Functional development of the digestive system in Atlantic cod (Gadus morhua L.) larvae.

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Aims of the study

1. To study functional development of the intestine and liver in cod larvae

2. To describe the effect(s) of early weaning and dietary composition on functional development and to evaluate tissue changes

3. To establish quantitative histological parameters suitable for evaluating nutritional status of developing cod larvae

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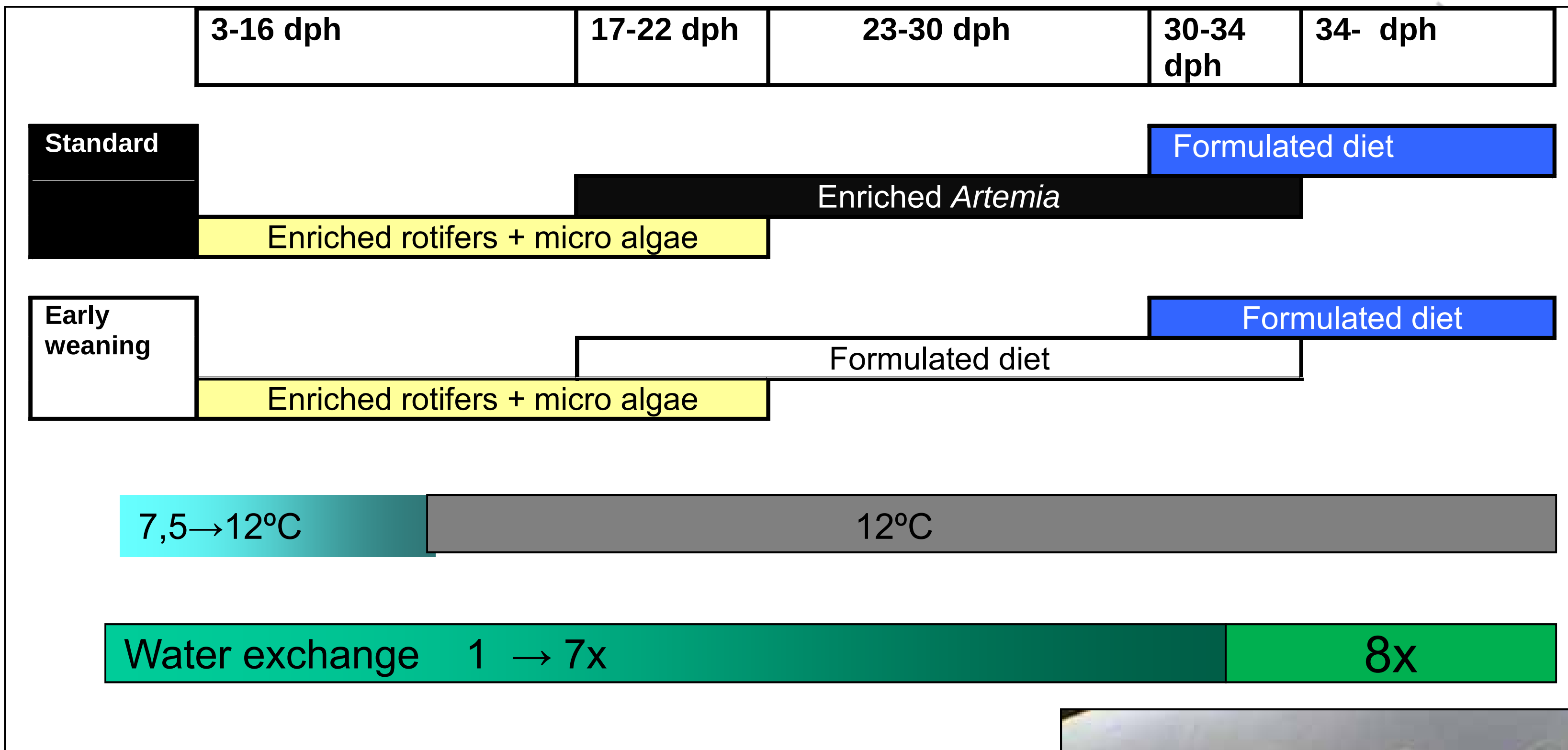
Methods- Start-feeding protocol

All start-feeding experiments were conducted according to the standard start-feeding protocol established at NTNU/SINTEF



