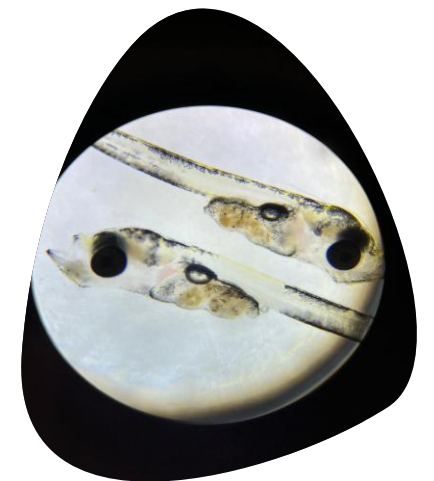


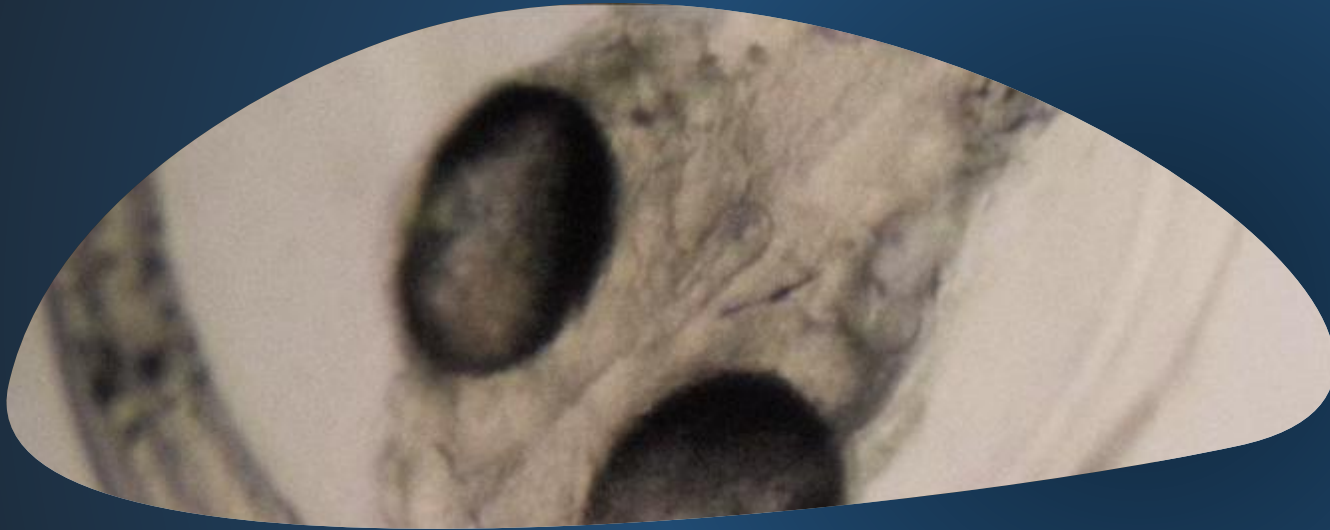
# Novel feeding protocols & microdiets for Atlantic cod larvae

# EarlyCOD trials:

- Trial **Cod3** (EarlyCOD project)
- Trial **Cod4** (EarlyCOD project)
- Trial **Cod5** (Aquaexcel project - NTNU)



# New feeding protocols combining Cryoplanton and microdiets



# Trial: Cod3

## Objective:

- Test the effect of a new live feed regime and two novel microdiets on Atlantic cod biological performance.

## Trial features:

- Atlantic cod larvae from 3-66 dph

CTRL	Rotifers + Cryo-L + CONTROL diet	Live Feeds → 3-45 dph
D1	Rotifers + Cryo-μ + Cryo-S + Cryo-L + D1 diet	Co-feeding → 27-45 dph
D2	Rotifers + Cryo-μ + Cryo-S + Cryo-L + D2 diet	Inert Feeds → 46-66 dph

- Microdiets: **D1** – enhanced in Vegetable phospholipids (Veg PL);  
**D2** – enhanced in Marine phospholipids (Mar PL);  
**CTRL** – SK Gemma Micro

# Trial: Cod3



## □ Histology Summary

15 DAH	D1	D2	CTRL
Anterior Intestine	3	2	2
Posterior Intestine	4	3	4
Liver	2	2	2
Eyes	3	3	3
Gills	3	3	1

30 DAH	D1	D2	CTRL
Anterior Intestine	4	3	4
Posterior Intestine	4	4	2
Liver	4	4	2
Eyes	4	3	3
Gills	3	2	2

66 DAH	D1	D2	CTRL
Anterior Intestine	5	4	3
Posterior Intestine	4	3	4
Liver	4	3	4
Eyes	3	3	3
Gills	4	2	4

□ At 15dph, **combination of Cryo-μ, Cryo-S and Cryo-L seems to have a positive effect on organ ontogeny**, with earlier cell development/differentiation

