Control of sexual maturity in cod farming – status and future solution

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Cod farming in the Nordic Countries, 21. sept. 2011, Reykjavik, Iceland

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Outline / status

- **Photoperiod** used to delay/arrest maturation.
- **Triploid** cod being tested, some experimental results from Norway and Canada.
- **All female** production possible using hormones or UV+pressure
- Alternative **sterility** models, molecular tools being tested at lab scale at IMR and NOFIMA

Sauaneset area at Austevoll

The sea cages at Austevoll

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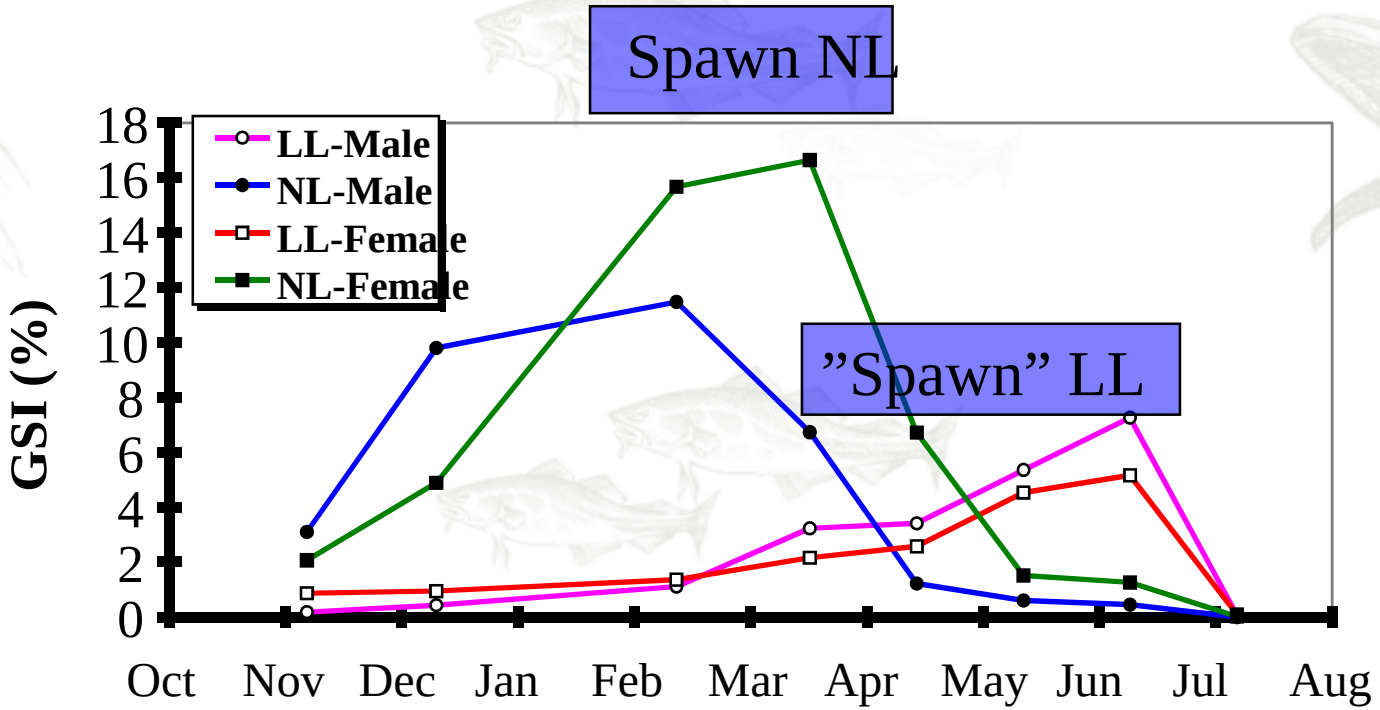
Photoperiod



Continuous light from summer solstice:

- **Netpens**: Maturation usually delayed, not arrested
- **Indoor tanks**: Arrested for at least 3 yrs

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Taranger et al., 2006

Why the difference between indoor and outdoor systems?

- Indoor true LL, no influence from ambient photoperiod
- Higher light intensity through water column
- Reflections produces light from all sides

→ The difference between natural and added light much larger in smaller systems, and usually much higher light intensity.

