

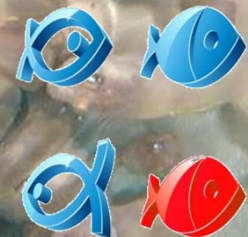
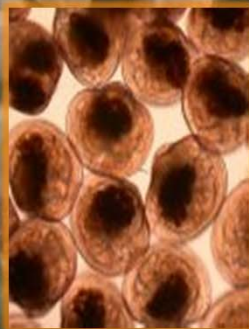
larvi 2013

6th fish & shellfish larviculture symposium
ghent university, belgium
2-5 september 2013

ARTEMIA AS MODEL ORGANISM IN LARVICULTURE RESEARCH

Patrick Sorgeloos

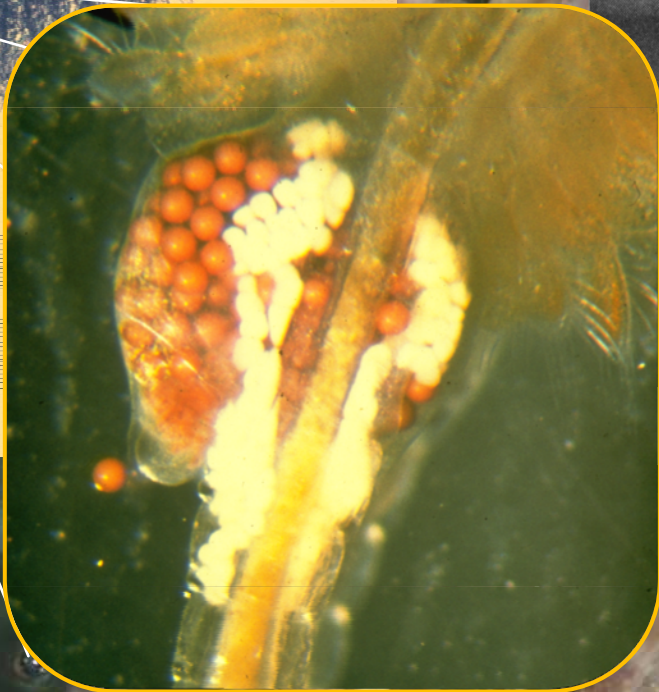
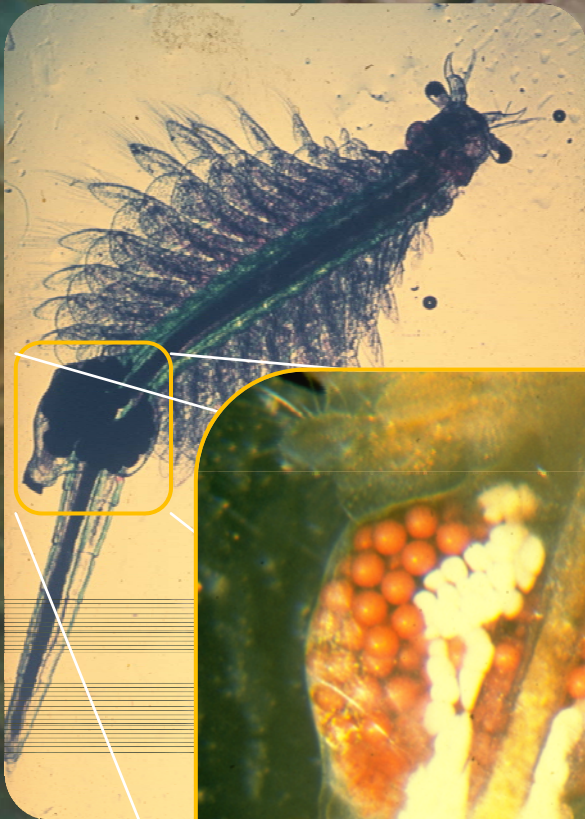
Conductor of the UGent Aquaculture Orchestra



1960s

State University of Ghent - Faculty of Medicine - Laboratory of Anatomy

brine shrimp *Artemia* as model organism in embryology research



Julien Fautrez



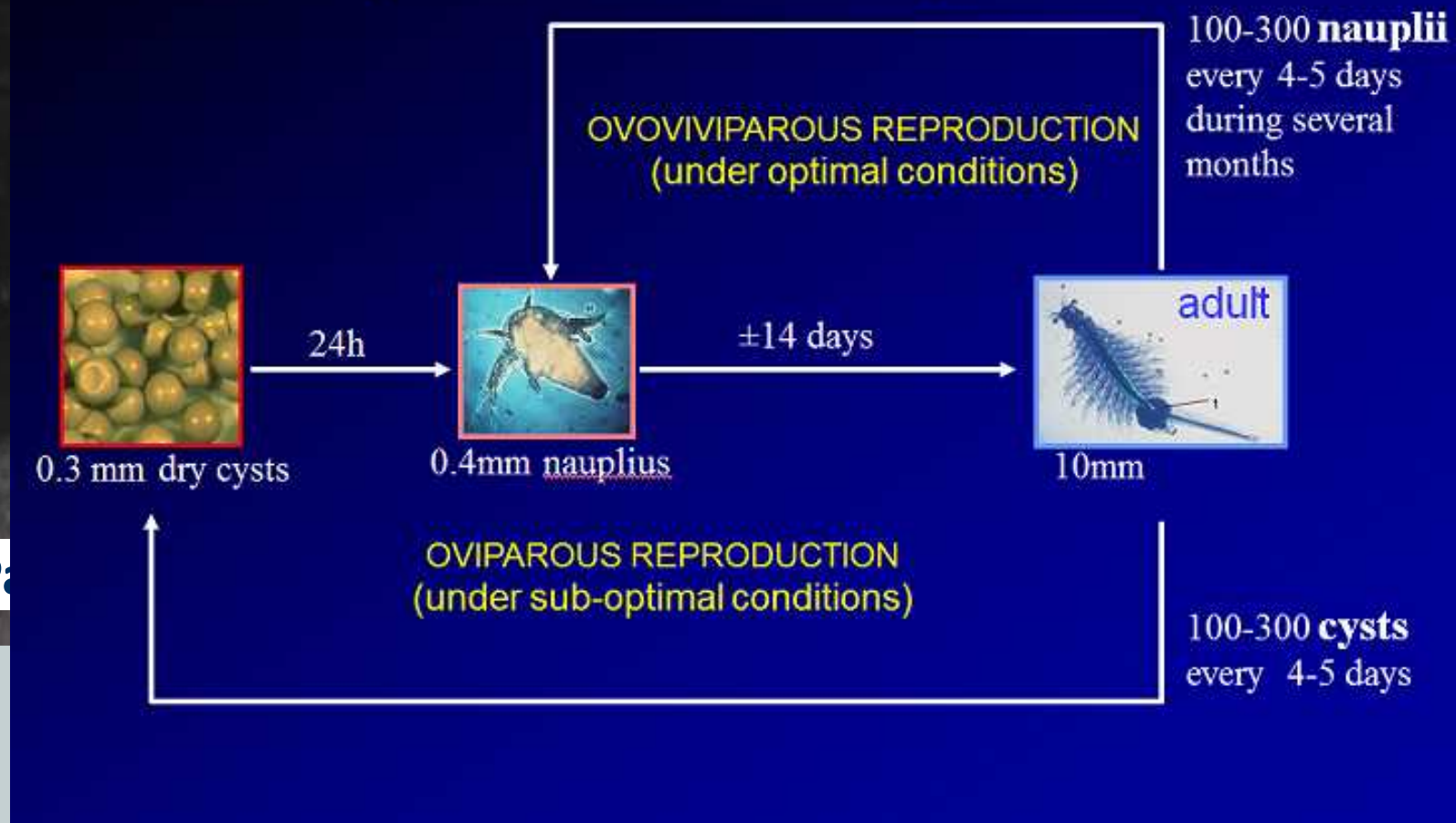
Lieve Criel

1969

State University of Ghent - Faculty of Sciences - Laboratory of Ecology

start research on *Artemia* culturing biology

Life cycle of brine shrimp *Artemia*



Duke University Marine Laboratory

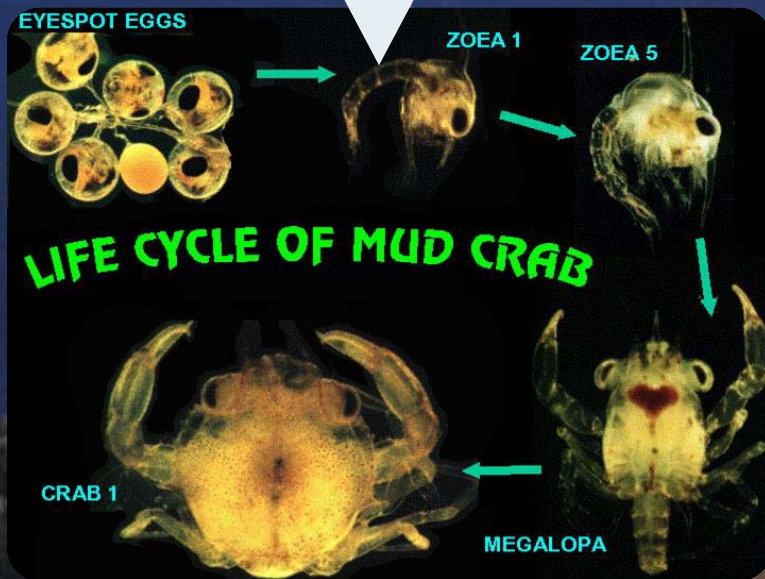
Beaufort, North Carolina-USA

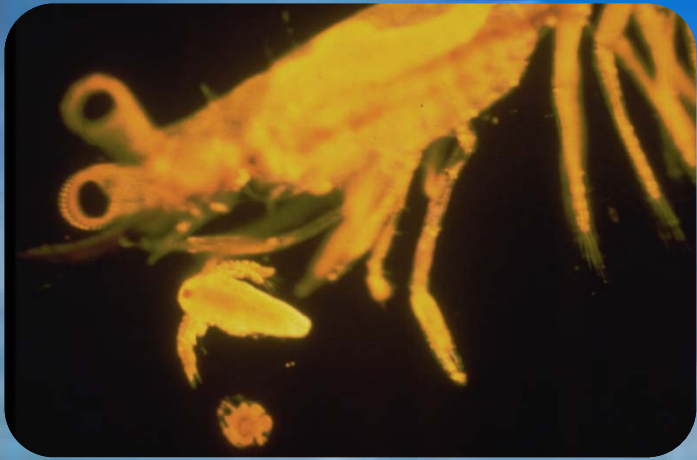


Artemia as food



C.G. Bookhout &
John D. Costlow

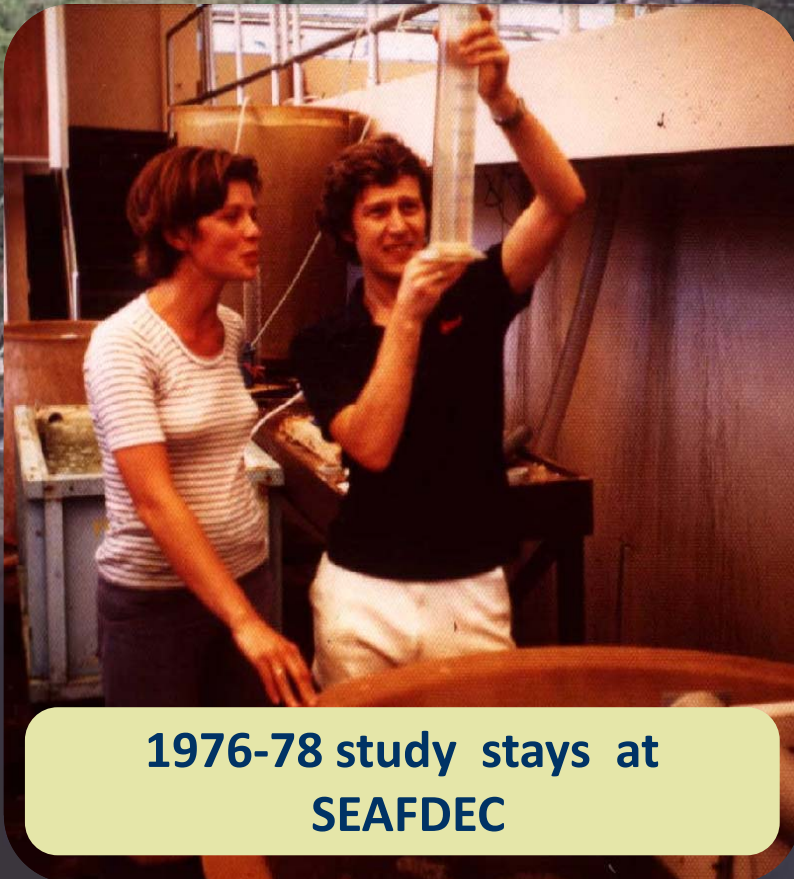




SouthEast Asian Development Center

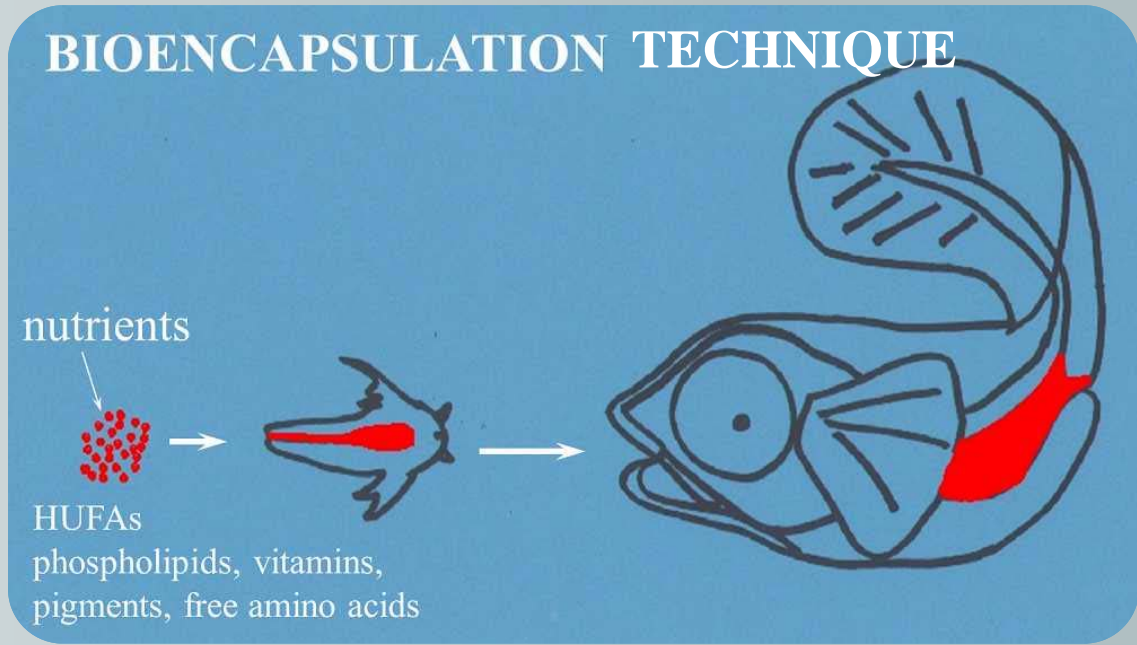
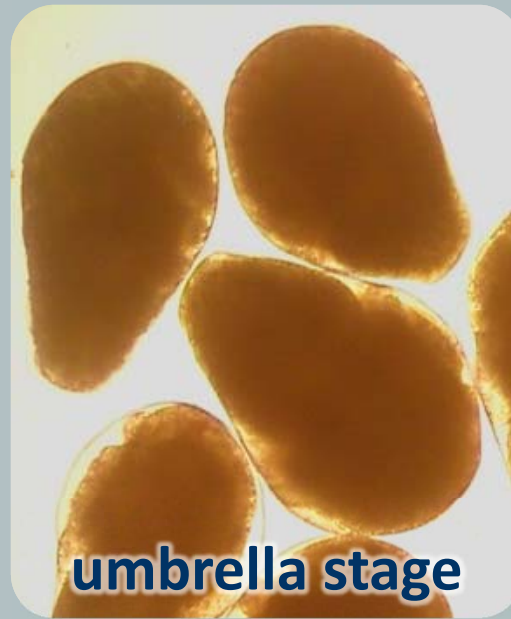


