# Hatchery production of the greater amberjack (*Seriola Dumerili*)

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- Data on <u>applicable</u> rearing conditions in captivity is scarce
  - particularly for the early developmental stages

**Aim**:

- Define (some of) the parameters related to the larval rearing in order to optimize the applied methodologies
  - larvae are optical predators
  - adequate foraging of appropriate preys

- **Objectives**: to obtain new information on the larval rearing parameters
  - Acquire basic biological information
    - Ontogeny of digestion and vision system
    - Ontogeny of Somatotropic axis
    - Skeletal Ontogeny
  - Feeding regime - Prey enriching diet
  - Husbandry parameters
    - tank type-shape,
    - stocking density,
    - light conditions

# Ontogenesis of key biological systems

## Mesocosm

- A semi-intensive technology that facilitates the rearing of several species providing the required "biological control"

## Intensive rearing

- The "pseudo-green" water methodology – closed hydraulic circuit
- Controlled conditions
  - temperature
  - photophase
  - feed distribution
    - computer based application

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# Methodology (HCMR)

## Mesocosm

- Water: filtered (5 μm) natural seawater (salinity 40 psu) UV treated
- T: 24 ± 0.7 °C
- pH: 8.0 to 8.2
- D.O. 5.8 to 6.8 mg $l^{-1}$.
- Photophase: 24L:00D from 3 to 25 dph then to 18L:06D.
- Light intensity: 500 lux to 1,000 lux,
  ~250 lux at night

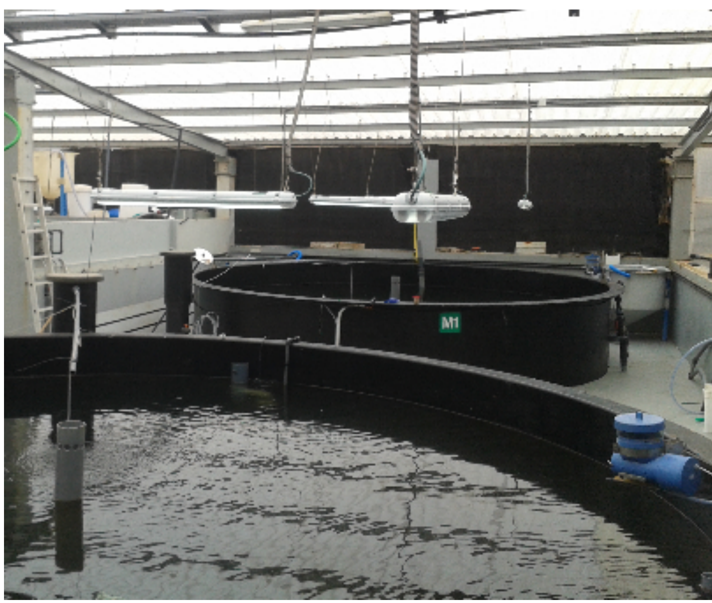
## Intensive

- Water: filtered borehole 35 psu-water.
- T: 22 ± 0.5°C until mouth opening
  24 ± 0.5°C afterwards.
- pH: 8.0 to 8.2
- D.O. 6.8 to 7.2 mg $l^{-1}$.
- Photophase: 24L:00D from 3 to 25 dph then 18L:06D
- Light intensity: 200 - 800 lux
  ~200 lux at night

- Feeding
  - 3 to 18 dph : Microalgae (*Chlorella sp*) and enriched rotifers (*Brachionus sp*)
  - 12 to 14 dph. *Artemia* AF $A_0$ nauplii and 14 to 30 dph enriched *Artemia* EG $A_1$ nauplii
  - 16 dph (MES) and 21 dph (INT) artificial feeds (grain size 200–300 μm; and 300–500 μm)
  - Frozen gilthead sea bream eggs in MES after 20 dph
  - Mesocosm tanks developed naturally zooplankton (harpacticoida copepods)

# Methodology (FCPCT)

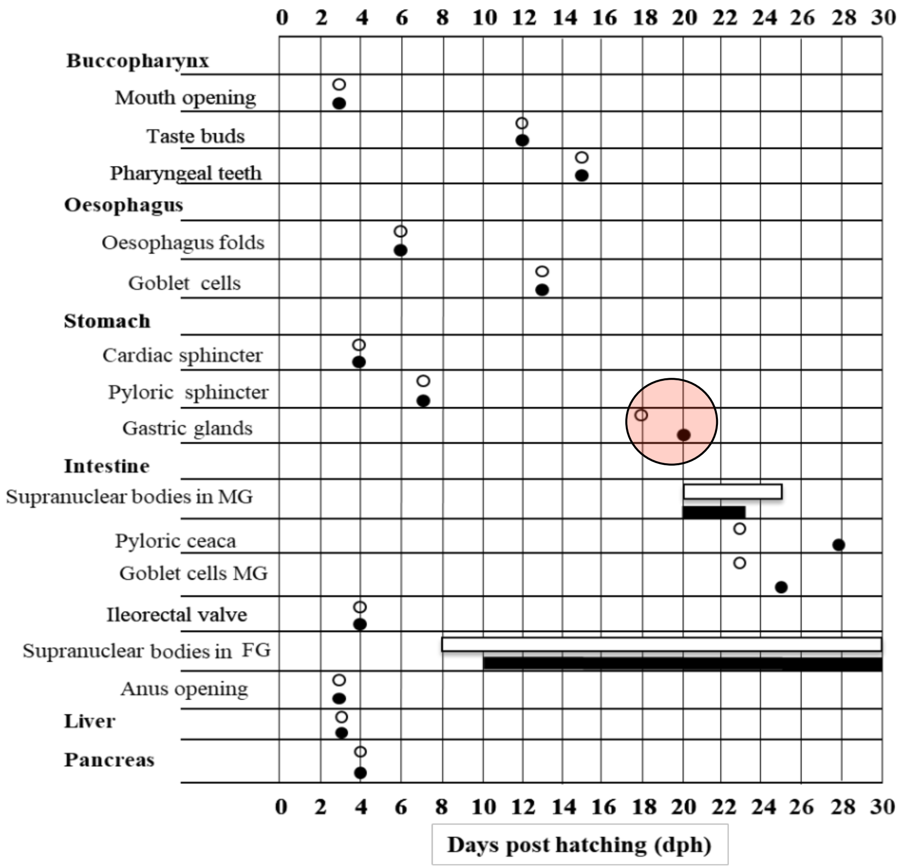
- Two different tanks types: 40,000 l and 2,000 l
  - Photoperiod was natural (14:10 h, L:D),
  - S=37 psu, and
  - T=25-27ºC
  - DO=6.78 ± 0.5 ppm
  - Feeding: live phytoplankton (*Nannochloropsis sp.* ), enriched rotifers (1-30 dph), enriched *Artemia* (12-30 dph), microdiets of 75, 150 and 300 µm (13-30 dph)



