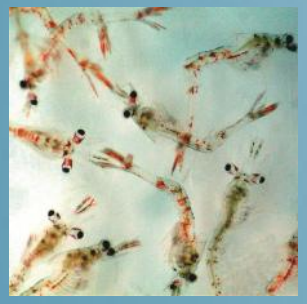
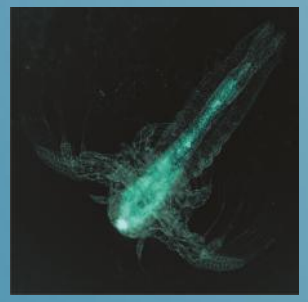
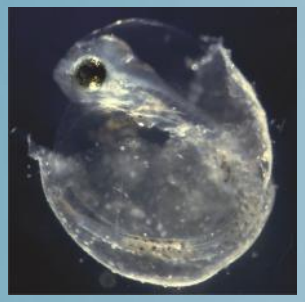
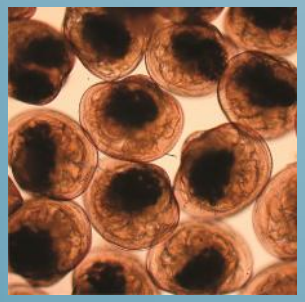




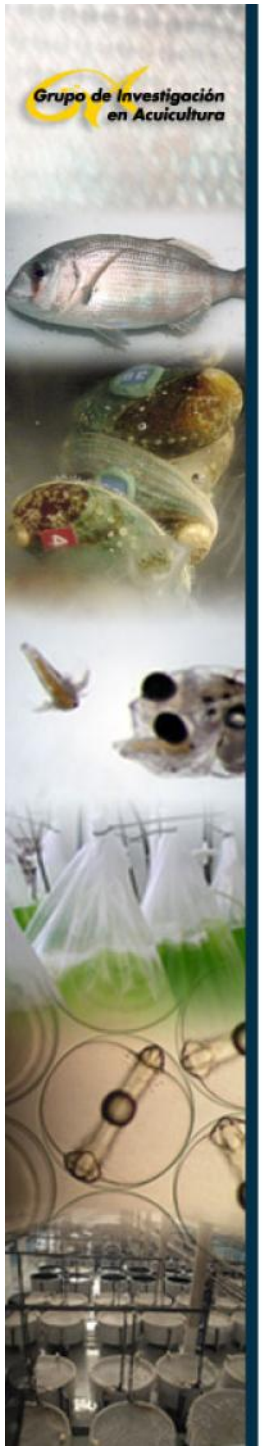
larvi 2013

6th fish & shellfish larviculture symposium

Optimum phospholipids and antioxidant levels in microdiets for gilthead seabream larvae

Reda Saleh & Marisol Izquierdo

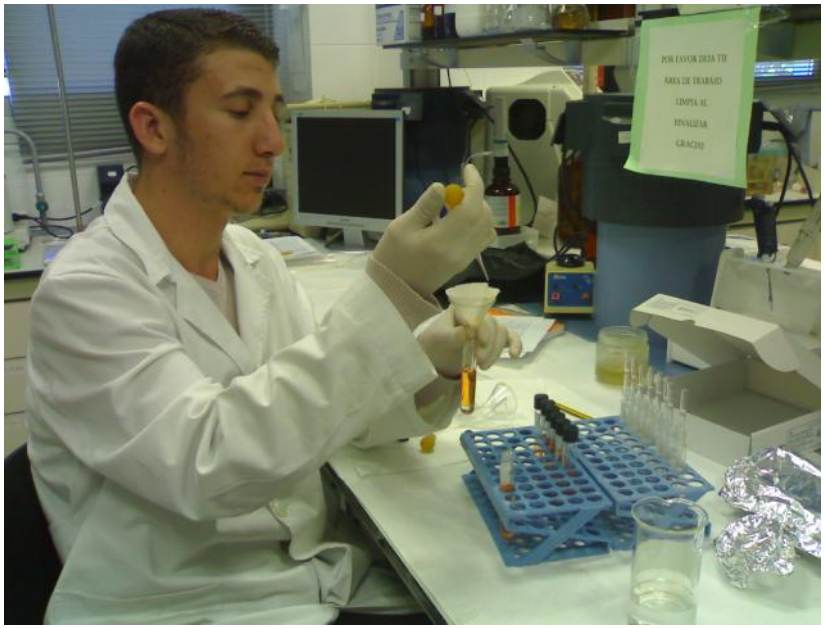
ghent university, belgium, 2-5 september 2013

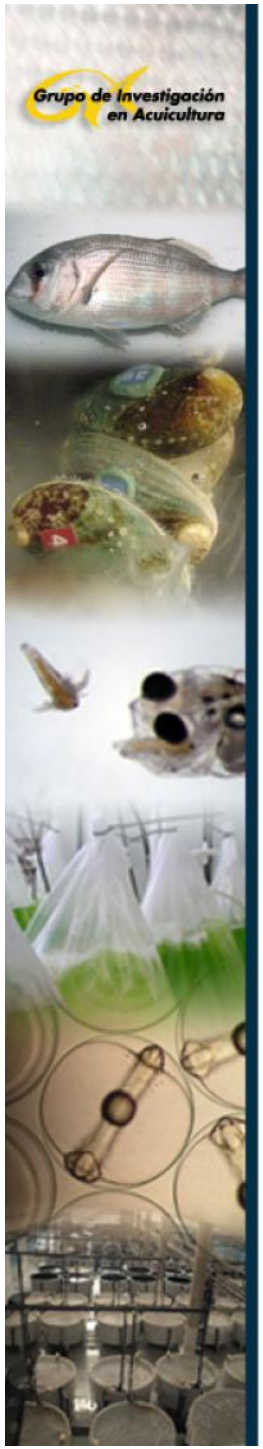


Optimum phospholipids and antioxidant levels in microdiets for gilthead seabream larvae

Reda Saleh[1,2] & Marisol Izquierdo[1]

[1]Grupo de Investigación en Acuicultura (GIA),
Gran Canaria, Spain.
[2]Oceanography Dpt., Faculty of Science,
Alexandria University, Egypt.





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- Effect of level and type of PL
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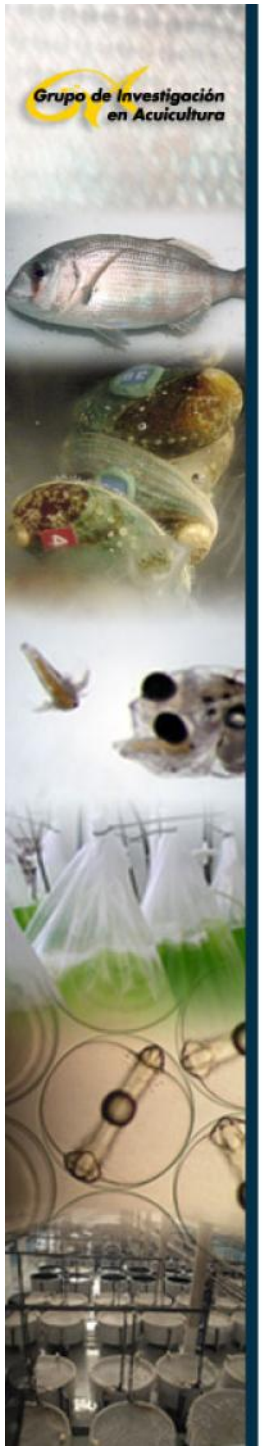
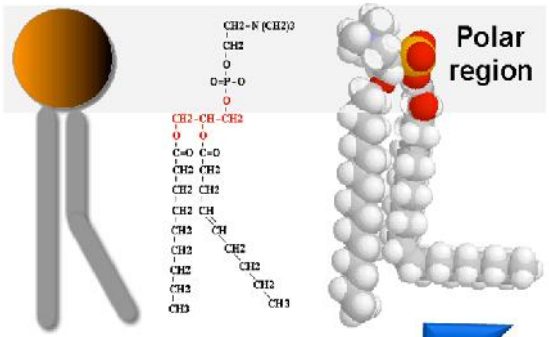


Introduction

Growth and survival (Kanazawa et al. 1983, Coutteau et al. 1997, Fontagne et al. 2000, Wold et al. 2007)

Source of fatty acids (Izquierdo & Koven 2010) and energy (Rainuzzo et al., 1992)

Phospholipids



Lipoproteins synthesis

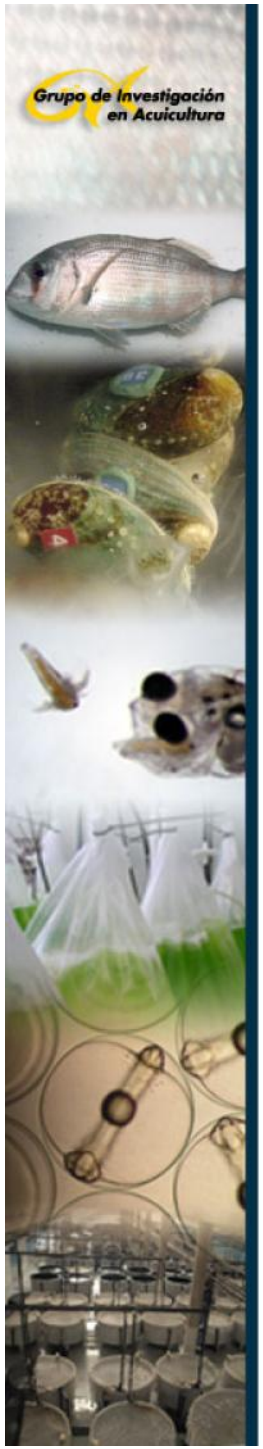
(Liu et al., 2002, Hadas et al., 2003)

Feeding activity (Koven et al., 1998)

Reduce malformations

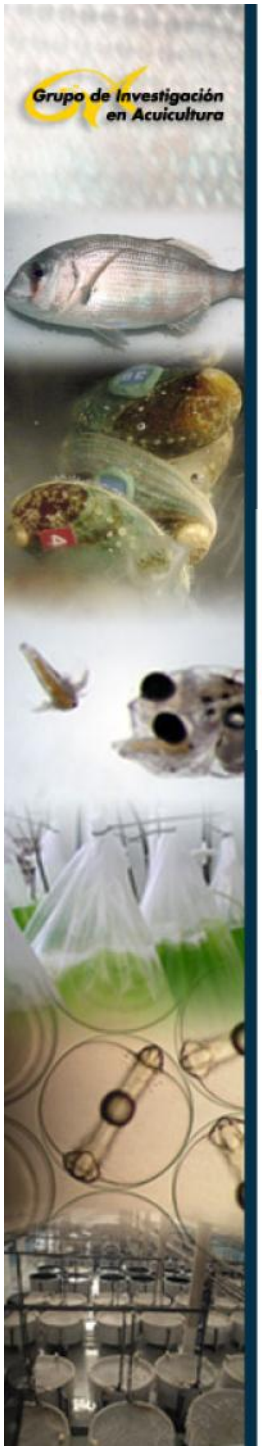
(Kanazawa *et al*., 1981, Guerden et al., 1998; Cahu et al., 2003)

Emusifiers (Olsen & Ringø, 1997) Digestion and transport of lipids (Izquierdo et al., 2001; Morais et al., 2005), gut maturation (MacQueen Leifson et al., 2003; Wold et al., 2007; Saleh et al., 2012)

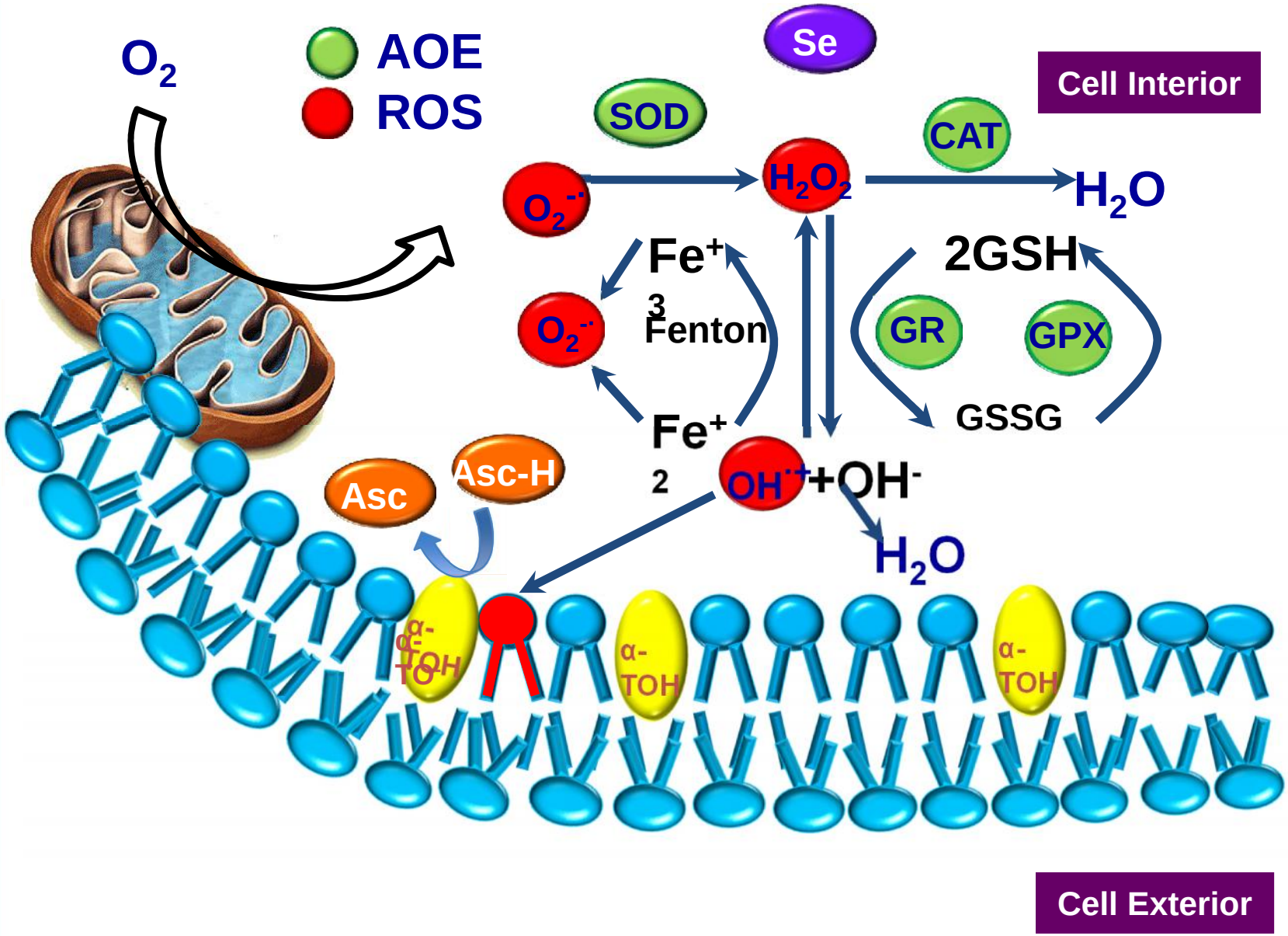


Introduction

Species	Phospholipid supplemented	Optimal requirement	Reference
Common carp	Hen egg lecithin	2%	Geurden *et al*. (1995)
Red seabream	soybean lecithin (SBL)	5%	Kanazawa *et al*. (1983a)
Knife jaw	SBL	7.4%	Kanazawa *et al*. (1983b)
Japanese flounder	SBL	7%	Kanazawa (1993)
European sea bass	SBL	12%	Cahu *et al*. (2003)
Seabream	SBL	15%	Seiliez *et al*. (2006)

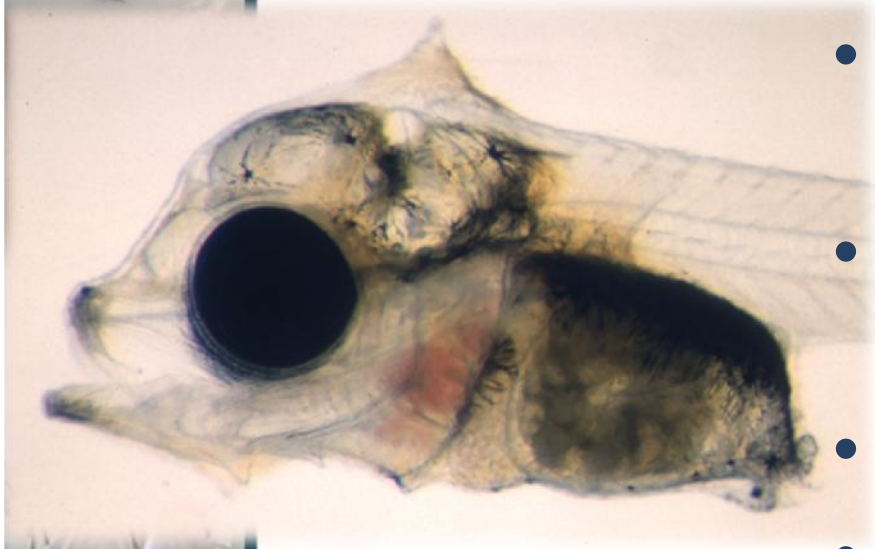


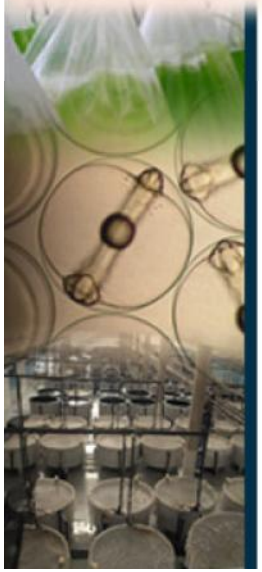
Introduction



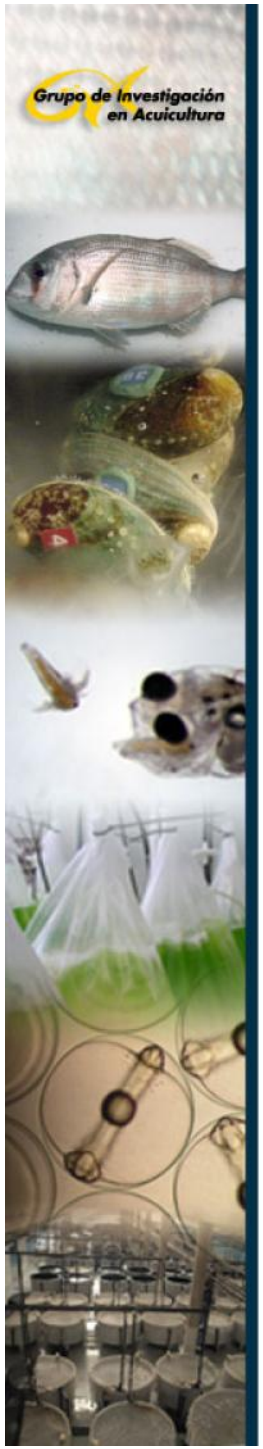
Betancoret al., Larvi 2013

Marine fish larvae are under a high oxidation risk



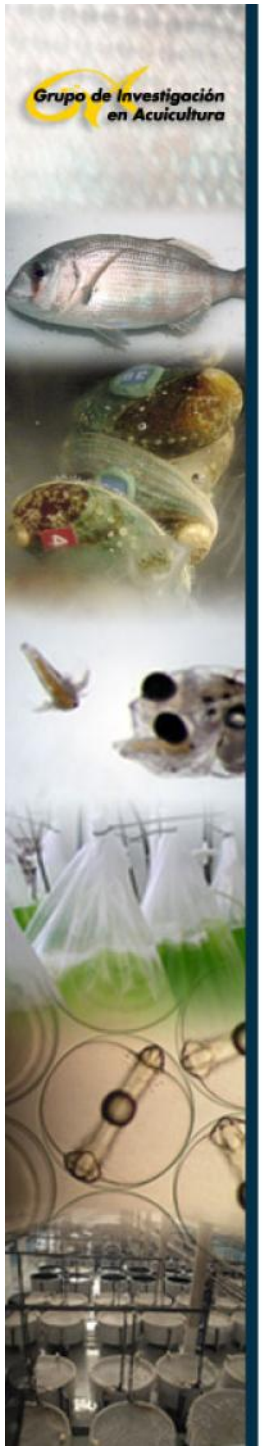


- High metabolic rate & oxygen requirements
- High water content and water reabsorption at metamorphosis
- High PUFA requirements
- Lipid content and lipid mobilization from yolk sac
- Feed with high surface/volume
- Long water exposure of feed…



Objectives

1. *How much? Which type? How will it affect digestive enzymes? How will it affect skeleton development? How will it affect oxidative status?*
2. *Will PL requirements be affected by the antioxidant vit E?*
3. *Se levels improve the performance of larvae fed optimum PL levels?*



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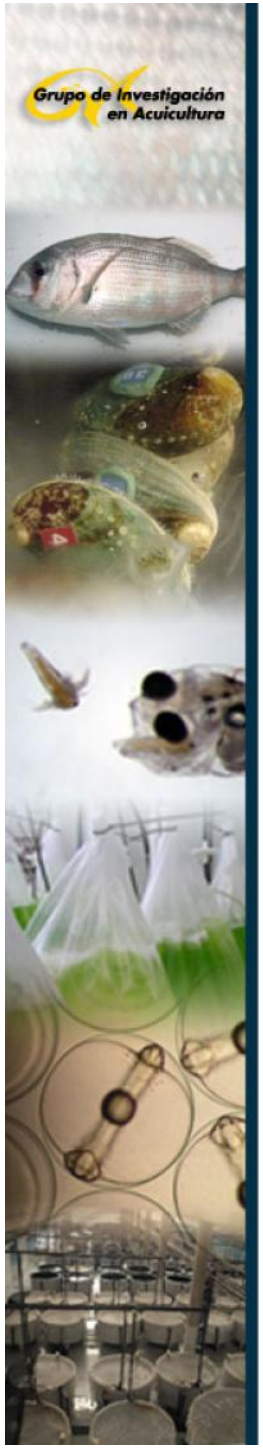


Materials & Methods

5 studies published in
Aquaculture Nutr. & Br. J. Nutr.

