Knowledge transfer workshops – Atlantic halibut
Hjelmeland, 11-12 September 2018

Reproduction & Genetics - GnRHa induction of "ovulation" in Atlantic halibut

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The Hellenic Center for Marine Research

Three research institutes

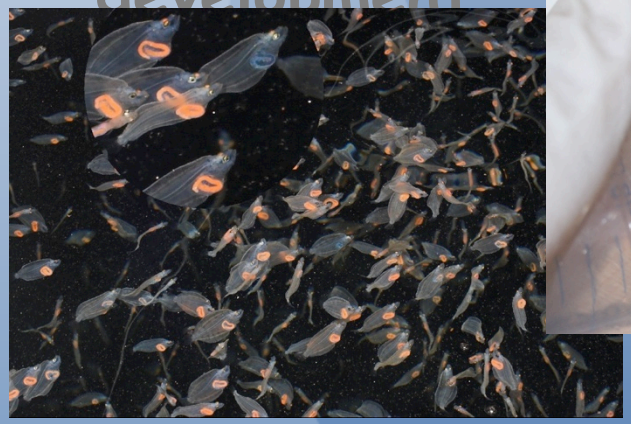
- Institute of Marine Biology, Biotechnology & Aquaculture
- Institute of Marine Biological Resources & Inland Waters
- Institu

Broodstock management
Spawning induction

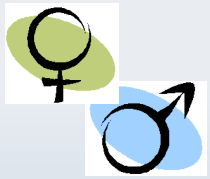
Atlantic halibut

-objectives

- Optimize ovulation kinetics and stripping, F1 vs wild breeders
- Larval rearing using ongrown *Artemia*, early weaning and improvement of juvenile quality
- Production of VNN capsid protein development



The reproductive axis



Environment
(photoperiod, lunar cycle, temperature, rainfall)

Brain

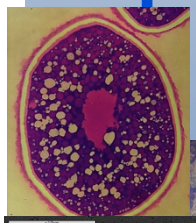
GnRH + | Dopamine -

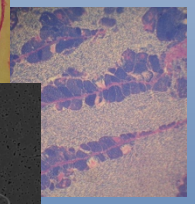
Pituitary

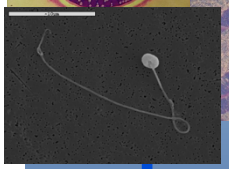
Follicle stimulating hormone (FSH)

Luteinizing hormone (LH)

Gametogenesis (spermatogenesis & vitellogenesis)







Androgens
Estrogens

Gonads

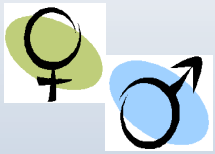
Maturation

Maturation inducing steroids (Progestagens)

Fully grown, differentiated gametes

Fertile gametes (spermiation, ovulation)

