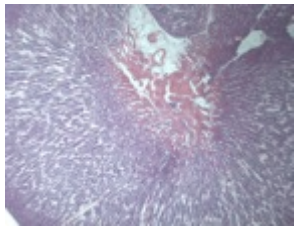
List of sampled specimens

Fish State	Sampling Area	FL (cm)	BM (kg)	TM (g)	GSI
APR-MAY					
wild	Lampedusa Island (Italy)	111	14	300	2.09
		112	20	450	2.31
		112	15	300	1.96
		113	19	400	2.11
		117	19	550	2.96
captive	Salamina Island (Greece)	92	12	65	0.54
		94	12	60	0.49
		94	13	60	0.47
		101	15	95	0.62
MAY-JUN					
wild	Lampedusa Island (Italy)	99	14	1150	7.99
		102	13	650	5.00
		115	19	2200	11.46
		124	22	1900	8.48
captive	Salamina Island (Greece)	90	9	370	3.91
		97	14	295	2.14
		98	13	600	4.59
		103	15	690	4.73
JUN-JUL					
wild	Lampedusa Island (Italy)	100	12	650	5.24
		102	14	700	5.05
		104	16	950	5.92
		99	11	577	5.29
		100	11	400	3.81
captive	Salamina Island (Greece)	91	10	70	0.74
		95	11	155	1.37
		96	13	140	1.12
		96	12	130	1.08

LABORATORY ANALYSES: Histology, IHC, TUNEL, Steroids

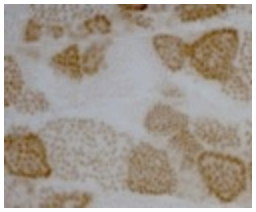
➢ HISTOLOGICAL ASSESSMENT OF MALE REPRODUCTIVE STATE

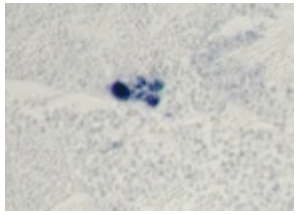
- Basic Histology
- Identification of spermatogenic cysts and assessment of luminal spermatozoa amount
- Mean diameter of seminiferous lobules



➢ DETECTION AND SEMI-QUANTITATIVE ANALYSIS OF PROLIFERATING AND APOPTOTIC CELLS

- Immunolocalization of a nuclear marker of proliferation (Proliferating Cell Nuclear Antigen, PCNA)
- Terminal deoxynucleotidyl transferase-mediated d'UTP nick end labelling (TUNEL method)
- Semi-quantitative analysis through Image Analysis





➢ DETERMINATION OF PLASMA STEROID CONCENTRATIONS

- Plasma obtained by blood centrifugation (5000 rpm for 5 minutes)
- ELISA assay for testosterone (T), 11-Ketotestosterone (11-KT) and 17,20β-dihydroxypren-4-en-3-one (17,20β-P) determination

LABORATORY ANALYSES: sperm quality

➢ SPERM CONCENTRATION

On site

- Dilution in tap water or 0,9 % NaCl; deposit on cell counter (hemocytometer); micrographs recording

Laboratory

- Analyse with imageJ using particle analysis function

➢ SPERM MOTILITY

On site

- Sperm aliquot dilution in modified Leibovitz
- Activation by mixing to 1 ml seawater + 2% BSA (final dilution of 1/500)
- 1 µl sperm dropped on a dedicated cell and video recording
- Recording interrupted at the cessation of any movement

Laboratory (Ifremer-Palavas)

- CASA analysis
- Variable parameters were motility (% of mobile spermatozoa) and velocity on smoothed trajectory (average path velocity; VAP)

➢ ATP CONTENT

On site

- Preparation of 1 and 10 µl aliquots of each sperm samples according to Boryshpolets et al (2009)

Laboratory (Ifremer-Brest)

- ATP content assessed by using ATPlite luminescence kit

➢ SPERM MEMBRANE INTEGRITY (SPERM VIABILITY)

On site

- Pre-fixation for 4 minutes in 4% glutaraldehyde as described by Beirao et al (2009)

