



Dysfunctional reproductive maturation in captive greater amberjack

WP3_Reproduction and Genetics
Greater amberjack



Presenter
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Second Annual Coordination Meeting
2-4 February 2016
Nancy, France

OBJECTIVE

Identify possible dysfunctions of gametogenesis in greater amberjack caught from the wild and reared in captivity

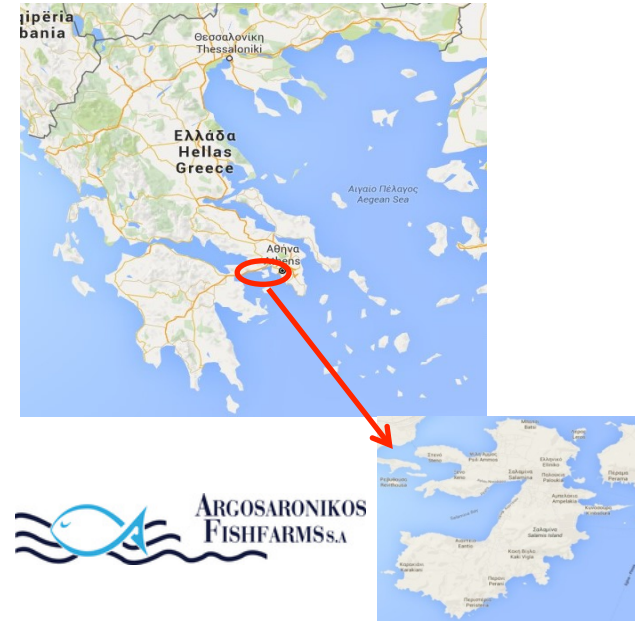
Task title	Deliverable title	Deliverable description	Delivery month
3.1_ Description of the reproductive cycle of greater amberjack (led by UNIBA)	D3.1_ Establishment of quantitative PCR assays to measure transcript levels of target genes in greater amberjack (i.e., LH β , FSH β , leptin, Vg and Vg receptor)	qPCR assay for Vg, VgR, LH β , FSH β , leptin	12 DELIVERED
	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)
	D3.5_ Description of the process of oogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of ovarian development, (c) pituitary levels of FSH and LH, (d) plasma levels of FSH, LH, leptin, sex steroid hormones and Vg, (e) nutritional status and (h) egg biochemical composition	First sexual maturity; captive fish body condition; reproductive cycle of captive fish to identify the optimal time for hormonal treatments; gonadotropins pituitary and plasma levels, and sex steroid plasma concentration; key metabolic hormones (e.g. leptin), Vg plasma level and oocyte yolk accumulation; egg composition	46
	D3.6_ Description of the process of spermatogenesis in captive greater amberjack, including (a) aspects of growth and body indices, (b) histological evaluation of testicular development, (c) pituitary levels of FSH and LH, (d) plasma levels of FSH, LH, leptin, sex steroid hormones, (e) proliferation and apoptosis of germ cells, (f) sperm quality, (g) fish nutritional status	First sexual maturity; captive fish body condition; reproductive cycle of captive fish in order to get a synchronized effect of the hormonal treatment; gonadotropins pituitary and plasma levels, and sex steroid plasma concentration; germ cells proliferation and apoptosis as well as sperm quality; key metabolic hormone (e.g. leptin)	46

SAMPLING AREA: LAMPEDUSA (Pelagic Islands, Sicily, Italy)



**33 adult wild specimens
(14 males and 19 females)**

SAMPLING AREA: Argosaronikos Fish Farm (ARGO), (Salamina Island, Greece)



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





Early gametogenesis (EG)

Advanced gametogenesis (AG)

Spawning (SP)

Wild (2014-2015)

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						

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				

Captive-reared (2015)

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		

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	

Biometric data

- Fork Length (FL, cm)
- Body Mass (BM, kg)
- Gonad Mass (GM, g)



Biological samples

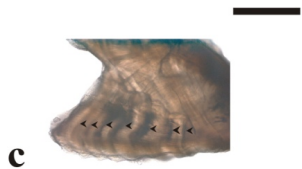
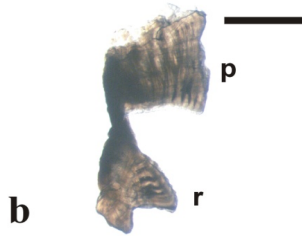
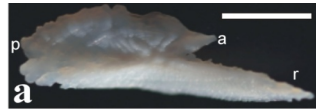
- **Blood**, brain, pituitary, **liver**, muscle, **gonads**, sperm
- Hard parts for age determination : first spiniform ray of the first dorsal fin (spine); caudal vertebrae; otoliths (sagittae); **scales**



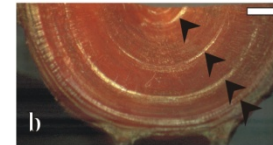
Spine



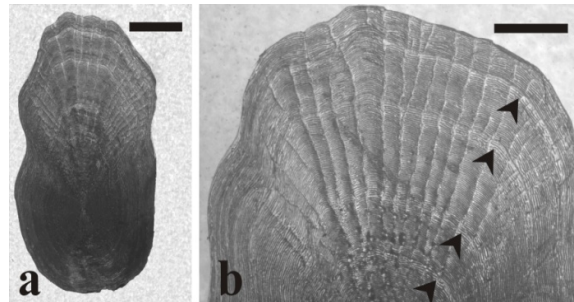
Otolith



Vertebra



Scale



THE EASIEST STRUCTURE FOR AGE DETERMINATION



Wild

Captive-reared

Sampling Date	Sex	Fork length (FL, cm)	Body Mass (BM, kg)	Gonad Mass (GM, g)	Age (years)
Early gametogenesis					
01/05/2015 (SST = 18.1 °C)	m	111	14	300	6
	m	112	20	450	6
	m	112	15	300	6
	m	117	19	550	6
	m	113	19	400	6
	f	103	14	100	5
	f	112	19	200	6
	f	116	20	300	6
	f	103	15	200	5
	f	106	13	100	5
Advanced gametogenesis					
31/05/2014 (SST = 19.3 °C)	m	124	22	1900	7
	m	102	13	650	5
	m	115	19	2200	6
	m	99	14	1150	5
	f	117	22	1650	6
	f	114	21	1600	6
Spawning					
29/06/2015 (SST = 23.8 °C)	m	100	12	650	5
	m	102	14	700	5
	m	104	16	950	5
	f	101	14	500	4
	f	114	19	1000	6
30/06/2014 (SST = 23.4 °C)	f	109	16	700	6
	m	100	11	400	5
	m	99	11	577	4
	f	99	11	500	5
	f	100	12	490	5
	f	97	12	450	5
	f	100	12	400	5
	f	98	12	500	4
	f	96	12	390	4
	f	102	13	600	5
f	104	14	950	5	
f	95	12	450	5	