Herminio Rabanal

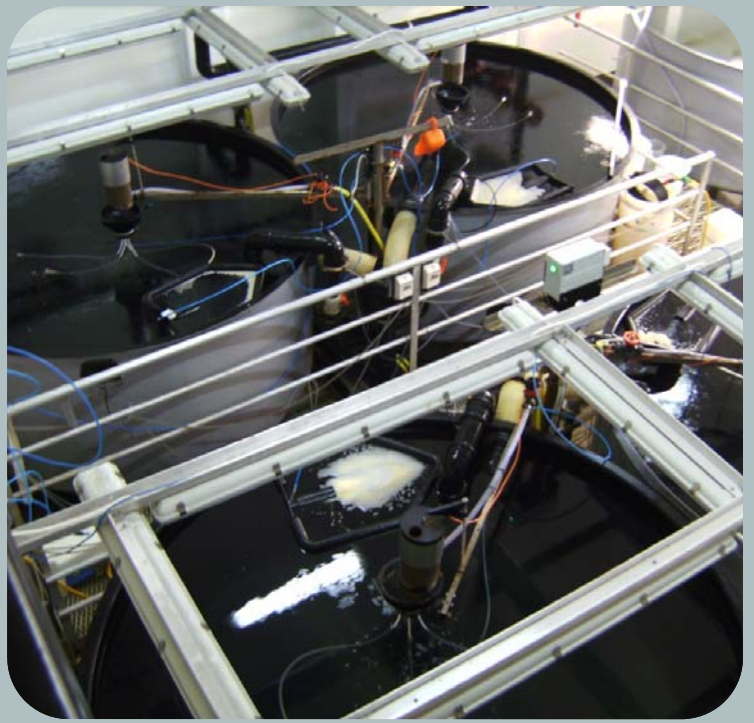
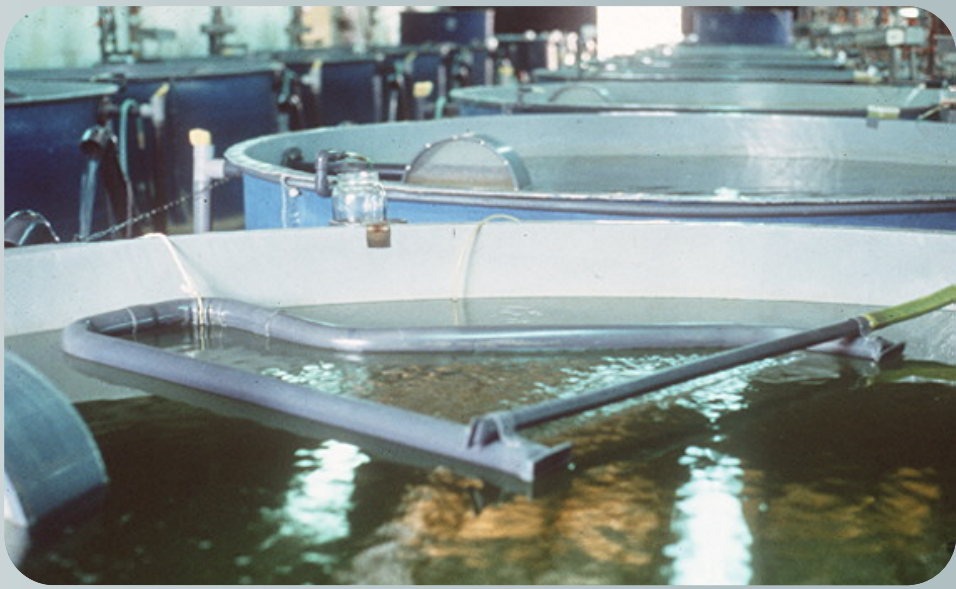