Methods – sample preparation

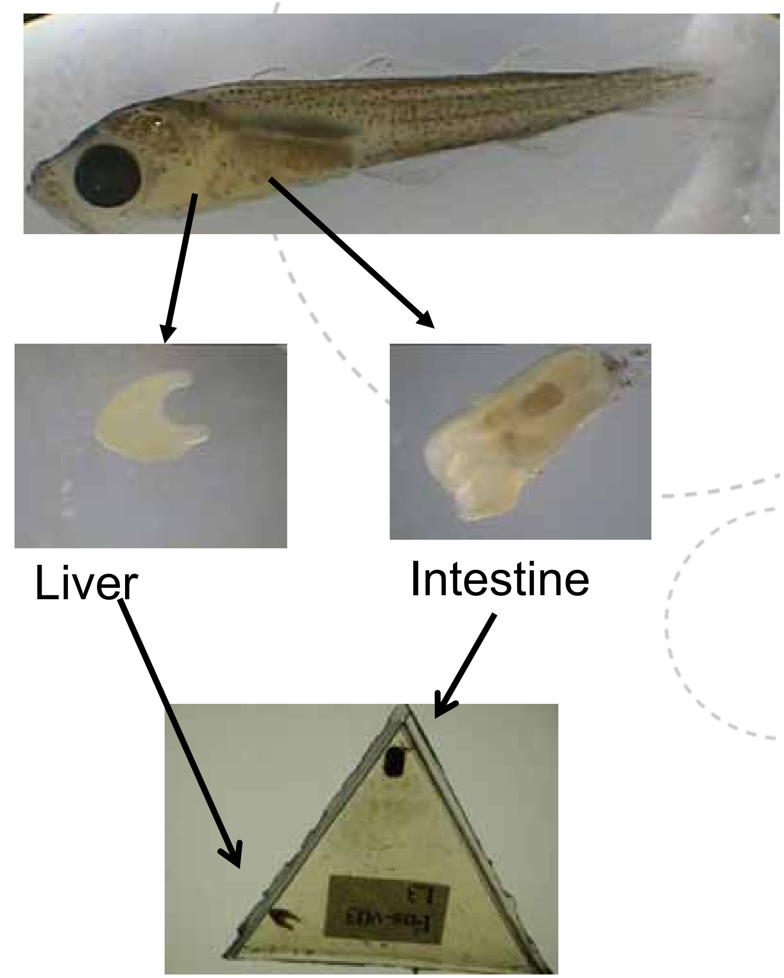
- Sampled larvae were fixed in glutharaldehyde and paraformaldhyde in cacodylic buffer.

-The intestine and liver were dissected out under a stereomicroscoope and then embedded in Epon

- Samples were sectioned according to a pre-calculated protocol designed to give an unbiased estimate of the chosen parametres.

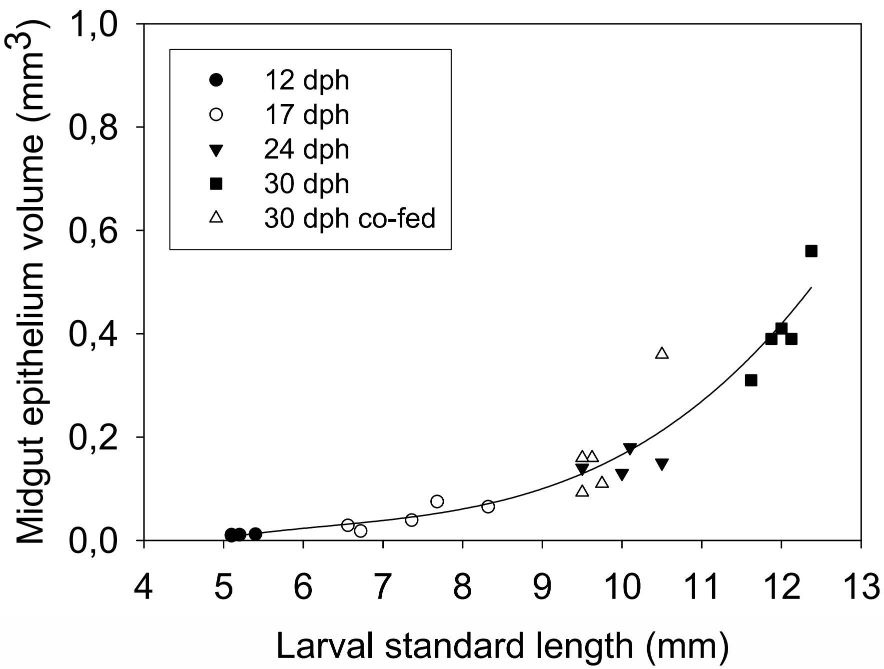
- Sections were made for LM and TEM.

