



UNIVERSITÉ
DE LORRAINE



Conselho Nacional de Desenvolvimento
Científico e Tecnológico

Study of the transcriptomic content of the Eurasian perch eggs: research of links with its quality



UR AFPA

UNITÉ DE RECHERCHE
ANIMAL
& FONCTIONNALITÉS
DES PRODUITS
ANIMAUX

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INRA

70
ans

VOTRE AVENIR EST NOTRE CULTURE

Reproductive cycle

Growth → Breeders



Gametes

PHOTOPERIOD
AND
TEMPERATURE

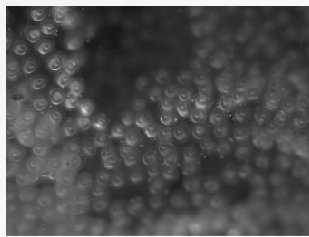
Gametes → Embryo

Embryo

Embryo → Larvae



Larvae → Growth



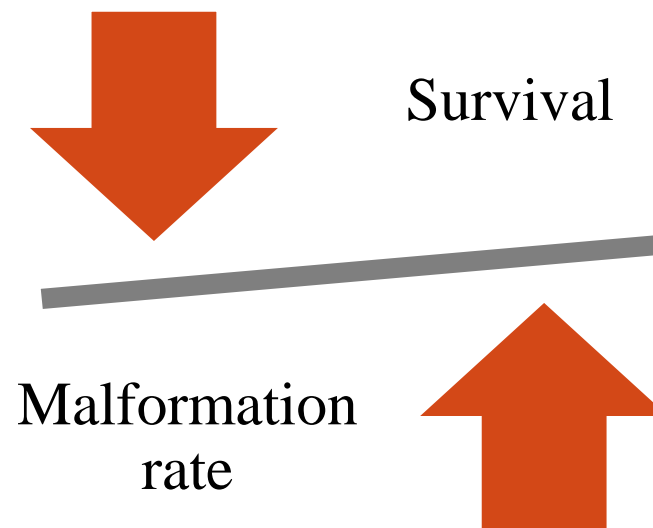
Modulating factors

Intrinsic

- Breeder size
- Rank of spawn
- Genetics
- Level of domestication

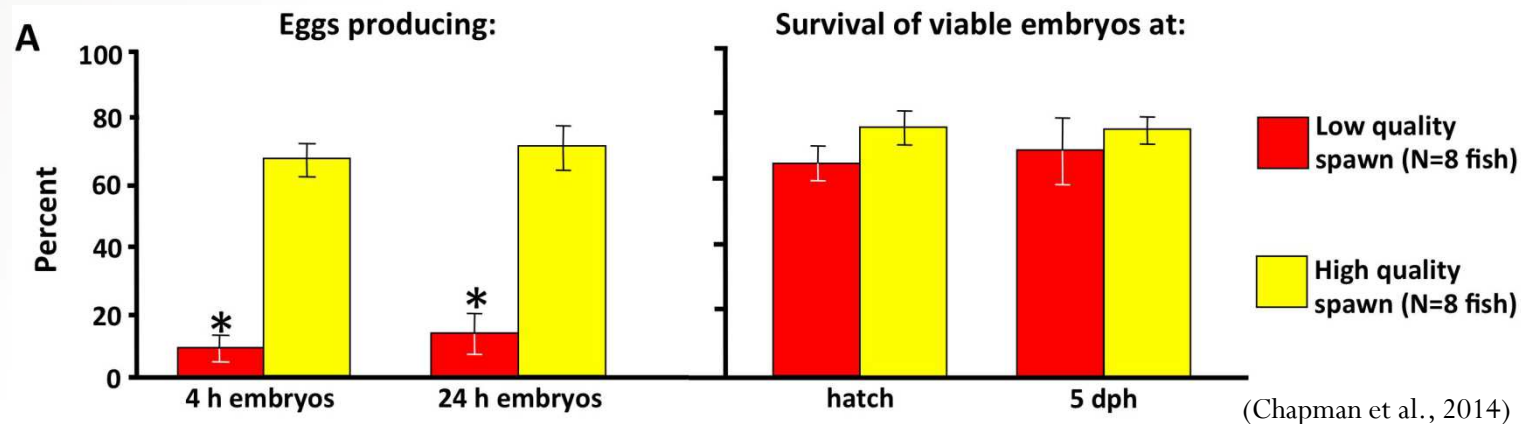
Extrinsic

- Environment
- Stress
- Nutrition

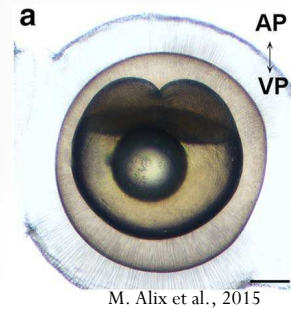


Early development failure

- Major problem in fish breeding programs
- Example Striped Bass:
 - >50% until 4h = high quality
 - <30% until 4h = low quality

