



Greater amberjack larval rearing in IEO

Effect of live prey enrichments and feeding regime



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Main Work Packages (WP) & research activities in Oceanographic Centre of Canary Islands (IEO-Canarias)




WP 3. Reproduction & Genetics - greater amberjack

- Task 3.4 Development of an optimized spawning induction protocols for F1 greater amberjack in the eastern Atlantic.  





WP 9. Nutrition - greater amberjack

- Task 9.1 Improve larval enrichment products to enhance production of larvae and juveniles   
 - Subtask 9.1.2 - Study the combined effect of PUFA-rich lipids and carotenoids
- Task 9.3 Design adequate feeding regimes for broodstock to optimize reproduction  


WP 15. Larval husbandry - greater amberjack

- Task 15.1 Effect of feeding regime and immune-stimulants  
- Task 15.4 Development of industrial protocol 

WP 21. Grow out husbandry - greater amberjack

- Task 21.2 Development of feeding methods.  
 - Action 21.2.3 Definition of feeding pattern for 200 g reared in 1000 l-tanks for 4 months
- Task 21.3 Development of appropriate husbandry practice.  
 - Action 21.2.3 Definition of feeding pattern for 200 g reared in 1000 l-tanks for 4 months

WP 25. Fish health - greater amberjack

- Task 25.4 Effectiveness of stocking density and anti-oncomiracidia attaching substances in the control of monogenean parasites  

Main Work Packages (WP) & research activities in Oceanographic Centre of Canary Islands (IEO-Canarias)

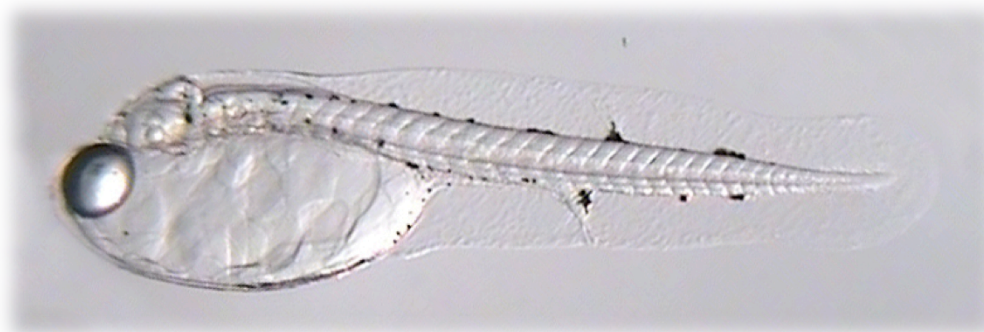
Greater amberjack larval rearing in IEO

WP 9. Nutrition - greater amberjack

- Task 9.1 Improve larval enrichment products to enhance production of larvae and juveniles
 - Subtask 9.1.2 - Study the combined effect of PUFA-rich lipids and carotenoids

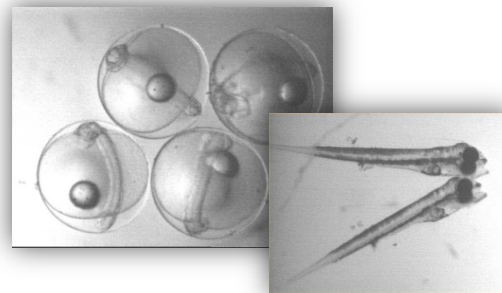
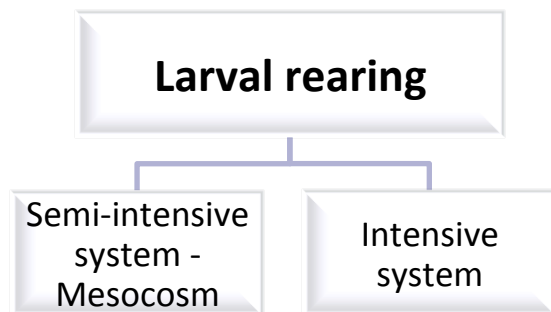
WP 15. Larval husbandry - greater amberjack

- Task 15.1 Effect of feeding regime and immune-stimulants
- Task 15.4 Development of industrial protocol



Introduction

Previous studies on greater amberjack larval rearing in IEO



Bottlenecks

- High early mortalities
- Scarce knowledge of nutritional requirements
- Need of new live food enrichments to improve health
- Development of appropriate feeding regimes
- Need to improve environmental factors

WP9 NUTRITION.

- Subtask 9.1.2 Combined effect of PUFA-rich lipids and carotenoids in enrichment products for live prey (rotifers) for greater amberjack



Scarce knowledge on larval nutritional requirements

■ Lipid requirements.

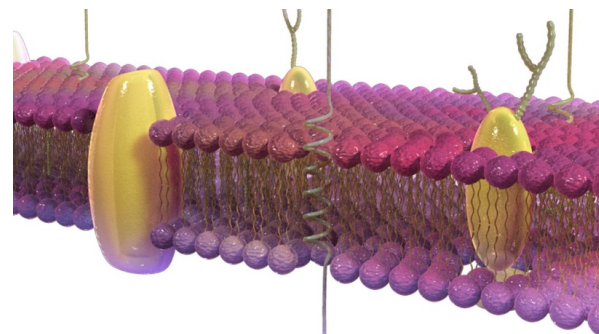
□ Long chain polyunsaturated fatty acids (LC-PUFA):

- essential components of cellular membranes
- modulate physiological processes: inflammatory and immune responses

Arachidonic Acid (ARA, 20:4n-6)

Eicosapentaenoic Acid (EPA, 20:5n-3)

Docosahexaenoic Acid (DHA, 22:6n-3)



□ Live food enrichment:

- triglycerides (TAG) or fatty acid ethyl esters. *Artemia* sp. incorporates LC-PUFA into TAG
- oils rich in **phospholipids (PL)** → dietary PL more efficient source of LC-PUFA for larvae.