□ At 66dph, **SPAROS D1 group showed overall improved organ ontogeny**

**Synergistic effects**  
of live feed and novel microfeeds

# Trial: Cod3

## DEFORMITIES



### Types of Anomalies

Scoliosis

Lordosis

Kyphosis

Jaw deformities

Deformed or reduced operculum

Reduced dental/malformed pre-maxillary and/or maxillary

Multiple vertebral fusions (3 consecutive or more)

Vertebral fusion

Vertebral slipping

Vertebral body malformation

Malformed neural arch and/or spine

Malformed haemal arch and/or spine and/or rib

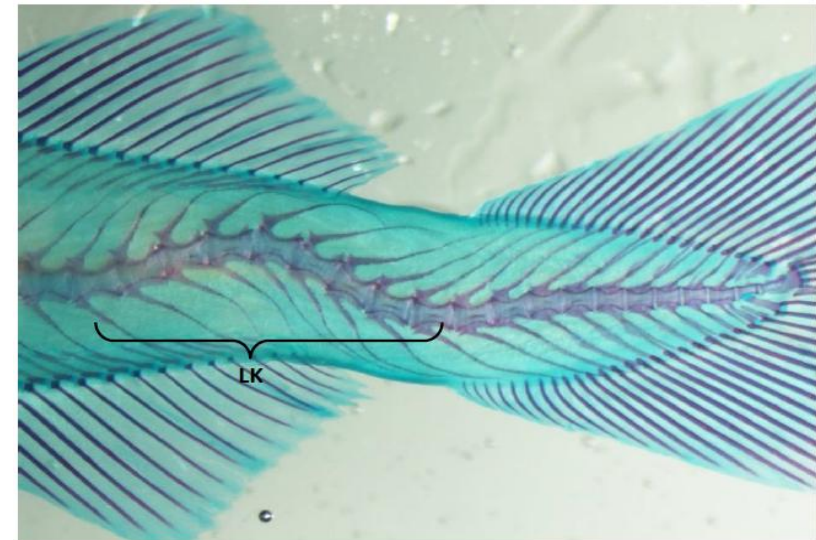
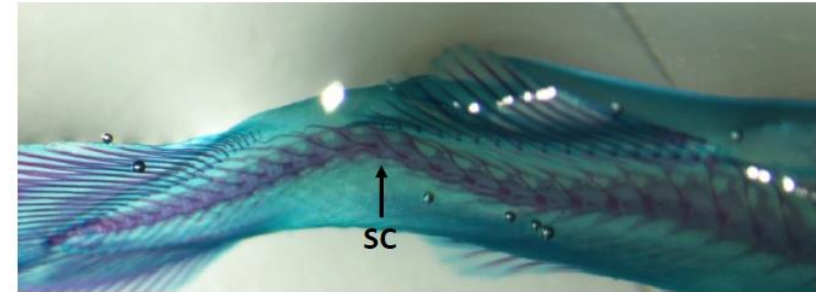
Malformed ray (deformed, absent, fused)

Malformed pterygiophores (deformed, absent, fused)

Malformed hypural (deformed, absent, fused)

Malformed epural (deformed, absent, fused)

**Severe anomalies:** skeletal malformations that modify gross morphology and may lead to an impairment of commercialization

