

Successful and unsolved cases in European fish larviculture: major gaps

Marisol Izquierdo

COLOQUIO INTERNACIONAL DE
LARVICULTURA
INLARVI
Puerto Montt 18-21 enero 2017



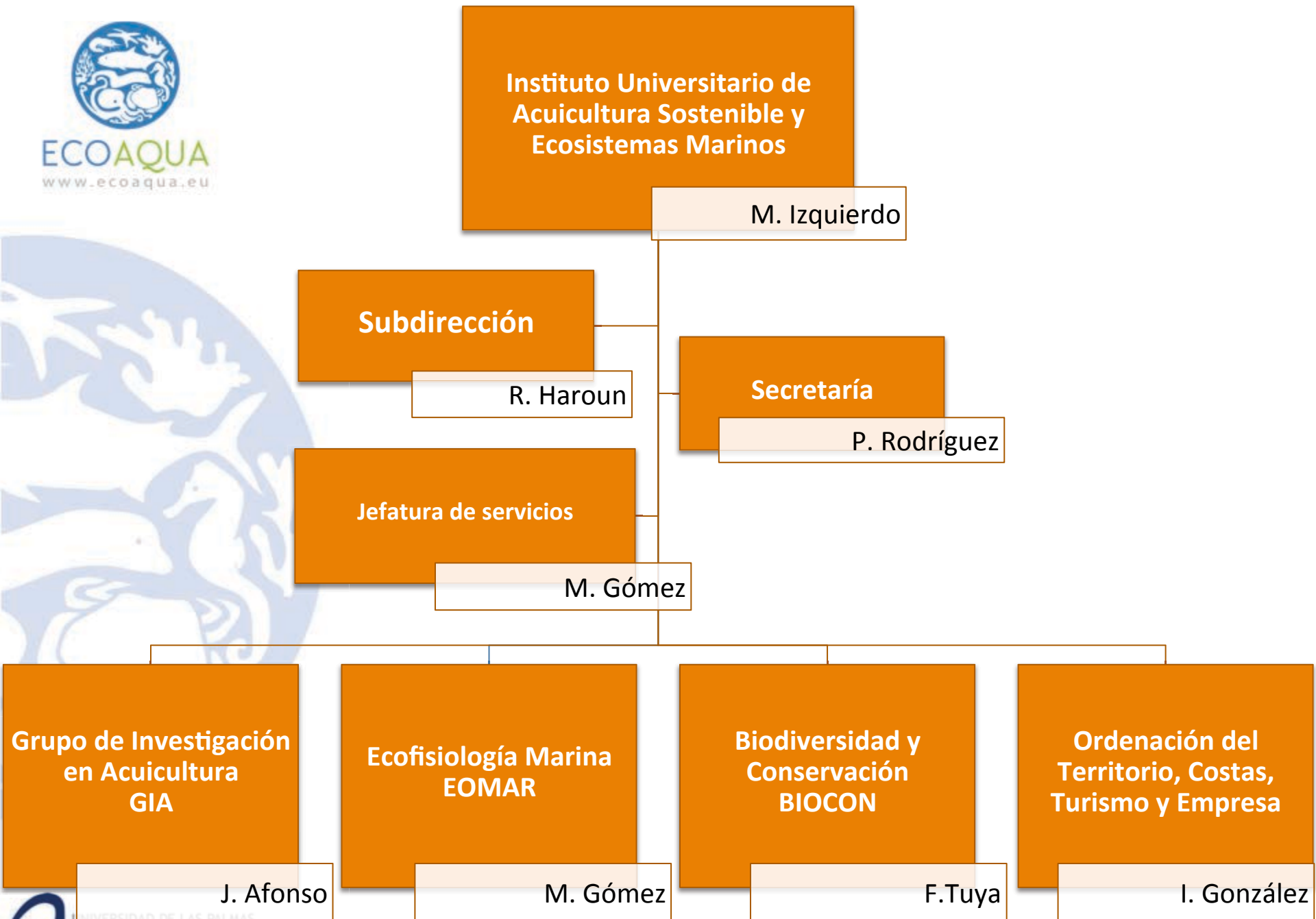
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Research and Technology to enhance excellence in Aquaculture development under an Ecosystem approach

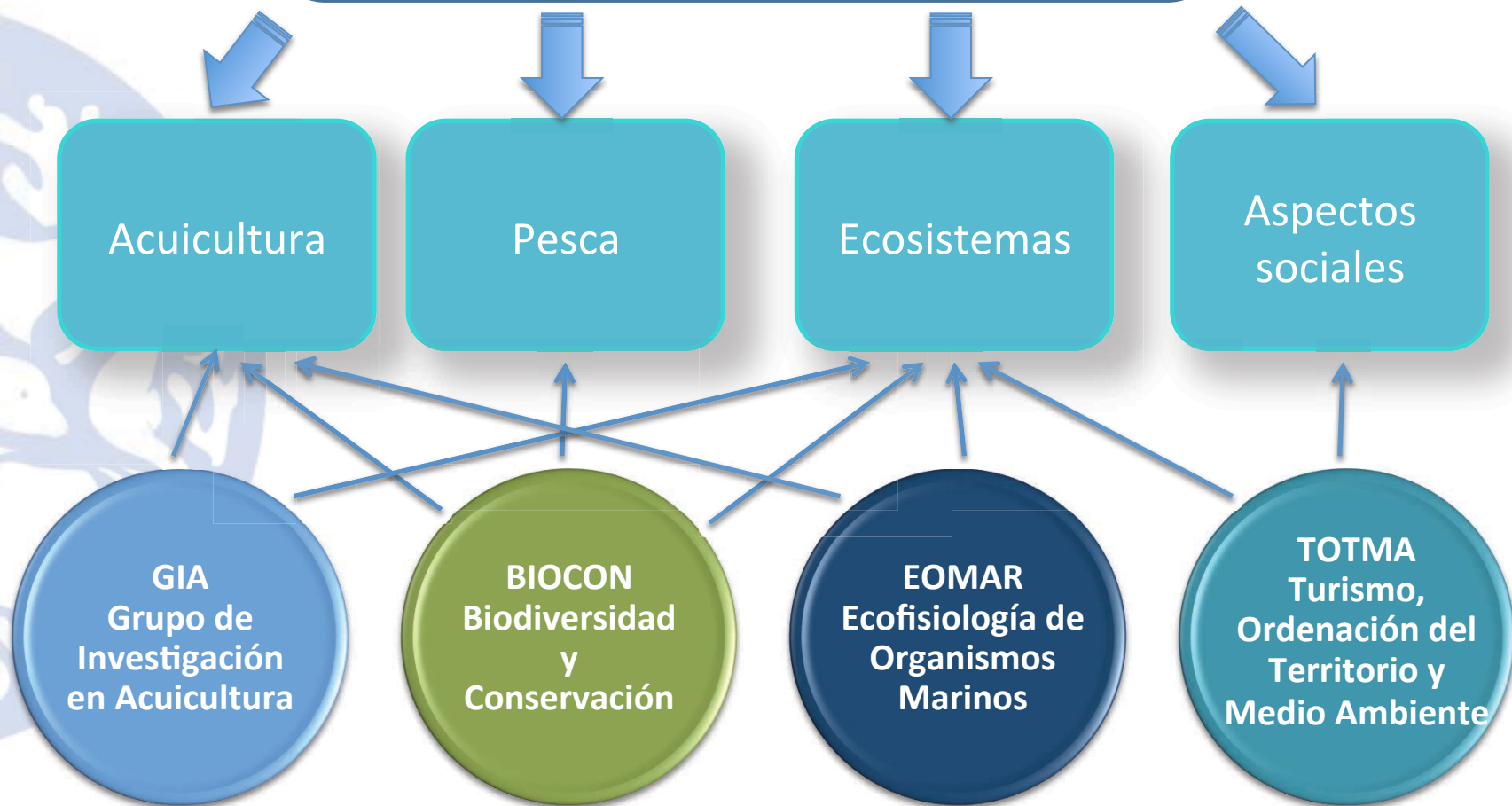
Contents

- **IU-ECOQUA®**
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- **ARRAINA**
- **DIVERSIFY**





Blue growth:
Gestión integral, sostenible, y socio-
económicamente coherente de
océanos y humedales (FAO, 2014)



GRUPOS DE
INVESTIGACIÓN
ULPGC



Recursos materiales



Docencia

3 Másteres



FP en acuicultura

Docencia
en 5
facultades

Cursos para
CIHEAM

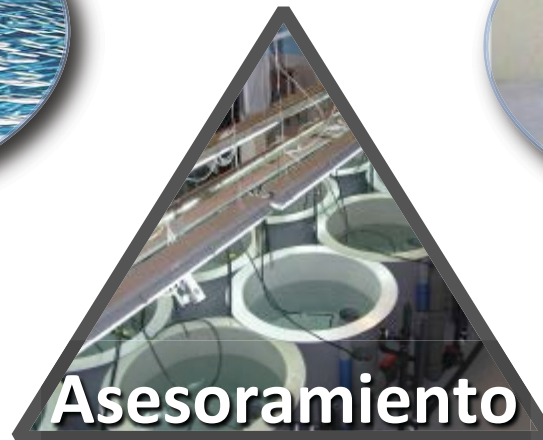


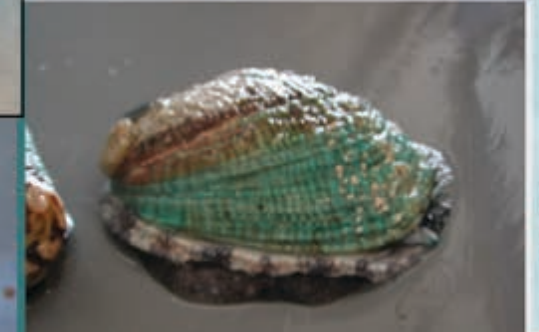
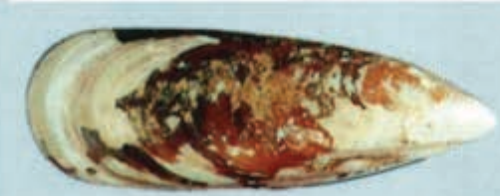
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Colaboración con la industria



Se ha colaborado con mas
de 80 empresas de
distintos sectores







ECOAQUA

Distribución de
género: 1,14

2% del profesorado de la ULPGC en el 2014

Alcanzó el 11% de la productividad científica de la ULPGC

Aportó el 23% de la financiación competitiva externa conseguida por la ULPGC

6 investigadores entre los 15 mejores índices h de la ULPGC

Three EU-funded projects related to larviculture during the last 6 years



Seabream, seabass, carp, salmon, trout

S. aurata, D. labrax, C. carpio, S. salar, O. mykiss

Meagre, seriola, pikeperch, wreckfish

A. regius, S. dumerili, S. rivoliana, L. lucioperca, P. americanus



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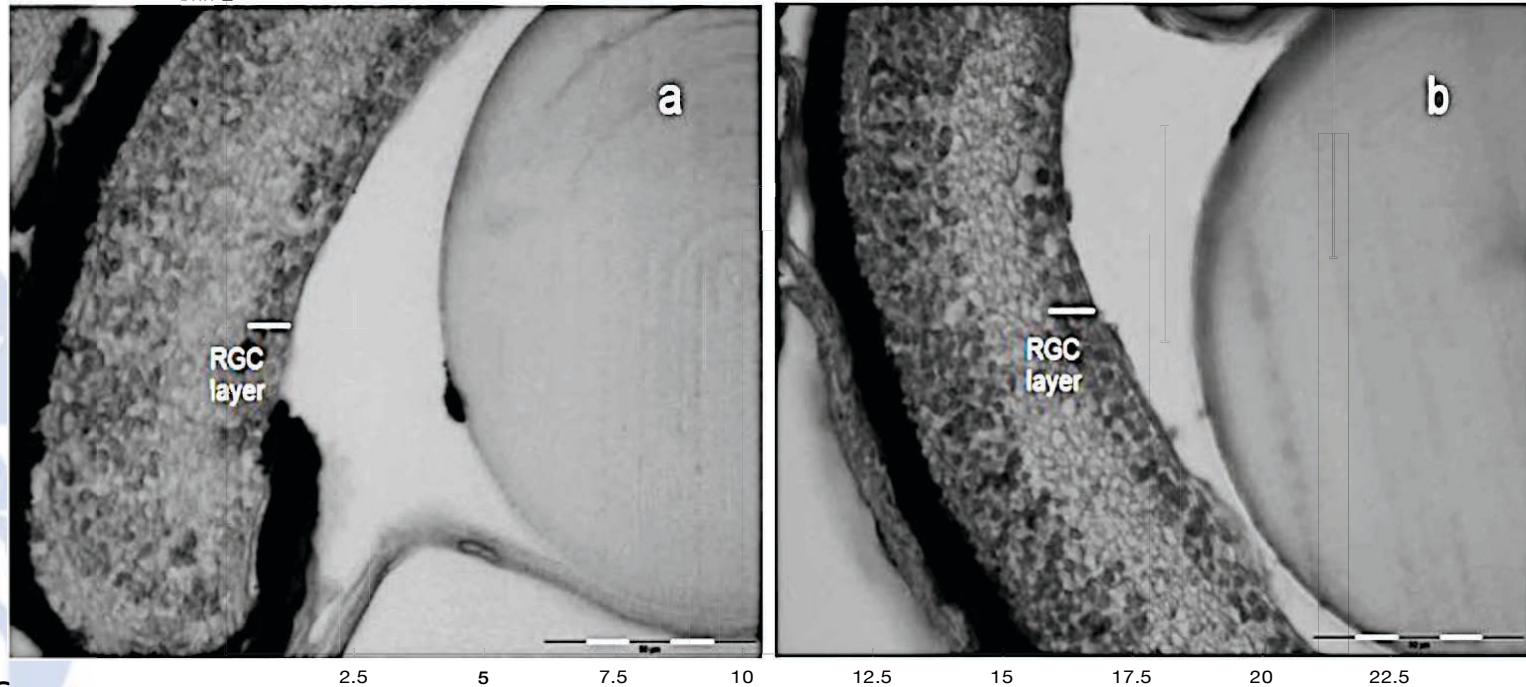


Contents

Larvanet: Critical success factors for fish larval production in European Aquaculture

- **Basic knowledge on fish larvae biology**
 - Neural development
 - Bone development
 - Digestive tract development
 - Nutritional requirements
- **Quality indicators for larval production**
 - Biological
 - Morphological
 - Molecular
 - Health
- **Improved production protocols and hatchery management**

Desarrollo neural

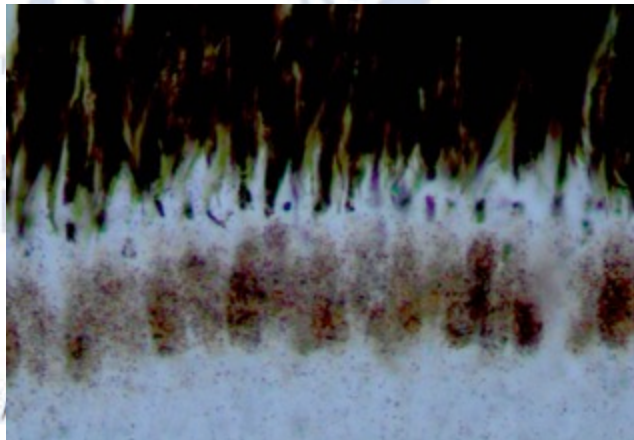


Castro et al., 2016

n-3 HUFA* Fatty Acids Content of the Diet

El incremento de ω -3 LC-PUFA en la dieta incrementa la densidad de células ganglionares en la retina (RGC)

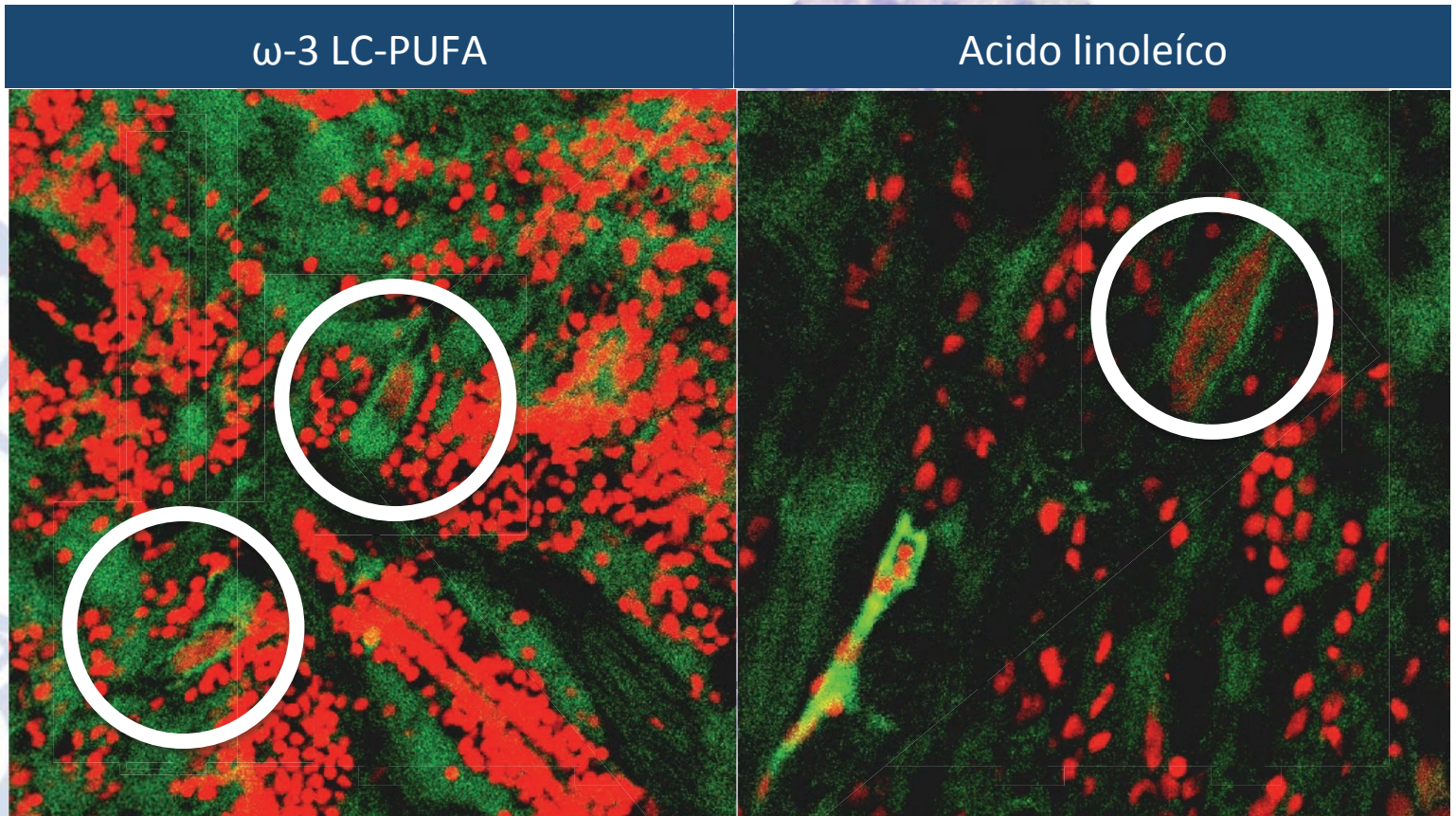
El tipo de Zn en la dieta afecta a su incorporación en la retina