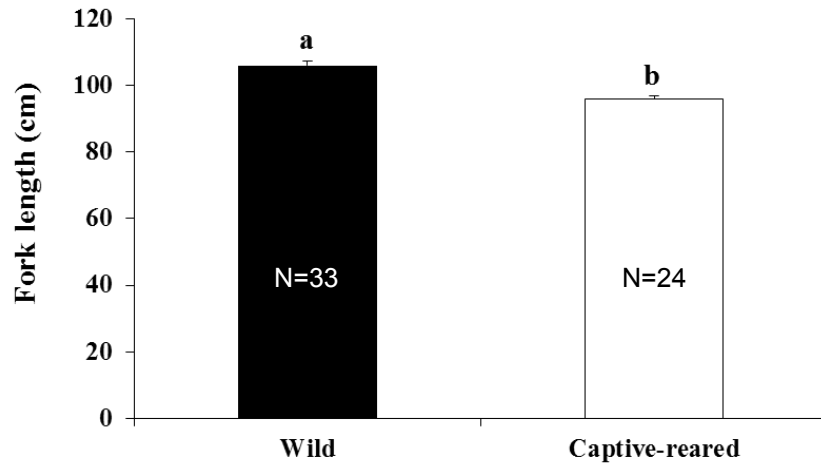
SST: Sea Surface Temperature

Sampling Date	Sex	Fork length (FL, cm)	Body Mass (BM, kg)	Gonad Mass (GM, g)	Age (years)
Early gametogenesis					
24/04/2015 (SST = 17.5 °C)	m	101	15	95	4
	m	94	12	60	4
	m	92	12	65	4
	m	94	13	60	4
	f	87	10	85	4
	f	97	14	155	4
	f	96	14	125	4
	f	100	14	160	4
Advanced gametogenesis					
04/06/2015 (SST = 20.0 °C)	m	90	9	370	4
	m	97	14	295	4
	m	98	13	600	4
	m	103	15	690	4
	f	97	13	335	4
	f	97	13	920	4
	f	106	17	305	5
	f	101	12	660	4
Spawning					
02/07/2015 (SST = 25.5 °C)	m	96	13	140	4
	m	95	11	155	4
	m	91	10	70	4
	m	96	12	130	4
	f	92	8	95	4
	f	96	12	130	4
	f	95	11	135	4
	f	97	12	140	4

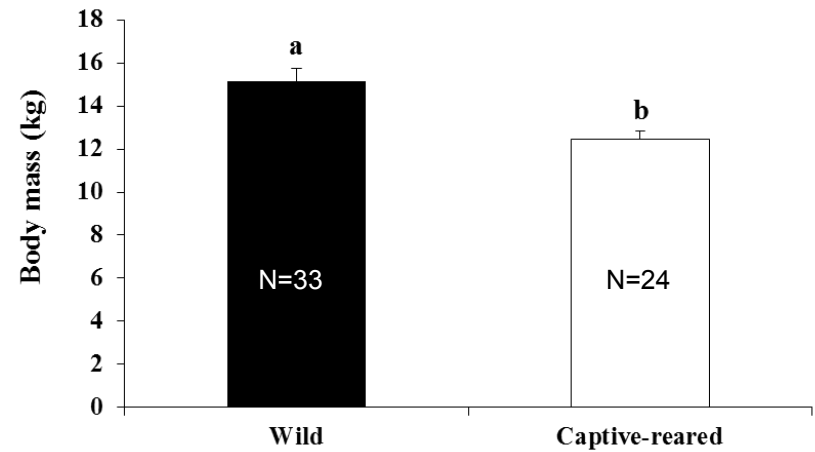
SST: Sea Surface Temperature



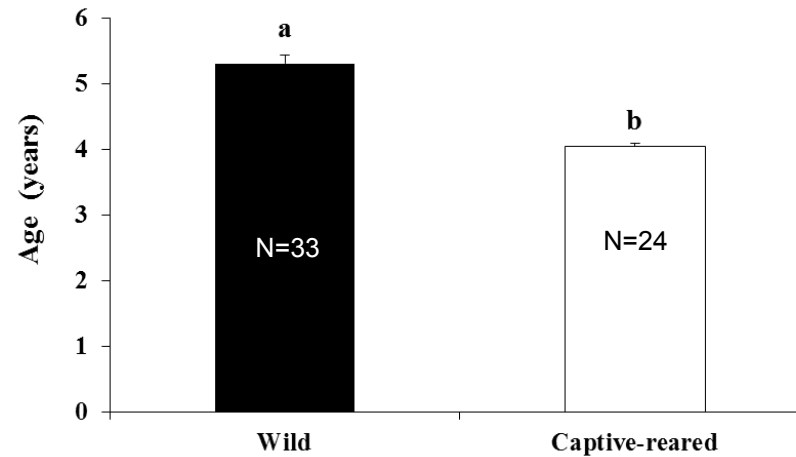
Fork length



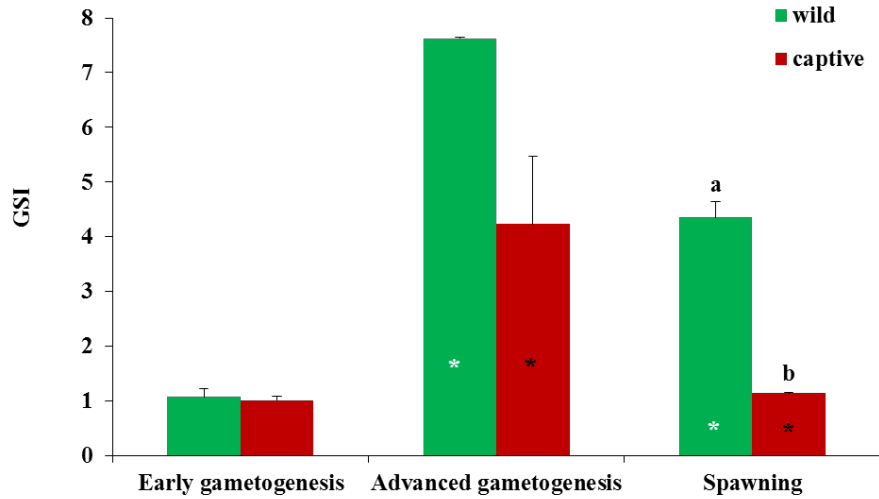
Body mass



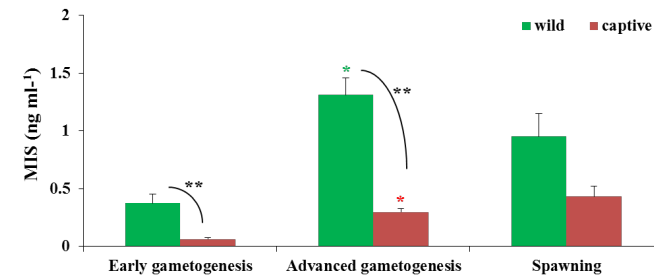
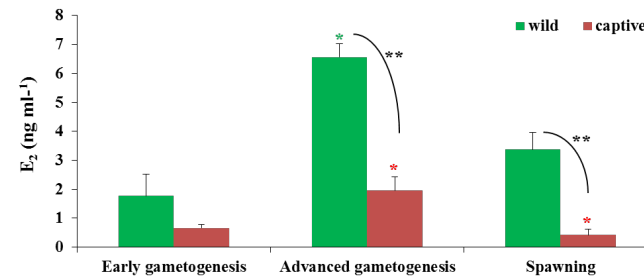
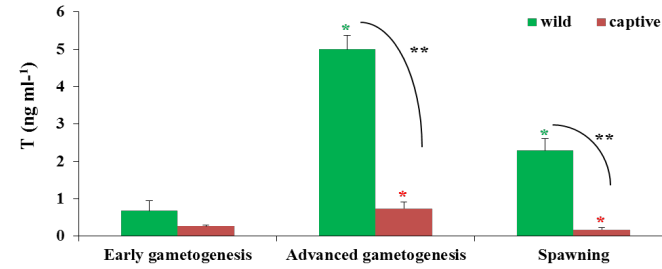
Estimated age