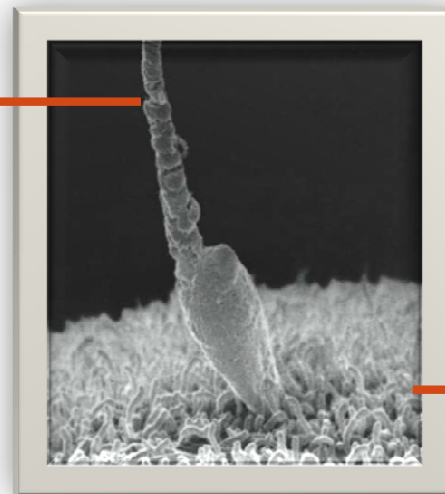


Fertilization and egg content



SPERMATOZOA

- Genetic material
- Centrioli



OOCYTE

- Genetic material
- Transcripts
- Proteins (enzymes)
- Lipids
- Organelles
- Metabolites

Oogenesis in the literature

- Primary oocyte growth
 - Cortical granule formation
 - Vitellogenesis
 - Other events (nuage, vitelline envelope formation, intercellular junctions, pigment formation, intramitochondrial crystals, annulate lamellae)
- (Wallace and Selman, 1990)

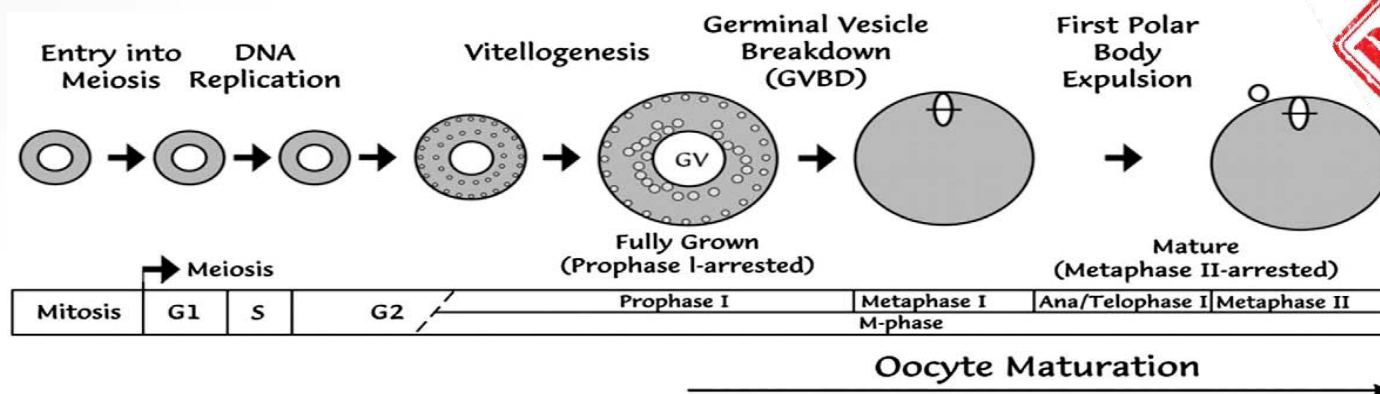
- Primary oocyte growth
 - Cortical alveolus stage
 - Lipids inclusion
 - Vitellogenesis
 - Maturation
 - Ovulation
- (Tyler and Sumper, 1996)

- Growth
- Previtellogenic
Vitellogenic (regulation, yolk protein formation, lipid deposition, vitelline envelope formation)
- Maturation (competence, MIH production, resumption of meiosis, cytoplasmic maturation)
 - Ovulation
- (Patino and Sullivan, 2002)

- Oogonia formation
 - Oogonia proliferation and transition into meiosis
 - Secondary growth
 - Vitellogenesis (lipid accumulation, vitellogenins, uptake of vitamins, egg envelope proteins)
 - Maturation and hydration
 - Ovulation
- (Lubzens et al., 2010)

