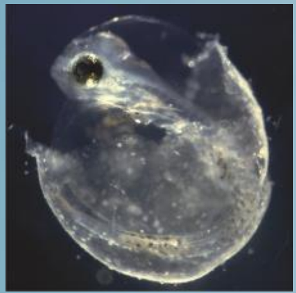
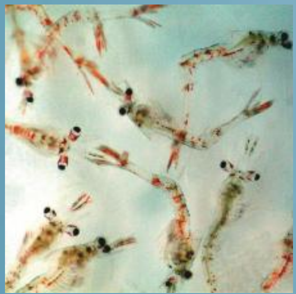
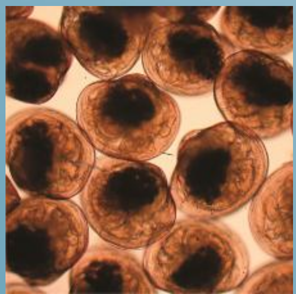
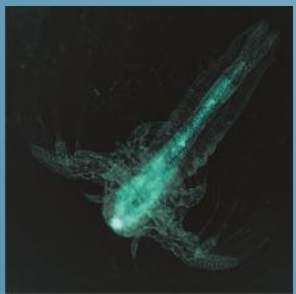








larvi 2013

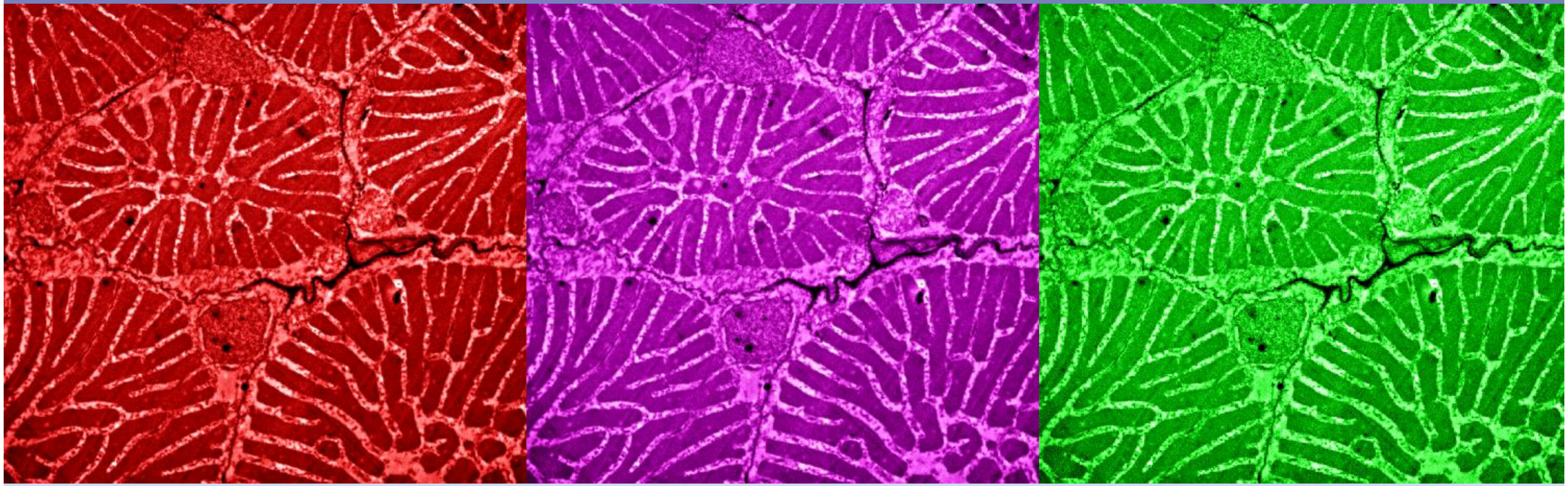
6th fish & shellfish larviculture symposium

Oxidative stress in sea bass (*Dicentrarchus labrax*) larvae interaction of high dietary DHA contents and several antioxidant nutrients

Monica Betancor

ghent university, belgium, 2-5 september 2013

Oxidative stress in sea bass (*Dicentrarchus labrax*) larvae fed on high DHA microdiets. Involvement of several antioxidant nutrients



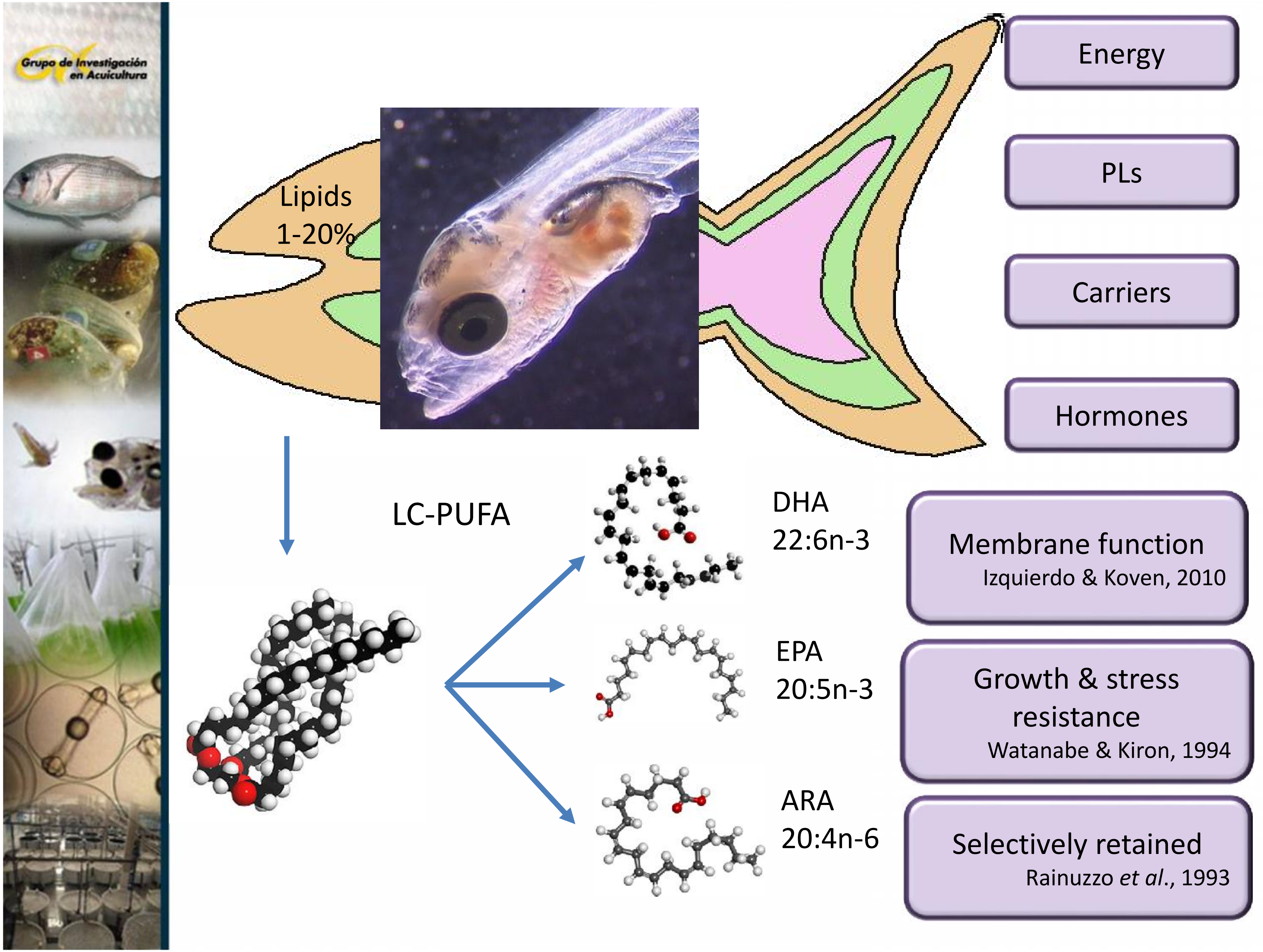
Grupo de Investigación en Acuicultura

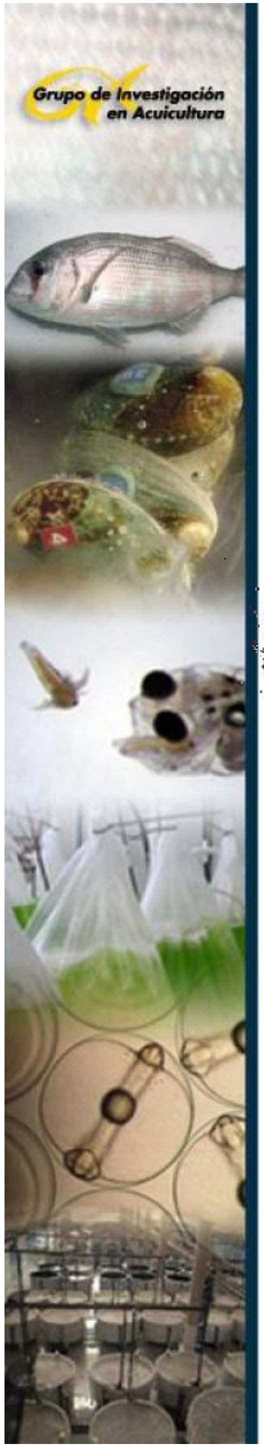
University of Las Palmas de GC,
Canary Islands, Spain

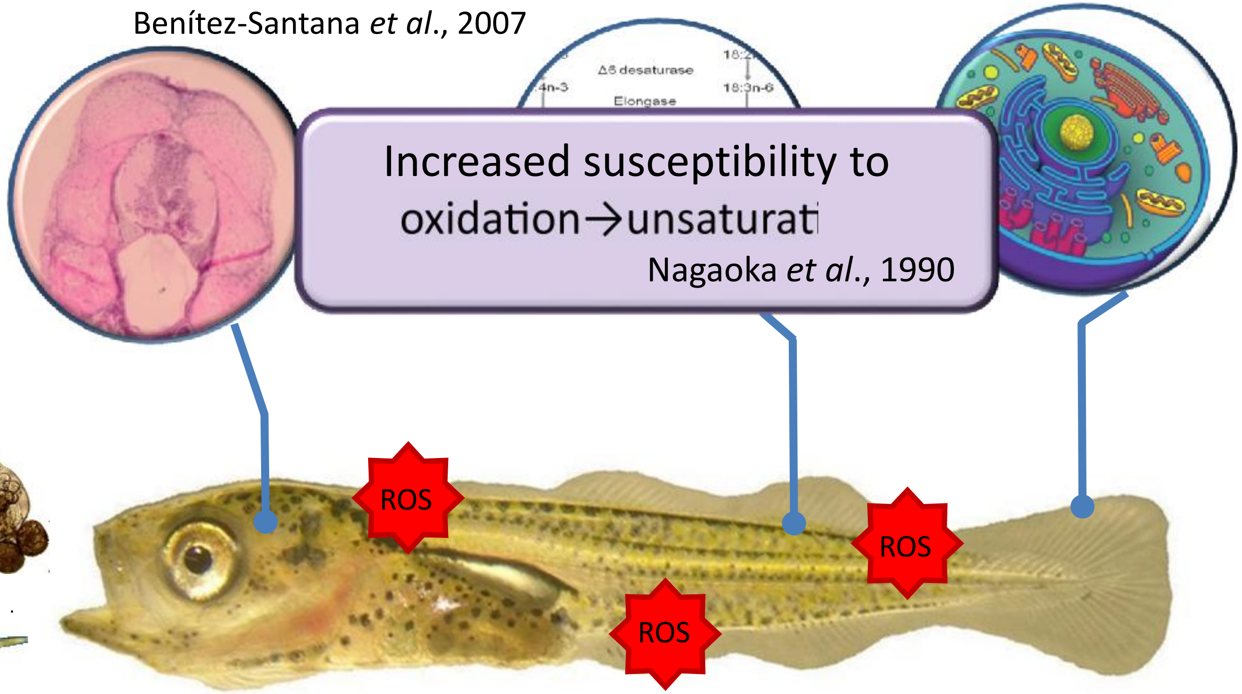
UNIVERSITY OF STIRLING

*Author present address

Mónica B Betancor*
Mª José Caballero
Marisol Izquierdo

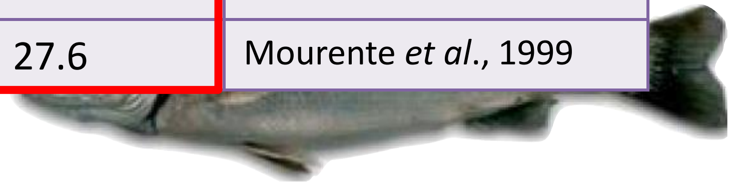

Grupo de Investigación en Acuicultura
Lipids 1-20%
Energy
PLs
Carriers
Hormones
LC-PUFA
DHA 22:6n-3
EPA 20:5n-3
ARA 20:4n-6
Membrane function
Izquierdo & Koven, 2010
Growth & stress resistance
Watanabe & Kiron, 1994
Selectively retained
Rainuzzo et al., 1993





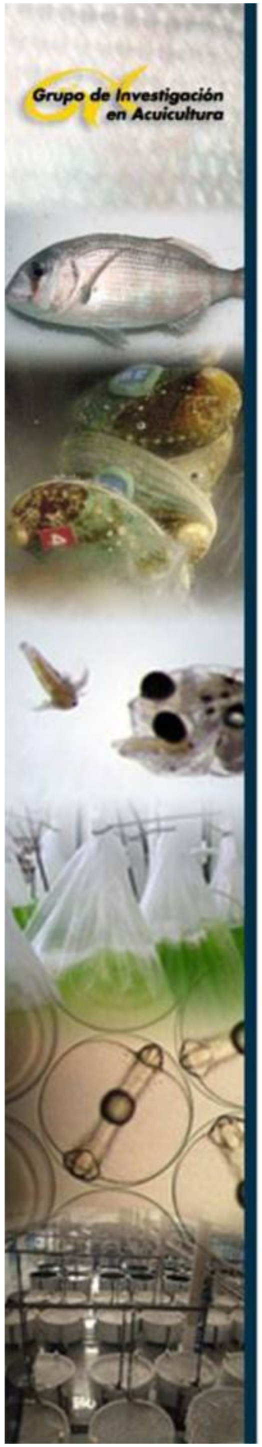
Species	Larvae DHA requirements (%)	Larvae DHA content (% total fatty acid)	Authors (Requirements)
Sparus aurata	>3	30.1± 0.4	Izquierdo, 2005
Scophtalmus maximus	3.2	27.0 ± 0.3	Le Milinaire, 1984
Seriola dumerilii	4	-	Izquierdo, 2005
Pagrus pagrus	3.4	26.9	Hernández *et al.*, 1999
Dentex dentex	4	27.6	Mourente *et al.*, 1999

2011



Laurel *et al.*, 2010

What is the effect of high DHA dietary levels on sea bass larvae?



