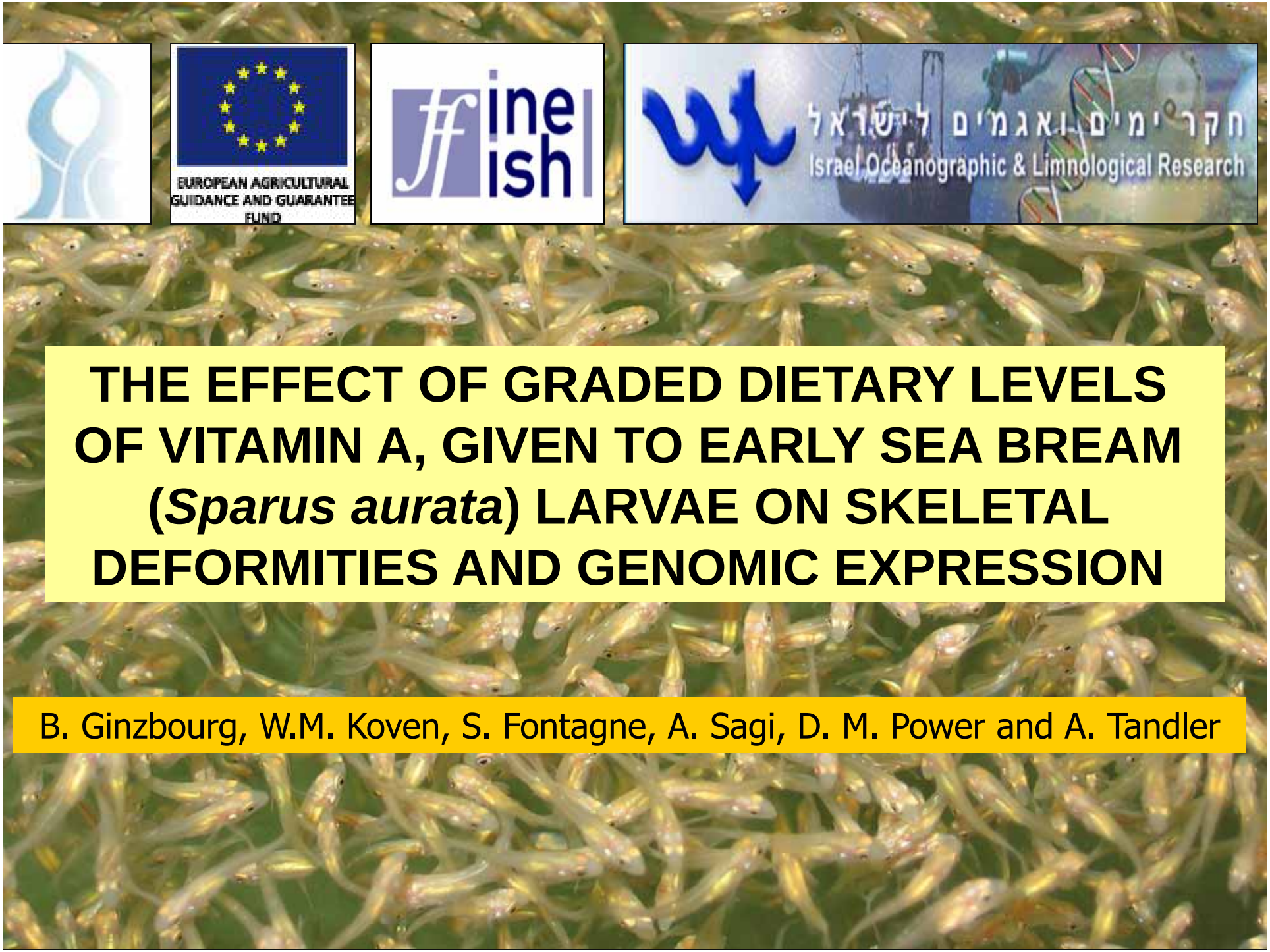
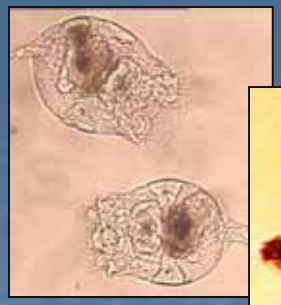
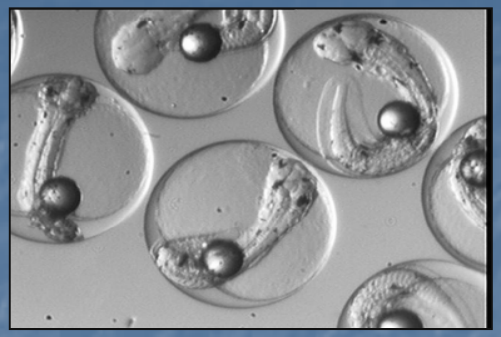
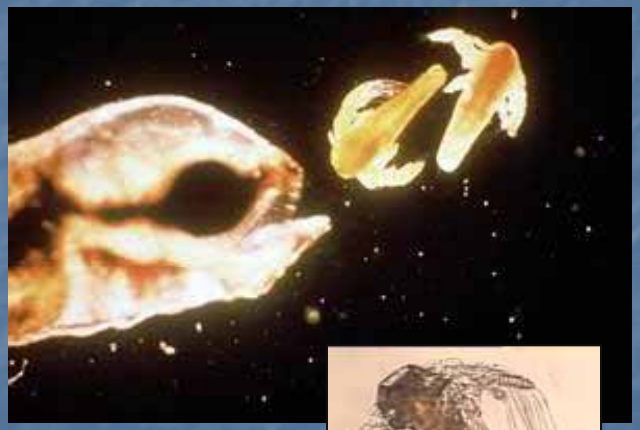


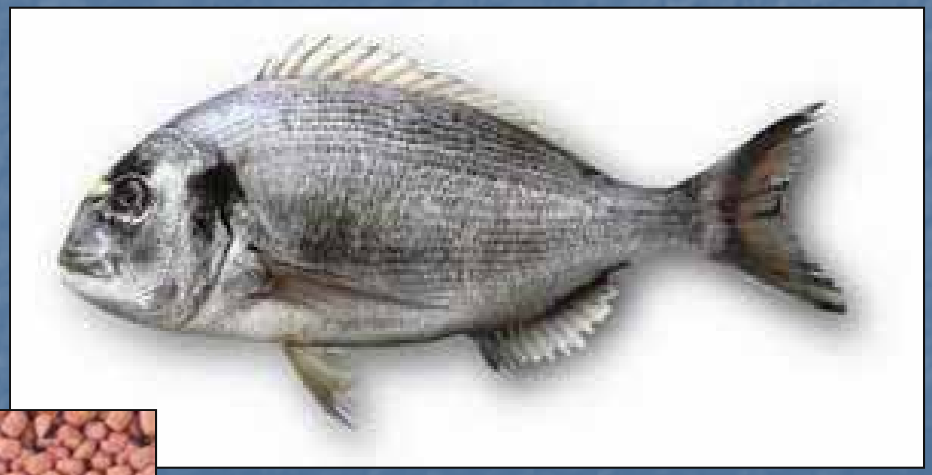
THE EFFECT OF GRADED DIETARY LEVELS OF VITAMIN A, GIVEN TO EARLY SEA BREAM (*Sparus aurata*) LARVAE ON SKELETAL DEFORMITIES AND GENOMIC EXPRESSION