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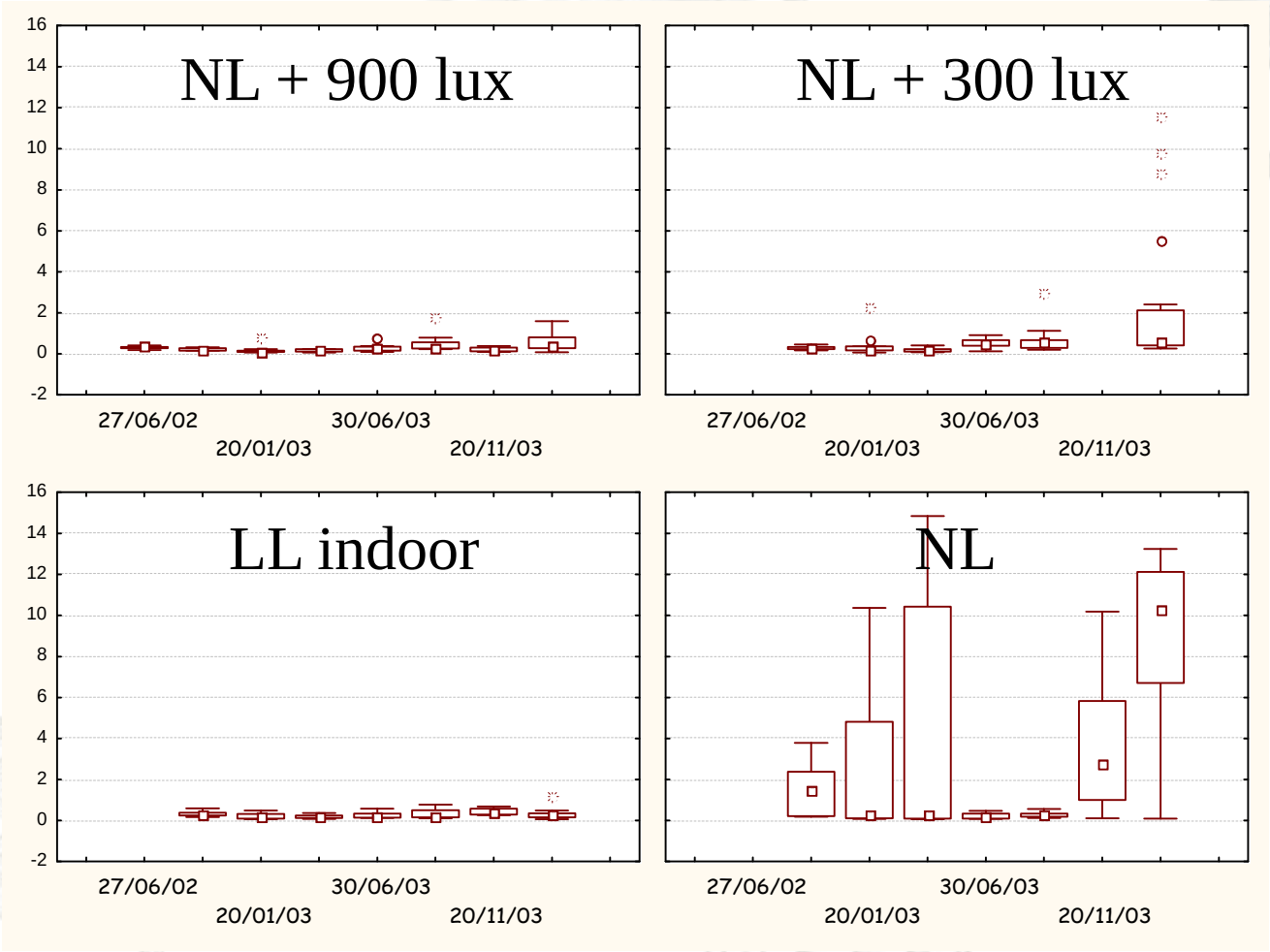
Relative light perception

- In cod at least, it appears that the definition of day and night is relative and dependent on the previously experienced daytime intensity (Vera et al., 2010)
  - (could be seasonal and adaptive variations)
- Gives options
  - Increased nightime intensity
  - Reduced daytime intensity
    - Shades
    - Submerged cages
  - Position the fish where the lamps is, or low in deep cages