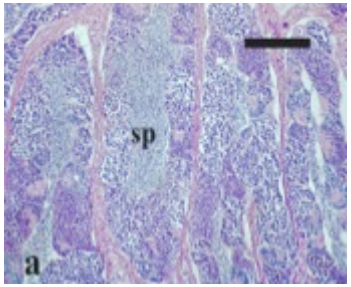
Laboratory (Ifremer-Palavas)

- Spermatozoa membrane integrity assessed by using the LIVE/DEAD Sperm Viability Kit

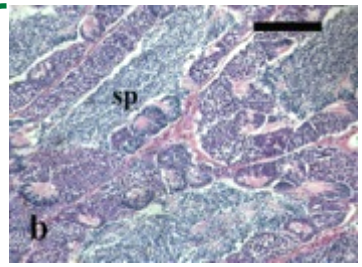
RESULTS

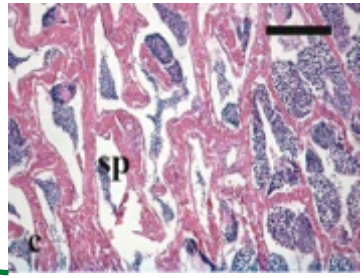
WILD

- **Early Gametogenesis (APR-MAY)**
  - all stages of spermatogenesis; luminal spermatozoa (n=5)

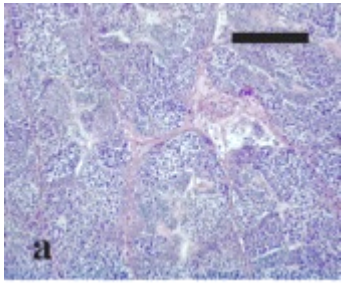


- **Advanced Gametogenesis (MAY-JUN) + Spawning (JUN-JUL)**
  - all spermatogenic stages; plenty of luminal spermatozoa (n=8)
  - partially spent (n=1)

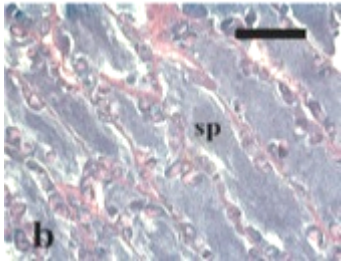




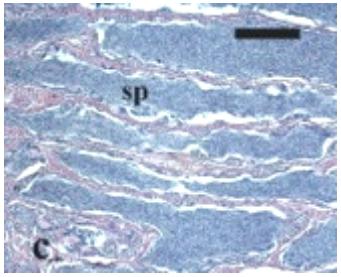
CAPTIVE-REARED



- **Early Gametogenesis (APR-MAY)**
  - all stages of spermatogenesis; rare luminal spermatozoa (n=4)



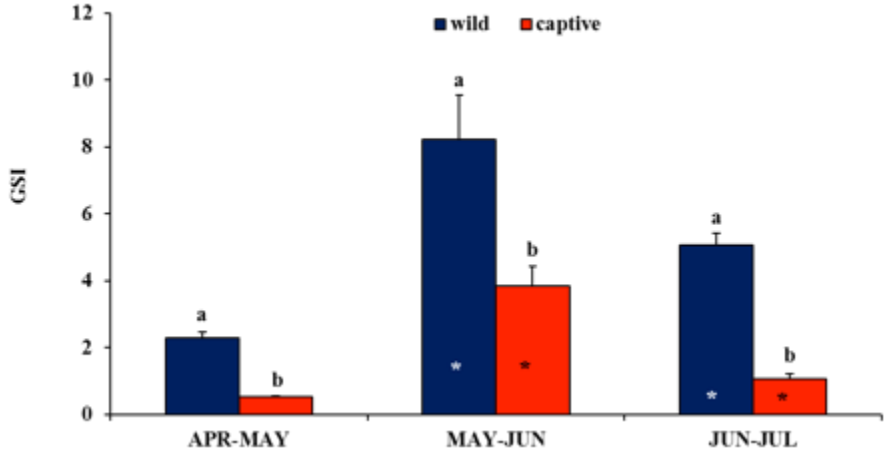
- **Advanced Gametogenesis (MAY-JUN)**
  - all spermatogenic stages; plenty of luminal spermatozoa (n=2)
  - residual sperm cysts; abundant luminal spermatozoa (n=2)