WP9 NUTRITION.

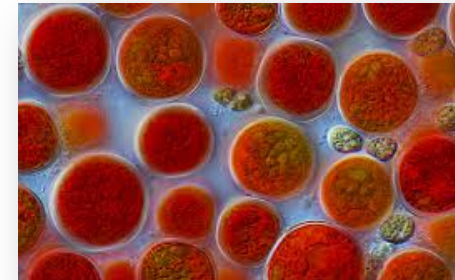
- Subtask 9.1.2 Combined effect of PUFA-rich lipids and carotenoids in enrichment products for live prey (rotifers) for greater amberjack



Scarce knowledge on larval nutritional requirements

■ Carotenoids requirements

- **Antioxidants:** To avoid the autoxidation of LC-PUFA and bioaccumulation of potentially toxic lipid peroxides into larvae
- **Astaxanthin**, high oxygen quenching abilities



Aim: To determine the combined effect of LC-PUFA-rich lipids and carotenoids in greater amberjack enrichment products evaluating their effects on survival, growth, welfare and lipid composition of greater amberjack larvae.

1. First preliminary rotifer enrichment assay

Rotifer enrichment to establish a good protocol for LC-PUFA enrichment accordingly to the lipid composition of wild seriola viable eggs.

- Four lipid enrichment treatments were tested by triplicate:
 - **E1**: polar rich emulsion, **marine natural lecithin** LC60 (PhosphoTech Laboratories, France) with up to 60% phospholipids.
 - **E2**: a blend of these lipid sources: **marine lecithin** + DHA-rich **TAG** oil + cod liver oil
 - **E3**: a combination of **different TAG sources** (DHA-rich TG oil and cod liver oil)
 - **C**: commercial enrichment product DHA-PROTEIN SELCO enrichment
- Experimental emulsions supplemented with free **arachidonic acid**.
- Culture conditions: Tank volume: 10 l, continuous light and aeration, temperature 20°C, initial density: 300 rot/ml., sampling at 0, 3, 6, 10 and 24 hours.



Ingredients of the experimental lipid emulsions.

	E1	E2	E3
<i>Ingredients (mg L⁻¹)</i>			
Marine lecithin	78	23.6	0
Incromega DHA 500	0	39.3	47.2
Cod liver oil	0	15.7	31.5
Arachidonic acid	2	1.4	1.3

1. First preliminary rotifer enrichment assay

Total Lipid (TL, % dry matter, DM), Triglycerides (TAG) and Total Polar Lipid (TPL, % TL) and main Fatty Acid composition (% of total FA) of TPL of rotifers enriched for 3, 6 and 10 h with the control and experimental lipid emulsions.

	Control			E1			E2			E3		
	3 h	6 h	10 h	3 h	6 h	10 h	3 h	6 h	10 h	3 h	6 h	10 h
TL (% DM)	12.8±1.1 a,B	16.6±1.7 b,B	17.6±1.2 b,A	19.9±1.4 b A	16.0±1.6 a,B	15.6±0.3 a,A	20.6±1.5 A	22.9±0.8 A	26.0±3.5 B	12.7±1.6 a,B	13.9±2.1 ab,B	16.5±1.0 b,A
TG (% TL)	33.5±0.7 a,B	39.8±1.8 b,A	46.1±1.9 c,B	24.5±0.9 a C	33.5±0.3 b,B	36.1±1.3 c,A	43.4±0.8 A	42.2±2.4 A	42.5±0.4 B	35.9±2.5 a,B	41.6±2.4 ab,A	44.9±3.2 b,B
TPL (% TL)	26.1±1.9 A	26.0±2.2 A	23.3±1.3 B	31.5±2.9 A	31.1±0.8 A	29.4±1.5 C	13.6±0.5 C	17.5±2.7 C	13.1±2.1 A	24.9±0.2 B	24.1±1.9 B	22.8±1.8 B
PL-fatty acids (%)												
20:4n-6	0.8±0.1 a,B	1.6±0.0 b,C	1.7±0.1 b,A	4.0±0.0 A	4.6±0.5 A	4.8±0.1 C	3.7±0.3 ab,A	4.4±0.3 b,A	3.5±0.3 a,B	3.7±0.2 A	3.6±0.0 B	3.6±0.1 B
20:5n-3	1.1±0.1 a,D	3.3±0.2 b,B	3.3±0.2 b,A	6.4±0.2 A	6.5±0.7 A	6.3±0.3 C	5.0±0.5 a,B	6.4±0.6 b,A	5.5±0.4 ab,BC	3.1±0.3 C	4.1±0.6 B	4.4±0.8 AB
22:6n-3	2.4±0.2 a,C	11.4±0.4 b,B	11.5±0.6 b,A	16.3±0.5 b A	16.5±2.8 ab,A	14.4±0.2 a,A	15.3±1.1 A	18.1±2.8 A	19.7±0.6 B	7.4±0.1 a,B	9.5±0.1 ab,B	12.2±2.1 b,A
∑ n-3 HUFA	4.3±0.3 a,C	16.0±0.7 b,B	16.8±0.9 b,A	23.2±0.5 A	23.7±2.4 A	22.2±0.5 B	21.0±1.7 a,A	25.6±2.2 b,A	26.9±0.6 b,C	11.2±0.4 a,B	14.9±0.5 b,B	19.0±2.9 b,AB