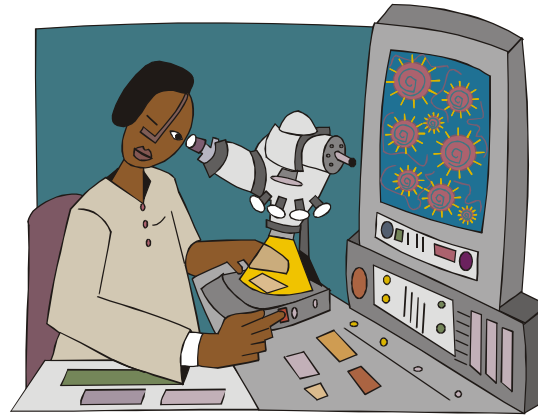
GSI = 100 Gonad Mass/Body Mass



Sex steroids (ELISA)



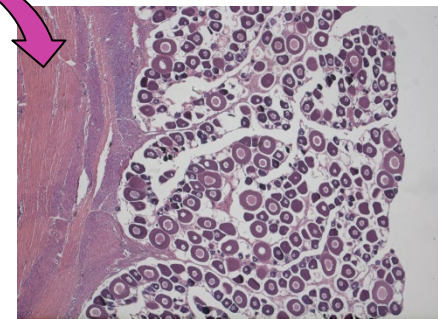
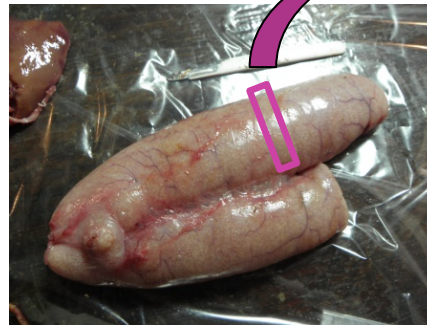
HISTOLOGICAL ASSESSMENT OF FEMALE REPRODUCTIVE STATE



most advanced oocyte stage

atretic follicles

post-ovulatory follicles (POFs)



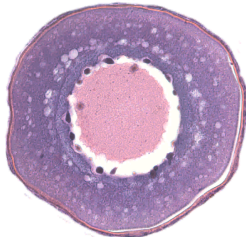
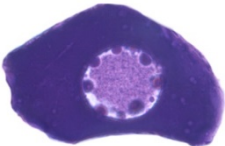
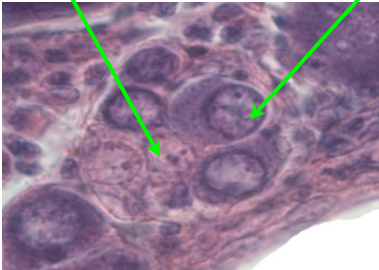
PRIMARY GROWTH

Oogonia
(8-13 μm)

Chromatin-nucleolus stage
(15-30 μm)

Perinucleolar stage
(30-120 μm)

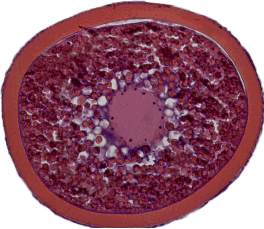
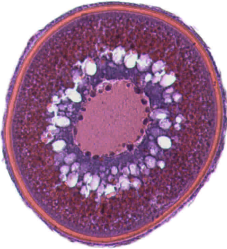
Lipid/Cortical alveoli stage
(120-200 μm)



VITELLOGENESIS

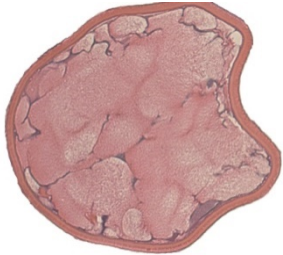
Early vitellogenesis stage
(200-400 μm)

Late vitellogenesis stage
(400-550 μm)



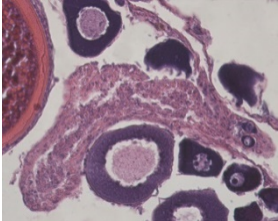
OOCYTE MATURATION

Hydrated stage
(≈ 800 μm)

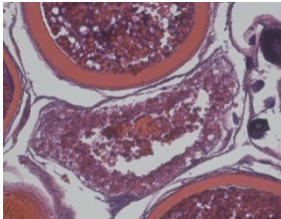


HIC_anti-Vg antibodies

Post-ovulatory follicles (POFs)

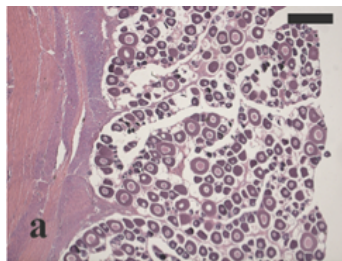


Atretic follicles

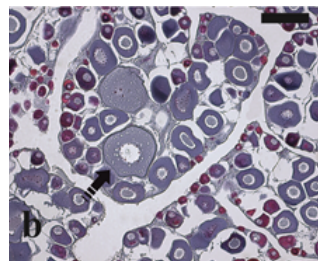




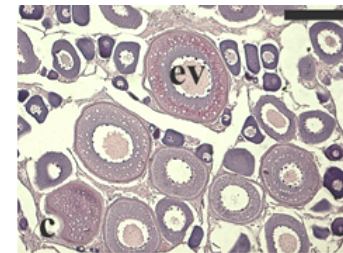
Early gametogenesis



WILD n=1
CAPTIVE n=1

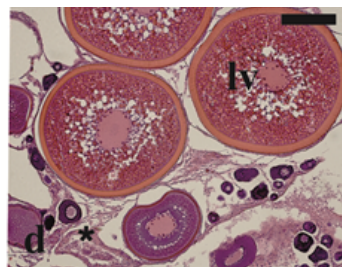


WILD n=2

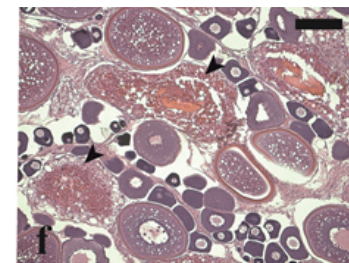


WILD n=2
CAPTIVE n=3 (few early Vg oocytes)

Advanced gametogenesis

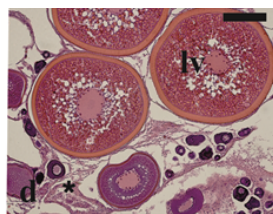


WILD n=2 (with POFs)

