

DELIVERING BIOACTIVE COMPOUNDS TO FISH LARVAE USING MICROCAPSULATED DIETS

M. Yúfera¹, S. Kolkovski², C. Fernández-Díaz¹,
J. Rinchard³, K.J. Lee³ & K. Dabrowski³

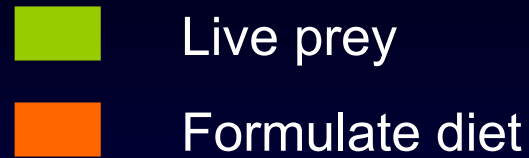
1. Instituto de Ciencias Marinas de Andalucía (CSIC), Cádiz, Spain

2. Mariculture Research and Advisory Group, Fisheries Western
Australia, Perth, WA Australia

3. School of Natural Resources, Ohio State University, Columbus,
Ohio, USA



LIVE PREY REPLACEMENT STATUS



- Early weaning



- Co-feeding



- Total replacement:

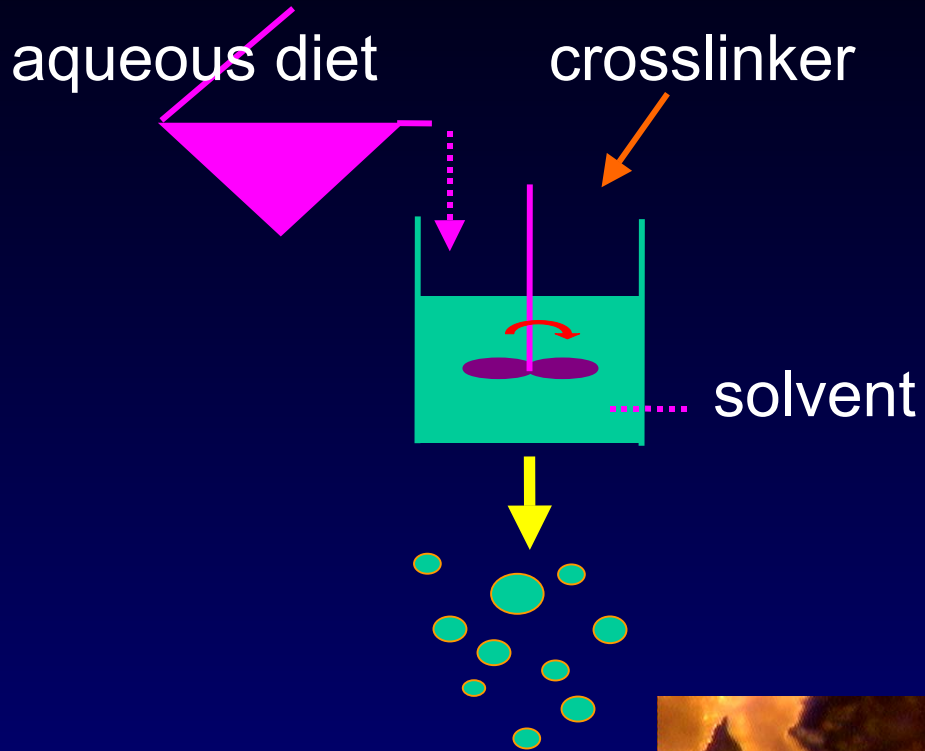
· Complete period



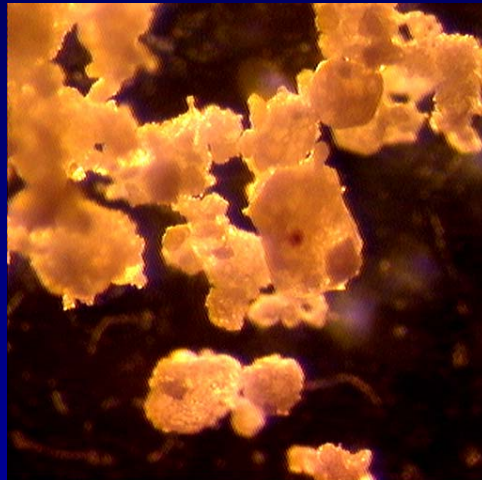
· Almost complete



Protein-walled microcapsules

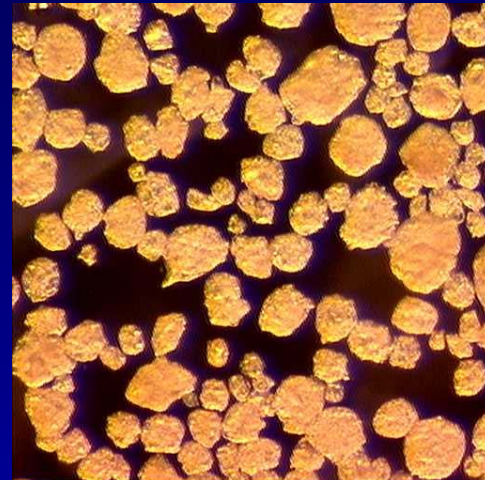


Dry capsules



Buoyancy
Stability
Digestibility

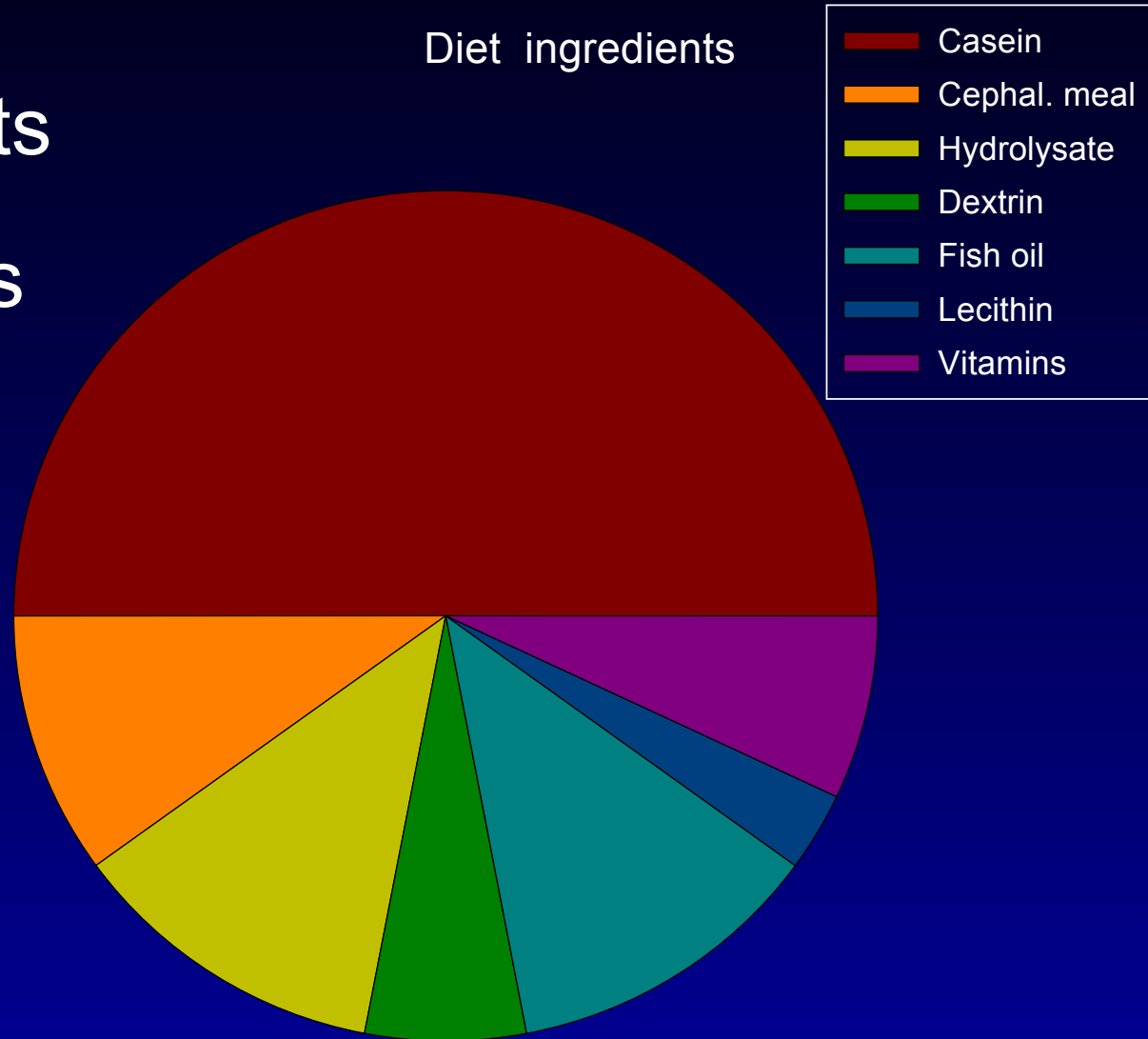
Shell is constituted
by dietary compounds



Rehydrated capsules

MICRODIET COMPOSITION

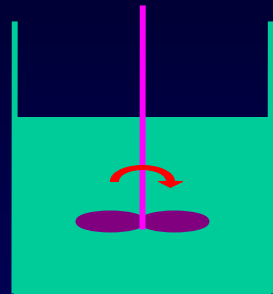
- Macronutrients
- Micronutrients
(specific compounds)



