



Does increasing the level of selenium in rotifers (*Brachionus plicatilis* 'Cayman') improve the health of cod (*Gadus morhua* L.) larvae?

Sam Penglase, Kristin Hamre, Terje van der Meeren, Synnove Helland
and Andreas Nordgreen





Cod larvae

- Intensively reared cod larvae are generally low in quality
 - Low growth rates
 - Highly variable survival
 - High rate of skeletal deformities
- Strongly linked to first feeding diet

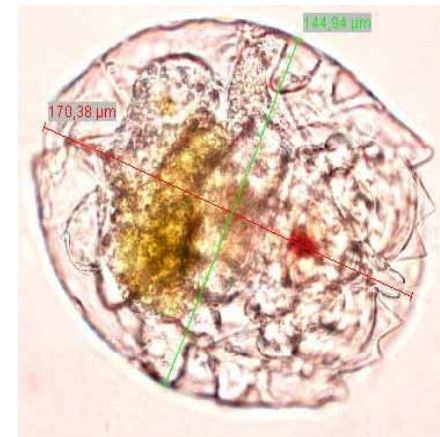


First feeding diet

- Intensive feed → Rotifers
- Wild feed → Copepods
- Large difference in nutritional composition

0.08-0.09 mg Se kg⁻¹
dry weight

3-5 mg Se kg⁻¹ dry
weight
(Hamre *et al.*, 2008)





Selenium

- Essential micronutrient for vertebrates

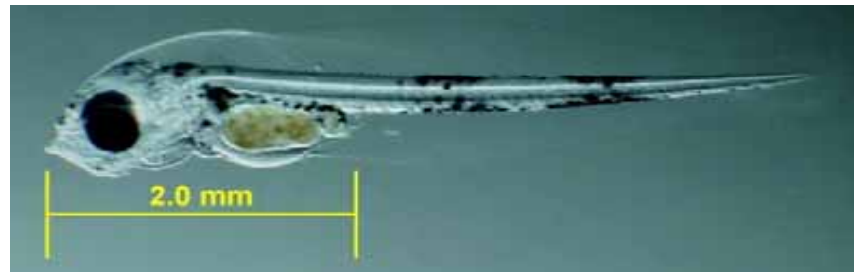
- Selenium dependent enzymes
 - Glutathione peroxidases
 - Antioxidant
 - Common Se status biomarker

 - Deiodinases
 - Thyroid hormone differentiation



Study aims

- Investigate whether rotifers enriched to 3 mg Se kg⁻¹ DW increase cod larvae quality





Trial design

- Eight tanks, $n=4$
 - 100 Larvae L^{-1} → 40,000 Larvae per tank
- Feeding
 - Treatment larvae fed Se-yeast enriched rotifers
→ 3 mg Se kg^{-1} DW (Se+larvae)
 - Control larvae fed normal enriched rotifers
 - Rotifer feeding period -> 3 to 29 days post hatch