**1976-78 study stays at
SEAFDEC**



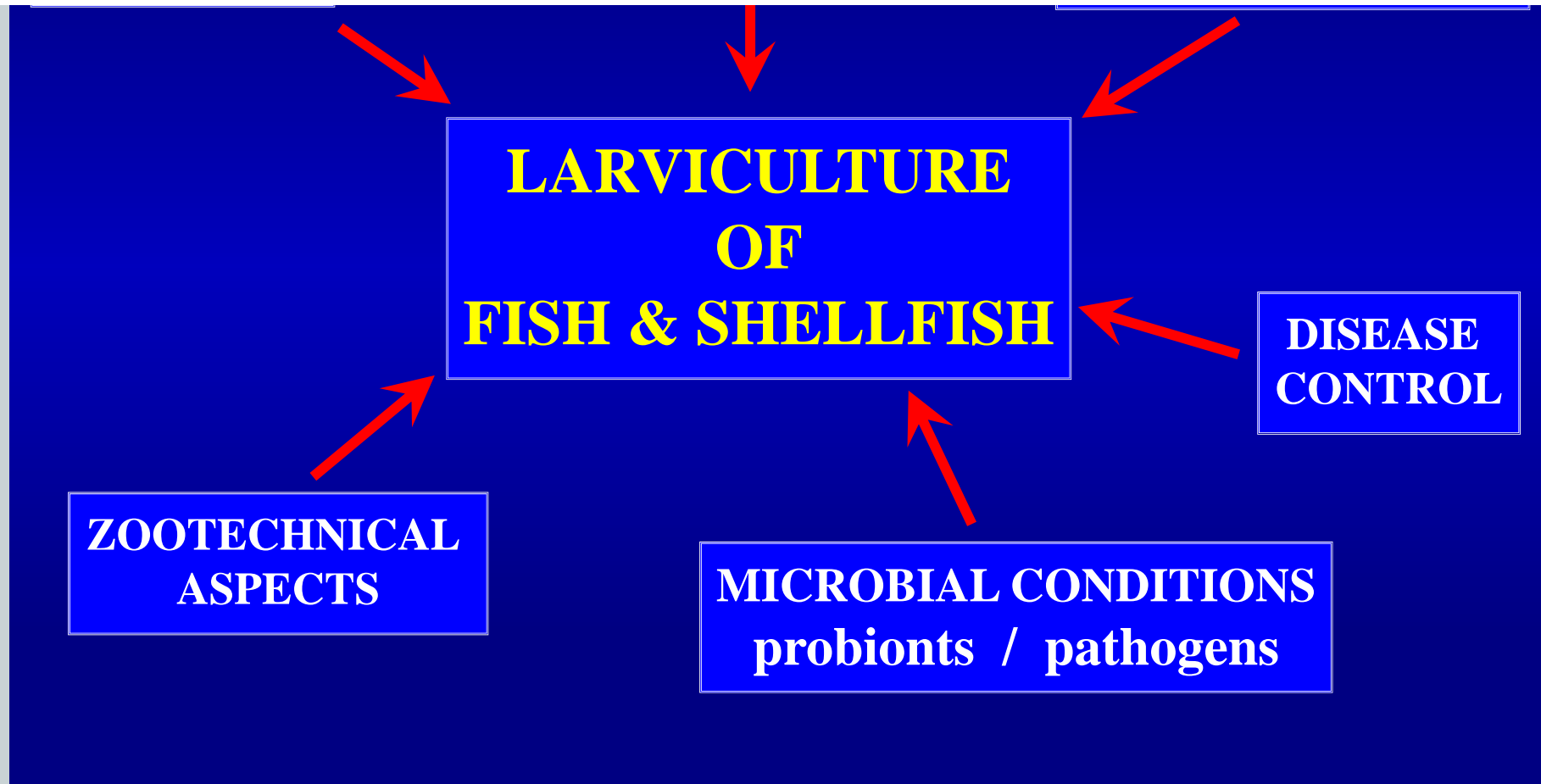




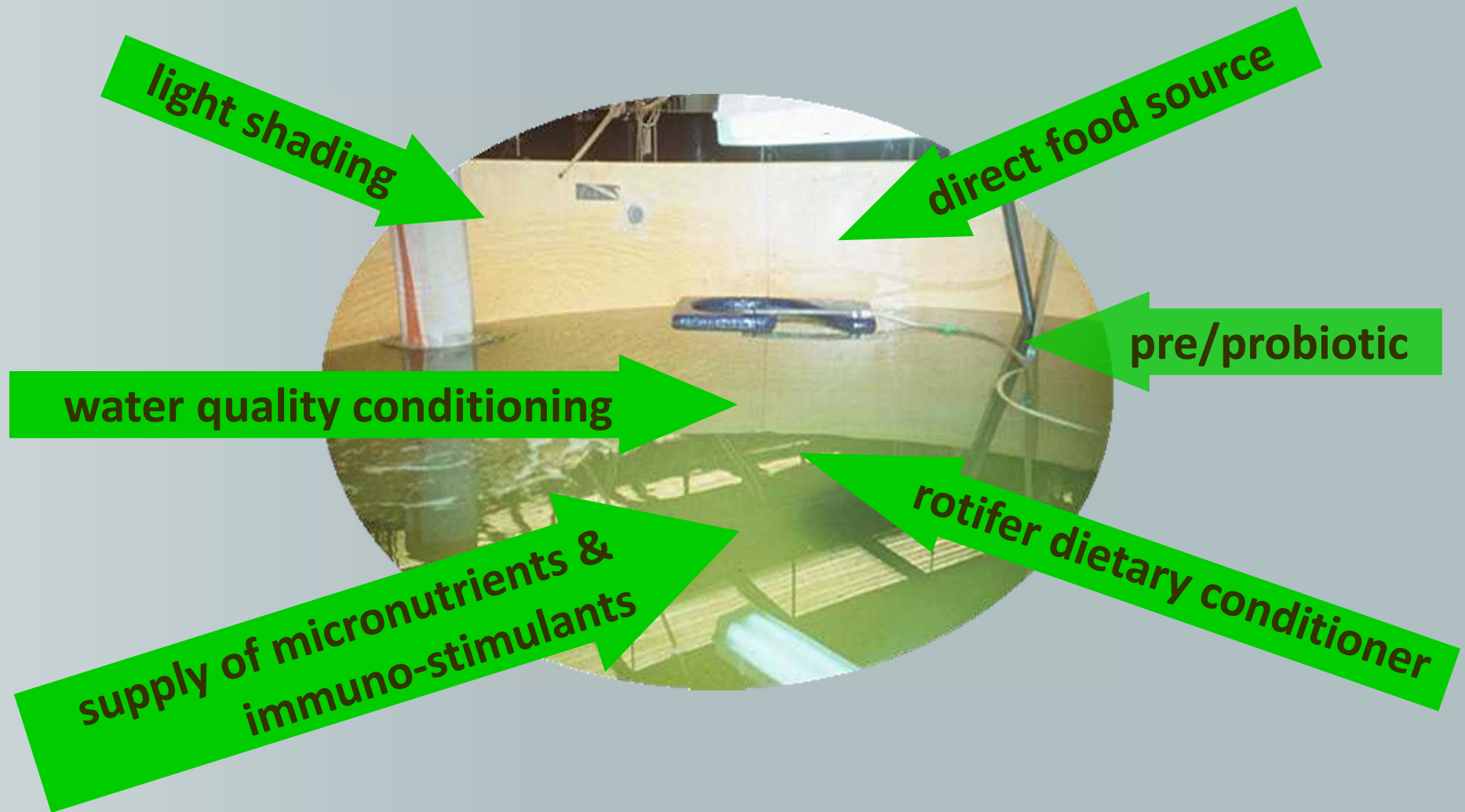


larvi '91

fish & crustacean larviculture symposium
august 27-30, 1991
gent, belgium

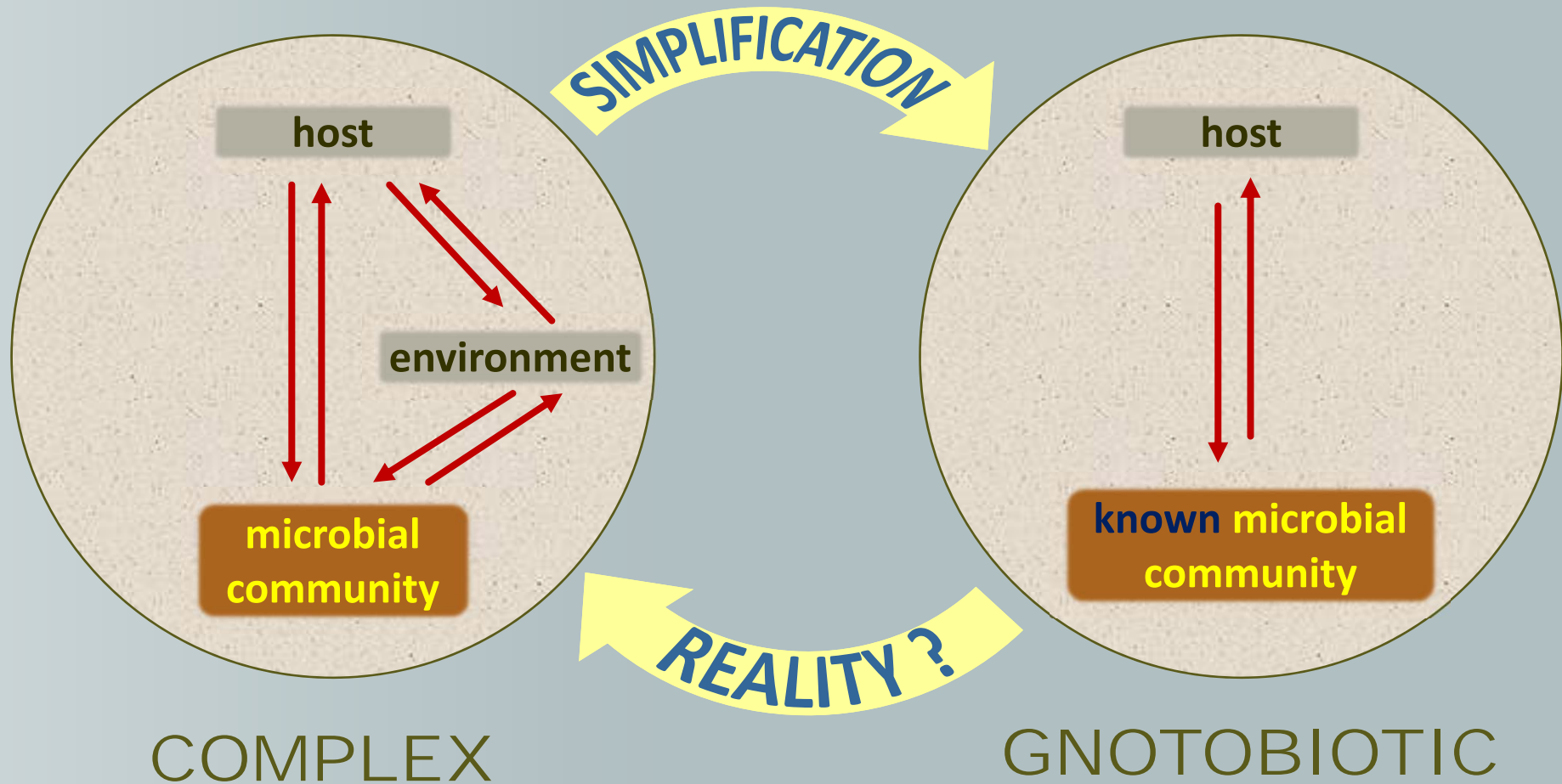


ADVANTAGES OF GREEN-WATER VERSUS CLEAR-WATER TECHNIQUE

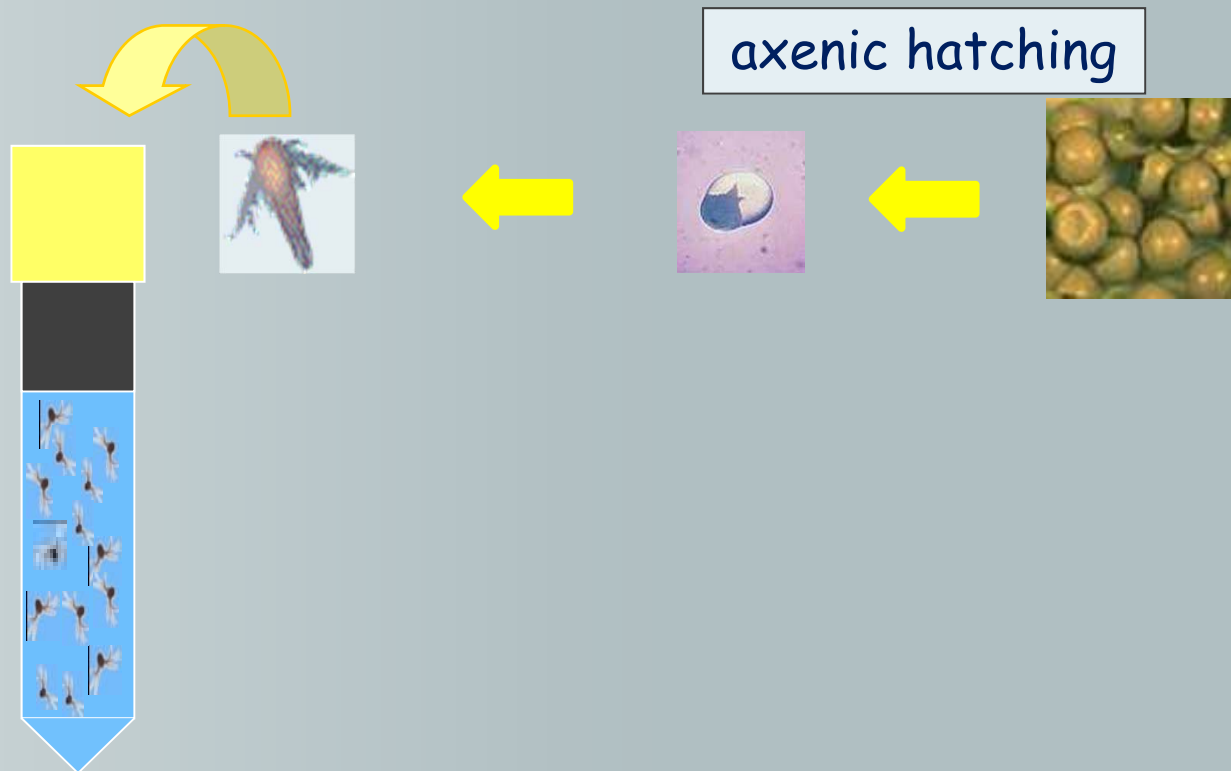


more hypotheses than proofs
black box approach – need for new research approach

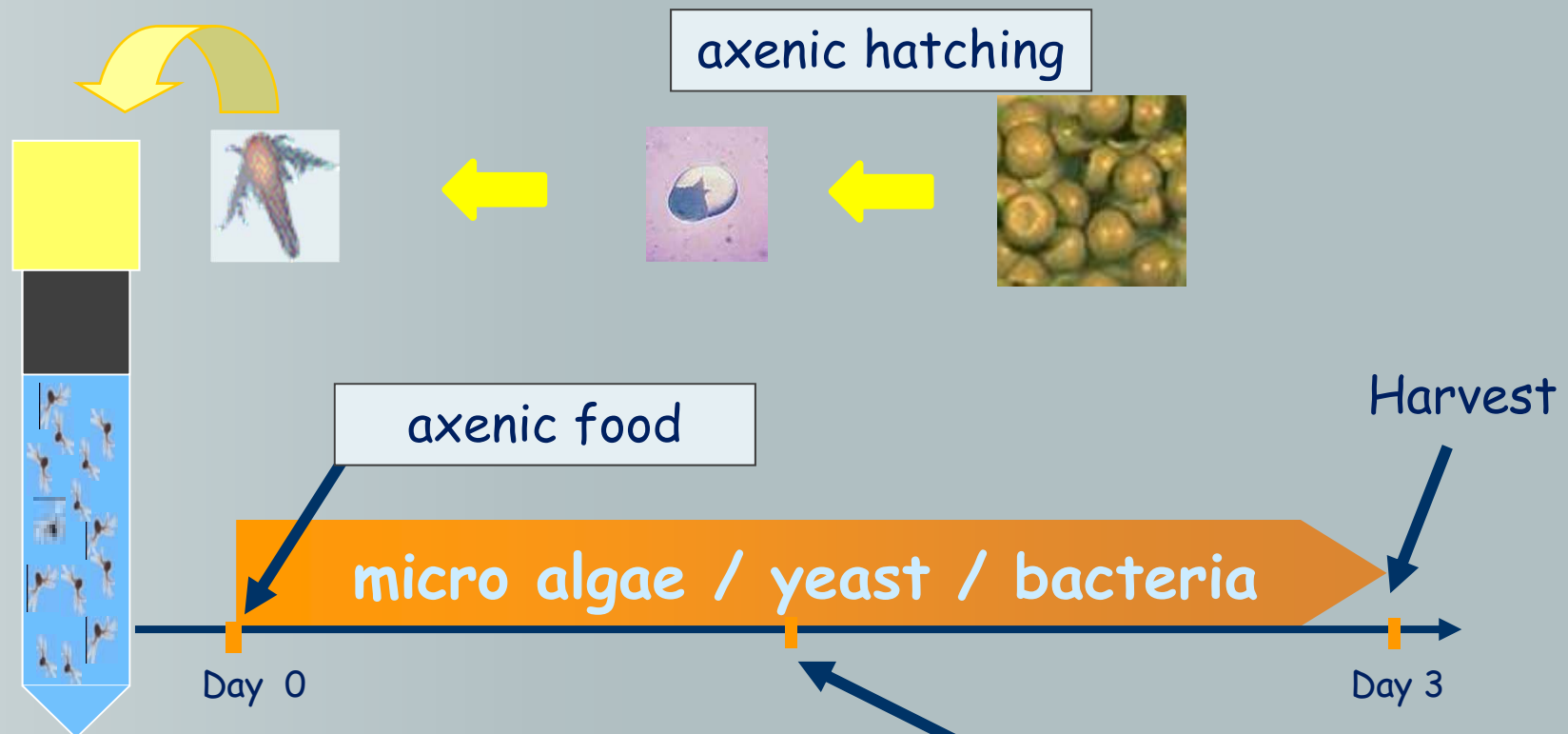
NEW APPROACH IN THE STUDY OF HOST-MICROBE INTERACTIONS



Gnotobiotic culture of *Artemia*



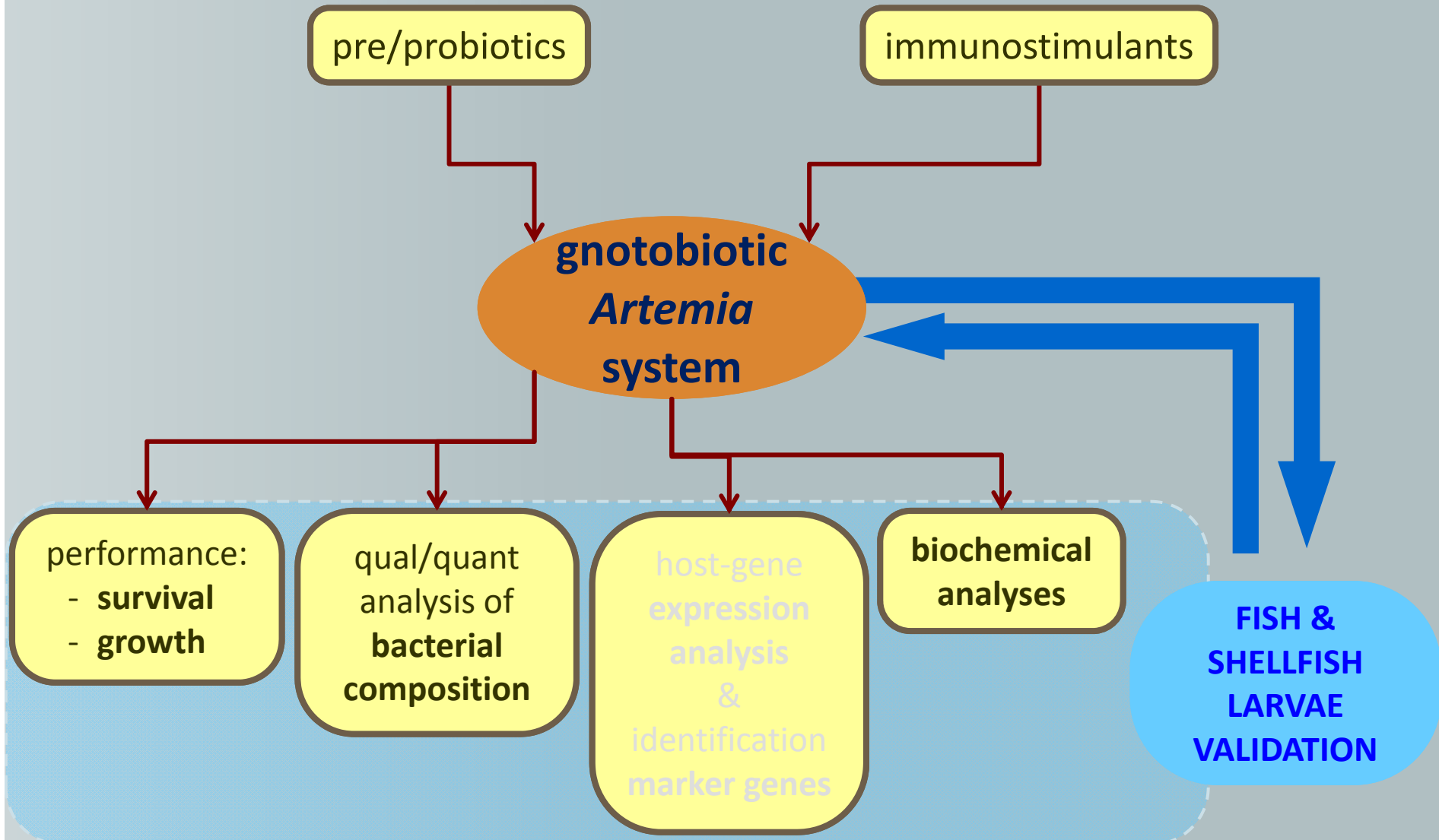
Gnotobiotic culture of *Artemia*



Evaluation criteria

- survival
- length
- total biomass
- immune parameters

Pathogen (*Vibrio campbellii*)



ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- **host-microbe interactions**
- breeding studies
- epigenetics
- nutrition studies – bioflocs



ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- **host-microbe interactions**

→ *Influencing microbial numbers or activity*

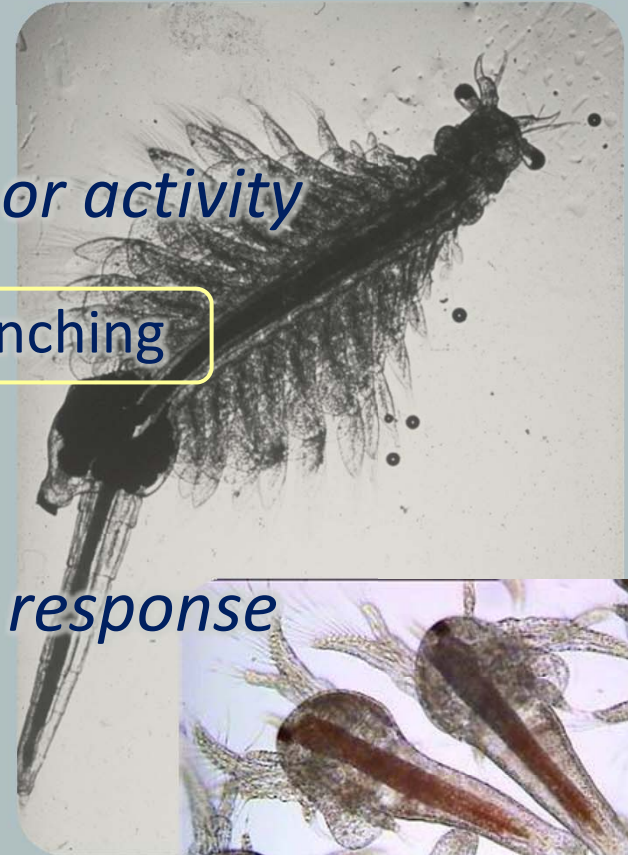
- quorum sensing / quorum quenching

- Poly- β -hydroxybutyrate

→ *Stimulating the host's immune response*

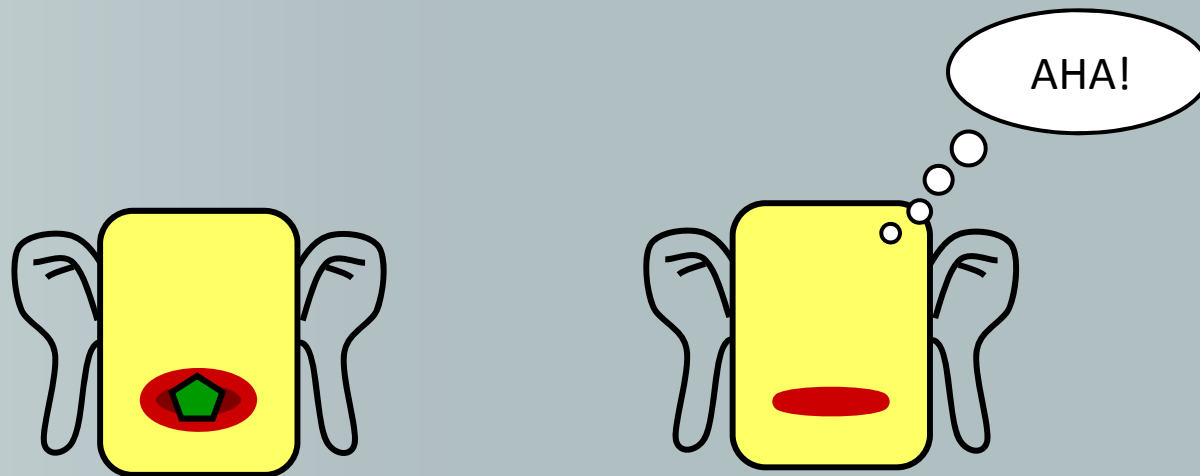
- heat shock proteins

- yeast cell wall-bound glucan



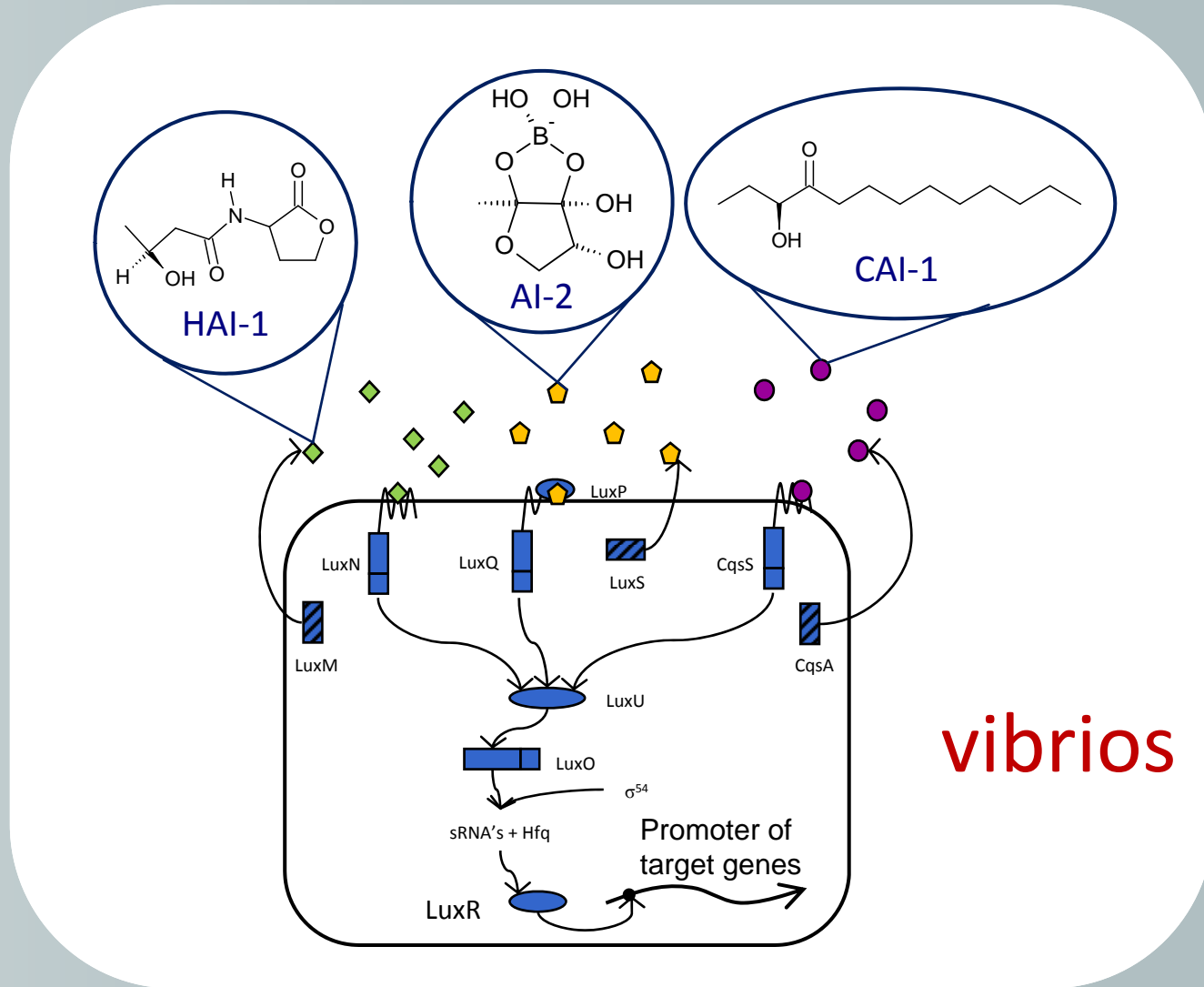
Quorum Sensing (QS)

bacteria **sense and respond** to environmental changes
and to each other through **extracellular**
signal molecules \approx hormones in higher organisms



