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SEVENTH FRAMEWORK PROGRAMME

OVERCOMING GREY MULLET (*MUGIL CEPHALUS*) REPRODUCTIVE DYSFUNCTION IN CAPTIVITY: AN EXPANDED TOOL BOX FOR SUCCESSFUL BREEDING

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WORKSHOP ON GREY MULLET AQUACULTURE
14th May 2018, Palace Hotel, Bari (Italy)

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Mullet: a good candidate for aquaculture

High environmental value:

- A warm water euryhaline marine teleost with a worldwide distribution

- An efficient aquaculture bioremediator

High economic value:

- Highly priced roe in addition to the fish flesh

Mullets do not spawn spontaneously in captivity

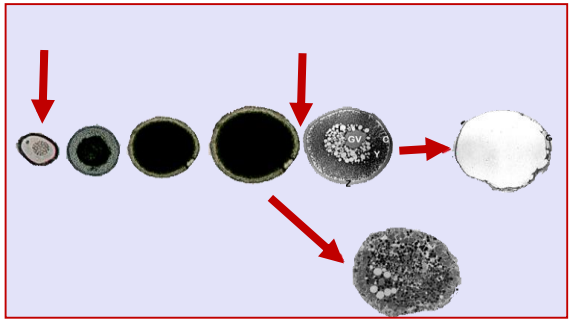
Lack of gamete release

Lack of reproductive development

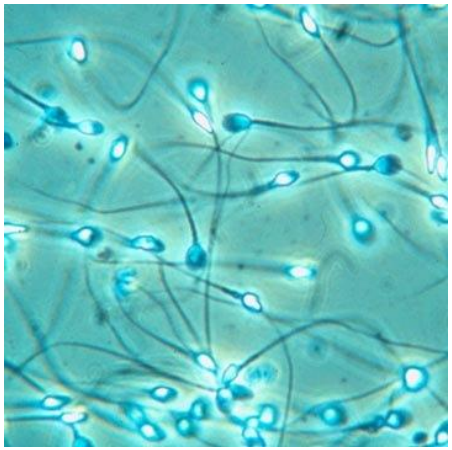
REPRODUCTIVE DISORDERS OF CULTURED FISHES

Mullets do not spawn spontaneously in captivity

- Females often “get stuck” at early stages of vitellogenesis
- Females do not undergo final maturation & spawning



- Spermiating males are rarely observed
- In most cases the produced milt is highly viscous and fails to fertilize the eggs



Mullets do not spawn spontaneously in captivity

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GENERAL AND COMPARATIVE ENDOCRINOLOGY

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Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition

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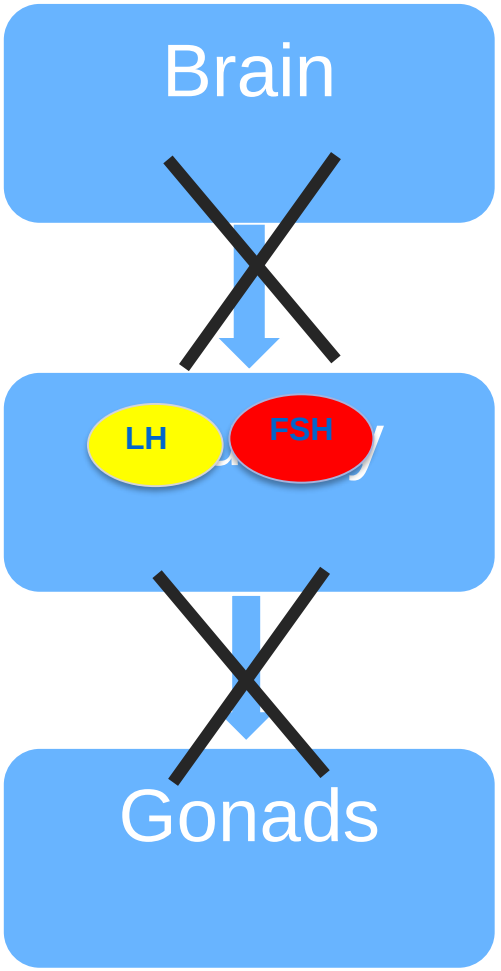
The implementation of hormone- therapy, which timed the administration of GnRH and an antagonist for dopamine D2 receptors with these critical stages, successfully enhance gonadal development and induced captive mullets to reproduce



Induction of early stages of gonadal growth, i.e., vitellogenesis and to a greater extent spermatogenesis, still need further optimization

