

DIVERSIFY meeting 2<sup>nd</sup> February 2016



# European eel breeding, larval culture and first-feeding attempts



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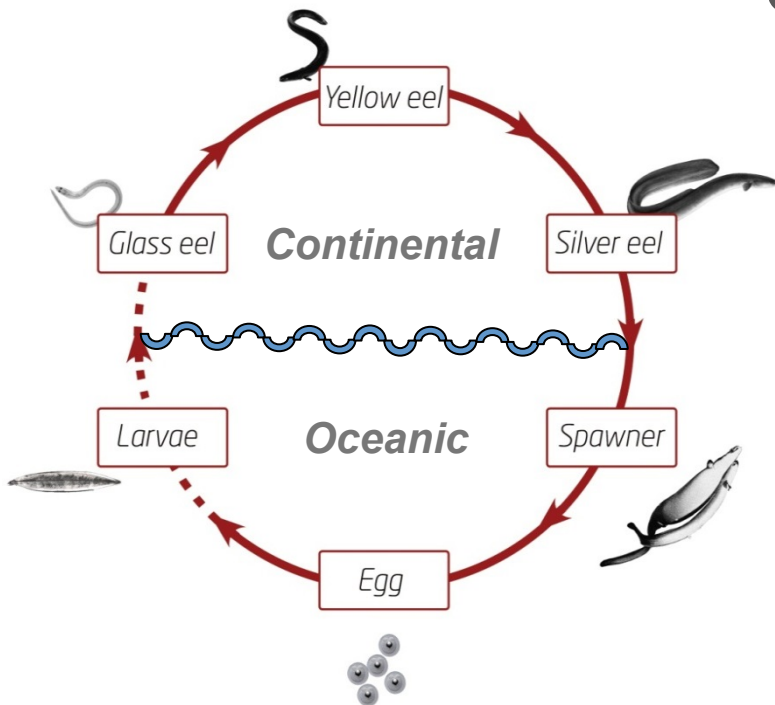
# PRESENTATION OUTLINE

- European eel obstacles and research approach
- Breeding: gamete production and fertilisation
- Incubation and larval culture technology
  - Ontogeny
  - Bio-physical culture conditions & physiological requirements
- Tailoring suited first feed – physiology, morphology and behaviour
- Challenges and perspectives

## European eel stock decline drives the need for a shift from to captive breeding of glass eels

### Challenges for glass eel production:

- Complex hormonal control and inhibition of maturation
- Lack of knowledge about wild eel reproduction and early life stages including larval diet and eco-physiology



# RESEARCH APPROACH



**Linking basic research to application and technology development**

**Broodstock nutrition and selection criteria**

**Hormonal control and treatments**

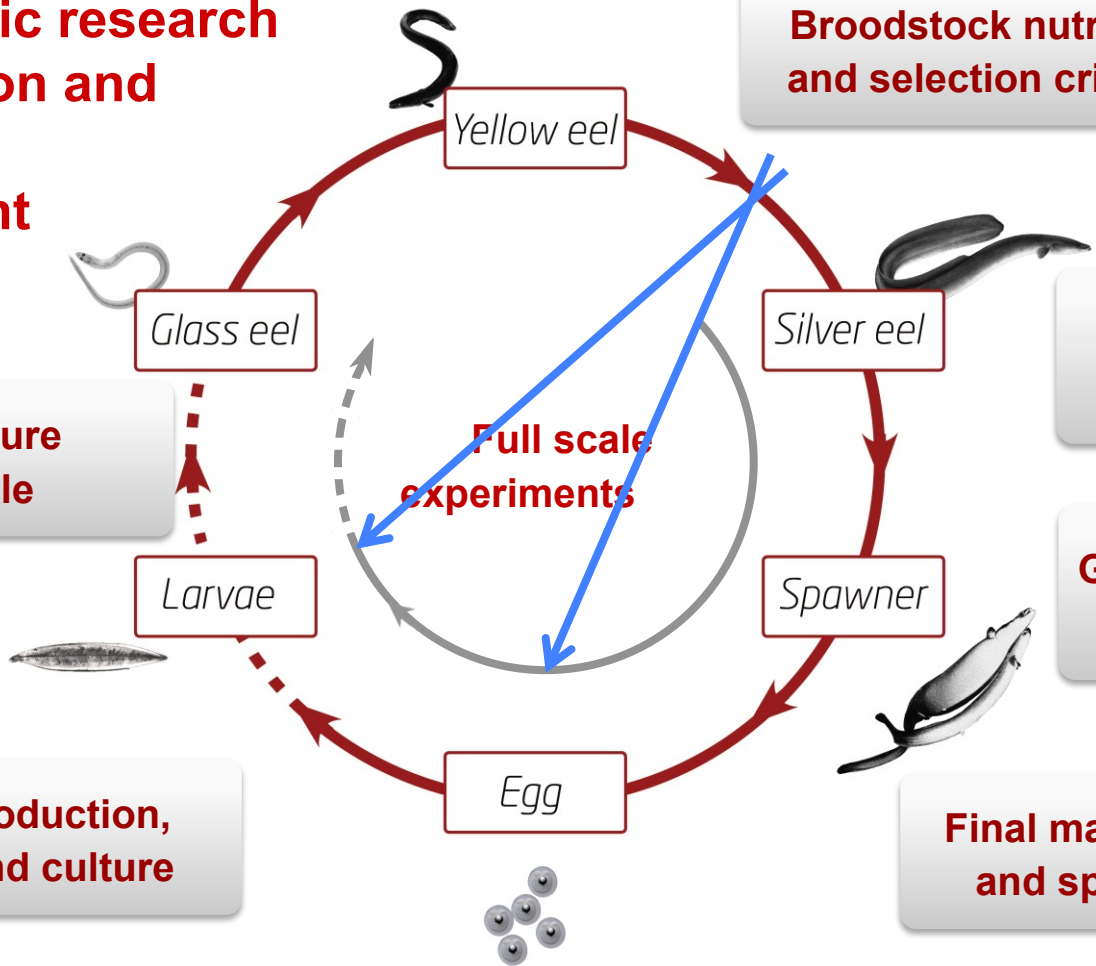
**Gamete development of females and males**