Reproductive dysfunctions of fish in captivity



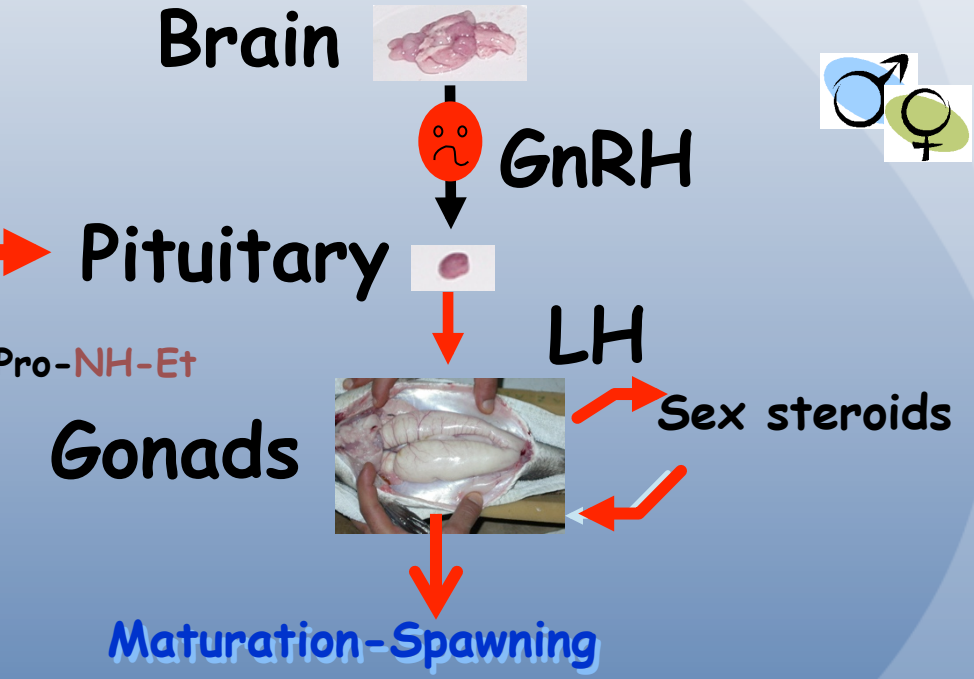
- No gametogenesis at all (females & males)
- No oocyte maturation-ovulation (females)
- Reduced sperm production/quality (males)
- Spawning, but no fertilization (males, flatfish)
- Ovulation, but no spawning (females)

in vitro fertilization

Use of GnRHa in spawning induction

synthetic GnRH agonists (GnRHa)