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Nutrición y desarrollo neural



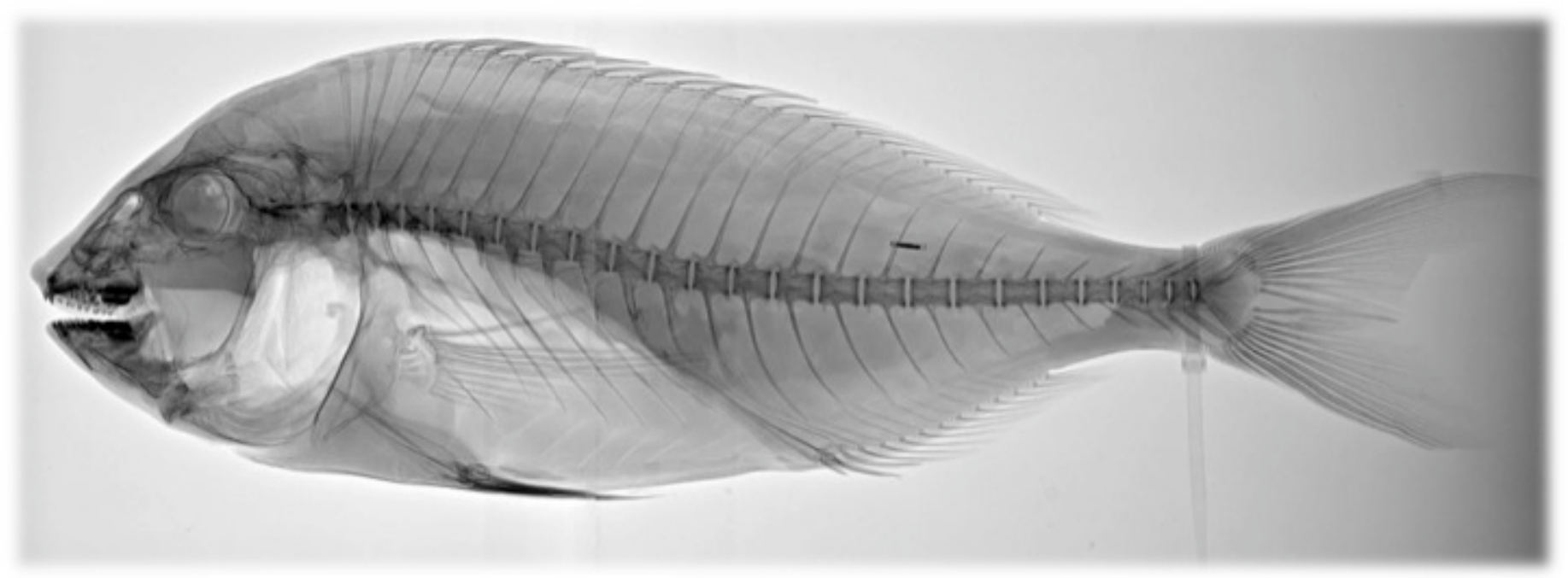
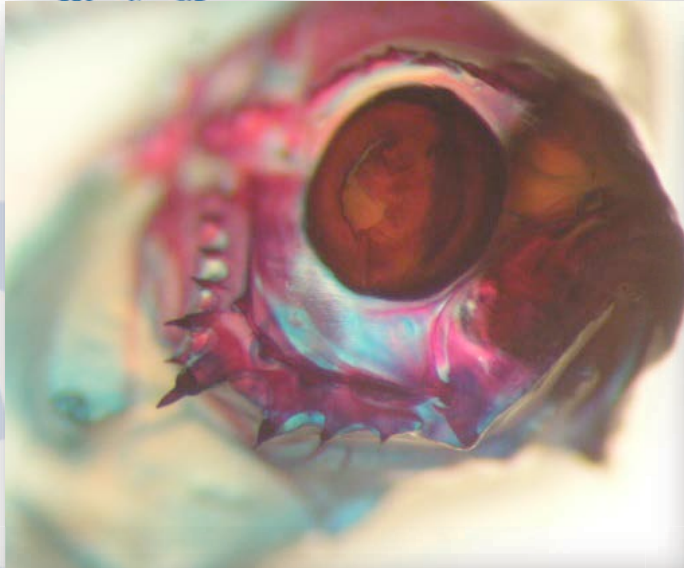
Benítez-Santana, et al., 2012
Br. J. Nutr.,

Benítez-Santana, et al., 2014
Physiology and Behavior

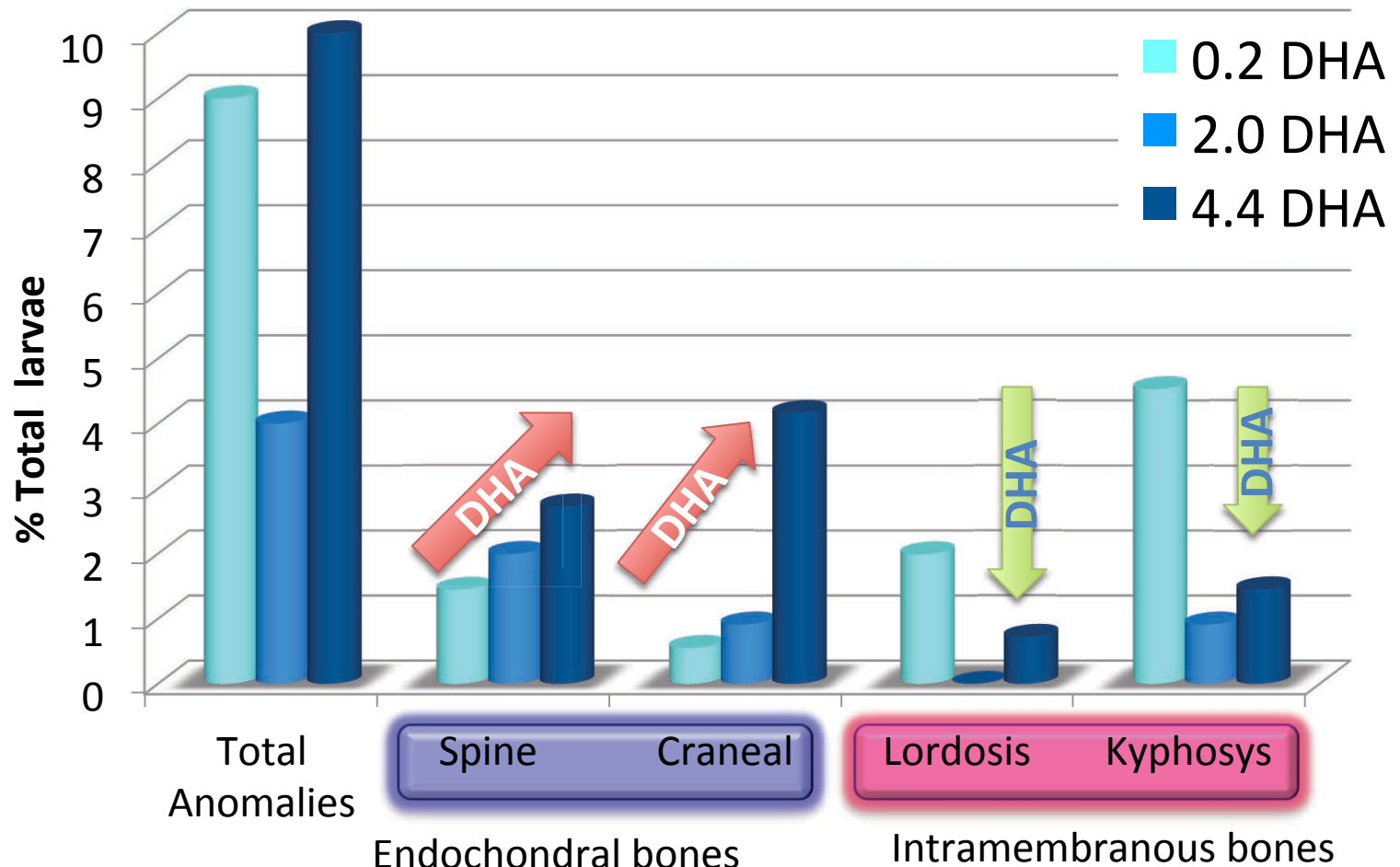
- Los ω -3 LC-PUFA dietéticos afectan el comportamiento, desarrollo neural y actividad neuronal de dorada, bocinegro y pez cebra



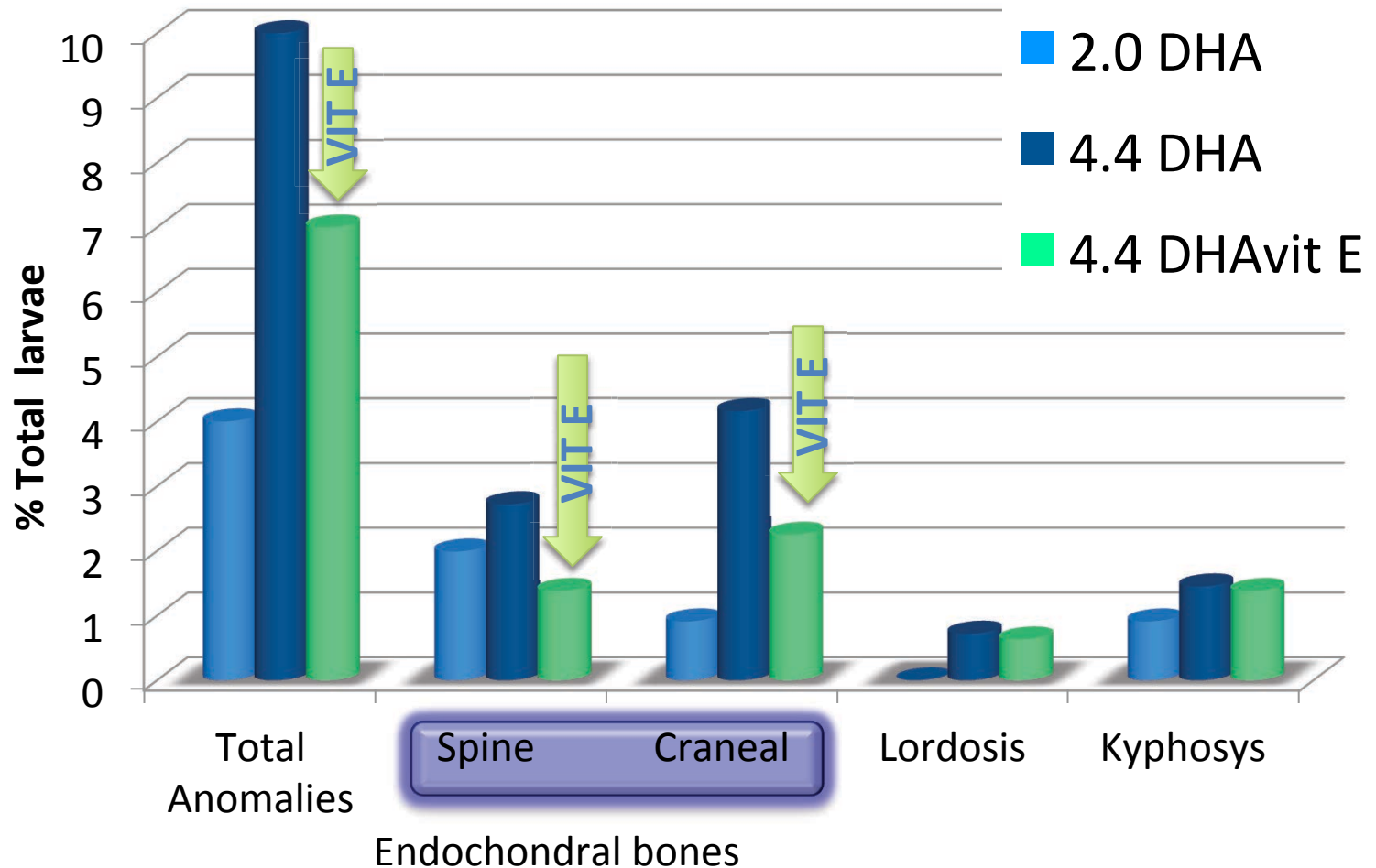
Desarrollo óseo



In 45 dph gilthead seabream fed rotifers with increase DHA contents (from 0.2 to 4.6% d.w.) from 3 to 21 dph: Craneal and spines deformities were increased and lordosis and kyphosis reduced



But α -tocopherol supplementation in rotifers high in DHA reduced craneal and spines deformities



Izquierdo et al., 2012 Br.J.Nutr.

DHA incrementa la mineralización en larvas de peces

2% DHA



0.2% DHA

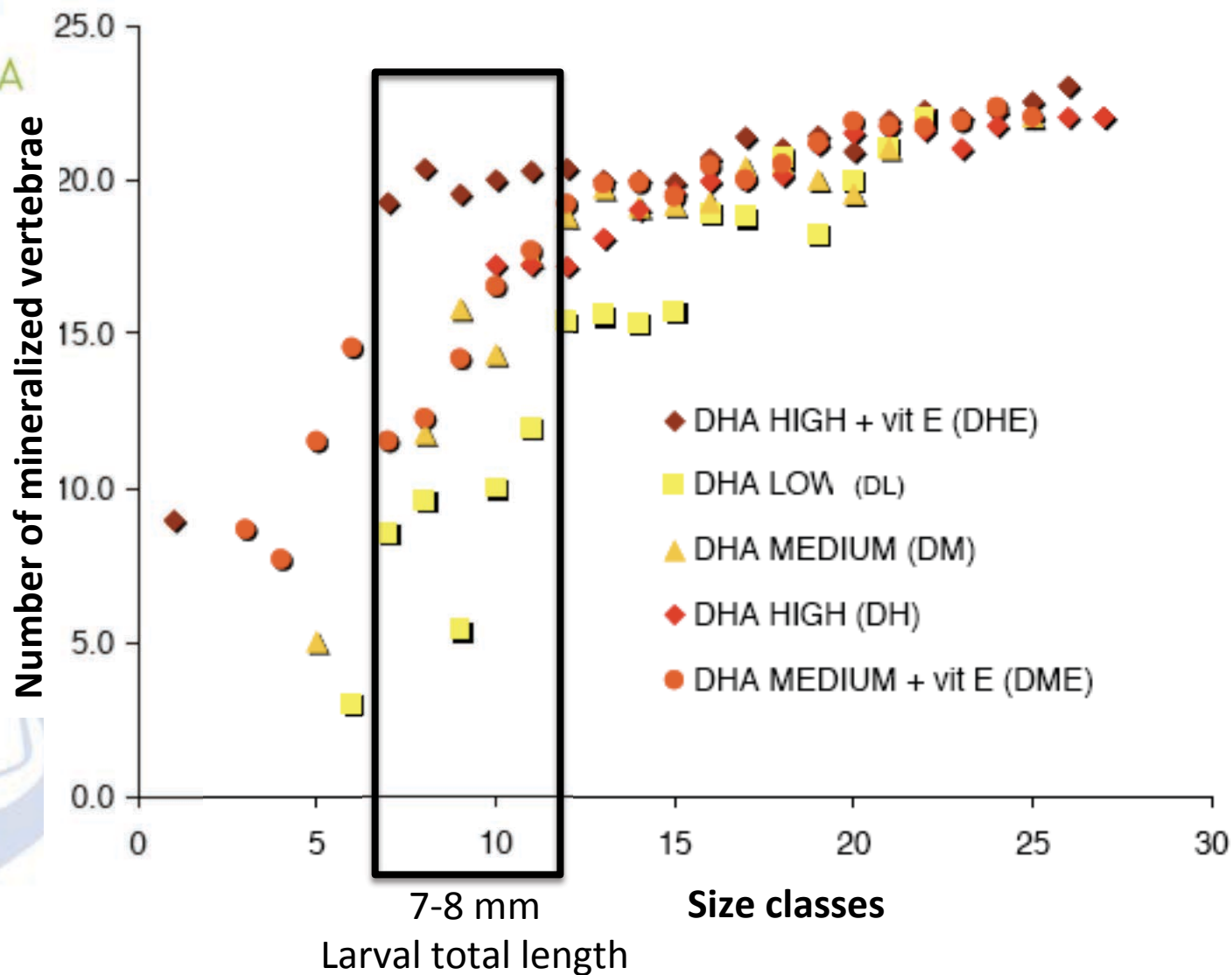


Izquierdo et al.,
2012 Br.J.Nutr.



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Distribution of the number of mineralized vertebrae for each size class of *S. aurata* fed different DHA and vit E levels



Izquierdo et al., 2012 Br.J.Nutr.