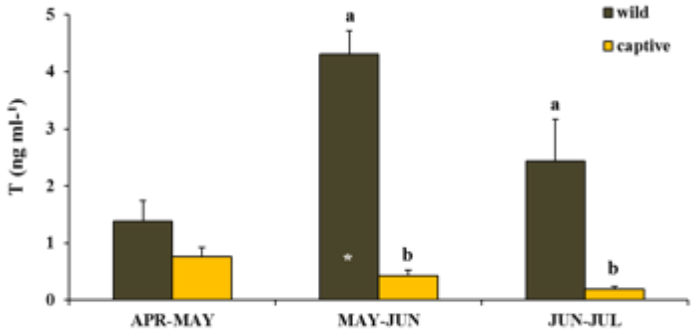
- **Spawning (JUN-JUL)**
  - residual luminal spermatozoa (n=4)

RESULTS

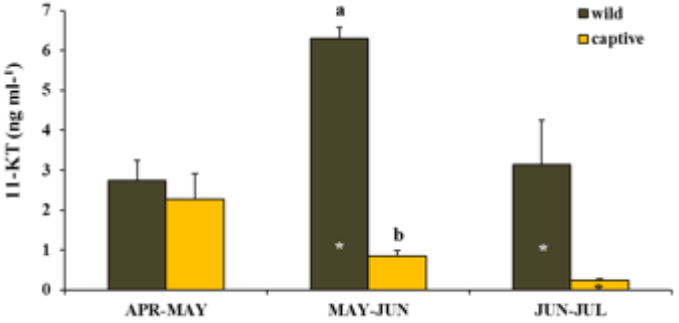
GONADOSOMATIC INDEX



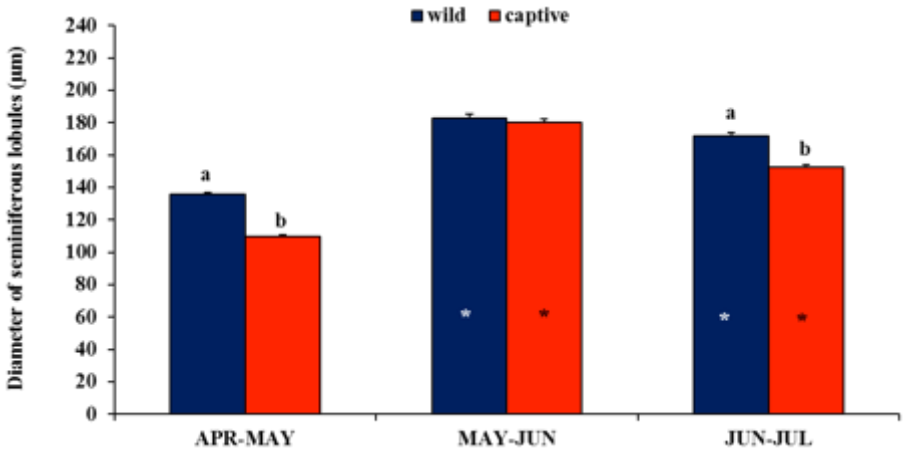
TESTOSTERONE



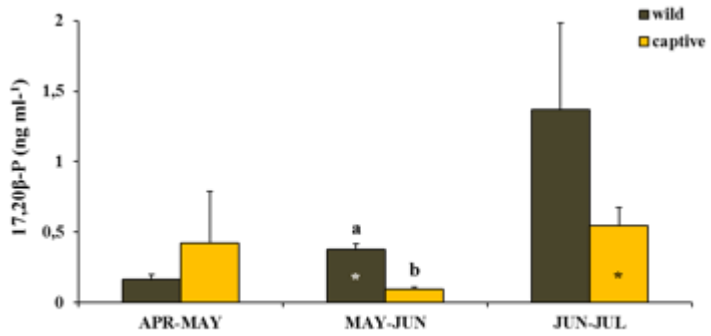