Increased light intensities tanks
GSI Males



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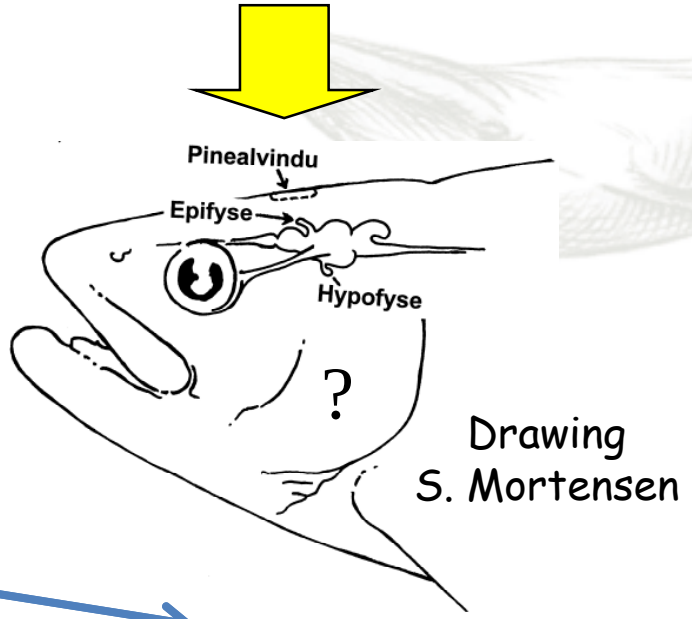
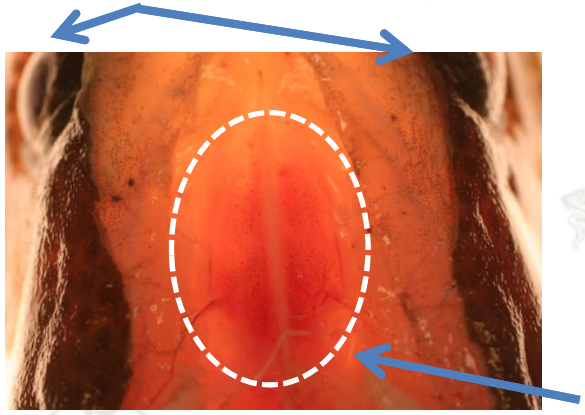
How the fish (cod) reads the light?

- Light sensitive receptors in pineal, retina and deep brain
- Produces (most of) the dark hormone melatonin
- Melatonin participates in translating light signals to physiological responses

- If the eyes is removed the melatonin production is reduced by about 50 % (Migaud et al., 2007).
- < 5% of the light passes through the pineal window



Drawing
S. Mortensen



Pineal window

Photo
OF Skulstad

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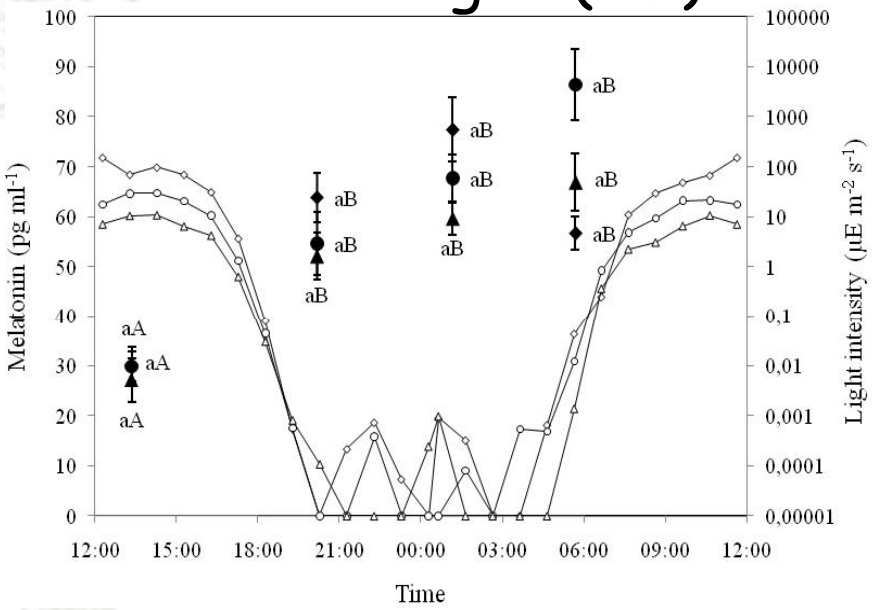
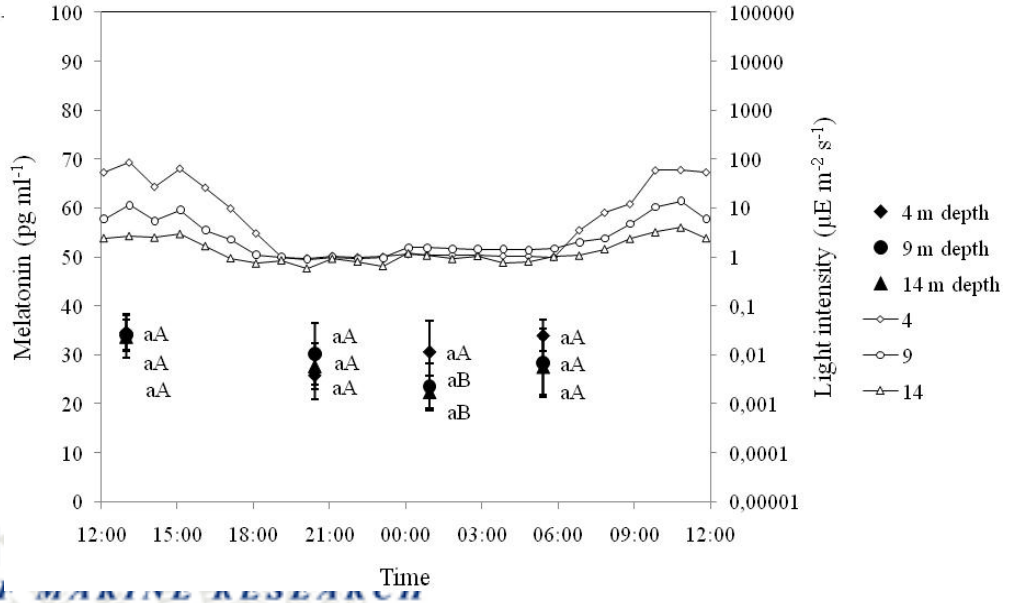
Melatonin in cod under NL and LL from lights at either 2, 7, 12 m depth

