Dietary mineral supply in Atlantic salmon – impact on skeletal development

Grete Bæverfjord, Synnøve Helland, Ståle Refstie, Kirsti Hjelde and Torbjørn Åsgård

Nofima Marin, Sunndalsøra

Nutritional aspects of vertebral deformities

- No limit to the number of potential nutritional factors
- In commercial diets, mineral supply is the most likely limiting factor
- Phosphorus deficiency impaires bone formation
- Mineral supply (dietary level X bioavailability) is a challenge in fast growing animals of any species
- Unpredictable dietary supply in formulated diets
  - Vegetable ingredients impairs absorption
  - Unforeseen variation in availability in fish meal
- Potential interaction between dietary minerals and reduced water quality in impact on bone mineralisation

- Minerals were suspected to be a cause of skeletal malformations early on (mid 1990-ies)
- A study from 1993-1994 demonstrated that a restricted dietary supply of P caused impaired bone development both in freshwater and seawater (Baeverfjord et al., 1998)
- Temperature took over as the number one causal factor as experimental research progressed
- After temperature adjustments were implemented by industry, the focus on mineralization as a possible second factor resurfaced

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The unpredictability of dietary P supply

- Digestibility of P depends on source
- Required dietary content depends on digestibility and feed conversion ratio (kg feed/kg weight gain, FCR)
- Whole body P content in fish with normal bone mineralization ~4000 mg/kg.

Dietary P content mg/kg (%)	Digestibility of P %	FCR (kg feed/ kg weight gain)	Available P mg/kg per kg weight gain
12 000 (1,2 %)	50	1	6000
12 000 (1,2 %)	30	0,8	2880

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2002-2005:

Deformities of vertebral column and jaw in Atlantic salmon

Effects of genetic susceptibility, mineral nutrition and production temperature

- Funded by Fiskeri- og Havbruksnæringens Forskningsfond (FHF), the Norwegian industry research foundation
- Study supported by
  - AquaGen: Supplied egg groups selected for genetic disposition
  - Ewos Innovation: Experimental feeds

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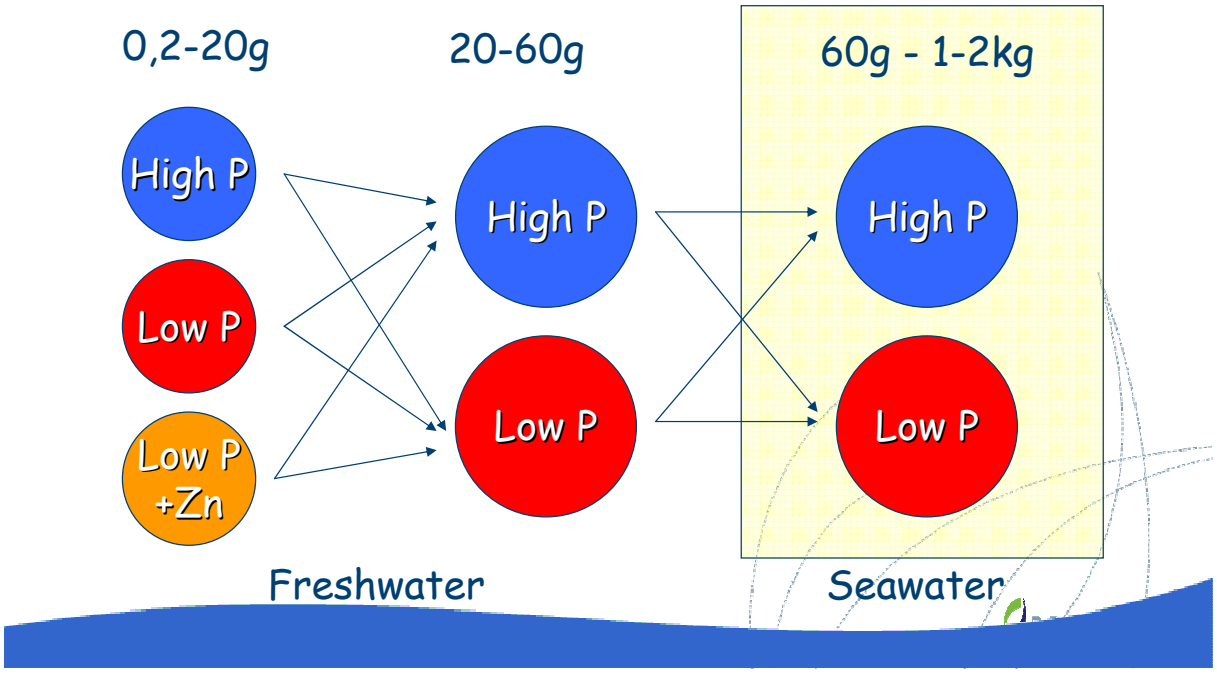
Long term experiment:

Restricted dietary phosphorus supply in salmon - Effects on skeletal development



Diets:

	Phosphorus (P) %	Zinc (Zn) mg/kg
High P	1,7-1,8	140-190
Low P	1,0-1,2	140-190
Low P + Zn	1,0-1,2	50-80

Low P: P levels comparable to published requirements
Low Zn: Zn levels " " "