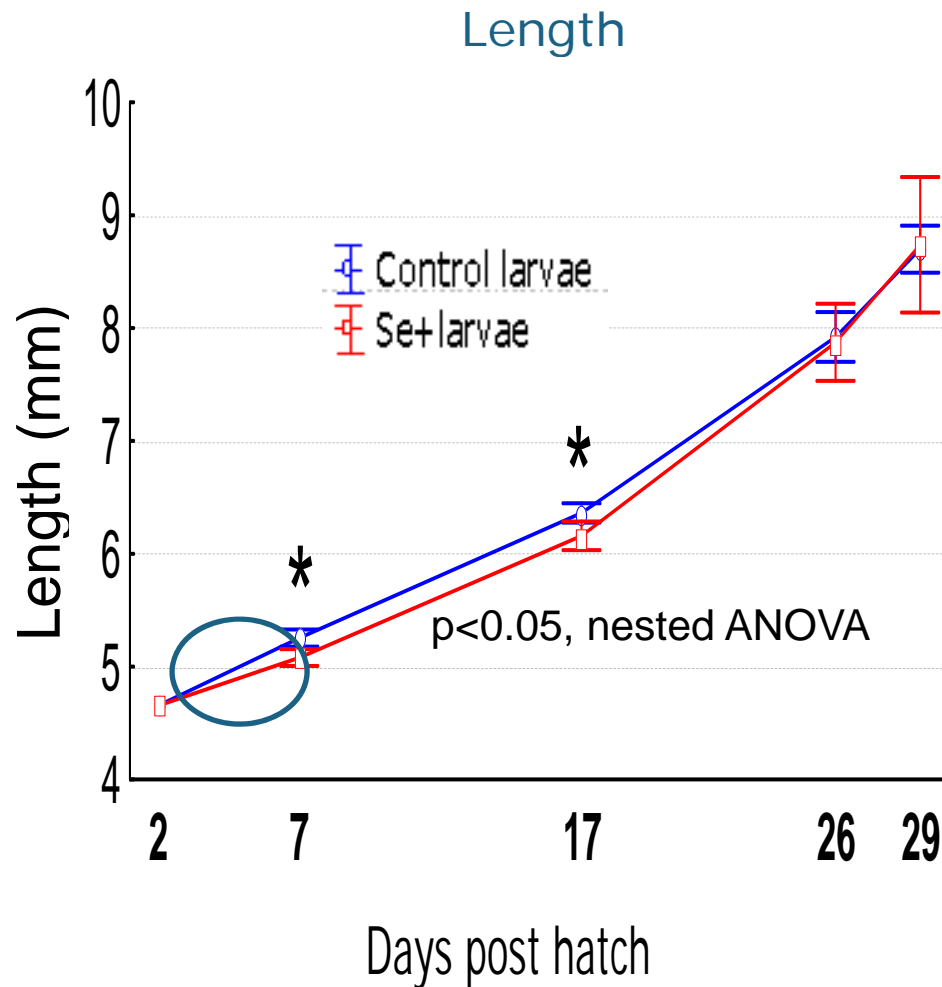


Sampling

- At 2, 7, 17, 26 and 29 dph
 - Growth
 - Length
 - Weight
 - Survival
 - Selenium concentration
 - Selenium dependent enzymes
 - Glutathione peroxidases
 - Deiodinases