Oogenesis

- Growth
 - 1st } Previtellogenic growth (mRNA, CHO, proteins)
 - 2nd }
 - Vitellogenesis (lipids, vitamins)
- Maturation
 - Meiosis resumption
 - Cytoplasmic maturation (synthesis of proteins from vitellogenin)
- Ovulation
 - Metaphase II oocyte released from its follicle



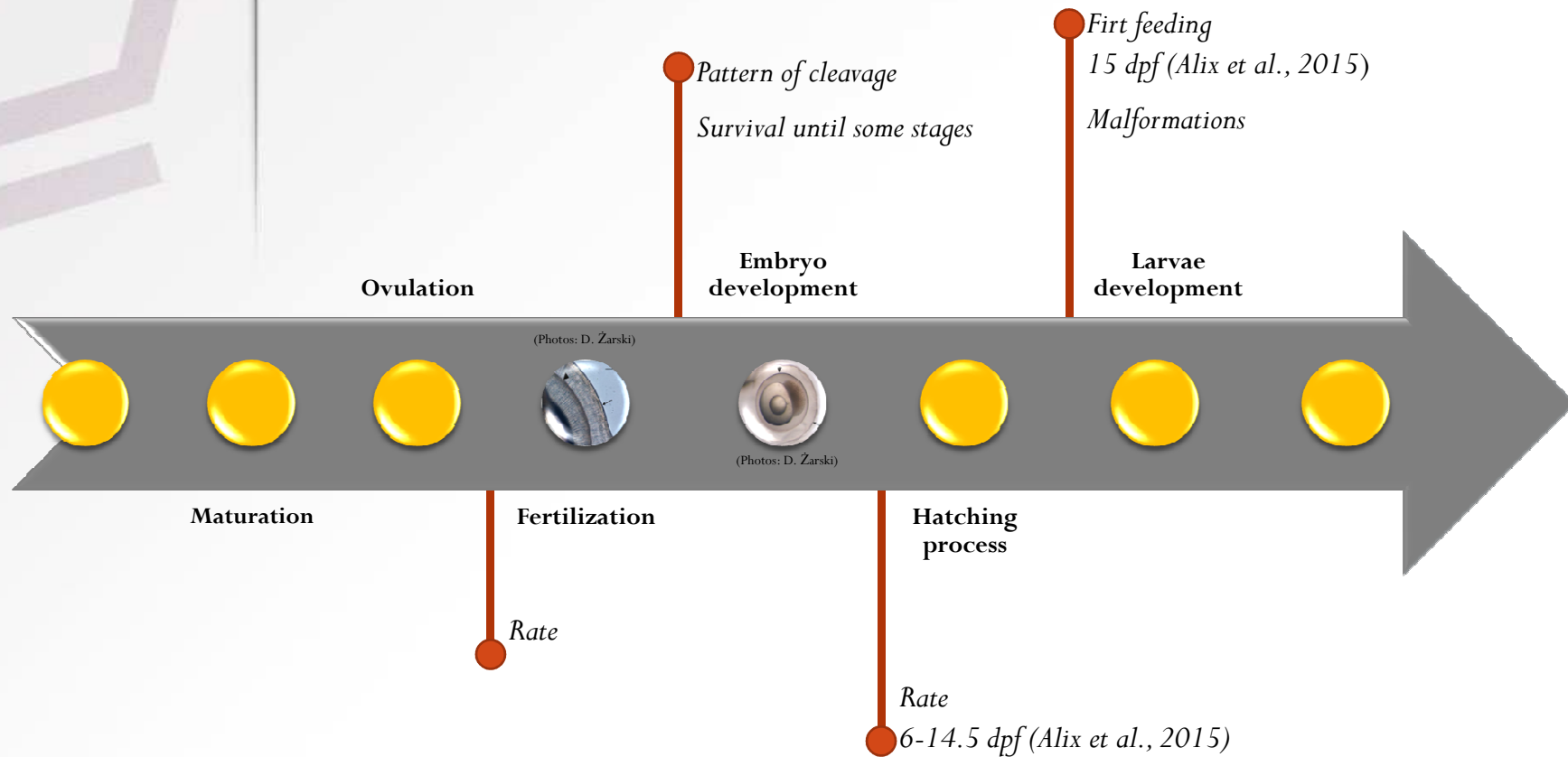
What is egg quality?

Good egg quality are usually defined as those which exhibit low levels of mortality at fertilization, eying, hatch and first-feeding and those which produce the fastest-growing and the healthiest fry and old fish (Bromage et al., 1992).