B. Ginzbourg, W.M. Koven, S. Fontagne, A. Sagi, D. M. Power and A. Tandler





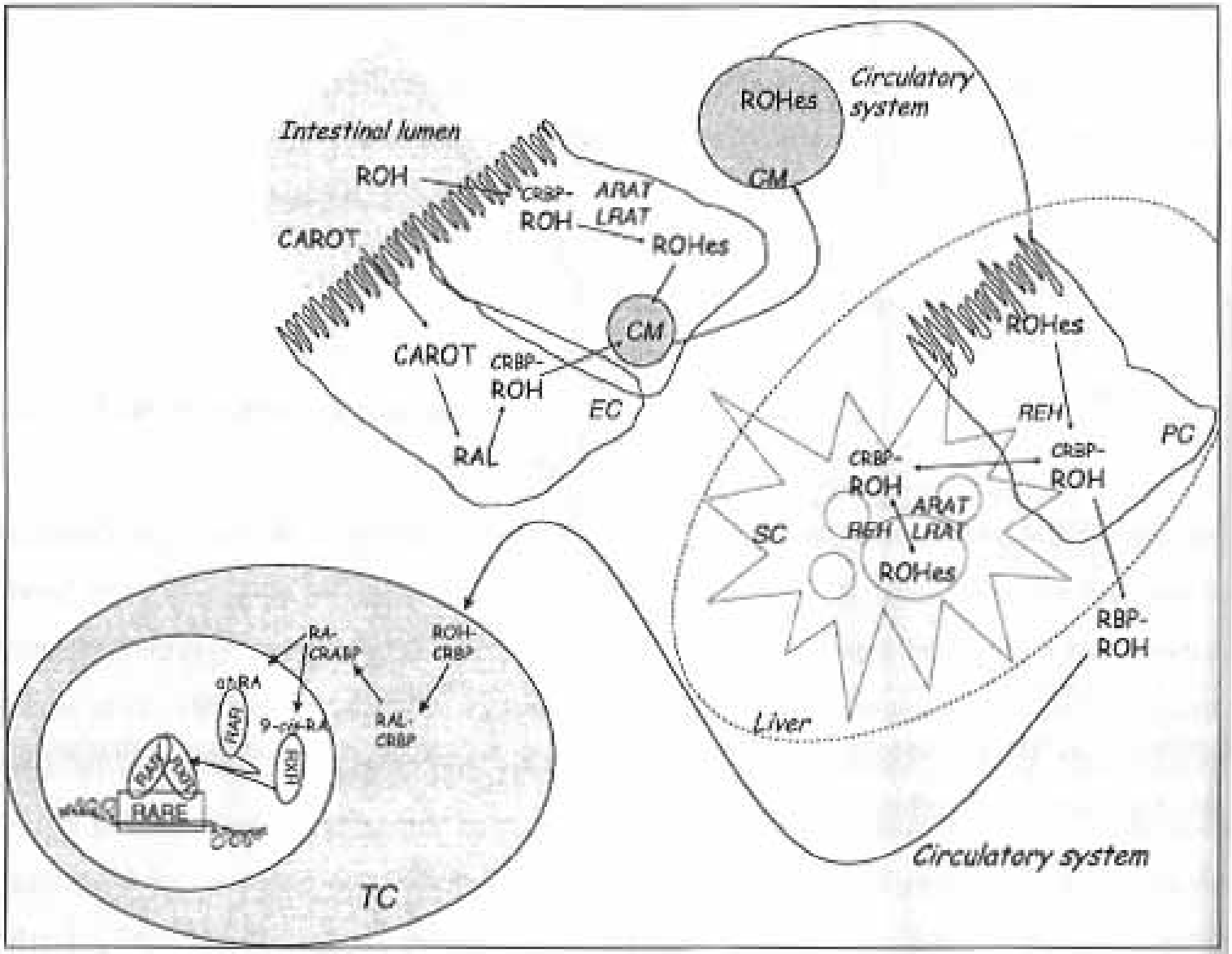




Aims of the study:

- To develop a vitamin A enrichment technology based on the use of liposomes.
- To evaluate the effect of different vitamin A dietary levels on fish development, in terms of optimal growth, and incidence of skeletal malformations.
- To provide an insight into dietary larval requirements.
- To study the relative mRNA gene expression abundance of RBP RALDH2, LRAT, STRA6 during Sea bream larval morphogenesis,

Metabolism

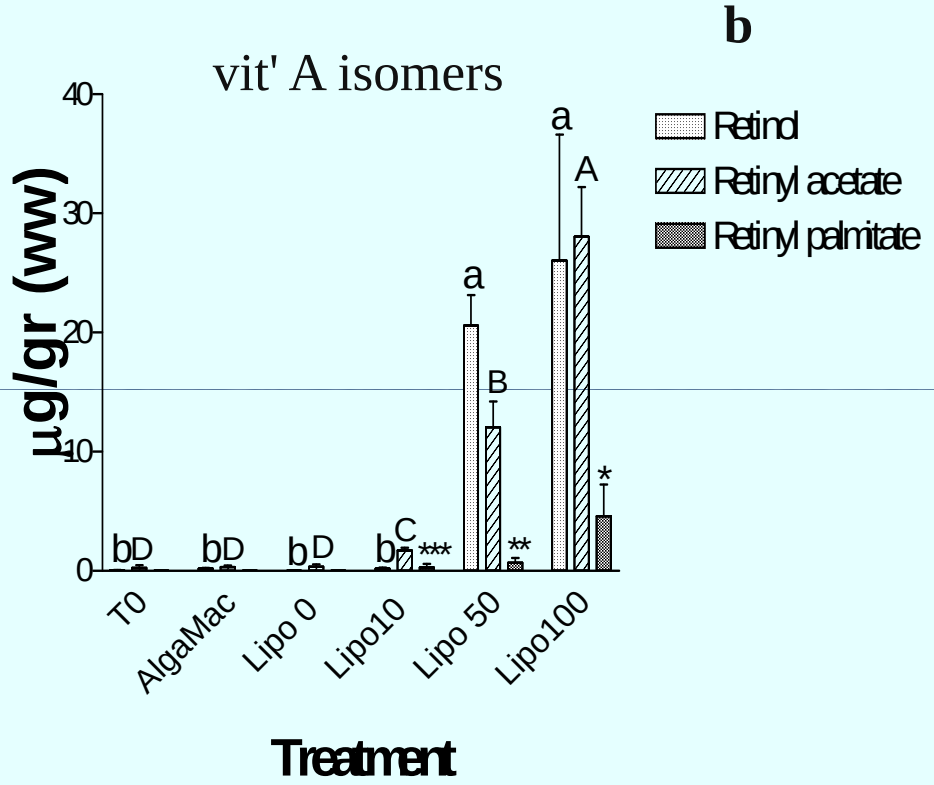


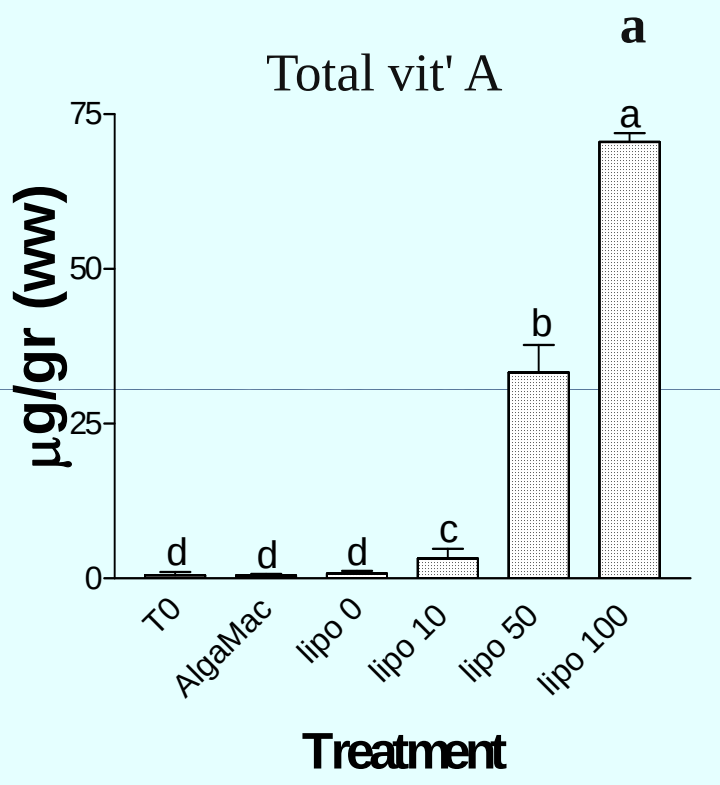
Mari Moren 2004

Materials and methods

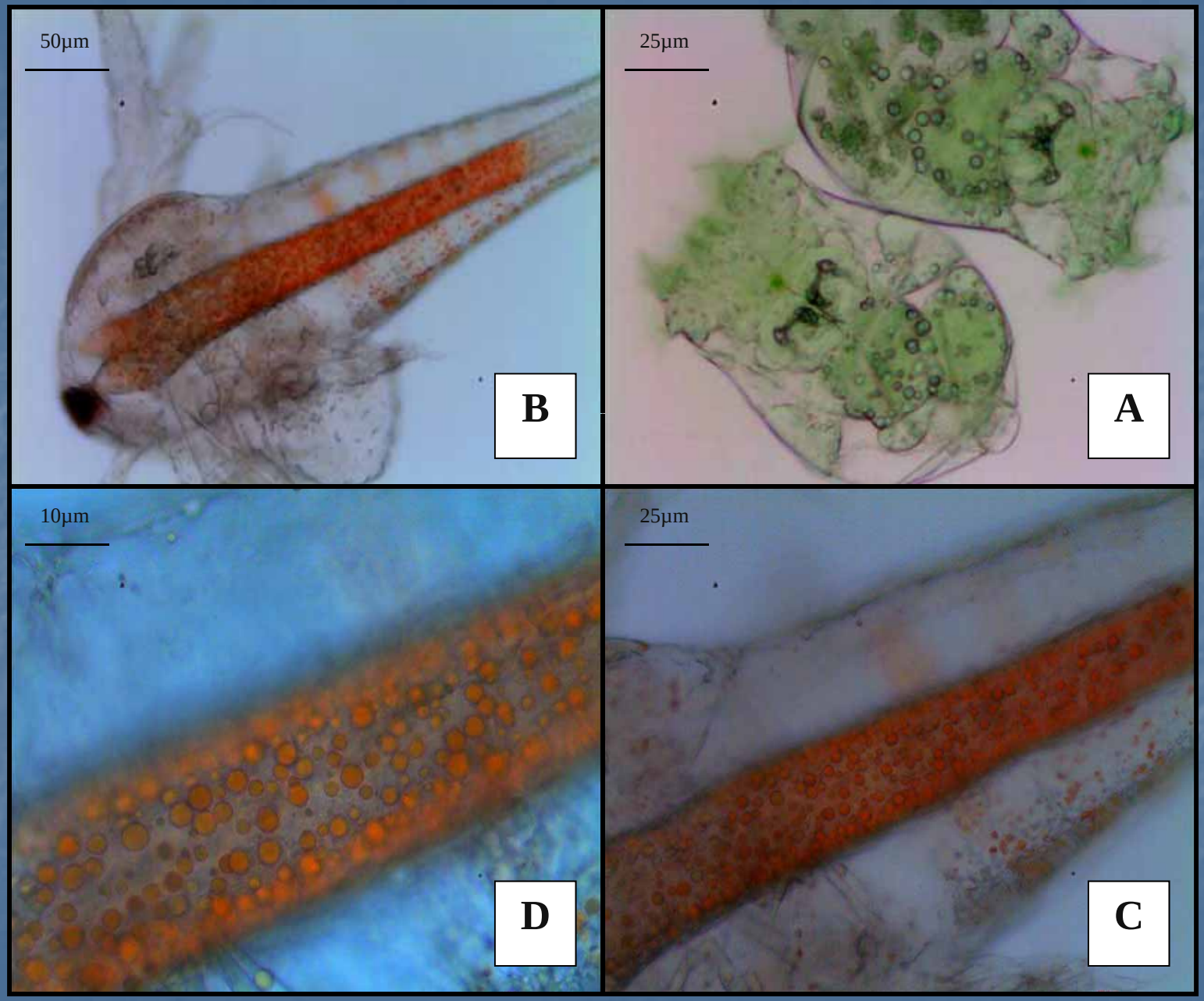
- Diets:
  - Artemia and rotifers enriched with increasing vitamin A levels with liposomes.
- Larval performance:
  - Measurement of optimal growth and survival rate.
- Analysis:
  - HPLC - vitamin A analysis.
  - Gas Chromatography – fatty acids analysis.
  - X RAY analysis for bone deformation.
  - Potthoff stain for cartilage and bone formation.
- Gene expression:
  - Semi-quantitative RT-PCR for RALDH2, LRAT, STRA6 and RBP expression.