Algunos resultados resumidos en Aquaculture Reviews

Fish larval nutrition and feed formulation - knowledge gaps and bottlenecks for advances in larval rearing

Hamre, Kristin^{1*}, Manuel Yúfera², Ivar Rønnestad³, Clara Boglione⁴, Luis E.C. Conceição⁵,
Marisol Izquierdo⁶

¹National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway

²Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Puerto Real, Cádiz, Spain

³Department of Biology, University of Bergen, Norway

⁴Department of Biology, University of Rome 'Tor Vergata', Italy

⁵CIMAR LA/CCMAR, Universidade do Algarve, Faro, Portugal

⁶Grupo de Investigación en Acuicultura, ULPGC & ICCM, Telde, Canary Islands, Spain

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- Basic knowledge on fish larvae biology
 - Nutritional requirements
 - Neural development
 - Bone development
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 - Morphological
 - Molecular
 - Health
- Improved production protocols and hatchery management
 - Nutritional programming
- IU-ECO AQUA®

EARLY MARKERS FOR FISH JUVENILES QUALITY

- Early quality parameters, from industry and research, used as predictors of fish larval and

Genómica

Transcriptómica

Metabolómica

Science-based certification to aquaculture

Nutrigenómica

Epigenética

Microbiota

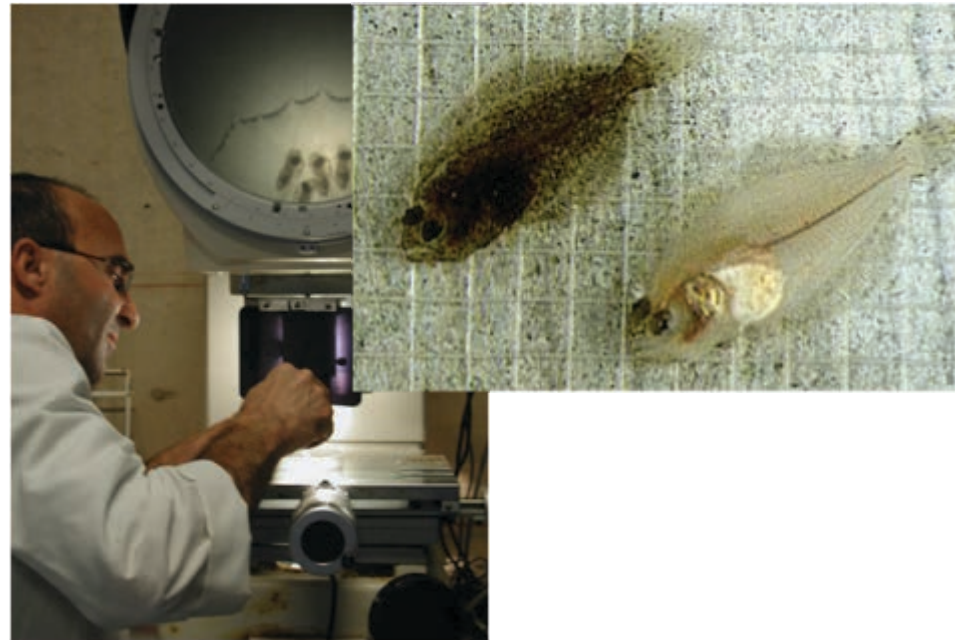
Metanálisis



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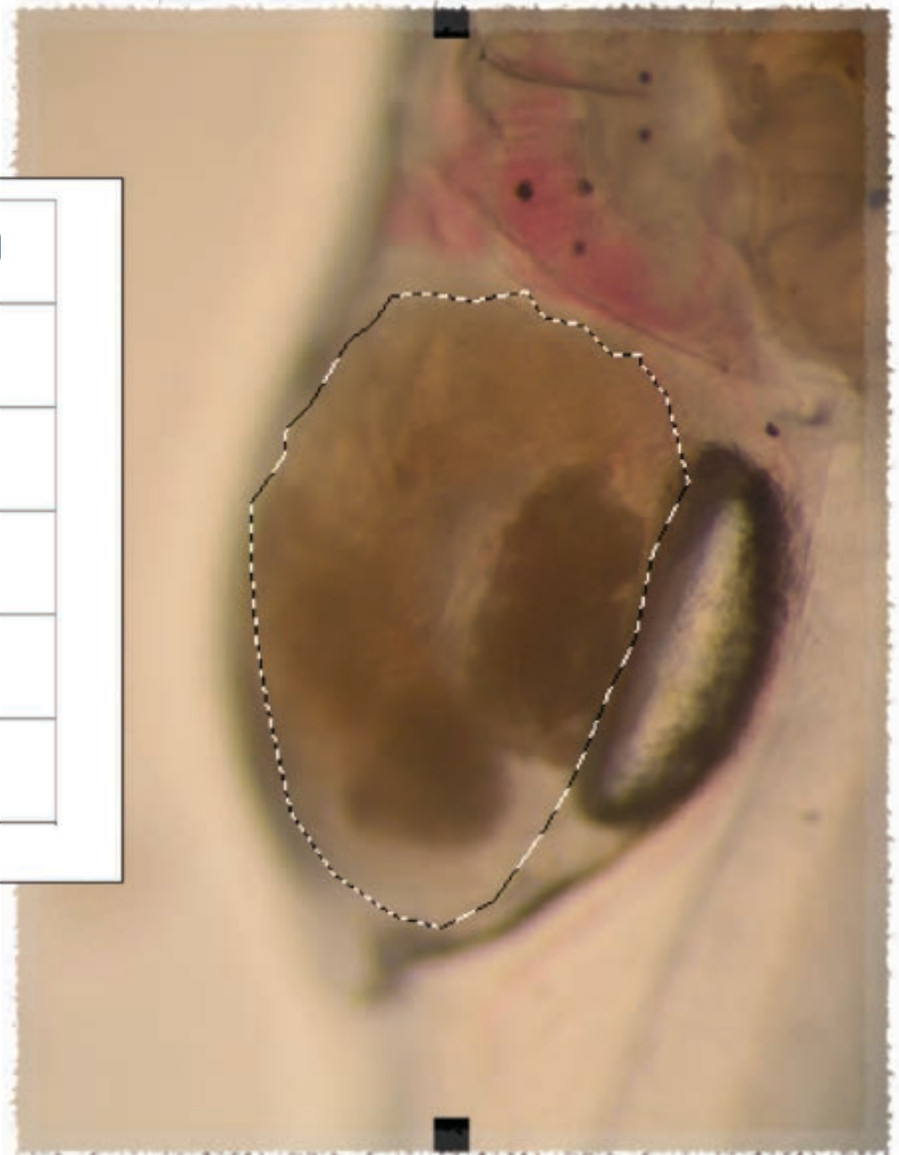
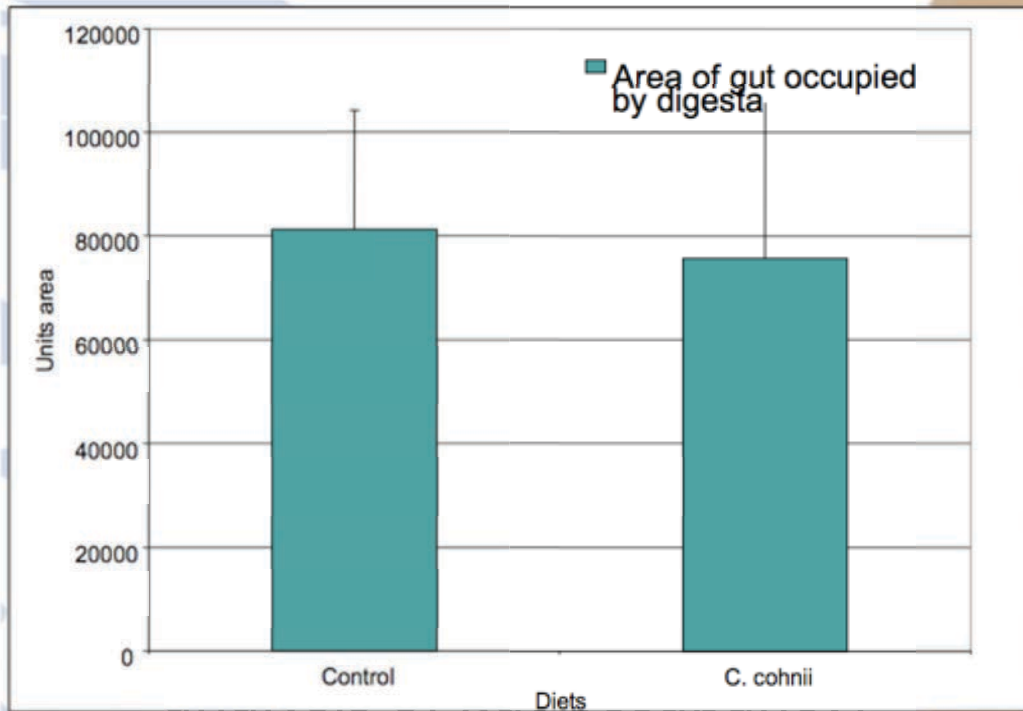
BIOLOGICAL INDICATORS

- Methodology: age, representative numbers, method:
 - Survival
 - Standard length
 - Whole body (dry) weight
 - Feed acceptance
 - Pigmentation
 - Development
 - Biochemical composition



BIOLOGICAL INDICATORS

Methodology: age,



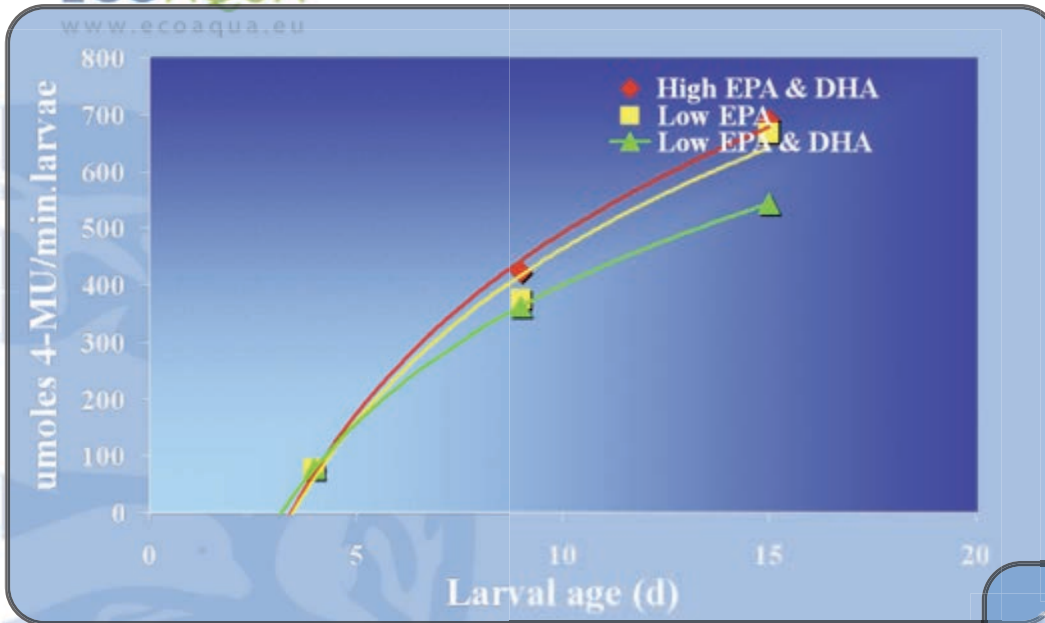
Liu et al., 2002; Izquierdo et al.,
in press



PHYSIOLOGICAL INDICATORS

ECO AQUA

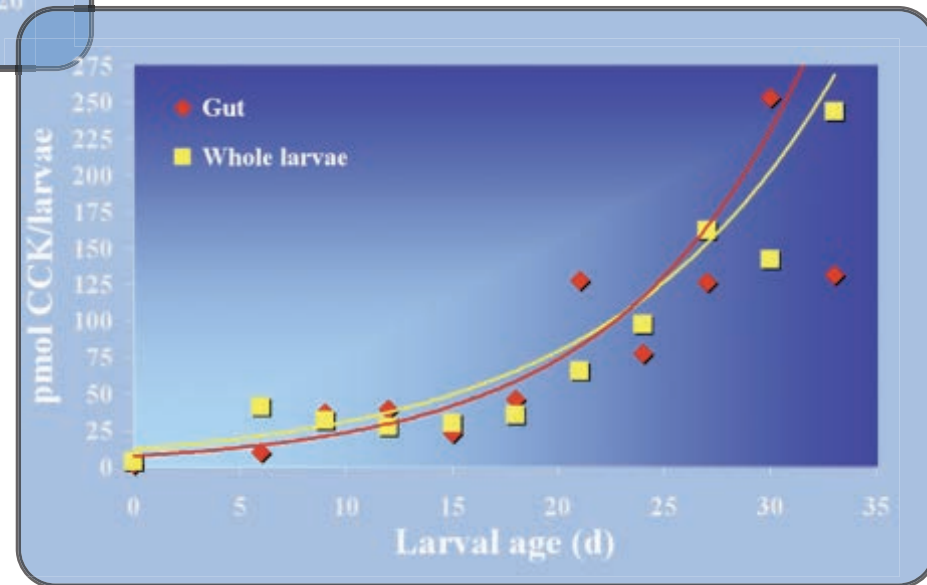
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Izquierdo et al., 2008

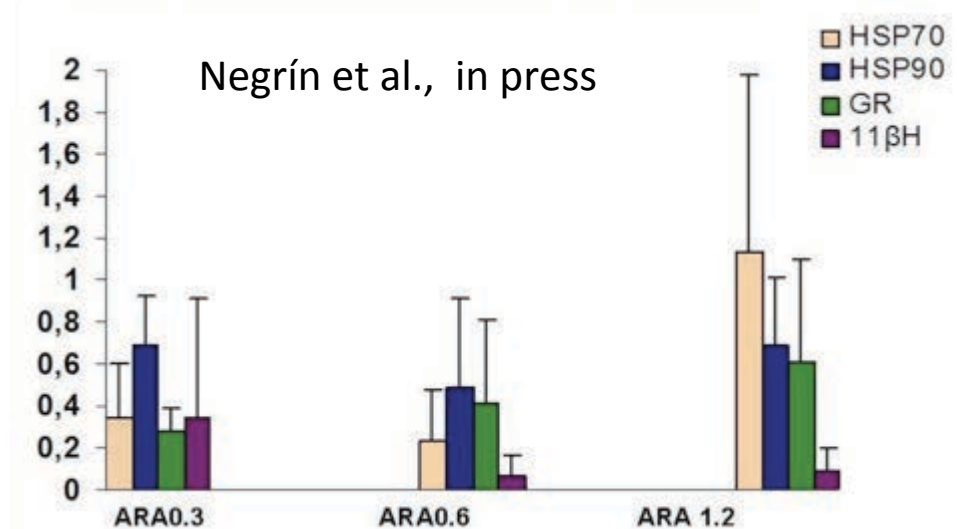
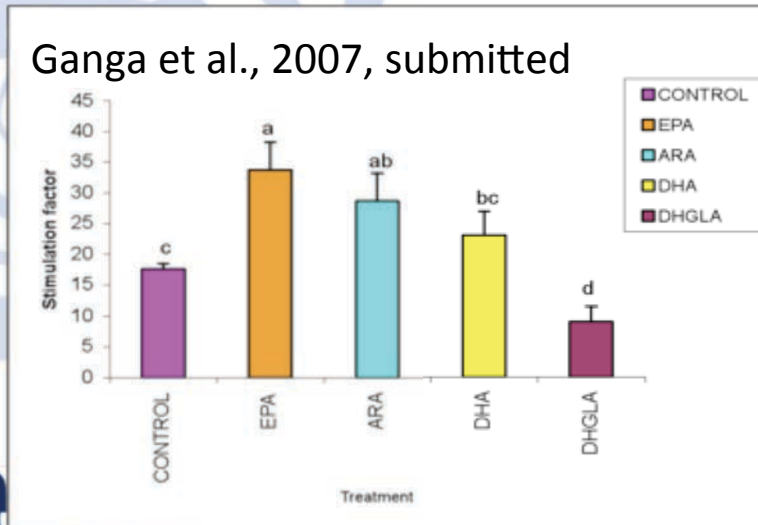
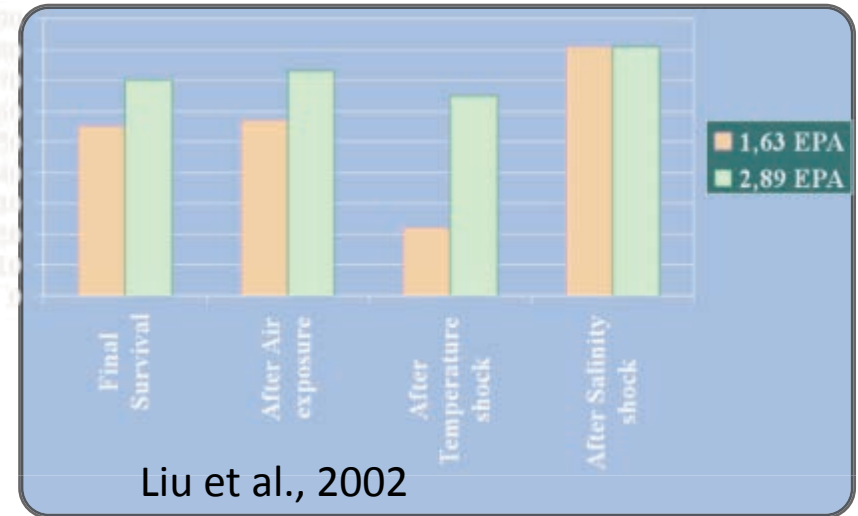


- Digestive enzymes
- Digestive hormones
- Reacilation enzymes
- Desaturase gene expression



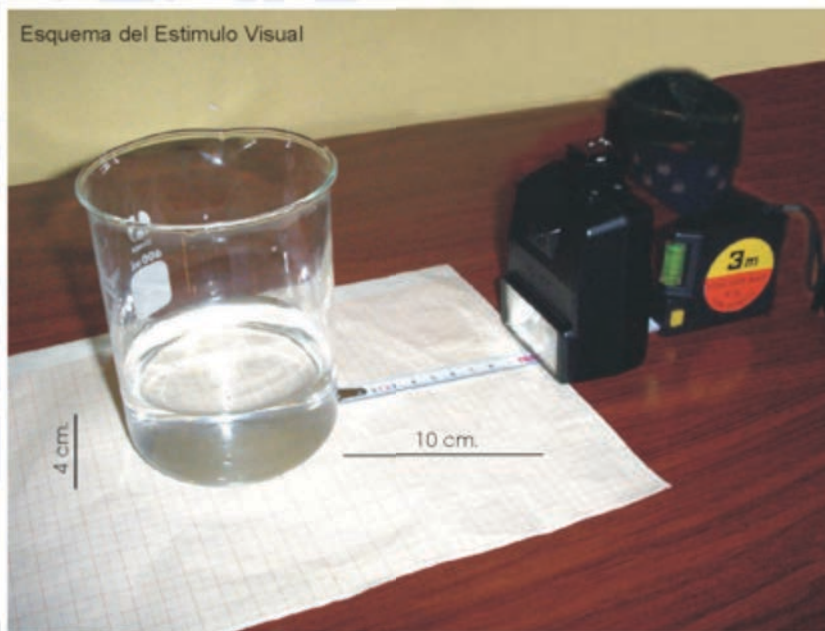
STRESS RESPONSE

- Air, temperature, salinity response
- HSP as molecular markers of stress response
- In vitro cell culture studies

