REPRODUCTIVE DEVELOPMENT IN WILD AND CAPTIVE-REARED GREATER AMBERJACK *Seriola dumerili* (RISSO, 1810)

Corriero A., Mylonas C. C., Zupa R., Pousis C., Fakriadis I., Papadaki M., De Virgilio C., Santamaria N., Bello G., Passantino L.

University of Bari Aldo Moro, Italy

Hellenic Center for Marine Research, Heraklion, Crete, Greece

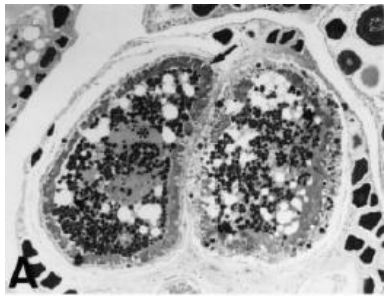
Reproductive maturation in greater amberjack

Greater amberjack Seriola dumerili (RISSO, 1810)

An excellent candidate to aquaculture thanks to its rapid growth, excellent flesh quality and worldwide market appreciation.

Before the beginning of **DIVERSIFY**, a few experiments had showed a poor capacity of females reared in tanks to mature oocytes and spawn, due to oogenesis interruption at early or advanced vitellogenesis.

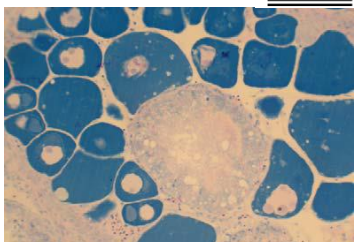
Aquaculture Research, 1999, **30**, 349–355



The reproductive biology of the amberjack, *Seriola dumerilii* (Risso 1810). I. Oocyte development in captivity

V Micale, G Maricchiolo & L Genovese

Aquaculture



Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants

Constantinos C. Mylonas[a,*], Nikos Papandroulakis[a], Andreas Smboukis[b], Maria Papadaki[a], Pascal Divanach[a]

WP 3 - Reproduction and Genetics - greater amberjack

Task 3.1 - Description of the reproductive cycle of greater amberjack

Identification of possible reproductive dysfunctions of greater amberjack reared in captivity based on the comparative evaluation of fish sampled in the wild.

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]*

Aquaculture Research

ORIGINAL ARTICLE

The observed oogenesis impairment in greater amberjack *Seriola dumerili* (Risso, 1810) reared in captivity is not related to an insufficient liver transcription or oocyte uptake of vitellogenin

Chrysovalentinos Pousis[1] | Constantinos C Mylonas[2] | Caterina De Virgilio[3] | Gemma Gadaleta[3] | Nicoletta Santamaria[1] | Letizia Passantino[1] | Rosa Zupa[1] | Maria Papadaki[2] | Ioannis Fakriadis[2,4] | Rosalia Ferreri[5] | Aldo Corriero[1]

J. Anim. Sci. 2017.95:4085–4100

Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack, *Seriola dumerili* (Risso, 1810)[1]

R. Zupa,* C. Fauvel,† C. C. Mylonas,‡ C. Pousis,* N. Santamaria,* M. Papadaki,‡ I. Fakriadis,‡ § V. Cicirelli,* S. Mangano,# L. Passantino,* G. M. Lacalandra,* and Aldo Corriero*[2]

SAMPLING OF WILD AND CAPTIVE-REARED GREATER AMBERJACK

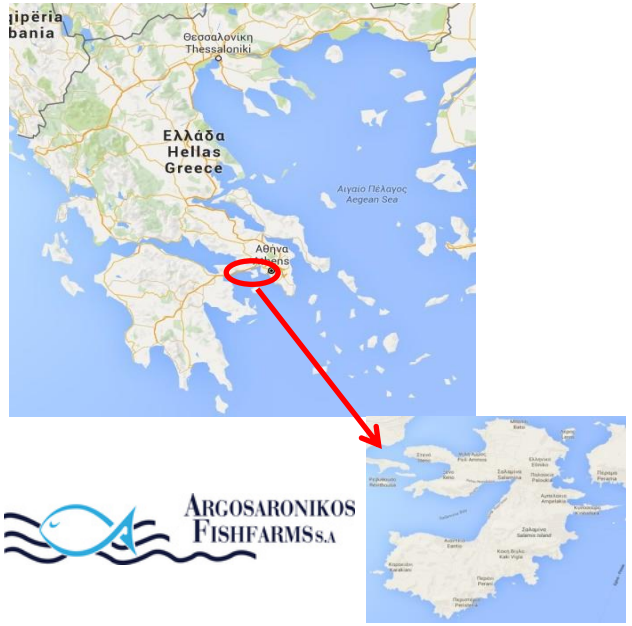
SAMPLING AREA: LAMPEDUSA

(Pelagic Islands, Sicily, Italy)

33 adult wild specimens
(14 males and 21 females)

SAMPLING AREA: Argosaronikos Fish Farm

(ARGO), (Salamina Island, Greece)



24 adult captive-reared specimens
(12 males and 12 females)

Diet: Vitalis-Cal (Skretting SA, Norway)

Wild (2014-2015-2016)

Captive-reared (2015)

Early gametogenesis

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		

Advanced gametogenesis

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				

Spawning

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); Gonad Mass (GM, g)

Sampling of wild fish

Purse seine vessel Graziella

Sampling of captive-reared fish

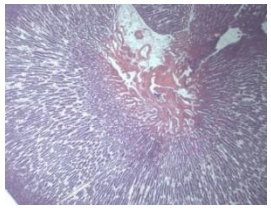
Argosaronikos Fish Farm

Biological samples: blood; gonads

LABORATORY ANALYSES: Histology, IHC, TUNEL, Steroids

➢ HISTOLOGICAL ASSESSMENT OF GONAD DEVELOPMENT

- Basic histological analysis

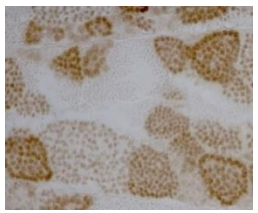


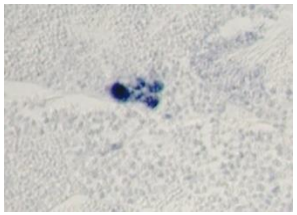
➢ PLASMA STEROID CONCENTRATIONS

- Plasma obtained by blood centrifugation (5000 rpm for 5 minutes)
- ELISA assay for 17β-estradiol (E_2), testosterone (T), 11-Ketotestosterone (11-KT) and 17,20β-dihydroxypren-4-en-3-one (17,20β-P) determination

