DIVERSIFY

New species for EU aquaculture

SEVENTH FRAMEWORK PROGRAMME

WP 3 : REPRODUCTION & GENETICS

Maturation and spawning induction of Grey Mullet (*Mugil cephalus*)

Israel Oceanographic & Limnological Research

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IRTA

Grey mullet (*Mugil cephalus*)

Characteristics:

A warm water euryhaline marine teleost with a worldwide distribution

High environmental value:

An efficient aquaculture bioremediator

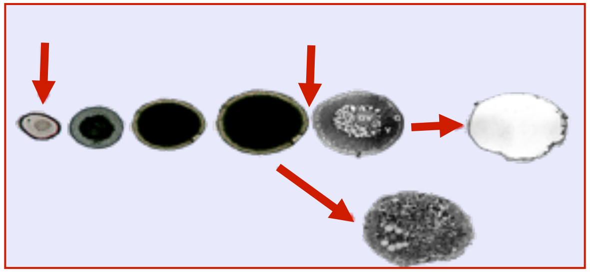
High economic value:

Highly priced roe in addition to the fish flesh value

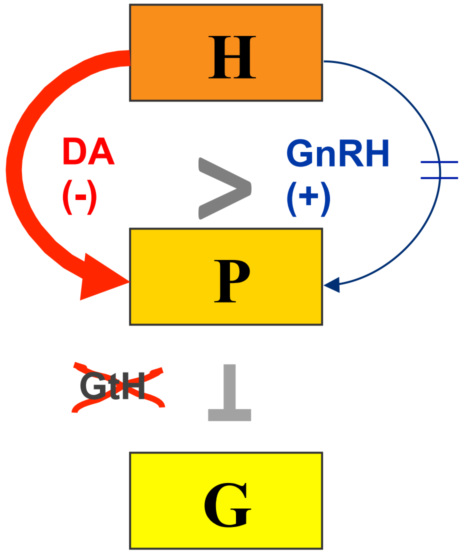
The problem: Mullet fingerling supply for aquaculture comes almost exclusively from the wild, as techniques for artificial spawning remain incomplete

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



Dopaminergic inhibition appears to have a role in two different junctures of the reproductive cycle of grey mullet, i.e. early stages of gametogenesis & the stage of final gamete maturation (Aizen et al., 2005)

Major aims

- **To increase the abundance of spermiating males exhibiting high quality milt**
- **To synchronize gonadal development in captive grey mullet females & males**
- **To induce spawning**

Specific objectives:

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

Production and purification of grey mullet recombinant gonadotropins

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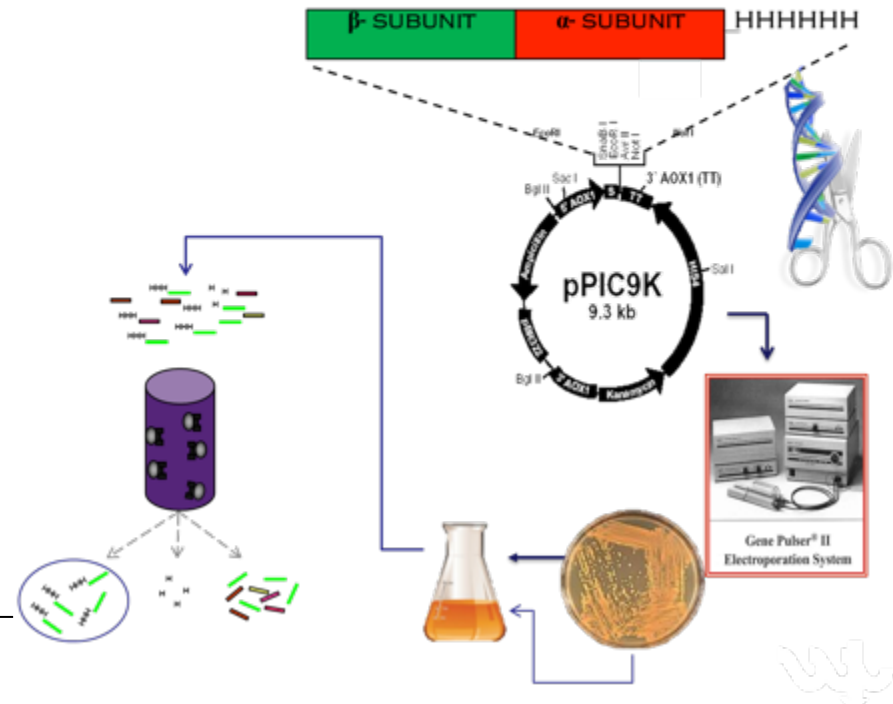
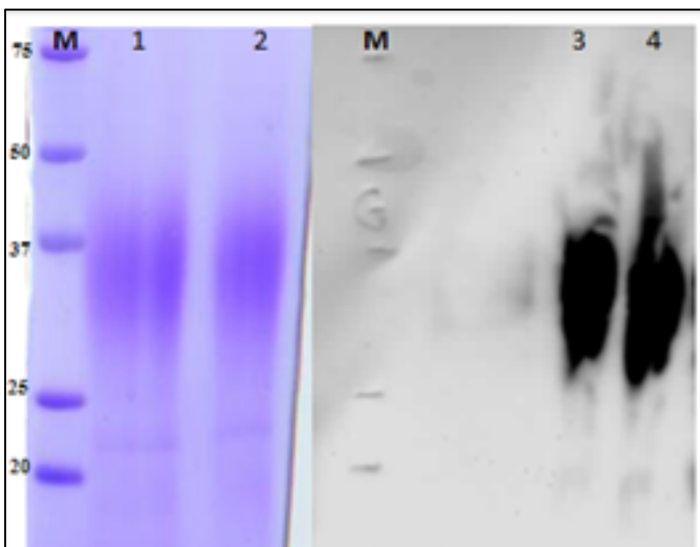
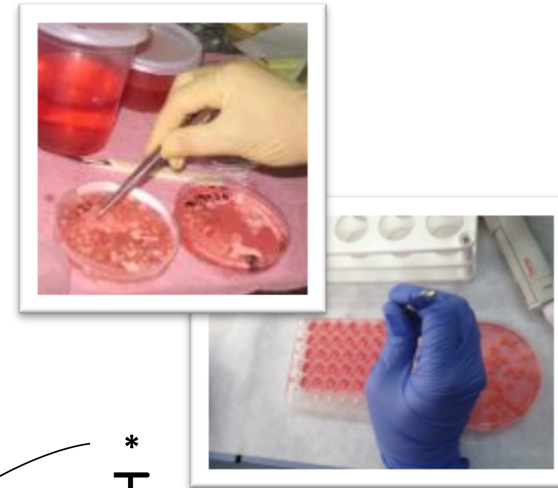


