



WT 3 : **REPRODUCTION & GENETICS**
WP 7 : **Grey Mullet (*Mugil cephalus*)**



Assessment of the effects of captivity on first sexual maturity of wild-caught and hatchery-produced fish

Iris Meiri Ashkenazi, Aldo Corriero, Oriya Nixon, Vered Zlatnikov, Chen Bracha, Eldad Toledano, Bill Koven and Hanna Rosenfeld



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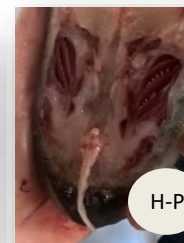
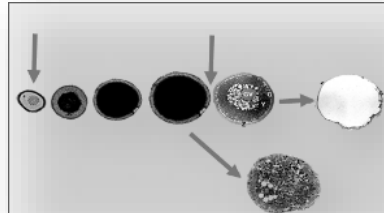
UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO

GREY MULLET KNOW-HOW TRANSFER WORKSHOP
14TH MAY 2018, BARI, ITALY

Puberty in Fish

Developmental period during which an individual becomes capable of reproducing sexually for the first time, and implies a functional competence of the **brain–pituitary–gonad** axis

It starts some time after sex differentiation and is associated with the initiation of germ cell maturation and full functional differentiation of the germ cell-supporting somatic cells of the gonads, and culminates in **first spermiation and sperm hydration** or **ovulation**



Why is puberty a big issue for the fish farming industry ?

Farming conditions

opposing
effects on fish
growth and
sexual maturity

- Improved food availability
- Domestication

Lack of appropriate
environmental stimuli

Why is puberty an issue for the Mullet farming industry?

Promotion of sexual maturation
at reduced age and size

Sexual maturity is being **blocked**

Endocrine disruption may have dramatic consequences either on the alteration or inhibition of the time of spawning and on the quality of eggs and larvae produced

Ripe mullet ovaries extracted for roe production

Sicilian and Sardinian **bottarga**



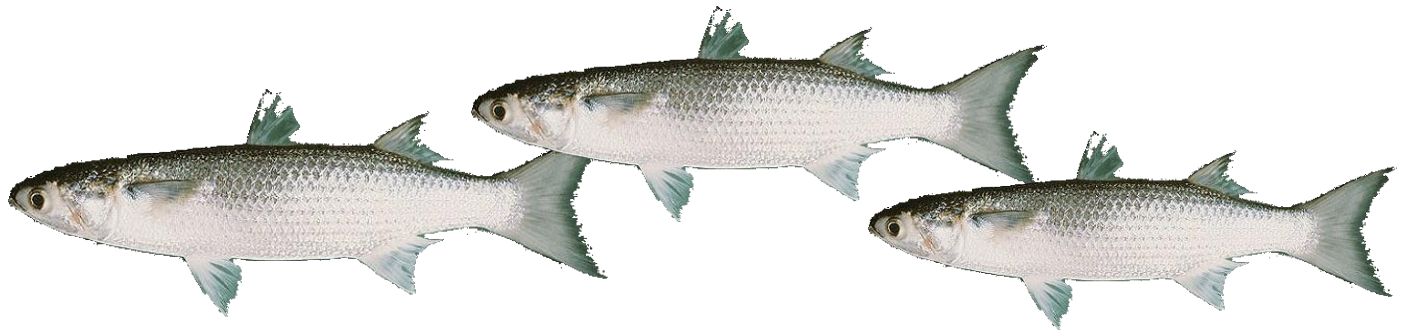
2 in 1

Picture from <http://www.bottargabrothers.com/what-is>

Bottarga is popular in Italy, France, Spain, Greece, Egypt, Japan, Taiwan, Portugal, Croatia, North Africa, Lebanon and Turkey

Work Task

**Characterize pubertal development
in **wild-caught** vs. **hatchery produced**
grey mullet subjected to captive
conditions**

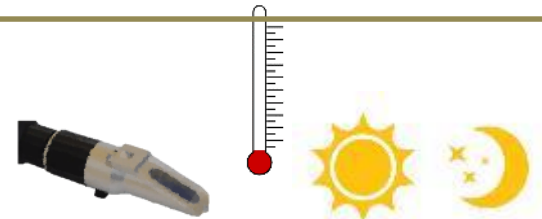




Wild caught **Vs.** Hatchery produced (G1) ≥4 yr fish

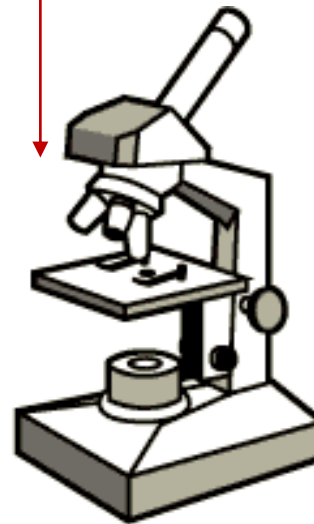
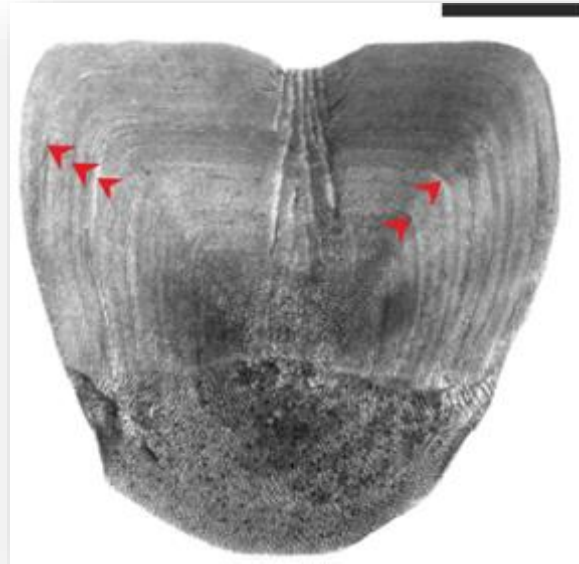
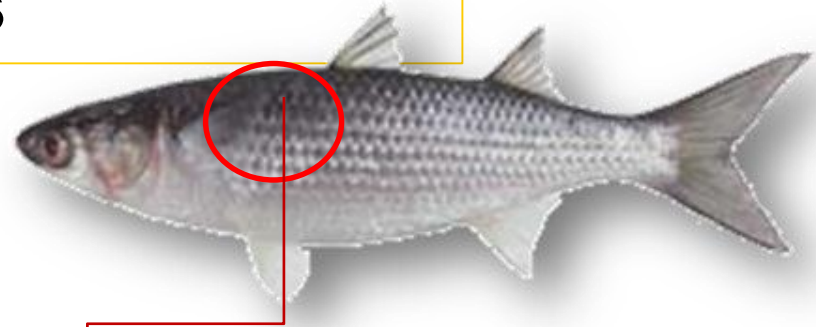
Caught in the Schiapparo Channel (Apulia, Italy) during their migration from the Lesina Lagoon to the South Adriatic Sea

IOLR – spawned in 2010



Ambient seawater salinity
and photo-thermal regime

Age of the fish was estimated based on the number of annuli counted on their scales



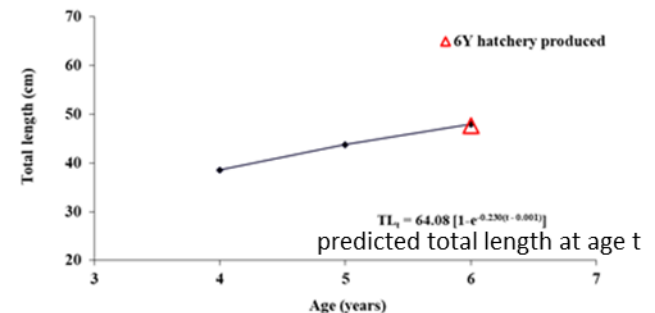
Scale from a 5-year old wild grey mullet.
Bar = 3 mm. Arrowheads point to annuli.