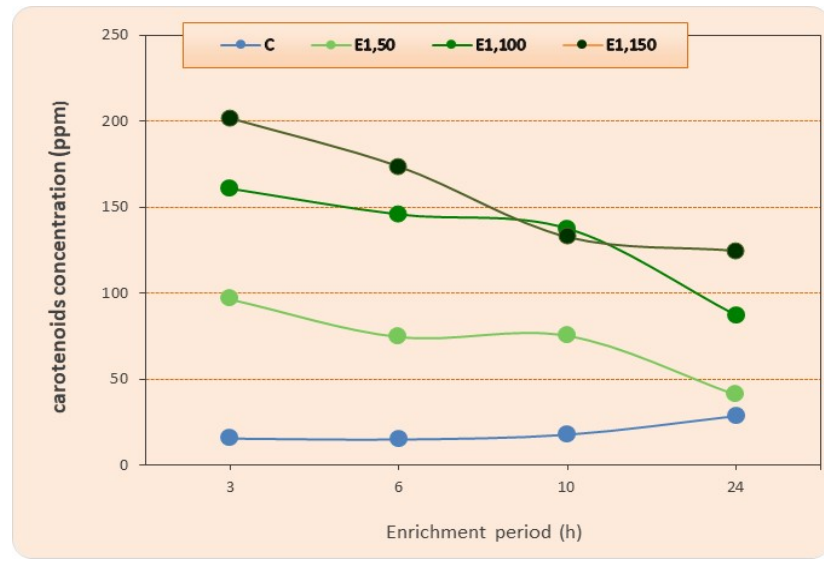
The best combination in terms of rotifer PL absolute contents and proportions of DHA, EPA and ARA was achieved with the marine lecithin used in treatment E1 for 3h.

2. Second preliminary rotifer enrichment assay

Treatment E1 (100% marine natural lecithin LC60 for 3 hours) was combined with three levels of carotenoids (astaxanthin-Naturese®) at 6% concentration:

- 50 ppm
- 100 ppm
- 150 ppm

Time course of **carotenoids content** (ppm) in rotifers enriched with lipid emulsion C or E1 supplemented with increasing levels of astaxanthin (50, 100 and 150 ppm).

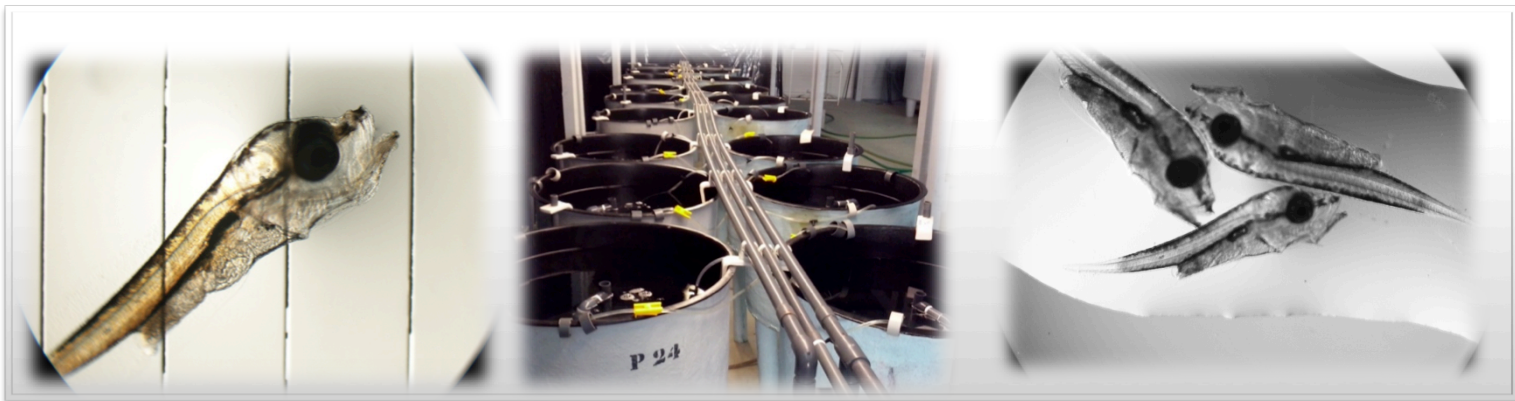


- ✓ Maximum absorption of carotenoids after 3h
- ✓ High concentration at the lower supplementation

➤ Rotifers enriched for short periods (3h) with 6% of the marine lecithin with a slight supplementation of AA (E1) in combination with a range of carotenoids well below 50 ppm

3. Effect on greater amberjack larval rearing.

- Commercial enrichment compared with best experimental emulsions at 6% and 3h:
 - E1 (100% marine lecithin)
 - E1,10 (E1+10 ppm carotenoids)
 - E3,10 (100% blend of oils rich in TAG+10 ppm carotenoids)
- 100-l triplicate tanks, 5000 larvae/tank, continuous water exchange and light
- 13 days