pGlu-His-Trp-Ser-Tyr-DAla-Leu-Arg-Pro-NH-Et



GnRHa injection or controlled release

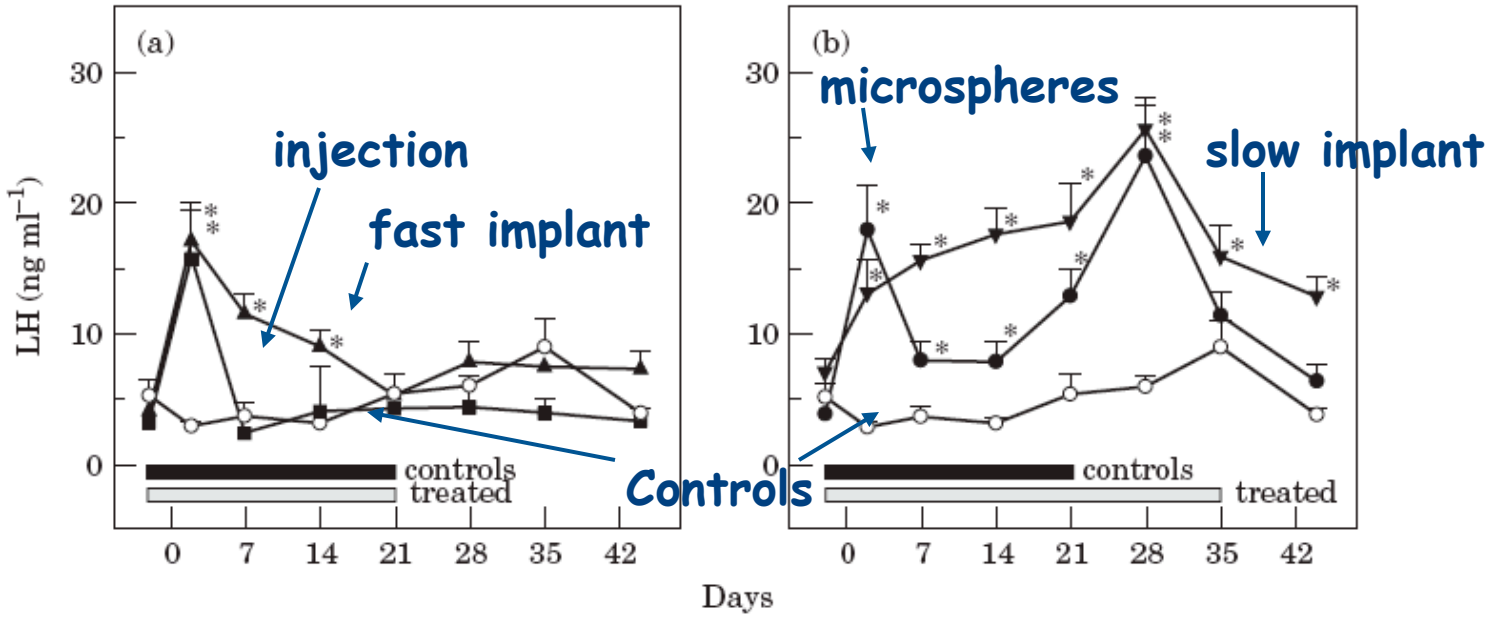


FIG. 2. Plasma luteinizing hormone (LH) levels, after treatment with different GnRHa-delivery systems. (a) Groups treated with GnRHa fast-release systems, ■, injection (IN) and ▲, EVAc implants; (b) groups treated with GnRHa slow-release systems, ●, microspheres (MC) and ▼ EVSL implants, in both cases compared with ○, controls. The horizontal bars indicate the duration of the spermiating period (expressible milt>0·6 ml kg^{-1} BM). *, Significant (P<0·05) differences from the corresponding control group, for each sampling point. Data are mean ± S.E.M. (n=8).

Mañanos *et al.* (2002) J. Fish Bio.

Ovulation and spawning of Senegalese sole (*Solea senegalensis*)

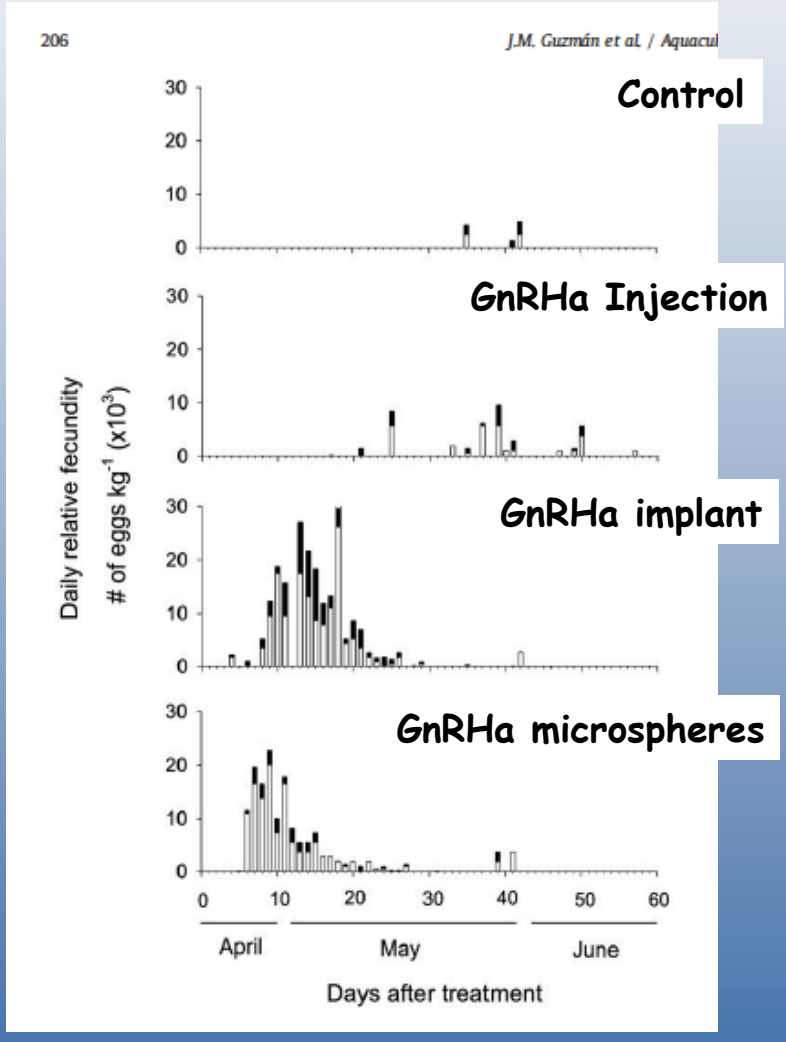


Table 2
Spawning characteristics of cultured Senegalese sole treated with, saline (controls, CNT), GnRHa injection (INJ), GnRHa implant (IMP) or GnRHa microspheres (MIC).