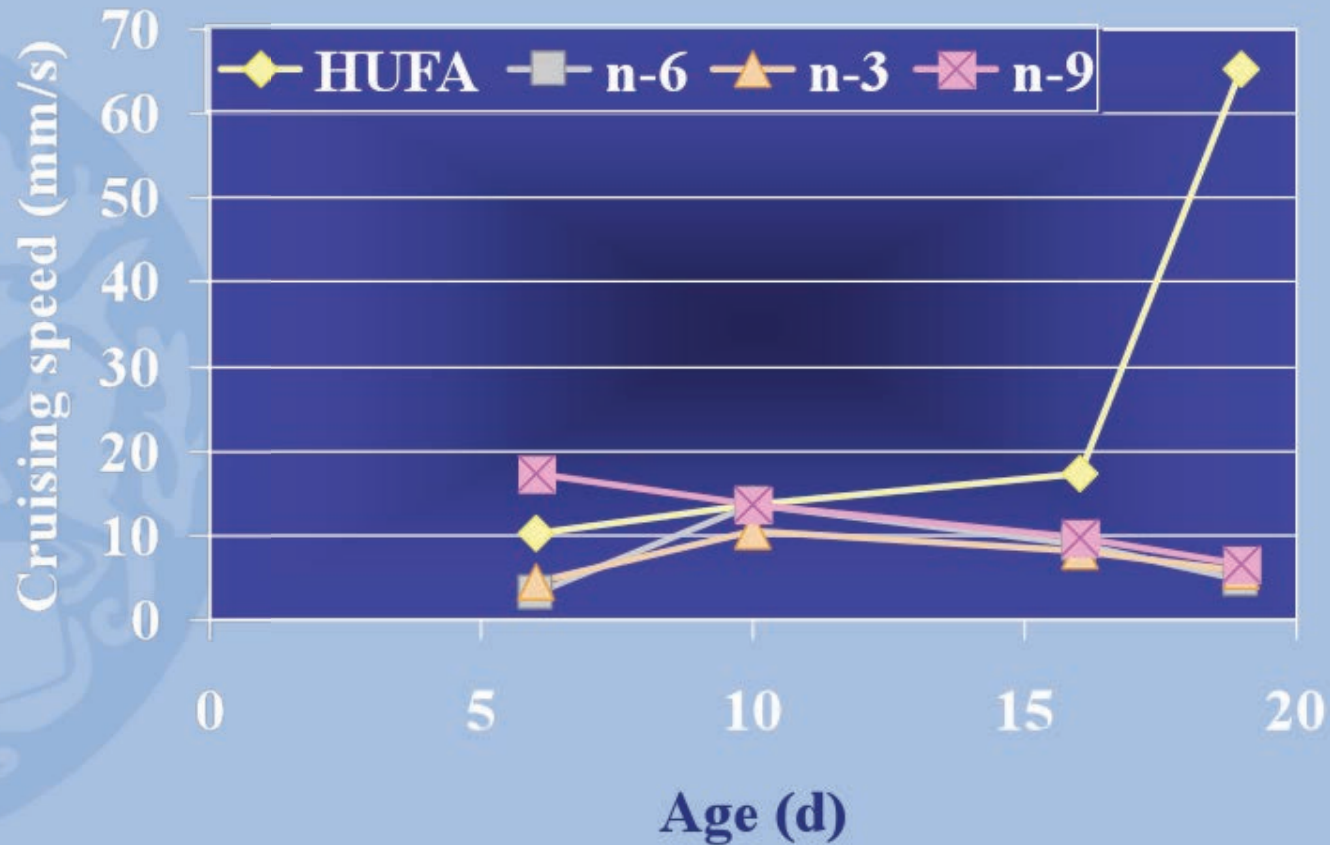


BEHAVIOUR INDICATORS

- Video-recorded swimming speed
- Response to visual or sound stimuli
- Video image analysis of quantifiable parameters

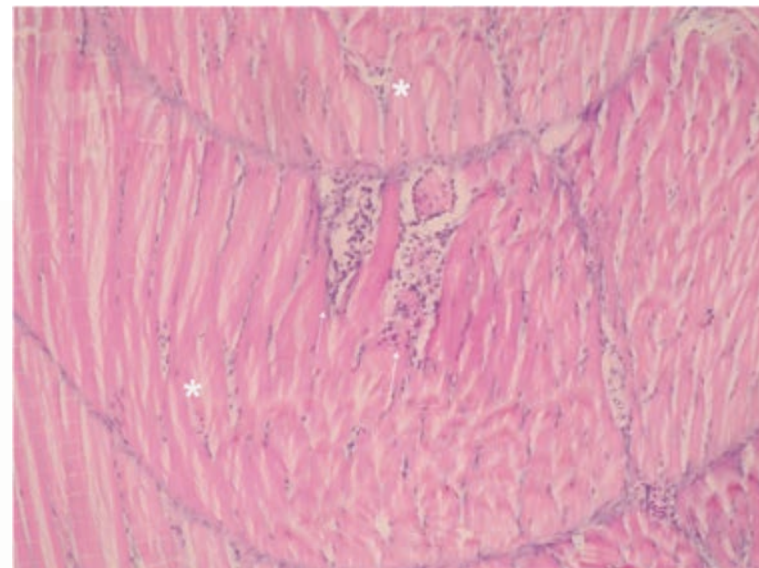
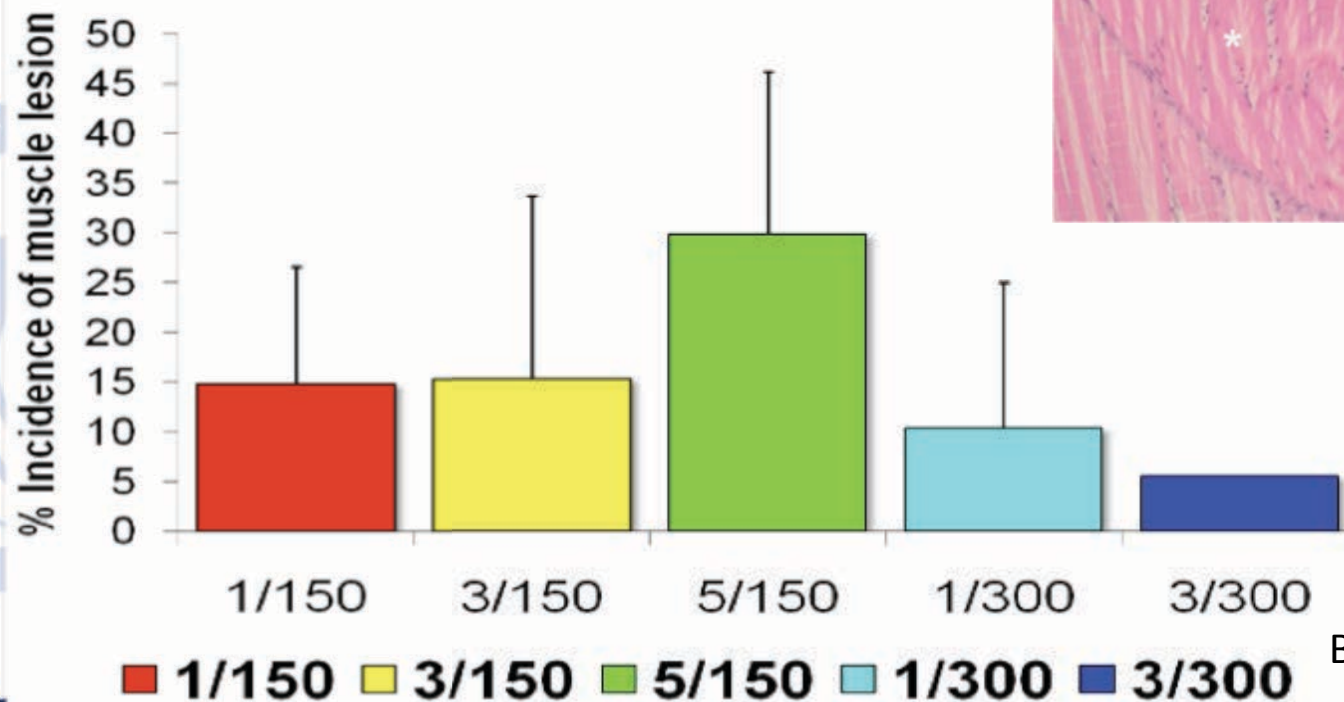


BEHAVIOUR INDICATORS



Benítez et al., 2007, in press

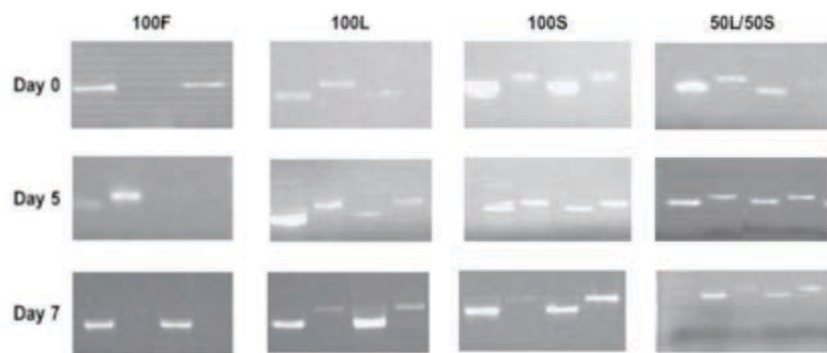
MUSCULAR DEVELOPMENT



Betancour et al., in press

MOLECULAR MARKERS OF IMMUNE RESPONSE

Markers of immune response



Detection of Mx transcripts
by Rt-PCR

Fig 4

Mx kinetic of response to
a sublethal dose of p.d.s.p

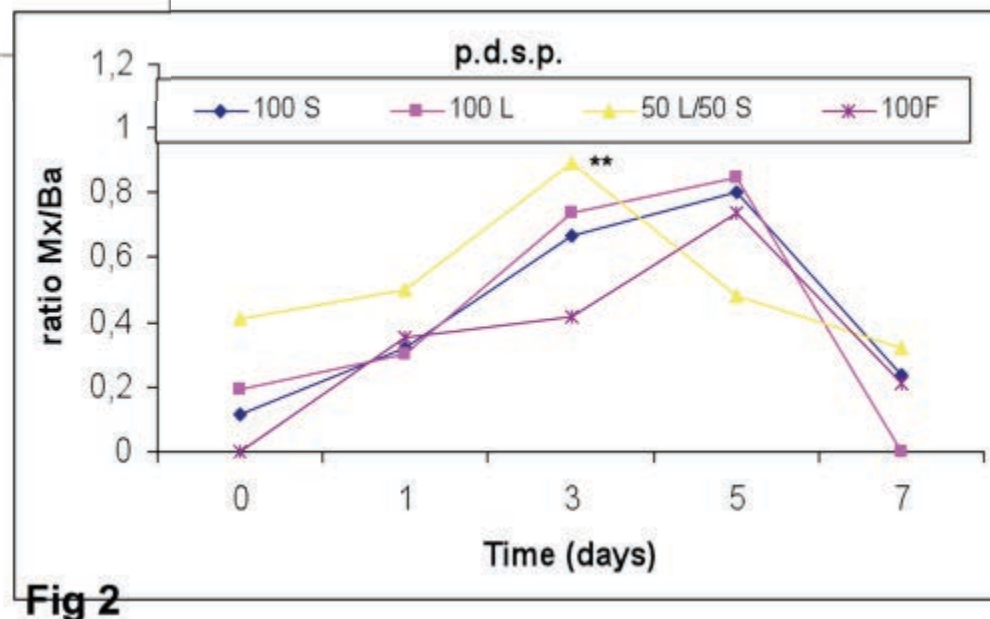


Fig 2

Montero et al., in press

DETECTION OF QTLS FOR SKELETAL DEFORMITIES IN GILT HEAD SEABREAM (*Sparus aurata* L).

- The aim of the present study was to analyze the existence of QTLs (Quantitative Trait Loci) affecting the most important skeletal deformities in this specie, which could be used as a tool to minimize the prevalence of these deformities in this species under genetic breeding programs
- A set of 13 multiplex PCR reactions (Negrín-Báez et al., 2015a) containing 106 microsatellite markers that covers 100% of the linkage groups was used for analyze fish from 3 different studies
- The QTLs detected for lack of operculum deformity in study 2, explain about 25% of phenotypic variation for this deformity
- QTLs detected for vertebral fusion, lordosis and jaw deformity showed low values of phenotypic variation.

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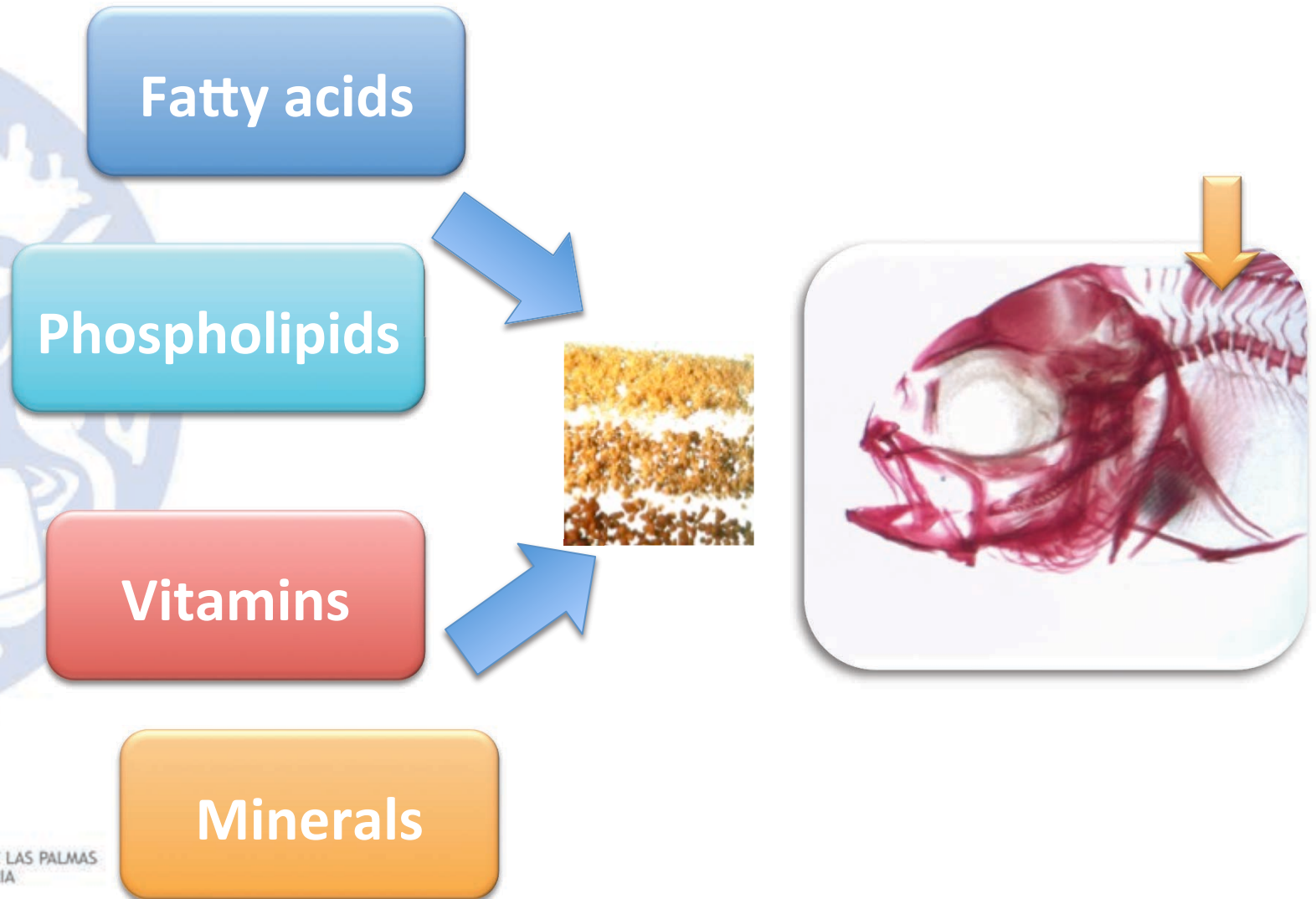


Contents

ARRAINA

- Transfer of nutrients to the embryo
- Transfer of nutrients to the larvae
- Development of microdiets
- Nutritional requirements of fish larvae
 - Phospholipids
 - Vitamins
 - Minerals
 - Interactions Vit E/VitC, PL/Vit E, Tau/Vit E/Vit C
- Nutritional Programing

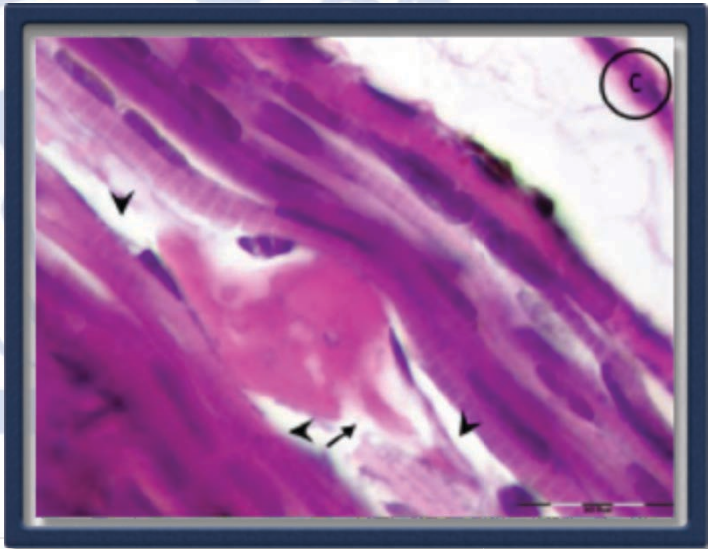
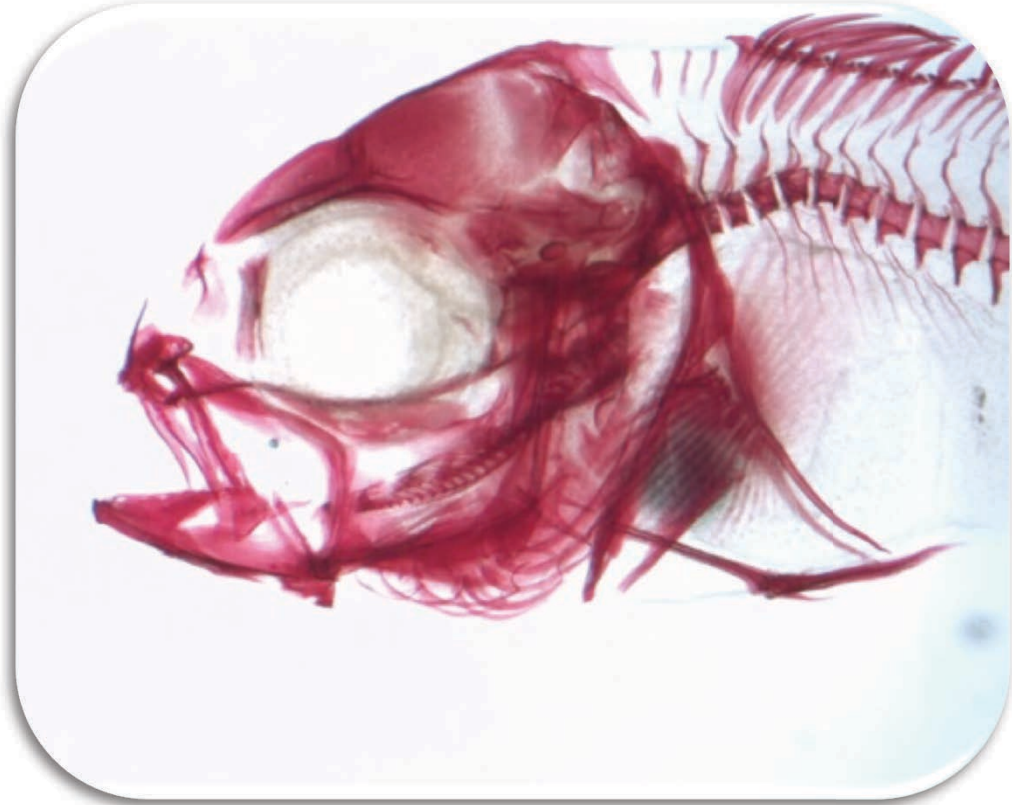
Specific requirements for marine fish larvae are not well known