If digestibility 50% and FCR 1,0: Sufficient
If digestibility 30% and FCR 0,8: Marginal deficiency

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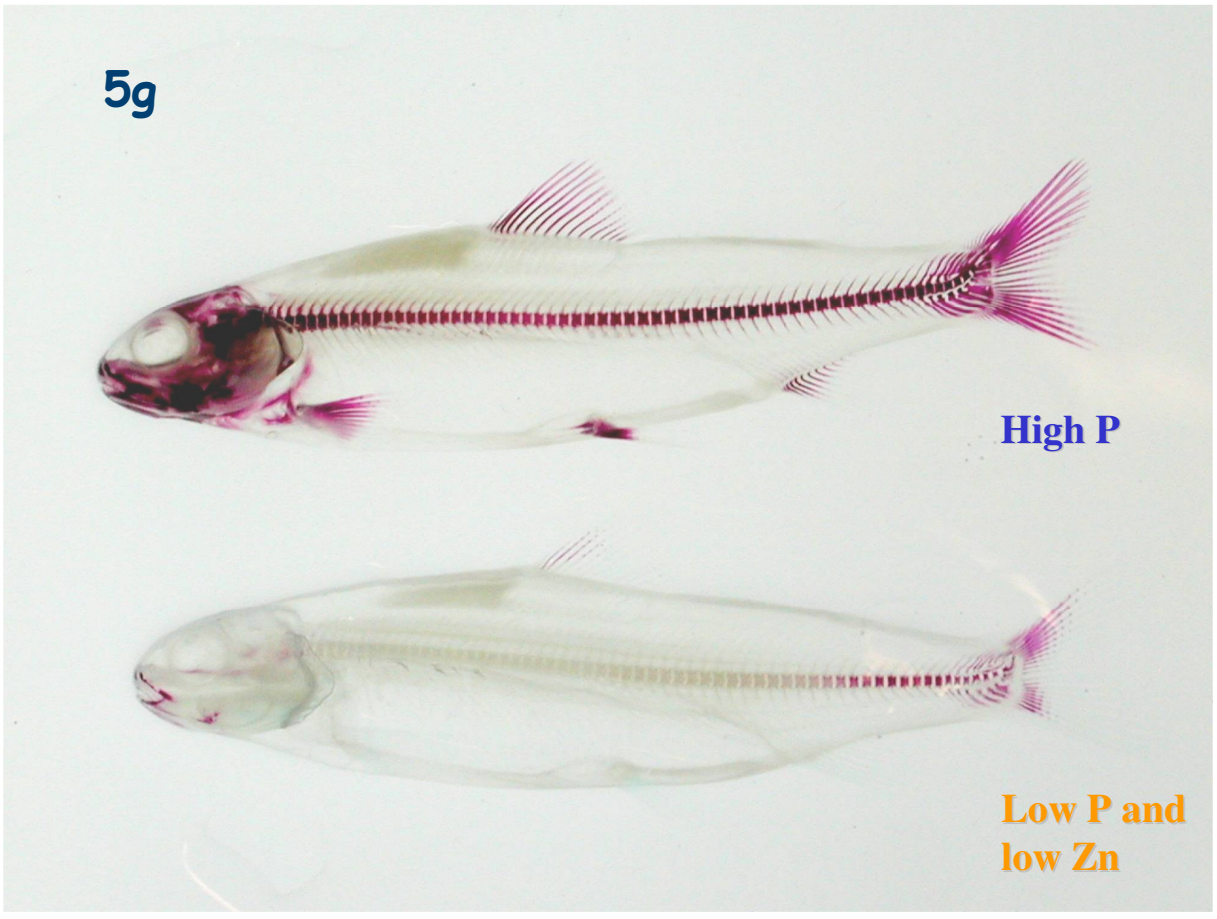
