# VITAMIN K IN FISH NUTRITION: AN INTEGRATIVE UPDATE OF FUNCTIONS AND REQUIREMENTS

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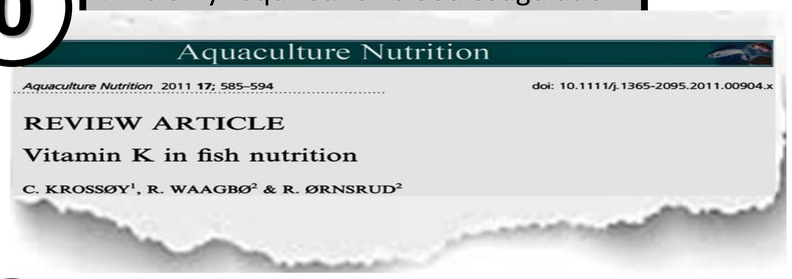
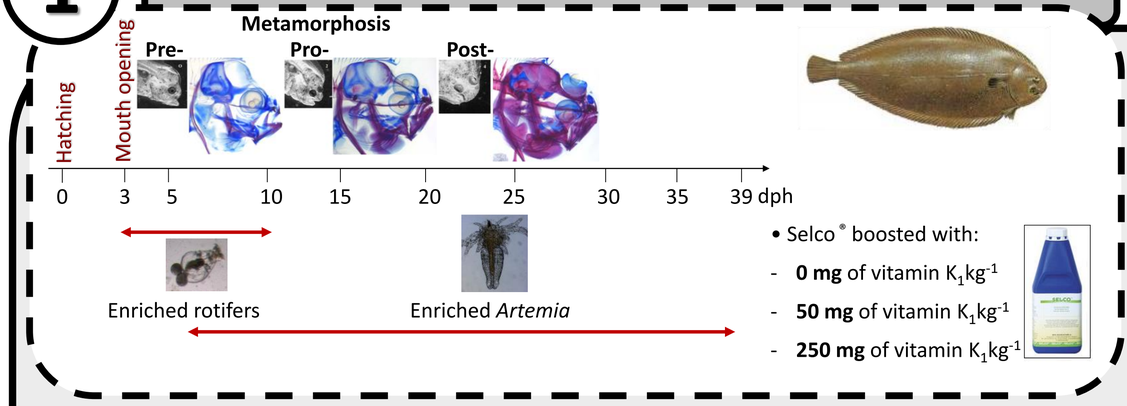
## Introduction

Vitamin K (VK) is a fat-soluble molecule known to be essential for blood coagulation and bone metabolism by two different pathways: (i) the γ-carboxylation of different VK dependent proteins (VKDPs) and (ii) the transcriptional control of several genes through the pregnane X receptor (PXR) signaling. Recently, new biological functions of VK have been suggested in fish. Here, we will review the molecular basis of VK metabolism and the recent literature on this issue, showing how VK has a higher impact on fish hemostasis and skeletal system than previously considered. New reports suggest a broader role in another biological functions such as brain, visual organs and reproduction.

## 0 VK is only required for blood coagulation

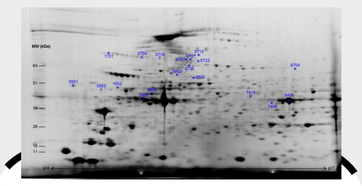



## 1 VK supplementation during larval development improved skeletogenesis




### Results

#### Proteome

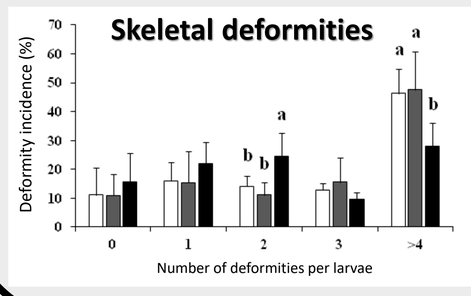


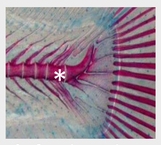
- **773 spots** detected and quantified
- **19 spots** differentially expressed between **Control diet** and **diet 250**

