GWP Grow out Husbandry WP20-21-22-23

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… DIVERSIFY will address the **main documented species specific bottlenecks in the production** of the selected species, in order to **develop adequate husbandry practices and technologies for the industry** to enable production …

Structure of the GWP

- WP 20: Meagre
- WP 21: G. amberjack
- WP 22: Pike perch
- WP 23: Grey mullet

WP 20. Meagre

- Technologies and practices used for grow out, similar to those for gilthead sea bream and European sea bass

  But Meagre is different!!

- Species-specific husbandry practices are needed

- The objectives of this WP are
  - **to develop method**
    - **to avoid size variability in juveniles**
    - **for feeding respecting the specific behaviors of meagre**
  - **to modify applied methods**
    - **for ongrowing in cages to maximize performance**

Task 20.1 **Size variability at juveniles**

Difference in growth depends on genetic origin?

Potential of low-growth fish for compensatory growth?

- Tank experiments at juvenile stage
  - Genetic characterization of juveniles for parental assignment (In Progress)
  - Growth studies
  - Economic analysis

- Result:
  - Identification of causes
  - Development of methodology

- Implementation: IRTA, HCMR

20.1.- Size variability in meagre juveniles

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Two trials

2014 with 6 families

- larvae separated per spawn (very diferent growth)

2015 with 4 families

- larvae mixed and distributed in four tanks

- at ~50 dph fish graded into S, M and L groups

2014:

- at 116 dph second grading
  - 3 groups of ~80 fish

2015:

- at 110 dph second grading
  - 3 groups (x3 tanks) with 100 ind

Family	Spawning Date (Tank)	Female	Male
1	24/04/2014 (V8-1)	5-wild	19-wild
2	01/05/2014 (V8-1)	5-wild	20-wild
3	01/05/2014 (V8-2)	1-wild	19-wild
4	24/04/2014 (C2)	16-cultured	21-wild
5	01/05/2014 (C1)	2-wild	22-wild
6	01/05/2014 (V6)	13-cultured	17-wild

Family	Spawning Date (Tank)	Female	Male
1	13/05/2015 (V7)	5-wild	19-wild
2	13/05/2015 (V6)	6-wild	23-cultured
3	13/05/2015 (V8-1)	1-wild	20-wild
4	13/05/2015 (V8-2)	8-wild	22-wild

2014

	53 dph 19/06/2014		88 dph 24/07/2014		SGR	116 dph 21/08/2014		SGR	144 dph 18/09/2014		SGR
S	0,43	0,4	9,42	1,8	2,27	17,855	1,786	2,80	31,488	6,052	3,35
M			15,47	3,9		22,682	1,288	3,02	41,214	4,488	3,61
L	5,26	3,4	43,23	17	3,72	27,184	1,544	3,17	47,768	5,311	3,75

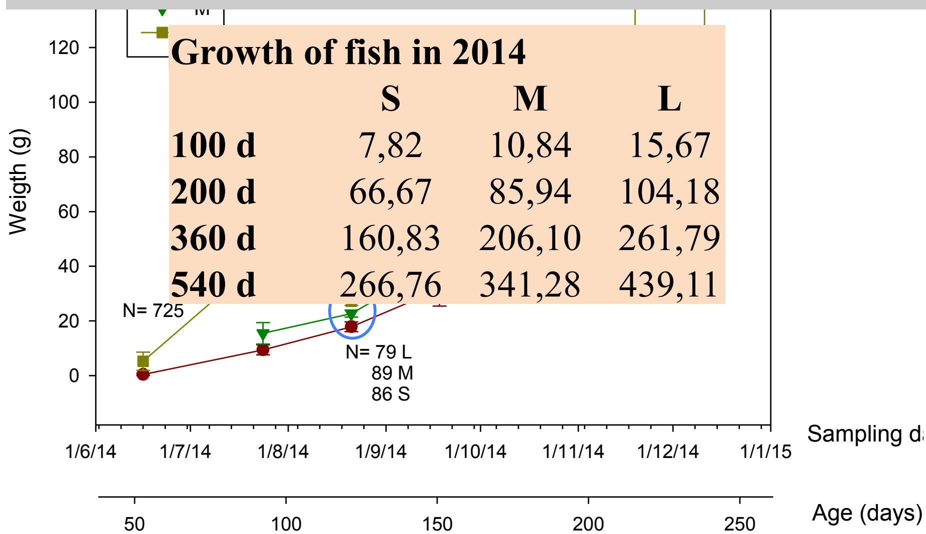
	164 dph 08/10/2014		SGR	185 dph 29/10/2014		SGR	205 dph 19/11/2014		SGR	227 dph 11/12/2014		SGR
S	45,55	8,57	3,65	61,01	15,14	4,04	71,58	21,31	4,07	79,47	24,40	4,18
M	58,59	8,55	3,88	78,13	14,81	4,28	94,18	19,79	4,33	101,33	23,48	4,41
L	65,26	9,06	3,99	94,39	13,46	4,47	113,93	21,00	4,51	125,55	26,49	4,62