	CNT	INJ	IMP	MIC
No. of spawns	5	13	26	26
Spawning period[a] (d)	9	32	39	37
Total relative fecundity[b] (eggs kg^{-1})	9720	41,960	208,800	141,880
Daily[b] relative fecundity (eggs kg^{-1})	1940 ± 9			5720 ± 1310
Egg buoyancy (%)	58 ± 10			27 ± 5
Egg size[c] (μm)	979 ± 3	971 ± 6	979 ± 1	978 ± 1
Hatching success (%)	0	0	0	0

No fertilization!

Guzman *et al.* (2009) Aquaculture

GnRHa injection or controlled release

Saline injections
1. easy to prepare, inexpensive

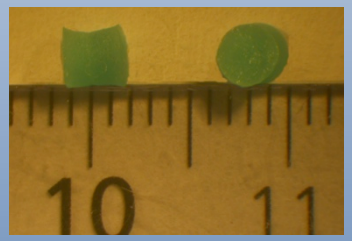
but

2. short term LH increase
3. multiple treatments
4. ineffective in some fishes

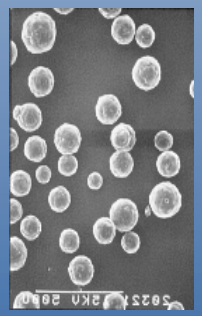
Controlled-release systems
1. difficult to prepare, expensive

but

2. long term LH increase
3. single treatment
4. effective in all fishes tested



Solid implants



Microspheres (biodegradable)

Atlantic halibut

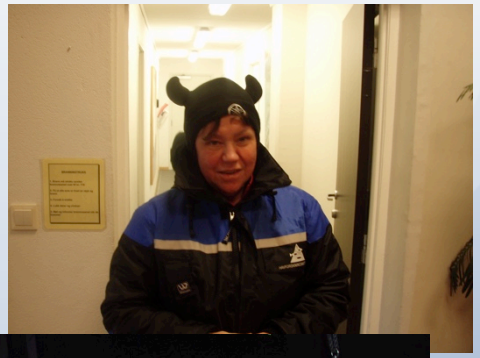
-problems with hatchery-produced breeders

