Development of Zn and Mn enrichment method in live feed and nutritional importance in marine fish larvae

By

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Introduction

- Growth and survival of hatchery-raised finfish are affected by the nutritional value of live feeds such as rotifers and *Artemia*.
- Manipulation of nutrient profiles of live feeds has been improved growth, survival and quality of hatchery-raised fingerlings (example: enrichment of live feeds with HUFAs, vitamin A, taurine….)
- So far, little effort has been directed toward the enrichment of live foods with minerals

Zn and Mn are important minerals for growth and normal skeletal development

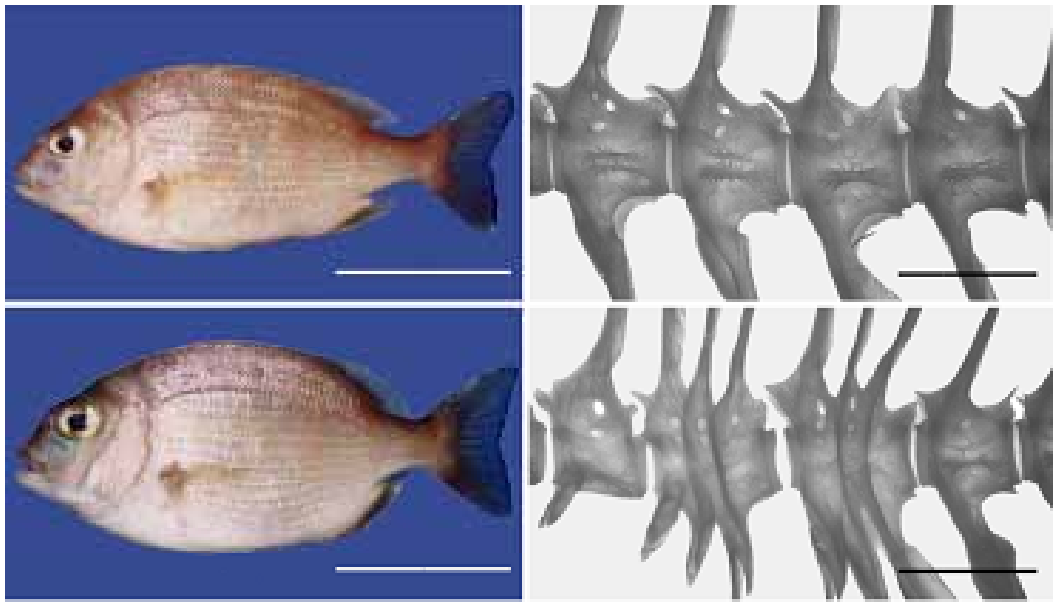
Zn

- Stimulates bone mineralization and bone protein synthesis
- Deficiency causes dwarfism and disturbance of skeletal formation

Mn

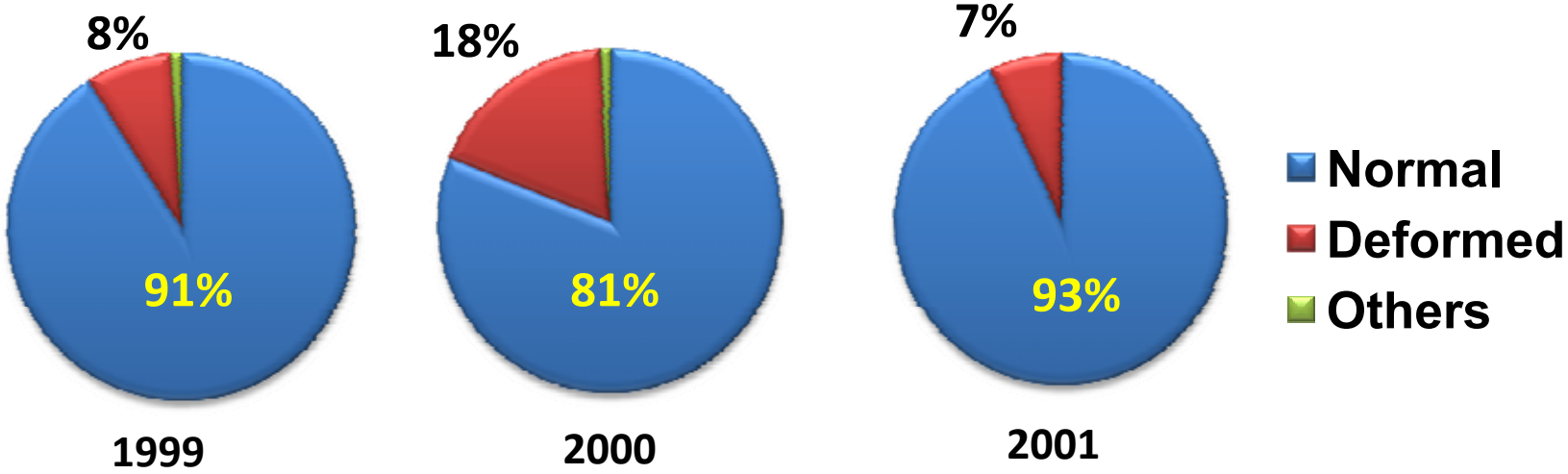
- Constitutes of enzymes and involves in carbohydrate, lipid and protein metabolism
- Deficiency causes impaired growth and abnormal skeleton

Vertebral deformity in hatchery-raised red sea bream Hattori *et al*., 2003



Normal

Deformed

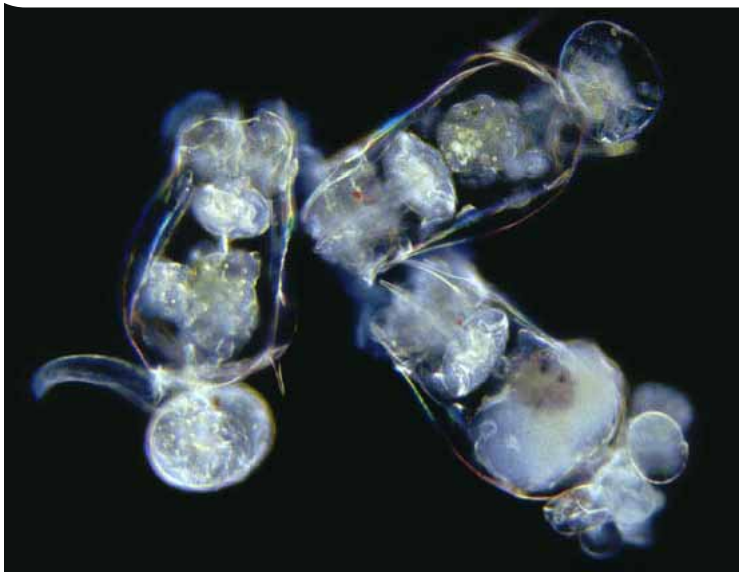


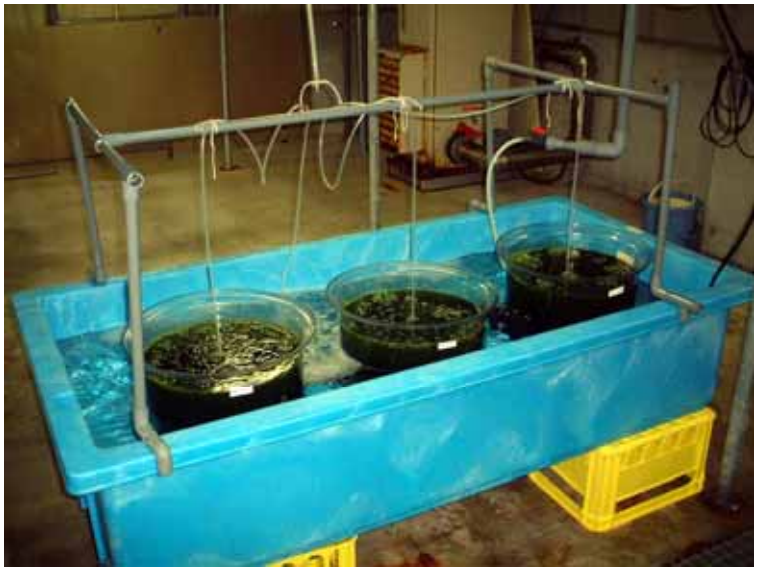
Objectives

- **Develop new method to enrich Zn and Mn in rotifers and *Artemia* nauplii to the level of these minerals in natural zooplanktons.**
- **To investigate the effects of Zn and Mn supplementation in *Artemia* on early growth, body compositions and skeletal deformity of red sea bream larvae**

Experiment 1: Examination direct absorption of Zn by rotifers

Can rotifer directly absorp Zn from enrichment media?