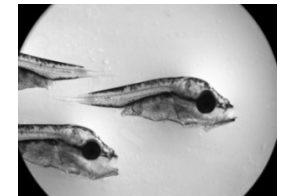
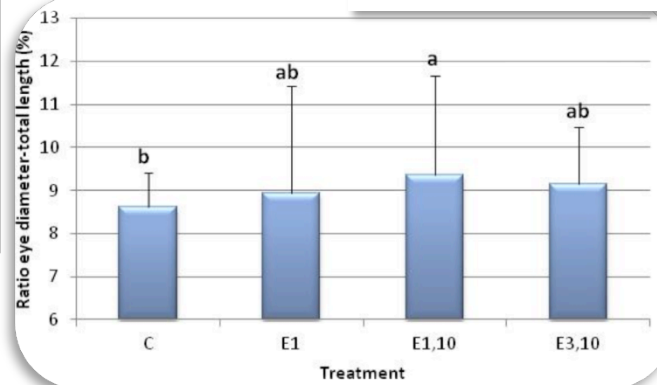
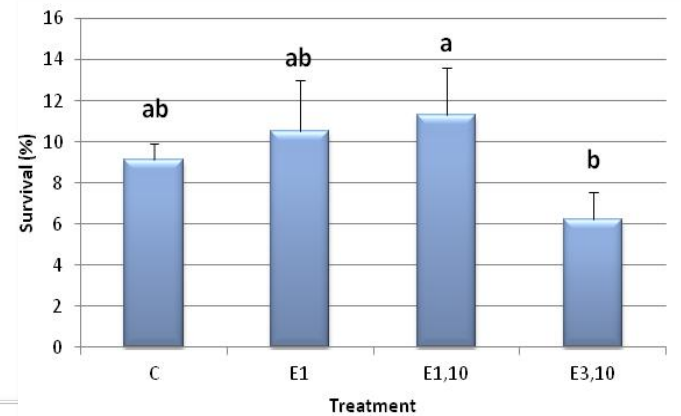
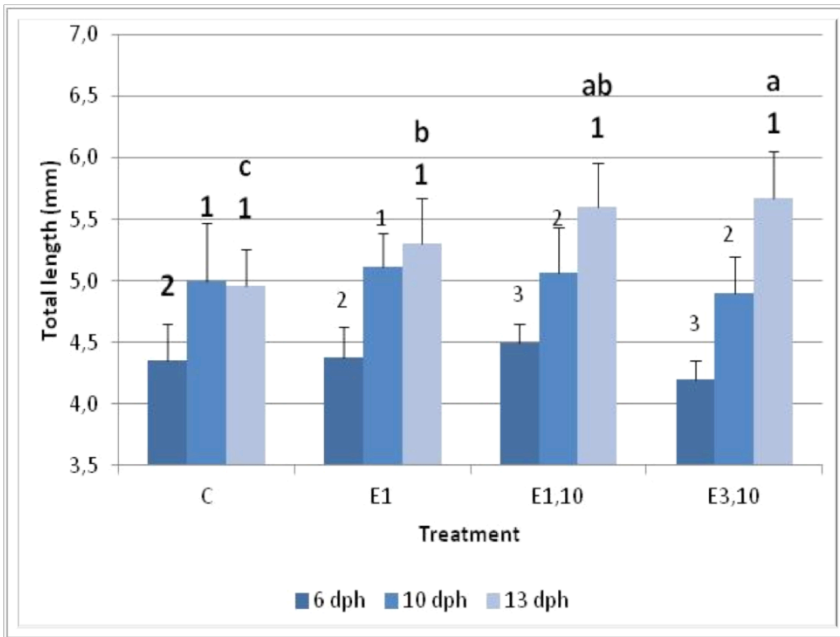


3. Effect on greater amberjack larval rearing.

■ Larval performance

- ✓ Fish **total length** was higher in E1,10 and E3,10 at 13 dph

- ✓ Higher **survival** in larvae from treatment E1,10 than E3,10
- ✓ E1,10 larvae showed the highest **eye diameter to total length ratio**



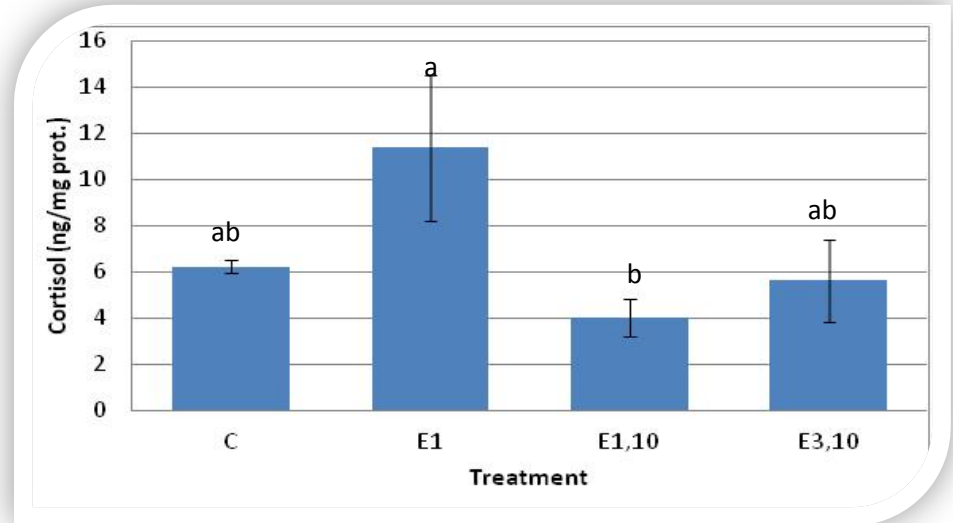
Total length (mm) of greater amberjack larvae, fed with rotifers enriched with commercial and experimental (E1, E1,10 and E3,10) emulsions at 6, 10 and 13 dph.

Final survival percentage (13 dph) and eye diameter to total length ratio (%) of greater amberjack larvae, fed with rotifers enriched with C and E1, E1,10 and E3,10 emulsions.

3. Effect on greater amberjack larval rearing.

■ Welfare

- Whole body cortisol lower for E1,10.
- Whole body lactate higher for commercial treatment



Whole body cortisol (ng/mg prot) levels in 13 dph greater amberjack larvae fed with rotifers enriched with commercial (C) and experimental (E1, E1,10 and E3,10) emulsions. Values are mean + S.D., n=3. ANOVA (P ≤0.05); Tukey's HSD).