Wild and Hatchery-reared grey mullet adults sampled in Italy and at IOLR.

Fish Origin	Sampling area	Sampling Date	Sex	Total Length (TL, cm)	Body mass (BM, g)	Gonad mass (GM, g)	Liver mass (LM, g)	Age (year)
Wild	Schiapparo channel	Early September 2016	f	38.0				
			f	40.0				
			f	40.0				
			f	42.0				
			f	43.0				
			f	45.0				
			f	46.0				
			f	47.0				
			f	49.0				
			f	50.0				
			m	37.0				
			m	38.0				
			m	38.0				
			m	41.0				
			m	41.0				
			m	45.0				
Captive-reared	P7. IOLR	03/11/2016	f	47.1				
Hatchery -produced			f	50.3				
			f	54.4				
			m	41.2				
			m	45.2				

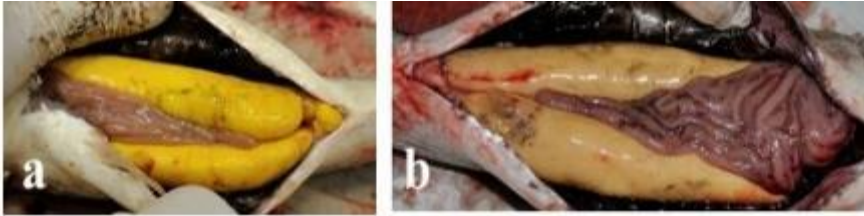
The mean length of 6 year old hatchery-produced grey mullets reared at IOLR were identical to same age wild specimens sampled in Italy

Von Bertalanffy growth curve of wild grey mullet

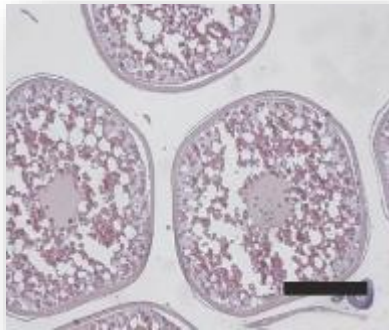


Rearing in captivity allowed similar body growth to individuals caught from the wild.

Ripe ovaries from wild grey mullets

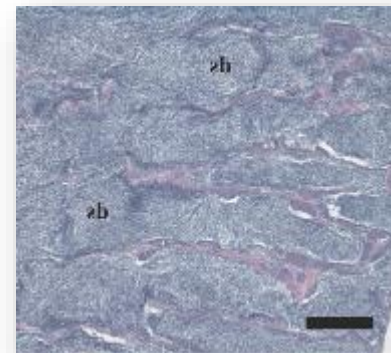


Differences in shape and colour



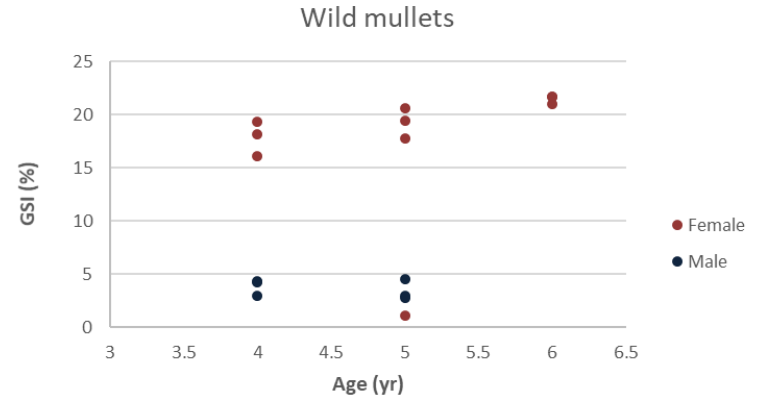
Bar=200 μ m

All showed late vitellogenic oocytes as the most advanced oocyte stage



Bar=150 μ m

All had mature testes, the lumen of seminiferous lobules filled with spermatozoa



* Sampled during their migration towards the spawning ground of the Adriatic Sea



31st October 2014





Growth (Body weight and Length)



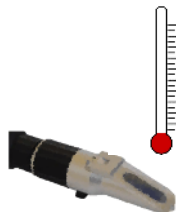
Gonadal Development



Wild-captive reared *Vs.* Hatchery produced (G2)

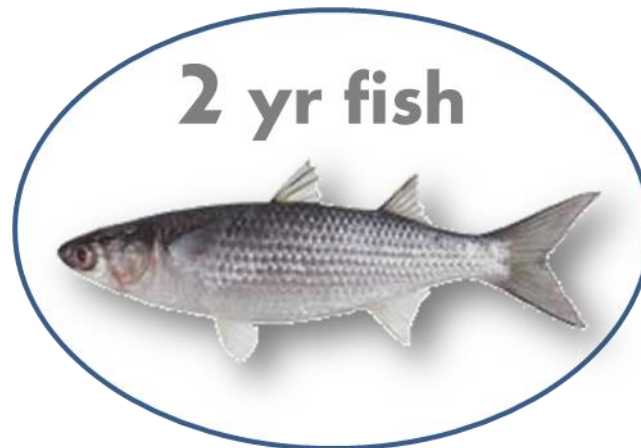
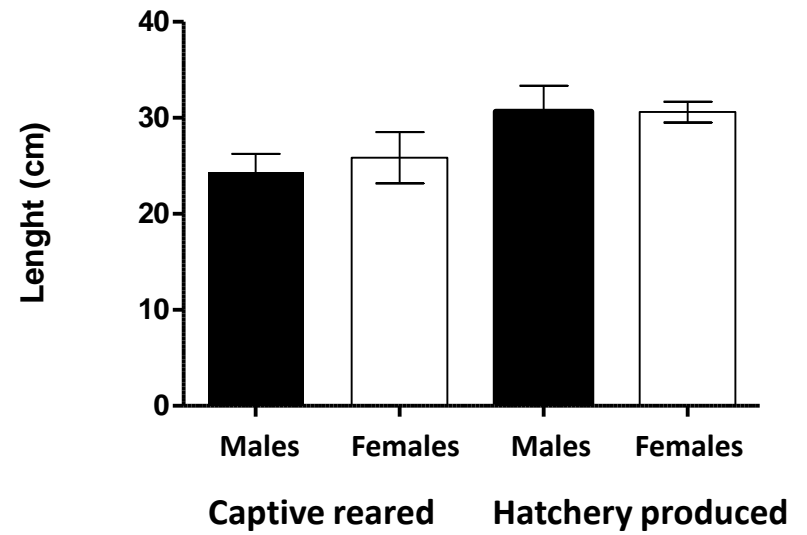
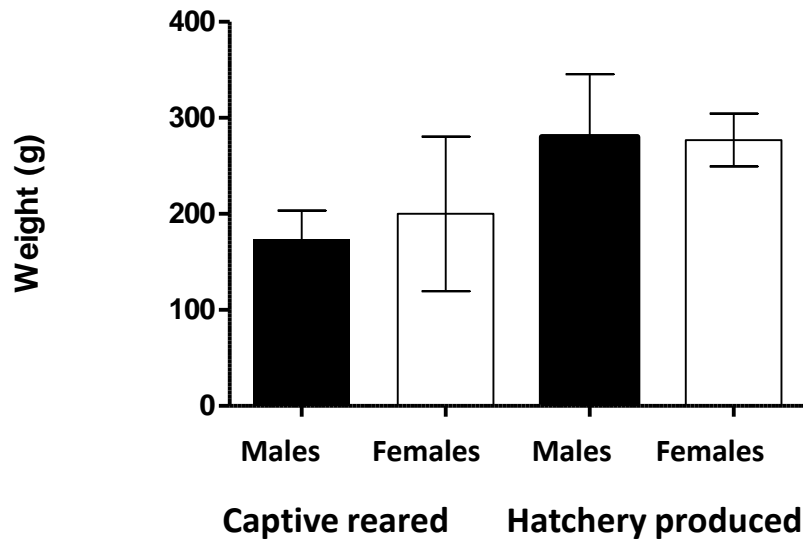
Caught in the Ebro River delta and imported from Spain to Israel

