

# larvi 2013

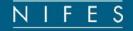
6th fish & shellfish larviculture symposium



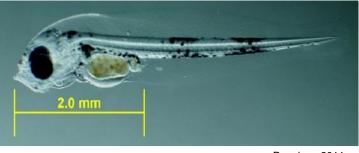


ghent university, belgium, 2-5 september 2013





## ONTOGENY OF THE REDOX BALANCE IN RELATION TO ORGANOGENESIS IN ATLANTIC COD (GADUS MORHUA) LARVAE

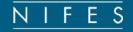


Penglase 2011

Kristin Hamre, Samuel J. Penglase, Josef D. Rasinger, Kaja H. Skjærven, Pål A. Olsvik NI FES, Bergen, Norway



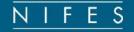




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## The redox balance

- Glutathione (GSH/GSSG) is present in all cells at high concentrations (1-10mM) and is considered important for determining the cell's average redox potential
- Other redox couples such as the CysSH/CysSSCys couple, the Thioredoxin couple, NADP(H) and reactive oxygen species (ROS) modulate the potential in microenvironments within the cells
- There are different redox potentials in
  - In different organelles within the cell
  - In different organs
  - Intra and extracellularly
- The redox potential is important for determination of metabolism and cell fate





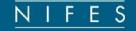
The redox potential can be calculated using the Nernst's equation

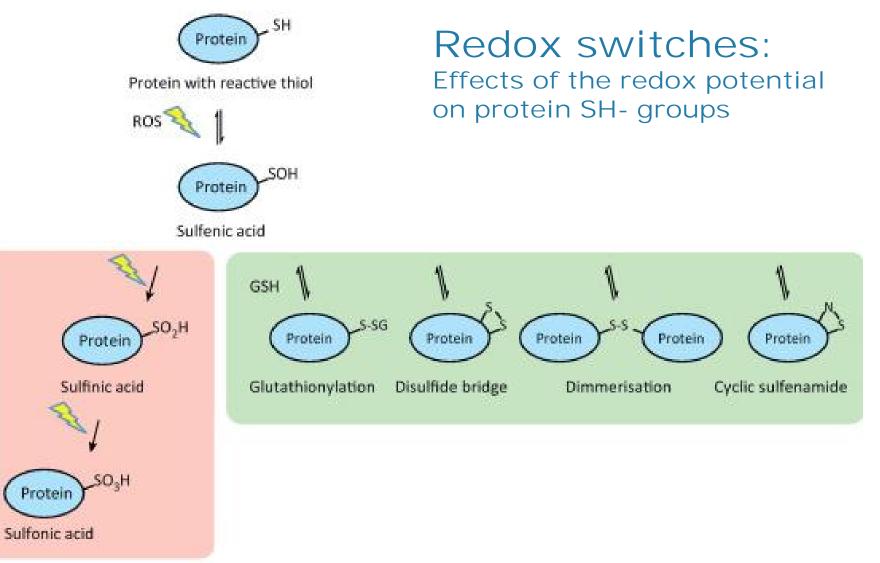
The potential of the GSH/GSSG couple :

 $E'=E'_0 - k * Log [GSH]^2$ [GSSG]

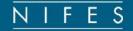
- E'<sub>0 GSH/GSSG</sub> = -240 mV (Scafer and Buettner 2001)
- The GSH/GSSG couple is not at equilibrium, the concentrations are at a steady state, which seems to be tightly regulated