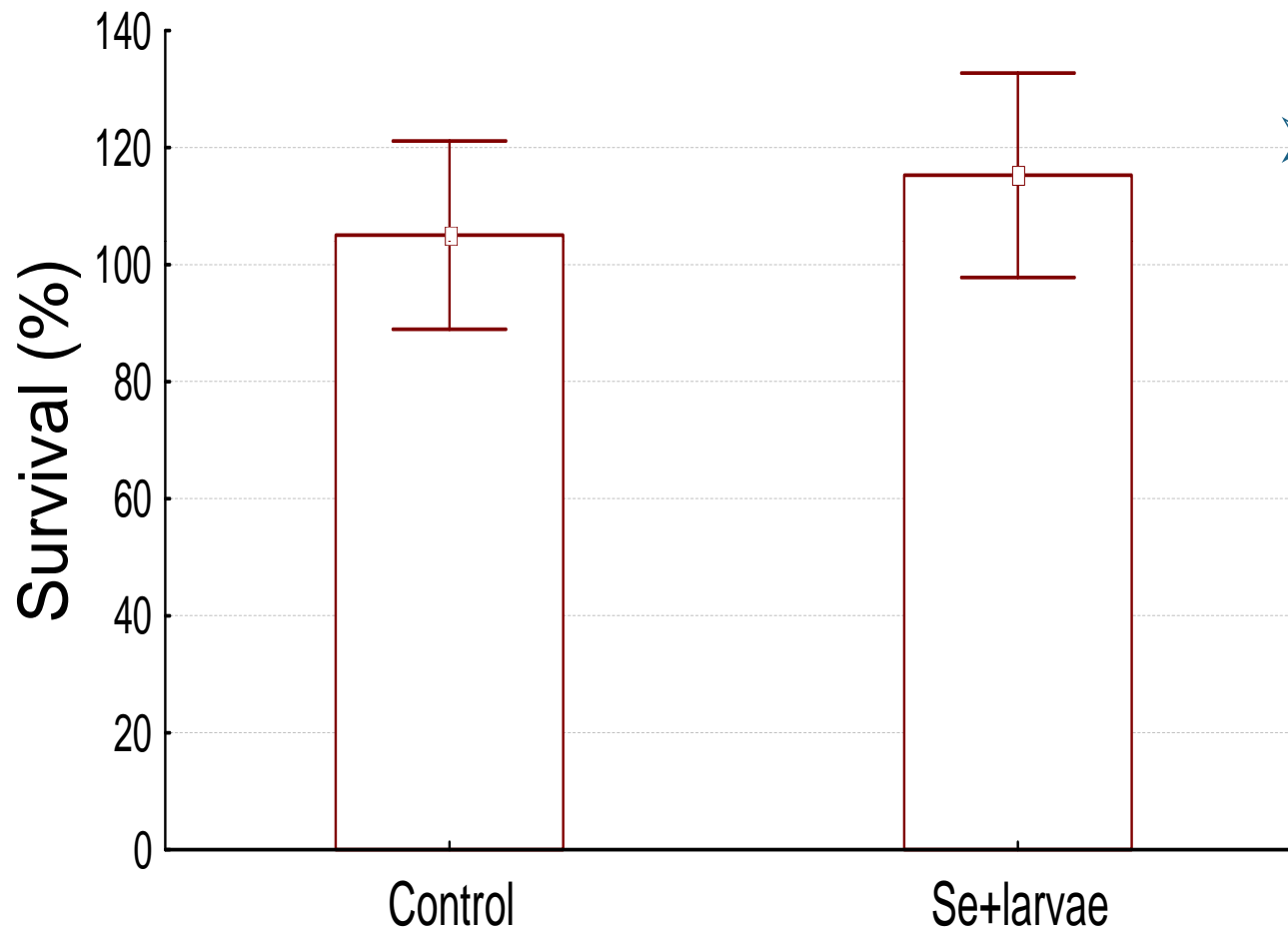


Cod larvae → Length and weight





Cod larvae → Survival at 29 dph

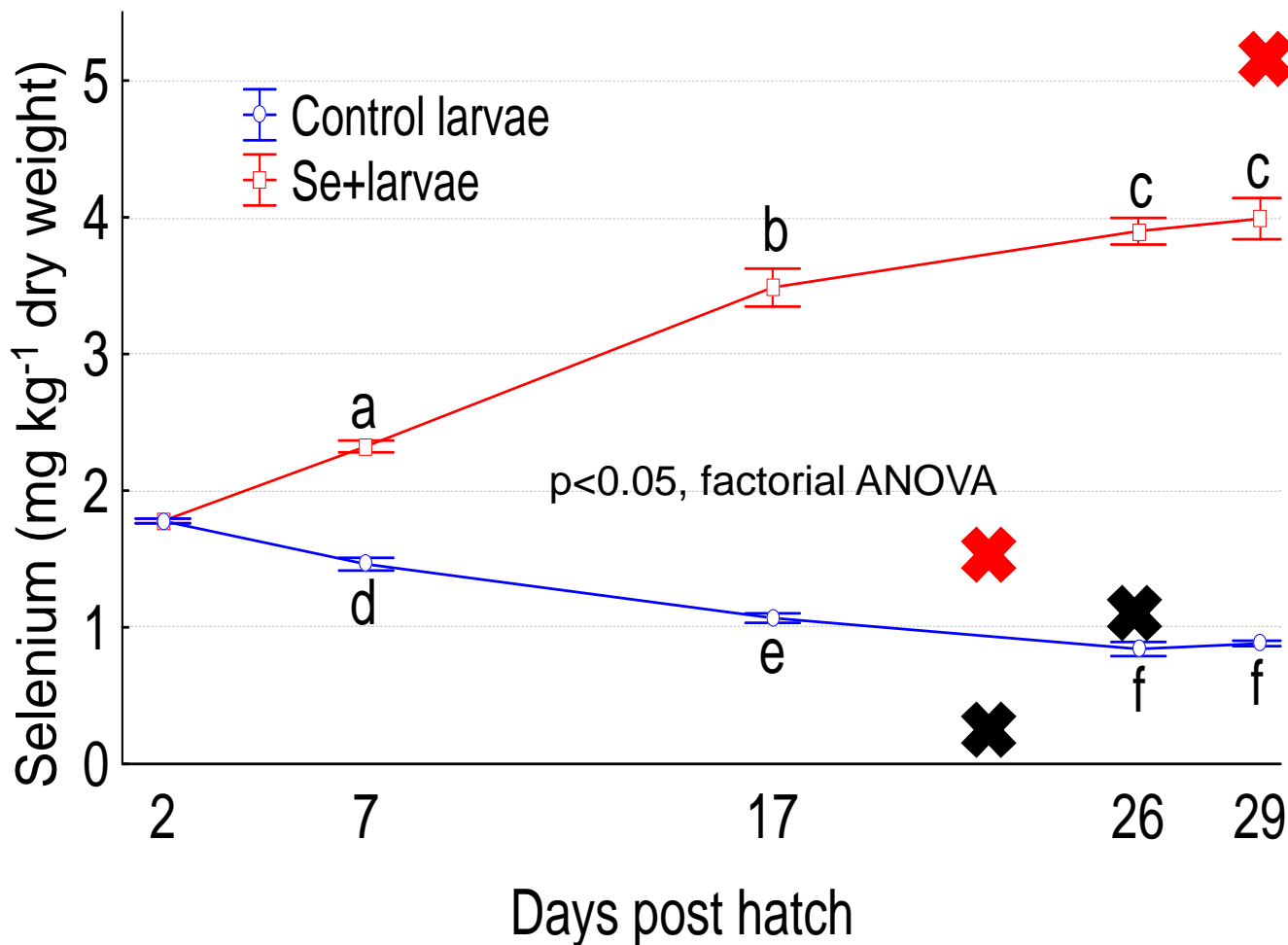


➤ Survival

➤ 100% at 29 dph

➤ No difference between treatments

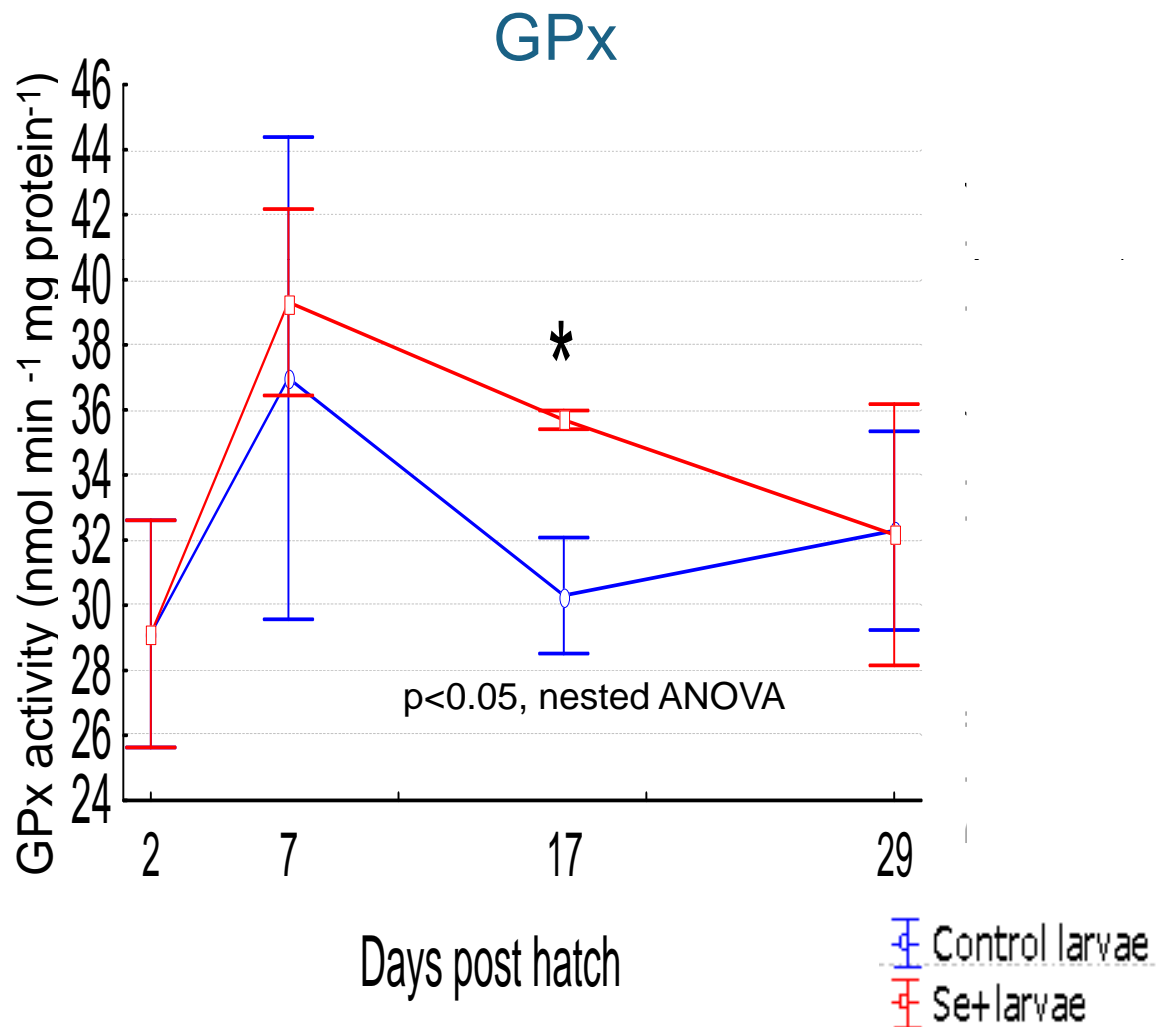
Cod larvae → Selenium uptake



➤ Cod larvae Se concentration

➤ Se+larvae 4.5× higher Se levels by 29 dph

Cod larvae → enzyme activity





Cod juveniles → 120 dph

		Control	Se+larvae
Growth	Weight (g)	12.23 ± 3.32	11.13 ± 3.19
	Length (cm)	10.89 ± 0.91	10.57 ± 0.95
Skeletal deformities (% of total population)	Bent neck	48.0 ± 8.0	54.0 ± 12.5
	Lordosis	3.3 ± 5.8	2.0 ± 0.0
	Vertebrate*	20.0 ± 3.5	27.3 ± 3.1
	Jaw	24.7 ± 6.4	22.0 ± 7.2
	Total fish	75.3 ± 11.0	76.0 ± 5.3

* Close to significant, ANOVA with post hoc Tukey test, p=0.0514



Summary → Cod larvae

➤ Se+larvae

➤ Differences

- Shorter than controls → 7 and 17 dph
- Se concentration → 4.5 fold 29 dph
- Higher GPx activity → 17 dph

