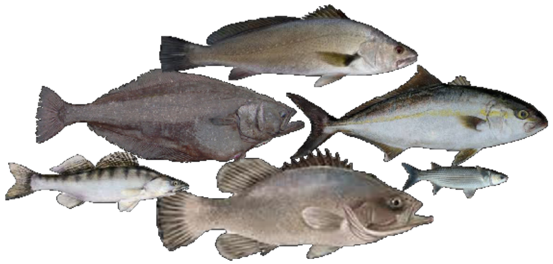


Summary of tasks carried out in 2015 in larval husbandry

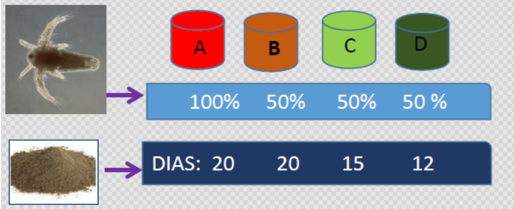
GWP Larval Husbandry
WP14: Meagre

IRTA, ULL
ACM 2016
Nancy, 2-4 February 2016

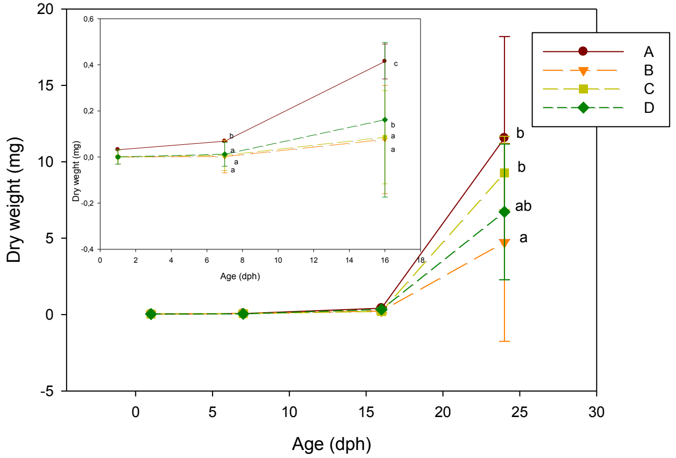
WP14.-Larval husbandry

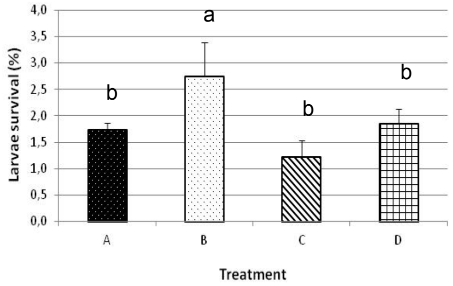
Task 14.1 Determining the earliest and most cost effective weaning period (IRTA).

Two trials were carried out at IRTA in 2014 and 2015.
In 2014 a high cannibalistic behaviour was observed, with (1) big differences in size among the larvae of the same tank and (2) a low survival rate



	Alimento			
Grupo	Rotífero días	Artemia días	Artemia %	Pienso días
A	2-14.	8-30.	100%	20
B	2-14.	8-30.	50%	20
C	2-14.	8-30.	50%	15
D	2-14.	8-30.	50%	12





Weight (mg)	Small	Medium	Large
A	4.52 ±0.97	16.06 ±9.58	38.28 ±8.41
B	1.28 ±0.16	8.00 ±7.69	18.67 ±9.75
C	3.73 ±1.55	12.27 ±3.79	23.17 ±5.11
D	3.01 ±1.29	8.91 ±3.89	26.41 ±2.77

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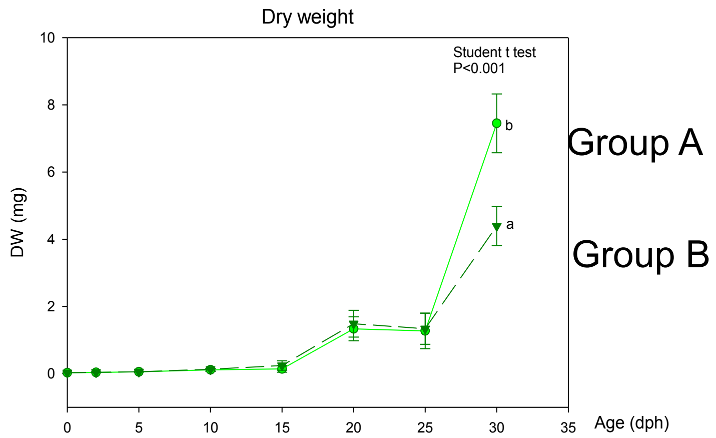
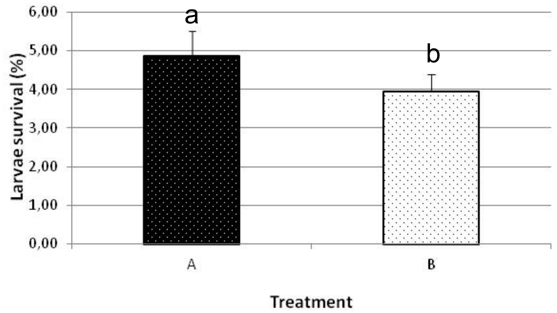
WP14.-Larval husbandry

Task 14.1 Determining the earliest and most cost effective weaning period (IRTA).

In 2015 only 2 groups (A –weaning at day 20- and B –early weaning at day 10-) were used, better results in survival rate were obtained. Care was taken to avoid cannibalism, reducing light intensity and increasing the number of doses of Artemia and dry feed



	Grupo A		Grupo B	
DÍAS	Average	Desvest	Average	Desvest
2	3,305	0,036a	3,243	0,021b
5	3,646	0,064	3,695	0,046
10	4,341	0,1432b	4,514	0,063a
15	6,227	0,086	6,260	0,107
20	7,994	0,359b	8,727	0,105a
25	11,032	0,393a	10,377	0,275b
30	13,948	0,308a	11,764	0,727b
Student t test P<0,001 (day 30)				



IRTA
INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIA

DIVERSIFY

ULL
Universidad de La Laguna

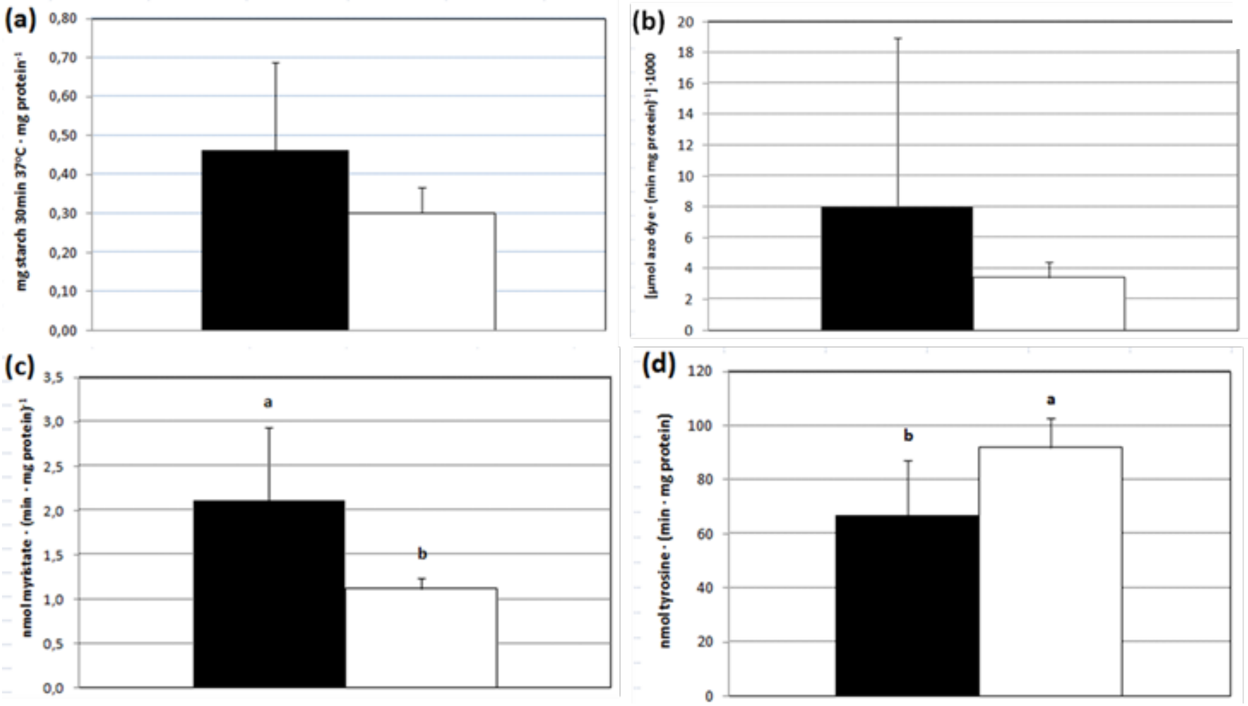
Meagre

WP14.-Larval husbandry

14.1.- Task 14.1 Determining the earliest and most cost effective weaning period (IRTA).

Digestive enzyme activity (ULL)

■ Early weaning (10dph)
□ Control weaning (20dph)



(a) amylase; (b) alkaline protease; (c) lipase; (d) pepsin

- Pancreatic enzymes tended to be more active in the early weaning larvae, with a higher ($P<0.05$) lipase activity compared to the control group.
- Capacity for pepsin digestion lower ($P<0.05$) in the early weaned larvae coinciding with the lower ($P<0.05$) growth rate achieved by this group.