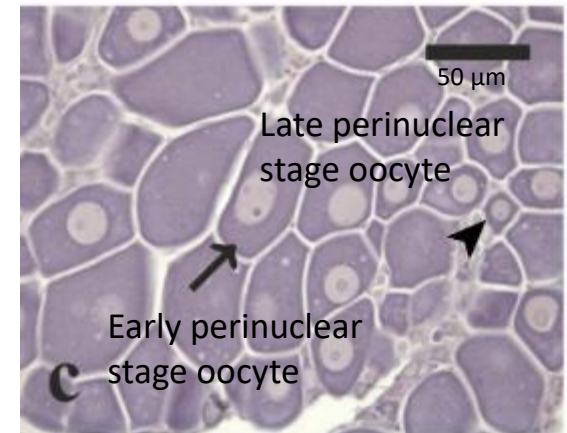
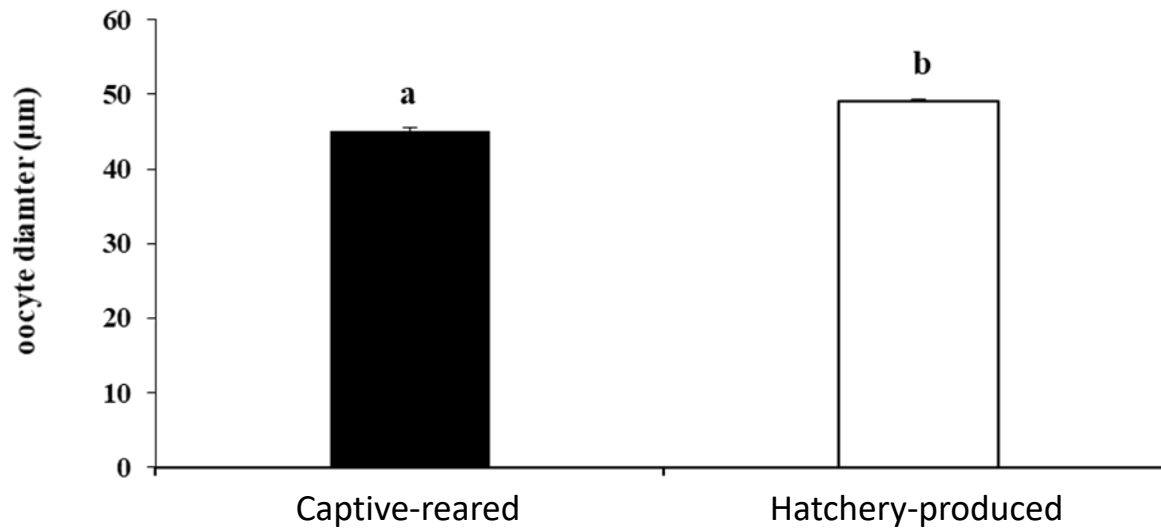
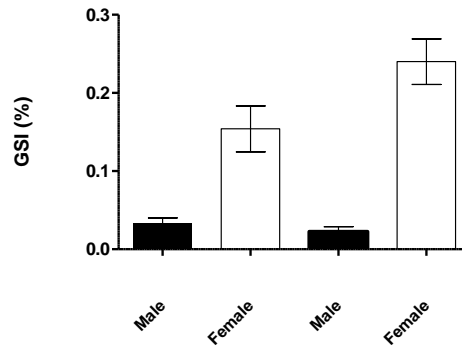
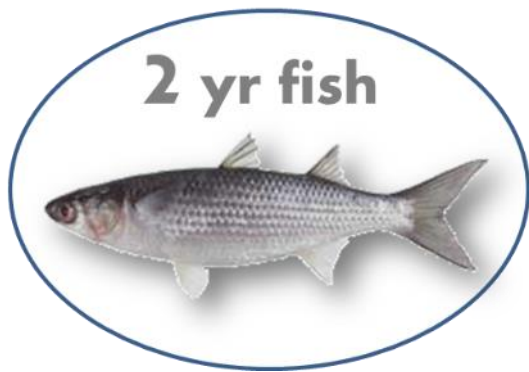
IOLR - spawning date:
31st October 2014



Ambient seawater salinity
and photo-thermal regime

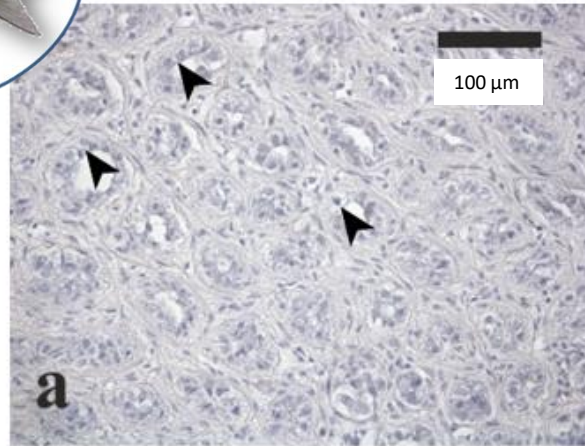




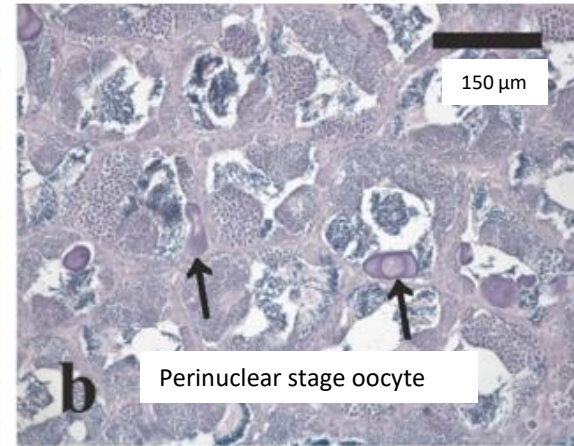


Hatchery-produced grey mullets had significantly larger oocytes than captive-reared specimens