Natural light (NL)



LL from all depths



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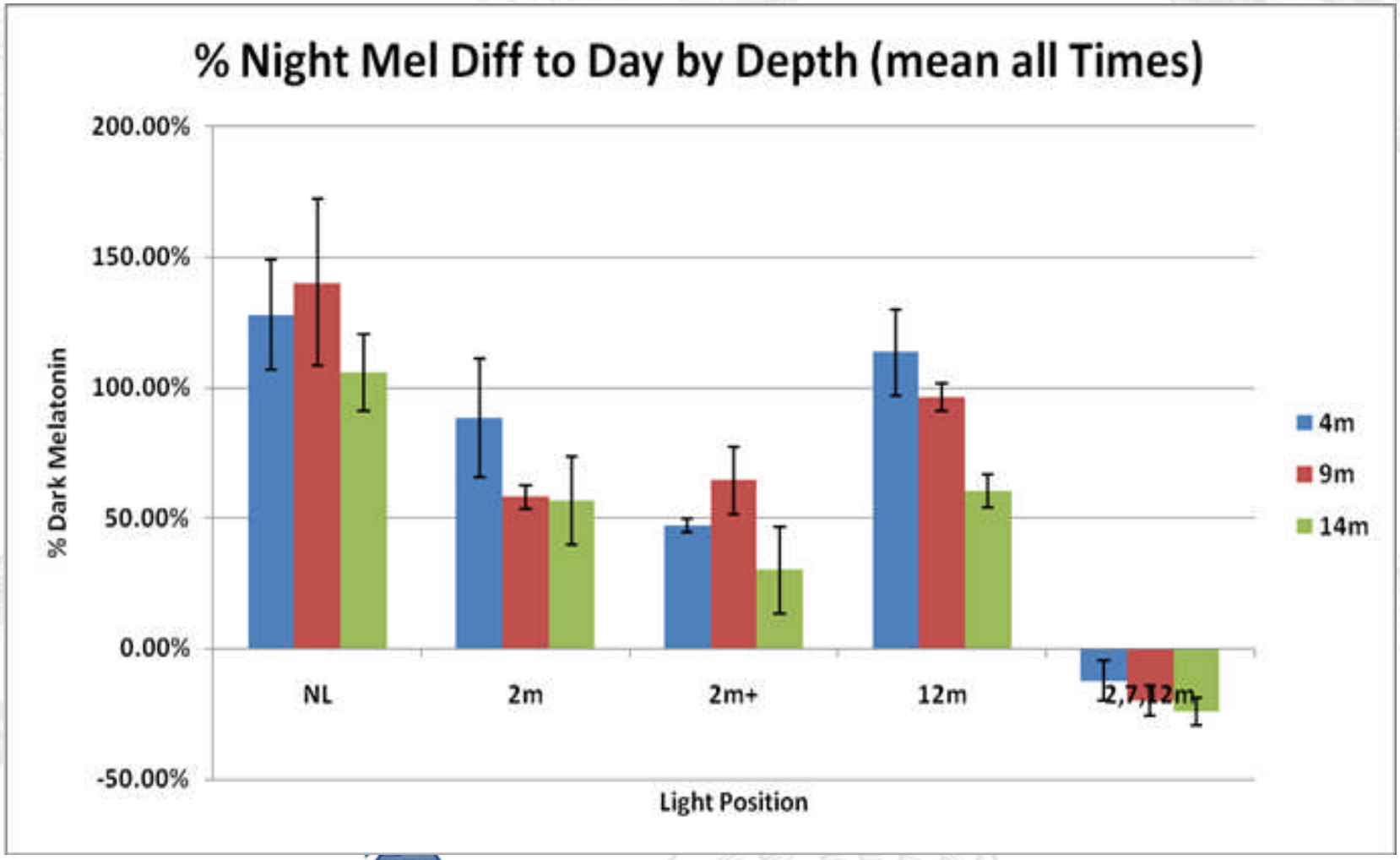
Skulstad et al., submitted