Tank volume 200 l

Water renewal 25%h
Aeration 125 ml/min

Photoperiod 12L:12D
1000-2000 lux

2100 Seabream larvae 14 dph

(T.L. 5 mm & 100 µg DBW)

Feeding
2.5-5 g/daily

Materials & Methods

Zootechnical Parameters **Total**

length, Dry body weight, Survival, Handling stress Test, Feed acceptance

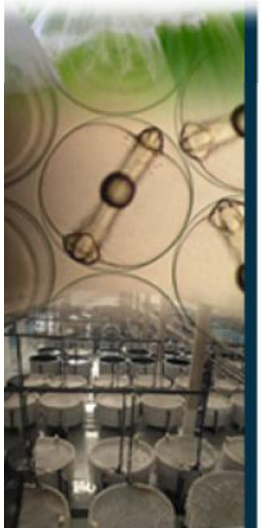
Exp 1. Optimum krill phospholipids

Exp 2. Optimum soy phospholipids

Exp 3. Comparison between KPL or SBL

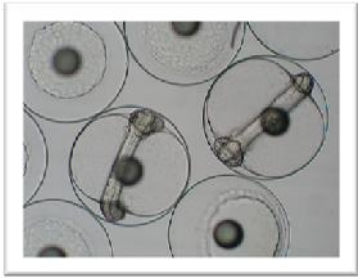
Exp 4. Combined PL/vit E levels

Exp 5. Ranged Se levels

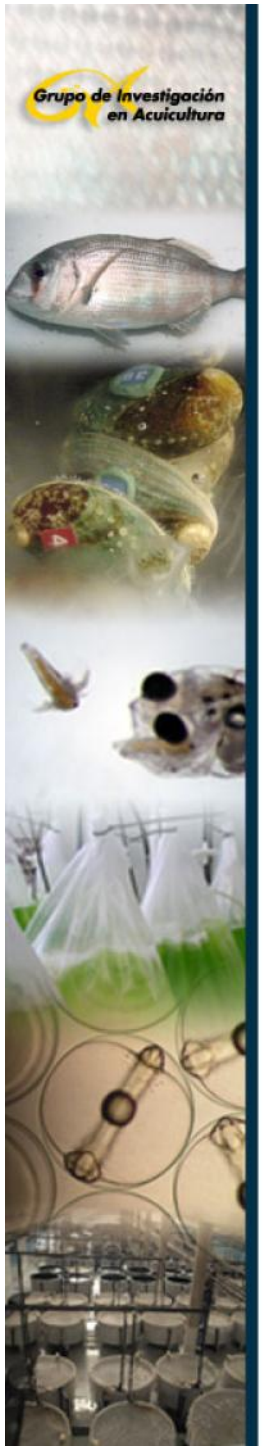


dph

0 3 15 22 31



Sparus aurata



Materials & Methods

Ingredients, feeds and larvae

Protein (AOAC, 1995)

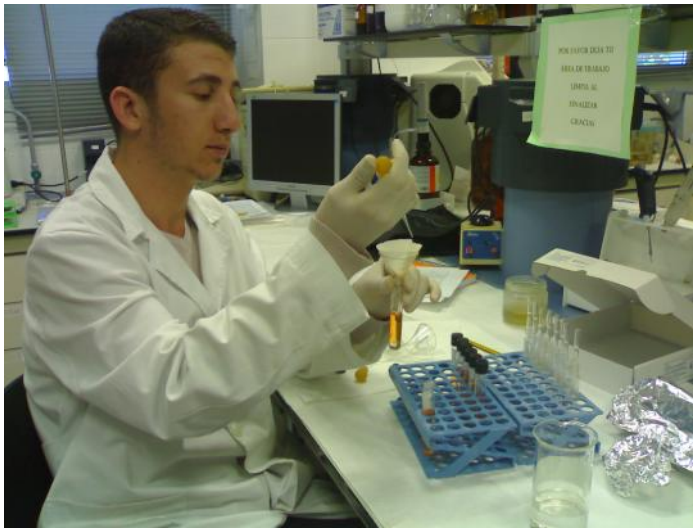
Moisture & Ash (AOAC, 1995)

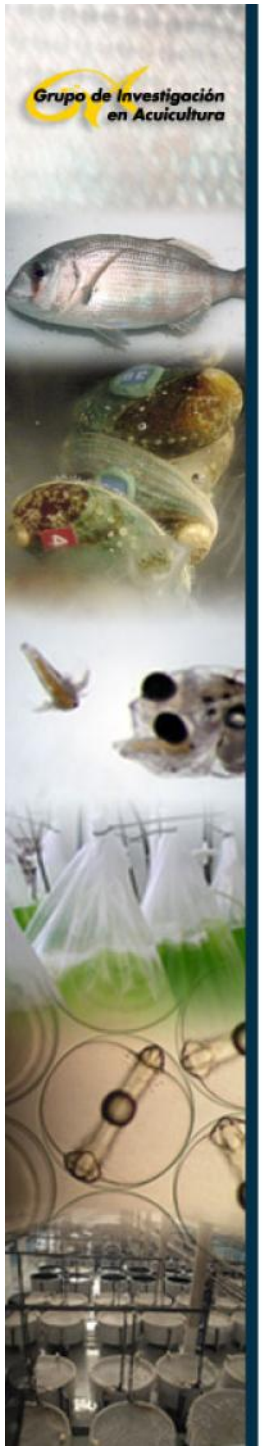
TBARS (Burk *et al.*, 1980)

Selenium Collision/reaction cell ICP-MS

Total Lipids (Folch *et al.*, 1957)

- **Neutral & polar lipids** (Olsen & Henderson, 1989)
- **Fatty Acids** (Christie, 1982, Izquierdo *et al.*,1990)



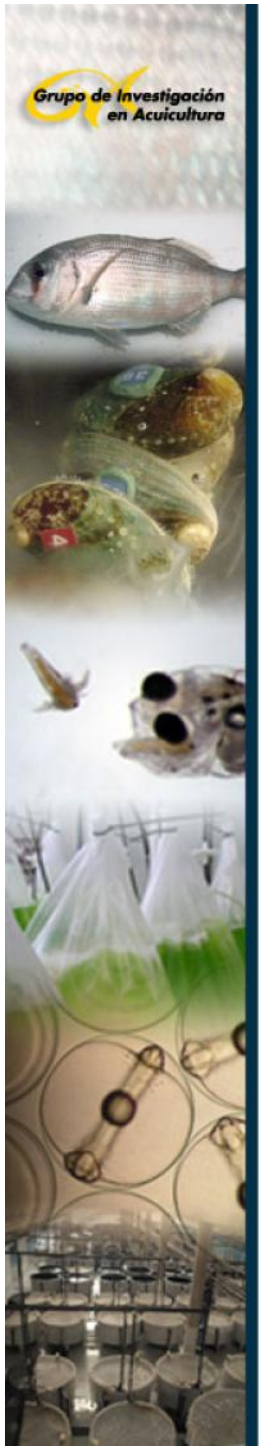


Materials & Methods

Digestive Enzymes

- **Alkaline Phosphatase** (Gee *et. al.*,1999)
- **Trypsin** (Rotllant *et al.*, 2008)
- **Lipase** (Rotllant *et al.*, 2008)
- **Phospholipase A2** (Huang *et. al.*, 2006)

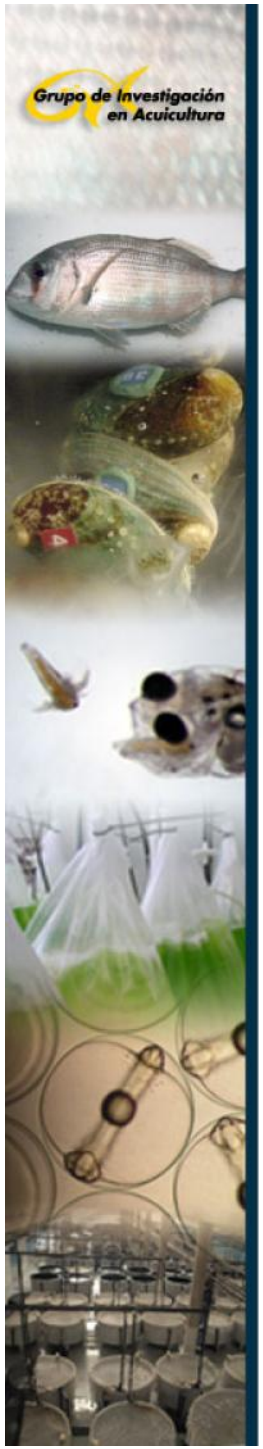
Spectrofluorometer



Whole mount staining

Alizarin red
(Izquierdo *et al*., 2012, modified from Vandewalle et al., 1998)

Deformities studied
according to Boglione et al. (2001)

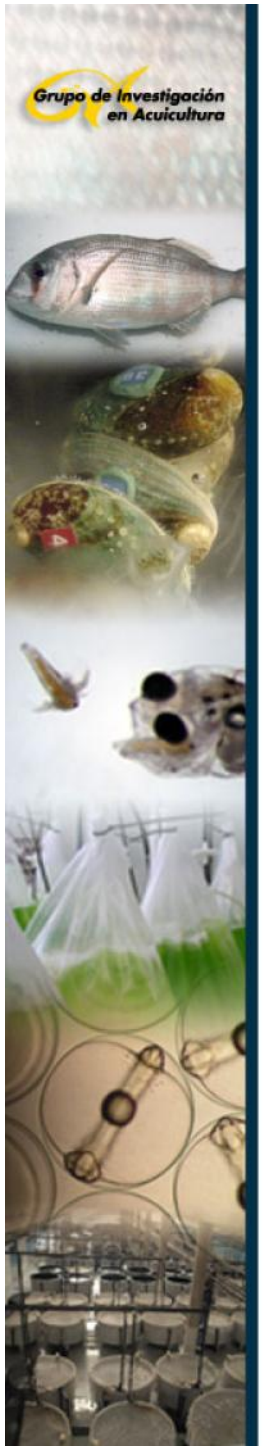


Materials & Methods

Gene expression (RT-PCR)

- AOE markers
  - CAT
  - SOD
  - GPX
- Osteo markers
  - BMP4
  - RUNX2
  - Alakaline phosphatase
  - Osteocalcin
  - Osteopontin
  - Osteonectin
  - Matrix Gla

Means compared by Duncan's test ($P < 0.05$) using SPSS software (SPSS for Windows 11.5; SPSS Inc., Chicago, IL, USA).



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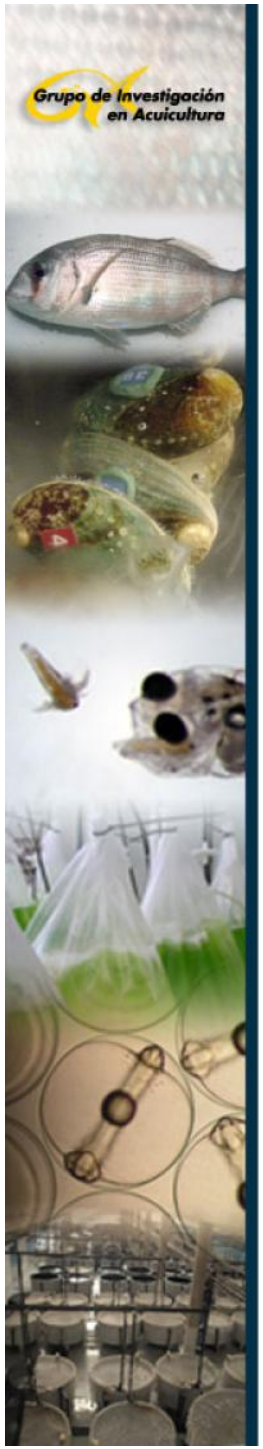
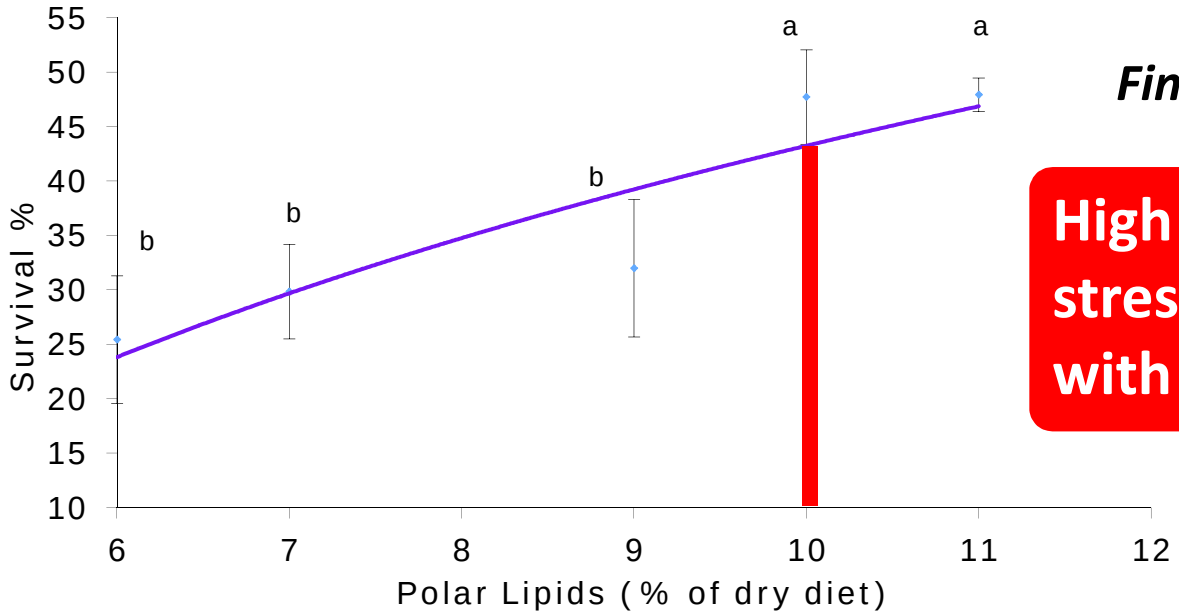
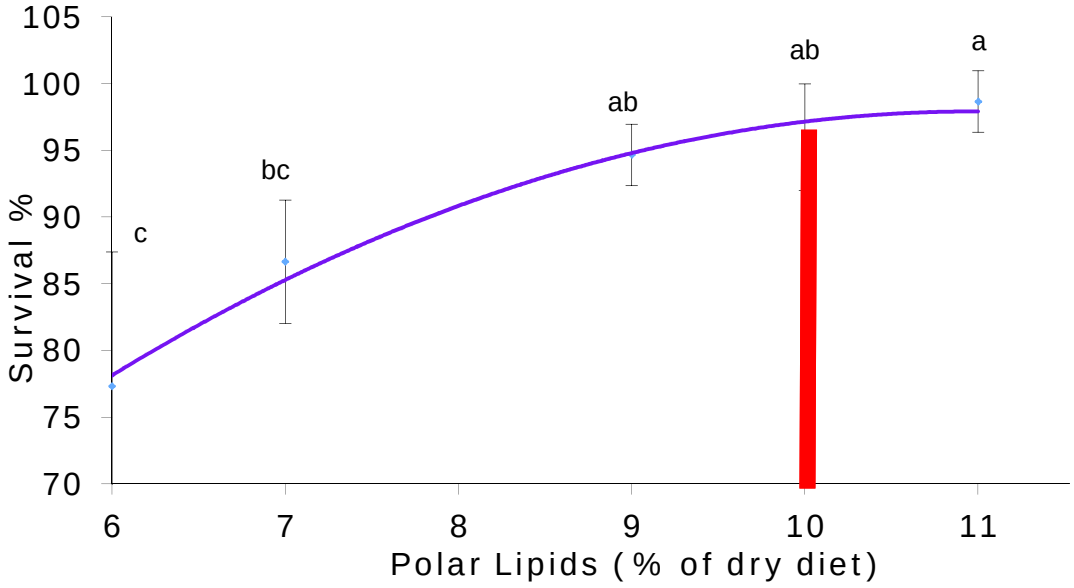


Exp. I Krill phospholipids



Final Survival

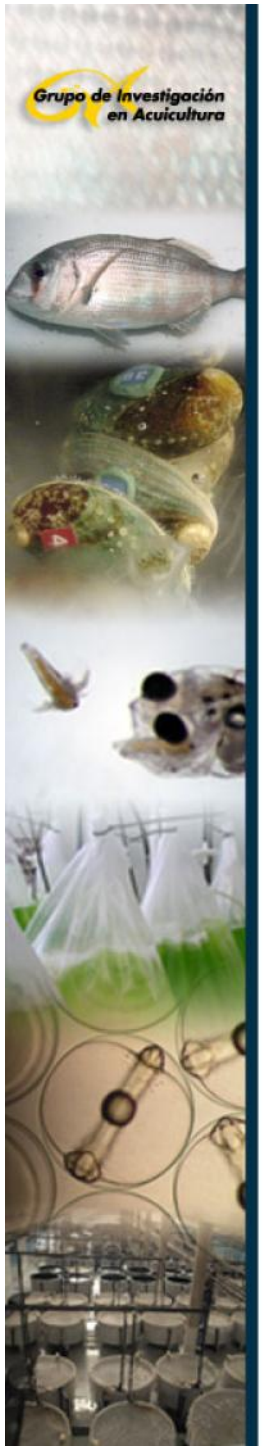
High survival & stress resistance with 9-10% KPL



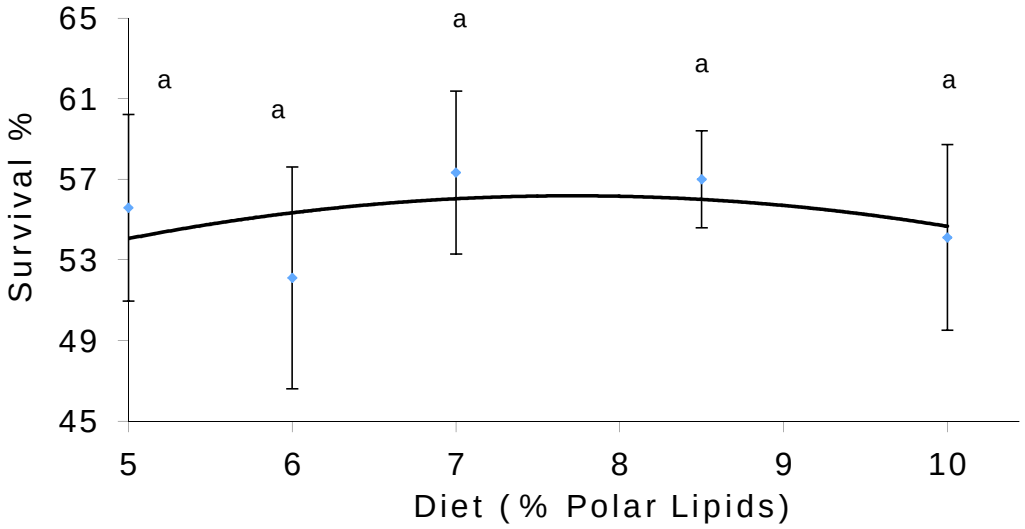
Survival after handling stress

Lower requirements than previous authors for European seabass using SBL (Cahu *et al.*, 2003)

Saleh et al., 2012. Aquaculture Nutr.