-7 spots under-expressed in diet 250:
- Myosin heavy chain
- 2 spots: keratin I isoform 2
- calreticulin

-12 spots over-expressed in diet 250:
- Muscular creatine kinase

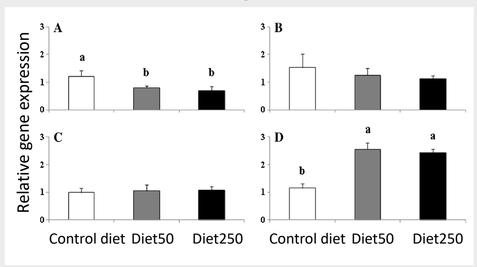
#### Skeletal deformities




*: fused vertebra

#### Gene expression




### Conclusions

- VK is required for more than hemostasis
- Dietary VK supplementation improves skeletal development
- VK might be involved in other processes (e.g. $Ca^{+2}$ regulation, muscle contraction and energy metabolism)

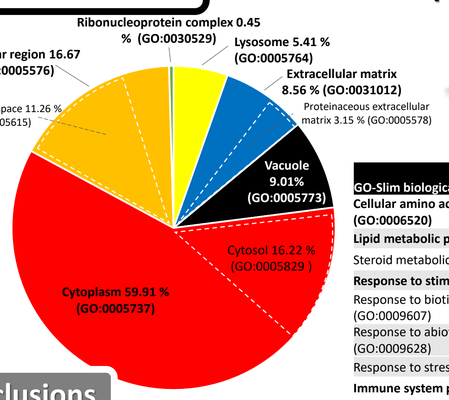
***Richard et al., 2014. Mar Biotechnol 16, 522-537.***

## 5 VK-induced deficiency altered early development: whole embryo mRNA transcriptome


- **Warfarin [ ]:**
  - **0 mg** $L^{-1}$
  - **125 mg** $L^{-1}$

### Results

#### Transcriptome




*HiSeq 2500*

GENEONTOLOGY Unifying Biology

| GO-Slim biological process | Fold enrich. | FDR |
|---|---|---|
| **Cellular amino acid metabolic process (GO:0006520)** | **+ 4.16** | **2.87E-08** |
| **Lipid metabolic process (GO:0006629)** | **+ 2.08** | **2.07E-02** |
| Steroid metabolic process (GO:0008202) | + 3.13 | 4.06E-02 |
| **Response to stimulus (GO:0050896)** | | |
| Response to biotic stimulus (GO:0009607) | + 6.55 | 3.93E-02 |
| Response to abiotic stimulus (GO:0009628) | + 5.04 | 3.40E-04 |
| Response to stress (GO:0006950) | + 1.98 | 2.09E-02 |
| **Immune system process (GO:0002376)** | **+ 2.04** | **9.82E-03** |

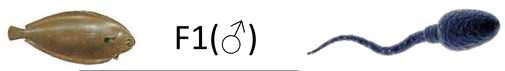
### Conclusions

- VK nutritional status might affect different biological processes

***Granadeiro et al., 2019. Ecotox Environ Safe 181, 559-571.***

## 4 VK supplementation improved sperm quality: sncRNA circulating transcriptome

Control
1250 mg $VK_1Kg^{-1}$



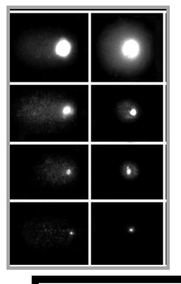
F1(♂)



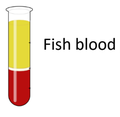
### Results

#### Sperm quality

| Variable: | Control | VK1250 |
|---|---|---|
| Sperm (M $mL^{-1}$) | 502 ± 210 | 684 ± 457 |
| DNA frag. (%) | 46.6 ± 5.4 % | 34.7 ± 8.7%* |