➤ No difference

- Dry weight
- Survival
- Deiodinase activity
- Growth or rate of skeletal deformities at 120 dph



Conclusion

- Se-yeast was effective for increasing selenium concentrations in rotifers to the desired levels
- Control rotifers appear to contain insufficient selenium to meet cod larvae requirements



Thank you for listening





Acknowledgments

- Lab technicians at NIFES and IMR





Bibliography

- Busch, K., Falk-Petersen, I. B., Peruzzi, S., Rist, N. and Hamre, K. (submitted). "Natural zooplankton as larval feed in intensive rearing systems for juvenile production of Atlantic cod (*Gadus morhua* L.)." Aquaculture Research.
- Gatlin, D. M., III and Wilson, R. P. (1984). "Dietary Selenium Requirement of Fingerling Channel Catfish." Journal of Nutrition **114**(3): 627-633.
- Folkvord, A., Koedijk, R., Grahl-Nielsen, O., Meier, S., Olsen, B. R., Blom, G., Hamre, K. and Imsland, A. K. (Manuscript). "Differences in nutritional composition of cod larvae reared on natural zooplankton and enriched rotifers."
- Hamre, K., Srivastava, A., Rønnestad, I., Mangor-Jensen, A. and Stoss, J. (2008b). "Several micronutrients in the rotifer *Brachionus* sp. may not fulfil the nutritional requirements of marine fish larvae." Aquaculture Nutrition **14**: 51-60.
- Hilton, J. W., Hodson, P. V. and Slinger, S. J. (1980). "The requirement and toxicity of selenium in rainbow trout (*Salmo Gairdneri*)." Journal of Nutrition **110**(2527-2535).
- Julshamn, K., Haugsnes, J. and Utne, F. (1978). "The contents of 14 major and minor elements (minerals) in Norwegian fish species and fish byproducts determined by atomic absorption spectrophotometry." Fiskeridirektoratets Skrifter, Serie Ernæring **1**: 117-135.
- Lin, Y.-H. and Shiau, S.-Y. (2005). "Dietary selenium requirements of juvenile grouper, *Epinephelus malabaricus*." Aquaculture **250**(1-2): 356-363.