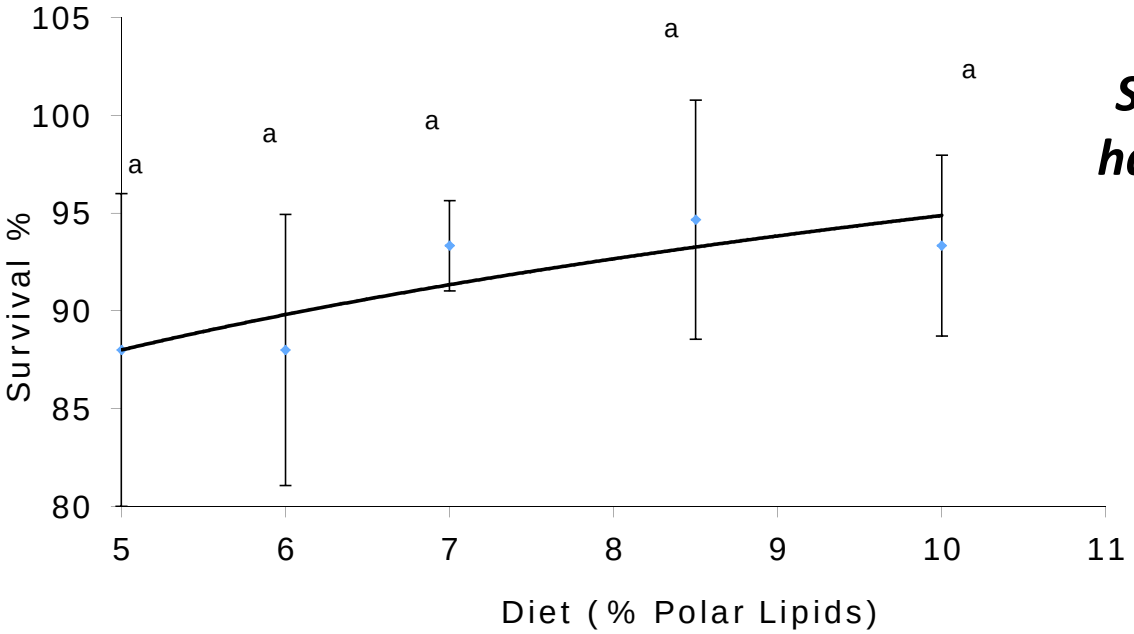


Exp 2. Soybean lecithin levels



Final Survival

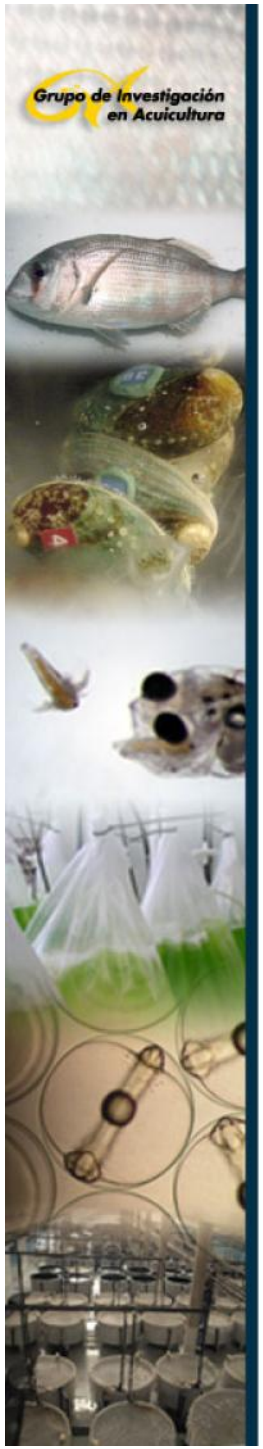
Poor effect of SBL on survival and stress resistance



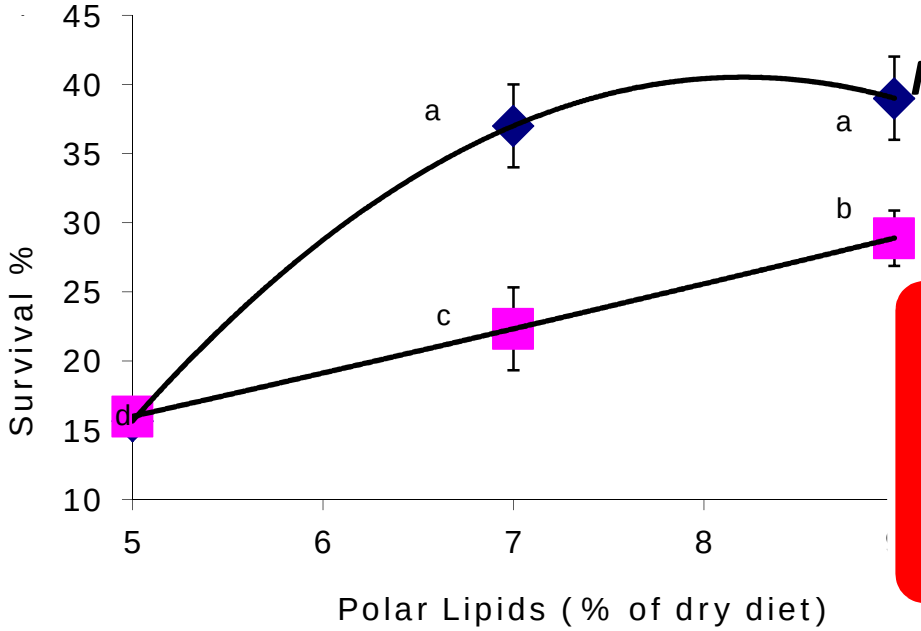
Survival after handling stress

Up to 12% SBL did not affect pikeperch larval survival (Hamza *et al.*, 2008)

Saleh et al, 2013. Aquaculture Nutr.

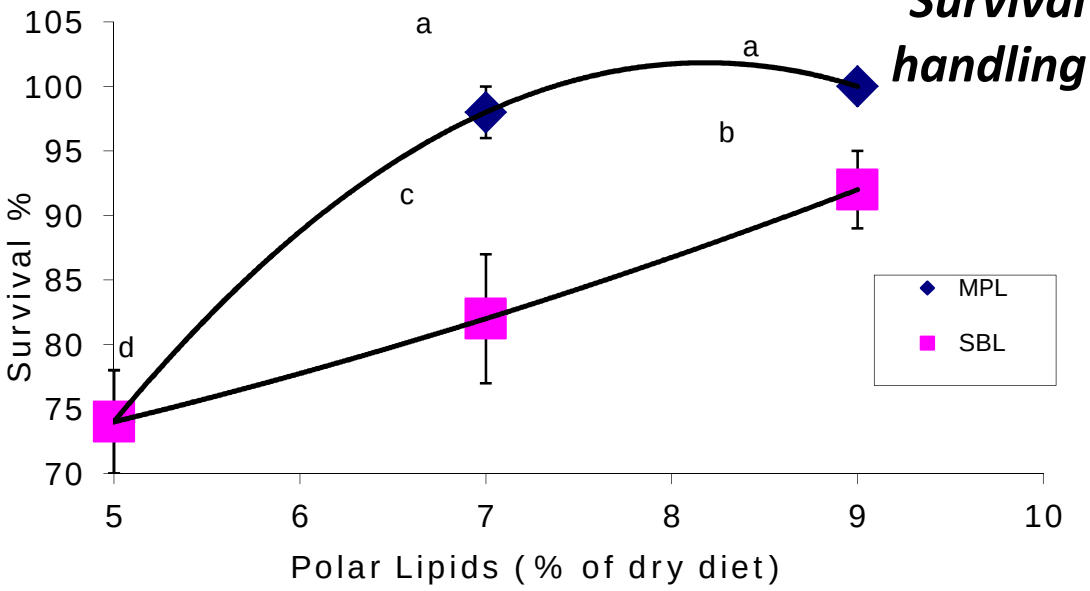


Exp 3. Krill phospholipids vs soybean lecithin



Final Survival

Better survival and stress resistance by KPL than SBL even at lower dietary levels

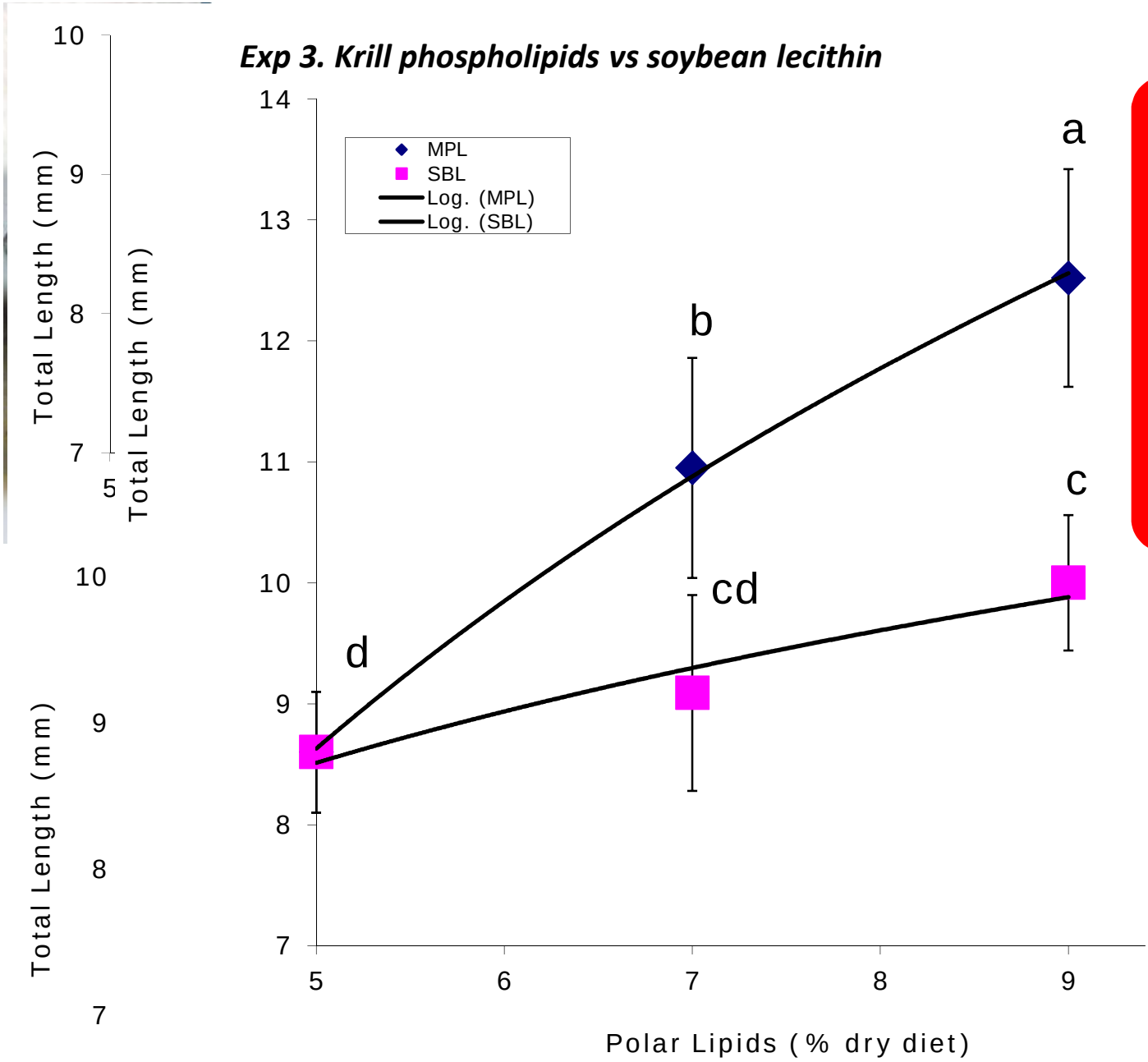


Survival after handling stress

Krill phospholipid as a good PL source (Betancor et al. 2012)

Saleh et al, in press. Aquaculture Nutr.

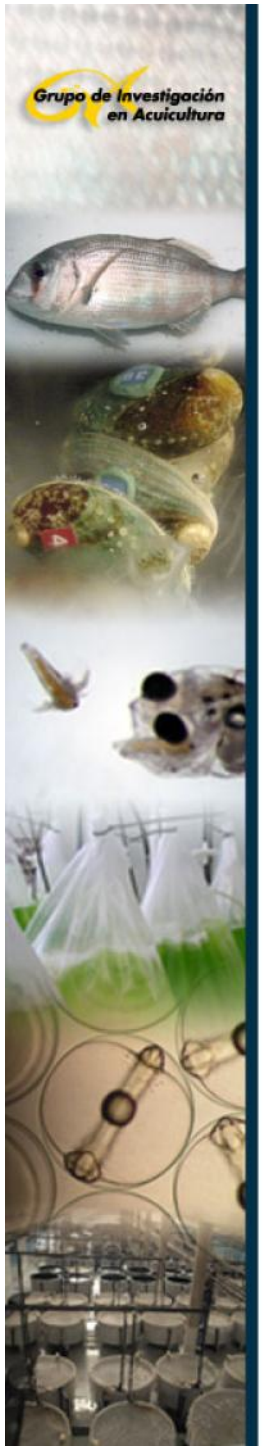
Exp 3. Krill phospholipids vs soybean lecithin



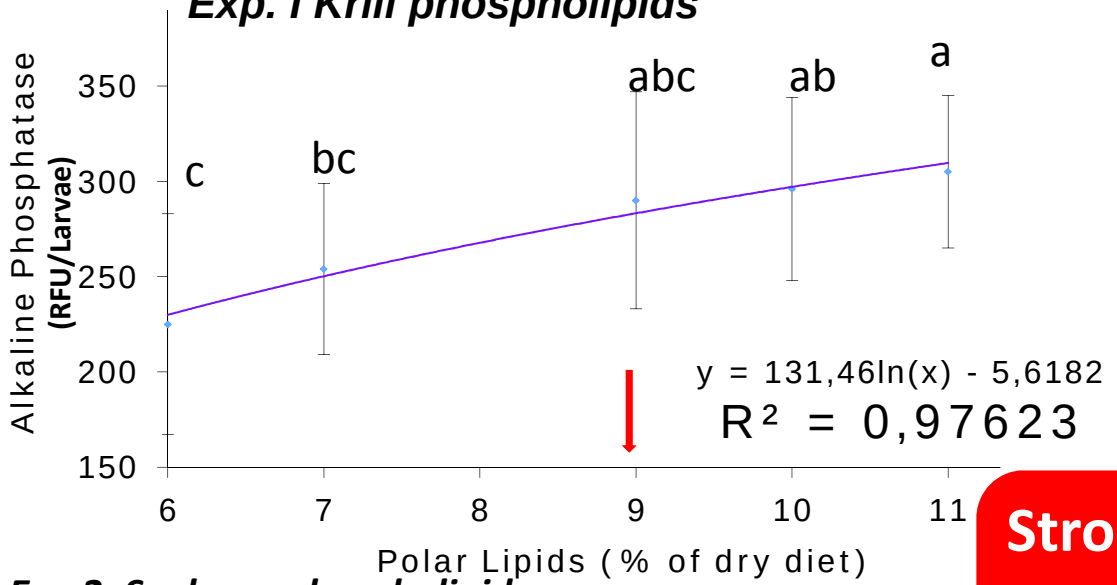
Better total length and body weight by KPL than SBL even at lower dietary levels

Higher effectiveness of marine PL (Salhi et al. 1999; Izquierdo et al. 2001; Wold et al. 2007)

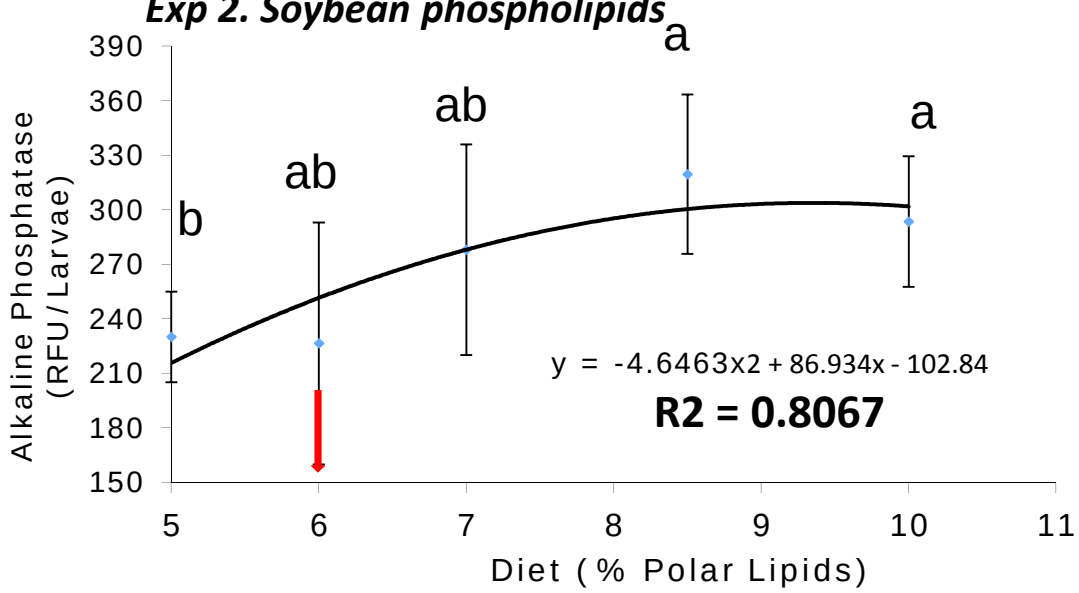
Saleh et al, in press. Aquaculture Nutr.