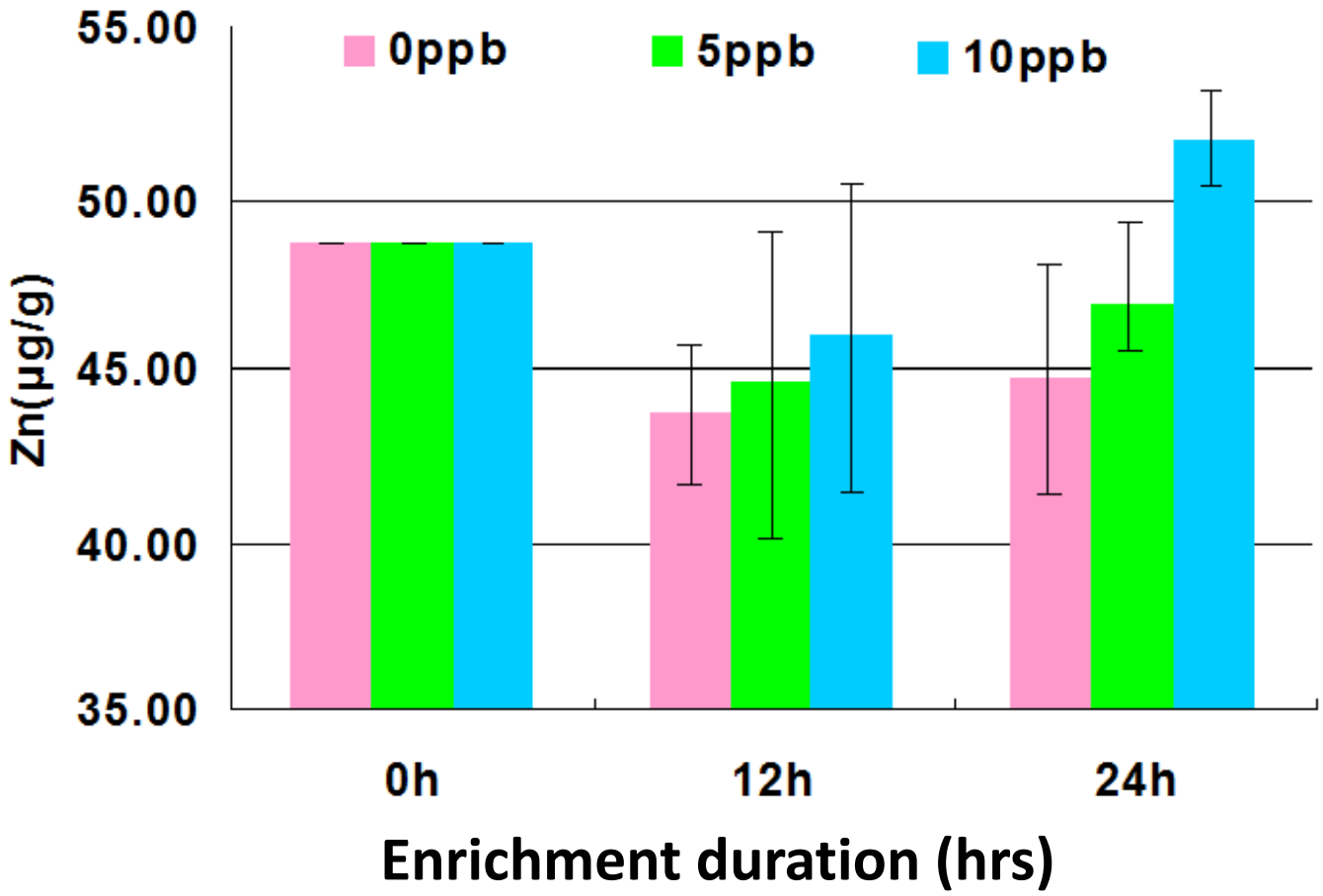




Experimental conditions

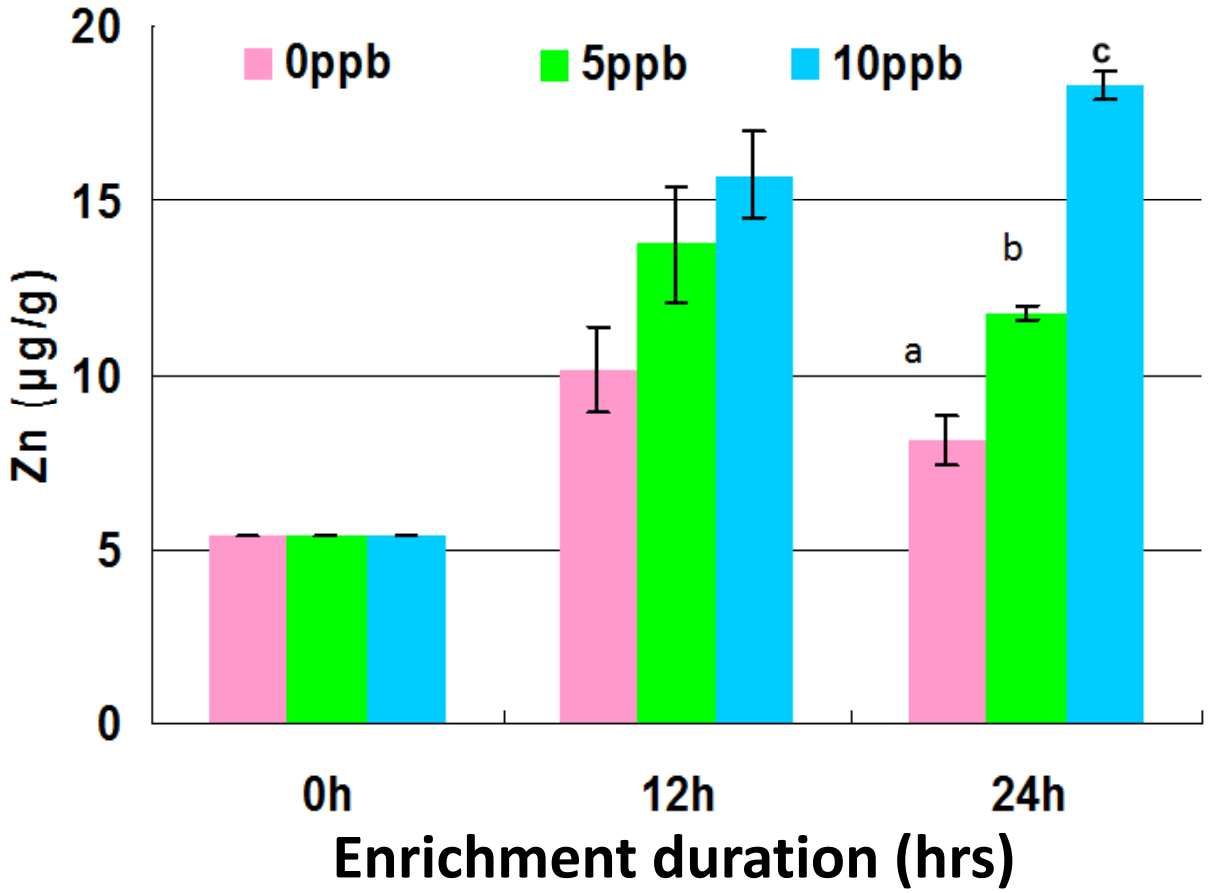
- Zinc source: $ZnSO_4$ (0, 5, 10 ppb)
- Organism: rotifer (*Brachionus plicatilis*) (L size)
- Stocking Density:
  - rotifer: 500ind/ml
  - chlorella: 3×10^6 cell/mL
- Sampling: Rotifer, Culture media at 0 h,12 hrs, 24 hrs
- Tank: 30 L round-shaped tank
- Water temperature: 27°C
- Replication: Duplicate

Fig 1. Zn accumulation in the rotifer (DM)



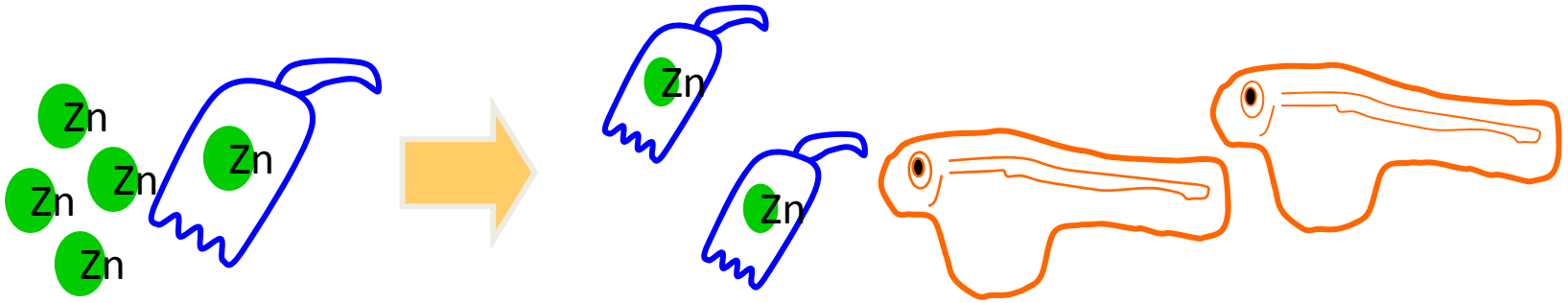
The rotifers could not absorb and retain zinc in their body from enrichment media effectively

Zinc concentration in *Chlorella*



Chlorella **could absorb water-bound zinc more effective than the rotifer**

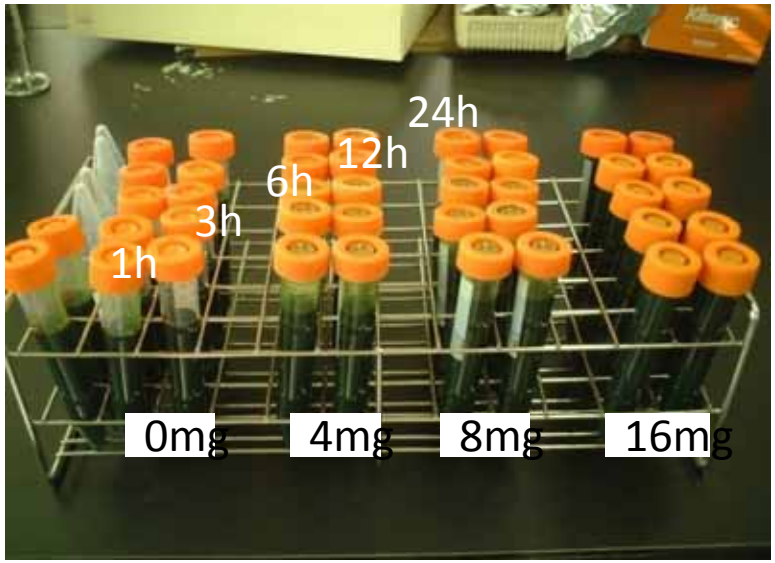
It is possible to enrich *Chlorella* with zinc as a first step of enrichment. The *Chlorella* thus produced contained high levels of zinc and can be fed to rotifers.