➢ MALE GERM CELL PROLIFERATION AND APOPTOSIS

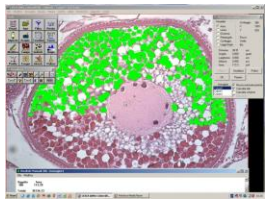
- Immunolocalization of a nuclear marker of proliferation (Proliferating Cell Nuclear Antigen, PCNA)
- Terminal deoxynucleotidyl transferase-mediated d'UTP nick end labelling (TUNEL method)
- Image Analysis



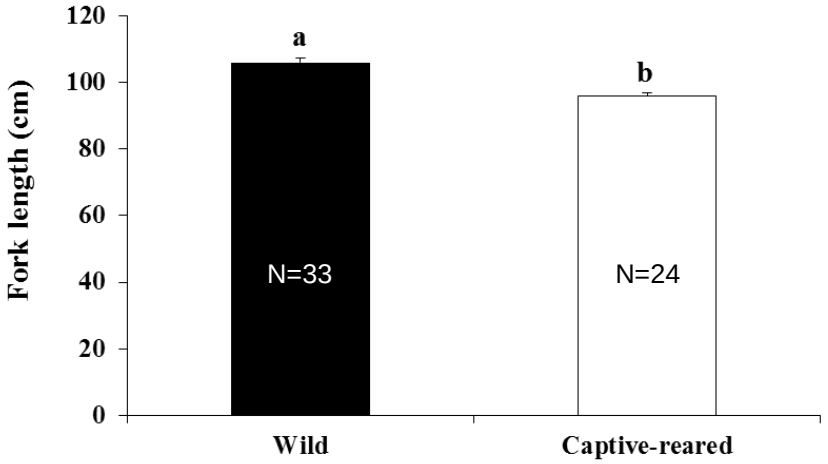


➢ VITELLOGENESIS

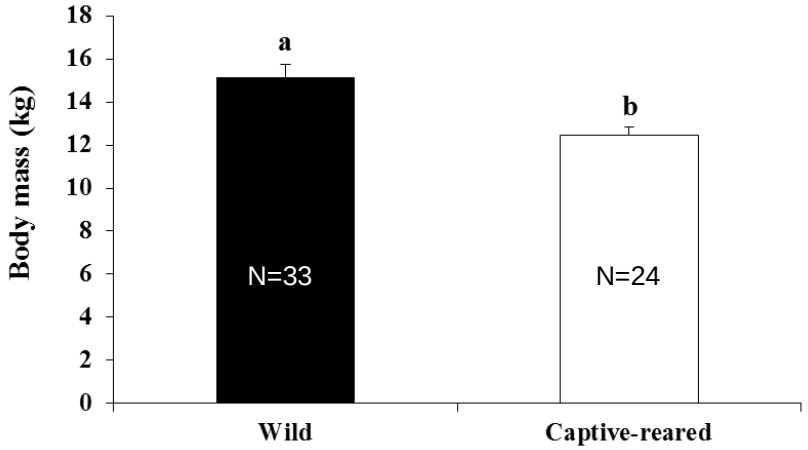
- Liver Vg expression (RT-PCR)
- Oocyte yolk accumulation (Image Analysis)



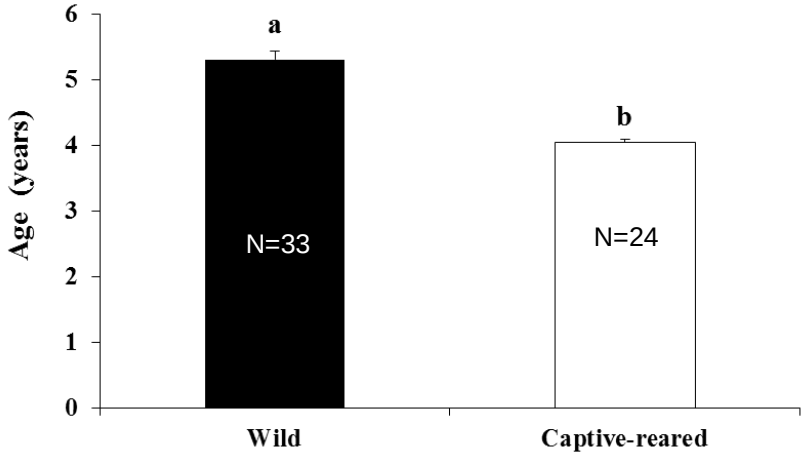
Fork length



Body mass



Estimated age

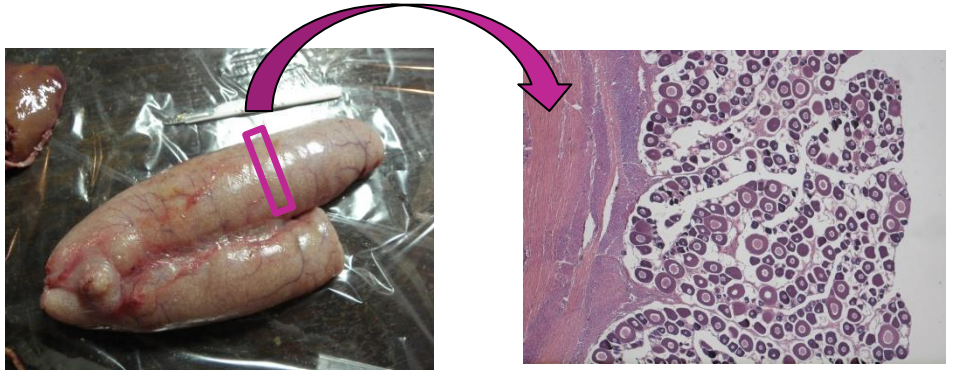


HISTOLOGICAL ASSESSMENT OF FEMALE REPRODUCTIVE STATE

Most advanced oocyte stage

Atretic follicles

Post-ovulatory follicles



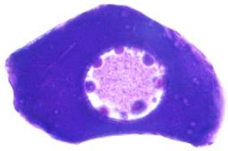
OOCYTE DEVELOPMENT STAGES

PRIMARY GROWTH

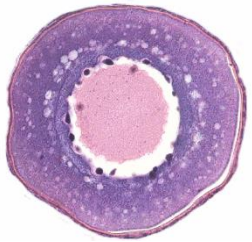
Oogonia
(8-13 µm)