Exp. I Krill phospholipids

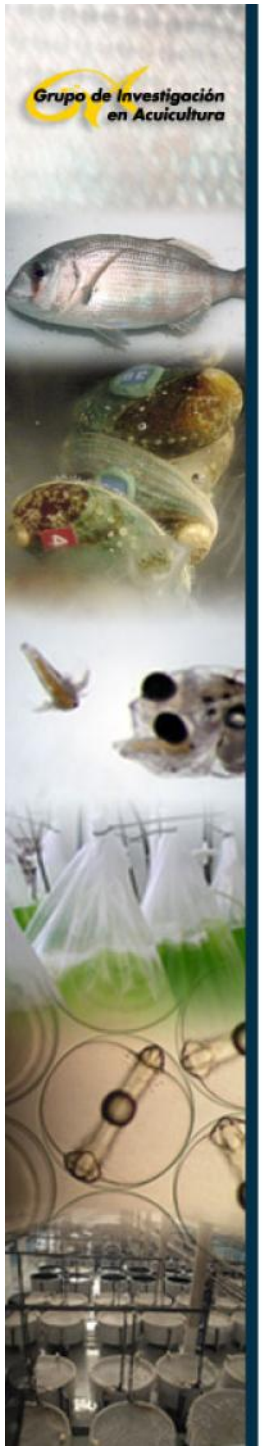


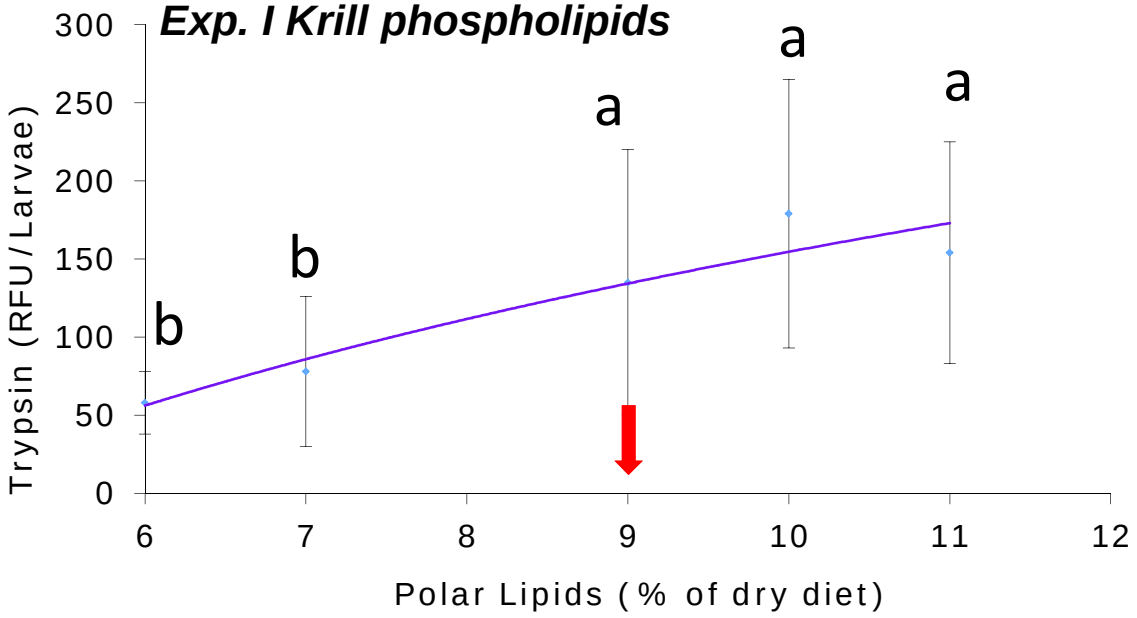
Exp 2. Soybean phospholipids

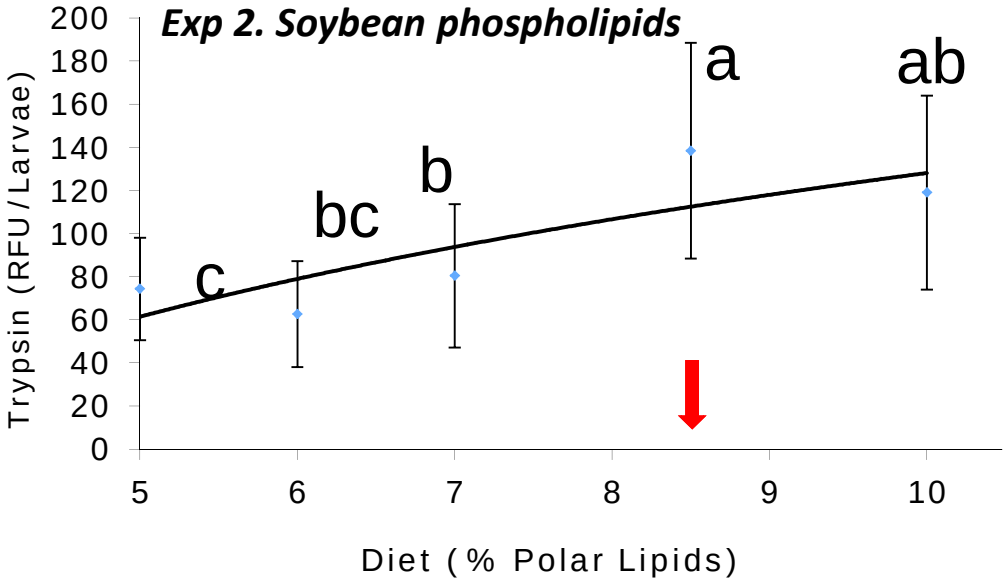


Stronger positive effect of KPL on Alkaline phosphatase and thus on maturation of digestive tract could contribute to the better growth

Saleh et al., 2012 & 2013. Aquaculture Nutr.

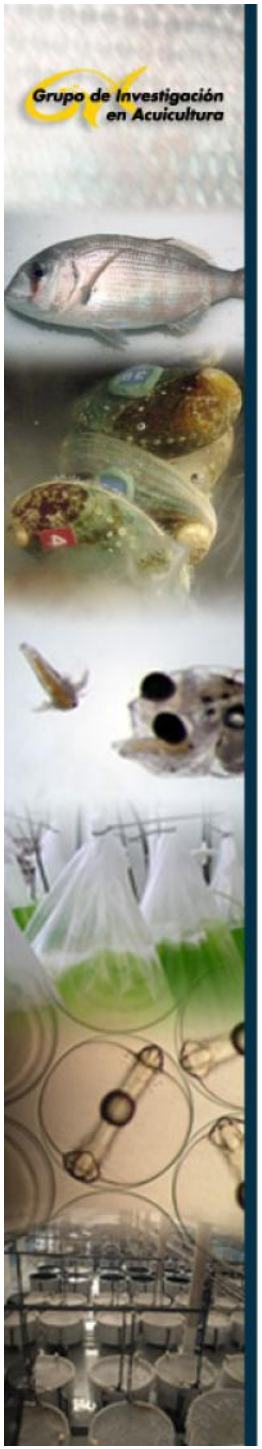






Very similar effect of both PL on Trypsin activity, so…not related with the HUFA content of the PL source

Saleh et al., 2012 & 2013. Aquaculture Nutr.



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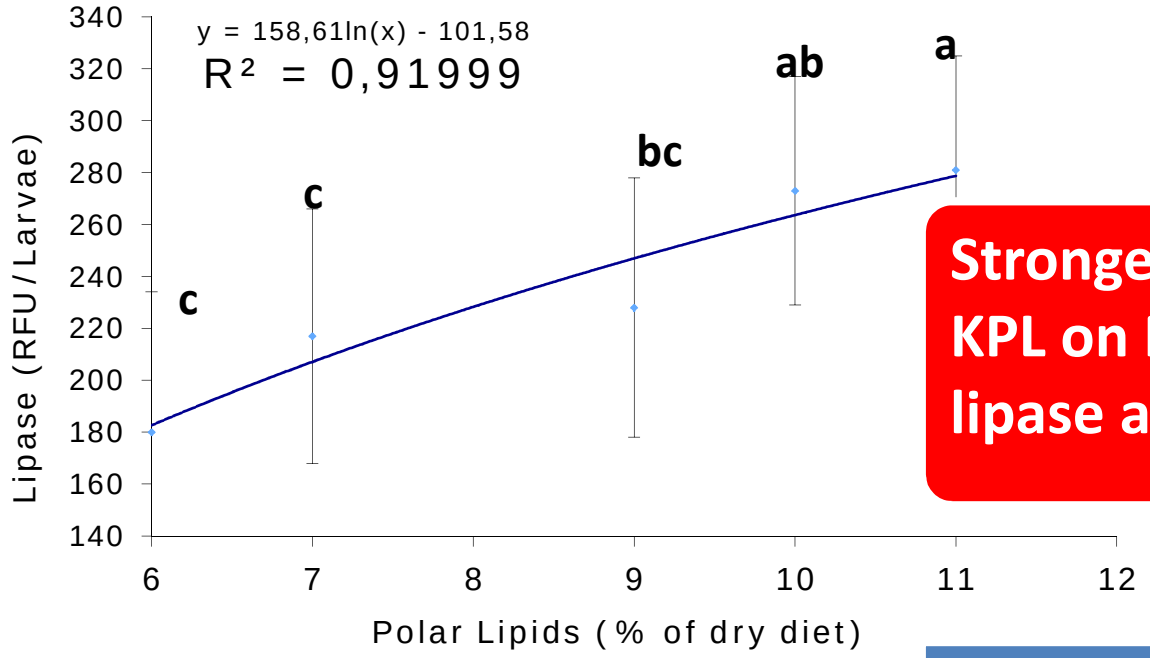
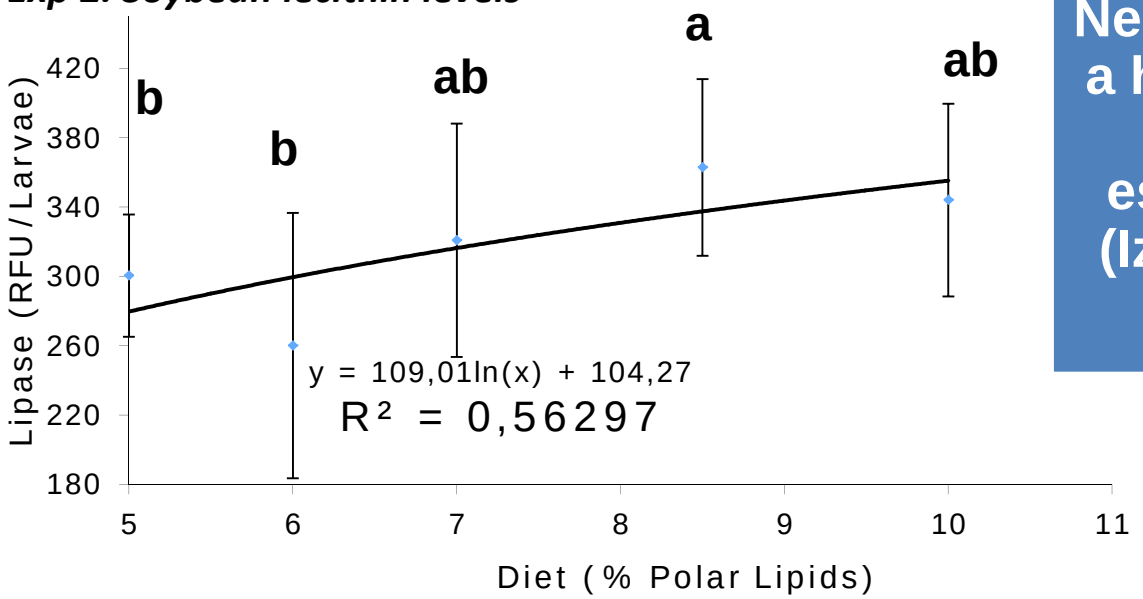


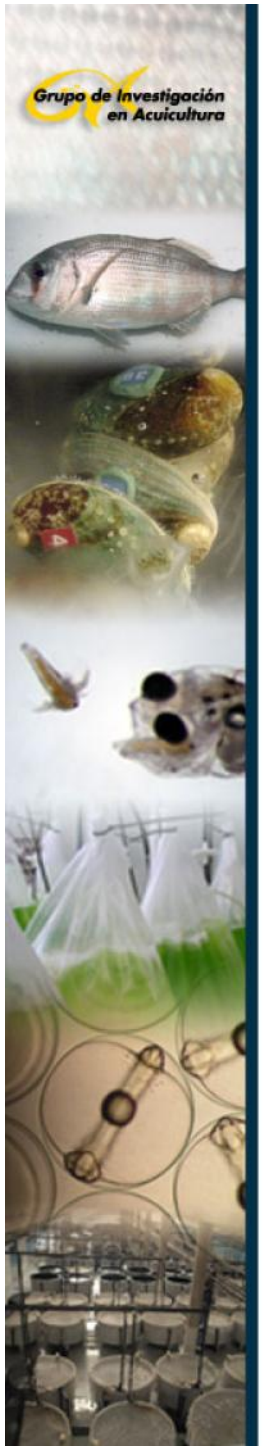
Stronger effect of KPL on Neutral lipase activity

Exp 2. Soybean lecithin levels



Neutral lipase has a high affinity for n-3 HUFA esterified lipids (Izquierdo et al., 2002)

Saleh et al., 2012 & 2013. Aquaculture Nutr.



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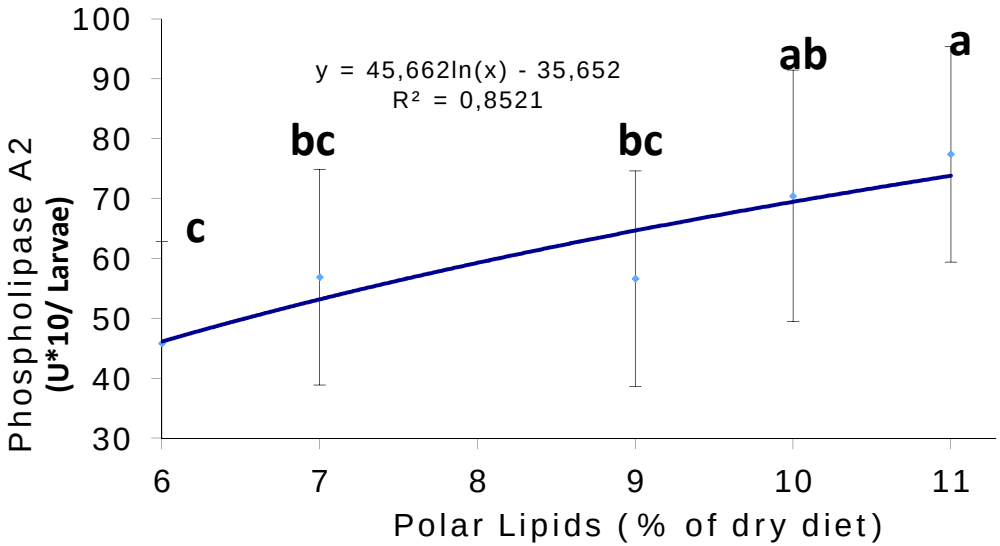
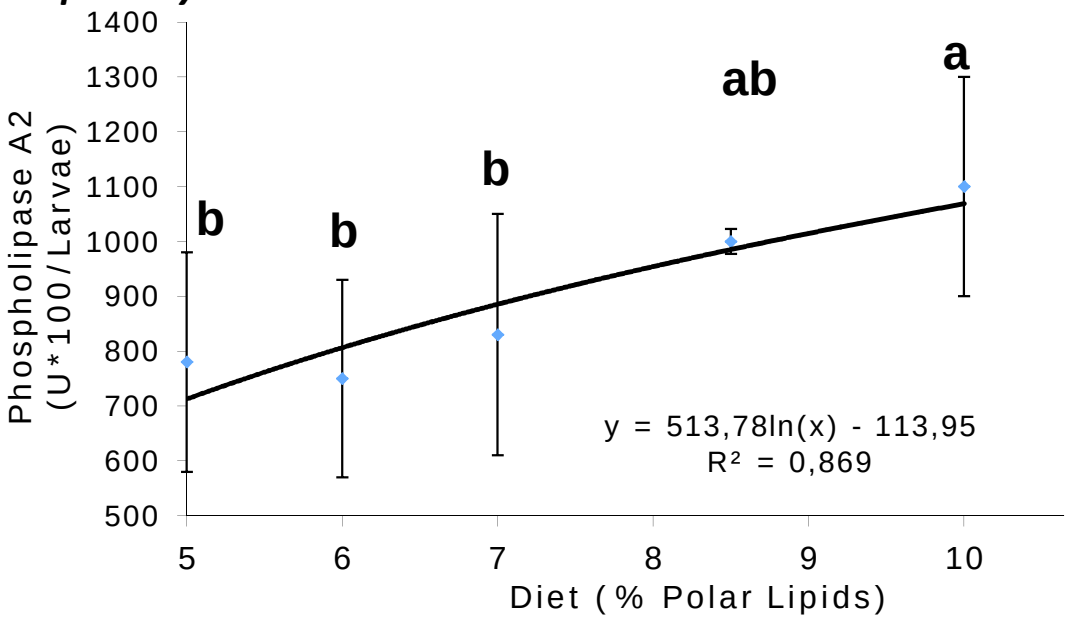


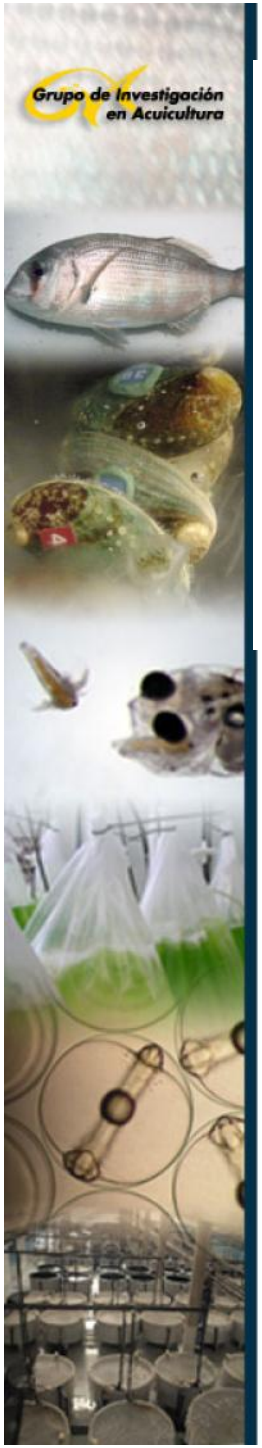
Very similar effect of both PL sources on PLA2…only depending on the level of PL

Exp 2. Soybean lecithin levels

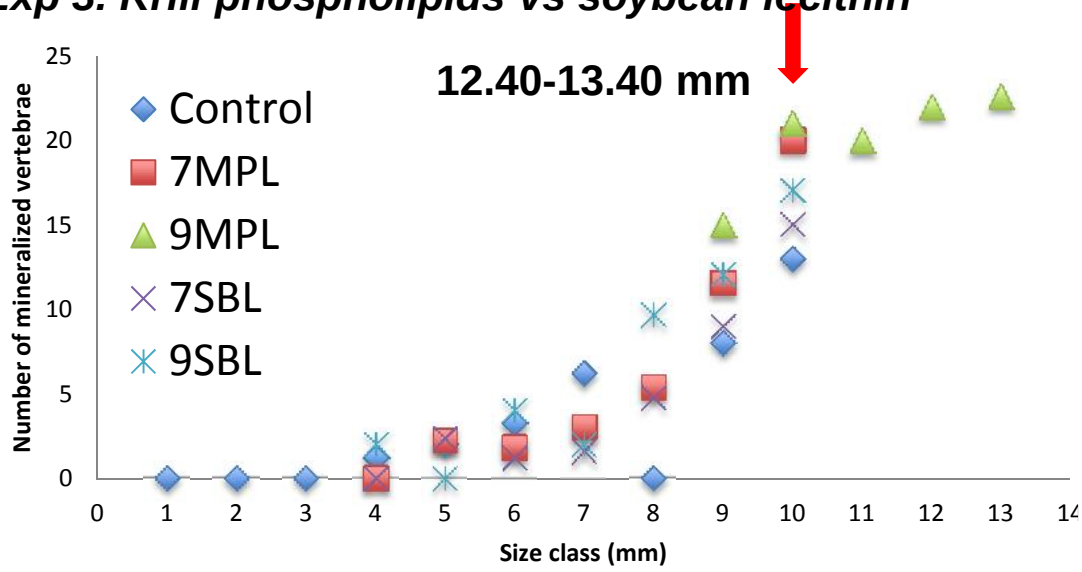


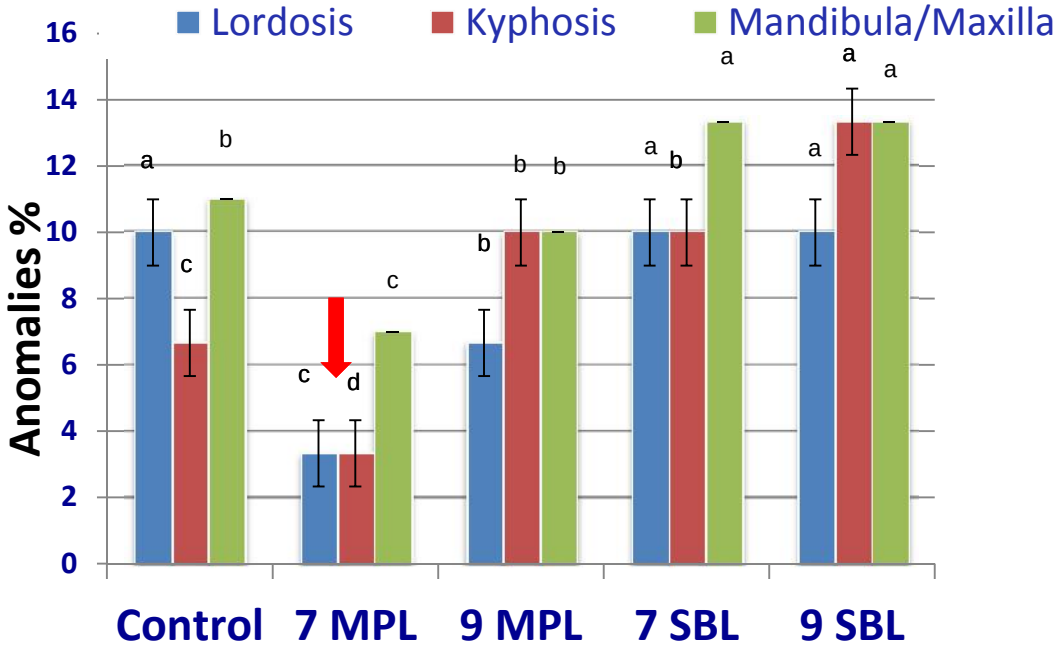
Transcriptional regulation of PLA2 by dietary PL (Zambonino-Infante and Cahu, 1999). SBL enhance gut and liver lipid transport activity (Liu et al., 2002)

Saleh et al, 2013. Aquaculture Nutr.