2 yr fish



Testis - **immature** age 2
hatchery-produced :
small seminiferous lobules



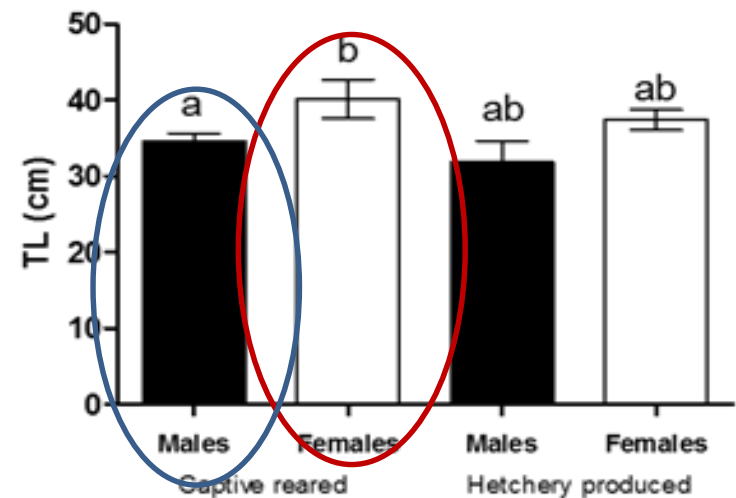
Gonad - age 2 hatchery-
produced: **intersex** with all
stages of spermatogenesis.

Both wild-caught and hatchery-produced, showed **immature testes**.

Two year old wild and hatchery produced mullets were still sexually immature. Hatchery produced fish demonstrated slightly better gonadal and somatic growth.

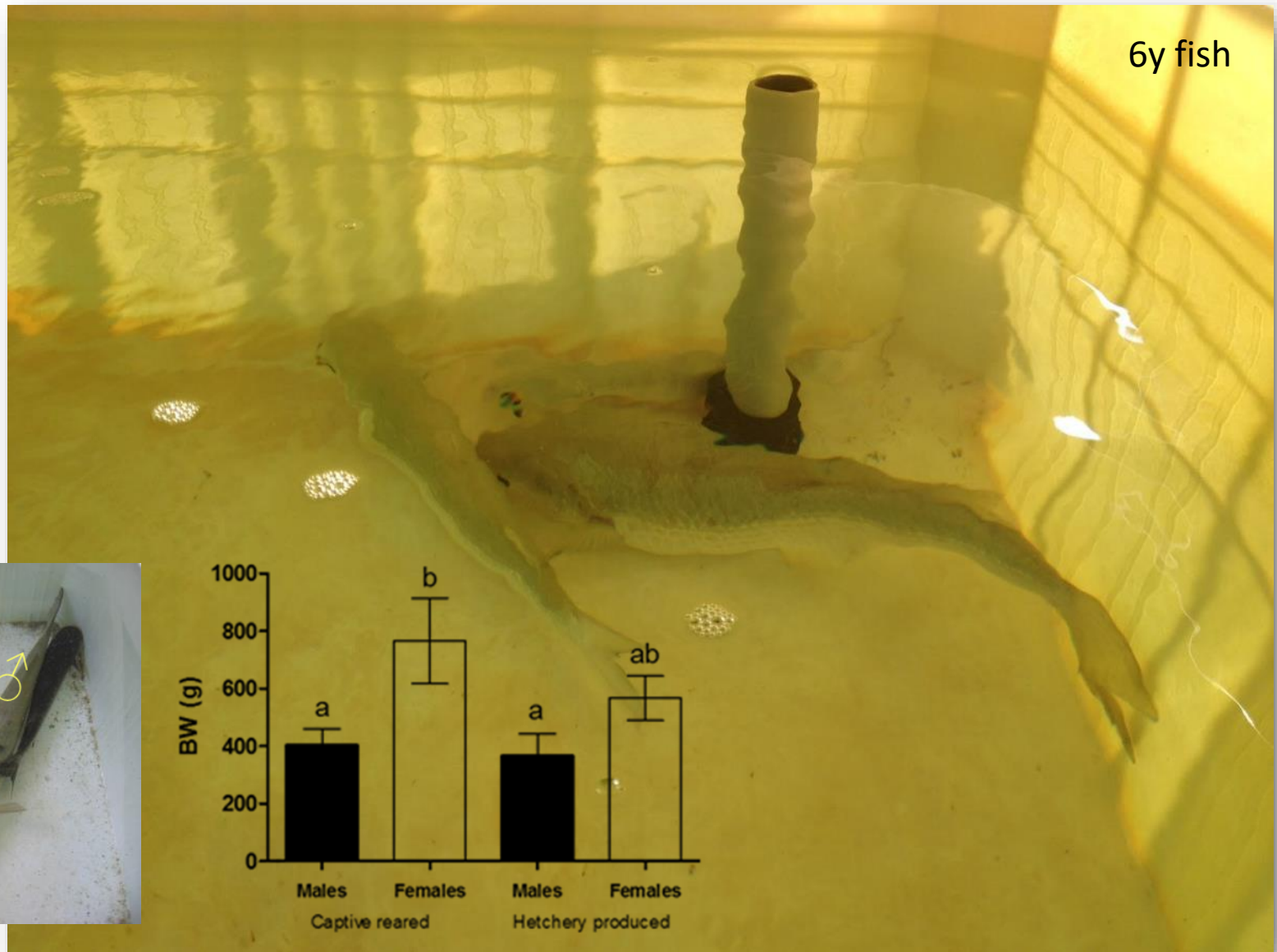
Body Weight and Total Length

3 yr fish



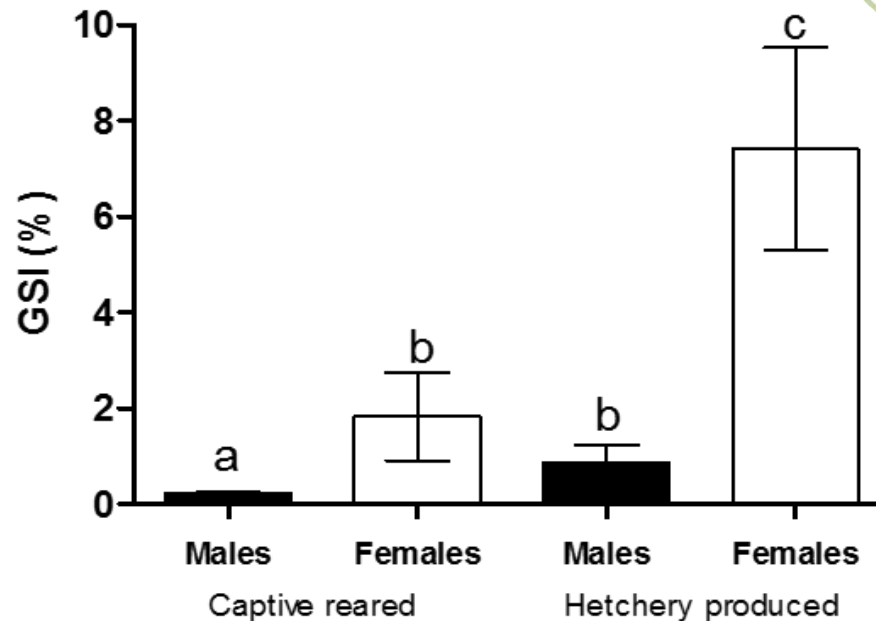
Captive-reared females are larger and heavier than cognate males. Although not significant, the hatchery-produced grey mullets appear to exhibit a similar trend