**Final maturation, egg and sperm quality**

**Fertilisation and embryonic developmental competence**

**Larval production, quality and culture**

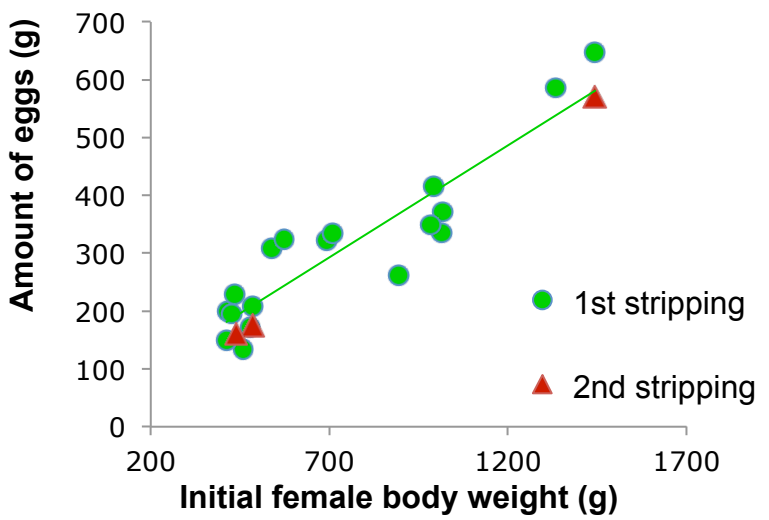
**Larval culture large scale**



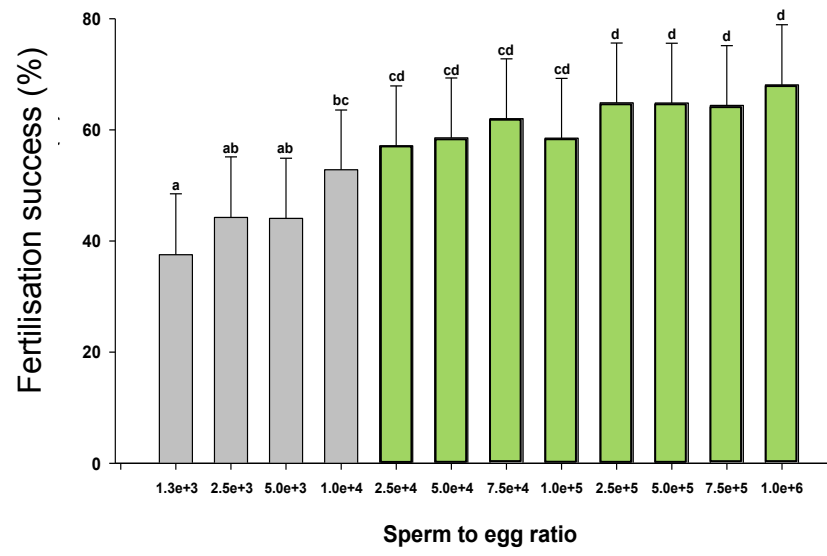
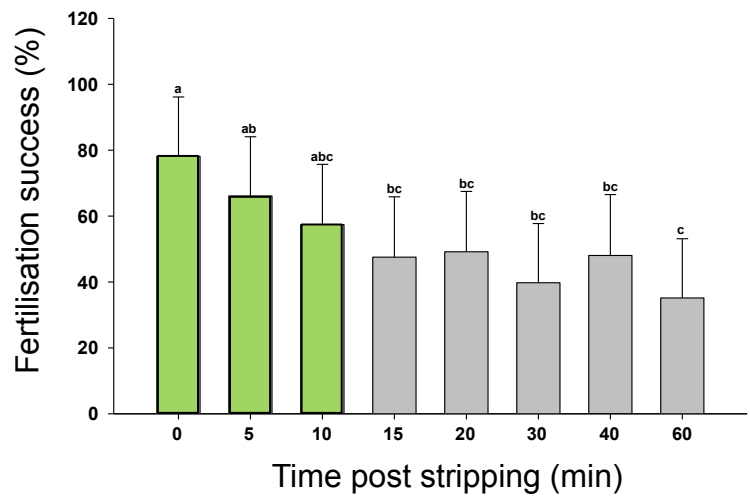
**Strategy: Incremental progress through controlled experiment**

# BREEDING: GAMETE PRODUCTION AND FERTILISATION

- ✓ **Enhanced broodstock feeds for improved quality eggs**
- ✓ **Assisted reproduction protocols**
- ✓ **Stable egg production: wild and farmed broodstock**
- ✓ **Fertilisation standardised protocols**



High fecundity: 1 g of eggs ~2000 eggs



# INCUBATION AND LARVAL CULTURE: ONTOGENY

## Embryonic development

Duration ~48 h at 20 °C

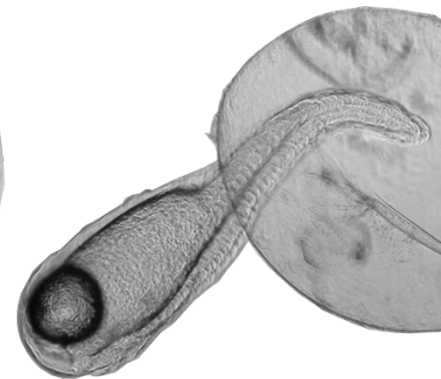
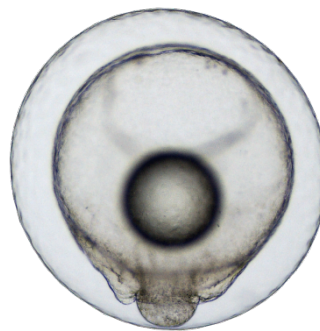
First  
cleavages  
~ 3 h