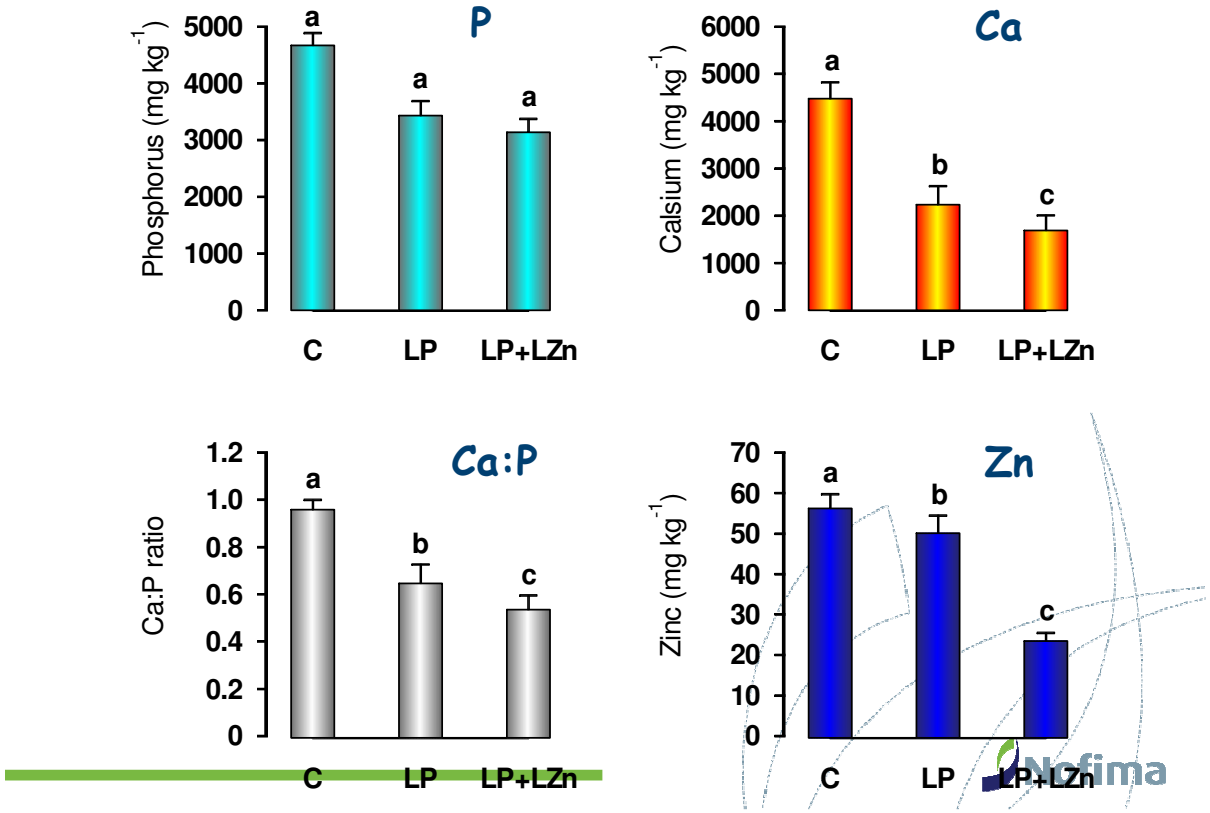
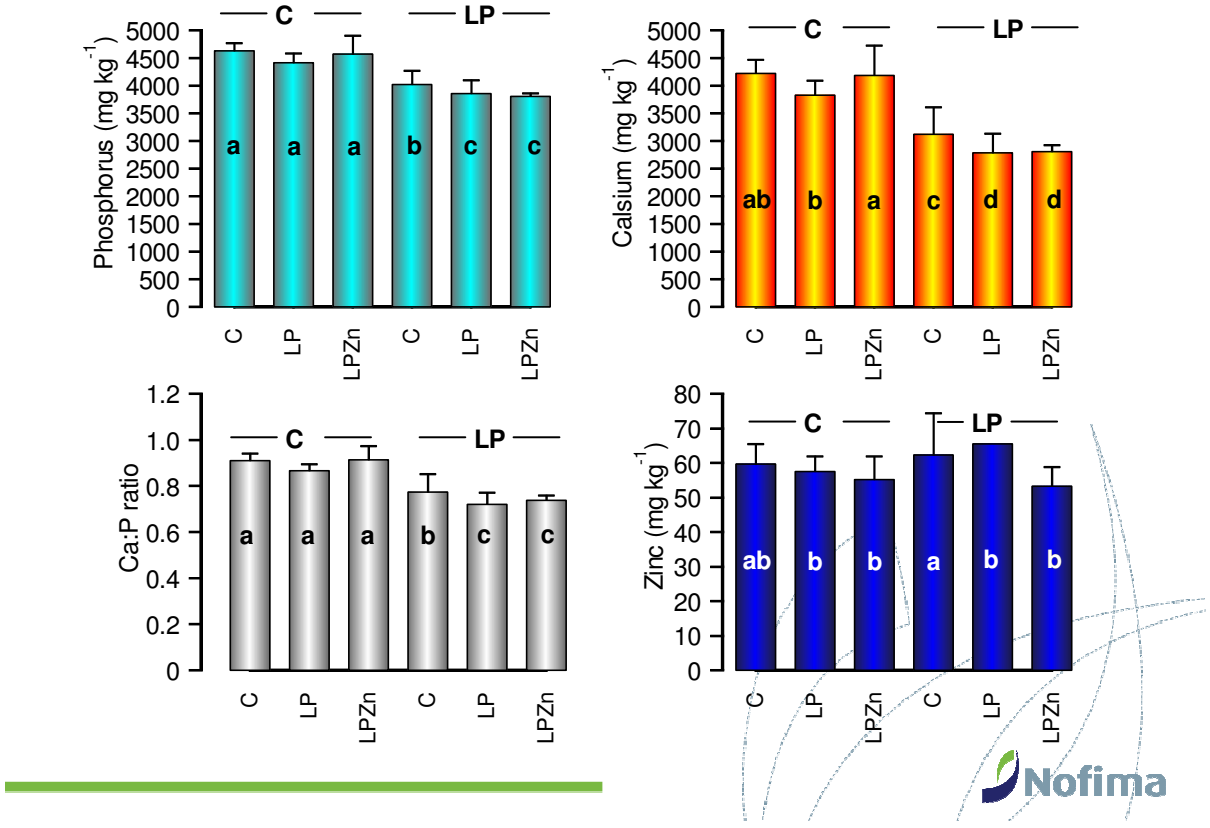
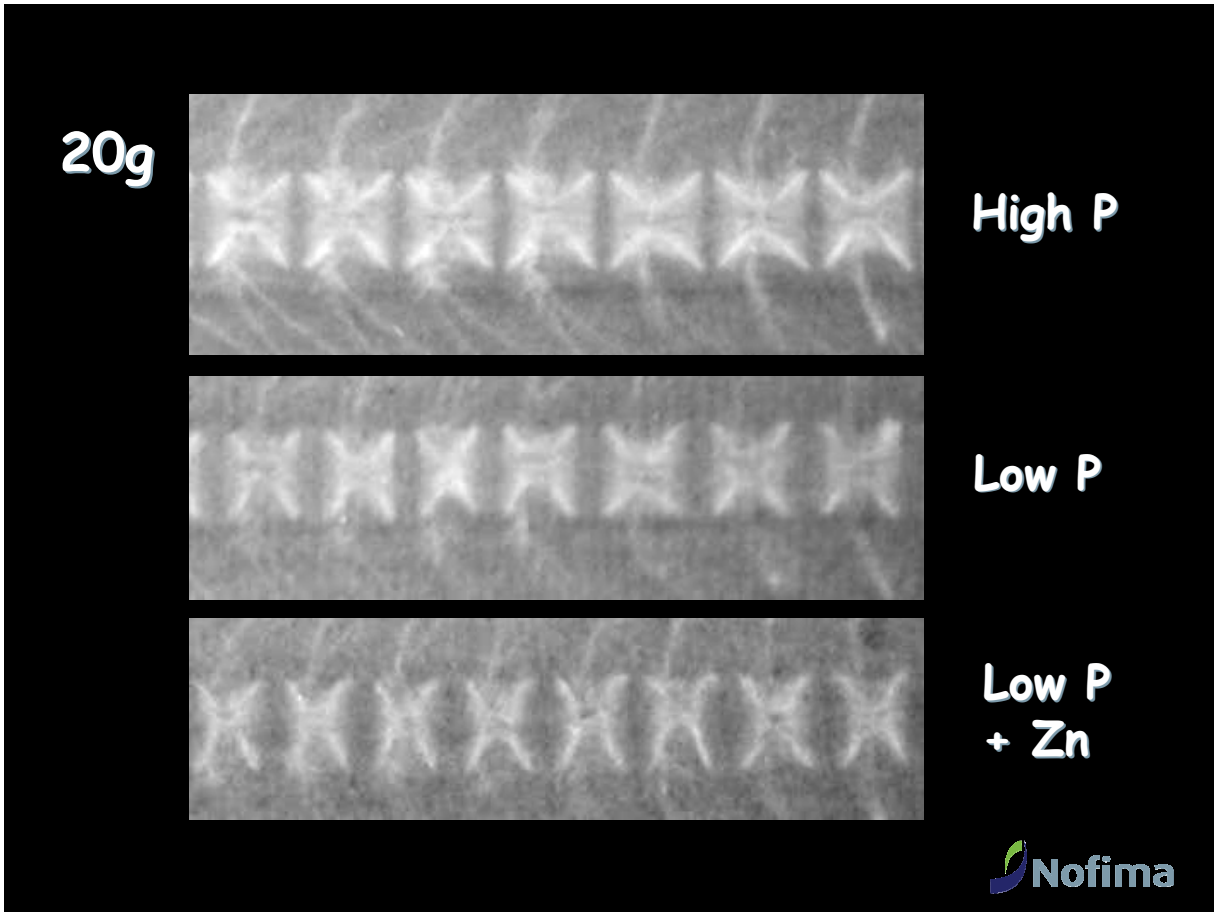