## Ontogeny of digestive and vision systems

- The digestive system
  - enables the fish to capture, ingest, digest and absorb nutrients from the food
  - is of special relevance to establish the larval feeding protocol
  - ontogenesis of related organs and digestive enzymes

# The *digestive system* ontogenesis is a rapid process




| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 15 | 17 | 20 | 23 | 25 | 28 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| INT | 3.52 | 3.72 | 3.73 | 3.81 | 3.96 | 4.01 | 3.94 | 3.98 | 4.23 | 4.23 | 4.55 | 5.26 | 5.19 | 6.01 | 6.35 | 7.77 | 8.47 | 8.20 |
| MES | 3.52 | 3.67 | 3.75 | 3.83 | 3.99 | 4.05 | 4.03 | 4.02 | 4.05 | 4.09 | 4.53 | 5.00 | 5.69 | 6.49 | 7.2 | 9.23 | 8.54 | 8.74 |

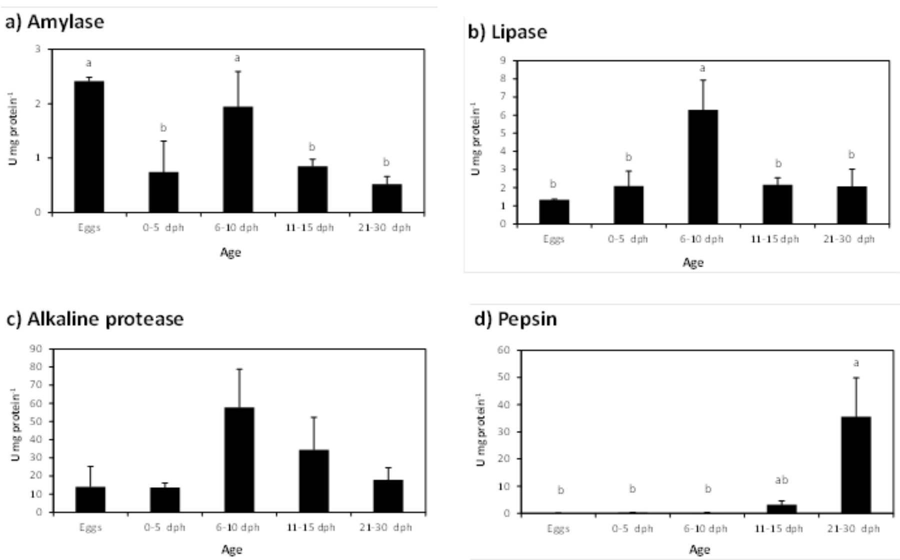
Total length in (mm)

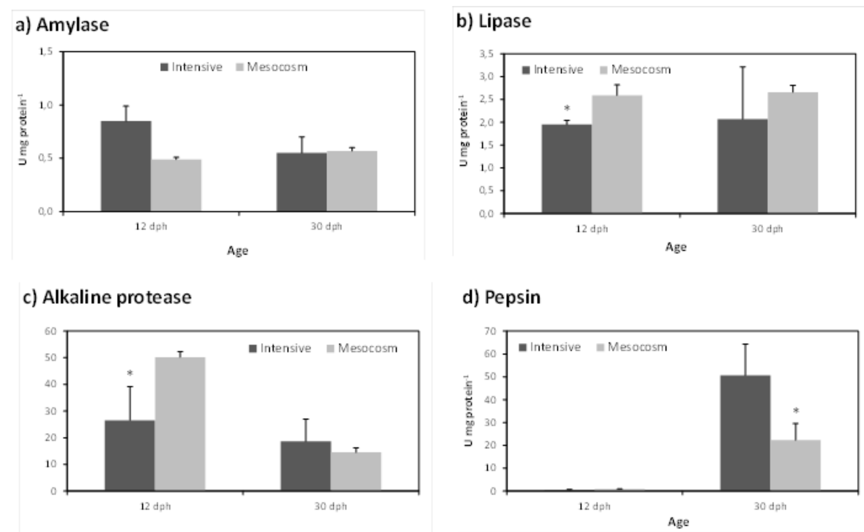
The **gastric glands** mark the transition from larval to juvenile and the development of a functional stomach

- in greater amberjack appeared after 5.5 mm of TL in all the rearing systems (16-20 dph).

| | Growth rate (exp) | Survival |
|---|---|---|
| Mesocosm | 0.065 $d^{-1}$ | 13.9±0.8% |
| Intensive | 0.047 $d^{-1}$ | 8.2±3.1% |