Egg quality can be defined as the egg's potential to produce a viable fry (Kjørsvik, 1990).

Egg quality or oocyte developmental competence can be defined as the ability of the egg to be fertilized and subsequently develop into a normal embryo (Bobe & Labbé, 2010).

Assessing egg quality



Indicators of egg quality

Marine fish:
sink X float

Freshwater fish:
translucid X
white or opaque

Morphological

Size (Kjorsvik and Lonning, 1983;
Kjorsvik et al., 1990; Brooks et
al., 1997; Bobe & Labbé, 2010)

Oil droplets (Żarski et al., 2011)

Cortical reaction (Żarski et al.,
2012)

Blastomere morphology

(Bromage et al., 1994; Shields et al.,
1997; Vallin et al., 1998; Kjorsvik et al., 2003)

Specie specific

Practices can interfere

Bad quality

Indicators of egg quality

Biochemical

Proteins (Nguyen et al., 2012)

Proteomic (Castests et al., 2012)

Lipids (Henrotte et al., 2010; Luo et al., 2015)

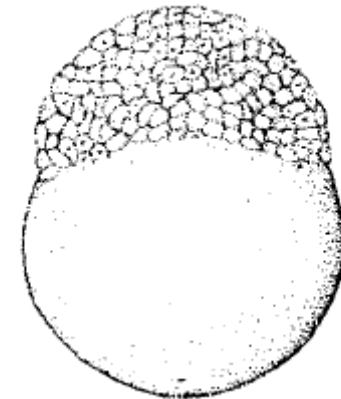
Hormones (Skaalsvik et al., 2015)

RNA (Aegerter et al., 2005)

Ovarian fluid analysis: **pH** (Fauvel et al., 1993); **proteins** (Rime et al., 2004); **osmolality**, **conductivity** (Skaalsvik et al., 2015); **enzymes** (Lahnsteiner et al., 1999; Lahnsteiner et al., 2001)

Early development

- Maternal-to-zygotic transition (MZT) / Midblastula transition (MBT)
 - Maternal transcript destabilization
 - Zygotic genome activation



512-cell
2.75 h

Image: Kimmel et al., 1995

Basic cellular
functions

Cellular metabolism

Nuclear and cellular divisions

Intercellular adhesion

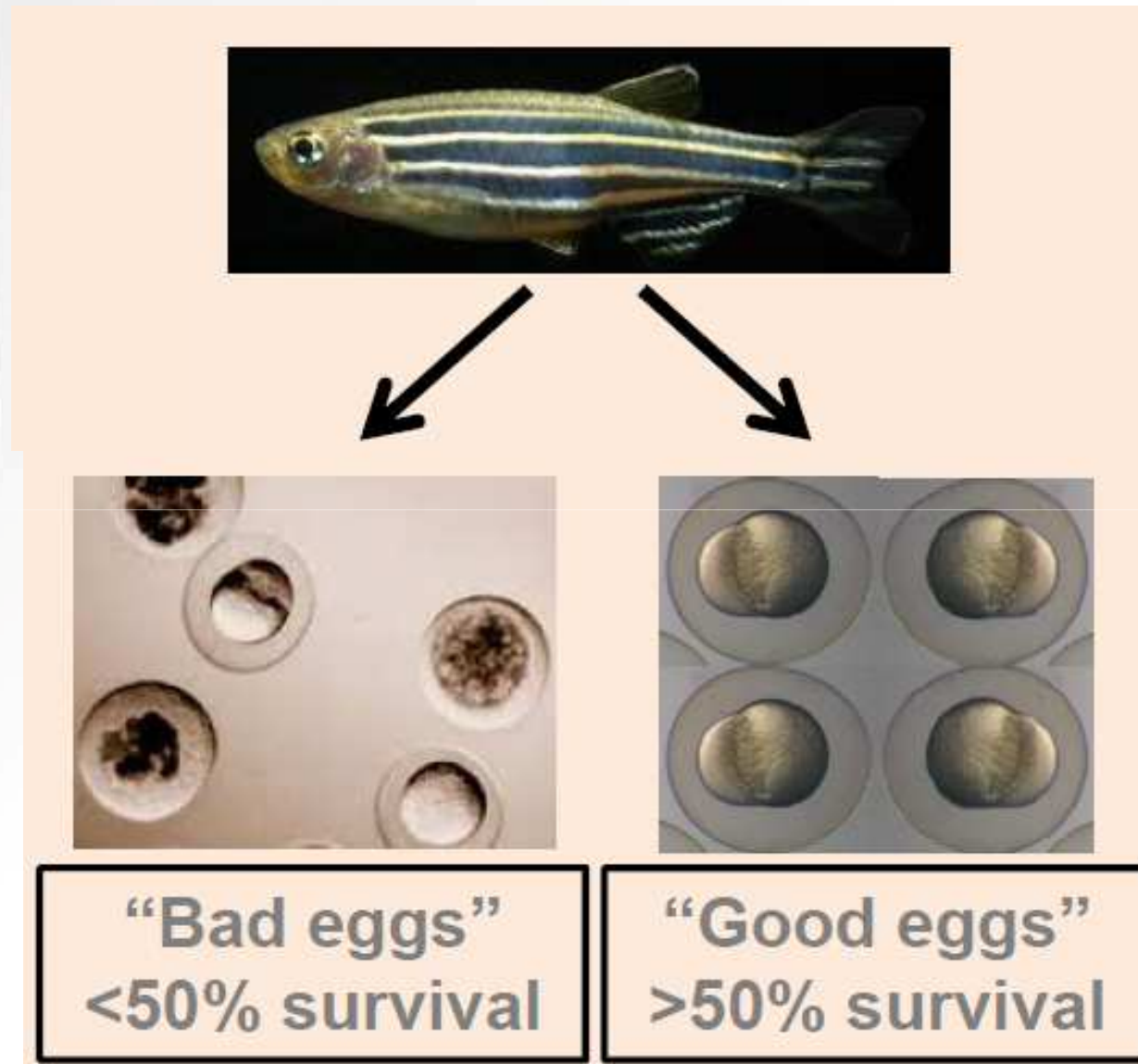
(Chapman et al., 2014; Tadros & Lipshitz, 2009; Pelegri, 2003)