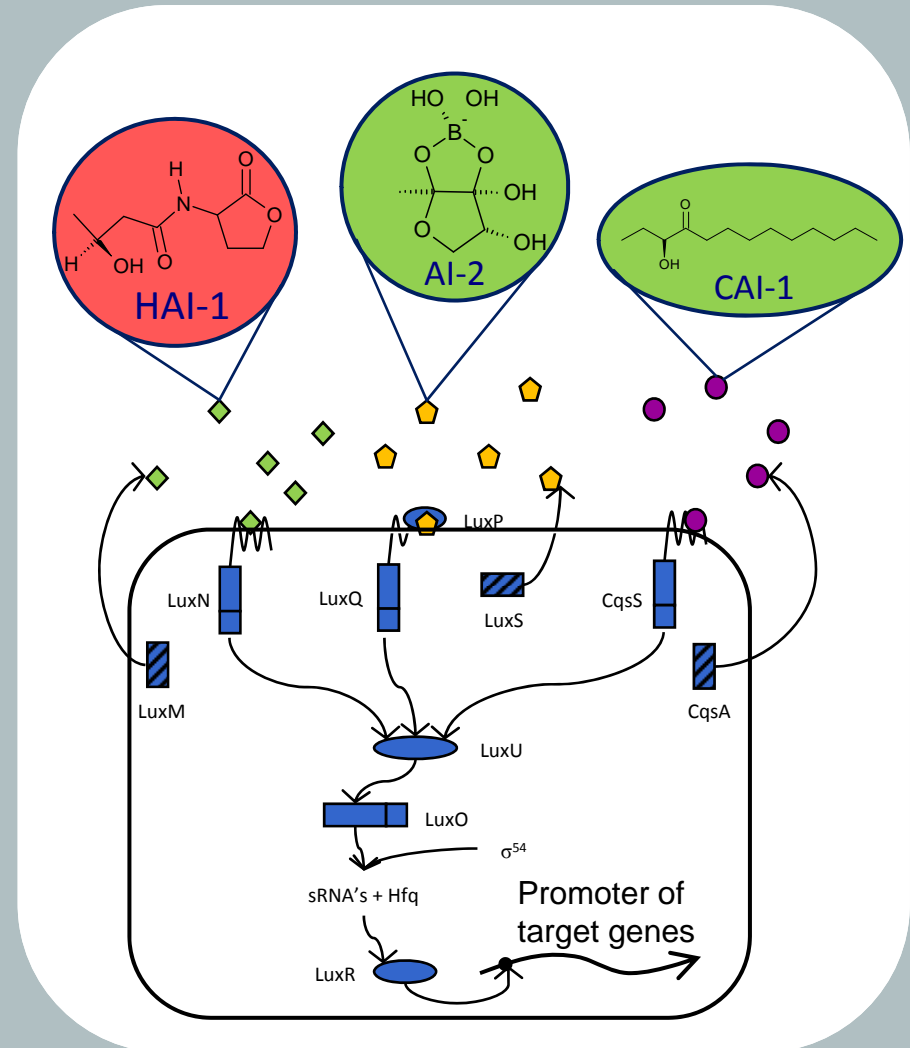
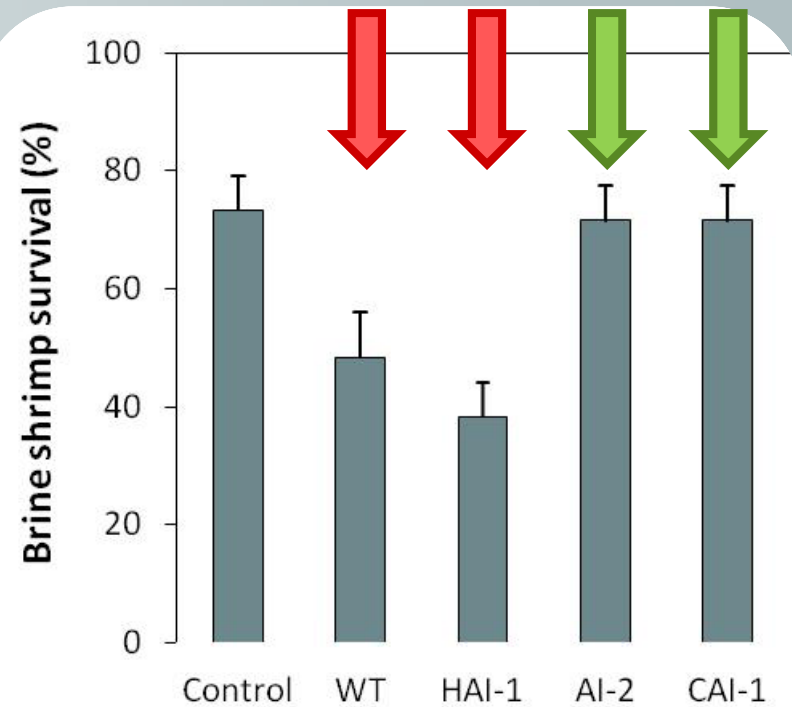
Presence of QS signal molecules affects gene expression

f.ex. virulence factors (biofilm formation, toxin secretion, etc.)



new concept: QS-disruption to control bacterial infections

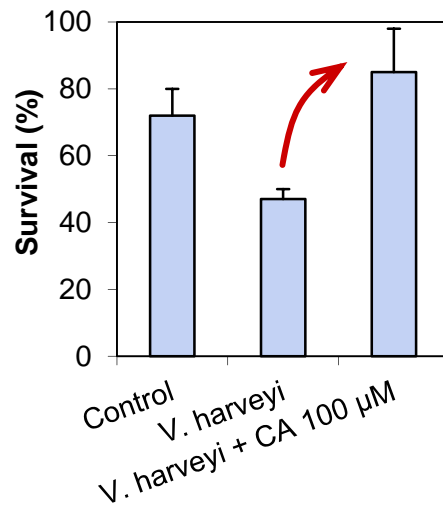
verification with *Vibrio harveyi* QS mutants - effect on *Artemia* survival



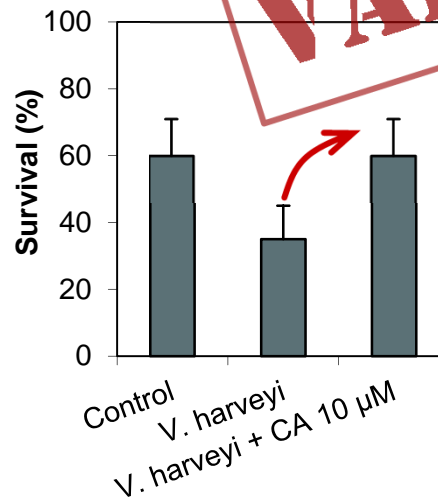
QS-disruption to control bacterial infections

- use of QS inhibitors (e.g. plant extracts)
- degradation of QS signals by other bacteria

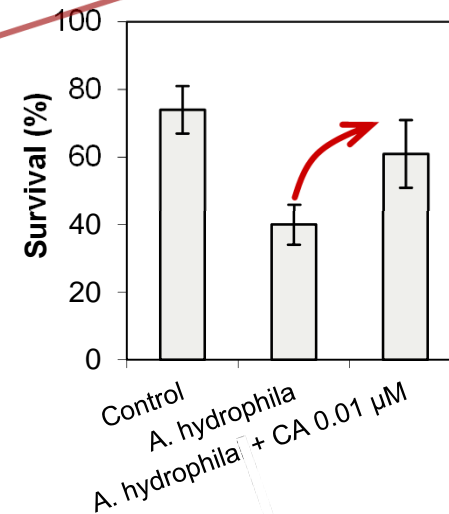
VALIDATED



Artemia
Vibrio harveyi



Macrobrachium
Vibrio harveyi



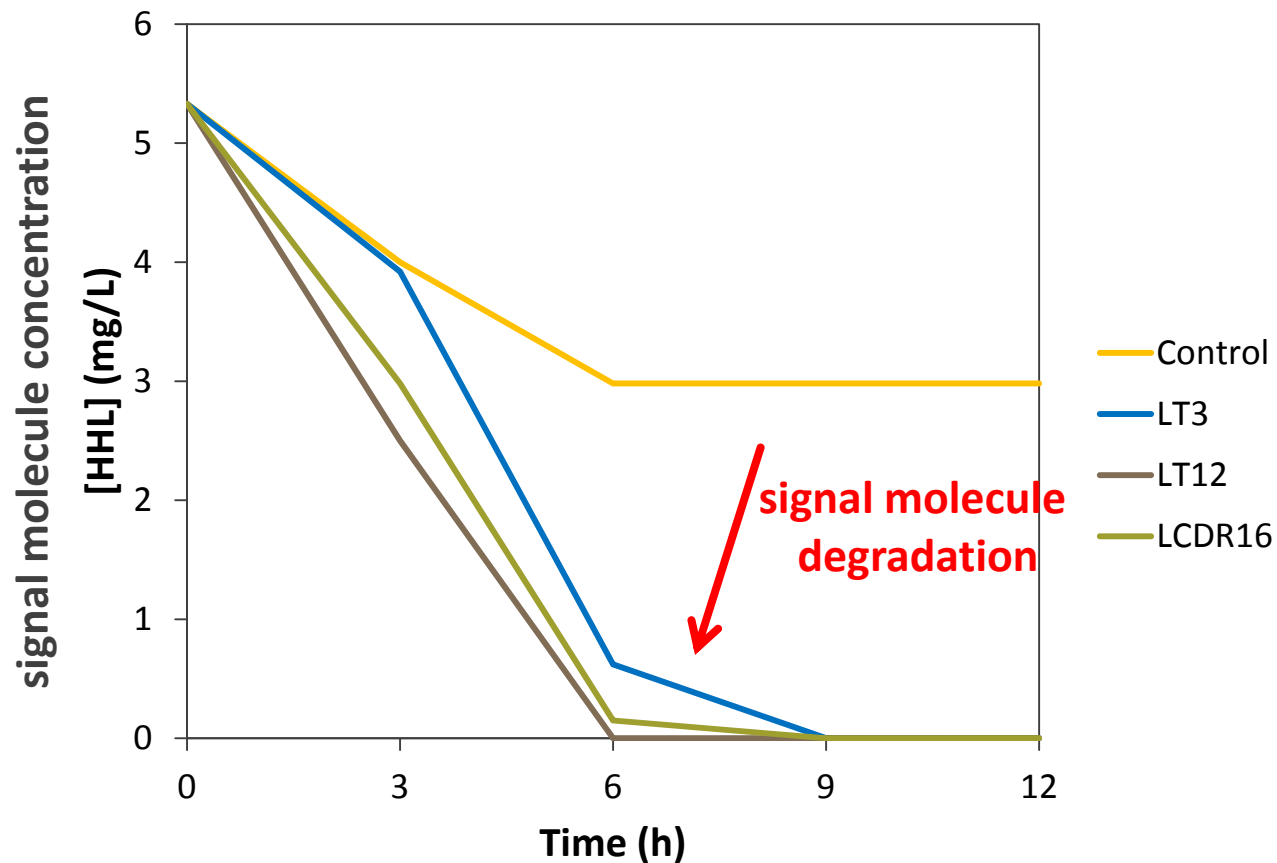
Burbot
Aeromonas hydrophila

Crustaceans: 10-100 µM

Fish: 0.01 µM

QS-disruption to control bacterial infections

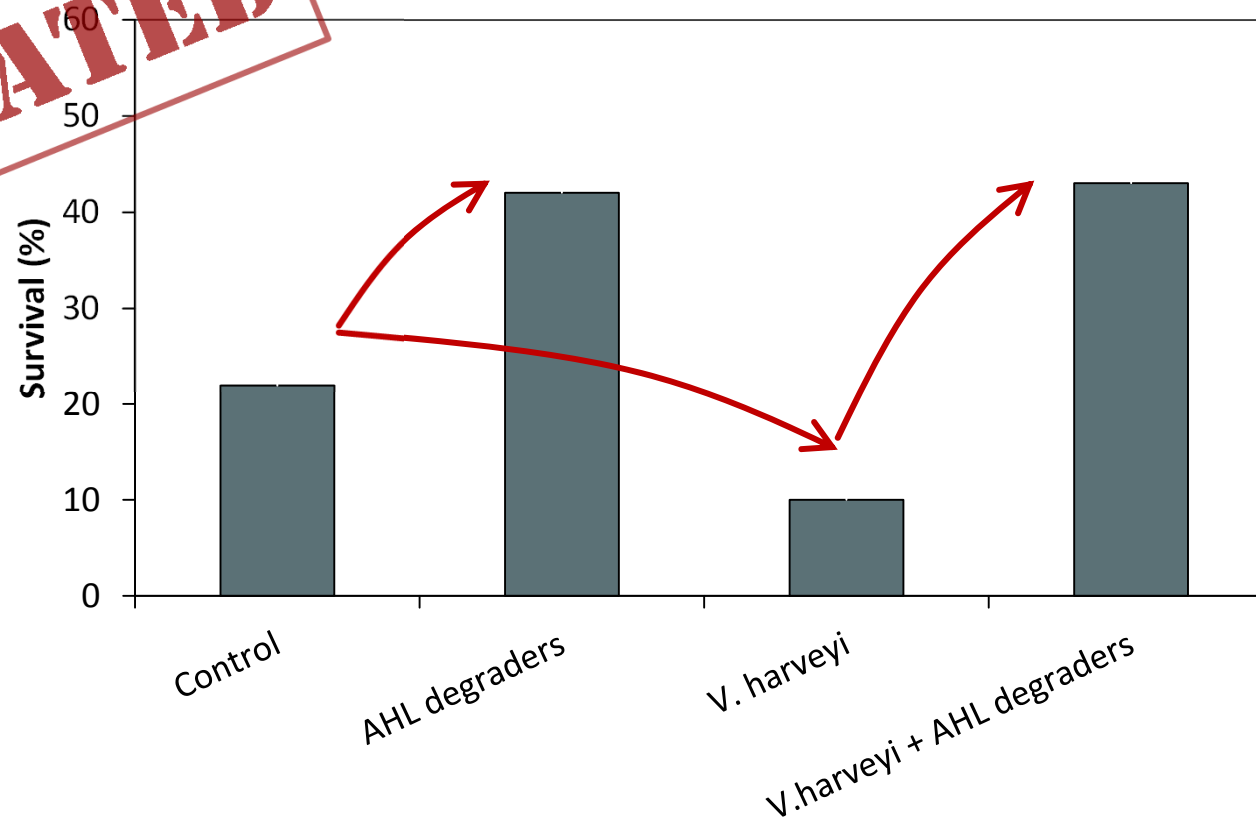
- ☑ use of QS inhibitors (e.g. plant extracts)
- ☐ degradation of QS signals by other bacteria
f.ex. *Bacillus* strains isolated from aquatic organisms



QS-disruption to control bacterial infections

- ☑ use of QS inhibitors (e.g. plant extracts)
- ☐ degradation of QS signals by other bacteria
 - use of signal-degrading probionts in *Macrobrachium* larviculture

VALIDATED



ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- **host-microbe interactions**

