Larval nutrition and programming in marine fish

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ECOAQUA University Institute

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Advanced Research Initiatives for Nutrition & Aquaculture

SEVENTH FRAMEWORK PROGRAMME

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FISH NUTRITION RESEARCH: RECENT ADVANCES AND PERSPECTIVES
13-16 June 2017

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Nutritional programing

- Nutritional programming is the process through which variation in the quality or quantity of nutrients consumed during critical epridos in life exerts permanent effects upon the developing fetus.
- Nutritional programming is a nutritional experience at critical periods in life that can programme a organism development, metabolism and health for the future.

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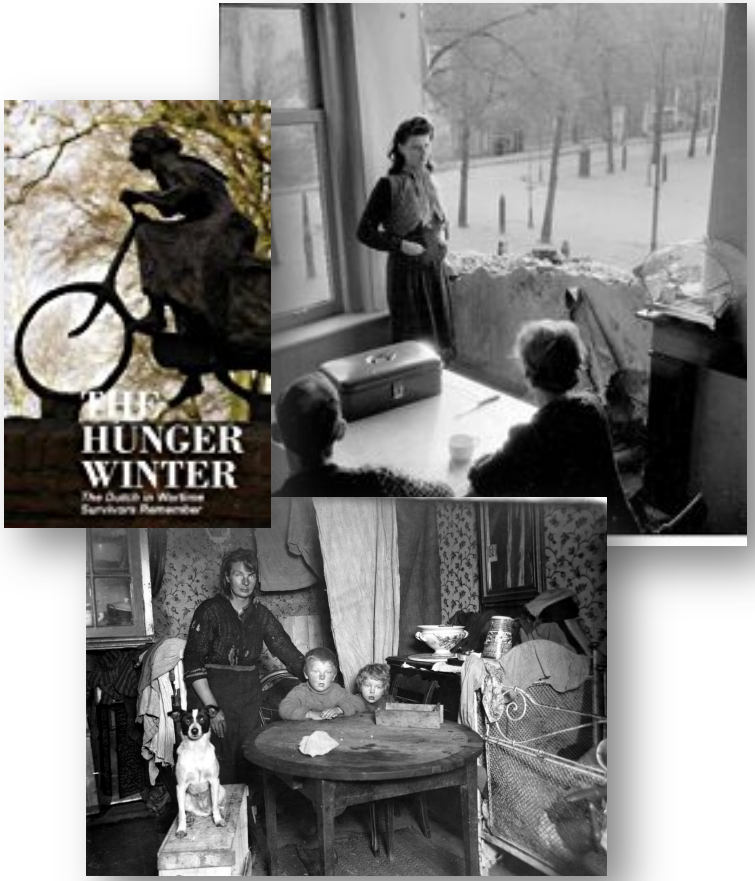
Dutch Hunger on 44-45 winter was reflected six decades later in the children of mothers exposed to famine early in gestation, by increased obesity, dyslipidemia and vascular-related deaths



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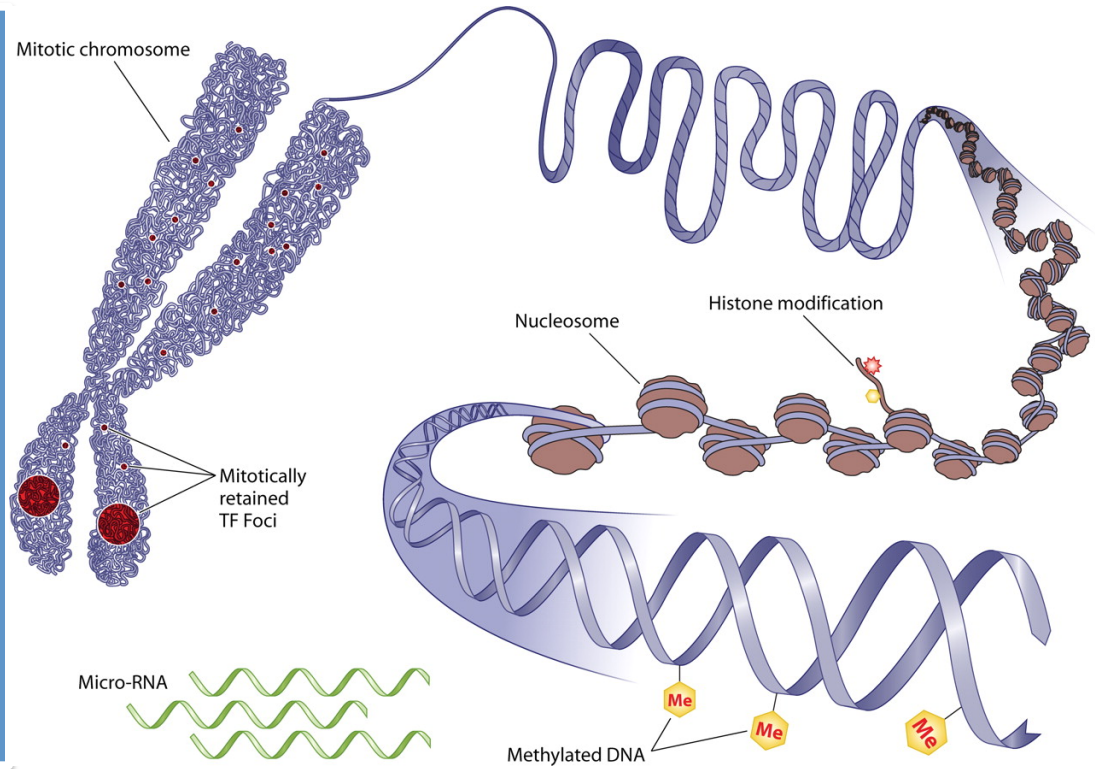
Epigenetics

- Epigenetics is the study of a series of semi-permanent changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself.
- Epigenetics is the study of potentially heritable changes in gene expression that does not involve changes in the DNA sequence. Implies changes in the phenotype without changes in the genotype.

Epigenetic factors (including nutrients) can modify gene expression in a stable and even heritable way that is not due to alterations in DNA sequence (epigenetic)

There are three main epigenetic mechanisms of gene expression regulation:

1. DNA methylation,
2. Histone modifications,
3. Non-coding RNAs



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In other animals PUFA have important Epigenetic properties

Cell specific lipid metabolism and expression of fatty acid-regulated transcription factors, play an important role in determining how cells respond to changes in PUFA composition

Offspring fatty acid composition in seahorses whose parents were fed either high (W) or low (C) n-3 LCPUFA diets

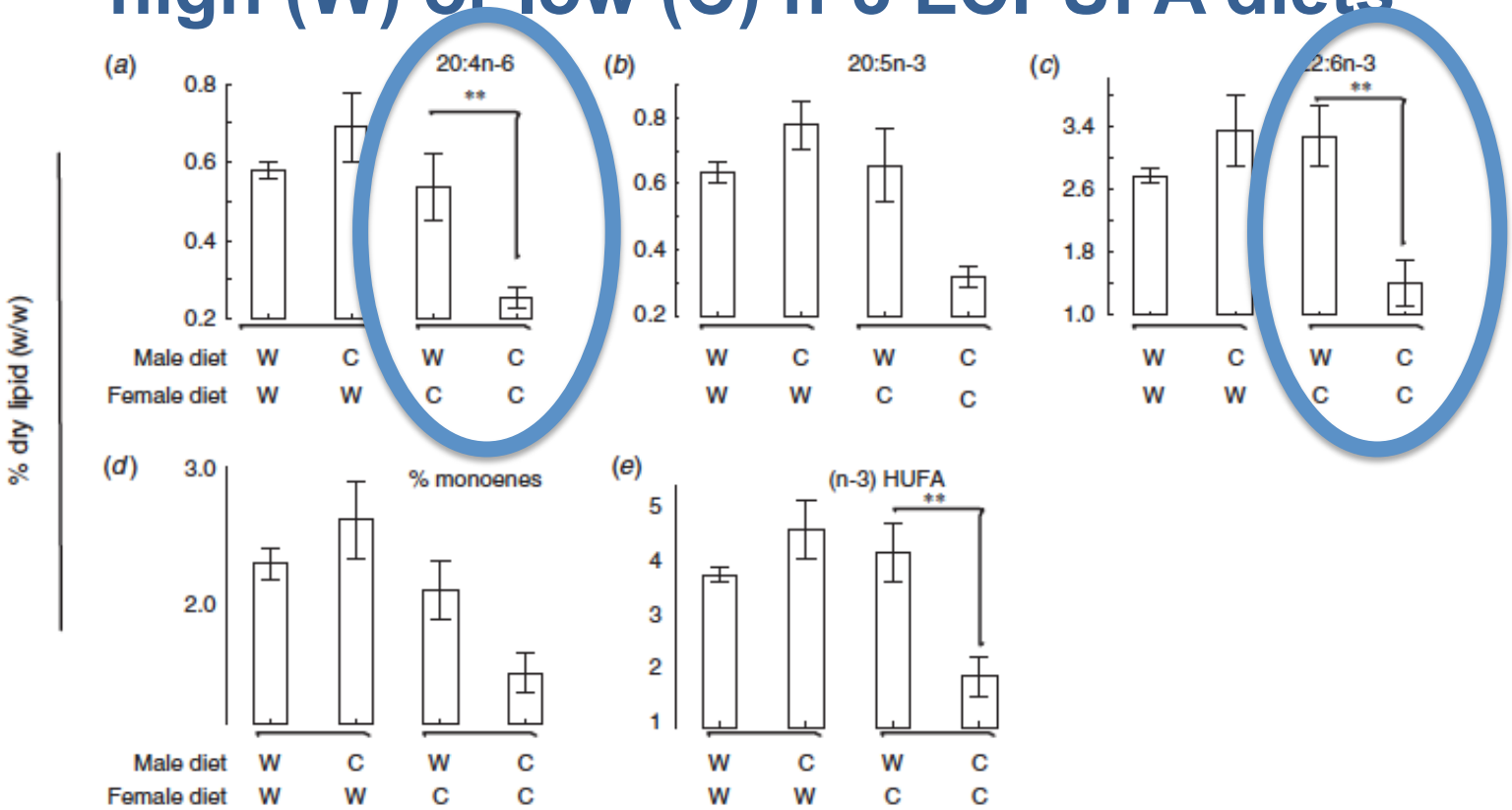

Fig. 5. Effects of dietary treatment on fatty acid concentrations (% dry lipid w/w) in newborn seahorses. (*a–c*) Data for individual fatty acids. (*d*, *e*) Percentage of (*d*) monoenes and (*e*) n-3 highly unsaturated fatty acids (HUFAs). Data are the mean ± s.e.m. $^{*}P < 0.05$,

ARA and DHA were high in offspring from mothers fed low n-3 LC-PUFA but fathers high

Dietary fatty acids in broodstock diets completely changed the morphology of the offspring