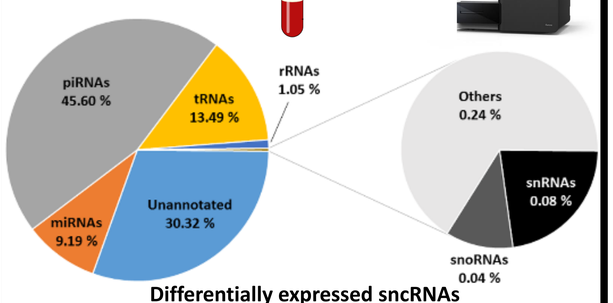


#### Transcriptome



Fish blood plasma

*MiSeq*




**Differentially expressed sncRNAs**

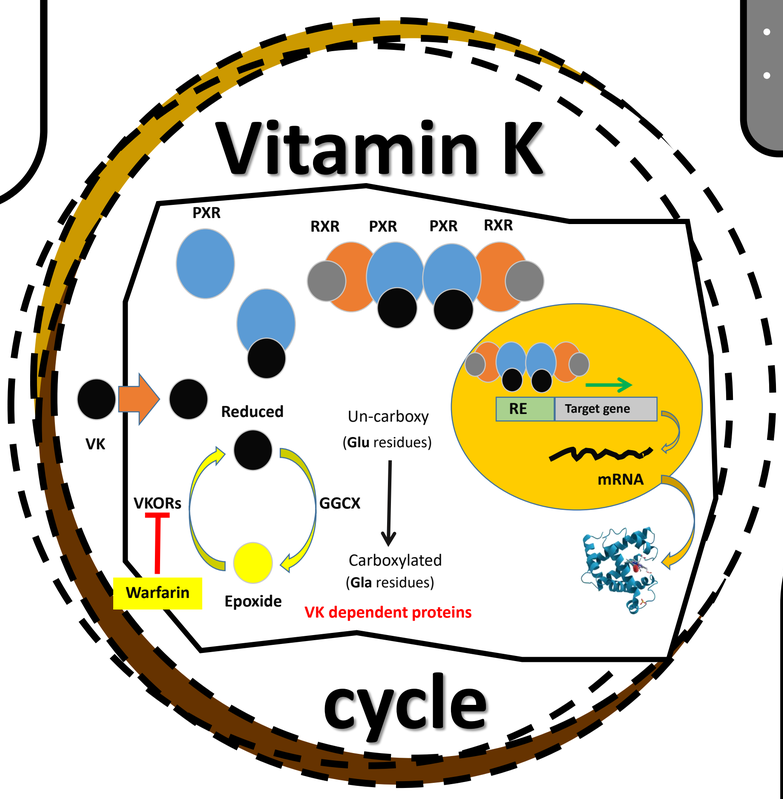
| Name | Low DNA (VK+) fragmentation Mean ± SD | High DNA fragmentation Mean ± SD | Q-value |
|---|---|---|---|
| let-7g | 959.4 ± 78.06 | 525.59 ± 103.63 | < 0.0001 |
| let-7e | 933.2 ± 26.07 | 625.46 ± 123.34 | 0.002 |
| miR-146a | 121.0 ± 44.79 | 237.53 ± 16.19 | 0.018 |
| let-7a-1 | 25408 ± 2201.25 | 17678.1 ± 2712.05 | 0.027 |