# Ontogenesis of *digestive enzymes*




0-3 dph: enzymatic activities before onset of exogenous feeding

importance of egg glycogen as energy during the embryonic development

3-5 dph: higher activity of alkaline protease than lipase

proteins the main energy source

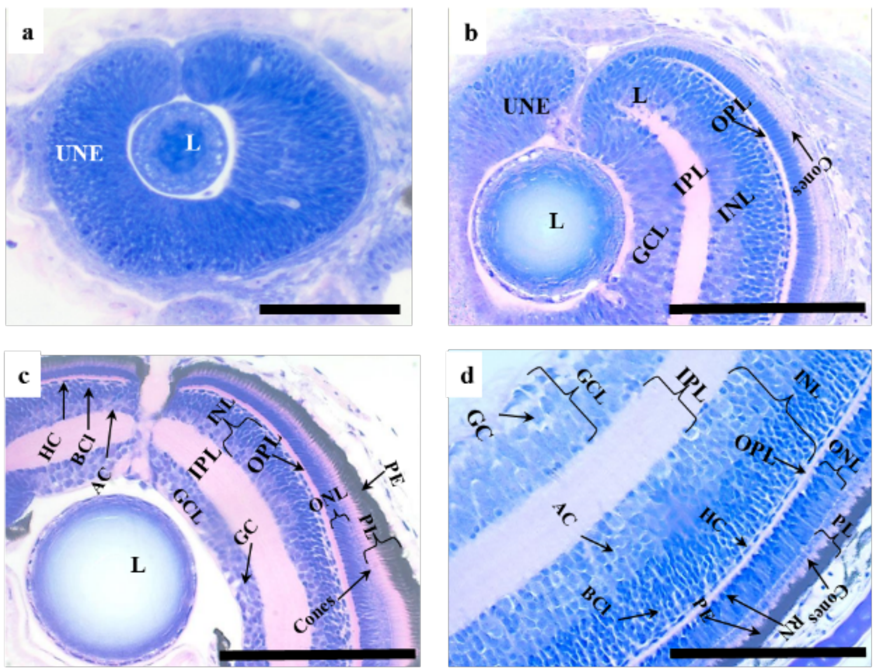
5-10 dph: period of rotifer feeding

proper nutritional condition of the larvae

10-15 dph: in both systems malnutrition

difficult acceptance of Artemia

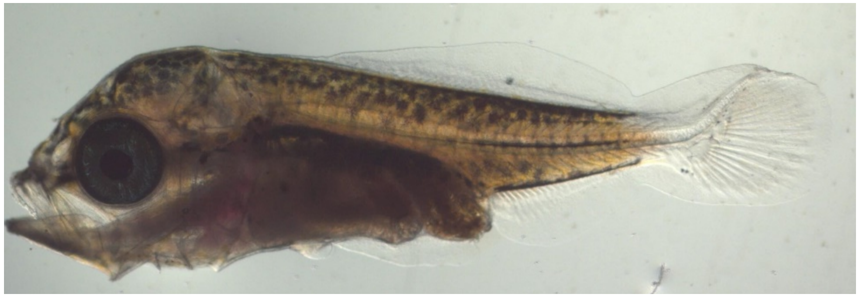
# Ontogenesis of *Vision system*



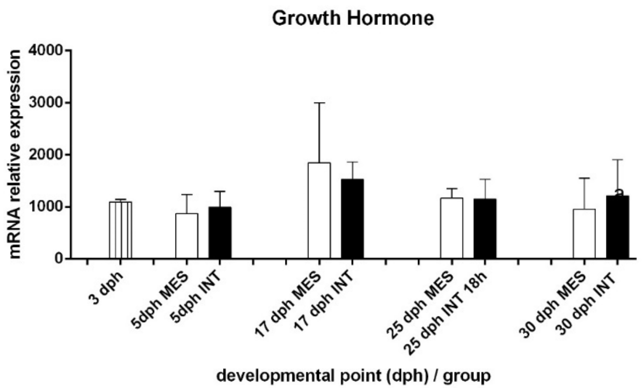

- 0 dph: retina an undifferentiated neural epithelium (UNE)
- 1-2 dph the first structures appeared
  - ganglia cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL) and the photoreceptor layer (PL)).
- 3 dph- onwards: the pigment epithelium PE appeared on the external area of the retina
  - the nucleus of the cone cells appeared in the outer nuclear layer
- Rods first appeared at TL 5.0±0.2 mm

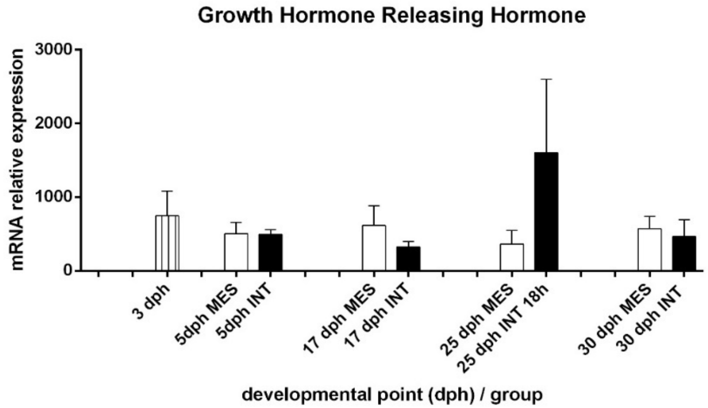
# Lessons learned

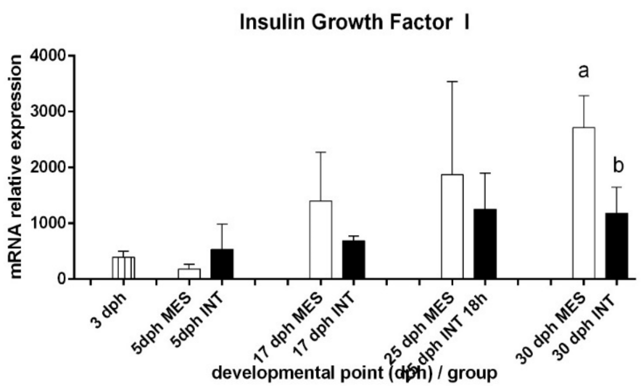
- Transitions to juveniles after 5.5 mm
- Protein main energy source since first feeding
- Night vision after 5.0 mm
- Rotifer appropriate as diet
- Difficult adaptation to Artemia

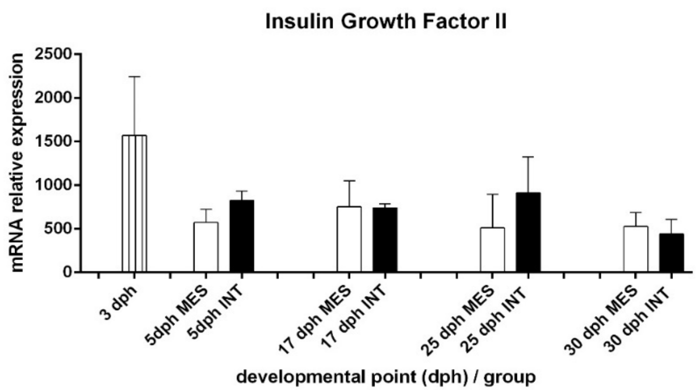


# Ontogenesis of the somatotropic axis



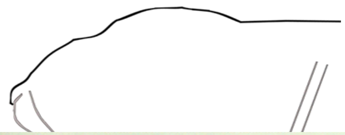

IGF Binding Proteins 1, 2, 3, 4, 5

Available for the first time information on the regulation of the various components of the IGF signaling pathway in greater amberjack