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IRTA INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIA

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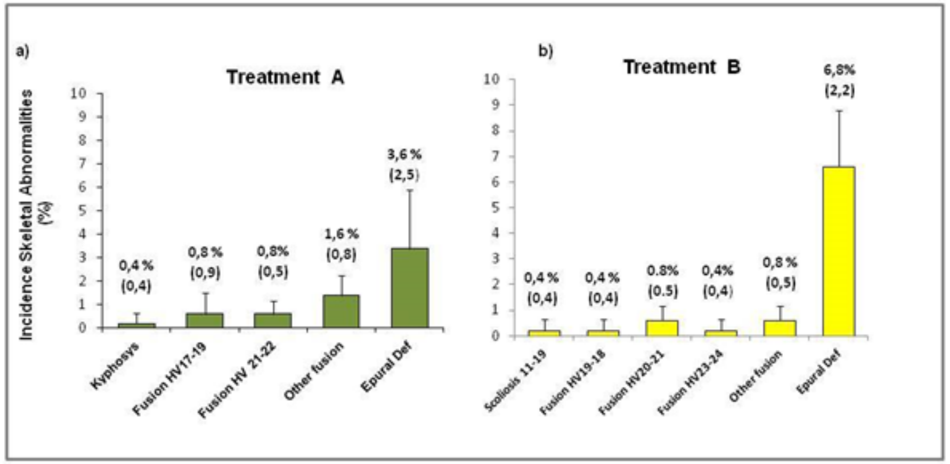
Meagre

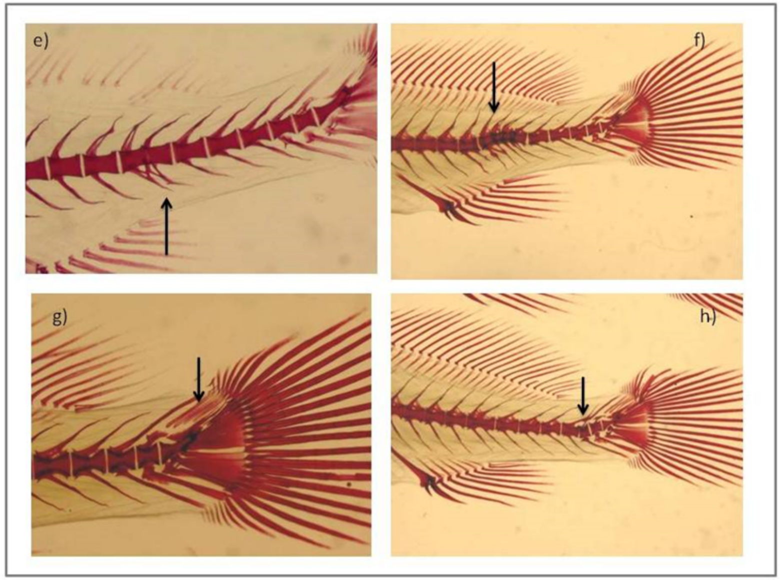
WP14.-Larval husbandry

Task 14.1 Determining the earliest and most cost effective weaning period (IRTA, ULL).

Skeletal Deformation



Different typologies of skeletal deformities (%) found in 36 dph A. regius larvae in two different feeding regimes, considering the number of abnormal skeletal elements per fish (mean±SD). HV haemal vertebrae; EP epural



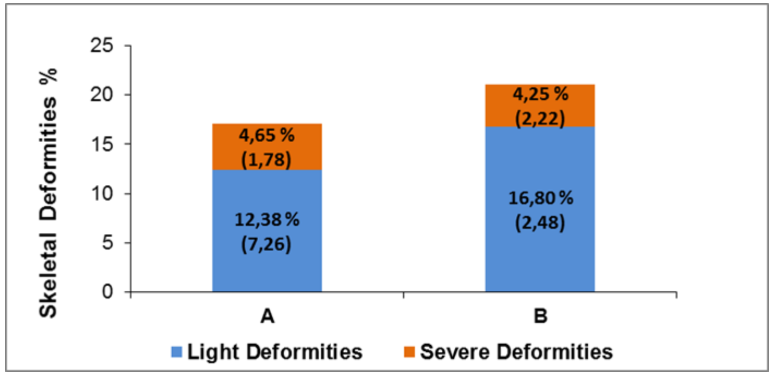
(e) Partial fusion in the haemal region 17-18 haemal vertebra (HV) and modification haemal spines (Hs). (f) Strong Fusion in the haemal region 17 to 19 HV and presence of fusion of two haemal spines closely parallels. (g) 2º Epural Modificated. (h) Torsion in caudal vertebra (23 -24 Ca V).

Generalitat Gobierno

WP14.-Larval husbandry

Task 14.1 Determining the earliest and most cost effective weaning period (IRTA, ULL).

Skeletal Deformation



Skeletal deformities (in %) in A. regius in different feeding regimes

- Skeletal deformations not a consequence of feeding regime.
- Since similar deformities observed in both treatments, other factors such as rearing environment, genetic background and broodstock feeding should be considered.

Conclusions

- Meagre larvae can be weaned from live feeds to artificial diet as early as 10 dph without compromising nutritional condition and skeletal deformities but others aspects such as growth and survival should be considered.
- Cannibalism can be controlled by increasing feeding frequencies, removing dominant individuals, performing regular gradings and keeping the larvae in the dark when food was unavailable or in short supply

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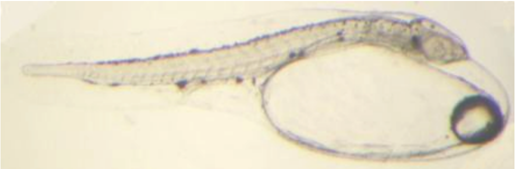
GWP Larval Husbandry
WP15: Greater amberjack

HCMR, ULL, FCPCT
ACM 2016
Nancy, 2-4 February 2016

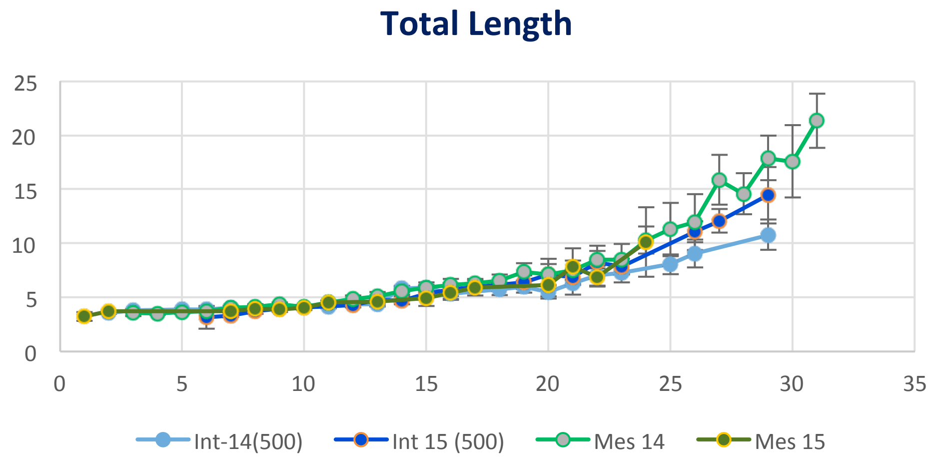
Comparison of semi-intensive and intensive rearing (task 15.2)



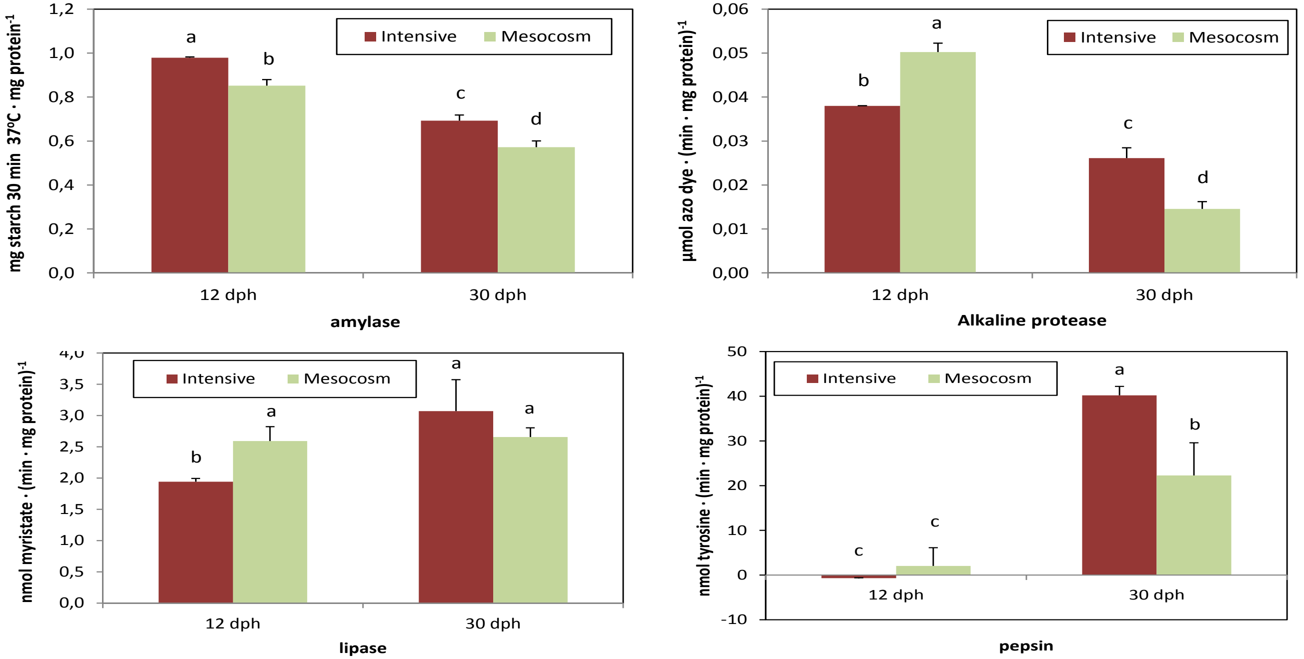
Growth performance (exp)

Mes 14: 0.0590 d^{-1}]
Int 14: 0.0490 d^{-1}] **

Mes 15: 0.0428 d^{-1}]
Int 15: 0.0638 d^{-1}] **



Digestive enzyme activity (RAS *vs.* Mesocosm)



- Amylase and alkaline protease were more active in the younger larvae, whereas lipase was similarly expressed in both aged-group larvae.
- Pepsin activity was almost null at 12dph, compared to that measured for 30 day-old larvae.
- Intensive rearing conditions favored amylase, alkaline protease and pepsin activities in the older larvae.
- Amylase activity was also higher at 12dph for the RAS system larvae, whereas the opposite trend was observed for alkaline protease and lipase activities.