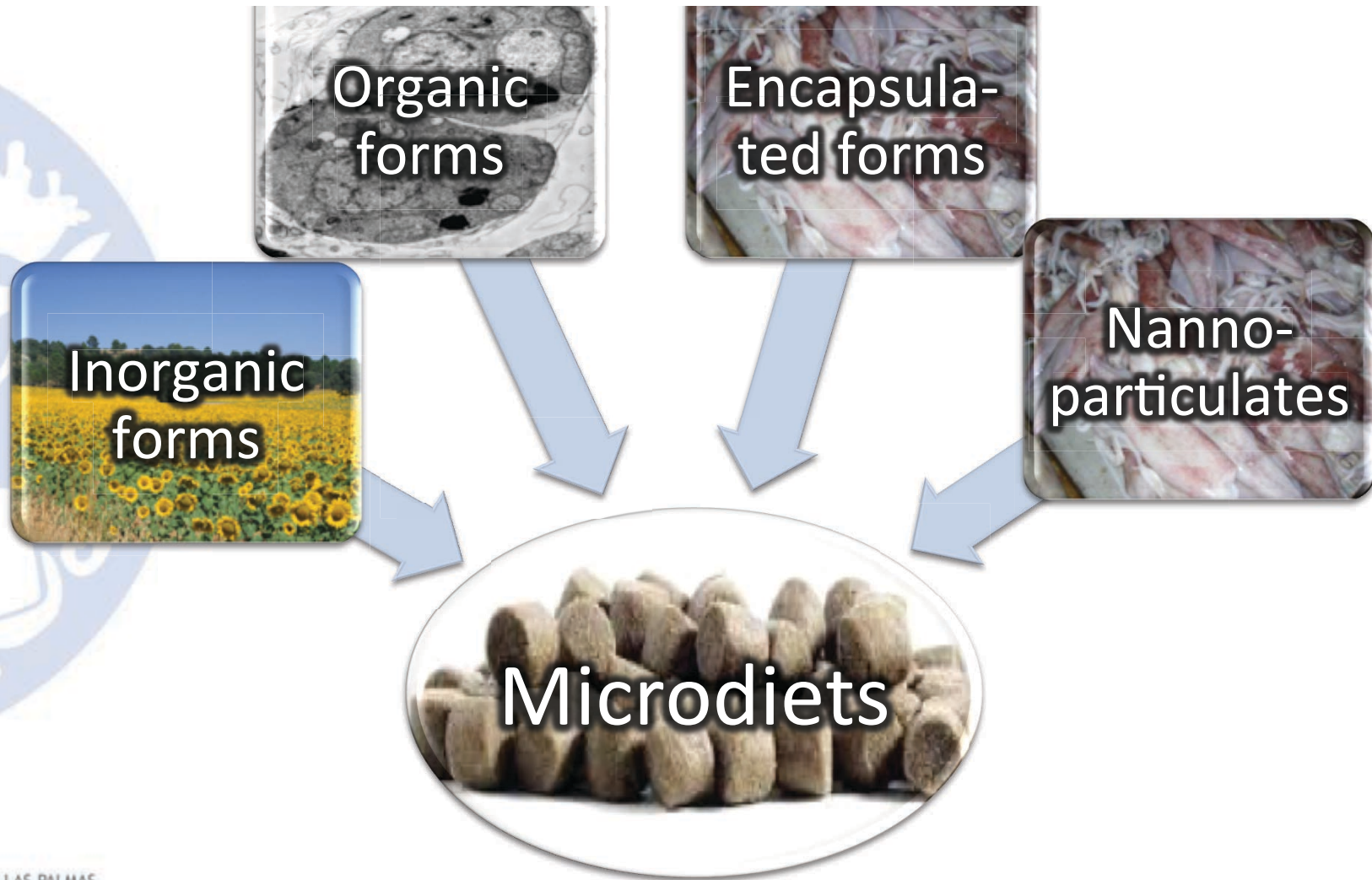
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Live preys are much lower in certain minerals than copepods, marine fish larvae natural prey, and even lower than the NRC requirements for juveniles

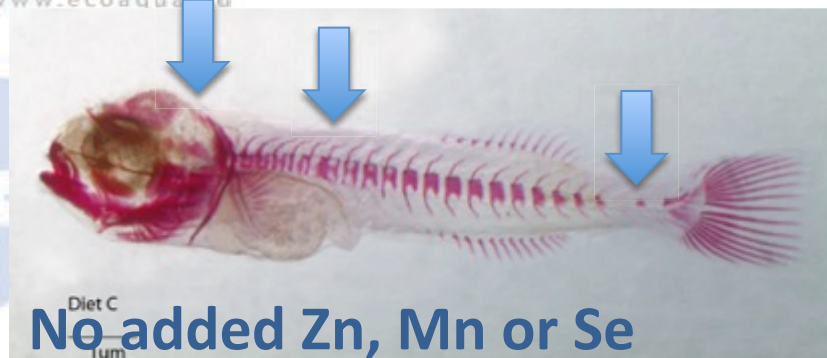


Marine fish larvae are exposed to a high oxidative risk

Effect of dietary Fe, Mn, Se or Zn in organic, inorganic, nanometals or encapsulated form for seabream larvae



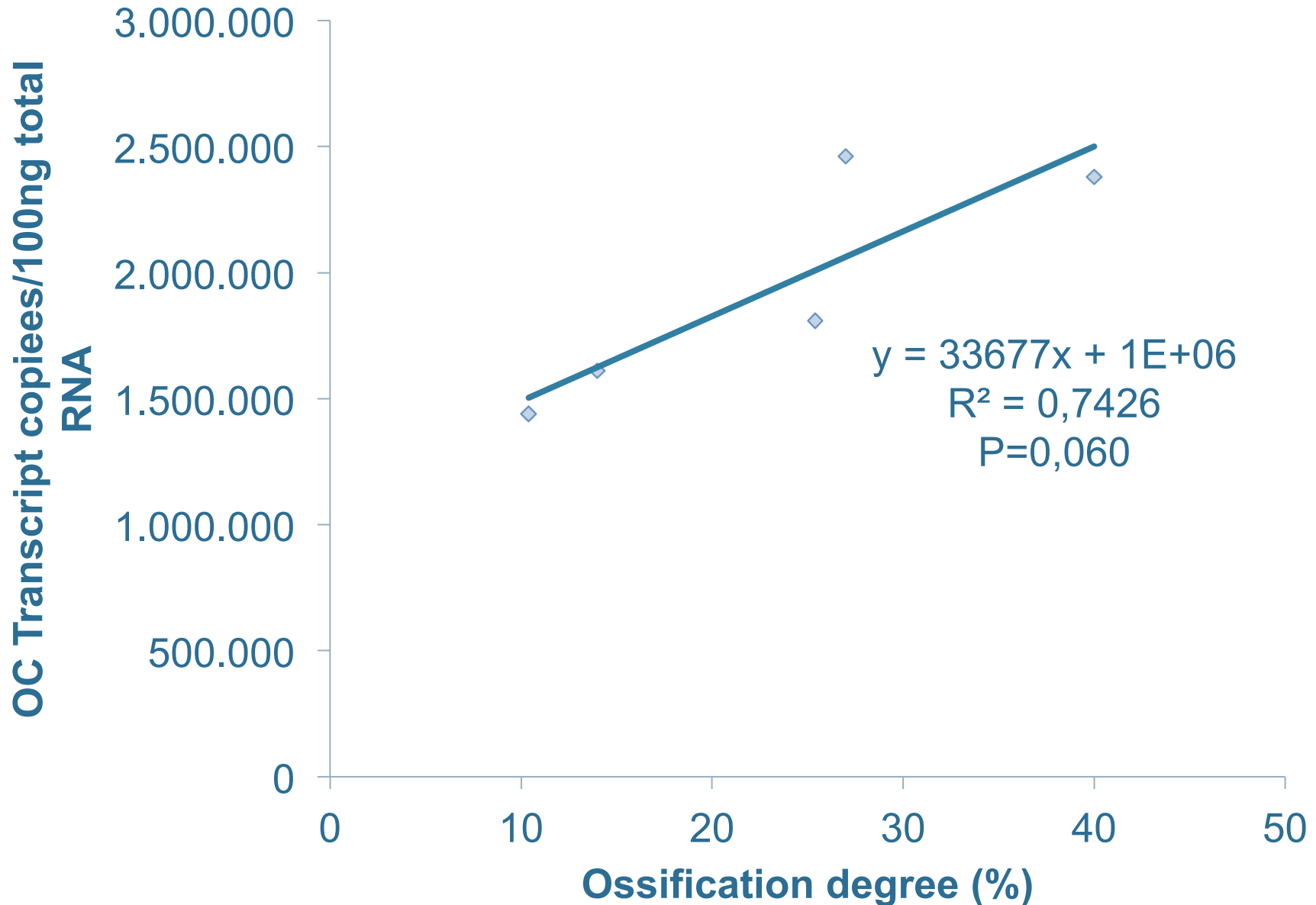
Bone mineralization of seabream larvae fed microdiets with different mineral sources



Organic and nannoparticulated minerals produced a better mineralization than inorganic and encapsulated minerals

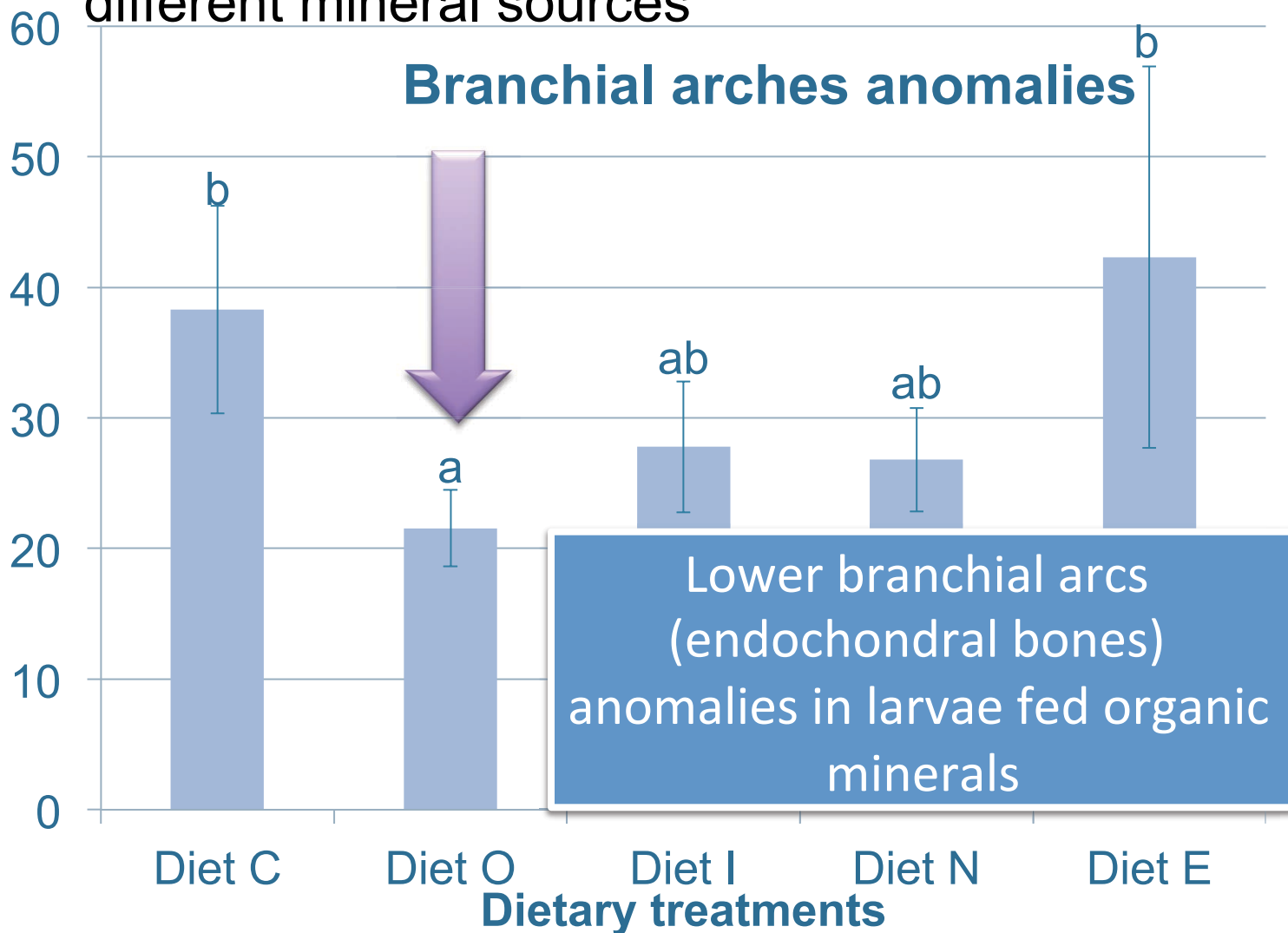


Ossification degree in seabream larvae fed 35 days different mineral sources

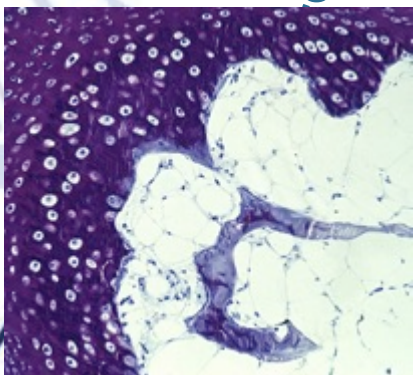


Branchial arcs anomalies in seabream larvae fed 35 days different mineral sources

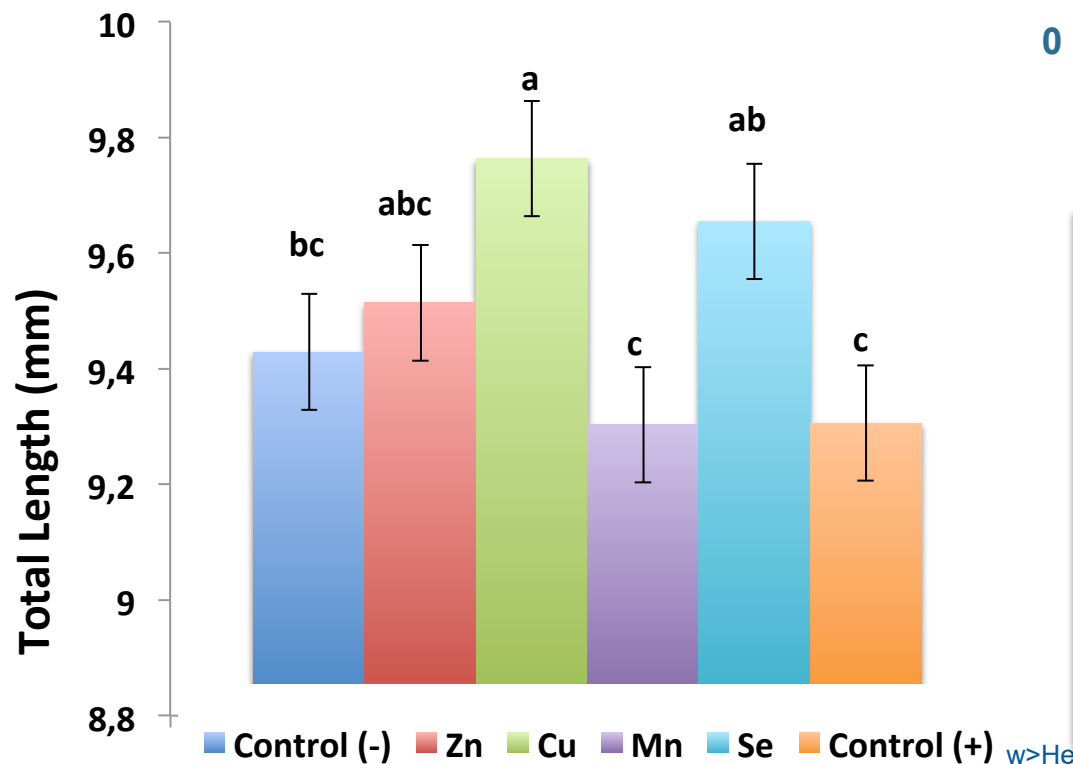
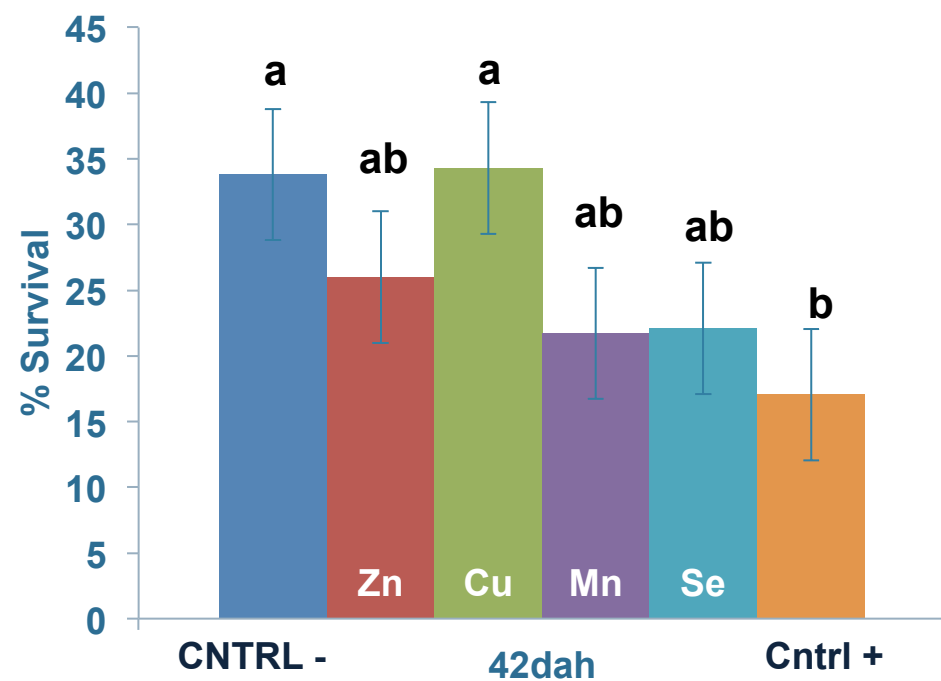
centages of sepecimens per treatment(%)



