# Nutrition **OF GREATER AMBERJACK (*Seriola dumerili*): Highlights at different stages of the life cycle.**

**Daniel Montero**, C.M. Hernández Cruz, S. Sarih, H. Fernández-Palacios, M. Izquierdo (**FCPCT-ULPGC**)
S. Jerez, M. Virginia Martín (IEO), J. Perez, C. Rodríguez (ULL)
Kotzamanis, Y., (HCMR)
Fontanillas, R., Rosenlund, G. (SARC)

Hellenic Centre for Marine Research
Institute of Marine Biology, Biotechnology & Aquaculture

Fundación Parque Científico Tecnológico
Universidad de Las Palmas de Gran Canaria (ULPGC)

Universidad de La Laguna

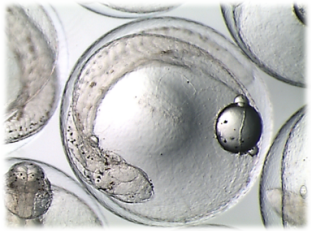
- Fast growing species, highly susceptible to marginal dietary deficiencies. Higher demand of certain nutrients compared to other aquaculture species. Nutritional Information on bottlenecks of different life-cycle stages is scarce.

**Aim**:

- Improve the quality of greater amberjack in different bottlenecks of the life-cycle through nutrition

- **Objectives**: to define the effect of different nutrients to optimize nutrition at:
  - Early larval stage
  - Grow out
  - Brood-stock to improve spawning quality

  - Nutrition related with essential fatty acids and amino acids.



# *Hatchery and grow out nutrition for greater amberjack*

- To determine the nutritional requirements and optimum levels of ***DHA, EPA, and combined PUFA-carotenoids*** in greater amberjack enrichment products at the time of both ***rotifer and Artemia*** stages

Material & Methods

## 1. Optimum docosahexaenoic acid (DHA) in enrichment products for live preys

- Five different emulsions with graded levels of DHA containing:

- ✓ High DHA content commercial methyl ester oil (DHA-70, Maruha Nichiro Foods, Tokyo, Japan)
  - ✓ containing 70% of Total Fatty Acid (TFA) as DHA, 12% as EPA and 2% as ARA;
  - ✓ Oleic Acid oil (Sigma-Aldrich; Madrid, Spain) including 77% of TFA as oleic acid
  - ✓ soya lecithin (SL, Korot SL, Alcoy, Spain) containing mainly 54% of TFA as linoleic acid (18:2n−6, LA) and trace amounts of EPA and DHA
- ✓ 3000 mg $kg^{-1}$ vitamin E (DL-α-tocopherol acetate) and 2500 mg $kg^{-1}$ vitamin C (L-ascorbic acid)

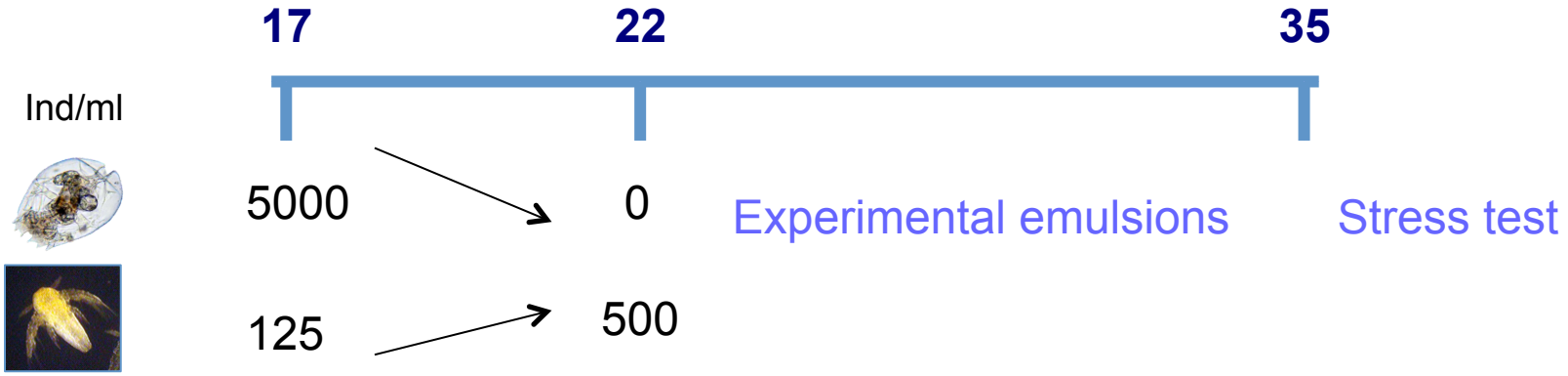
| *DHA (%TFA)* | 0.5 | 10 | 20 | 30 | 50 |
|---|---|---|---|---|---|
| *Experimental Emulsion* | *DHA-0* | *DHA-1* | *DHA-2* | *DHA-3* | *DHA-4* |
| *Ingredients (g $kg^{-1}$ diet)* | | | | | |
| *DHA-7*[a] | 0 | 300 | 450 | 600 | 900 |
| *Oleic acid* | 900 | 600 | 450 | 300 | 0 |
| *Soy bean lecithin* | 100 | 100 | 100 | 100 | 100 |



## Material & Methods

Larvae from natural spawning at ULPGC facilities culture in Mesocosm system

- ✓ 17 dph, at a total density of 1000 larvae per tank (mean total length 6.4 mm)
- ✓ 15 experimental tanks of 200 L

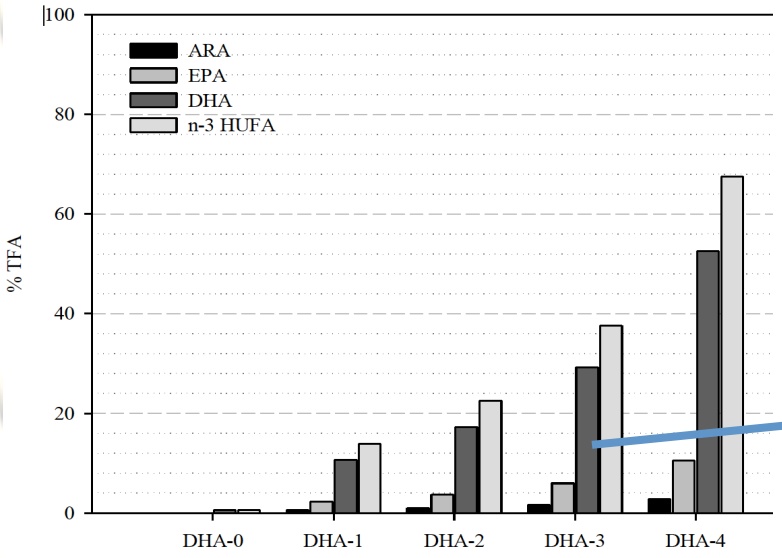



- ✓ Thirty larvae per tank at 35 dph were submitted to acute stress, handling them out of the water for 60 seconds and returning them to a bucket with aerated seawater.
- ✓ Survival rate was determined 24 hours later, counting all the surviving larvae.
- ✓ To determine the skeletal anomalies incidence, 100 larvae were studied per tank at 35 dph.