Deliverable Number: **15.4**
Sub-task 15.2.1

Ontogeny of greater amberjack larval visual and digestive system reared in mesocosm and intensive rearing system.

- The deliverable has been completed

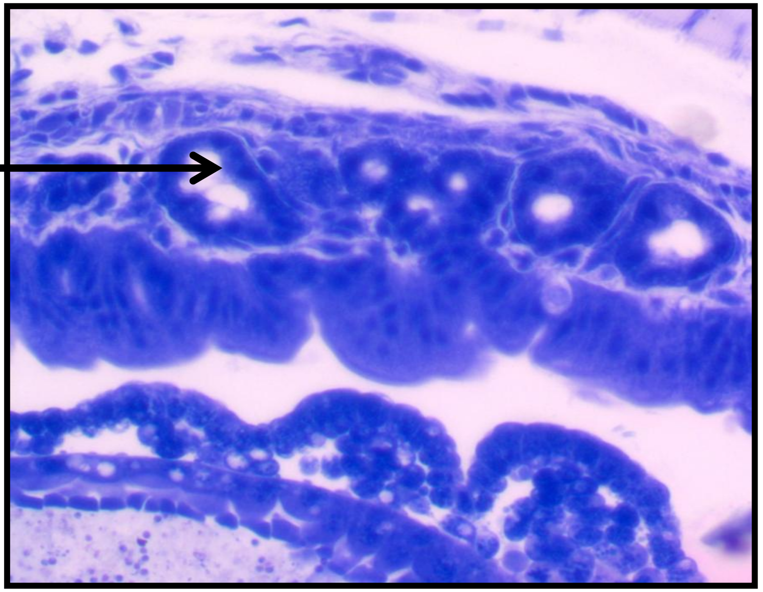
The study focused on the :

a) Ontogeny of the digestive system and the eye, and
b) Feeding preferences during larval rearing,

The final aim of the study was to evaluate the rearing protocols, based on the above information for both species.

Digestive system ontogeny :

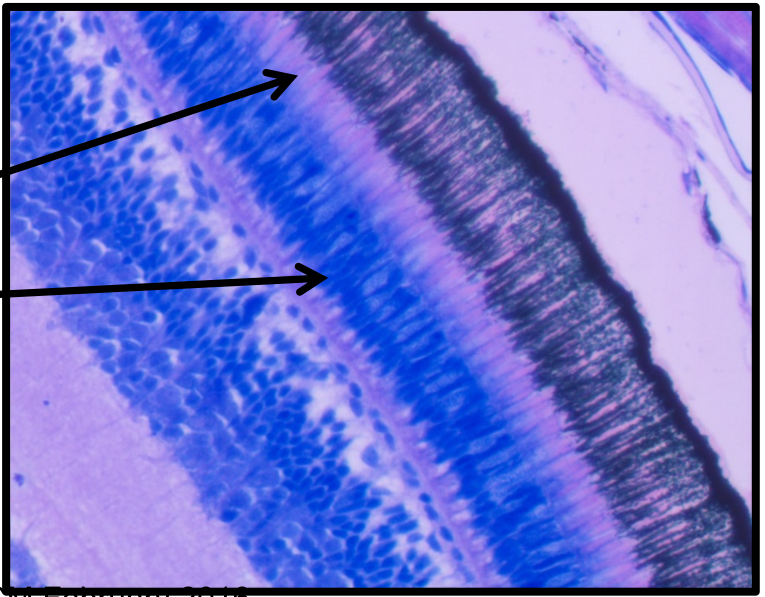
The appearance of **gastric glands** happened at the Intensive rearing system at **19** days after hatching (dah) and at the mesocosm at **18** dah (5.8 ± 0.4 mm total length of larvae).



Stomach

Vision system ontogeny :

In both rearing systems **cones** appeared at 3 dah and **rods** appeared at 15 dah (5.3 ± 0.2 mm total length of larvae).

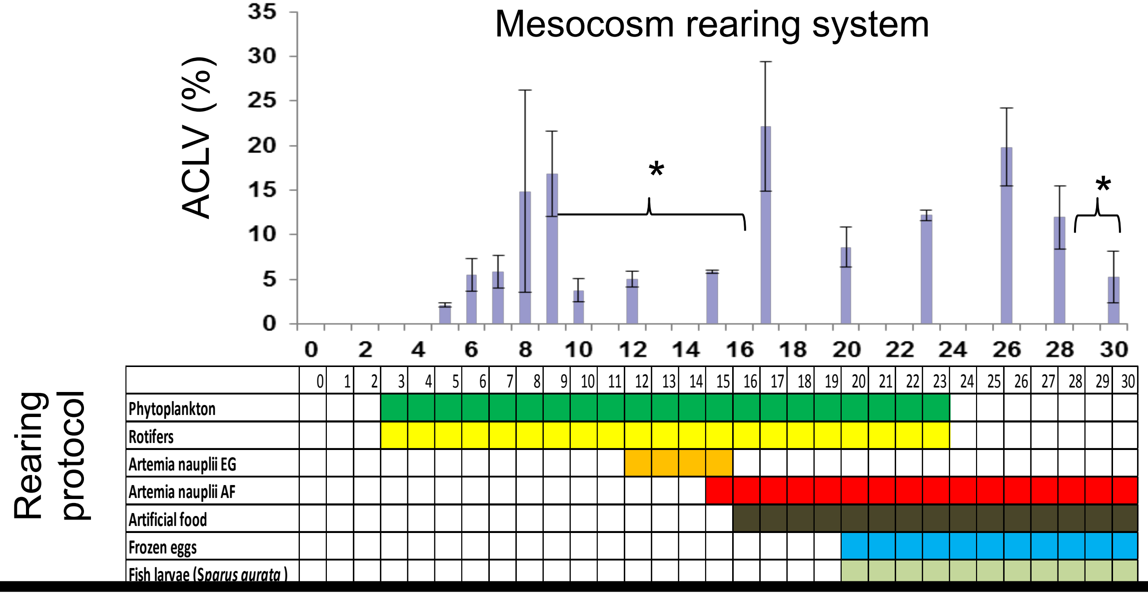


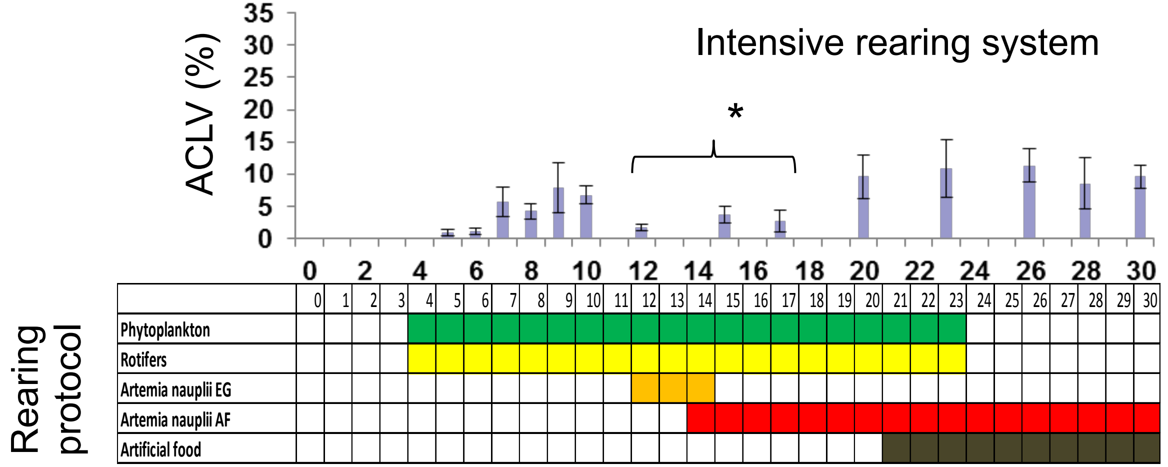
Retina

Results

Evolution of Area Covered with Lipid Vacuoles (ACLV) in the liver (%) during the rearing procedure

Changes in the liver lipid content are considered as indicators for periods of malnutrition in fish larvae.





The asterisks -brackets indicate the malnutrition periods.