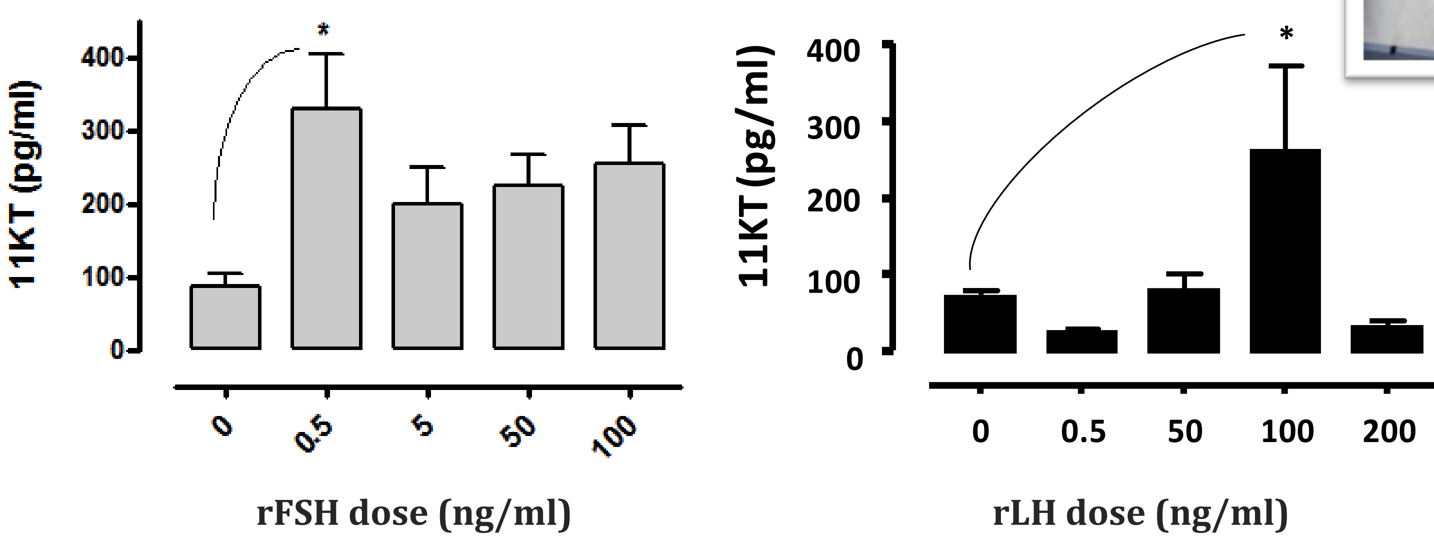


The lab-scale *Pichia pastoris* fermentation generated over 100 mg/L

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



A. B.



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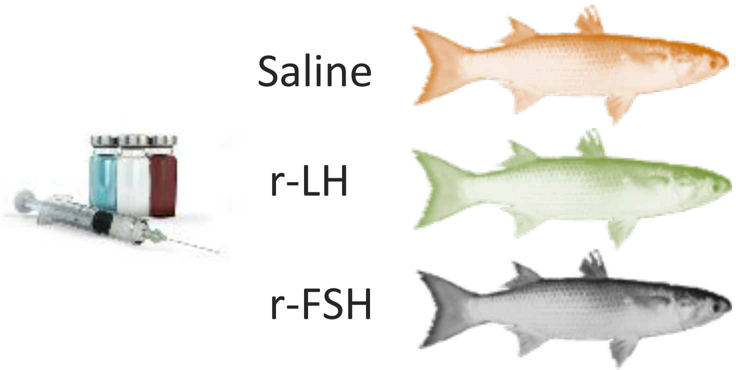
In vivo functional characterization of r-FSH and r-LH

Experimental design

During the onset of the reproductive season (early August, 2014) 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:

1) Body and gonad mass (BW and GSI values);
2) Testicular development;
3) Endogenous pituitary-gonad endocrine axis (i.e., gonadotropin β-subunit synthesis and sex steroid circulating levels).

In vivo effects of r-FSH and r-LH:

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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]

No significant difference in BW across all treatment groups.

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

In vivo effects of r-FSH and r-LH:

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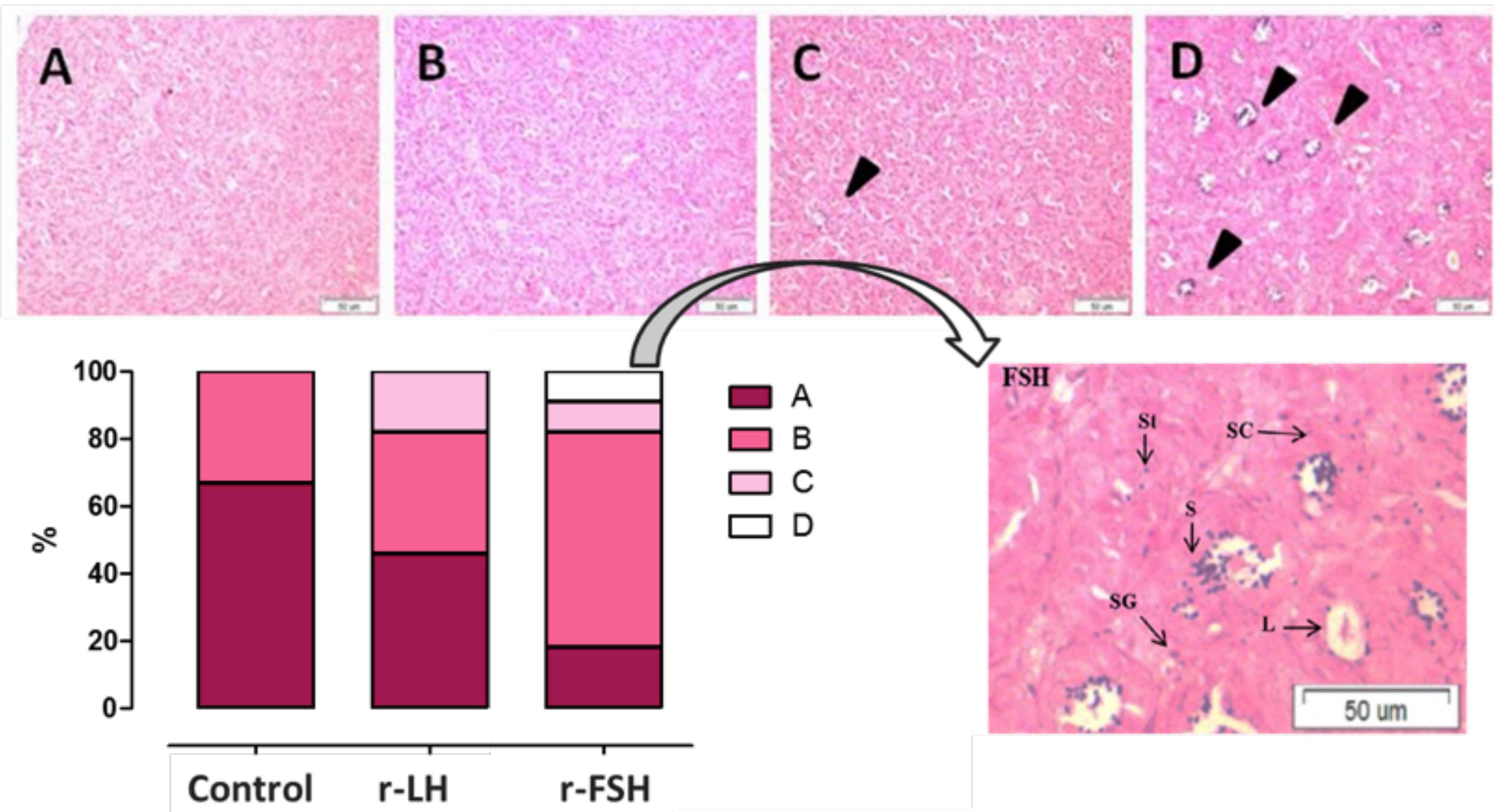
II. Spermatogenic development



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

In vivo effects of r-FSH and r-LH:

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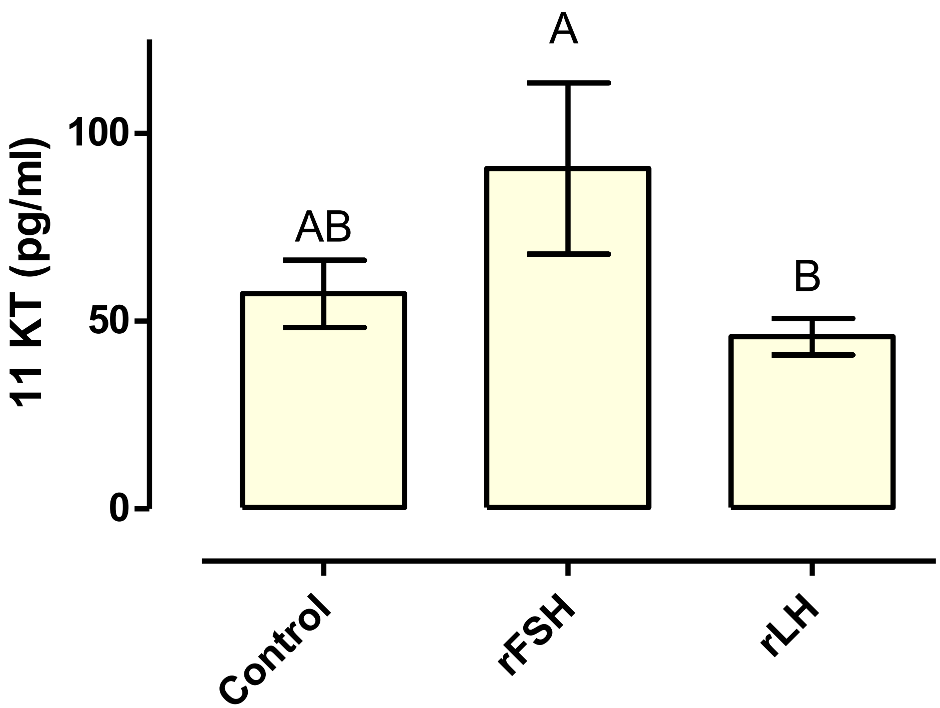
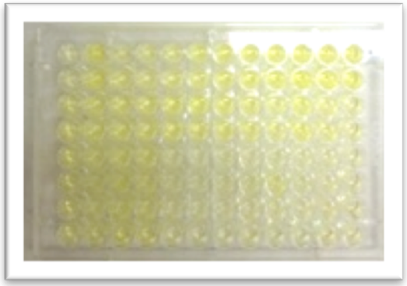
III. Plasma levels of 11-KT





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

In vivo effects of r-FSH and r-LH:

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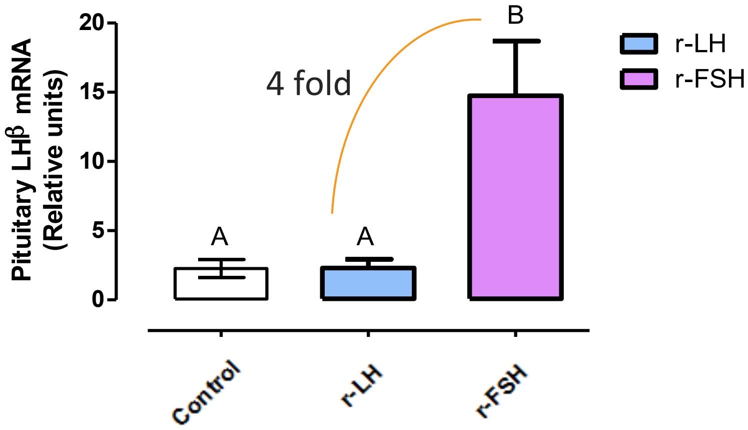
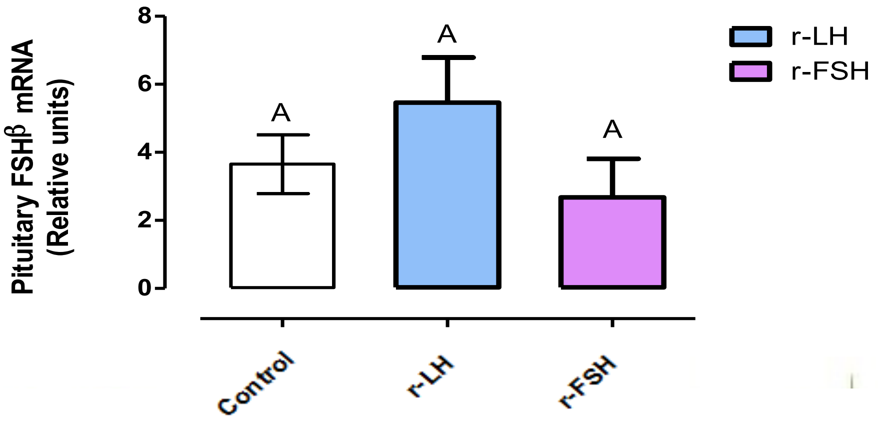
IV. Pituitary expression of LH and FSH β-subunits