DIVERSIFY

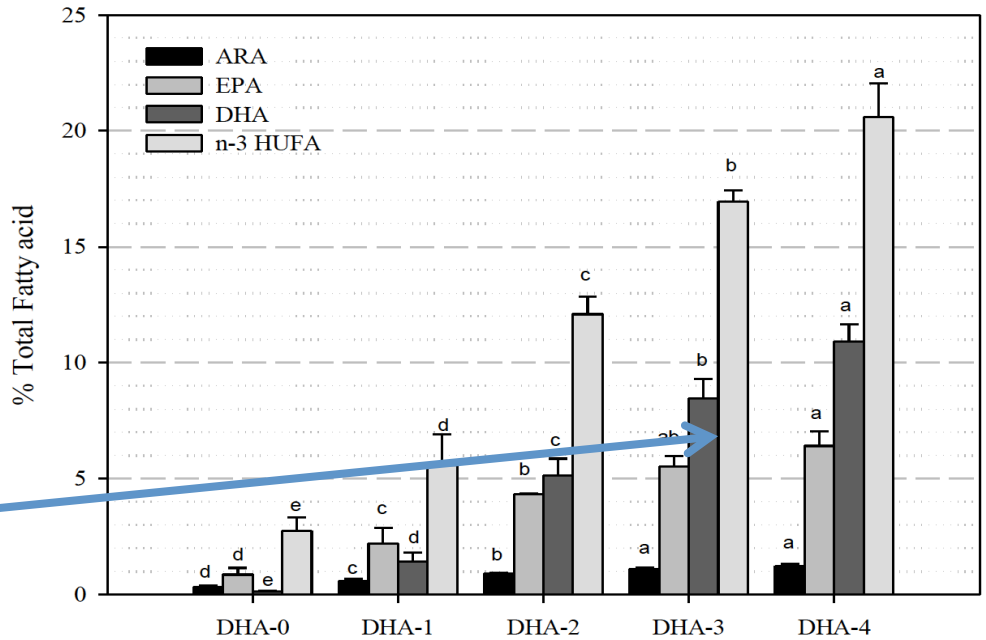
# Results

## Fatty acids in emulsions



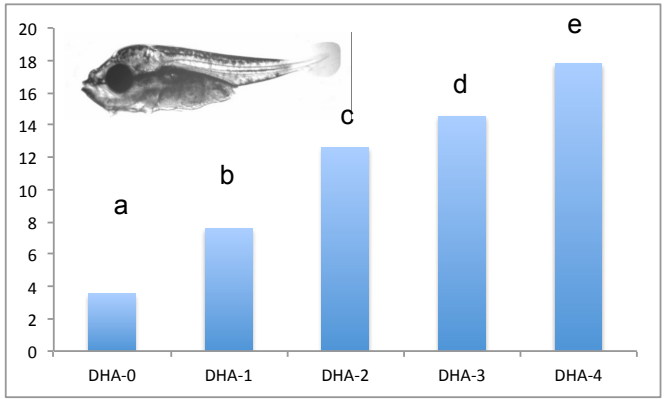

| %TFA | 0.5 | 10 | 20 | 30 | 50 |
|---|---|---|---|---|---|

## Fatty acids in Artemia




| | 0.1 | 1.4 | 5.0 | 8.5 | 11.0 |
|---|---|---|---|---|---|

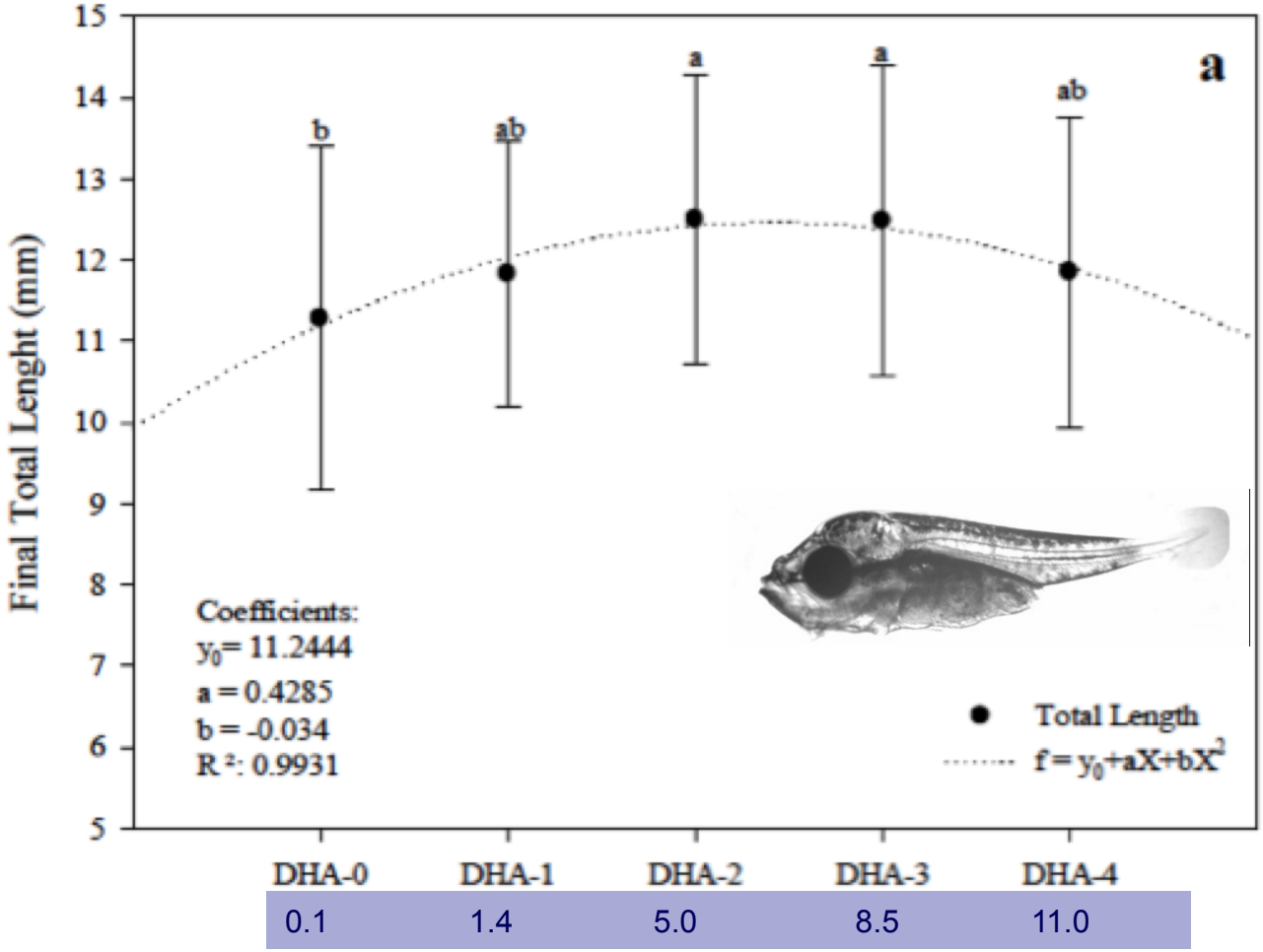
DHA in 35dph larvae (%) TFA





Results

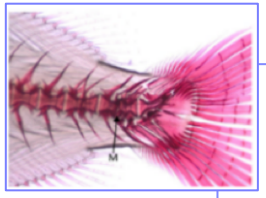
## *Larval growth at 35dph*

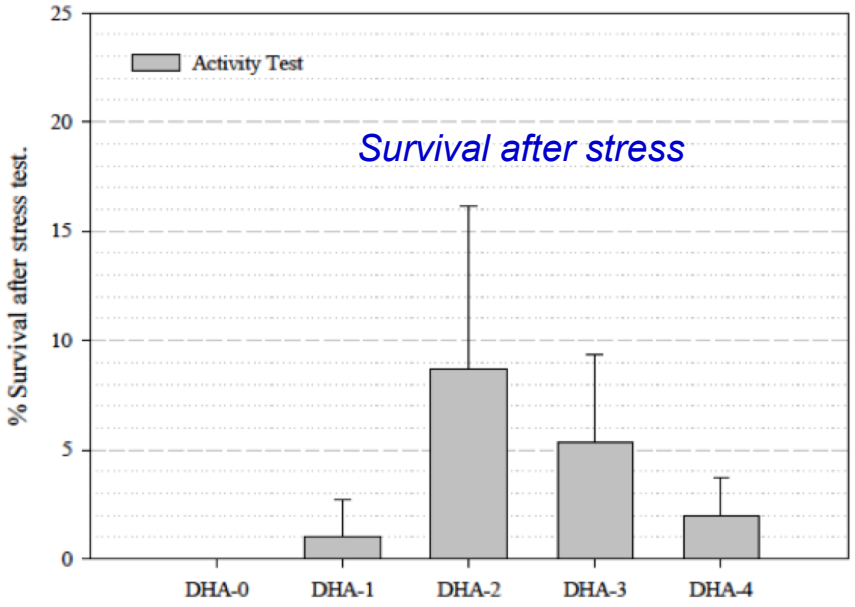


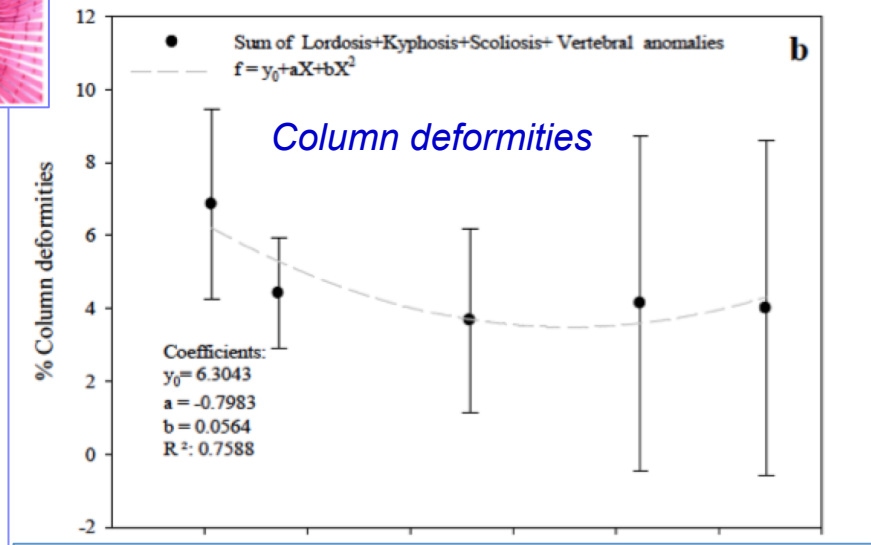

DIVERSIFY

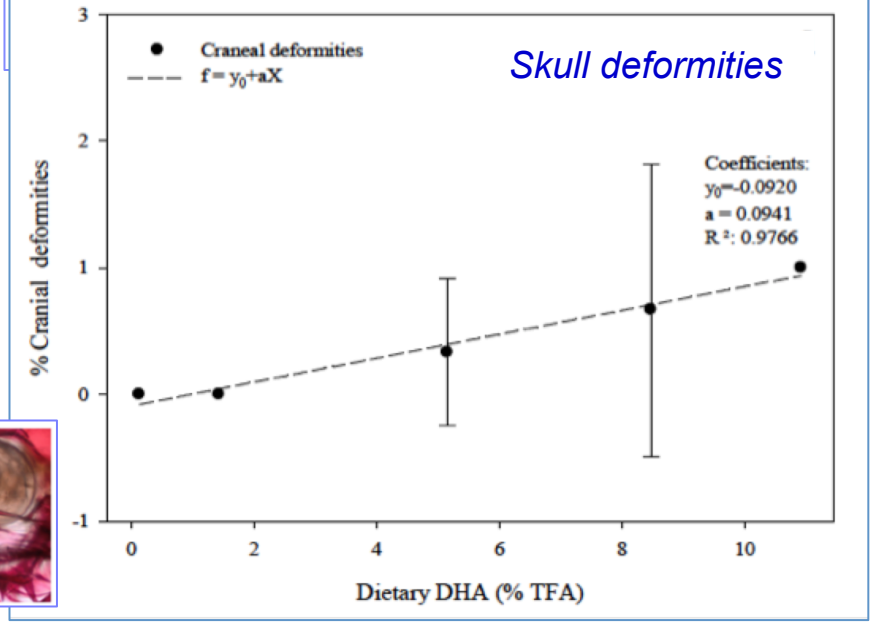
Results

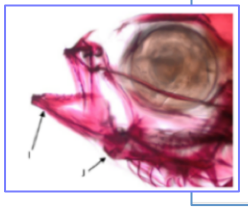
# Larval quality at 35dph





DIVERSIFY

## 2. Optimum Eicosapentaenoic acid (EPA) in enrichment products for live preys

Material & Methods

- Five different emulsions with graded levels of EPA containing:

✓ high EPA content commercial triglycerides oil (Incromega EPA 500 TG, Croda, Barcelona, Spain)
  - ✓ containing 63% of Total Fatty Acid (TFA) as EPA, 8% as DHA and 3% as ARA;
  - ✓ Oleic Acid oil (Sigma-Aldrich) including 77% of TFA as oleic acid
  - ✓ soya lecithin (SL, Korot SL, Alcoy, Spain) containing mainly 54% of TFA as linoleic acid (18:2n−6, LA) and trace amounts of EPA and DHA

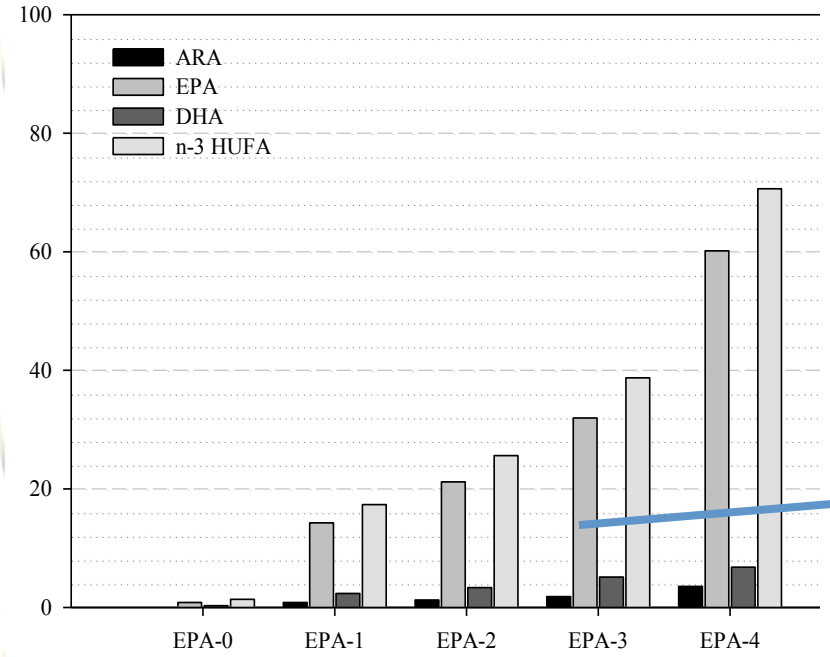
✓ 3000 mg $kg^{-1}$ vitamin E (DL-α-tocopherol acetate) and 2500 mg $kg^{-1}$ vitamin C (L-ascorbic acid)

| *EPA (%TFA)* | 0.8 | 15 | 20 | 30 | 60 |
|---|---|---|---|---|---|
| *Experimental Emulsion* | *EPA-0* | *EPA-1* | *EPA-2* | *EPA-3* | *EPA-4* |
| | *Ingredients (g $kg^{-1}$ diet)* | | | | |
| *EPA 500TG*[a] | 0 | 300 | 450 | 600 | 900 |
| *Oleic acid* | 900 | 600 | 450 | 300 | 0 |
| *Soy bean lecithin* | 100 | 100 | 100 | 100 | 100 |