Values are mean ± SE

Dah

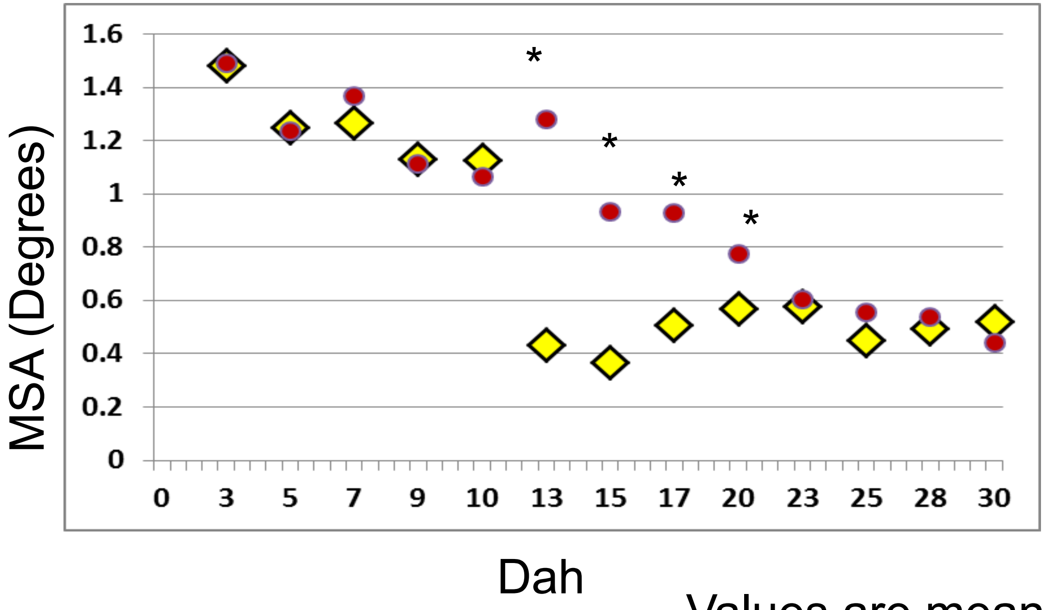
Results

Evolution of Histological visual acuity during the rearing procedure

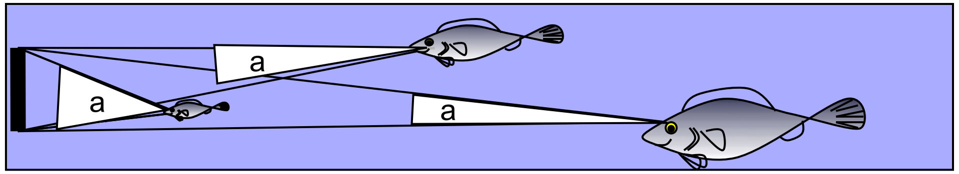
Histological visual acuity was expressed as the Minimum Separable Angle a (MSA) and is defined as the minimum angle, with which two parallel objects can project at the eye and still be resolved as separate.

As the Minimum Separable Angle is reduced, the visual acuity increases.

Asterisks indicate statistical differences between the two rearing systems (P<0.05)



Values are mean ± SE



Visual acuity (visual distance) increases during development

Mesocosm rearing system   Intensive rearing system

Results – Conclusions

- The development of the digestive system of greater amberjack is a rapid process.
- The ability for nocturnal vision developed from 15 dah.
- Visual acuity increased continuously during larvae development.
- The rearing protocols need to be modified during the malnutrition periods that were identified by histological procedure. In both rearing systems (mesocosm and intensive) malnutrition periods were identified:
  1. during the transition from rotifers to *Artemia* nauplii.
  2. during the transition from *Artemia* nauplii to artificial food.

Sub-task 15.2.1; Investigating the larval Somatotropic axis

Primer design for *S. dumerili*.

Results after primer check with 2% agarose gel electrophoresis and sequencing

- **IGF-I**

FWD 5´ TGTTGACGAATGCTGCTTCC 3´
REV 5´ GTCTTGTCTGGCTGCTGTG 3´ Tα = 60 °C

- **IGF-II**

FWD 5´ GTGGGATCGTAGAGGAGTGTTGT 3´
REV 5´ CATCACGGGAATGACCTGTAGAGA 3´ Tα = 60 °C

- **IGF_Binding Protein 1**

FWD 5´ CCCTTTGACCACCATGACACT 3´ Tα = 60 °C
REV 5´ GGGTCCCTGTTGTTCCAGTTT 3´ Pedroso et al. 2009

- **IGF_Binding Protein 2**

FWD 5´ TCCAGGGTTTAGGTCGATGTG 3´ Tα = 60 °C
REV 5´ GTTGCCTGGTGGTCCAGACT 3´ Pedroso et al. 2009

IGF_Binding Protein 3

FWD 5´ CCGAGAGGCTTCCGCATA 3´ Tα = 60 °C
REV 5´ ACGGCACTGTTTTTTCTTGTAGAA 3´ Pedroso et al. 2009

- **IGF_Binding Protein 5**

FWD 5´ GCCCATCGACAAGCATGAT 3´ Tα = 60 °C
REV 5´ CGTCCTTCATCCCCTGAATG 3´ Pedroso et al. 2009

- **Growth Hormone**

FWD 5´ CTGAACCAGAACCTGAACTTGAAC 3´
REV 5´ CTGTCTGTGATTGGCTGAGA 3´ Tα = 60 °C

- **Growth Hormone Releasing Hormone**

FWD 5´ GCATTCTCTGATGGCAAA 3´
REV 5´ CTGTAGCTGTCTGTGAAG 3´ Tα = 59 °C

Sub-task 15.2.3 **(ULL)** Ontogeny of the digestive system of greater amberjack larvae

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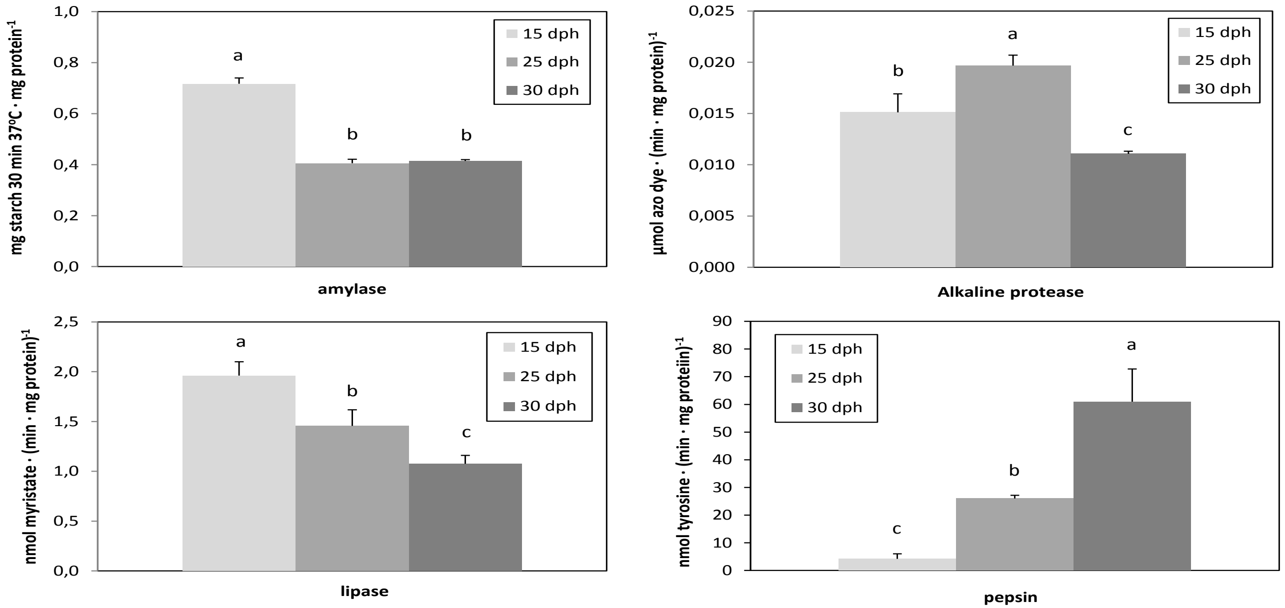
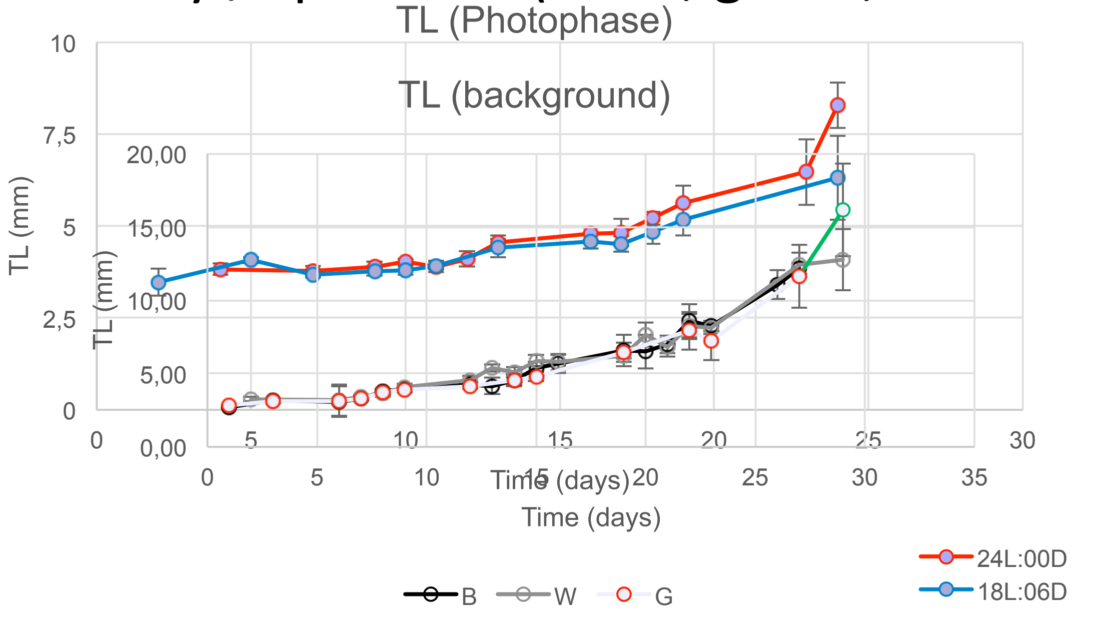
Results 15.2.2 FCPCT. Digestive enzyme activity (25 eggs · l^{-1})



- The three pancreatic enzymes were more active in the youngest larvae compared to the 30 days-old larvae, whereas pepsin followed the opposite trend, displaying an almost null activity at 15 dph.
- Further studies are necessary to elucidate if larval rearing density has any influence in their digestive capacity development.