- All measurements, and quantitative estimates by stereology, were calculated with the software CAST2

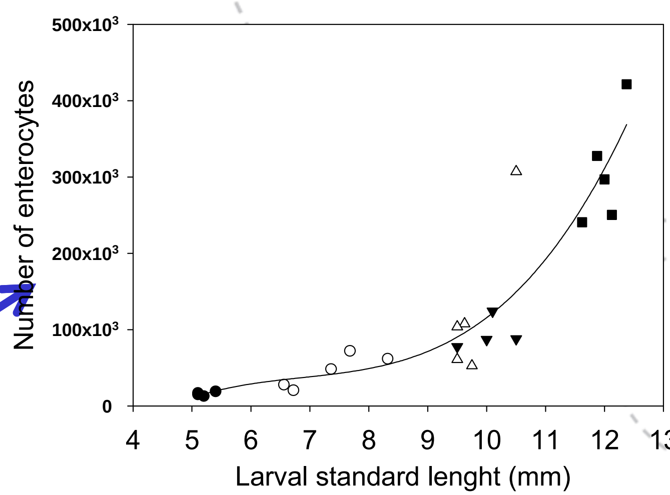


Functional development of the intestine- size and growth

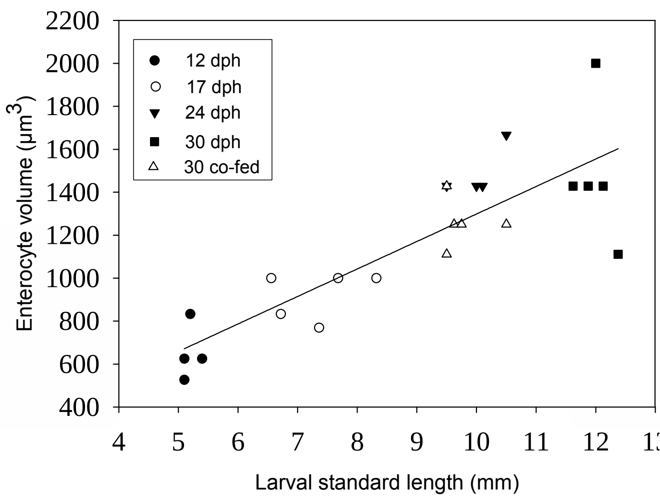
The development of the intestinal size was positively correlated to larval standard length.



Hyperplasia



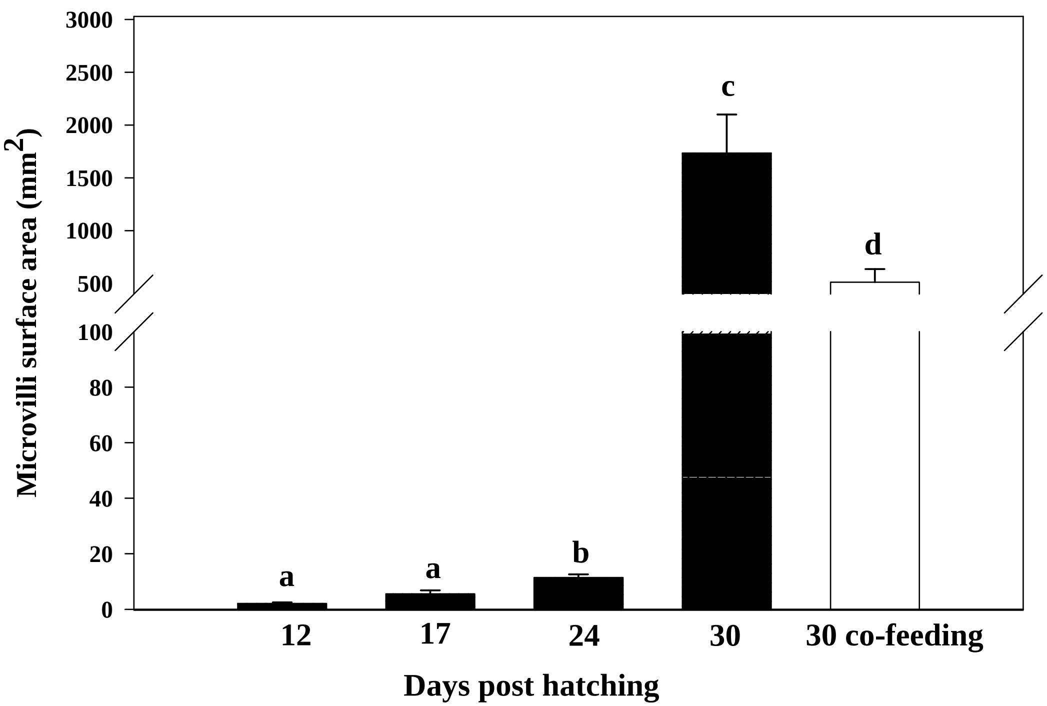
Hypertrophy



Midgut volume increased 40X on 18 days (12-30 dph)- dry weight increased 28X.