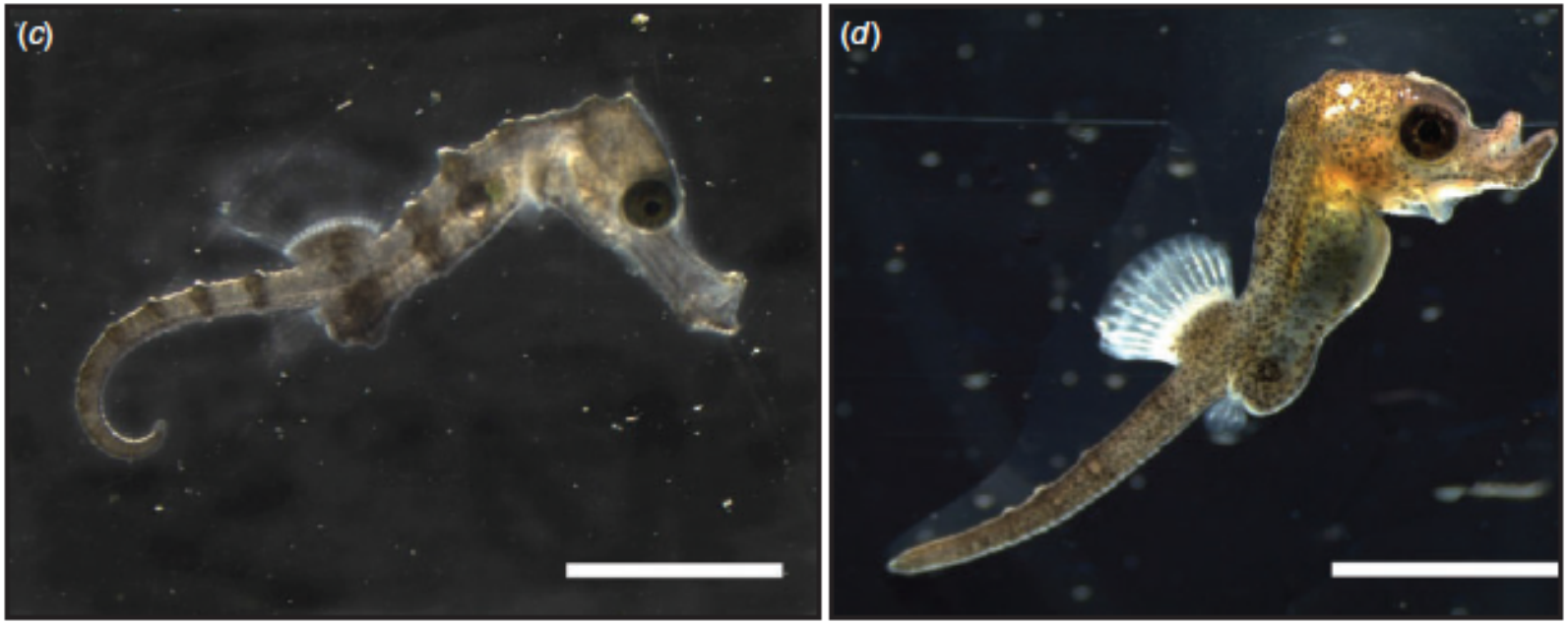

Fig. 4. (*a*, *b*) Scatter plots of the snout length (SnL) : head length (HL) ratio plotted against trunk length (TrL) in the first brood for offspring from all experimental females fed either the wild (*a*) or commercial (*b*) diet. A positive linear regression was observed for the commercial diet (*b*), but not for the wild-caught diet (*a*). (*c*, *d*) Representative images of newborn seahorses derived from females fed either the wild (*a*) or commercial (*b*) diet. The newborn from the female fed the commercial diet (*d*) has an abnormally short snout and therefore a low SNL : HL ratio compared with the newborn from the female fed the wild diet (*c*). Scale bar = 2 mm.

Seahorse offspring were larger when males were fed low n-3 LC-PUFA if females were fed high n-3 LC-PUFA

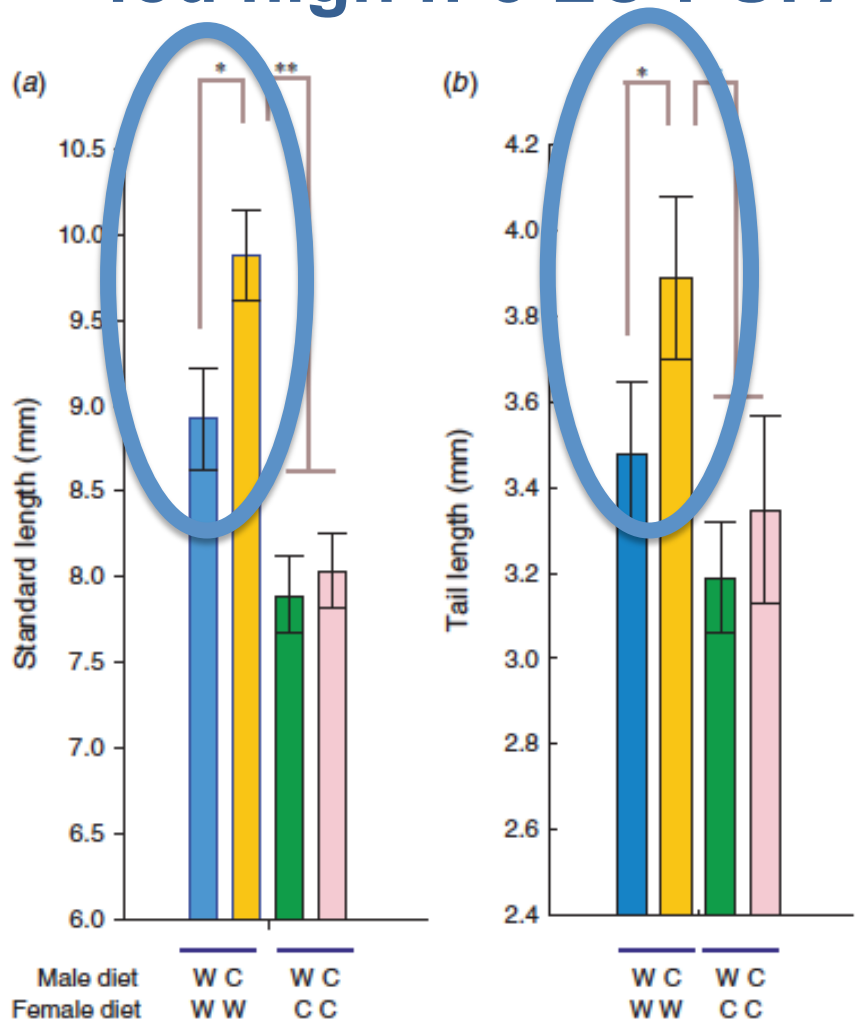

Fig. 1. (*a*) Standard length and (*b*) tail length of newborn seahorses from the first brood born to males and females fed either a commercial (C) or wild-caught (W) diet. Data are the mean ± s.e.m. $^{*}P < 0.05$ and $^{**}P < 0.01$.

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Izquierdo MS

Nutritional programming and progeny performance in marine fish

Seafood has a high nutritional value for human health and its demand is increasing, being expected to reach over 150 million MT/year in 2030. However, world fisheries production remained stagnant for the last 20 years with over 85% of the stocks being over-exploited or fully-exploited. Only aquaculture can meet this seafood demand, being the food production sector with the fastest growing rate. Fishmeal and fish oil were main ingredients in aquafeeds, but they are unsustainable and insufficient to maintain aquaculture growth. Thus, these ingredients have been reduced by 50% in aquafeeds during the last decade. Nevertheless, the complete removal of fishmeal and fish oil is required to promote the sustainable development of aquaculture and for that, fast growing high quality fish that are better adapted to utilize diets without fishmeal and fish oil are necessary. Two different strategies are investigated to achieve this goal: nutritional programming and inheritance.
Fish oil is rich in omega 3 highly-unsaturated fatty acids (n-3HUFA), essential for marine species, due to their low bio-conversion ability. Plant oils lack n-3HUFA but contain their precursors. The genes of enzymes bio-converting these precursors may be nutritionally regulated. Nutritional conditioning would allow fish genome adaptation for a better utilization of diets without fishmeal or fish oil. To define the dietary factors that influence the epigenetic profile, the most sensitive time-windows and the lenght of intervention we conducted three types of programs by early exposure of embryos, larvae or postlarvae to different diets. After several months of monitoring growth and metabolism, fish were challenged with either plant diets or fishmeal diets. The studies showed the very long-term effects resulting from parental epigenetic control of embryogenesis, perhaps along the whole life cycle of the fish, as well as the potential of nutritional conditioning during early larval stages.

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Proceedings of the EPICONCEPT Workshop 2013
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IFIC EFFECTS OF PARENTAL DIET DURING PREGNANCY ON EMBRYO IN THE LONG SNOUT SEAHORSE (*HIPPOCAMPUS REIDI*; GINSBURG, 1933)

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vestigación en Acuicultura, University of Las Palmas Gran Canaria, Telde, Las Palmas, Spain;
Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, UK

os develop internally but, unlike in mammals, this process occurs within a paternal structure (the brood pouch). pports developing embryos through placenta-like interactions, but as egg quality is determined by the female's diet, unities to study the effect of the male's diet on embryo development while varying the female's diet independently. hypothesis that development of seahorse embryos is affected differentially by independent manipulation of the male males and females were fed separately with either wild-caught crustaceans (Male-W or Female-W, respectively) or -C or Female-C, respectively) for 1 month before conception and during the subsequent pregnancy (approximately ty acid content (18:3n-3) and (20:4n-6) in the W diet was approximately double that in the C diet. In total, 5231 d from 4 treatment groups formed from (1) Male-W × Female-W; (2) Male-C × Female-W; (3) Male-W × Female-C; ch treatment was replicated with 4 couples. Newborns (10 from each brood) were weighed and dimensions survival rates were determined from 40 offspring/couple (N at Day 0 = 160/treatment) and fatty acid profiles lysed by nested analyses of covariance (ANCOVA); replicates were nested within treatments and individual sted within replicates (male and female parental sizes were used as covariates). Offspring produced by the Male-C × oth as standard length and tail length; $P < 0.05$) than those produced by Male-W × Female-W couples but their % *v.* 39%; $\chi^2 = 39.19$, 1 DF; $P < 0.001$). Fifteen-day survival of the other groups was 0% in both cases. When both l the commercial diet, their offspring were considerably smaller than those from all the other treatments ($P < 0.05$). -W × Female-C couples showed distortion of the snout:head length ratio, a phenotypic feature that was highly ale-W treatment group. Fatty acid profiles of the offspring showed significant dependence on the preconception 2:6n-3 contents were significantly lower in Male-C × Female-C than in Male-W × Female-C ($P < 0.01$) offspring,

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Embryonic developmental plasticity in the long-snouted seahorse (*Hippocampus reidi*, Ginsburg 1933) in relation to parental preconception diet

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[B]Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK.
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Abstract. The aim of the present study was to investigate the hypothesis that parental periconception nutrition in adult seahorses affects the development and growth of their offspring. We tested the hypothesis that because seahorse embryos develop inside the male's brood pouch, manipulation of the male's diet would affect offspring growth and development independently of the female's diet. Adult males and females were fed separately with either wild-caught crustaceans or commercial aquarium diet for 1 month before conception to influence the periconception environment. Approximately

Otero-Ferrer F, Izquierdo M, Segade A, Fazeli A, Holt W

Seahorses: A new and unique epigenetic model

Environmental influences (including diet and feeding conditions) experienced during development modify animals' phenotype, in a way possibly 'predictive' of future conditions, and may be essential for their survival and reproduction. With their particular reproductive behaviour, where males not only become pregnant but also support spermatogenesis, seahorses constitute an excellent model. The aims of this work were to (1) investigate the hypothesis that parental nutrition in adult seahorses (Hippocampus reidi) affects the growth and development of offspring, much as in mammals, and (2) validate the proposal that seahorses may be a valuable and effective epigenetic model.
The experimental design involved using either wild or commercial mysids to influence the peri- and post-conception environment. Sixteen seahorse breeding pairs were divided into separate aquariums. We manipulated the male's and female's diets separately prior to mating, obtaining offspring from animals from all combinations of dietary treatments (male x female x wild x commercial) over 5 months. Approximately 10,000 offspring were born.
In the first experiment brood pairs in which males received commercial and females received wild diet, produced significantly larger offspring than controls (males and females both receiving wild diet). Offspring produced by commercial-fed females were consistently smaller than the controls, suggesting that maternal nutrition directly affects offspring development. These results demonstrated that the growth and survival of offspring is dependent on the quality of diet received by both parents prior to conception. It is of interest that commercial-fed males (commercial diet is lower in polyunsaturated fatty acids) had abnormally large offspring. The results support the hypothesis that the male's brood pouch does more than simply provide a suitable environment that sustains embryonic growth and supports the hypothesis that seahorses could be used as an experimental model for studying the relationships between parental nutrition and offspring fitness.