■ Effect of light (Task 15.3, subtask 15.3.2)

- Duration (photophase 18L:06D vs 24L:00D)
- Intensity / spectrum (black, green, white background)

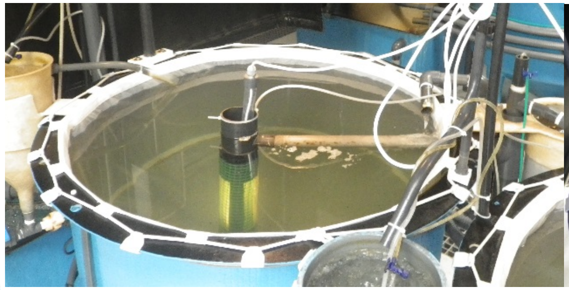
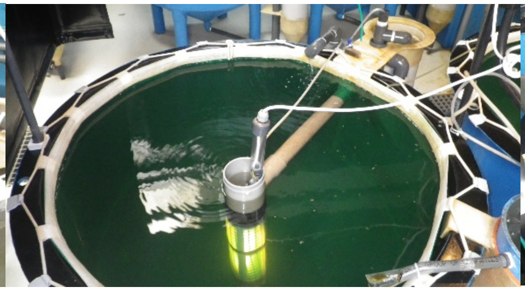


Growth performance (exp)

18L:06D	0.0244 d^{-1}
24L:00D	**0.0364** d^{-1}

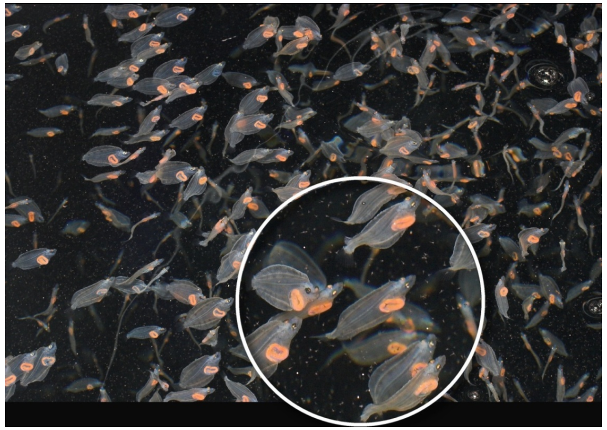
Growth performance (exp)

Black	0.0603 d^{-1}
White	0.0582 d^{-1}
Green	0.0915 d^{-1}


GWP Larval Husbandry
WP16: Pikeperch

IRTA, UL, DTU, ASIALOR
ACM 2016
Nancy, 2-4 February 2016



WP 16 : Larval husbandry - pikeperch

1. Improvement of pikeperch larval rearing protocols by using a **multifactorial** approach

2. Reduction of **cannibalism** rate to increase survival

3. Development of an **industrial protocol** to improve larval performance during rearing

Four experiments are planned

WP16 - 1 : Environmental factors

WP16 - 2 : Nutritional factors

WP16 - 3 : Populational factors

WP16 - 4 : Optimal combination of factors

WP 16-1 => Four factors tested

Light intensity:
❑ sensitive to high LI (above 200 lx) (Hamza et al., 2008; Steenfeldt, 2011; Lund, 2012; Francesconi, 2014)

→ **5 vs 50 lx**

Water renewal rate:
❑ based on previous works (Szkudlarek & Zakes, 2007; Lund & Steenfeldt, 2011; Lund *et al.*, 2012; Ott *et al.*, 2012)

→ **50 vs 100 % / hour**

Water current direction:
❑ impacts the position of larvae in the water column
❑ responsible of mortality, deformities (Summerfelt, 1996)

→ **Water arrival: Surface vs Depth**

Siphoning tank period:
❑ a time of stress for larvae: impact behaviours (foraging and swimming) and water quality

→ **Morning vs Evening**

Objective:
study simultaneously the effect of different factors and their interactions

Conclusion

For environmental factors, the best combination is:

Light intensity: 50 lx
Water renewal rate: 100 % / hour
Siphoning tank period: morning
Water current direction: surface water arrival

- ✓ Larger larvae
- ✓ Heavier larvae
- ✓ More homogenous group larvae

Last results on histological analysis received from IRTA last week => Deliverable D16.1 will be finalized in February

Experiments in 2016 :

Effect of nutritional factors / feeding strategy (February – March):

Factors tested : Food distribution (continuous or discontinuous)
Co-feeding or not
Early or late weaning
Weaning duration (3 or 9 days)

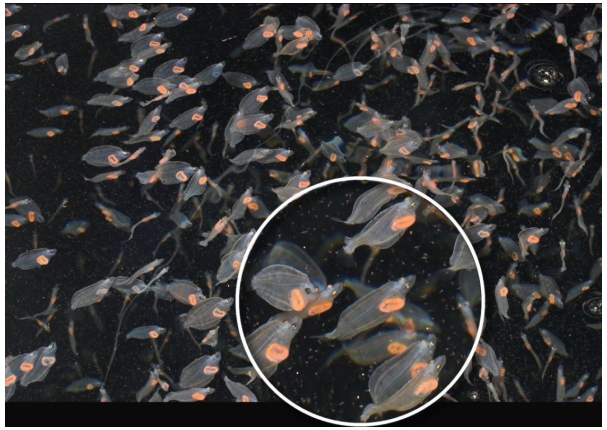
Larvae supplied by Asialor (40 000/tank), out-of-season spawning

Effect of population factors (April – June):

Factors tested : Must be specified (fish density, single or mixed siblings, geographical origins, size grading or not …)

GWP Larval Husbandry
WP17: Atlantic halibut

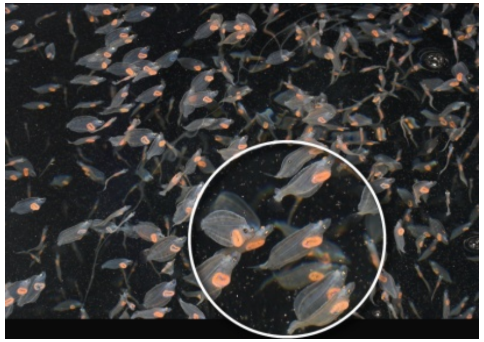
IMR, NIFES, SWH
ACM 2016
Nancy, 2-4 February 2016



Tasks

Task 17.1
Recirculation (RAS) vs Flow through (FT) systems during yolk sac and first feeding stages and the effects on larval survival, quality and growth.

Task 17.2
The effect of probiotics on larval microbiota and survival and development of an industrial protocol.



Task 17.3
Production of on-grown Artemia.

Task 17.4
Comparison of feeding on-grown Artemia versus Artemia nauplii on larval performance.

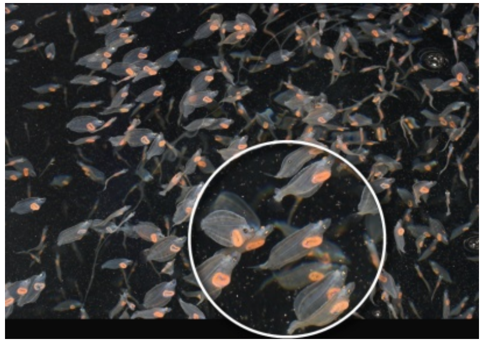
Tasks

Task 17.1

✓ Recirculation (RAS) vs Flow through (FT) systems during yolk sac and first feeding stages and the effects on larval survival, quality and growth.

Task 17.2

The effect of probiotics on larval microbiota and survival and development of an industrial protocol.



✓ Task 17.3

Production of on-grown Artemia.

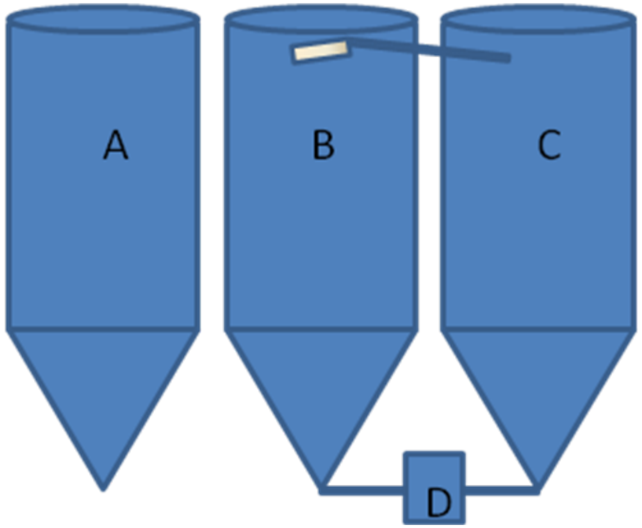
✓ Task 17.4

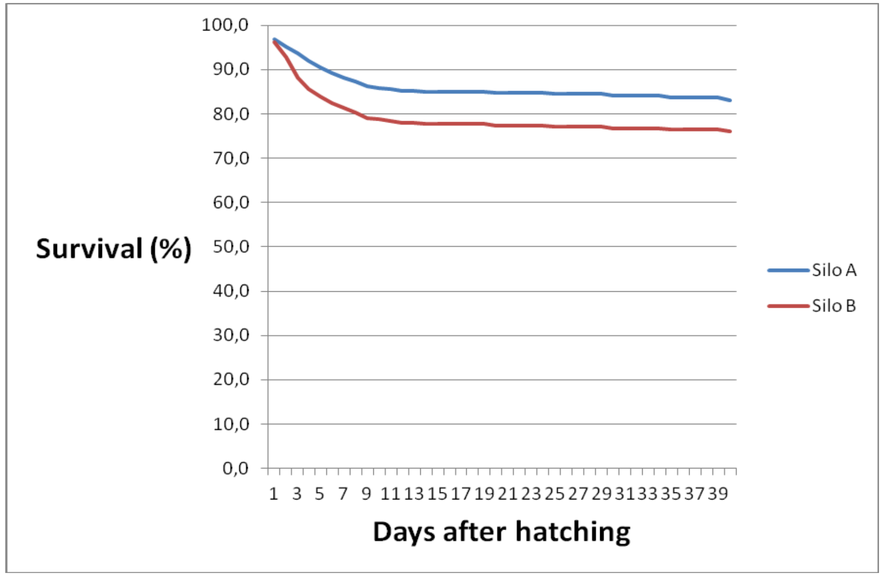
Comparison of feeding on-grown Artemia versus Artemia nauplii on larval performance.