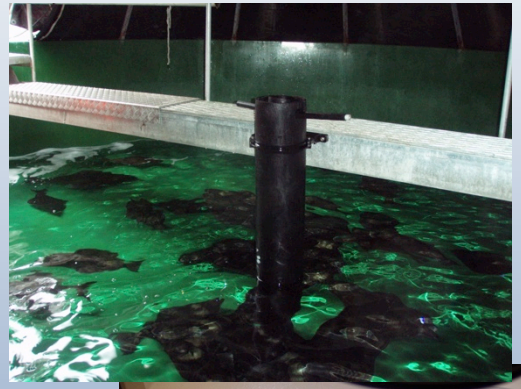
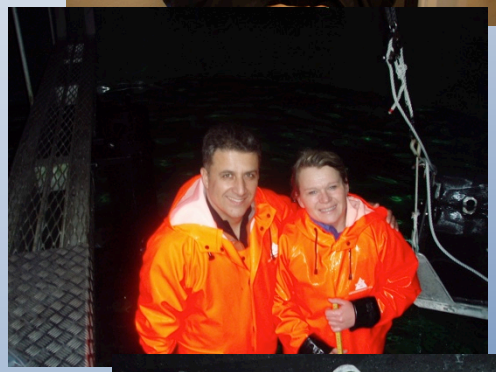
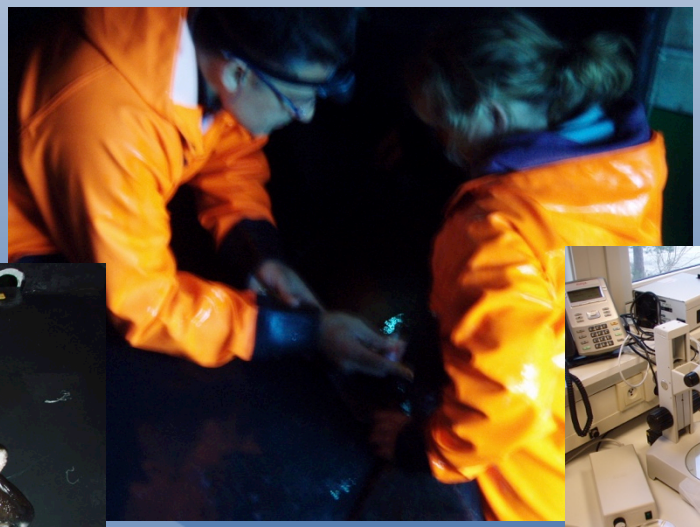
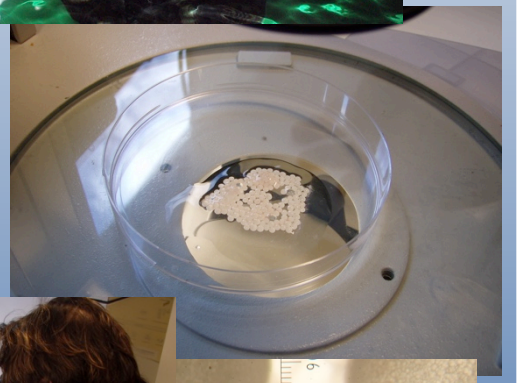
Mature and ovulate, but

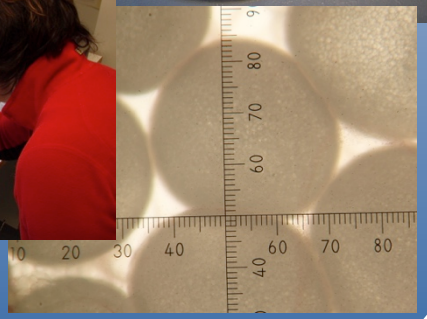
- irregular spawning cycles,
- low and unstable fertilization,
- low gamete survival and lower realized fecundity than wild females