2015

	49 dph 02/07/2015		83 dph 05/08/2015		SGR	110 dph 01/09/2015		SGR
S	0,263	0,030	4,806	1,20	1,61	17,841	5,646	2,82
M	0,434	0,093	7,030	1,83	1,97	22,171	5,776	3,03
L	1,202	0,494	12,359	5,69	2,51	37,950	14,961	3,54

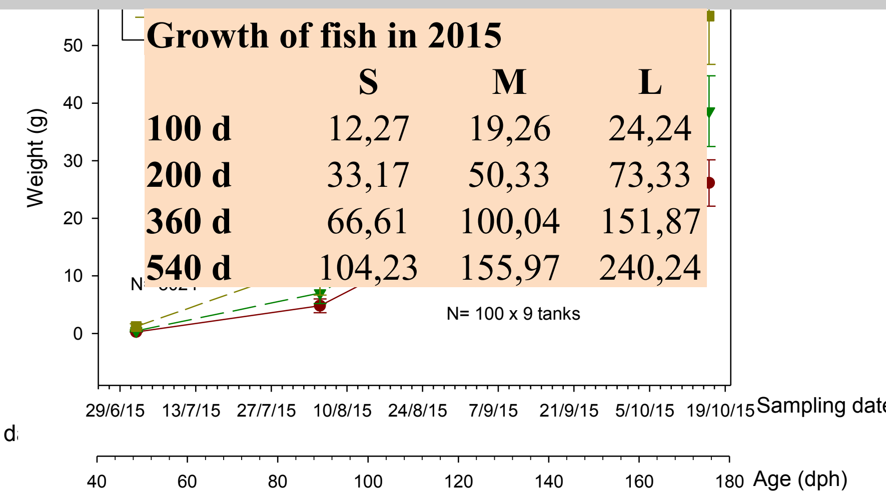
	112 dph 03/09/2015		SGR	134 dph 25/09/2015		SGR	155 dph 16/10/2015		SGR	190 dph 05/11/2015		SGR
S	13,96	1,39	1,20	19,07	2,79	2,83	26,13	4,04	3,12	29,89	5,31	3,30
M	21,50	1,47	1,52	30,43	3,53	3,28	38,61	6,13	3,49	45,83	8,79	3,72
L	29,18	1,56	1,56	39,76	5,40	3,53	55,12	8,40	3,83	66,62	11,88	4,08

SGR was always higher for L fish and lower for S fish



Growth of fish in 2014

	S	M	L
100 d	7,82	10,84	15,67
200 d	66,67	85,94	104,18
360 d	160,83	206,10	261,79
540 d	266,76	341,28	439,11



Growth of fish in 2015

	S	M	L
100 d	12,27	19,26	24,24
200 d	33,17	50,33	73,33
360 d	66,61	100,04	151,87
540 d	104,23	155,97	240,24

Growth results allow an extimation of production cost for S and L groups

PRODUCTION COST OF L- AND S- GROUPS (1000 juveniles)		
	L-	S-
Juveniles (0.6€/unit)	600	600
Food: 10-30 gr (2.4€/Kg)	90	136,8
30-250 gr (2.04€/Kg)	1526	2557,7
250-500 gr (2.04€/Kg)	1943,1	3243,6
Total	4159,1	6538,1
Market price (9,3€/Kg)	4650	4650

General conclusion:
In the case of meagre large fish always show a higher growth rate than Medium and Small fish

No compensatory growth occur after grading.

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Task 20.2 **Effect of rearing environment**

Which is proper environment for meagre rearing?

- Effect of cage depth (sT 20.2.1)
- Effect of light intensity (sT 20.2.2)

- Result:
  - Definition of optimal depth
  - Definition of optimal light intensity

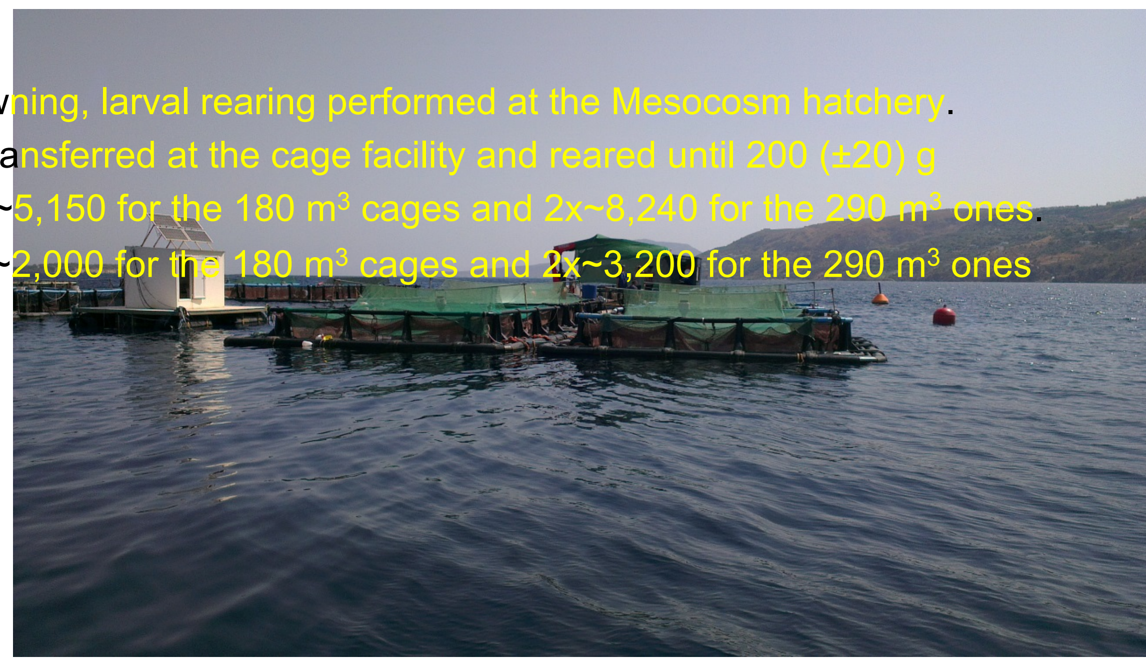
- Implementation: HCMR, Argosaronikos SA