Experimental conditions:

-Sea bass larvae

- 170 L grey cylinder fibre glass tanks

-Temperature 19.5-20°C

-Oxygen 5-8 g L^{-1}

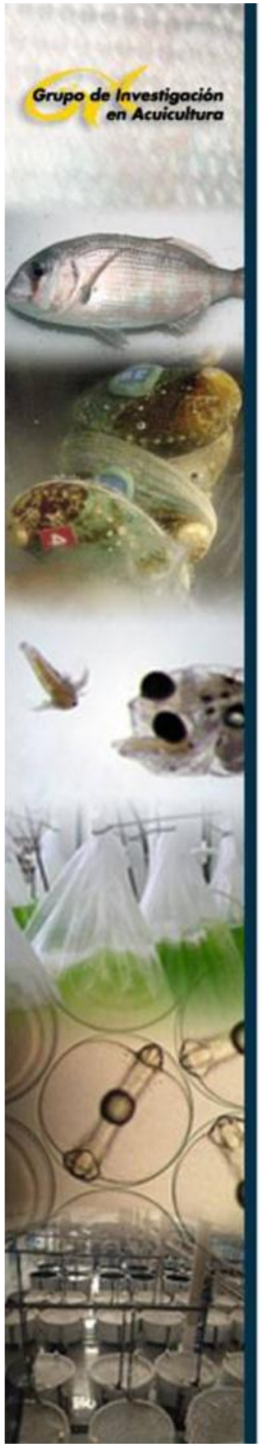
-Salinity 34 g L^{-1}

-Photoperiod 12:12

-Manually cleaned

-Water flow 1.0-1.5 L min^{-1}

Grupo de Investigación en Acuicultura



Diets and Experimental Design

CRODA

-Five experiments

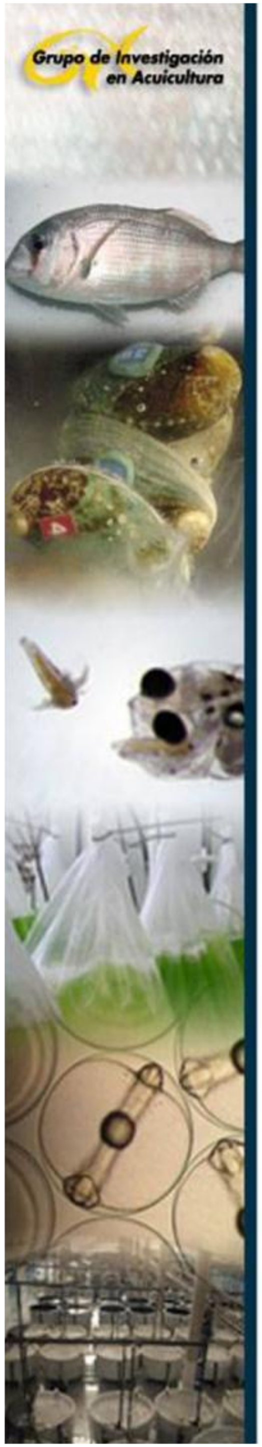
-Two, three or five weeks

SIGMA-ALDRICH

α-TOH (mg/100g) \ DHA (g/100g)	1	3	5
150	1/150	3/150	5/150
300	1/300	3/300	5/300

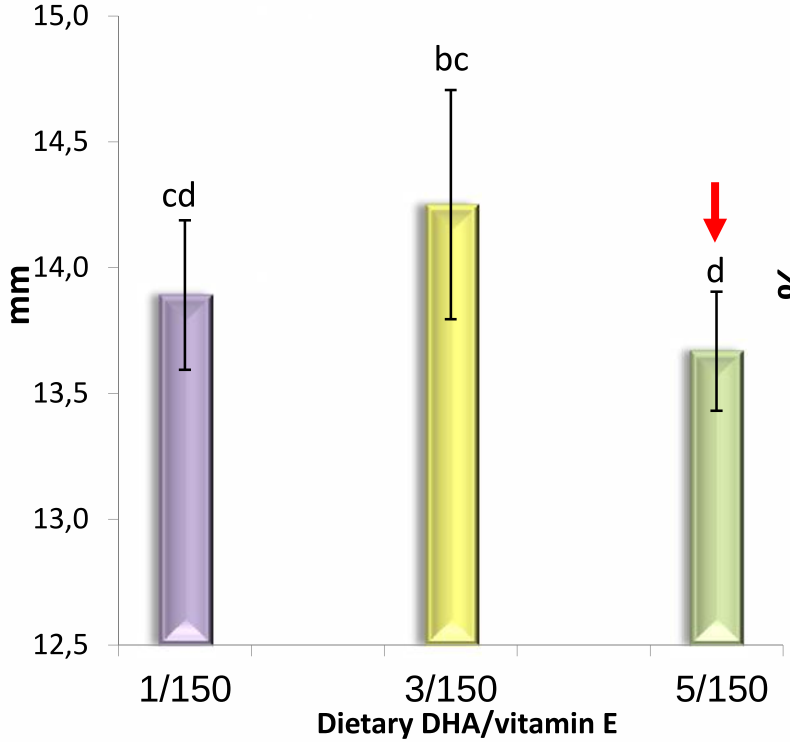
5/300+ Selenium

5/300+ Vitamin C

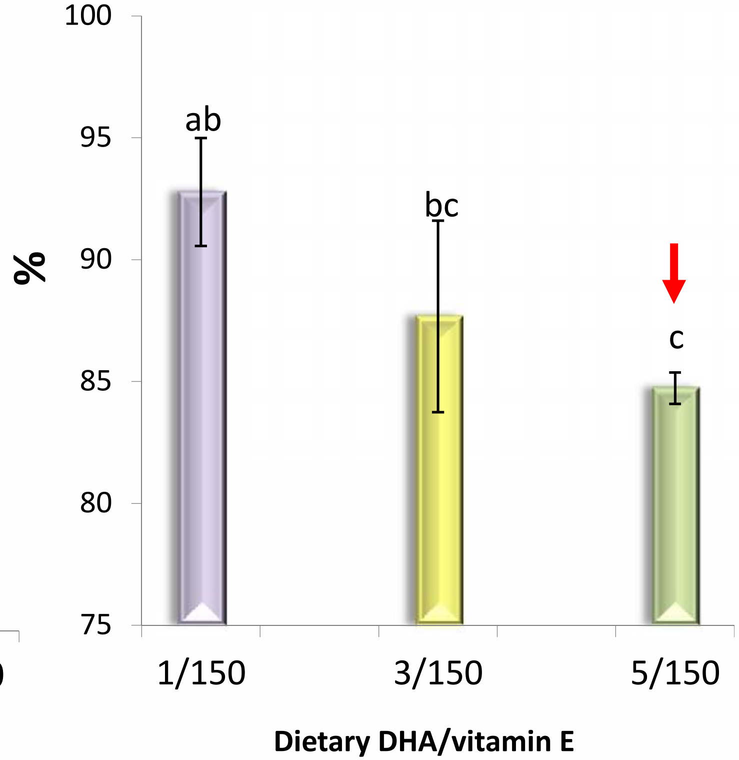


Betancor *et al.*, 2011. Aquacult Nutr

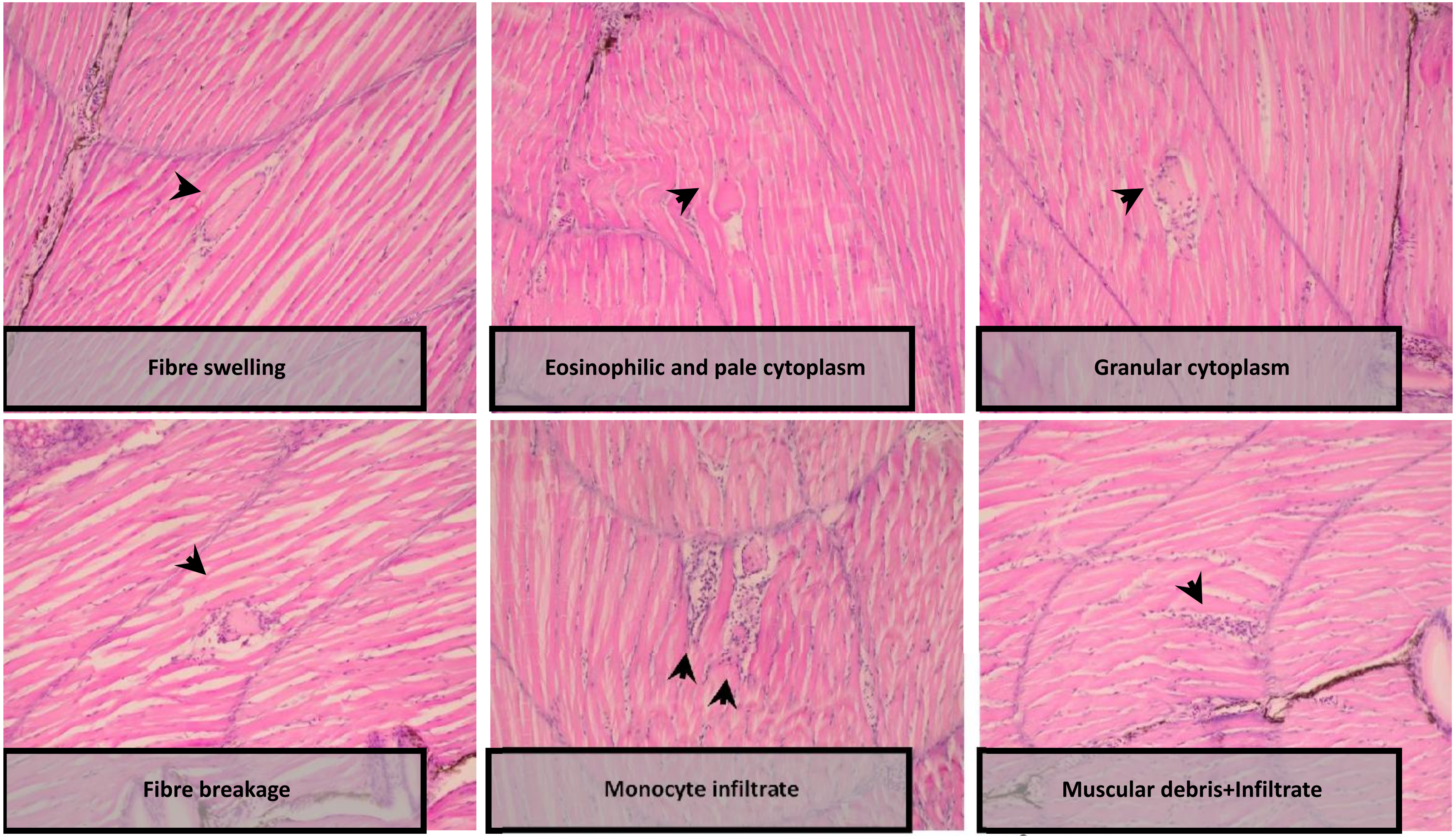
Total length



Final survival

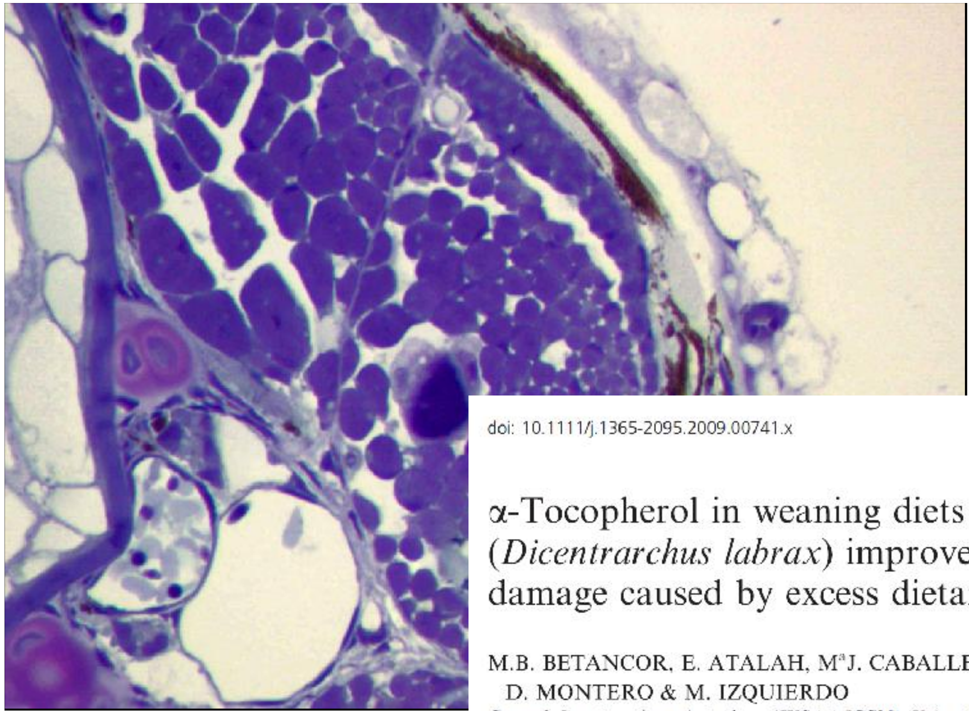


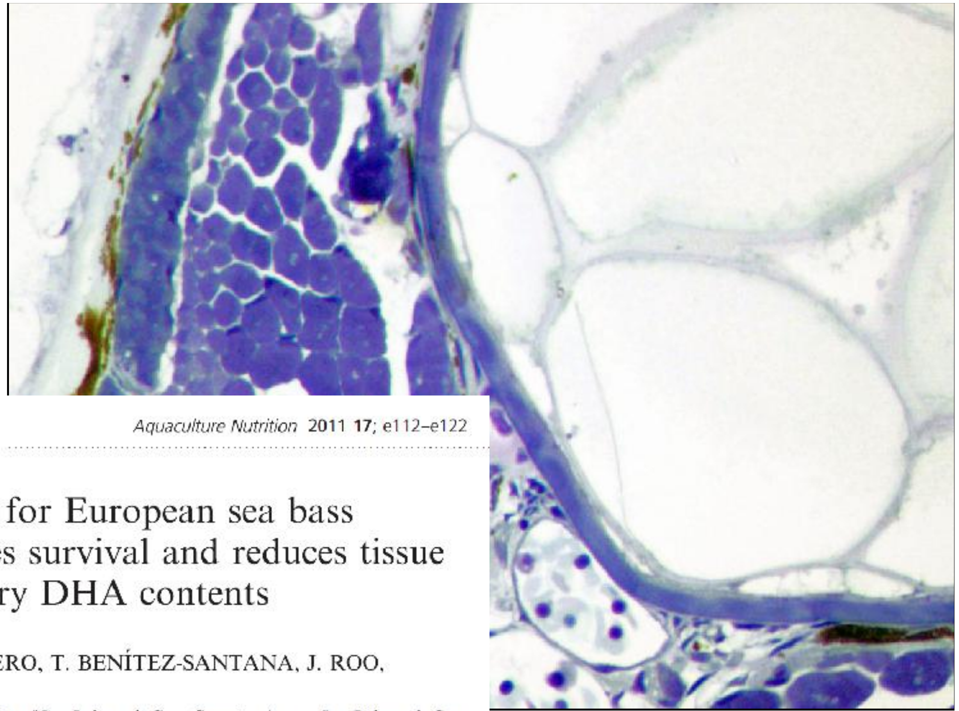
High DHA
↓
Reduced growth and survival



Muscular dystrophy

Lovell *et al*., 1984; Gatlin *et al*., 1986; Frischknecth et al., 1994; Bowater & Burren, 2007; Lebold *et al*., 2013





doi: 10.1111/j.1365-2095.2009.00741.x

Aquaculture Nutrition 2011 **17**; e112–e122