Atlantic halibut

-treated females with GnRHa implants

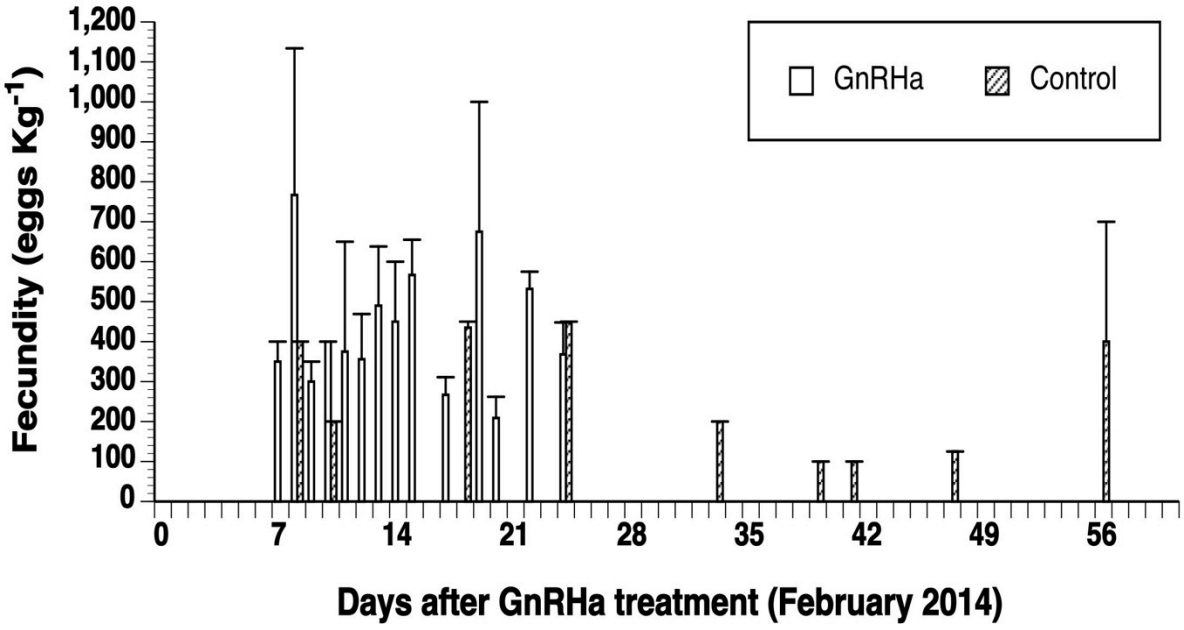


Atlantic halibut

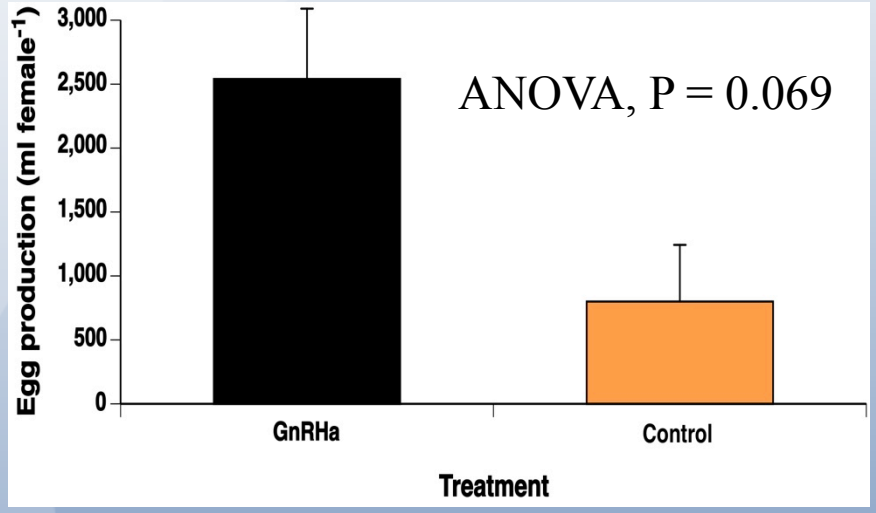
- trials at IMR with GnRHa implants



- All GnRHa implanted fish ovulated, one Control did not
- Two Control females ovulated after 18-24 days, when most GnRHa implanted females completed ovulation
- GnRHa implants synchronized ovulation (3 vs 8 weeks in controls)