Major aims

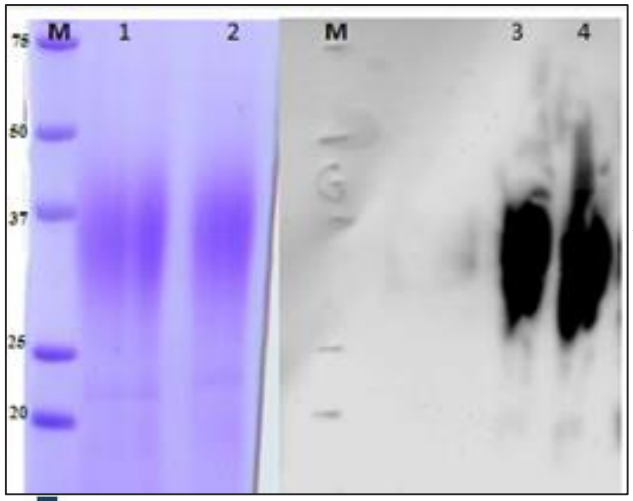
1. **To increase the abundance of spermiating males exhibiting high quality milt**
2. **To synchronize gonadal development in- and between sexes**
3. **To induce spawning**

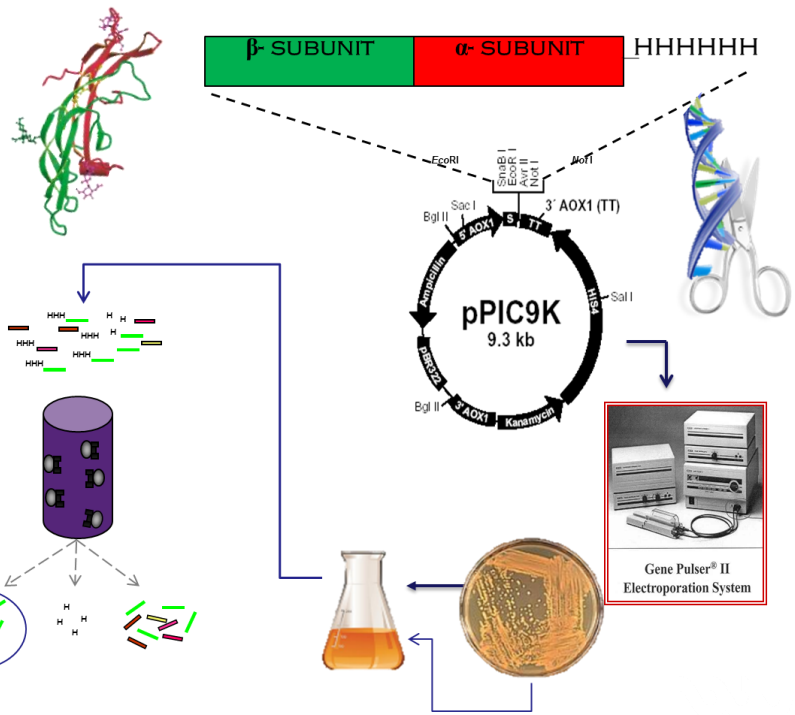
Specific objectives:

1. To produce bio-potent recombinant gonadotropins (r-LH and r-FSH)
2. To evaluate their potential to act as therapeutic agents alleviating reproductive dysfunction in captive mullet

Production and purification of grey mullet recombinant GtHs

- Recombinant GtHs (rLH and rFSH) were produced as a single chain chimera, utilizing the yeast, *Pichia pastoris*, expression system.
- The *P. pastoris* expression system was found to produce up to 4 mg recombinant GtHs per liter of culture supernatant
- Purity and integrity of the recombinant proteins were verified by SDS-PAGE and Western blot analyses