α-Tocopherol in weaning diets for European sea bass (*Dicentrarchus labrax*) improves survival and reduces tissue damage caused by excess dietary DHA contents

M.B. BETANCOR, E. ATALAH, Mª J. CABALLERO, T. BENÍTEZ-SANTANA, J. ROO, D. MONTERO & M. IZQUIERDO

Grupo de Investigación en Acuicultura (IUSA & ICCM), University of Las Palmas de Gran Canaria, Arucas, Las Palmas de Gran Canaria, Canary Islands, Spain

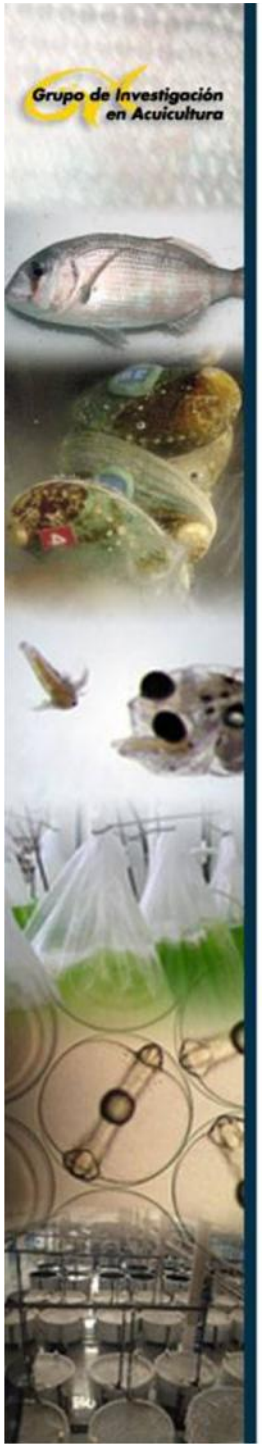
Journal of Fish Diseases 2013, 36, 453–465

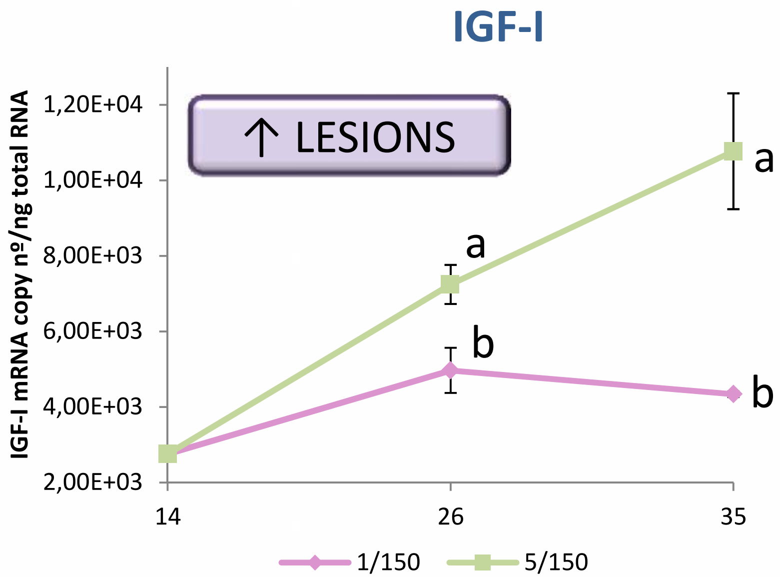
doi:10.1111/j.1365-2761.2012.01447.x

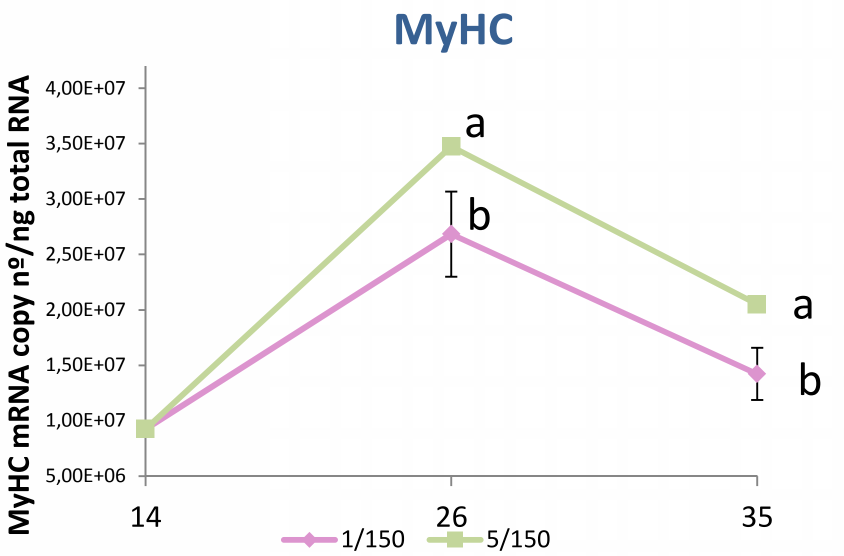
Oxidative status and histological changes in sea bass larvae muscle in response to high dietary content of docosahexaenoic acid DHA

M B Betancor[1,*], M J Caballero[1], T Benítez-Santana[1], R Saleh, J Roo[1], E Atalah[1] and M Izquierdo[1]

1 Grupo de Investigación en Acuicultura, University of Las Palmas de Gran Canaria, Instituto Universitario de Sanidad Animal, Las Palmas de Gran Canaria, Spain





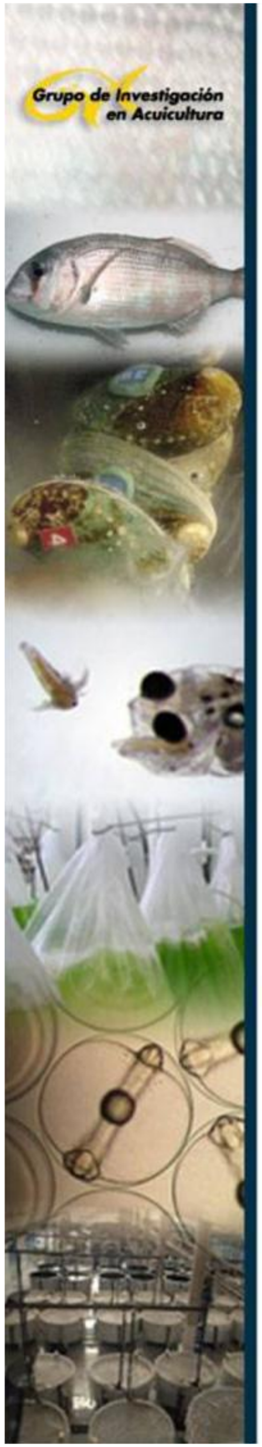


Regenerating fibres
Rowlerson *et al.*, 1997

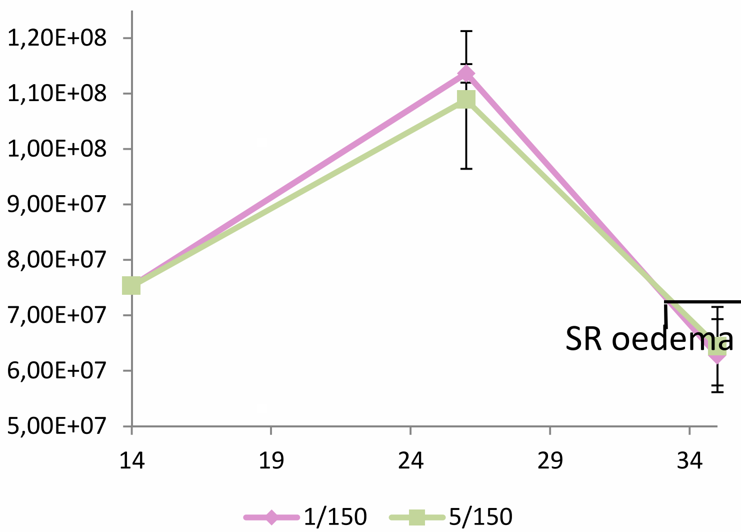
Satellite cell pool control
Grounds ,1999; Seale & Rudnicki, 2000