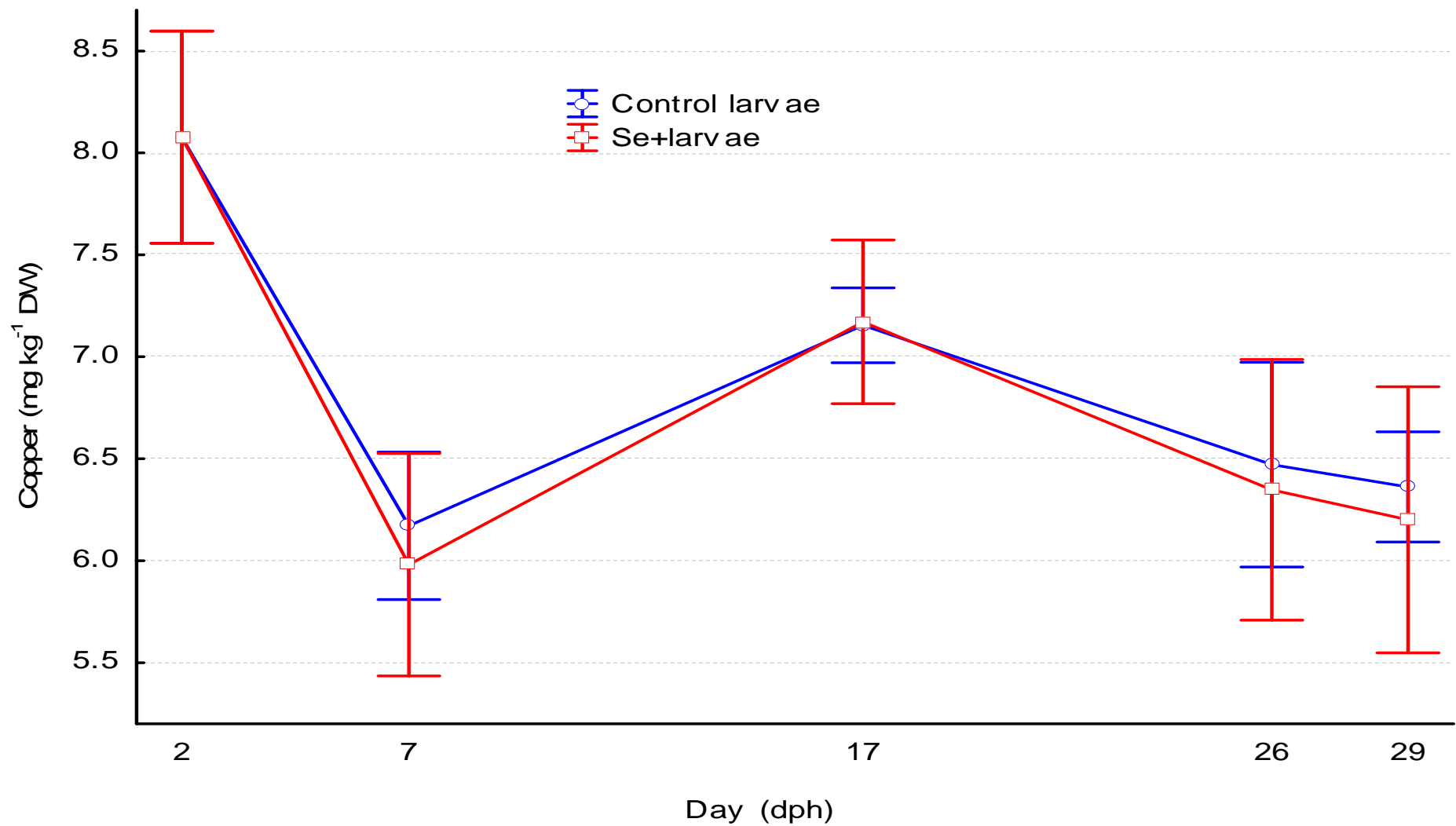
Selenium concentration (mg kg^{-1} DW) in Se+ and control rotifers with time after enrichment

Time (hrs)	Se+rotifers	Control rotifers
0	2.95 ± 0.48^a	0.66 ± 0.02^b
2.5	2.86 ± 0.48^a	0.63 ± 0.05^b
8	1.95 ± 0.36^c	0.56 ± 0.02^b
18.5	1.73 ± 0.23^c	0.54 ± 0.02^b
18.5*	2.71 ± 0.34^a	0.62 ± 0.01^b

* Rotifers after 18.5 hrs storage in holding tanks without the presence of algae after ST enrichment