Task 20.2.1 **Effect of cage depth**

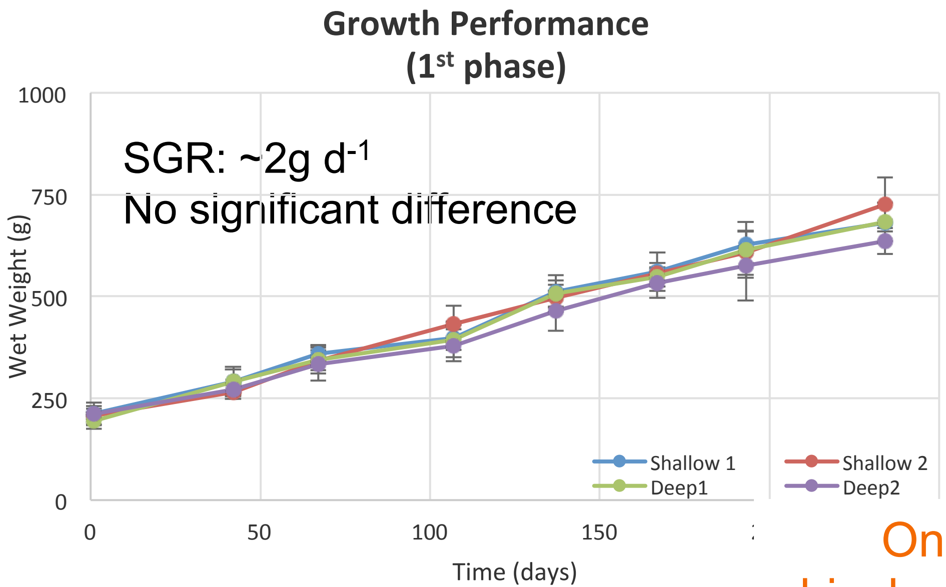
Test performance of meagre in cages of different depth

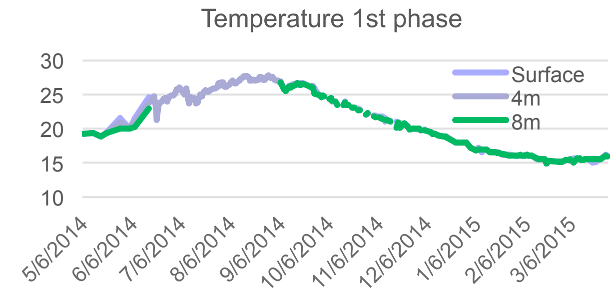
(started May 2014 – finished January 2016)

- Methodology
  - Cages of 180 (6x6x5-Shallow) and 290 (6x6x8-Deep) m^3 at the HCMR pilot farm in duplicates
  - Fish origin from HCMR.
    - Eggs form a single spawning, larval rearing performed at the Mesocosm hatchery.
    - Juveniles of 2 gr were transferred at the cage facility and reared until 200 (±20) g
    - 1st phase: 4 groups 2x~5,150 for the 180 m^3 cages and 2x~8,240 for the 290 m^3 ones.
    - 2nd phase: 4 groups, 2x~2,000 for the 180 m^3 cages and 2x~3,200 for the 290 m^3 ones
  - Duration of trial 8 months