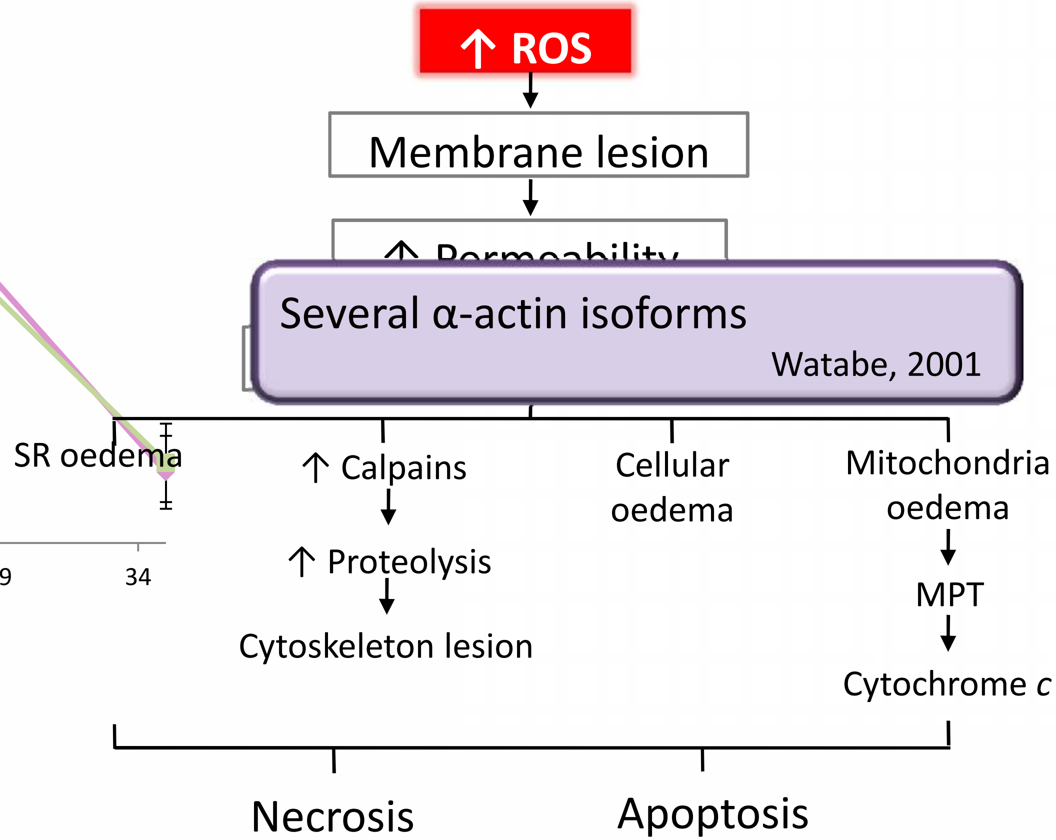
Muscle regeneration process

Betancor *et al.*, 2013. CBPA

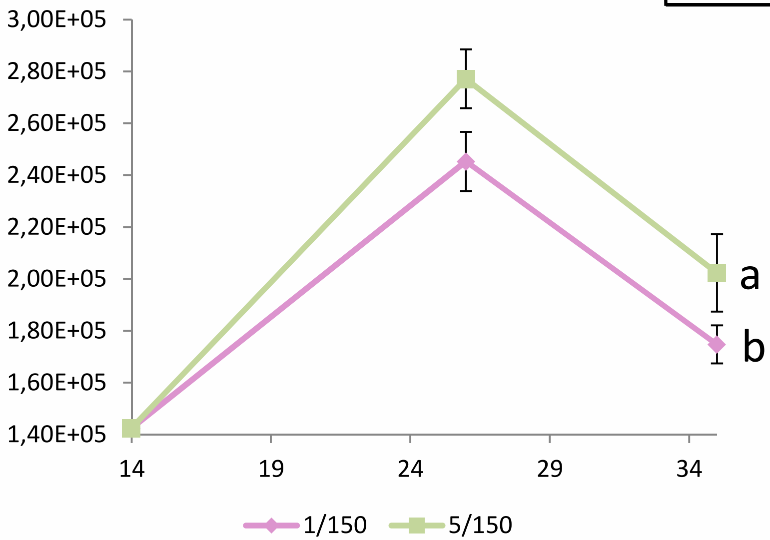


α-actin



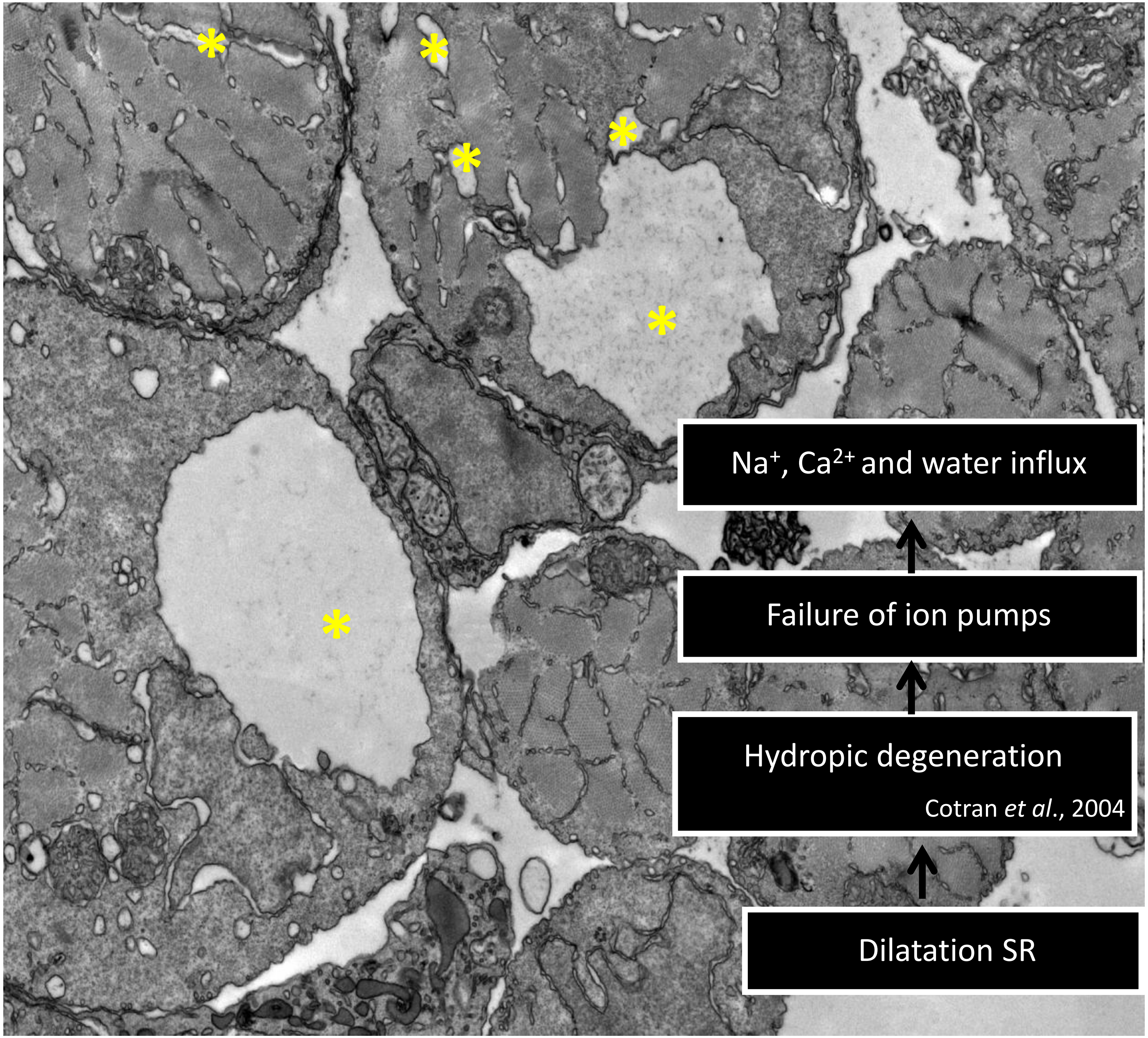


Capn1



Betancor *et al.*, 2013. CBPA

THIN SECTIONS



1. High DHA (5%) microdiets lead to reduced growth and survival and favoured the appearance of muscular lesions in sea bass larvae

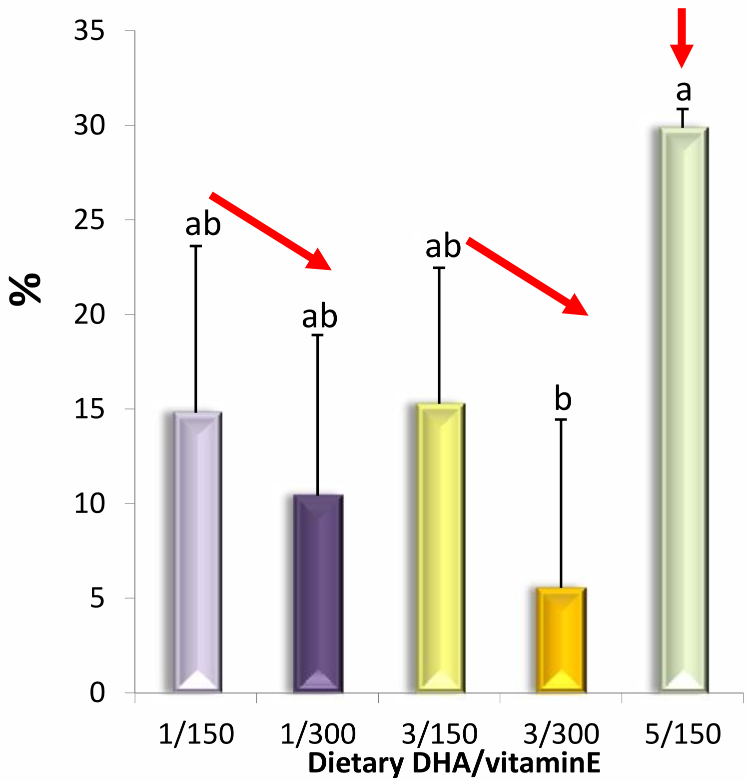
2. One of the first alterations takes place at the cell membrane, probably due to the direct attack of ROS to phospholipids, causing alteration of its permeability and leading to cell and organelles swelling. Massive influx of calcium occurs and a consequent activation of µ-calpains causes proteolysis

3. A compensatory muscle response was found, as shown by the increase in insulin-like growth factors and myosin heavy chain gene expression, as well as the abundant activated satellite cells

What is the effect of high DHA dietary levels on sea bass larvae?

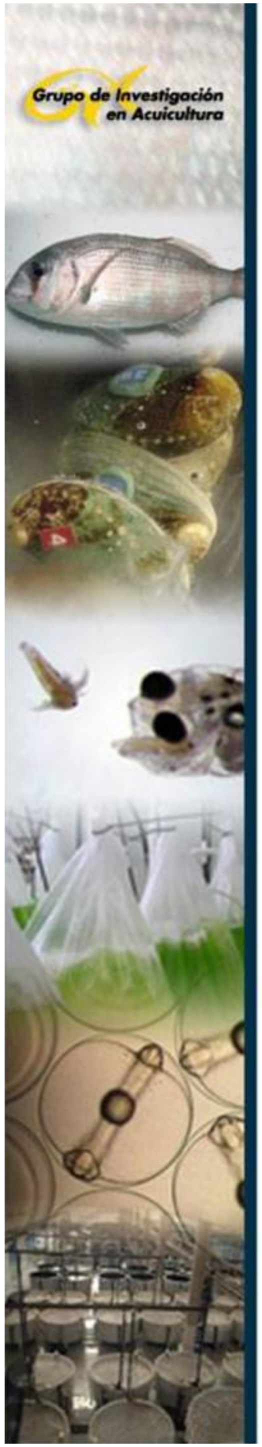
Is there an *in vivo* oxidation?? Could it be prevented with increased levels of vitamin E?