Whole body mineral content at 20g



Whole body mineral content at smolt transfer (60-80g)




20g
High P
Low P
Low P
+ Zn
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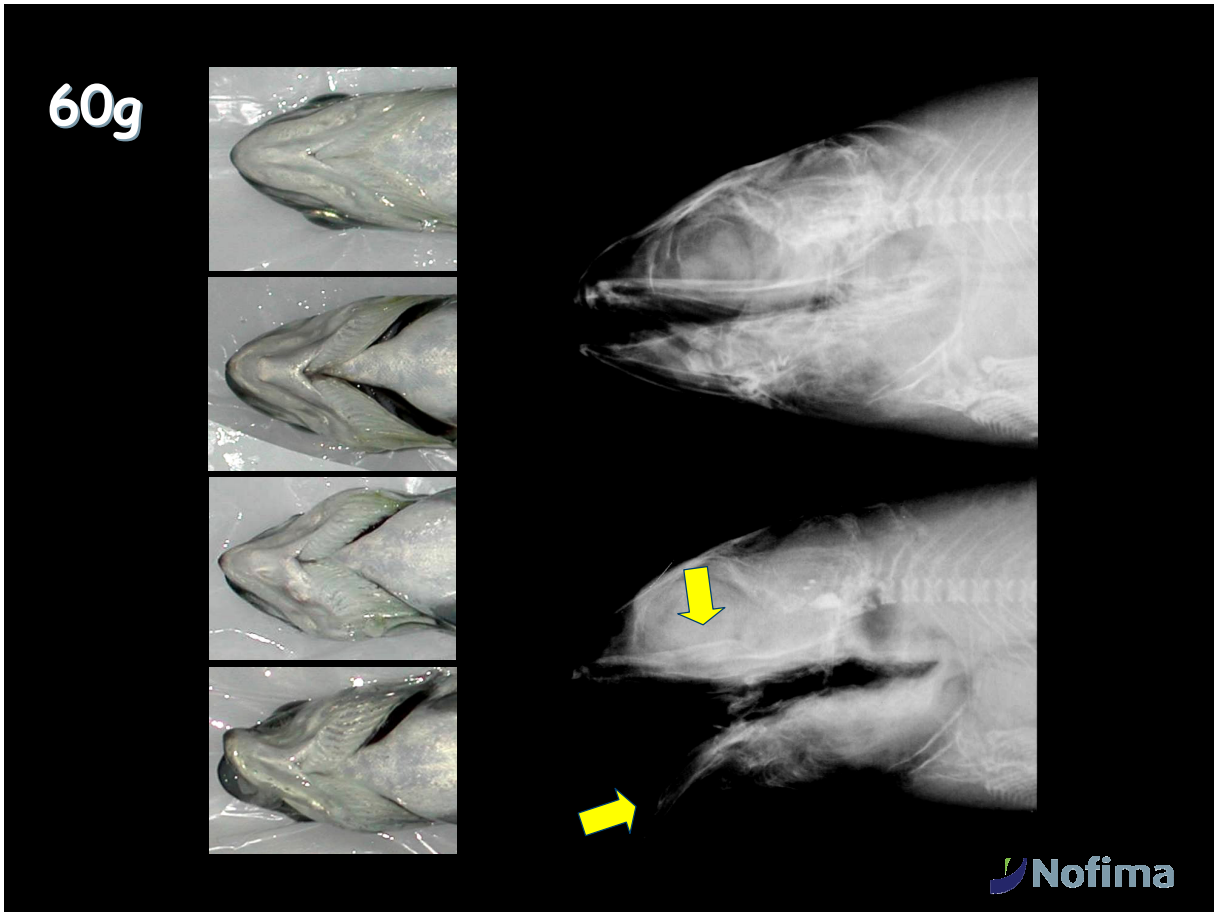
60g
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End of seawater rearing:
- Abnormally soft fish
- Short fish

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Summary, long term P study:

- Even a moderate P deficiency may induce long term effects
- P deficiency is aggravated by low Zn
- Periods of negative P balance may induce irreversible structural changes in bones, even though mineral content will return to normal if supply is increased
- The early juvenile stages are most vulnerable, but..
- The skeletal structures are susceptible to mineral deficiency throughout freshwater and seawater stages

- Long term effects of undermineralisation include
  - Malformations of the lower jaw
  - Platyspondylia of the spinal column

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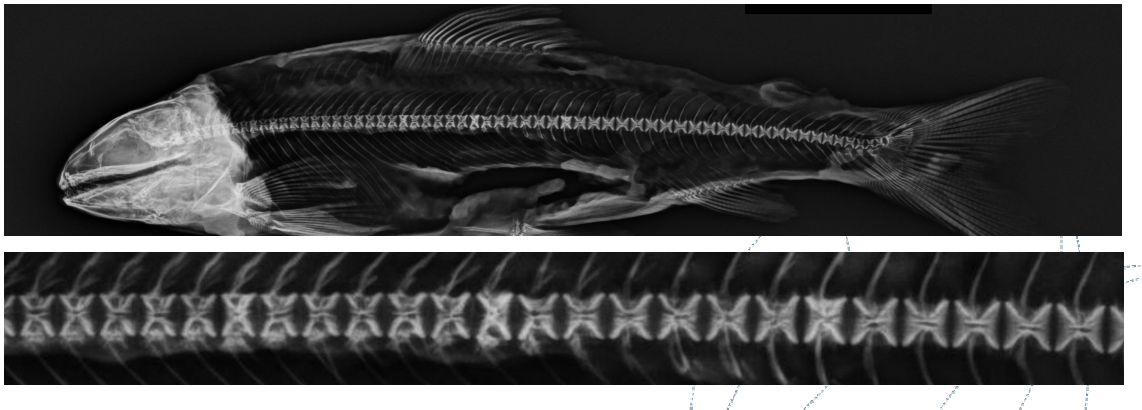
Hyper dense vertebrae in Atlantic salmon parr

- **Experiment with graded levels of phytic acid (Denstadli m.fl., 2006)**
- **Phytic acid in high levels led to reduced levels of Ca og P in fish**
- **Presence of hyper dense vertebra at start of experiment**