Experiment 2: Zn absorption by *Chlorella*

Aims

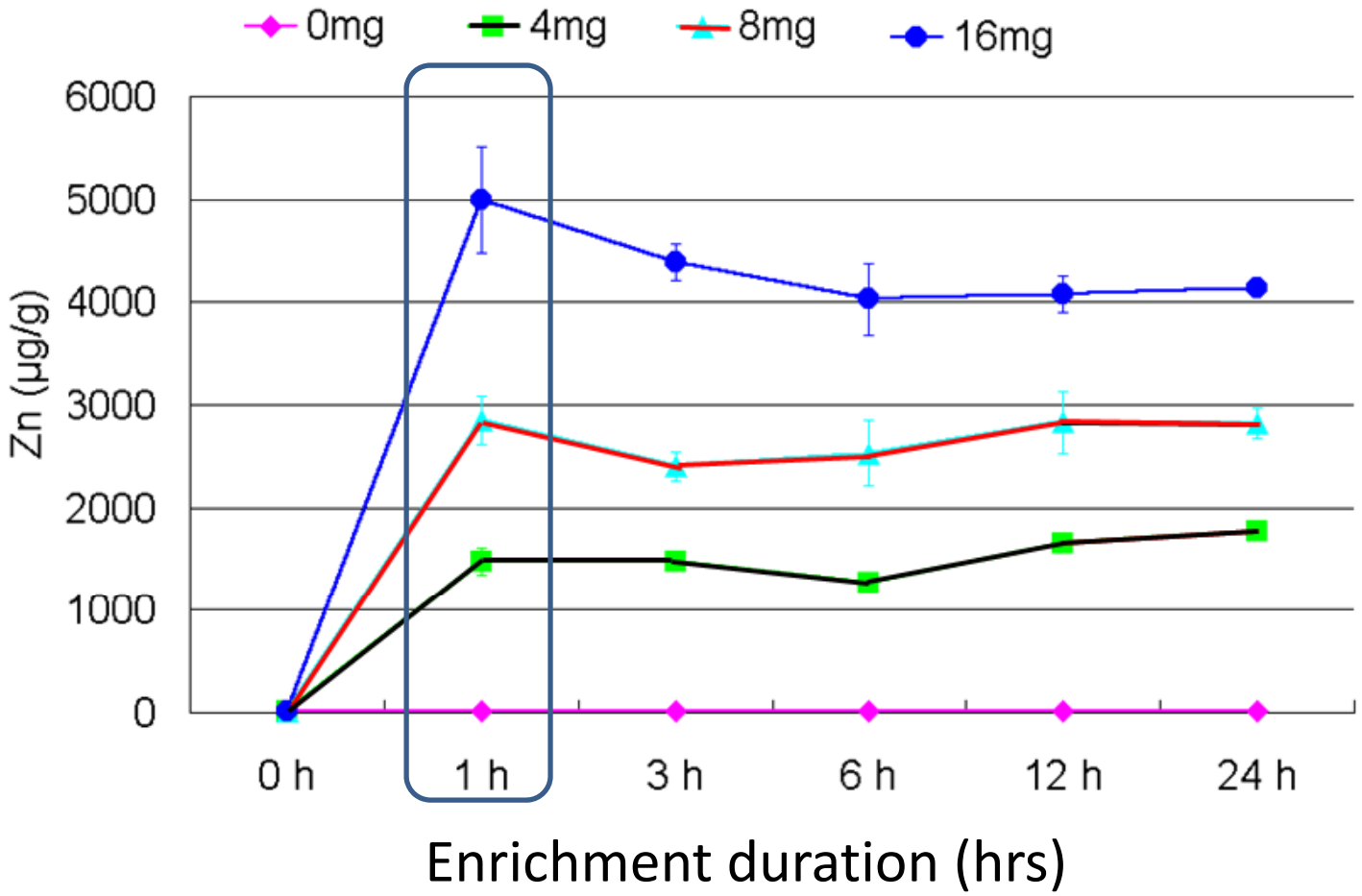
- **To examine the zinc accumulation ability of the *Chlorella***
- **To determine the suitable duration for *Chlorella* enrichment with Zn**



Experimental conditions

- **Zinc source: $ZnSO_4$ (0, 4, 8 and 16 mg/10 g of condensed *Chlorella regularis***
- **Sampling: 0h, 1h, 3h, 6h, 12h, 24h**
- **Replication: duplicate**
- **Temperature: 27°C**

Zn accumulation in the *Chlorella* (DM)

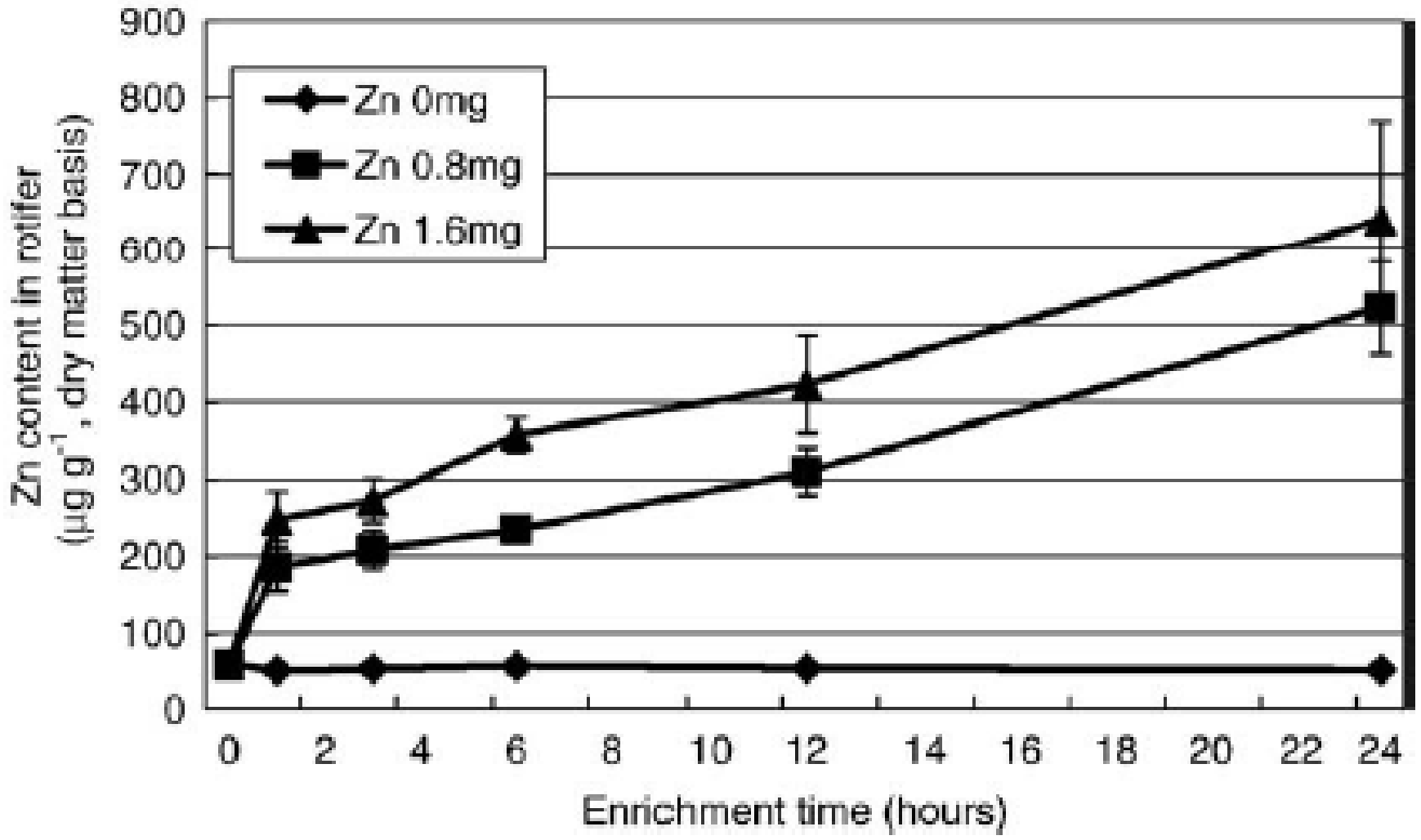


***Chlorella* could absorb water-bound zinc effectively and quickly within the first 1 h of enrichment.**

Experiment 3: Enrichment of rotifer with Zn-enriched *Chlorella*

- **Objective:**
  - **To investigate incorporation ability of Zn in rotifer by feeding with Zn enriched *Chlorella***
- **Experimental conditions**
  - **Condensed freshwater *Chlorella vulgaris***
  - **Supplemented with 0.0; 0.8 and 1.6 mg Zn g^{-1}**
  - **Incubation duration was 12 hours at 25°C**
  - **Organism: rotifer *(Brachionus plicatilis)* (L size); 300 ind/mL**
  - ***Chlorella* density $1.8x10^6$ cell/mL**

Zn content in enriched rotifer



Summary experiment 3

- **Zn in rotifer was successfully improved by enrichment with *Chlorella* that pre-accumulated with Zn.**
- **Zn content in the rotifer was elevated with Zn supplementation in the Chlorella.**
- **The Zn content in the 1.6 mg Zn group after 24 h enrichment was 640.3 µg g^{-1}, almost equivalent to Zn content in natural *Acartia clausi* which contains 700 µg g^{-1} zinc (DM)**

Exp 4: Enrichment *Artemia* with Zn and Mn

Objectives

- **To determine the incubation duration of Marine ω A® with Zn and Mn**
- **To investigate incorporation ability of Zn and Mn in *Artemia***