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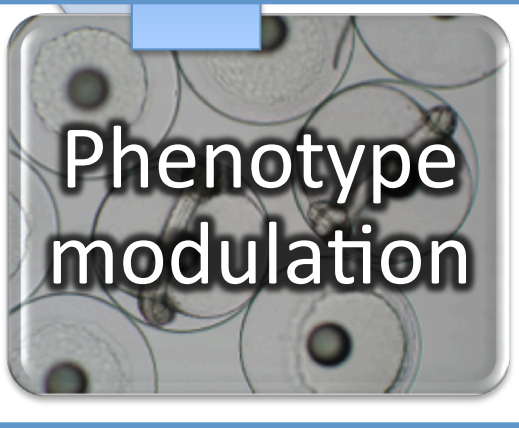
Fast growing high quality fish able to use feeds without fishmeal and fish oil

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



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Raw materials, particularly FO & FM constitute a main challenge for aquaculture development

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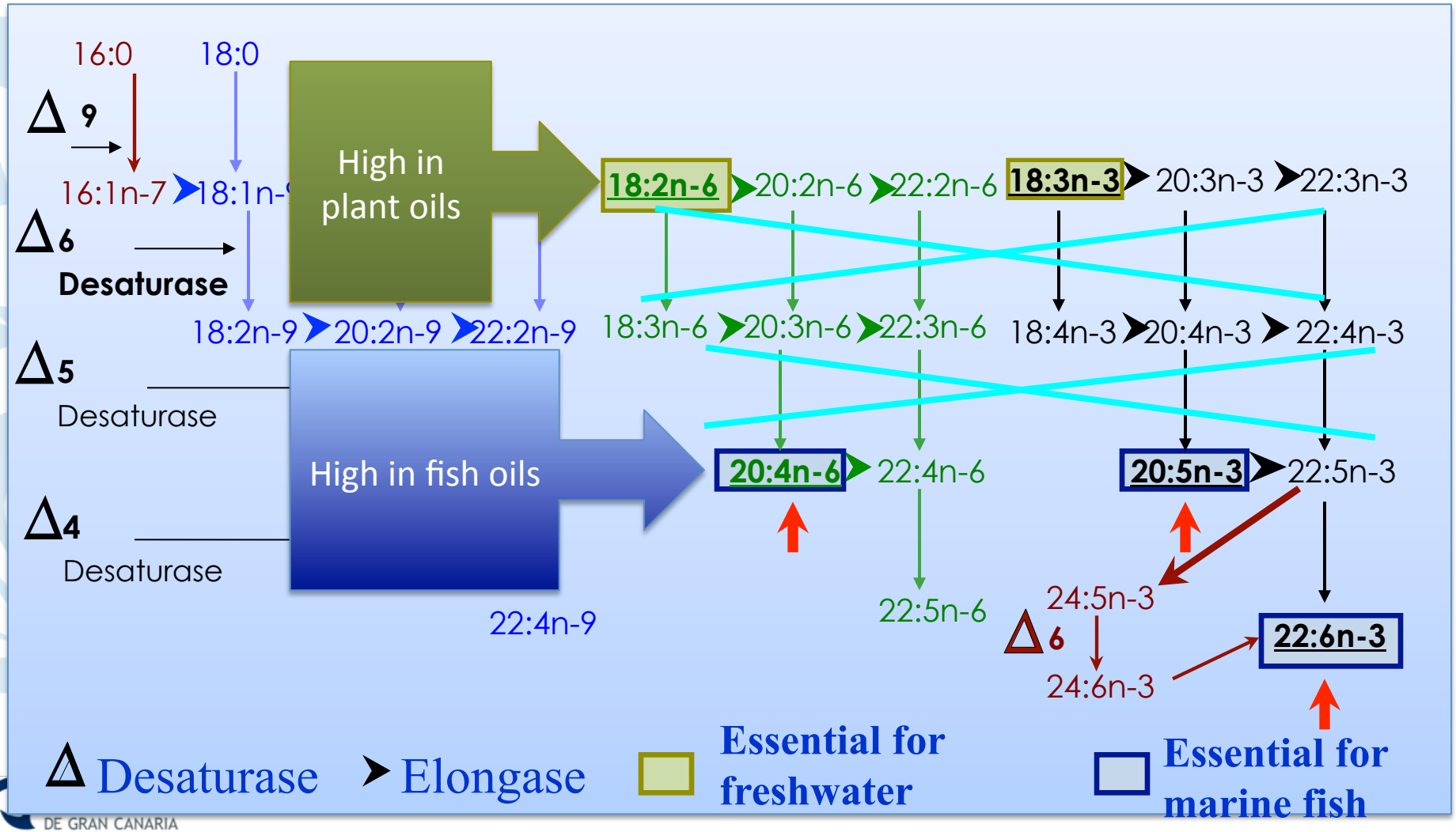
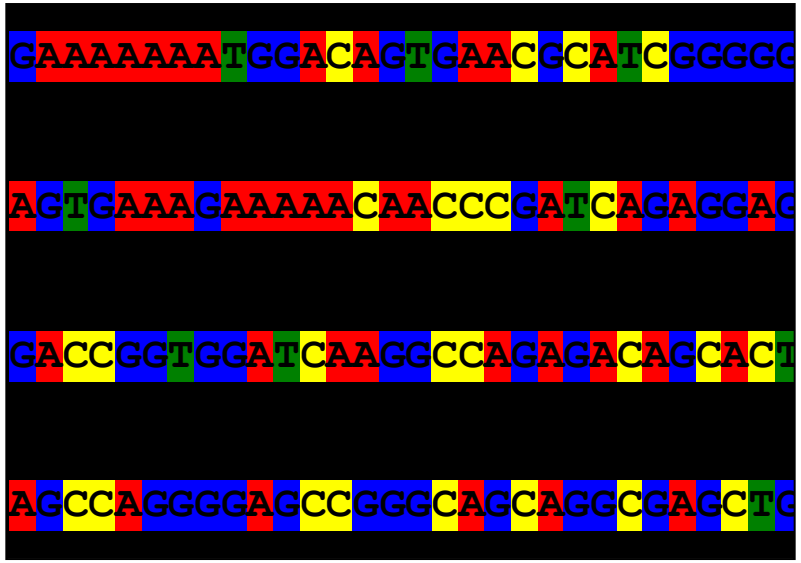
Essential fatty acids synthesis pathways

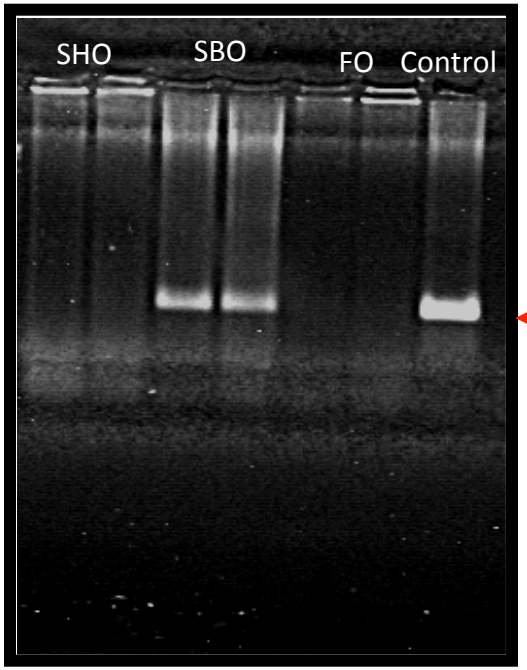


Δ-6/Δ-5 Desaturase gene (*fads2*) isolated from seabream larvae



Part of Δ-6 Desaturase Multiple sequence Alignment

(Seiliez *et al*., 2003)



Δ-6 Desaturase

(Izquierdo *et al*., 2007)

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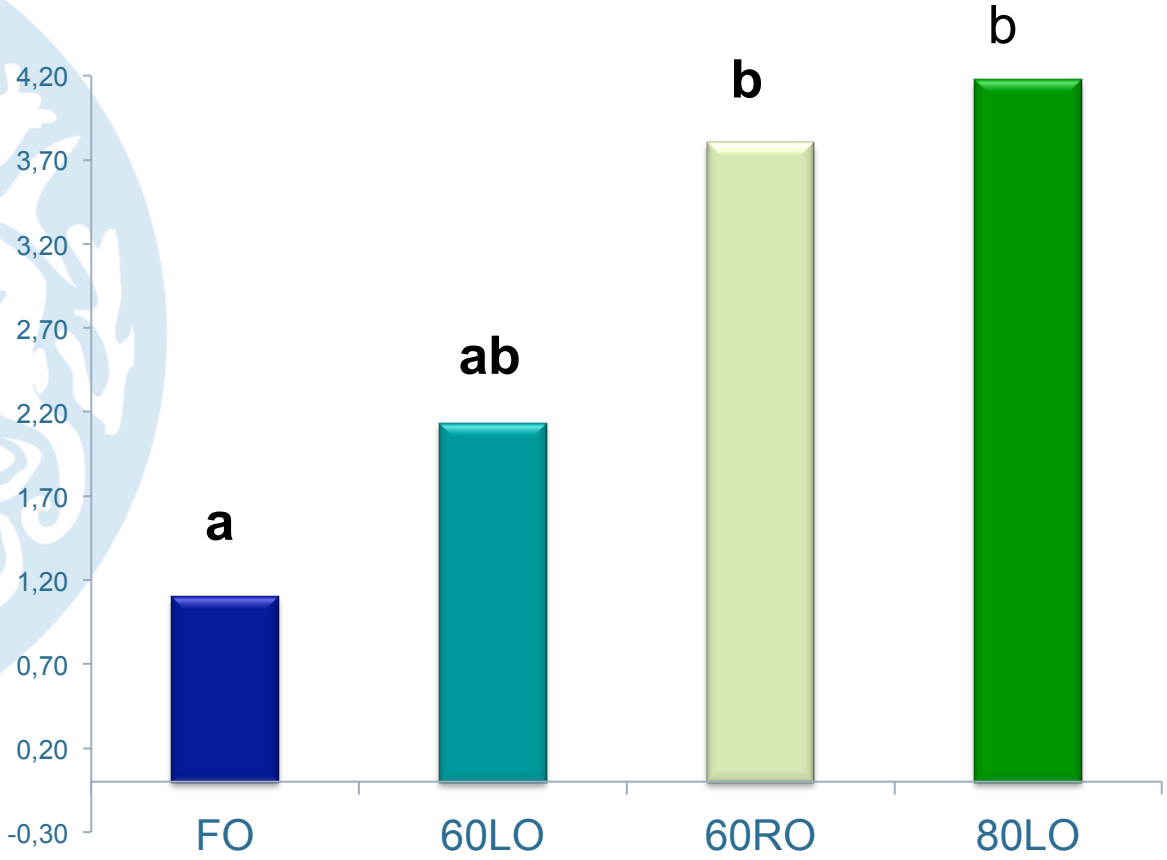
fasd2 expression in liver of gilthead seabream juveniles fed different plant oils for three months



(Izquierdo *et al*., 2008)

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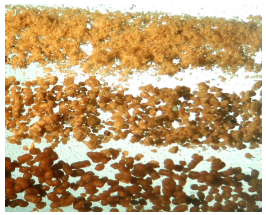
Embryogenesis, early and late larval development are critical moments in marine fish life cycle

Parental diet

Maternal diet

Individual diet



Life spam

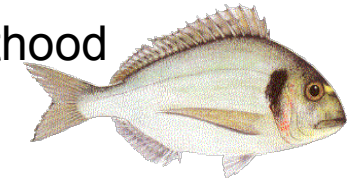
EP

EP

EP

EP

Adulthood



Fertilization

Hatching

Puberty

Gametes

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PROGENIE

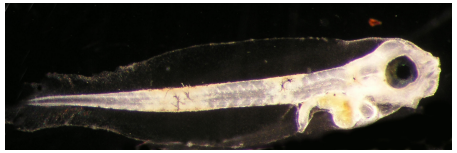
INTERVENTION

EP

Is it feasible the early programming of seabream for a better utilization of vegetable oils during on-growing ?

Life spam

F1 EARLY DEVELOPMENT



EP

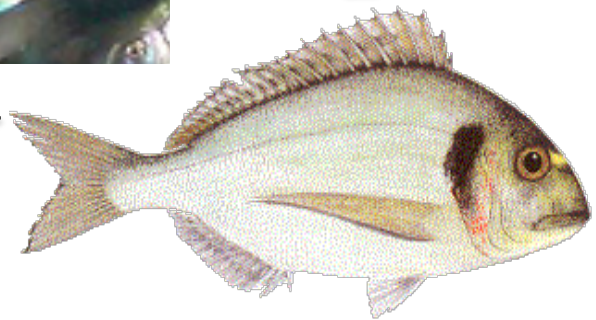
F1 LATE DEVELOPMENT

EP

Better performance when fed plant oils?