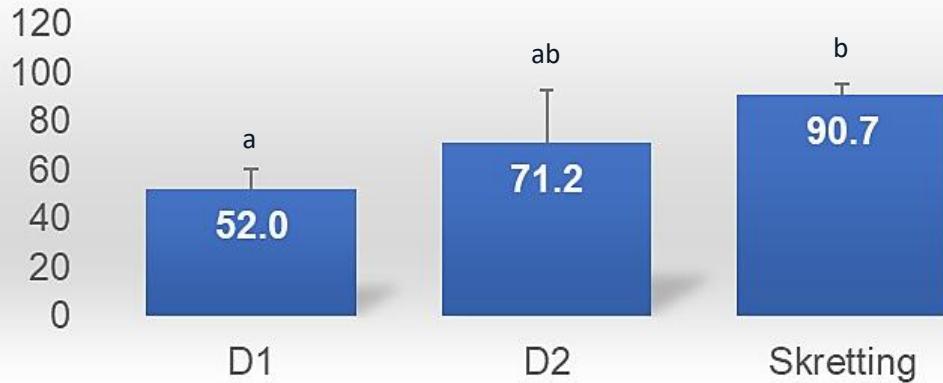


# Trial: Cod3

## DEFORMITIES

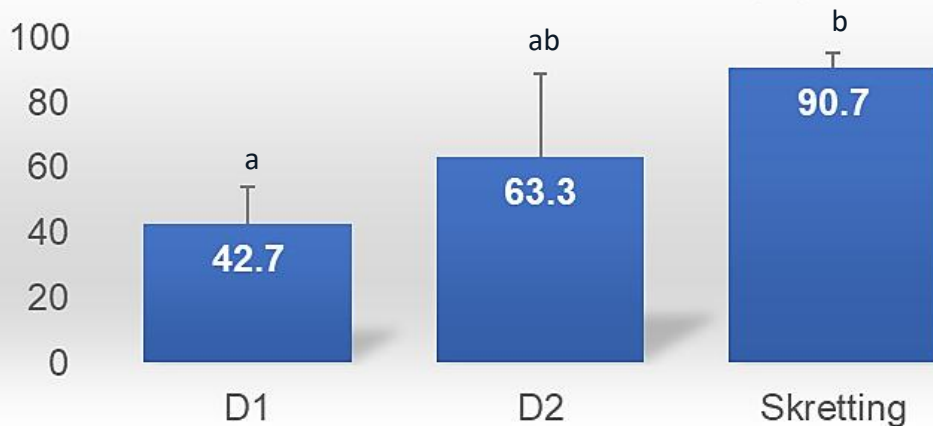


Incidence of anomalies (%)



D1 had lower proportion of individuals with at least one skeletal anomaly, when compared to fish fed CTRL diet

Severe anomalies (%)



**D1 presented a lower incidence of severe anomalies**, in comparison with CTRL group

# Final remarks – Cod3



## HISTOLOGY

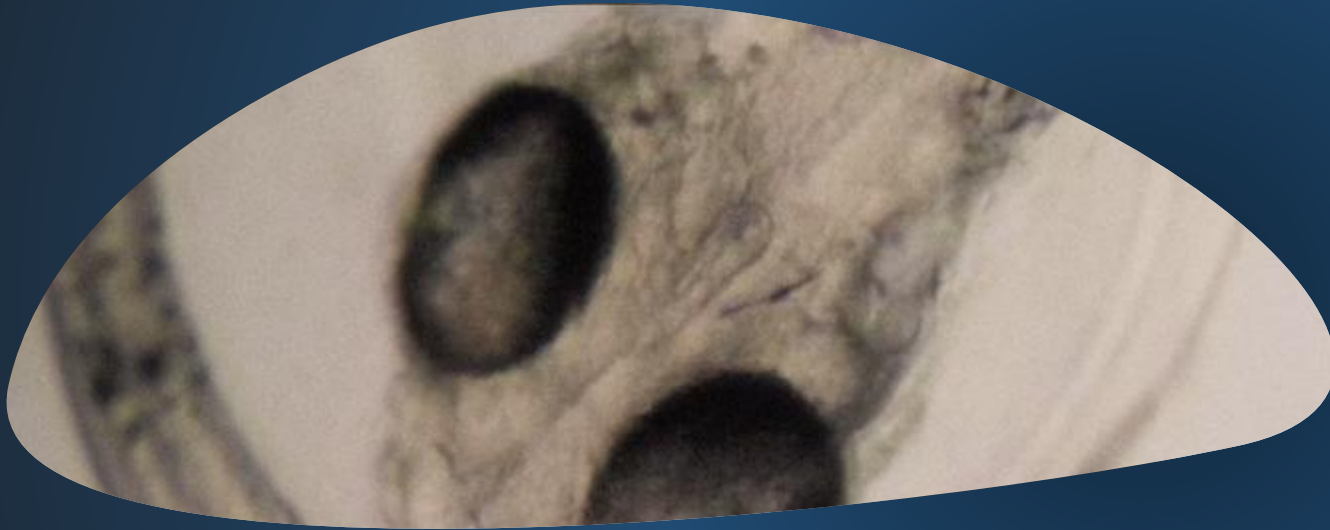
- ❑ **Cryo-μ, Cryo-S and Cryo-L** seems to have a positive effect on organ ontogeny
- ❑ **Diet D1** (Veg PL) lead to better organ ontogeny, than diet D2 or CTRL



## DEFORMITIES

- ❑ Cod larvae fed the **D1 diet** presented the lowest incidence of skeletal anomalies

# New feeding protocols combining Cryoplanton and microdiets



# Trial: Cod4

## Objective:

- Test the effect of two novel microdiets on Atlantic cod biological performance.