Exp 3. Krill phospholipids vs soybean lecithin

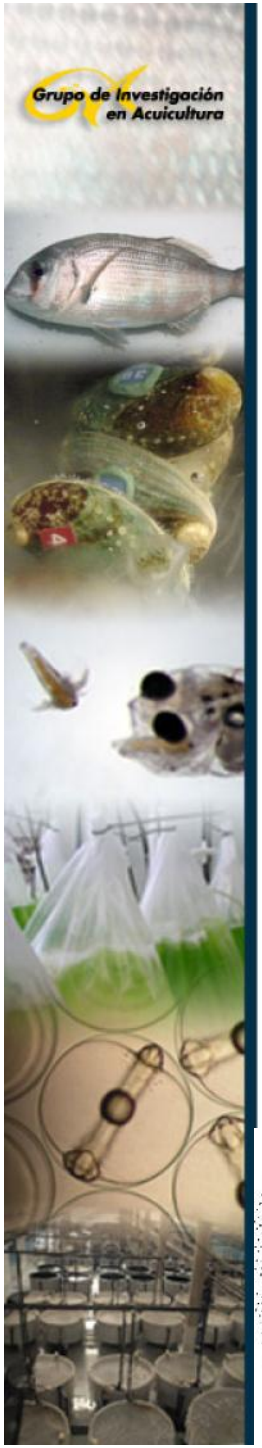




Saleh et al, in press. Aquaculture Nutr.

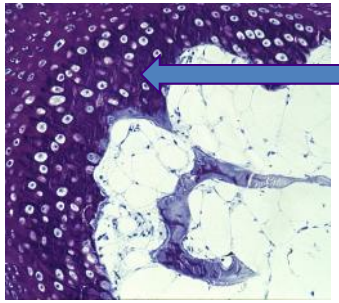
Both PL enhanced bone mineralization, but KPL was more effective and at 7% reduced bone anomalies

Improved mineralization and reduced deformities by n-3 HUFA but very high levels increased craneal deformities (Izquierdo et al., 2012 Br.J.Nutr.)

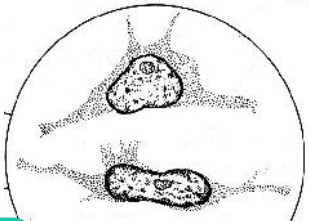


Endochondral bone

cartilage



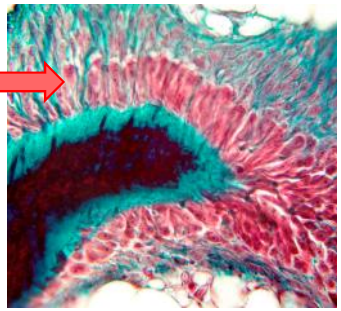
IGF



Mesenchymal stem cell

Intramembranous bone

osteoblasts



Sox9

Runx2

PPARϒ

BMP-2

Collagen II

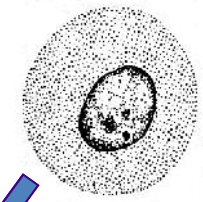
Collagen I

Osteopontin

Osteocalcin

ALP

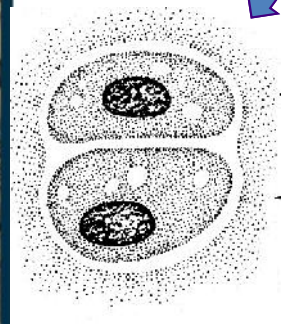
Matrix GLA

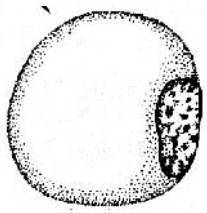


chondroblast

osteoblasts

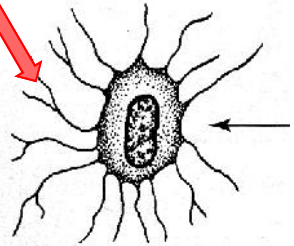
chondrocyte

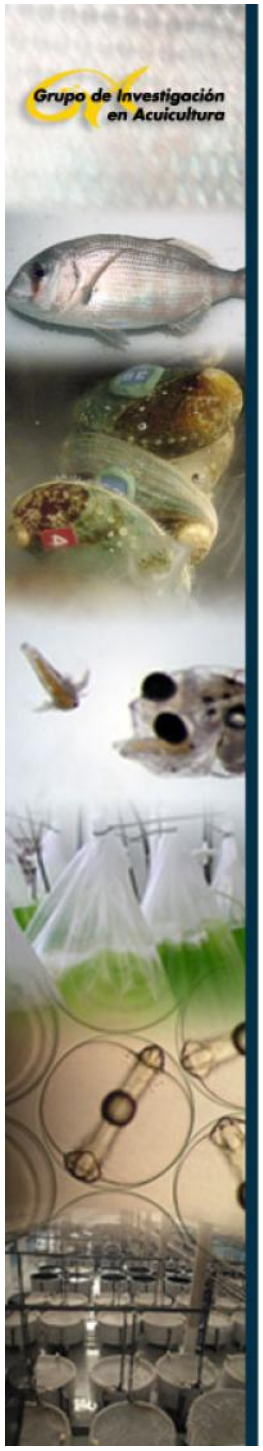




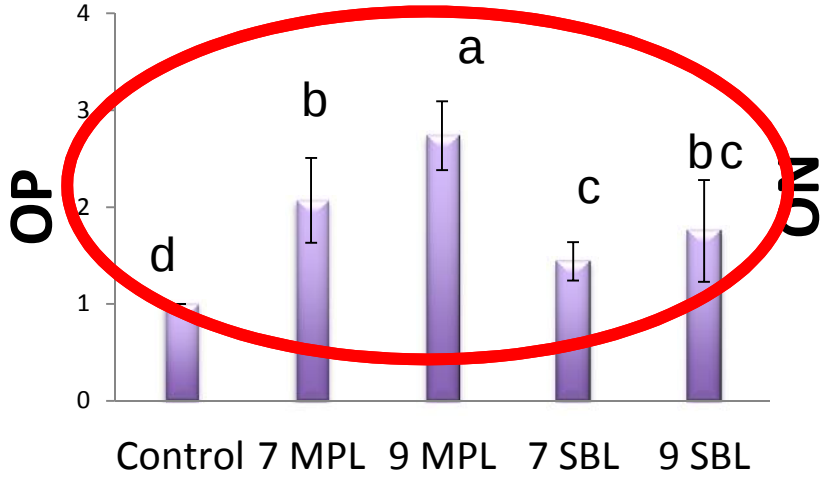
adipocyte

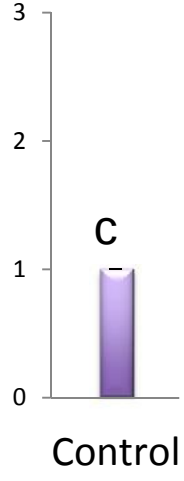
osteocyte



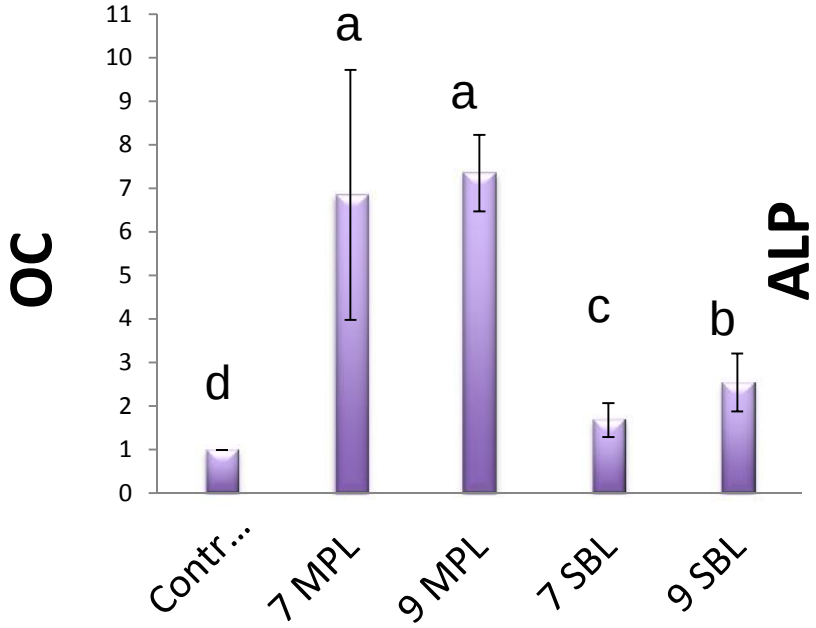


Exp 3. Krill phospholipids vs soybean lecithin



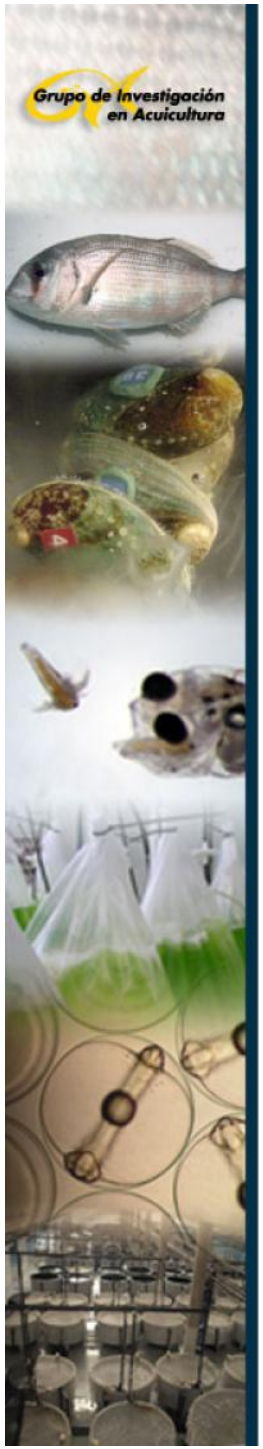


At 30 dph OP (SPP1) is more sensitive to DHA levels than in other bone molecular marker studied (Izquierdo unpublished data)

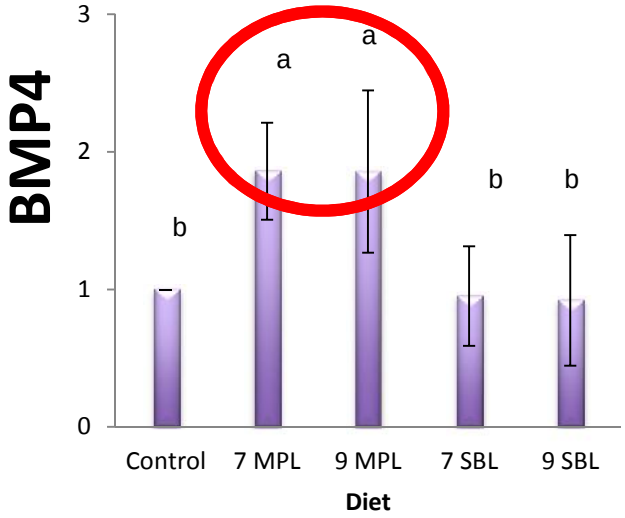


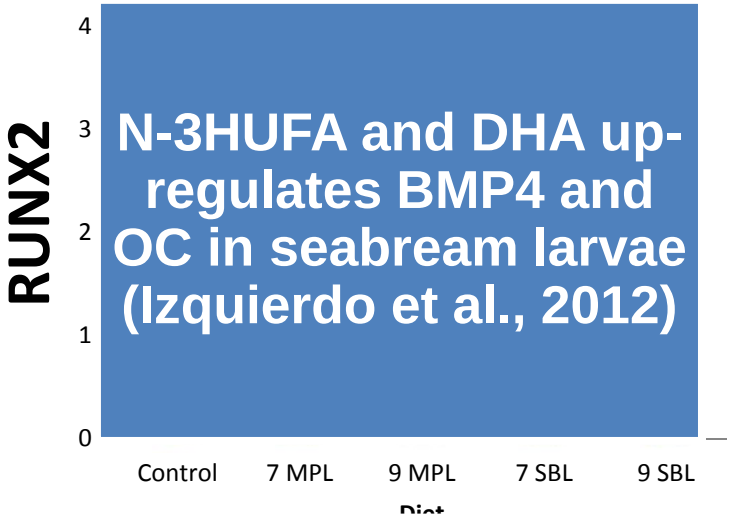
The three mineralization protein genes were up-regulated by KPL and to a lower extent by SBL, but Osteopontine expression was better correlated with the mineralization observed

Saleh et al, in press. Aquaculture Nutr.

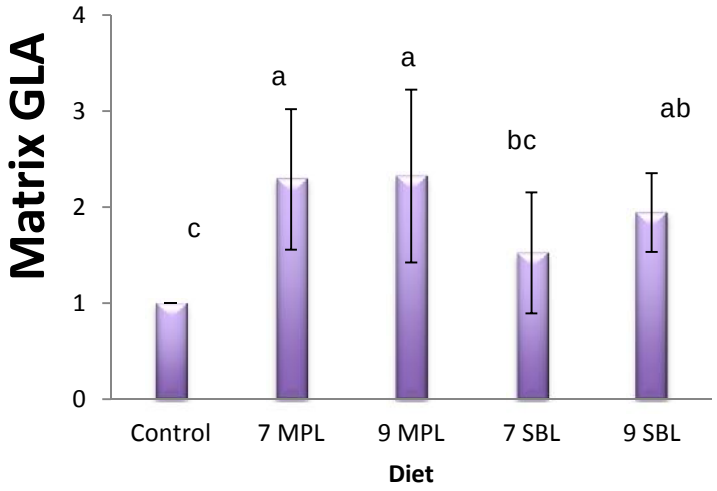


Exp 3. Krill phospholipids vs soybean lecithin



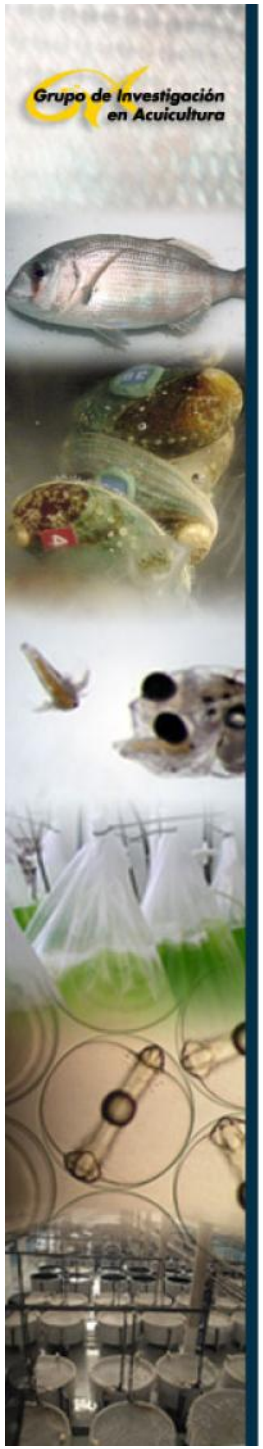


N-3HUFA and DHA up-regulates BMP4 and OC in seabream larvae (Izquierdo et al., 2012)

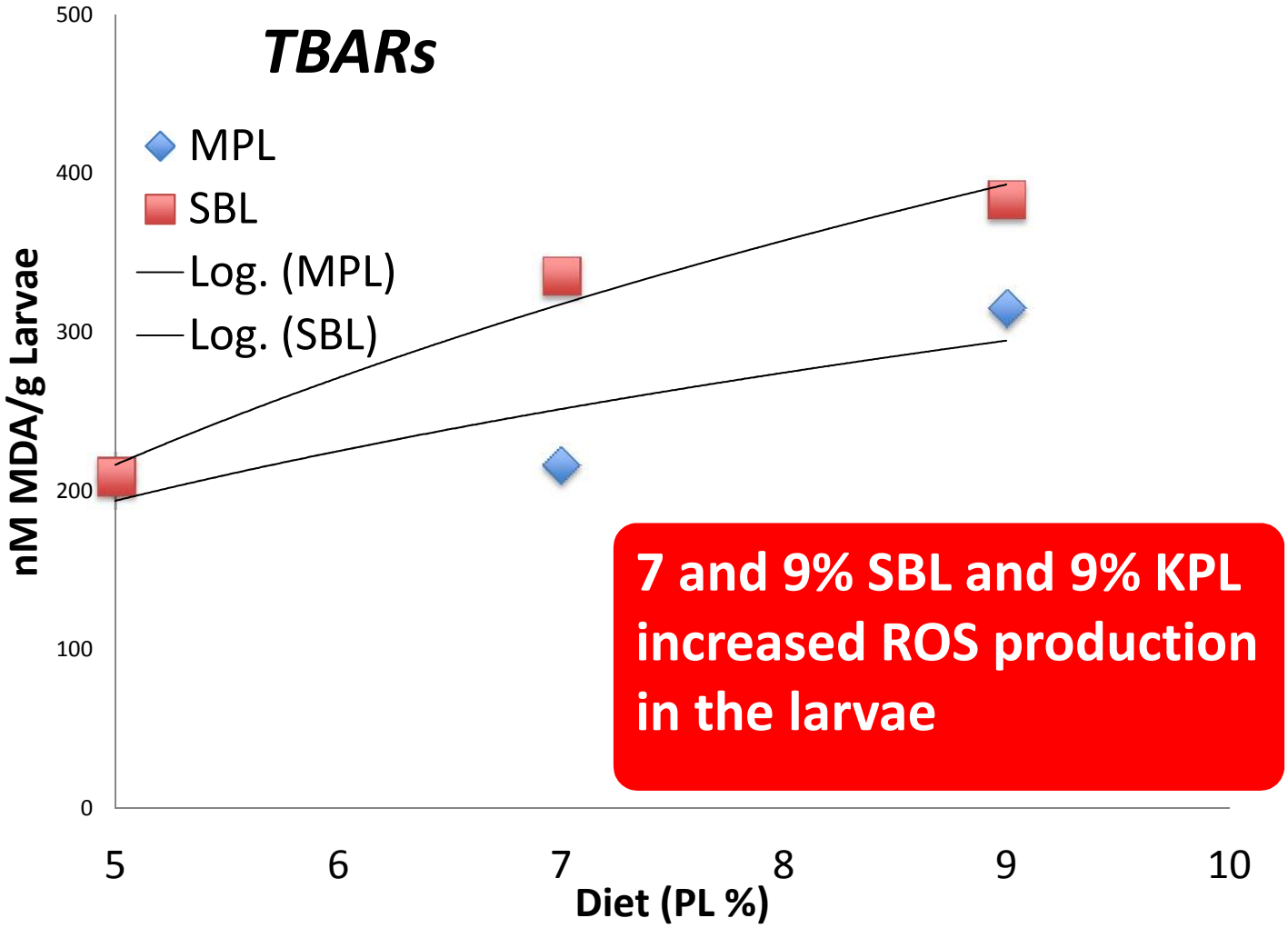


BMP was up-regulated by KPL and it was negatively related to lordosis incidence. The other two markers of early and late bone differentiation were also affected by KPL

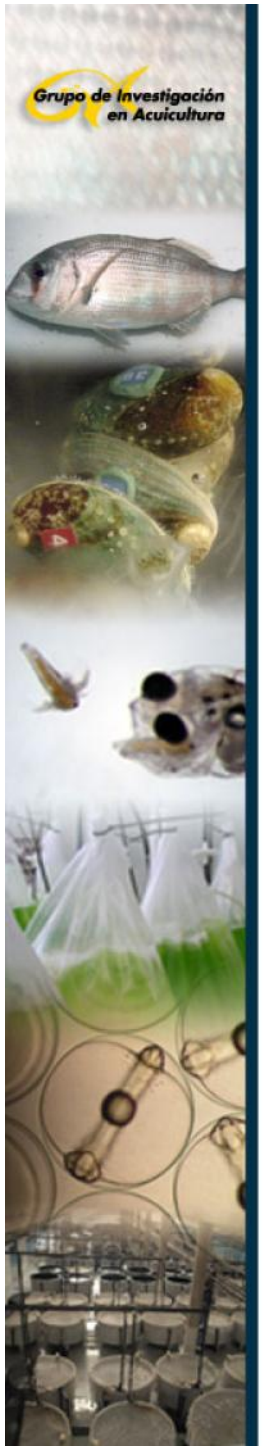
Saleh et al, in press. Aquaculture Nutr.