Whole body glucose (mg mg protein⁻¹), lactate (mg mg protein⁻¹), Na⁺ (mg mg protein⁻¹) and K⁺ (mg mg protein⁻¹) in 13 dph greater amberjack larvae fed with rotifers enriched with commercial (C) and experimental (E1, E1,10 and E3,10) emulsions. Values are mean + S.D., n=3. ANOVA (P ≤0.05); Tukey's HSD).

	C	E1	E1,10	E3,10
Glucose	0.14 ± 0.05	0.10 ± 0.04	0.09 ± 0.05	0.15 ± 0.06
Lactate	1.76 ± 0.06 a	0.68 ± 0.13 b	0.65 ± 0.35 b	0.75 ± 0.14 b
Na ⁺	250.22 ± 26.05	160.12 ± 124.18	104.46 ± 6.09	261.78 ± 74.17
K ⁺	17.22 ± 3.77	11.36 ± 4.51	8.83 ± 1.41	7.69 ± 2.23

3. Effect on greater amberjack larval rearing.

■ Total lipid and fatty acid contents

Main FA composition (% of total FA) of total lipid (TL) and total polar lipid (TPL) of larvae enriched with the three experimental emulsions (E1; E1,10; E3,10). Values are mean + S.D., n=3.

TL	E1,0		E1,10		E3,10		TPL	E1,0		E1,10		E3,10	
18:1n-9	10,38	± 0,27	12,20	± 1,71	11,83	± 0,88	18:1n-9	10,02	± 0,34	10,82	± 0,63	11,32	± 0,34
18:2n6	1,92	± 0,22	2,54	± 0,52	3,00	± 0,37	18:2n6	1,92	± 0,24	2,43	± 0,45	2,76	± 0,48
20:4n6	6,87	± 0,22	6,38	± 0,56	6,25	± 0,41	20:4n6	6,86	± 0,28	6,47	± 0,37	6,06	± 0,25
20:5n3	4,91	± 0,46	5,73	± 1,37	8,72	± 0,74	20:5n3	4,80	± 0,66	5,55	± 1,44	7,97	± 0,93
22:6n3	24,77	± 1,32	21,99	± 2,54	17,79	± 1,38	22:6n3	25,28	± 1,32	23,22	± 1,97	18,13	± 1,05
SFA	29,01	± 0,20	29,21	± 1,48	28,11	± 0,10	SFA	29,54	± 0,25	29,57	± 0,89	29,60	± 0,74
MUFA	22,35	± 0,52	23,85	± 1,46	24,32	± 0,25	MUFA	21,25	± 0,75	22,28	± 0,87	22,78	± 0,39
PUFA	44,70	± 0,62	43,24	± 2,22	43,41	± 0,15	PUFA	44,84	± 0,04	44,11	± 0,42	42,23	± 0,30
n3/n6	2,95	± 0,02	2,87	± 0,04	2,85	± 0,12	n3/n6	3,07	± 0,04	3,01	± 0,04	2,94	± 0,06
n3 HUFA	31,69	± 0,83	29,69	± 1,75	29,54	± 0,73	n3 HUFA	32,19	± 0,45	30,88	± 0,49	29,02	± 0,52

- Higher contents of DHA in both E1 and E1,10 larval TL and TPL. E1 better resembling wild egg DHA/ARA/EPA ratios.

CONCLUSION: Rotifers enriched for 3h with a polar rich emulsion containing a marine natural lecithin (LC60) and 20:4n-6, combined with 10 ppm of Naturose (E1,10), resulted in a significant advantage for seriola larval growth, survival and welfare

WP 15. LARVAL HUSBANDRY

- Task 15.1. Effect of feeding regime and immune-stimulants



New live food enrichments to improve health

The stimulation of the larval immune system is a promising tool to increase survival rates at early stages of fish

Echium plantagineum seeds oil: decrease stress symptoms and improve responses to disease in several fish species



Black cumin *Nigella sativa* seed, enhance growth performance and immunity in fish



Feeding regimes

Appropriate management of feeding regimes leads to the improvement of larval fish production efficiency.

Nutrition of fish larvae depends on prey concentration → A direct correlation between rotifer concentration and larval survival.

AIM

- To test the combined effect of enrichment products containing immune-stimulants, *Echium* and black cumin oils using live preys as vectors, and feeding regime, prey concentrations and feeding frequency.

1- Product and enrichment time selection (preliminary assay)

□ Rotifers culture: 10-l tanks, 300 rot ml⁻¹, light, sampling at 1, 3, 6, 10 & 24 hours

□ Treatments:

T1: commercial protocol (S.presso®)

T2: marine lecithin LC60+AA+10 ppm carotenoids.

T3: T2+20% *Echium* oil

T4: T2+10% *Echium* oil



✓ The enrichment protocol based on LC60+AA+10 ppm carotenoids supplemented with 20% *Echium* oil for 3 hours was selected

Results

Rotifer population characteristics and culture media conditions during enrichment for 24 hours.