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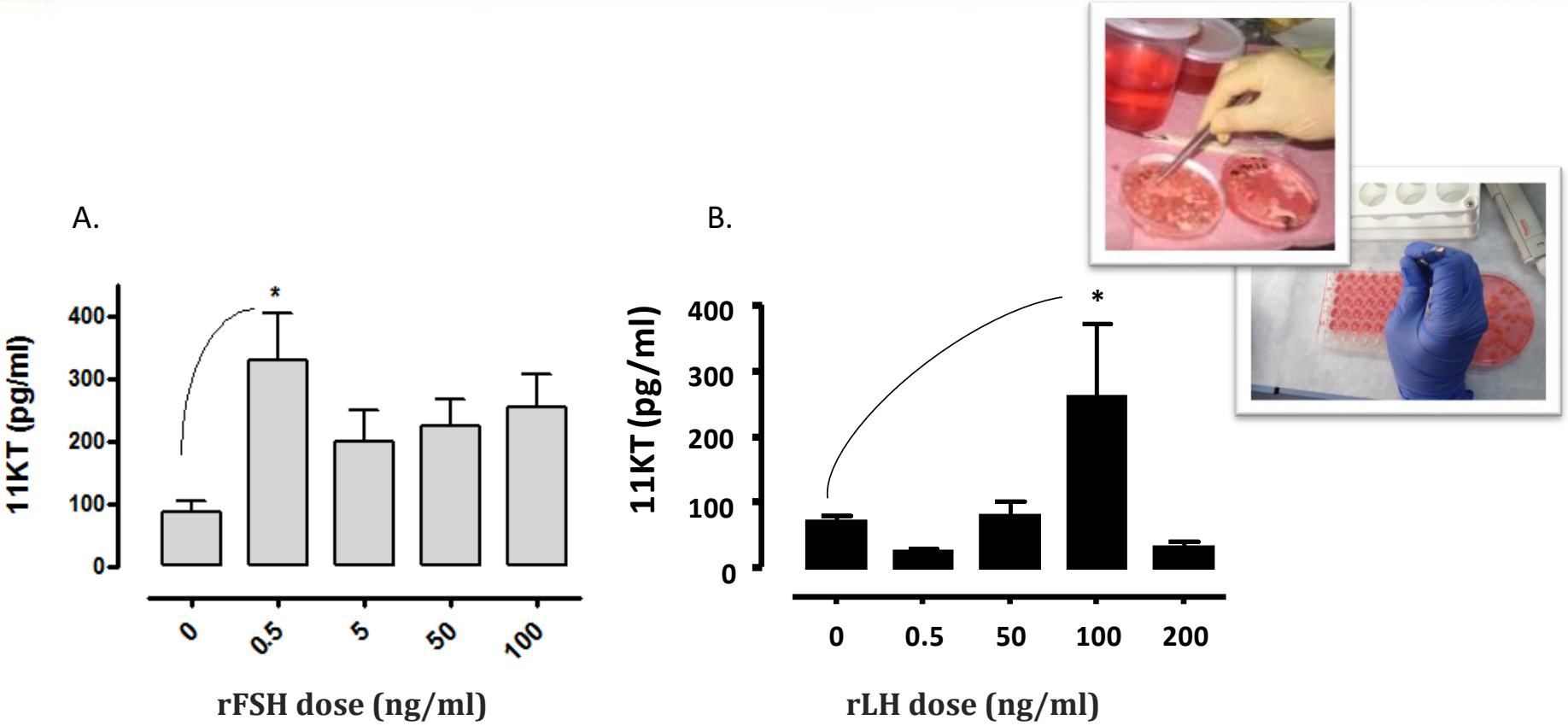
In vitro functional characterization of r-FSH and r-LH



Both r-FSH and r-LH stimulated 11-KT secretion 4-folds higher than the controls. Yet, r-FSH appears to be more potent than r-LH.

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In vivo functional characterization of r-FSH and r-LH

During the onset of the reproductive season (i.e., early August) grey mullet males received a single injection containing either: r-FSH, r-LH or saline (control).

Three weeks later, fish were sampled to evaluate treatment effects on:

- Body and gonad mass (BW; GSI)
- Testicular development
- Endocrine parameters

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r-FSH vs. r-LH *in vivo* effects

I. Body weight (BW) and Gonadosomatic Index (GSI)

Treatment Group	BW (g)	GSI (%)
C	950 ± 49	0.045 ± 0.010^{a}
r-FSH	863 ± 41	0.088 ± 0.012^{b}
r-LH	890 ± 52	0.072 ± 0.014^{ab}