Incidence of muscular lesions

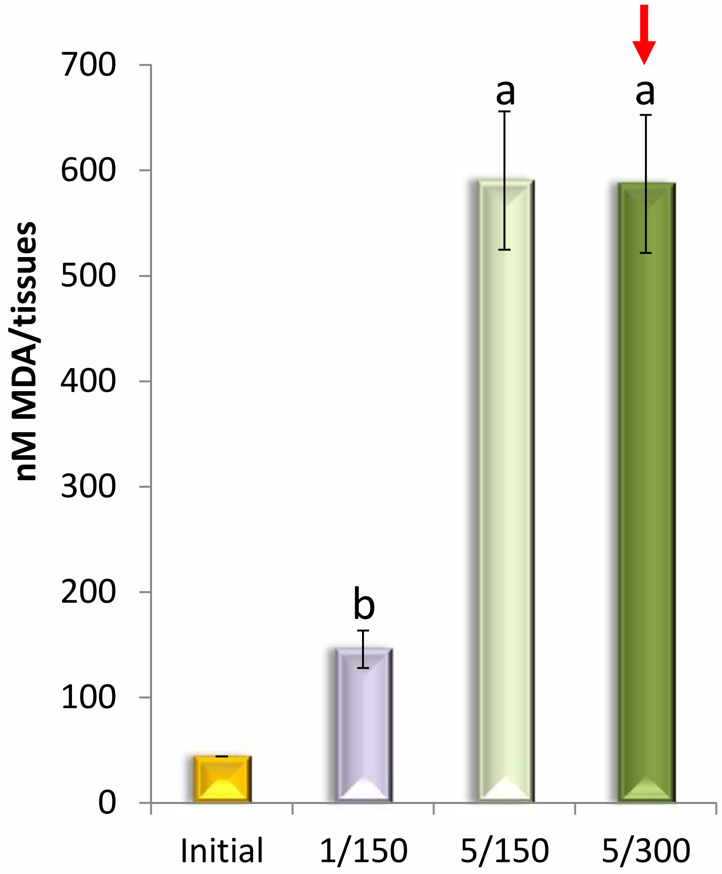


Vitamin E increase can protect sea bass larvae from increased DHA contents

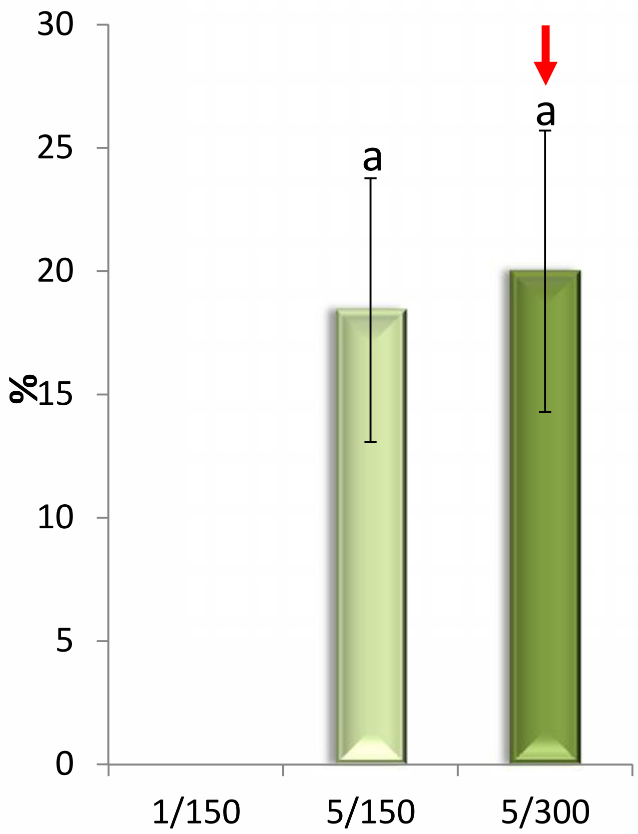
Betancor et al., 2011. Aquacult Nutr



TBARS content

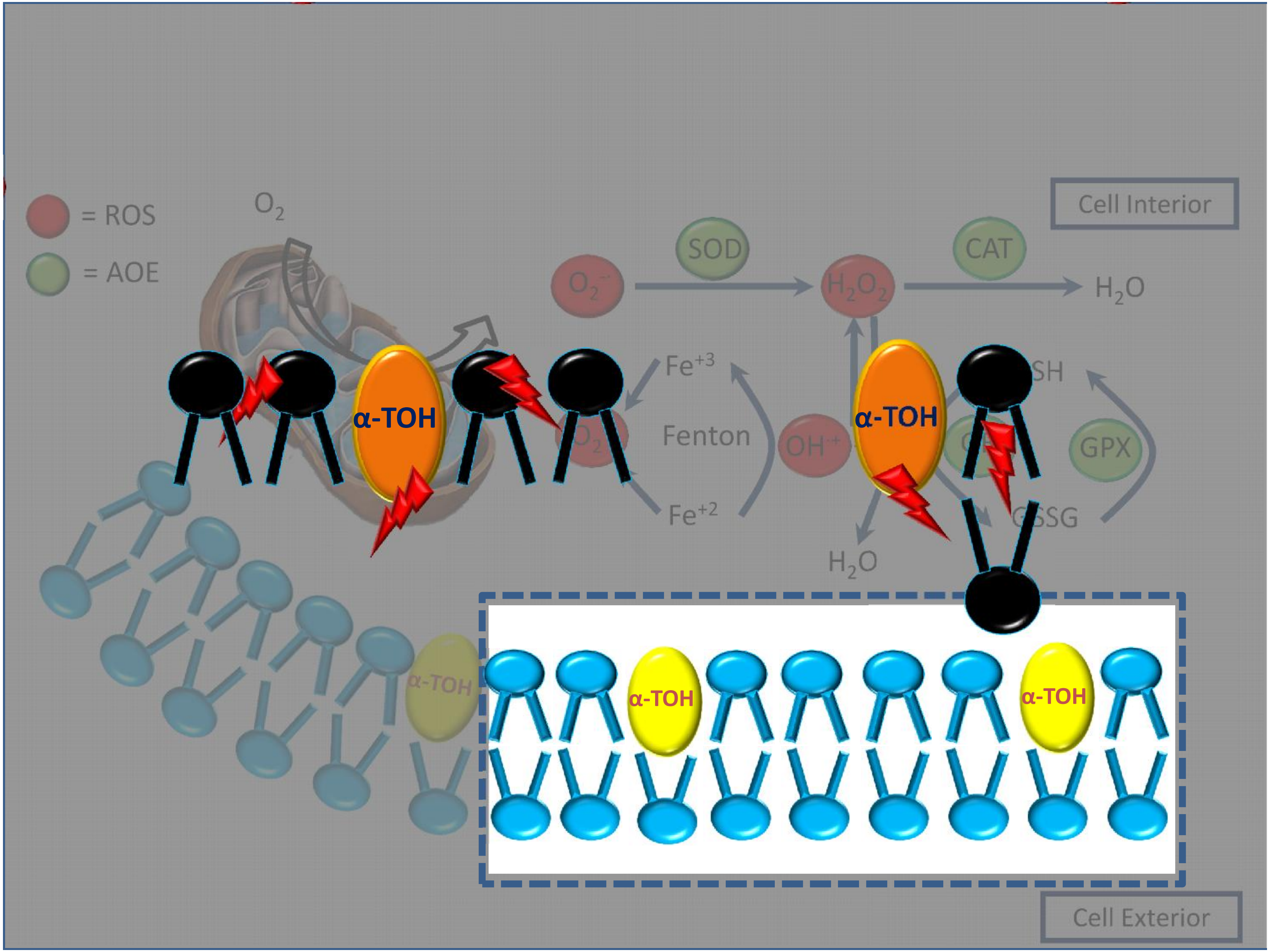


Incidence of muscular lesions at 35 dph



High DHA+High VitE
↓
Increased oxidation

Betancor *et al*., 2012. J Fish Dis



= ROS
= AOE
O2
Cell Interior
SOD
CAT
H2O
Fe+3
Fenton
Fe+2
α-TOH
α-TOH
SH
GPX
GSSG
H2O
α-TOH
α-TOH
α-TOH
Cell Exterior

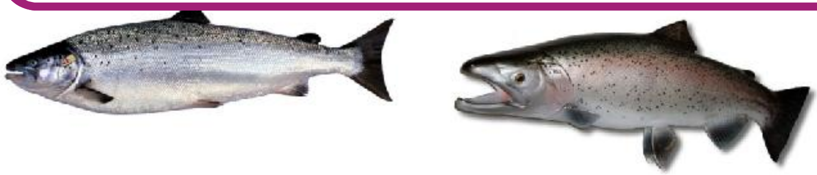
Which other nutrients could protect sea bass larvae when high levels of DHA are used in their diets?

Active sites of the GPX

Felton *et al.*, 1996

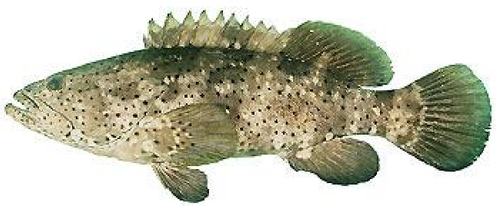
Synergistic action between α-TOH and Se

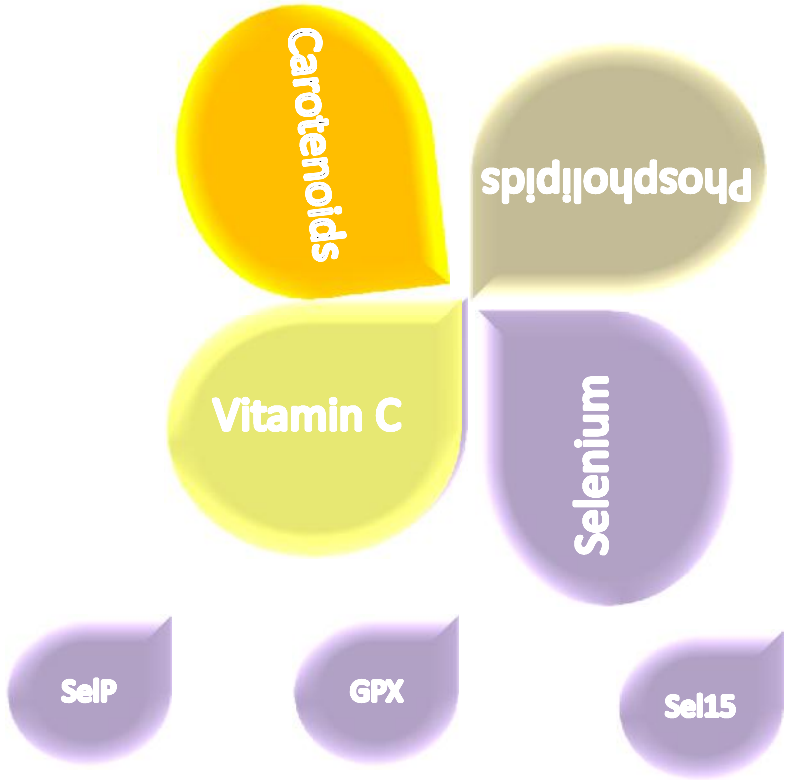
Poston *et al.*, 1976; Bell & Cowey, 1985



Sparing effect between α-TOH and Se

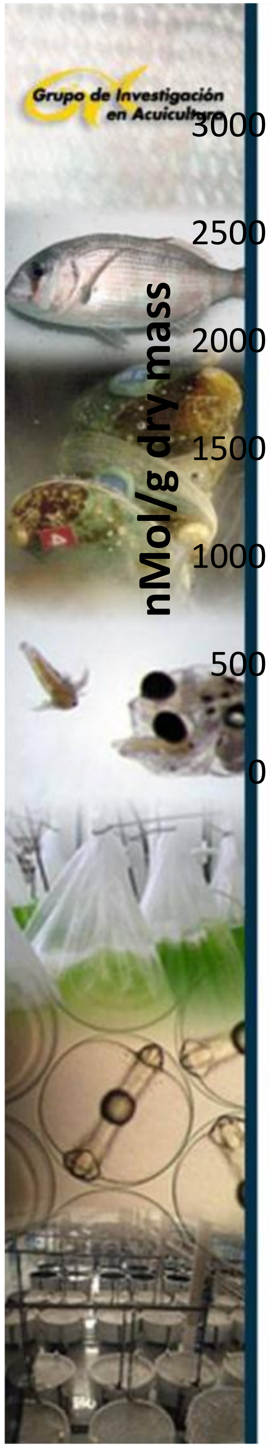
Lin & Shiau, 2009

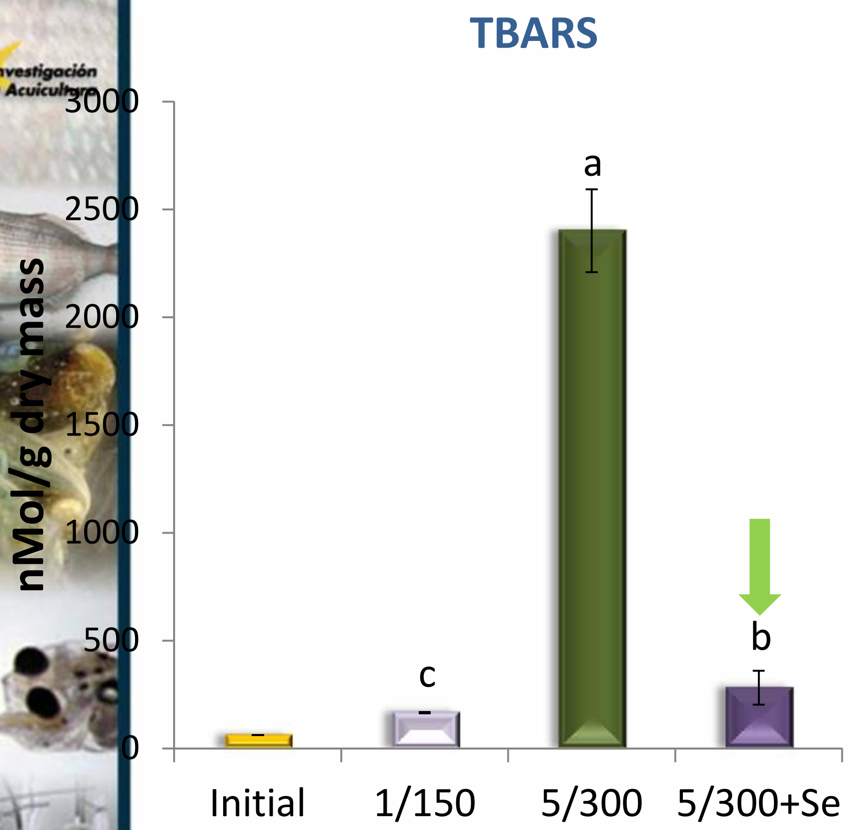


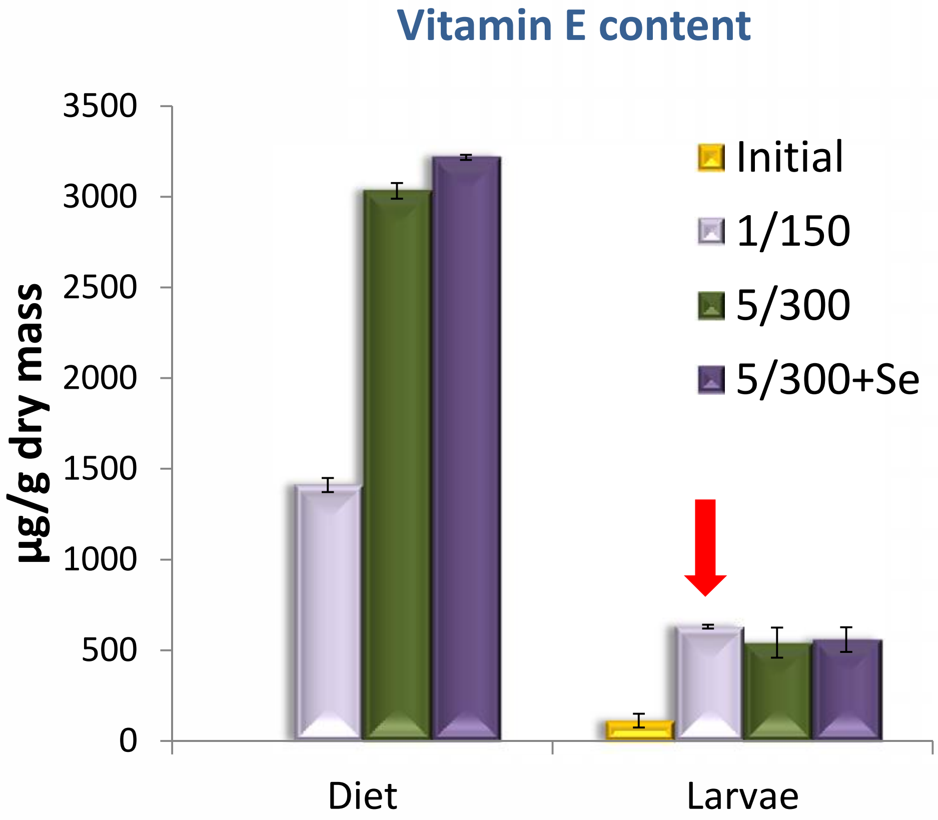


Involved in thyroid hormone and insulin function, maintenance of fertility and regulation of cell growth