Rotifers enrichment

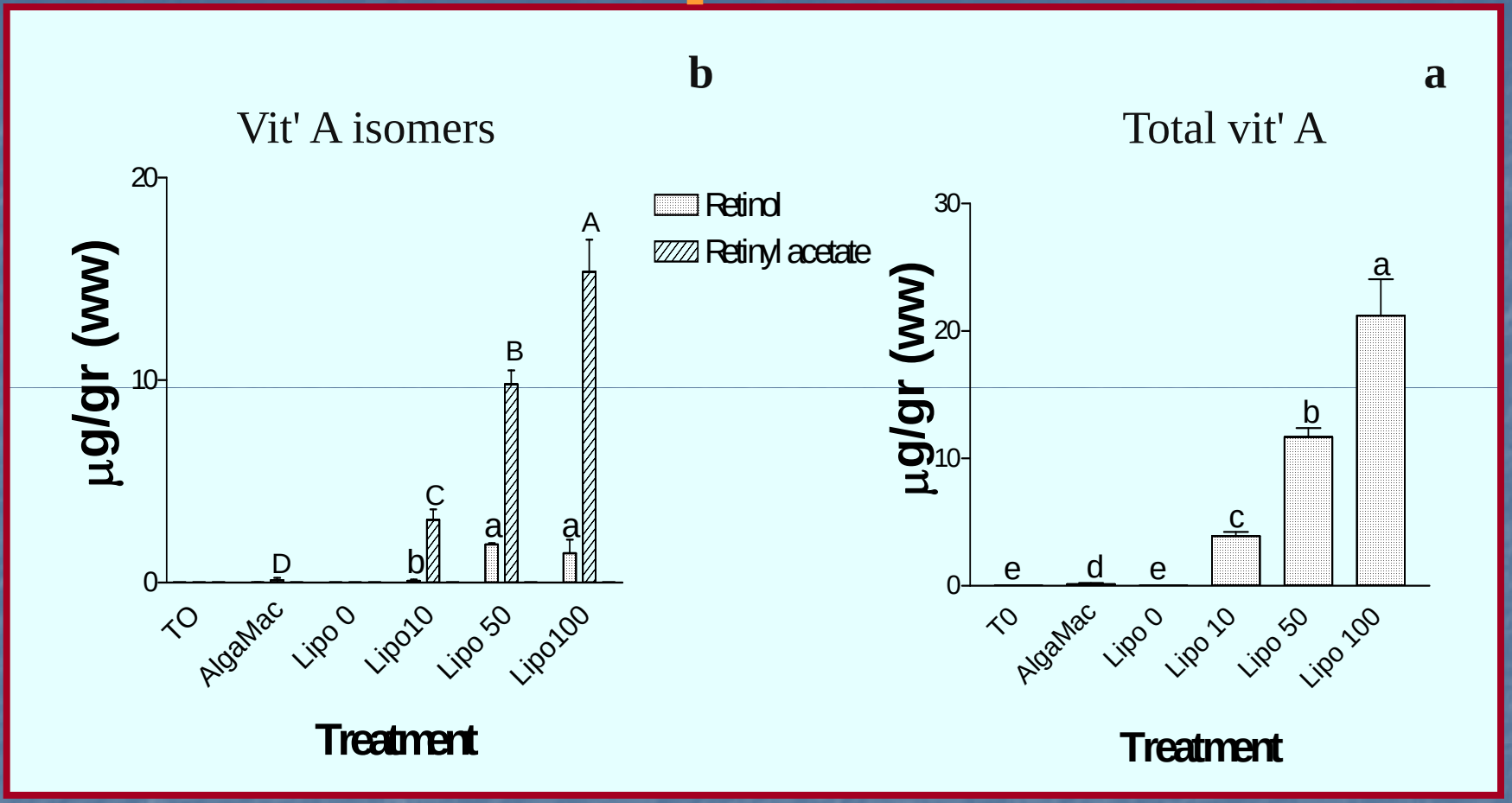




The pattern of use and accumulation of vitamin A isoforms in rotifers (*B. rotundiformis*) , as a function of its concentration in the culturing medium, after 6 hours of enrichment. (a) Vitamin A accumulation in the rotifers. (b) Vitamin A isoforms distribution in the rotifers. (Statistical analysis: Retinol- a-b, Retinyl acetate- A-B, Retyinl palmitate- * - ***).


50µm
B
25µm
A
10µm
D
25µm
C

Artemia nauplii enrichment



The pattern of accumulation of vitamin A isoforms in artemia nauplii as a function of its concentration in the culturing medium, after 8 hours of enrichment. (a) Vitamin A accumulation in artemia nauplii. (b) Vitamin A isoforms distribution in artemia naplii after the above conditions. (Statistics: Retinol -a-b ,Retinyl acetate -A-D).

Experimental

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

Yolk sac

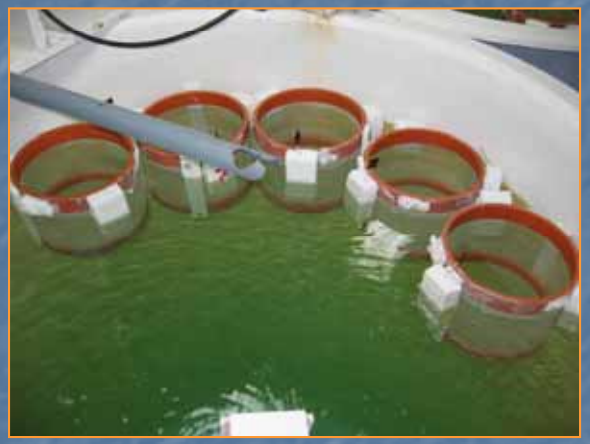
Rotifers

Artemia

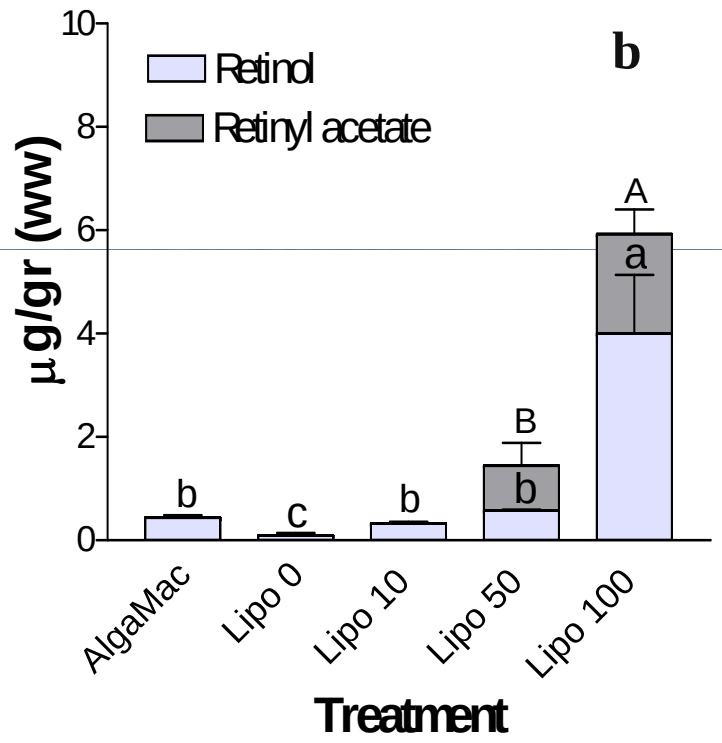
	 4 – 19 DPH	 20 – 34 DPH
Early	Rotifers enriched with increasing vitamin A levels	Artemia NO vitamin A enrichment
Late	Rotifers NO vitamin A enrichment	Artemia enriched with increasing vitamin A levels
Entire	Rotifers enriched with increasing vitamin A levels	Artemia enriched with increasing vitamin A levels