F1 JUVENILE

F1 ADULT



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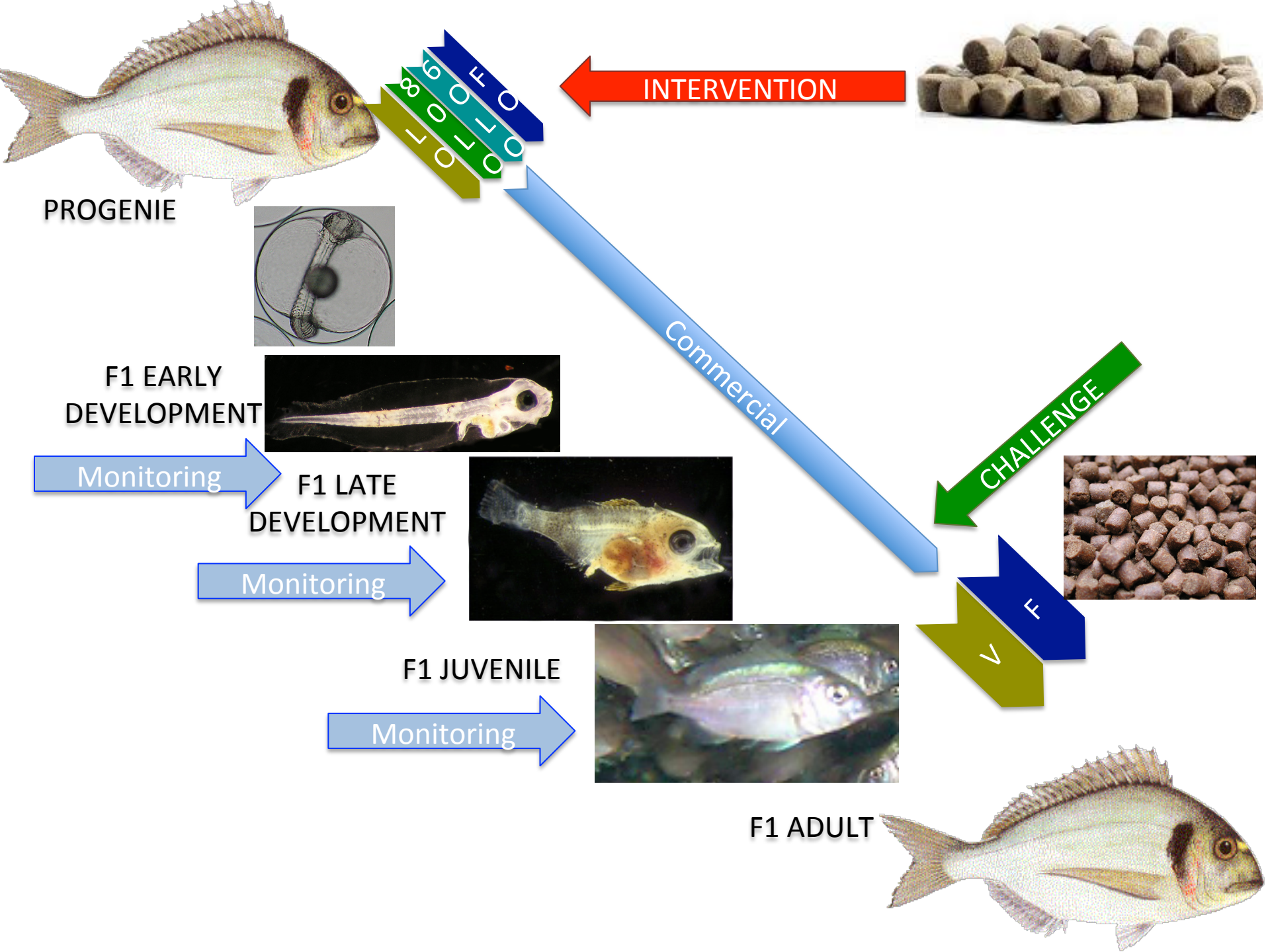
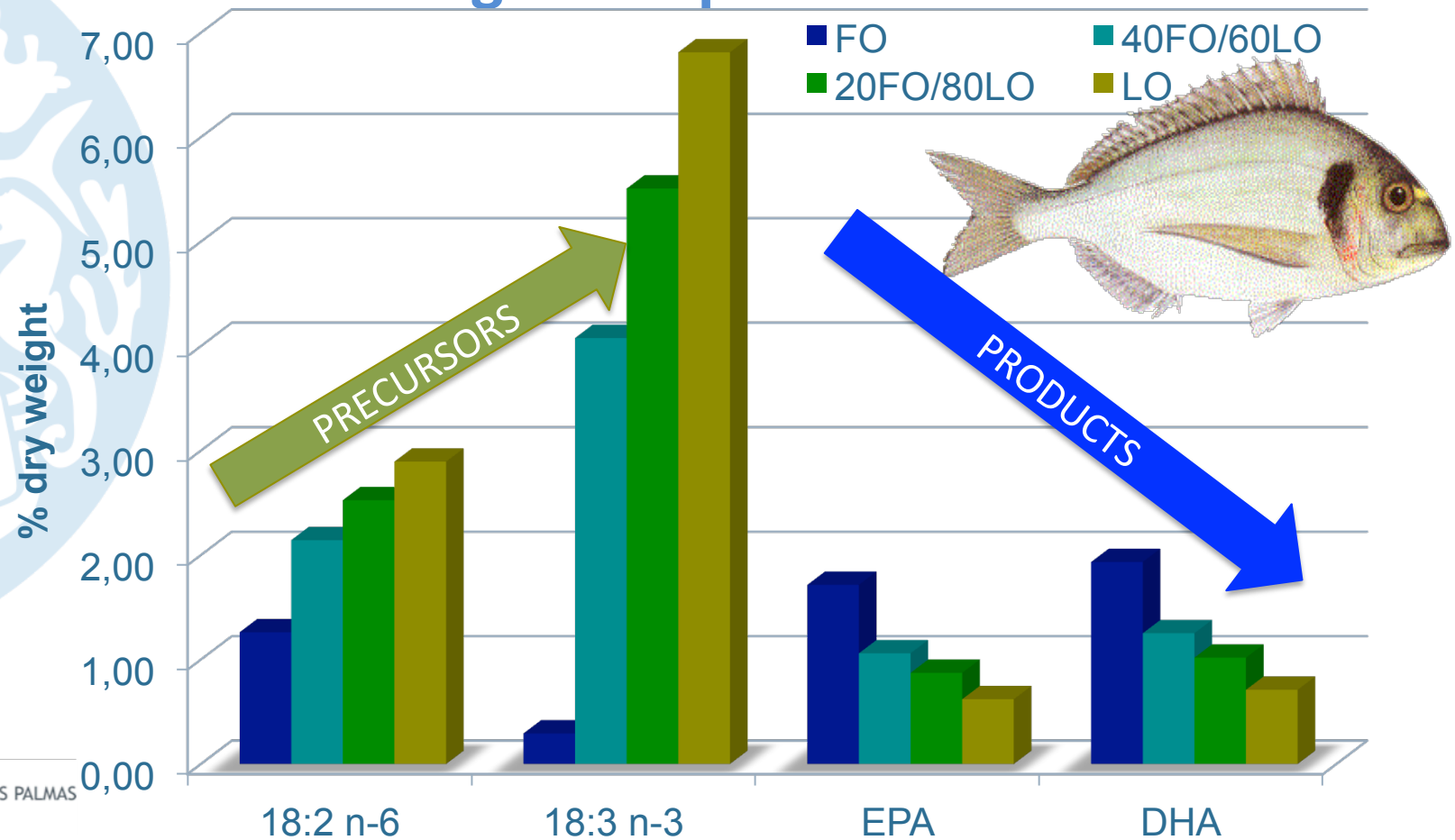

PROGENIE
F1 EARLY DEVELOPMENT
Monitoring
F1 LATE DEVELOPMENT
Monitoring
F1 JUVENILE
Monitoring
F1 ADULT
INTERVENTION
Commercial
CHALLENGE
F
V

Fatty acids from parental diets with 4 LO levels for epigenetic reprogramming during gametogenetic and embriogenetic peridods



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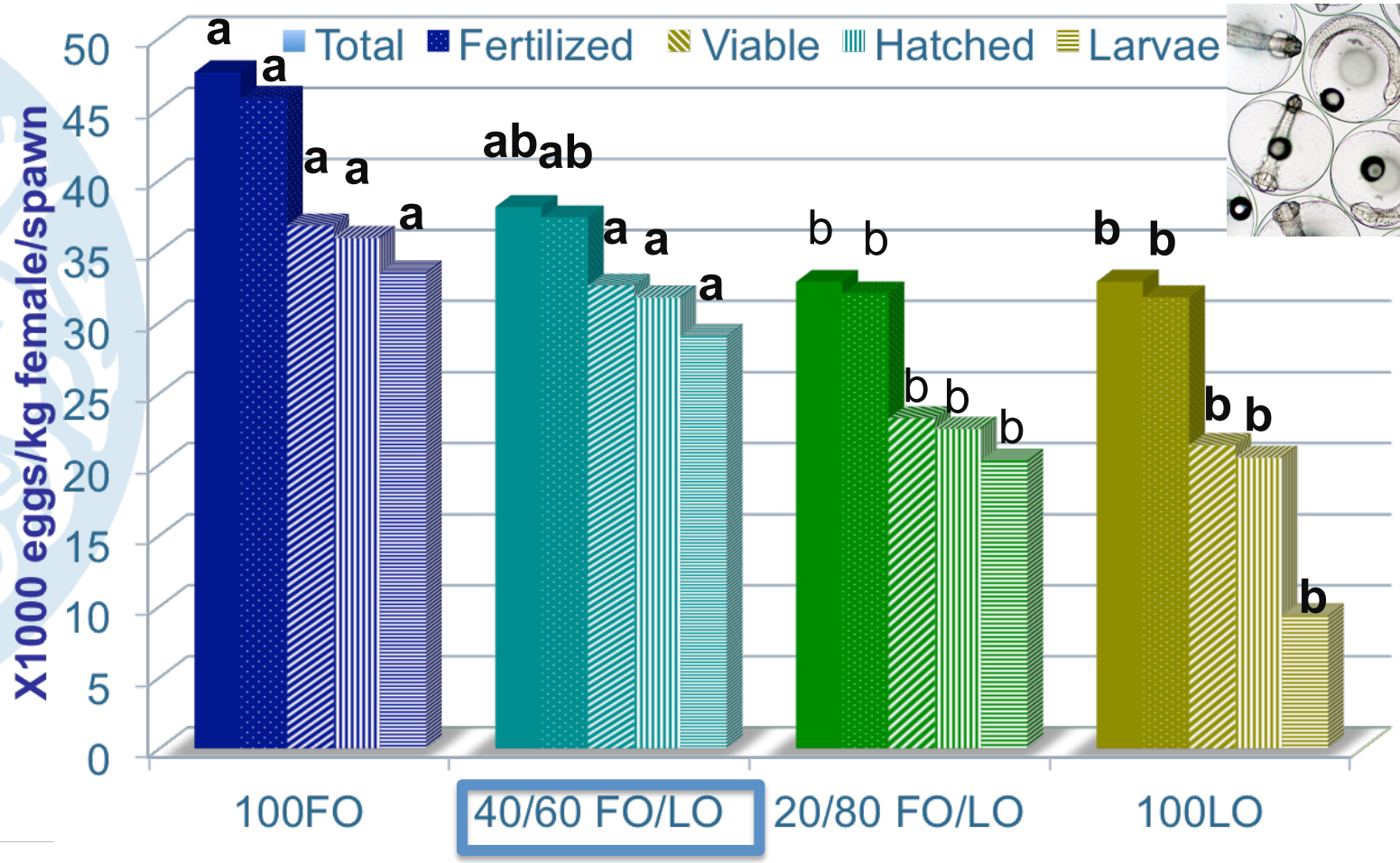
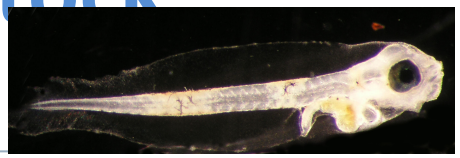
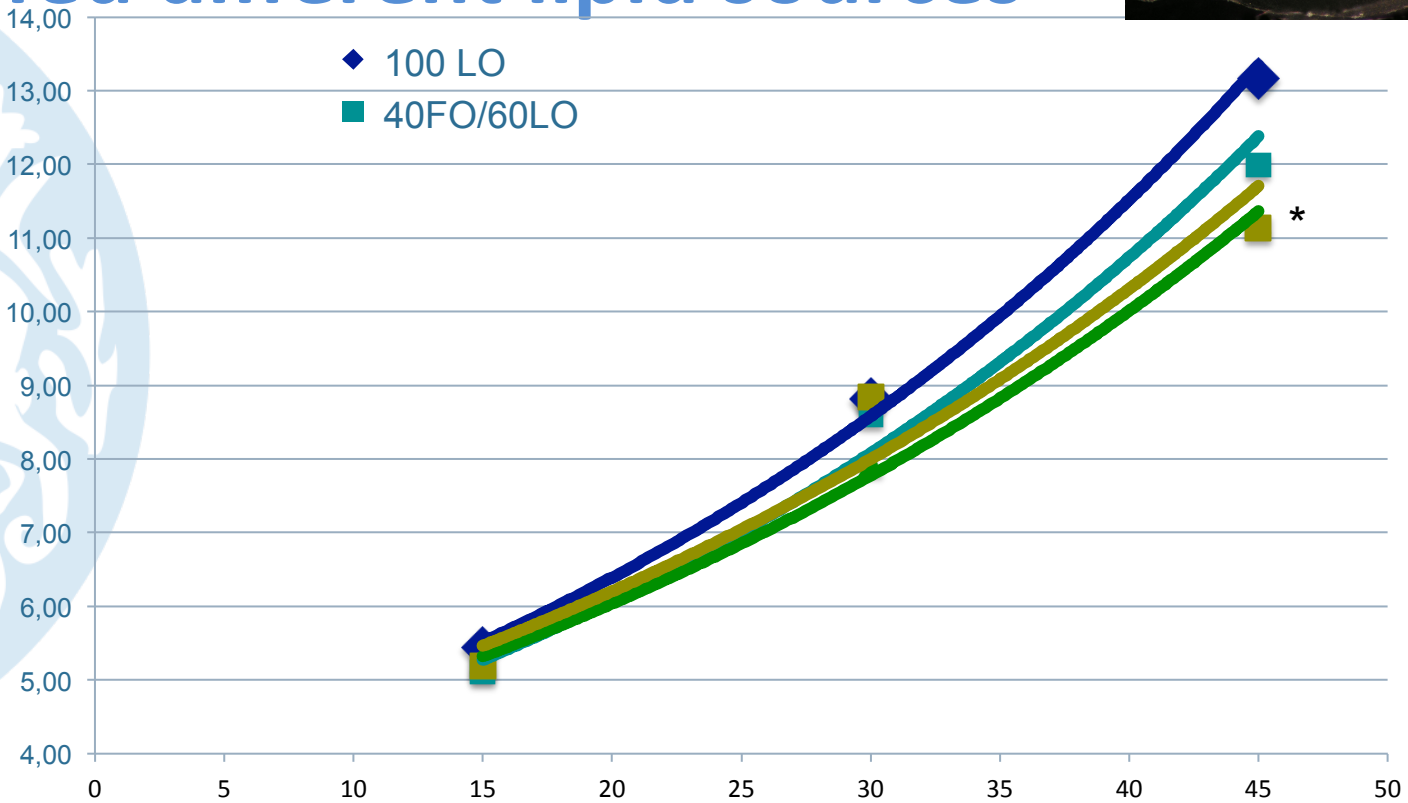
Fecundity of seabream broodstock conditioned with three LO inclusion levels