Endochondral branchial arcs staining



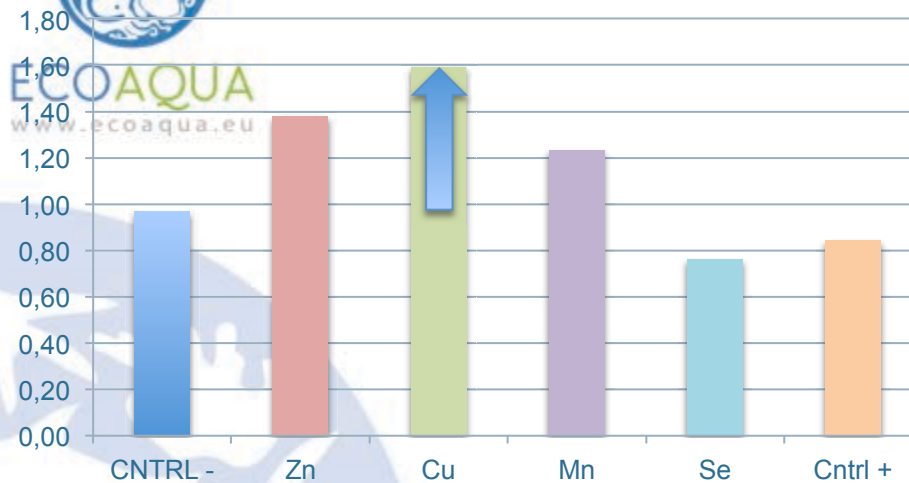
Specific minerals supplementation in weaning diets for gilthead seabream inorganic Mn, Cu and Zn and organic Se



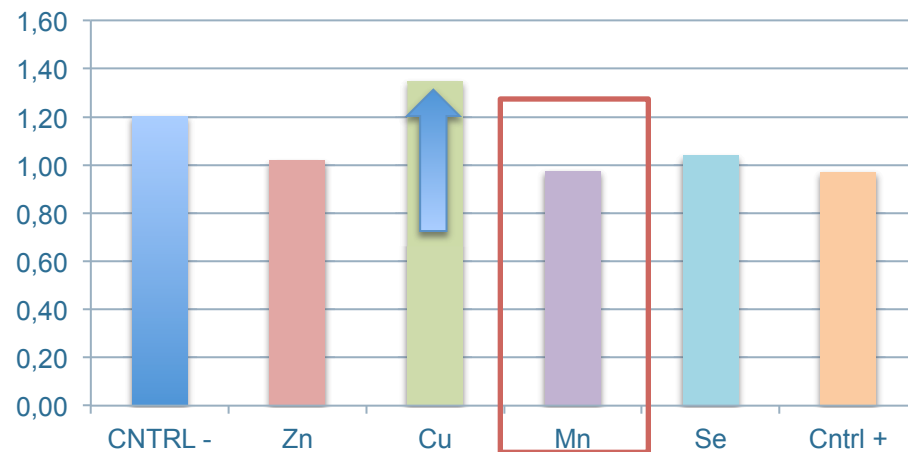
Inclusion of Zn, Mn and Se significantly reduced survival and only Cu and Se increased larval growth



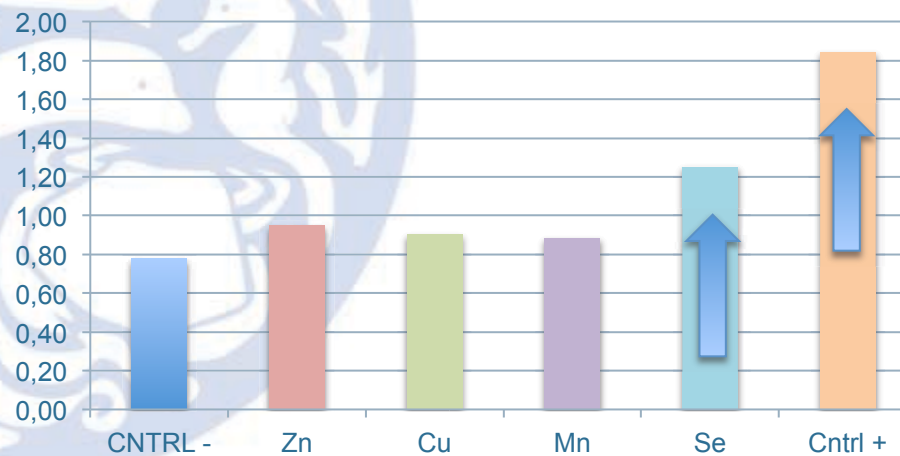
CuZnsod



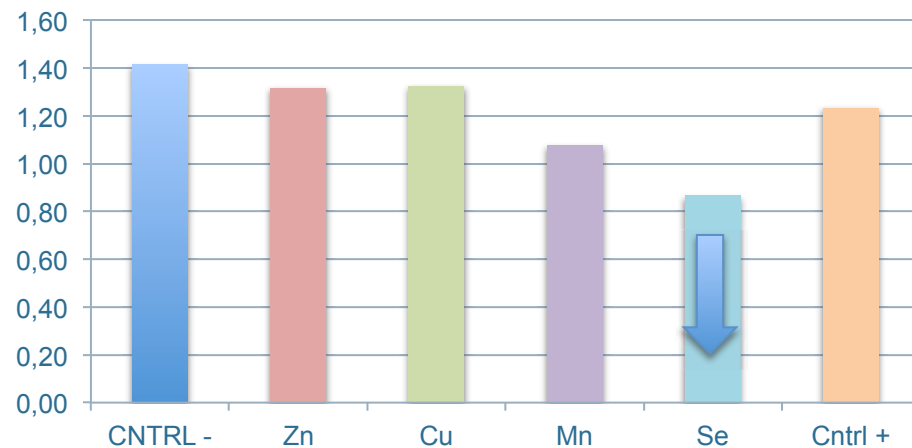
Mnsod



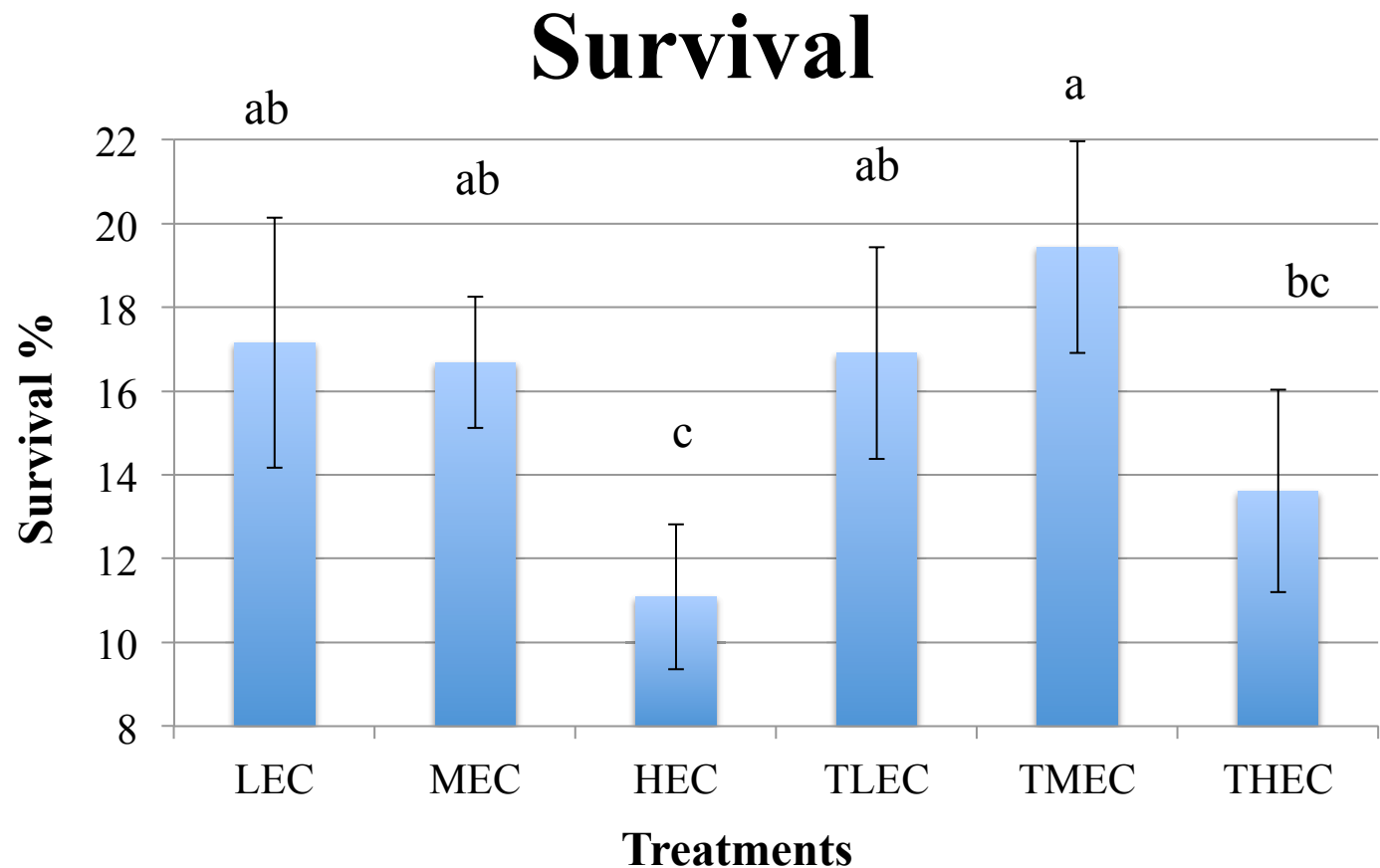
gpx



cat

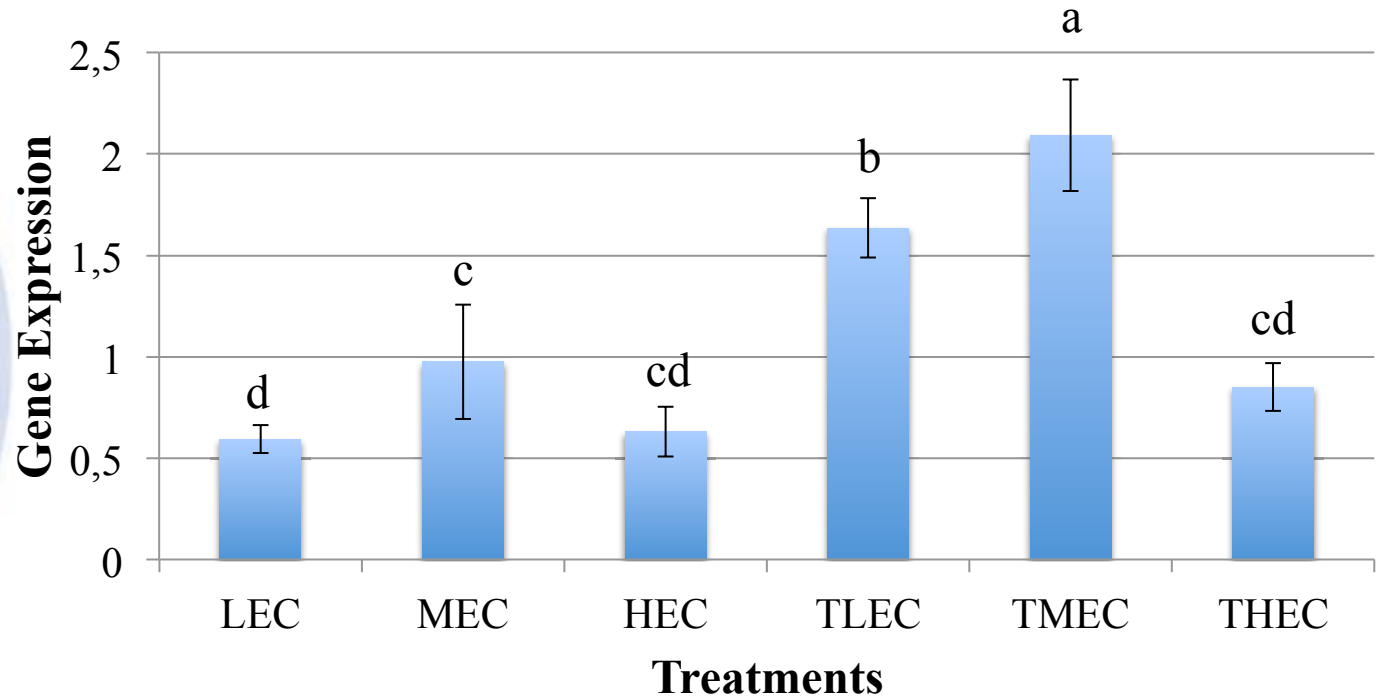


Very high levels of dietary vit E and C, significantly reduced survival, suggesting, as in the previous meagre study the pro-oxidant effect of high level of vit E



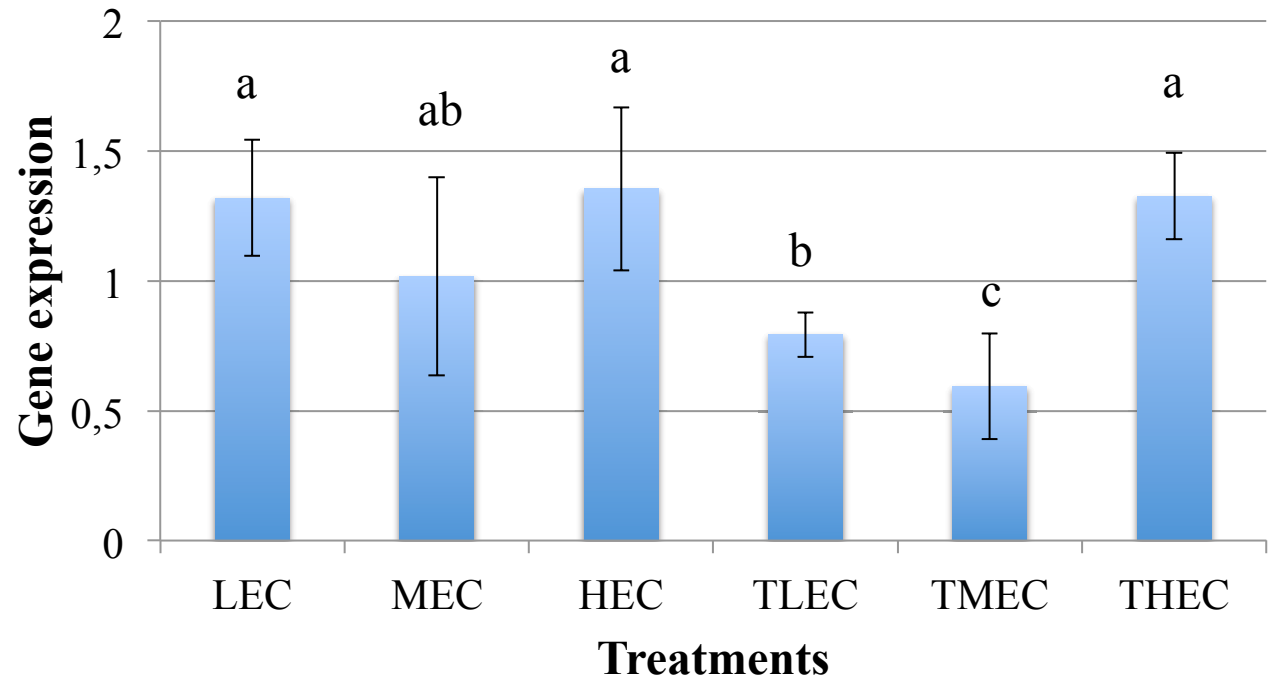
Intermediate levels of vit E and C up-regulate *oc* gene expression, and Taurine enhanced this effect.