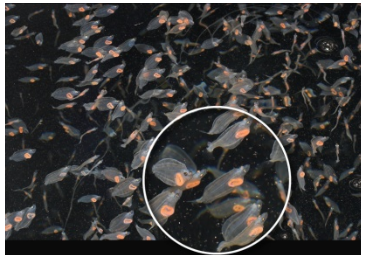
Task 17.1

Recirculation (RAS) vs Flow through (FT) systems during yolk sac and first feeding stages and the effects on larval survival, quality and growth.

Yolk sac stage:







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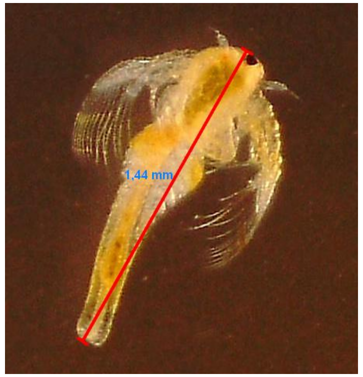
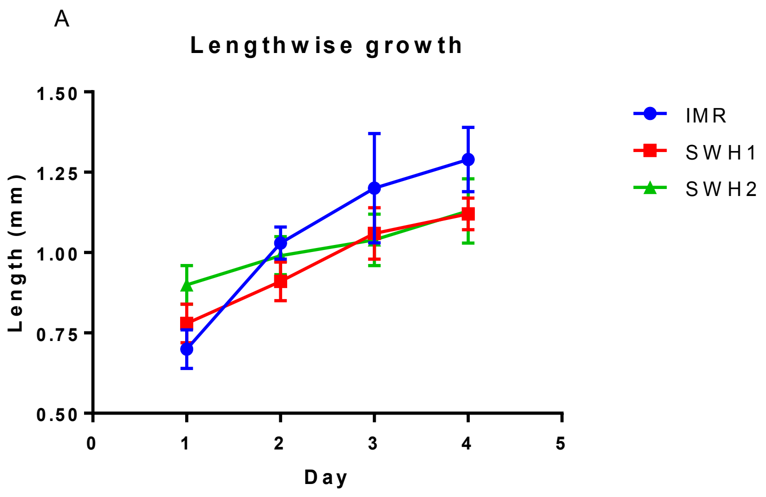
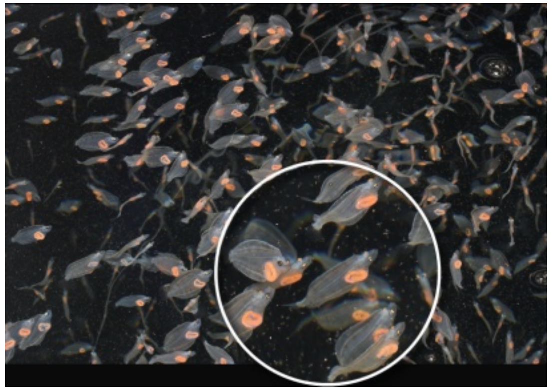
Task 17.3
Production of on-grown Artemia.

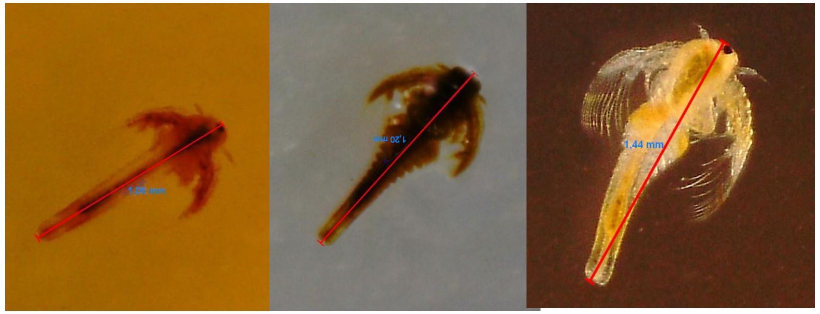




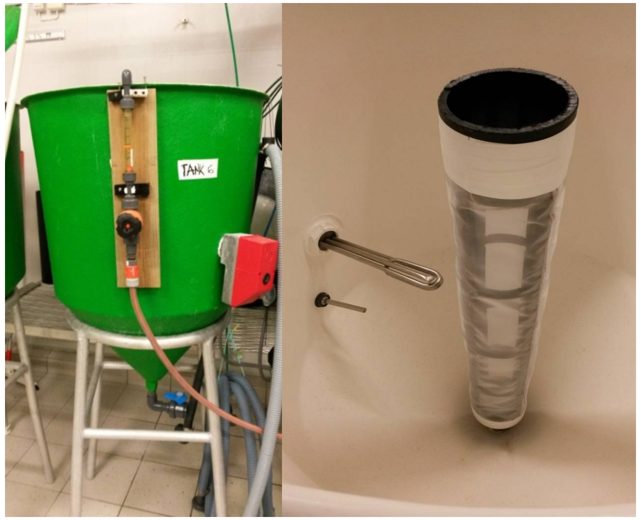


Sterling White Halibut

Production of on-grown Artemia

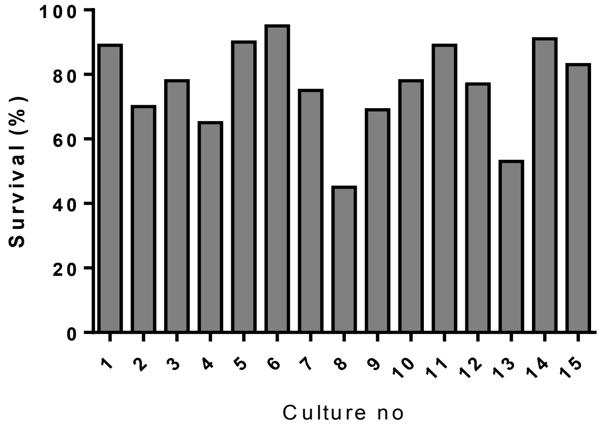


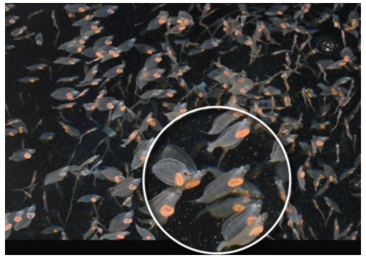
Artemia grown from nauplii for 2, 3 and 4 days. Length: 1,06, 1,2 and 1,4mm respectively.



Artemia tank showing water supply and outlet sieve.

Artemia survival from incubation to harvest

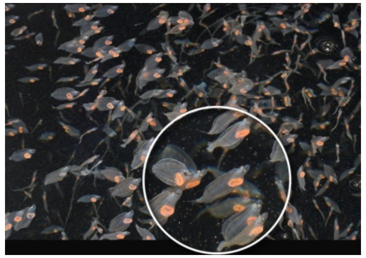




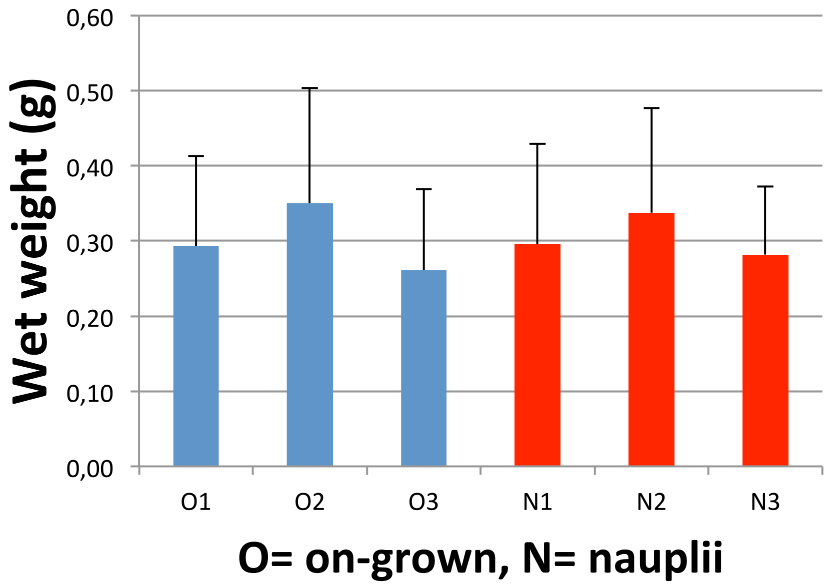
Task 17.4

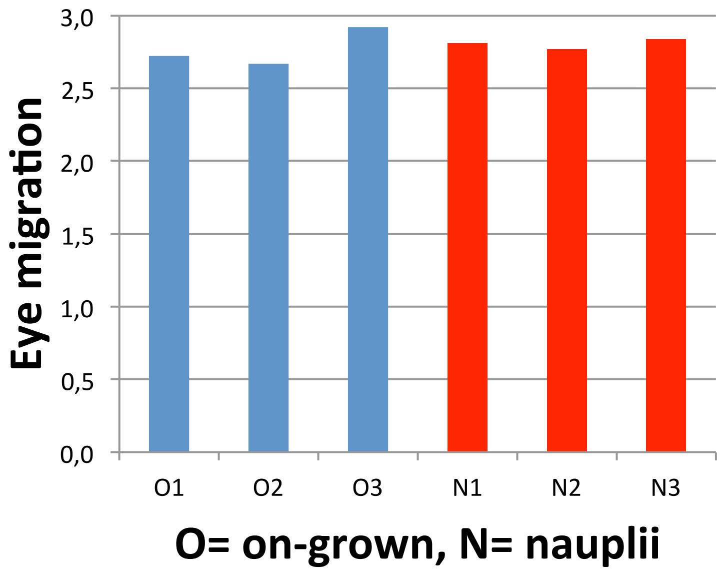
Comparison of feeding on-grown Artemia versus Artemia nauplii on larval performance.