11-KETOTESTOSTERONE



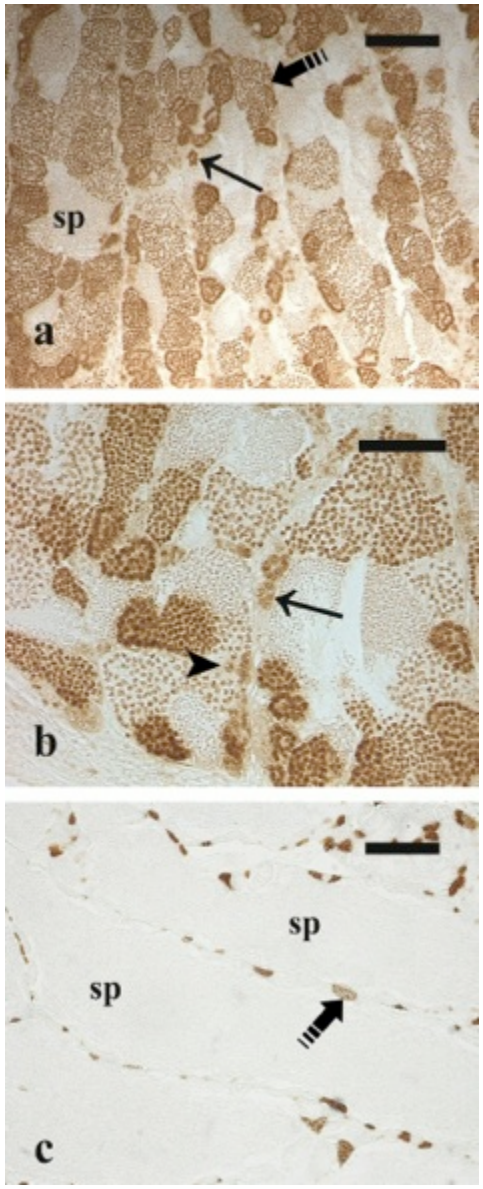
DIAMETER OF SEMINIFEROUS LOBULES



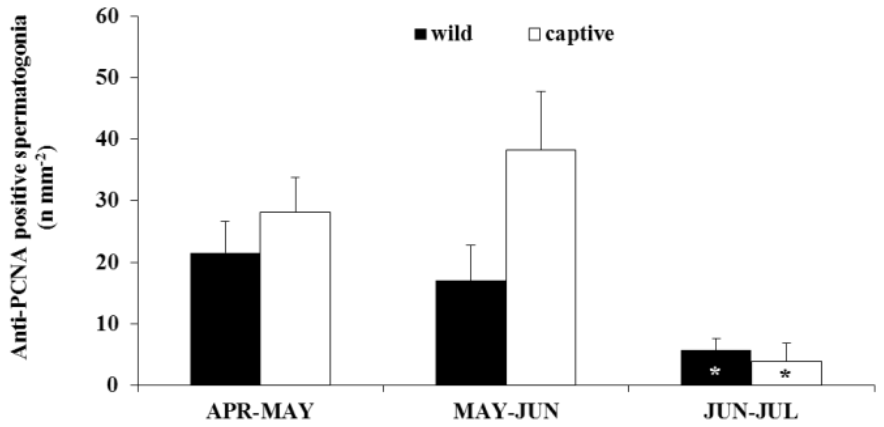
17,20B-DIHYDROXYPREN-4-EN-3-ONE



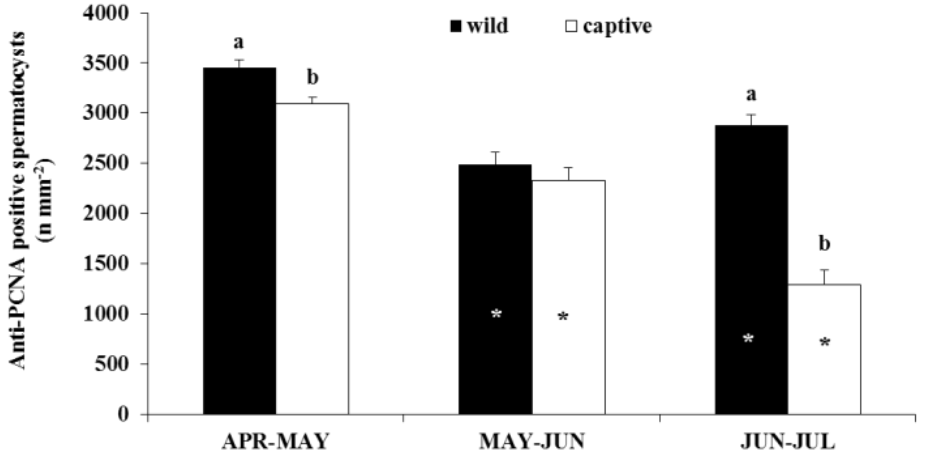
PROLIFERATING GERM CELLS



(a)

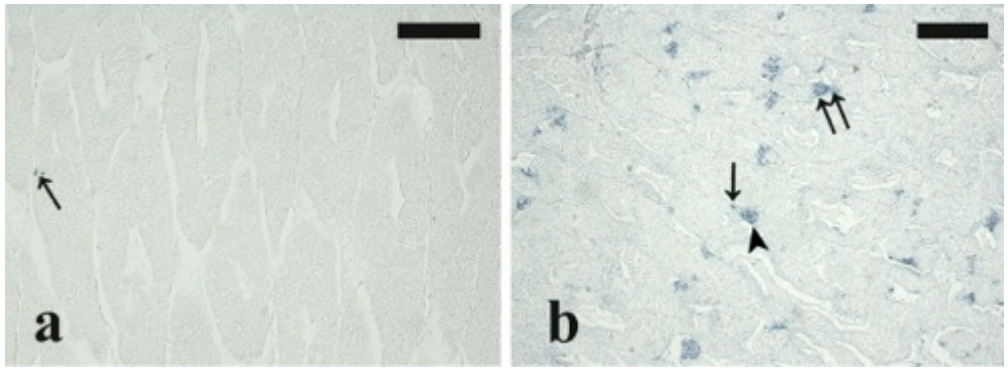


(b)