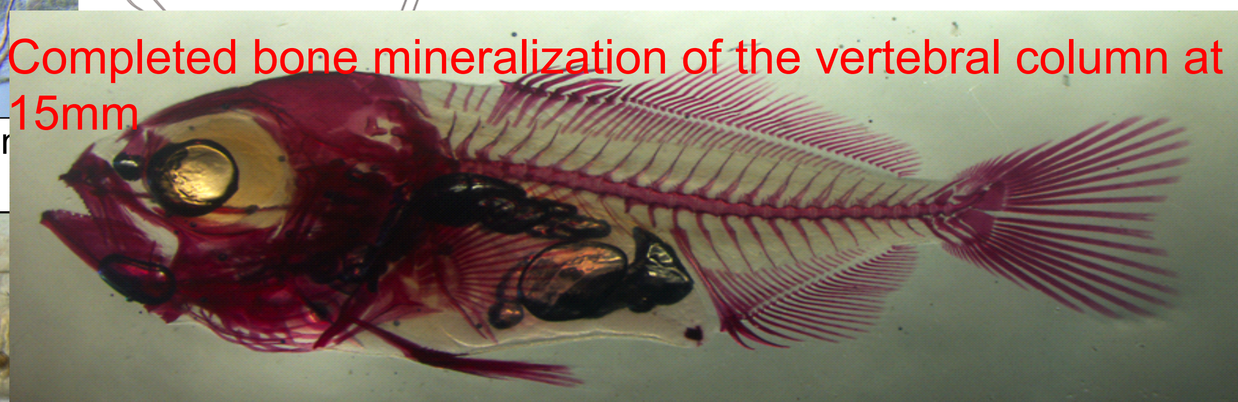
# Skeletal Ontogeny

## Cranial development

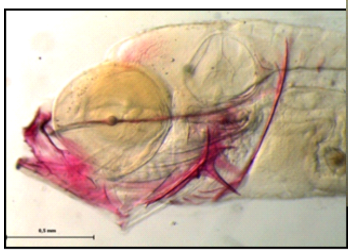
## Vertebral column and fin ontogeny

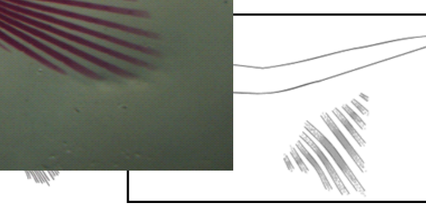


TL: 3.38 ± 0.15r



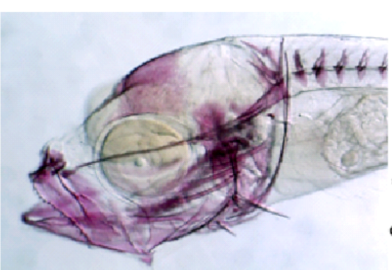
Completed bone mineralization of the vertebral column at 15mm

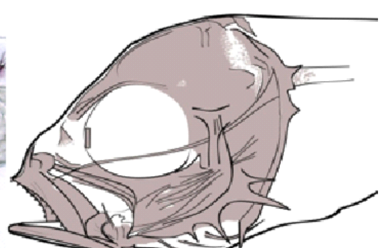


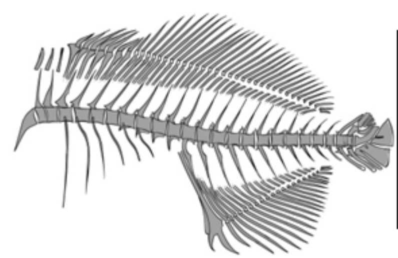


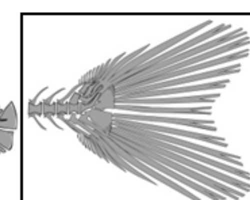
TL: 4.49 ± 0.14mm

TL: 5.36±0.2mm







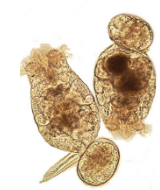


TL: 10.15 ± 1.86mm

TL: 13.03±0.09mm

# Effect of feeding regime and probiotics

- Effect of
  - prey concentration and supply frequency
  - use of immune modulators substances during the rotifer administration

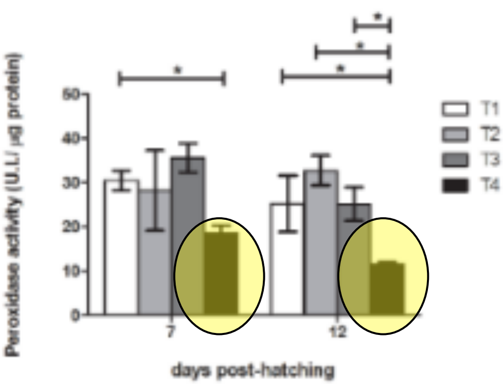


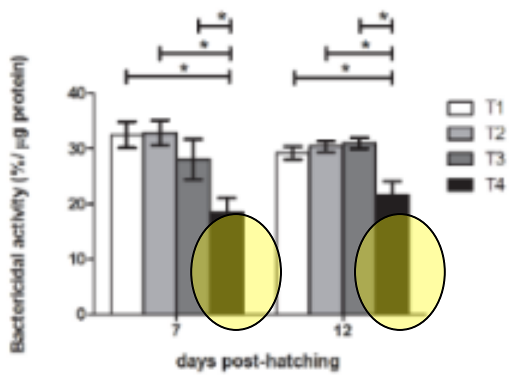
- Rotifers
  - Density: 5 vs 10 rot $ml^{-1}$
  - Distribution 2 vs 3 times $d^{-1}$

- Four enriching diets
  - A commercial diet (T1)
  - LC60/20:4n-6/10ppm carotenoids basic emulsion (T2),
  - LC60/20:4n-6/10ppm carotenoids basic emulsion with 20% *Echium* oil (T3)
  - LC60/20:4n-6/10ppm carotenoids basic emulsion with 20% black cumin oil (T4)

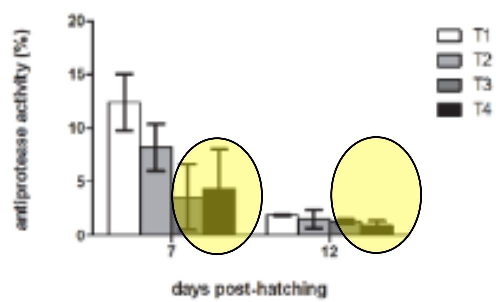
# Larval growth and survival

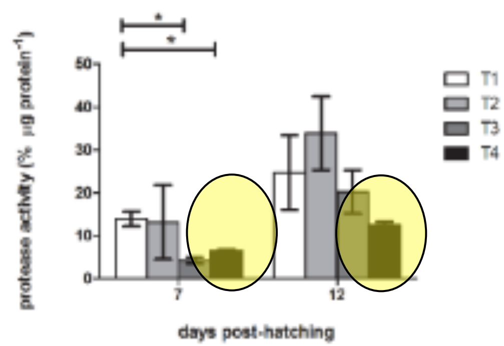
- Similar regardless rotifer density
  - slightly better at 5 rot $ml^{-1}$
- Better for feeding 3 times $day^{-1}$
- Better with T4