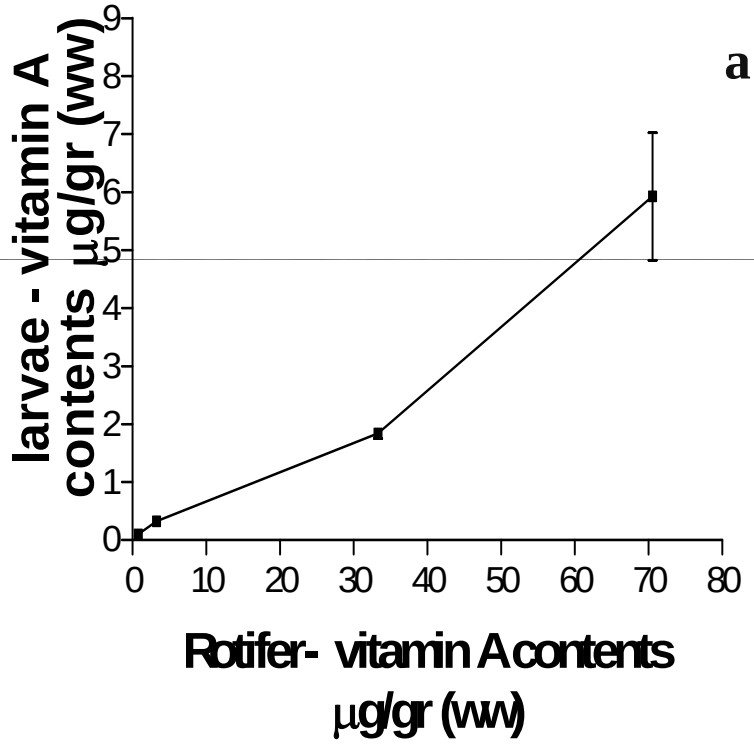
Materials and methods

Experimental systems



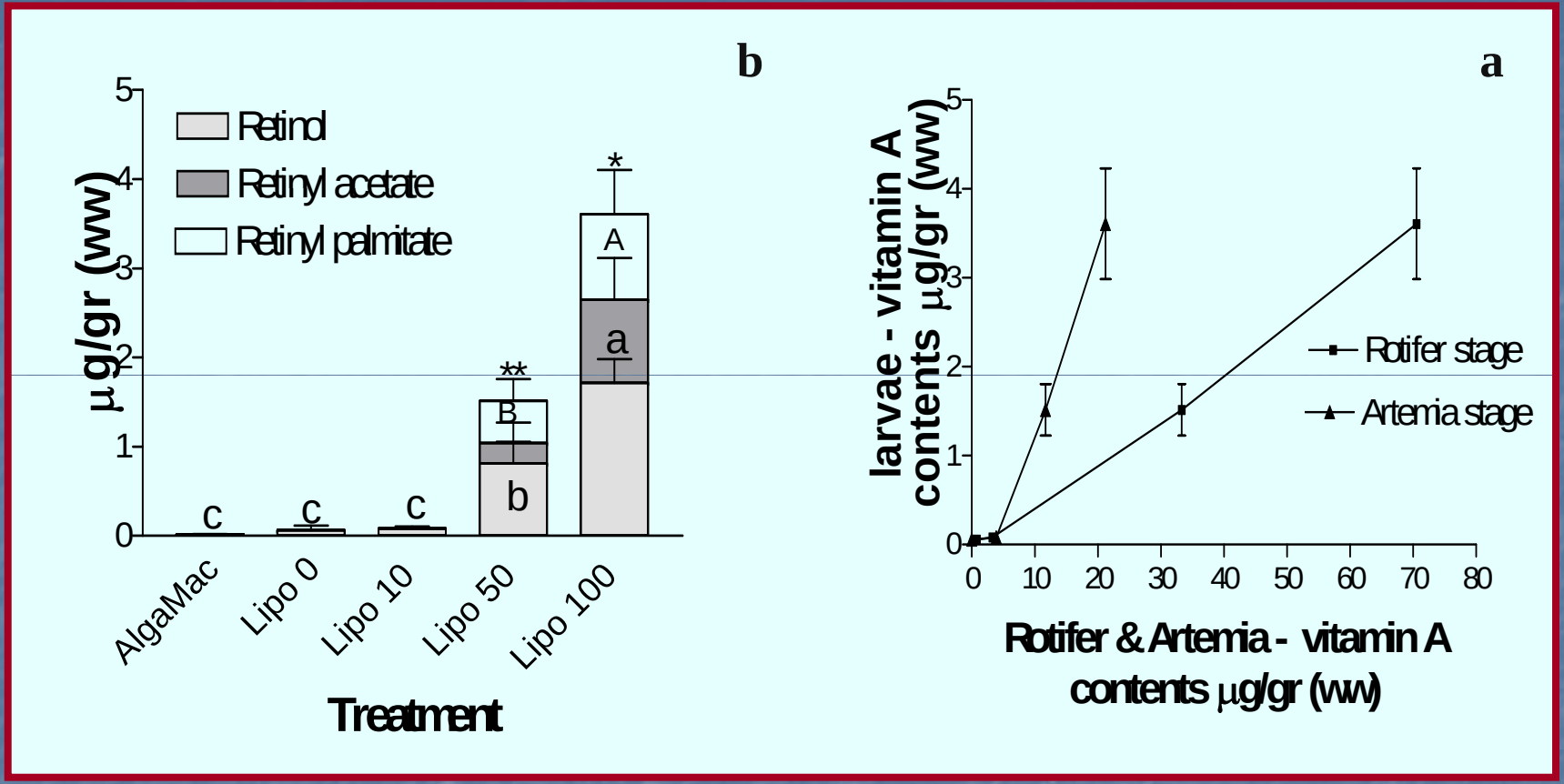
Early larval bodily accumulation of vit A and it's isoforms





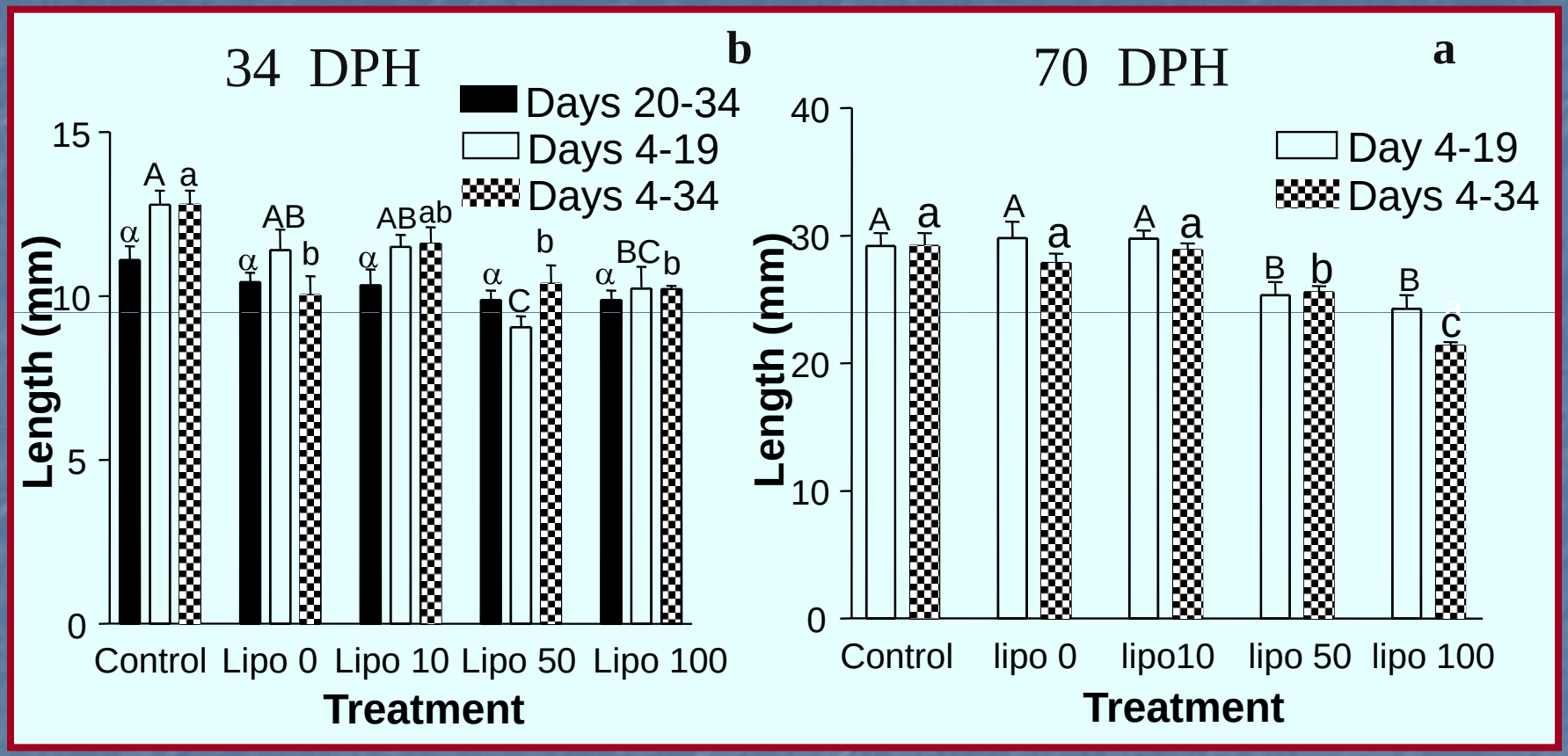
The relationship between rotifers vitamin A content on larval body accumulation of Vit A and its isoforms in 19 dph sea bream larvae, (*Sparus aurata*) after 15 days of feeding (4-19 DPH). (a) Vitamin A accumulation in 19 dph larvae. (b) Vitamin A isoforms distribution in the larvae. (Statistics: A-B Retinyl accetate, a-c Retinol).

Entire larval bodily accumulation of vit A and it's isoforms



The relationship between vitamin A content in rotiers and artemia to the accumulation and isoforms distribution of this vitamin in 34 dph sea bream (*Sparus aurata*) larvae, after 30 days of feeding (4-34 DPH) with rotifers and artemia containing increasing vitamin A levels. (a) Vitamin A accumulation in 34 DPH larvae. (b) Vitamin A isoforms distribution in the 34 DPH larvae.(Statistics: a-c - Retinol, A-B - Retinyl acetate, *-** -Reyintl palmitate).