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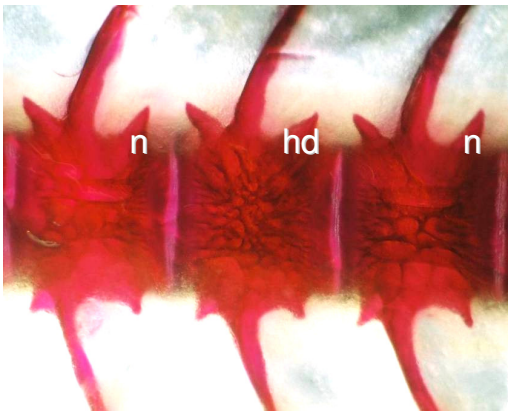
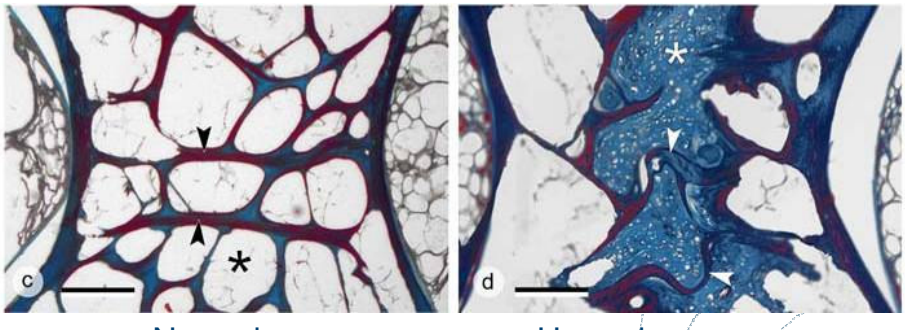
Structure of hyper dense vertebrae



(Whole mount staining, Alizarin red)



(Massons Trichrome)

Normal Hyperdense

Helland m. fl., 2006

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Restricted dietary levels of phosphorus and zinc induces specific skeletal deformities in juvenile Atlantic salmon (*Salmo salar L.*)

G. Baeverfjord, K. Hjelde, S. Helland and S. Refstie
Nofima Marine
N-6600 Sunndalsøra, Norway

The study was funded by:
FINE FISH - Improving sustainability of European fish aquaculture by control of malformations (EU, CCL-CT-2005-012451)

Background and objective

- Previous experiments demonstrated the importance of adequate supply of P and Zn for preventing skeletal deformities in long term development
- Ca and P levels are low in a significant number of samples
  - Ca or P < 3500 mg kg^{-1}, Ca and P should be > 4000 mg kg^{-1}
  - Ca << P, should be Ca > or = P
- Zn levels are low in commercial samples
  - Whole body Zn values of 30 mg kg^{-1} are typical, and lower values are commonly observed
  - Reference values are in the range of 40 – 60 mg kg^{-1}
- Mg levels are highly variable in samples of commerially reared fish
- In the present study, the specific effects of restricted P and Zn supply during early juvenile development was examined.
- The potential effects of reduced dietary Mg supply was also addressed

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Objective:
To determine the specific effects of restricted, suboptimal dietary supply of P, Zn and Mg during early skeletal development

- First feeding experiment A. salmon
- Start weight 0,2g (start of exogenous feeding)
- Final weight 25-30g
- Feeding period 20 weeks
- 4 diets x 3 replicates = 12 tanks
- Continous feeding, continous light, flow through water supply

- Sampling of fish at 1g, 5g and 25g (end of feeding experiment)
- Chemical contents and radiography
- Analyses of gene expression (not presented here)

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Diets

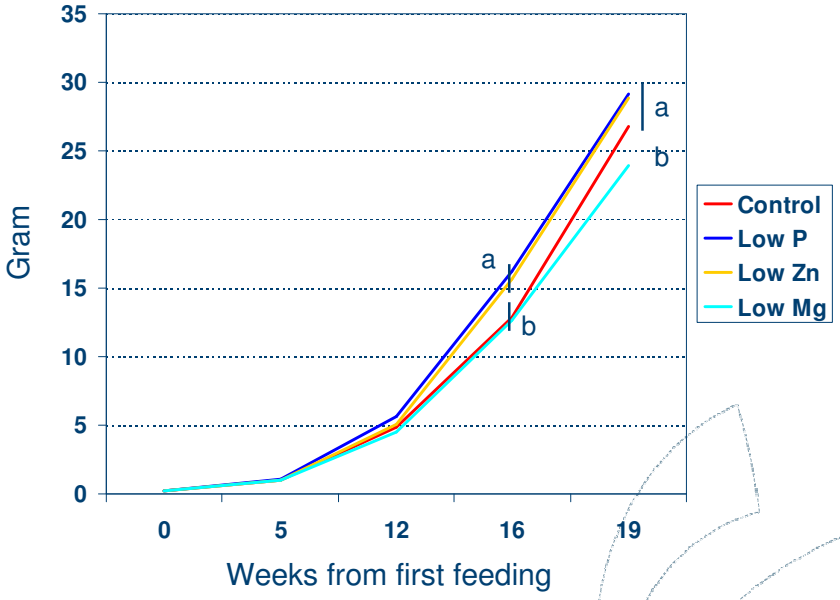
- Four diets with differing mineral contents were made by Nofima marine Bergen (then Fiskeriforskning Bergen)
- The basic diet was formulated to contain
  - 53 % protein
  - 18 % lipid
  - 10 % carbohydrate
- Mineral content was differentiated by excluding mineral supplementation selectively for P, Zn and Mg
- Diets were aimed at producing subclinical mineral deficiency comparable to the lower end of commercial values
- Four different particle sizes were produced per diet, from the same basic feed batch

Mineral analyses of experimental diets

Diet	P (mg kg^{-1})	Ca (mg kg^{-1})	Zn (mg kg^{-1})	Mg (mg kg^{-1})
Control	16500	15600	194	2375
Low P	**13300**	**13200**	181	2350
Low Zn	16800	15500	**85**	2440
Low Mg	15900	14800	182	**1860**