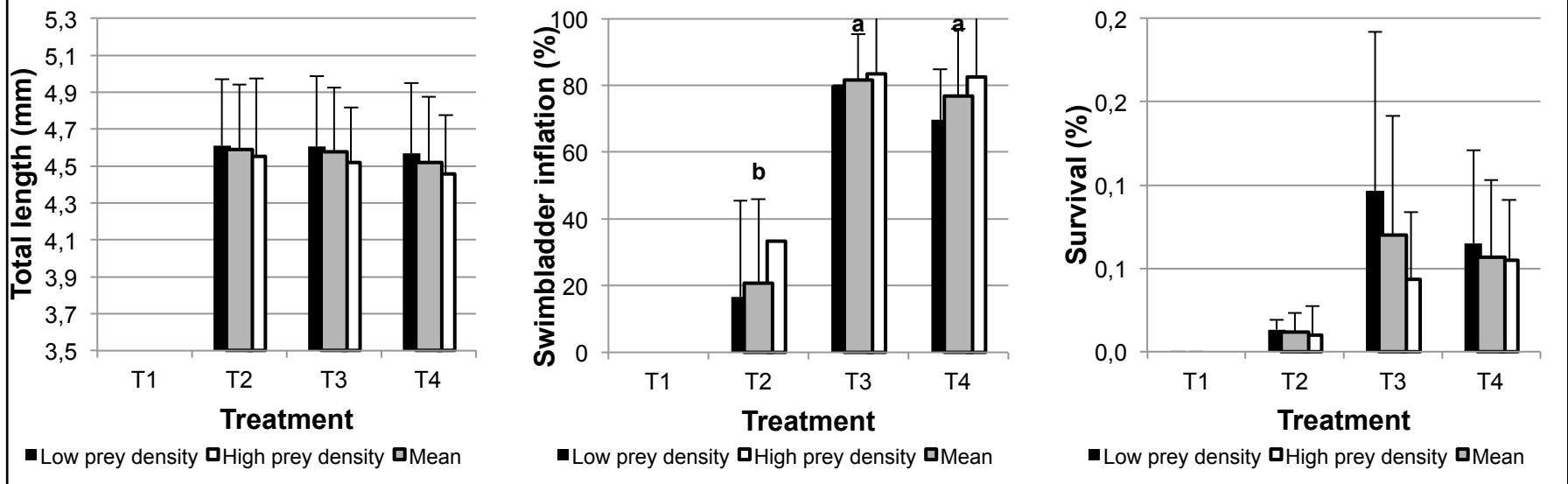
T	M	Rotifers ml ⁻¹	Survival (%)	Ovigers (%)	Temp. (°C)	Oxygen (%)
T1	1	241 ± 24 a	80.3 ± 8.0 a	21.4 ± 4.6 a	22.9 ± 0.1 a	87.5 ± 6.0
	3	236 ± 12 a	78.6 ± 4.1 a	18.8 ± 3.3 ab	22.5 ± 0.0 b	85.3 ± 5.5
	6	205 ± 24 ab	68.5 ± 8.1 ab	15.6 ± 6.2 ab	21.9 ± 0.1 c	86.4 ± 8.7
	10	178 ± 22 ab	59.4 ± 7.4 ab	12.2 ± 5.4 ab	21.6 ± 0.0 d	86.5 ± 6.2
	24	153 ± 35 b	51.1 ± 11.8 b	6.86 ± 2.1 b	20.2 ± 0.1 e	95.3 ± 1.0
T2	1	266 ± 15 a	88.6 ± 5.0 a	22.6 ± 3.2 a	22.8 ± 0.1 a	85.9 ± 2.4 b
	3	219 ± 25 ab	73.2 ± 8.3 ab	20.5 ± 3.5 a	22.4 ± 0.1 b	80.9 ± 5.6 b
	6	164 ± 16 bc	54.8 ± 5.6 bc	9.46 ± 4.1 b	21.9 ± 0.1 c	83.8 ± 4.3 b
	10	141 ± 7 c	47.1 ± 2.5 c	8.60 ± 3.6 b	21.5 ± 0.1 d	81.6 ± 5.3 b
	24	124 ± 41 c	41.5 ± 13.9 c	4.16 ± 1.8 b	19.9 ± 0.1 e	96.0 ± 1.5 a
T3	1	265 ± 17 a	88.5 ± 5.9 a	25.0 ± 1.3 a	22.8 ± 0.1 a	86.2 ± 4.2 bc
	3	259 ± 26 a	86.5 ± 8.8 a	20.2 ± 1.2 ab	22.5 ± 0.1 a	84.4 ± 5.4 c
	6	230 ± 14 ab	76.8 ± 4.8 ab	16.0 ± 4.9 bc	21.9 ± 0.1 b	88.9 ± 2.5 ab
	10	220 ± 12 ab	73.3 ± 4.0 ab	13.1 ± 2.9 bc	21.6 ± 0.1 c	88.6 ± 5.1 ab
	24	179 ± 47 b	59.8 ± 15.8 b	9.50 ± 2.6 c	20.0 ± 0.1 d	97.7 ± 0.7 a
T4	1	257 ± 28	85.6 ± 9.5	20.6 ± 5.6 a	22.8 ± 0.1 a	87.7 ± 4.8 b
	3	256 ± 29	85.4 ± 9.9	19.2 ± 0.9 a	22.5 ± 0.1 a	89.3 ± 3.5 ab
	6	252 ± 14	84.1 ± 4.9	17.1 ± 3.2 ab	21.9 ± 0.1 b	93.5 ± 3.0 ab
	10	243 ± 31	81.2 ± 10.3	17.1 ± 2.6 ab	21.5 ± 0.2 b	92.9 ± 3.2 ab
	24	209 ± 31	69.7 ± 10.5	9.00 ± 3.9 b	20.0 ± 0.2 c	97.9 ± 2.3 a

Means ± SD. Different letters significant differences ($P < 0.05$) at each time for each treatment; different numbers significant differences ($P < 0.05$) among treatments for each time.