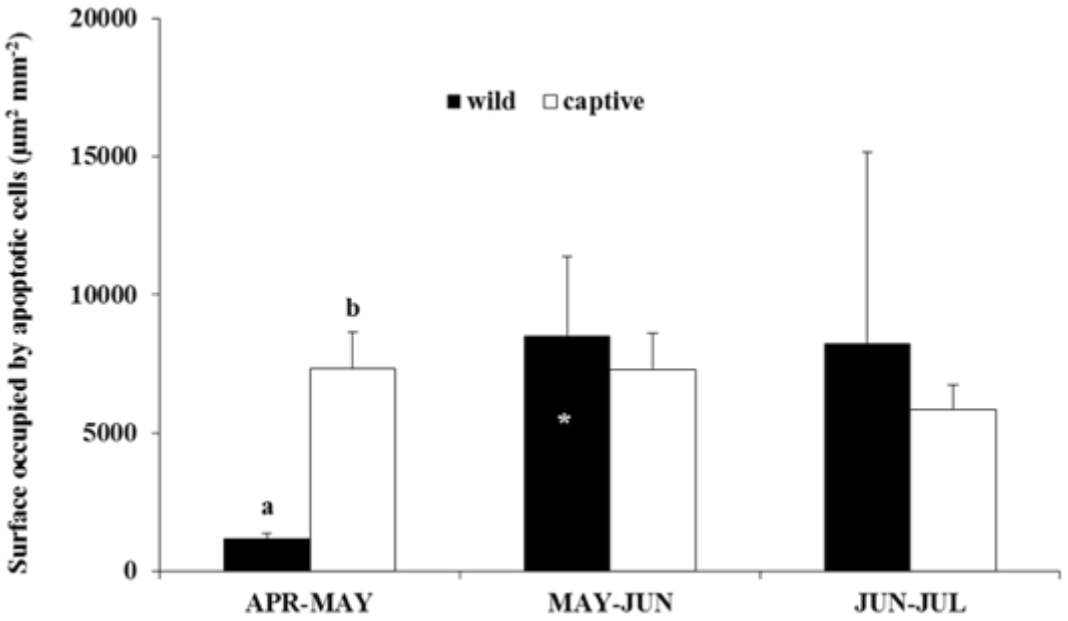


APOPTOTIC GERM CELLS

WILD

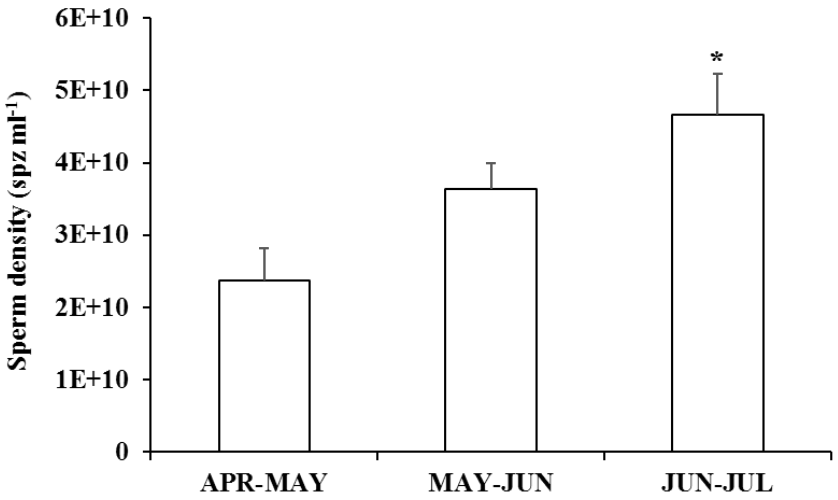


CAPTIVE-REARED

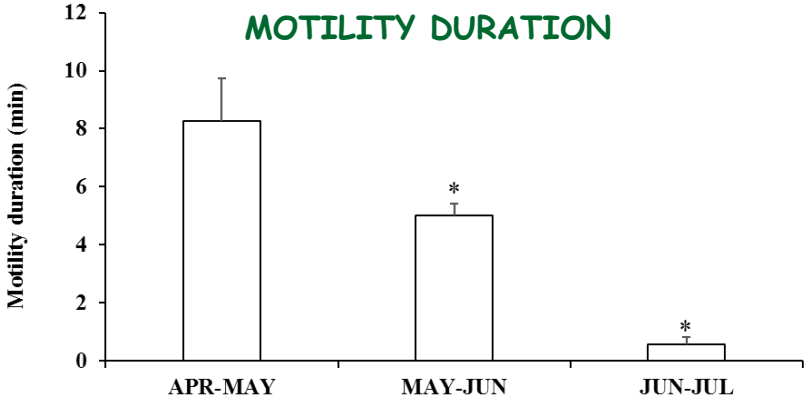


RESULTS