(Izquierdo *et al.*, 2013, 2015)

Total length of larvae from broodstock fed different lipid sources





(Izquierdo *et al.*, 2013, 2015)

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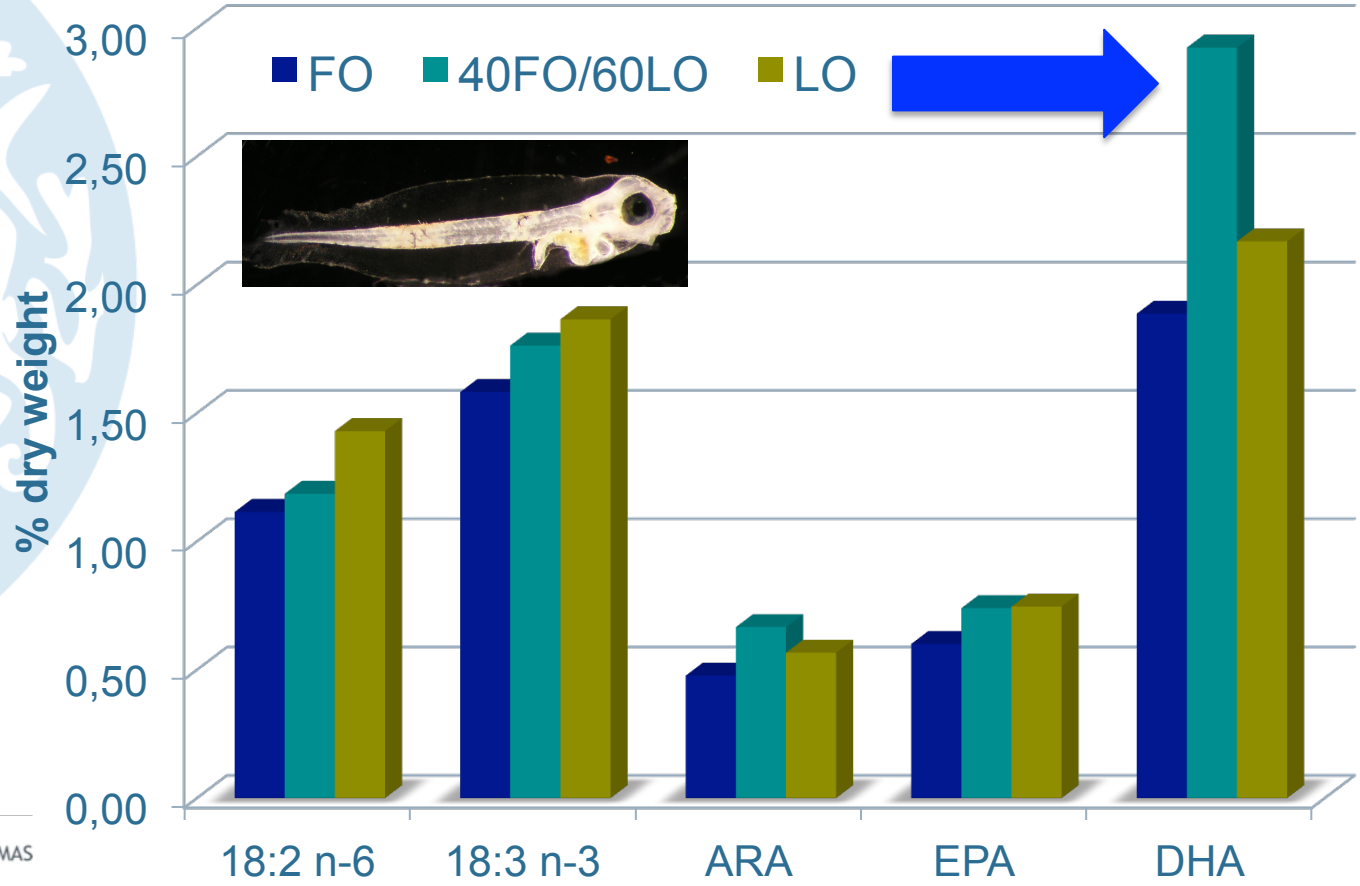
Fatty acids from of 30 dah larvae after programming during embryogenesis with parental diets with 4 LO levels



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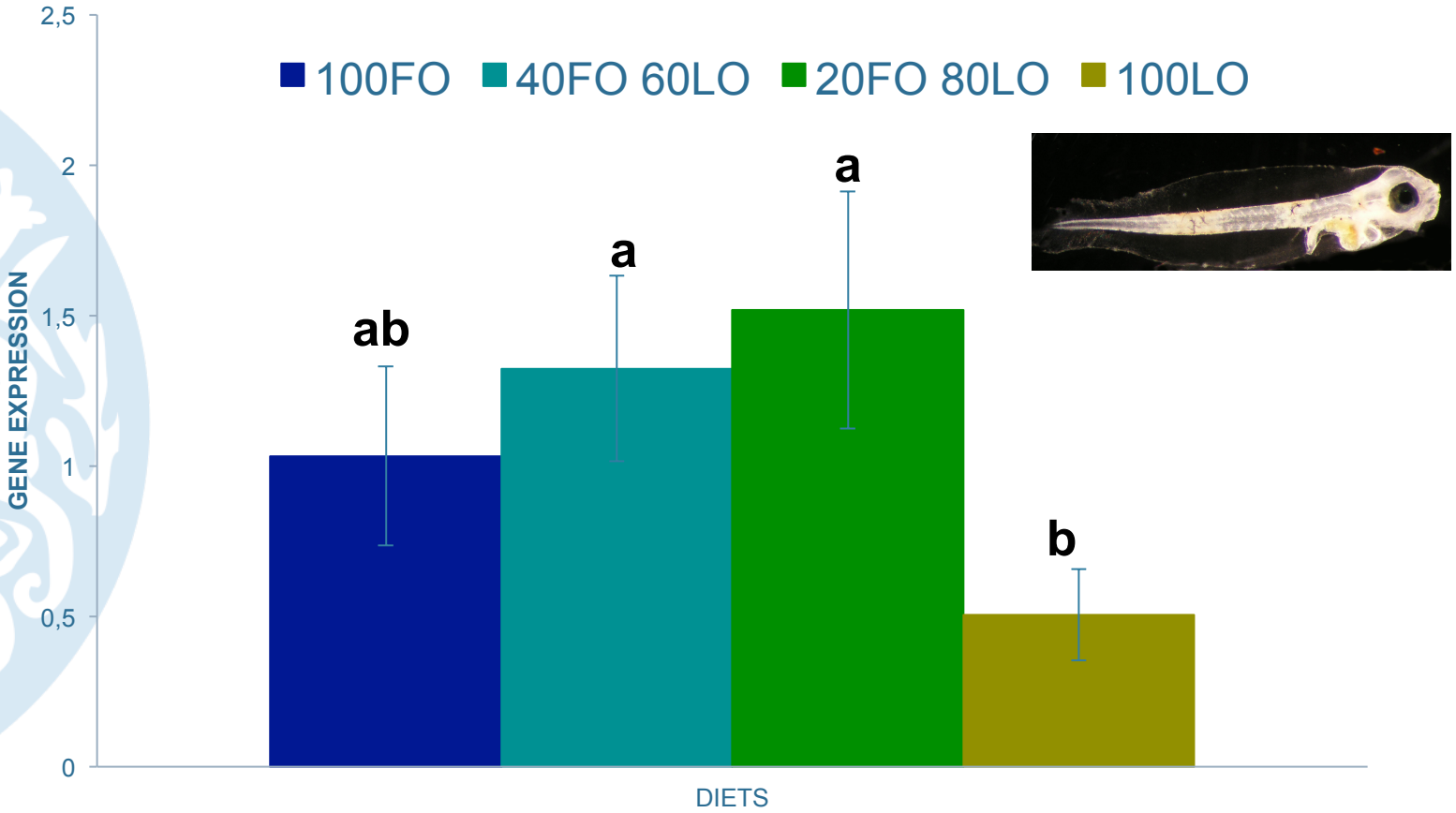
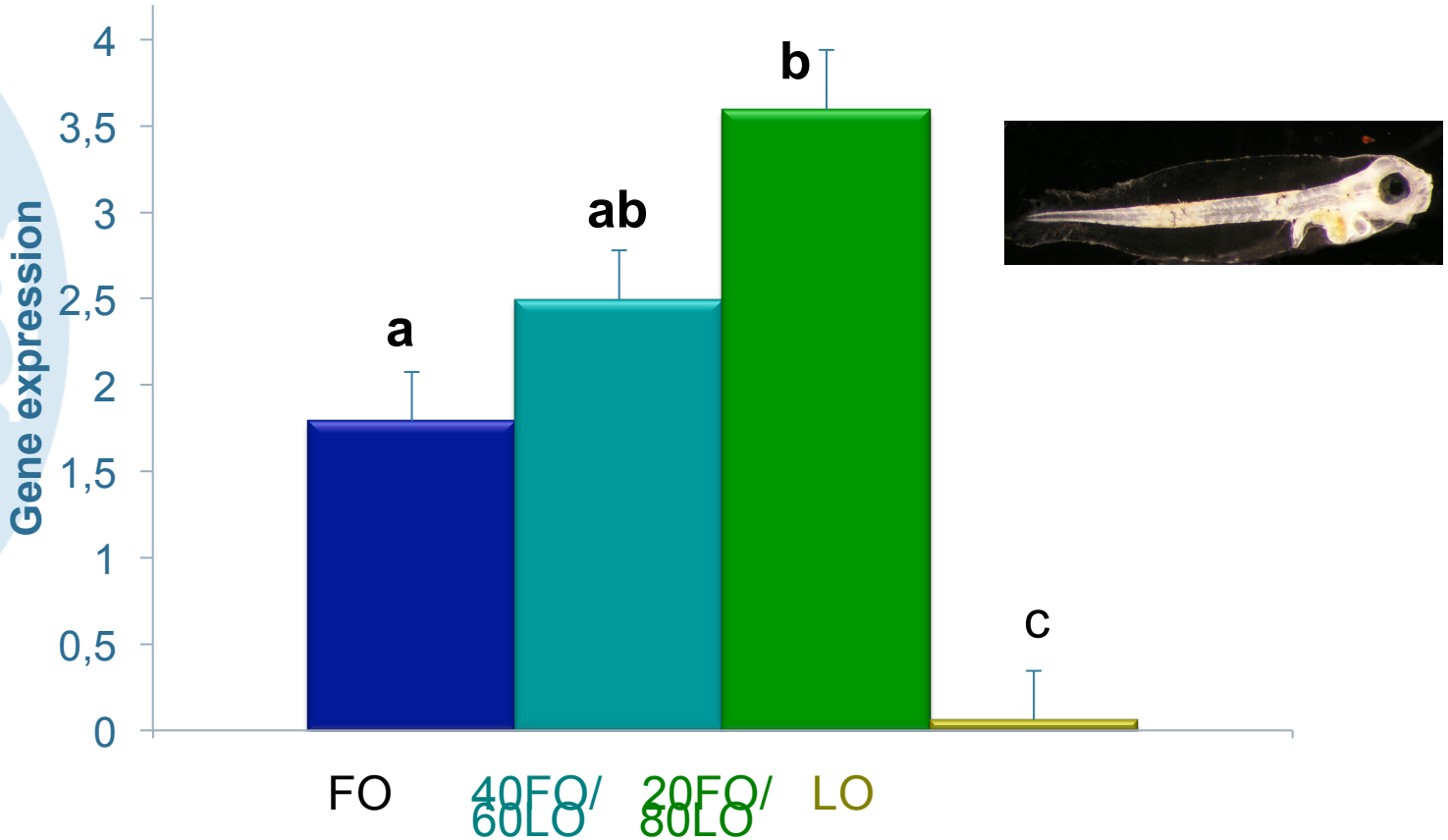
Δ6 desaturase gene in 30 dah fish is up-regulated by parental feeding with 80% LO and down-regulated by 100%