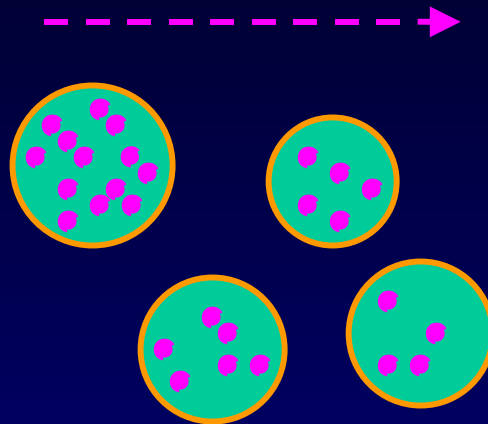
From -----
ingredients mixture

To
positive response

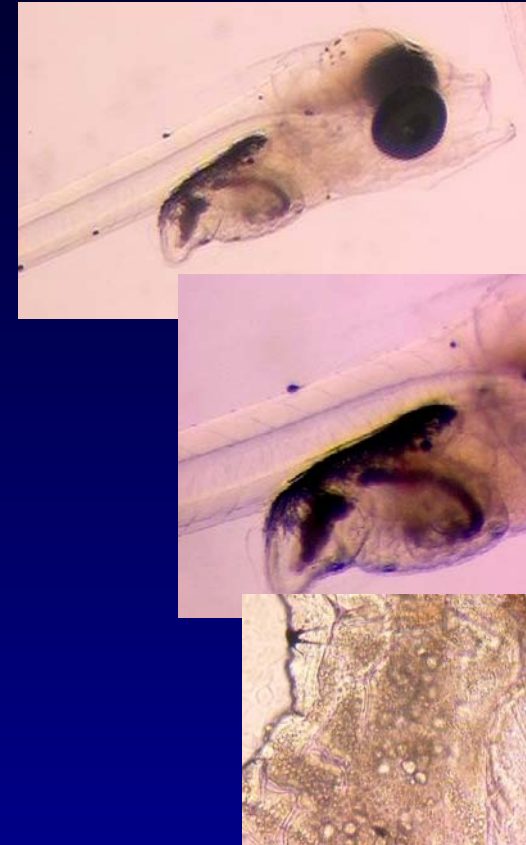
manufacturation



water immersion



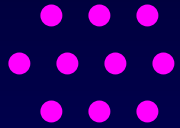
digestion



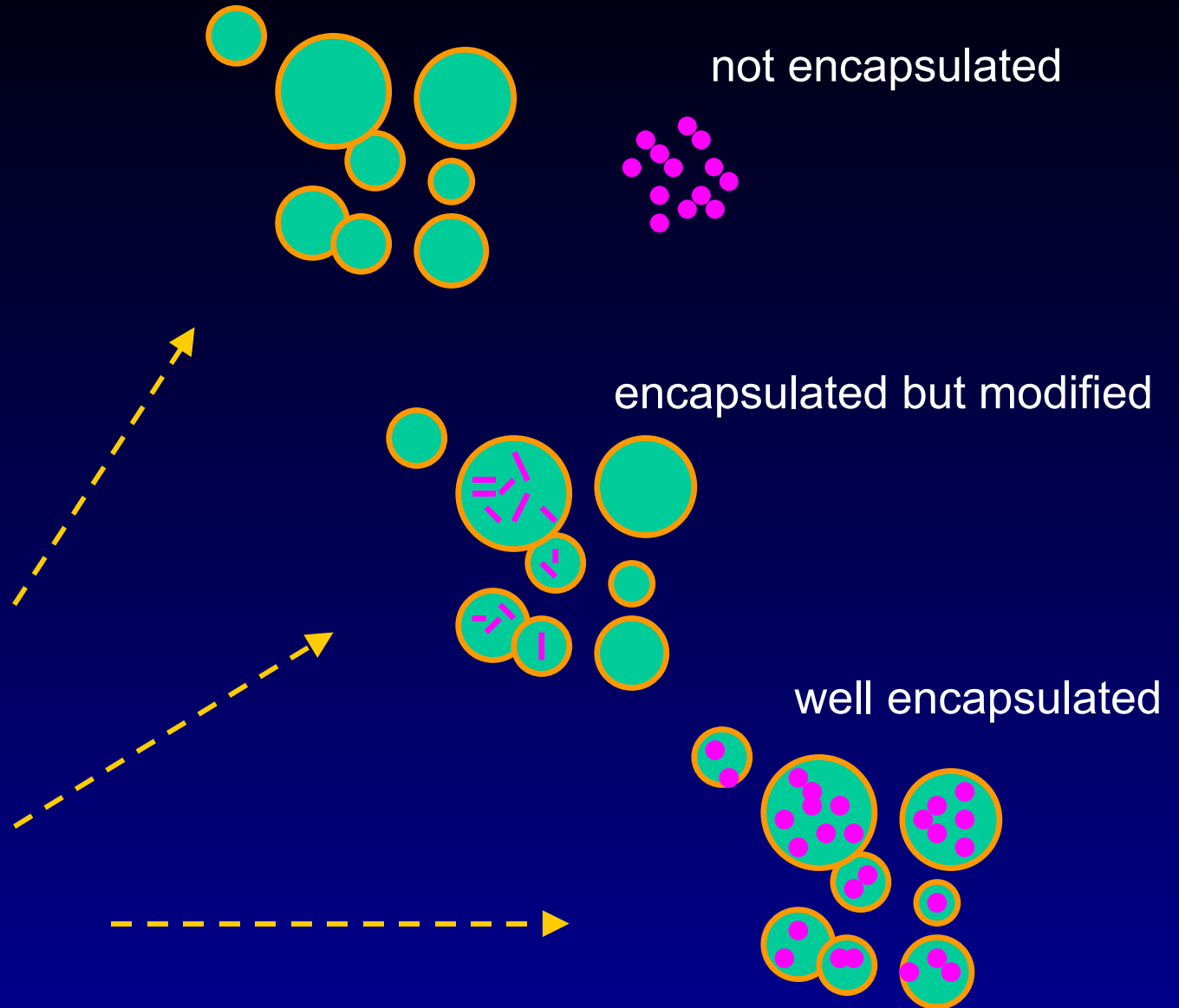
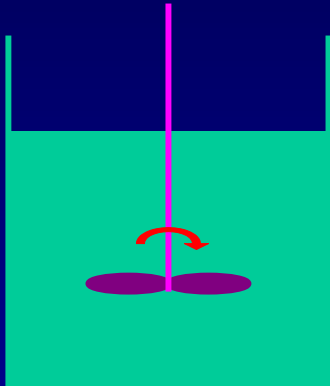
Manufacturation

water immersion
ingestion

Substance



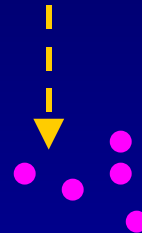
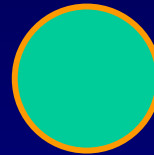
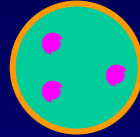
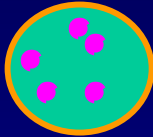
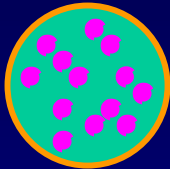
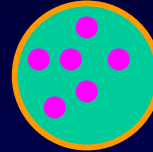
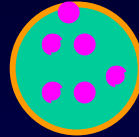
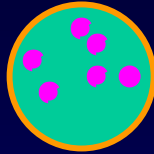
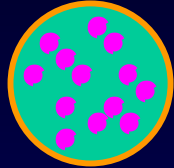
Diet
ingredients



manufacturation

Water immersion

ingestion

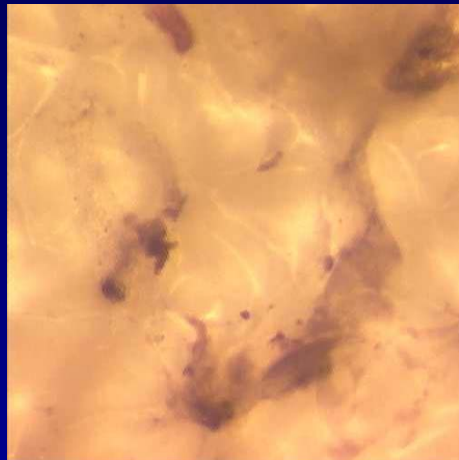
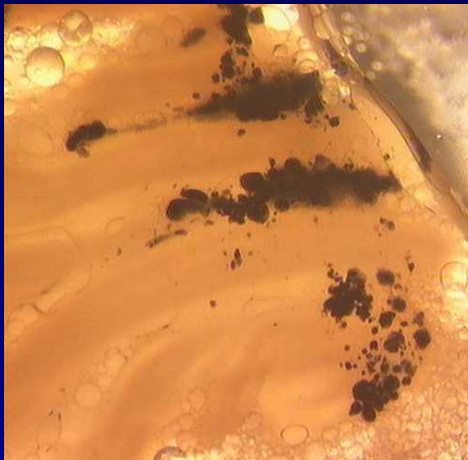
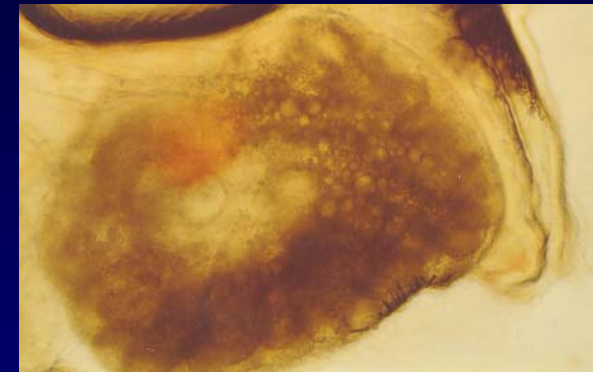


manufacturation
water immersion
Digestion

No digested



Digested and assimilated



Substance destroyed

TESTING RESPONSES

(causes of negative response)

- **Manufacturation:**
 - substance not included
 - included but altered
- **Water residence period:**
 - loss by leaching
 - particle not ingested
- **Digestion and assimilation:**
 - not digested
 - digested but destroyed
- **Larval experiments** (growth & survival)
 - inadequate concentration
 - deficiency in other nutrient
 - no response is achieved