Growth of A. salmon juveniles

fed diets differing in mineral contents



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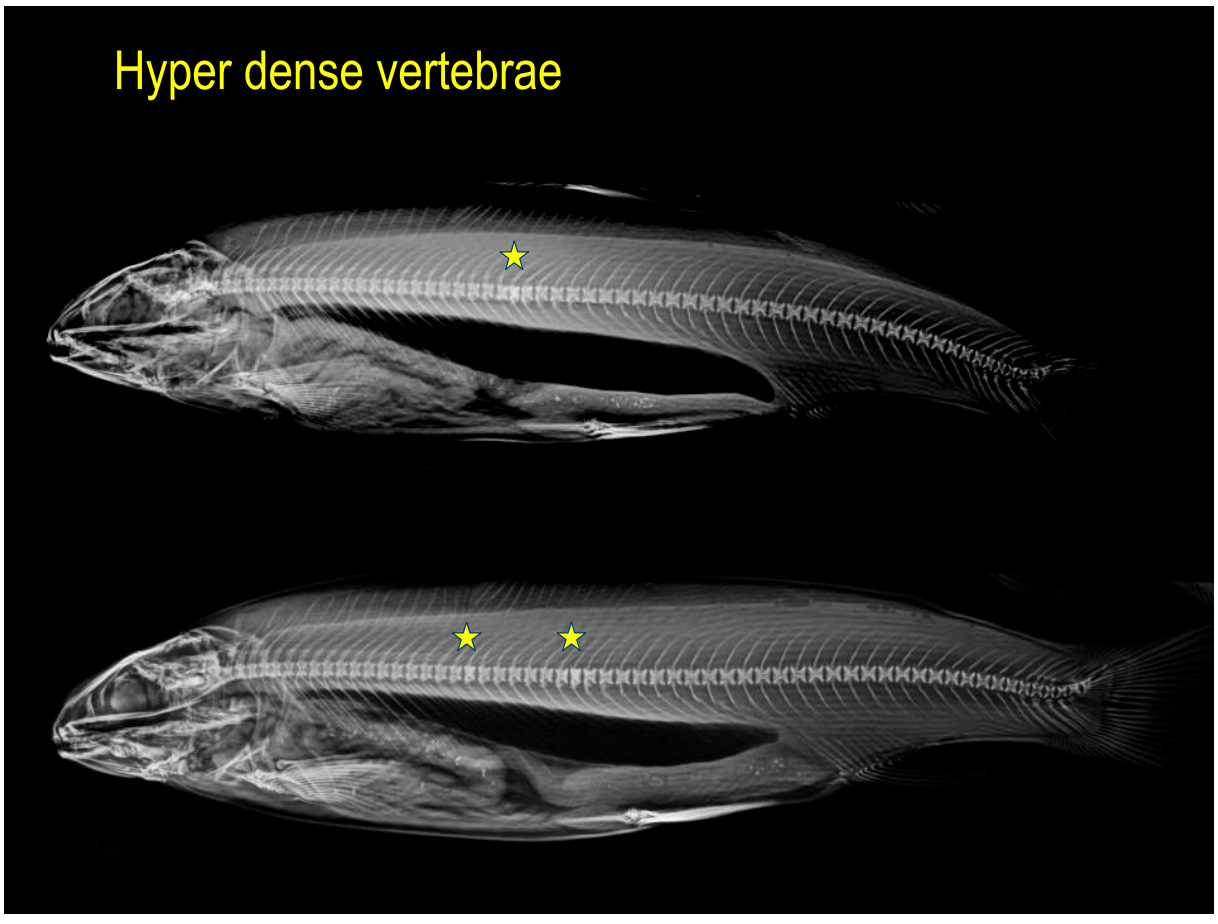
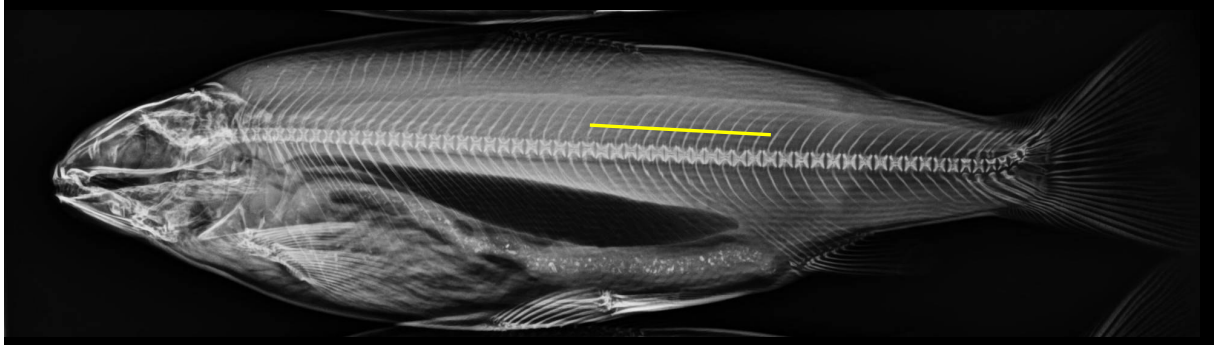
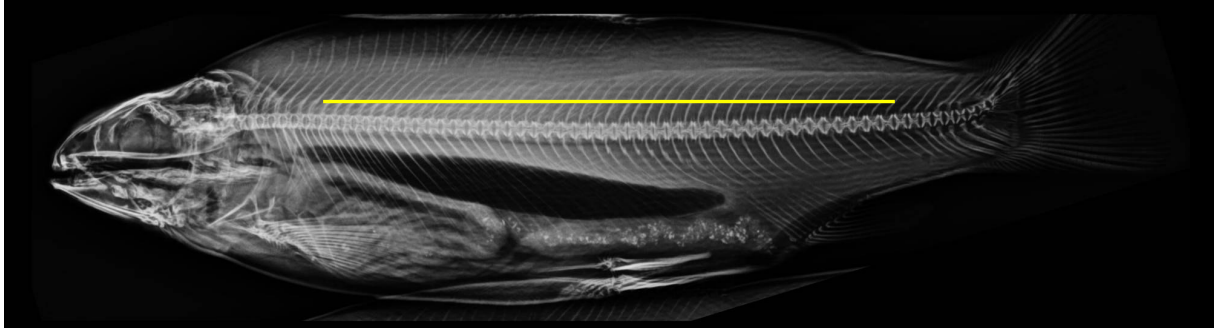
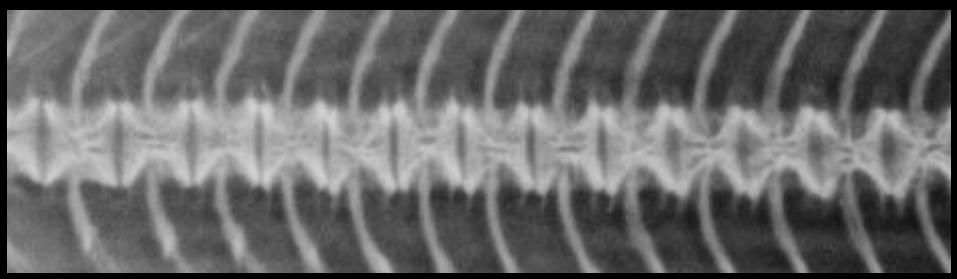