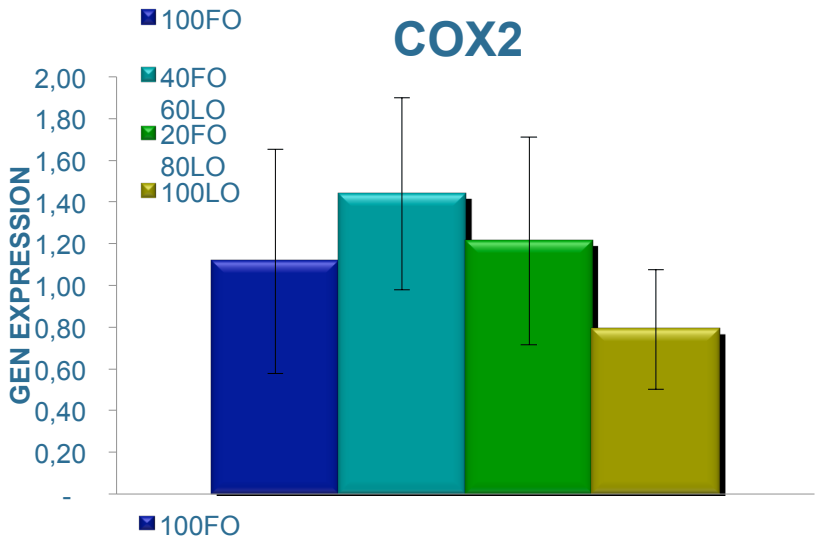
(Fernández-Palacios *et al.*, submitted)

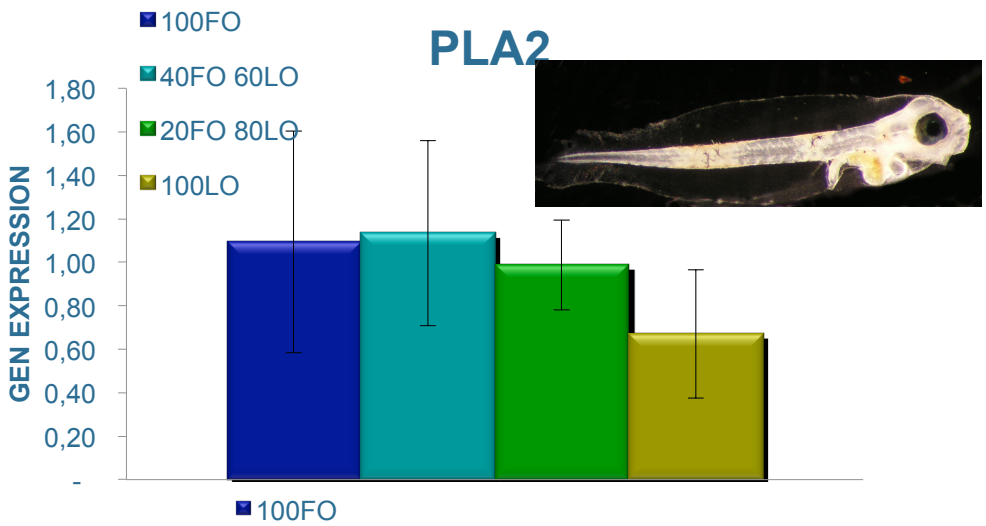
Δ6 Desaturase expression of 45 dah larvae from broodstock conditioned with several LO levels

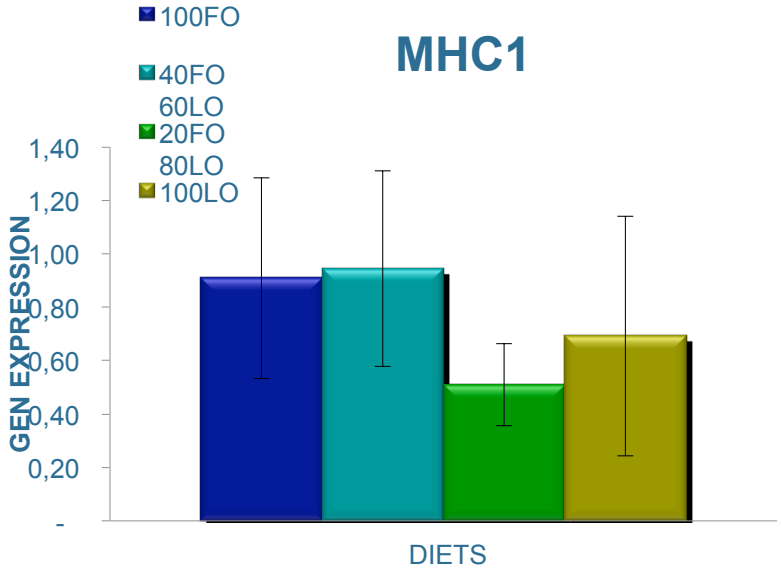


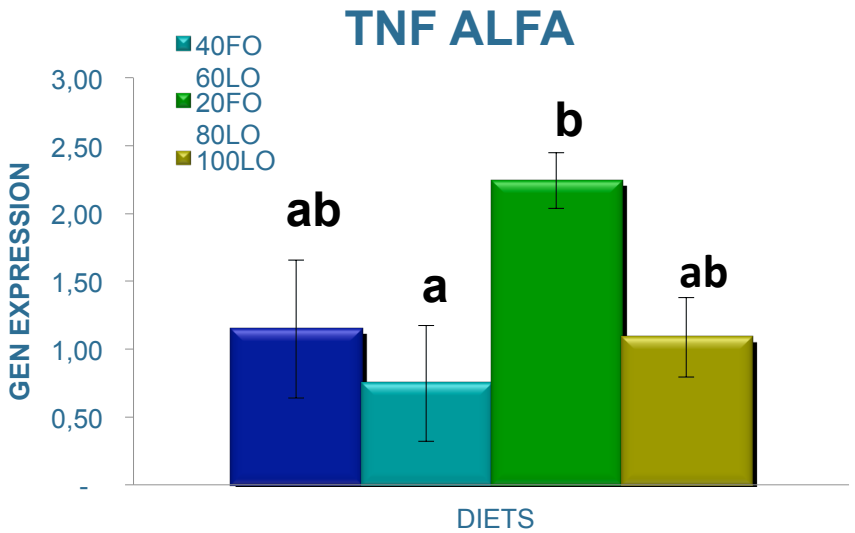
(Izquierdo et al., 2015)

Expression of specific fatty acid related genes in 30 dah larvae larvae after programming during embryogenesis with parental diets with 4 LO levels









Expression of specific fatty acid related genes in 30 dah larvae larvae after parental programming

