Genetic implications in the production of rotifers in commercial finfish hatcheries

EU project

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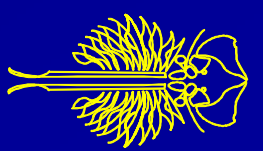
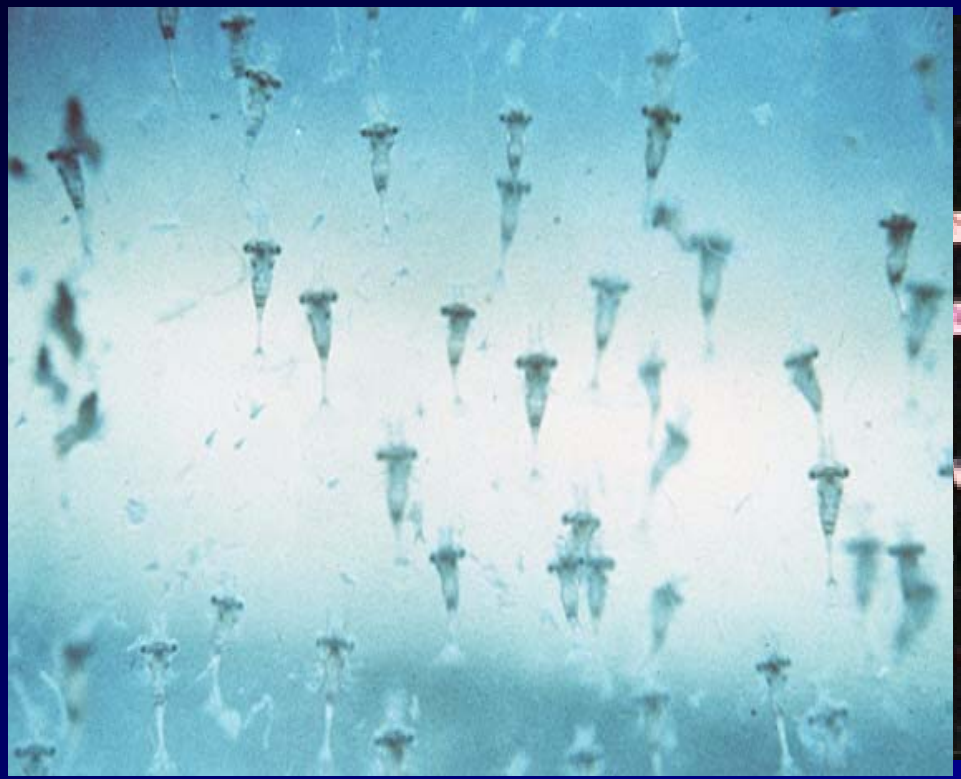
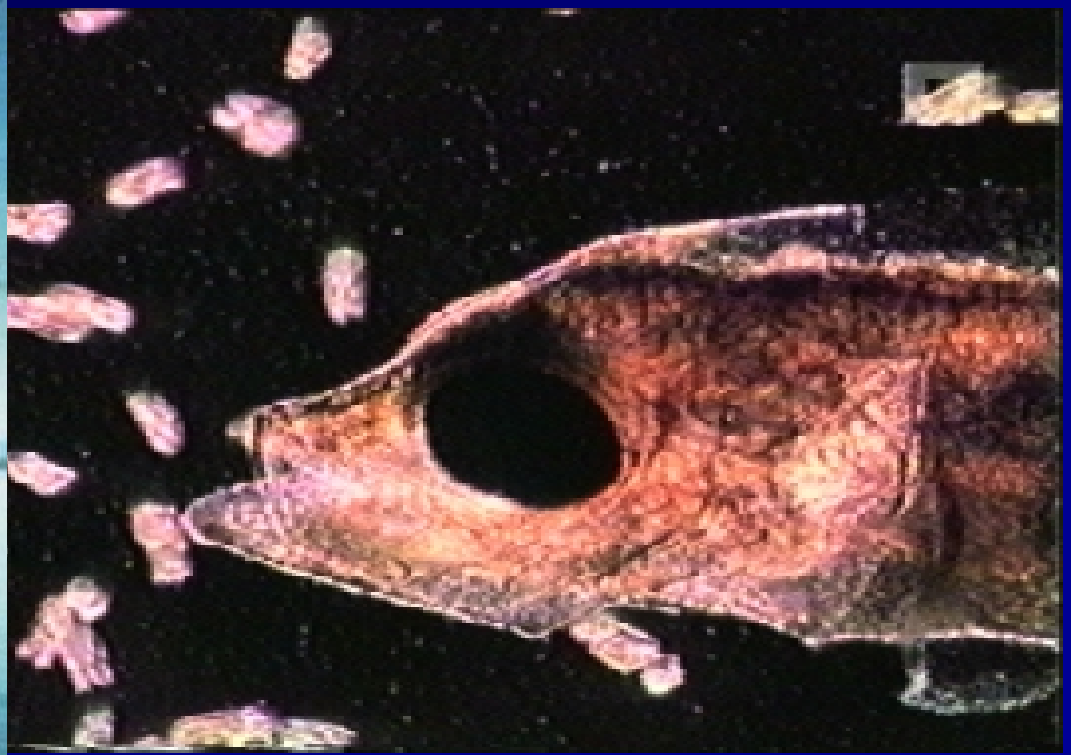
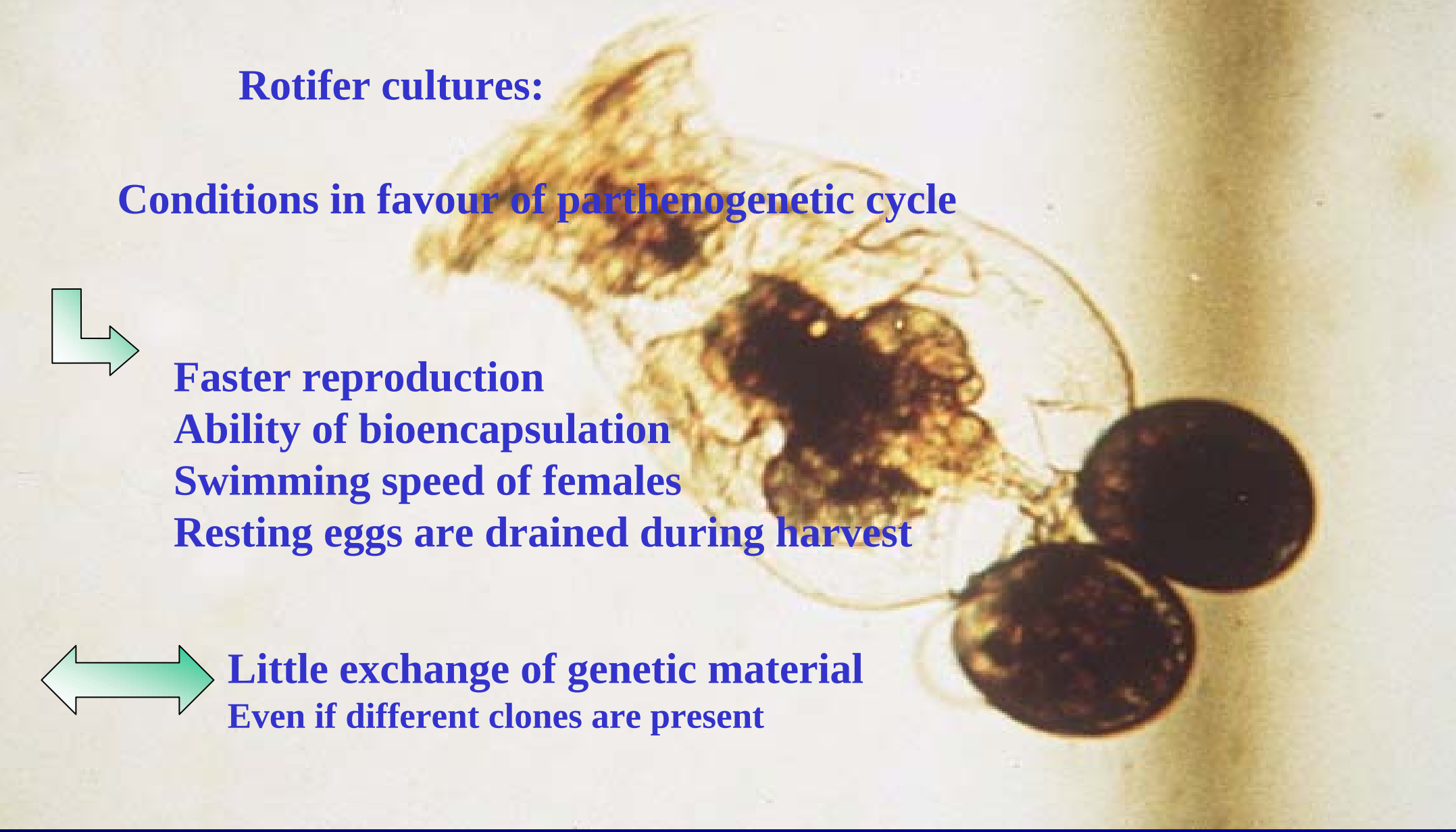