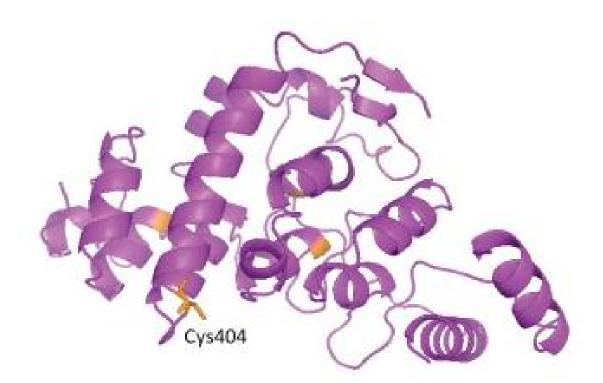


#### Chiu and Dawes 2012





SS bonds and glutathionation change the 3-dimensional conformation of proteins

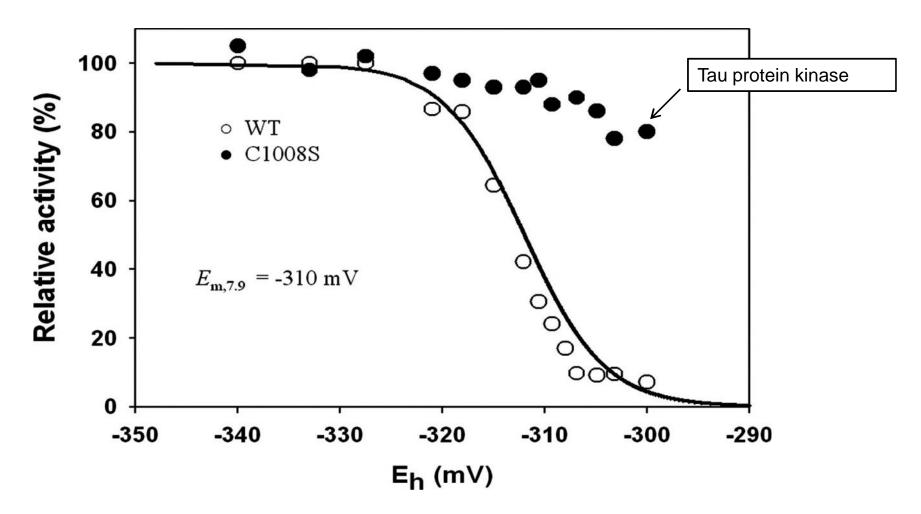


Enzymes Structural proteins Transcription factors More...

Chiu and Dawes 2012

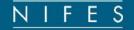
# Enzyme activity affected by the redox potential $\frac{N I F E S}{P}$

Glucan, water dikinase (GWD): A plastidic enzyme with redox-regulated and coordinated catalytic activity and binding affinity

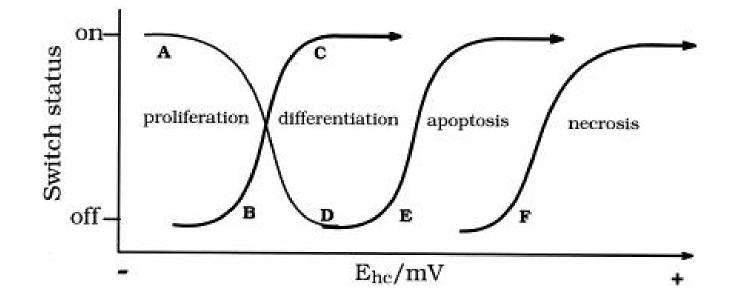


Mikkelsen et al., 2005





## Redox potential and cell fate



The normal range of potential in healthy cells is -200 to -260 mV

Schafer and Buettner 2001



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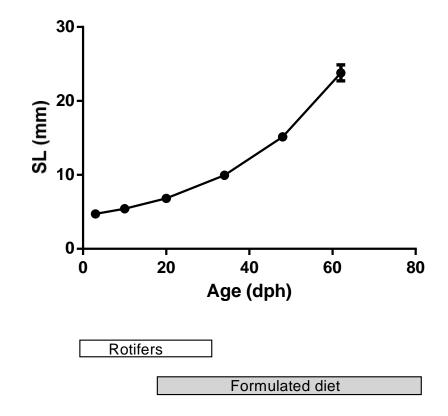
How does this work in cod larvae?

- Cod larvae from triplicate tanks were sampled between 3 and 63 days post hatch from a commercial hatchery
- Whole larvae were pooled, and prepared for GSH, antioxidant enzyme analyses and qPCR

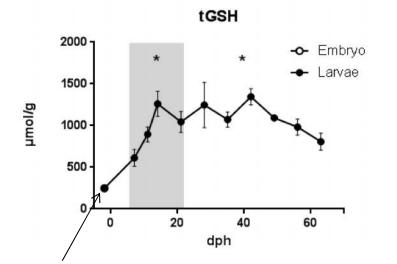


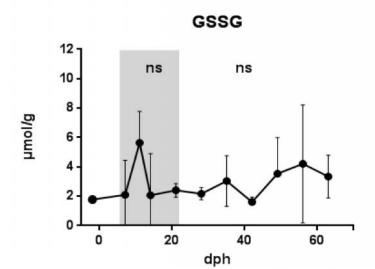


# Standard length (SL) of sampled larvae and feeding regime



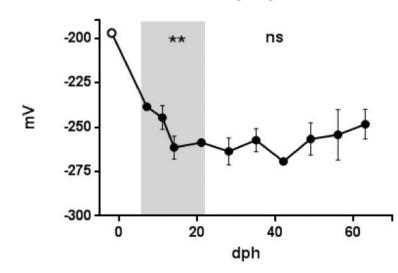
## The GSH based redox potential (E) in whole body of cod larvae I F E S