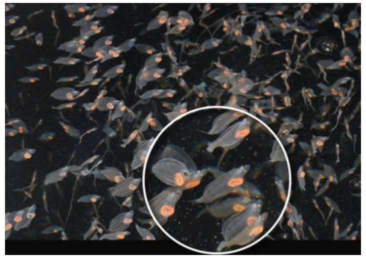
- Atlantic halibut larvae fed Artemia nauplii until 14 dpff (days post firstfeeding)
- One group was fed nauplii, and the other ongrown Artemia until 28 dpff (2+ out of 3 meals)
- No difference in larval performance. 100% pigmentation and good eye migration (score: more than 2.5/3) in both groups.
- Samples taken for nutrient analyses (NIFES) and analyses of digestive capasity (ULL) after end of feeding ongrown Artemia



Task 17.4
Comparison of feeding on-grown Artemia versus Artemia nauplii on larval performance.







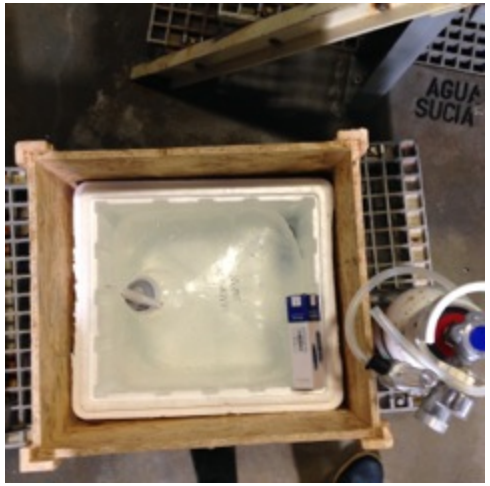
GWP Larval Husbandry
WP18: Wreckfish

HCMR, IEO, CMRM, MC2
ACM 2016
Nancy, 2-4 February 2016

Origin of eggs

Aquarium FINISTERRAE (A Coruña)

- Transport in polystyrene boxes (24 hours of transport)
- 2000 larvae in 5 l seawater
- Arrival at 19.5 ˚C, 19.5 mg/l O_2, 8 pH
- Incubation in 500 l tanks



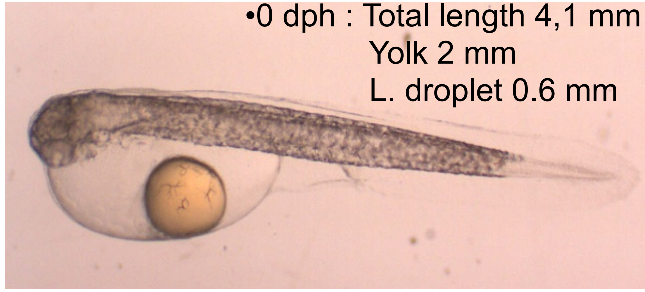
HCMR brood stock

- Induced spawning
- ~4000 eggs
- At 16.5 ˚C, 7.2 mg/l O_2, 8.2 pH
- Incubation in 2,000 l tank

Two “rearing” trials in closed recirculating systems

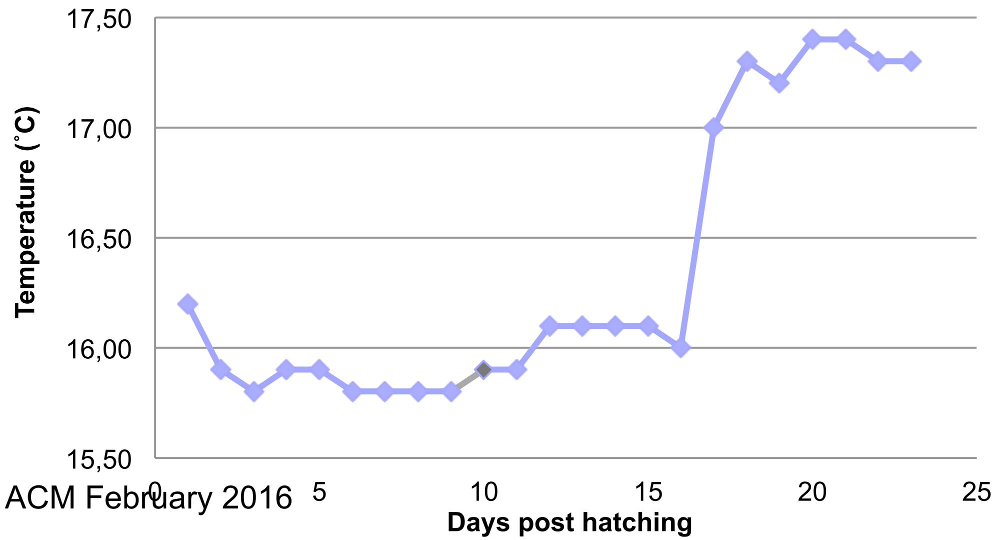
Tanks of same shape different depth

- 2000 l tanks (May 2015)
- 500 l tanks (June (2015)

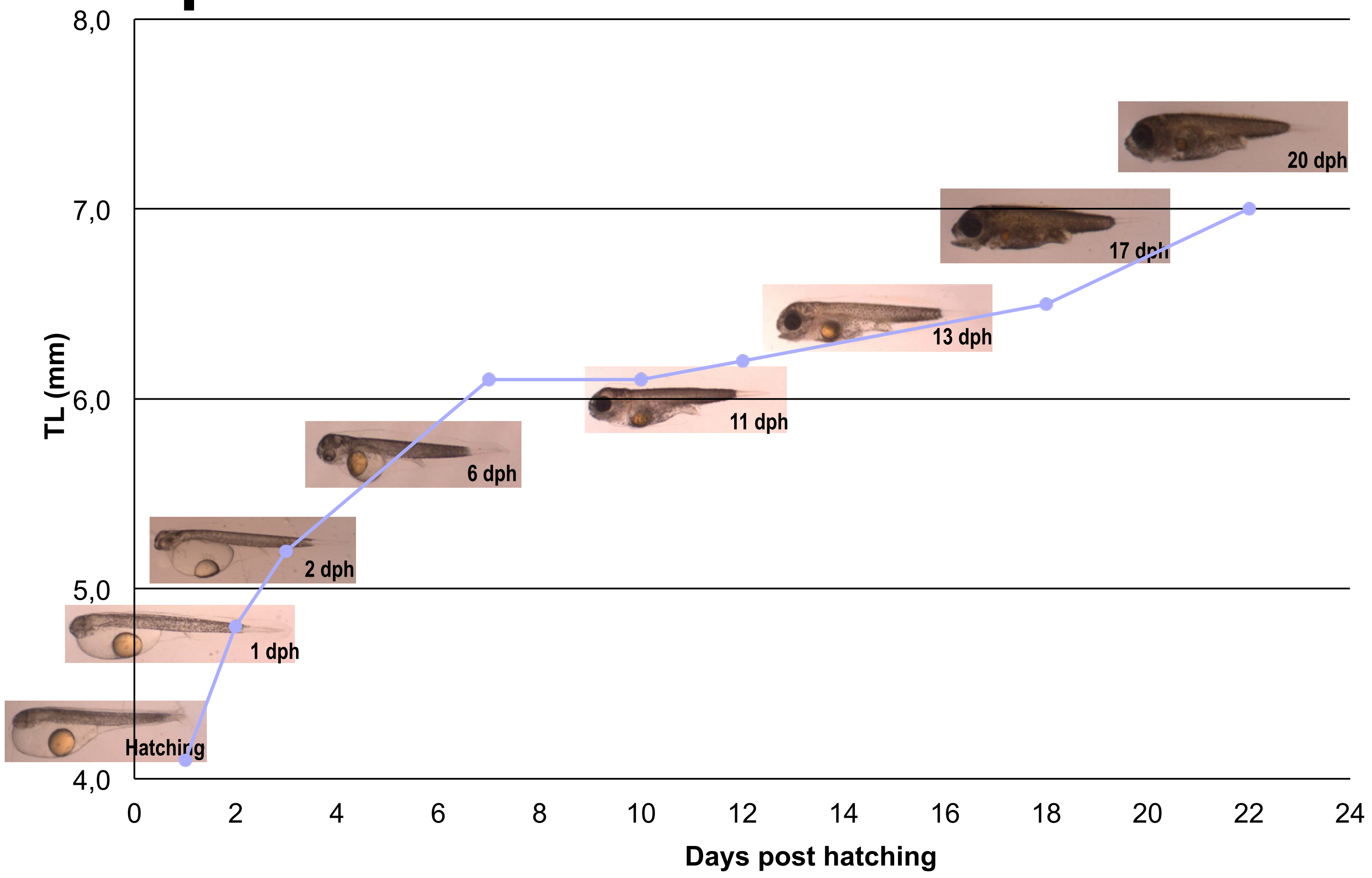


Conditions

- ~ 2 ind l^{-1}
- First feeding: 10 dph
- Feed with:
  - •Rotifers,
  - •Artemia AF (since 13 dph)
  - •Artemia EG (since 24 dph)