The 3 year old grey mullet exhibit sex related growth patterns



Gonadal Development

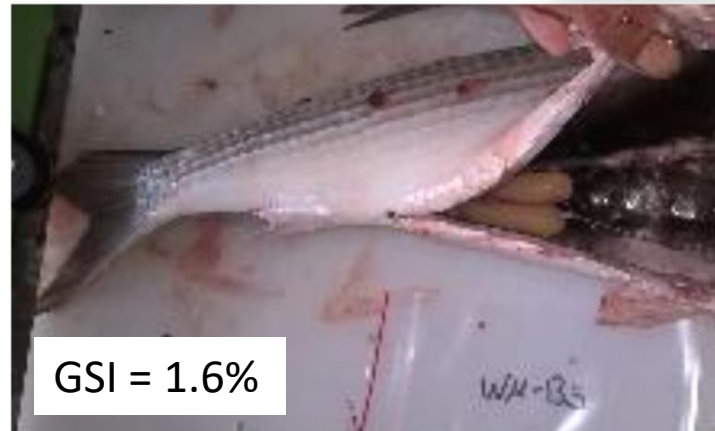
3 yr fish



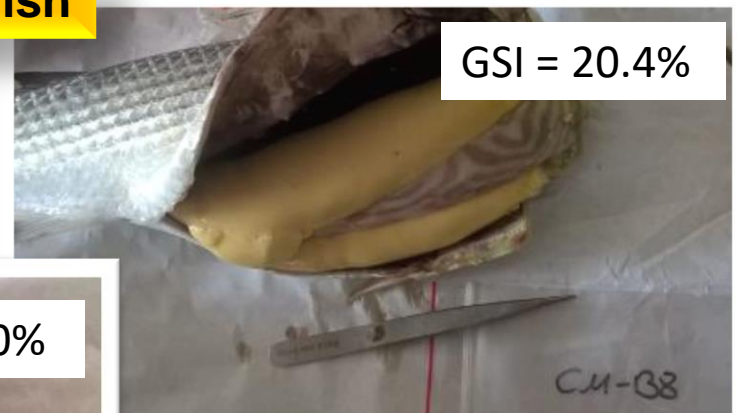
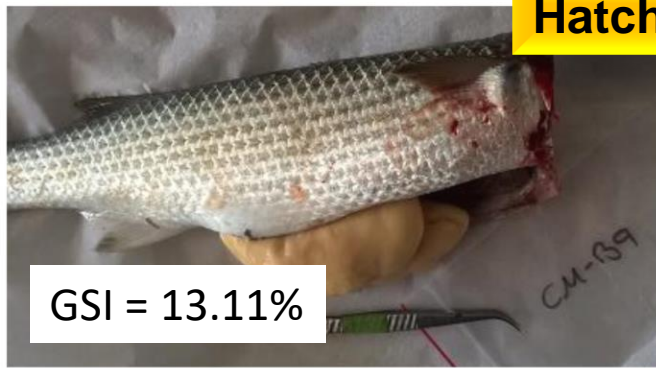
The 3 year old grey mullet exhibit sex related gonadal development patterns.

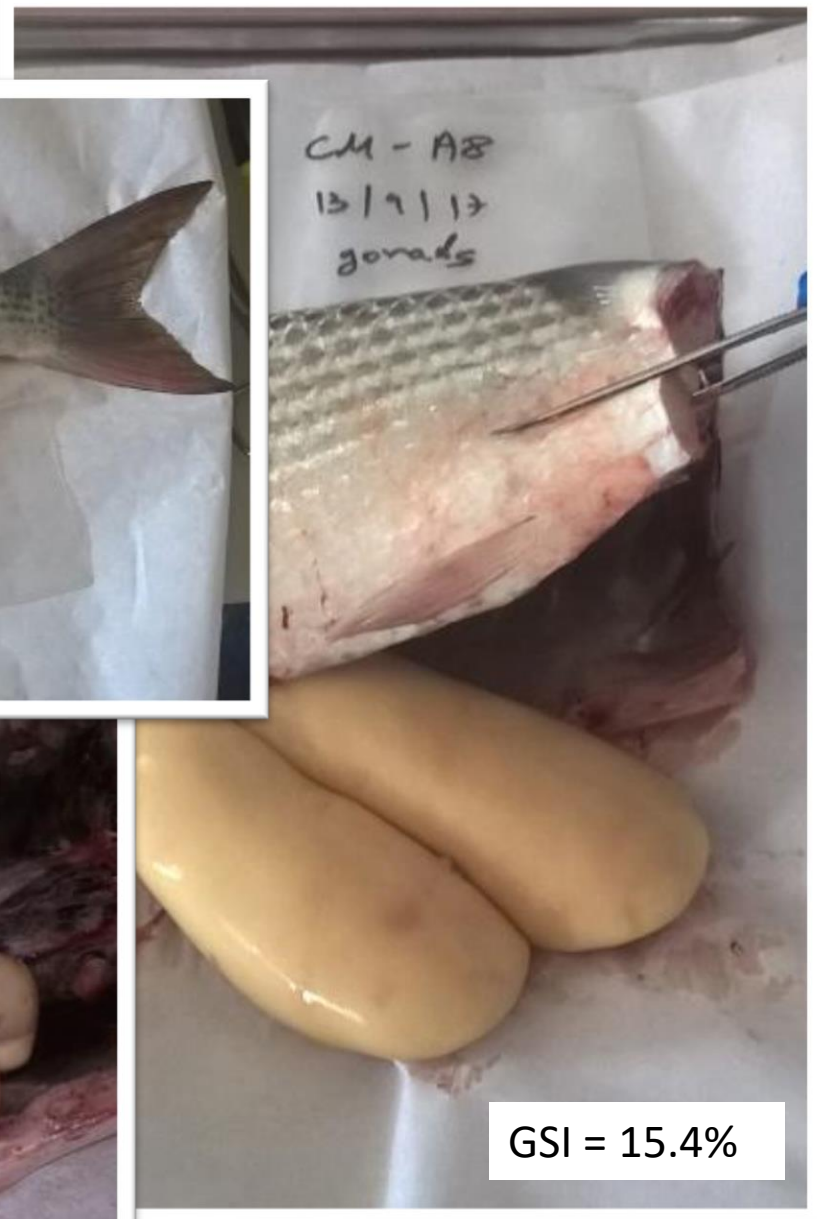
The GSI values in hatchery produced females and males were significantly higher than those of the captive reared females.