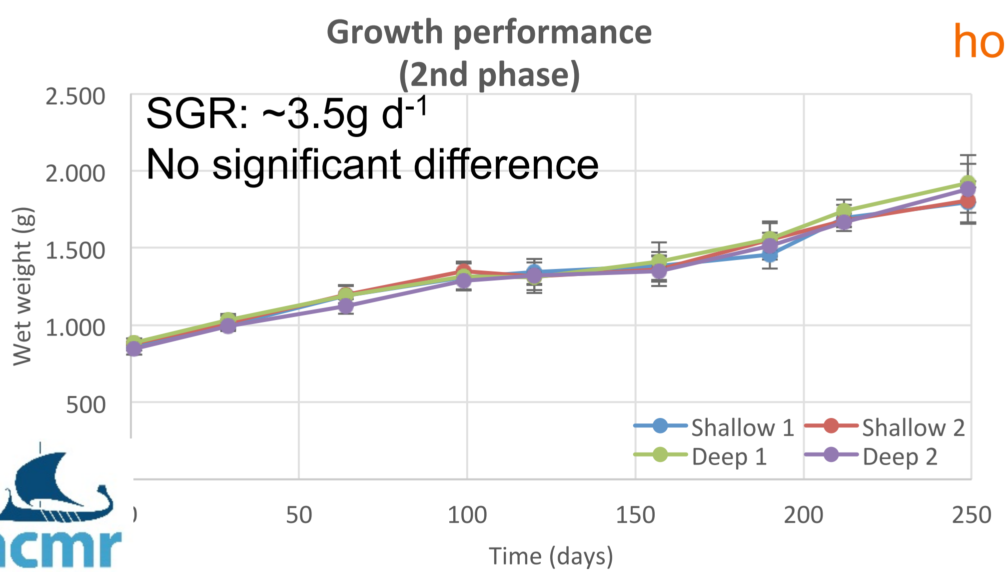
Results (so far)

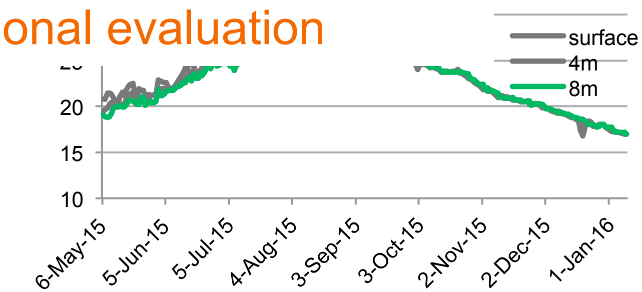




	S1	S2	D1	D2
Mortality(%)	23,5	24,2	12,1	13,9
FCR	1,92	1,92	1,58	1,60

On going hematological, biochemical, immunological and hormonal evaluation





	S1	S2	D1	D2
Mortality(%)	10.8	9.7	7.9	8.1
FCR	1.67	1.70	1.50	1.47

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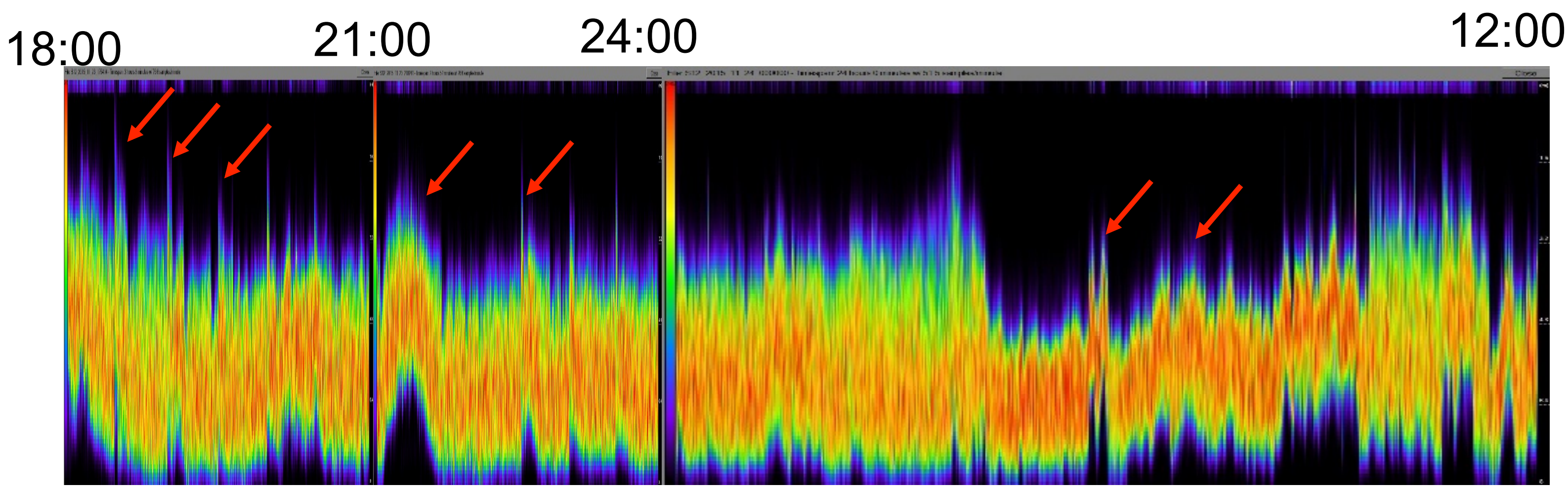
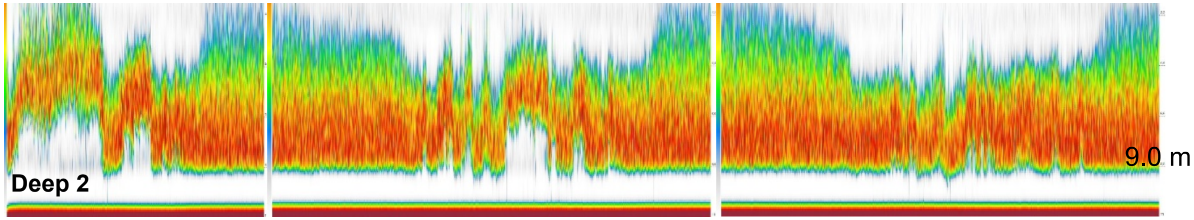
Results (so far)