### Conclusions

- VK might improves sperm quality through sncRNAs

***Fernández et al., 2019. BBA Gen Subj 1863, 39-51.***

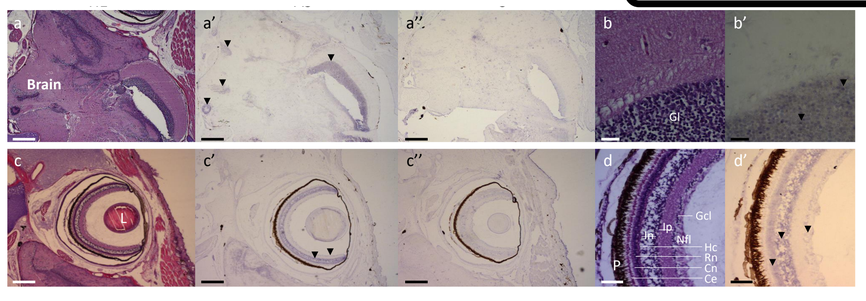
## Vitamin K cycle




## 3 VK control different biological functions through PXR activation

### Results

#### In *situ* hybridization



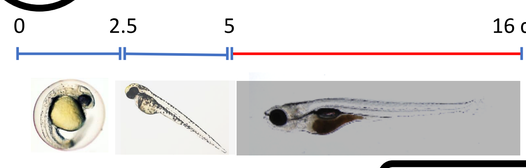

***ISH – PXR:*** *HE*, Hematoxylin-eosin (a, b, c and d); *AS*, anti-sense probe (a', b', c' and d'); *S*, sense probe (a'' and c''). *Ce*, cone ellipsoids; *Cn*, cone nucleus; *Gcl*, ganglion cell layer; *Gl*, granular layer; *Hc*, horizontal cells; *In*, inner nuclear (layer); *Ip*, inner plexiform (layer); *L*, lens; *P*, pigment epithelium; *Nfl*, nerve fiber layer; *Rn*, rod nucleus. Scale bars: 200 µm (a, c), 50 µm (d), and 20 µm (b).

### Conclusions

- VK might be required for cognitive capacities and visual function

***Marques et al., 2017. Mar Environ Res 130, 187-199.***

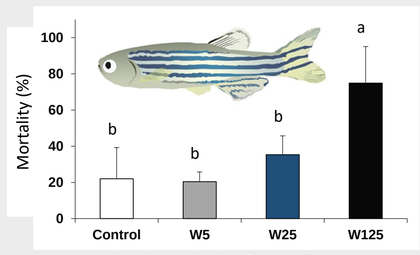
## 2 VK-induced deficiency during larval stage led hemorrhages and skeletal deformities



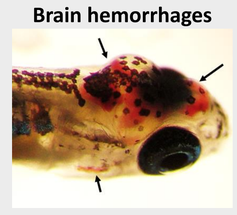

- **Warfarin [ ]:**
  - **0 mg** $L^{-1}$
  - **5 mg** $L^{-1}$
  - **25 mg** $L^{-1}$
  - **125 mg** $L^{-1}$

### Results

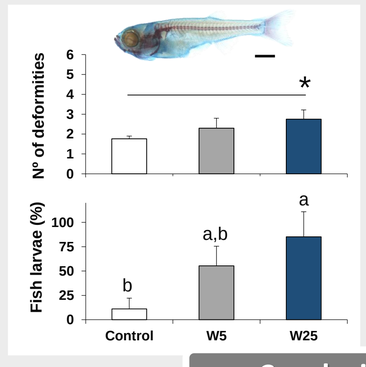
#### Survival



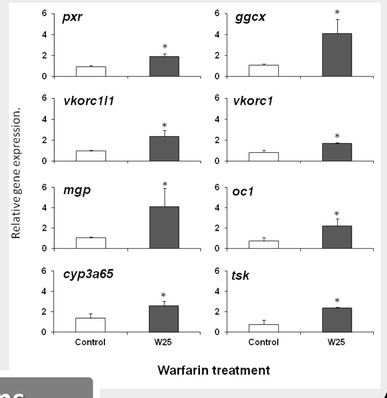

#### Brain hemorrhages



#### Skeletal deformities




#### Gene expression




### Conclusions

- VK-induced deficiency provokes high incidence of hemorrhages and pathological calcification of soft tissues
- VK-induced deficiency reduces the skeletal quality of larvae
- VK-induced deficiency alters the VK recycling, bone development and PXR signaling at transcriptional level

***Fernández et al., 2014. Environ Pollut 194, 86-95.***

## Acknowledgements

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