The r-FSH- treated males exhibited significantly ($P < 0.05$) higher GSI values

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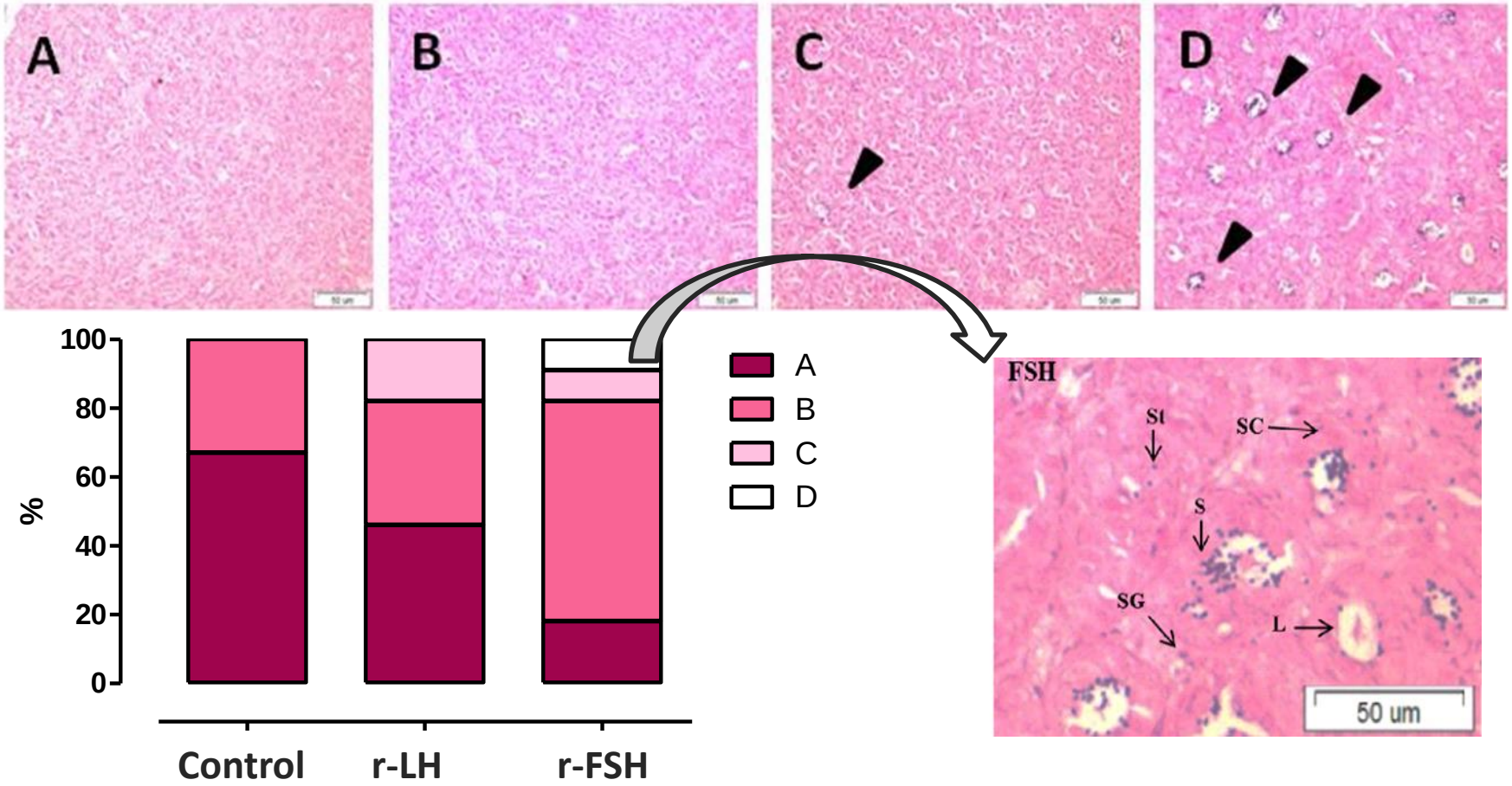
r-FSH vs. r-LH *in vivo* effects

II. Spermatogenic development

H&E staining



r-FSH and to a lesser extent r-LH enhanced spermatogenic development among captive mullet males.