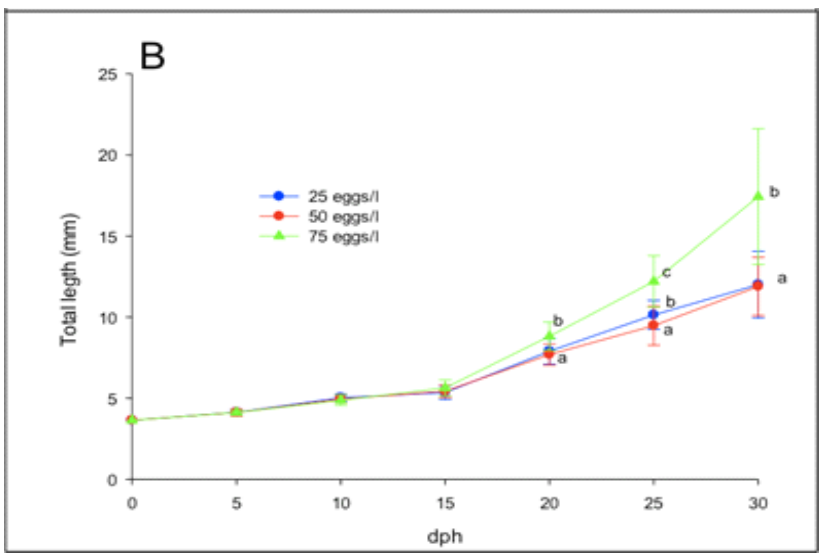

Humoral innate immune activities
7 dph and 12 dph larvae

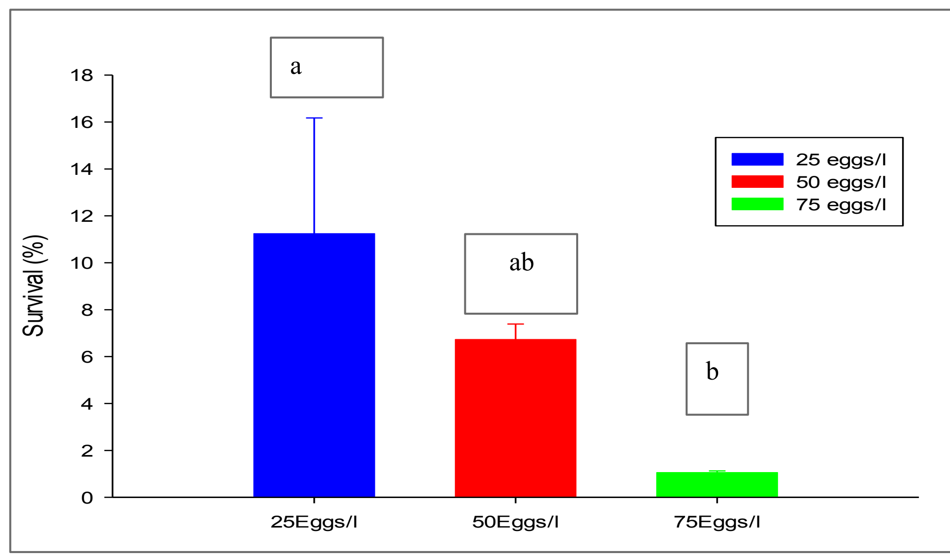


# Effect of larvae density

- 3 densities: 25, 50 and 75 eggs $l^{-1}$
  - Two different tanks types: 40,000 l and 2,000 l tested for 30 days
    - Photoperiod natural (14:10 h, L:D),
    - S: 37 psu,
    - T: 25-27ºC
    - DO: 6.78 ± 0.5 ppm
    - Feeding: live phytoplankton (*Nannochloropsis sp.* ), enriched rotifers (1-30 dph), enriched *Artemia* (12-30 dph), microdiets of 75, 150 and 300 µm (13-30 dph)