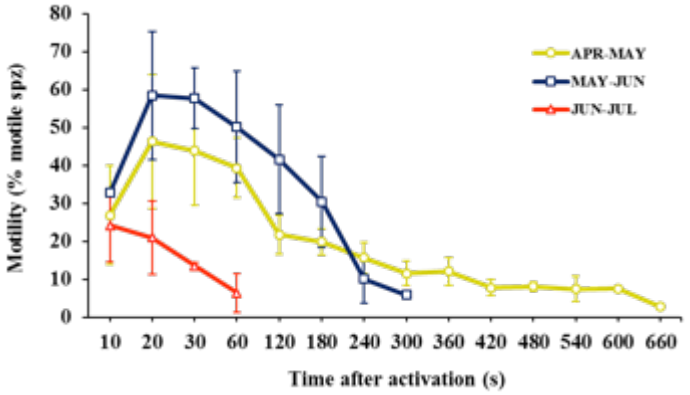
SPERM CONCENTRATION



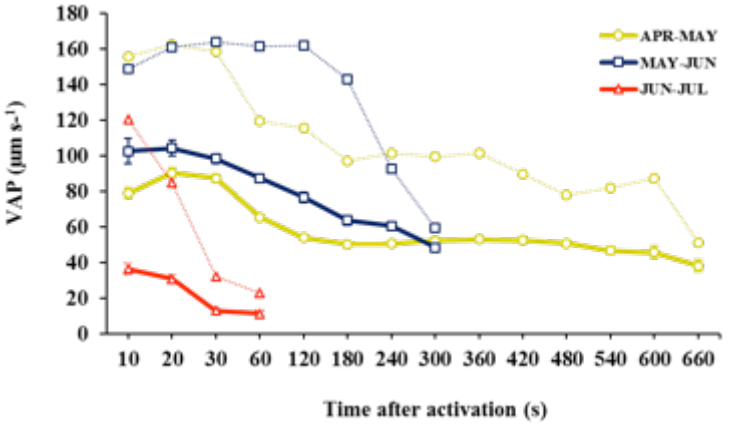
MOTILITY DURATION



% MOTILE SPERMATOZOA



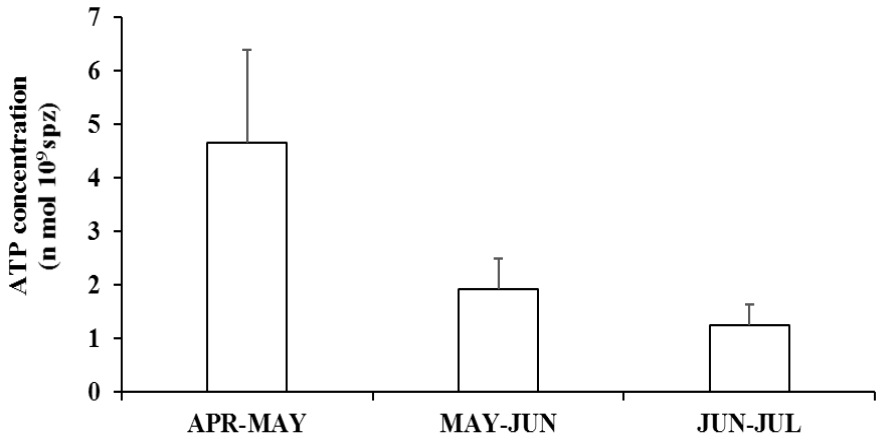
AVERAGE PATH VELOCITY