## Trial features:

- Atlantic cod larvae from 3-67 dph

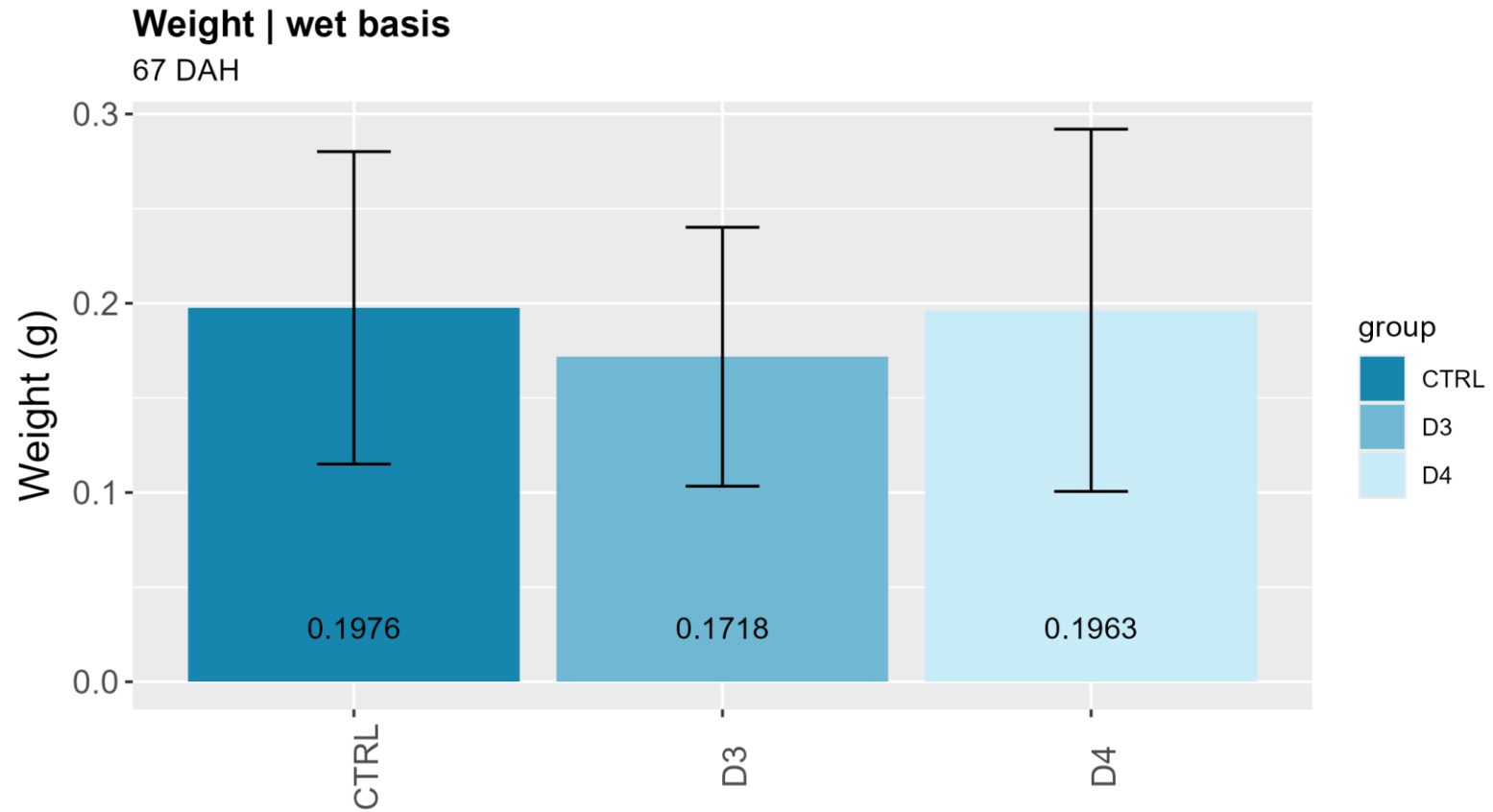
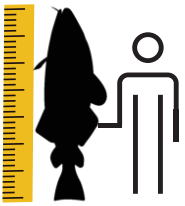
CTRL	Rots + Cryo- $\mu$ + Cryo-S + Cryo-L + CTRL diet
D3	Rots + Cryo- $\mu$ + Cryo-S + Cryo-L + D3 diet
D4	Rots + Cryo- $\mu$ + Cryo-S + Cryo-L + D4 diet