→ *Influencing microbial numbers or activity*

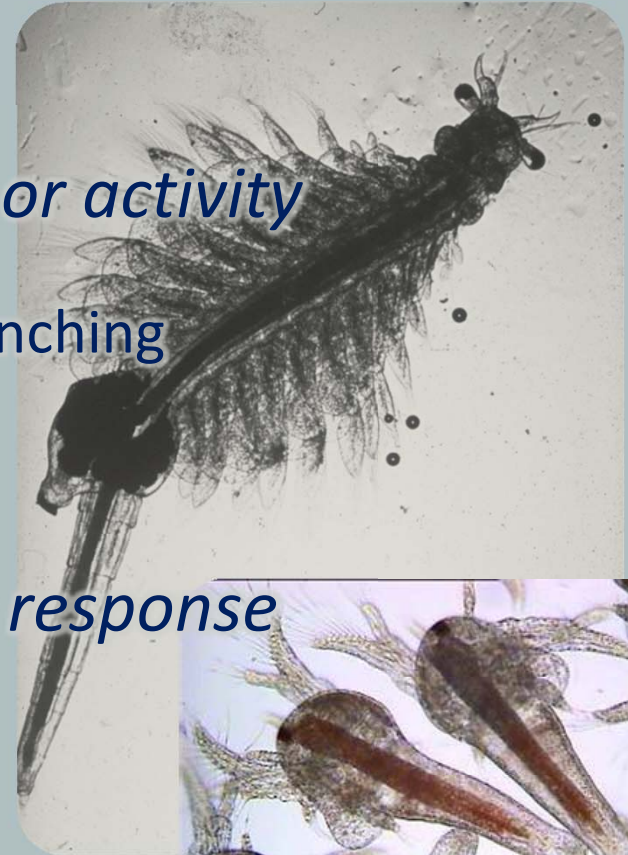
- quorum sensing / quorum quenching

- Poly- β -hydroxybutyrate

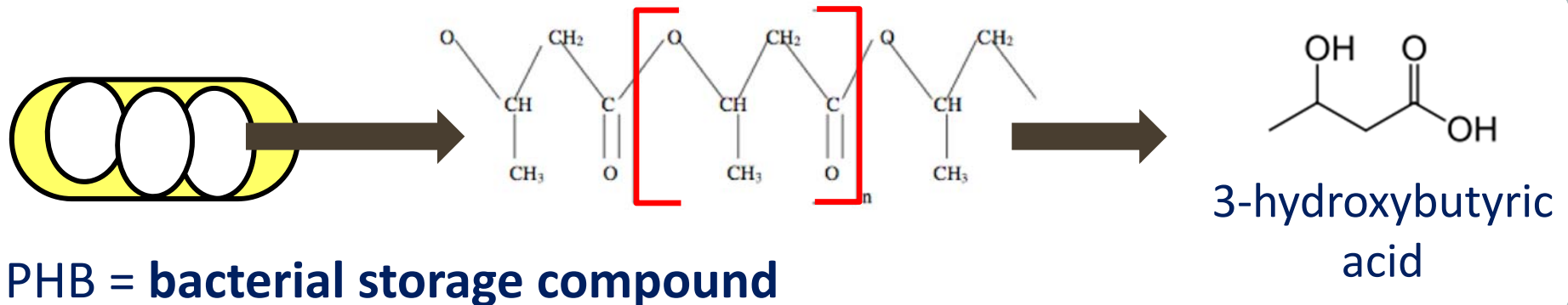
→ *Stimulating the host's immune response*

- heat shock proteins

- yeast cell wall-bound glucan



Poly- β -hydroxybutyrate PHB as a bio-control strategy in larviculture

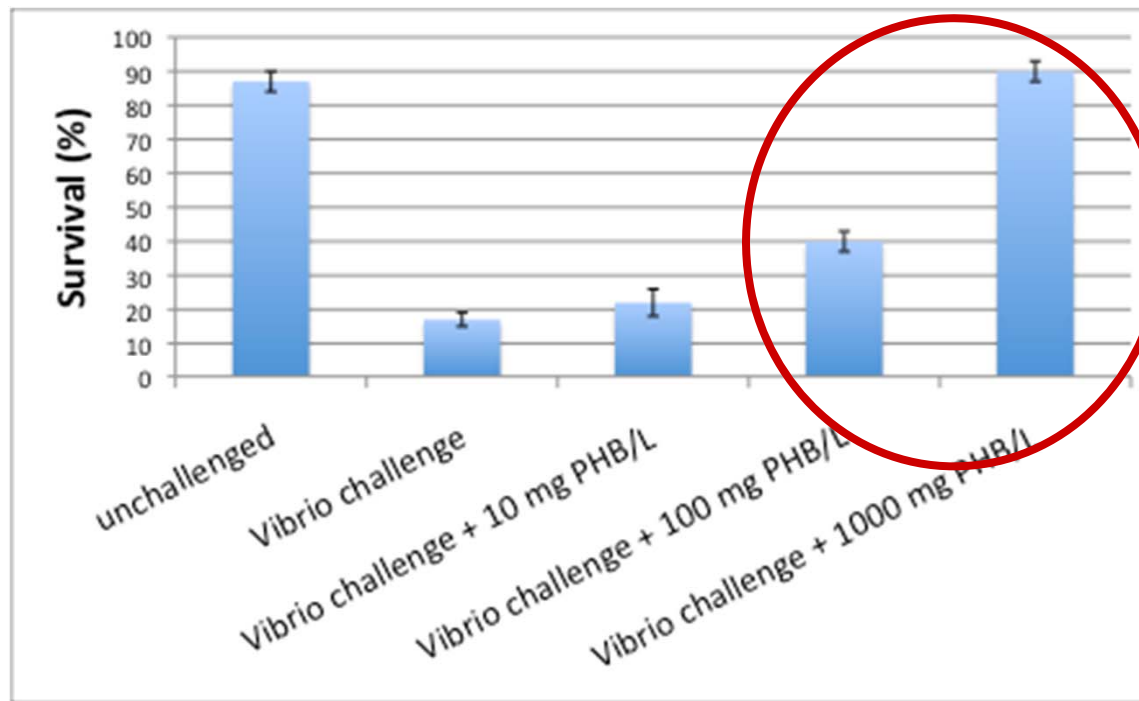


Short chain fatty acids (SCFA) and organic acids:

- ⇒ bacteriostatic and bacteriocidal effects on pathogens
- ⇒ influence invasion capacity of pathogens
- ⇒ beneficial effects on host intestinal cells

→ Does PHB have a protective effect against *Vibrio* infection?

PHB as dietary ingredient for *Artemia* challenge test with pathogenic *Vibrio campbellii*

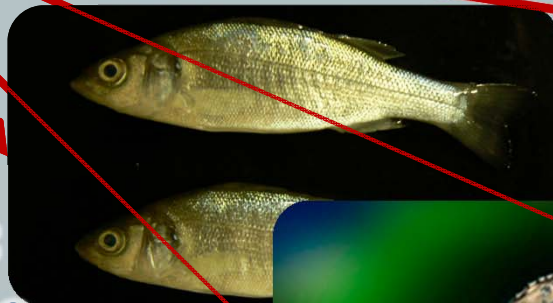
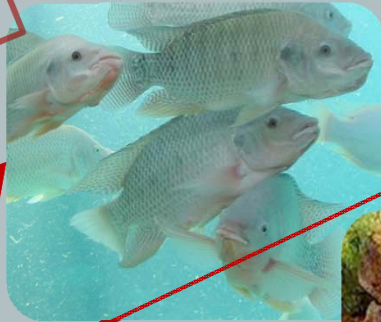


significant increase of
survival at 100 mg/L
and 1000 mg/L PHB



Application of PHB in fish and shellfish larviculture

VALIDATED



- Improved parameters:
- growth performance
 - larval survival
 - infection resistance

ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- **host-microbe interactions**

→ *Influencing microbial numbers or activity*

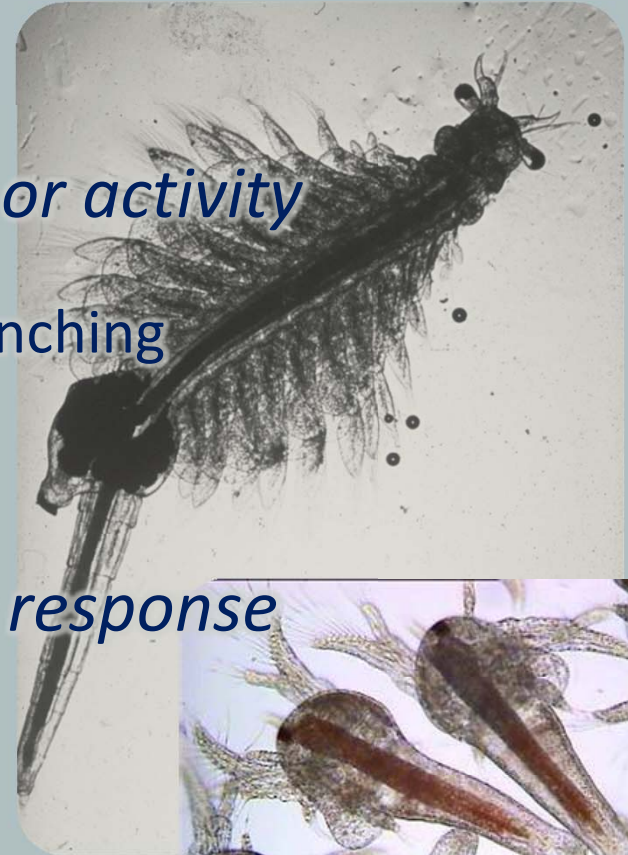
- quorum sensing / quorum quenching

- Poly- β -hydroxybutyrate

→ *Stimulating the host's immune response*

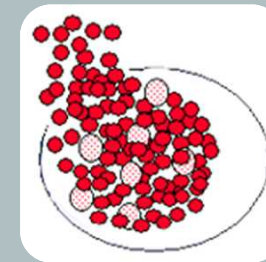
- heat shock proteins

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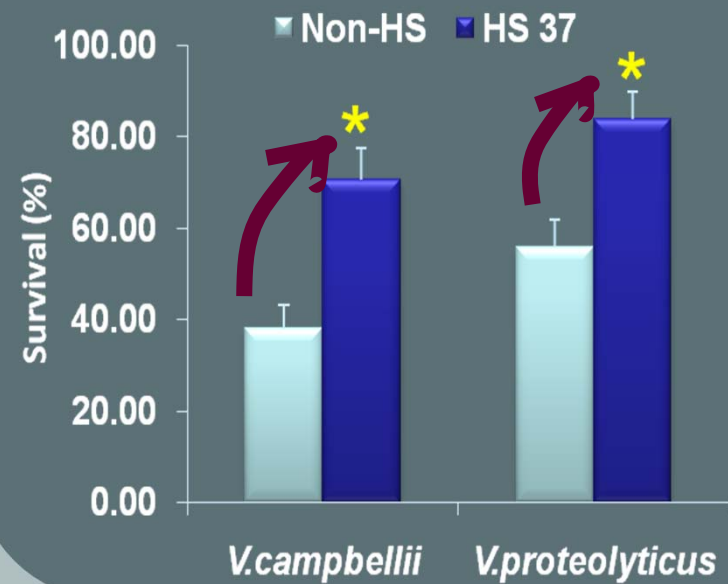
Heat shock proteins (Hsps)

- ✓ highly conserved proteins, available in all living cells
- ✓ Induced after exposure to stressors (heat, cold, O₂ deprivation, radicals, disease etc)
- ✓ Inside the cell, act as molecular chaperones - assist in protein biogenesis and degradation
- ✓ Extracellular Hsps serve as danger signals and modulate both innate and adaptive immune responses



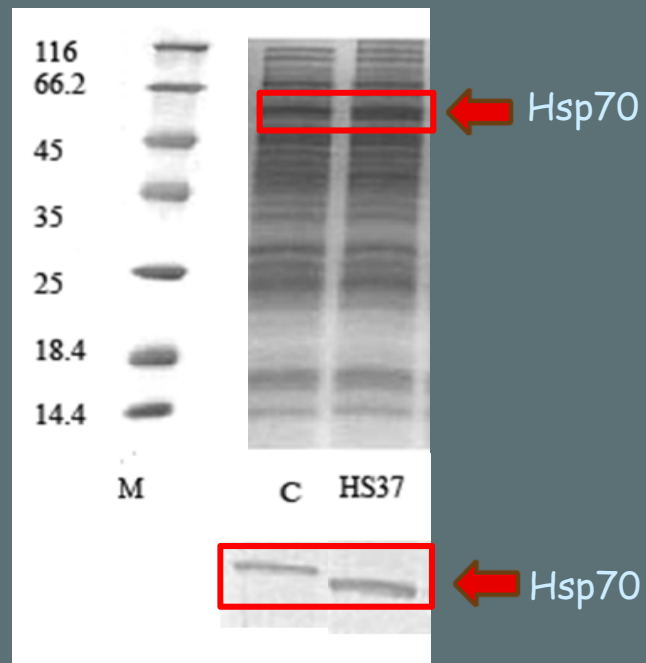
Hsps effects in *Artemia* - *Vibrio* challenge test

Survival after *Vibrio* challenge

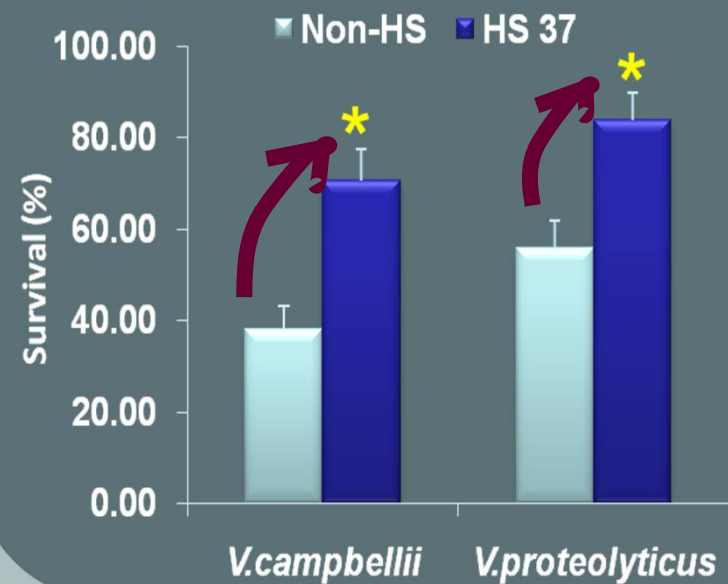


Hsps effects in *Artemia* - *Vibrio* challenge test

Endogenous Hsp accumulation



Survival after *Vibrio* challenge



Correlation exists between enhanced protection and Hsp70 accumulation

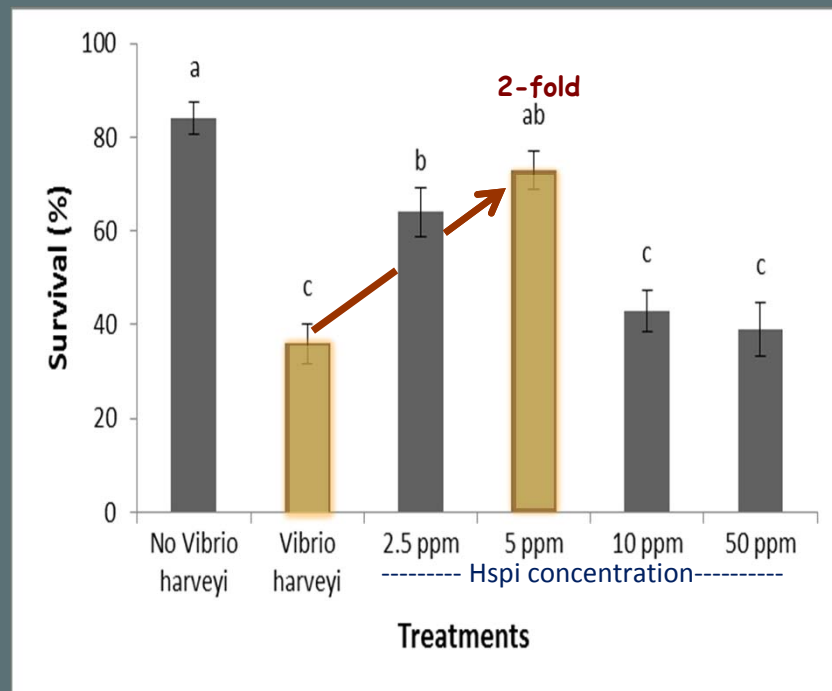
new concept: use of Hsp-inducing compounds

- ✓ heat shock is not an ideal way to enhance Hsps in aquaculture
- ✓ less traumatic approaches are needed to manipulate Hsps expression
- ✓ can compound(s) extracted from plants induce Hsp70 in aquaculture animals?
- ✓ can they confer protection against stress and disease ?