DIVERSIFY

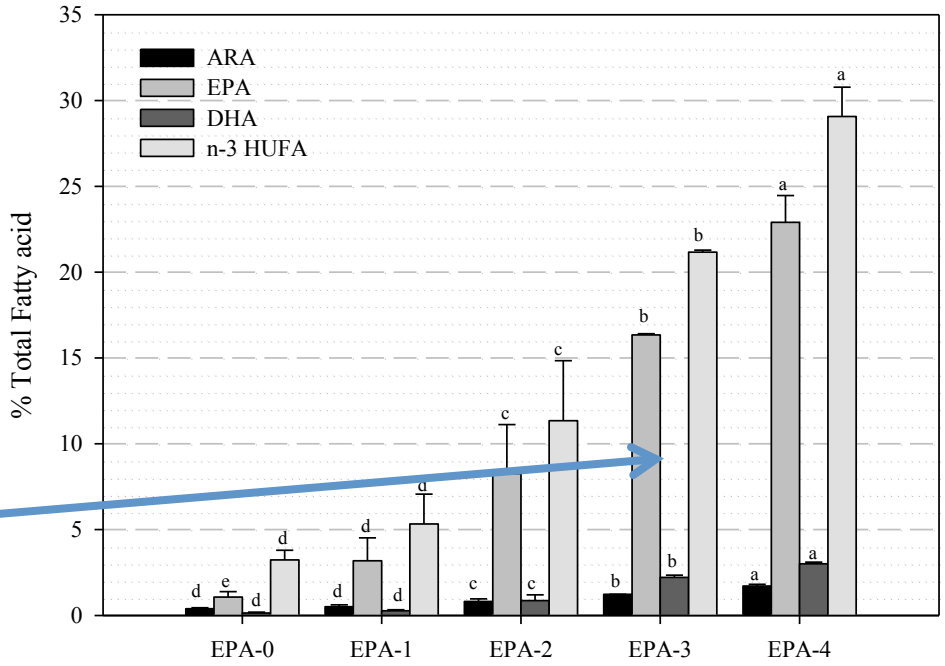
# Results

## Fatty acids in emulsions



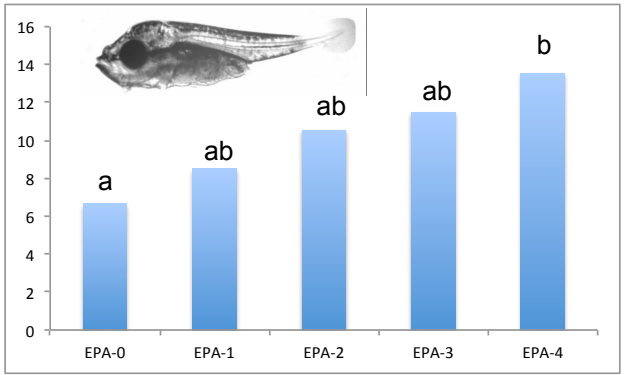

| %TFA | 0.8 | 15 | 20 | 30 | 60 |
|---|---|---|---|---|---|

## Fatty acids in Artemia




| | 1.0 | 3.0 | 8.0 | 16 | 23 |
|---|---|---|---|---|---|

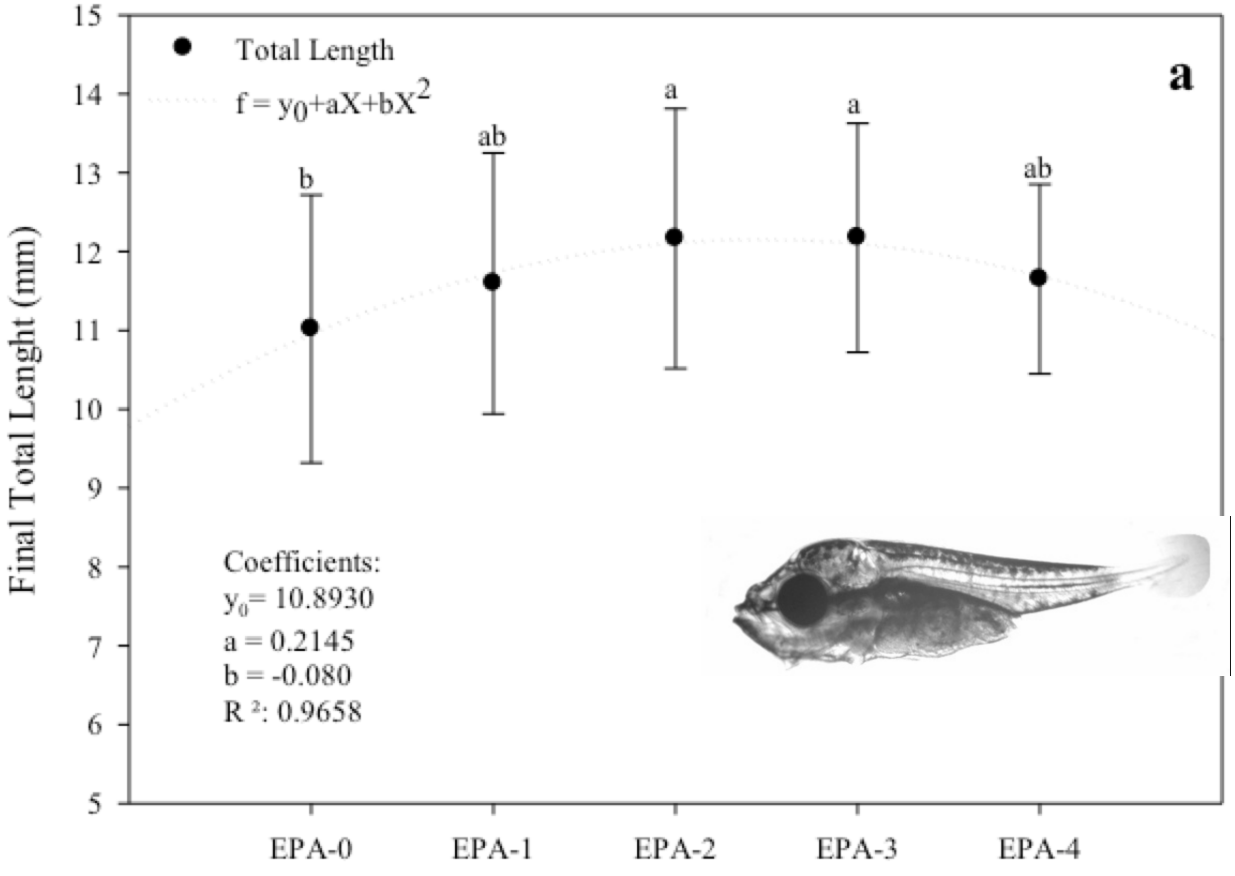
EPA in 35dph larvae (%) TFA




DIVERSIFY

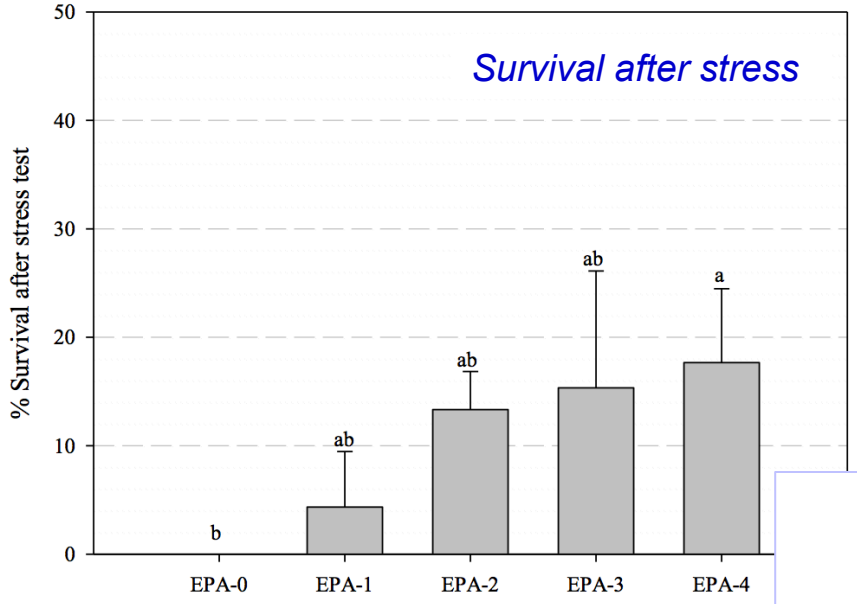
# Results

## *Larval growth at 35dph*



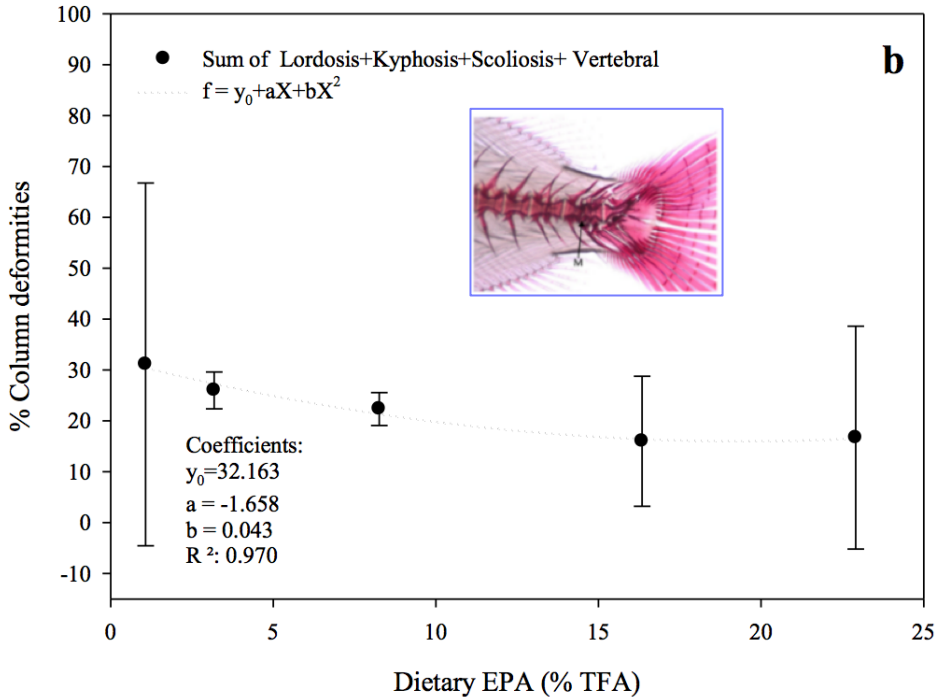

| 1.0 | 3.0 | 8.0 | 16 | 23 |
|---|---|---|---|---|

Results



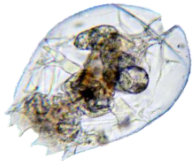

# Larval quality at 35dph

*Column deformities*




DIVERSIFY

## 3. Combined effect of PUFA-rich lipids and carotenoids in enrichment products for rotifers



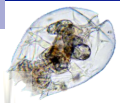
Material & Methods

- Three different emulsions:
  - ✓ **E1** based on a polar rich (PL-rich) emulsion containing a marine natural lecithin LC60 (PhosphoTech Laboratories, France) with up to 60 % phospholipids (40% PC + 20 PE) rich in DHA, and a DHA/EPA ratio of 2.5/1.
  - ✓ **E3** was based on a mixture of different TAG sources (Incromega DHA500 TAG and cod liver oil) although a slight supplementation with soybean lecithin was performed to help emulsification and absorption of lipids. Finally,
  - ✓ **E2** emulsion was formulated on a blend of these three lipid sources.