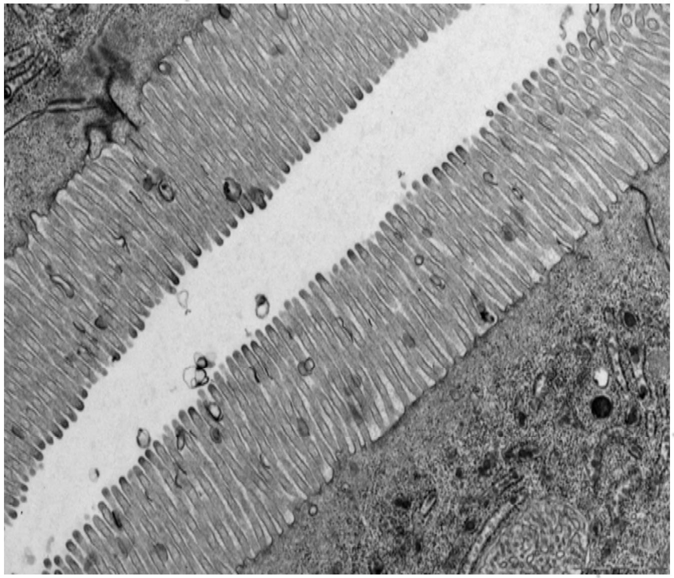
Data from Wold et al. 2008

Functional development of the intestine – microvilli surface

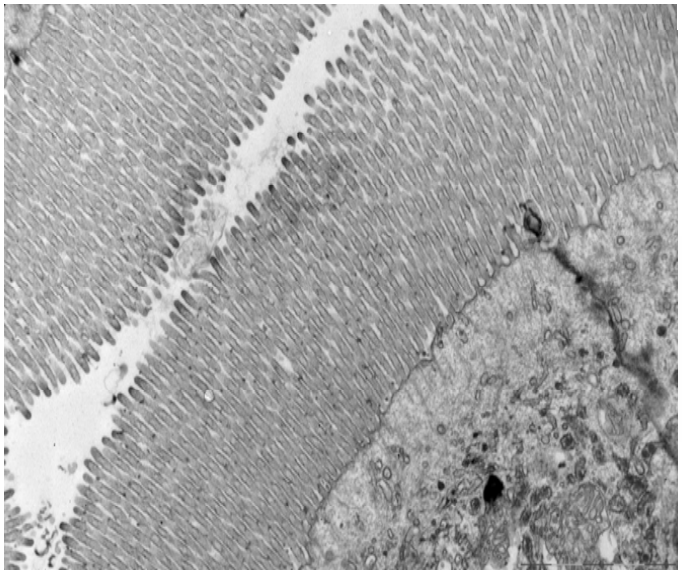


The microvilli surface increased more than 800 times from 12 to 30 days post hatching

12 days post hatching (20K)



30 days post hatching (20K)