Live Feeds	→	3-27 dph
Co-feeding	→	20-45 dph
Inert Feeds	→	46-67 dph

Earlier intro of inert diets: 7 days earlier than previous trial

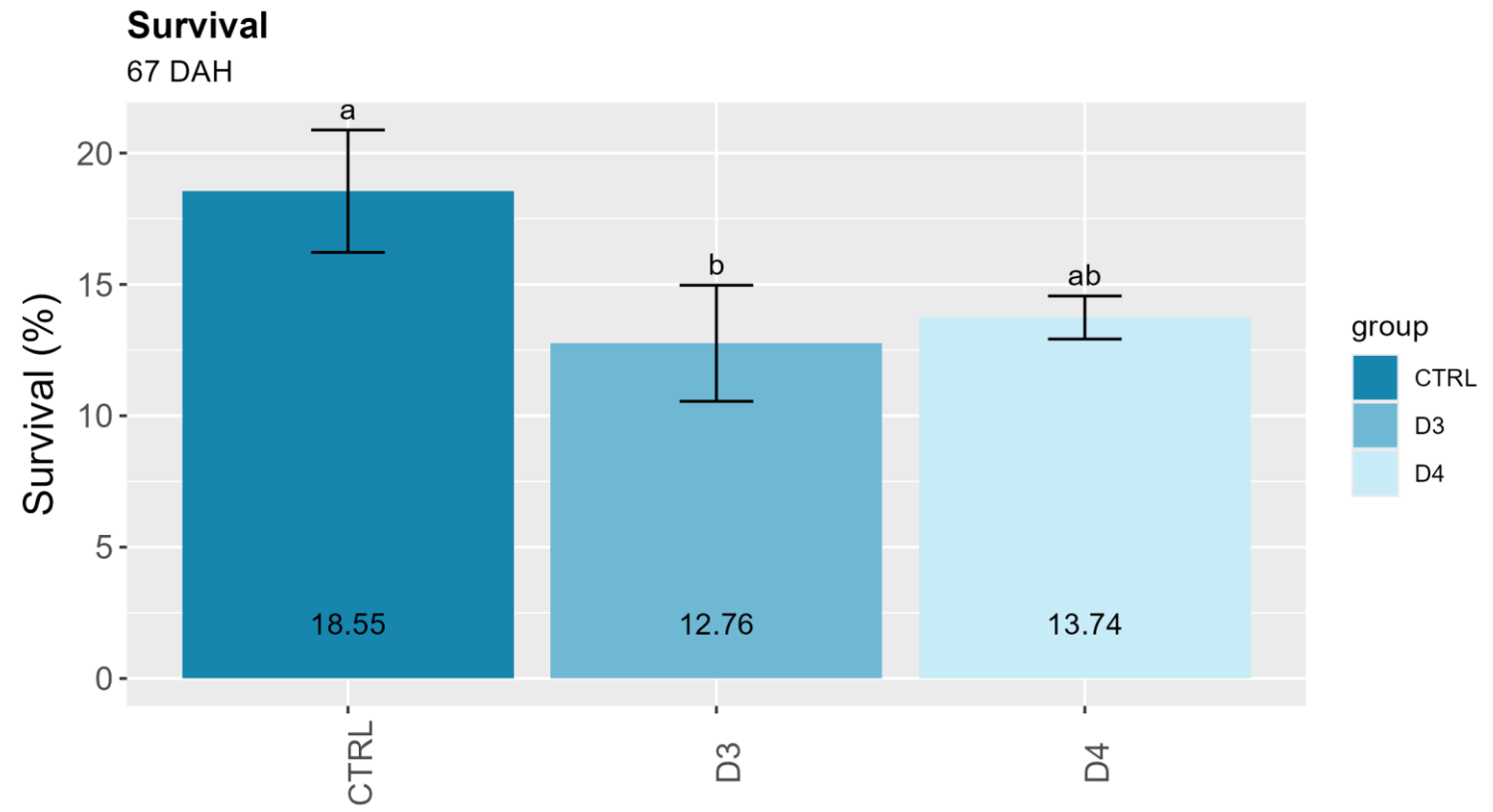
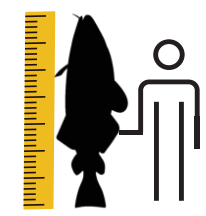
- Microdiets: **D3** slightly higher in Veg PL  
**D4** slightly higher in Mar PL  
**D3 & D4** with moderate fat level (12-13%), in comparison with D1 & D2 (14-16%)

# Trial: Cod4



☐ At 67dph there were no significant differences in WW between treatments

# Trial: Cod4



❑ CTRL group showing slightly higher survival but not statistically significant from D4 group

# Trial: Cod4



## □ Histology Summary

35 DAH	Control	D3	D4
Anterior Intestine	3	5	2
Posterior Intestine	3	4	3
Liver	2	4	3
Eyes	3	4	3
Gills	3	4	2

67 DAH	Control	D3	D4
Anterior Intestine	3	5	3
Posterior Intestine	3	4	3
Liver	3	4	2
Eyes	3	4	3
Gills	3	4	2

□ At 35dph, introduction of **SPAROS D3** (15 days co-feeding) already associated with improved organ ontogeny.