A commercial booster rich in TAG was also used as a control (**C**) Espresso product, followed by DHA-PROTEIN SELCO enrichment (INVE-AQUACULTURE, Belgium).

| | E1 | E2 | E3 |
|---|---|---|---|
| *Ingredients (mg $L^{-1}$)* | | | |
| Marine lecithin | 78 | 23.6 | 0 |
| Incromega DHA 500 | 0 | 39.3 | 47.2 |
| Cod liver oil | 0 | 15.7 | 31.5 |
| Arachidonic acid | 2 | 1.4 | 1.3 |

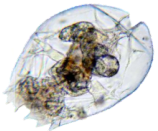
DIVERSIFY



Material & Methods

Larvae from induced spawning at IEO facilities culture in Mesocosm system

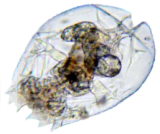
- ✓ rotifers enriched for short periods (3-6h) with 6% of the marine lecithin with a slight supplementation of AA (E1) in combination with a range of carotenoids well below 50 ppm
- ✓ Newly hatched larvae, at a total density of 5000 larvae per tank (mean total length 3.14± 0.08 mm), were randomly distributed in 12 experimental tanks of 100 L.
- ✓ From 3 to 11 dph, rotifers in the tanks were adjusted to 5 individuals ml-1 and increased to 10 individuals ml-1 until the end of the trial.
- ✓ rotifer enrichment commercial protocol (C) was compared with three experimental emulsions (**E1**; **E1,10** and **E3,10**. (E1,10 and E3,10 = +10 ppm (mg l-1) of Naturose (~2% astaxanthin)



# 3. Combined effect of PUFA-rich lipids and carotenoids in enrichment products for rotifers

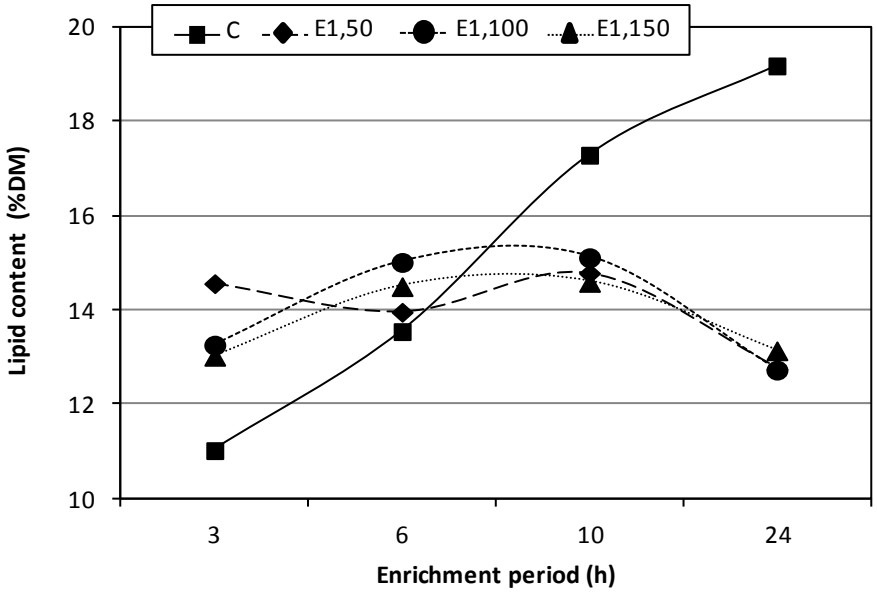
Results

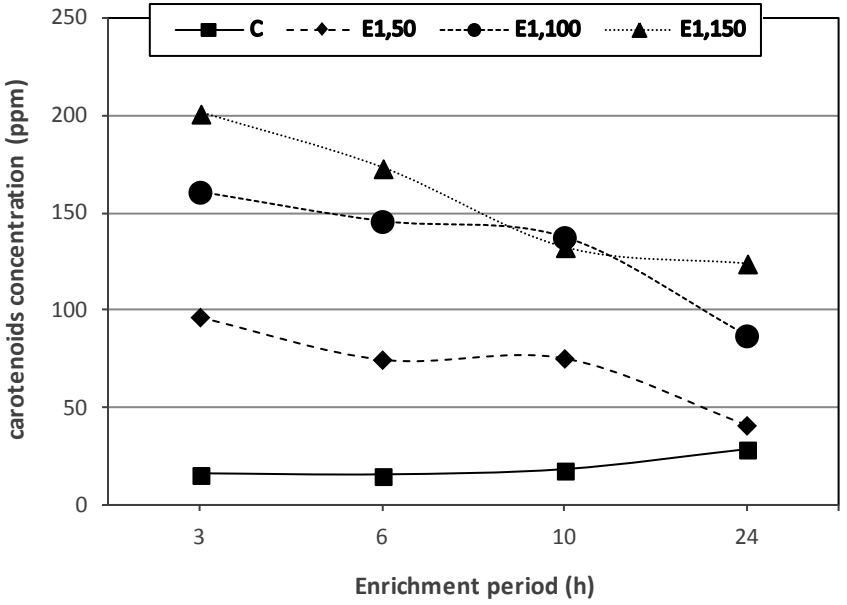
| | Control | | | E1 | | | E2 | | | E3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3h | 6h | 10h | 3h | 6h | 10h | 3h | 6h | 10h | 3h | 6h | 10h |
| *TL* | 12.8±1.1$^{a,B}$ | 16.6±1.7$^{b,B}$ | 17.6±1.2$^{b,A}$ | 19.9±1.4$^{b,A}$ | 16.0±1.6$^{a,B}$ | 15.6±0.3$^{a,A}$ | 20.6±1.5 $^{A}$ | 22.9±0.8 $^{A}$ | 26.0±3.5 $^{B}$ | 12.7±1.6$^{a,B}$ | 13.9±2.1$^{ab,B}$ | 16.5±1.0$^{b,A}$ |
| *TAG* | 33.5±0.7$^{a,B}$ | 39.8±1.8$^{b,A}$ | 46.1±1.9$^{c,B}$ | 24.5±0.9$^{a,C}$ | 33.5±0.3$^{b,B}$ | 36.1±1.3$^{c,A}$ | 43.4±0.8 $^{A}$ | 42.2±2.4 $^{A}$ | 42.5±0.4 $^{B}$ | 35.9±2.5$^{a,B}$ | 41.6±2.4$^{ab,A}$ | 44.9±3.2$^{b,B}$ |
| *TPL* | 26.1±1.9 $^{A}$ | 26.0±2.2 $^{A}$ | 23.3±1.3 $^{B}$ | 31.5±2.9 $^{A}$ | 31.1±0.8 $^{A}$ | 29.4±1.5 $^{C}$ | 13.6±0.5 $^{C}$ | 17.5±2.7 $^{C}$ | 13.1±2.1 $^{A}$ | 24.9±0.2 $^{B}$ | 24.1±1.9 $^{B}$ | 22.8±1.8 $^{B}$ |
| *TPL-Fatty acids* | | | | | | | | | | | | |
| ∑ SFA | 14.3±0.9$^{a,A}$ | 16.2±0.3$^{b,A}$ | 17.0±0.5$^{b,B}$ | 22.5±0.3$^{a,C}$ | 19.7±1.2$^{b,B}$ | 19.8±0.2$^{b,C}$ | 18.5±0.9 $^{B}$ | 18.4±1.8 $^{AB}$ | 17.6±0.5 $^{B}$ | 14.5±0.3 $^{A}$ | 14.7±1.7 $^{A}$ | 14.9±0.7 $^{A}$ |
| ∑ MUFA | 61.6±0.3$^{c,D}$ | 44.1±0.5$^{b,B}$ | 38.3±0.8$^{a}$ | 37.6±1.2$^{a,A}$ | 39.1± 0.4$^{b,A}$ | 36.9±0.4$^{a}$ | 42.1±0.8$^{b,B}$ | 38.7±0.8 $^{a,A}$ | 35.8±2.9$^{a}$ | 49.8±0.6$^{c,C}$ | 46.3±0.3$^{b,C}$ | 41.6±2.3$^{a}$ |
| ∑ n-6 PUFA | 8.9±0.1$^{a,A}$ | 15.7±0.3$^{b,B}$ | 16.4±0.3$^{b,C}$ | 9.7±0.0$^{a,B}$ | 11.3±0.4$^{b,A}$ | 11.0±0.3$^{b,B}$ | 10.7±0.4$^{b,C}$ | 11.3±0.2 $^{b,A}$ | 9.1±0.9$^{a,A}$ | 15.6±0.6 $^{D}$ | 16.1±0.3 $^{B}$ | 14.8±1.1 $^{C}$ |
| 20:4 | 0.8±0.1$^{a,B}$ | 1.6±0.0$^{b,C}$ | 1.7±0.1$^{b,A}$ | 4.0±0.0 $^{A}$ | 4.6±0.5 $^{A}$ | 4.8±0.1 $^{C}$ | 3.7±0.3$^{ab,A}$ | 4.4±0.3 $^{b,A}$ | 3.5±0.3$^{a,B}$ | 3.7±0.2 $^{A}$ | 3.6±0.0 $^{B}$ | 3.6±0.1 $^{B}$ |
| ∑ n-3 HUFA | 4.3±0.3$^{a,C}$ | 16.0±0.7$^{b,B}$ | 16.8±0.9$^{b,A}$ | 23.2±0.5 $^{A}$ | 23.7±2.4 $^{A}$ | 22.2±0.5 $^{B}$ | 21.0±1.7$^{a,A}$ | 25.6±2.2 $^{b,A}$ | 26.9±0.6$^{b,C}$ | 11.2±0.4$^{a,B}$ | 14.9±0.5$^{b,B}$ | 19.0±2.9$^{b,AB}$ |
| 20:5 | 1.1±0.1$^{a,D}$ | 3.3±0.2$^{b,B}$ | 3.3±0.2$^{b,A}$ | 6.4±0.2 $^{A}$ | 6.5±0.7 $^{A}$ | 6.3±0.3 $^{C}$ | 5.0±0.5$^{a,B}$ | 6.4±0.6 $^{b,A}$ | 5.5±0.4$^{ab,BC}$ | 3.1±0.3 $^{C}$ | 4.1±0.6 $^{B}$ | 4.4±0.8 $^{AB}$ |
| 22:6 | 2.4±0.2$^{a,C}$ | 11.4±0.4$^{b,B}$ | 11.5±0.6$^{b,A}$ | 16.3±0.5$^{b,A}$ | 16.5±2.8$^{ab,A}$ | 14.4±0.2$^{a,A}$ | 15.3±1.1 $^{A}$ | 18.1±2.8 $^{A}$ | 19.7±0.6 $^{B}$ | 7.4±0.1$^{a,B}$ | 9.5±0.1$^{ab,B}$ | 12.2±2.1$^{b,A}$ |