Melatonin night/day
100 = no response



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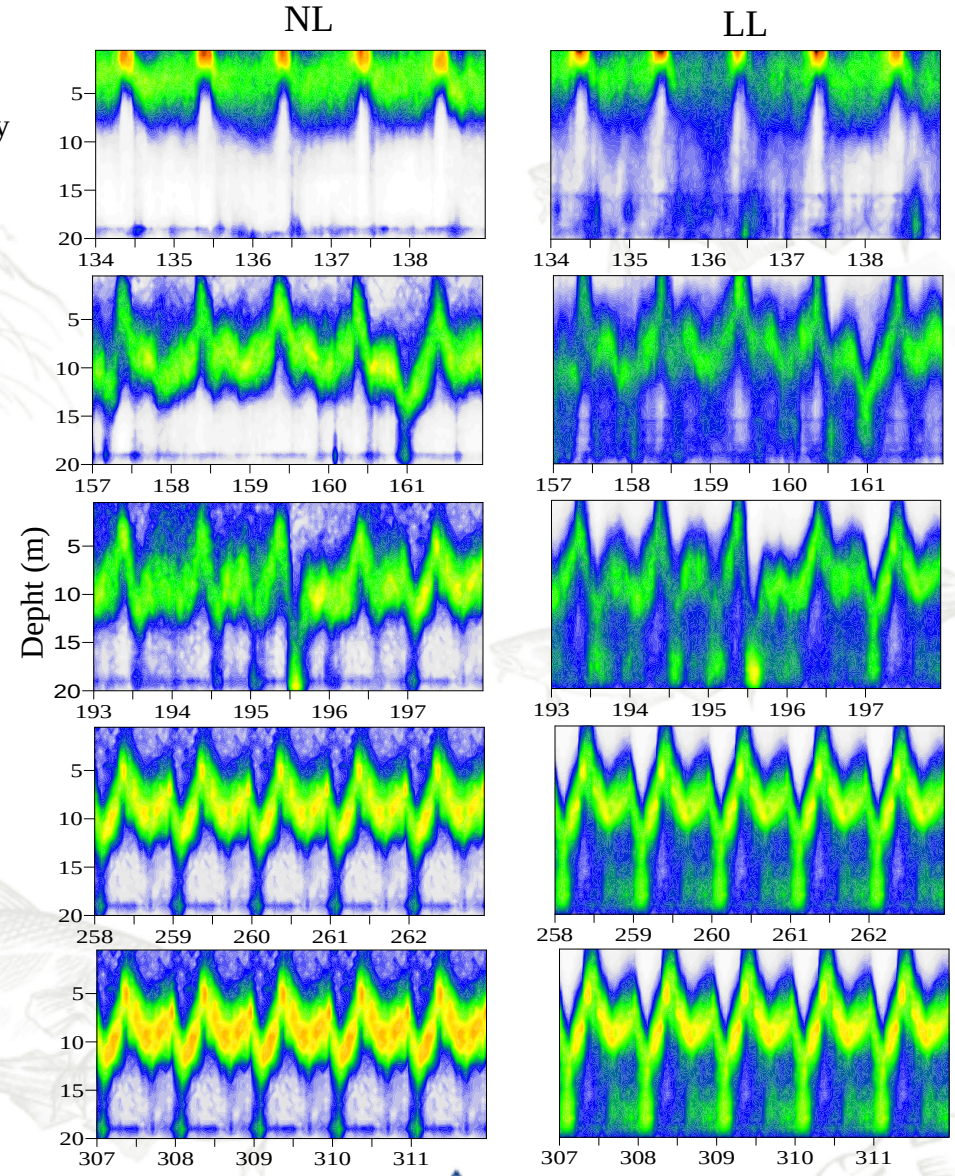
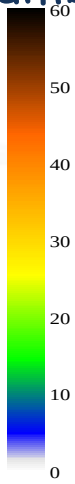
Skulstad et al., submitted

Density / distribution

Numbers indicate midnight





Light affects the vertical distribution of cod in net pens

But cod does not seem to be attracted to the lights

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Skulstad et al., submitted

Conclusions triploid cod Austevoll

- 100% 3N; fertilisation and hatching good
- Slower growth and far more deformities using this intensive method
- 3N males mature at 2 yrs age
- Few mature females
- The fish is sterile