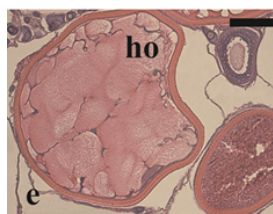


CAPTIVE n=4

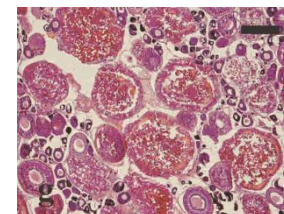
Spawning



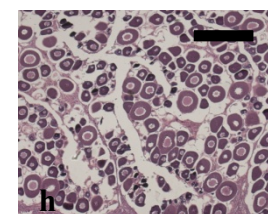
WILD n=10
(with POFs)



WILD n=2

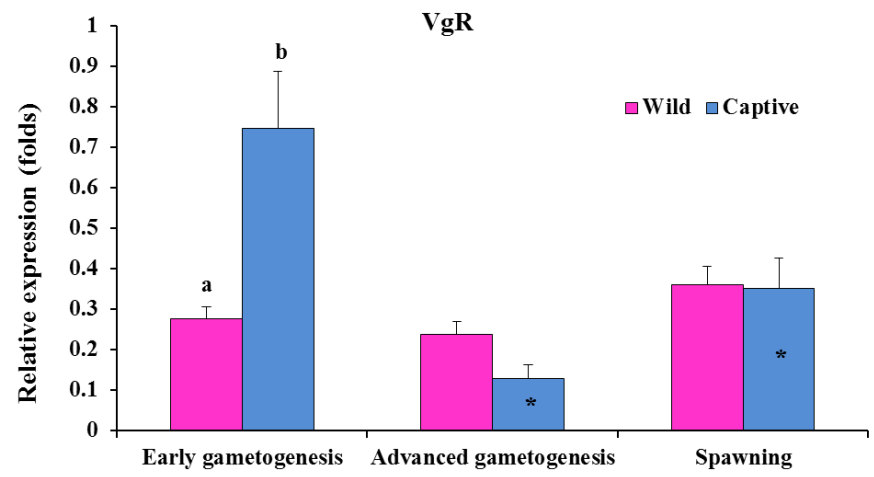
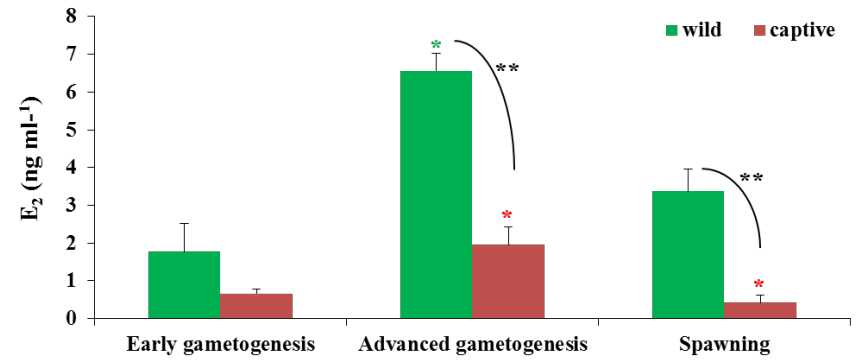
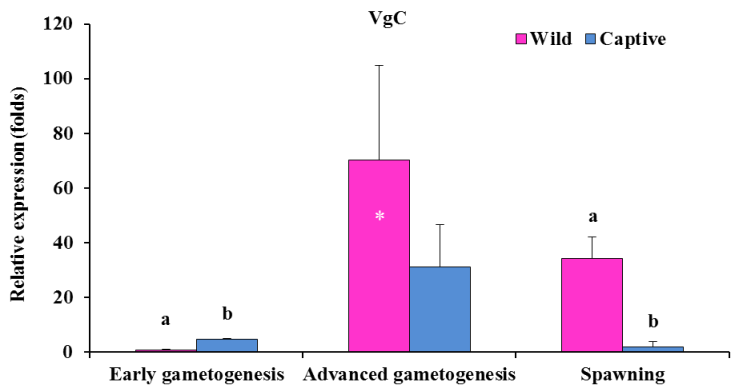
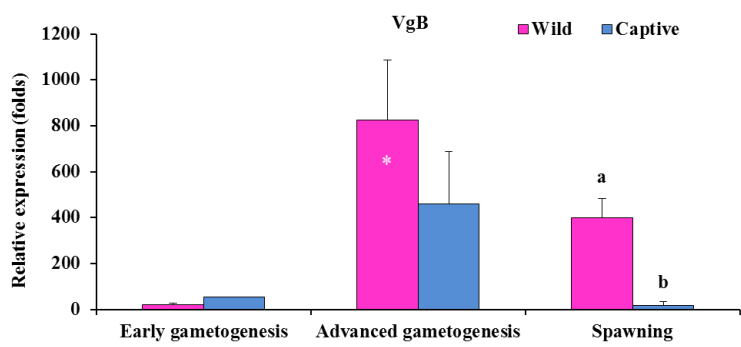
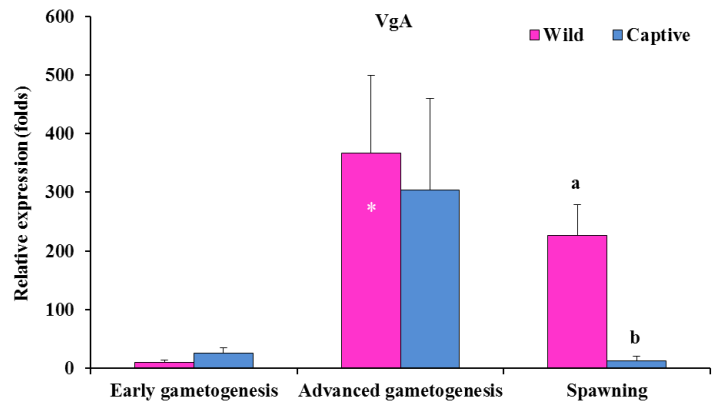


CAPTIVE n=3



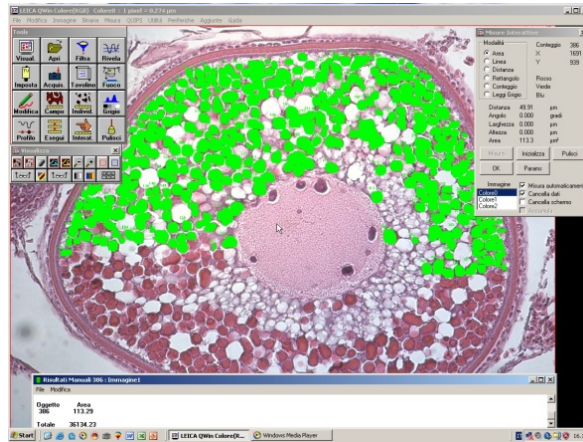
CAPTIVE n=1

LIVER VITELLOGENINS AND OVARIAN VITELLOGENIN RECEPTOR EXPRESSION



VgA, VgB, VgC and Vg receptor sequences obtained in Y1 (D3.1)
 Vgs and VgR gene expression analysis by qRT-PCR