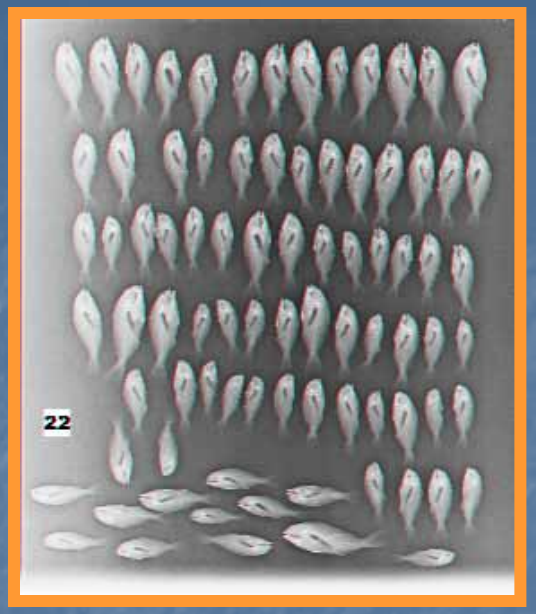
The effect of dietary Vit. A supplementation on late larval performance: Length

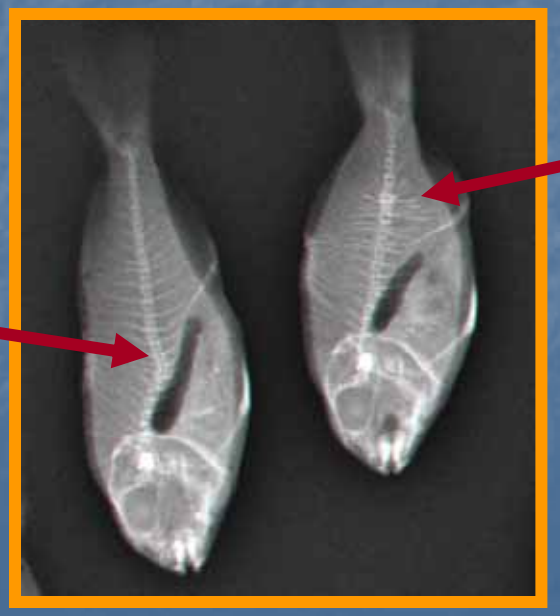


Length (mm) of 34 DPH (Graph b) and 70 DPH (Graph a), after feeding sea bream larvae, with increasing vitamin A levels, at 3 different time periods during larval metamorphosis. (Statistics: A-B: 4-19 DPH, α-β: 20-34 DPH, a-b: 4-34 DPH)

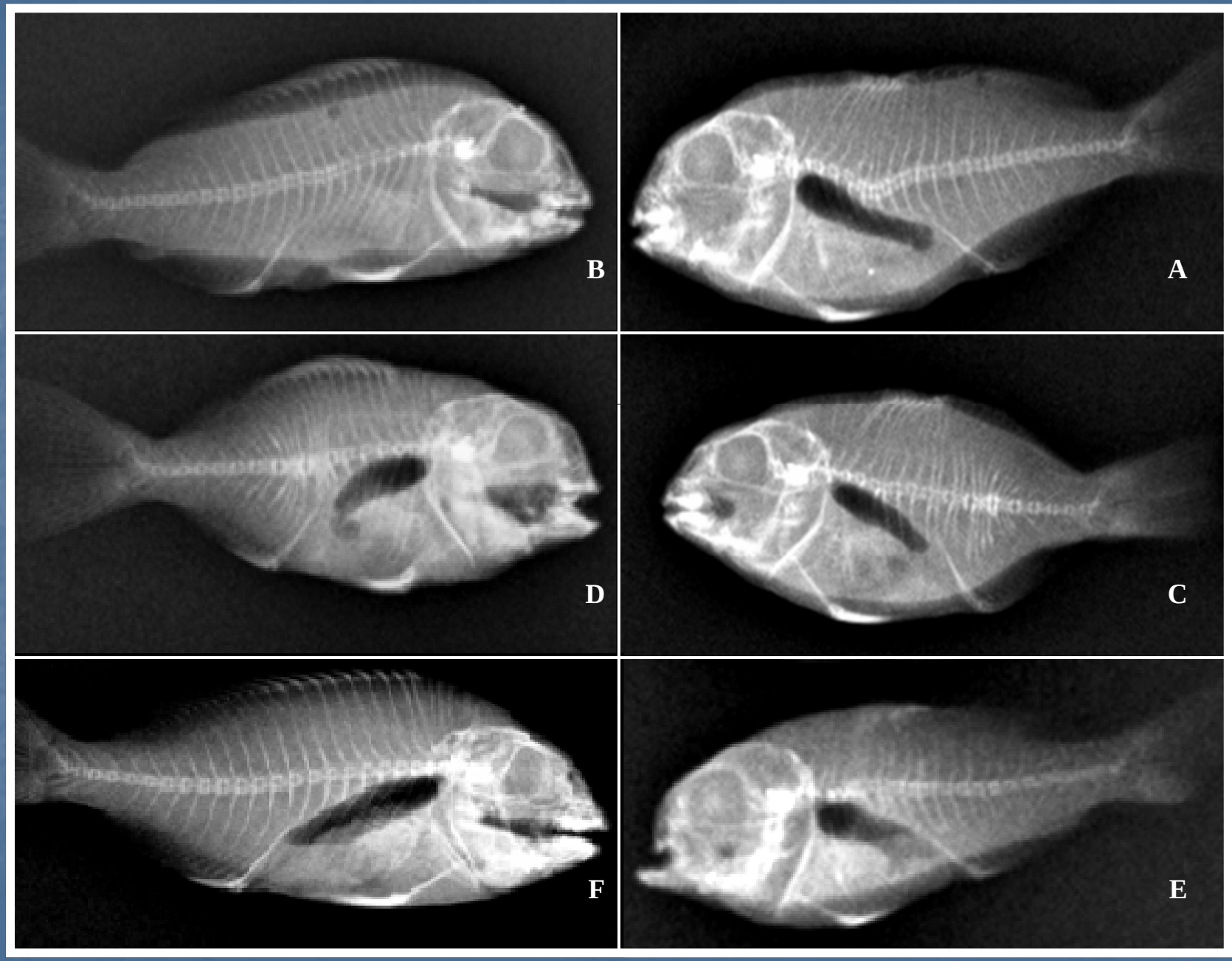
The effect of larval dietary vit. A supplementation on late skeletal deformities in seabream fingerlings