OBJECTIVES

- Assess protein-walled microcapsules (prototype 2; Yúfera et al., 2000) as a tool for delivering compounds of interest in fish larvae research
- Advance in developing a complete formulate microdiet for fish larvae

SPECIFIC EXPERIMENTS

- Hormones (17 β -Estradiol)
- Free amino acids (L-lysine)
- Vitamin C (Ascorbyl mono-/ poly-phosphate)

Hormones

Experiments:

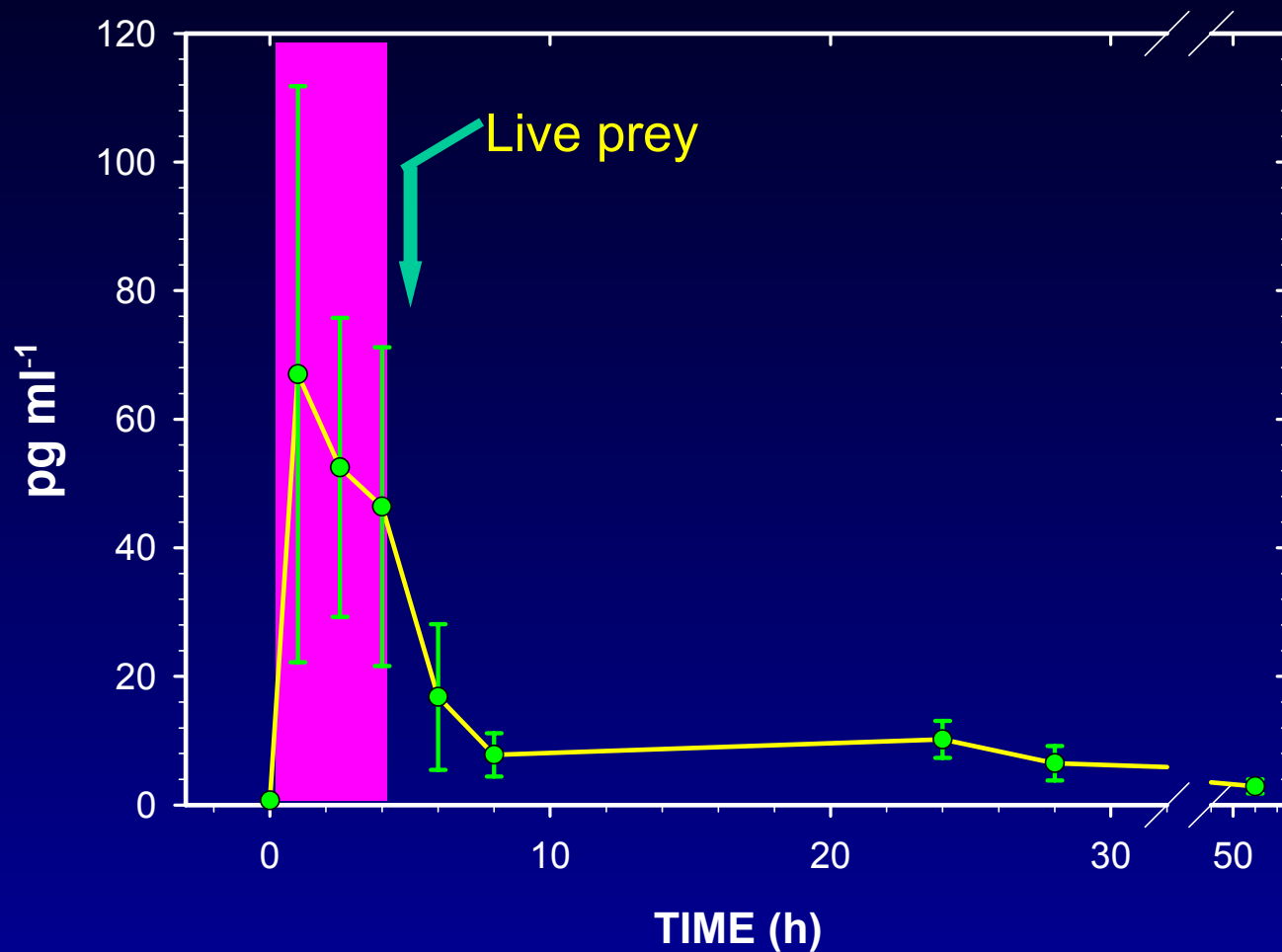
- Microencapsulation of diet including 17β -Estradiol
- Feeding of 32 DAH *Sparus aurata* larvae during 4 h with MC-E2
then replaced with live prey

Measurements:

- Determination by RIA of 17β -Estradiol in larval tissues throughout the feeding experiment

Hormones

17 β -estradiol in larvae tissues



Sparus aurata

32 DAH

Free amino acids

Experiments:

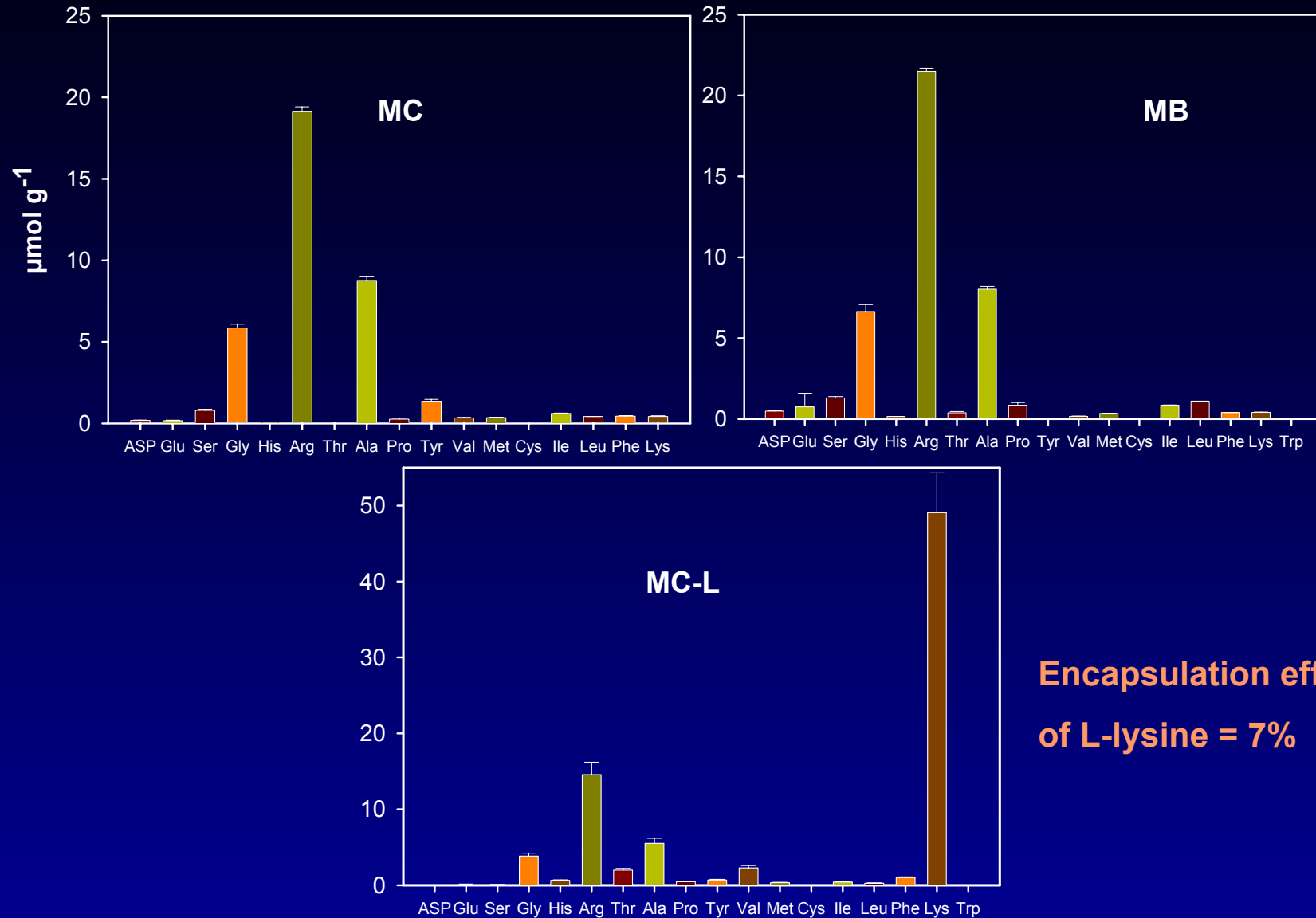
- Microencapsulation of a diet including 12% of L-Lysine
- Comparison of free amino acids leaching (FAA) pattern in protein-walled microcapsules and gelatin-microbound particles

Measurements:

- Analysis of FAA in microparticules
- Total FAA leaching pattern during 60 min of water immersion