2. Effects of selected enrichment products supplemented with **immune modulators** substances and **rotifers density** in larval rearing of *S. dumerili*

- ❑ **Larval culture:** 18 tanks (100 l), 100 larvae l⁻¹, water exchange (0.20 l min⁻¹), light (24 h), temperature (24.4±0.1°C) and oxygen (6.7±0.1 mg l⁻¹).
- ❑ **Rotifers** enriched with: **T1**=commercial; **T2**=LC60+AA+10 ppm carot.; **T3**=T2+20% **Echium oil**; **T4**=T2+20% **black cumin oil (*Nigella sativa*)** .
- ❑ **Rotifers** added at **two prey density** (5 and 10 rot ml⁻¹) twice a day (8:00 & 16:00)

Larval performance at 12 dph

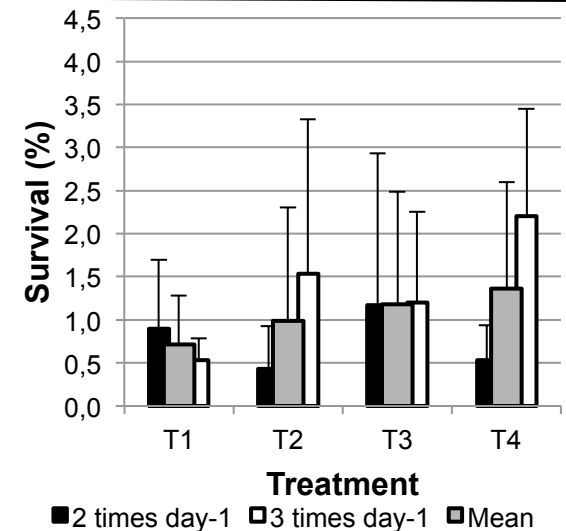
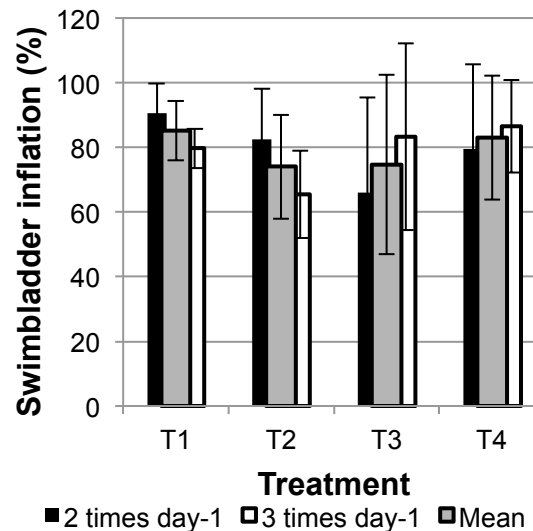
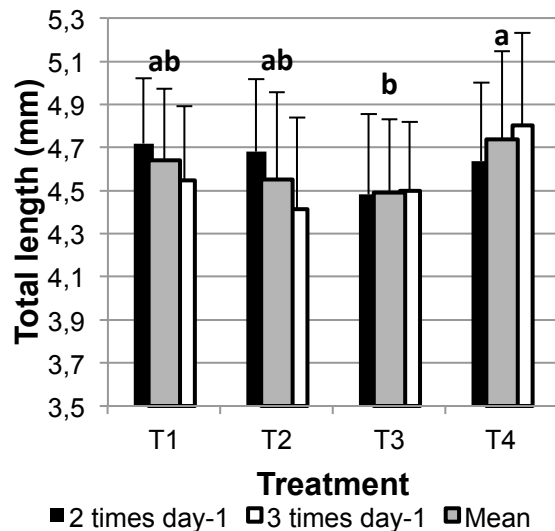


- ✓ **The commercial treatment (T1) showed the worst results**
- ✓ **Rotifer's density (5-10 rot ml⁻¹) did not affect larval performance**
- ✓ **Positive effect of the experimental emulsions on larval performance, particularly when combined with immune-promoter oils**

3. Effects of selected enrichment products supplemented with **immune modulators** substances and **feeding frequency** on *S. dumerili* larval rearing

- ❑ **Larval culture:** 24 tanks (100 l), 50-80 larvae l⁻¹, water exchange (0.20 l min⁻¹), light (24 h), temperature (22.1-23.5°C) and oxygen (6.7-6.5 mg l⁻¹).
- ❑ **Rotifers** enriched with: **T1**=commercial; **T2**=LC60+AA+10 ppm carot.; **T3**=T2+20% *Echium* oil; **T4**=T2+20% **black cumin oil**.
- ❑ **Rotifers** (5 rot ml⁻¹) **twice** (10:30 & 20:30) or **three times a day** (10:30, 15:30 & 20:30)