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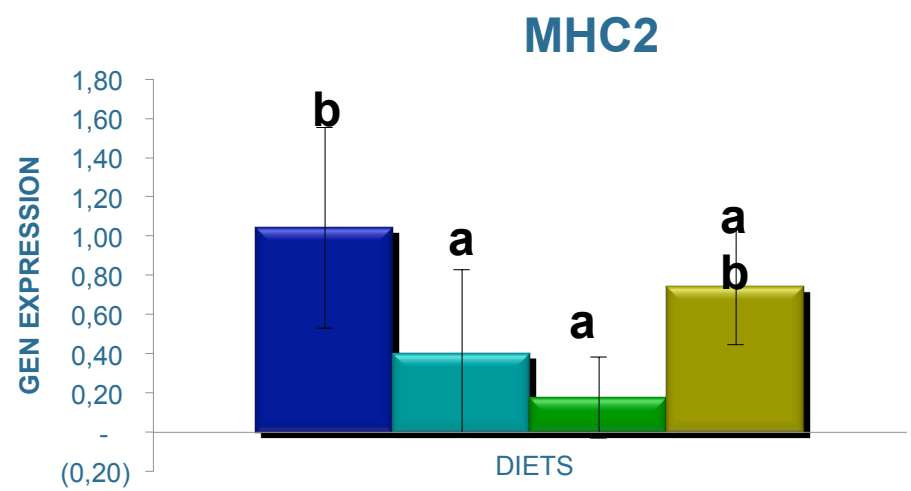
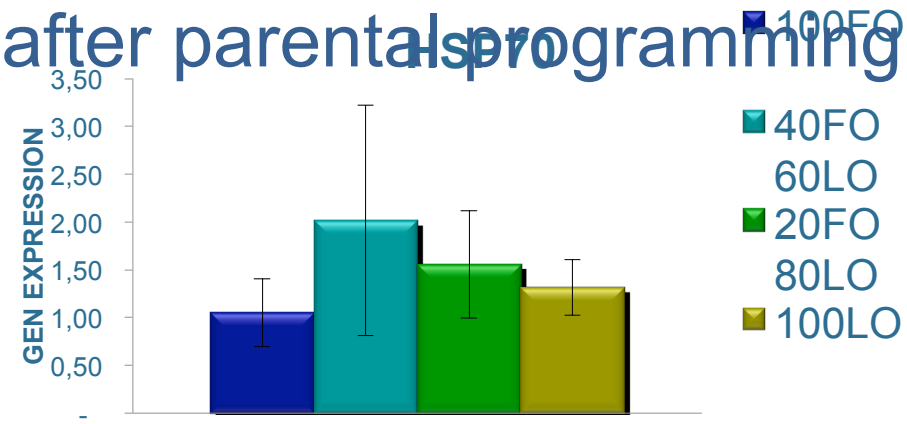
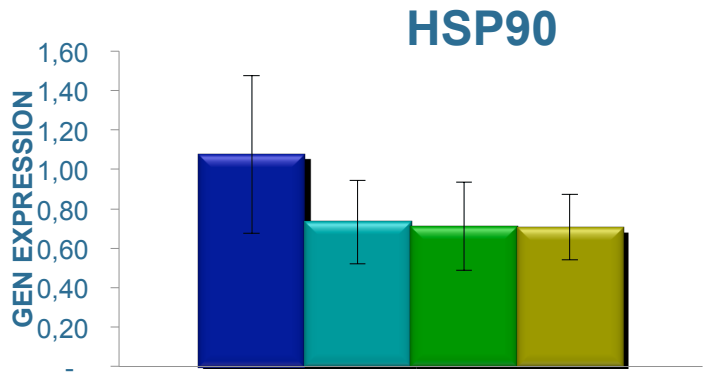
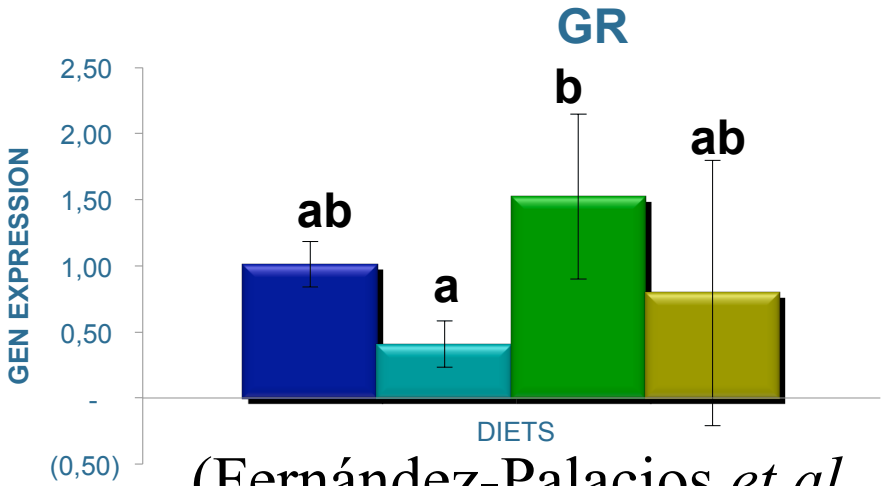
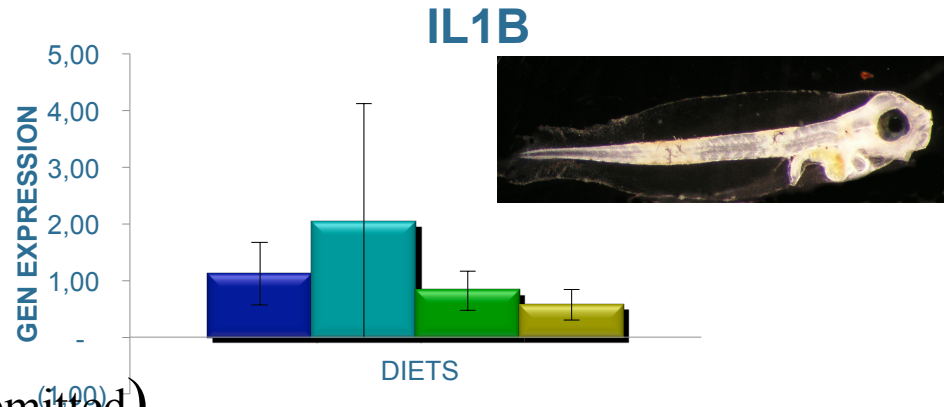




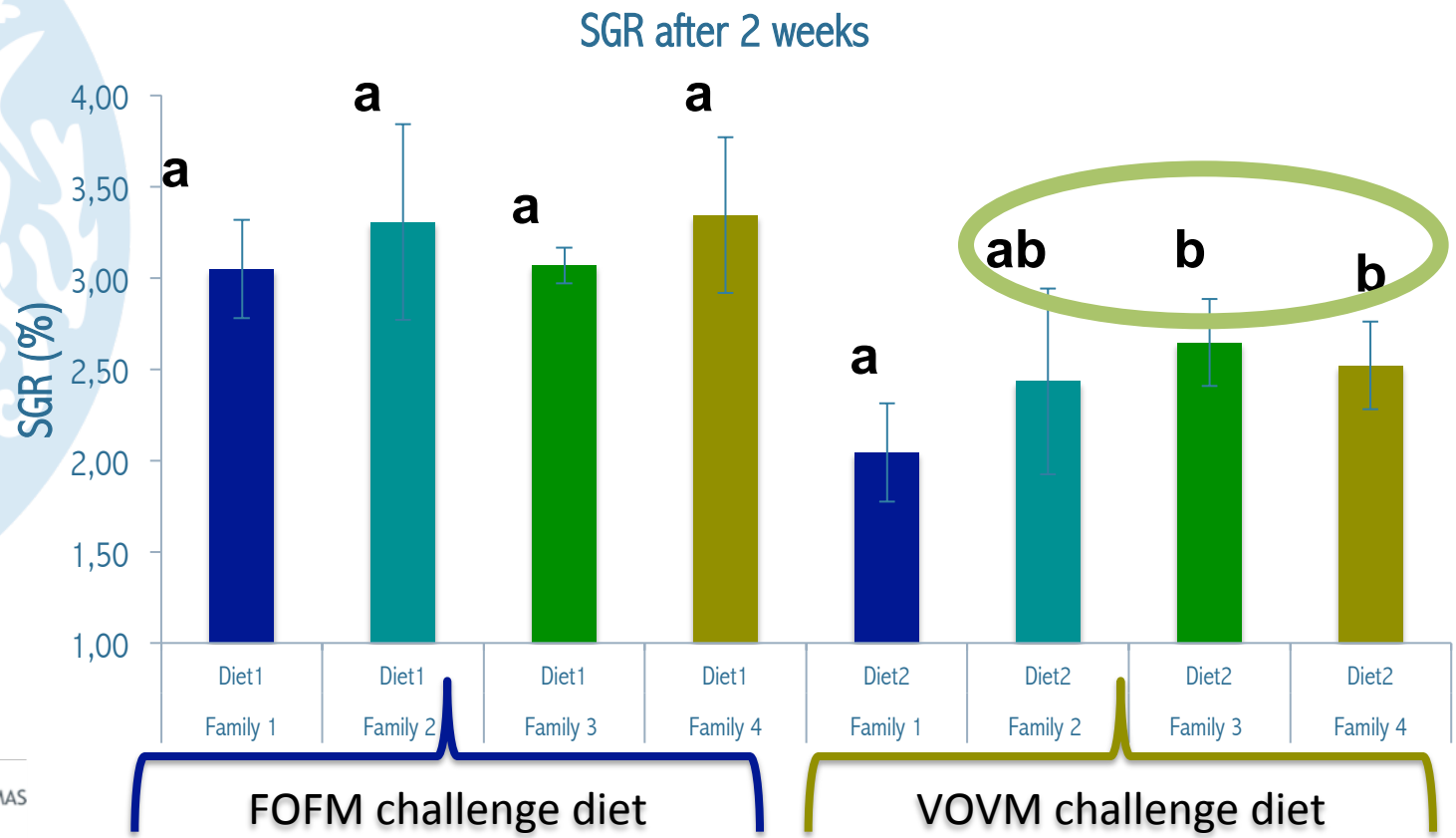






(Fernández-Palacios *et al.*, submitted)

Growth after feed challenge of 4-months juveniles programmed during embryogenesis with parental diets with 4 LO levels



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Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream

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ABSTRACT

The complete removal of fishme
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Nutritional stimuli of gilthead seabream (*Sparus aurata*) larvae by dietary fatty acids: effects on larval performance, gene expression and neurogenesis

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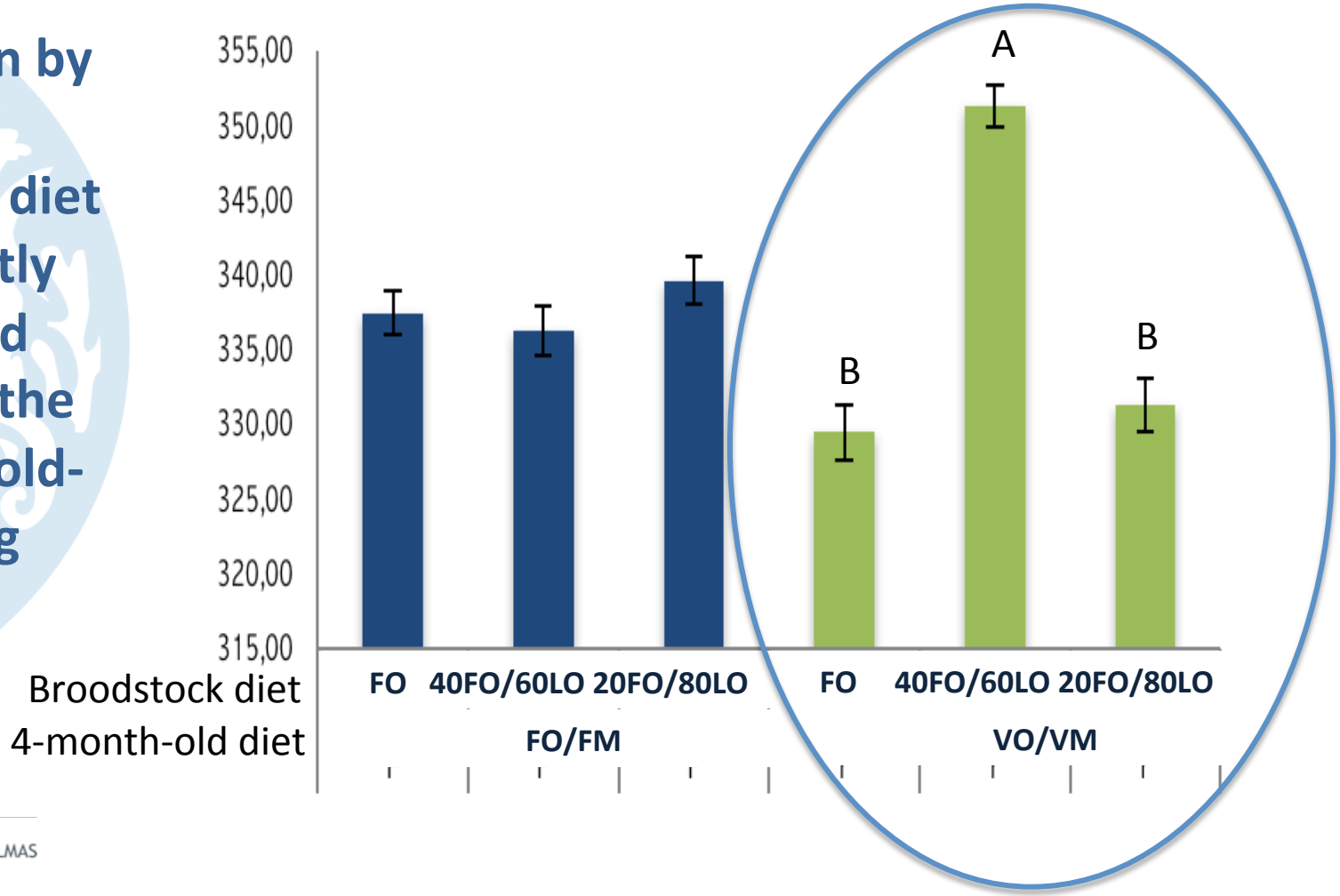
Growth after feed challenge of 16-months juveniles programmed during embryogenesis with parental diets with 4 LO levels

Groups		Initial Body Weight (g)	Final Body Weight (g)	SGR[1] (%/day)	FCR[2]
Ff		243.0±1.9	337.4±3.3	0.7±0.06	^{A}1.5±0.1
LLf		244.1±0.9	^{B}336.3±2.6	^{B}0.6±0.03	^{B}1.7±0.1
HLf		242.5±2.0	339.6±7.2	0.7±0.05	1.6±0.1
Fv		243.6±0.9	319.5±5.5^{b}	0.5±0.07^{b}	^{B}1.8±0.1^{b}
LLv		246.4±0.4	^{A}351.3±1.2^{a}	^{A}0.7±0.03^{a}	^{A}1.4±0.2^{a}
HLv		242.3±1.3	321.3±4.0^{b}	0.6±0.07^{b}	1.9±0.2^{b}
Two-way ANOVA*					
Broodstock Diet	*(B)*	*n.d.*	***p<0.05***	*n.d.*	*n.d.*
Reminder Diet	*(R)*	*n.d.*	*n.d.*	***p<0.05***	*n.d.*
B × R		*n.d.*	***p<0.01***	***P<0.05***	***p<0.01***