Types of skeletal deformities

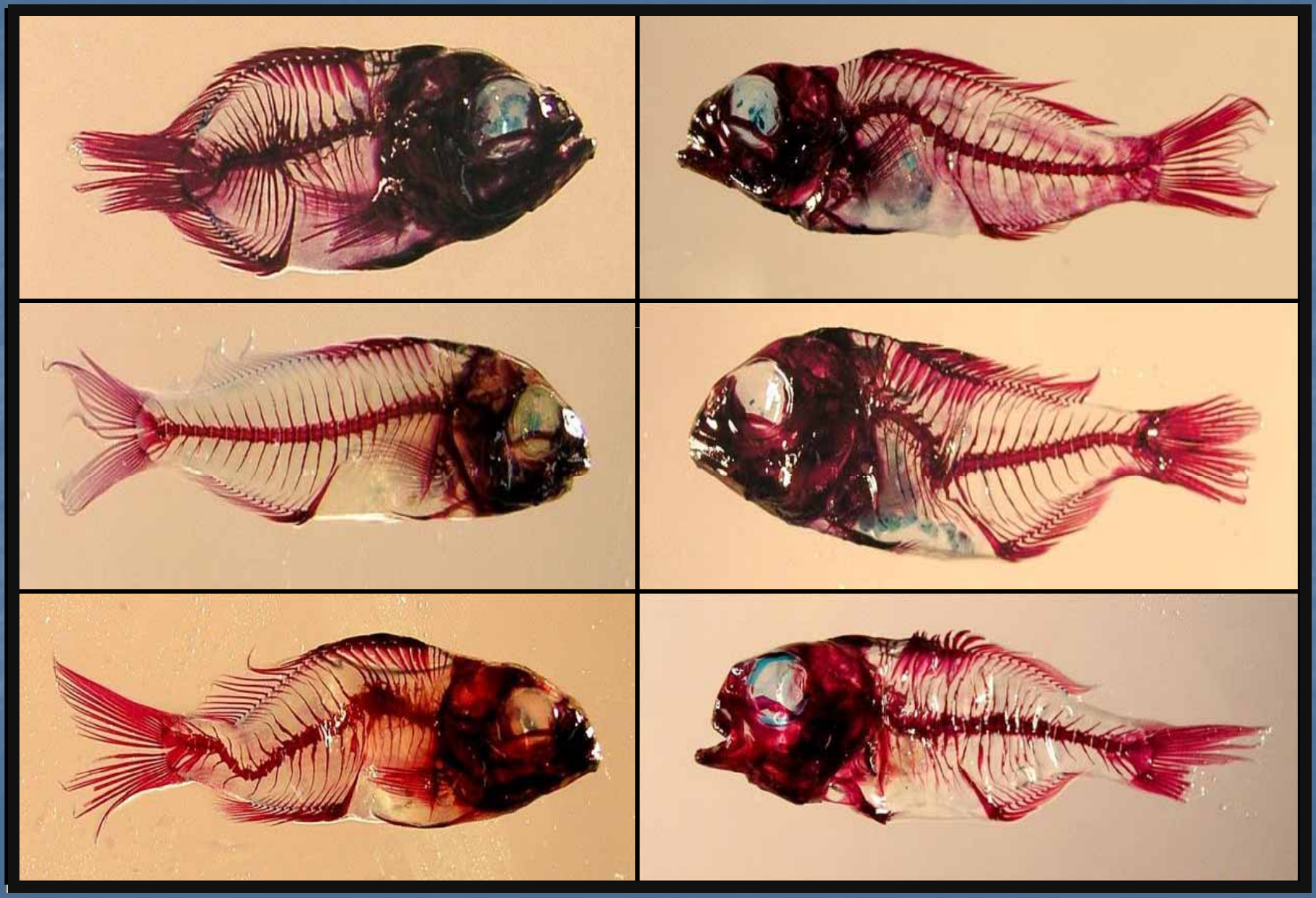




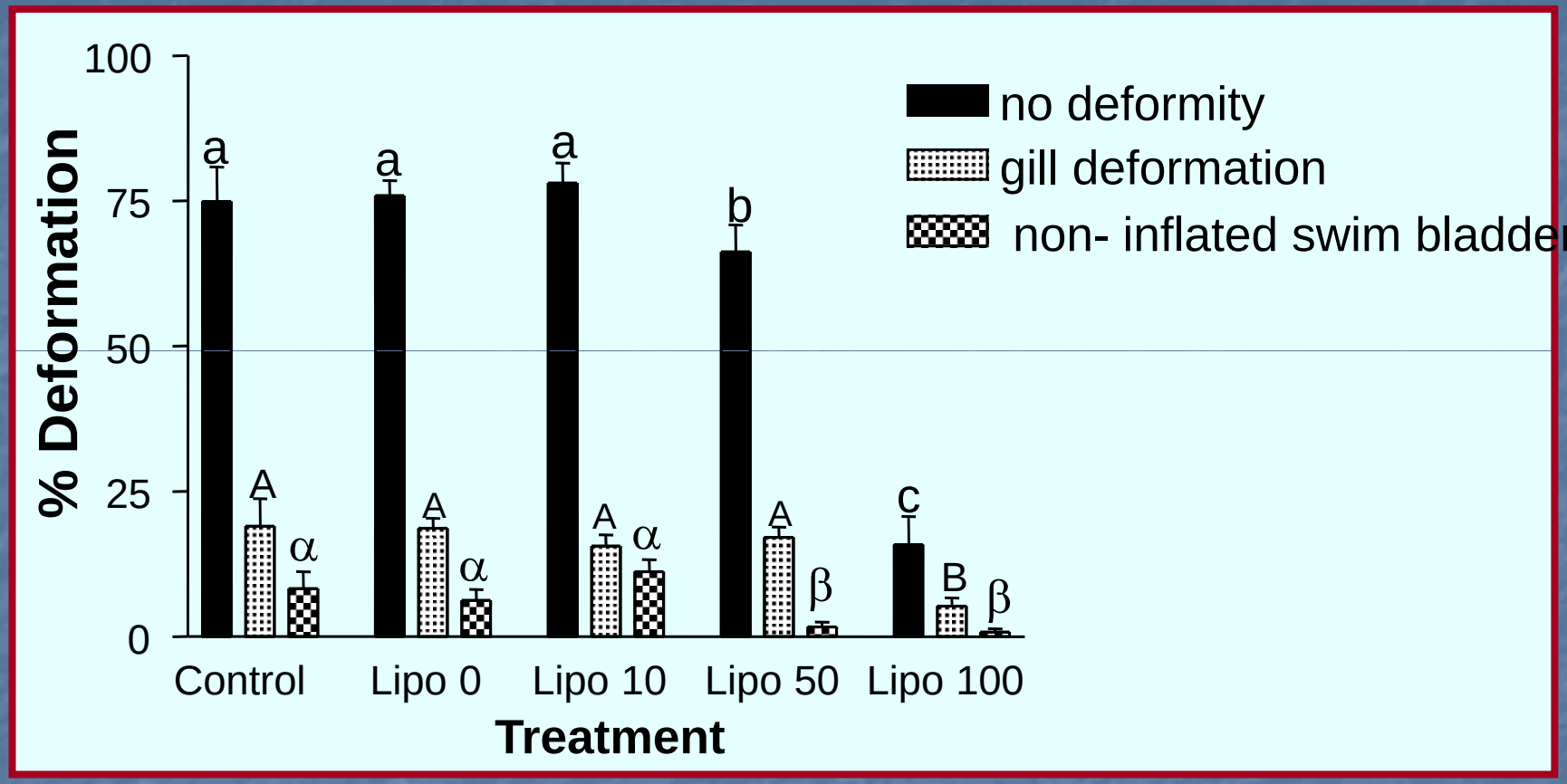
X ray analysis


B
A
D
C
F
E

Skeletal staining

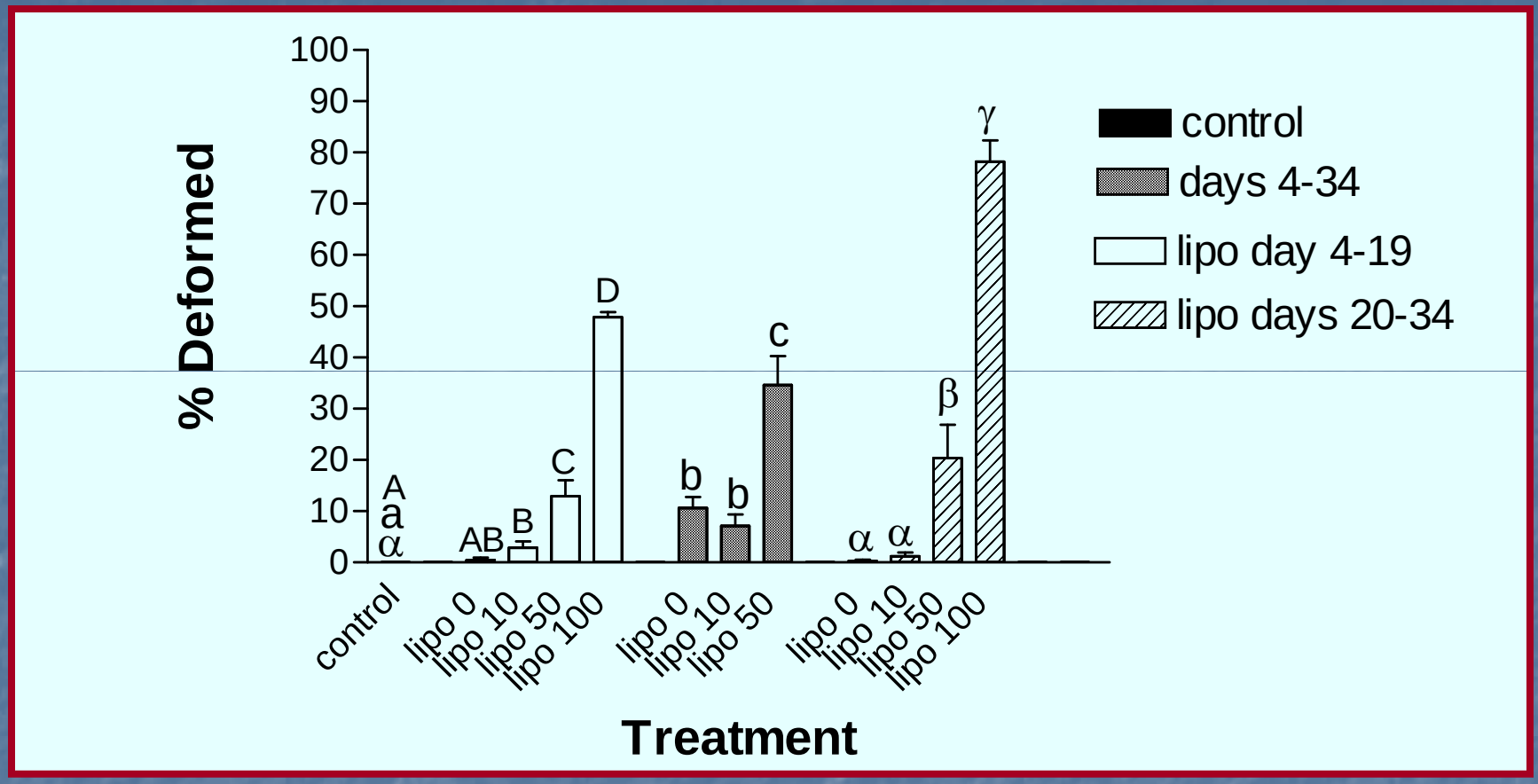


Gill deformities and swimbladder inflation



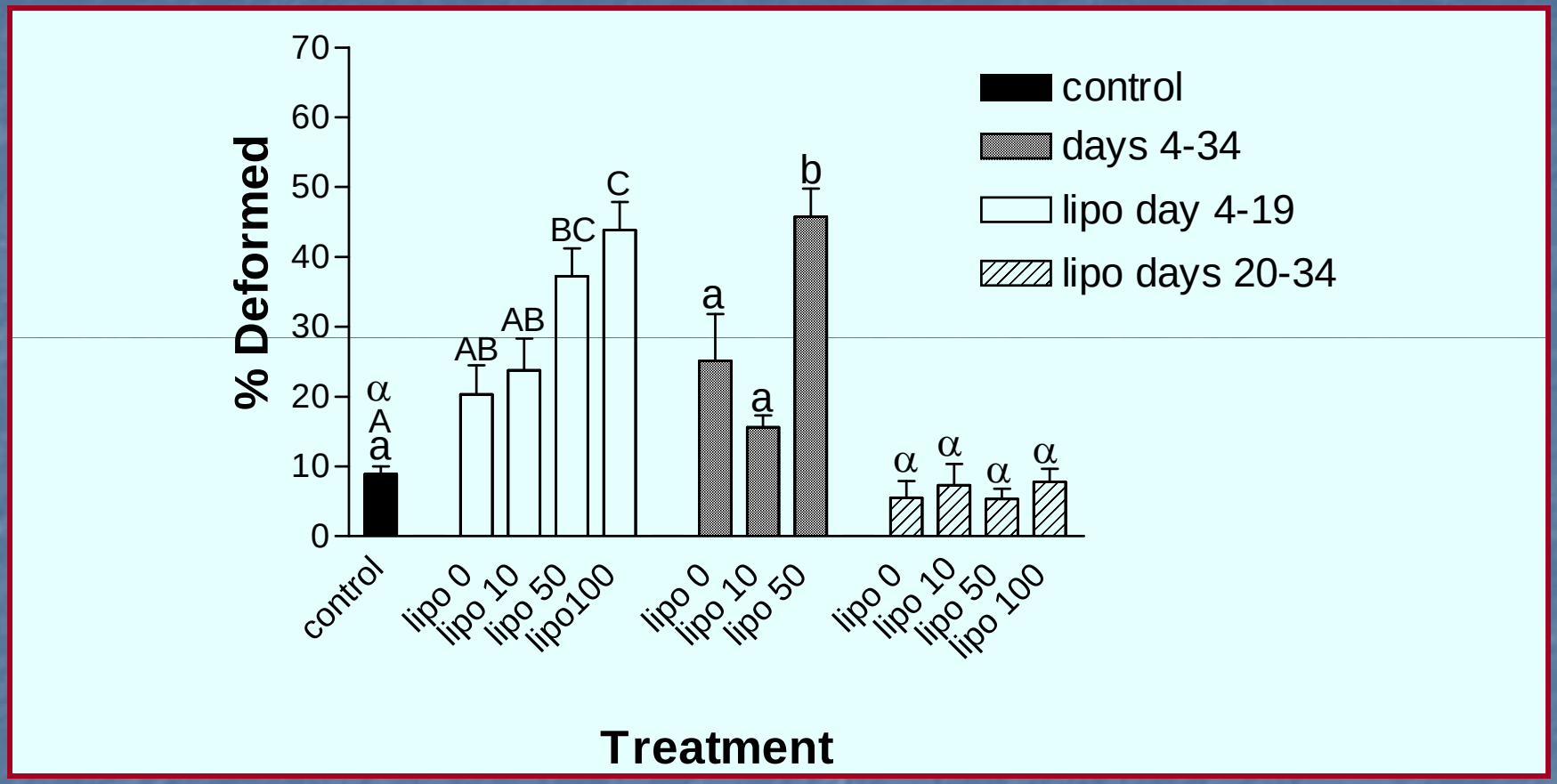
Gill deformities distribution and non inflated swim bladder distribution versus undamaged fingerling at 115 DPH, after feeding sea bream larvae, with artemia containing increasing vitamin A levels, at 19-34 DPH, (A-B: Gills deformity, α-β: non inflated swim bladder, a-b: undamaged fingerling)

Skeletal deformities