- Evidence for nigh feeding behavior
  - observation independent of the cage depth
  - first time that such a behavior has been observed
  - to be evaluated in the next period

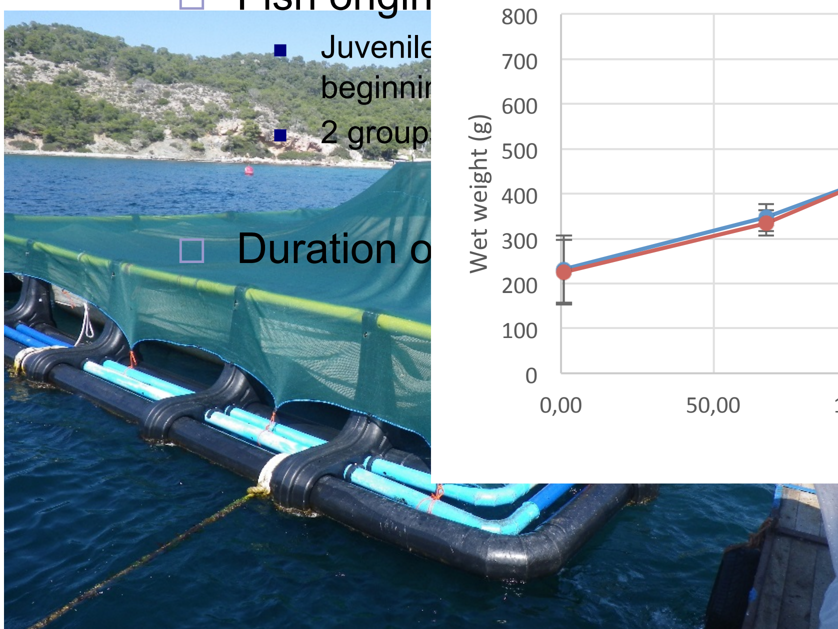


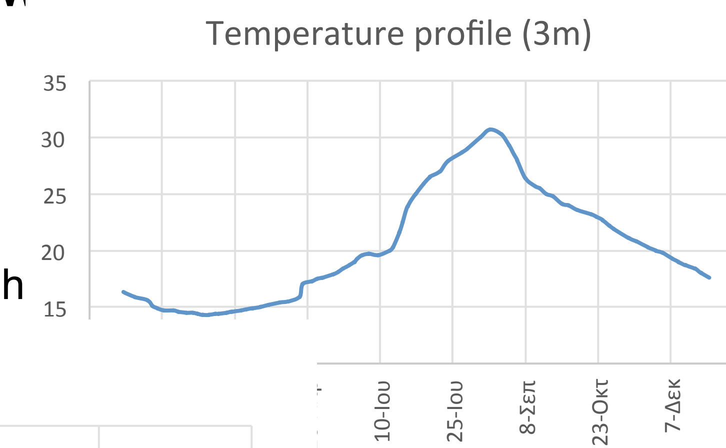
Task 20.2.2 **Effect of light intensity**