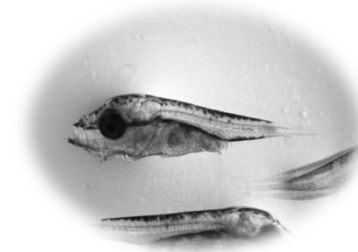
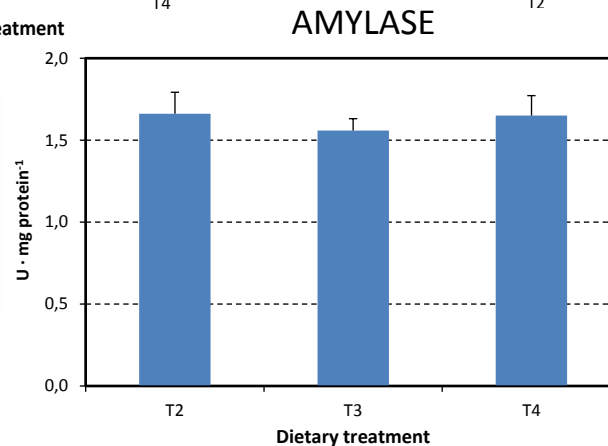
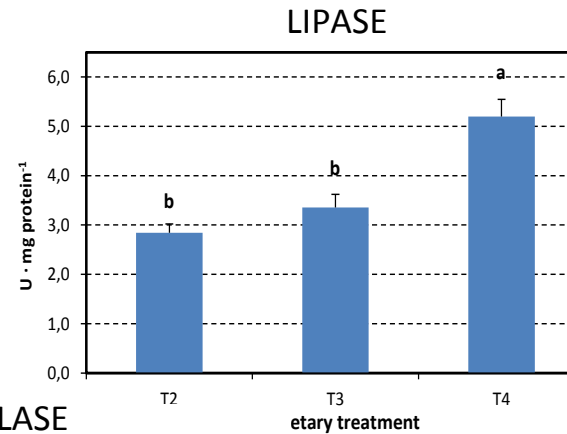
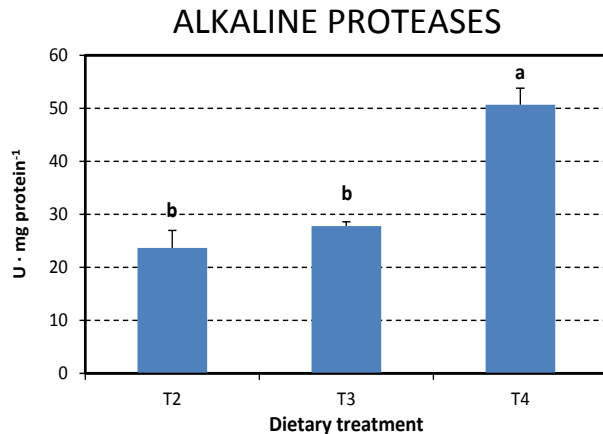
Larval performance at 12 dph



- ✓ Feeding frequency neither affect larval growth, swim bladder and eye development, nor survival
- ✓ Dietary regime significantly affected larval growth at 12 dph (P < 0.05).

3. Effects of selected enrichment products supplemented with **immune modulators** substances and **feeding frequency** on *S. dumerili* larval rearing

Digestive enzyme activities



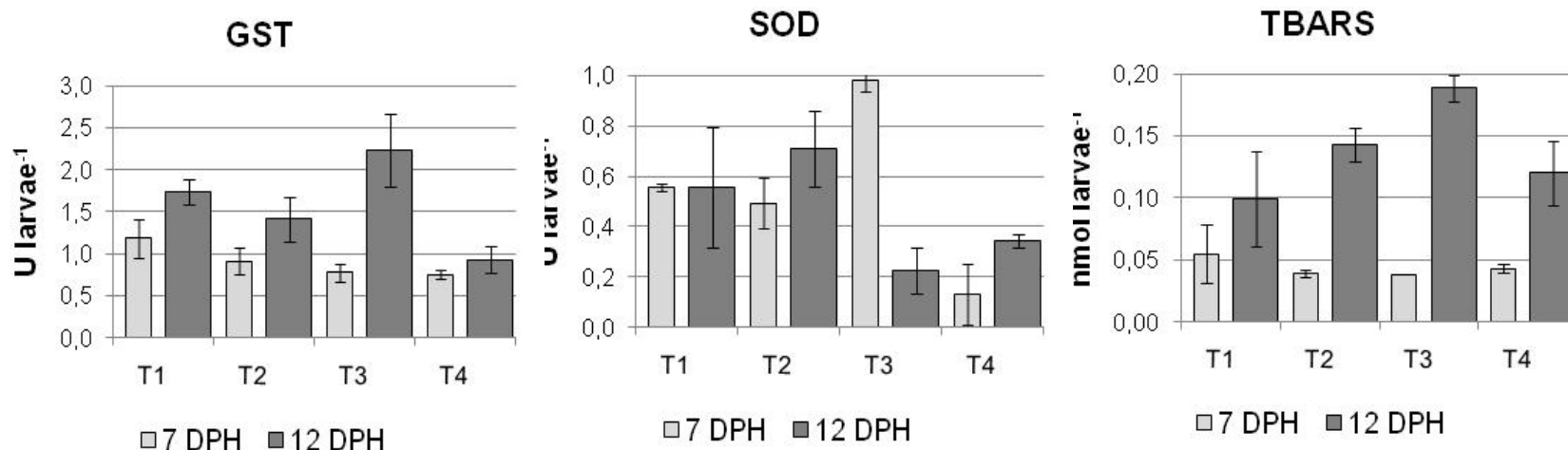
Digestive enzymes activities of 12 dph greater amberjack larvae fed rotifers. Values are mean \pm SD (n=3). (ANOVA, $P < 0.05$).

✓ The protease alkaline and lipase activities were higher in T4 (black cumin oil)

3. Effects of selected enrichment products supplemented with **immune modulators** substances and **feeding frequency** on *S. dumerili* larval rearing

Antioxidant enzymes and lipid peroxidation

- The activities of the enzymes involved in the oxidative stress response were affected by immunostimulants depending on larval age



Antioxidant enzymes Glutathione S-transferase (GST) and Superoxide dismutase (SOD) and of 7 and 12 dph greater amberjack larvae.

In summary: The results suggest the positive effect of experimental live prey enriching emulsions supplemented with immune-modulators such as *Echium* oil and black cumin oil compared to commercial emulsions on larval performance of *Seriola dumerili*.



Take home message

Propose protocol for greater amberjack larval rearing

- Mesocosm tank, Initial density of 3 larvae /L.
- Phytoplankton (*Chlorella sp*) until 25 dph (~15000 cell cm³).
- Rotifers (*Brachionus plicatilis*) from 3 to 25 dph:
 - Enriched with **6% of marine lecithin LC60 + Arachidonic acid + 10 ppm carotenoids supplemented with 20% black cumin oil for 3 hours**. DHA 14% TFA, EPA 6% TFA.
 - Rotifer density: 5 rotifers/ml.
 - Feeding frequency: two times a day.

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