Exp 3. Krill phospholipids vs soybean lecithin



Saleh et al, in press. Aquaculture Nutr.



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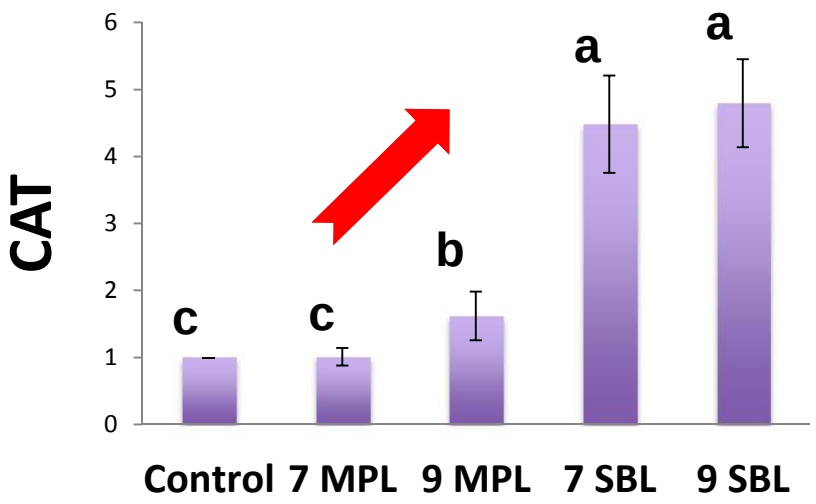
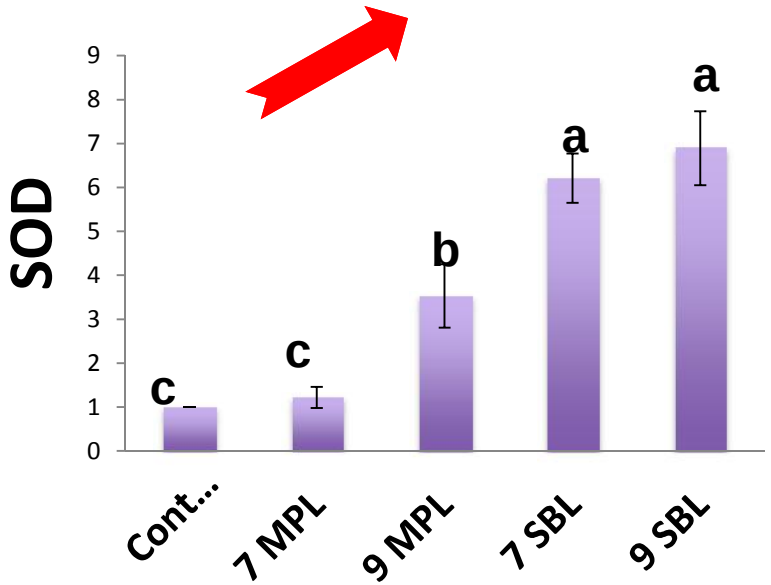
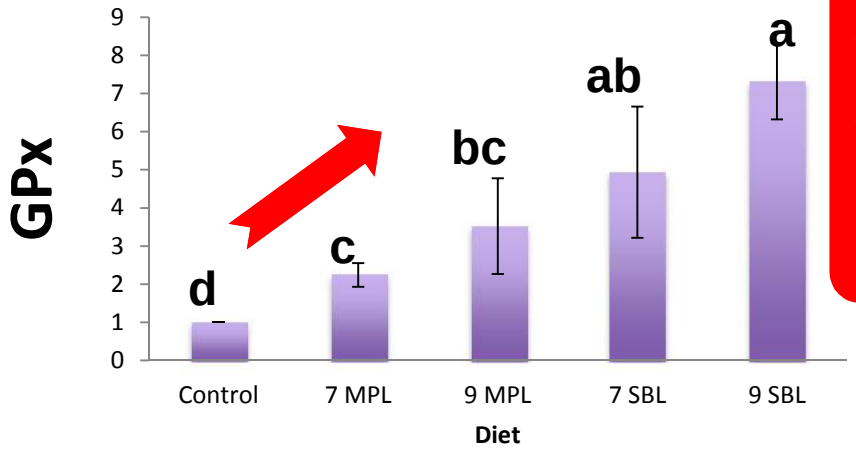
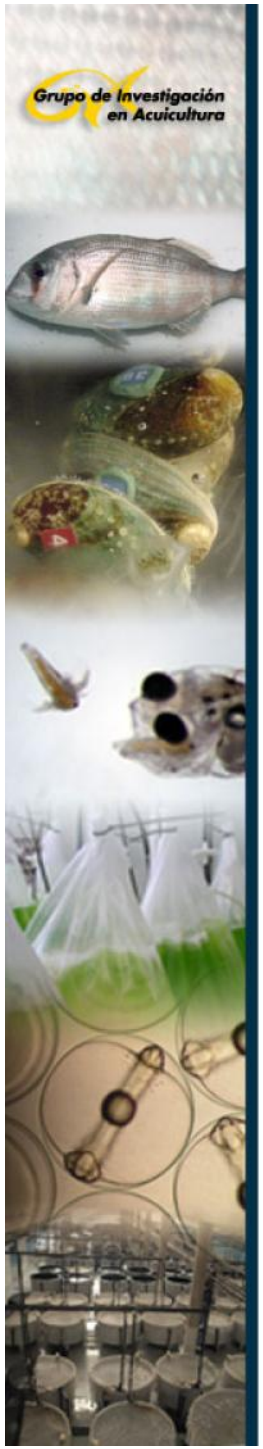






AOE, particularly CAT, gene expression was up-regulated by SBL and 9% KPL

Saleh et al, in press. Aquaculture Nutr.



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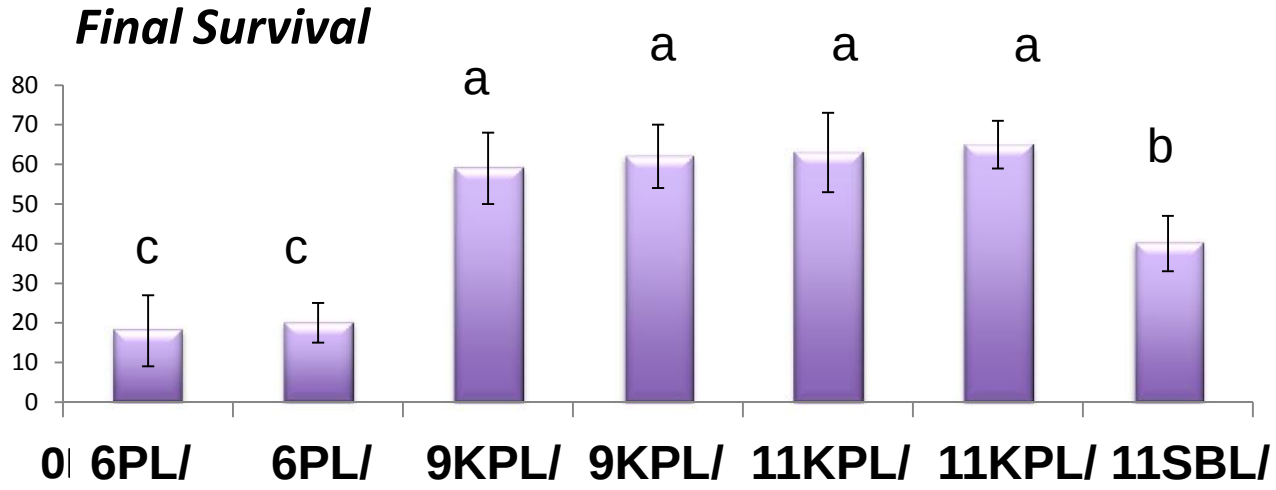
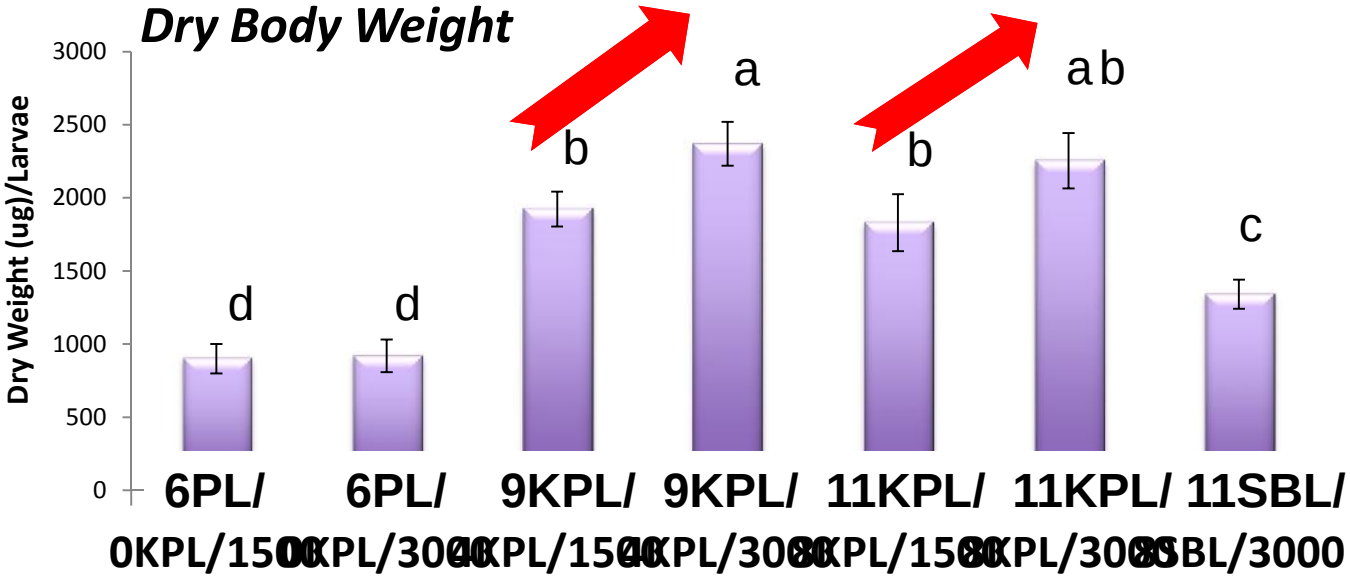
Exp 4. Combined Vit E and PL levels

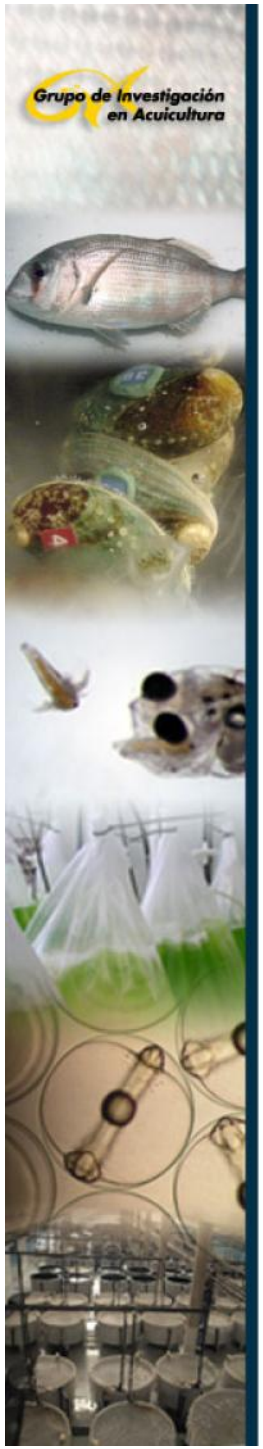
Vit E addition was not able to improve survival or growth in SBL diets

Dietary vit E improved growth in KPL diets



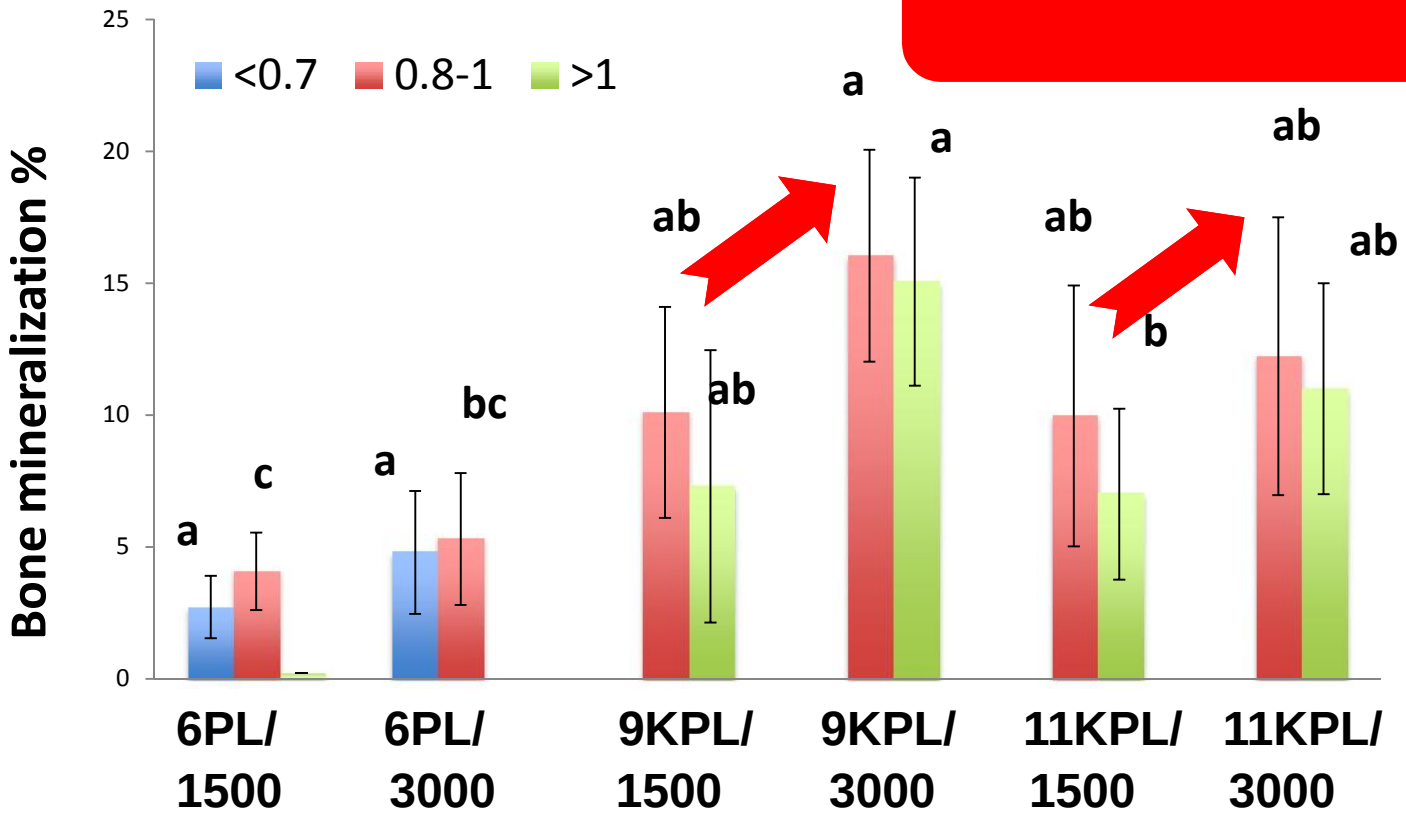


Saleh et al, submitted. Aquaculture Nutr.