Free amino acids

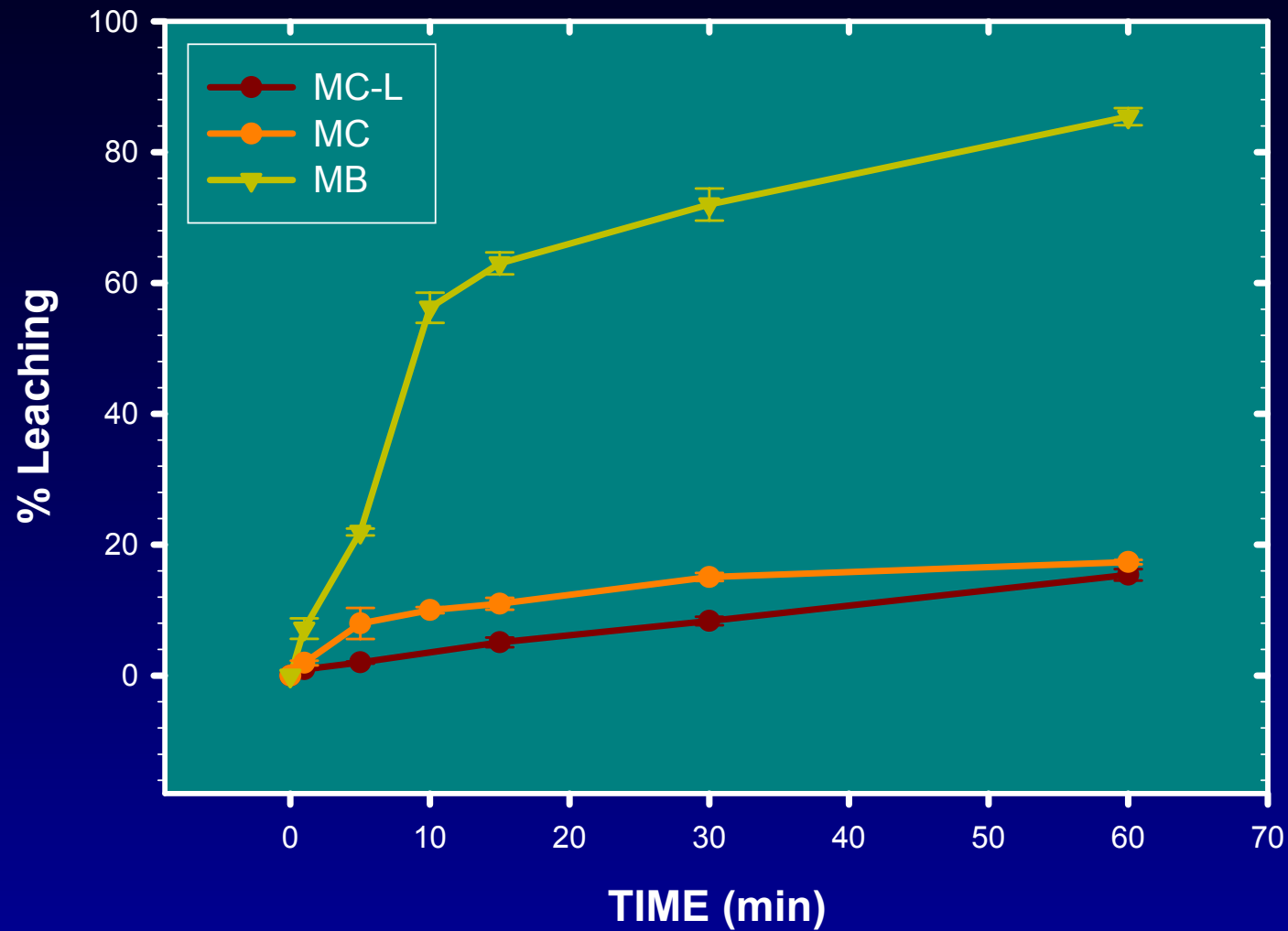
FAA Content in microdiets



**Encapsulation efficiency
of L-lysine = 7%**

Free amino acids

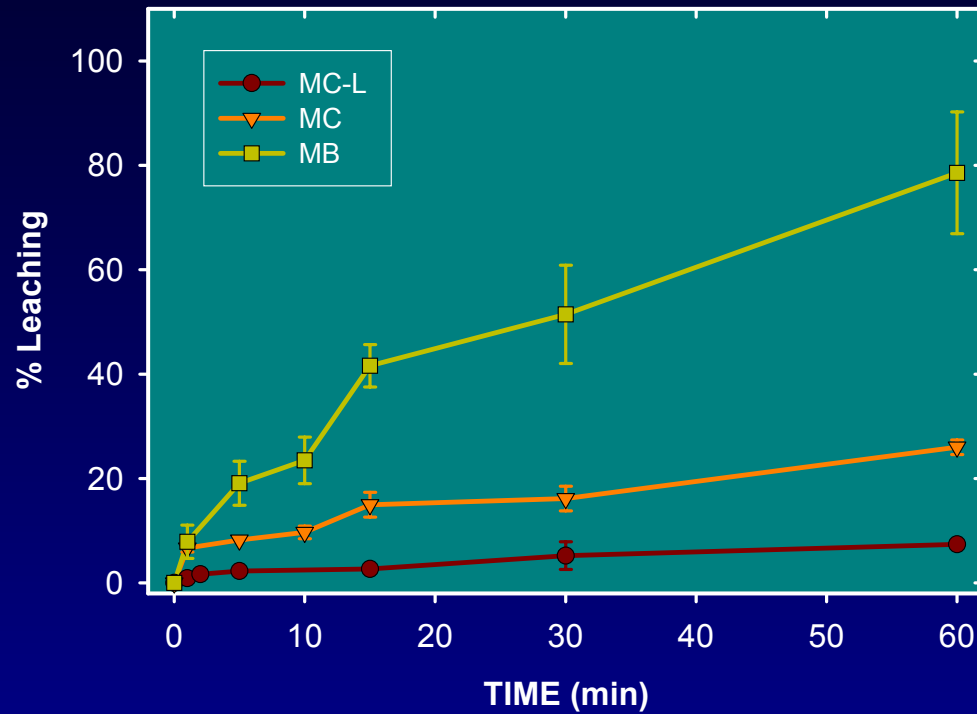
Total FAA leaching pattern



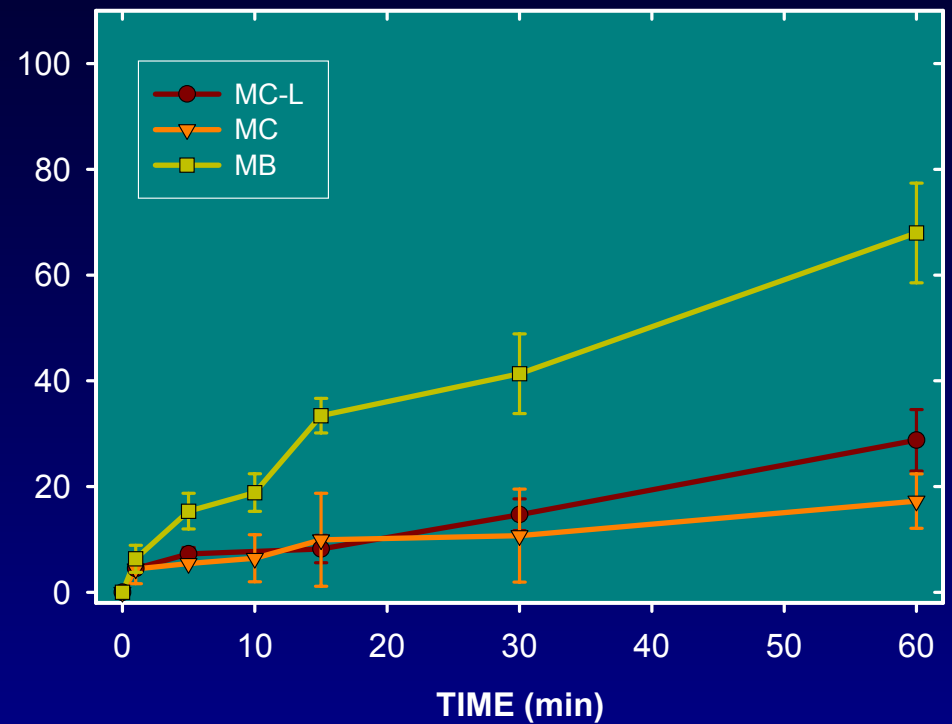
Free amino acids

FAA leaching

Glycine



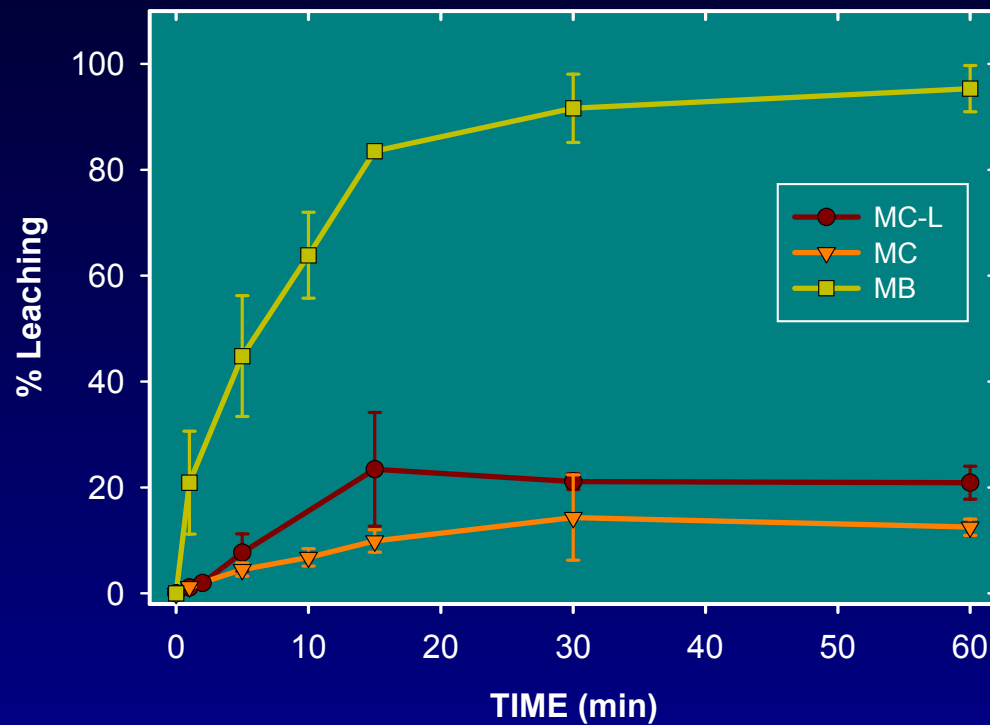
Alanine



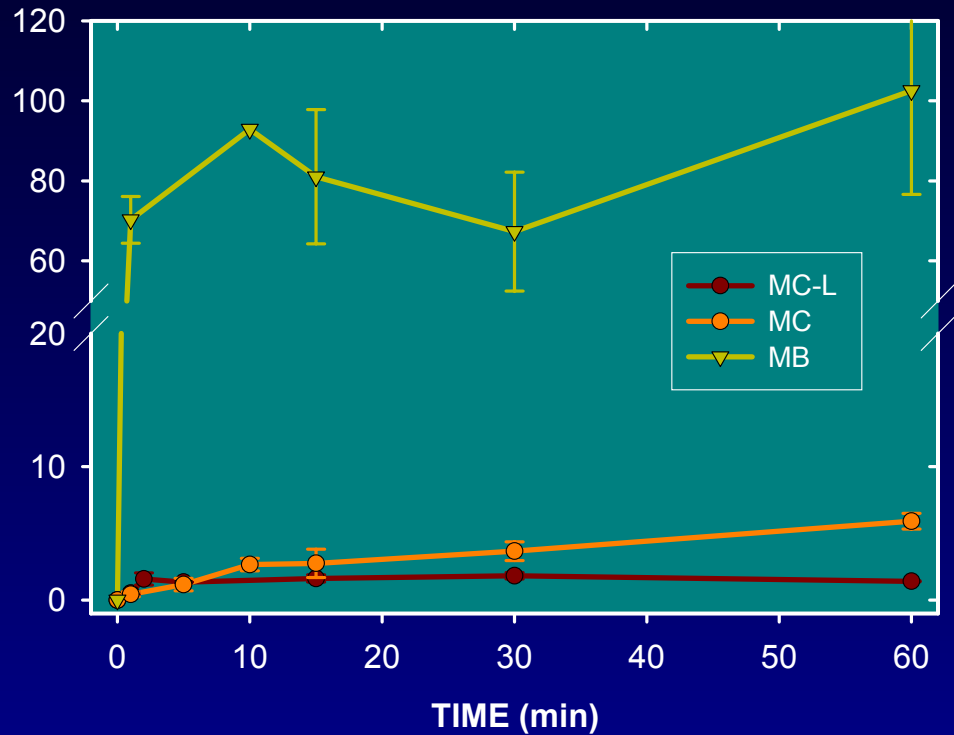