# 3. Combined effect of PUFA-rich lipids and carotenoids in enrichment products for rotifers

Results

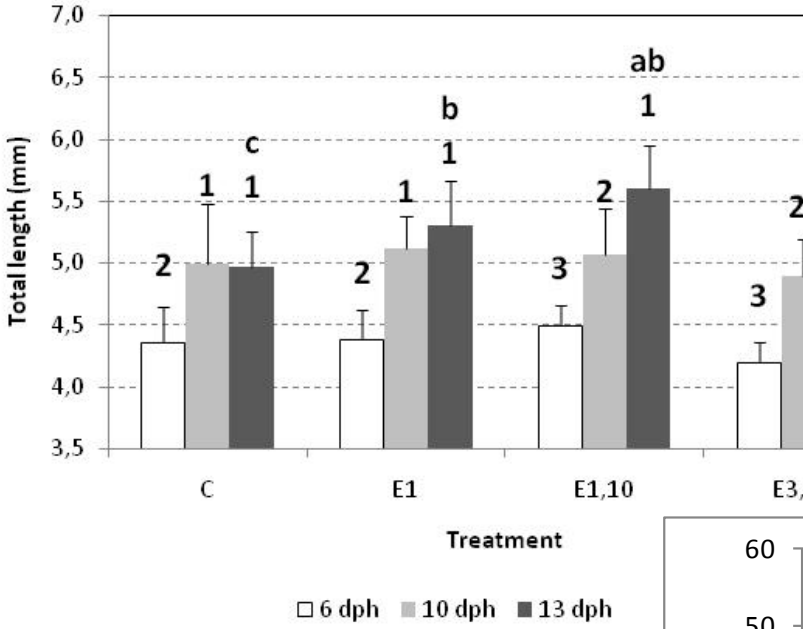


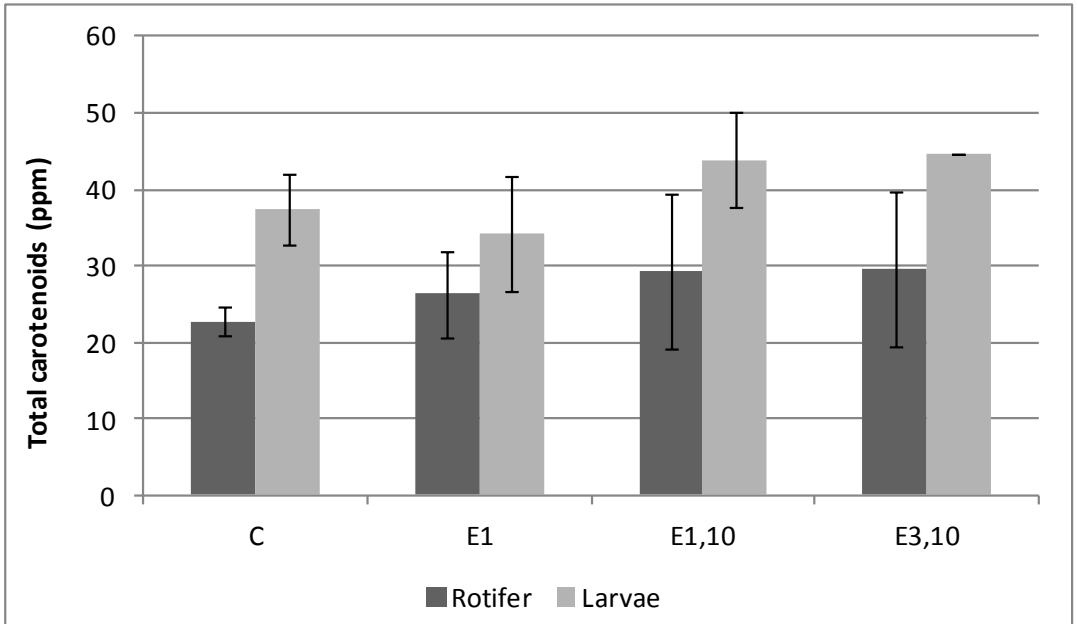



# 3. Combined effect of PUFA-rich lipids and carotenoids in enrichment products for rotifers

Results

## Larval growth (13 dph)

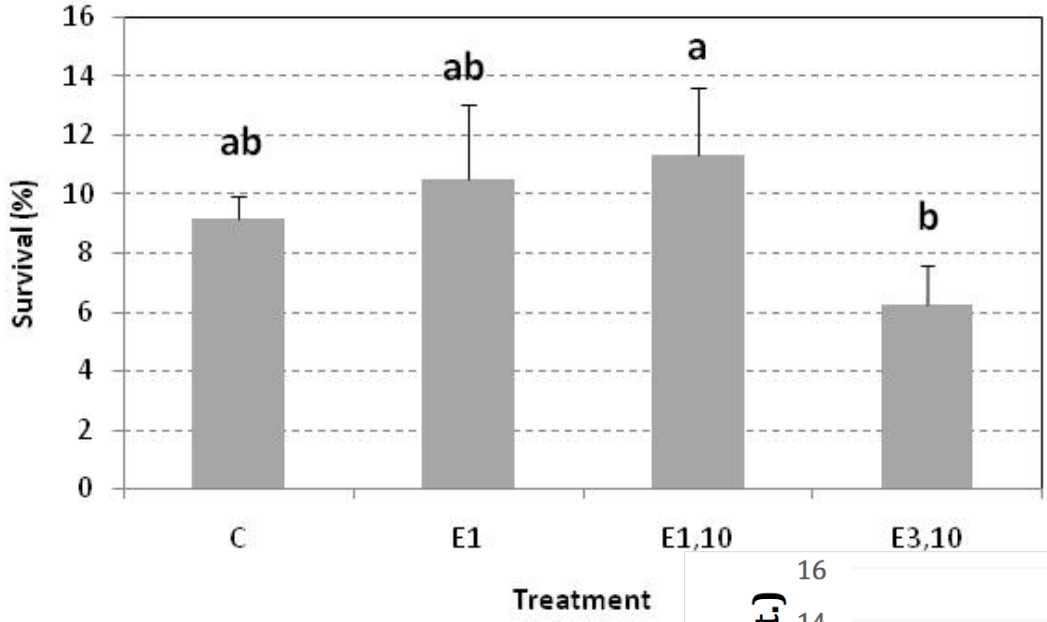


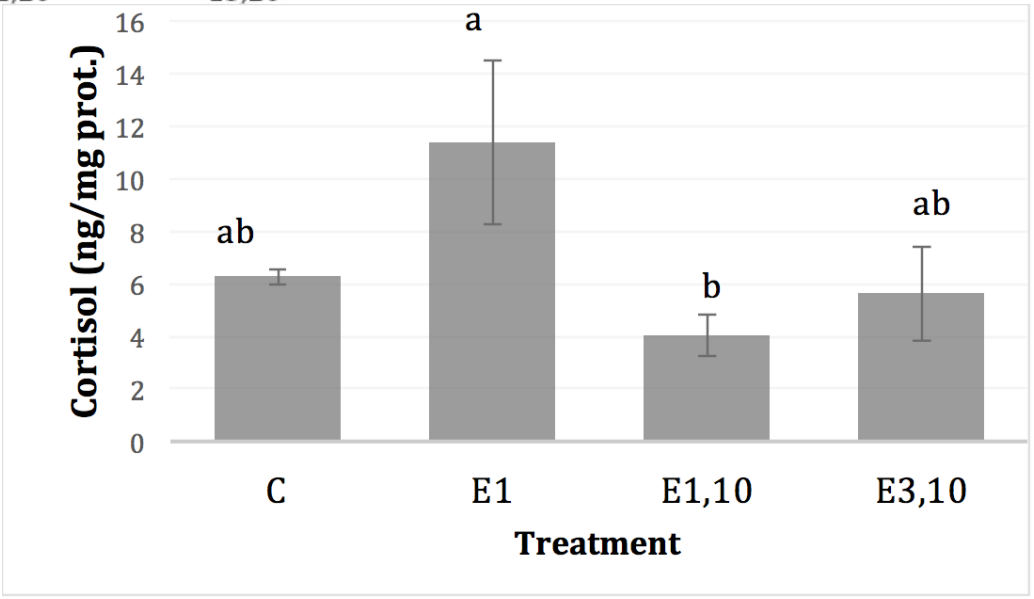

DIVERSIFY

# 3. Combined effect of PUFA-rich lipids and carotenoids in enrichment products for rotifers

Results




## Larval quality




DIVERSIFY

# Lessons learned

Results

- DHA in enrichment products for *Artemia* 10-17% TFA
- EPA in enrichment products for *Artemia* 14-20% TFA
- DHA/EPA in enrichment products for *Artemia* 1-5
- DHA in enrichment products for rotifers 14% TFA
- EPA in enrichment products for rotifers 6% TFA
- DHA/EPA in enrichment products for rotifers 2.3
- Carotenoids levels in enrichment products 10 ppm

Material & Methods

## 4. Levels of Lysine in grow-out diets

| *Ingredients* (% diet) | L1 | L2 | L3 | L4 | L5 | L6 |
|---|---|---|---|---|---|---|
| Fish meal (71%)[a] | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Wheat meal | 28.65 | 28.55 | 28.40 | 28.30 | 28.20 | 28.10 |
| Corn gluten | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Wheat gluten | 21.95 | 21.95 | 21.95 | 21.95 | 21.95 | 21.95 |
| Soya concentrate | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |
| Fish oil | 12.33 | 12.33 | 12.33 | 12.33 | 12.33 | 12.33 |
| Lysine HCl | ***0.00*** | ***0.10*** | ***0.21*** | ***0.31*** | ***0.41*** | ***0.52*** |
| Dicalcium phosphate | 0.61 | 0.61 | 0.61 | 0.61 | 0.61 | 0.61 |
| Mineral & Vitamin premix | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| *Analyzed chemical composition of diets (% or specified)* | | | | | | |
| Protein | 44.58 | 44.83 | 44.63 | 44.52 | 44.53 | 44.68 |
| Fat | 17.65 | 17.47 | 17.24 | 17.19 | 17.01 | 17.38 |
| Ash | 5.14 | 5.34 | 5.31 | 5.23 | 5.16 | 5.15 |
| Moisture | 7.87 | 8.66 | 8.41 | 8.65 | 8.52 | 8.13 |
| Carbohydrate* | 24.76 | 23.70 | 24.21 | 24.41 | 24.78 | 24.66 |
| Gross energy (MJ $kg^{-1}$) | 21.90 | 21.63 | 21.55 | 21.58 | 21.52 | 21.78 |