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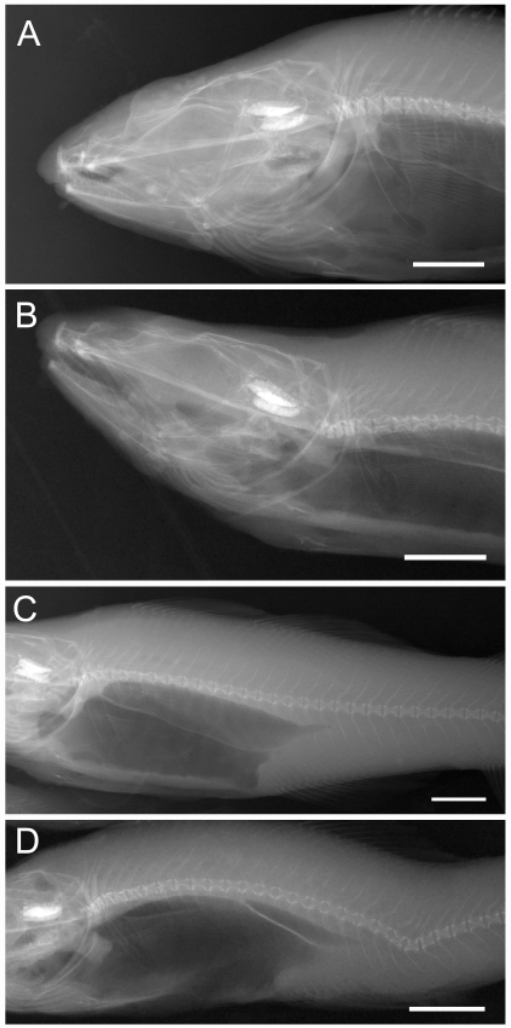
Opstad et al., submitted

Deformities in 3N cod
Intensively produced



Group	Neck deformities (%)	Lordosis (%)
Diploid	40	2
Triploid	56	42

Photo: PG Fjelldal IMR

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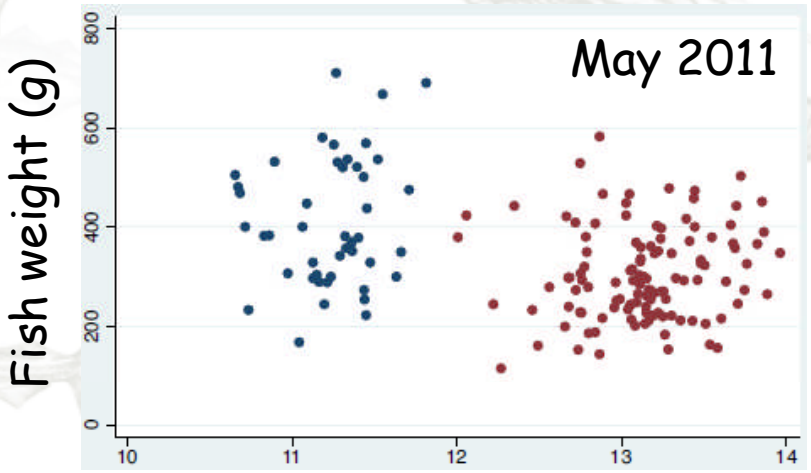
Opstad et al. submitted

Large scale production of trploid cod in the pond Parisvannet, 2010 - 2011

- Produced about 150 000 (2010) and 200 000 (2011) in the pond using high pressure, eggs stripped.
- Start-feeding on natural zooplankton
- Good growth, few deformities,
- Slightly uncertain whether 100% 3N
- 2010: Transferred 122 000 to farmer in Gulen
- 2011: Transferred > 150 000 to two farmers
- Bags with "all-female" not as successful



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H Otterå, A Thorsen, J Pedersen, GL Taranger

"All-female" cod

- No males – no spawning – no genetic environmental impact
- Females grows better?
- Eggbound?
- Triploid females shows no gonad growth, while triploid males does!
- Therefore triploid all female production?

Methods to produce all female
A: Masculinisation during sex differentiation

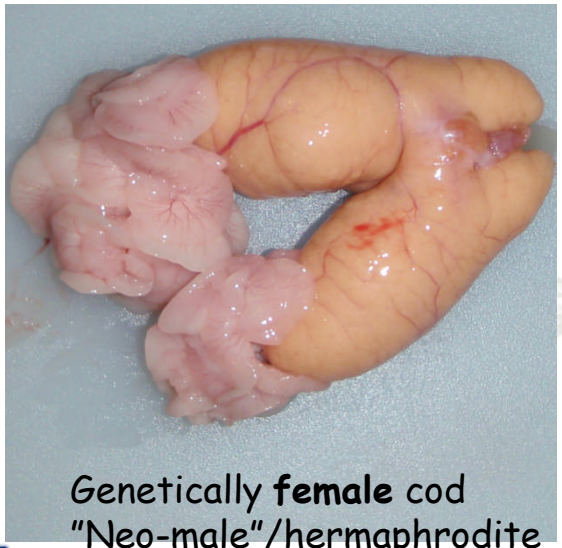
12mm larvae (480d°)

Testosteron $\xrightarrow{\text{P450 aromatase}}$ Estrogen

Uses either hormones (testosterone – like), or inhibitors to prevent the normal conversion of testosterone to estrogen, necessary for normal female development

→ Neo males (or females with sperm or hermaphrodites)