Lall, 2002; Kohlmeier, 2003







Antioxidant protection

Betancor *et al.*, 2012. Br J Nutr

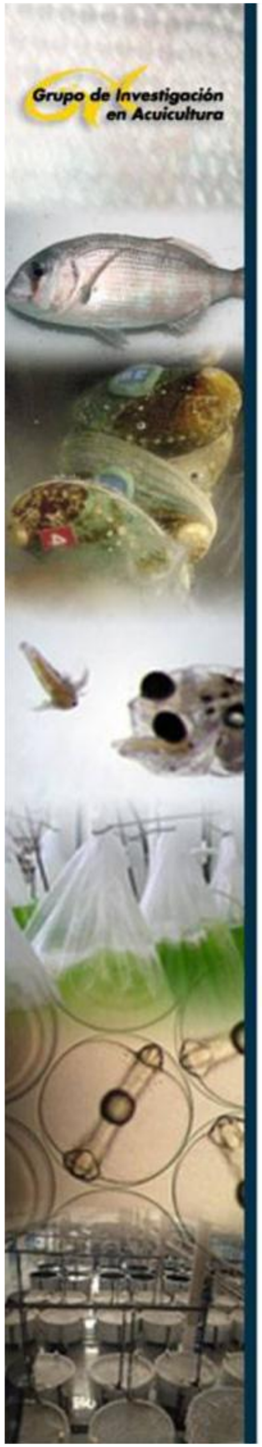
No dose dependant effect

Kiron *et al.*, 2004

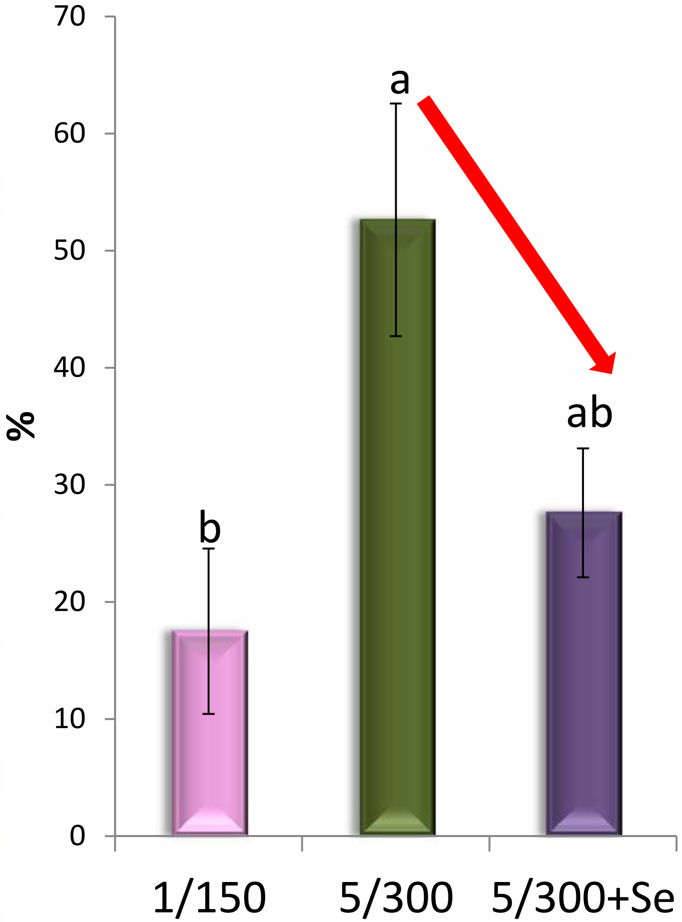
Antioxidant

↓ Tissue vitamin E

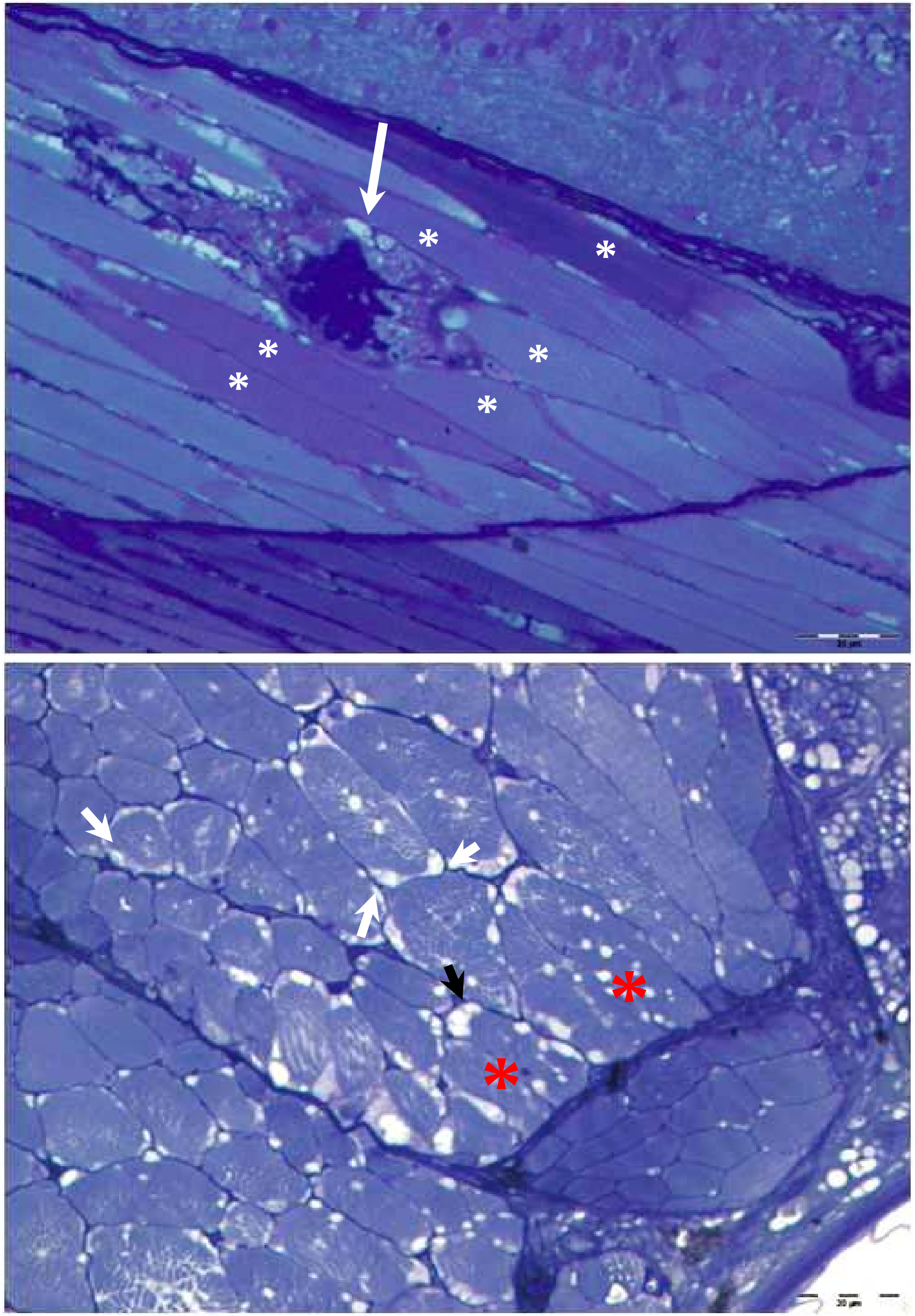
Puangkaew *et al.*, 2005

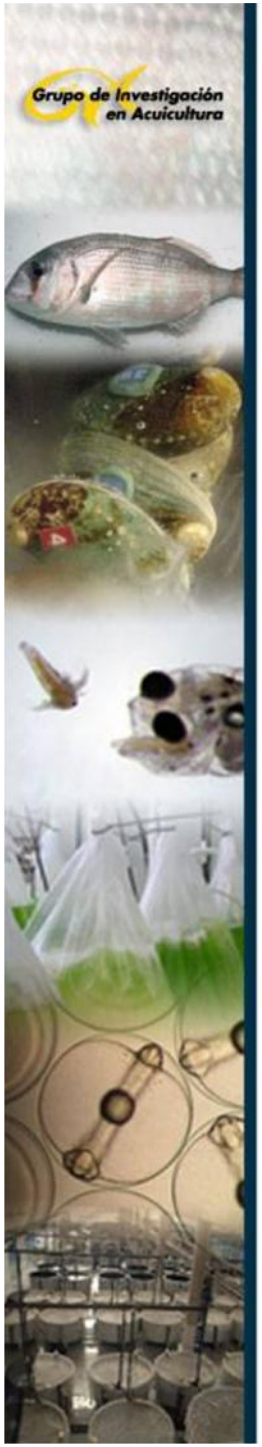


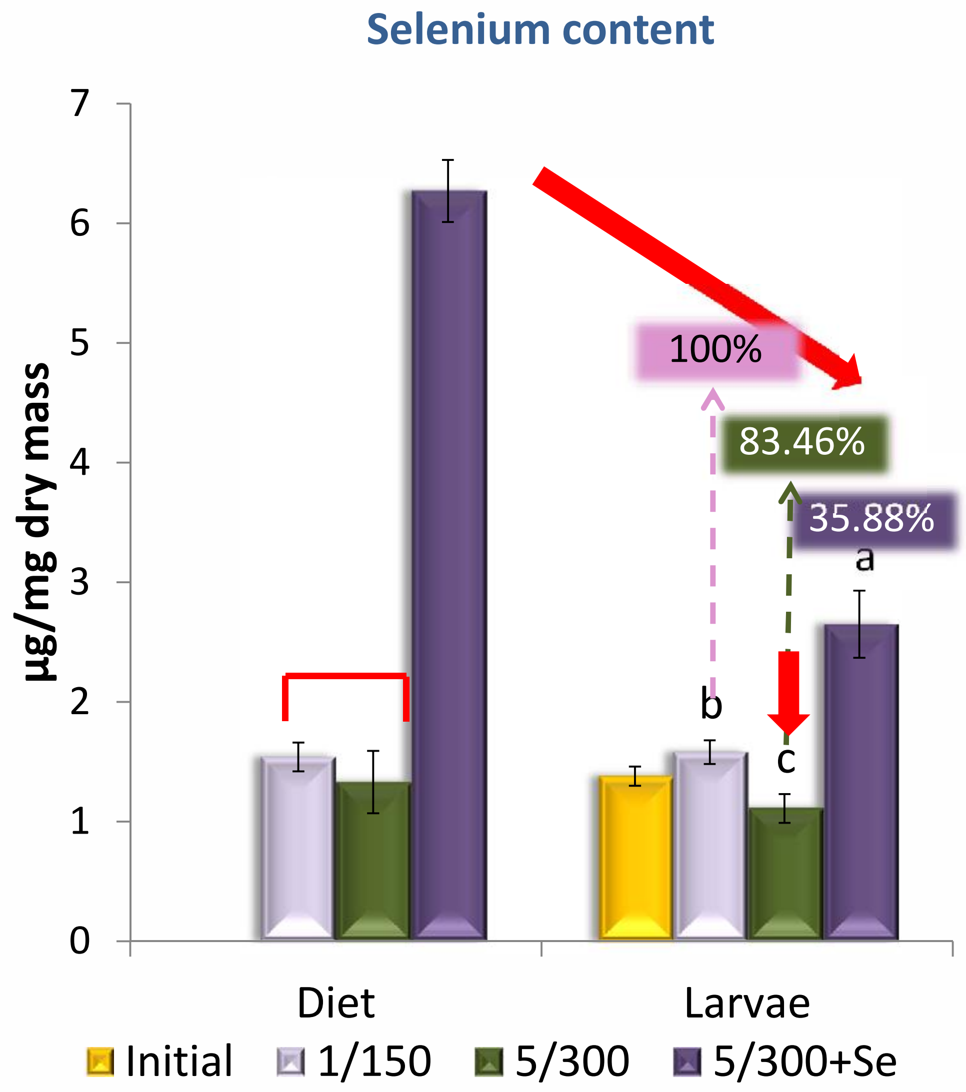
Incidence of muscular lesions



↓ Se accumulation in muscle
Monteiro *et al*., 2009; Elia *et al*., 2011





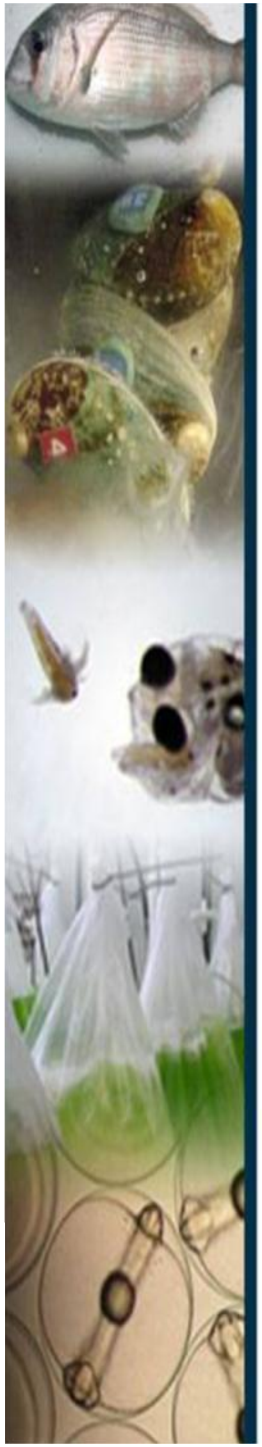


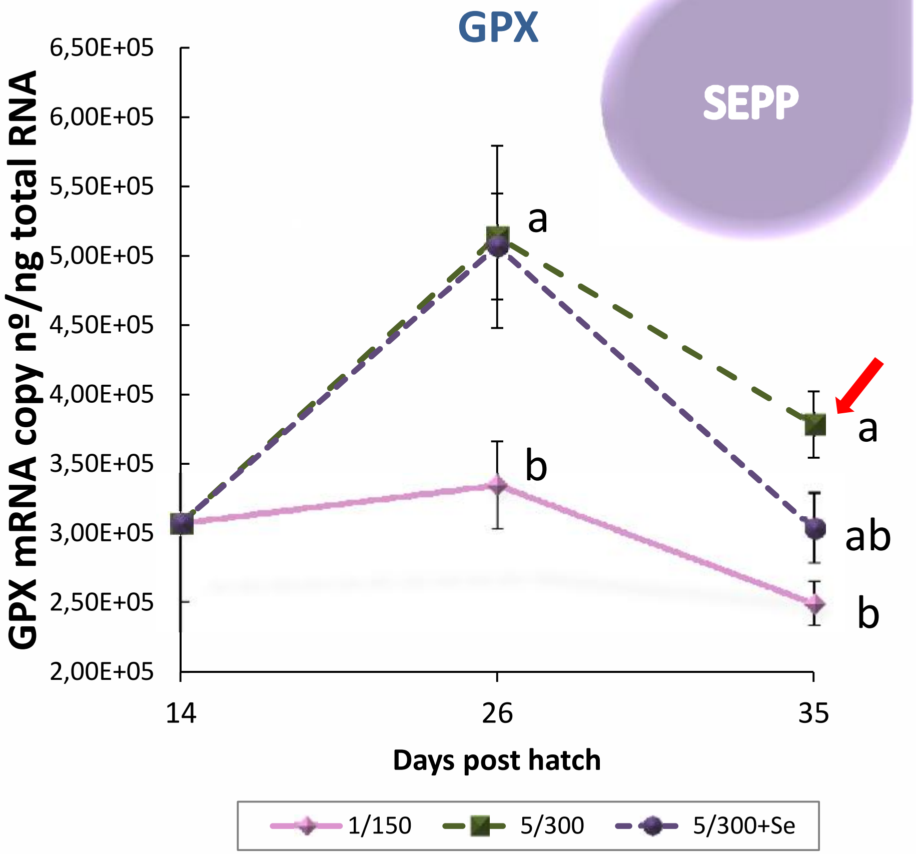
↓ Tissue concentration

Antioxidant

Low retention rate – GPX??