Effect of broodstock feeding with reduced FO contents on final weight in the 16-months offspring fed at 4-months-old with FO (blue) or LO (green)

60%FO substitution by LO in broodstock diet significantly improved growth in the 16 month-old-offspring



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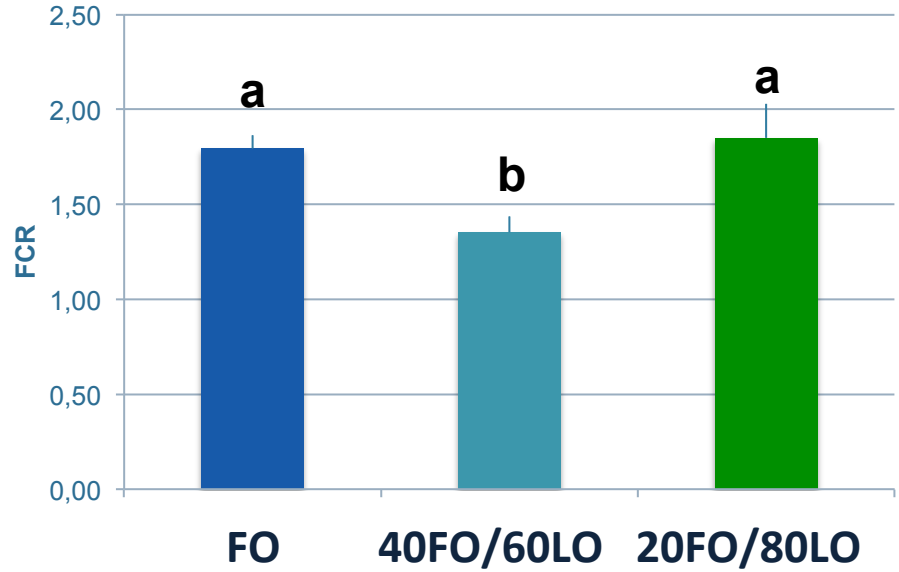
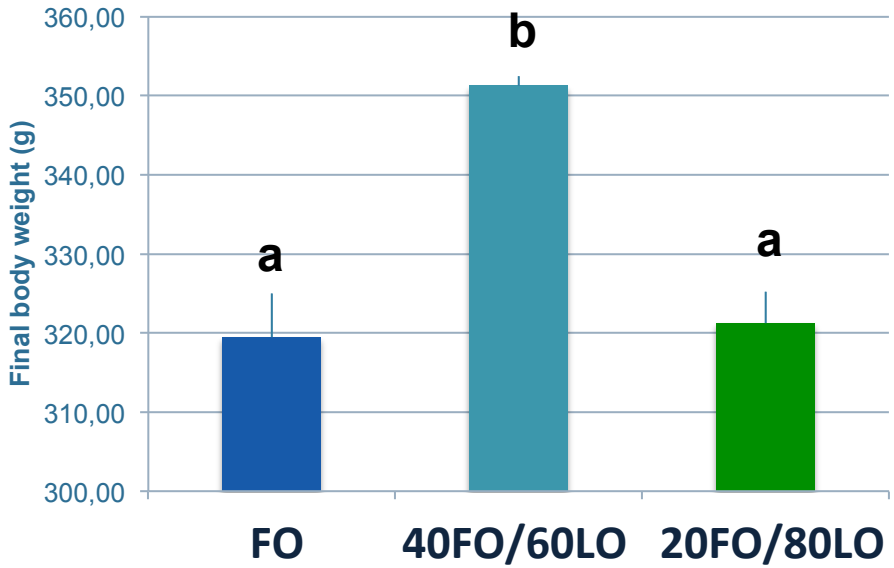
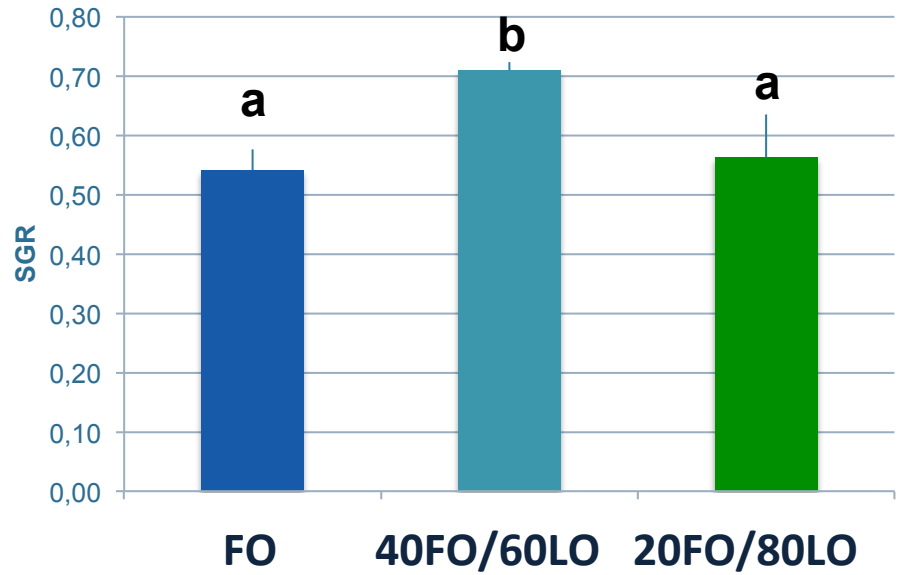
Nutritional programing through broodstock diets allows in only 2 months of feeding low FM&FO diets a 24% improvement in FCR, a 12% improvement in SGR

Effect of broodstock feeding on 16 month-old gilthead seabream challenged at 4-months-old with VM/VO diet







Effect of broodstock feeding with reduced FO contents on expression of selected genes in the liver of 16-months offspring

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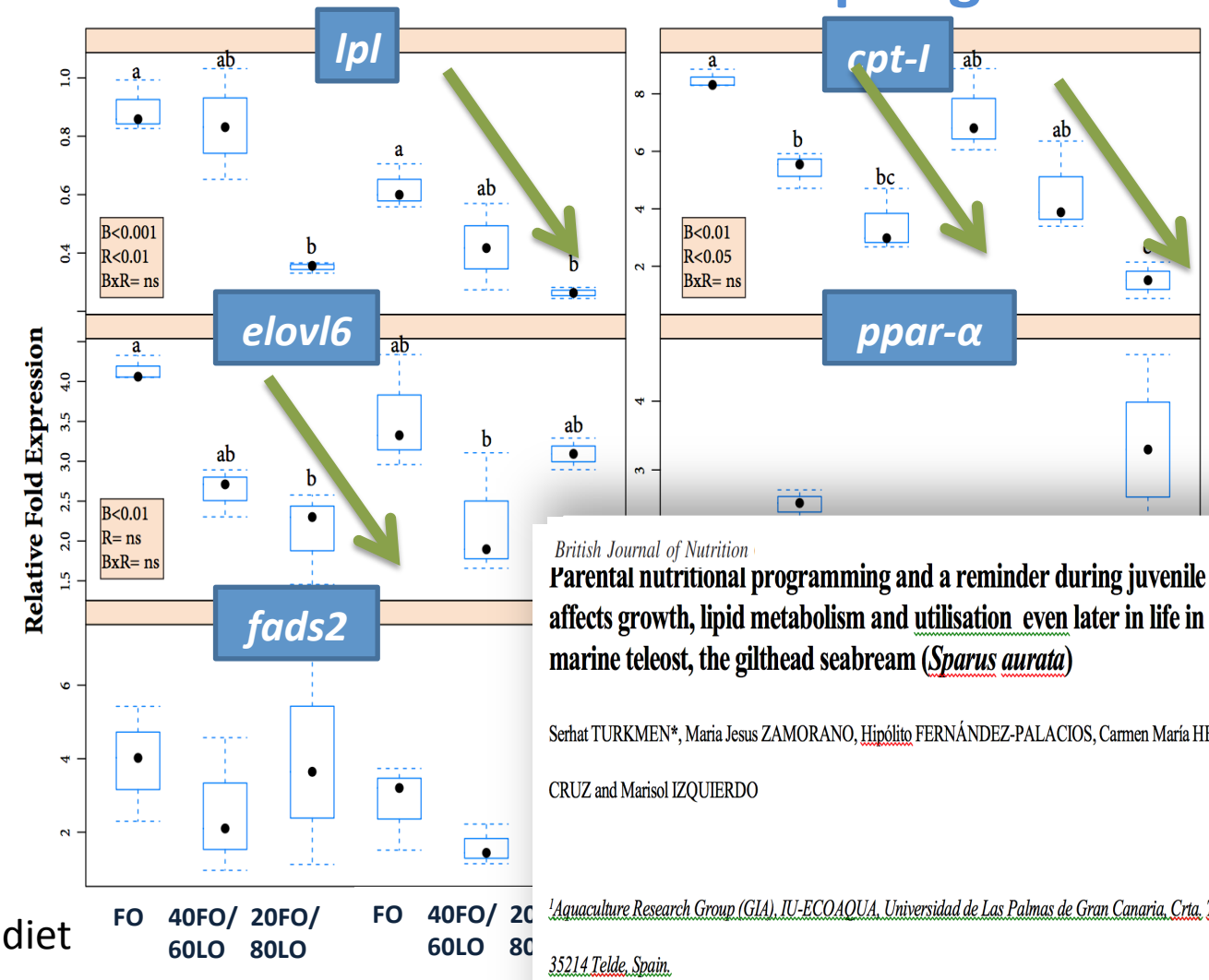
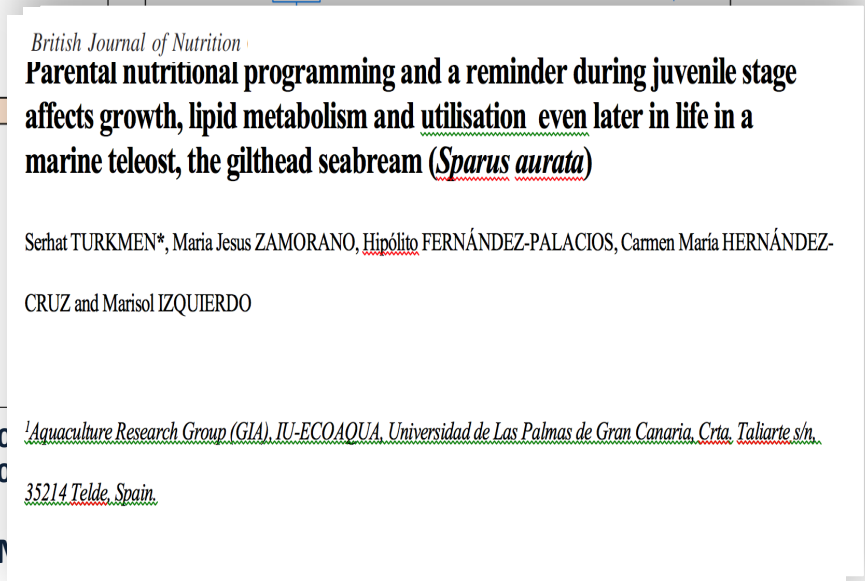
Nutritional programing through regulation of *lpl, elovl6 and cpt-I*, may prepare the offspring for a better utilisation of VM and VO diets and reducing the risk of hepatosteatosis



Broodstock diet

4-month-old diet



British Journal of Nutrition

Parental nutritional programming and a reminder during juvenile stage affects growth, lipid metabolism and utilisation even later in life in a marine teleost, the gilthead seabream (*Sparus aurata*)

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Conclusions

- Broodstock feeding with moderate FO replacement by LO combined with juvenile feeding with low FM and FO diets improves offspring growth and feed utilisation of low FM/FO diets even when they are 18 months-old
- Offspring from fish selected for high fads2 expression showed faster growth regardless the diet fed to the broodstock

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Thanks for your attention!

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