Fish and Crustacean culture depend on the production of rotifers







Rotifer cultures:

Conditions in favour of parthenogenetic cycle

- Faster reproduction
- Ability of bioencapsulation
- Swimming speed of females
- Resting eggs are drained during harvest

Little exchange of genetic material

Even if different clones are present

PROBLEM: Unpredictable culture crashes

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Unreliable live feed source

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Unpredictable fish/shrimp larviculture

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Unpredictable income

Solution: **Farmers double the amount of rotifer culture tanks**
Nearby hatcheries help each other in case of emergency

Solutions: only dealing with consequences not with causes

ROTIGEN project

↓

Shed light on the possible causes of rotifer crashes

Hypotheses:

1) Does the controlled mass culture of rotifers lead to an impoverishment in the genetic diversity of the cultured rotifers?

2) Does this fact in its turn make the rotifer culture more susceptible to crashes if there is a change, controlled or uncontrolled, in the biotic or abiotic conditions prevailing in the culture?

Overview (Part 1)

Topic 1: Genetic characterisation of rotifer clones

Topic 2: Effect of culture methods on the genetic stability of rotifers

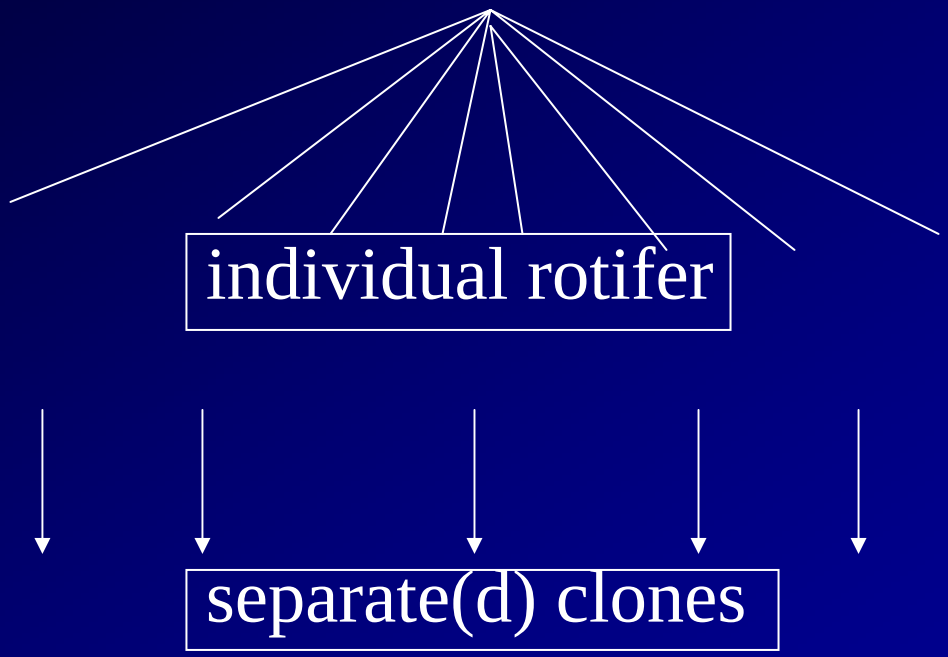
Topic 3: Effects of environmental conditions on the genotypic diversity of cultured rotifers