Compressed vertebrae

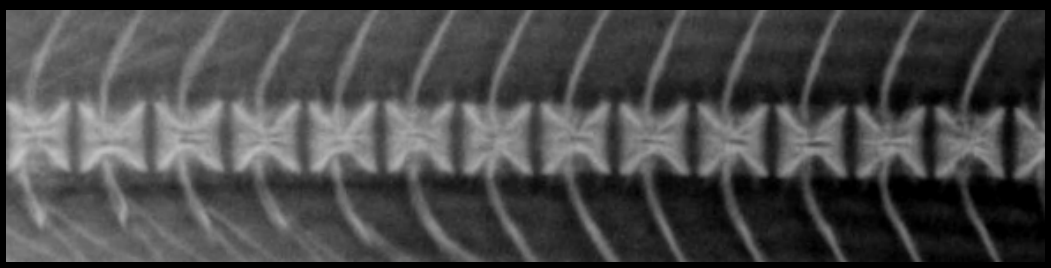




Detail of compressed vertebrae



Compressed



Normal

Hyper dense versus compressed vertebrae

- Hyper dense vertebrae were observed in all dietary treatments except control
- The observations confirm that hyper dense vertebrae can be used as indicator for disturbances in early mineralization
- Highest number of fish with hyper dense vertebrae observed in low P groups
- Low P groups were particularly low in Ca and P around 1g size

- Compressed vertebrae were typical for Zn deficiency
- The observations are comparable to older studies of "short body dwarfism" related to Zn deficiency in other species
- Zn deficiency was consistent in low Zn groups throughout the experiment
- Early-life subclinical hypomineralization was previously linked to development of late-onset platyspondylia

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Fate of HD-vertebrae

- High density vertebrae (HD-vertebrae) were identified in 20 fish on X-ray at seawater transfer.
- HD-vertebrae were seen in fish from all diet groups
- At harvest, same vertebrae were identified, X-rayed and sampled for histology

- Of 20 HD vertebra identified at seawater transfer
  - 10 were normal by termination of experiment
  - 10 were developed into fusions at termination

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Conclusions, P, Zn and Mg

- Dietary phosphorus supply may still be a limiting factor for normal skeletal development in A. salmon early rearing. The low P diet in this experiment was higher in P than many samples of commercial diets
- Supply of Zn in diets for juvenile salmon should receive more attention. The estimated Zn requirement of 37-67 mg kg^{-1} feed is clearly too low.
- Low dietary Mg may interfere with mineralization, although how and why remains unanswered by this study

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Summary and recommendations

- Minerals most important dietary factors, in particular P and Zn
- Still reason for concern about mineral supply from commercial feeds
- Present and future challenges
  - Reduced mineral availability with vegetable meals
  - Variable and unpredictable availability of minerals from fish meals

- Subclinical mineral deficiency does not affect growth
- Morphological effects are delayed
- Mineral status can be assessed through chemical anlyses of fish
- Remember that mineralization is dynamic
- You will see the full effect of undermineralization only as the fish approach harvest size

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