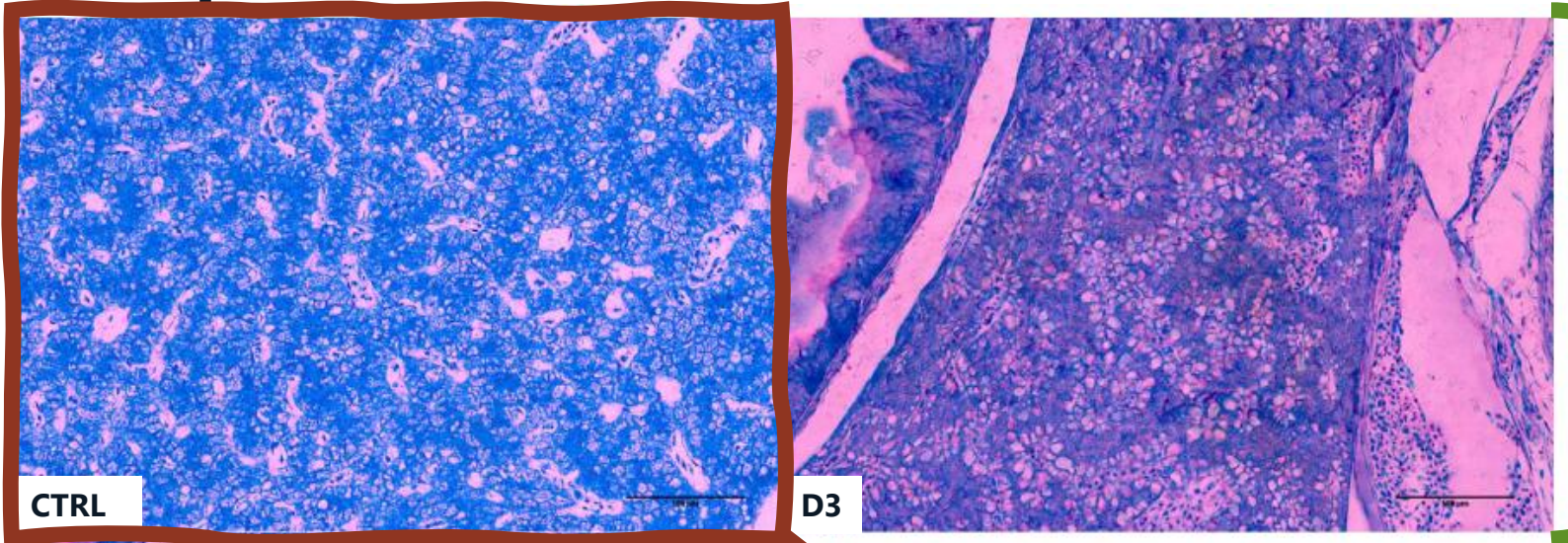
□ At 67dph, **D3 group showed overall the best organ ontogeny.**

Marine fish larval and juvenile quality scoring system (Pacorig et al, 2022):  
Scale from 1 (very poor development) to 5 (very well developed)