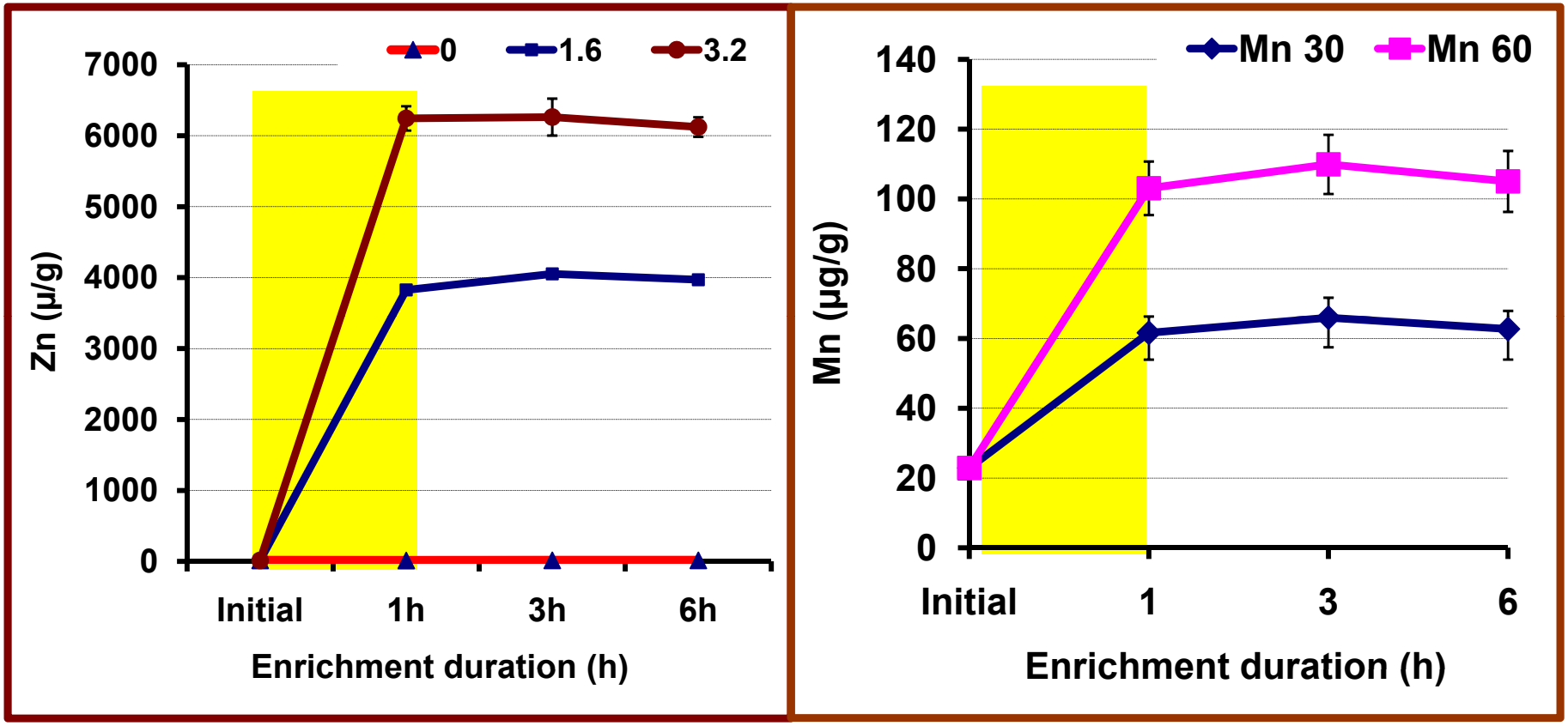
Materials

- **Marine ω A ®: A commercial enrichment material made by microalgae that had been removed the cell-wall (Nisshin Marine Tech Co., Ltd., Japan)**
- ***Artemia*: Newly-hatched nauplii *Artemia franciscana* (E.G. grade, INVE, Belgium)**
- **Minerals: Zn and Mn**

Methods

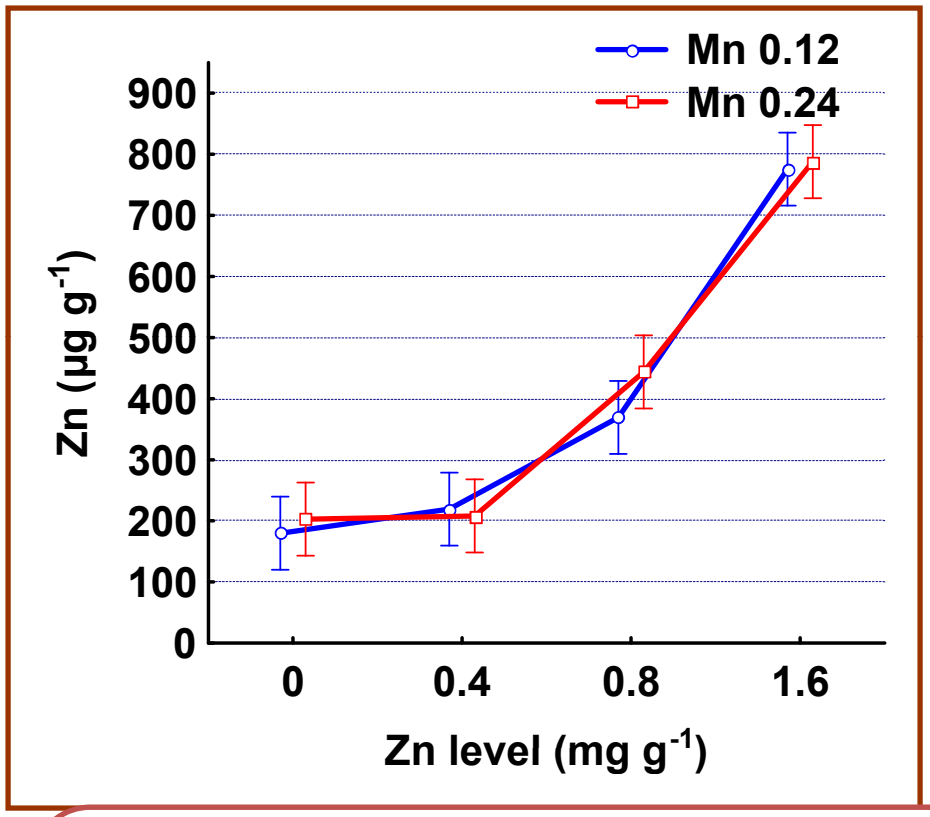
- **Incubation of marine ω A®.**
  - **Zn levels: 0, 1.6, 3.2 mg/10g marine ω A®**
  - **Mn levels: 30 and 60 µg/g marine ω A®**
  - **Incubation duration: 0, 1, 3 and 6 hours**
- ***Artemia* enrichment**
  - **Zn levels: 0, 0.4, 0.8 and 1.6 mg/g marine ω A®**
  - **Mn levels: 0.12, 0.24 mg/g marine ω A®**
  - **Incubation duration: 2 hours**
  - **Enrichment duration: 24 hours**

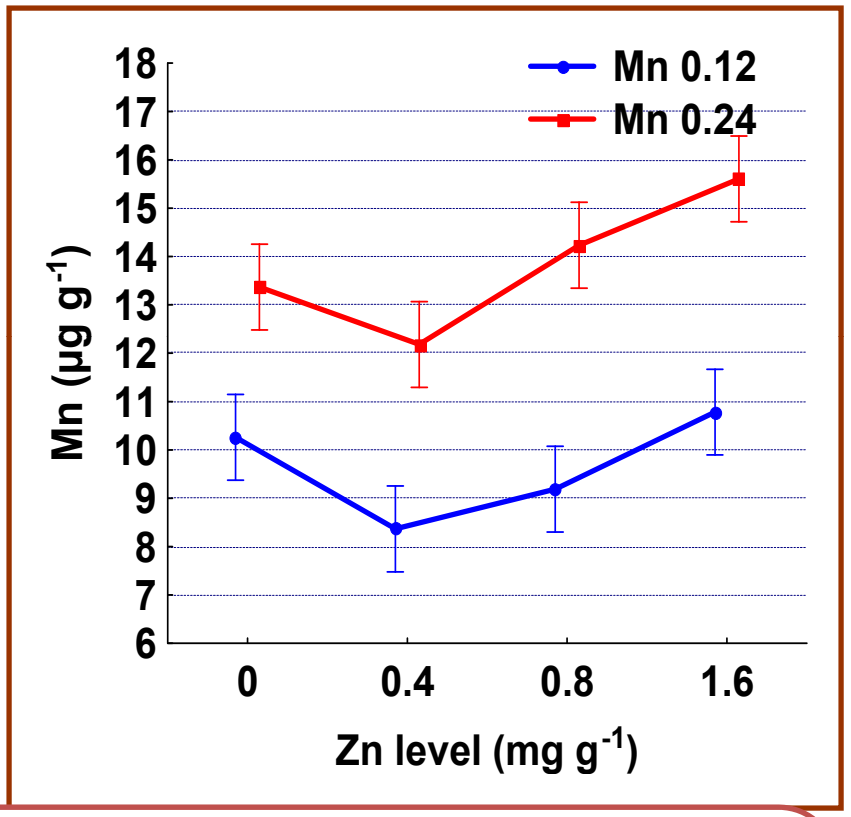
Incorporation of Zn and Mn in Marine ω®



Incubation 1-2 hrs was enough for Marine ω A® to incorporate Zn and Mn

Zn and Mn content in *Artemia* at 24 hrs enrichment (DW)





Enrichment *Artemia* with Marine ω A® supplemented with Zn and Mn could increase Zn and Mn contents in the nauplii

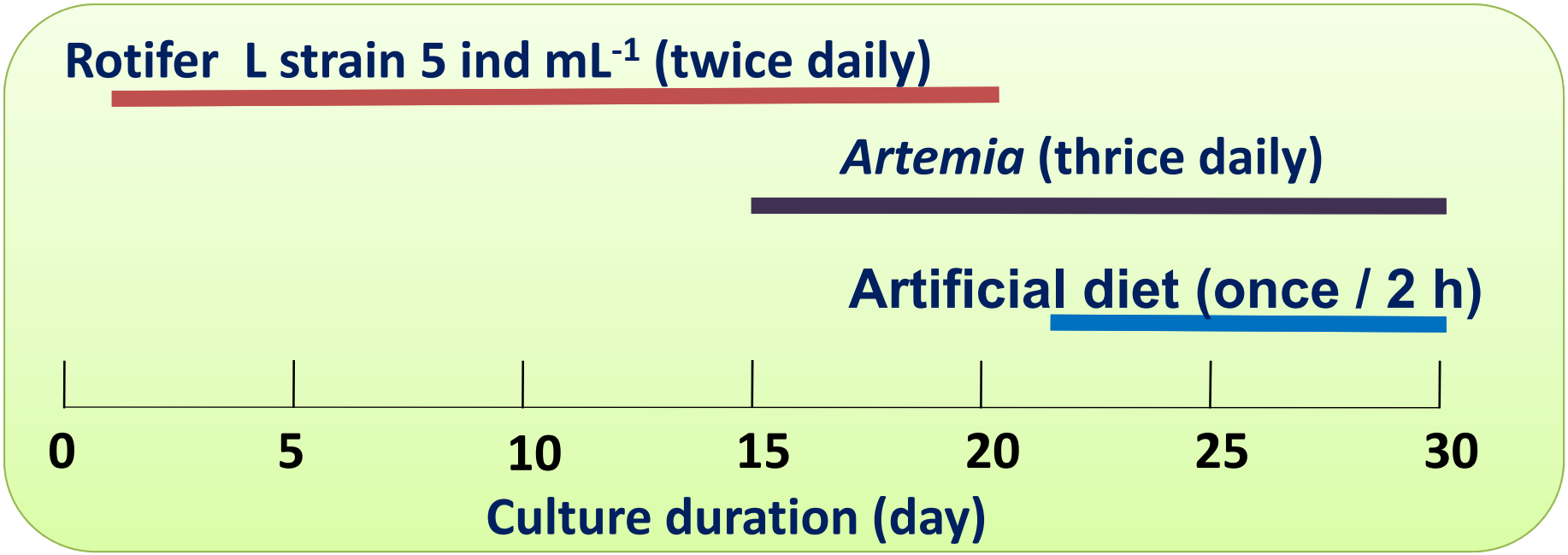
Nutritional important of Zn and Mn in marine fish larvae