→ Sperm from neo males used to fertilise normal eggs, producing all-female



Genetically **female** cod "Neo-male"/hermaphrodite

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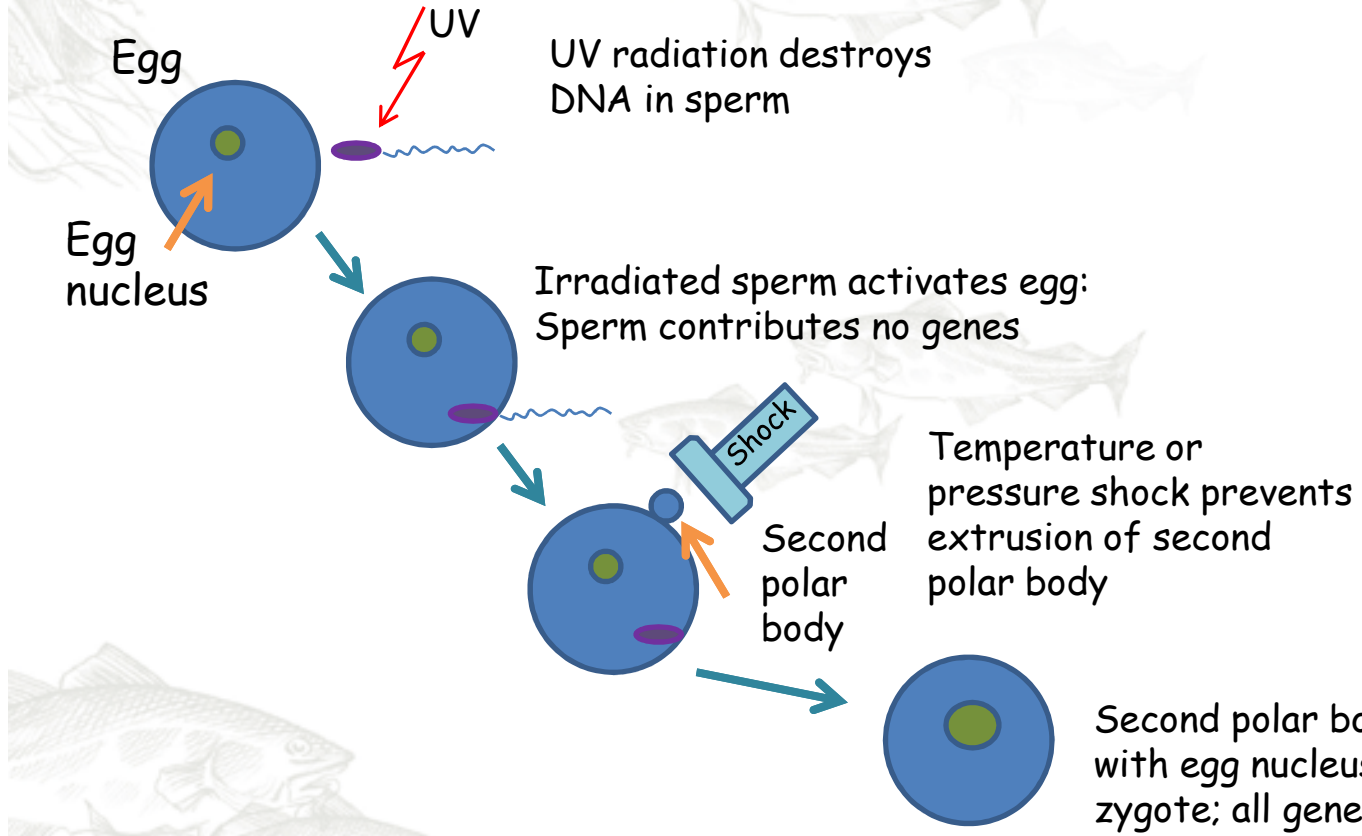
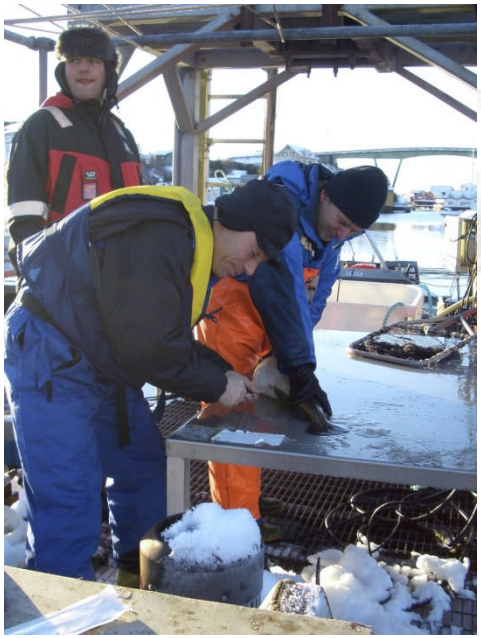
Trine Haugen, IMR