Chromatin-nucleolus stage
(15-30 µm)

Perinucleolar stage
(30-120 µm)

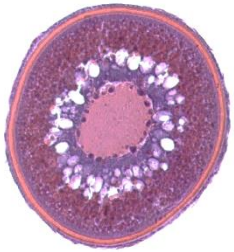


Lipid/Cortical alveoli stage
(120-200 µm)

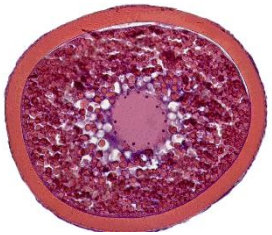


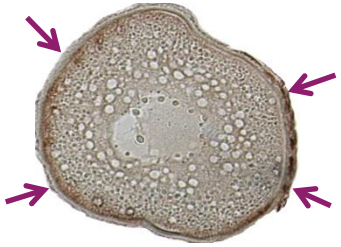
VITELLOGENESIS

Early vitellogenesis stage
(200-400 µm)



Late vitellogenesis stage
(400-550 µm)

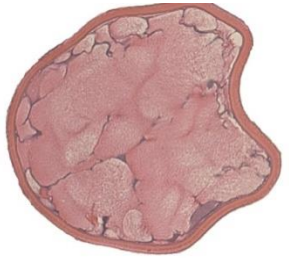




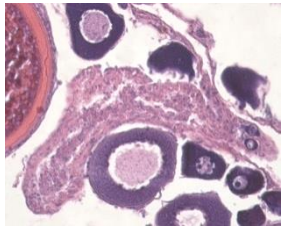
IHC anti-Vg antibodies

OOCYTE MATURATION

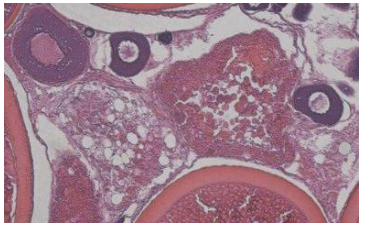
Hydrated stage
(≈ 800 µm)



Post-ovulatory follicles (POFs)