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# Deformities observed

**A**: Lordosis

**B, G**: Vertebral fusion

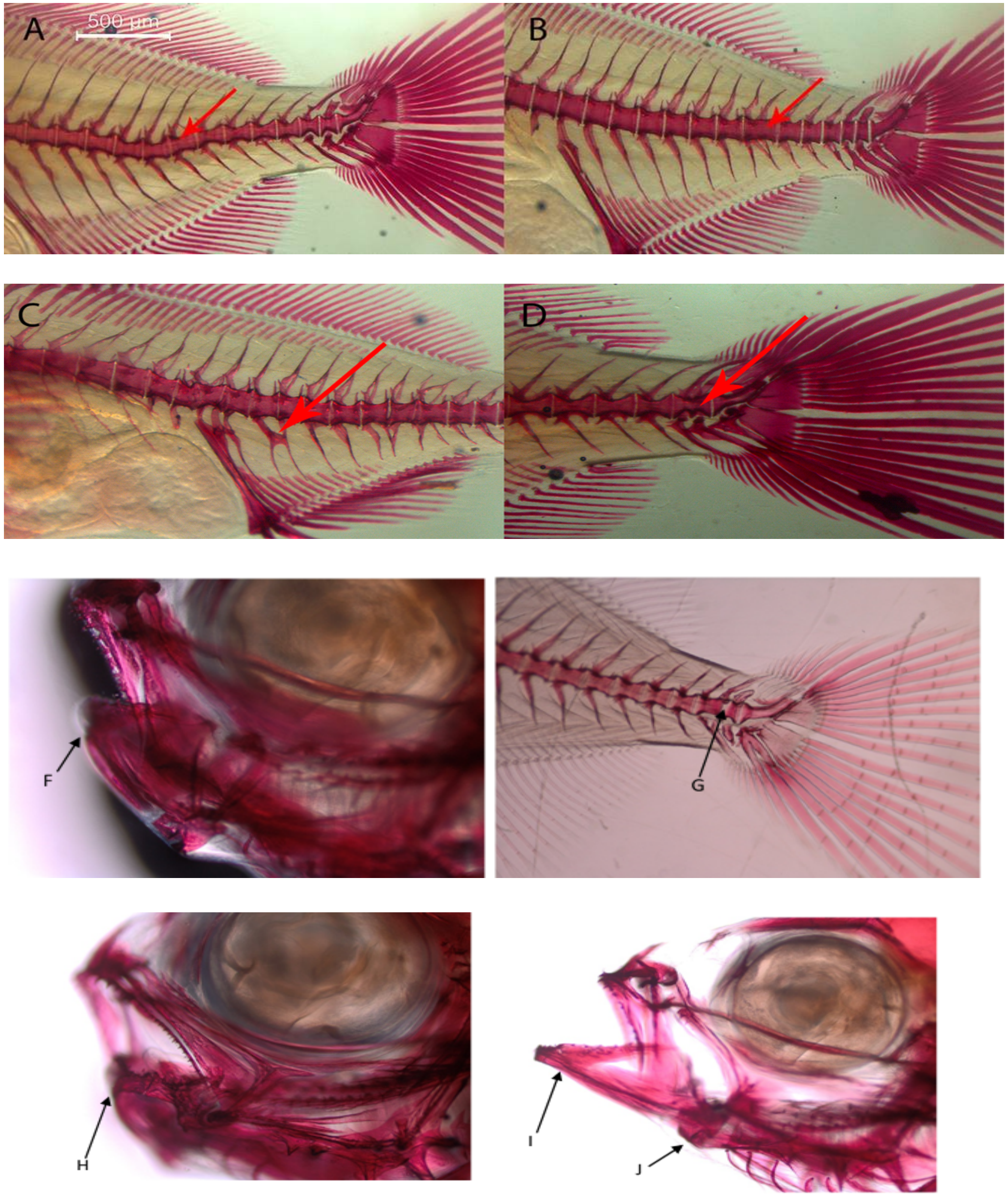
**C**: Fusion of neural arch and spines

**D**: Fusion of caudal vertebrae

**F, H, I**: Anomalous dentary

**G**: Partial vertebral fusion in caudal vertebrae

**J**: Cephalic anomalies (glossohyal)

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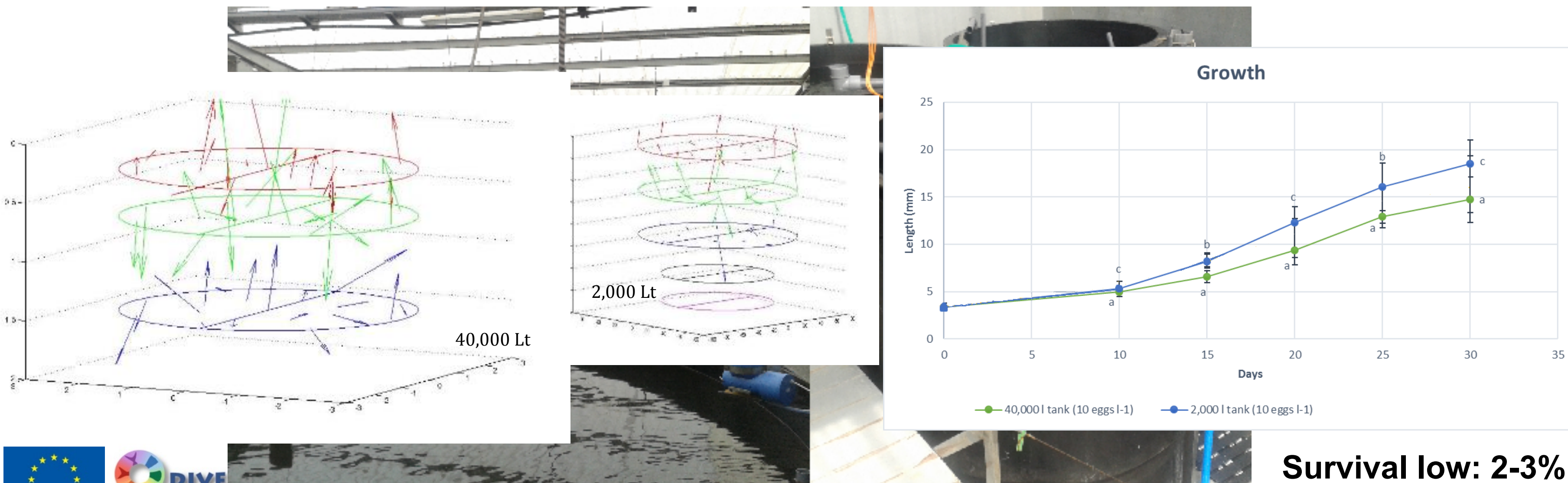
# Effect of environmental parameters

## Tank hydrodynamics

- Two different tanks types: 40,000 l and 2,000 l were tested
  - eggs from natural spawning (10 eggs $l^{-1}$)
  - photoperiod natural (14:10 h, L:D),
  - S: 37 psu,
  - T: 25-27ºC.
  - DO: 5–8 g $l^{-1}$.
  - Feeding: live phytoplankton (*Nannochloropsis sp.* ), enriched rotifers (1-30 dph), enriched *Artemia* (12-30 dph), microdiets of 75, 150 and 300 µm (13-30 dph)




**Survival low: 2-3%**

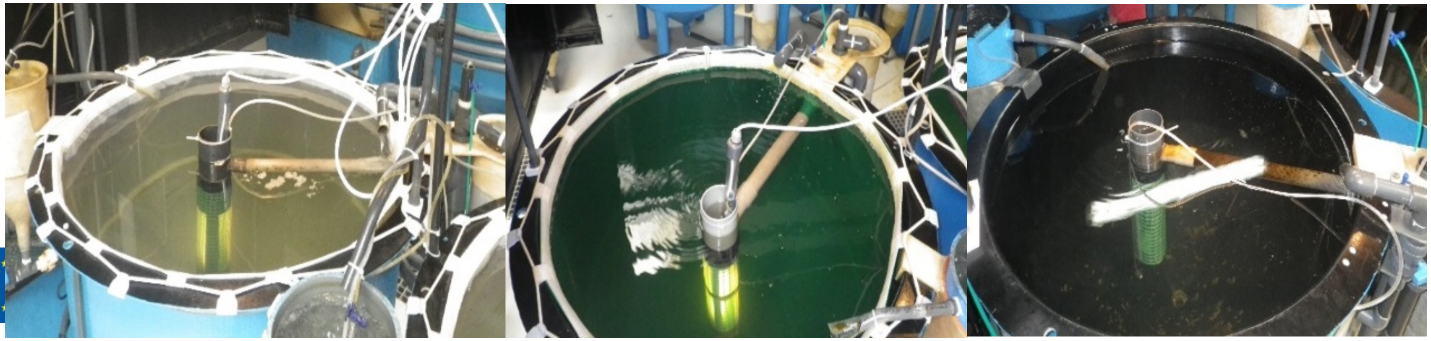
Differences in current profiles (generally higher in the 2,000 l tanks)

# The effect of light (intensity and duration)

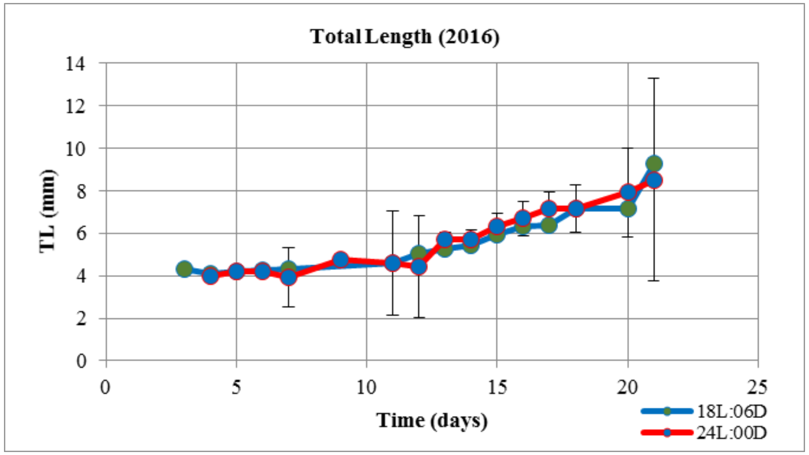
- Photophase 18L:06D vs 24L:00D)
- Background color (black, green, white)