First goal of the project

Defining the molecular portrait of a
developmentally competent egg

Zebra fish; Rainbow trout, Eurasian perch, and European
seabass

First goal of the project



Steps of the analysis

```

AGTAAACCC ACCGACTTC TTTCCTGGG CAGTTGAGT
TTTCACAGG CATTACACGC TGACCCCTCA CCCTCGGGA
TCTCTATCTC GCTAGCAAGG AAGATTGCT CAGACCTGA
AATCCCTATG TAAGTTGCCT ATTTTGCCT TATCTGAAA
XXXXXXXXX XXCATGGGTA TGACAGAGG TCTCTGTTT
CCGCGAGGT GAAGCATCAG GGCCTGAA CAGAATCA
CGGATG
GCAGM
GTTGG
GTGACT
TCATM
    
```

Genes

- Tilapia
- Perch

•16 genes from zebrafish
•13 perch homologous

```

AGTAAACCC ACCGACTTC TTTCCTGGG
TTTCACAGG CATTACACGC TGACCCCTC
TCTCTATCTC GCTAGCAAGG AAGATTGCT
AATCCCTATG TAAGTTGCCT ATTTTGCCT
XXXXXXXXX XXCATGGGTA TGACAGAGG
TCGCGAGGT GAAGCATCAG GGCCTGAA
CGGAT
GCAG
GTTG
GTGAC
TCAT
    
```

Primers

- Genes of interest
- Reference genes

•13 homologous in perch
•5 of reference

```

TCTCTATCTG GCTAGCAAGG
AATCCCTATG TAAGTTGCCT
XXXXXXXXXX XXCATGGGTA
TCGCGAGGT GAAGCATCAG
TCGGATGGGA TGCCAGTGGC
CGGAT
GCAG
GTTG
GTGAC
TCAT
    
```

Training

- RNA extraction
- RT
- PCR
- qPCR

```

TCGGATGGGA
GGCAGAGCCA
TGTTGGCCAG
GC
TC
    
```

Primers optimization

```

TGTTGGCCA
GGTGACTT
TC
    
```

qPCR

Genes we know

Samples available

2013

- 3 wild
 - 28 spawns
- 1 domesticated
 - 8 spawns

2014

- 2 wild
 - 14 spawns
- 1 mixed
 - 7 wild
 - 23 domesticated

2015

- 1 wild
 - 25 spawns
- 1 domesticated
 - 23 spawns

Categories

- ◇ **I - No fertilization**
- ◇ **II - Fertilization rate lower than 30%**
- ◇ **III - Early mortality (more than 50% between 24 and 48 hours post fertilization - hpf)**
- ◇ **IV - Low hatching rate (0-30%)**
- ◇ **V - Medium hatching rate (30-60%)**
- ◇ **VI - High hatching rate (higher than 60%)**