Cells over  
yolk-mass  
~ 14 h

Evident  
embryo  
~ 24 h

Segments  
& tail free  
~ 40 h

Time of  
hatch  
~ 48 h



1 mm

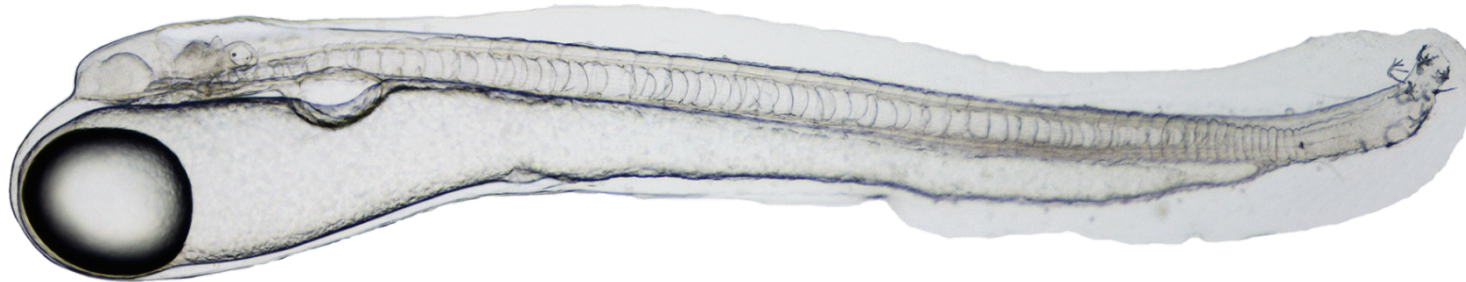
# LARVAL DEVELOPMENT



**2-3 hours**



**6-7 hours**

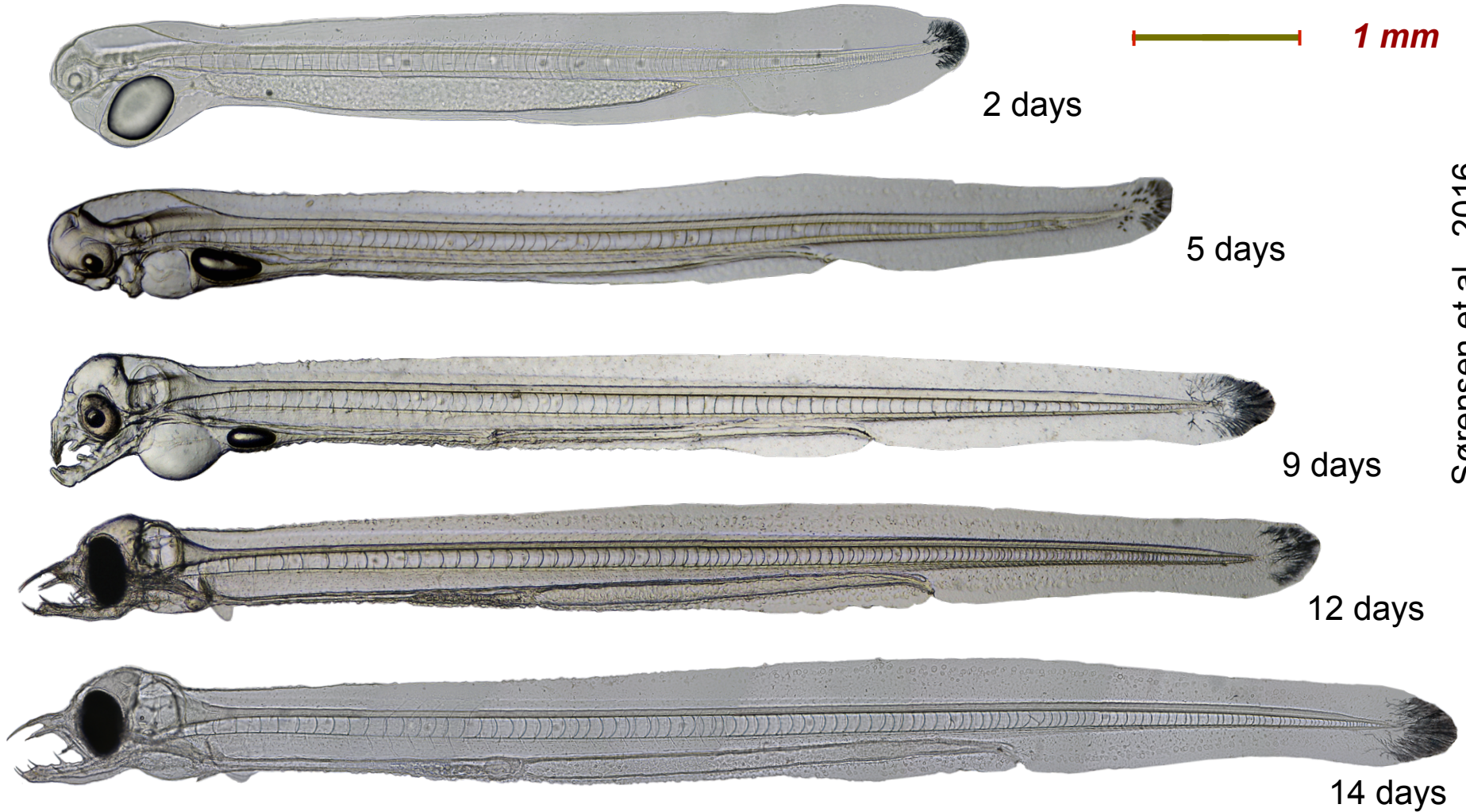


**10-11 hours**

 **500  $\mu$ m**

# LARVAL DEVELOPMENT

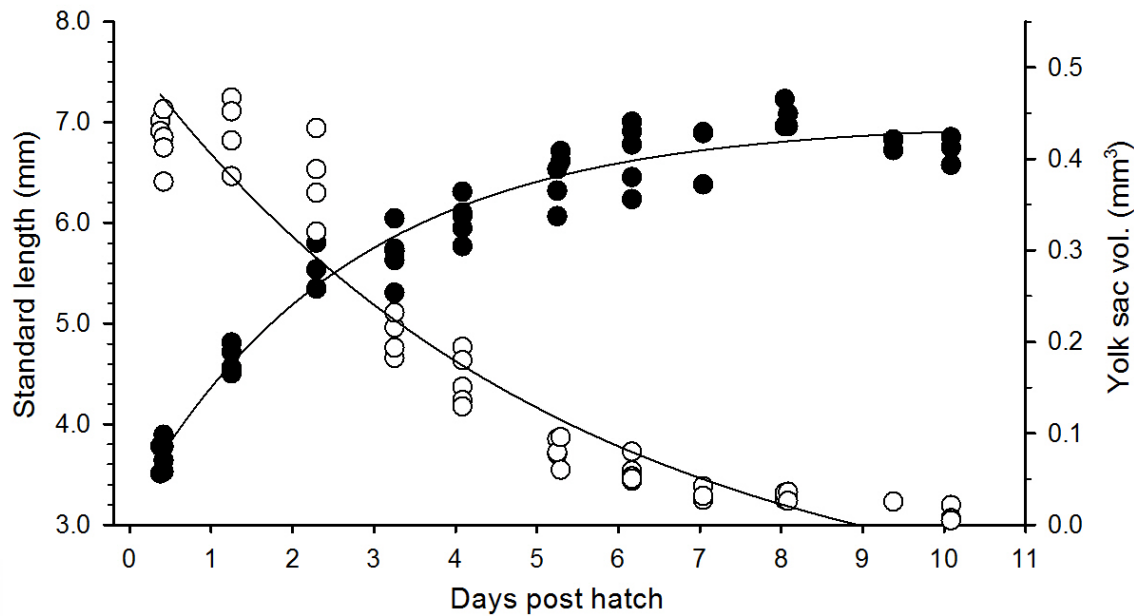
**Duration to larval feeding stage ~12 days at 20 °C**