Experiment 5: Effect of Zn and Mn enrichment in *Artemia* on growth and vertebral deformity in red sea bream larvae

Objective

To investigate the effects of Zn and Mn supplementation in *Artemia* on growth, body compositions and skeletal deformity of red sea bream larvae

Feeding scheme & experimental conditions



- **Tank volume:** 1000 L, triplicates
- **Density:** 20,000 larvae per tank
- **Water T°C:** 20-23 °C
- **Photoperiod:** 12 h dark : 12 h light
- **Culture duration:** 30 days

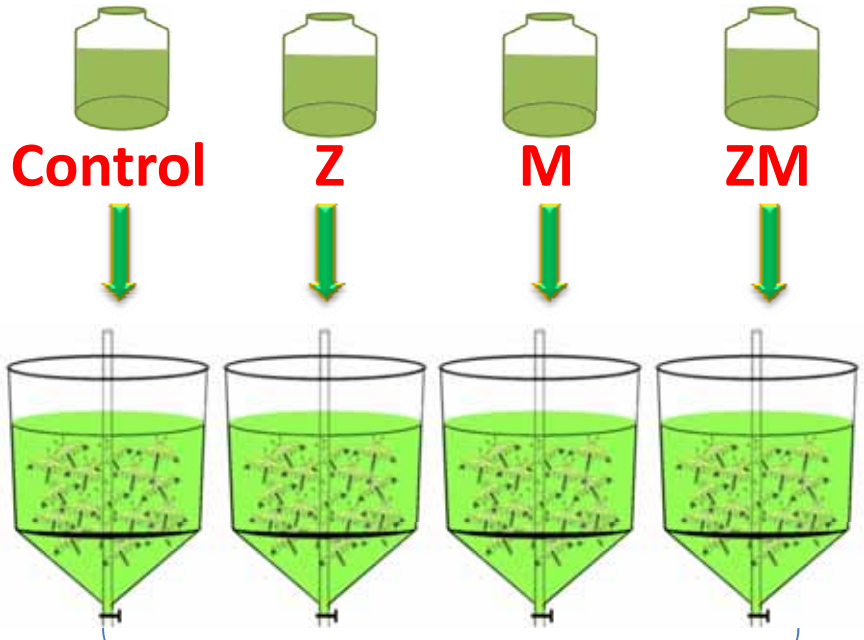
Artemia enrichment with Zn (0.1 mg/g) and Mn (2.4 mg/g)