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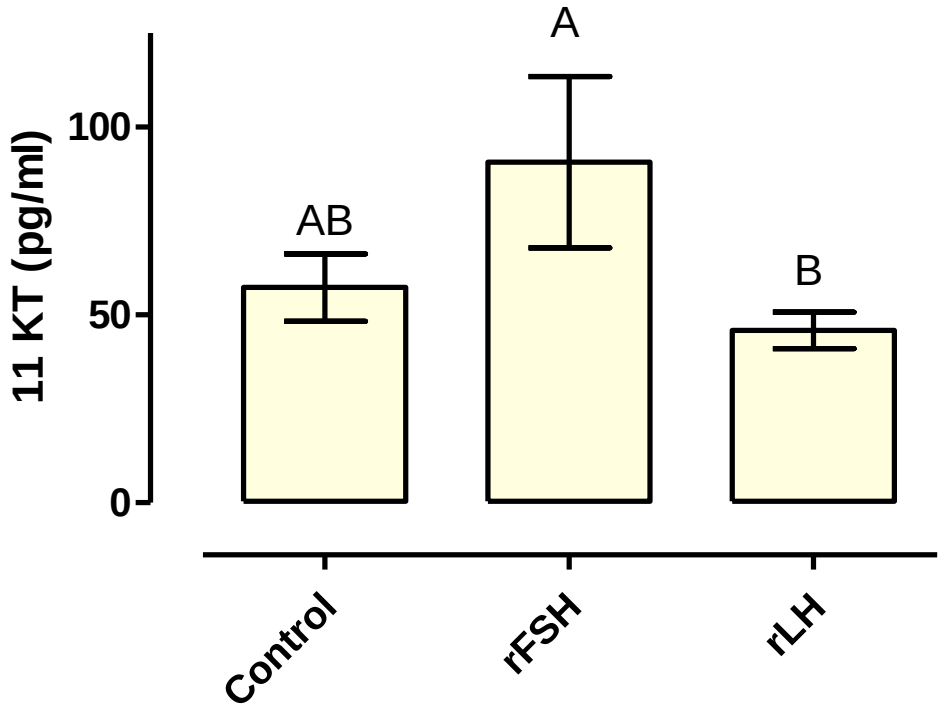
r-FSH vs. r-LH *in vivo* effects

III. Plasma levels of 11-KT



r-FSH treated males exhibited significantly ($P < 0.05$) higher plasma 11-KT levels as compared to those treated with r-LH.

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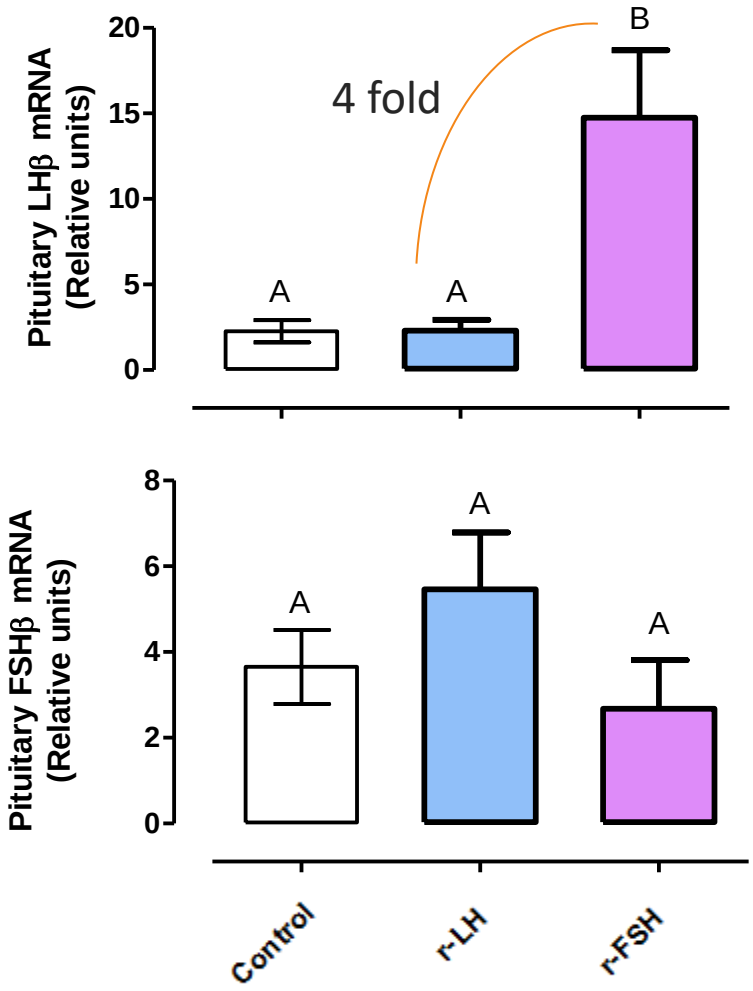
r-FSH vs. r-LH *in vivo* effects