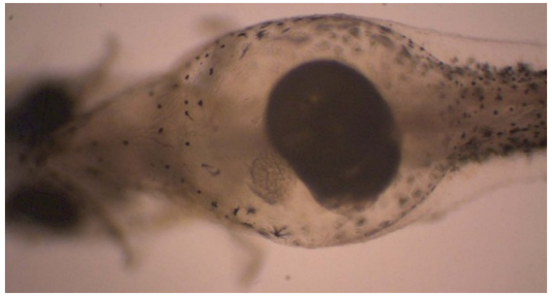


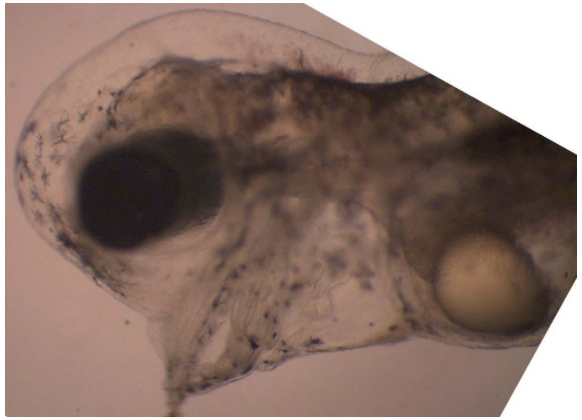
Growth performance

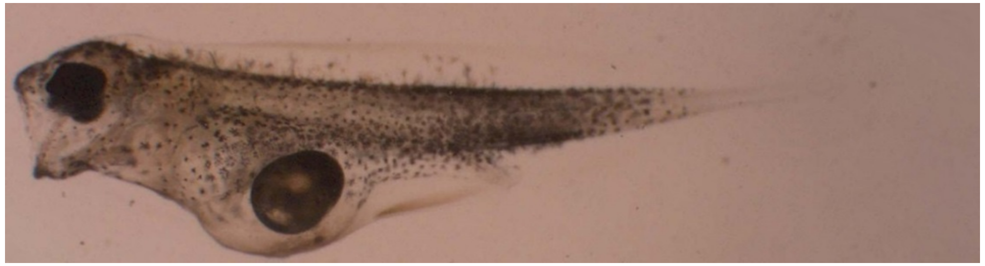


End of trial: 24 dph

Malformed individuals







A syndrome related to swollen yolk sac.

- Blue Sac Disease – (BSD) common in trout. Several reasons suggested; most common toxicity from Nitrogen compounds such as ammonia , oxidative stress plays significant role.
- Swollen Yolk Sac Syndrome (SYSS) described in Murray cod, (freshwater fish in Australia). Related to inadequate nutrition of the broodstock.

GWP Larval Husbandry
WP19: Grey mullet

IOLR, IRTA
ACM 2016
Nancy, 2-4 February 2016

Israel Oceanographic & Limnological Research

IRTA RESEARCH & TECHNOLOGY FOOD & AGRICULTURE

Larval husbandry (Task 19.1)

DIVERSIFY

Determine the effect of algal type and turbidity on grey mullet larval performance

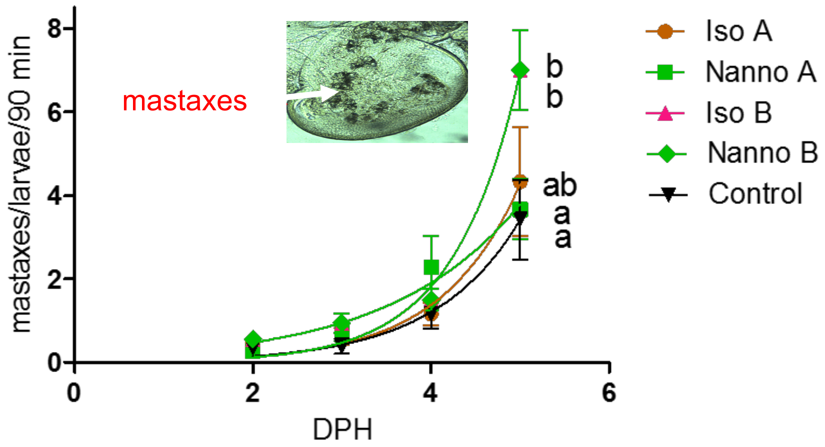
Aims:

- Determine if microalgal concentration provide background to facilitate prey hunting
- Compare the turbidity effect of *Nannochloropsis oculata* and *Isochrysis galbana* on larval performance

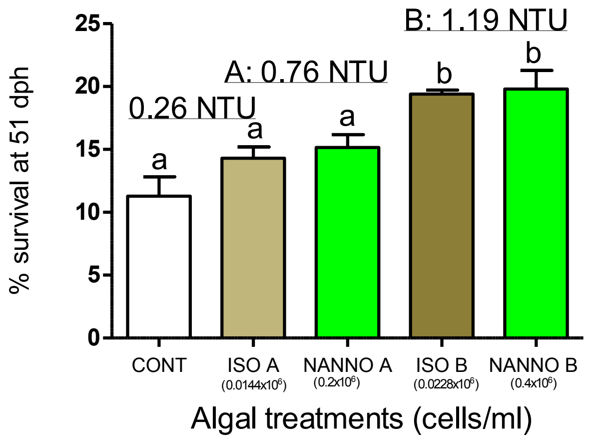
Treatments	Turbidity (NTU)
Control	0.26
Isochrysis A (0.0144x10^6cells\ml)	0.77
Nannochloropsis A (0.2x10^6 cells/ml)	0.75
Isochrysis B (0.0228x10^6cells\ml)	1.18
Nannochloropsis B (0.4x10^6cells\ml)	1.20

Results and Conclusions

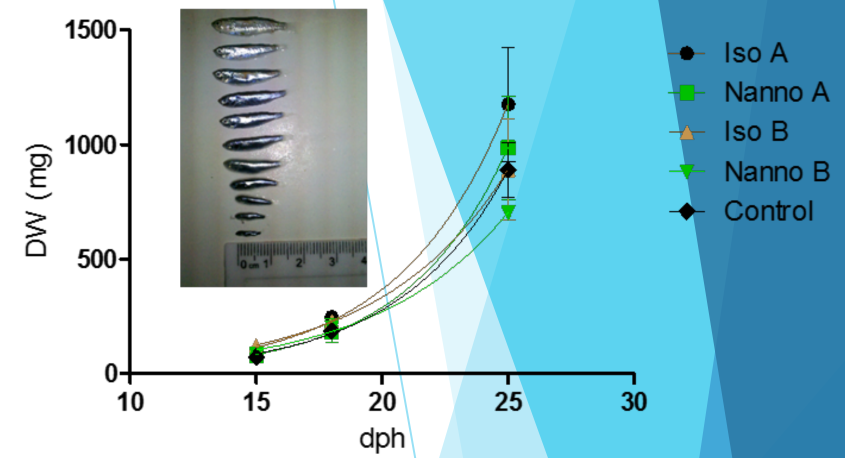
Rotifer consumption



Survival



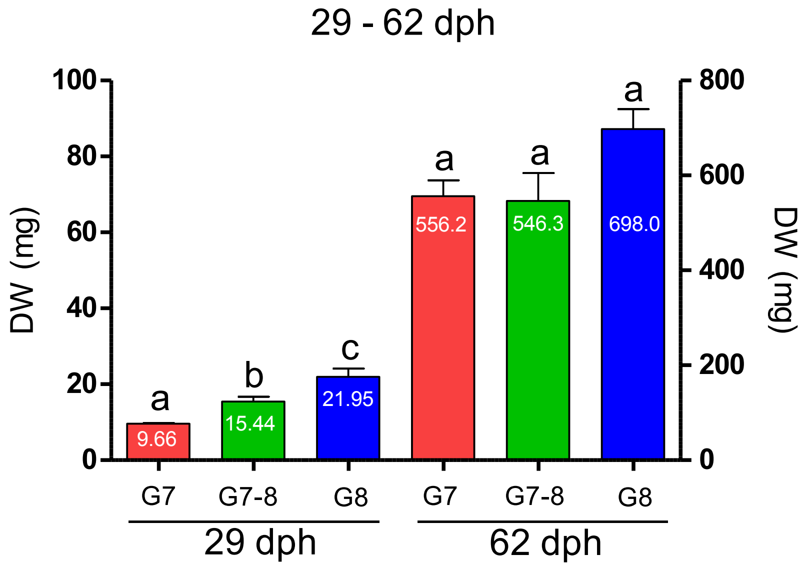
Growth

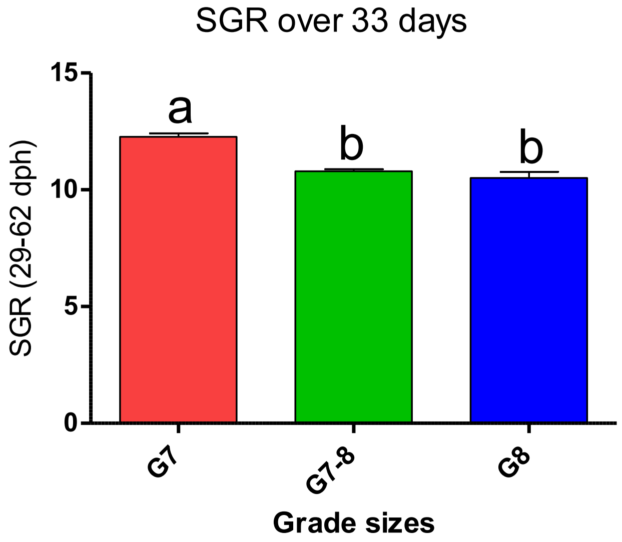


- Rotifer consumption and survival were dependent ($P<0.05$) on algal turbidity but independent of algal type.
- Suggests that rotifer consumption in early development markedly influences juvenile survival
- Higher survival resulted in higher levels of smaller fish which reduced average fish weight.
- Deliverable D19.1 submitted but final version will include further analysis that is being performed now (FA, digestive enzyme ontogeny)

Growth compensation occurs after grading

- Used fish from Iso B treatment.
- 3 replicates/treatment.
- Stocked 150 fish/replicate
- Grown from 29-62 dph in 400 l exp systen





- Growth compensation was demonstrated in graded small fish

Thank you