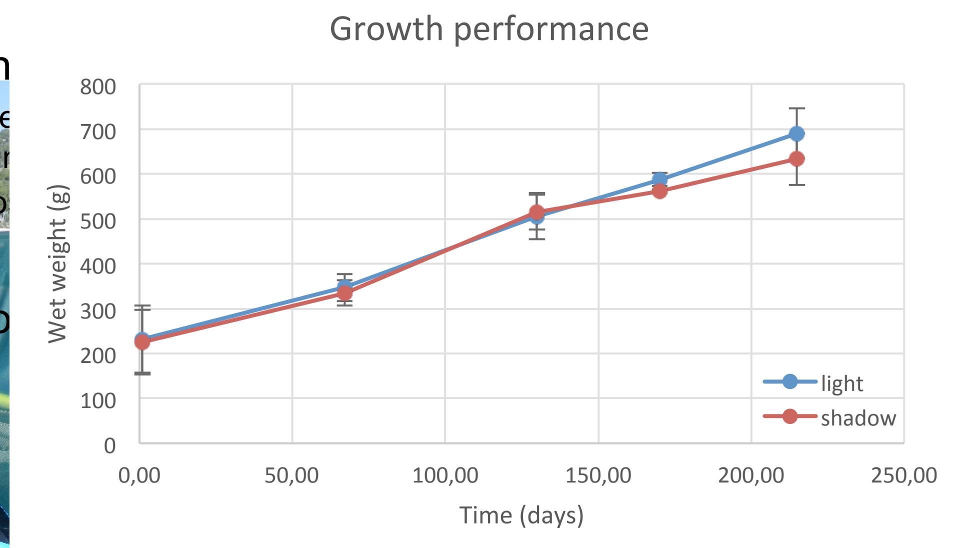
Test performance of meagre in cages w

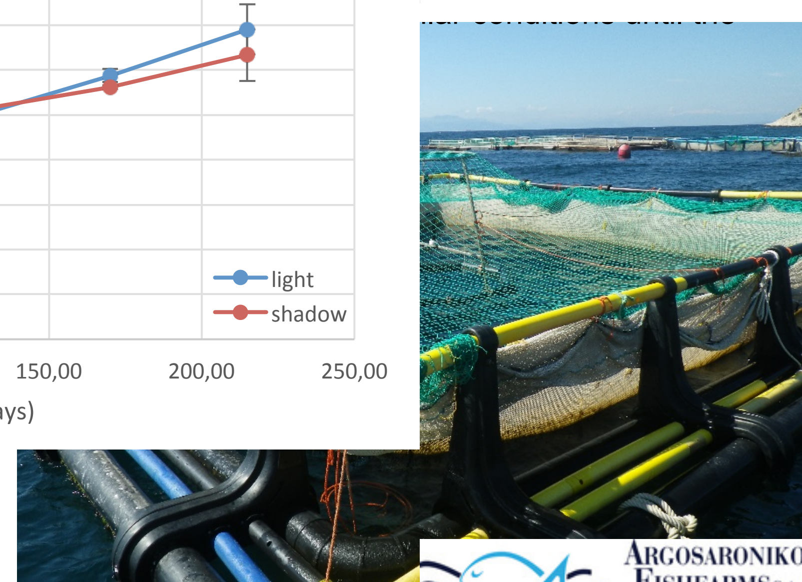
(started May 2014)

- Methodology
  - Cages of (10x10x6) m³ -Shaded and -Not-Sh
  - Fish origin
    - Juvenile
      beginni
    - 2 group
  - Duration o



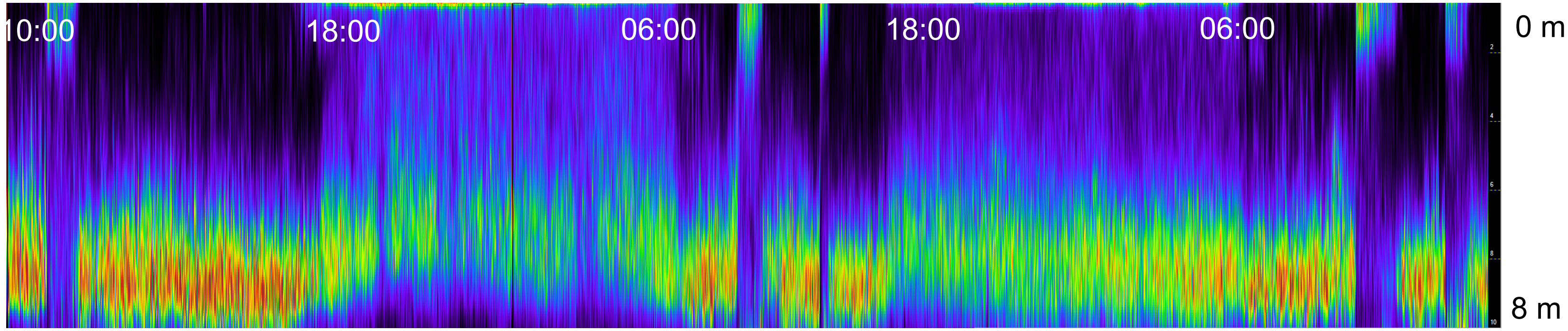






ARGOSARONIKOS FISHFARMS S.A

Not shaded



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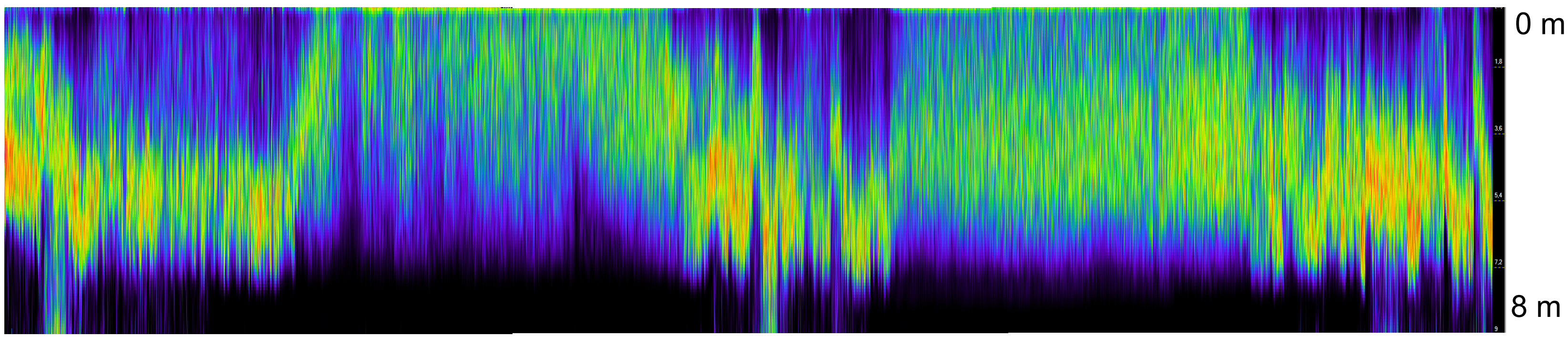


- Vertical distribution in cages
  - Repeated pattern!!