Osteocalcine

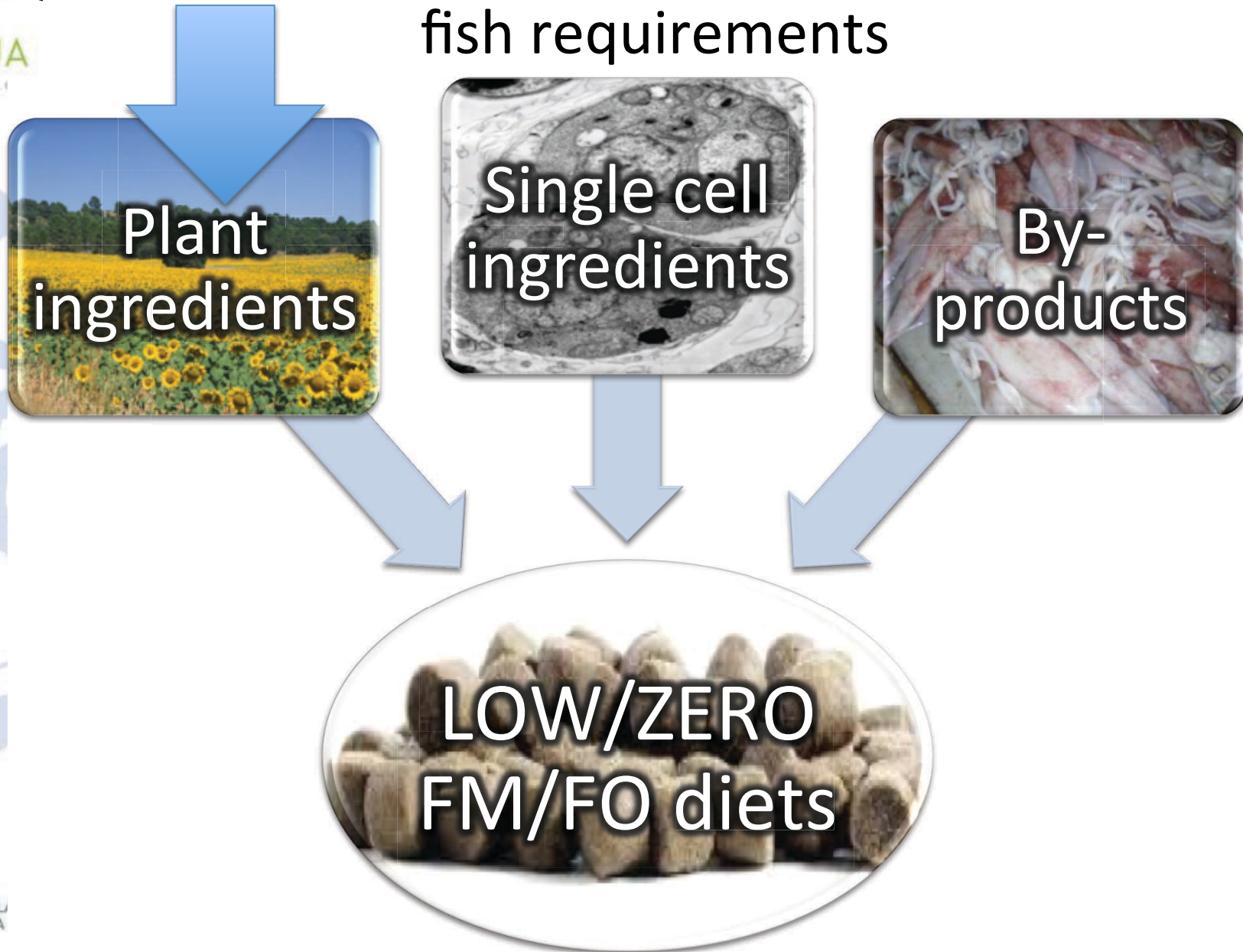


Intermediate levels of vit E and C down-regulate *cat* gene expression, and Taurine enhanced this effect

Catalase



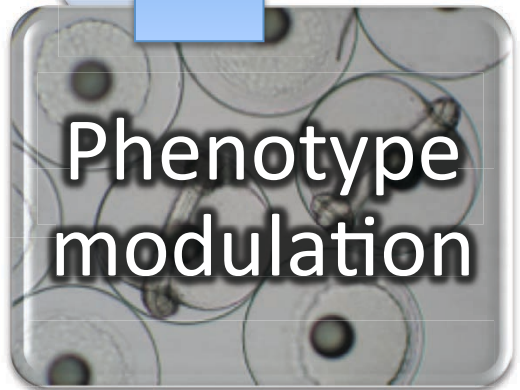
Low FM/FO formulations adapted to fulfil fish requirements



Fast growing high quality fish able to use feeds without fishmeal and fish oil



Persistent (life long & inheritance) modifications of the phenotype during sensitive “windows” along development:
EPIGENETICS



Phenotype modulation

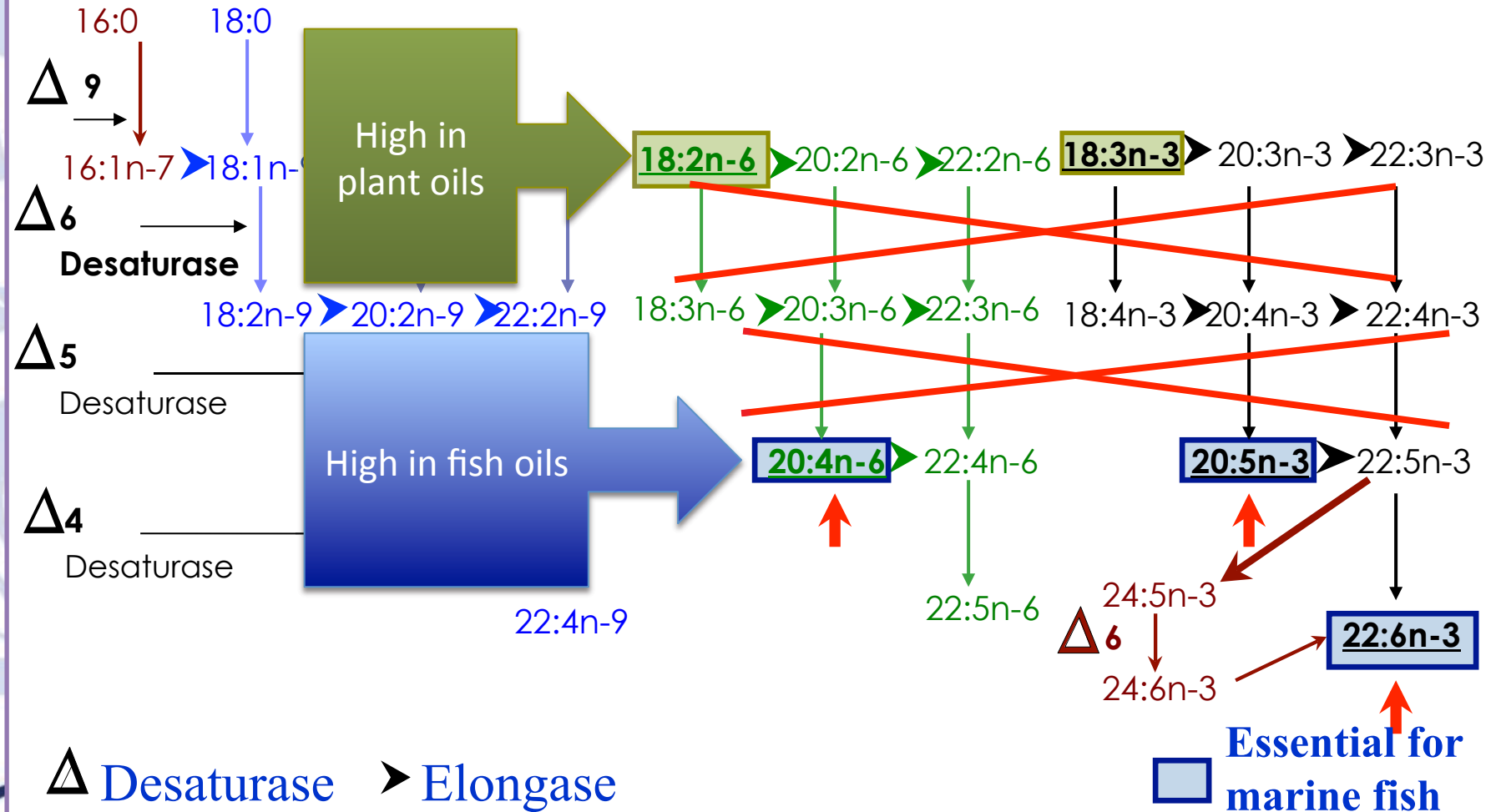
Vegetable oils are sustainable lipid sources for aquafeeds, but lack LC-PUFA essential for marine fish....



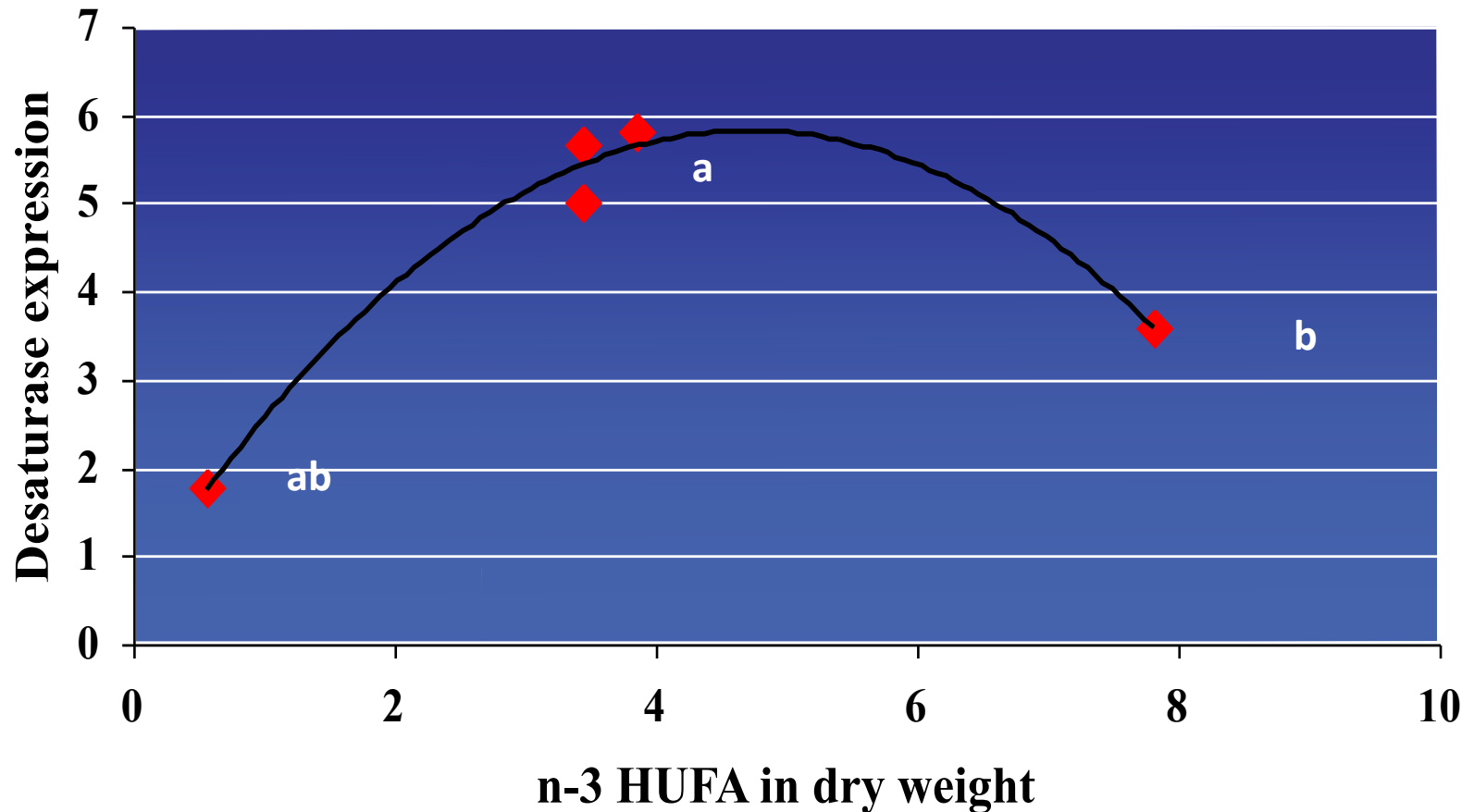
Low Survival
Slow Growth Rates
Low EPA and DHA in the fillet



$\Delta 6$ desaturase is a key enzyme in the synthesis of essential fatty acids



Gene expression of $\Delta 6$ desaturase can be modulated by the fatty acid composition of the diet, but it is not enough to cover essential fatty acid requirements of the fish



(Izquierdo *et al.* 2007)

Embryogenesis, early and late larval development are critical moments in marine fish life cycle