Atretic follicles

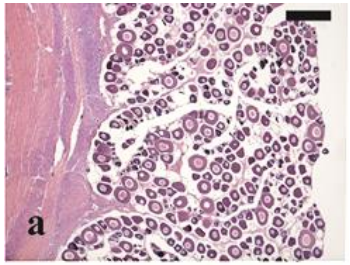


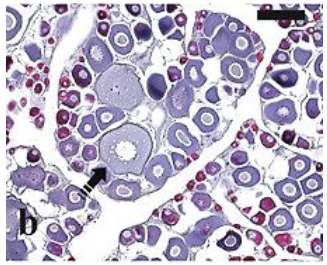
HISTOLOGICAL ANALYSIS

WILD / CAPTIVE-REARED

Early gametogenesis

Late April-early May



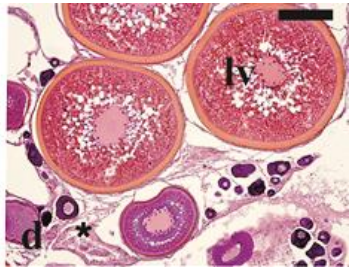


Perinucleolar, cortical alveoli, early vitellogenesis

Advanced gametogenesis

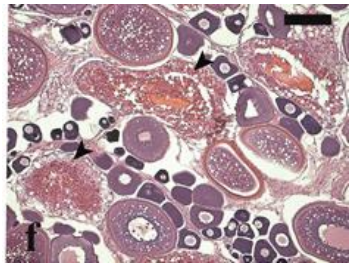
Late May-early June

WILD



Advanced vitellogenesis POFs

CAPTIVE-REARED

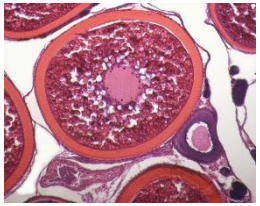


Atretic vitellogenic follicles

Spawning

Late June-early July

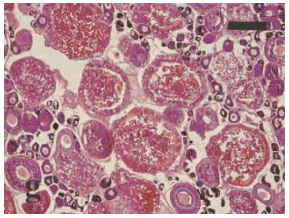
WILD



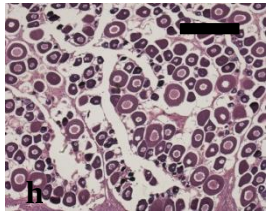
Advanced vitellogenesis POFs

Hydrated oocytes

CAPTIVE-REARED



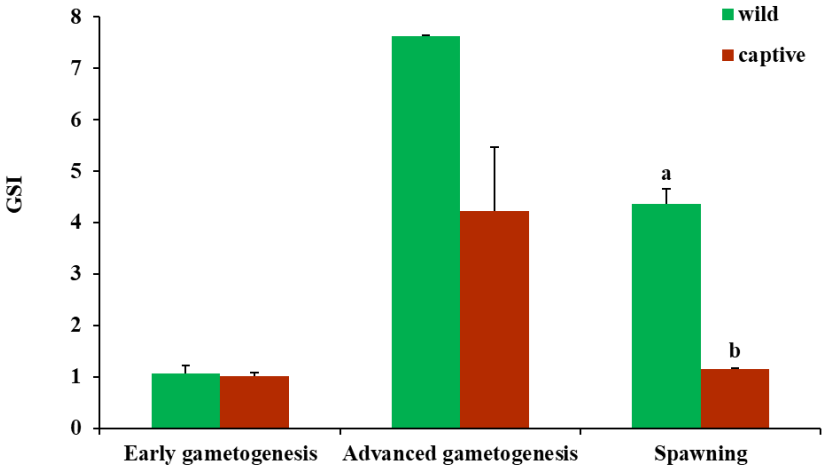
Atretic vitellogenic follicles



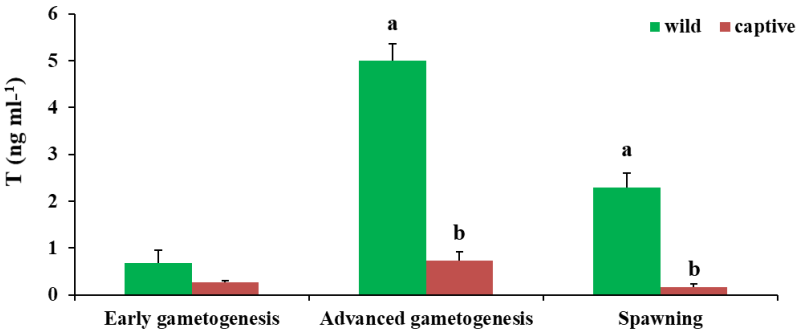
Spent

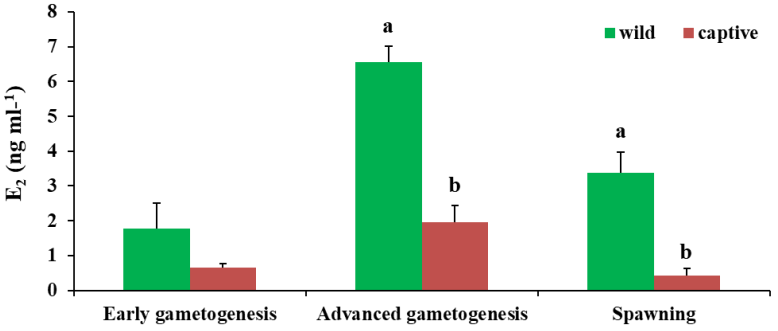
GONADOSOMATIC INDEX AND PLASMA STEROID CONCENTRATIONS

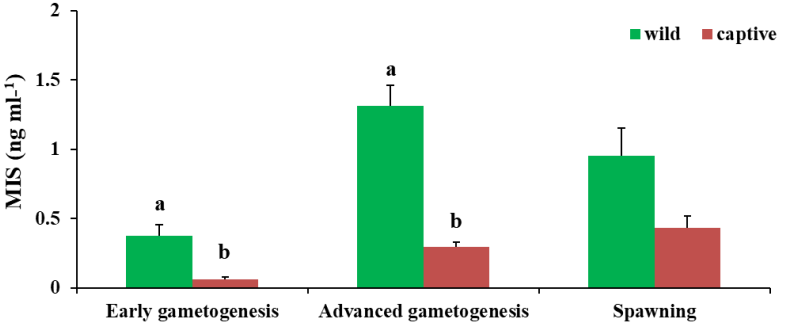
GSI = 100 Gonad Mass/Body Mass



Sex steroids (ELISA)