Exp 4. Combined Vit E and PL levels

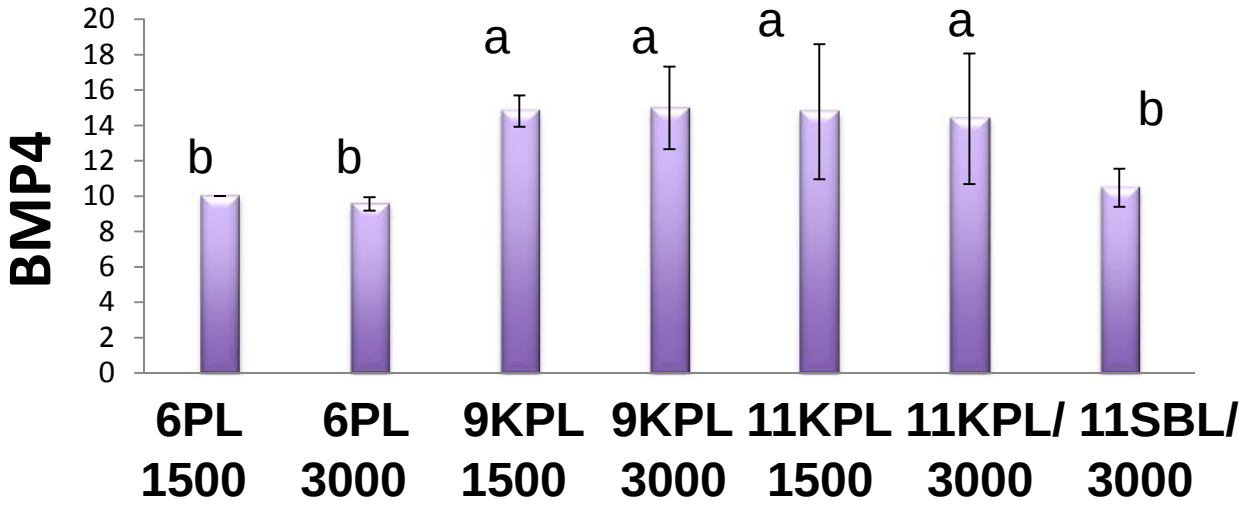
Increased dietary Vit E raised the percentage of mineralized bone in a given size class

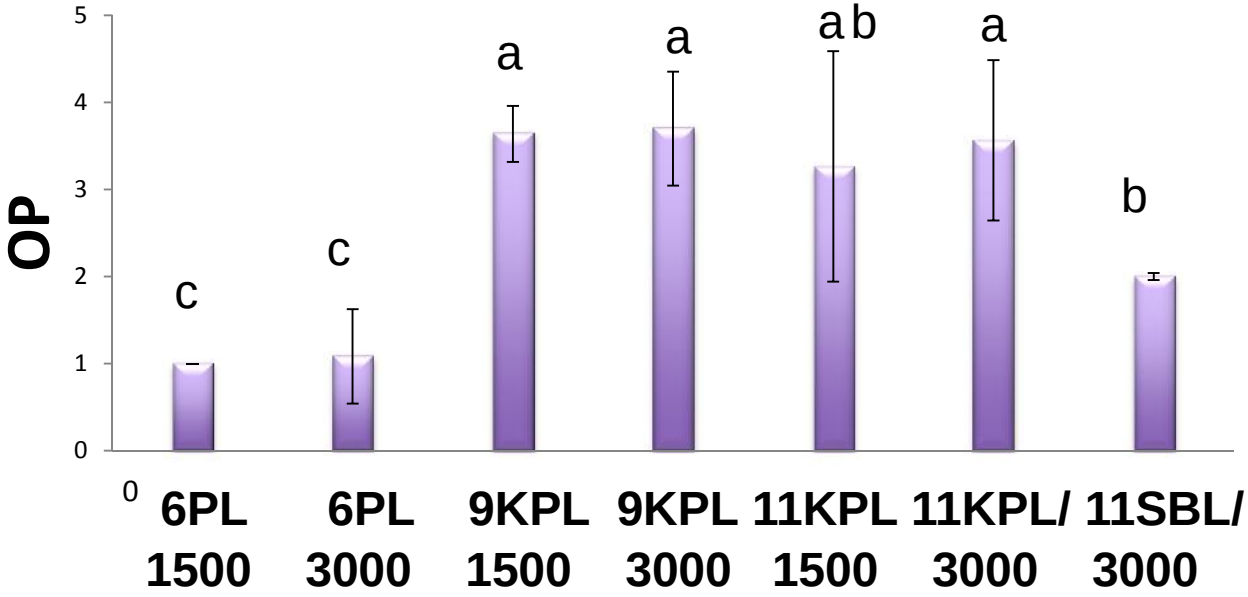


Saleh et al, submitted. Aquaculture Nutr.

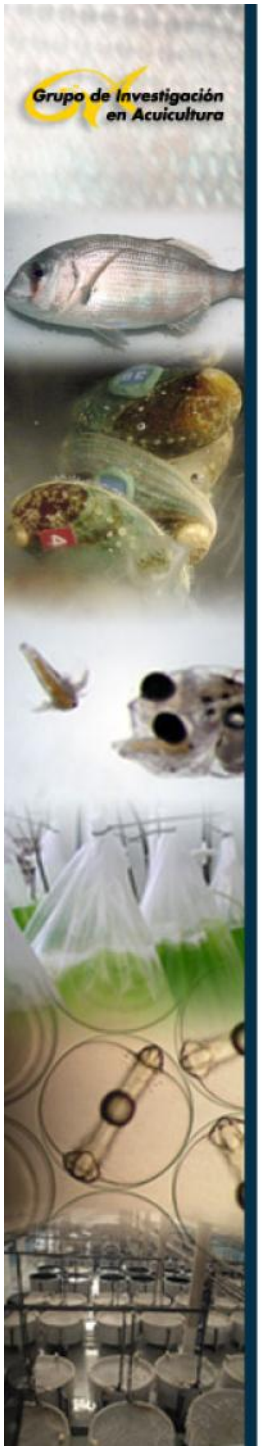
Exp 4. Combined Vit E and PL levels

Vit E addition did not affect any of the 7 bone biomarkers studied, although KPL and, thus n-3 HUFA, up-regulated them, but not through ROS!!





Saleh et al, submitted. Aquaculture Nutr.



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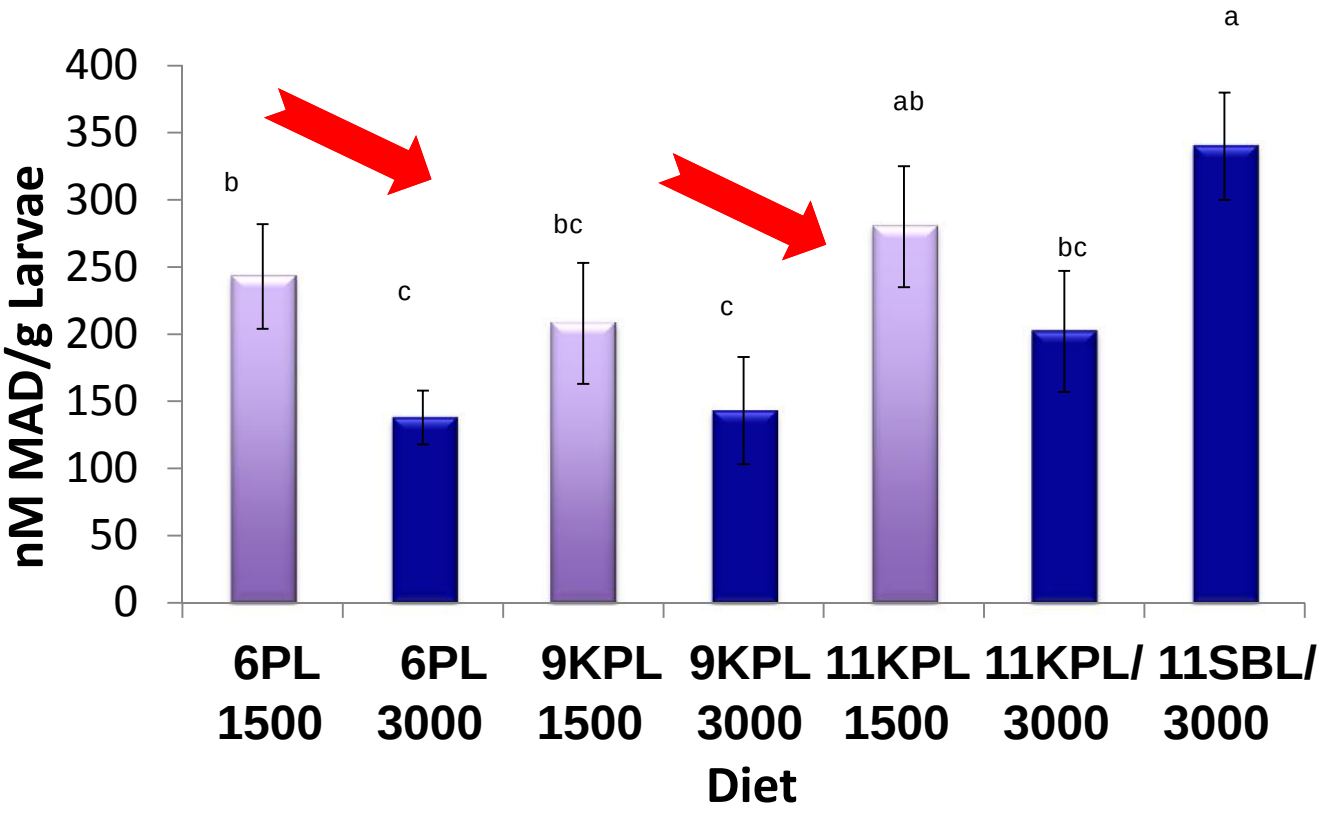
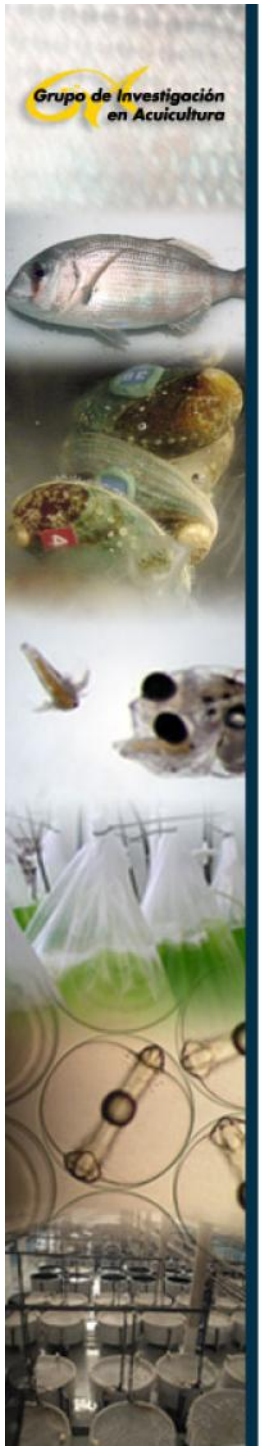


Saleh et al, submitted. Aquaculture Nutr.



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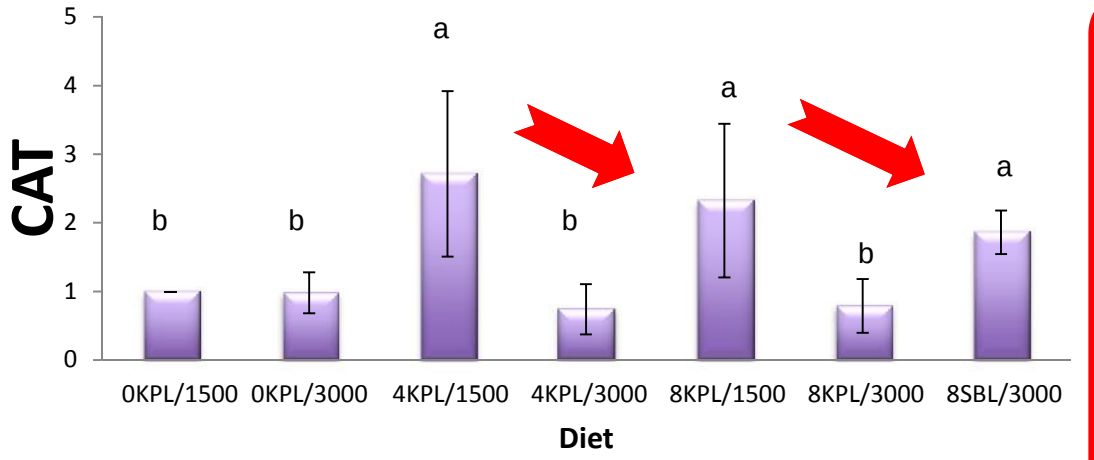
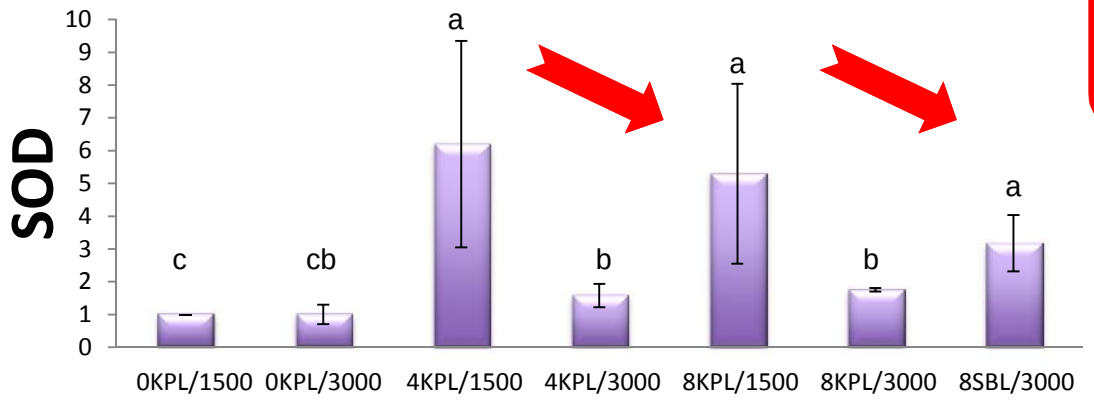
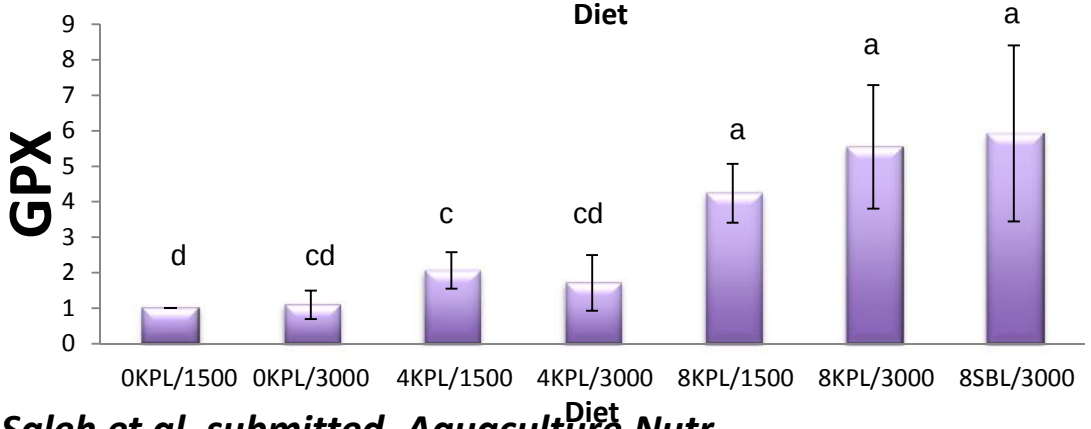
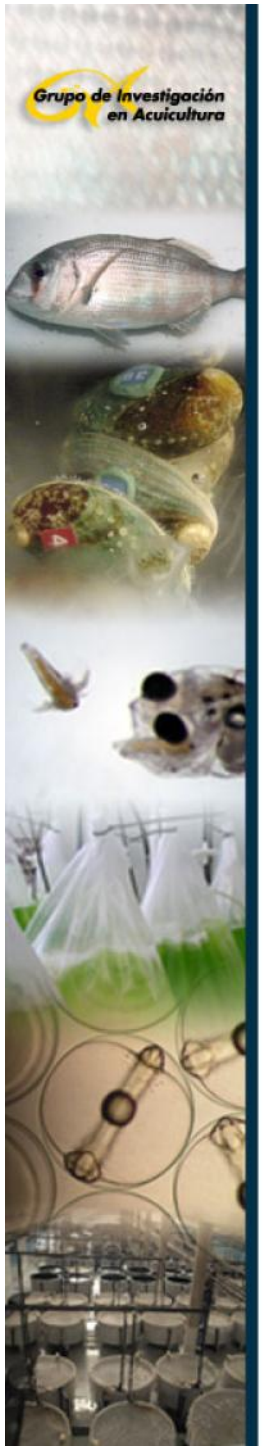






CAT gene expression is better correlated than GPX to oxidative risk by DHA (Izquierdo *et al*., 2012).

Saleh et al, submitted. Aquaculture Nutr.



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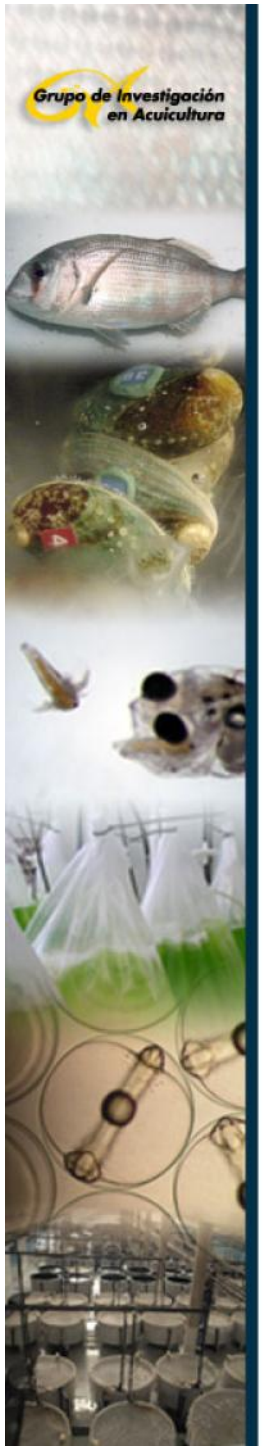
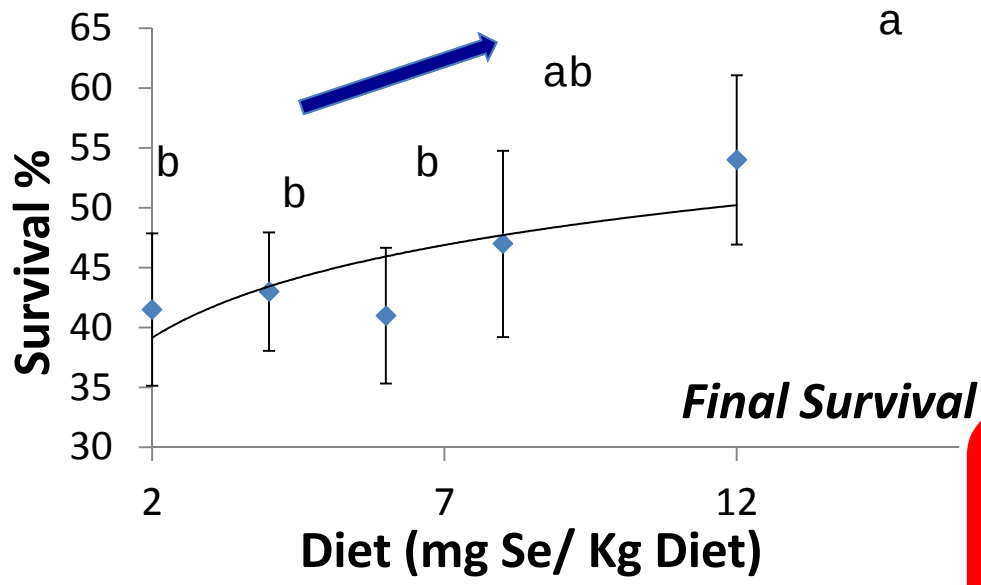
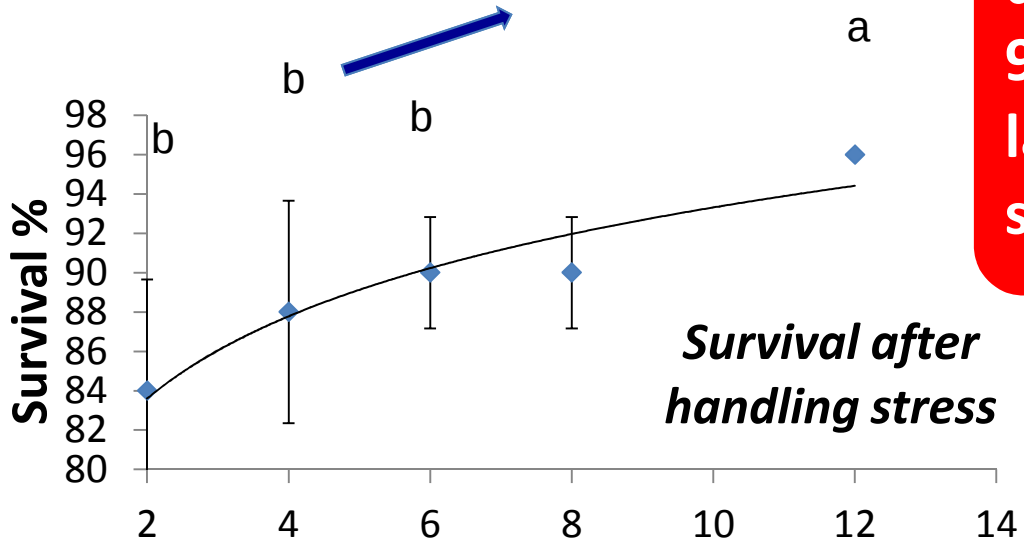