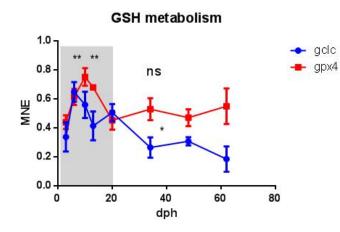


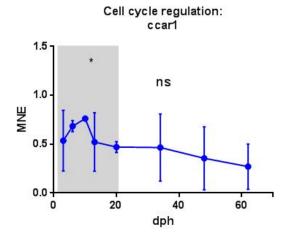


Skjærven et al 2013



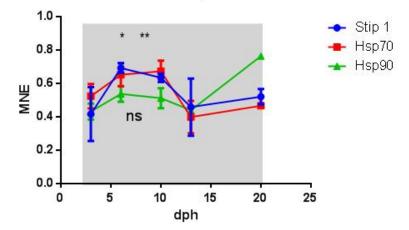






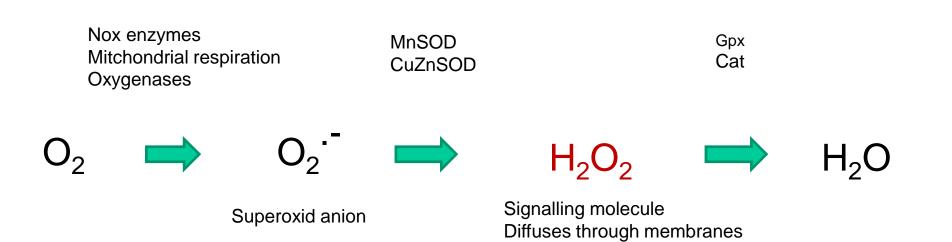
E Zn and Cu metabolism 1.5 1.0 0.5 0.0 0.0 20 40 40 60dph

Heat shock proteins





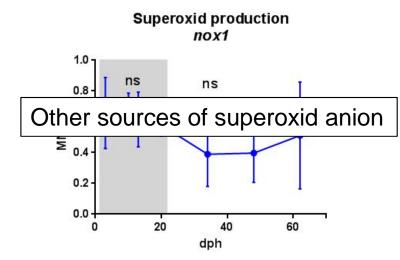
## Metabolism of reactive oxygen species (ROS)

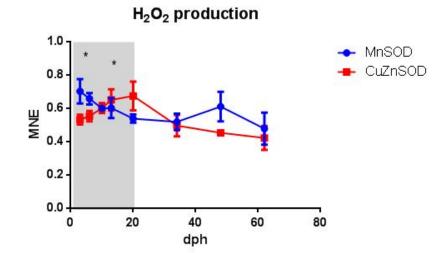




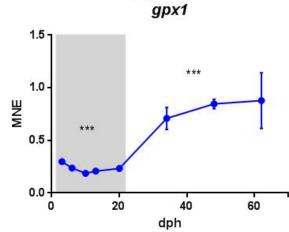
## ROS metabolism

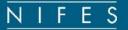
### NIFES



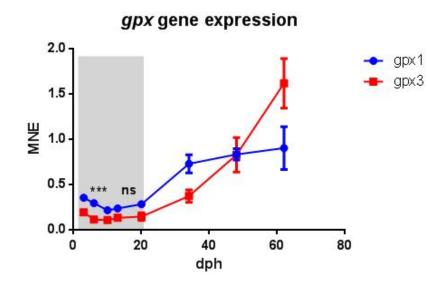


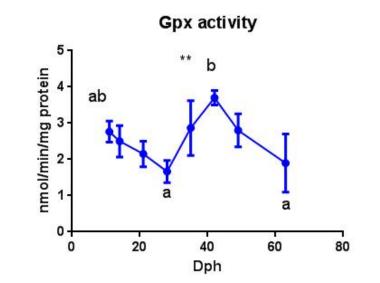
H<sub>2</sub>O<sub>2</sub> removal





# Not always correlation between gene expression and protein function





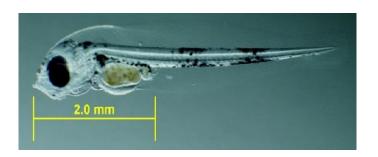


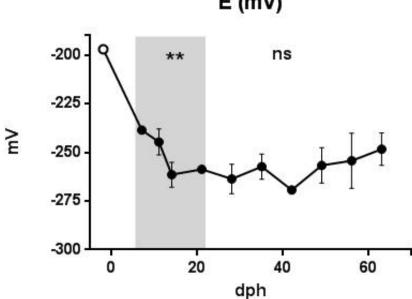
## Proliferation and differentiation of cod embryos

Stage:		9.5	11	11.5	14	16	17	20	23	
	Clevage Stage	Early Blastula	Late Blastula	Early Gastrula	25% Epiboly	50-70% Epiboly	100% Epiboly	40 Somites	Hatching gland	
Milt 6°	3.2	6.6	9.8	12.5	18.4	24.3	30.5	54.1	83.6	
Egg		(10°C	12.1	I	18.2	25.2	30.5	49.1	75.0	
DD										

Skjærven 2013

## Proliferation, differentiation and apoptosis in cod larvae





E (mV)

We conclude with a hypothesis:

The early change in redox potential correlates with a change of main cellular mode from differentiation at hatching to proliferation after 13 dph





## Thank you for your attention



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  - The Research Council of Norway.
    Projects 79016/S40 and
    99482/S40 Cod Development
    CODE
  - Larvanet



- Thanks to Marine Harvest Cod for samples
- Thanks to NIFES technicians
  for analyses