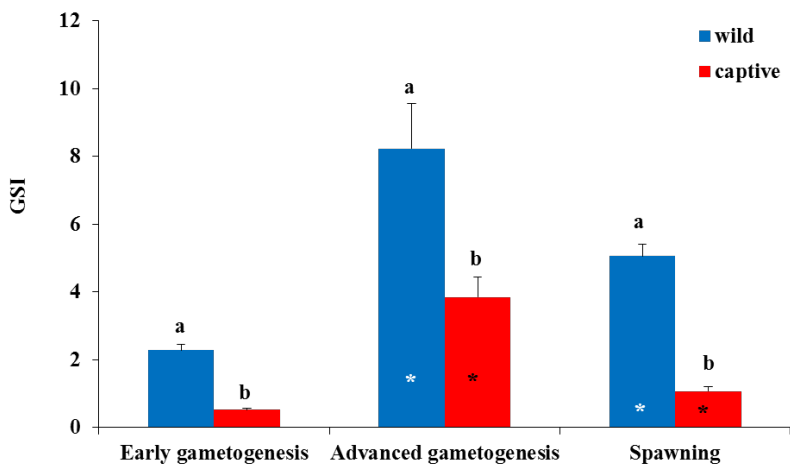
OOCYTE YOLK ACCUMULATION IN EARLY AND LATE VITELLOGENIC FOLLICLES



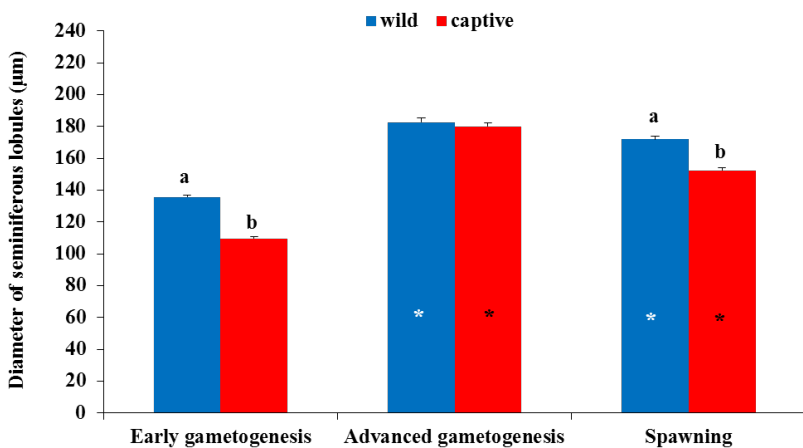
Oocyte stage	Fish condition	Oocyte diameter (μm)	Yolk surface (μm ²)
Early vitellogenesis	Wild	362.5 ± 3.5	55584.9 ± 1513.4
	Captive-reared	356.5 ± 6.9	55760.8 ± 3238.2
Late vitellogenesis	Wild	453.7 ± 3.5	84660.1 ± 1368.3
	Captive-reared	453.0 ± 9.3	90790.6 ± 3650.1



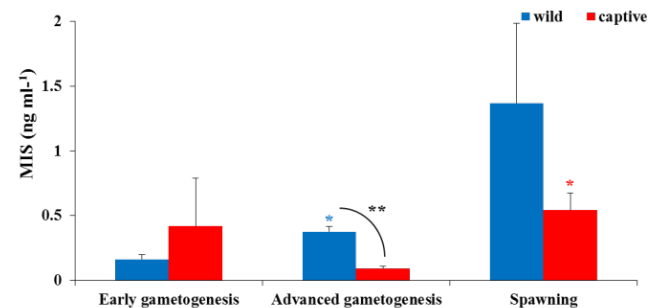
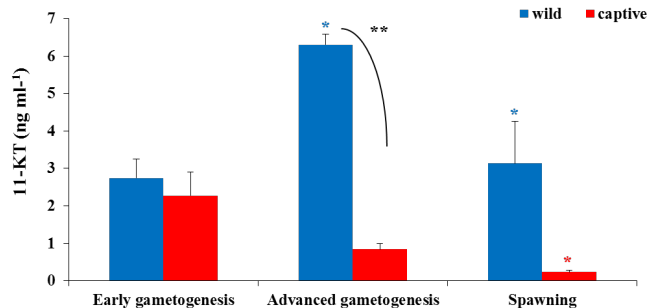
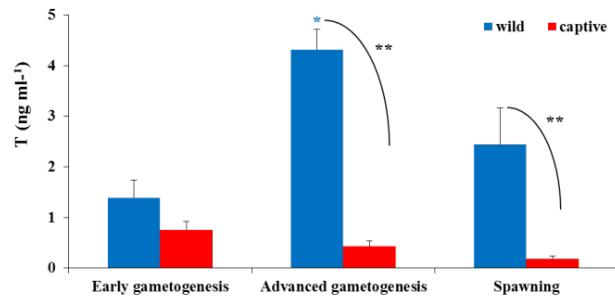
GSI = 100 Gonad Mass/Body Mass



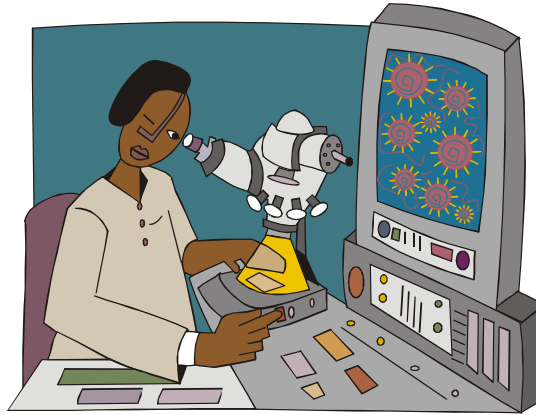
Seminiferous lobule diameter



Sex steroids (ELISA)

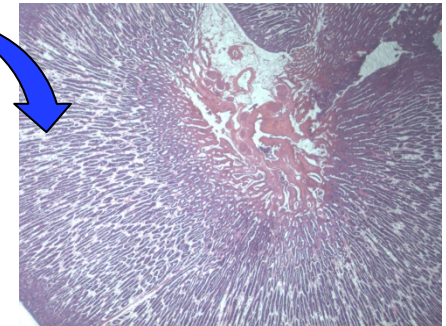
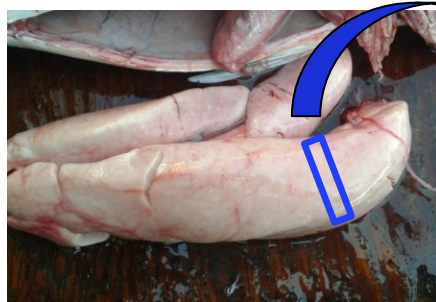


HISTOLOGICAL ASSESSMENT OF MALE REPRODUCTIVE STATE



spermatogenic cyst types

luminal spermatozoa amount

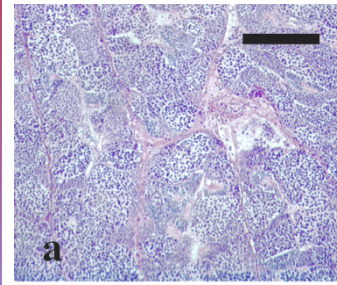
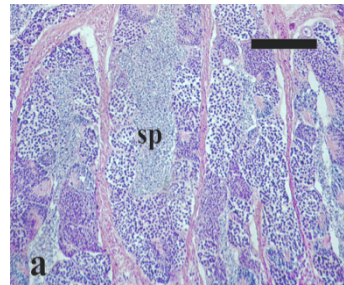