## Intensive rearing

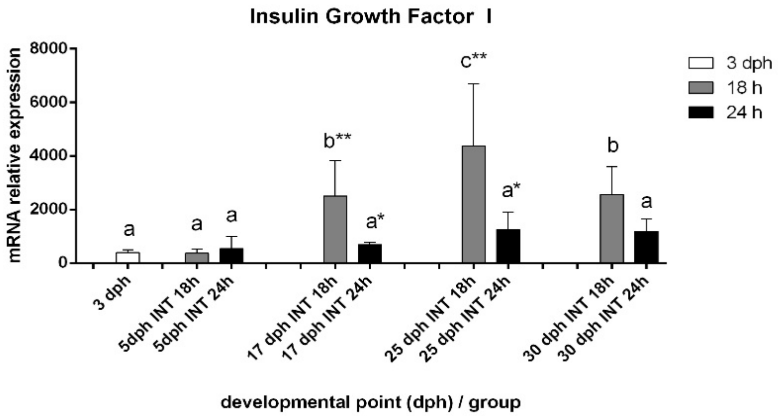
- The “pseudo-green” water methodology – closed hydraulic circuit
  - Water: filtered borehole 35 psu-water.
  - T: 22 ± 0.5°C until mouth opening
    24 ± 0.5°C afterwards.
  - pH: 8.0 to 8.2
  - D.O. 6.8 to 7.2 mg $l^{-1}$.
  - Photophase: 24L:00D from 3 to 25 dph then 18L: 06D
  - Light intensity: 200 - 800 lux
    ~200 lux at night



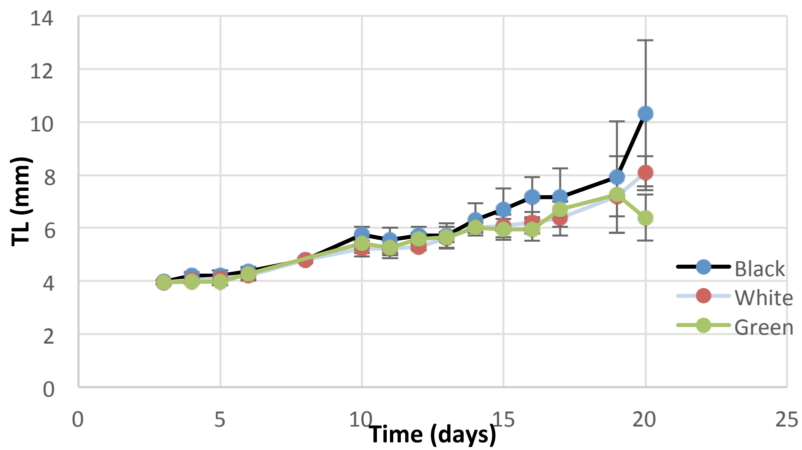
# Results - Photophase




| | Growth rate (exp) | Survival |
|---|---|---|
| 18L:06D | 0.031 $d^{-1}$ | 10.6±4.2% |
| 24L:00D | 0.031 $d^{-1}$ | 8.2±3.1% |

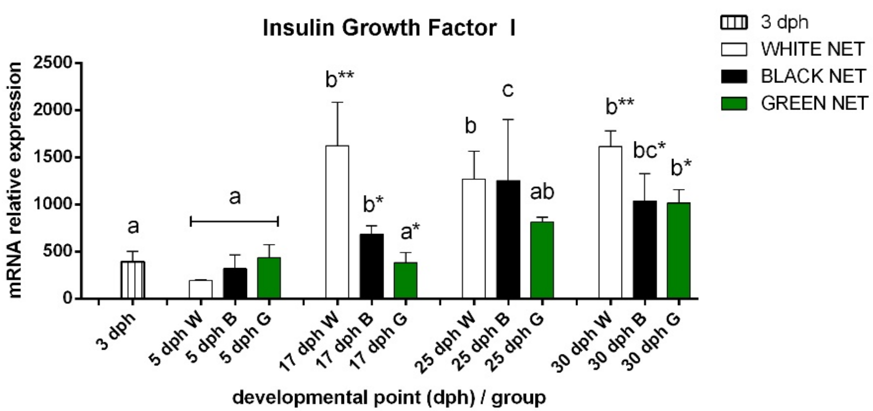


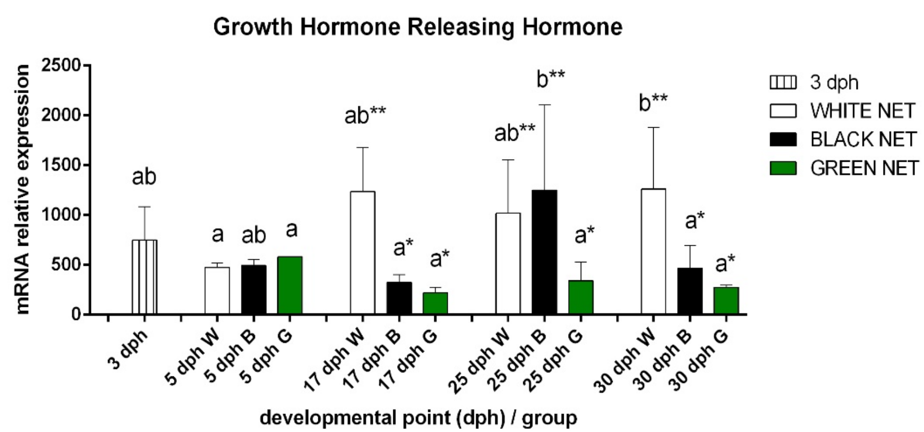

# Results - Background color




**Growth rate (exp)**: White: 0.0393 $d^{-1}$
Black: 0.0481 $d^{-1}$
Green: 0.0355 $d^{-1}$

**SURVIVAL:** WHITE: 22,2%
BLACK: 8,2%
GREEN: 16,5%




- Different expression of genes IGF-I, IGF-II and IGF Binding Proteins 2,3 & 4
  - higher levels for **WHITE** background

- Max light intensity in the water column at 30 cm ~50 lux
- 3-4x higher than without light