Methods to produce all female
B: Meiotic gynogenesis





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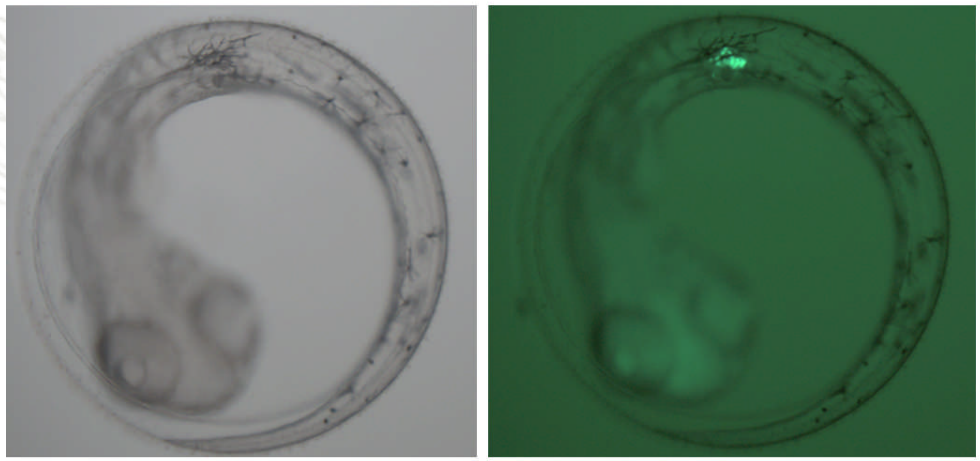
Håkon Otterå & Anders Thorsen, IMR

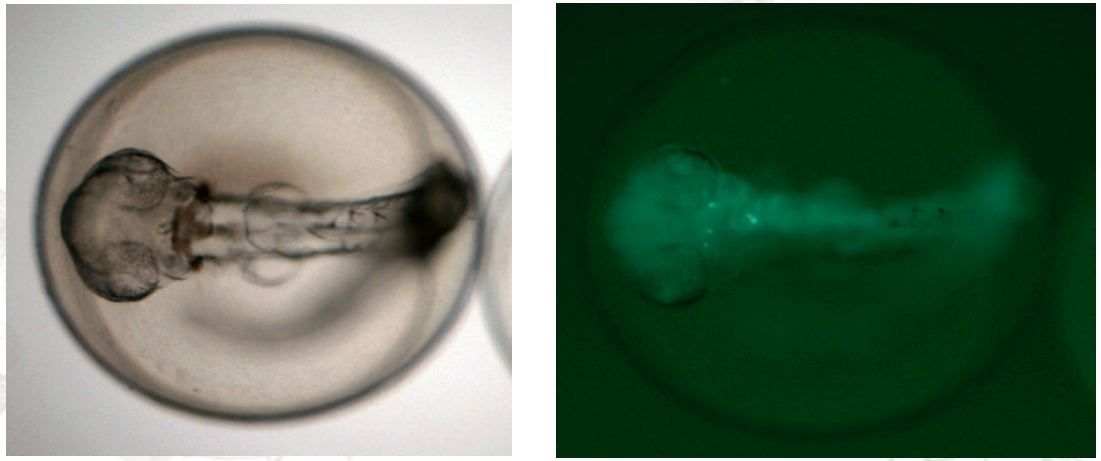
Plans for triploid cod

- All female in bags in Parisvannet (IMR)
- Intensive start feeding protocols for 3N fish (Nofima)
- Respiratory physiology (Nofima and NVH)
- Hypoxia og temp. tolerance (IMR)
- Deformities (IMR and Nofima)
- New triploidization protocols (IMR)
- Implementations in breeding programme (Nofima)
- International conference on sterile fish (March 2012)

Alternative sterility models using gene knockdown techniques



Visualization of primordial germ cells (PGC) in Atlantic cod embryos using GFP-nos1 3`UTR mRNA



PGC`s fail to migrate in Nos1 depleted Atlantic cod embryos

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Ø Drivenes, A Wargelius, R Edvardsen, in prep

"Sterility genes"

Gene	Function	Mutant
Dead-end	**Survival and migration of PGC's in zebrafish , RNA binding protein**	**Homozygous mutant mice sterile, Knockdown experiment in zebrafish show lack of PGC's (Weidinger et al., 2003)**
Nanos 1(Nos-1)	**PCG survival and migration RNA-binding zinc finger proteins**	**Homozygous mutant females maintains oocyte production in adult zebrafish (mutant zebrafish) but offspring from females (only females) are 100% sterile (Doitsidou et al., 2002; Draper et al., 2007)**
Ziwi/Zili	**Apoptosis of germ cells. Small RNA guide (miRNA or siRNA)**	**Loss of Ziwi function in zebrafish results in a progressive loss of germ cells due to apoptosis during larval development. (Houwing et al., 2007)**


Foto: J.E. Fosseidengen
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