WILD

CAPTIVE-REARED

➤ EG

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

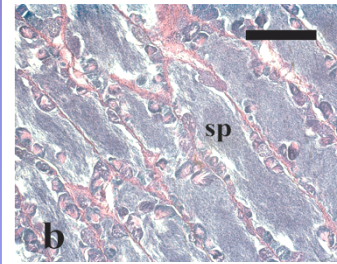
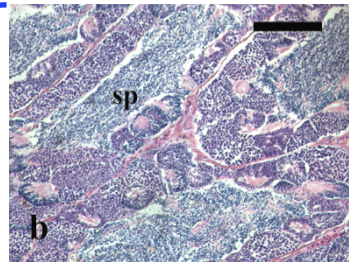


➤ EG

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

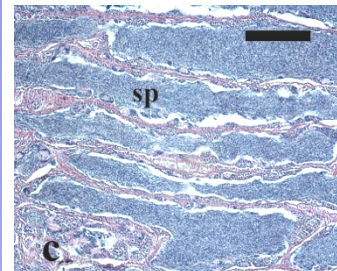
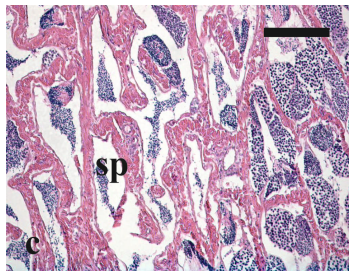
➤ AG + SP

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



➤ AG

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



➤ SP

- residual luminal spermatozoa (n=4)

Germ cell proliferation decrease
Germ cell apoptosis increase

GTHs
11-KT

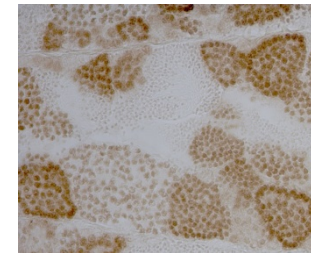


GTHs
11-KT



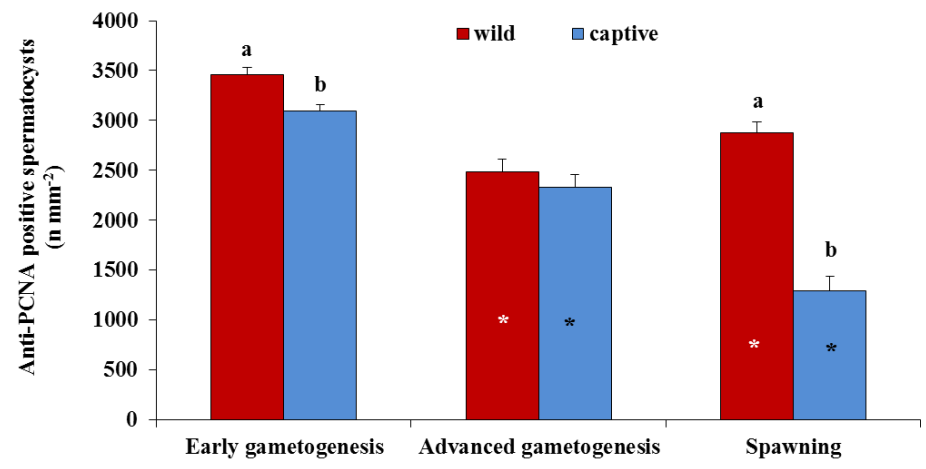
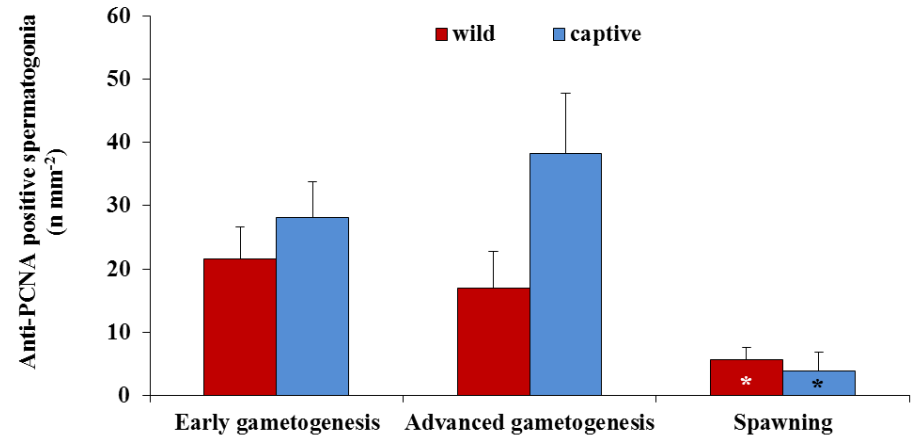
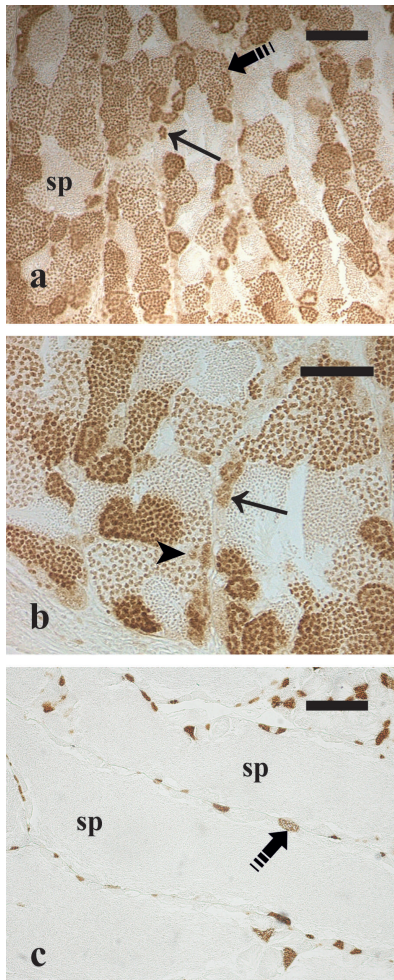
Germ cell proliferation increase
Germ cell apoptosis decrease