Betancor *et al.*, 2012. Br J Nutr





SEPP

Antioxidant defence
(Burk *et al.*, 1997)

17 selenocysteine residues
(Tubajeva *et al.*, 2000)

Largely used as an antioxidant

Saturated at normal nutritional intakes

Betancor *et al.*, 2012. Br J Nutr

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	RO	RO+Se	DHA	DHA+Se
DHA (% total fatty acids)	0.7	0.2	19.0	18.5
Se (ppm)	1.7	7.0	1.3	6.3

Two ages **2 months trial**

7 months 20 months

Glutathione peroxidases: GPX3, GPX4b

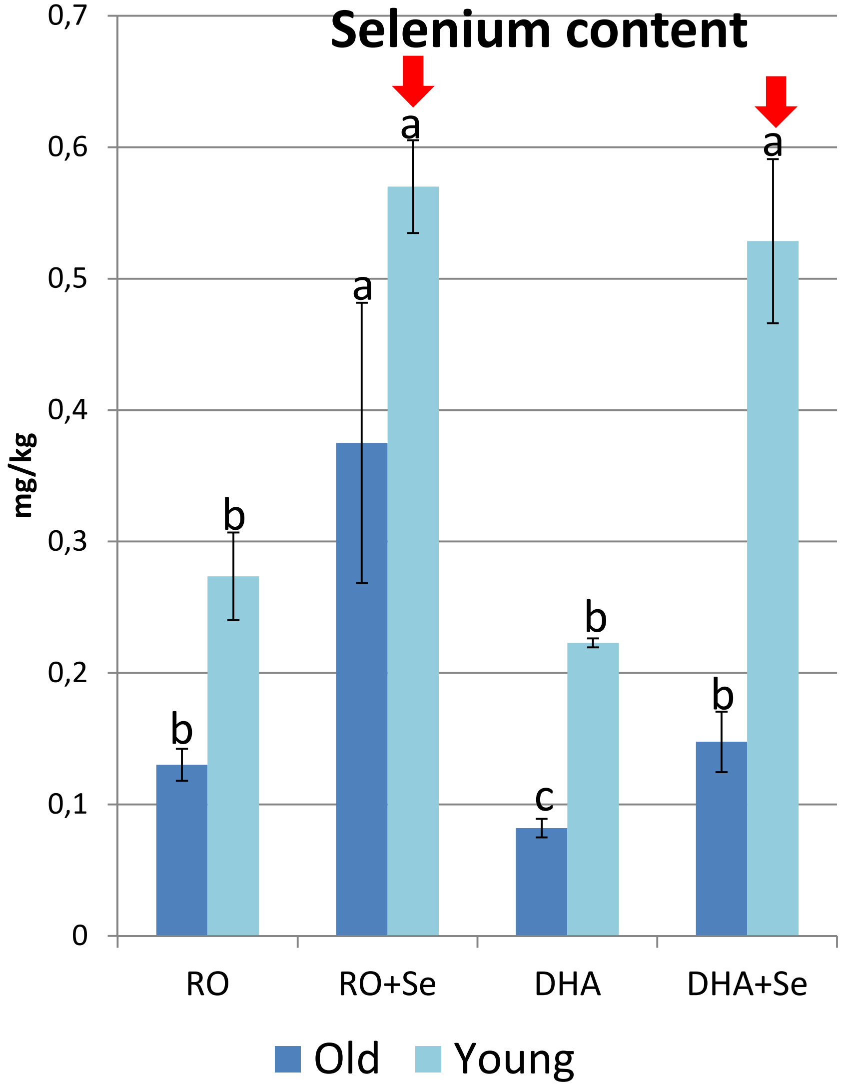
Thyroid Hormone Deiodinases: ID2

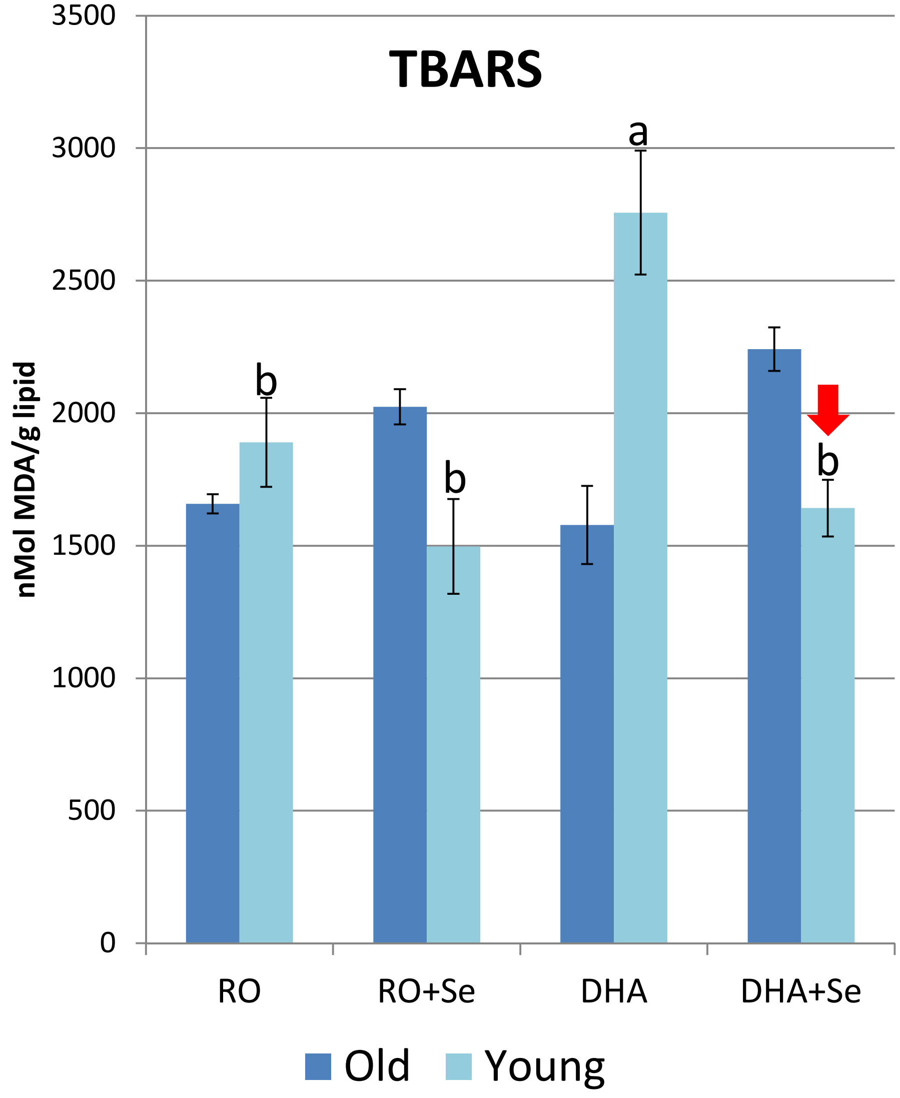
Thioredoxin reductases: TR1

Selenoproteins: SEPP1, SEPW, 15 KDa

Selenocysteine biosynthesis: SPS2, SEC43

Young fish more sensitive to oxidative stress





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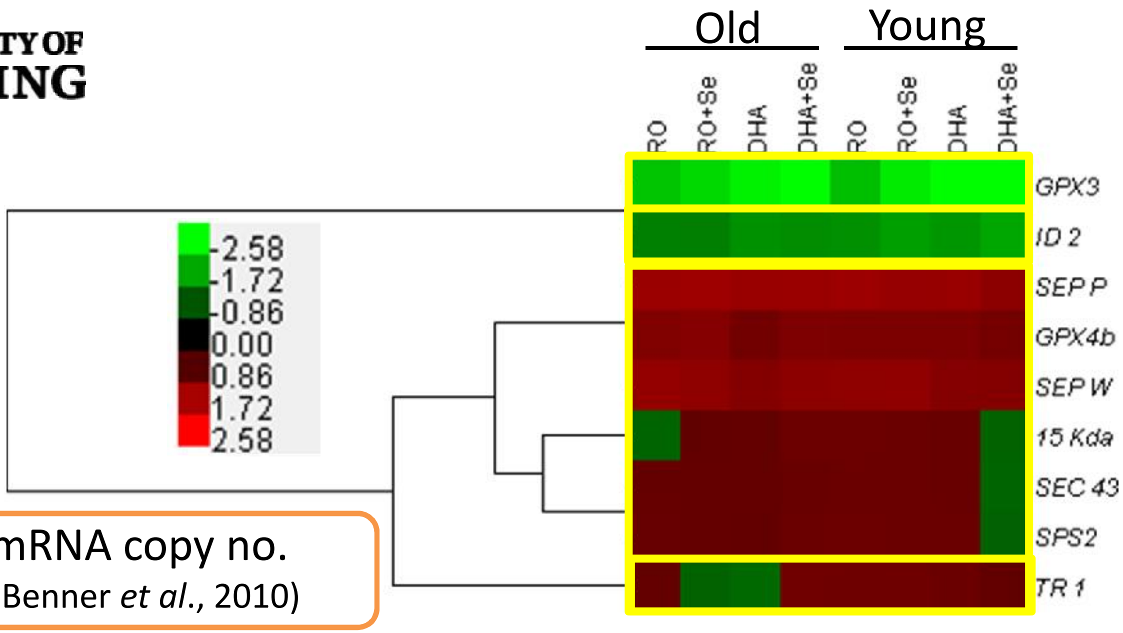

Reduced mRNA copy no.
(Benner *et al*., 2010)

Hierarchichal regulation of selenoprotein expression
(Schomburg and Schweizer, 2009)

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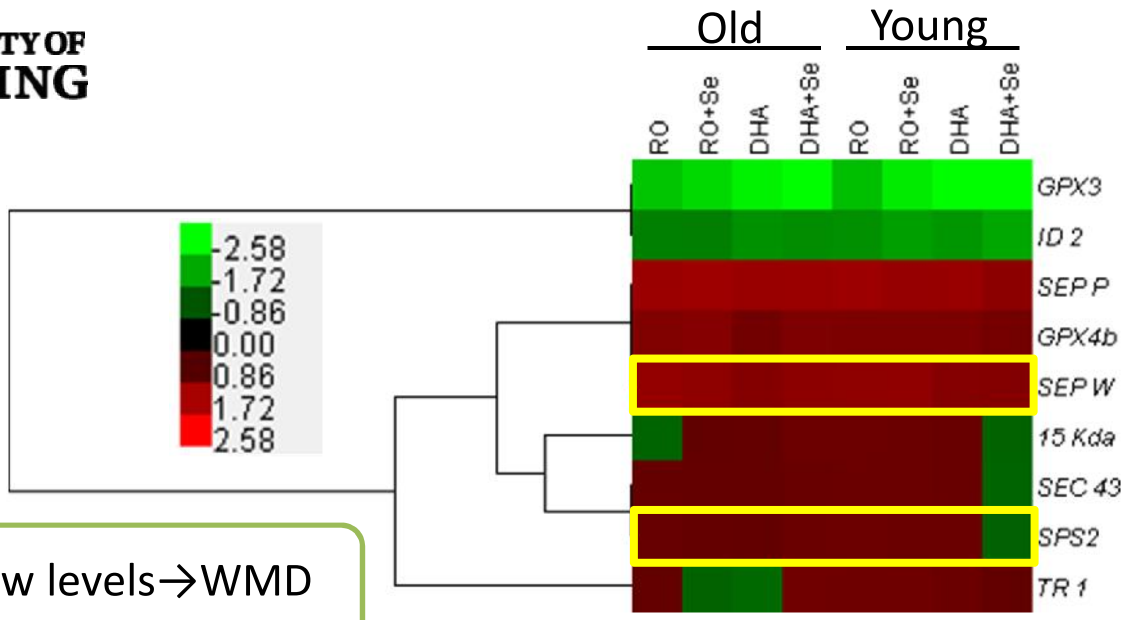