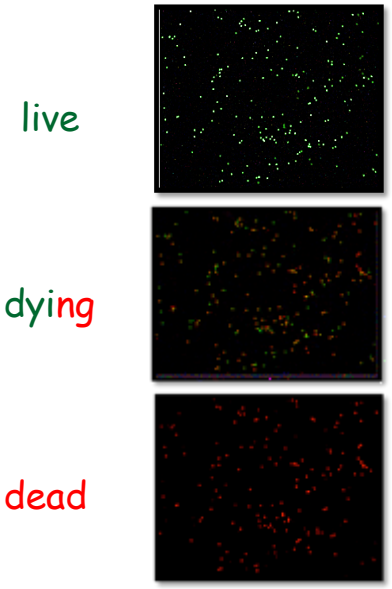
Solid lines: mean VAP of sperm population;

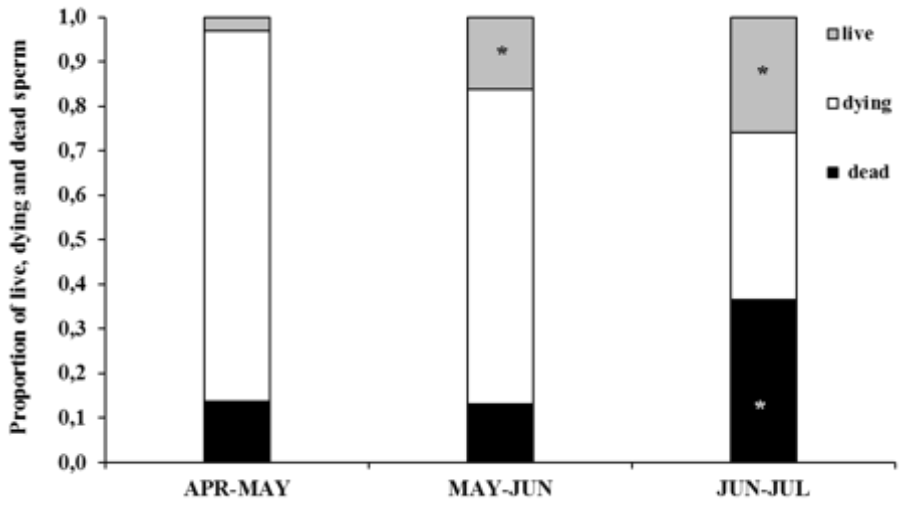
Dotted lines: the highest individual sperm velocity recorded