Captive-reared fish



Hatchery produced fish





Differences in shape and colour

3 yr fish



%

**Captive -
reared**

**Hatchery
produced**

UD

20

5

**Un-differentiated
gonad**

%

**Captive -
reared**

**Hatchery
produced**

**Early and late
perinuclear oocytes**

67

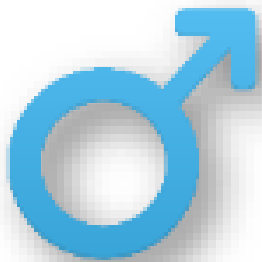
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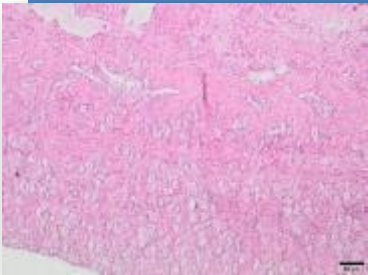
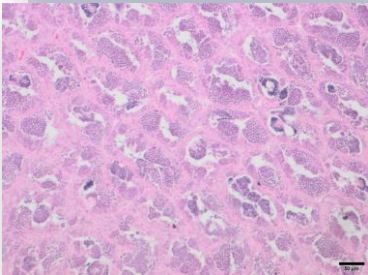
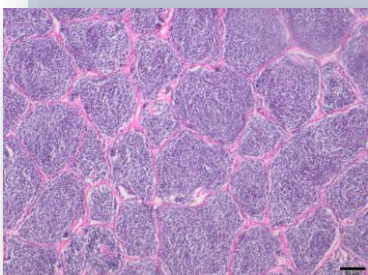
**Vitellogenic
oocytes**

33

54





	%	Captive - reared	Hatchery produced
Small seminiferous lobules		0	33
Stages of spermatogenesis		100	0
Mature sperm		0	67

Captive reared

	BW (g)	GW (g)	GSI (%)
28.10.2017	800	3.4	0.43
28.10.2017	400	un developed	

Hatchery produced

	BW (g)	GW (g)	GSI (%)
29.10.2017	650	120	18.5 **
26.10.2017	730	110	15.1 *
26.10.2017	630	163	25.9 **



- * Oocyte diameter > 600µm
- ** Ovulatory eggs

CALENDAR 2018

January 2018					
W	M	T	W	T	F
1	1	2	3	4	5
2	8	9	10	11	12
3	15	16	17	18	19
4	22	23	24	25	26
5	29	30	31		

February 2018					
W	M	T	W	T	F
5				1	2
6	5	6	7	8	9
7	12	13	14	15	16
8	19	20	21	22	23
9	26	27	28		

March 2018					
W	M	T	W	T	F
9				1	2
10	5	6	7	8	9
11	12	13	14	15	16
12	19	20	21	22	23
13	26	27	28	29	30

April 2018					
W	M	T	W	T	F
14	2	3	4	5	6
15	9	10	11	12	13
16	16	17	18	19	20
17	23	24	25	26	27
18	30				

May 2018					
W	M	T	W	T	F
18		1	2	3	4
19	7	8	9	10	11
20	14	15	16	17	18
21	21	22	23	24	25
22	28	29	30	31	

June 2018					
W	M	T	W	T	F
22					1
23	4	5	6	7	8
24	11	12	13	14	15
25	18	19	20	21	22
26	25	26	27	28	29

July 2018					
W	M	T	W	T	F
27	2	3	4	5	6
28	9	10	11	12	13
29	16	17	18	19	20
30	23	24	25	26	27
31	30	31			

August 2018					
W	M	T	W	T	F
31			1	2	3
32	6	7	8	9	10
33	13	14	15	16	17
34	20	21	22	23	24
35	27	28	29	30	31

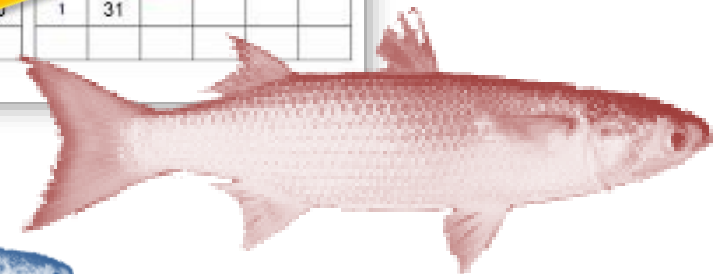
September 2018					
W	M	T	W	T	F
36	3	4	5	6	7
37	10	11	12	13	14
38	17	18	19	20	21
39	24	25	26	27	28

October 2018					
W	M	T	W	T	F
40	1	2	3	4	5
41	8	9	10	11	12
42	15	16	17	18	19
43	22	23	24	25	
44	29	30			

November 2018					
W	M	T	W	T	F
44					
45	5	6	7	8	9
46	12	13	14	15	16
	19	20	21	22	23
	26	27	28	29	30

December 2018					
W	M	T	W	T	F

Hierarchy



Summary
The size of 6 year old hatchery-produced specimens is equal to that of wild individuals of the same age class

Two year old mullets analysed were still **sexually immature**. Body growth and gonadal development of the hatchery-produced mullets proceeded slightly faster compared with wild-caught specimens

Three year old grey mullet exhibit **sex related growth** and **gonadal development** patterns

Three year old hatchery produced females and males exhibited **enhanced gonadal maturation** than that of wild-caught captive-reared fish, probably as a result of **domestication**