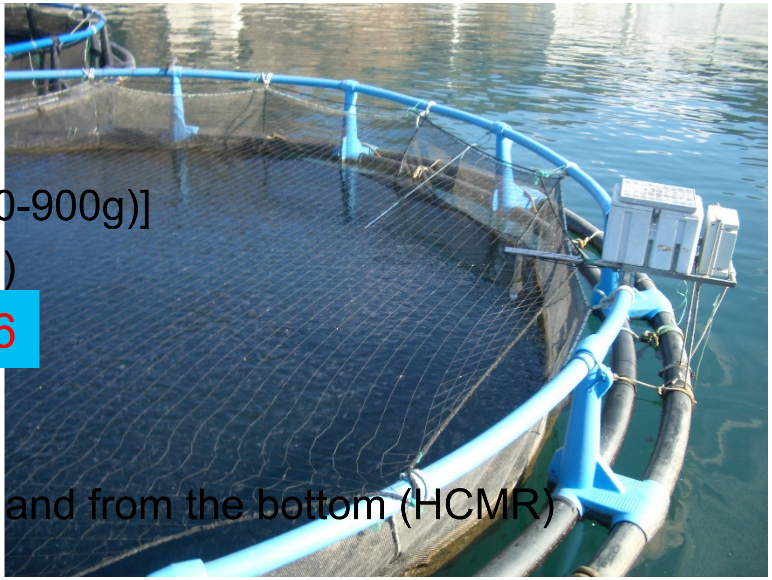
  - observation independent of the cage depth
  - correlated with the light and dark period of the day
  - repeated pattern during the experimental period

ARGOSARONIKOS FISHFARMS S.A

Task 20.3 **Development of feeding methodology**

Is the feeding method applied adequate for meagre?

Can we work towards the development of an "industrial" feeding system?



- Test in tanks
  - 2 different size groups [(50 – 100g) and (700-900g)]
  - different feeding stimuli (mechanical, optical)
  - different feeding methods To start in 2016
- Test in cages
  - 2 feed distribution methods from the surface and from the bottom (HCMR)
  - Test of distribution period?
- Result: Information towards the development of feeding system for industrial application
- Implementation: HCMR, IRTA, Argosaronikos SA

Sub-task 20.3.1. Test of different feeding stimuli

A. **Mechanical (**aeration **before** feeding**)**

B. **Optical –Light (**light at the feeding area **before** feeding**)**

Results

- Meagre is able to learn, and be trained to feeding stimuli
- Both stimuli (mechanical and optical) can be used in industrial scale

WP21. Greater amberjack

- Preliminary data for grow out of greater amberjack suggested that growth performance is high

- Further studies are required to develop
  - rearing method in cages
    - volume - depth and
    - test the application of submersible cages
  - feeding methods
  - appropriate husbandry practices
    - specific thermal ranges for optimal growth and health,
    - optimum rearing density

Task 21.1 **Development of rearing method in cages**

Which is the proper volume (depth) of cages? (sT21.1.1)

Can we use submerged cages? (sT21.1.2)

- Trials for 12 months in
  - commercial cages (10m and 6m depth)
  - commercial cages (20m diam; 10m depth)

To start in 2016

- Result
  - definition of optimal depth
  - definition of optimal technology
- Implementation: FORKYS, CanexMar, FCPCT, HCMR

Task 21.2 **Development of feeding methods**

Do we feed properly the greater amb

Which is the feeding pattern

Under implementation

- Test in tanks for 4 months with fry (5g) and juveniles (200g)

- Result: definition of optimal feeding method – feeding pattern
- Implementation: IEO , FCPCT

Task 21.2.2 **Definition of feeding pattern for 200 g juveniles**

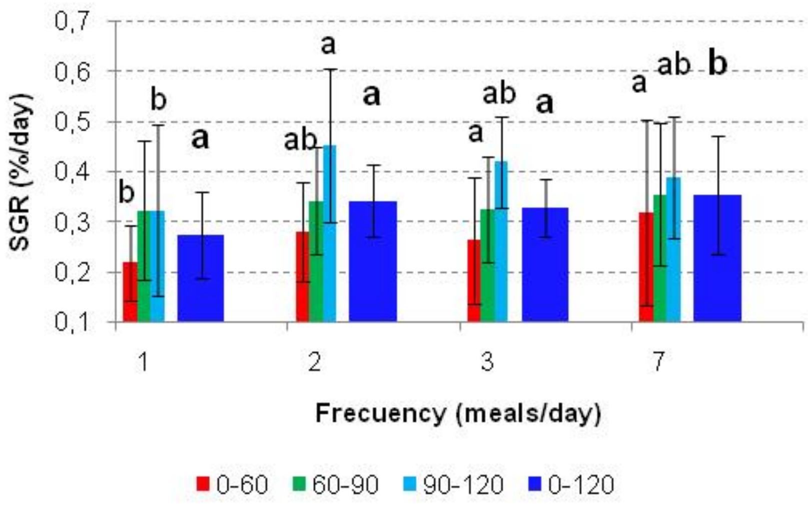
- Different feeding methods
  - Four different feeding frequencies: 1, 2, 3 and 7 meals d^{-1}
  - Triplicate tanks for a period of 120 days