# LARVAL DEVELOPMENT

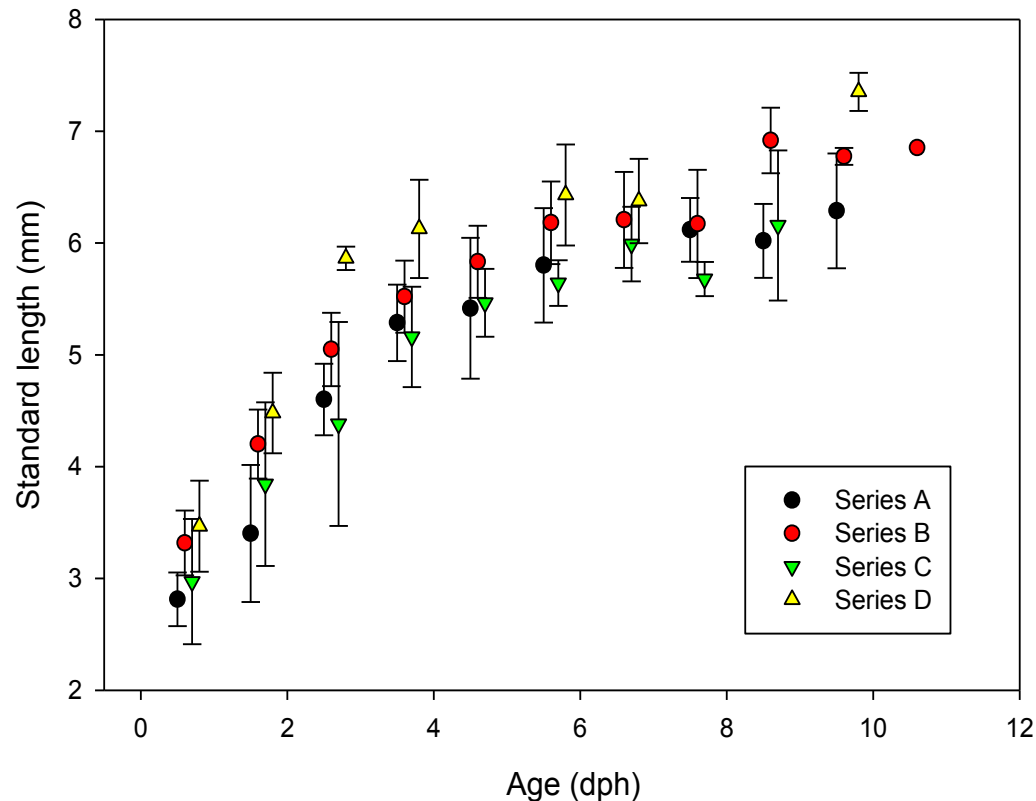
**Max. size of yolk sac larvae ~ 8 mm**



**Larval size increase and yolk resorption at 20 °C**

# LARVAL QUALITY – LENGTH INCREASE

**Difference in egg and larval quality between farmed & wild broodstock apparent from hatch**



**Differences in larval size**

**Farmed:**

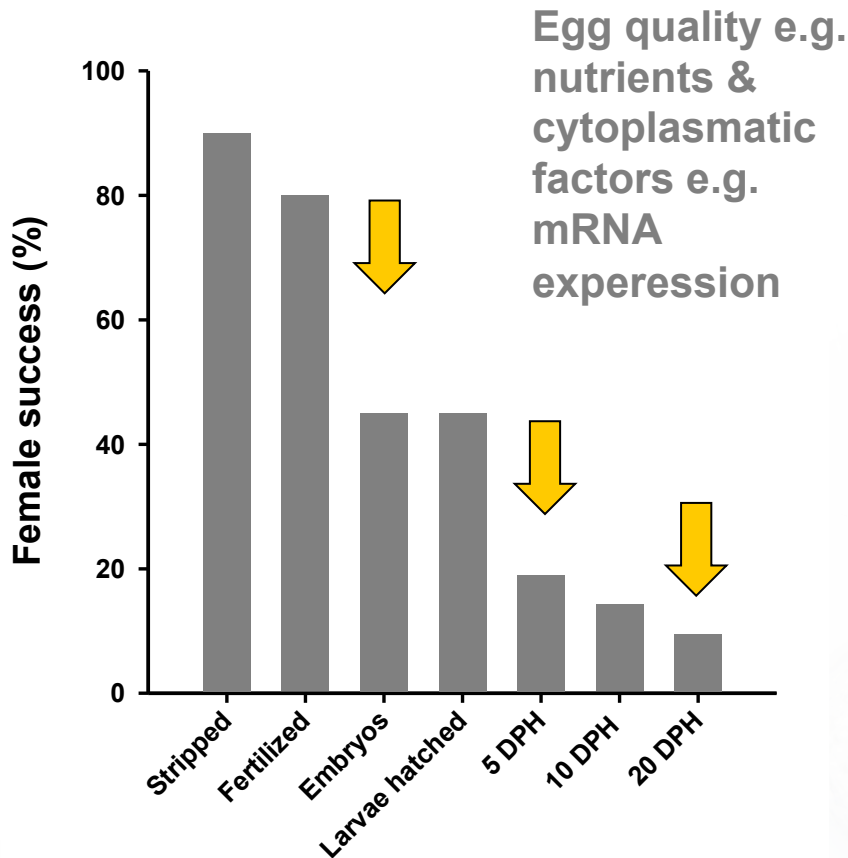
- A
- ▼ C

**Wild:**

- B
- ▲ D

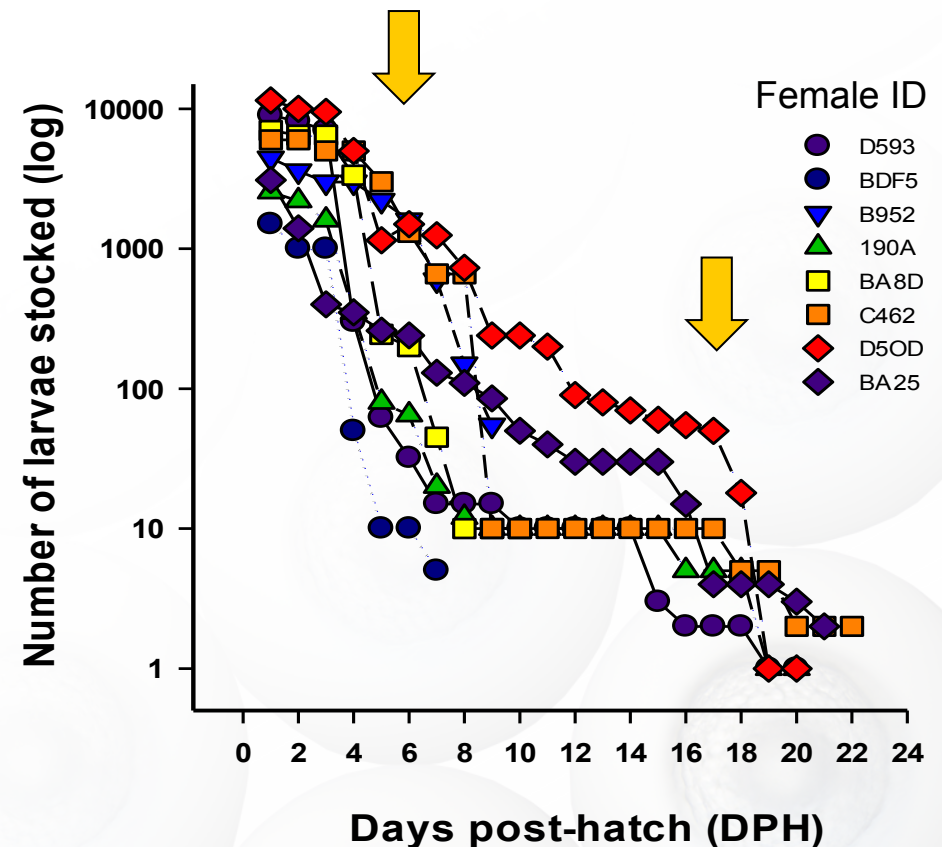
# MAJOR BOTTLENECKS IN OFFSPRING CULTURE

## Mortality during embryonic development



Tomkiewicz et al., in prep.