Cod larvae --> copper



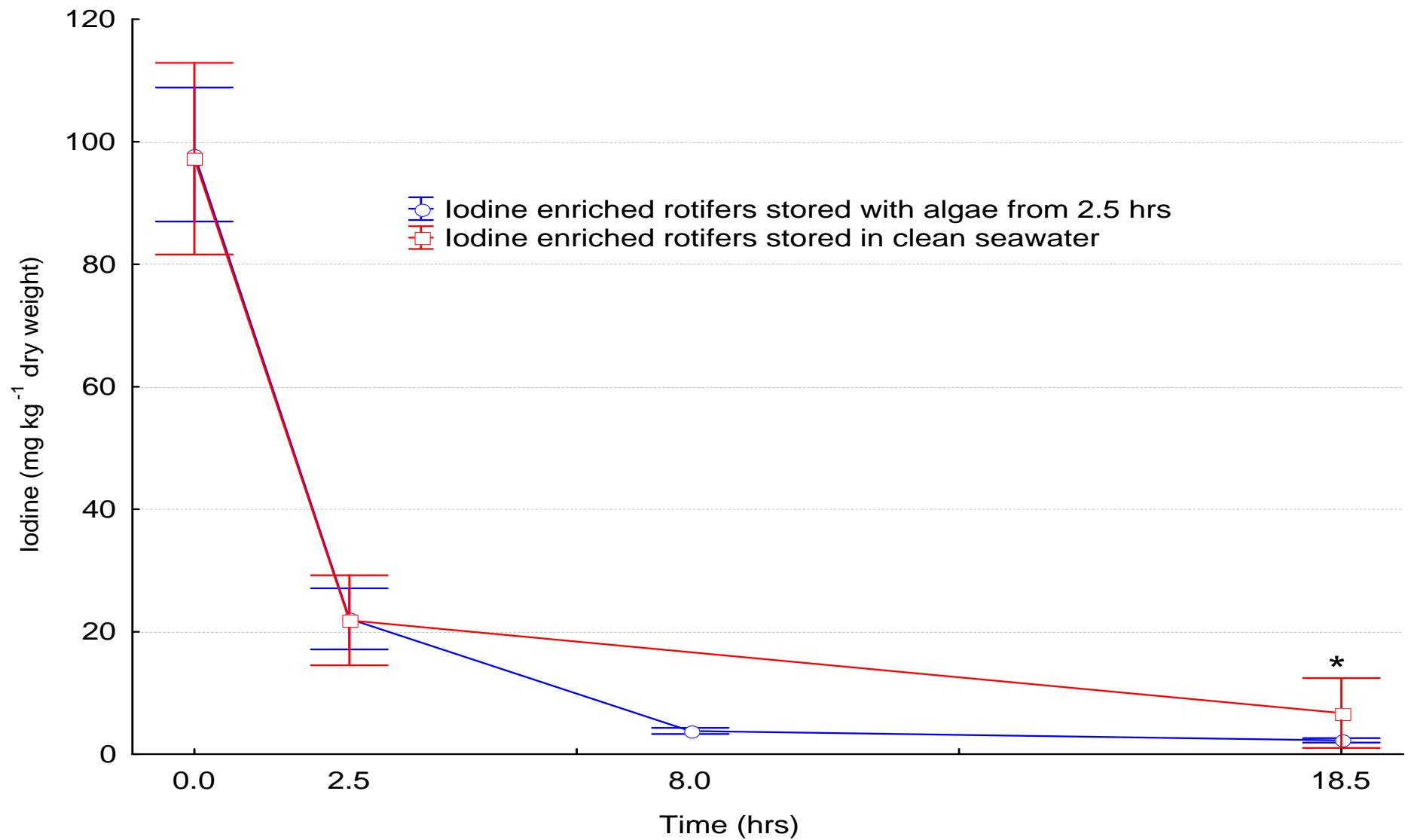


Cod larvae --> copper and Se

Mineral (mg kg ⁻¹ DW)	DPH	Se+larvae	Controls
Selenium	2	1.78 ± 0.02 ^a	1.78 ± 0.02 ^a
	7	2.32 ± 0.04 ^b	1.46 ± 0.05 ^e
	17	3.49 ± 0.14 ^c	1.07 ± 0.03 ^f
	26	3.90 ± 0.10 ^d	0.84 ± 0.05 ^g
	29	3.99 ± 0.15 ^d	0.88 ± 0.02 ^g
Copper	2	8.08 ± 0.52 ^a	8.08 ± 0.52 ^a
	7	5.98 ± 0.55 ^b	6.17 ± 0.36 ^b
	17	7.17 ± 0.40 ^c	7.15 ± 0.18 ^c
	26	6.35 ± 0.64 ^b	6.47 ± 0.50 ^b
	29	6.20 ± 0.65 ^b	6.36 ± 0.27 ^b