Marine ω A®
incubation
2 hours

Artemia
enrichment

Harvesting

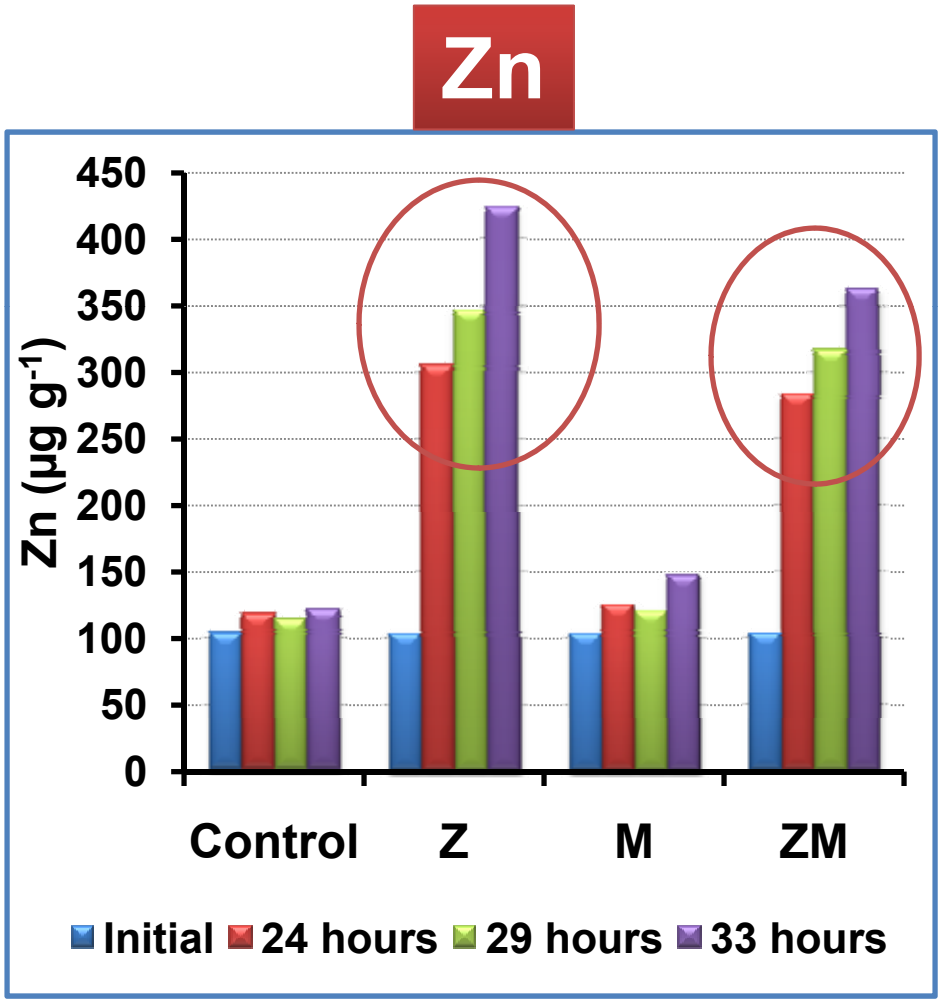
Feeding to larvae

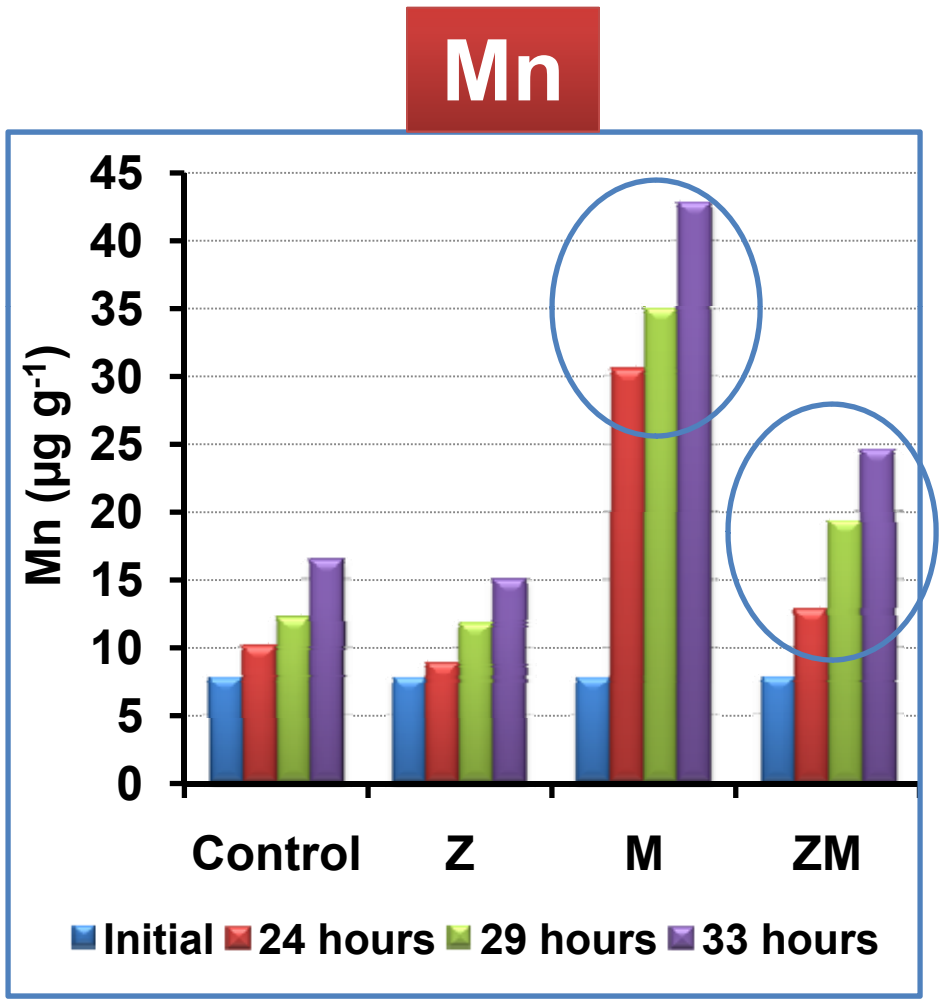


150 nauplii/mL
26 °C
4 g marine ω®/L

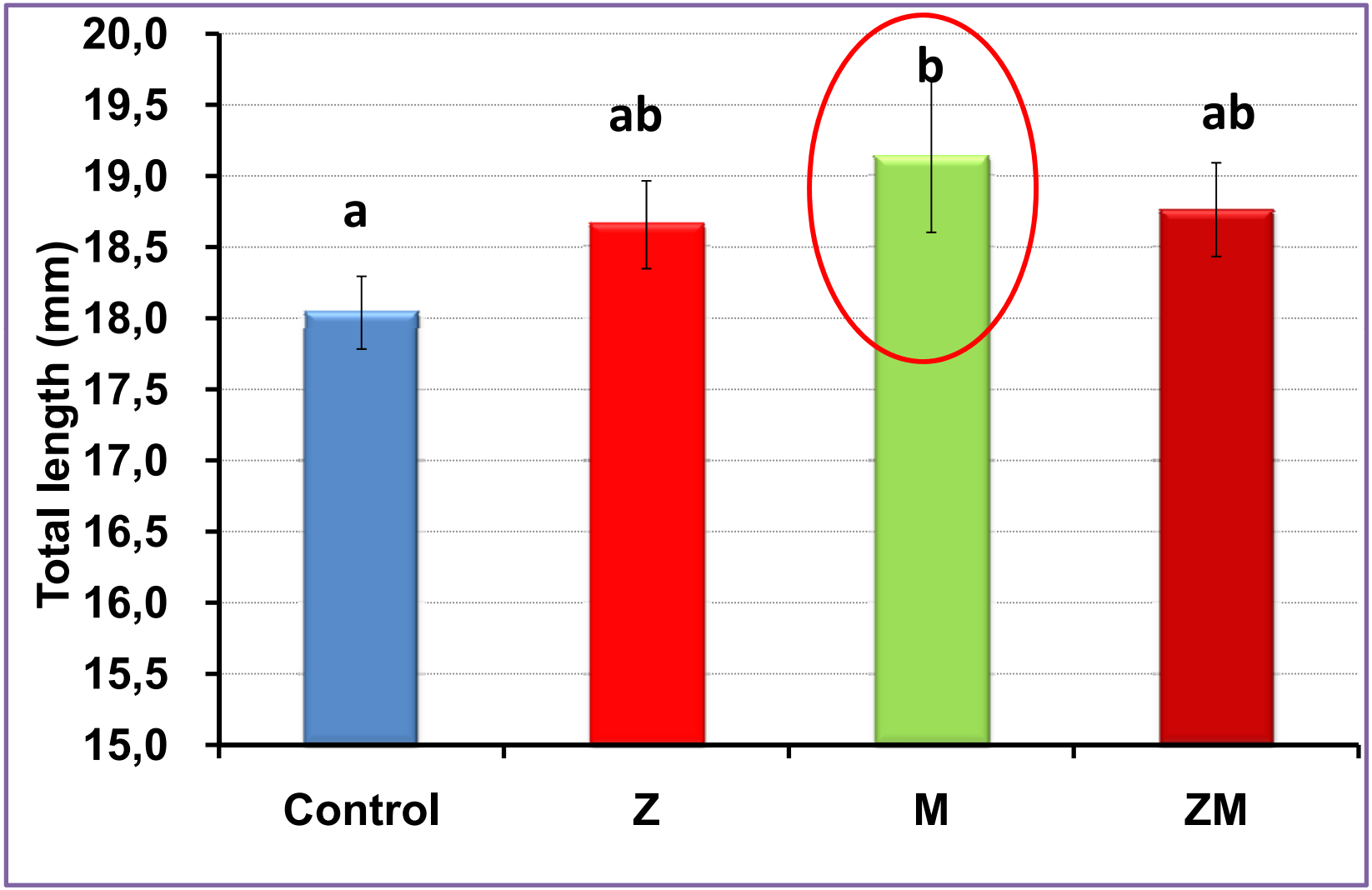
24, 29, 33 hours

Zn and Mn contents in enriched *Artemia* (dry-matter basis)