## Mortality Day 3 to 8 Starvation Day 15+

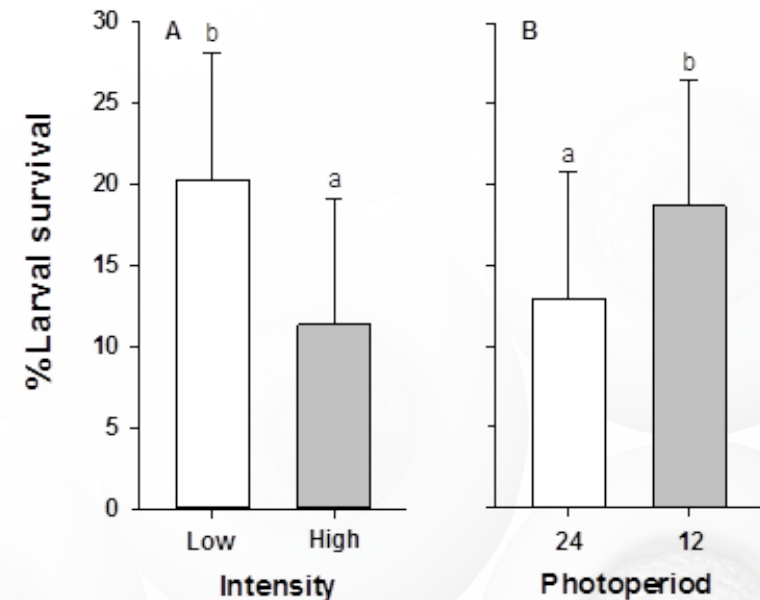
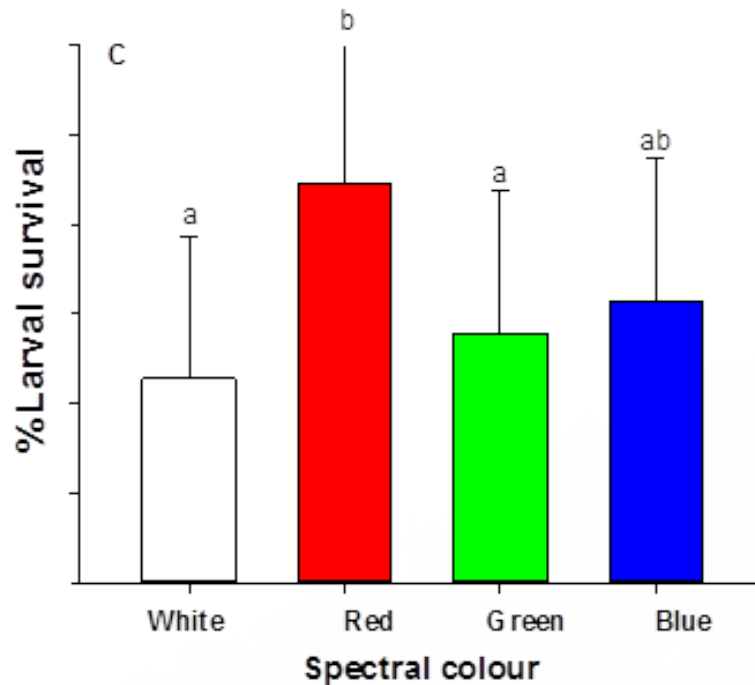


Stocking 3000 larvae per 100 l aquaria - aquaculture recirculation systems (RAS)

# LARVAL CULTURE TECHNOLOGY AND FEEDING TRIALS

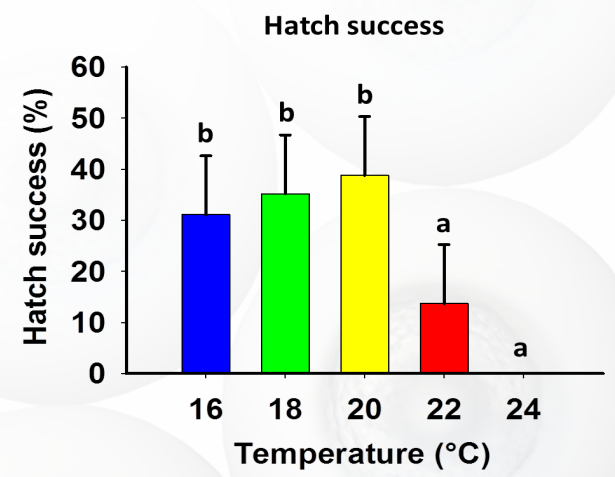
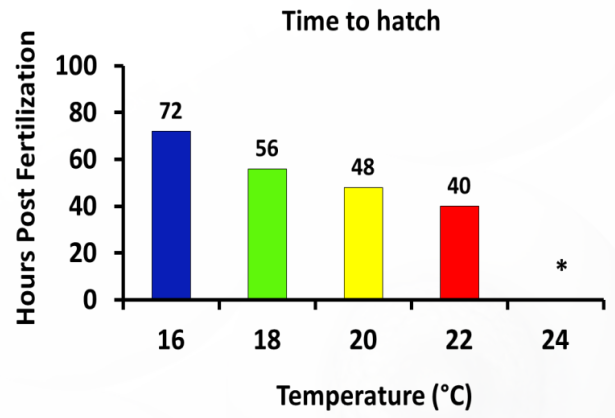
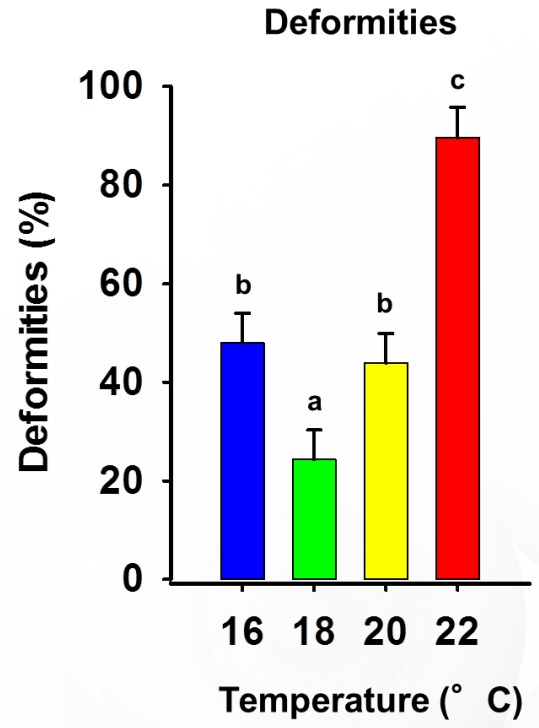
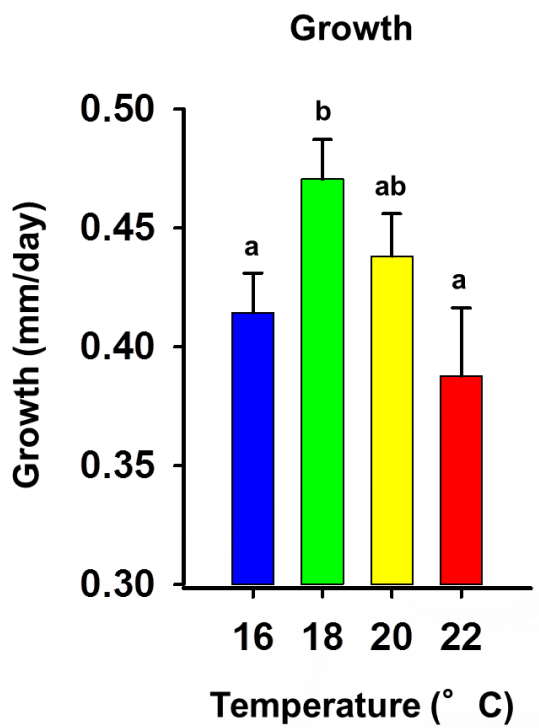
- Incubation and larval culture technology
  - Studies of ontogeny
  - Bio-physical culture conditions
    - Light regime, temperature, salinity
    - Microbial control
    - Rearing systems
  
- Tailoring suited first feed and feeding trials
  - Physiology & morphology
  - Feeding behaviour & ingestion