Rotifers --> Iodine





Cod larvae --> Se uptake, trial comparison

Trial	Se source used for rotifer enrichment	Rotifer Se concentration (mg Se kg ⁻¹ DW)	Cod larvae Se concentration at 26 dph (mg Se kg ⁻¹ DW)	Cod larvae Se versus rotifer Se concentration ratio
Current study	Se-yeast	3.0 ± 0.5	3.9 ± 0.1	1.3
Hamre <i>et al.</i> (2008a)	Na-Se	4.8 ± 0.5	3.5 ± 0.1	0.73



Cod larvae --> Se uptake

Feeding time	Se concentration (mg kg ⁻¹ DW)	Rotifer feeding rate (% of total daily feeding)	Time rotifers are available for larvae to ingest	Observation of cod larvae feeding activity	Assumed intake %	
13:00 Batch feeding	2.95 ± 0.48	40	3.5 hrs	At time of feeding, larvae guts were only partly full (30- 50%), fill up fully with rotifers	20%	
15:30 Batch feeding	2.86 ± 0.48	20	3 hrs	Rotifer guts still 70-90% full, fill up with new rotifers	10%	
15:30 – 10:00 next day Continuous feeding	2.86 ± 0.48 start 1.95 ± 0.36 8 hrs 1.73 ± 0.23 18.5 hrs (end)	40	17.5 hrs	At start, larvae guts are 100% full, at 10:00 larvae guts are 40-70% full	2.86 ± 0.48	10%
Average Se level in Se+rotifers ingested by Se+larvae.					1.95 ± 0.36	35%
					1.73 ± 0.23	25%
					2.3 mg Se kg ⁻¹	



Skeletal deformities

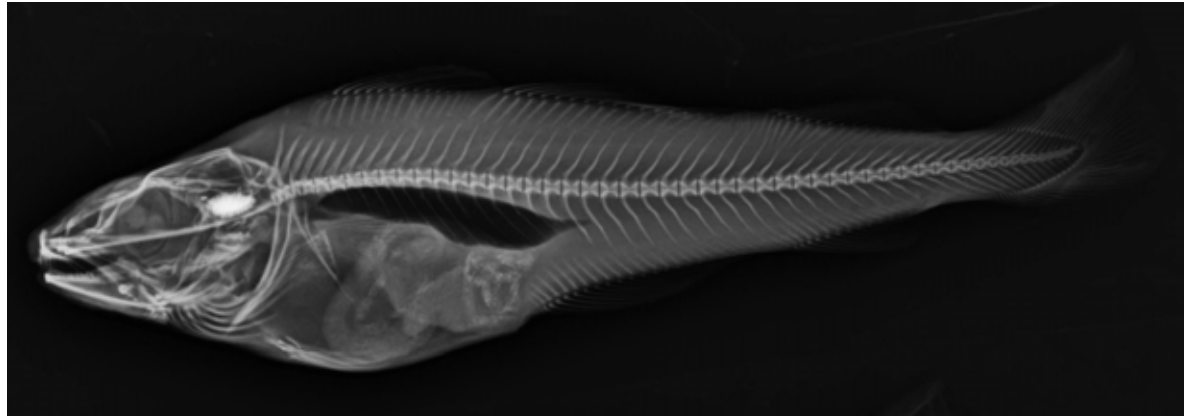


Figure 1. Normal cod

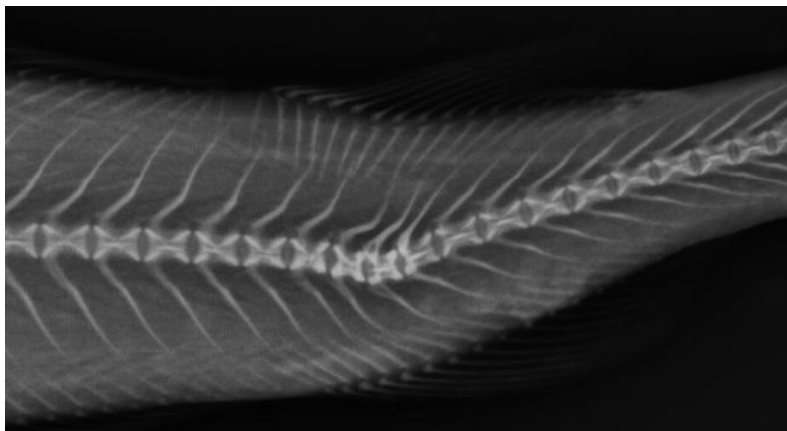


Figure 3. Lordose in backbone of grade 2 cod juvenile



Figure 2. Bent neck of grade 1 cod juvenile