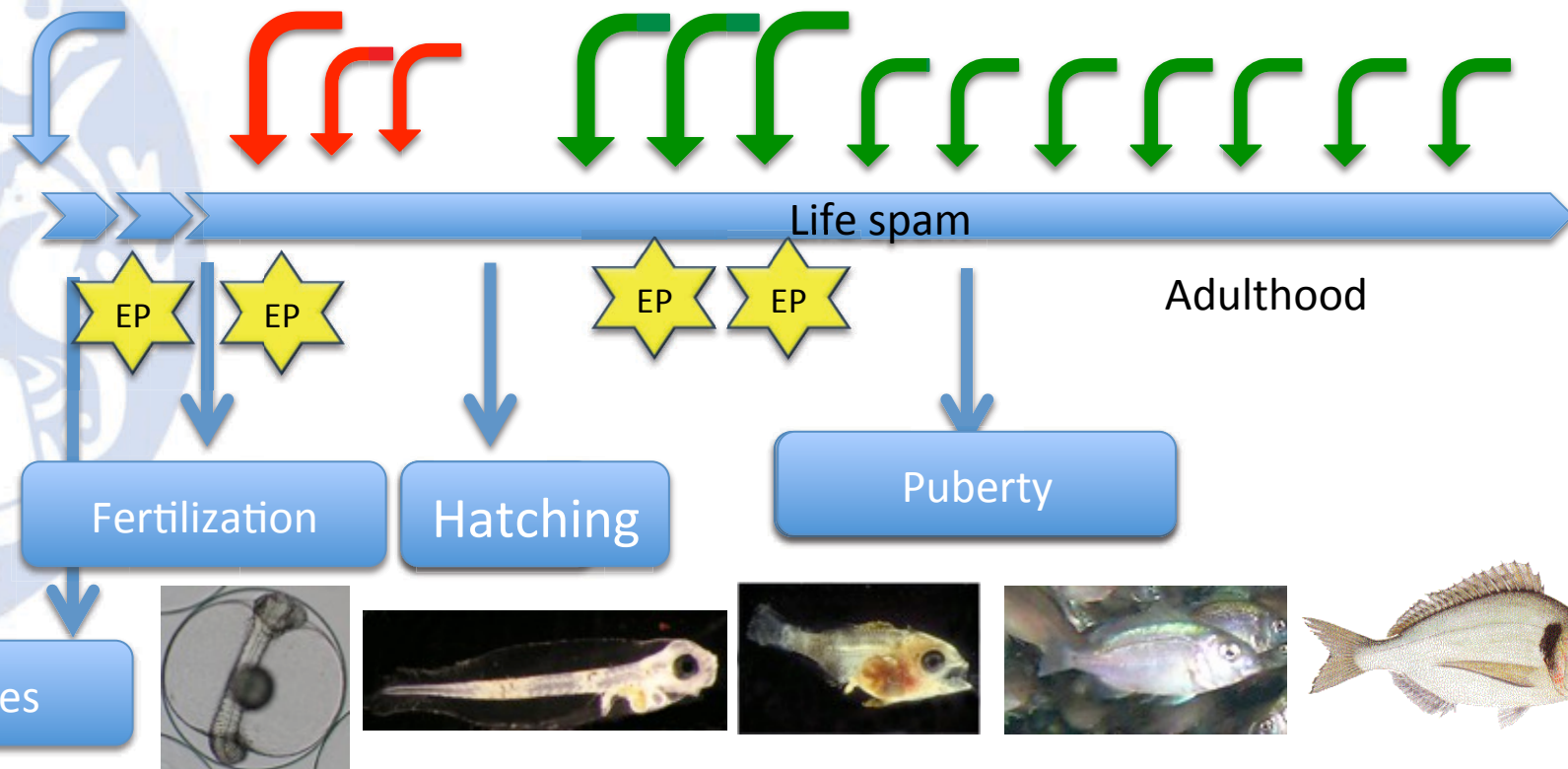


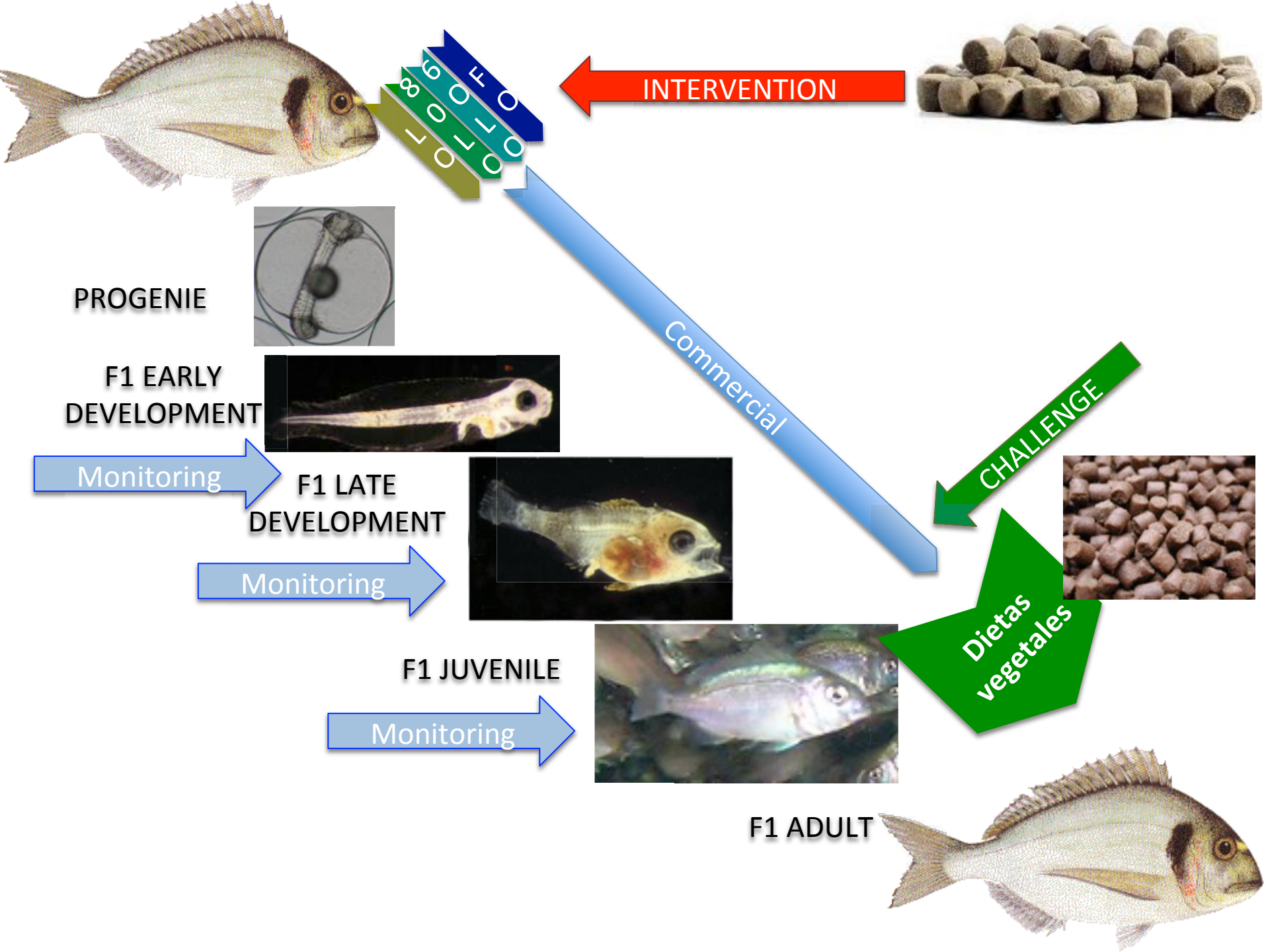
Parental diet



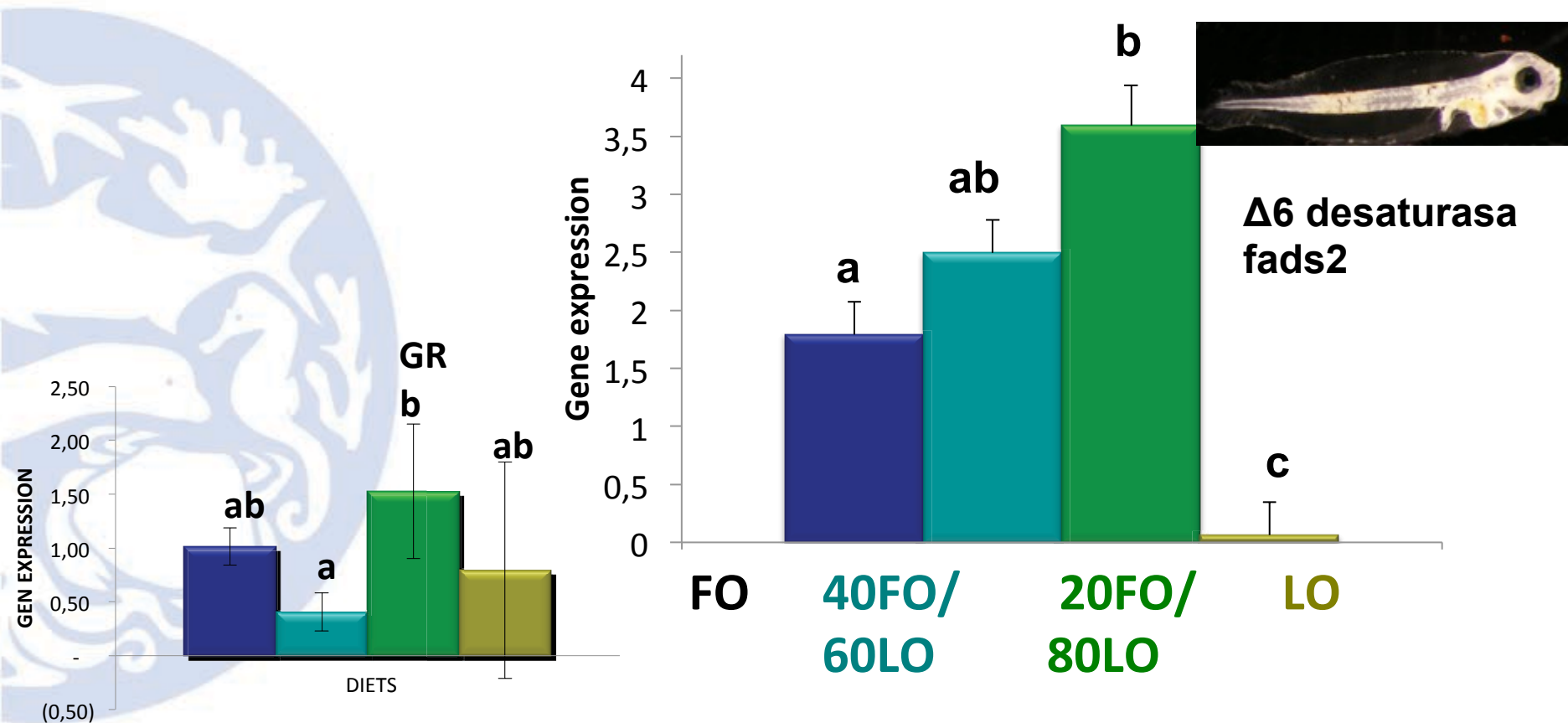
Maternal diet

Individual diet

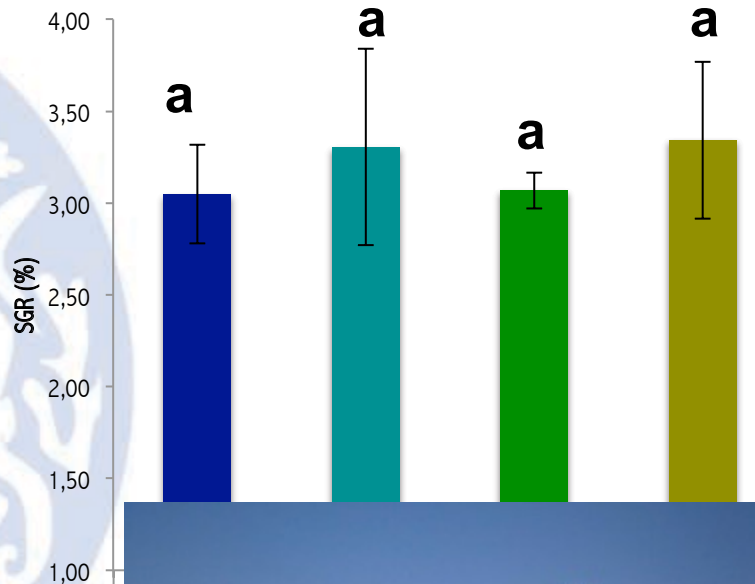




La alimentación de los reproductores con LC-PUFA afecto la expresión de genes del metabolismo de los descendientes



El crecimiento de las descendencia de 3 meses (pubertad) no se ve afectado si alimentamos con harinas de pescado, pero si la dieta es rica en ingredientes vegetales los descendientes de reproductores alimentados con aceite de lino es mejor



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Diversify

Seriola

Effect of broodstock nutrition on larval performance

Optimum larval density

Fatty acid requirements

Tau requirements

Argyrosomus regius

Microdiet development

Fat soluble vitamin requirements (Vit A, Vit E, Vit K, Vit D)

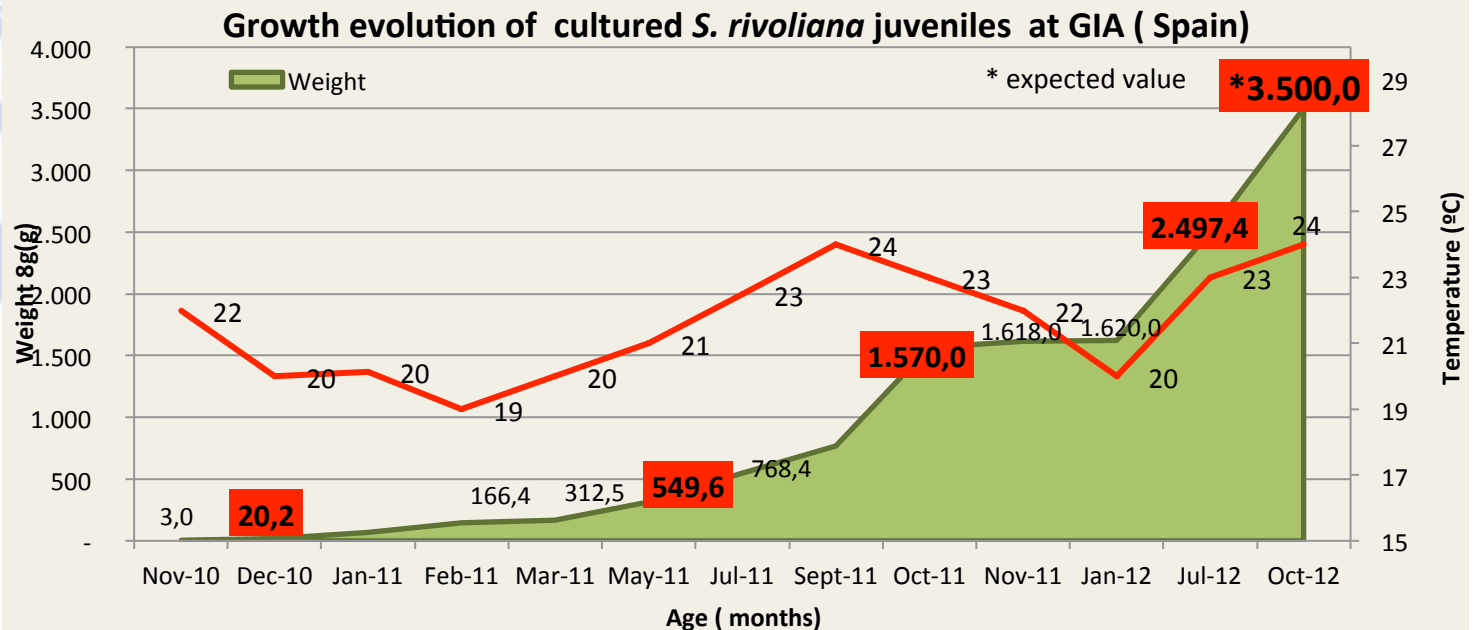
Water soluble vitamin requirements (Vit C)

Tau requirements





- Weight up to 59kg
- Aquaculture: Australia, Japan, USA,
- South America, Spain.
- Fast growth species (1,5-2kg one year in tanks)
- Lack juveniles supplies (2.6 ± 0.45)
 - poor spawns (number and quality).
 - low hatchery survival=collection of fingerlings from the wild.



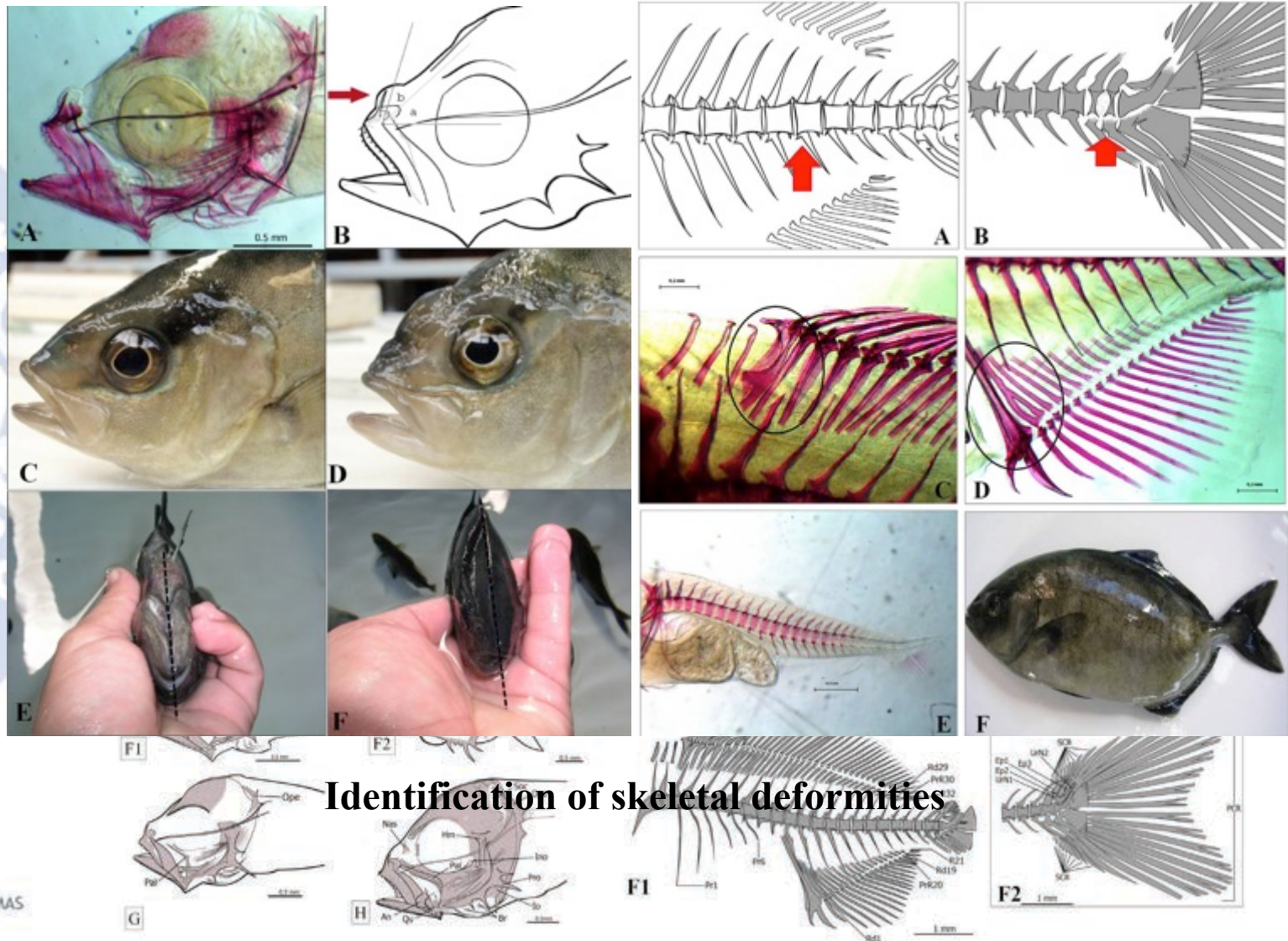




Basic knowledge for larval rearing

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Skeleton ontogeny (Mesa et al., 2009ab).



Identification of skeletal deformities



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Broodstock: Meagre

- **Broodstock from F1 population in 2006.**
- **First report of F1 maturation and spawning from 2008-2012.**
- **Maturation evolution.** (Fernandez-palacios et al, 2009 a, b in *XII Congreso Nacional de Acuicultura*).
- **Protocols for hormonal treatment** (Fernandez-palacios et al, 2009 , 2011 ab , b in *XII, XIII Congreso Nacional de Acuicultura*).





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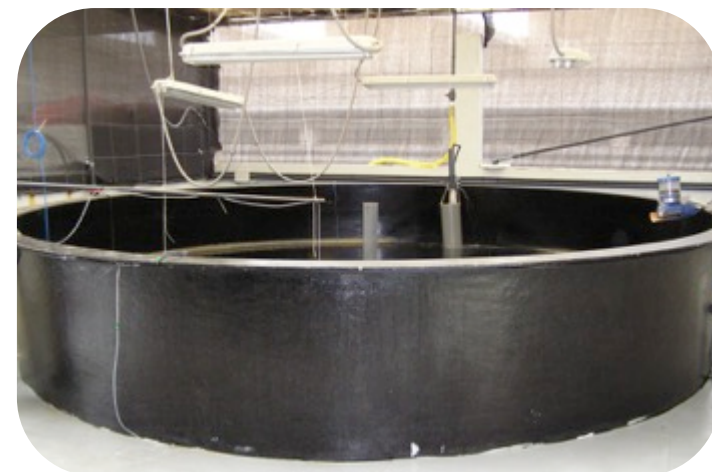
*Larval rearing of *Argyrosomus regius**

Larval rearing

1. **Comparison of culture systems** (Roo et al 2009, in Larvi symposium).
2. **Nutritional studies: Feeding sequence**, Roo et al, 2010. Aquaculture).
3. **Assignment of paternity and Heretability studies on production traits.** (Soula et al 2012).
4. **SME,s collaboration in juveniles production at pilot scale from 2008-2010.**

Ongrowth.

1. P/E studies. ACUISOST program.
2. Protein quality variation. ACUISOST program.
3. Inclusion of Antioxidants in diets. (Robaina et al 2012, Master thesis)



Diversification

Variety of cultivated species

Improve
aquacultured
production

Fish
health

Nutrition

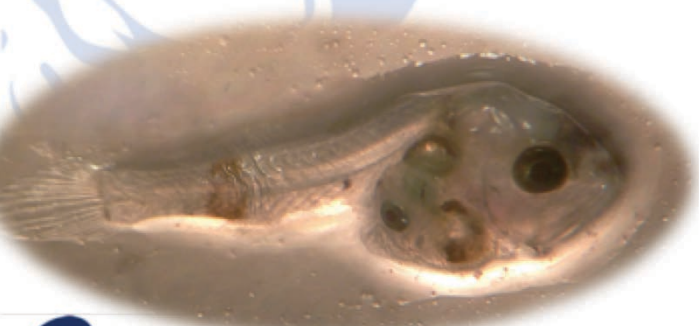
Larvae
culture

Reproductive
technology

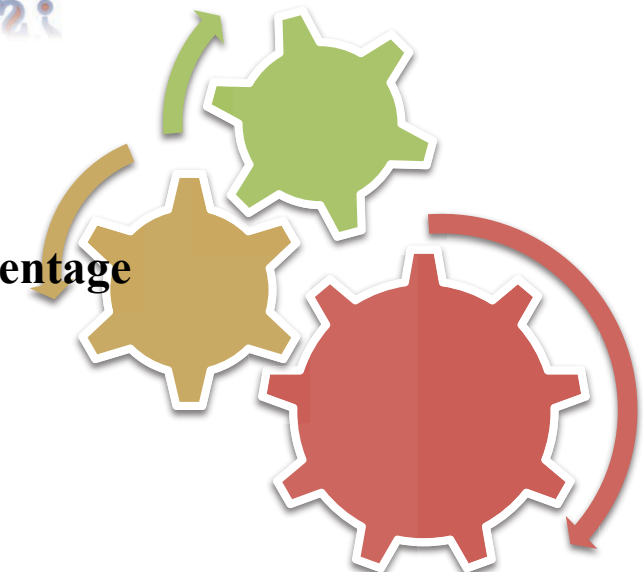


Why *Argyrosomus regius*?

- Rapid growth
- Low adiposity
- High dressing percentage
- Tolerant captivity
- Eurihaline
- High protein levels
- Low lipid content



Larva *Arggyrosomus regius* (24 dah)



THE IMPORTANCE OF DIETARY CONTENT OF VITAMINS K AND D FOR MEAGRE (*Argyrosomus regius*) LARVAE



Contents

- Basic knowledge on fish larvae biology
 - Nutritional requirements
 - Neural development
 - Bone development
 - Digestive tract development
- Quality indicators for larval production
 - Biological
 - Morphological
 - Molecular
 - Health
- Improved production protocols and hatchery management
 - Nutritional programming
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Muchas gracias por su atención