IV. Pituitary expression of LH and FSH β-subunits



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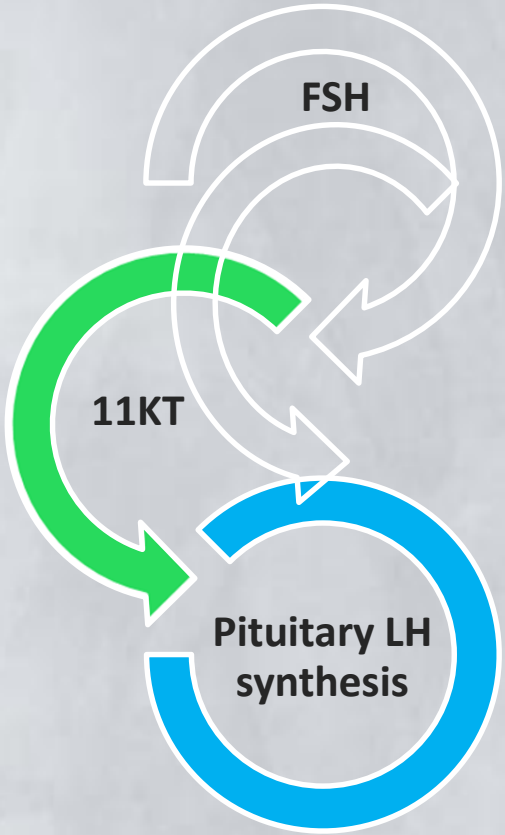
Interim conclusion

The obtained results demonstrate positive effects of r-FSH on pituitary LH synthesis and on 11-KT secretion giving rise to enhanced gonadal growth and development.

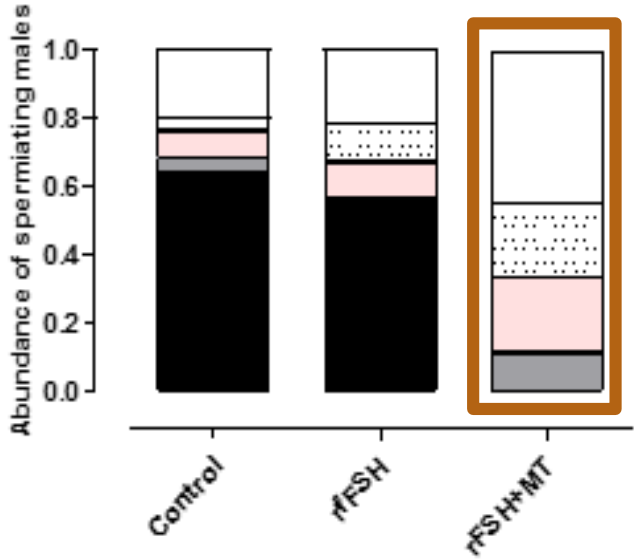


rFSH vs. MT treatment

1) rFSH (5 µg/kg rFSH))
2) rFSH (5 µg/kg rFSH) + MT-EVAc implantation (2-weeks apart)
3) Saline only (control group)

Treatment effect on sperm production

0- no milt,
1- only traces
2- small amounts
3- fluid milt
4- flowing fluid, easily spread in the water



MT-EVAc

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Hormonal treatment effects on synchronized ovarian development

DA-Dopamine Antagonist (15 mg/KgBW)
rFSH- recombinant FSH (5 µg/kg BW)
GnRH- GnRH-EVAc implant (36 µg per implant)

Relative abundance of post-vitellogenic females

	Control	DA	DA+GnRH	DA+rFSH
Exp. I	27%	58%	71%	
Exp. II	29%			91%

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Spawning induction

- **Spawning trials were carried out from Mid-September throughout November.**
- **Fully mature fish (i.e., post vitellogenic female and spermiating males) were selected and received two injections consisting of GnRH & DA given 22.5 h apart.**

Control				Treatment			
No. of induction trials	Spawning success (%)	Fecundity (million eggs /KgBW)	Fertilization rate (%)	No. of induction trials	Spawning success (%)	Fecundity (million eggs /KgBW)	Fertilization rate (%)
2	0	0	0	2	0	0	0
1	0	0	0	3	100	2.6 ± 0.55	0-98
0	0	0	0	2	0	0	0
				3	66	2.1±0.39	0-80
				1	100	0.6	30
6	50	1.99±0.9	50-100				
5	0	0	0	2	0	0	0
				1	0	0	0
1	100	1.64	0				
2	50	2.75	90				
17	29.4			14	42.9		

Spawning successes has been improved in the pre-treated vs. control groups (42.9% and 29.4%, respectively).