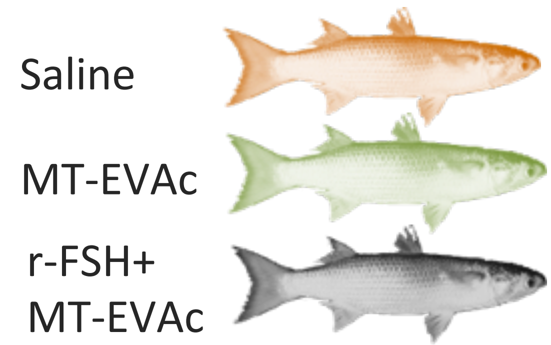
Interim conclusion

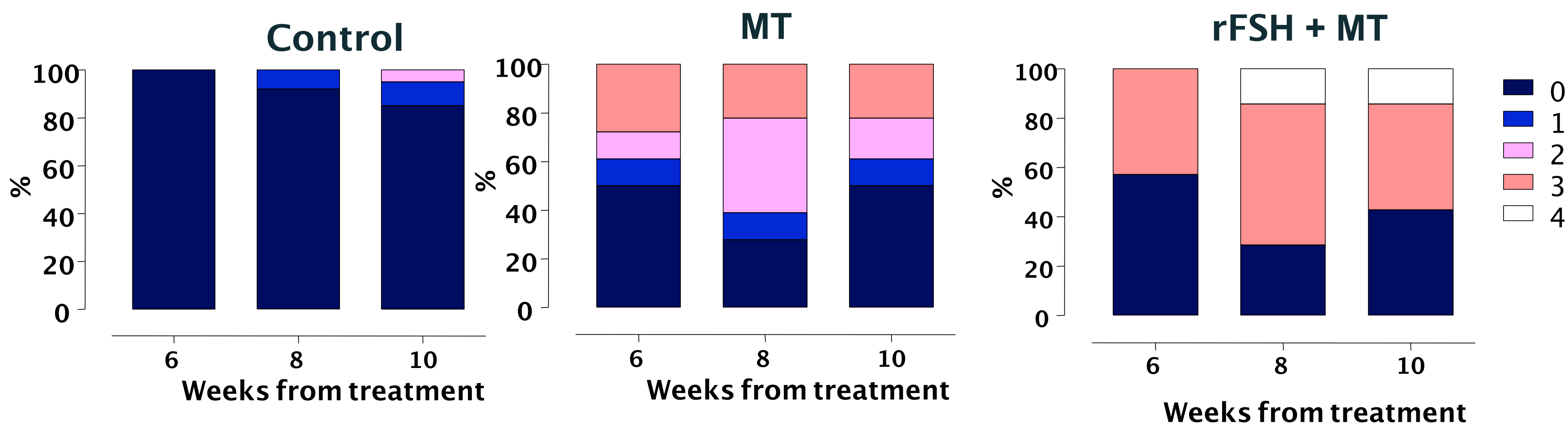
The obtained results demonstrate positive effects of r-FSH on pituitary LH synthesis and on 11-KT secretion giving rise to enhanced gonadal growth and development.

rFSH vs. MT treatment

First series of trials

1) 17α-methyltestosterone-EVAc (4 mg/Kg BW)
2) rFSH (5 µg/kg rFSH) + MT-EVAc implantation (2-weeks apart)
3) Saline only (control group)





The combined rFSH+MT treatment and to a lesser extent the MT- EVAc implantation increased the abundance of spermiating males and enhanced steady milt production.