# Trial: Cod4



## 67dph – Liver



### D3 group showing:

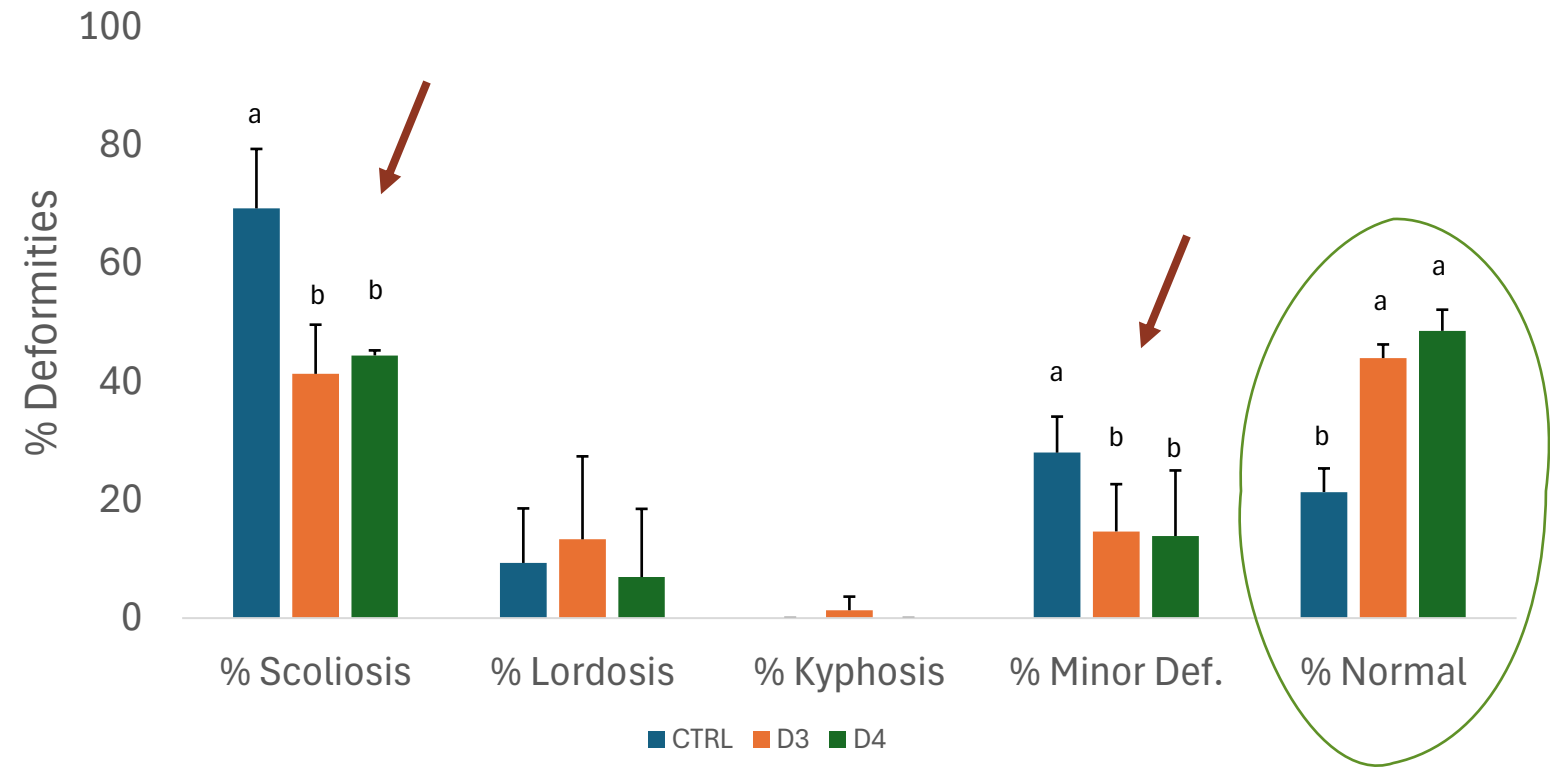
- normal fat deposition in the liver (~33%)
- uniform hepatocyte distribution
- no signs of cell degeneration

### CTRL showing:

- ~27% lipid accumulation;
- some uneven cell distribution
- potential necrotic areas

# Trial: Cod4

## DEFORMITIES



□ D3 and D4 groups showing significantly higher % of normal fish when compared to CTRL (44 & 49% vs 21%)

# Final remarks – Cod4



## ZOOTECHNICAL

- ❑ Cod larvae fed on both SPAROS diets showed good growth performance parameters
- ❑ **CTRL group** with slightly higher survival, but high deformity % → **Increased eFCR & higher losses**