# Material & Methods

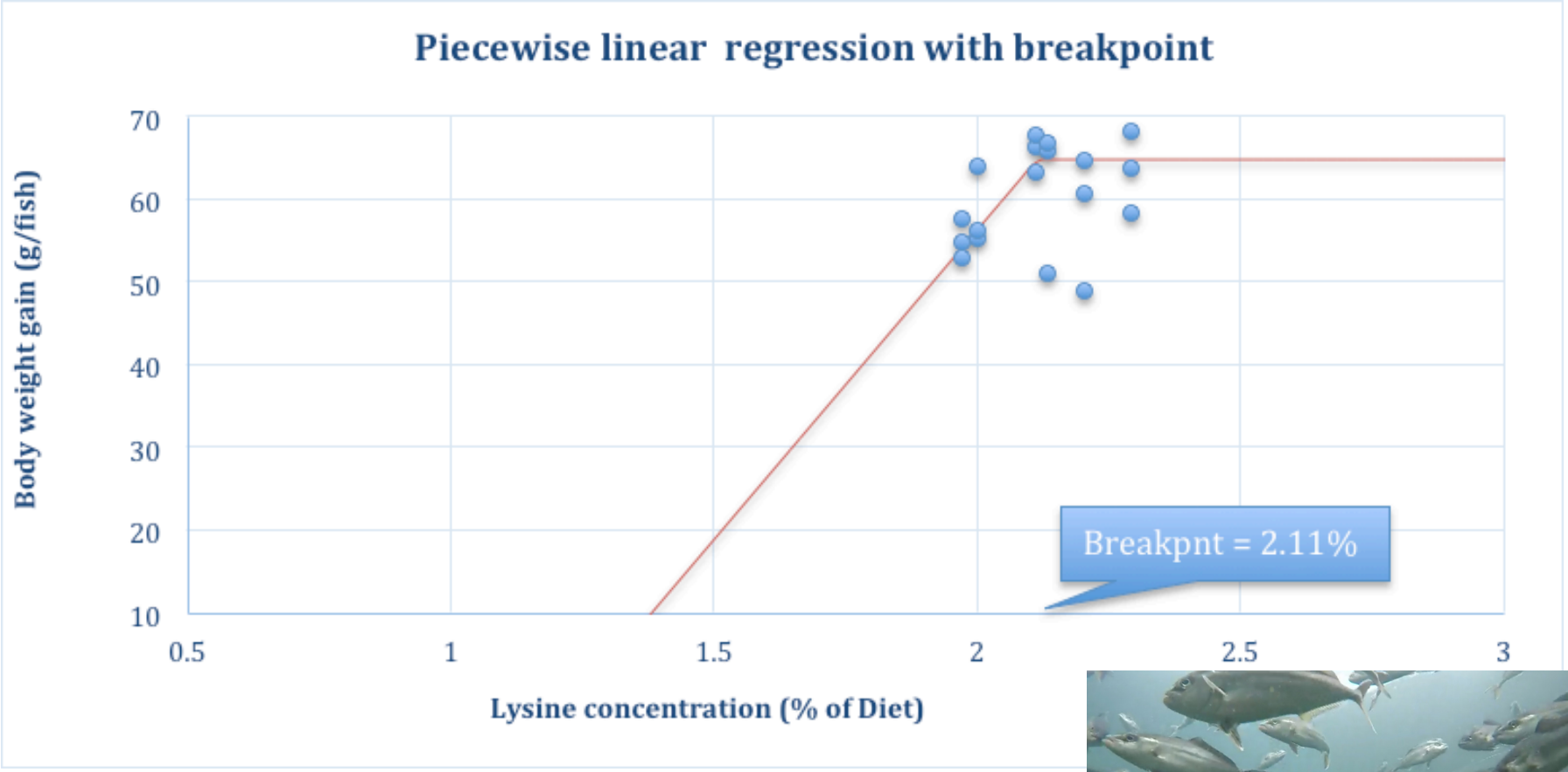
- Initial average body weight (BW) of 32.8 ± 3.0 g (n = 450)
- 18 experimental small cages (1.0 x 5 x 1.0 m; 5 m3), at a density of 25 fish per cage (3 replicates/cages per diet).
- All cages were placed in two large rectangular concrete tanks of 36 $m^3$ water capacity that were continuously supplied with filtered sea water (salinity 35 ppt) at 400 L/h and aerated to over 80% oxygen saturation.
- Water temperature 19.8 ± 1.7 $^0$C. The photoperiod followed the natural cycle of the season. Water quality was regularly checked and total ammonia levels were always below 0.3 mg/L.
- Fish were hand-fed ad libitum twice a day (09:00 and 15:00 h) to apparent satiation, six days a week with the experimental diets for a period of 55 days.

Results

**DIETS**

| | L1 | L2 | L3 | L4 | L5 | L6 |
|---|---|---|---|---|---|---|
| Survival | 93.5 ±6.6 | 98.0 ± 0.0 | 97.4 ± 4.4 | 88.6 ± 15.1 | 95.6 ±7.7 | 97.8 ± 3.8 |
| Initial body weight (g) | 32.8 ± 0.5 | 32.9 ± 0.4 | 33 ± 0.6 | 32.6 ± 0.4 | 32.8 ± 0.4 | 32.7 ± 0.3 |
| Final Body weight (g) | 88 ± 1.9 | 92 ± 3.0 | 99 ± 2.9 | 94 ± 3.2 | 91 ± 3.6 | 96 ± 3.6 |
| WG | 55 ± 2.3 | 59 ± 4.8 | 66.0 ± 2.3 | 61.4 ± 8.9 | 58.3 ± 8.2 | 63.7 ± 5.0 |
| DGI % | 2.31 ± 0.08 | 2.41 ±0.13 | 2.62 ± 0.05 | 2.50 ± 0.26 | 2.40 ± 0.26 | 2.57 ± 0.15 |
| TFI | 72.8 ± 2.6 | 71.0 ± 3.41 | 79.3 ± 7.4 | 82.3 ± 7.5 | 75.9 ± 4.3 | 79.5 ± 10.1 |
| FCR | 1.25 ± 0.05 | 1.21 ±0.05 | 1.18 ± 0.05 | 1.22 ± 0.10 | 1.27 ± 0.11 | 1.22 ± 0.04 |
| PER | 1.73 ± 0.05 | 2.06 ± 0.09 | 2.34 ± 0.14 | 2.13 ± 0.46 | 2.16 ± 0.28 | 2.25 ± 0.11 |
| SGR | 1.83 ± 0.07 | 1.90 ± 0.08 | 2.03 ± 0.02 | 1.96 ± 0.17 | 1.89 ± 0.18 | 2.00 ±0.10 |
| TGC x 1000 | 1.16 ± 0.04 | 1.22 ± 0.07 | 1.33 ± 0.02 | 1.26 ± 0.13 | 1.21 ± 0.13 | 1.3 ± 0.08 |

# Results


Piecewise linear regression with breakpoint
Body weight gain (g/fish)
70
60
50
40
30
20
10
0.5
1
1.5
2
2.5
3
Lysine concentration (% of Diet)
Breakpnt = 2.11%

# *Feeding regimes for broodstock to optimize reproduction*

- Three different dietary experiments were conducted in the facilities located at Canary Islands (Spain).
  - ✓ Effect of increased **protein, histidine and taurine** dietary levels on egg quality,
  - ✓ Optimum **DHA and EPA** levels as essential fatty acids for reproductive success,
  - ✓ effects of an experimental diet with a potentially improved formula of lipids, on reproductive development of hatchery produced greater amberjack

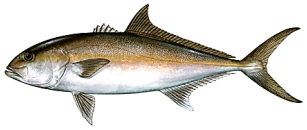
# Experimental design

Material & Methods

Mar. | Apr. | May | June | Jul. | Aug. | Sept. | Oct.

12 broodstock
3x 40 m3 circular tanks
(2♀ and 2 ♂ in each tank).

**Weigh & Size**



**Intramuscular injection**
**GnRHa: 20 µg kg-1 ♀ & ♂**

**Common diet**
**Egg quality**

**Experimental diets**
**Egg quality**

Twice a week: commercial diet at 1%
(13 mm, Vitalis CAL, Skretting)
Once a week: Atlantic mackerel at 2%

SKRETTING

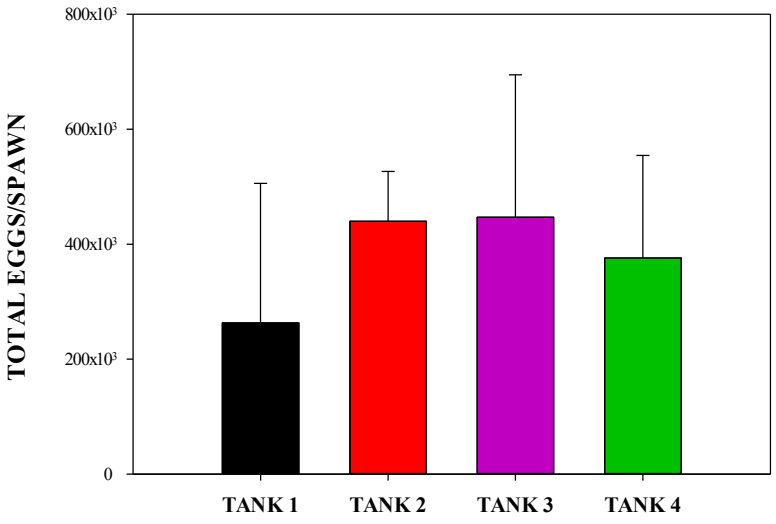
DIVERSIFY

# Results

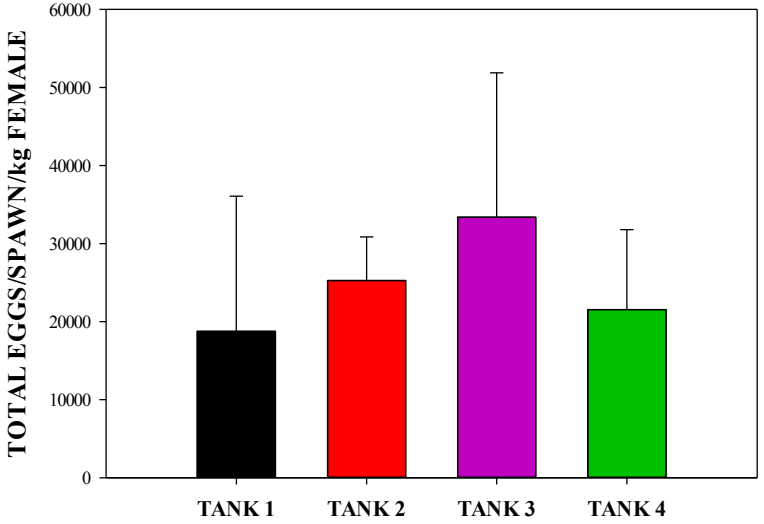
| Mar. | Apr. | May | June | Jul. | Aug. | Sept. | Oct. |
|---|---|---|---|---|---|---|---|

Common diet

## Number of eggs per spawn




## Number of eggs per spawn and kg female



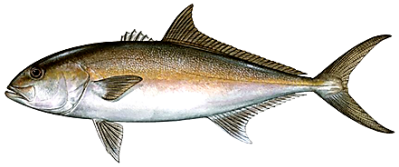

DIVERSIFY

# Experimental design

Material & Methods

Mar. Apr. May June Jul. Aug. Sept. Oct.