rFSH vs. MT treatment

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Second series of trials

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

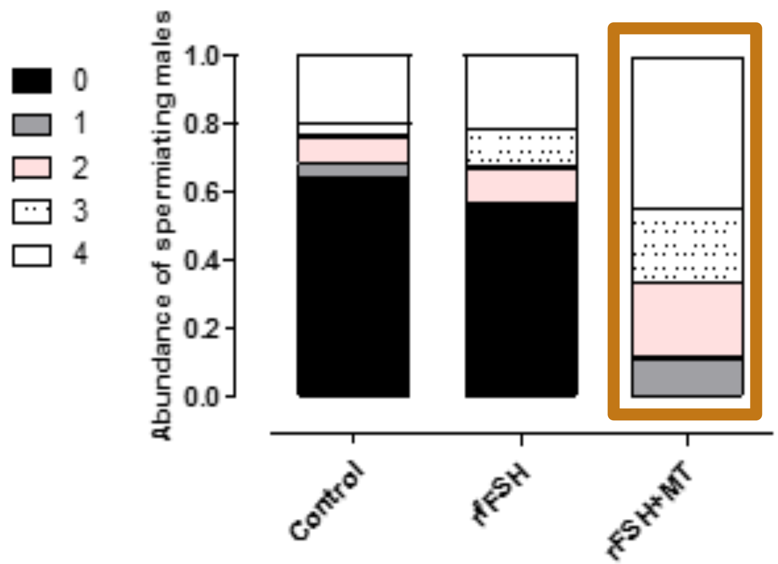
Saline

r-FSH

r-FSH+ MT-EVAc

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



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)

Saline

DA

DA+
GnRH--EVAc

DA+
r-FSH+

Relative abundance of post-vitellogenic females

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

The DA+r-FSH was found to be the best performing treatment, giving rise to 91% post vitellogenic females

Hormonal treatment effects on spawning success

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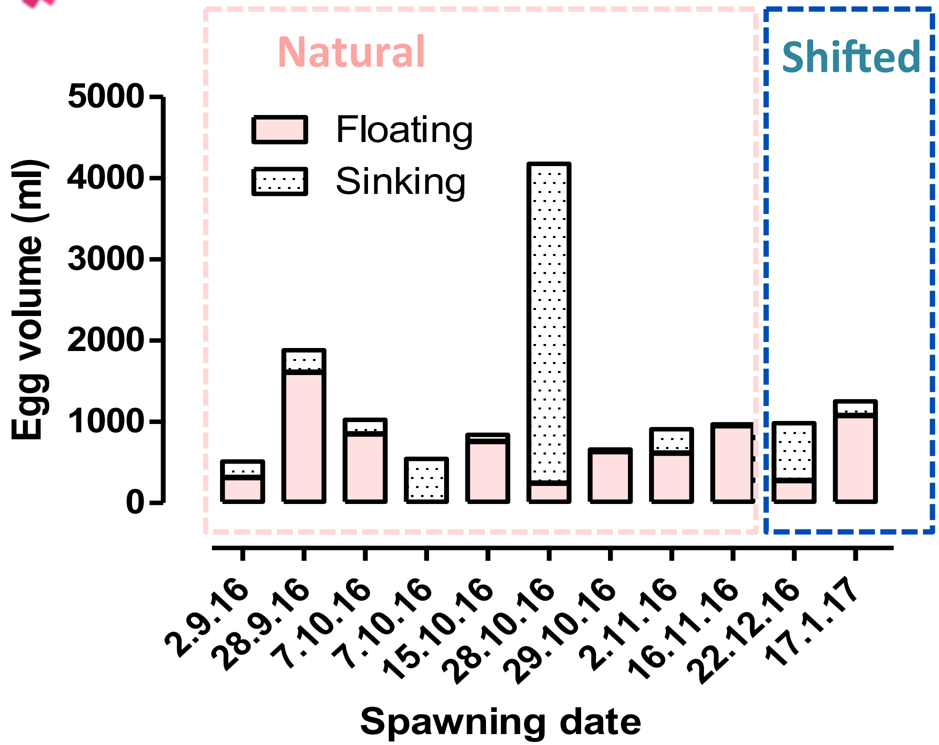
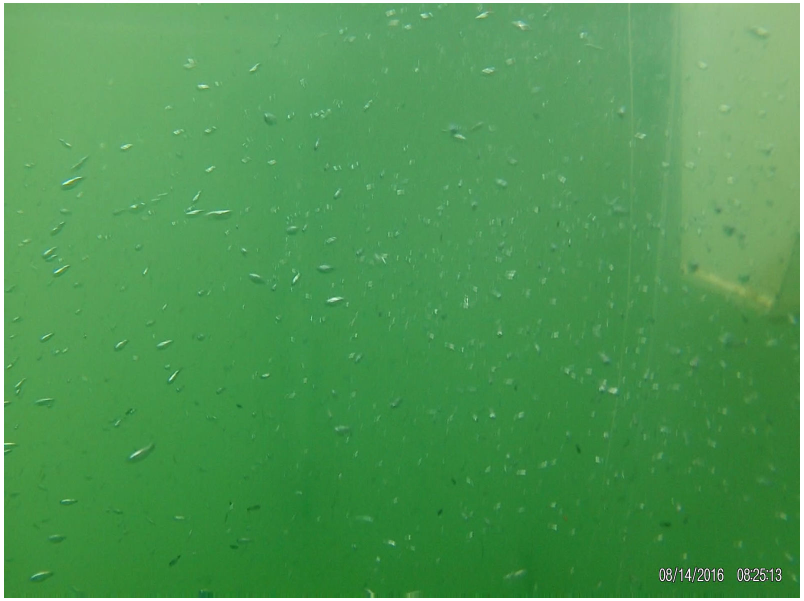
Spawning induction protocol:
Two injections consisting of **GnRH & DA** given **22.5 h** apart.