## Impact of light regime on survival of yolk sac larvae



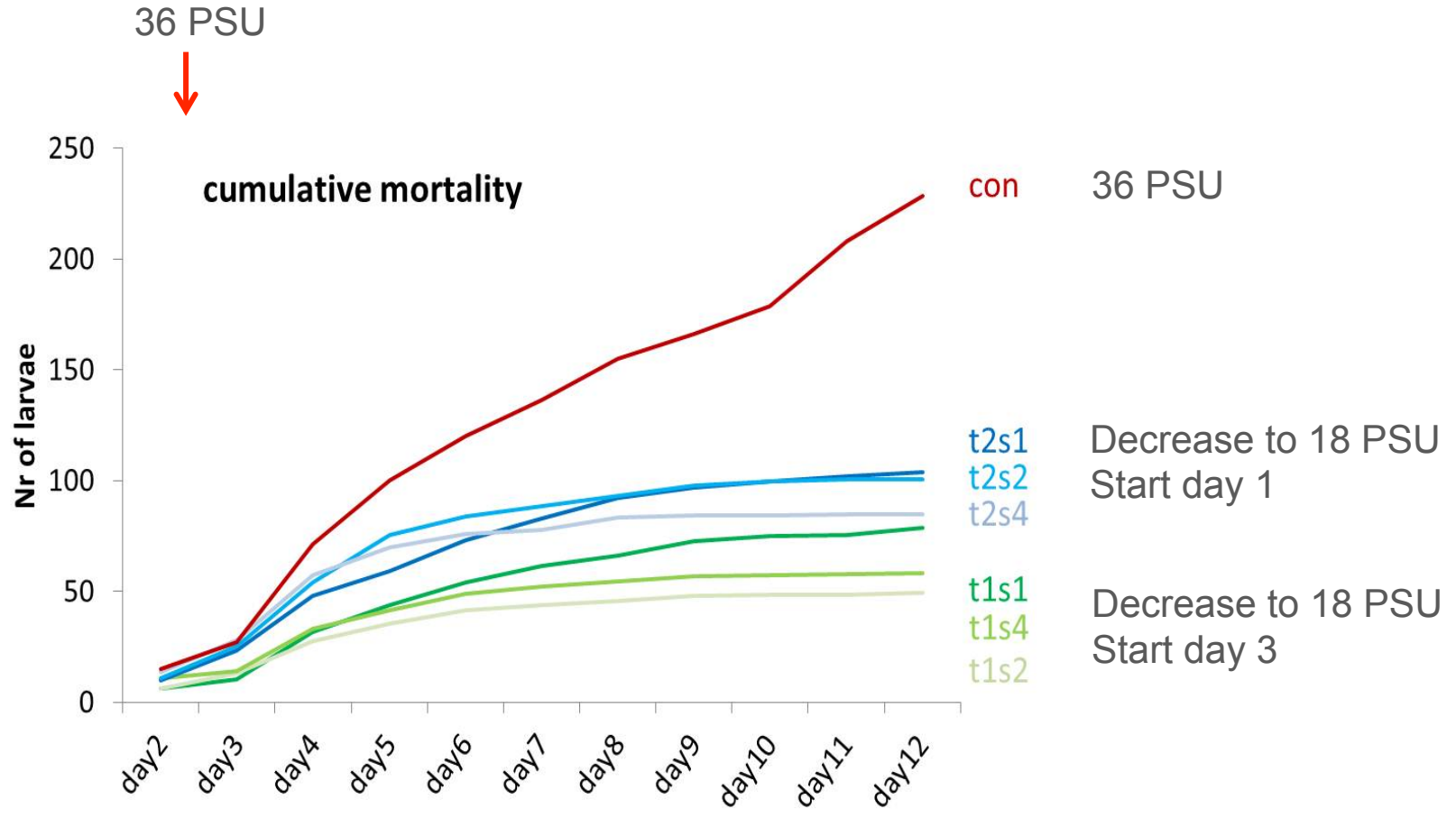
## Impact of temperature on larval hatch and survival

*~ 18 °C appears optimal*

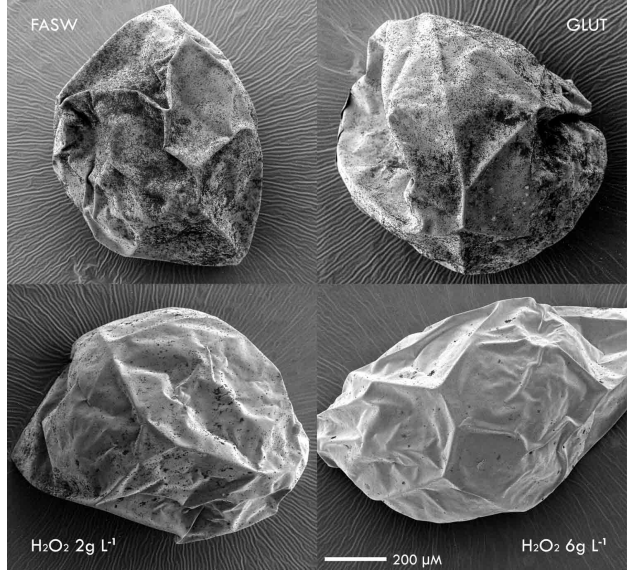
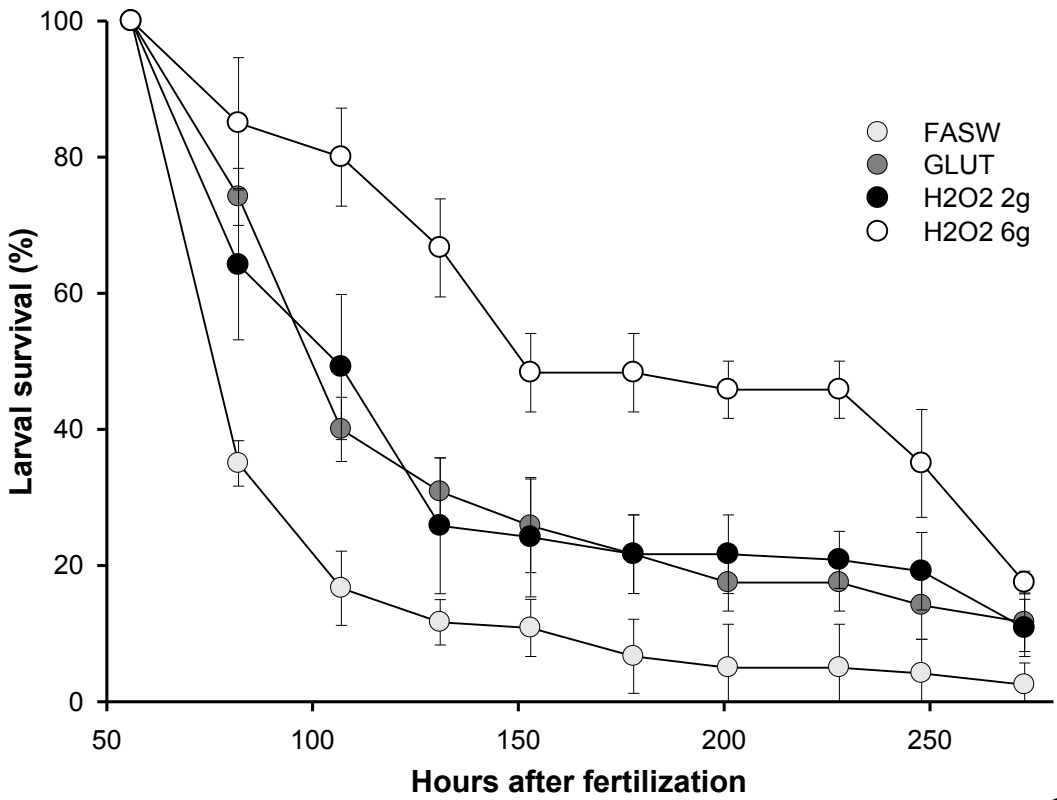