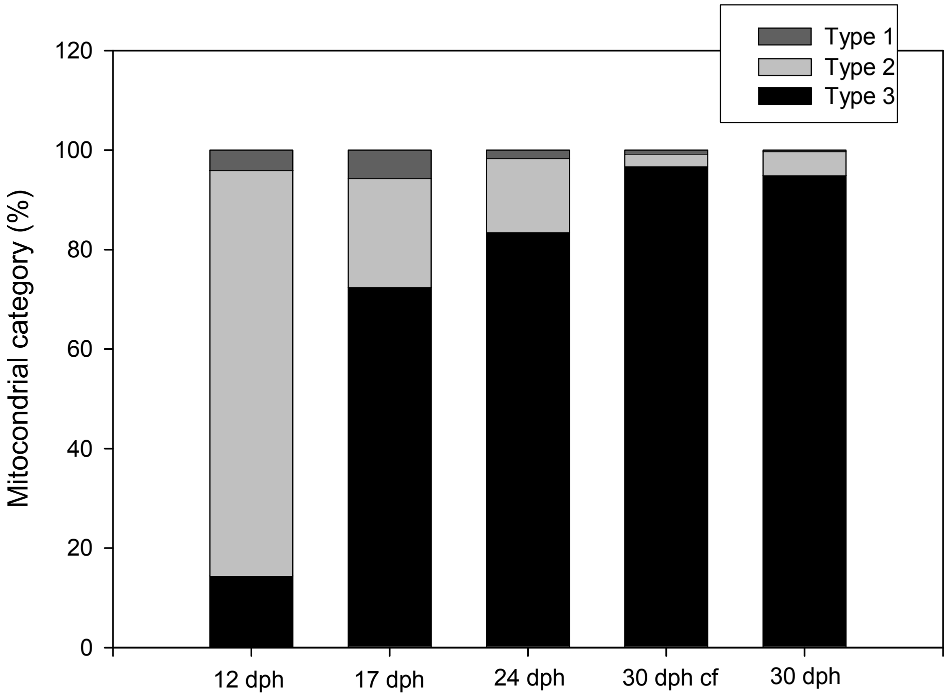
Data from Wold et al. 2008

Functional development of the intestine- mitochondria

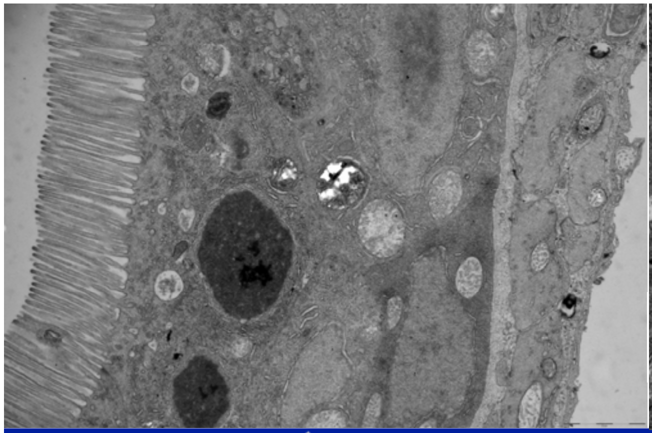
Estimated numbers of mitochondria per enterocyte increased from 53 (12 dph) to 99 (30 dph)

Three types of mitochondria were observed in enterocytes according to their membrane structures...

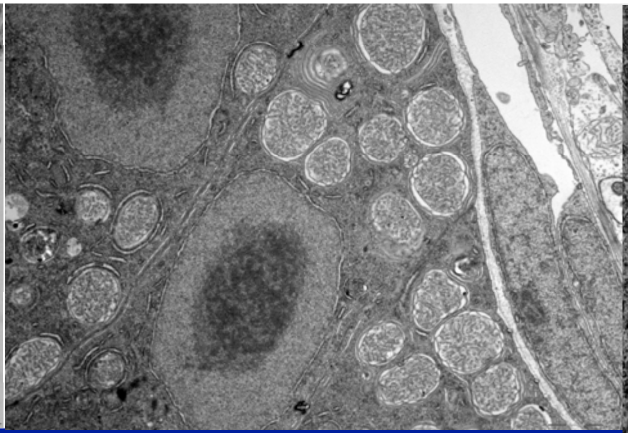
.....distribution changed during larval development