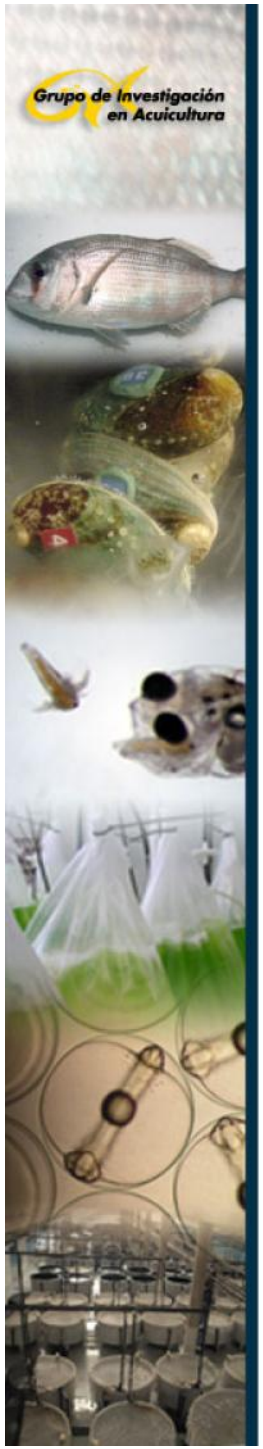
Exp 5. Effect of Se levels





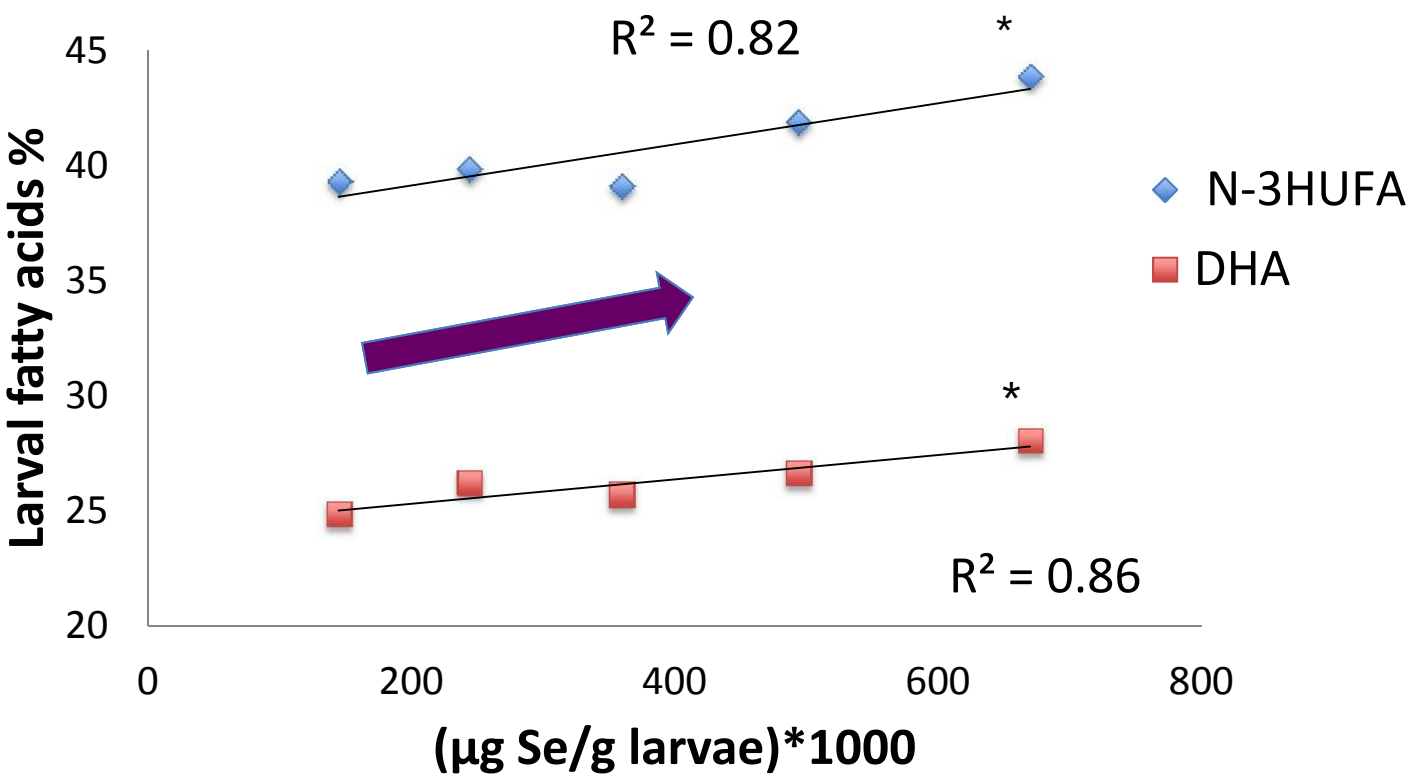
Addition of Se up to 8-12 mg/kg in diets containing 9% KPL increased larval survival and stress resistance

Saleh et al, submitted. Br. J. Nutr.

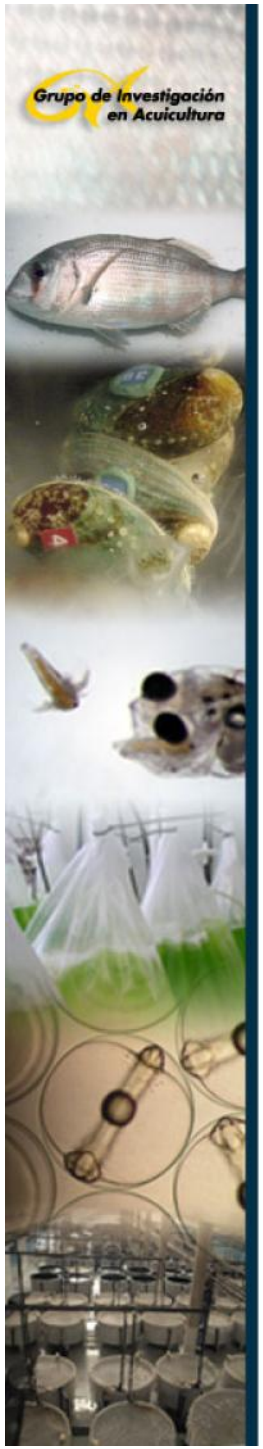


Exp 5. Effect of Se levels

Larval Se and n-3 HUFA were correlated



Saleh et al, submitted. Br. J. Nutr.



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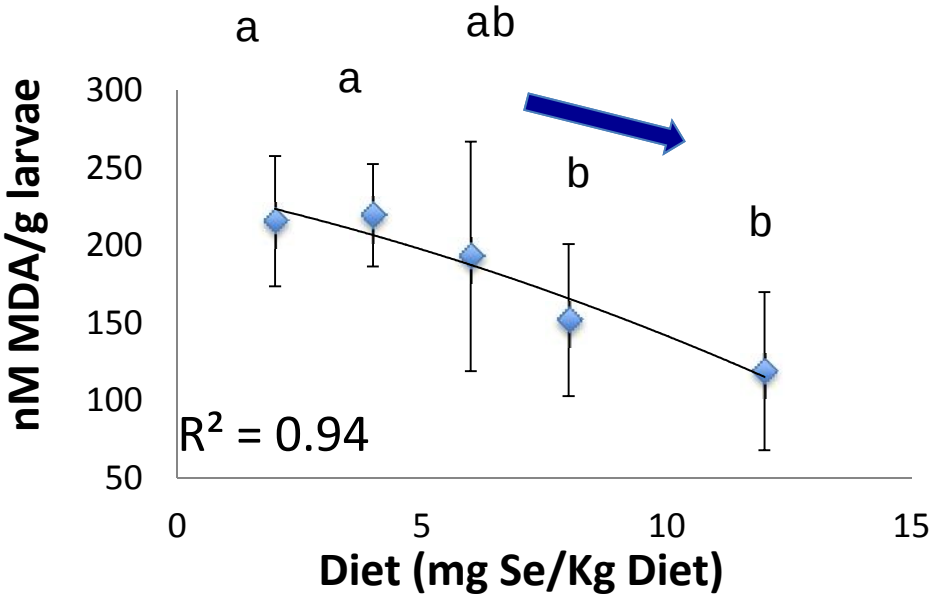
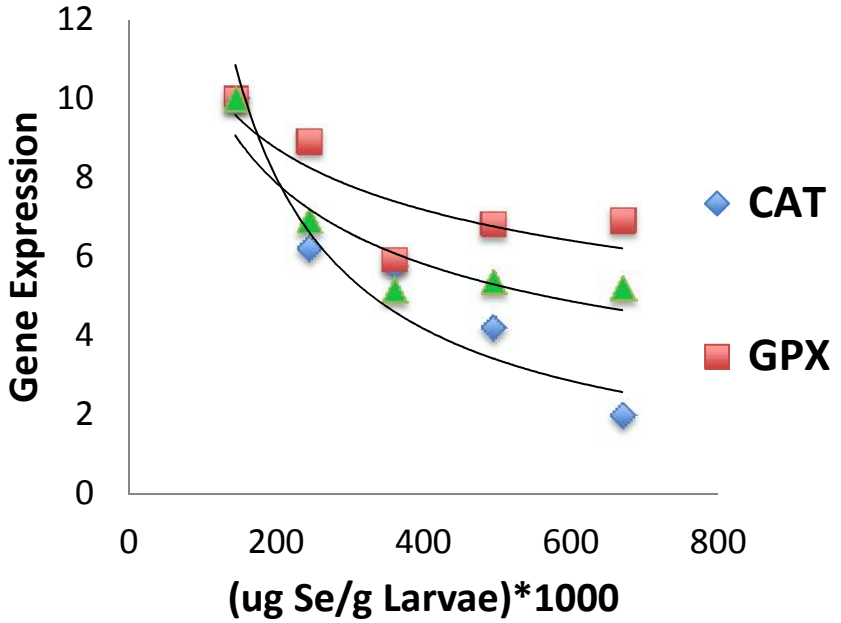
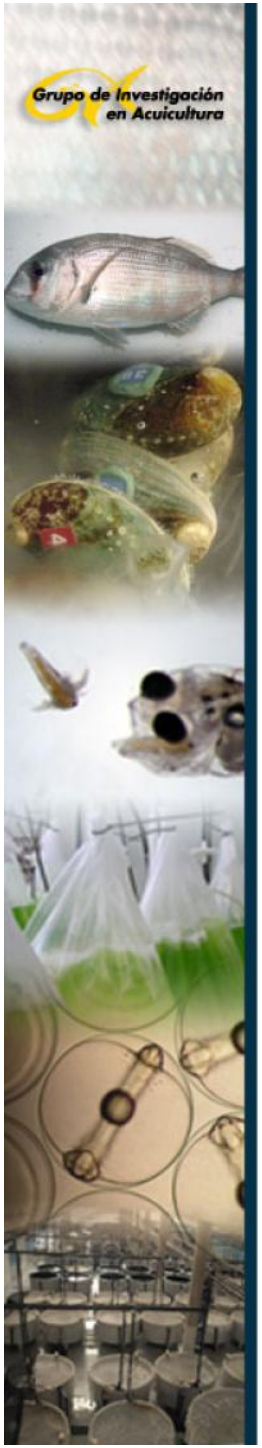




Dietary and larval Se was negatively correlated with TBARs and AOE gene expresion

Saleh et al, submitted. Br. J. Nutr.



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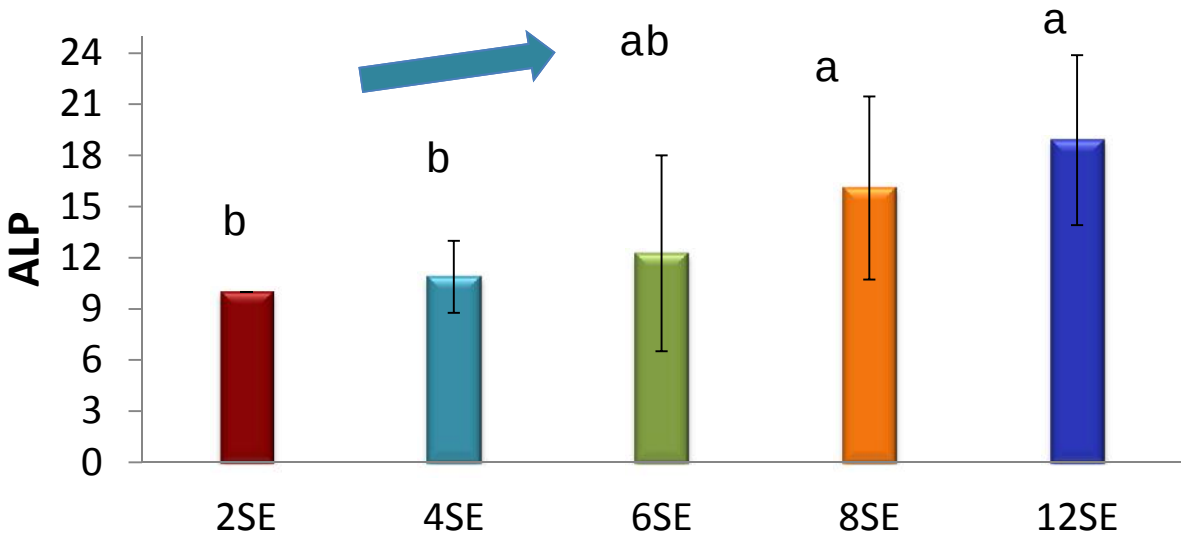
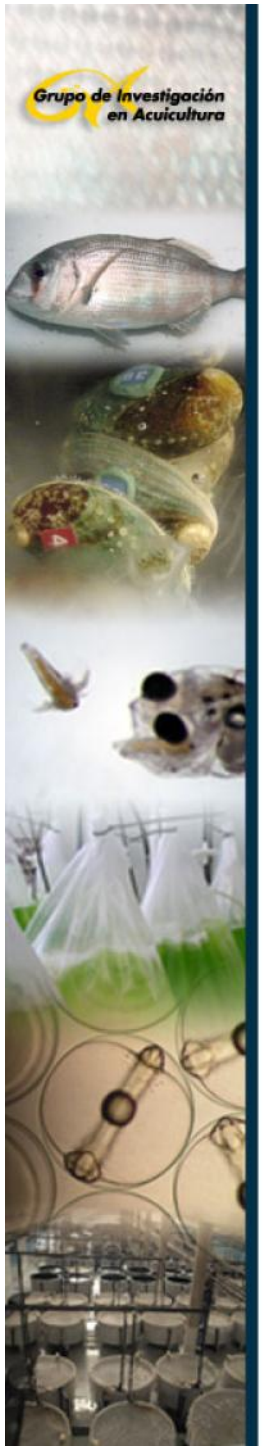


Dietary and larval Se, or reduction in ROS was better correlated with ALP gene expresion than the other 6 bone molecular markers

In agreement , there was an increased mineralization and reduced scoliosis incidence (Poster session, Bénitez et al.)

Saleh et al, submitted. Br. J. Nutr.



Oxidative balance in marine larvae

ROS

ROS

α-TOH

Vit C

PUFA

Fe

Stress

Light

Photoperiod

Canibalism

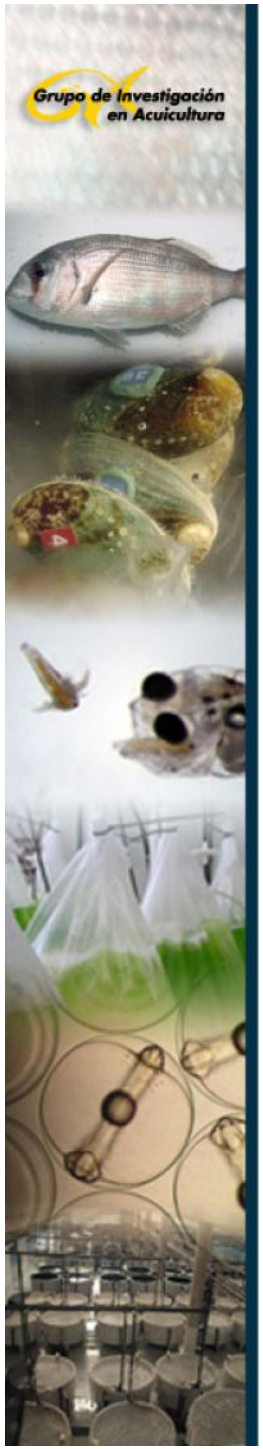
Pro-oxidants

Anti-oxidants

α-TOH

Se

carotenoids



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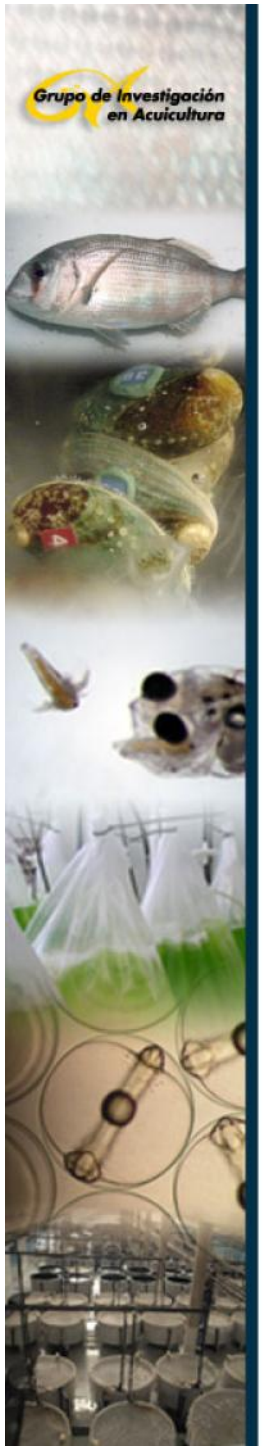
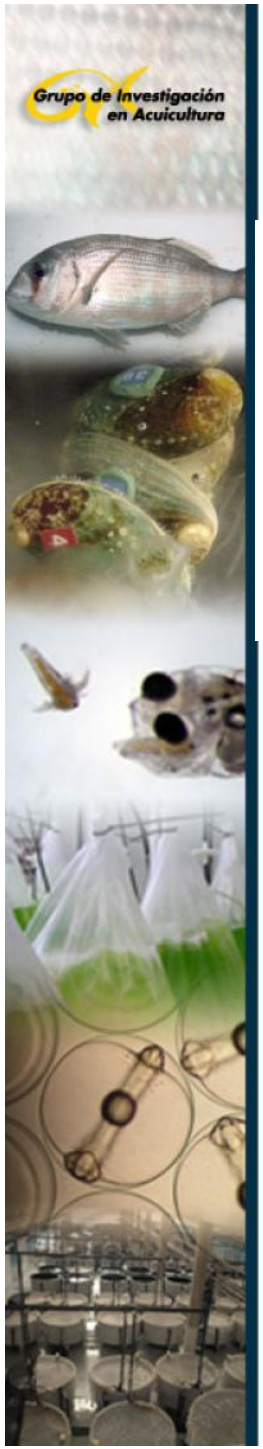


1. Seabream from 16-45 dph require about 9-10% HUFA rich PL to completely substitute live preys. KPL improves digestion, transport and deposition of dietary lipids and contribute to reduce skeleton anomalies by up-regulating bone molecular markers, particularly OP and BMP, inducing early mineralization and resistance of vertebral bodies to reduce anomalies such as lordosis and kyphosis.
2. SBL is not able to promote survival and growth as effectively as KPL, increases oxidative risk in the larvae and up-regulates AOE genes.
3. Dietary α-tocopherol promoted the beneficial effects of dietary PL, promoting growth, denoting its protective role against oxidation and reducing larval TBARs and gene expression of SOD and CAT. In relation to oxidative risk by HUFA, CAT gene expression is a better molecular marker.
4. Increase Se up to 8-12 mg/kg improved larval survival and stress resistance, protecting the larval tissues from oxidative risk



Acknowledgments

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