Low levels→WMD

Selenide+ATP
↓*SPS2*
Selenophosphate

Protects low density LP

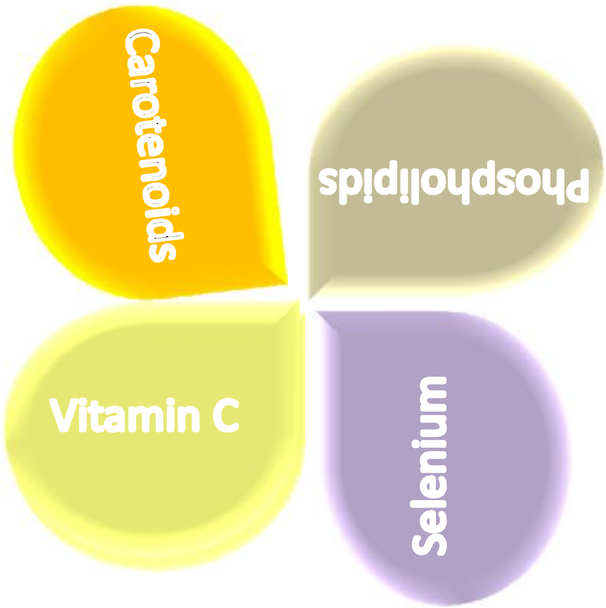
Levine, 1986

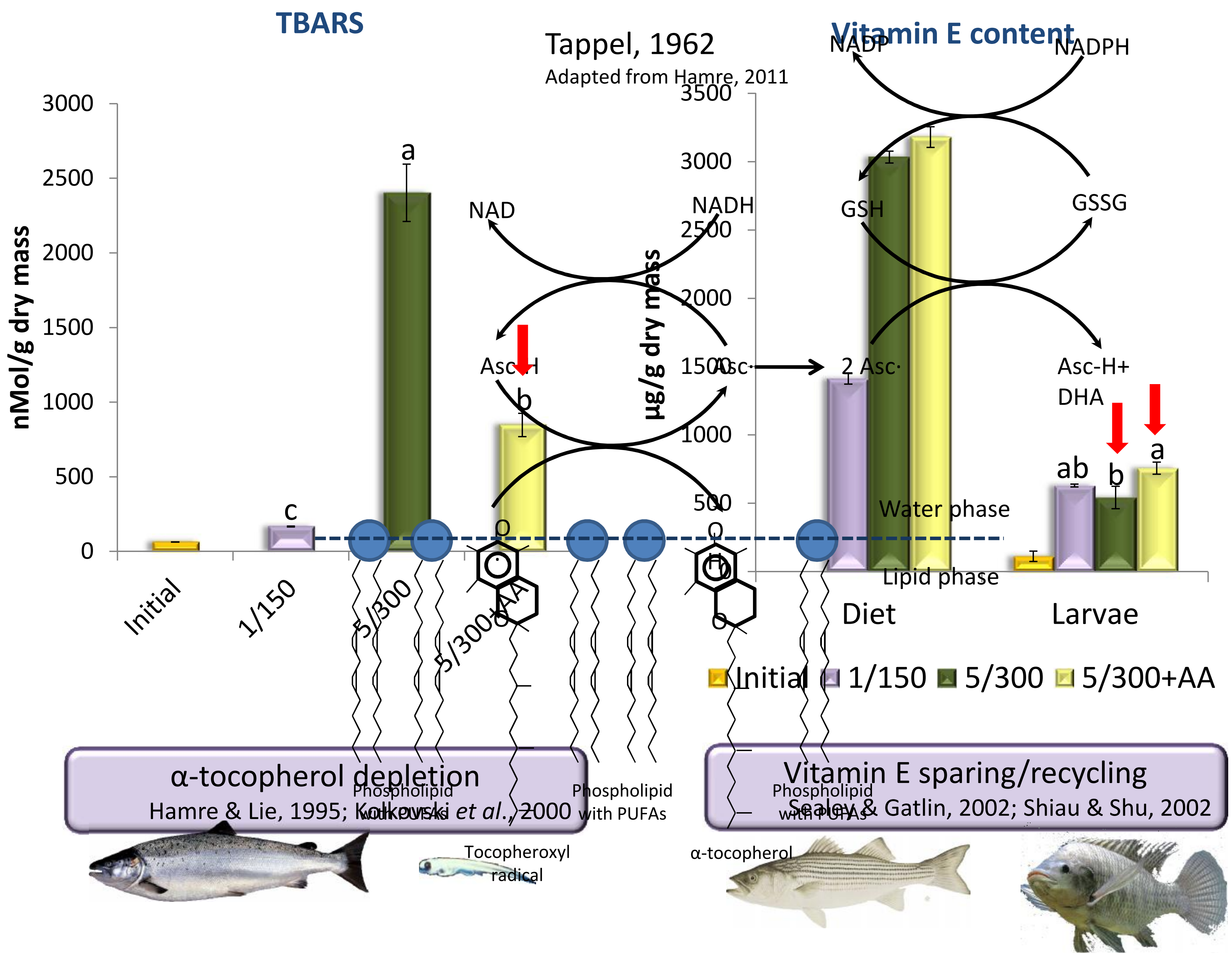
Recycling of α-TOH

Hamre *et al*., 1997; Sealey & Gatlin, 2002

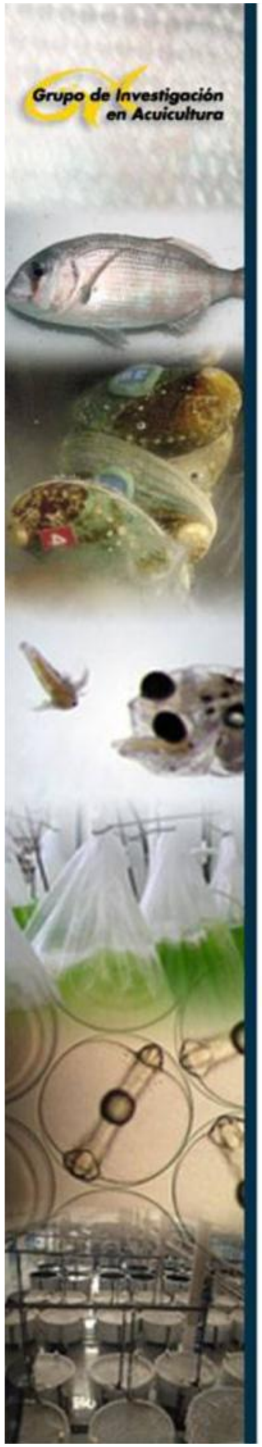
Sparing effect between α-TOH and AA

Shiau & Shu, 2002; Yildirim-Aksoy *et al*., 2008

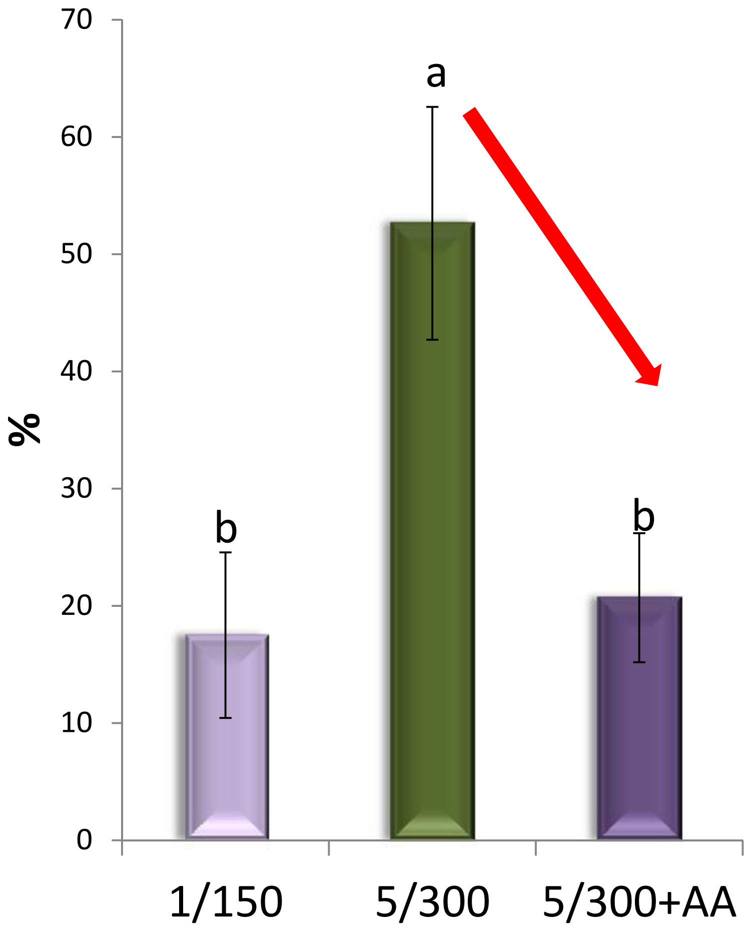


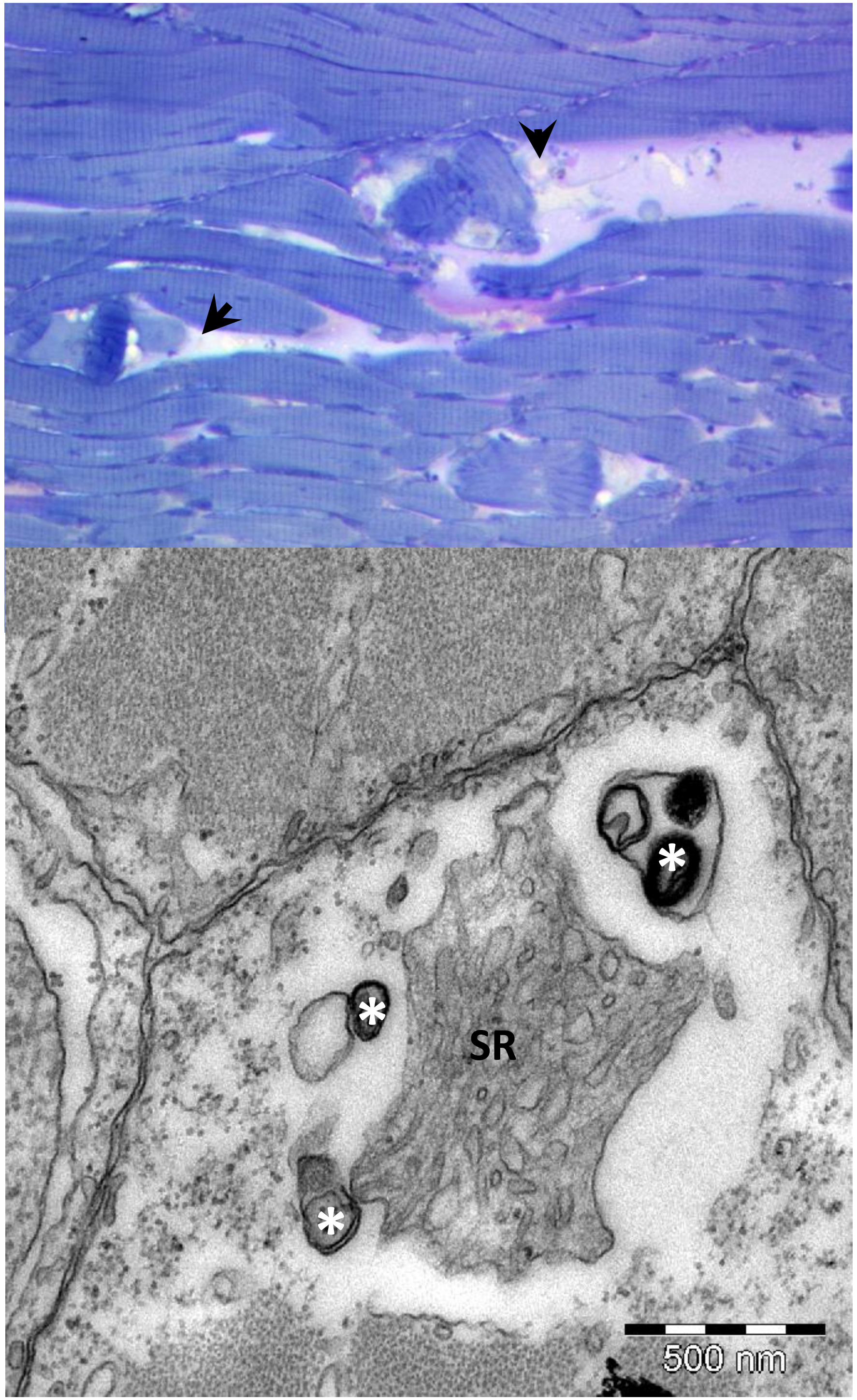


TBARS
nMol/g dry mass
3000
2500
2000
1500
1000
500
0
a
b
c
Initial
1/150
5/300
5/300+AA
Tappel, 1962
Adapted from Hamre, 2011
Vitamin E content
μg/g dry mass
3500
3000
2500
2000
1500
1000
500
Diet
Larvae
ab
b
a
Initial
1/150
5/300
5/300+AA
NAD
NADH
NADP
NADPH
GSH
GSSG
Asc-H
Asc·
2 Asc·
Asc-H+
DHA
Water phase
Lipid phase
Phospholipid with PUFAs
Phospholipid with PUFAs
Phospholipid with PUFAs
Tocopheroxyl radical
α-tocopherol
α-tocopherol depletion
Hamre & Lie, 1995; Kolkowski et al., 2000
Vitamin E sparing/recycling
Sealey & Gatlin, 2002; Shiau & Shu, 2002



Incidence of muscular lesions



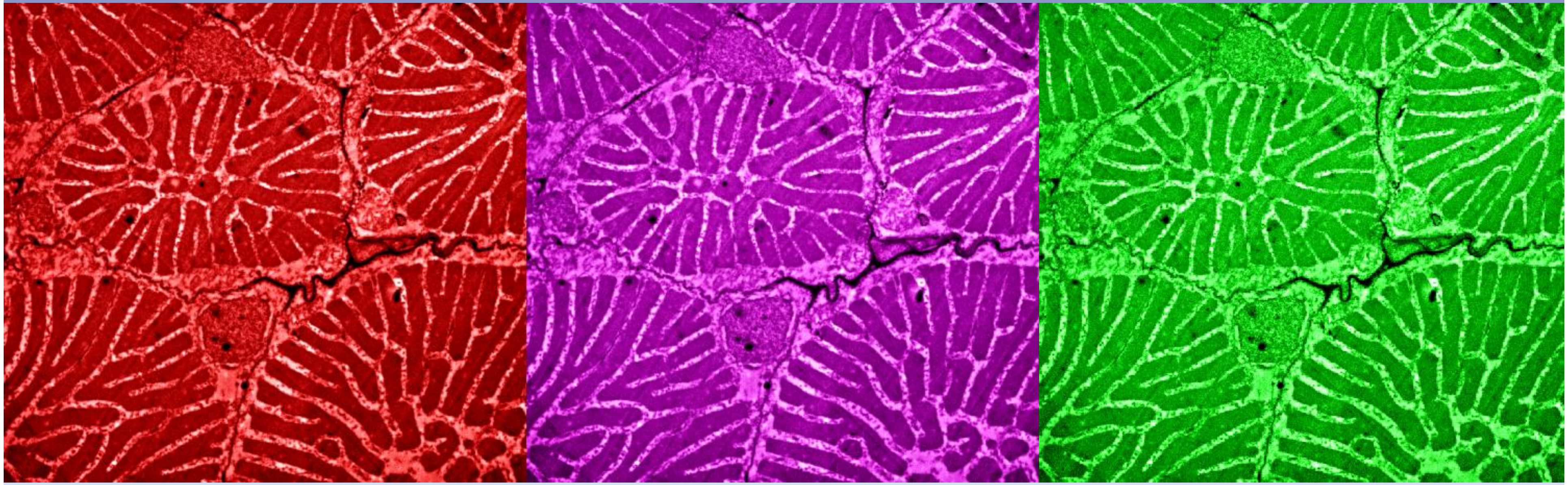


Inclusion of both organic selenium (5 mg kg^{-1}) and ascorbic acid (180 mg 100 g^{-1}) to diets with a 5% of DHA and 300 mg 100 g^{-1} of vitamin E controlled *in vivo* lipid peroxidation and decreased the incidence of muscular lesions when compared to the unsupplemented diets

Which other nutrients could protect sea bass larvae when high levels of DHA are used in their diets?

In zebrafish muscle TR1, ID2, SEPW and SPS2 expression is affected by the addition of 7 ppm of Se when high DHA diets are used, indicating that these could be good biomarkers of the oxidative status in teleost fish

Thanks for your attention



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