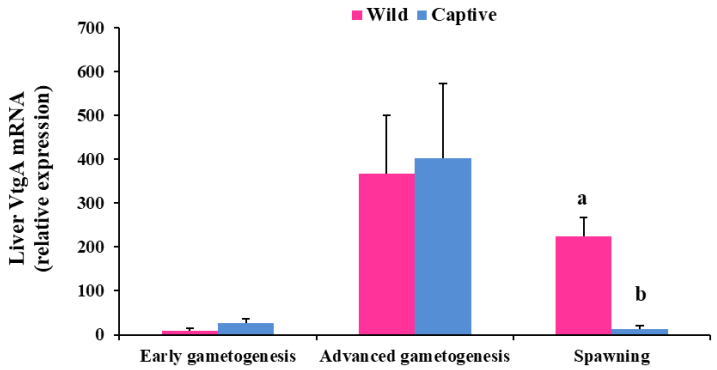


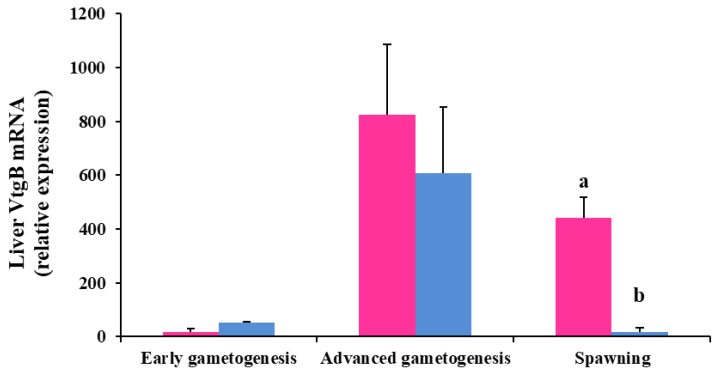


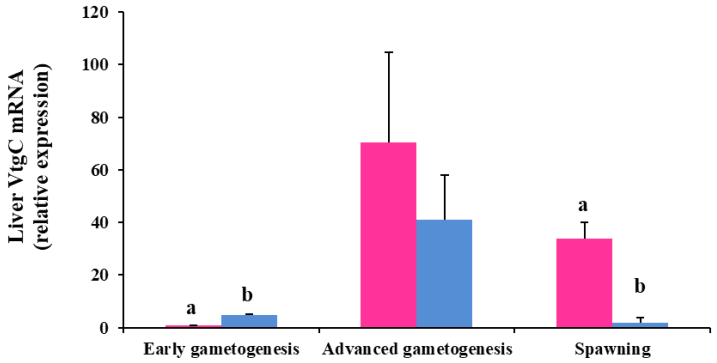


LIVER VITELLOGENIN GENE EXPRESSION

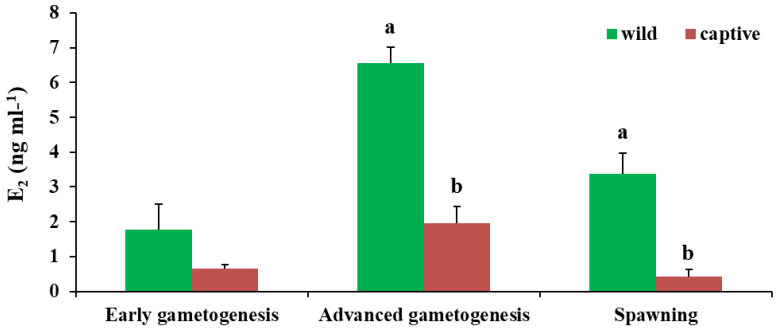
Vitellogenin gene expression

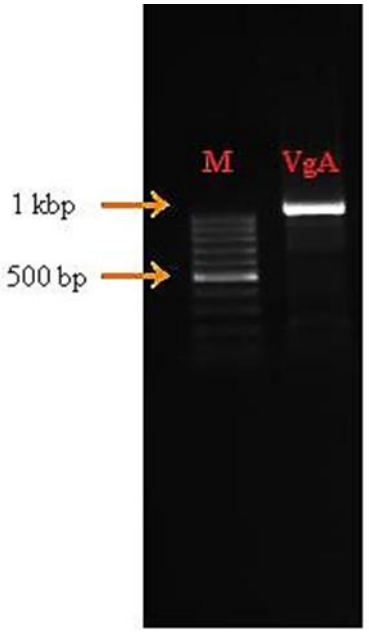






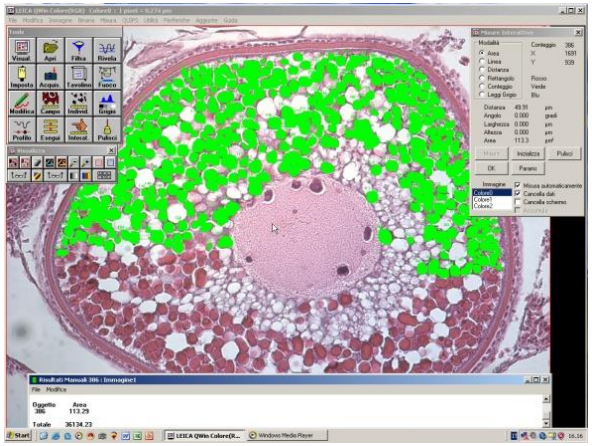
Plasma 17-β estradiol





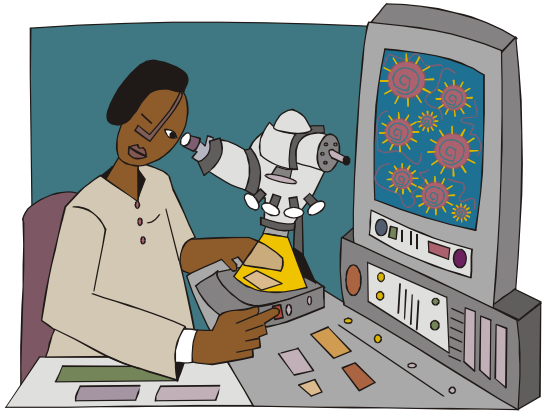
Agarose gel showing Vitellogenin A band (VgA) in a wild female greater amberjack. M = marker.

OOCYTE YOLK ACCUMULATION



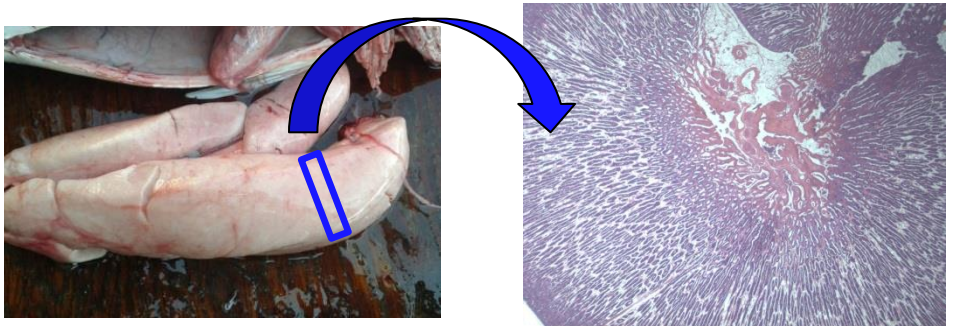
Oocyte stage	Fish condition	Oocyte diameter (µm)	Yolk surface (μm^2)
Early vitellogenesis	Wild	362.5 ± 3.5	55584.9 ± 1513.4
	Captive-reared	356.5 ± 6.9	55760.8 ± 3238.2
Late vitellogenesis	Wild	453.7 ± 3.5	84660.1 ± 1368.3
	Captive-reared	453.0 ± 9.3	90790.6 ± 3650.1