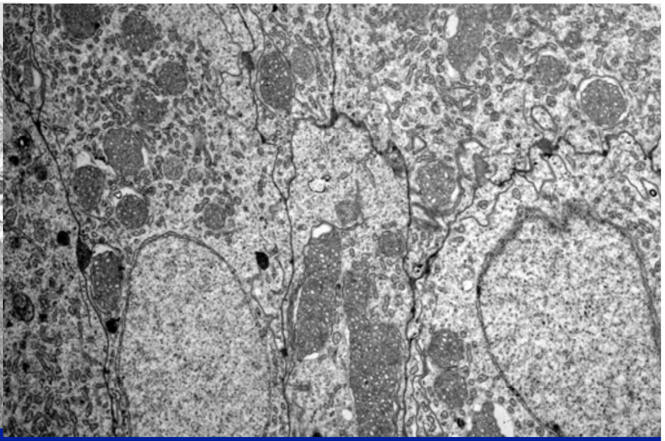
Type1



Type 2



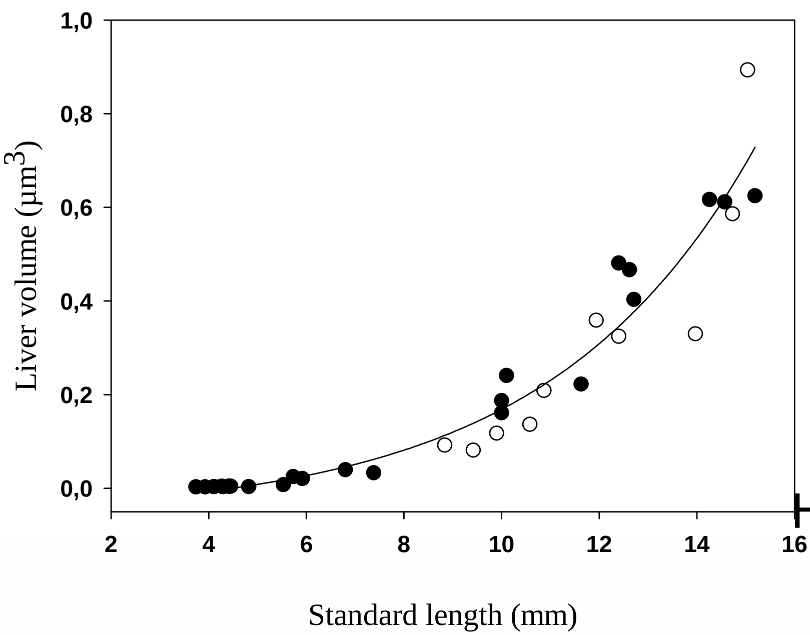
Type 3



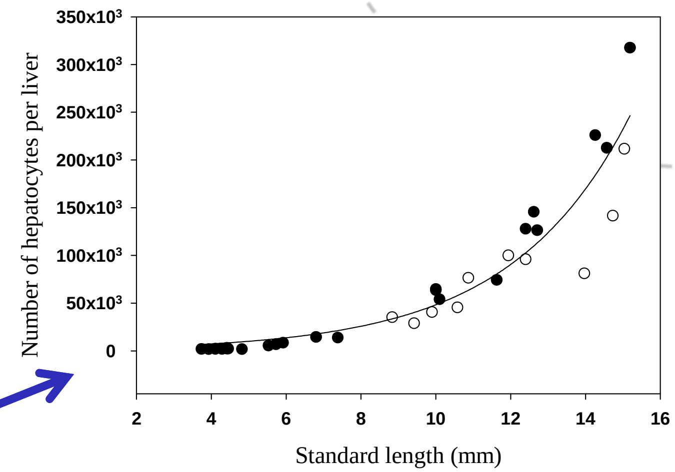
Data from Wold et al. 2008

Functional development of the liver- size and growth

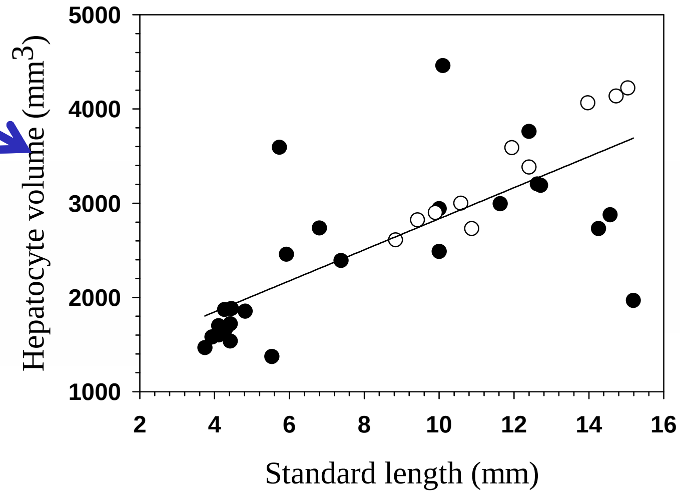
Growth and development of the liver showed similar pattern as observed for the intestine.



Hyperplasia



Hypertrophy



Liver size increased 160X between 3 and 39 dph (dry weight 50X)

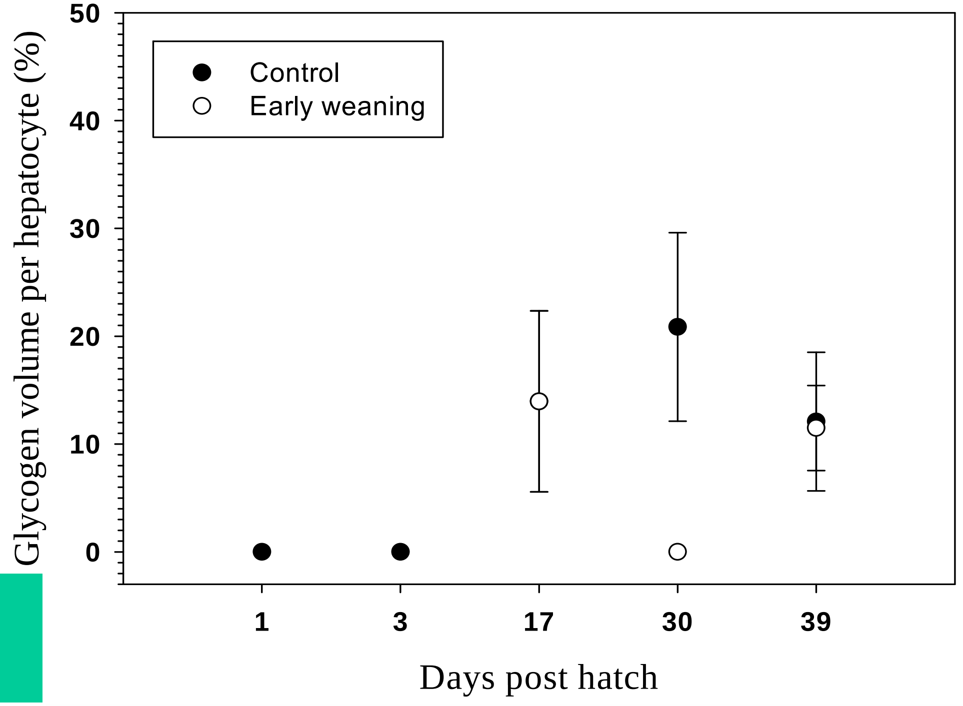
Data from Høvde et al. In prep

Functional development of the liver- energy storage

Glycogen was observed in hepatocytes from 17 days post hatching.

