EFFECT OF PHOTOPERIOD MANIPULATION ON RAINBOW TROUT EGG QUALITY
A GENOMIC STUDY

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Fish egg quality can be defined as the egg ability to be fertilized and to subsequently lead to the development of a normal embryo.

Egg quality is subjected to high variability, in the wild or in hatcheries.
Low egg quality has previously been correlated with oogenesis conditions such as:

- **Diet**
  - Izquierdo et al., 2001

- **Stress**
  - Schreck *et al.*, 2001

- **High water temperature**
  - Davies and Bromage, 2002

- **Egg post-ovulatory ageing**
  - Aegerter *et al.*, 2003
Photoperiod is the major factor affecting the timing of ovulation in many species

in Sole
*Solea solea*
(Devauchelle et al., 1987)

Sea Bass
*Dicentrachus labrax L.*
(Carrillo et al., 1989)

yellow perch
*Perca flavescens*
(Ciereszko et al., 1997)

rainbow trout
*Oncorhynchus mykiss*
(Davies and Bromage, 2002)
In rainbow trout, photoperiod is also known to modulate fecundity and egg size.

However...

Little is known about its direct effect on egg quality in terms of survival and embryonic development.
HIGH variability in egg quality

Oogenesis history
- stress
- diet
- photoperiodic manipulations
- water Temperature
- synchronisation of ovulation
- Post-ovulatory ageing
  *etc*

Egg composition
- trophic stores
- Proteins
- Messenger RNAs
  *etc*

...Context...

** Stored maternal mRNA **

** Fertilization **

** Transition **

** Early Embryo **

** Zygote mRNAs **

** time **
1- Does a long-short photoperiod regime affect egg quality?

2-Does it modulate expression of some maternally inherited mRNAs?
1. To test photoperiod effects, we use a Long-short photoperiod regime widely used in hatcheries.

2. To analyze egg maternal mRNA, we used rainbow trout cDNA microarrays.
...Methods...

**3-4 weeks before expected ovulations**

**Photoperiod (PM) group**

- Daylength (hours)
- Temperature (°C)

**Control group**

- Daylength (hours)
- Temperature (°C)

INRA facilities

- Water temperature 12°C
- 8L:16D PM/ natural C
- Ovulation checked three times a week
**Methods**

Eggs stripped 5 days after ovulation

- Semen pool
- Fertilization (10°C)
- Eyeing (10°C)
- Yolk-sac resorption (10°C)
  - Survival
  - Malformations and survival

**Egg quality Trial**

**Transcriptome analysis**

**QUALITY**

- 2 Survival index
- Malformation percentage

**MICROARRAYS**

- nylon membrane
- 2*9152 trout cDNA pool-tissue library
- 14 PM /4 Control
  - Statistical Differential analysis

**Survival**

- Fertilization (10°C)
- Eyeing (10°C)

**MALFORMATIONS AND SURVIVAL**

- Unfertilised and frozen -80°C
Only 49% survival in PM group at eying

High variability between females
Confirmed at YSR
Alive Normal Fry less 40%
malformations >15%

Photoperiod manipulation induces a decrease of egg quality with high individual variability & increase of malformations
Types of malformation were different between two groups

No Cyclops type in PM

High proportion of DYSR

Egg post-ovulatory ageing more Cyclops (Aegerter, 2004)

Photoperiod manipulation more DYSR

Is there a Malformation signature dependent on oogenesis history??
Photoperiod manipulation is associated with differences in egg mRNA levels

- Hybridizations: 8422 detected clones
- Differential analysis: 6 maternal mRNA significantly less abundant in PM group eggs
MAK10 is an auxiliary subunit of N-terminal acetyl transferase C (NAT C), in yeast.

- In humans, ovarian NAT activity was correlated with the synthesis of melatonin and serotonin (Itoh et al., 1999).

- In cockroaches (Periplaneta americana), NAT could be a clock-controlled gene in the brain functioning as an output regulator of the circadian clock (Bembenek et al., 2005)

Photoperiod manipulation could directly affect MAK10 expression in regards to its correlation with melatonin or its clock-controlled role.
Glucagon/glucagon-like peptides (GLP) are involved in the regulation of hepatic glycogenolysis and gluconeogenesis (reviewed by Plisetskaya and Mommsen, 1996; Moon, 1998).

- In mammals, glucagon plays an important role in the correction of hypoglycemia.

  Interestingly, glucose insufficiencies are involved in abnormal embryonic development in mice (Smoak, 2002).

- Glucagon has also been shown to be essential for the paracrine induction of differentiation of other pancreatic components in the early embryonic pancreas in mice (Prasadan et al., 2002).

These observations are consistent with the decrease of survival observed at YSR in PM group for which glucagon was less abundant.
A long-short photoperiod regime commonly used to advance spawning date in rainbow trout can induce significant egg quality defects

- Lower survival at eying and yolk-sac resorption
- Higher malformation percentage
- Higher individual variation between females.

In addition, new clues indicate a potential link between specific malformation signatures and oogenesis history.

Finally, this study suggests that the egg transcriptome before fertilization can be influenced by environmental perturbations.
In fish, these conclusions are original and illustrate the importance to

Study the relation between quality and mRNA in PM group

Study effects of other factors on egg quality
  synchronisation of ovulation

Do a functional analysis
  To detail the 6 maternal mRNA identified in this study

To Use rainbow trout cDNA microarrays as a powerful tool to characterize fish egg transcriptome
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