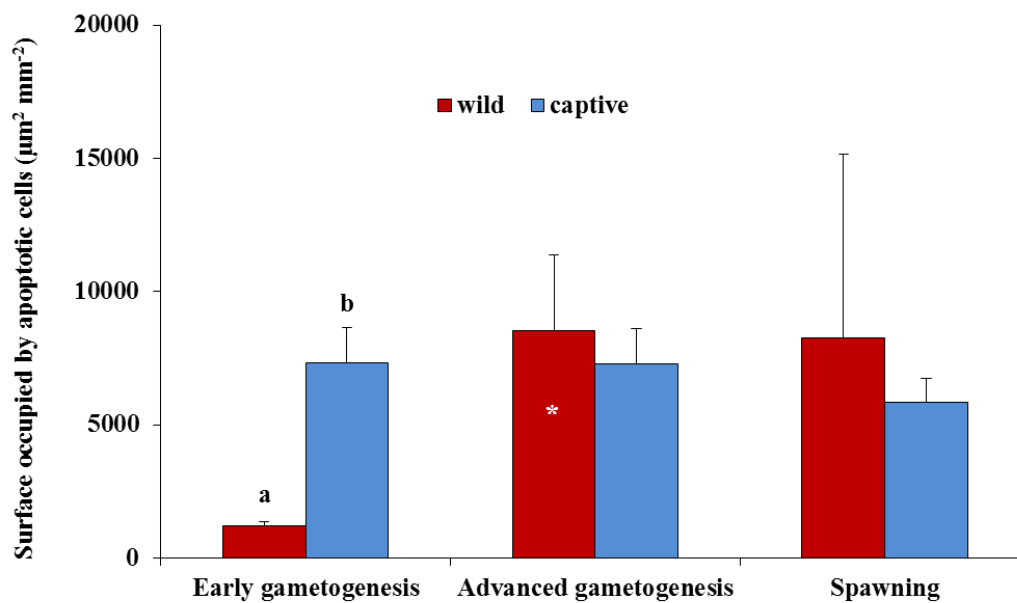
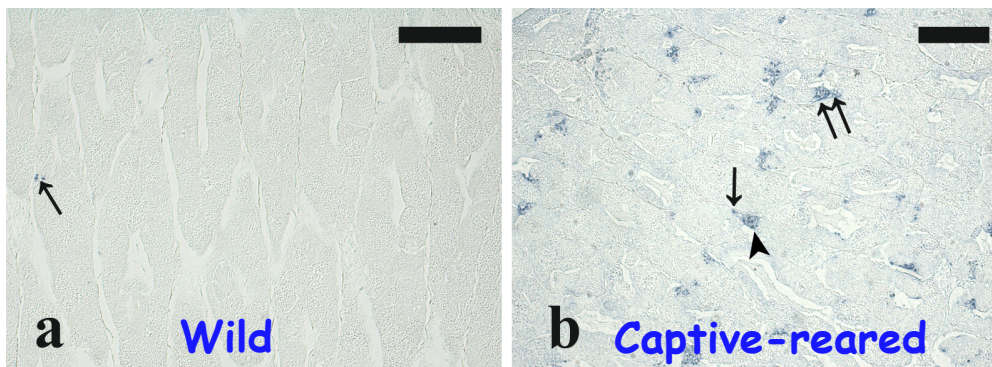
- Immunolocalization of Proliferating Cell Nuclear Antigen (PCNA)



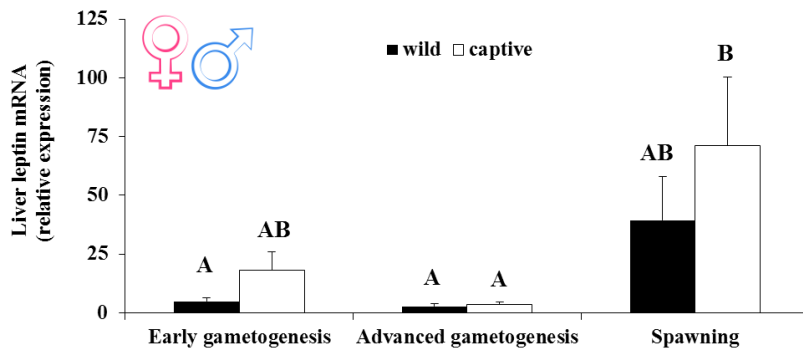
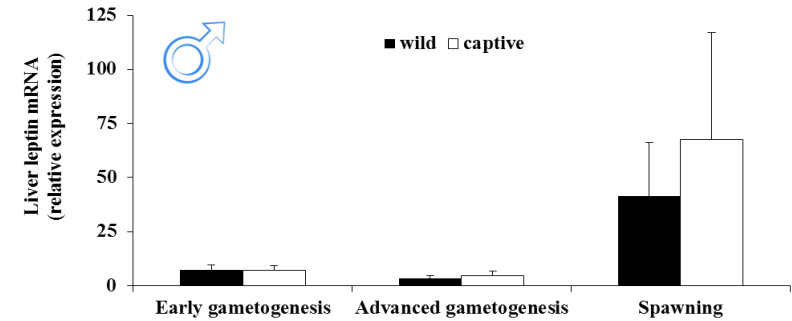
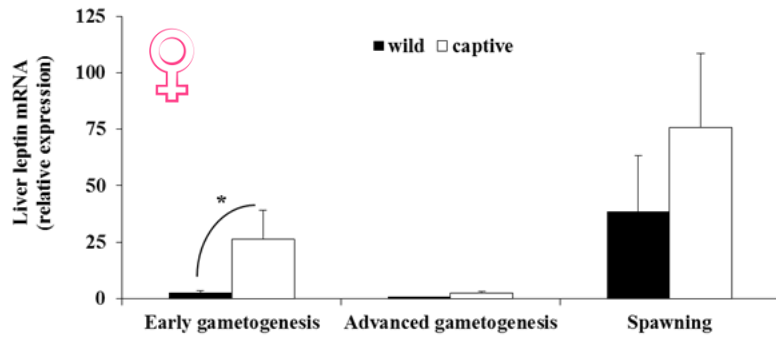
- Terminal deoxynucleotidyl transferase-mediated d'UTP nick end labelling (TUNEL)







ASSESSMENT OF NUTRITIONAL STATE/LIVER LEPTIN GENE EXPRESSION

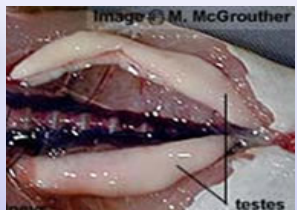


Leptin sequence obtained in Y1 (D3.1)
Leptin gene expression analysis by qRT-PCR



Wild ♀ ♂

Gonads



Early Gametogenesis (EG)

Advanced Gametogenesis (AG)

Spawning (SP)

Captive ♀ ♂

Total Protein and
Moisture
(AOAC, 2002)

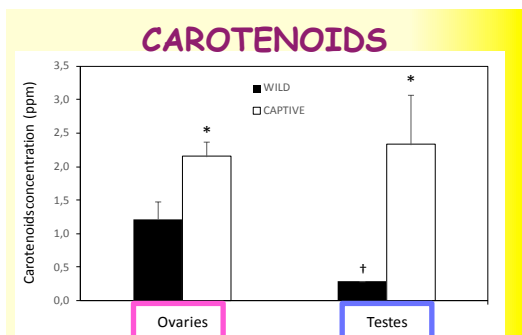
Total Carotenoids
(Barua et al., 1993)

Total Lipid
(Christie, 1982)

Lipid Classes Profile
(Olsen y Henderson, 1984)

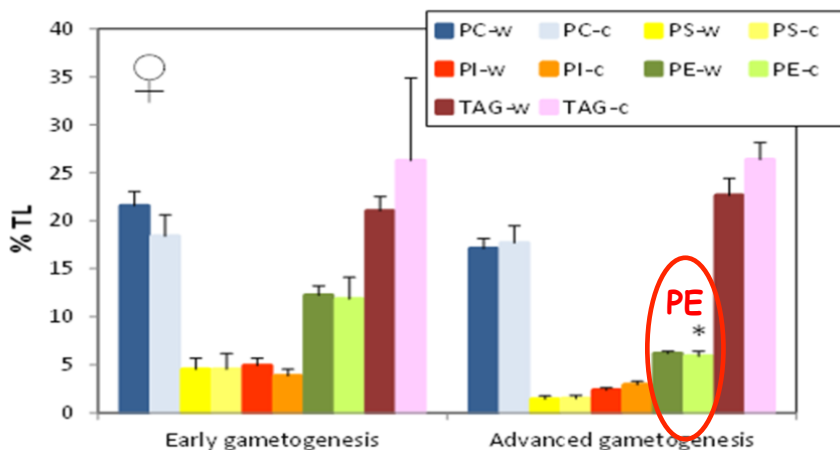
Fatty Acid Composition
(Christie, 1982)