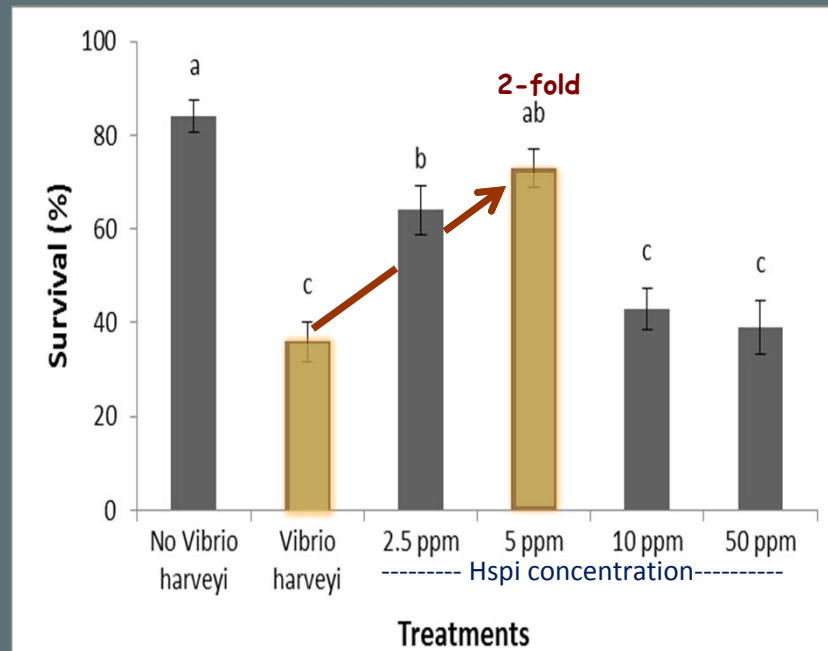
Protective effect of Hsp-inducing compounds against *Vibrio harveyi*

Survival after *Vibrio* challenge

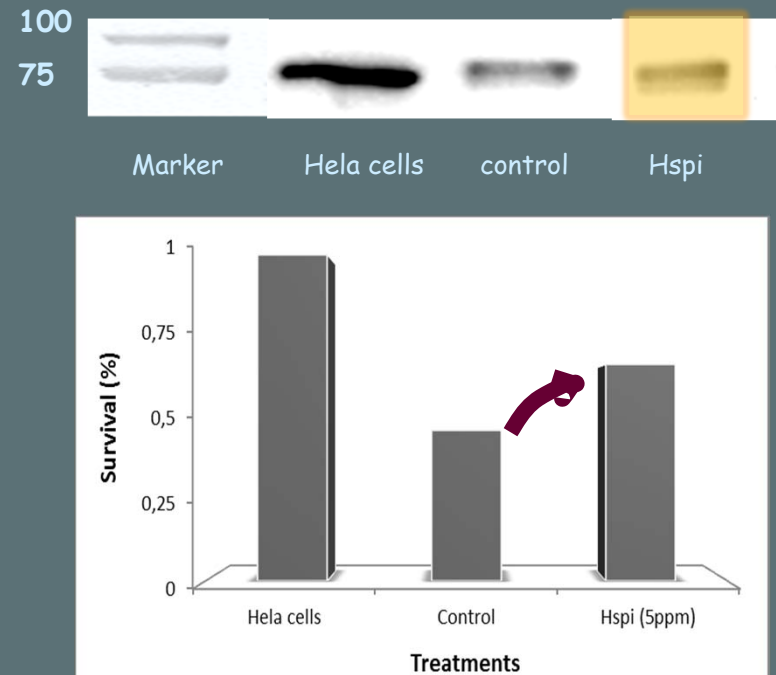


Protective effect of Hsp-inducing compounds against *Vibrio harveyi*

Survival after *Vibrio* challenge

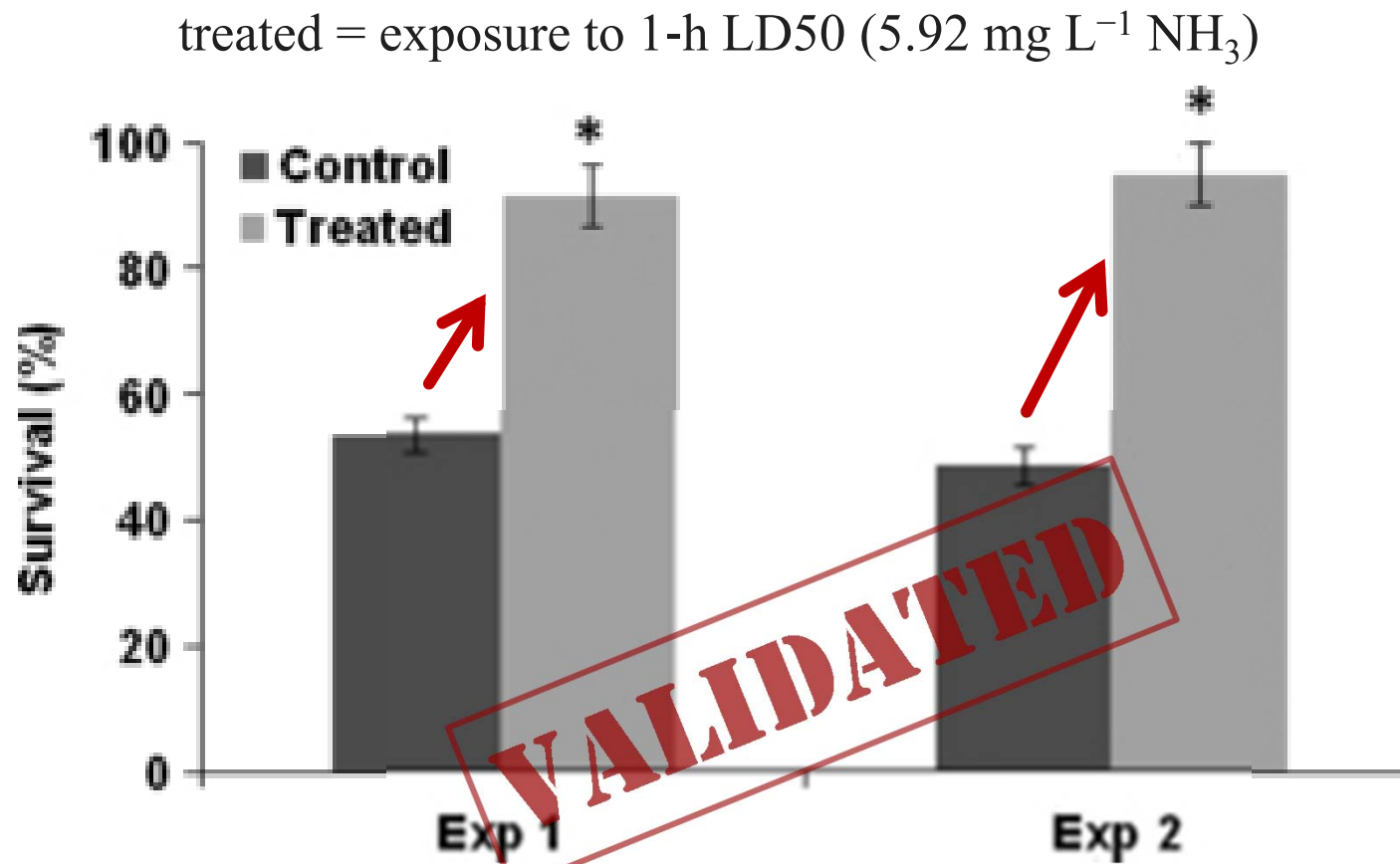


Induction of Hsp70

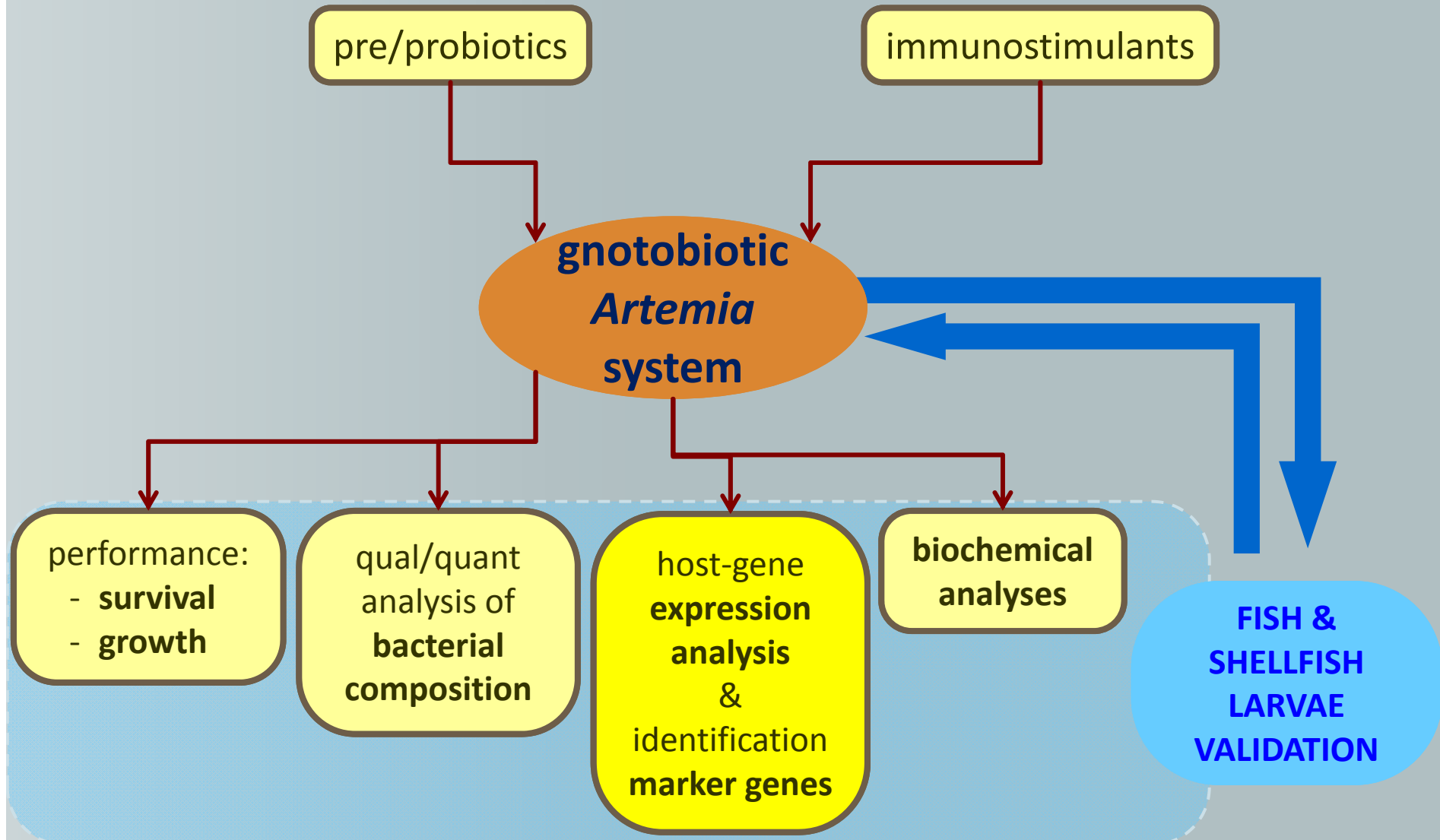


Validation with common carp *Cyprinus carpio*

Hsp-inducing plant extract protects against lethal ammonia toxicity

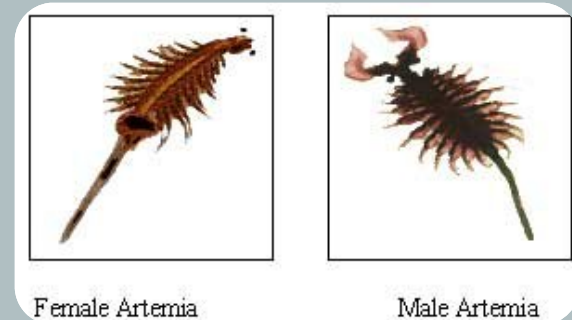


Development of innovative microbial management systems

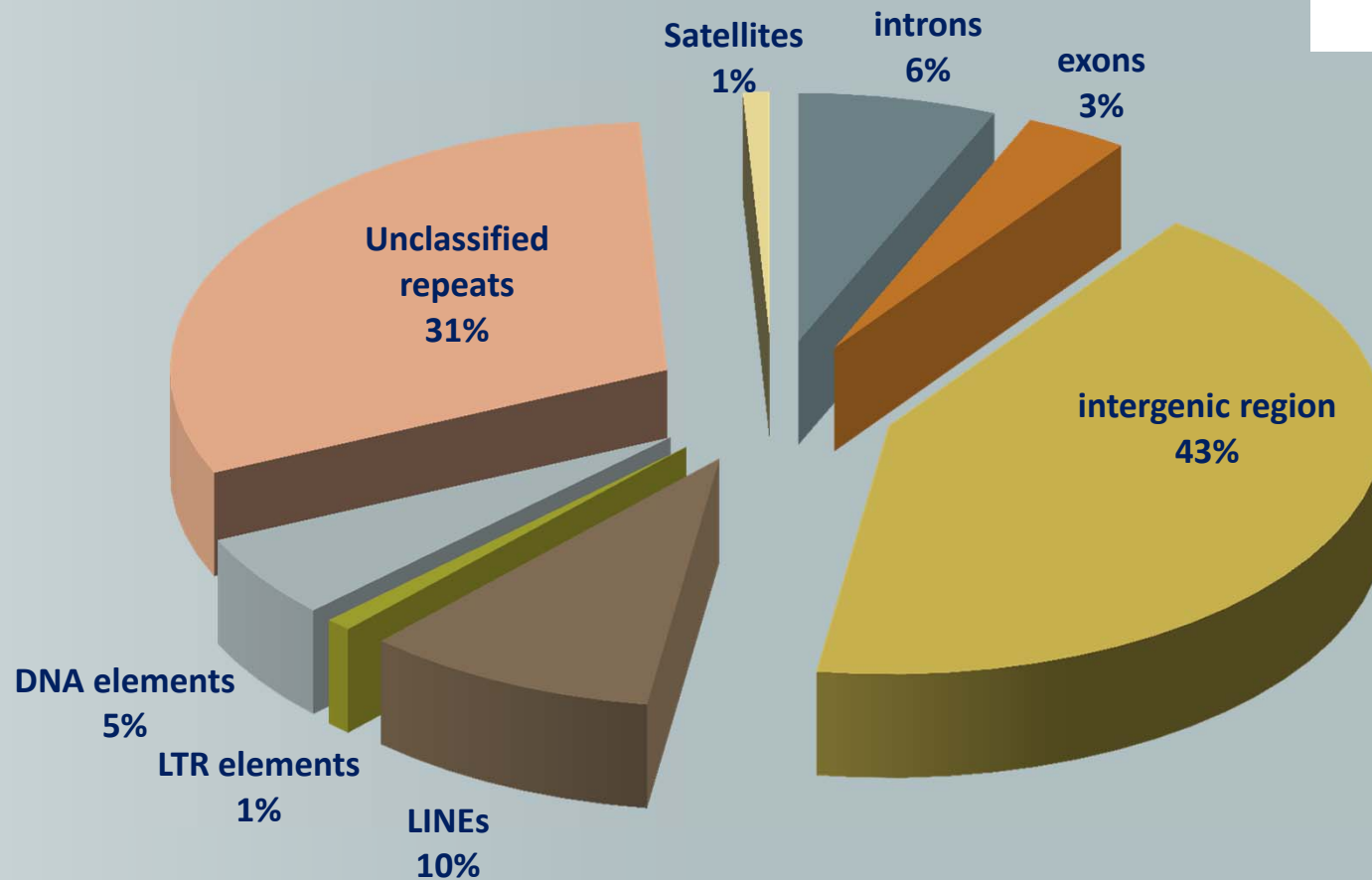
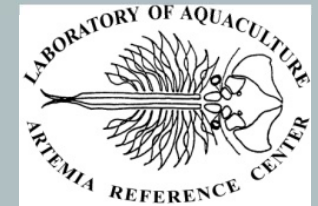


The *Artemia* genome so far

- **mtDNA** sequenced
(Valverde et al., 1994)
- **$2n = 42$** (*A. persimilis* 44)
(Abreu-Grobois, 1987; Badaracco et al., 1987)
- Genome size estimation ~ **1 Gb**
(De Vos et al., 2013)
- AFLP-based **linkage map**
with 433 AFLP markers
(De Vos et al., 2013)



The *Artemia* genome today



23,860 coding genes identified

ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- ✓ host-microbe interactions
- **breeding studies**
- epigenetics
- nutrition studies – bioflocs



ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

✓ host-microbe interactions

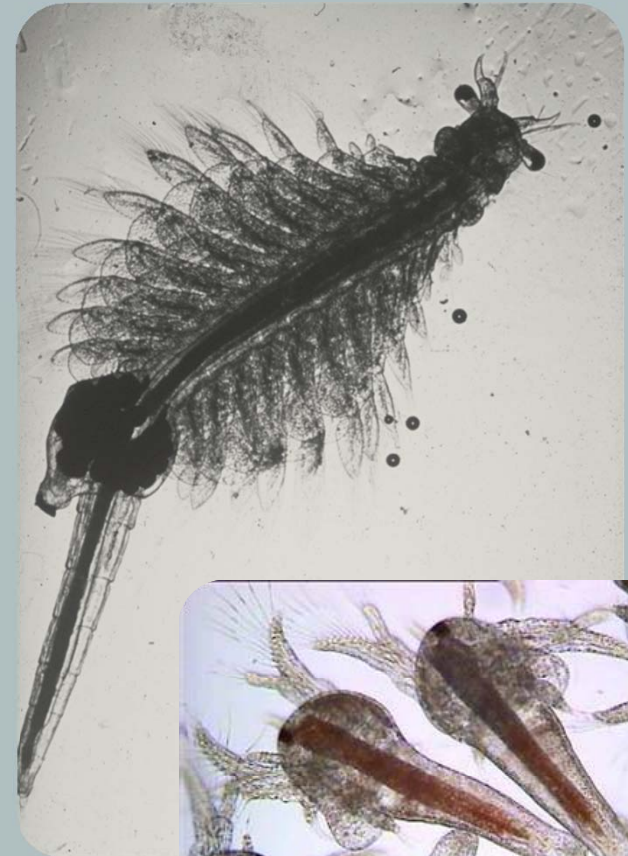
• **breeding studies**

→ *Artemia*: suitable tool as
gene discovery platform

for crustaceans ?