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Breeding unit effects on spawning success

During the natural spawning season three breeding units varying in ratios of female : male and tank size, were tested

	X10 1 female 3 males 1 m^3	X5 2 females 3 males 1 m^3	X2 3 females 6 males 3 m^3
Egg release/ induction trials	**50%**	**80%**	**100%**
Fertilized eggs/ spawning event	**80%**	**75%**	**50%**
Hatching success	92.6 ± 3.5%	72.9 ± 11.6%	73.4 ± 7.3%

The basic unit consisting of a single female and 2-3 males leads to better fertilization rate and hatching success.

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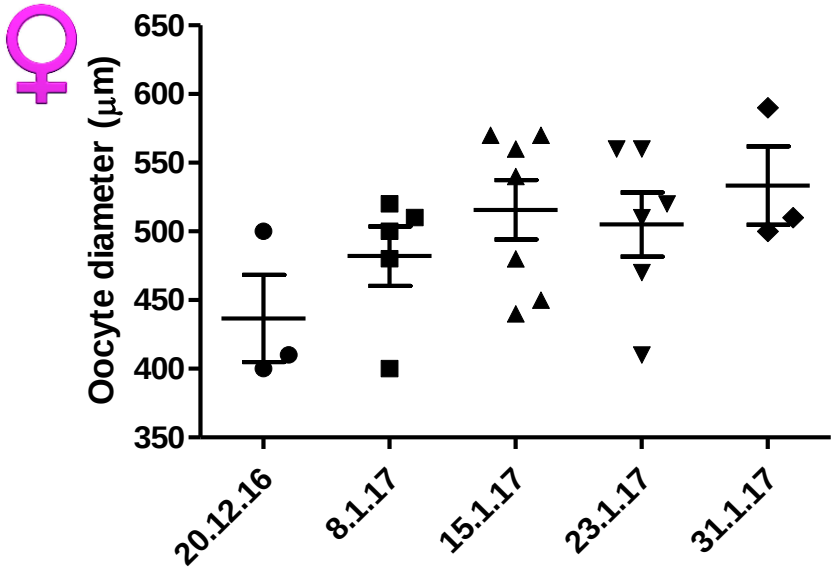
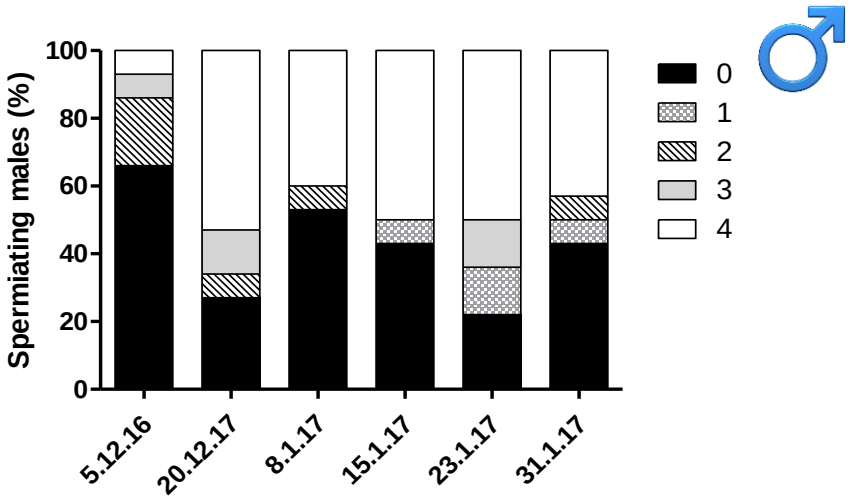
Breeding success during artificially shifted spawning season

4-month shifted photo-thermal regime





Date	Female BW (g)	Floating eggs (ml)	Sinking eggs (ml)	Fecundity (10^6 eggs/Kg BW)
20.12.2016	1660	272	710	2.1296
15.1.2017	2310	1070	180	1.9481
15.1.2017	2470			
23.1.2017	1930	1310	70	2.5741

The established breeding protocol for captive grey mullet was effectively applied during artificially shifted spawning seasons