Free amino acids

FAA leaching

Arginine

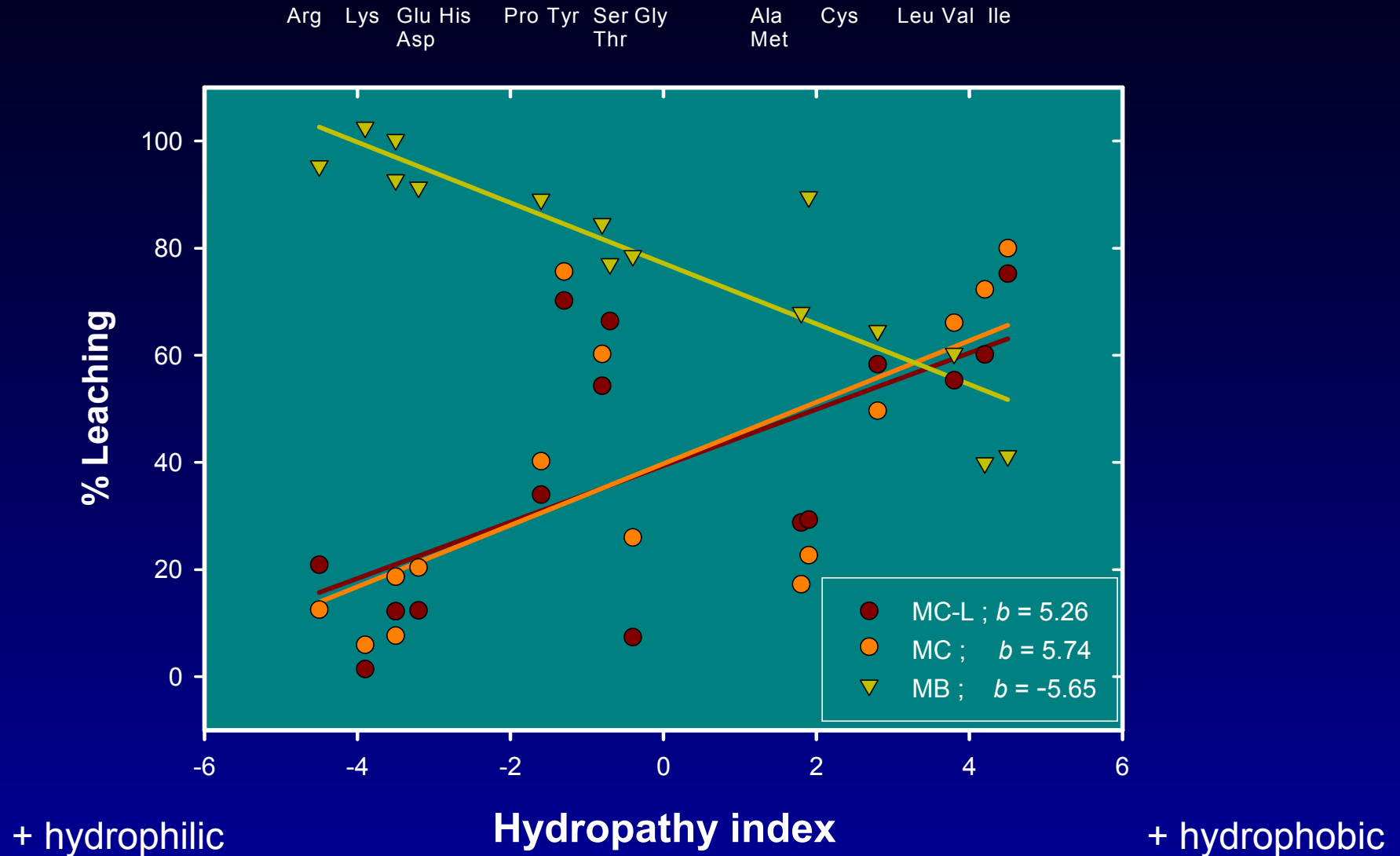


Lysine



Free amino acids

FAA leaching in 60 min



Vitamin C

Experiments:

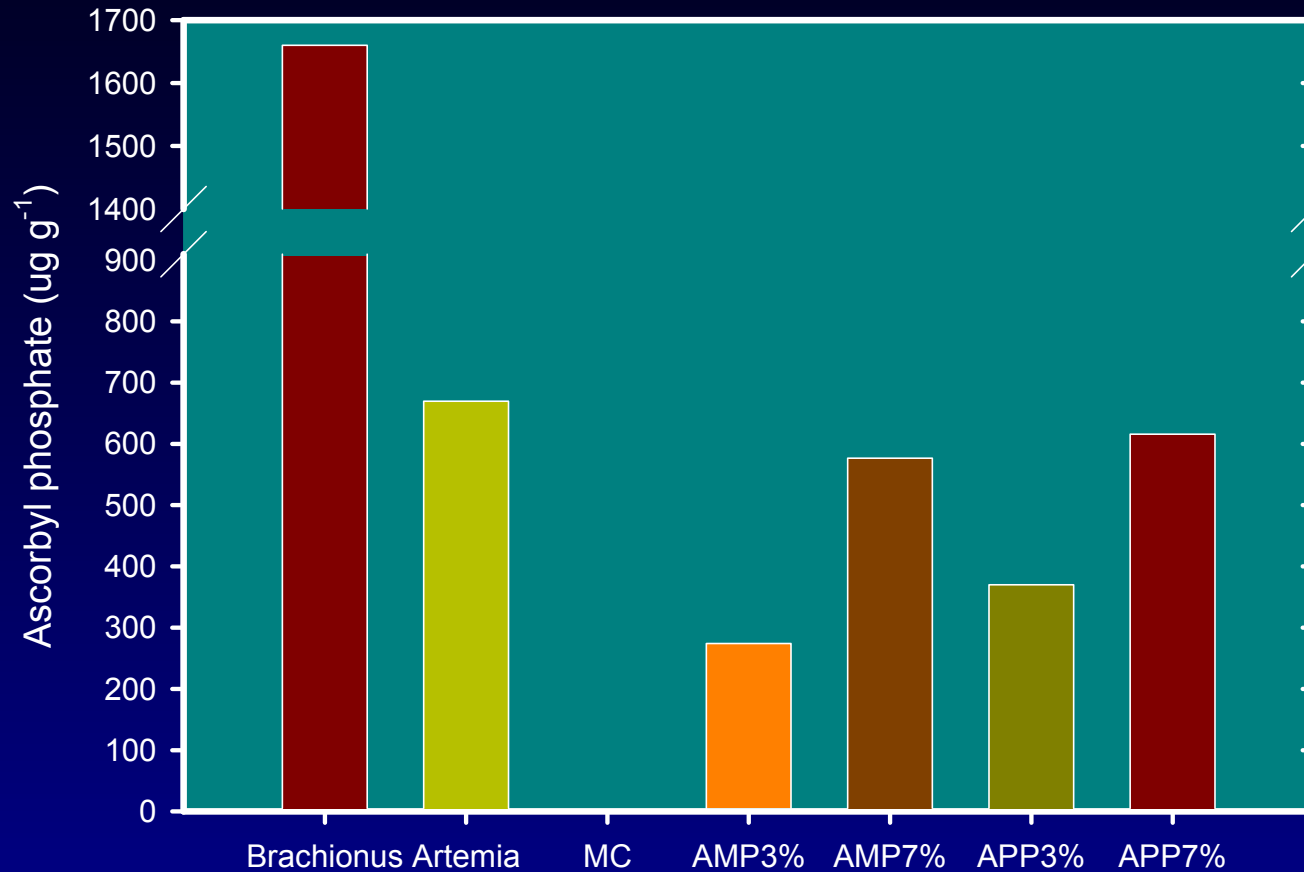
- Microencapsulation of a diet including 3% & 7% of Ascorbyl mono- & poly-phosphate
- Rearing of fish larvae (*Sparus aurata* & *Solea senegalensis*) with microencapsulated diet (3% AMP)