**Weigh & Size**

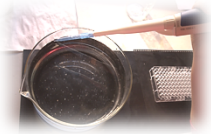


**Intramuscular injection**

**Common diet**
**Egg quality**

**Experimental diets**
**Egg quality**

- ***Indices of the spawning quality***
  - % Fertilization
  - % Viable eggs at 24 hours
  - % Hatching
  - % Larval survival:1, 3 & 5 dph



# 1. Effect of increased protein, histidine and taurine dietary levels on egg quality

Material & Methods

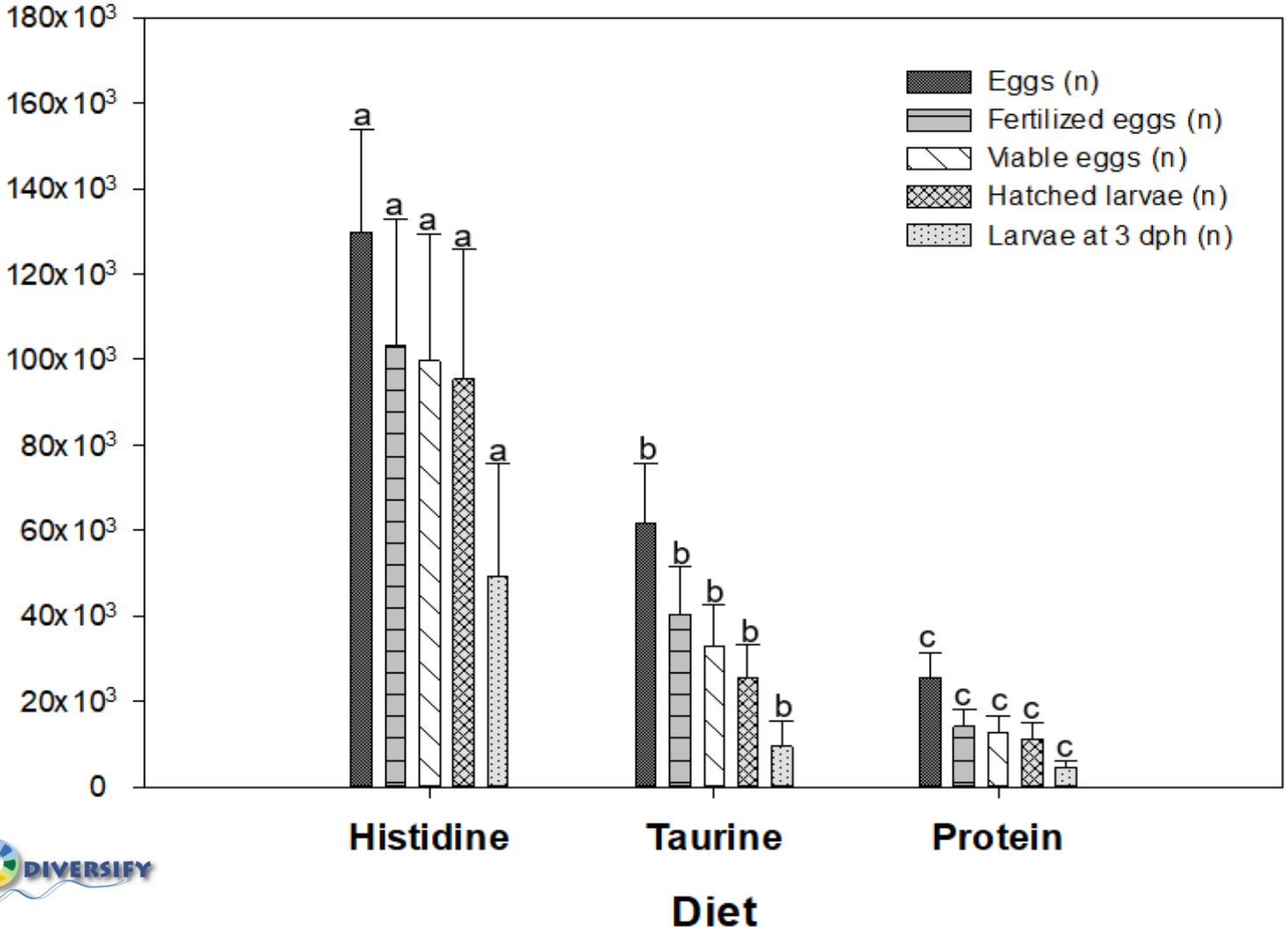
SKRETTING

| Diet | Histidine | Taurine | Protein |
|---|---|---|---|
| **Raw material (%)** | | | |
| Wheat | 17.94 | 18.29 | 11.81 |
| Wheat gluten | 13.00 | 13.00 | 17.00 |
| Fish meal | 45.14 | 44.64 | 48.36 |
| Squid meal | 10.00 | 10.00 | 10.00 |
| Fish oil | 12.47 | 12.50 | 12.18 |
| Taurine | 0.00 | **0.93** | 0.00 |
| Histidine HCl | **0.81** | 0.00 | 0.00 |
| Premix incl. vitamins & minerals | 0.64 | 0.64 | 0.64 |
| **Proximate composition (%)** | | | |
| Dry matter | 92.40 | 93.00 | 94.10 |
| Moisture | 7.60 | 7.00 | 5.90 |
| Crude protein | 51.30 | 51.50 | **56.10** |
| Crude fat | 17.80 | 18.50 | 18.30 |
| Ash | 8.40 | 8.40 | 8.40 |

DIVERSIFY

# 1. Effect of increased protein, histidine and taurine dietary levels on egg quality

Results




DIVERSIFY

## 2. Optimum DHA and EPA levels as essential fatty acids for reproductive success

SKRETTING

Material & Methods

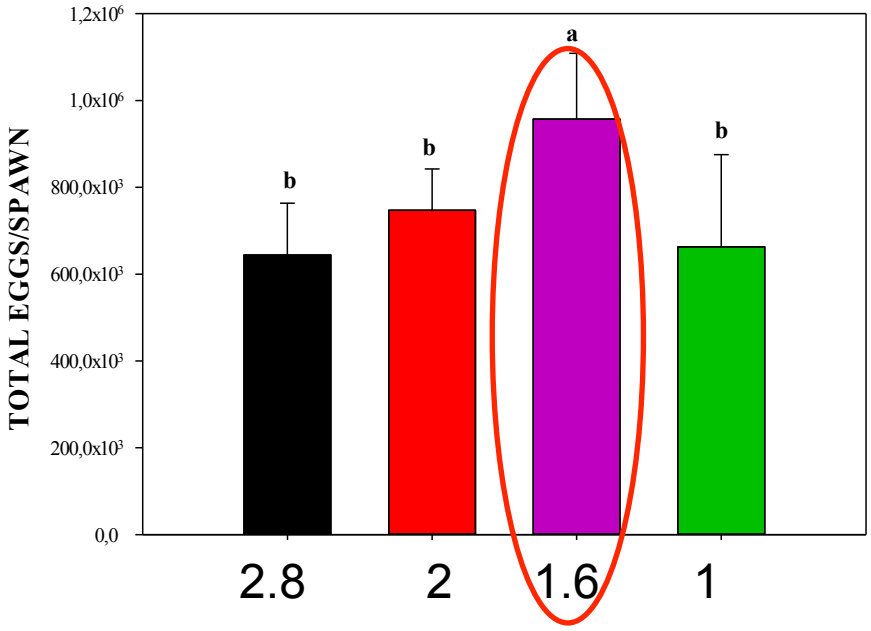
| Diet | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Lineseed oil | 0.00 | 1.52 | 3.01 | 4.50 |
| Wheat | 19.09 | 19.13 | 19.13 | 19.13 |
| Wheat gluten | 13.62 | 14.99 | 14.99 | 14.99 |
| Fish meal | 44.97 | 43.46 | 43.46 | 43.46 |
| Squid meal | 10.00 | 10.00 | 10.00 | 10.00 |
| Fish oil | 10.93 | 7.48 | 4.04 | 0.61 |
| Palm oil | 0.00 | 2.03 | 3.98 | 5.93 |
| Premix vit. Min. | 0.64 | 0.64 | 0.64 | 0.64 |
| **EPA+DHA (% total fatty acids)** | **2.80** | **2.17** | **1.57** | **0.96** |
| **Proximate composition (%)** | | | | |
| Crude protein | 58.50 | 58.91 | 58.91 | 59.06 |
| Crude fat | 24.25 | 24.89 | 24.35 | 25.61 |
| Moisture | 7.27 | 5.41 | 7.22 | 8.30 |
| Ash | 7.46 | 7.19 | 7.25 | 7.30 |

DIVERSIFY

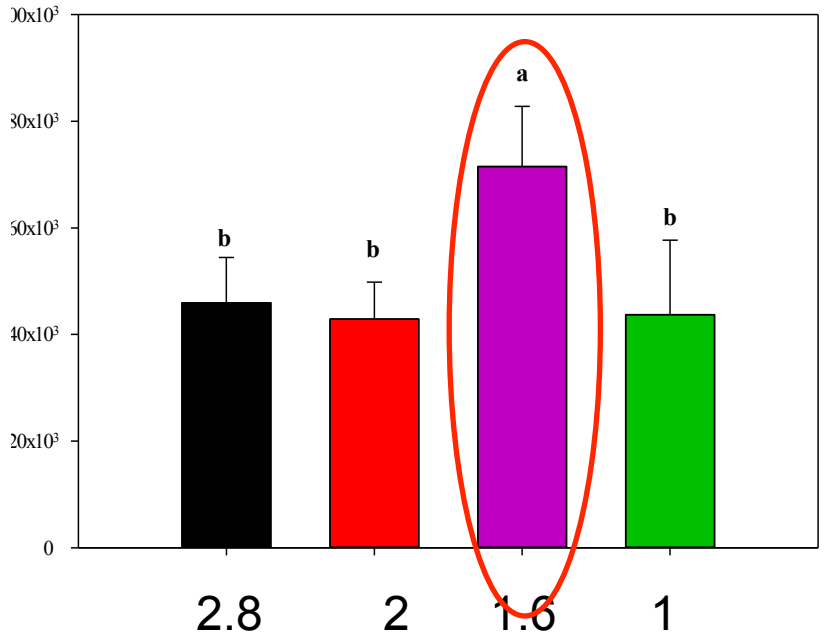
# 2. Optimum DHA and EPA levels as essential fatty acids for reproductive success

Results

## Number of eggs per spawn




## Number of eggs per spawn and kg female




· Bars, of the same shade, with the same letter were not significantly different ($P < 0.05$).