ATP CONTENT



SPERM MEMBRANE INTEGRITY (SPERM VIABILITY)





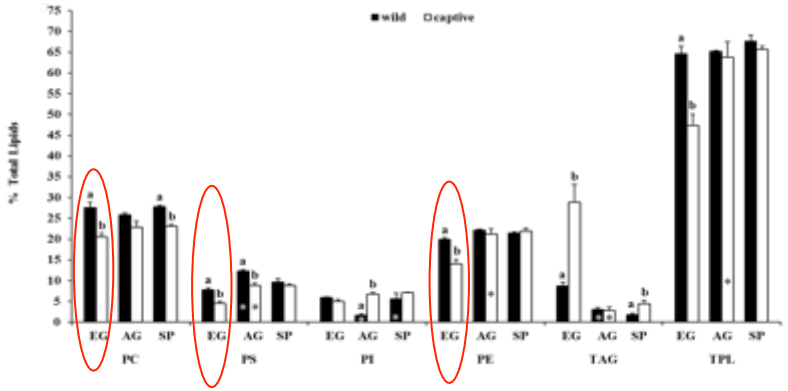
CONCLUSIONS

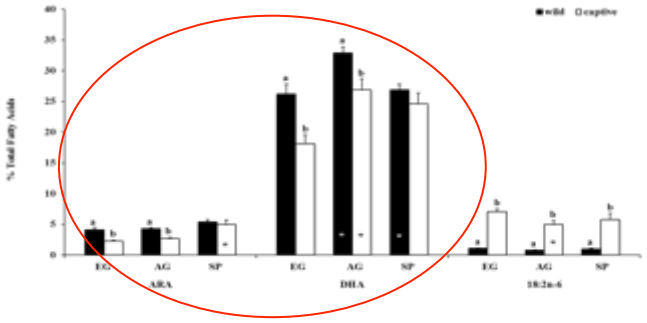
Spermatogenesis impairment and low sperm quality in captive-reared greater amberjack

- Low gonadosomatic index and diameter of seminiferous lobules
- Progressive T and 11-KT decrease during the reproductive season
- Early decrease of germ cell proliferation and cessation of spermatogenesis during the spawning period of the wild counterpart
- Very high germ cells apoptosis during early gametogenesis
- Progressive increase of sperm concentration during the reproductive season
- Mobile sperm hardly reach 60%
- Short motility duration possibly due to very low ATP content
- Very low percentage of alive spermatozoa

POSSIBLE CAUSES OF SPERMATOGENESIS IMPAIRMENT

- Acute stress due to repeated samplings?
- Unsuitable environmental conditions?
- Nutritional deficiency?





IN TESTES OF CAPTIVE-RERAED FISH:

- Lower proportion of lipid classes essential for spermatogenesis (PC, PS and PE) during the early gametogenesis period (APR-MAY)
- Lower content (30-40%) of fatty acids (ARA, DHA), all crucial factors for reproductive success

PLOS ONE

RESEARCH ARTICLE

Comparative Study of Reproductive Development in Wild and Captive-Reared Greater Amberjack *Seriola dumerili* (Risso, 1810)

Rosa Zupa[1], Covadonga Rodríguez[2], Constantinos C. Mylonas[3], Hanna Rosenfeld[4], Ioannis Fakriadis[3], Maria Papadaki[3], José A. Pérez[2], Chrysovalentinos Pousis[1], Gualtiero Basilone[5], Aldo Corriero[1]*

Gracias por
su atención