## HISTOLOGY

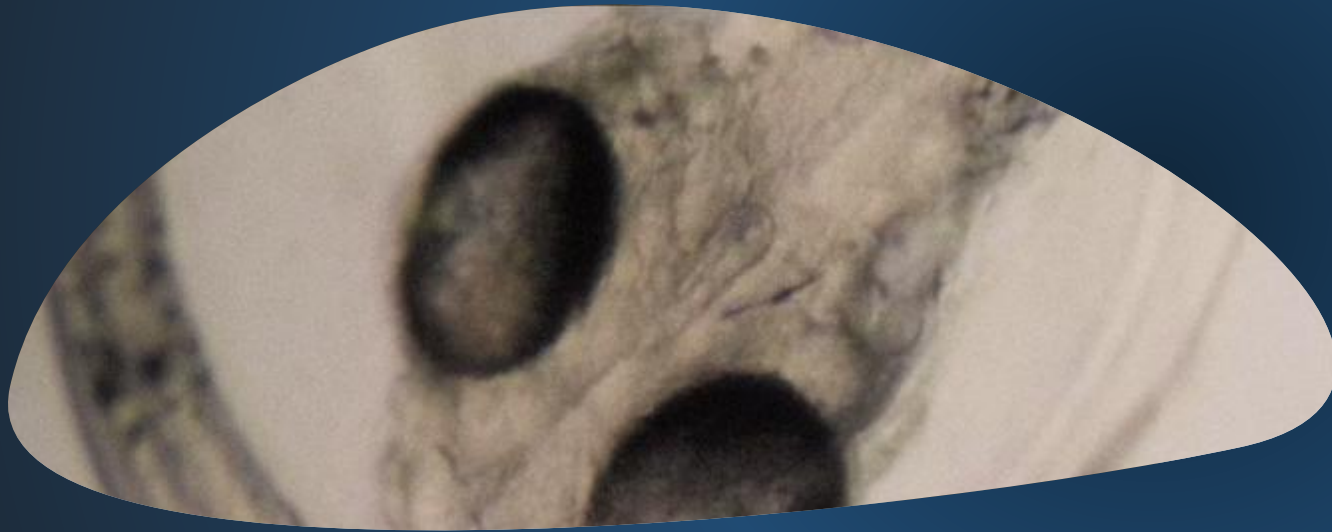
- ❑ Overall, **D3** presented the best organ ontogeny (67dph)



## DEFORMITIES

- ❑ **D3 and D4** groups presented the highest % of normal fish

# Dietary levels of Calcium , Phosphorus and Ca:P ratio, :impact on cod larvae skeletal development



# Trial: Cod5

@NTNU SeaLab

## Objective:

- Study the effect of **dietary levels of Calcium (Ca), Phosphorus (P) and Ca:P ratio** on cod larvae skeletal development.

## Trial features:

- Atlantic cod larvae from 3-50 dph

CTRL	Rotifers + Artemia AF + P2/0.6 diet
P2/0.4	Rotifers + Artemia AF + P2/0.4 diet
P2/0.8	Rotifers + Artemia AF + P2/0.8 diet
P1.5/0.4	Rotifers + Artemia AF + P1.5/0.4 diet
P1.5/0.6	Rotifers + Artemia AF + P1.5/0.6 diet

Live Feeds → 3-30 dph

Co-feeding → 15-30 dph

Inert Feeds → 31-50 dph

Earliest intro of inert diets in all trials;  
Cod3: 27dph  
Cod4: 20dph

- Microdiets: two levels of **P** (1.5 and 2.0%) and three **Ca:P ratios** (0.4, 0.6, 0.8)

# Trial: Cod5



## MAIN HIGHLIGHTS



- Tested **phosphorus** levels and **Ca:P** ratios have no impact on growth of Atlantic cod larvae
- Ca:P ratio seems to have little impact** on skeletal mineralization and skeletal anomalies of cod
- Dietary Phosphorus level of 2% in feed is recommended to **improve skeletal mineralization** and **reduce skeletal anomalies**

# Final remarks – Cod trials (EC & AQEXCEL)



## ZOOTECHNICAL

- ❑ **Combination of CryoPlankton & SPAROS diets linked to good growth performance** in Atlantic cod larvae, *in line with commercial hatchery production.*



## HISTOLOGY

- ❑ **Live feed: Cryo-μ, Cryo-S and Cryo-L** show a positive effect on organ ontogeny, when compared to Cryo-L only.
- ❑ **Microdiets: SPAROS D1 and D3 associated with** overall better organ ontogeny (namely gut, eye and liver), in comparison with SKRETTING diet.

## ❑ DEFORMITIES

- ❑ There was a clear reduction in skeletal deformities in cod fed SPAROS diets.
- ❑ 2% Phosphorus in feed is recommended for improved bone mineralization.

# Final remarks – Cod trials (EC & AQEXCEL)

## ZOOTECHNICAL

- ❑ Combination of CryoPlankton & SPAROS diets linked to good growth performance in Atlantic cod larvae, *in line with commercial hatchery production.*

## HISTOLOGY

- ❑ Live feed: Cryo-L compared to Cryo-L on organ ontogeny, when
- ❑ Microdiets: SPAROS associated with better organ ontogeny (namely gut, eye and liver) in comparison with Cryo-L diet.

There's NO "one size fits all..."

TAILORED NUTRITION for fish larvae is key!

## DEFORMITIES

- ❑ There was a clear reduction in skeletal deformities in cod fed SPAROS diets.
- ❑ A 2% Phosphorus in feed is recommended for improved bone mineralization.