Molecular analyses ongoing

## Salinity experiment preliminary results



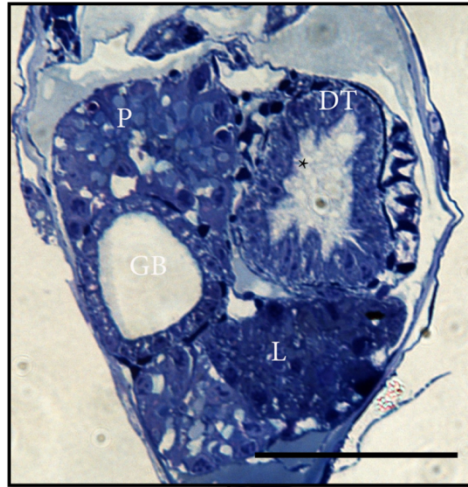
**Embryonic survival is not enhanced**  
**Larval survival using disinfection treatment increased**



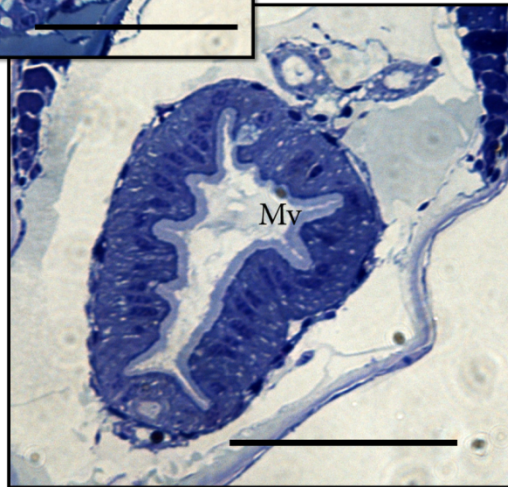
**Ongoing:  
Combination with  
matured water  
technology**



## What can larvae digest?



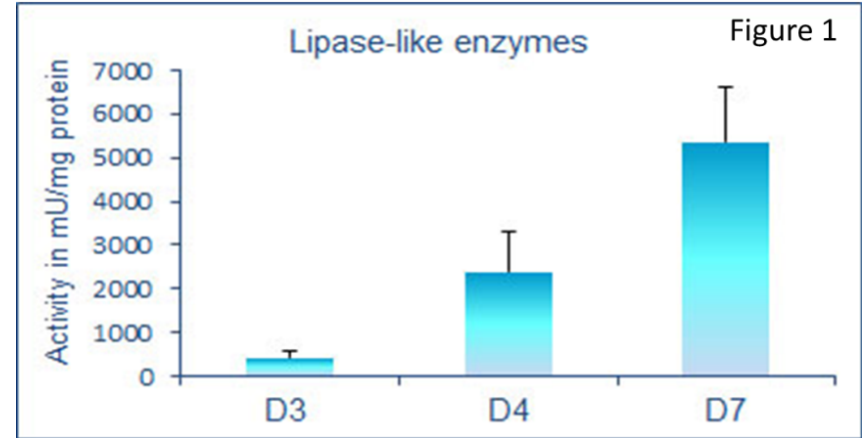
## Digestive histology



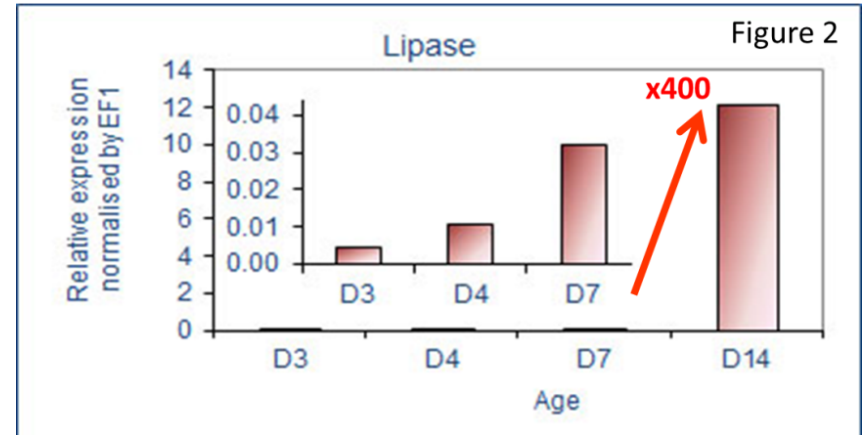
Kjørsvik et al. (in prep.)

Relatively immature liver/pancreas at 12 DPH

## Digestive enzymes day 3-14



Enzyme activity (biochemical study)



Enzyme gene expression

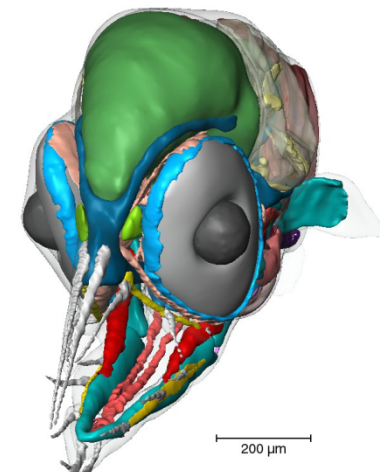
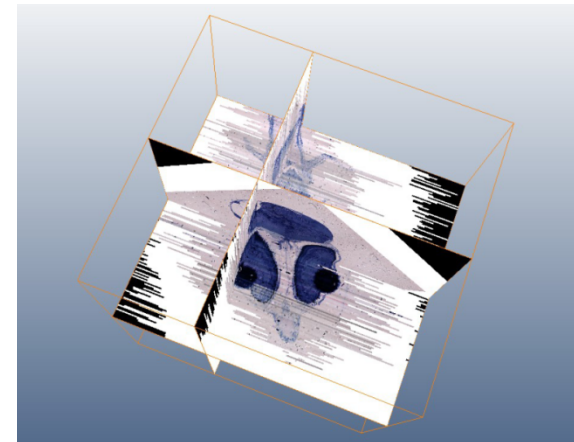
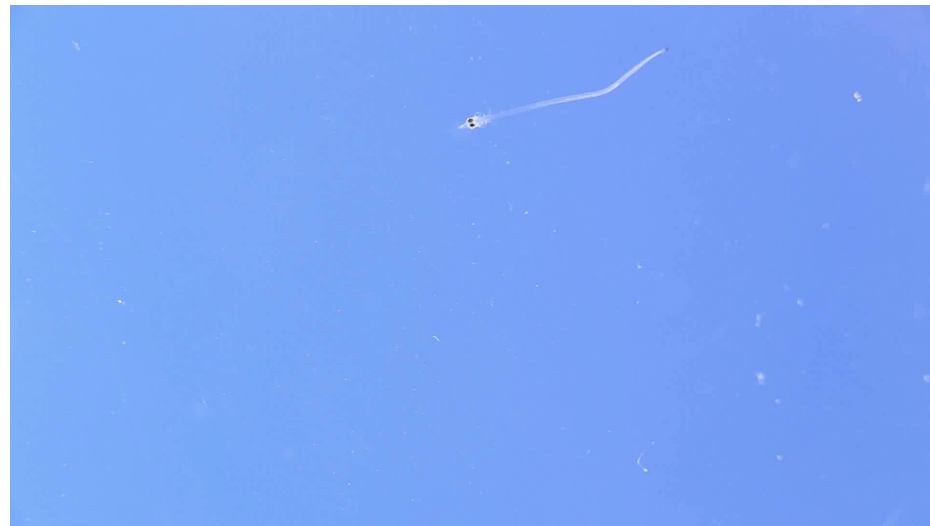
# LARVAL FEEDING CAPABILITIES

## Analysis of larval head morphology

What can they ingest?