Glycogen storage seemed to be related to larval growth rates- rapid growing larvae stored glycogen (DWI > 10%)

Low levels of lipids were observed (<5%).



Days post hatch	DWI Control	DWI Early weaning
5-17	11.6 ± 0.5	
17-30	13.14 ± 1.36	8.74 ± 0.19
30-39	5.65 ± 1.55	14.12 ± 0.50

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Data from Høvde et al. In prep

Organ development vs. larval dry weight

Larval characteristics	SL-interval (mm)	Increase	Dry weight increase
Wold et al. 2008			
Midgut volume	6.6-12.4*	40x	28x
Enterocyte number		6.7x	
Microvilli surface		840x	
Høvde et al in prep.			
Liver volume	6.2-13.7	20x	8.7x
Hepatocyte number		16x	
Wold et al. 2007			
Alkaline phosphatase activity	7.9-13.3	60x	6.5x
Kjørsvik et al. 2009			
No. of ossified vertebrae	7.9-13.3	from 2 to 44 vertebrae	6.5x

Larval response to dietary treatment

The liver was the organ that responded most rapidly to dietary changes.

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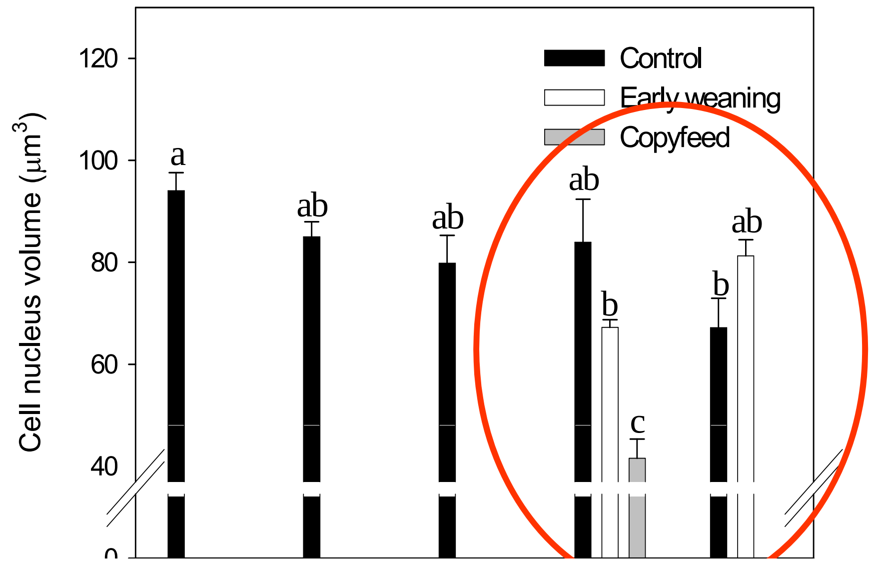
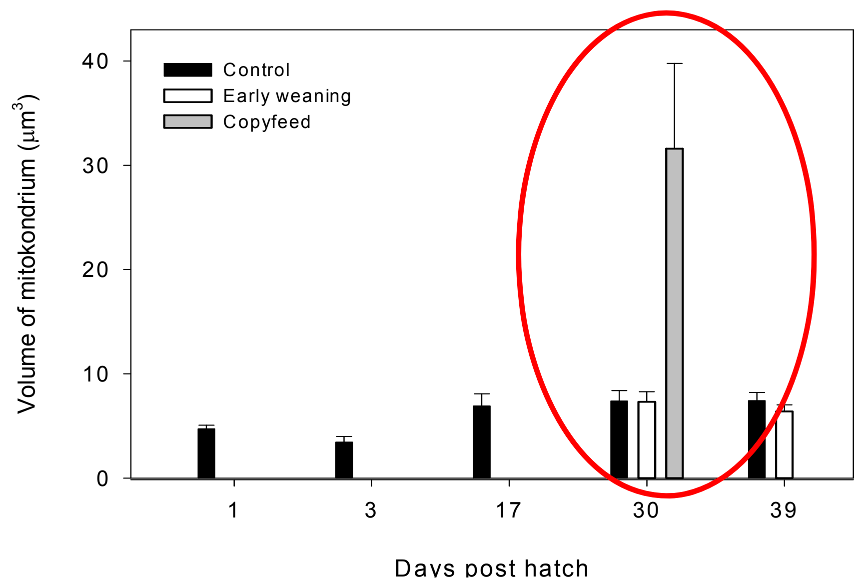


In larvae suffering from malnutrition/starvation, enlarged, swollen hepatocyte mitochondria were observed.