Second goal of the project – Microarray analyze

- New categorization
 - Maximize the differences
 - Easier to associate with the phenotype
 - Reliable results

Microarray analyze

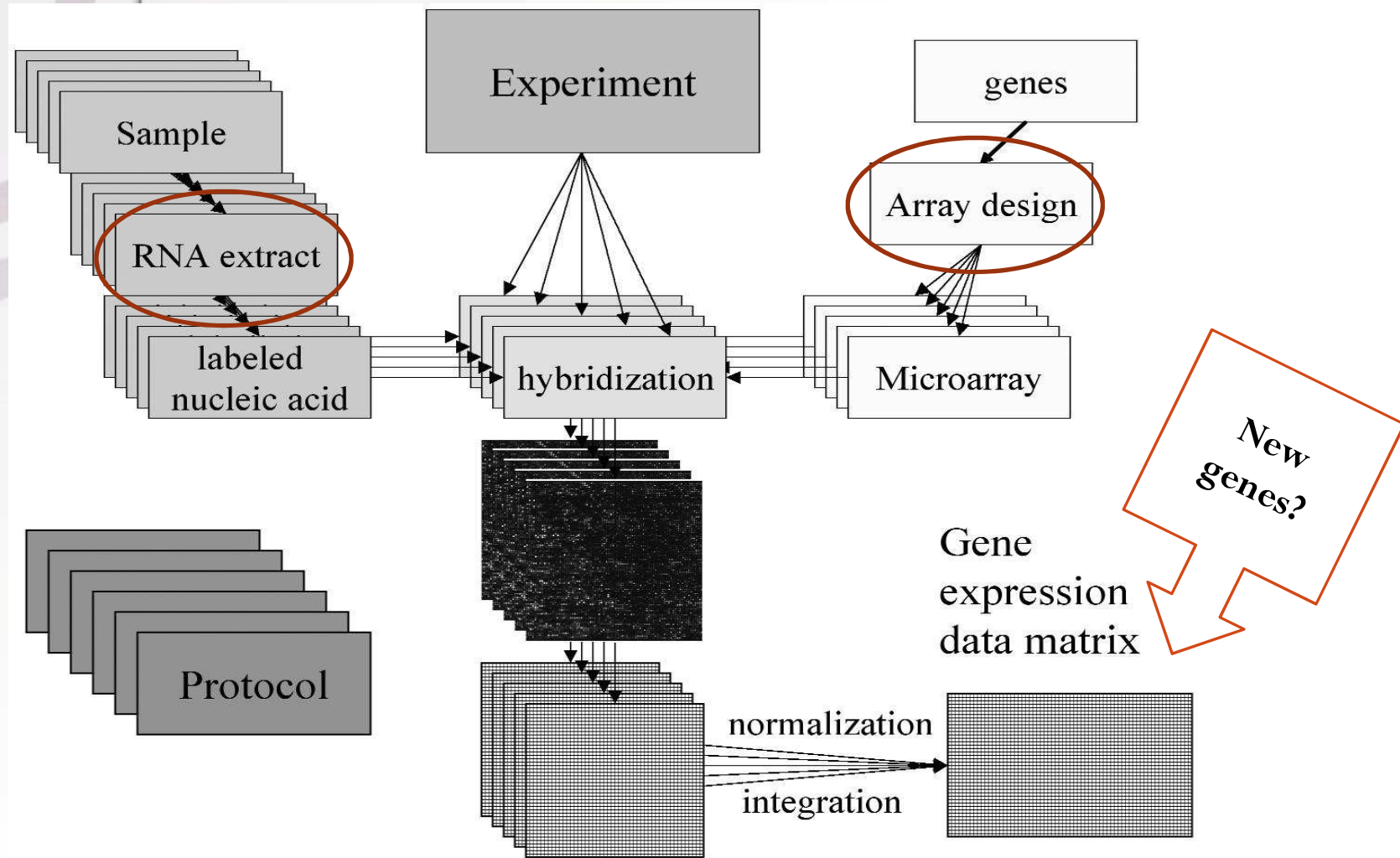


Image: Ball and Bazma, 2006

Samples
available

- Genes from Maternal Legacy Project
Are the molecular mechanisms conserved?

Samples
available

- Genes from microarray analysis
*There are new genes that are specific for egg
quality on perch?*

Perspectives

- Modulating factors affecting the
gene expression



Thank you for your attention

Questions? Suggestions?

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