## Study of larval behaviour

European eel larvae 14-15 DPH



# LARVAL FEEDING AND BEHAVIOUR EXPERIMENTS

**A**

Shading illustrates gut area



Representative gut fullness of 11.6%

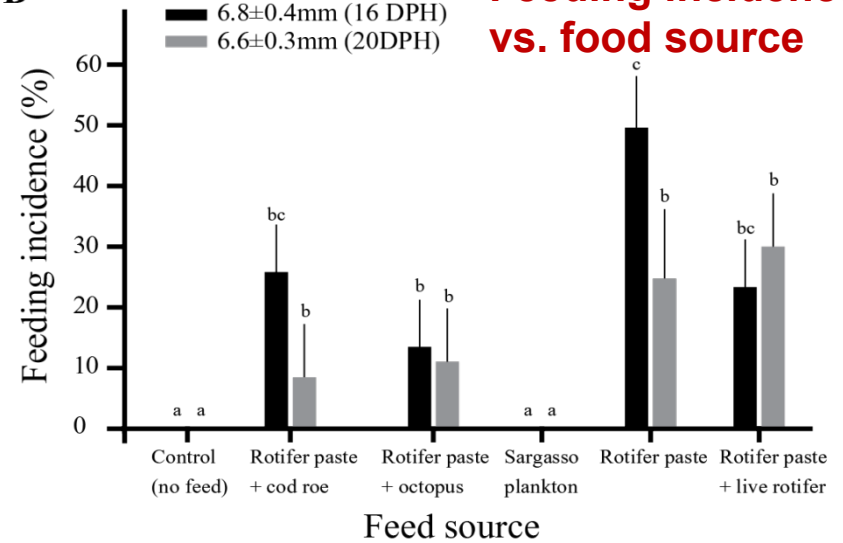


Representative gut fullness of 32.9%



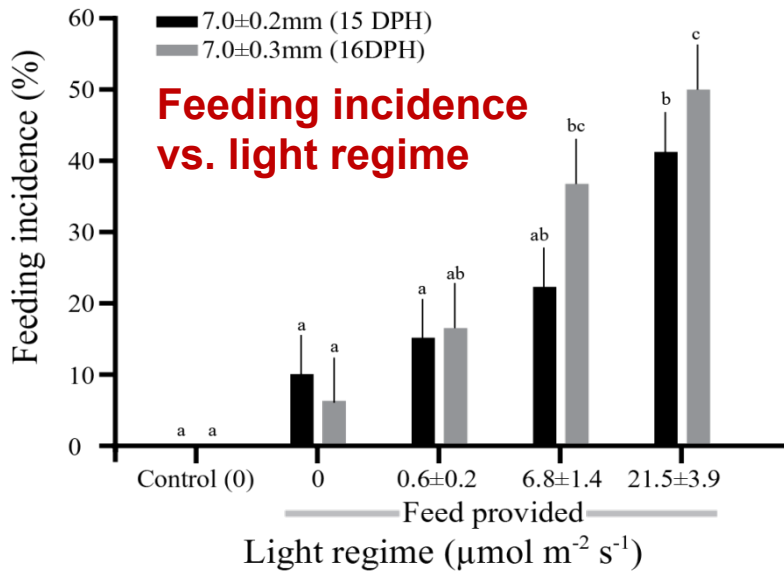
**B**

**Feeding incidence vs. food source**



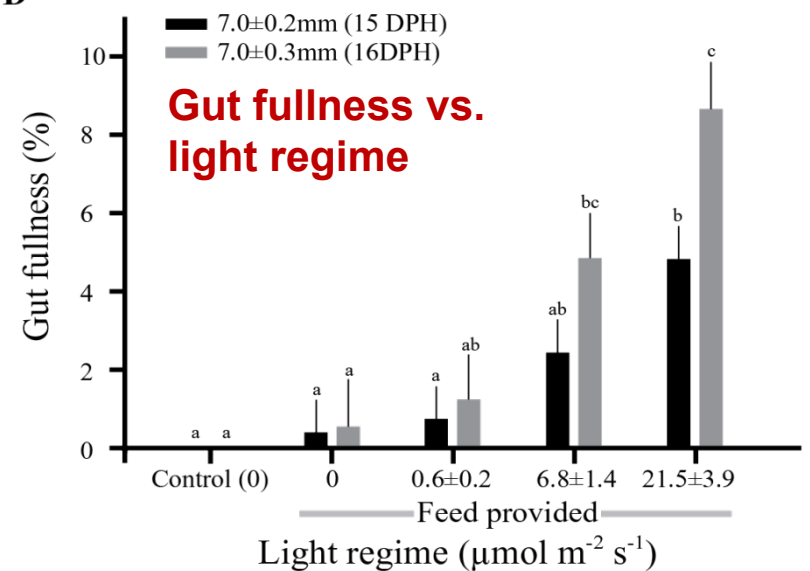
**C**

**Feeding incidence vs. light regime**



**D**

**Gut fullness vs. light regime**



Butts et al. (in prep.)

# MAIN CHALLENGES AND PRESENT FOCUS

## ➤ Culture conditions and ontogeny

- Temperature & salinity (incl. molecular study)
- Egg disinfection, matured water, systems & density
- Immune system development (molecular study)
- Vision and olfaction (TEM)

## ➤ Insight into larval nutritional requirements

- Molecular studies
- Force feeding study (C14-labelling)

<http://funfrd.com>

## ➤ Feeding trials medium scale

- Behavioural studies (+light)
- Algae, peptides etc.
- Diets, enrichments & attractants
- Ontogeny during ongrowing



Leptocephalus larvae –  
unprecedented in hatchery  
technology

# ACKNOWLEDGMENTS



[www.pro-eel.eu](http://www.pro-eel.eu)

## PRO-EEL 2010 -2015 Partners

	<b>DTU</b>	Technical University of Denmark
	<b>IMARES</b>	Institute for Marine Research and Ecosystem
	<b>LU</b>	University of Leiden
	<b>CNRS</b>	National Center for Scientific Research
	<b>ICTA UPV</b>	Institute for Animal Science and Technology, Polytechnic University of Valencia
	<b>NOFIMA</b>	Nofima Akvaforsk – Fiskeriforskning A/S
	<b>UGENT</b>	Ghent University
	<b>KU</b>	University of Copenhagen
	<b>INRA</b>	National Institute for Agronomic Research
	<b>BA</b>	Billund Aquaculture Service Aps.
	<b>WU</b>	Wageningen University
	<b>INSTM</b>	National Institute of Sciences and Technologies of the Sea
	<b>IMR</b>	Institute for Marine Research
	<b>NTNU</b>	Norwegian University of Science and Technology
	<b>BIOMAR</b>	BioMar A/S

# ACKNOWLEDGMENTS



[www.eel-hatch.dk](http://www.eel-hatch.dk)

## EEL-HATCH Consortium 2010-2017

Technical University of Denmark  
Billund Aquaculture Service  
BioMar  
Bioneer  
Danish Aquaculture Organisation  
North Sea Science Park  
STMI



**THANK YOU FOR YOUR ATTENTION!**