The relationship between vitamin A body content in seabream larvae at 3 different time periods during the larval metamorphosis on relative presence of skeletal deformities in 115 dph fingerlings. (Statistics: A-D: 4-19 DPH, α-γ: 20-34 DPH, a-c: 4-34 DPH).

Cranial deformities



The relationship between vitamin A body content in seabream larvae at 3 different time periods during the larval metamorphosis on relative presence of cranial deformities in 115 dph fingerlings. (Statistics: A-D: 4-19 DPH, α-γ: 20-34 DPH, a-c: 4-34 DPH).

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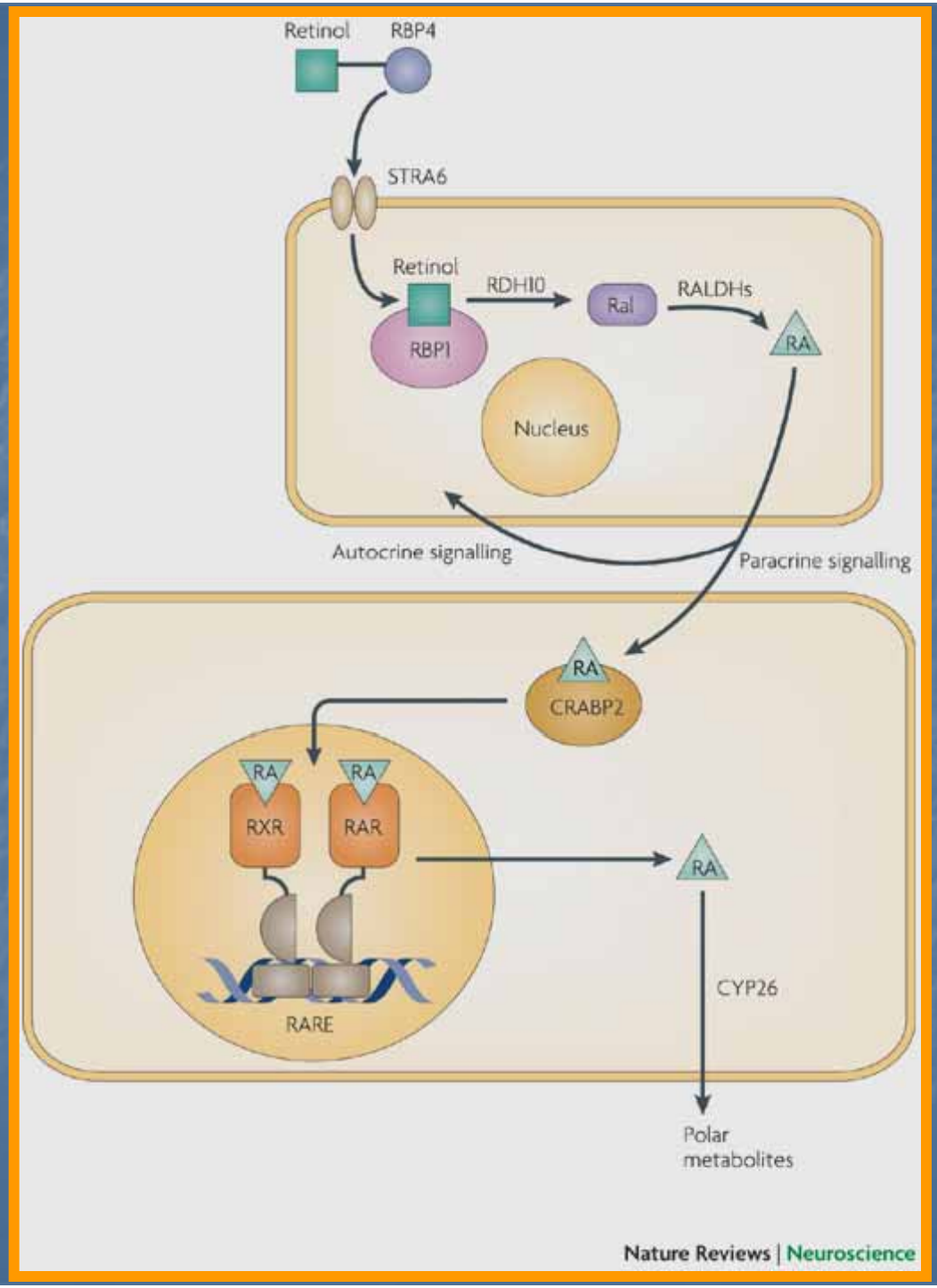
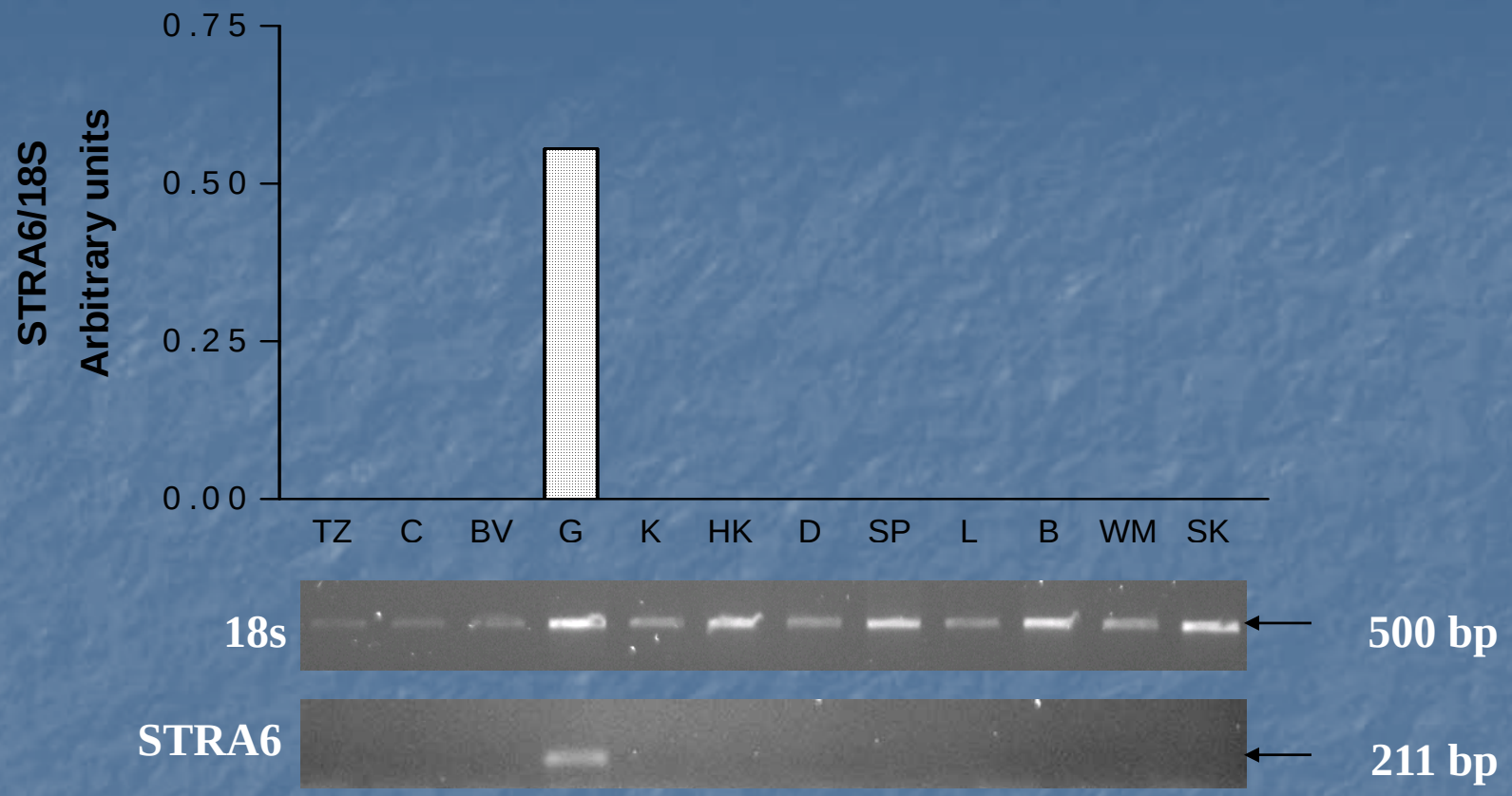
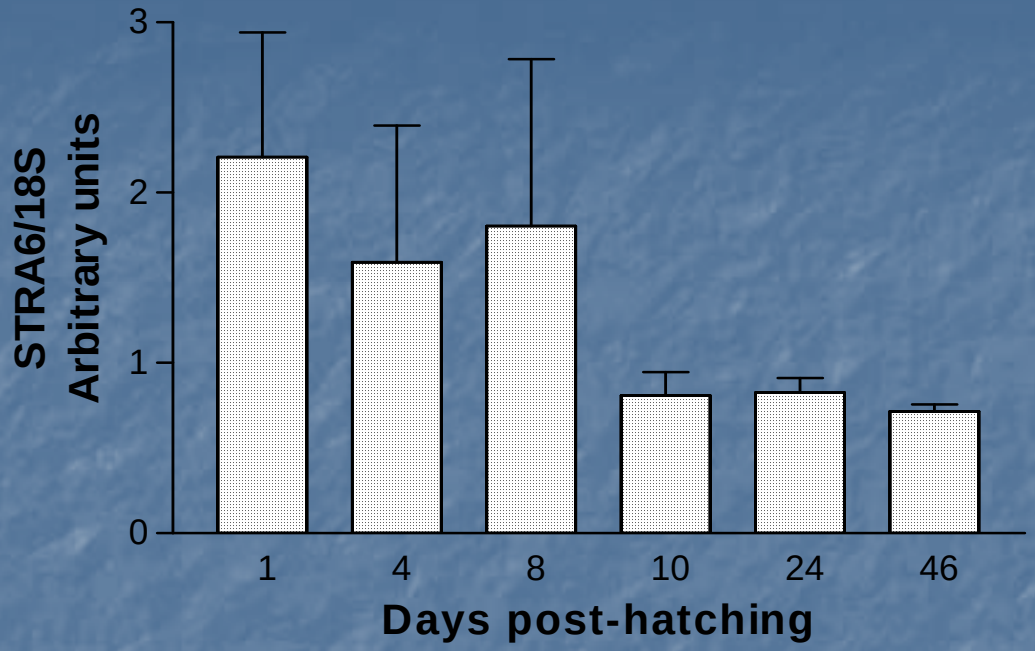
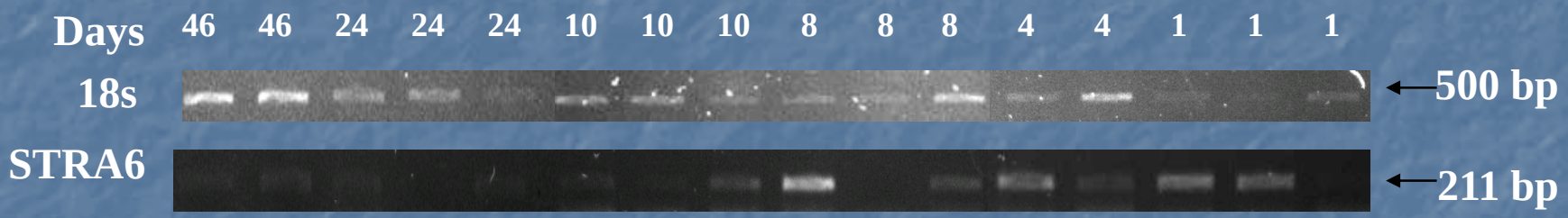
STSM funded by the fish welfare COST action 867