HISTOLOGICAL ASSESSMENT OF MALE REPRODUCTIVE STATE

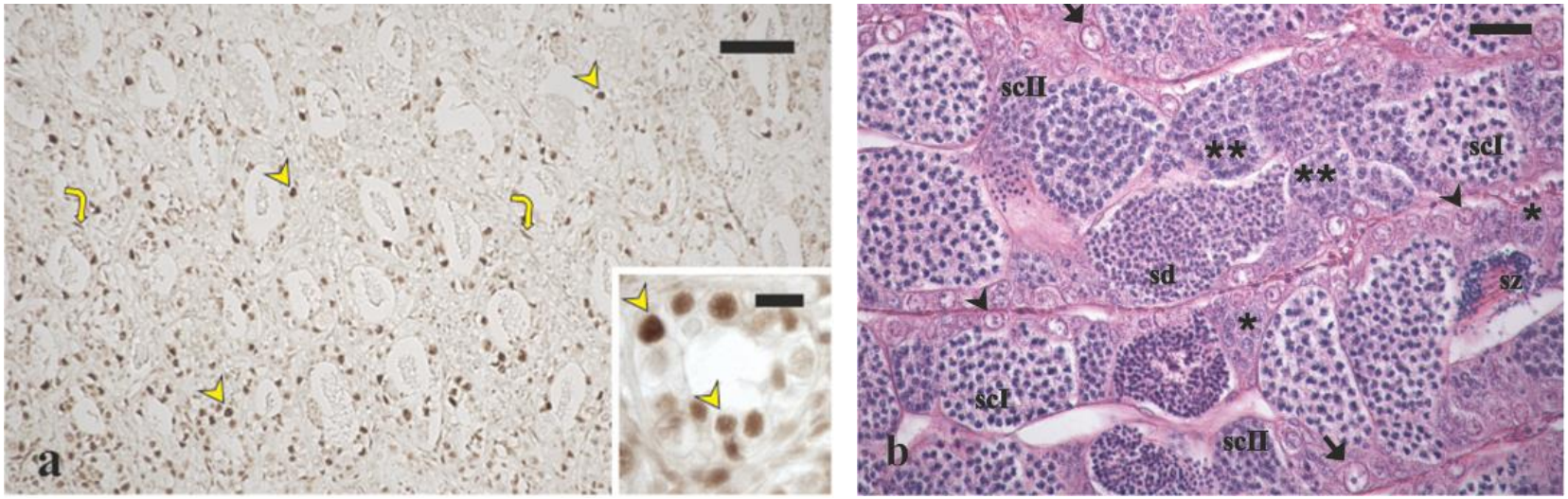


Germ cell types

Luminal spermatozoa amount



HISTOLOGICAL ASSESSMENT OF MALE REPRODUCTIVE STATE

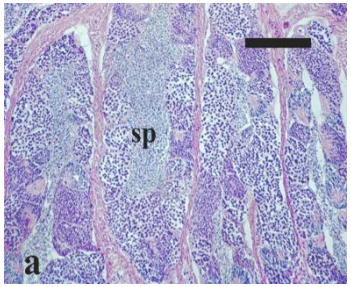


Identification of germ cell types through basic histological analysis and immunohistochemical labelling of undifferentiated stem spermatogonia (anti-Pou5f1).

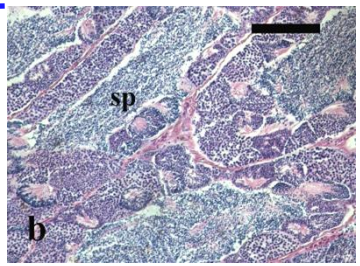
HISTOLOGICAL ANALYSIS

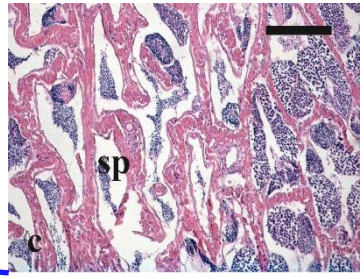
WILD

- **EG**
  - all stages of spermatogenesis; luminal spermatozoa (n=5)

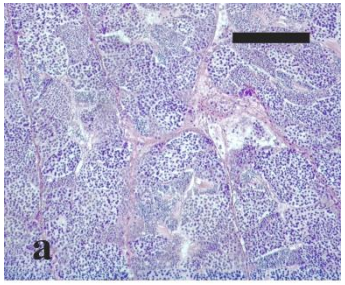


- **AG + SP**
  - all spermatogenic stages; plenty of luminal spermatozoa (n=8)
  - partially spent (n=1)

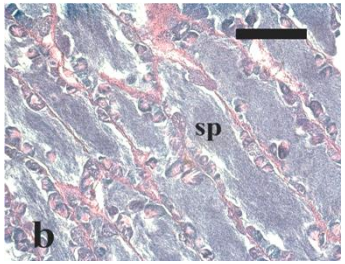




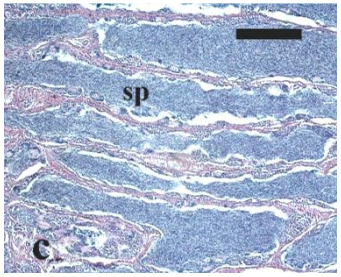
CAPTIVE-REARED



- **EG**
  - all stages of gametogenesis; rare luminal spermatozoa (n=4)



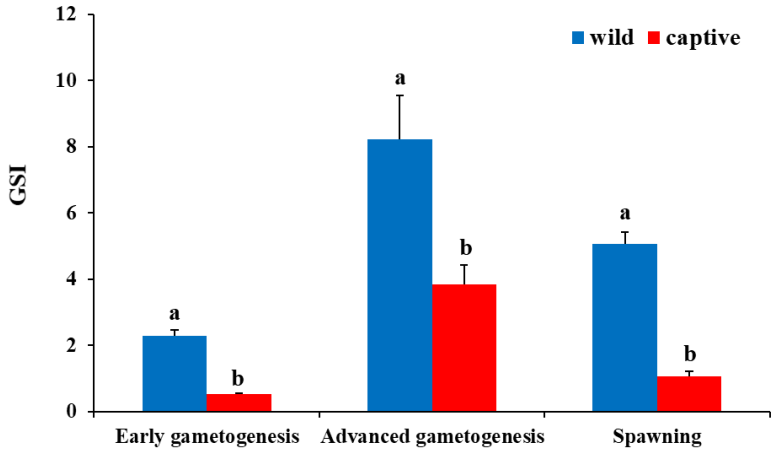
- **AG**
  - all spermatogenic stages; plenty of luminal spermatozoa (n=2)
  - residual sperm cysts; abundant luminal spermatozoa (n=2)