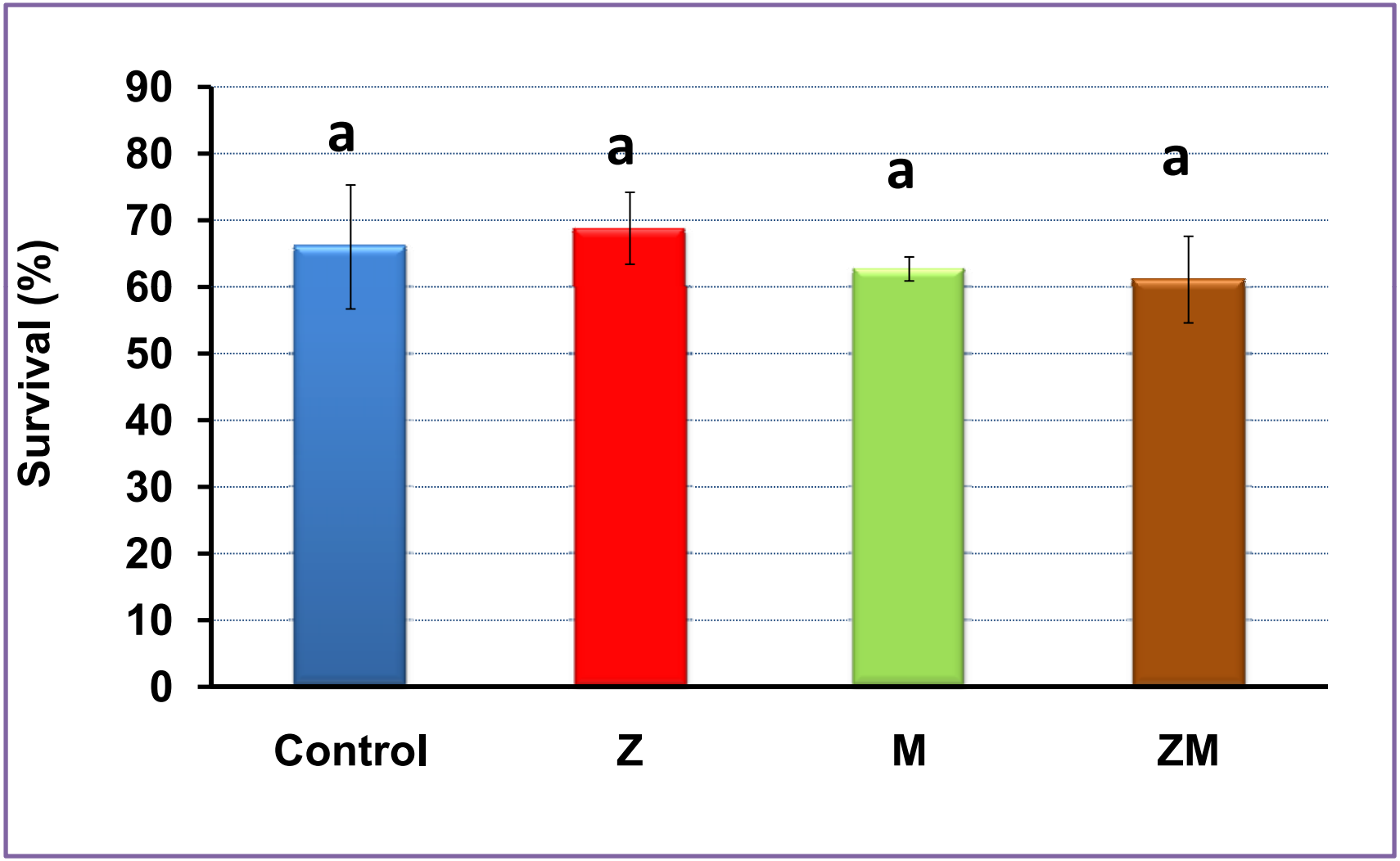




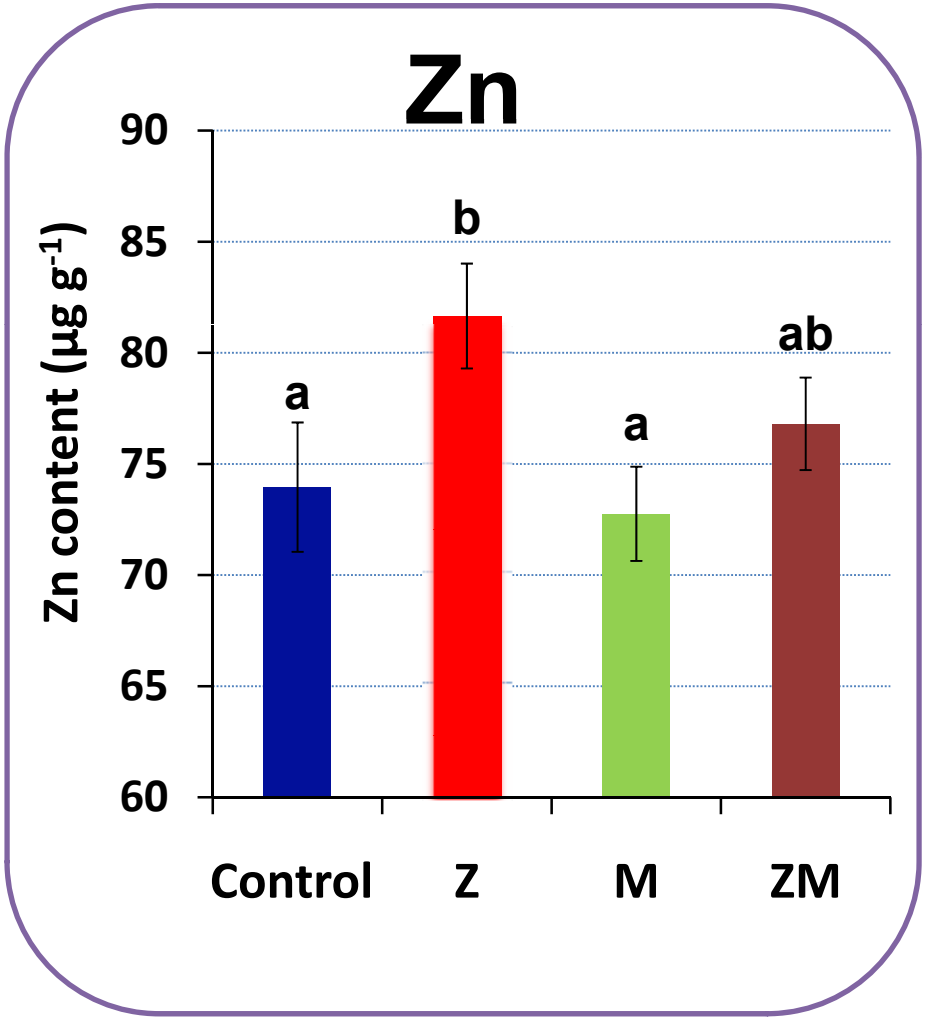
Growth of red sea bream at 30 dph

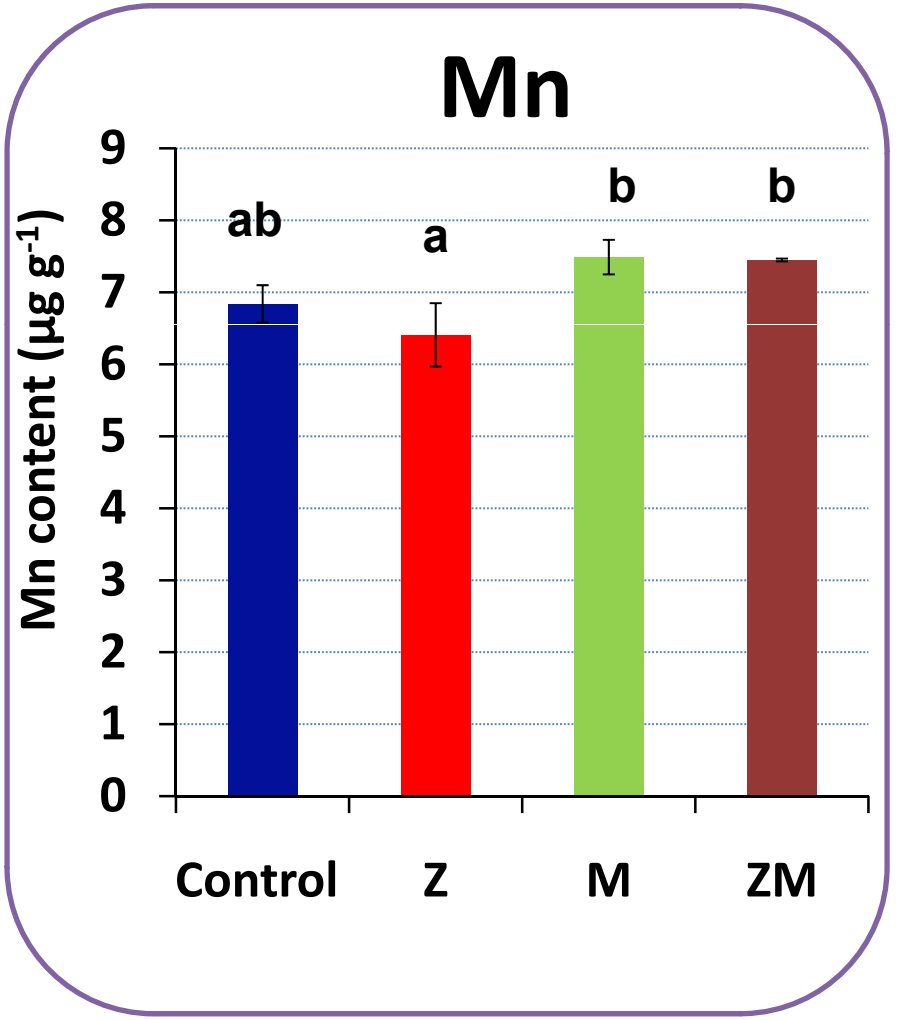


Survival of red sea bream during 15-30 dph

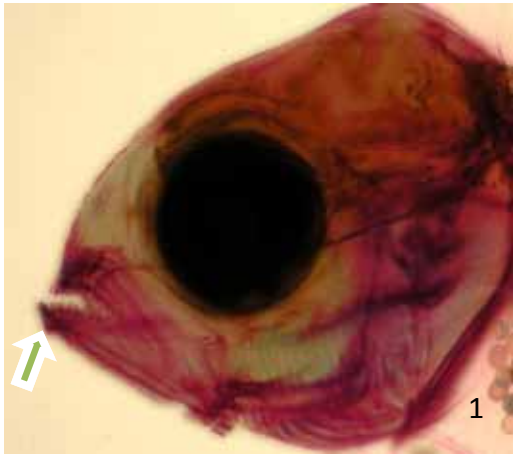


Zn and Mn content in whole body of red sea bream at 30 dph (dry-matter basis)

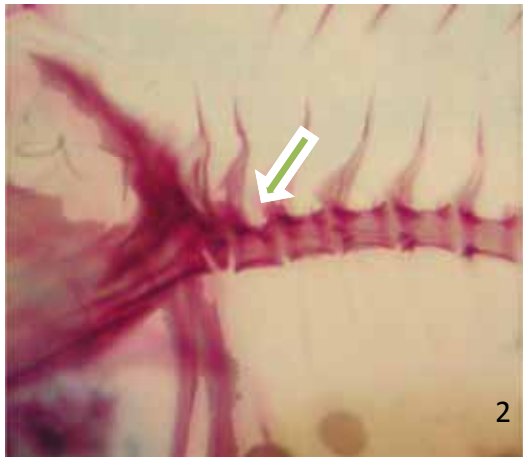




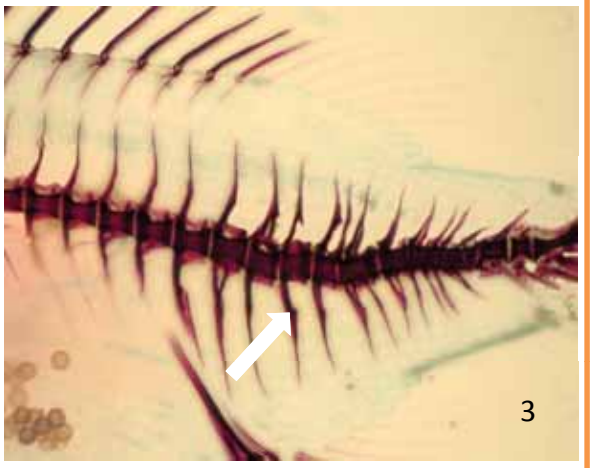
Typical vertebral deformities in red sea bream at 30 dph