Thank you

Eldad Toledano



Aldo Corriero



Hanna Rosenfeld Iris Meiri Ashkenazi



Bill Koven

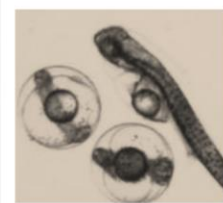


Oriya Nixon



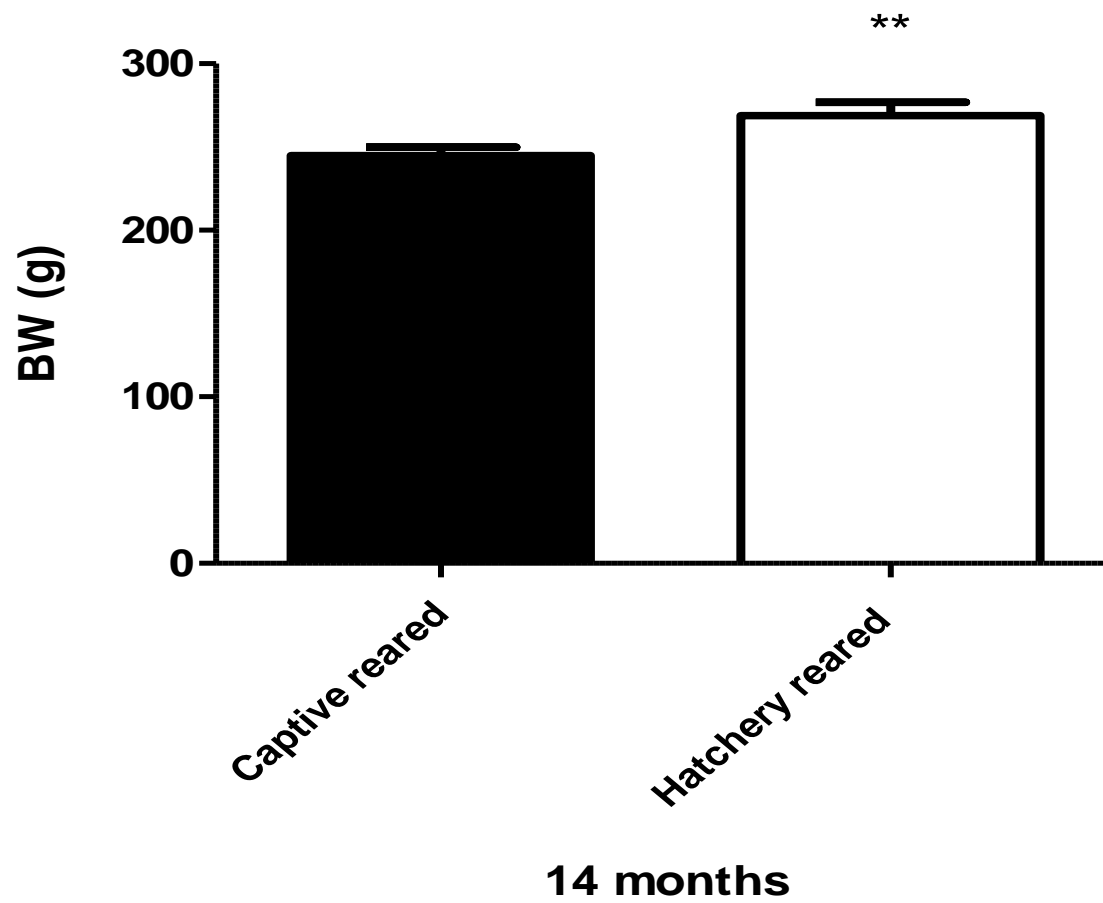
Barbara Colorni

Chen Bracha Vered Zlatnikov

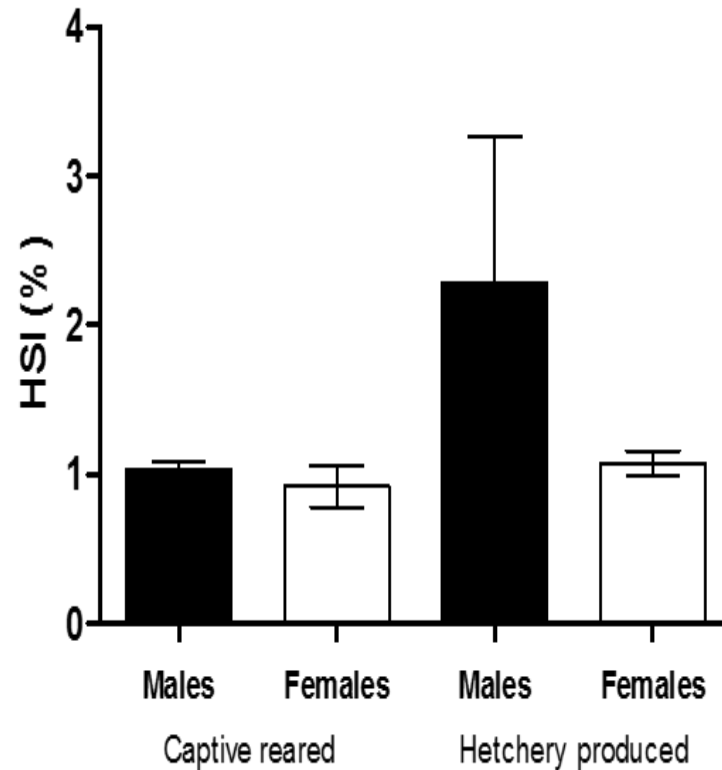


This project has received funding from the European Union's Seventh Framework Programme for Research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY)





Hepato Somatic Index



No difference was observed in HIS among the different experimental groups

Puberty in Fish

Developmental
of reproducing
competence of

comes capable
a functional

It starts some time
initiation of germ
the germ cell-s
in **first spermi-**



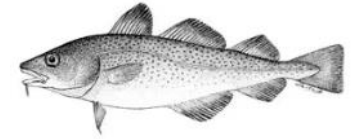
associated with the
differentiation of
and culminates

A variety of biotic and abiotic factors (e.g., availability
of food, population density, predators, photoperiod, temperature,
water quality, pollutants) are involved in the timing of puberty.

Puberty is a key component in life-history strategies of many fish species and has evolved to allow maximal reproductive fitness.

Why is puberty a big issue for the fish farming industry?

Early puberty might be a major **problem** in farmed fish. It might affect **growth, feed utilization, health** and **welfare**. Early puberty can also increase the risk for **negative genetic effects** of escapees on wild stocks or after spawning in sea cages



Under farm conditions some species (e.g. cod, sea bass, salmon) enter puberty precociously, leading to decreased flesh quality, growth performance and feed conversion, as well as low resistance to infectious diseases and an unattractive appearance to the consumer - all resulting in a lower commercial value.



An improved knowledge of the processes controlling puberty will aid in **enabling the maintenance of captive broodstock**, for restocking dwindling natural populations and for a sustainable fish farming industry.



Why is puberty a big issue for the fish farming industry?

Farming conditions

opposing
effects on fish
growth and
sexual maturity

- Improved food availability
- Domestication

Lack of appropriate
environmental stimuli

Promotion of sexual maturation
at reduced age and size

Sexual maturity is being **blocked**

Endocrine disruption may have dramatic consequences either on the alteration or inhibition of the time of spawning and on the quality of eggs and larvae produced



19 m3 tanks

IOLR spawning date: 31st October 2014

Wild-caught grey mullet of the equivalent cohort - originally caught in the Ebro River delta and imported from Spain

Five captive-reared specimens belonging to a 6-year old stock were sampled by P4. IOLR in early November 2016 and seventeen wild specimens, caught by traditional trap nets (lavoriera) in the Schiapparo Channel (Apulia, Italy) during their migration from the Lesina Lagoon to the South Adriatic Sea, were sampled by P13. UNIBA in early September 2016.

Ambient seawater salinity (40 ppt; C and photo-thermal regime

Scales

**Weight,
Length
and
Height**

Liver

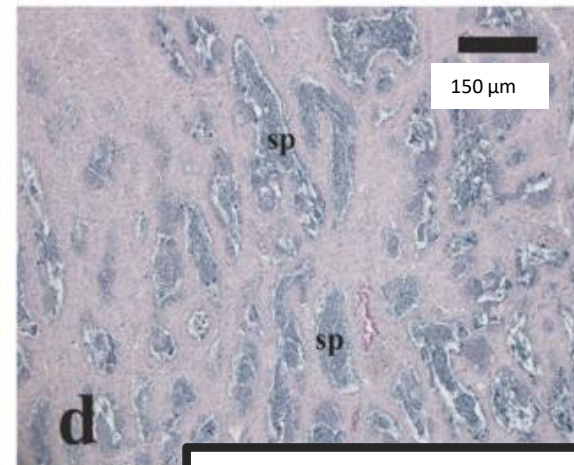
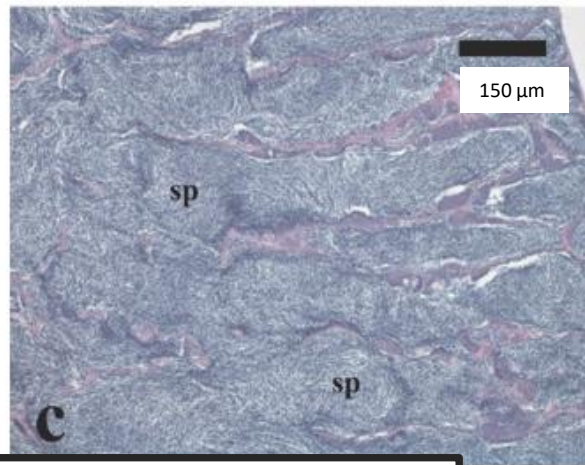
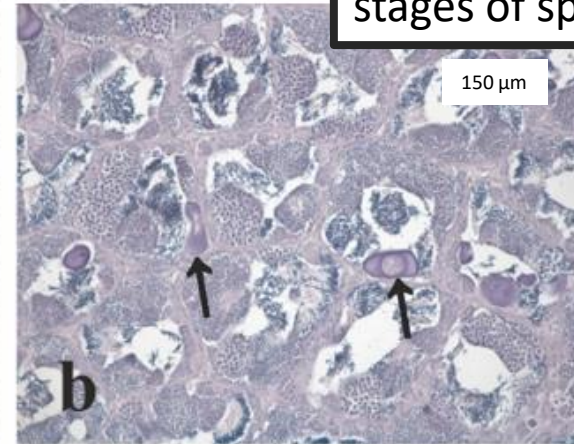
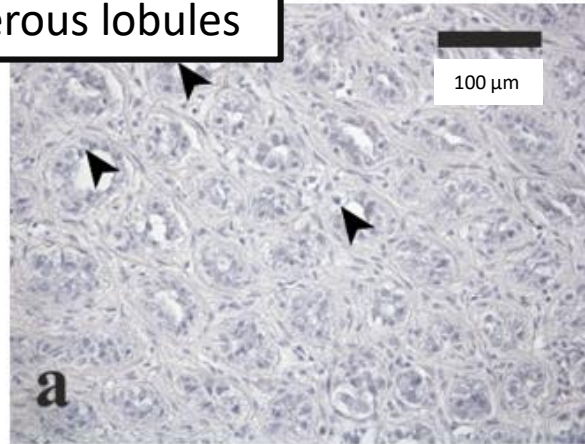
Pituitary