ASSESSMENT OF NUTRITIONAL STATE/KEY NUTRIENT ANALYSIS

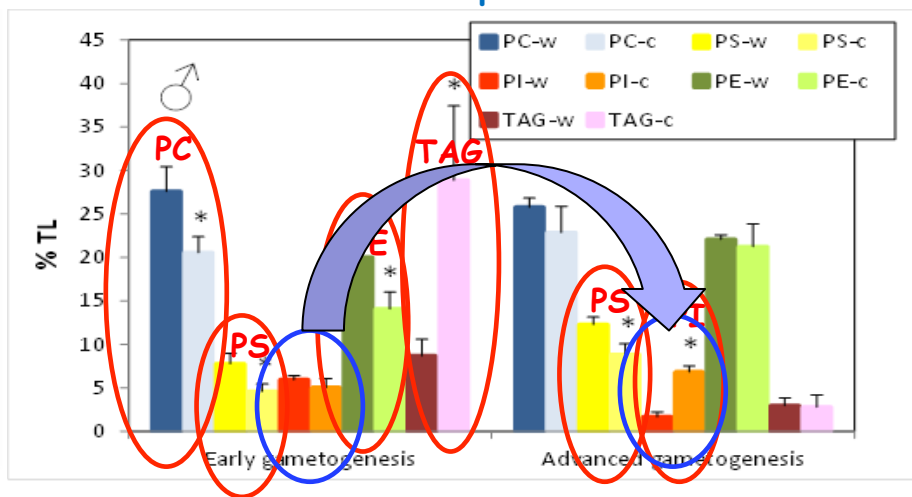


- Carotenoids overabundance in captive fish
- Natural condition inverted in captive-reared fish, with lower content of carotenoids in ovaries than in testes

ovary lipid classes



testis lipid classes

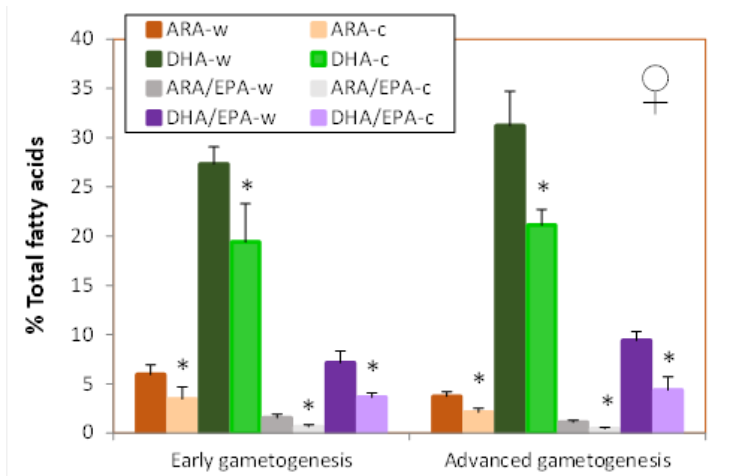


PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; TAG= triacylglycerols

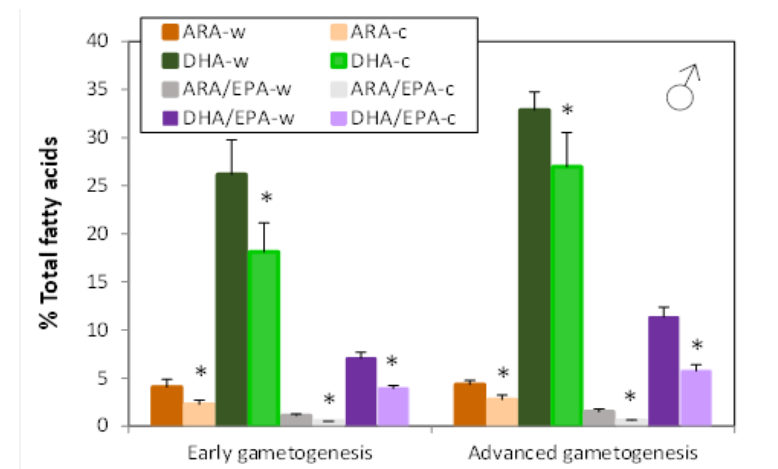
- Lower proportion of lipid classes essential for spermatogenesis (PC, PE and PS) during the early gametogenesis period
- Absence of the expected decrement of phosphatidylinositol (PI) from early to advanced gametogenesis as a possible mechanism to obtain arachidonic acid (ARA) for steroidogenesis

ASSESSMENT OF NUTRITIONAL STATE/KEY NUTRIENT ANALYSIS

ovary fatty acids



testis fatty acids



ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid

- Lower content (30-40%) of fatty acids (DHA, ARA, DHA/EPA and ARA/EPA ratios), all crucial factors for reproductive success



Lower gonadosomatic index and plasma sex steroid levels in captive-reared fish

➤ In captive-reared females:

- *Lower Vgs expression*
- *Extensive atresia of vitellogenic oocytes*
- *No oocyte maturation*

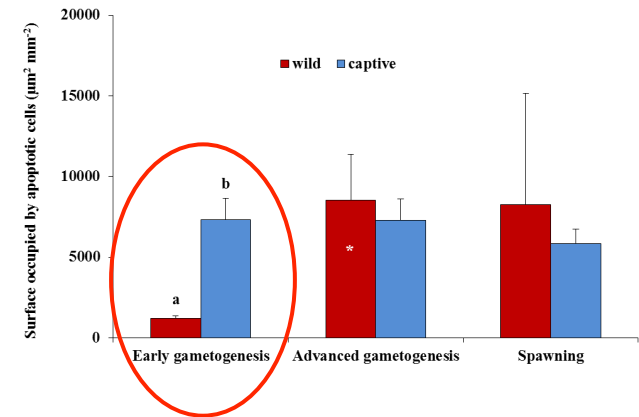
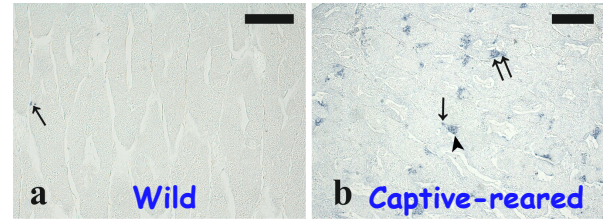
➤ In captive-reared males:

- *Progressive T and 11-KT decrease during the reproductive season*
- *Smaller seminiferous lobules*
- *Early decrease of germ cell proliferation and cessation of spermatogenesis during the spawning period of the wild counterpart*
- *Very high germ cells apoptosis during the early gametogenesis*

Severe gametogenesis impairment!!!!

POSSIBLE CAUSES OF GAMETOGENESIS IMPAIRMENT

- Acute stress due to repeated samplings?



- Chronic stress?
- Unsuitable environmental conditions?
- Nutritional deficiency?





Merci pour votre attention