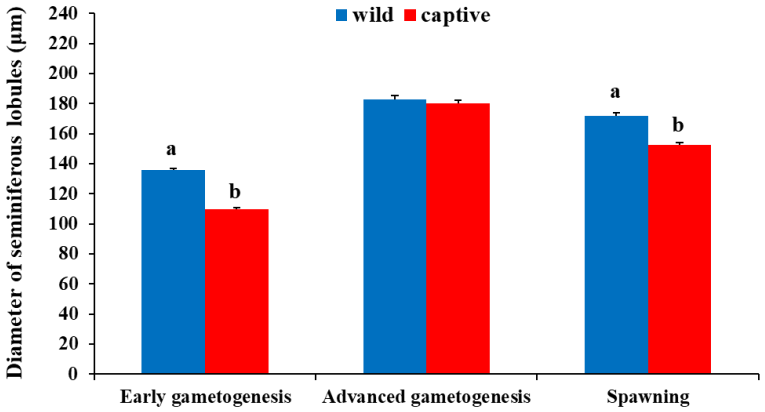
- **SP**
  - residual luminal spermatozoa (n=4)

GONADOSOMATIC INDEX AND PLASMA STEROID CONCENTRATIONS

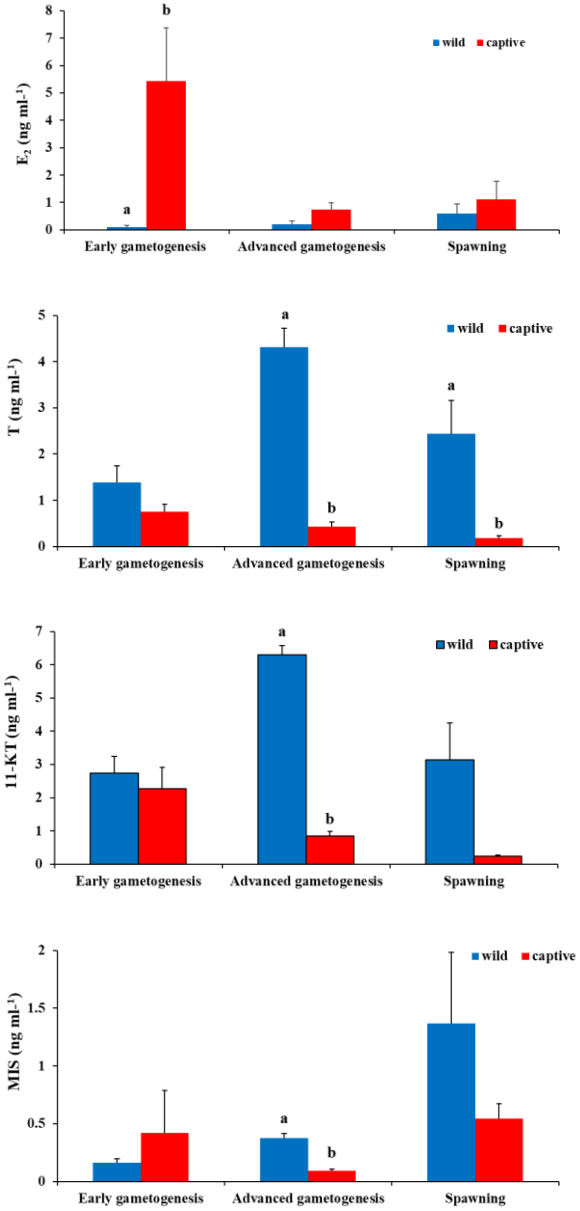
GSI = 100 Gonad Mass/Body Mass



Seminiferous lobule diameter



Sex steroids (ELISA)



GERM CELL PROLIFERATION AND APOPTOSIS

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).