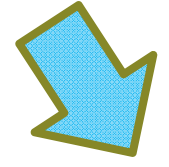
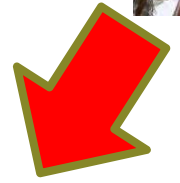
1st test case:

breeding for thermo-tolerance



lethal heat shock **T**

C iso-thermic conditions

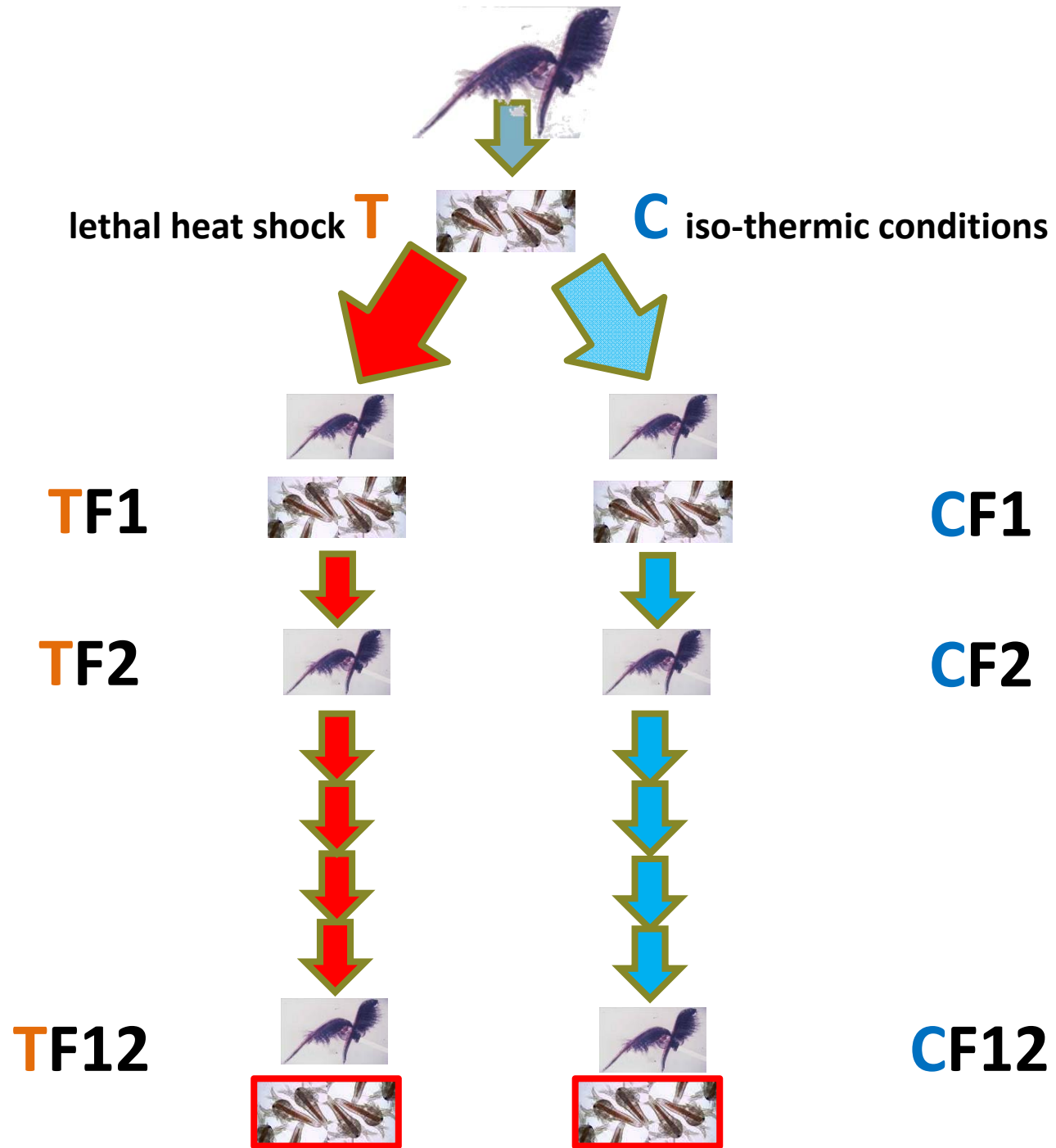


TF1

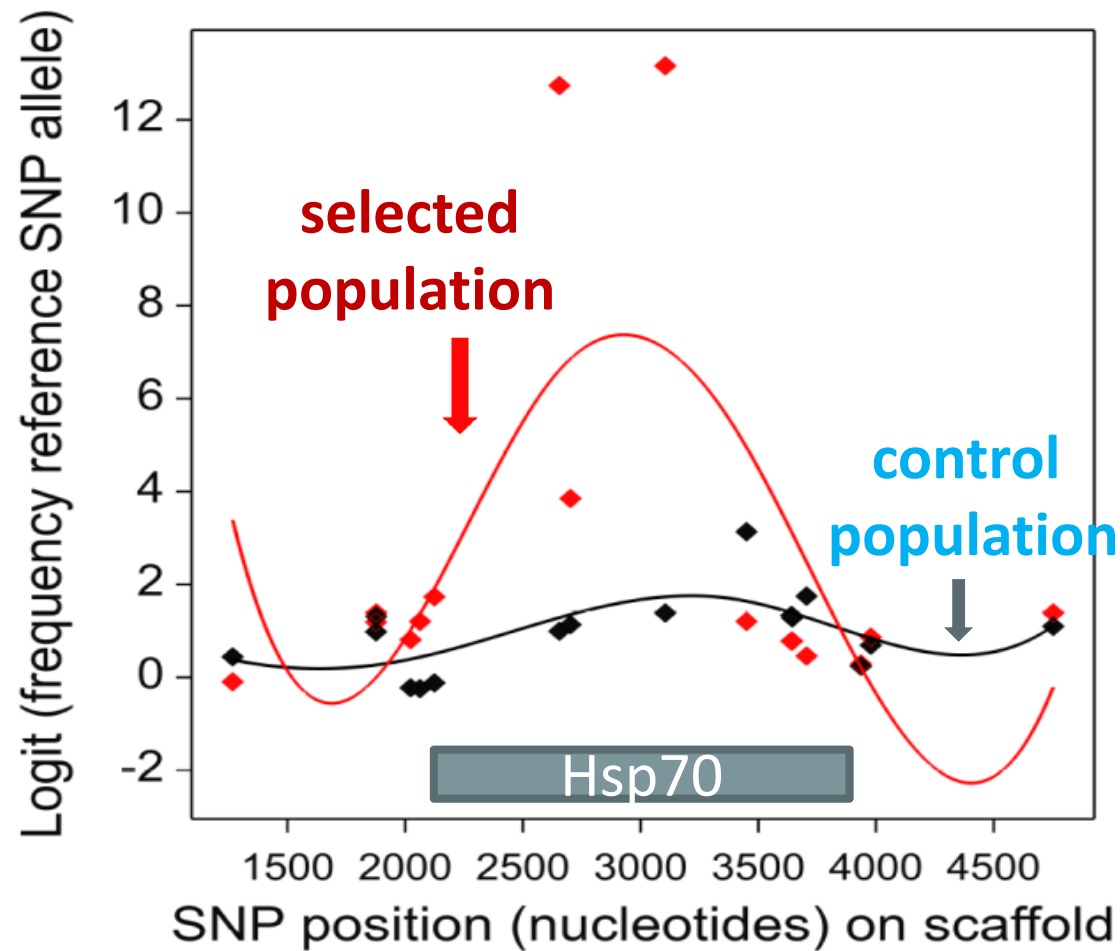


CF1





Allele shift detection in relevant genes by whole genome re-sequencing



ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- ✓ host-microbe interactions
- ✓ breeding studies
- **epigenetics**
- nutrition studies – bioflocs



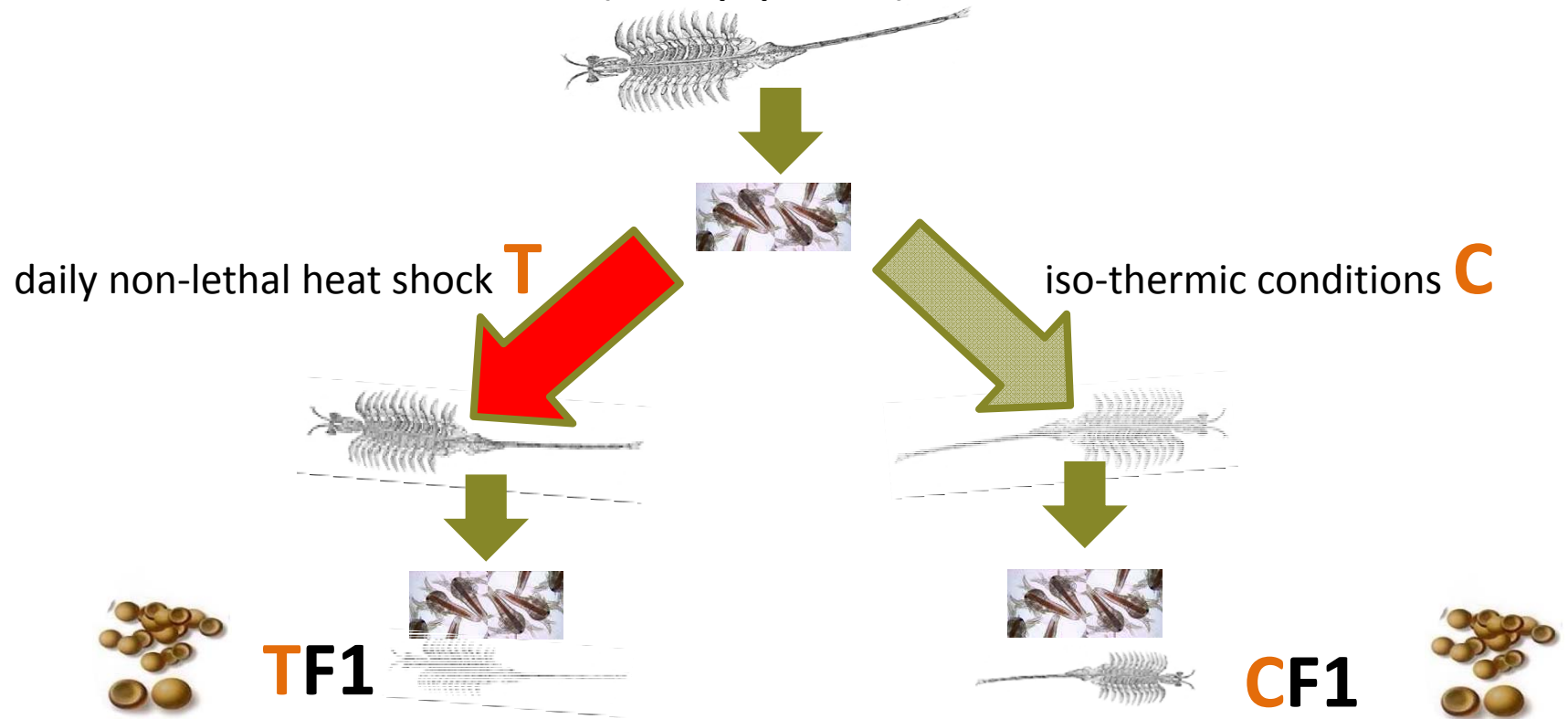
ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- ✓ host-microbe interactions
 - ✓ breeding studies
 - **epigenetics**
- heritable modification of phenotypes
without modification at genotype level
(modification of DNA/histones)



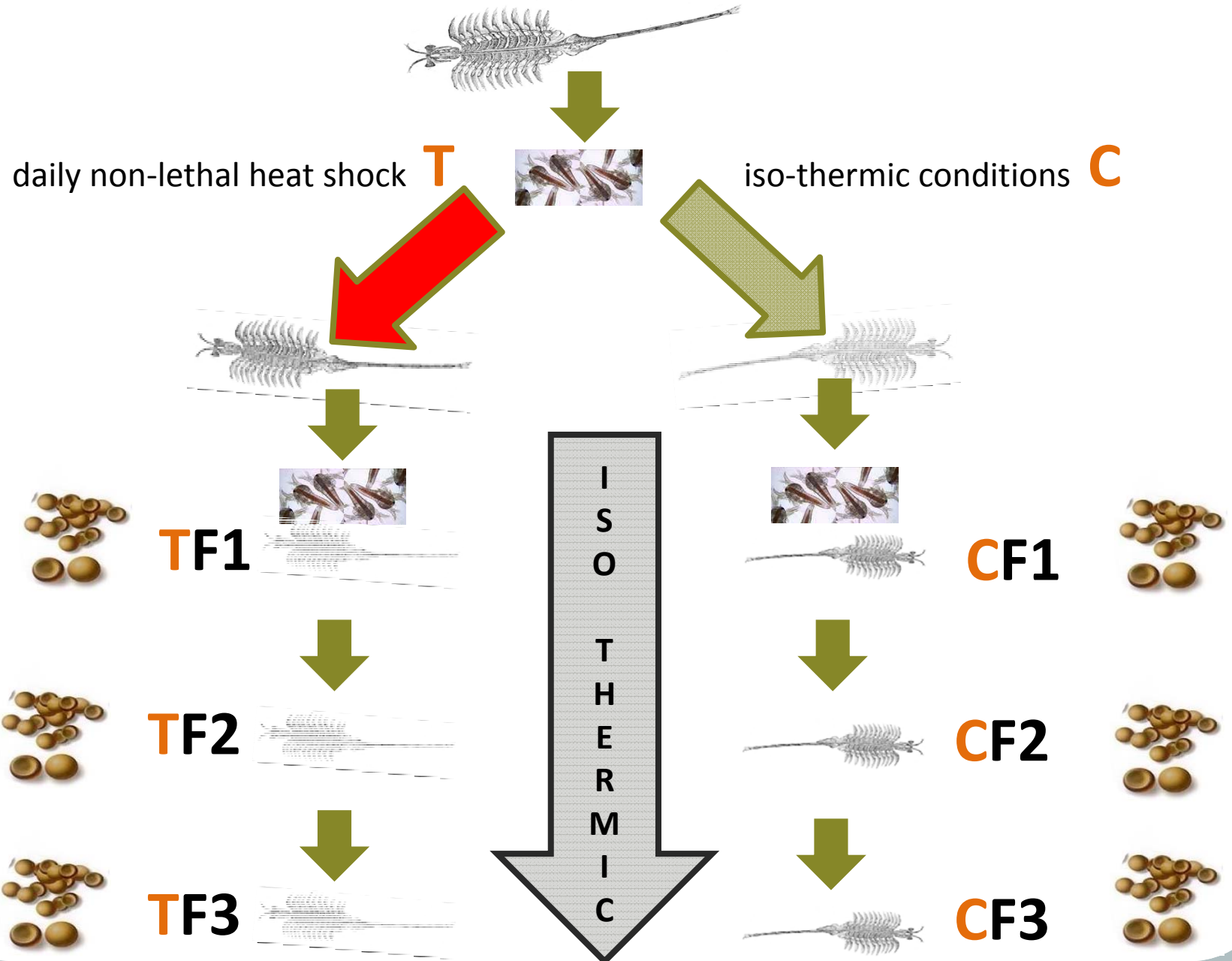
Parthenogenetic *Artemia* strain

(clonal population)