**Table 3. Measured light intensity (Lux) at several points in both tanks of each treatment. Values are means ± standard deviation.**

| Measurement point | | Green-no light | White-no light | Green-light | White-light | Black |
|---|---|---|---|---|---|---|
| Under surface | Centre (n=2) | 334 ± 4 | 303 ± 14 | 255 ± 11 | 234 ± 1.1 | 295 ± 24.6 |
| | Side (n=8) | 86 ± 102 | 78 ± 98 | 64 ± 10 | 66 ± 82 | 68 ±11 |
| Depth ~30cm | Angle 45° (n=8) | 15 ± 7.4 | 13 ± 6.2 | 11 ± 4.5 | 11 ± 7.7 | 12 ± 2 |
| | Towards centre (n=8) | 11 ± 1.7 [a] | 12 ± 1.1 [a] | 31 ± 1.7 [b] | 27 ± 4.8 [b] | 9 ± 3 |

- This study showed a catalytic effect of the light conditions on larval survival
  - may refle… e of the species

- The prese… environme… **itude** of any previo…

- Indicate a… larval rearing of…

# Towards an industrial protocol

## Validation in Commercial hatcheries

- Hatchery 1
  - Larvae density 75 ind $l^{-1}$
  - Light intensity
    - 800 lux on 3-6 dph, 1200 lux on 6-12 dph 1000 lux 15 dph 500 lux 20 dph
  - Photophase: 24L:00D from 3-20 dph then 18L:06D
  - Feeding: phytoplankton, enriched rotifers, Artemia and dry feeds. Frozen eggs after 20dph
  - Size selection during weaning
  - In 2017
    - 15.000 juveniles classified in 4 size-classes between 0.3 and 2.5 g to nursery
  - In 2018 similar method
    - until 20 dph survival ~10%
    - between 20-30 dph: no sorting → mortality >65% due to cannibalism
    - 30 dph 15.000 individuals were transferred to nursery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## Validation in Commercial hatcheries (2)

- Hatchery 2
  - Direct incubation of eggs in the larval rearing tanks
  - T: 24.5 to 25.0 ºC.
  - pH: 7.8
  - Light conditions : modified in some tanks
    - intensity on the surface >1000 lux
  - Photophase: 24L:00D from 3-20 dph then 18L:06D
  - Feeding: enriched rotifers, instar I (TL >5mm) and enriched instar II (TL >6mm) Artemia nauplii and artificial diets (TL >7mm).
  - Tanks with high light intensity performed better
  - Sorting in size from 20 dph, improves significantly survival
    - 48.300 juveniles of 25-50 g transferred in cages

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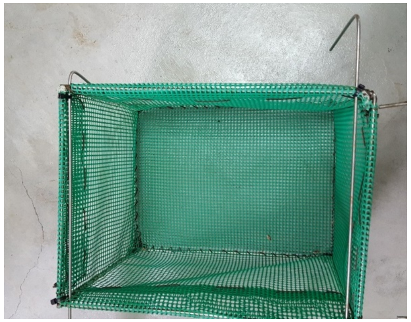
# Recommendations (1)

- Rearing parameters
  - large tank improves growth performance and survival
  - egg stocking densities ~ 25 eggs $l^{-1}$
  - DO: 5.0 to 8. mg $l^{-1}$, preferably >6.0 mg $l^{-1}$
  - S: 37 - 40 psu
  - pH: 7.8-8.5
  - T: 22 to 27ºC, preferably between 23.5 and 25.0ºC
  - Photophase 18L:06D from 1 to 25 dph
  - Light intensity: Defused light of 800-1200lux, 3-12 dph
    gradually 800 lux until 20 dph
- Feeding
  - coordinate rearing conditions and larval development
  - live microalgae at 150-300 x $10^3$ cell $ml^{-1}$ since 2 dph
  - enriched rotifers min 2x $d^{-1}$, 3-15 dph, at 3-7 rot $ml^{-1}$; Artemia AF (TL>5mm), for 2-3 days; enriched Instar II (TL> 6.0 mm); dry diet (200-800 µm) ) (TL>6.5 mm)
  - immune modulators (*Echium* and black cumin oil) together with optimized enriching emulsion and astaxanthin as carotenoid improve larval performance

# Recommendations (2)

- Husbandry
  - high size variability (at 20-30 dph) in all rearing systems tested until today (unknown reasons)
    - early sorting to appropriate size classes
    - standard methods and equipment available in all hatcheries
    - significant higher survival compared to unsorted groups although the losses
    - mortality for unsorted groups >90%, for sorted groups 10-15%.
  - Transportation of individuals
    - larvae with TL <15 mm <u>do not tolerate netting,</u> transfer performed with care.
    - larvae with TL >20 mm can be netted normally
  - individuals >0.5-1 gr are handled easier
    - light anesthesia may help

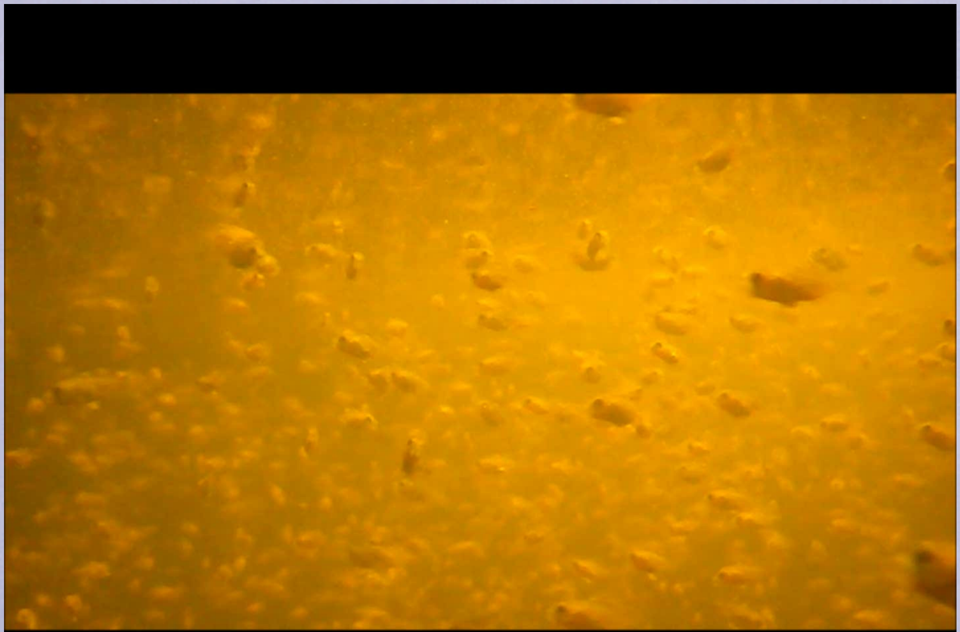
2mm



5mm

20mm

# Thank you!



Greater Amberjack larval rearing, HCMR 2016

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