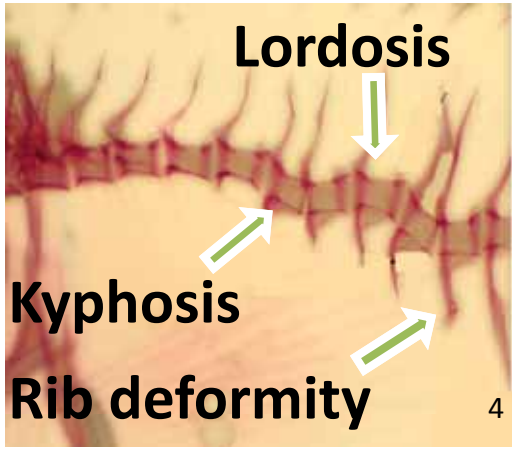
Dentary

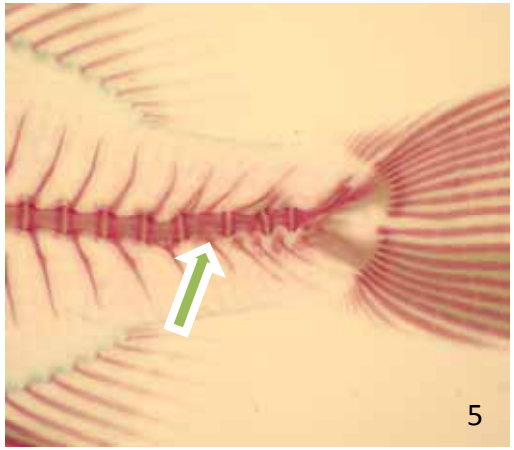


Pugheadness

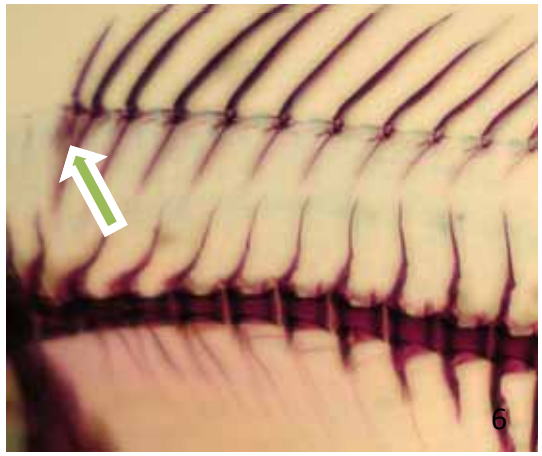


Vertebral fusion



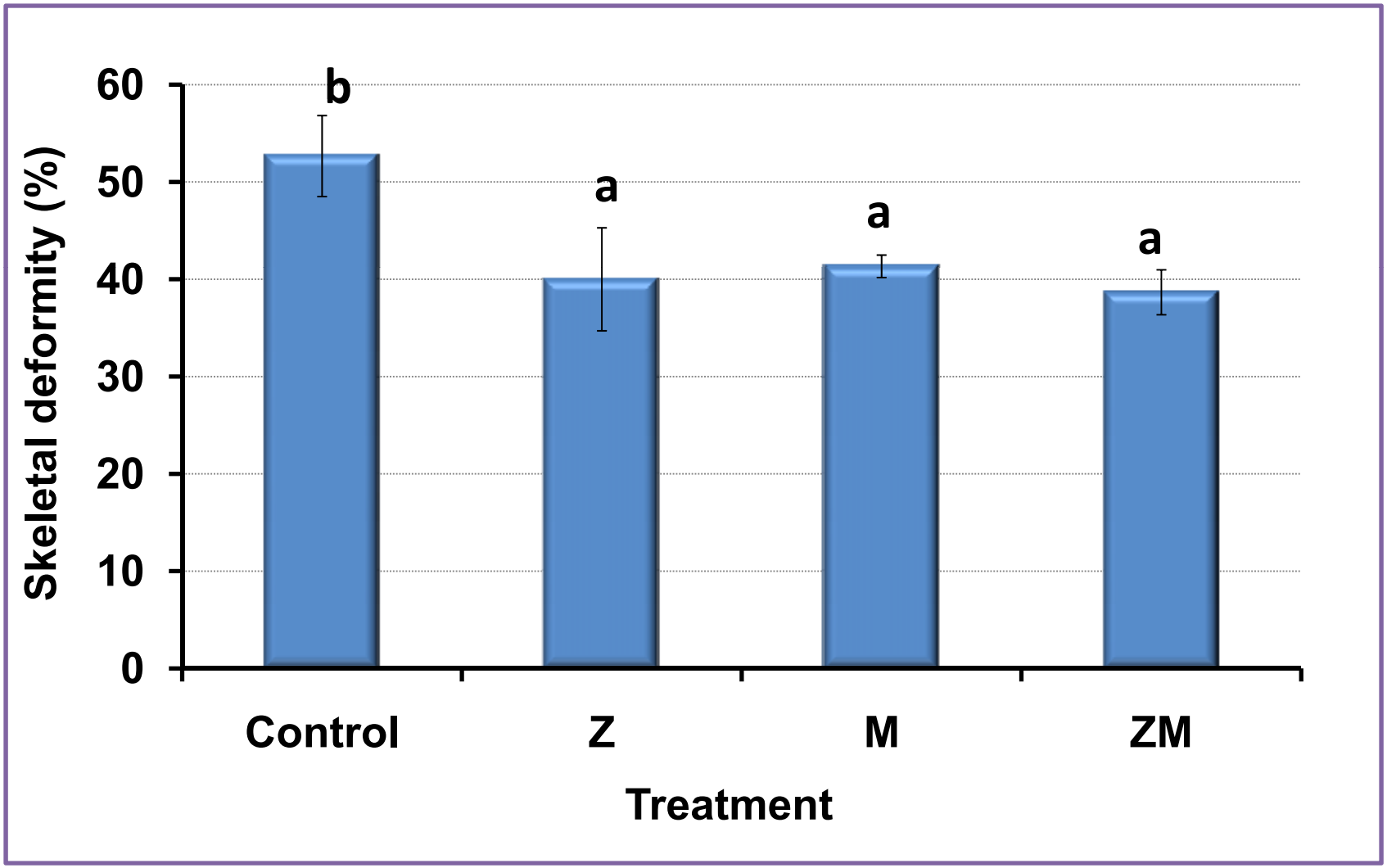


Caudal vertebrate

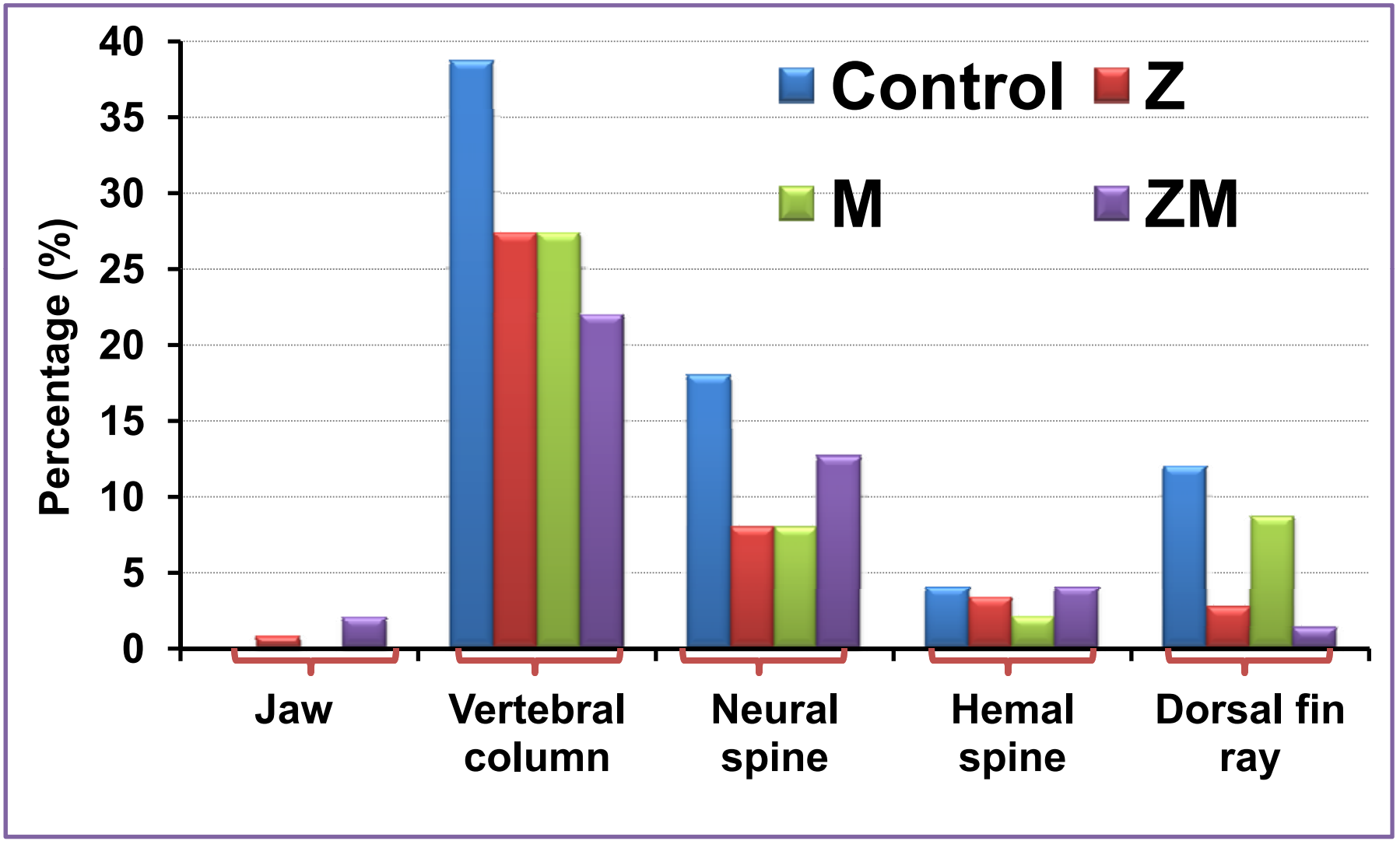


Fin support

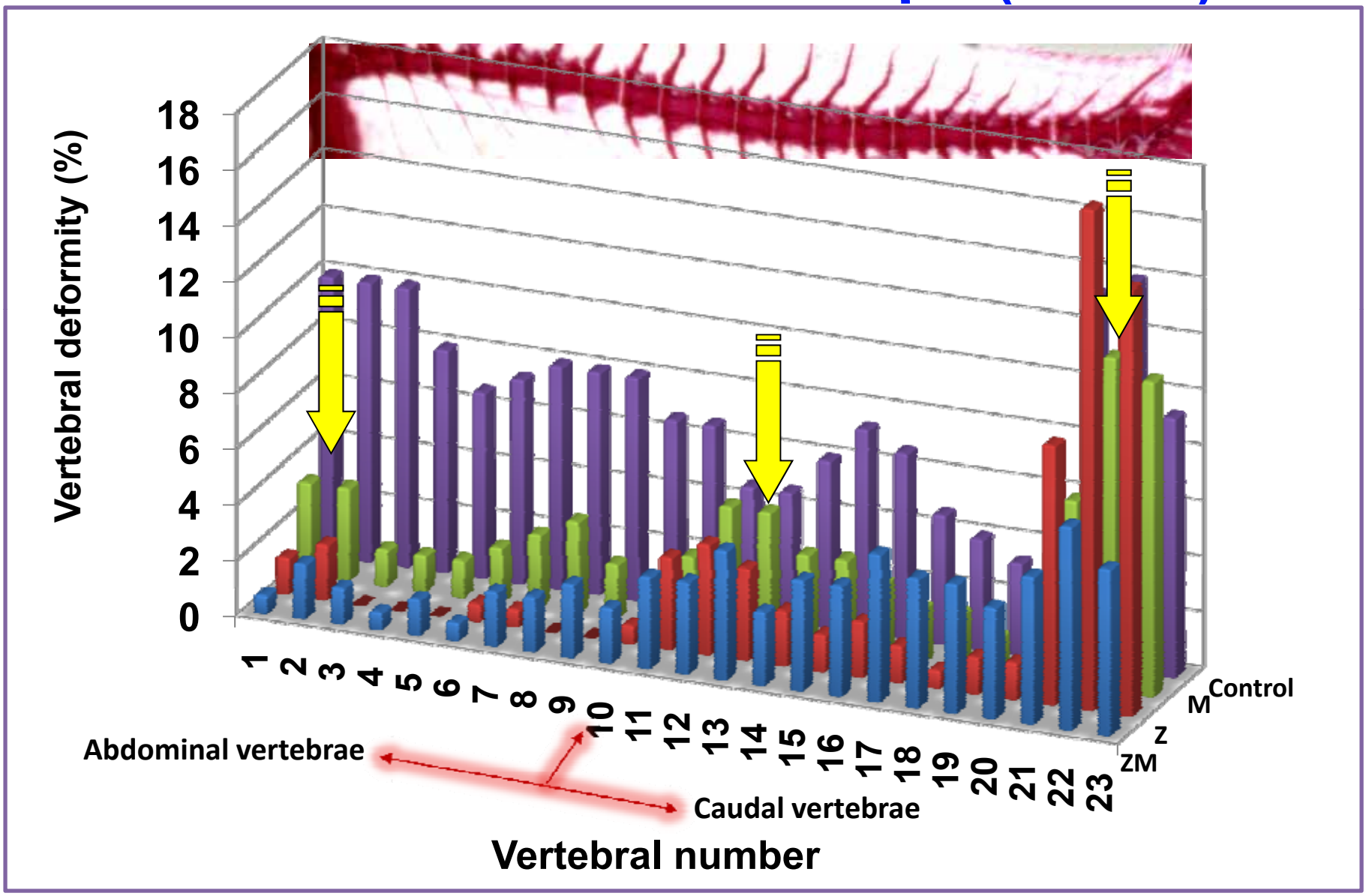
Average percentage of fish with at least 1 skeletal deformity at 30 dph (%)



Percentage of major skeletal deformities of red sea bream at 30 dph (n=600)



Occurrence of vertebral deformity in red sea bream at 30 dph (n=600)



Summary experiment 5

- **Mn supplementation in *Artemia* 12-42.8 µg g^{-1} (dry-matter) improves growth performance of the red sea bream larvae**
- **Zn and Mn supplementation in *Artemia* promotes normal skeletal development of red sea bream larvae**
- **Zn and Mn contents in whole body of red sea bream were affected by Zn and Mn content in *Artemia***
- **Zn and Mn should be supplemented together to prevent decline of Mn in enriched *Artemia***

Conclusion

- **The Zn concentration in rotifers was significantly increased indirectly by feeding zinc-enriched *Chlorella* for 6 to 12 h.**
- **Zn and Mn contents in *Artemia* were significantly improved indirectly by feeding Zn and Mn enriched marine ω A®**
- **Supplementation of Zn and Mn in *Artemia* improves growth performance and promotes normal skeletal development of red sea bream larvae**

Further readings

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Examination of a practical method for zinc enrichment of euryhaline rotifers (*Brachionus plicatilis*)

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Effect of zinc and manganese supplementation in *Artemia* on growth and vertebral deformity in red sea bream (*Pagrus major*) larvae

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