Blood

Gonads

Muscle

Testis - **immature** age 2
hatchery-produced :
small seminiferous lobules



Gonad - age 2 hatchery-
produced: intersex with all
stages of spermatogenesis.

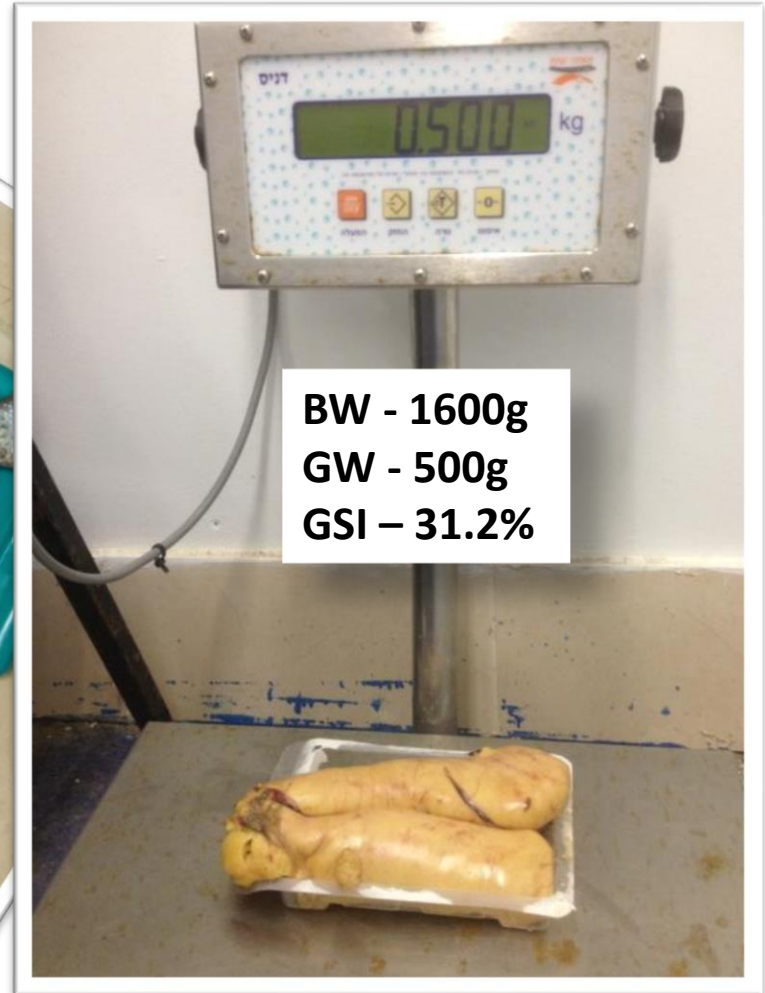
Testis - mature wild specimen sampled
during migration from the Lesina Lagoon
to the spawning ground of the Adriatic
Sea. Seminiferous lobule lumina are
filled with spermatozoa.

Spent testis from a hatchery-produced,
age 6 grey mullet showing residual
spermatozoa in the lumen of
seminiferous lobules.

6 yr old (G1) females and male



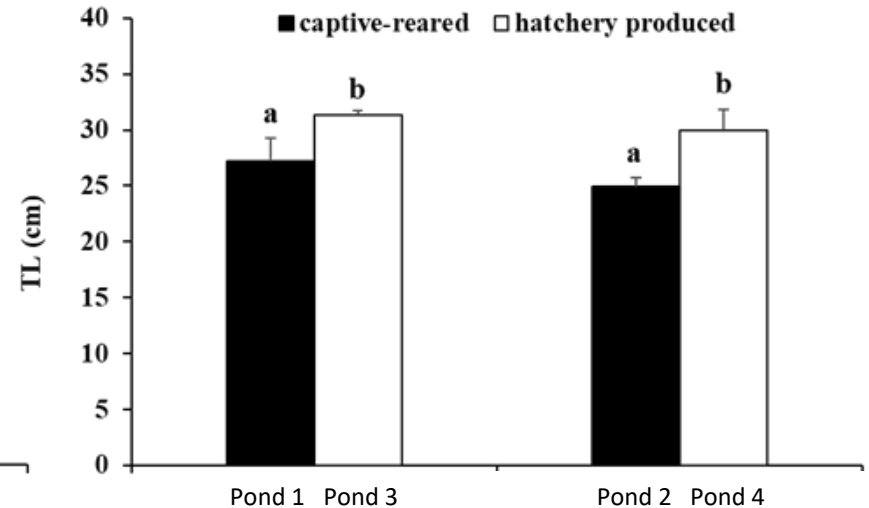
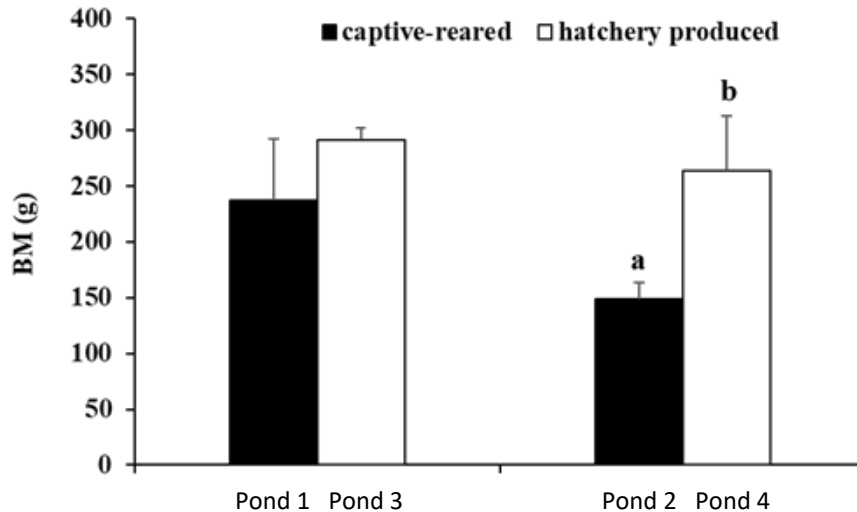
BW - 1760g
GW - 250g
GSI - 14.2%



- Differences in shape and colour
- Late vitellogenic oocytes, final maturation and ovulation

Body Weight and Total Length

2 yr fish



Hatchery produced 2 year old grey mullets appeared to be larger and heavier than cognate captive-reared individuals