## 2. Optimum DHA and EPA levels as essential fatty acids for reproductive success

Results

### Quality of egg and larvae obtained after feeding period with the experimental diets

| Diet | % Fertilization | % Viable 24h | % Hatching |
|---|---|---|---|
| 2.8 | 52.42±10.64$^{c}$ | 90.28±3.28$^{b}$ | 76.99±8.94$^{b}$ |
| 2 | 69.02±7.38$^{b}$ | 85.07±1.73$^{c}$ | 79.68±3.74$^{b}$ |
| 1.6 | 91.76±3.12$^{a}$ | 95.99±2.81$^{a}$ | 94.22±3.62$^{a}$ |
| 1 | 86.32±1.67$^{a}$ | 93.88±2.48$^{a}$ | 92.51±2.27$^{a}$ |

| Diet | % 1 dph survival | % 3 dph survival | % 5 dph survival |
|---|---|---|---|
| 2.8 | 57.44±3.08$^{b}$ | 16.15±4.96$^{b}$ | 1.56±1.04$^{b}$ |
| 2 | 59.85±2.94$^{b}$ | 11.59±2.22$^{c}$ | 2.95±1.98$^{a}$ |
| 1.6 | 85.25±9.97$^{a}$ | 28.33±8.01$^{a}$ | 3.98±1.52$^{a}$ |
| 1 | 87.04±2.92$^{a}$ | 28.12±2.05$^{a}$ | 3.73±1.08$^{a}$ |

* Means ± SD. Different superscripts in the same column indicate significant differences ($P < 0.05$).

DIVERSIFY

Results

## Selected fatty acid composition (% total fatty acids) of eggs

| Fatty acid | 2.8 | 2 | 1.6 | 1 |
|---|---|---|---|---|
| 14:0 | 2.61 ± 0.33 | 2.30 ± 0.12 | 2.05 ± 0.04 | 1.81 ± 0.37 |
| 16:0 | 18.73 ± 0.86 | 18.18 ± 0.62 | 18.52 ± 0.23 | 18.50 ± 0.45 |
| 18:0 | 5.78 ± 0.48 | 6.16 ± 0.22 | 4.90 ± 0.15 | 5.48 ± 0.66 |
| 18:1n-9 | 20.88 ± 2.43 b | 20.85 ± 0.12 b | 24.05 ± 0.57 a | 24.43 ± 2.21 a |
| 18:1n-7 | 4.09 ± 0.29 | 3.26 ± 0.17 | 3.04 ± 0.07 | 3.41 ± 0.48 |
| 18:2n-6 | 6.82 ± 0.45 c | 9.02 ± 0.56 ab | 10.39 ± 0.01 a | 8.66 ± 0.21 b |
| 18:3n-6 | 0.21 ± 0.01 a | 0.17 ± 0.01 b | 0.16 ± 0.01 b | 0.19 ± 0.01 ab |
| 18:3n-3 | 1.02 ± 0.06 c | 4.67 ± 0.21 b | 6.54 ± 0.40 a | 4.07 ± 0.44 b |
| 20:4n-6 (ARA) | 1.33 ± 0.01 a | 1.16 ± 0.08 ab | 0.93 ± 0.01 c | 1.07 ± 0.07 bc |
| 20:5n-3 (EPA) | 6.35 ± 1.10 a | 6.22 ± 0.07 a | 4.86 ± 0.11 b | 4.98 ± 0.97 b |
| 22:6n-3 (DHA) | 16.88 ± 0.84 a | 14.47 ± 0.35 b | 12.43 ± 0.04 c | 15.05 ± 0.28 ab |
| Total n-3 | 28.40 ± 2.36 | 28.80 ± 0.14 | 26.98 ± 0.54 | 27.46 ± 1.80 |
| Total n-6 | 8.94 ± 0.40 b | 10.84 ± 0.61 a | 11.97 ± 0.01 a | 10.48 ± 0.36 ab |
| Total n-9 | 21.50 ± 2.40 | 21.38 ± 0.13 | 24.56 ± 0.58 | 24.99 ± 2.16 |
| Total n-3 HUFA | 26.24 ± 2.01 a | 22.97 ± 0.37 ab | 19.63 ± 0.07 b | 22.57 ± 1.06 ab |
| DHA/EPA | 2.69 ± 0.34 | 2.33 ± 0.08 | 2.56 ± 0.06 | 3.08 ± 0.54 |
| DHA/ARA | 12.79 ± 0.71 | 12.57 ± 1.12 | 13.38 ± 0.13 | 14.08 ± 1.20 |
| EPA/ARA | 4.81 ± 0.86 | 5.39 ± 0.28 | 5.23 ± 0.08 | 4.68 ± 1.22 |
| EPA+DHA | 23.22 ± 1.94 a | 20.69 ± 0.29 b | 17.29 ± 0.09 c | 20.02 ± 1.24 b |
| -6 | 3.19 ± 0.41 | 2.66 ± 0.16 | 2.26 ± 0.05 | 2.63 ± 0.26 |

DIVERSIFY

# Lessons learned

Results

- Raising histidine contents in broodstock diets from 1 to 1.5% to optimize the reproductive performance.
- Taurine levels in broodstock diets increase fecundity.
- Increasing protein contents over 51% lead to the lowest number of egg and larvae produced.
- Lowest fertilization and egg viability were obtained from broodstock fed 2.8% EPA+DHA
- Best spawn quality and production parameters were obtained from broodstock fed diet 1.57% EPA+DHA.

## 3. Effects of an experimental diet with a potentially improved formula of lipids, on reproductive development

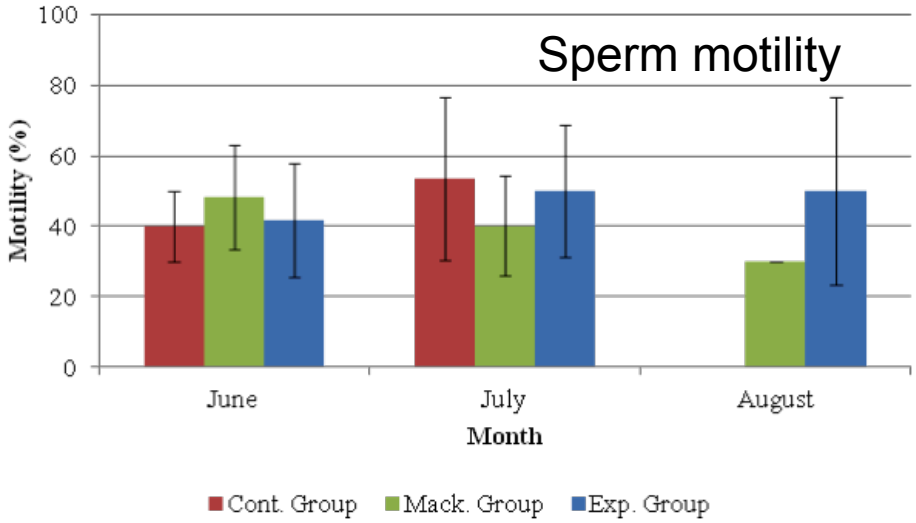
Material & Methods

| Groups | Mackerel | Control | Experimental |
|---|---|---|---|
| 18:1n-9 | 5.5±1.6 | 26.0±0.5 | 11.5±0.2 |
| 18:2n-6 | 1.3±0.3 | 10.4±0.2 | 7.8±0.2 |
| 20:4n-6 (ARA) | 2.8±0.5 | 0.5±0.0 | 1.4±0.0 |
| 20:5n-3 (EPA) | 5.5±1.2 | 6.8±0.2 | 11.8±0.0 |
| 22:6n-3 (DHA) | 36.0±6.3 | 7.1±0.1 | 14.6±0.3 |
| DHA/EPA | 6.5±0.6 | 1.0±0.0 | 1.2±0.0 |
| ARA/EPA | 0.5±0.1 | 0.1±0.0 | 0.1±0.0 |
| Total Lipids | 9.6±2.5 | 19.3±1.8 | 15±0.3 |
| Polar Lipids | | | +++ |
| Marine-origin ingredients | | | +++ |

commercial pellet for turbot (52/20)

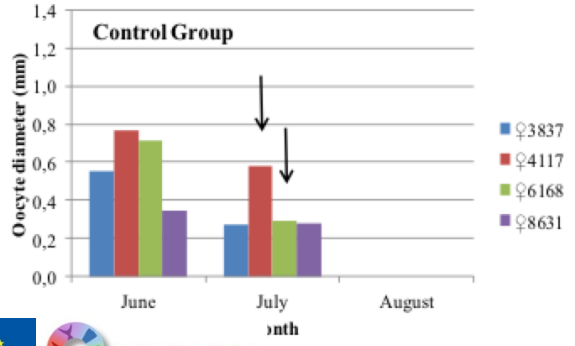
## 3. Effects of an experimental diet with a potentially improved formula of lipids, on reproductive development

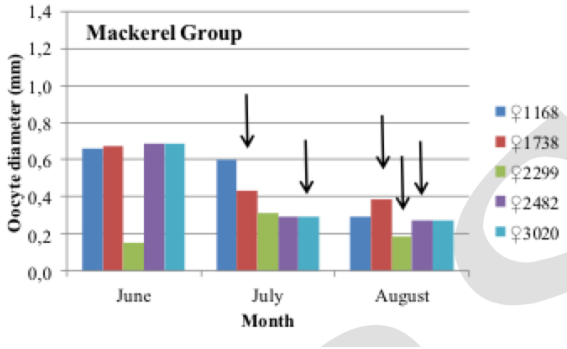
Results

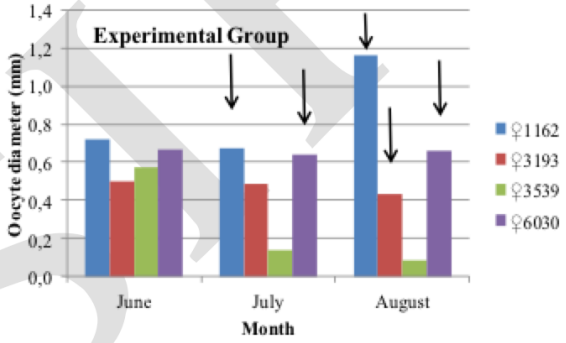



Currently in progress.
Un-suscessful spawning

Oocyte diameter



# Thanks for your attention

hcmr
ΕΛΚΕΘΕ
Hellenic Centre for Marine Research
Institute of Marine Biology, Biotechnology & Aquaculture

SKRETTING

ULL
Universidad de La Laguna

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Fundación Parque Científico Tecnológico
Universidad de Las Palmas de Gran Canaria (ULPGC)

DIVERSIFY

INSTITUTO
ESPAÑOL DE
OCEANOGRAFÍA