Retinol
RBP4
STRA6
Retinol
RDH10
Ral
RALDHs
RA
RBP1
Nucleus
Autocrine signalling
Paracrine signalling
RA
CRABP2
RA
RA
RXR
RAR
RA
CYP26
RARE
Polar
metabolites
Nature Reviews | Neuroscience

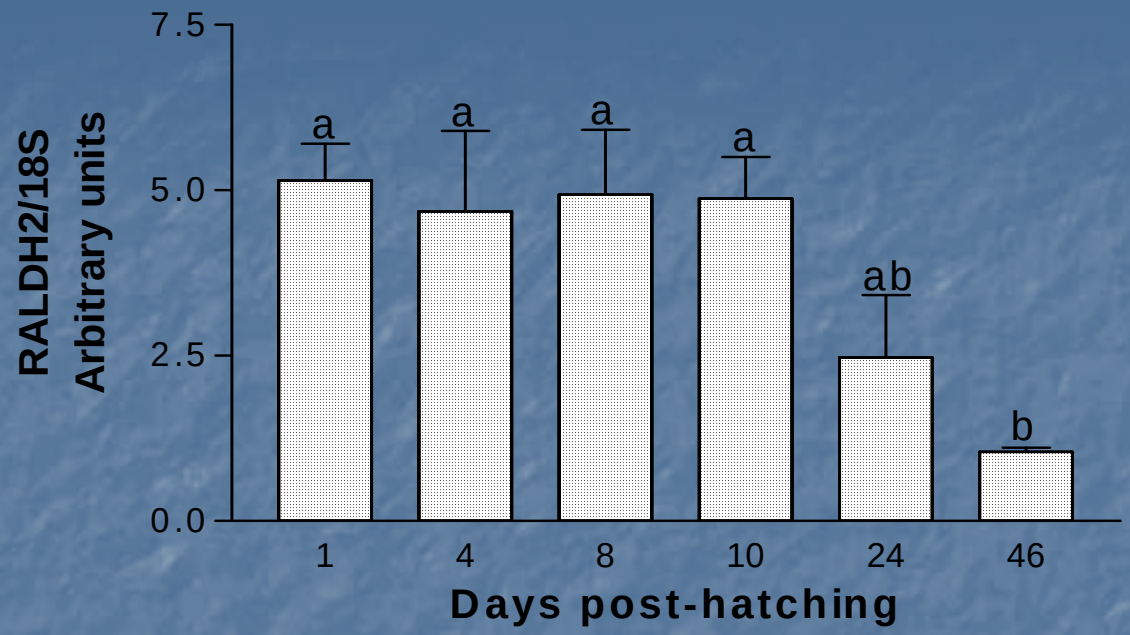


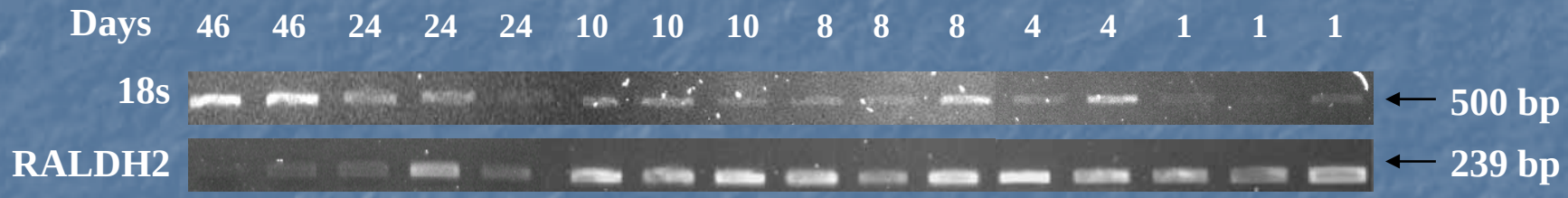
STRA6 expression in adult tissues. Relative abundance of STRA6 determined by semi-quantitative RT-PCR, with 18S to normalize quantity of total RNA in the different tissues. Tissues: TZ, thyroid zone; C, cartilage; BV, bone vertebral; G, gonad; K, kidney; Hk, head-kidney; D, duodenum; S, spleen; L, liver; B, brain; WM, white muscle; S, skin





STRA6 expression in larvae. Relative abundance of STRA6, determined by semi-quantitative RT-PCR, with 18S to normalize quantity of total RNA in different larvae stage: 1, 4, 8, 10, 24, 46 days post hatching.





RALDH2 expression in larvae. Relative abundance of RALDH2, determined by semi-quantitative RT-PCR, with 18S to normalize quantity of total RNA in different larvae stage: 1, 4, 8, 10, 24, 46 days post hatching.

Conclusions

- This study developed a vitamin A enrichment technology based on the use of liposomes.
- No relationship was found between the relative presence of opercular deformities or swim bladder presence and dietary vitamin A.
- This study demonstrated that during the first stages of the metamorphosis (4-19 DPH) higher dietary level of vitamin A caused a high number of cranial deformations, while during the late stages of the metamorphosis (20-34 DPH) extreme level of vitamin A caused a high number of abnormal vertebrae.
- These results indicate that in *Sparus aurata* larvae the effect of vitamin A is development stage specific.

Conclusions

- The relative mRNA gene expression abundance of RALDH2, LRAT, STRA6 was found to be higher during the first 3 weeks of Sea bream larval morphogenesis.

- STRA6, a multi-transmembrane domain protein, is widely expressed in embryonic development and in adult ovary which emphasizes the importance of vitamin A in the maternal yolk sac and during the embryonic development.

- The level of vitamin A that is associated with the best performance in terms of growth promotion and reduced rate of deformities is within the range of 0.5-3.9 μg retinoid/gr ww in the live food and 0.4 μg retinoid/gr ww in the larvae

Thanks