Date	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 (%)
10.9.14	2	0	0	0	2	0	0	0
29.9.14	1	0	0	0	3	100	2.6 ± 0.55	0-98
6.10.14	0	0	0	0	2	0	0	0
22.10.14					3	66	2.1±0.39	0-80
25.10.14					1	100	0.6	30
30-31.10.1	6	50	1.99±0.9	50-100				
5.11.14	5	0	0	0	2	0	0	0
14.11.14					1	0	0	0
21.11.14	1	100	1.64	0				
22.11.14	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).

Spawning season (2016)





- 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%).
- Fifty million eggs in total (average fecundity: 1.76 ±0.52 million eggs/kg)
- Hatching rate: 78.84 ± 11.93 %
- Over two hundred thousand fingerlings were produced

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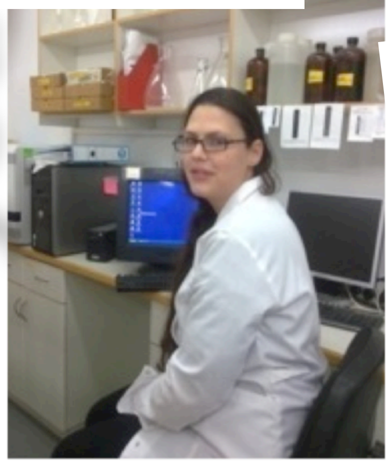
Thank you

Chen Bracha



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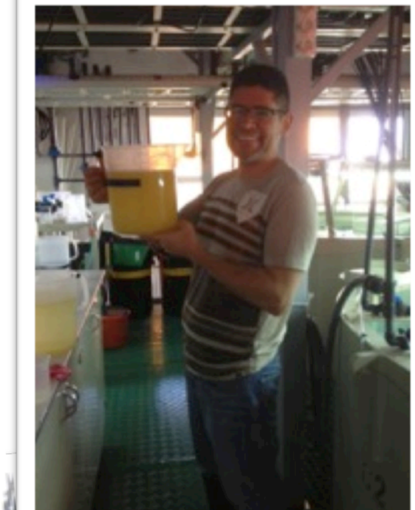

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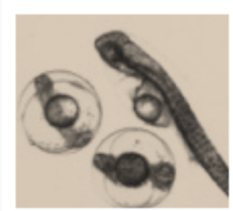

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