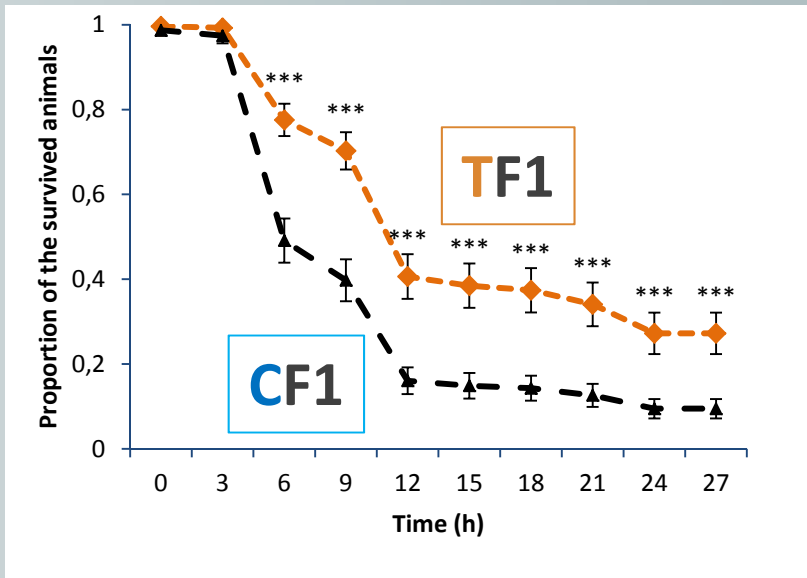


Parthenogenetic *Artemia* strain

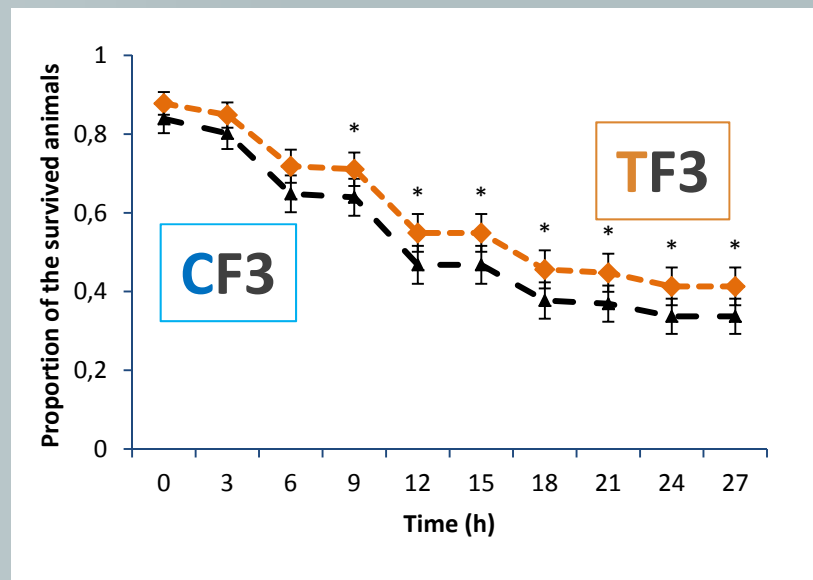
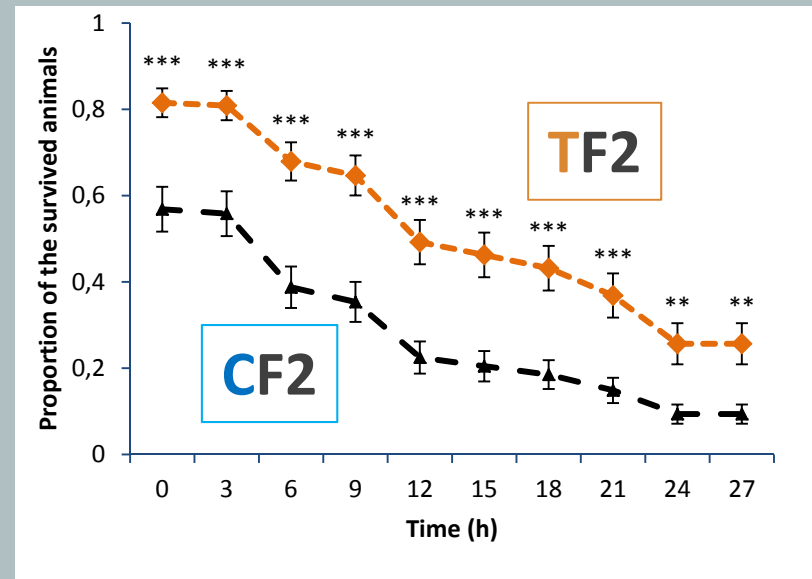
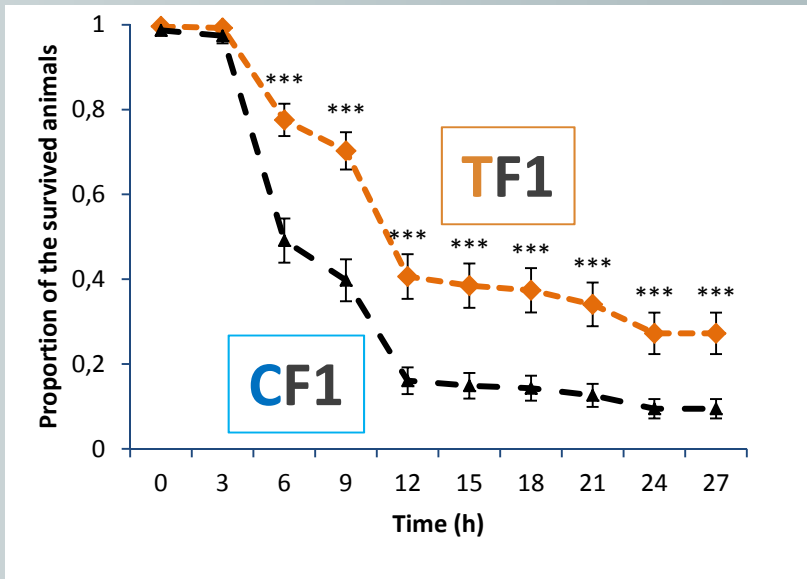
(clonal population)



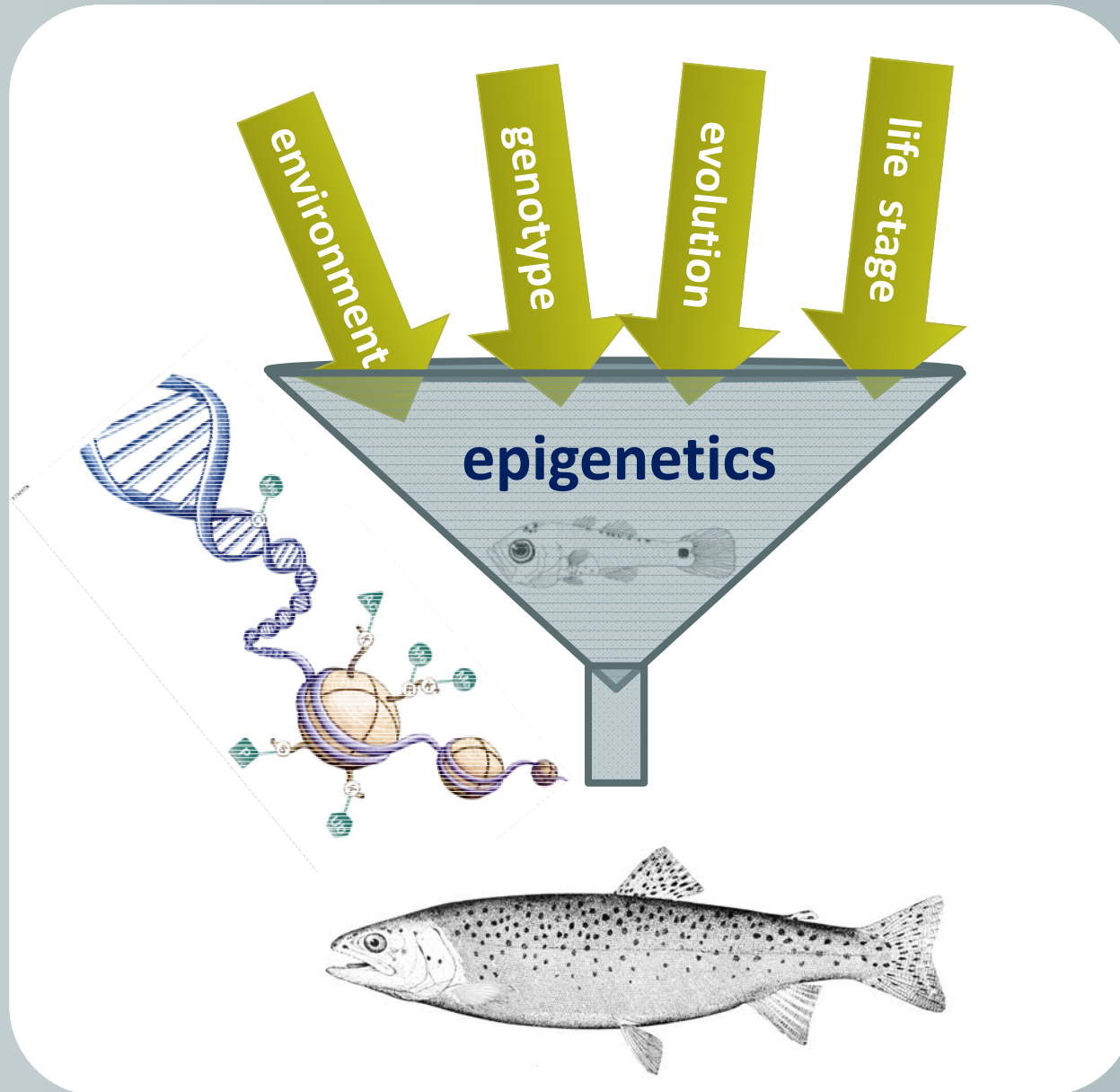
Thermo-tolerance test (common garden experiment)



Thermo-tolerance test (common garden experiment)



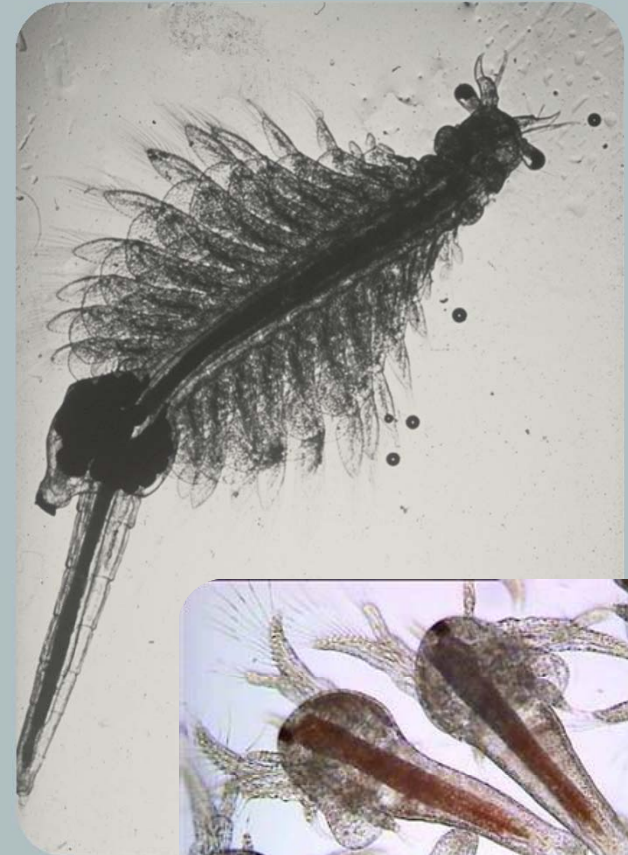
Opportunities for application of epigenetics in aquaculture



Pittman et al., 2013

ARTEMIA AS MODEL SYSTEM IN LARVICULTURE RESEARCH

- ✓ host-microbe interactions
 - ✓ breeding studies
 - ✓ epigenetics
 - **nutrition studies - bioflocs**
- *Artemia* as a model for
microbe-based feeding




ARTEMIA REFERENCE CENTER
State University of Ghent
J. Plateastraat 22
B-9000 Ghent, Belgium

1ST INTERNATIONAL TRAINING
COURSE ON THE BIOLOGY AND
PRACTICAL USE OF THE BRINE
SHRIMP *ARTEMIA* IN AQUACULTURE

MAY 15-26, 1978



Industry

- yields & quality ↗
- disease ↘

Society

- sustainability
- food security

Morphological
development

Life cycle analysis

Aquatic veterinary
medicine

Environmental
monitoring

Nutritional
research

Microbial
management

Genomics





Faculty of Bioscience Engineering

Animal Production - Patrick Sorgeloos and Peter Bossier

Biochemical and Microbial Technology – Nico Boon and Tom Van de Wiele

Ecotoxicology and Environmental Sanitation – Colin Janssen

Environmental Sustainability Assessment – Jo Dewulf

Faculty of Veterinary Medicine

Morphology – Wim Van den Broeck and Annemie Decostere

Virology, Parasitology and Immunology – Hans Nauwynck

Faculty of Sciences

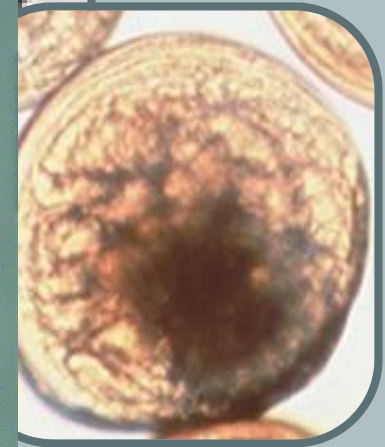
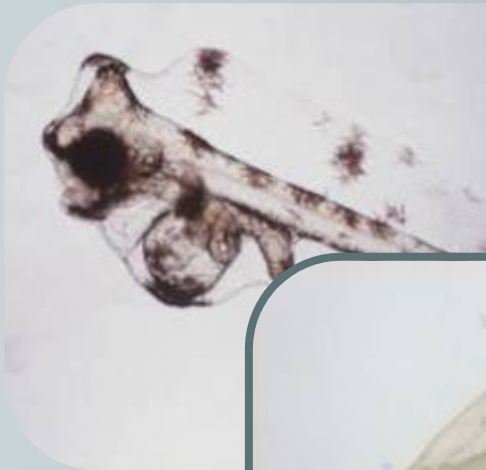
Biology – Dominique Adriaens and Magda Vincx

Molecular Genetics – Marnik Vuylsteke, Yves Van de Peer and Dirk Inzé

Algeria
Bangladesh
Belgium
Brazil
Chili
China
Colombia
Ecuador
Egypt
Greece
Indonesia
Iran
Morocco



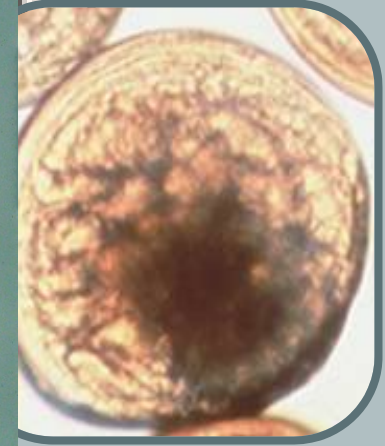
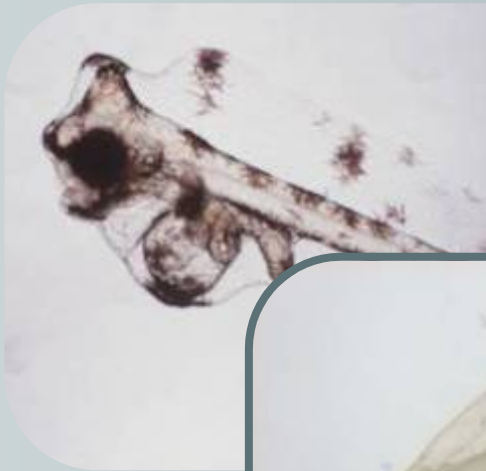
Thank you



slide layout courtesy Jean Dhont

www.aquaculture.ugent.be

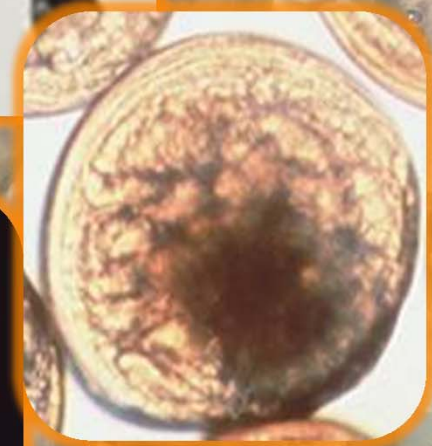
Thank you



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www.aquaculture.ugent.be

Thank you



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slide layout courtesy Jean Dhont

Thank you



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