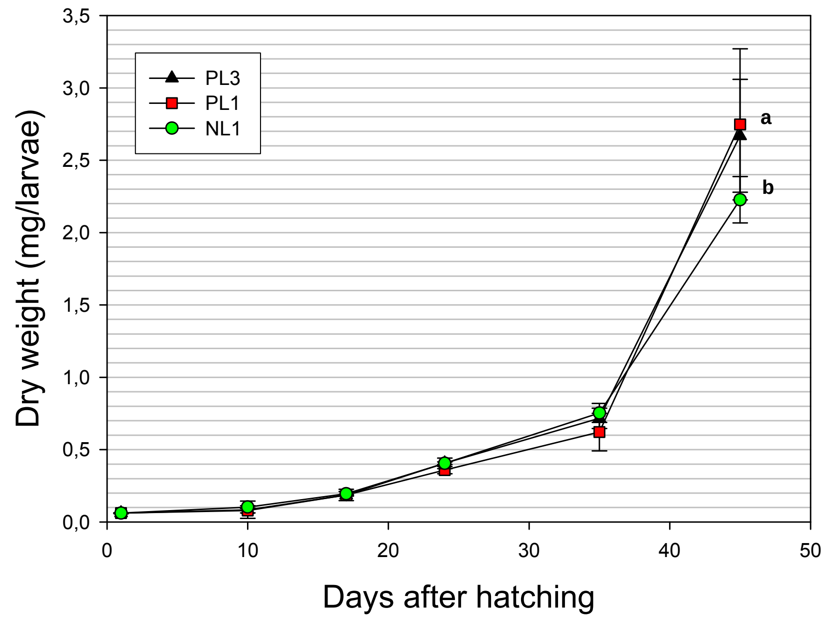
Data from Høvde et al. In prep

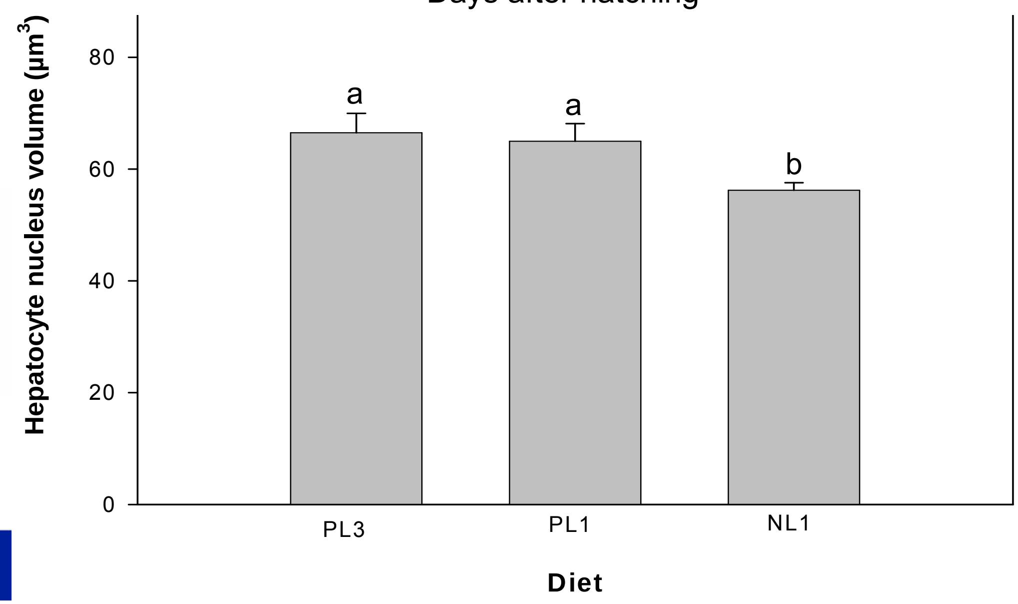
Larval response to dietary treatment

In an early-weaning experiment with three different formulated diets, containing different levels of marine phospholipids (PL);

-hepatocyte nucleus size were significantly larger in larvae fed PL- diets than in larvae fed a diet with no marine PL (NL1).

-hepatocyte mitochondria in larvae fed the NL1 contained a more open intermembranous matrix with more visible space between the christae.





Data from Wold et al. 2009

Conclusions

- **Cod larval digestive system is more related to larval size rather than age.**
- **The rapid larval development suggest a maturation period during the mixed-feeding period and furthermore a possible explanation why larvae are sensitive to suboptimal nutrition during the early days of development.**
- **The liver was more sensitive to dietary changes than the gut, and hepatocyte nucleus size was the most suitable parameter for evaluating larval nutrition status.**

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Acknowledgements

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