Atlantic halibut

-trials at IMR with GnRHa implants



- A clear trend towards higher fecundity
- Similar egg quality characteristics

Atlantic halibut

-trials at SWH with GnRHa implants

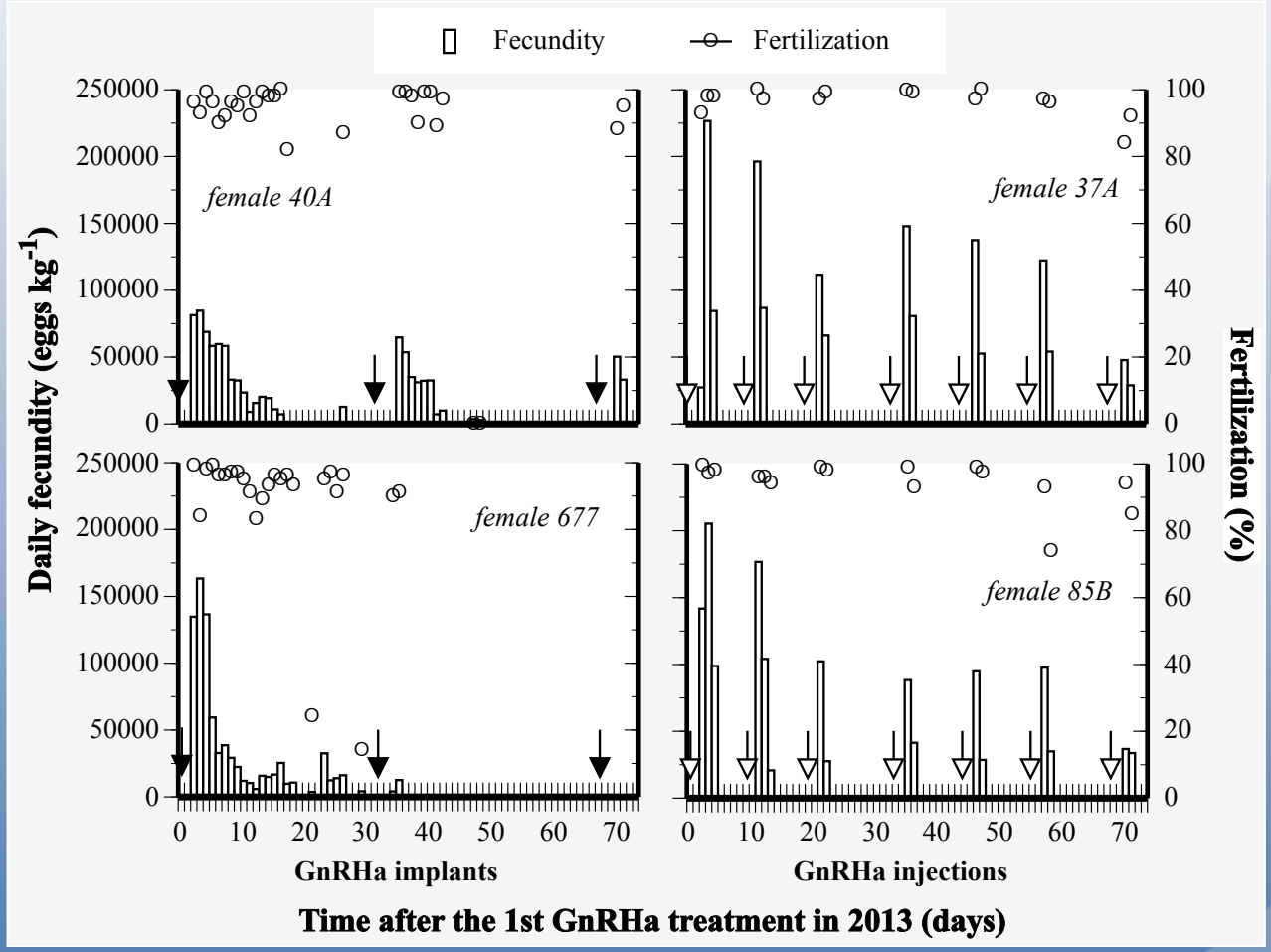
- All GnRHa implanted fish ovulated, whereas some Controls did not
- GnRHa implants synchronized ovulation (17-23 days, vs 17-37 days in Controls)
- Similar fecundity and fertilization success (186-400 ml kg^{-1}, 36-53%, respectively)