Measurements:

- Analysis of ascorbic acid in the microcapsules
- Analysis of ascorbic acid in larvae
- Growth & survival of larvae

Vitamin C

Content of Total Ascorbate



Brachionus

Artemia

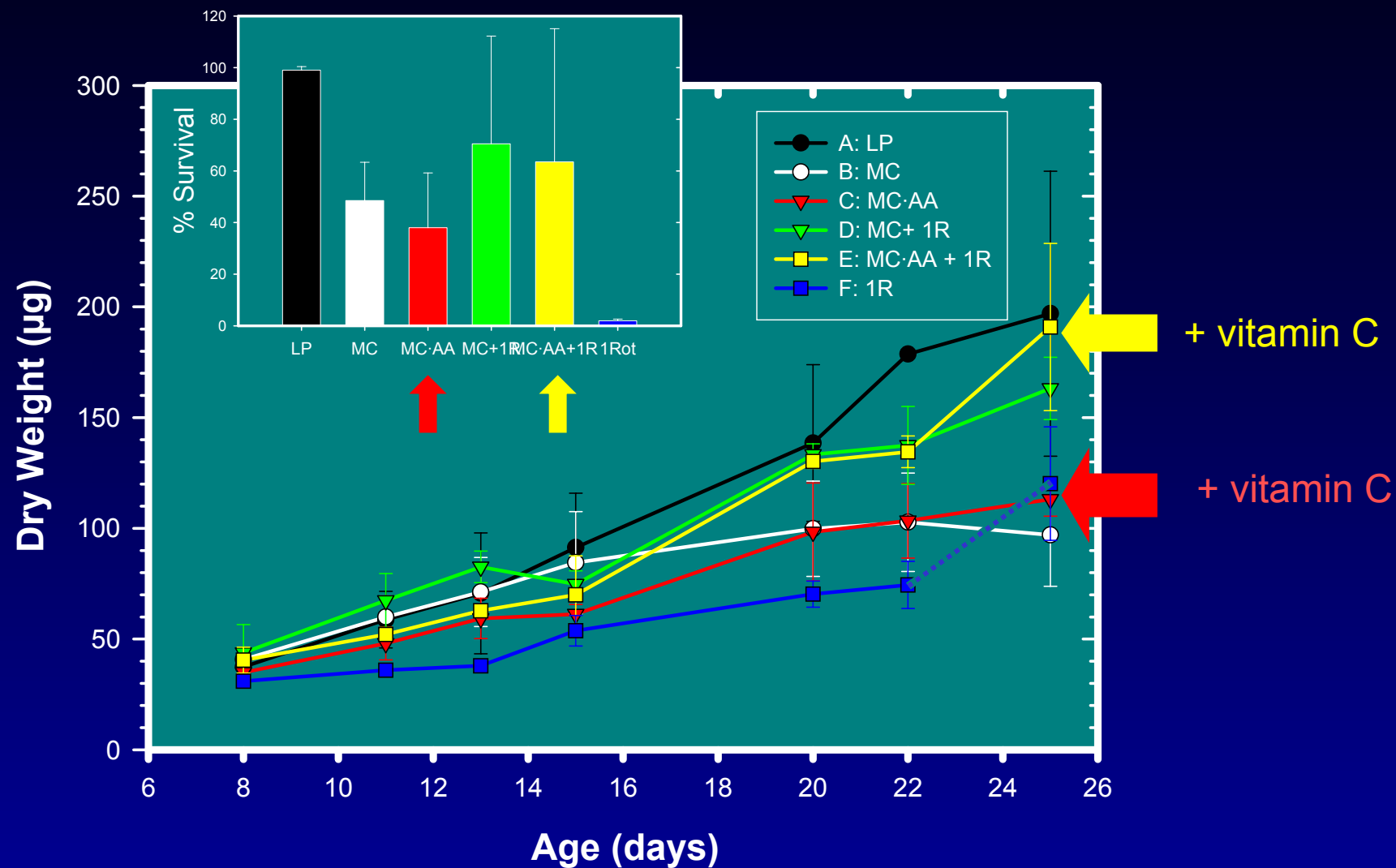
Capsules 3%

Capsules 7%

**Encapsulation efficiency
of vitamin C = 3%**

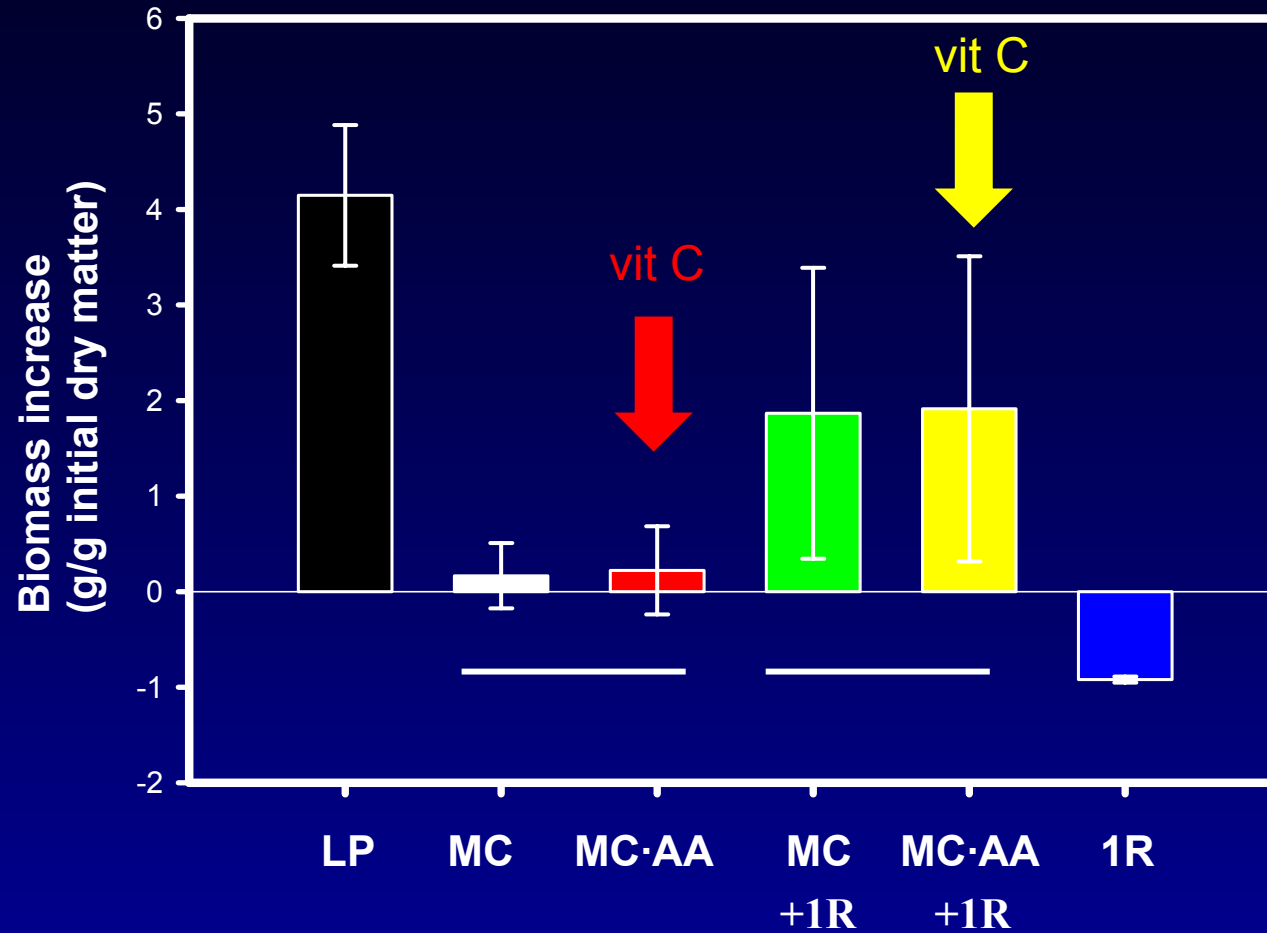
Vitamin C

Larval Growth
Sparus aurata



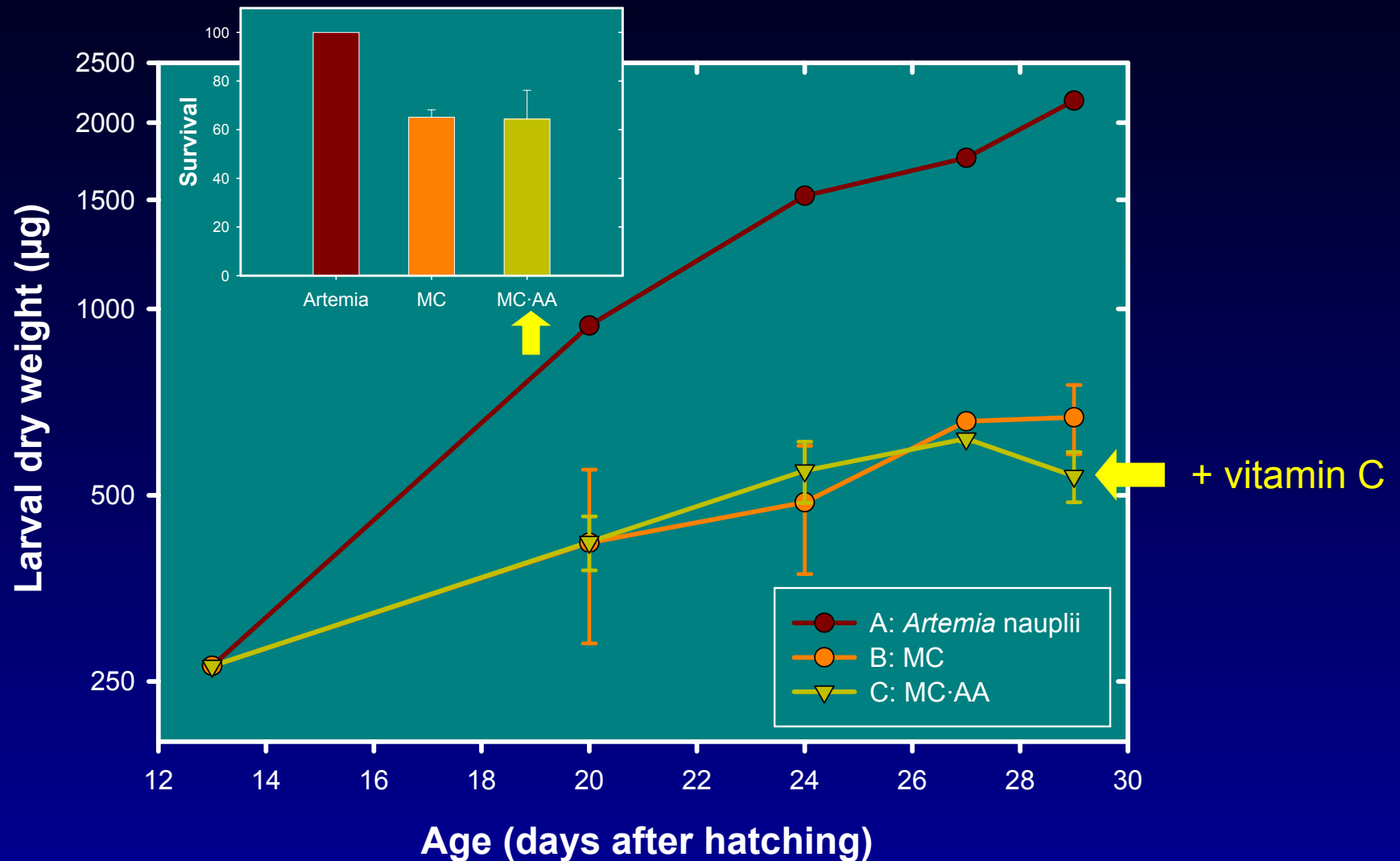
Vitamin C

Total Biomass *Sparus aurata*



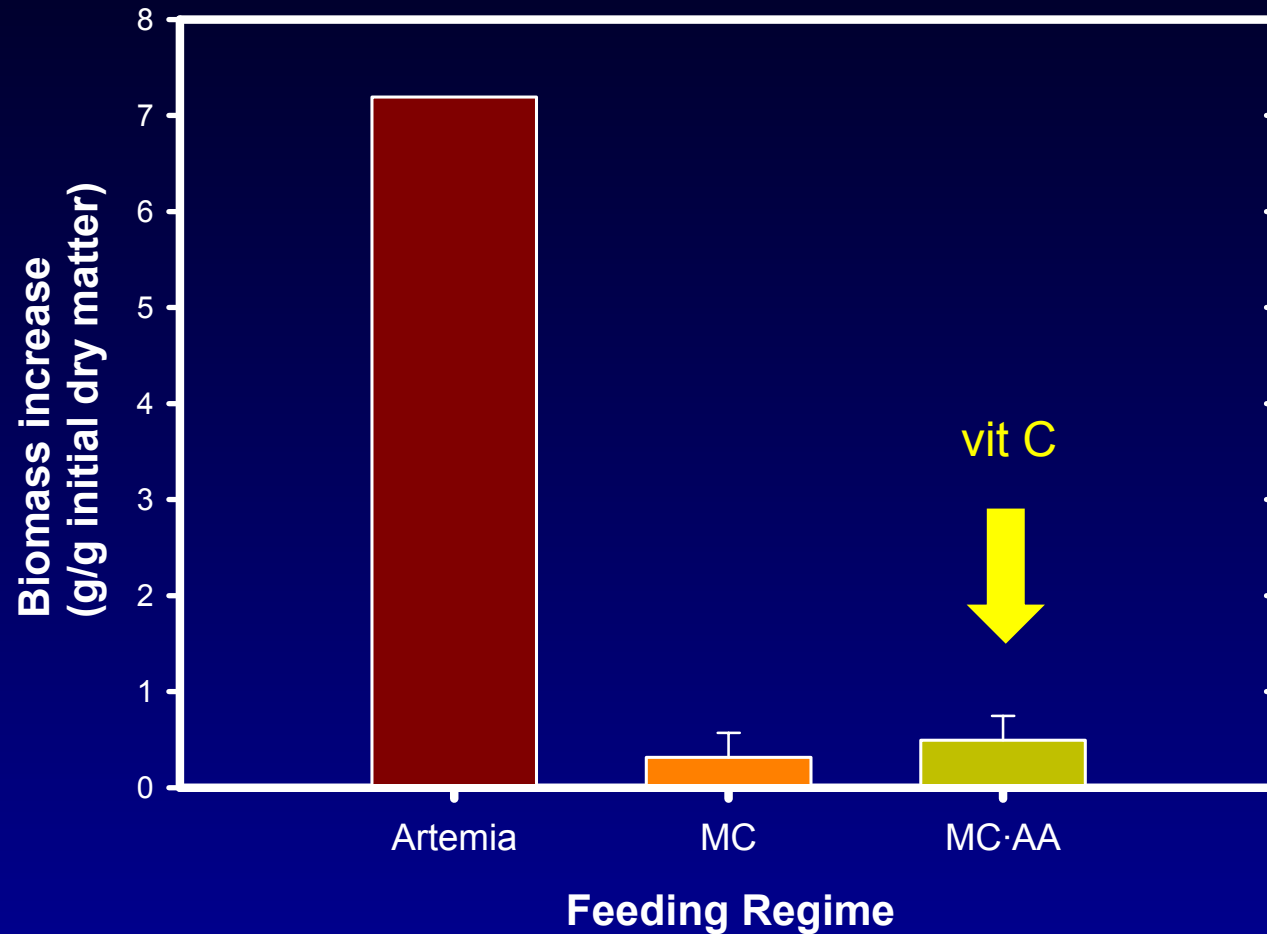
Vitamin C

Larval Growth *Solea senegalensis*



Vitamin C