Topic 4: Effect of microbial stability on the genotypic diversity of cultured rotifers

Topic 1: Genetic characterisation of rotifer clones

Isolation and maintenance of rotifer clones

Samples from different hatcheries/labs



Maintenance of living rotifer clones:

Falcon tubes of 50 ml

Culture period: 1 week

Autoclaved sea water

Feed: *Chlorella sp.*

Salinity: 25 ppt

Temp.: 25°C

Standardisation of protocols for: sampling & storage
transport

Building of a reference collection

Clones genetically characterised with:

- 16 S rDNA gene
- HSP 60 gene
- Microsatellite markers

(Results on the genetic characterisation: see poster section)

Topic 2: Effect of culture methods on the genetic stability of rotifers

1) Interclonal selection for different culture methods

Batch culture
(Belgium): 80 L

4 day culture period

Constant volume

25 ppt, 25°C

Feed: different kinds

Inoculation:500 ind/ml

or 50 ind/ml

Recirculation culture (Belgium)

80 L

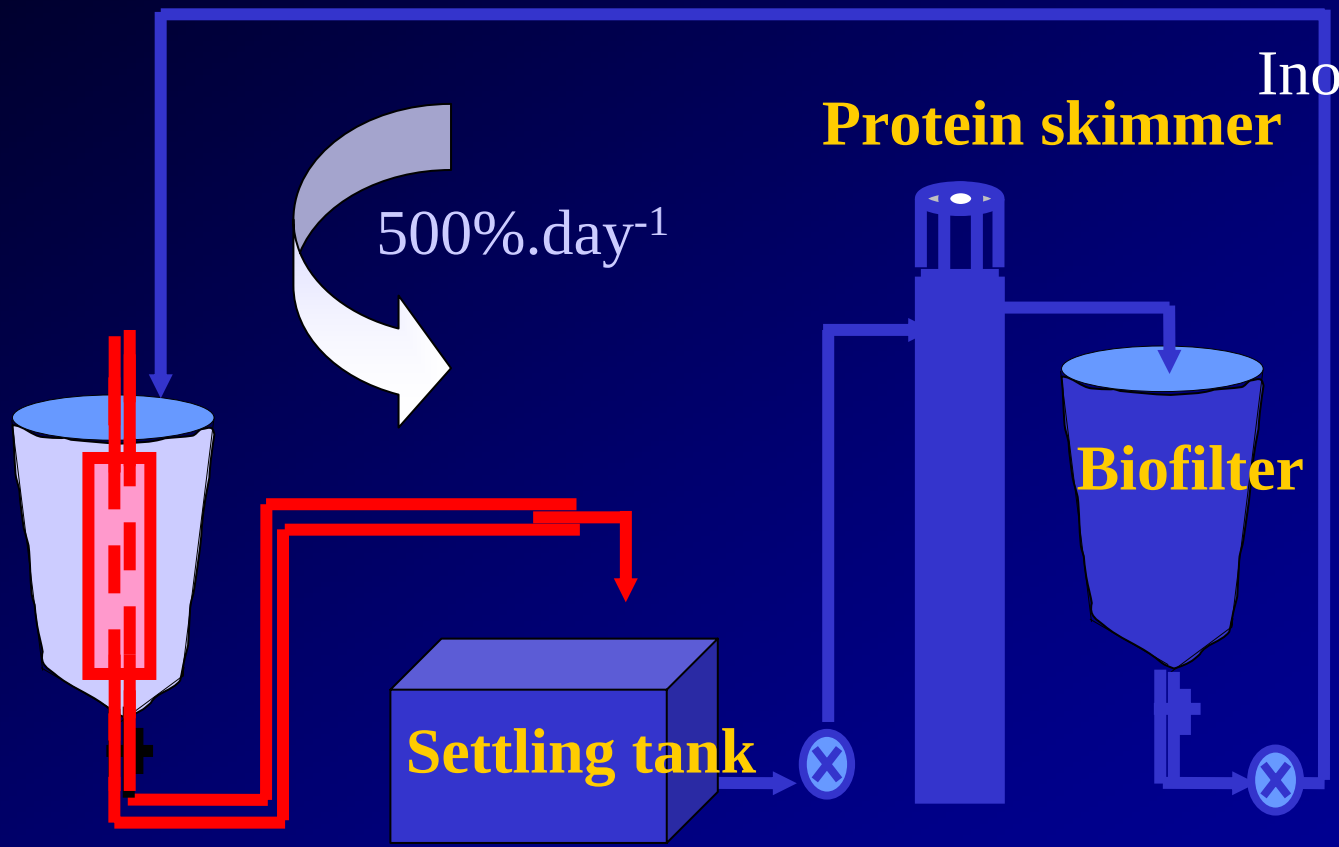
3 – 4 weeks culture period

25 ppt, 25°C

feed: different diets

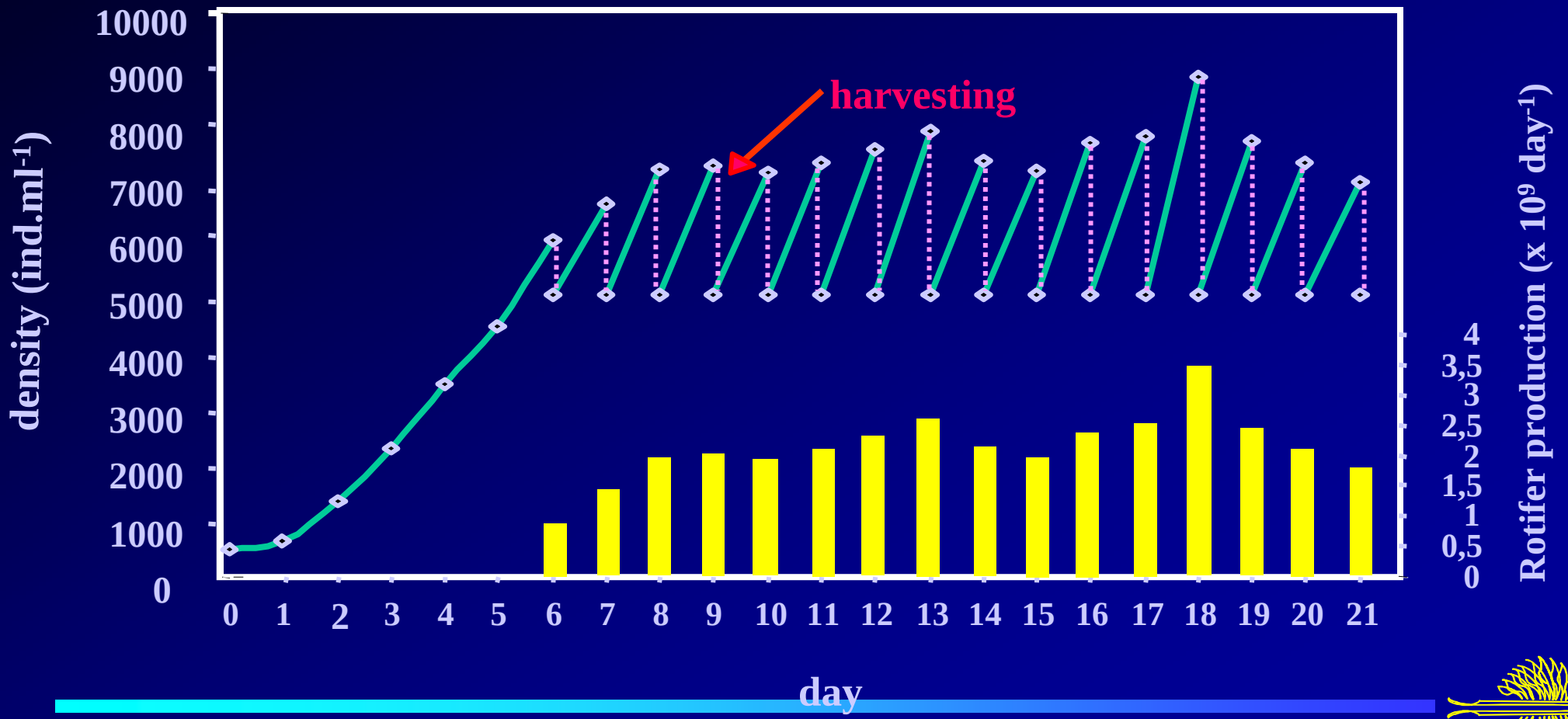
Inoculation: 500 rotifers/ml

or 50 rotifers/ml



Semi-continuous culture: Norway

Constant rotifer density: daily harvests
+ addition of new water



A mixture of identifiable rotifer clones at equal inoculation densities

Different culture methods

Sampling: Start, after every run (for batch), every week (for recirc)

Selection of rotifer clones dependent on the culture method

Topic 3: Effects of environmental conditions on the genotypic diversity of cultured rotifers

Axenic parthenogenetic eggs ⟶ Axenic cultures

Stable conditions

temperature

feed

salinity

Topic 3: Effects of environmental conditions on the genotypic diversity of cultured rotifers

Axenic parthenogenetic eggs ⟶ Axenic cultures

Variable conditions

Temperature

feed

Within 1 culture cycle

Variability between culture cycles

Topic 4: Effect of microbial stability on the genotypic diversity of cultured rotifers

1) Effect of selected bacterial strains on the interclonal selection

Axenic rotifer culture + 5 bacteria

Feed: Gamma-irradiated

axenic culture of algae

Sampling: daily: rotifer density + egg ratio

weekly: genetical analyses

↳ community:PCR-DGGE

ARC + NTNU: different salinity + temperature

2) Effect of selected bacterial strains on the interclonal selection in xenic cultures

Back to reality:

xenic rotifer culture + 5 bacterial strains

ARC: batch cultures

NTNU: semi-continuous culture

Expected results: Protocol for addition of selected bacteria resulting in high production and low selection

Overview (Part 2)