Atlantic halibut

-GnRHa induction of ovulation

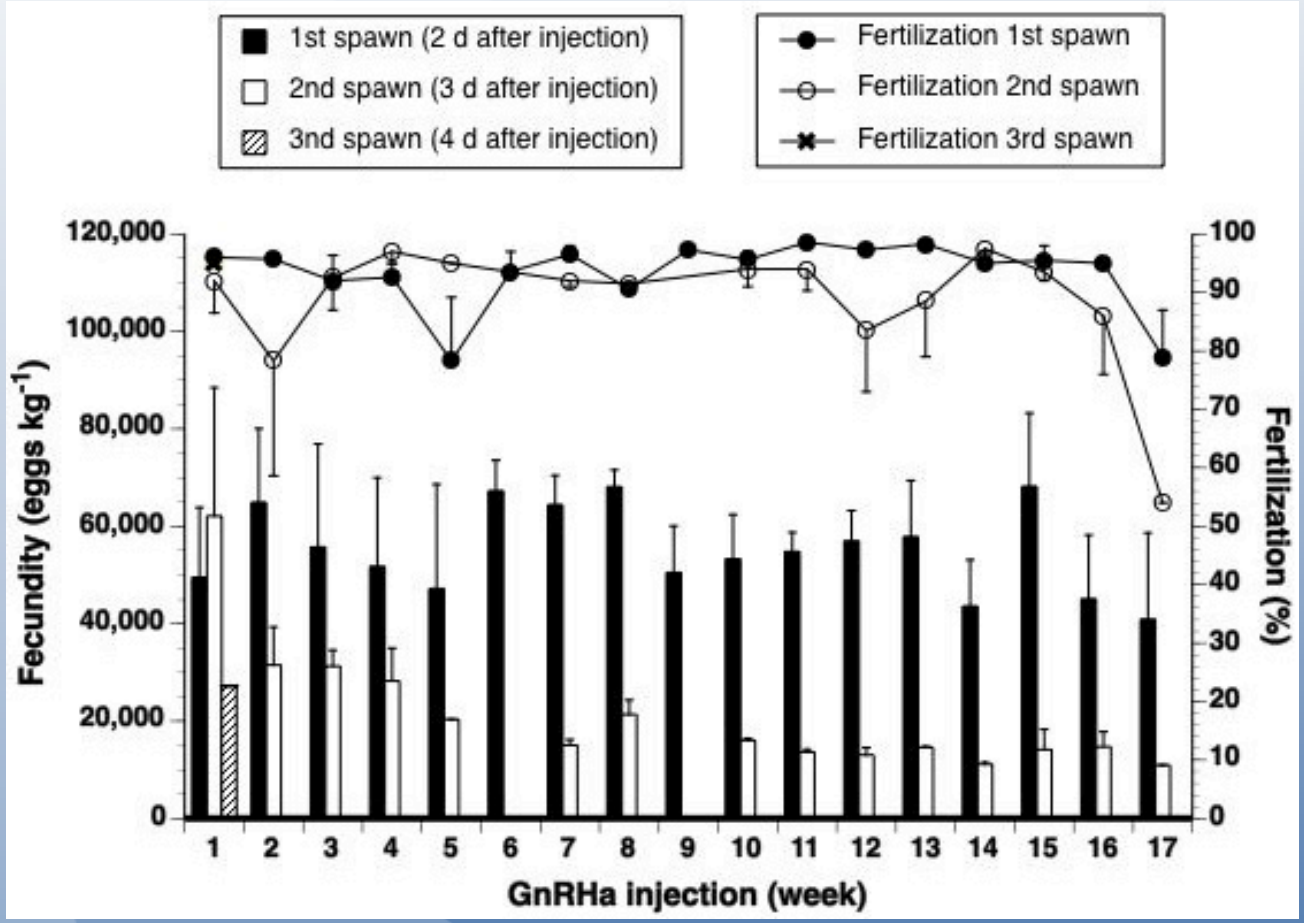
- GnRHa implants ensured that all fish ovulated multiple times (>3 per fish), while some Controls in all 3 trials never ovulated
  - But, perhaps "well-timed" multiple GnRHa injections may be more suitable??
- GnRHa implants synchronized ovulation
  - Earlier completion of egg collection activities
  - Less handling of the fish, less labor required

Fecundity and fertilization in meagre: GnRHa injection vs implants



Mylonas et al. (2015) Aquaculture

Multiple controlled spawning with GnRHa injections



Mylonas et al. (2016) Aquaculture

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