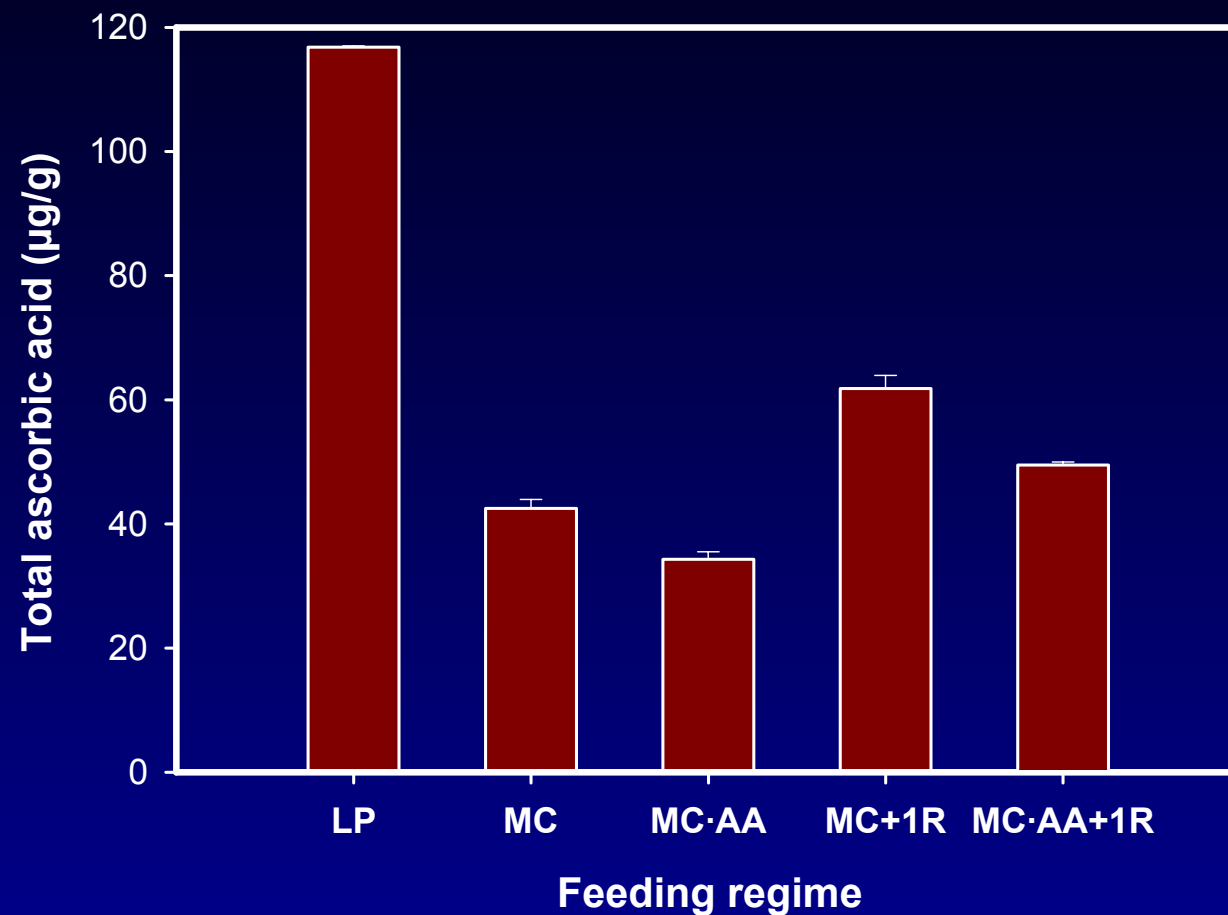
Total Biomass *Solea senegalensis*



Vitamin C

Ascorbate in larval tissues

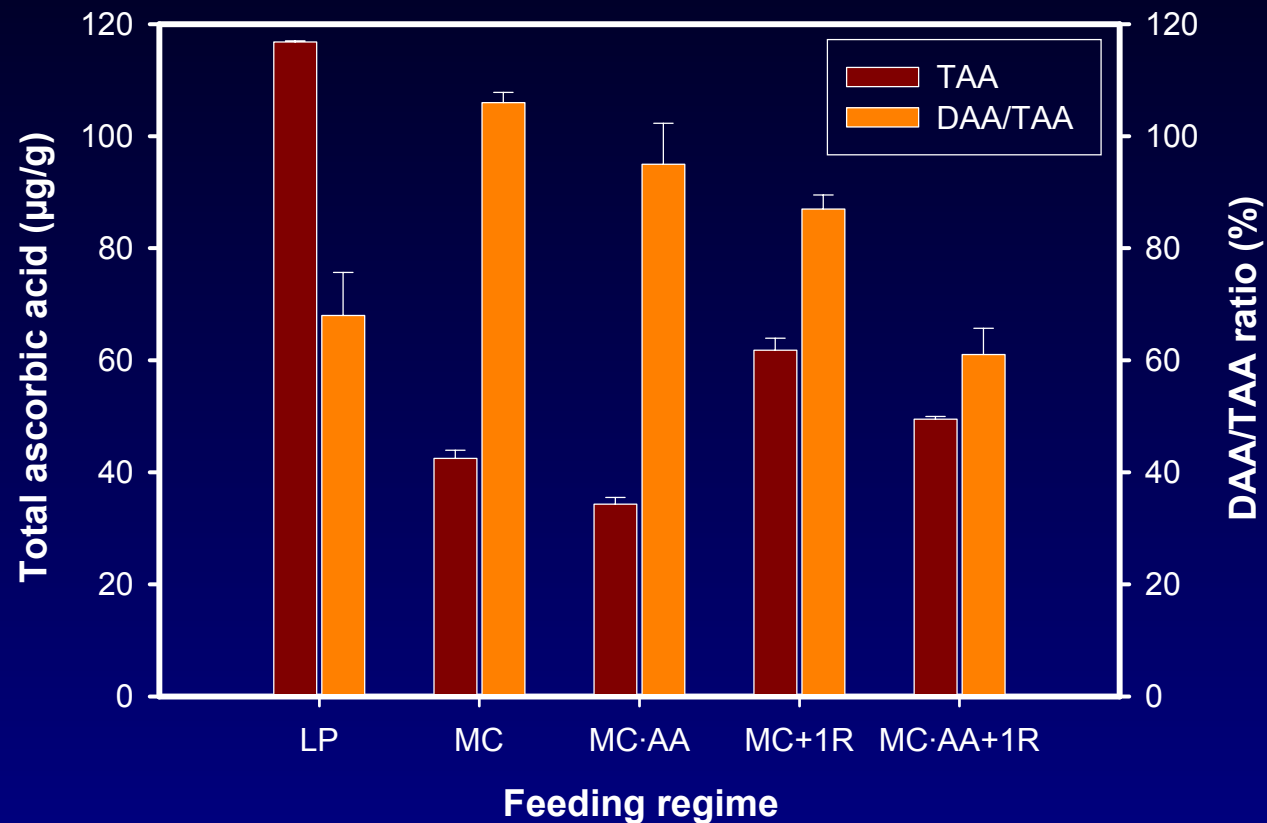
Sparus aurata



Vitamin C

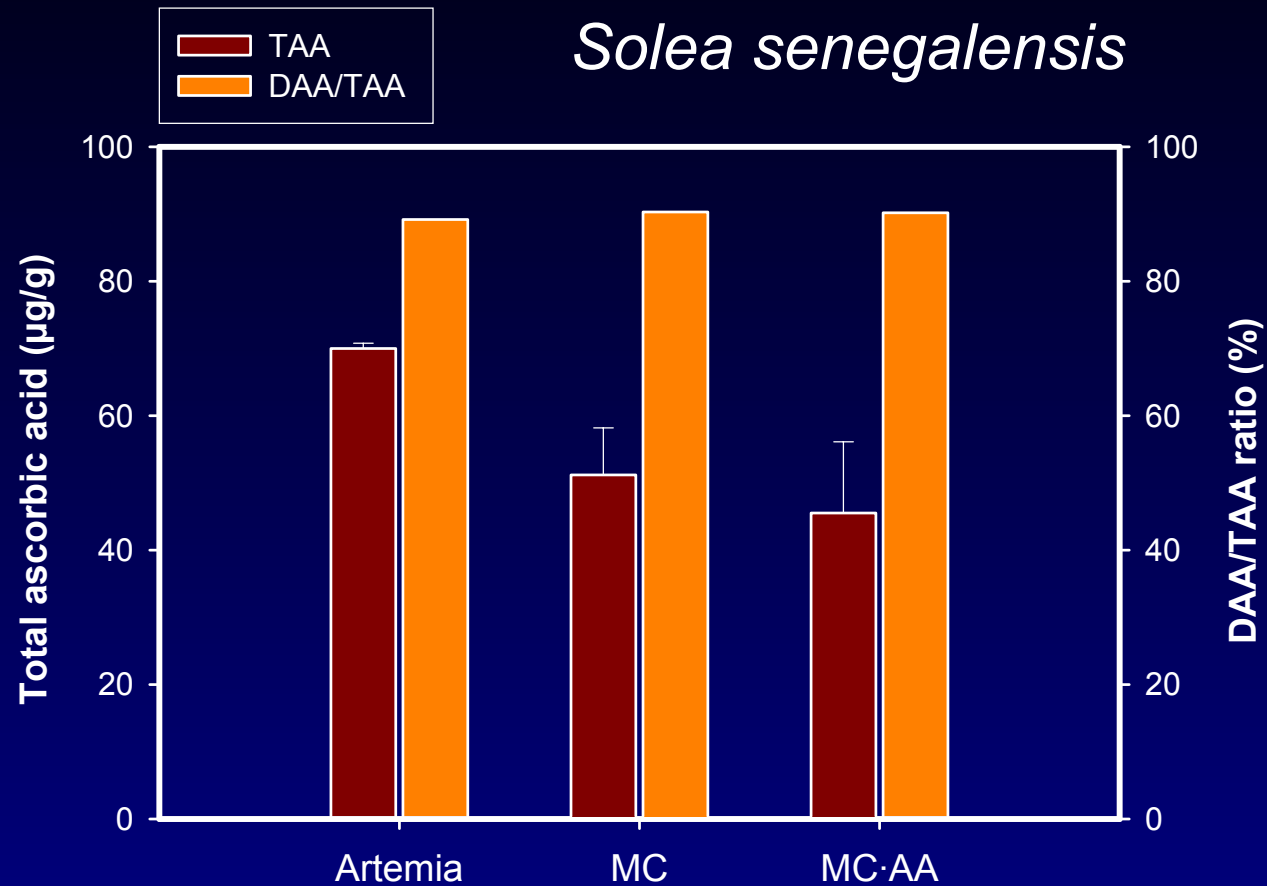
Ascorbate in larval tissues

Sparus aurata



Vitamin C

Ascorbate in larval tissues



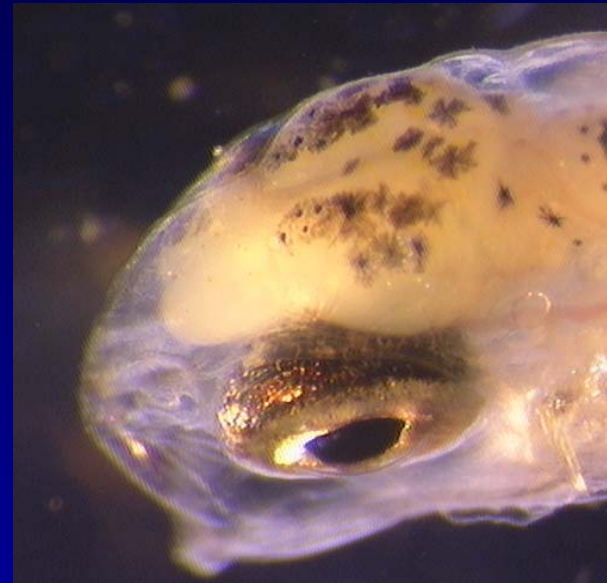
SUMMARY OF RESULTS

	Capsules	Water	Larvae	Response
Hormones	--	--	yes	--
FAA	yes	yes	--	--
Vitamin C	yes	--	yes	yes ?

CONCLUSIONS

- Protein-walled microencapsulated diets can be used for delivering of a variety of micronutrients and specific compounds in the digestive track of larval fish
- Particular research and adjustments are required for each new substance to improve its encapsulation efficiency
- To examine properly larval response it is necessary to check that the substance is effectively delivered into the digestive track

Sparus aurata



ACKNOWLEDGEMENTS

- Commission for Cultural, Educational and Scientific Exchange between the United States of America and Spain (The Fulbright Program), supported this work.
- We thank CUPIMAR SA (San Fernando, Cádiz, Spain) the supply of fish eggs.