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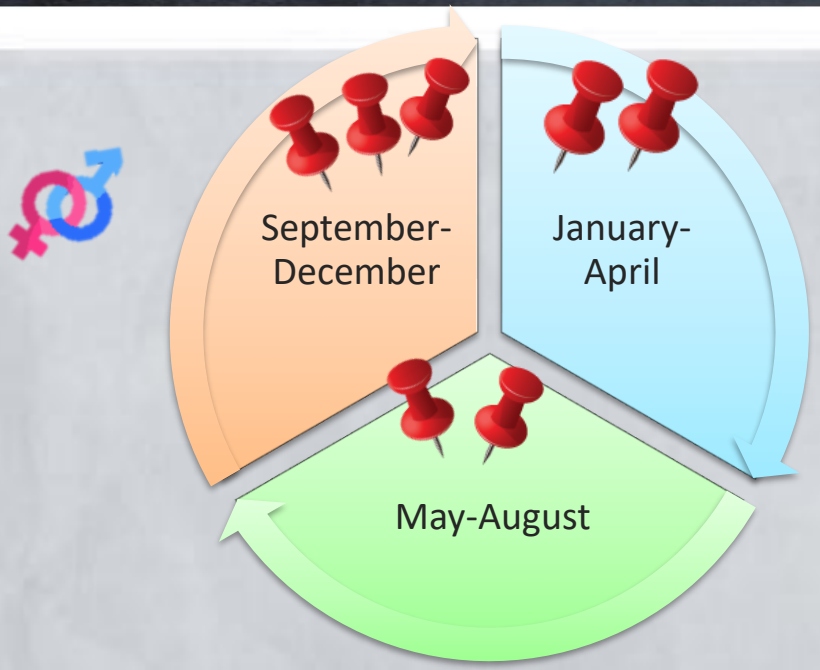
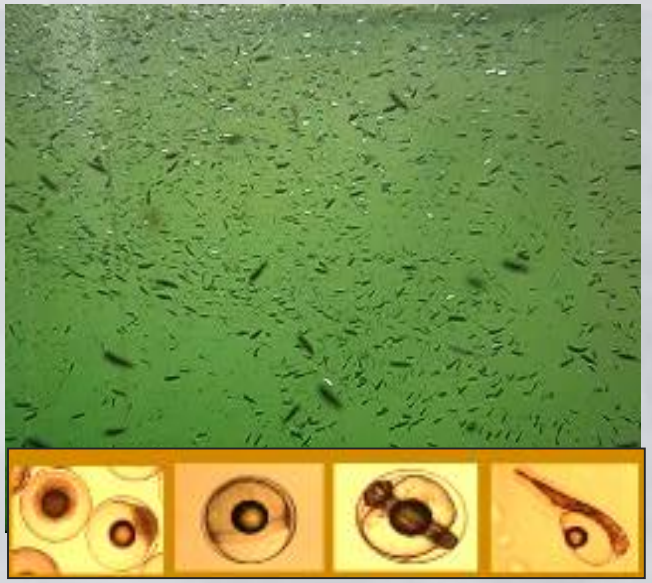
Summary of the spawning data





- A relatively extended natural spawning season (~3 months).
- Shifted spawning season can be easily achieved via photo-thermal manipulation.
- Improved spawning success (60%) among hormonally induced females.
- Improved synchronization among breeding units increased fertilization rate (>70%).
- Relatively high fecundity (Av. 1.76 ±0.52 million eggs/kg)
- Hatching rate: 78.84 ± 11.93 %
- Over two hundred thousand fingerlings were produced

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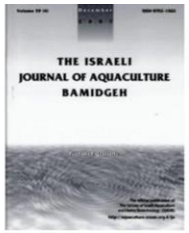

Genetic Stock Identification of the Flathead Grey Mullet (*Mugil cephalus*) in Lake Tiberias Based on Parent-Offspring Relationship

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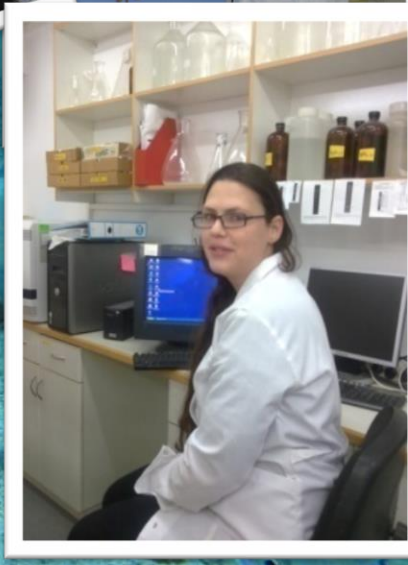
Thank you



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