Topic 5: Verification of genetic selection under pilot scale conditions

Topic 6: Sexual reproduction to provide resting eggs of selected strains

Topic 7: Follow up of cultures in commercial hatcheries

Final : Workshop for the industry

Topic 5: Verification of genetic selection under pilot scale conditions

Strains from Mediterranean hatcheries → ARC
→ NTNU

ARC: batch or recirculation culture

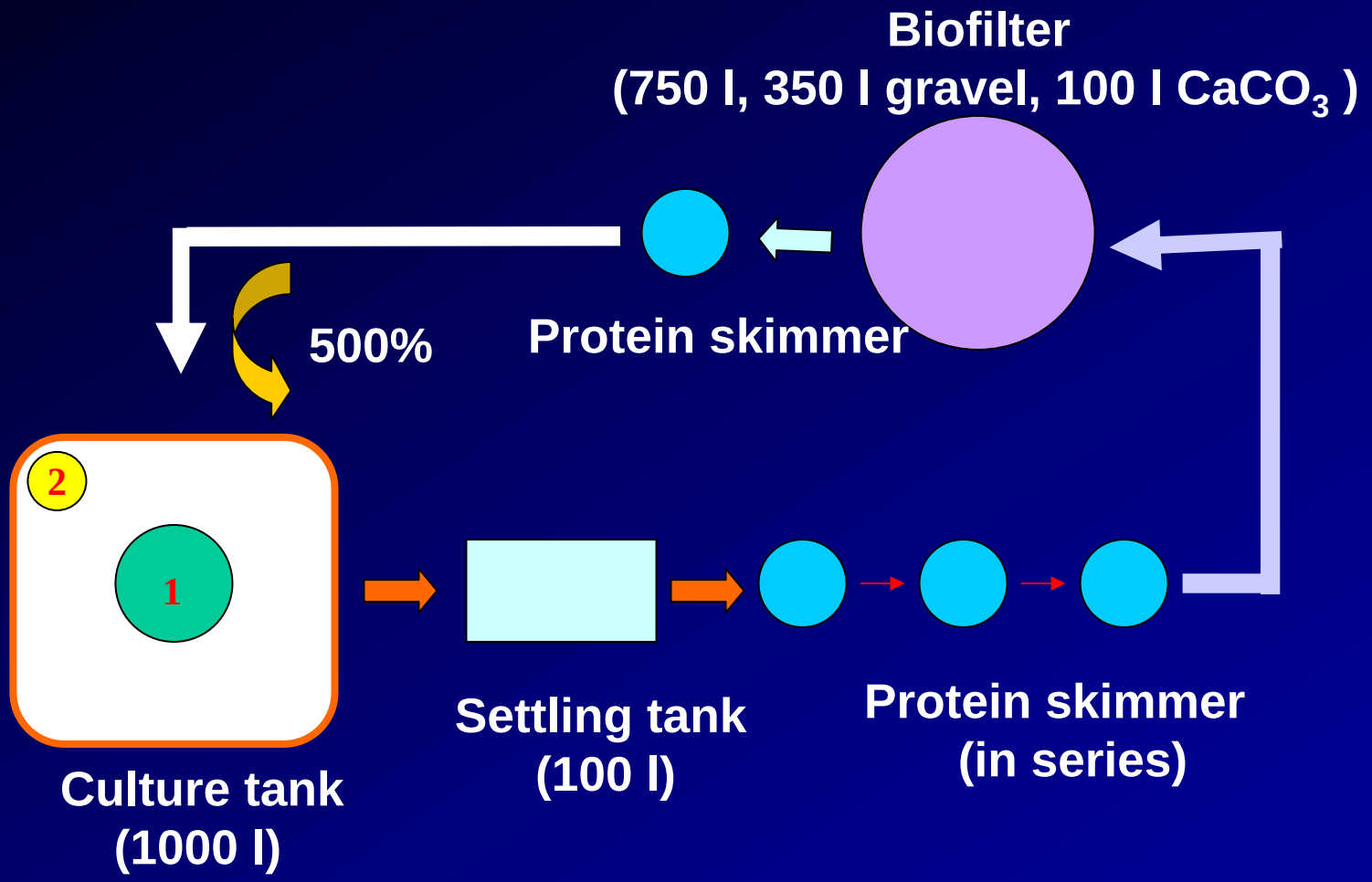
NTNU: semi-continuous culture

Methods: according to WP2

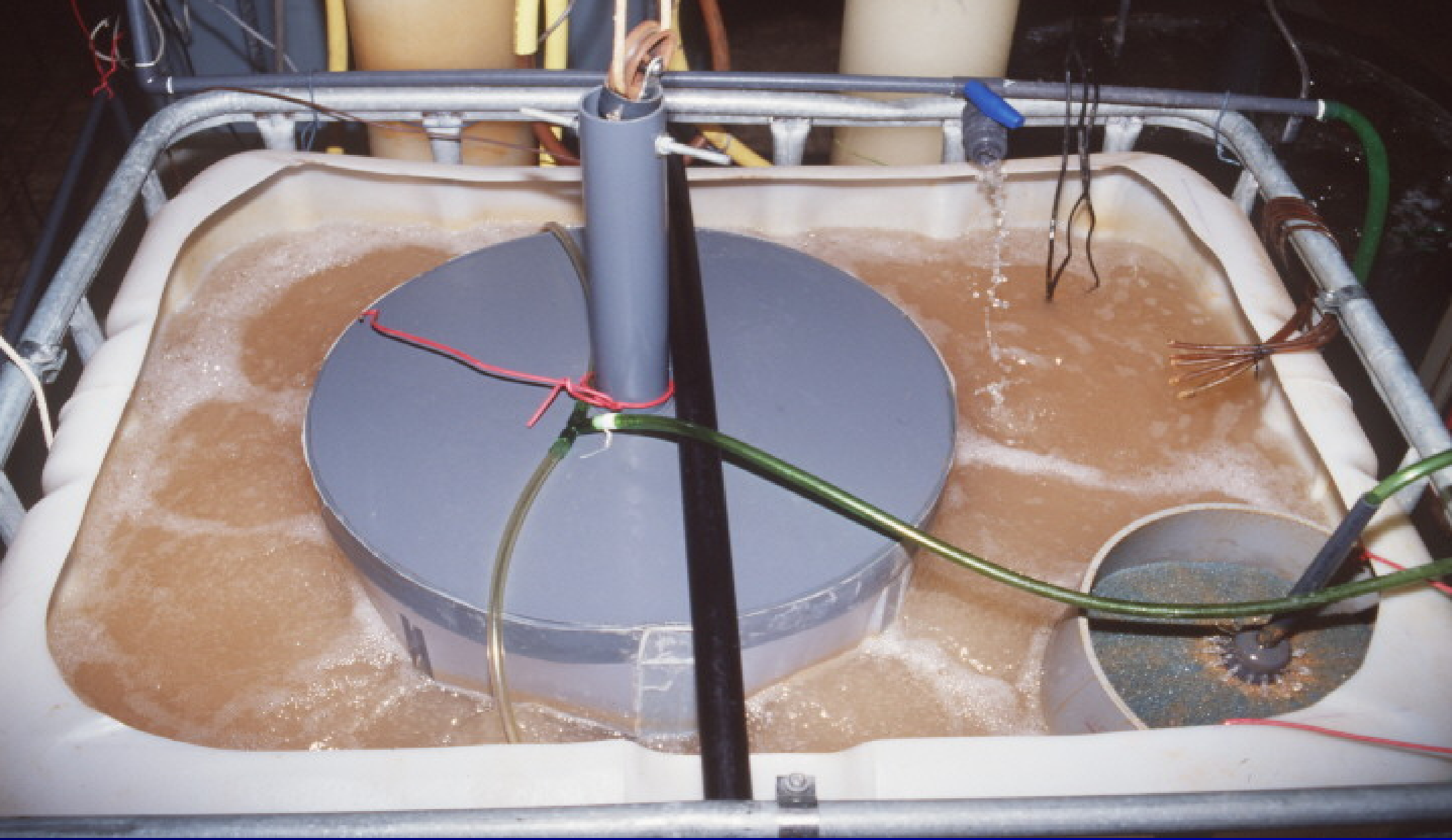
Deliverables: Adaptability of rotifers from commercial hatcheries to other culture conditions

Expected results: Data on clonal selection in rotifer populations in commercial cultures

Schematic outline of the pilot scale system



1 = filter (30 µm)
2 = air water lift



Topic 6: Sexual reproduction to provide resting eggs of selected strains

1) Methodology for resting egg production, harvest and storage

   Temperature, salinity, feed quantity, photoperiod, population density

   Triggers will vary among the different clones

2) Selection of competent strains

   Screening of genetically characterized clones for ability of resting egg production

3) Genetic characterisation of resting egg banks

Resting eggs produced in 2) will be checked for diversity on microsatelite scale

4) Application of resting eggs for aquaculture

Resting eggs as inoculum

as remedy against genetic impoverishment

Evaluation of the use of resting eggs for aquaculture

5) Cryopreservation of amictic eggs

Advantage: easier production

genetically identical

Topic 7: Follow up of cultures in commercial hatcheries

Rotifer samples from MRS → from stockcultures

from start of cultures

at onset of a crash or at the end of a culture cycle

Rotifer samples from other hatcheries through connections of the RTD partners (Greece, France, Norway)

Final : Workshop for the industry

ARC will organise a workshop on mass production of rotifers and the genetic consequences

AUTH and NTNU will also invite farmers

Expected results: Dissemination of the results to the industry


The end