Germ cell proliferation decrease

Germ cell apoptosis increase

GTHs

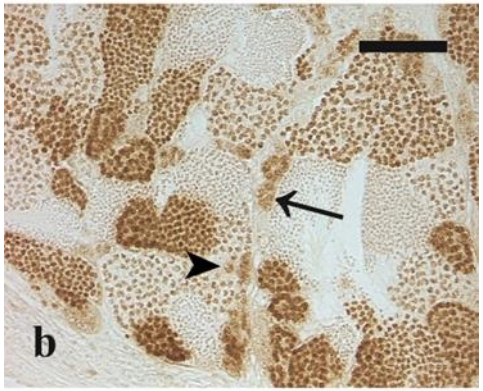
11-KT

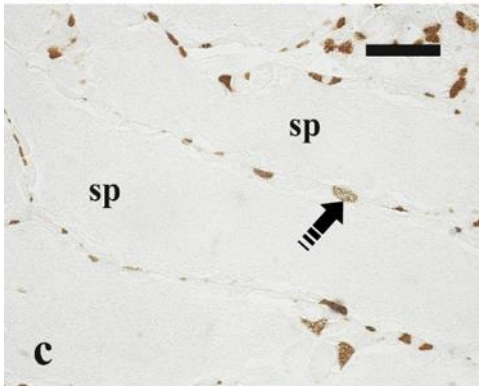
GTHs

11-KT

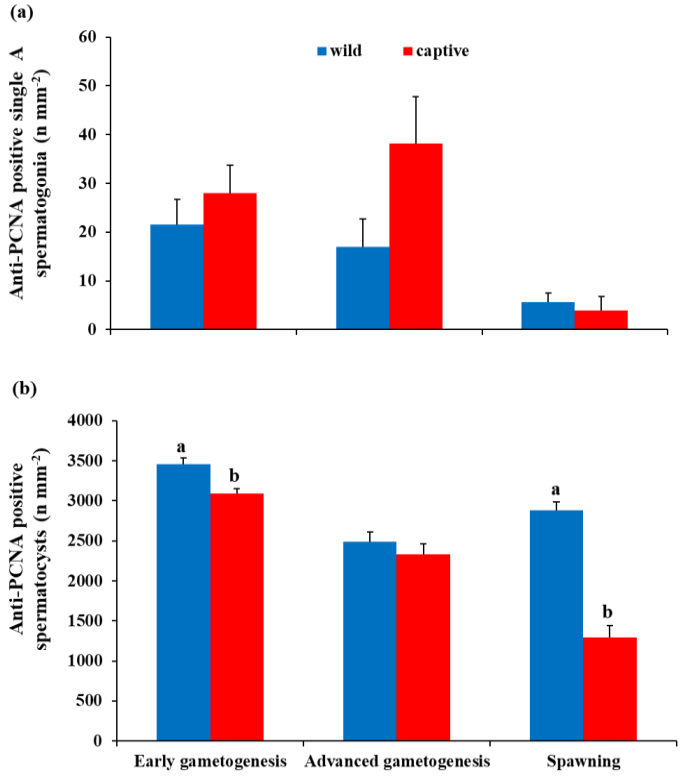
Germ cell proliferation increase

Germ cell apoptosis decrease





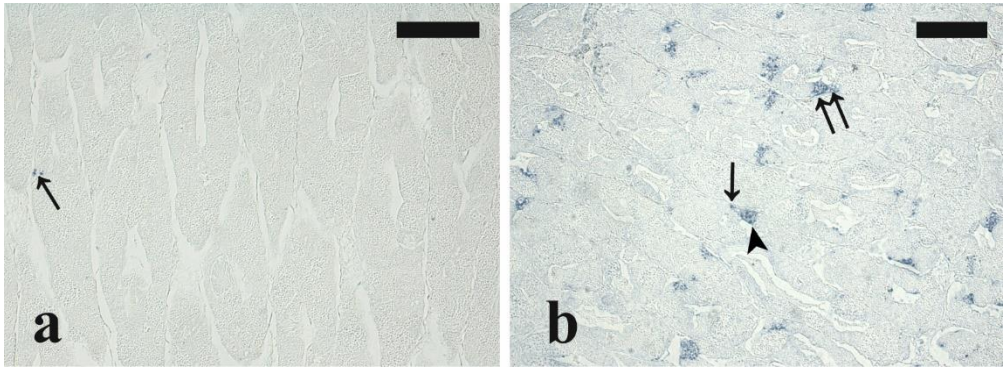
Dividing germ cells



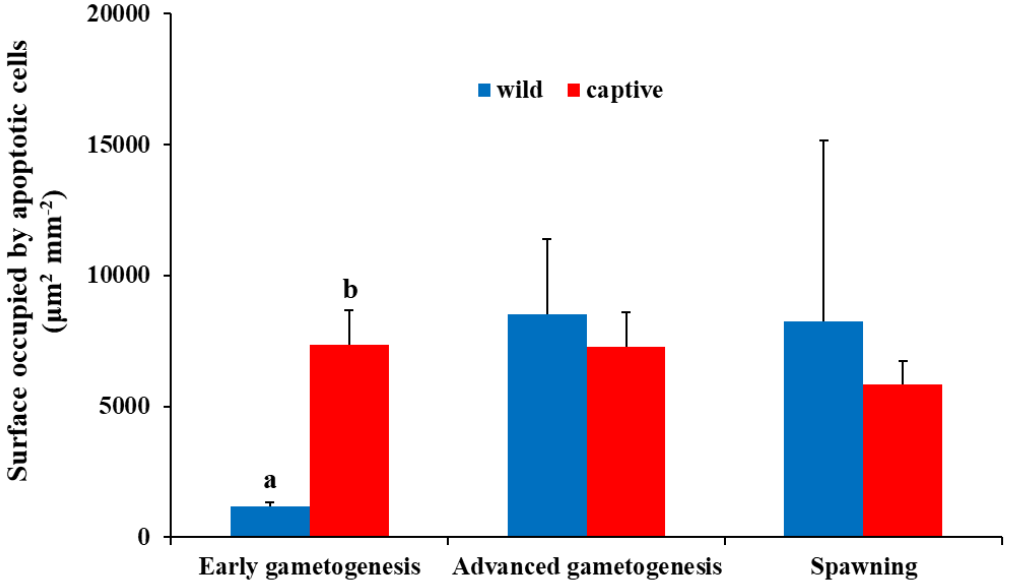
GERM CELL PROLIFERATION AND APOPTOSIS

WILD

APOPTOTIC GERM CELLS



CAPTIVE-REARED



CONCLUSIONS – Effects of rearing in captivity on gametogenesis

Females

- Low gonadosomatic index
- Progressive steroid decrease during the reproductive season
- Oogenesis arrest and atresia of vitellogenic follicles

Males

- Low gonadosomatic index and diameter of seminiferous tubules
- Very high estradiol level during early spermatogenesis
- Low androgen levels
- High germ cells apoptosis during early gametogenesis
- Early decrease of germ cell proliferation and early cessation of spermatogenesis

- Progressive increase of sperm concentration during the reproductive season
- Low percentage of alive spermatozoa
- Low percentage of mobile sperm (⩽60%)
- Short motility duration (low ATP content)

Ifremer

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ΕΛΚΕΘΕ

J. Anim. Sci. 2017.95:4085–4100

Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack, *Seriola dumerili* (Risso, 1810)[1]

R. Zupa,* C. Fauvel,† C. C. Mylonas,‡ C. Pousis,* N. Santamaria,* M. Papadaki,‡ I. Fakriadis,‡ § V. Cicirelli,* S. Mangano,# L. Passantino,* G. M. Lacalandra,* and Aldo Corriero*[2]

CONCLUSIONS

- ✓ Reproductive dysfunctions often occur in fish caught from the wild and reared in captivity due to captivity-induced stress, lack of environmental conditions suitable for reproduction, improper diet (Mylonas et al., 2010).
- ✓ In captive-reared greater amberjack, a reproductive axis malfunction had already manifested at the beginning of the reproductive cycle; however, handling/management stress might have exacerbated gametogenesis impairment.
- ✓ In fact, another greater amberjack broodstock, maintained in the same facility under the same rearing conditions, reached advanced stages of gametogenesis and was successfully induced to spawn using a GnRHa therapy (Mylonas et al., 2017).

Nutritional unbalances caused by the artificial diet - evidenced by a different lipid and fatty acid gonad content compared with wild spawners- might have also contributed to the described gametogenesis impairment.

ULL Universidad de La Laguna

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]*

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Hvala vam na pozornosti

Thank you for your attention

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

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