- Evaluate growth, condition and welfare of fish
  - Specific growth rate and fish condition (K)
  - Feed intake
  - Hematological and biochemical parameters
  - Immunological studies

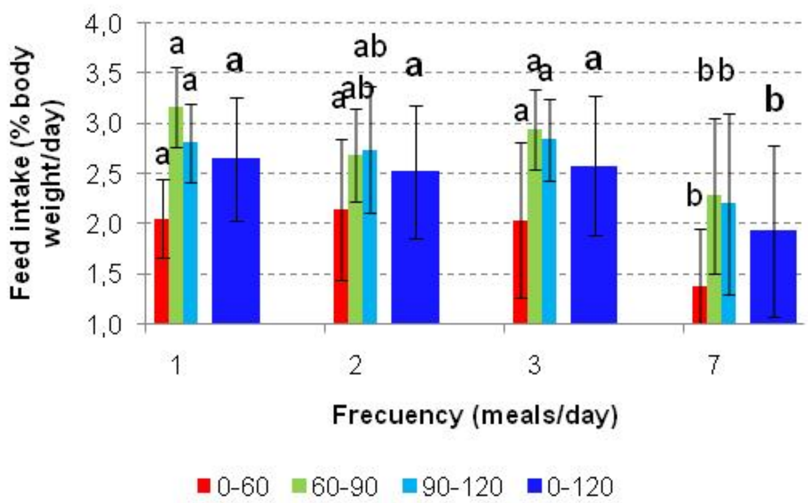
- Implementation: IEO

Task 21.2.2 **Results**

- Specific growth rate (SGR) and fish condition
  - 1 meal d^{-1} lower SGR, and 2, 3 and 7 meals d^{-1} similar SGR
  - 1 meal d^{-1} lower Condition index (K) at 120 days, and similar during the first 90 days.



- Feed intake (% bw)
  - 7 meals d^{-1} significantly lower

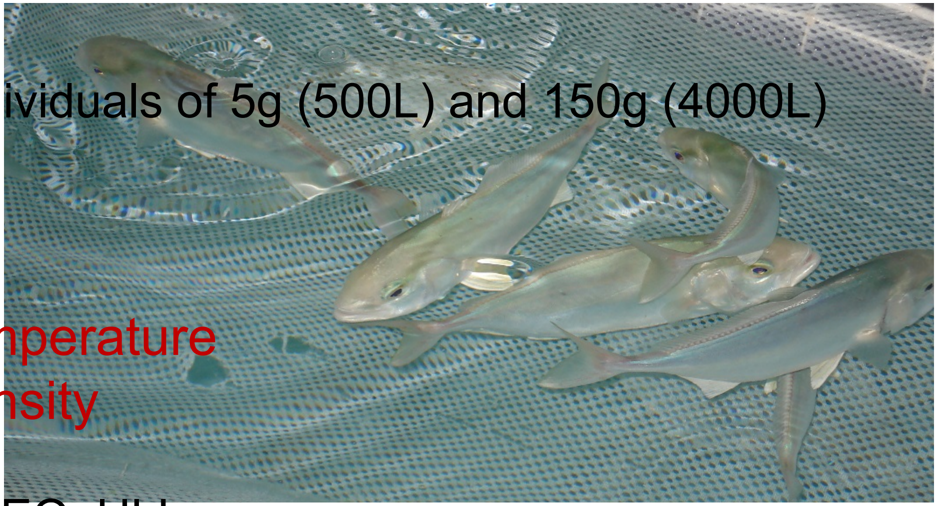


Task 21.3 **Development of appropriate husbandry practice**

Which is the optimum temperature range?

Which stocking density is optimal?

- Test in tanks for 4 months with individuals of 5g and 200g
  - 2 different temperature ranges 14-17 ^{o}C and 26-29 ^{o}C



- Test in tanks for 4 months with individuals of 5g (500L) and 150g (4000L)
  - 3 different stocking densities

Result:

- definition of optimal rearing temperature
- definition of optimal rearing density

- Implementation: FCPCT; HCMR, IEO, ULL

Task 21.3 **Temperature Tolerance of Juveniles**

- Better growth performance at 26ºC than at 22ºC and 17ºC
  - Best feed utilization at 26ºC
  - Morphologically, led to elongated shape of fish body,
    - clear head difference , and
    - better efficiency of the caudal fin propulsion
  - Faster gastric evacuation (temperature dependent) important parameter to define optimum feeding strategies

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Task 21.3.2 **Definition of optimal stocking density for 5 g (started Sep 2015) and 150 g juveniles (planned for 2016)**

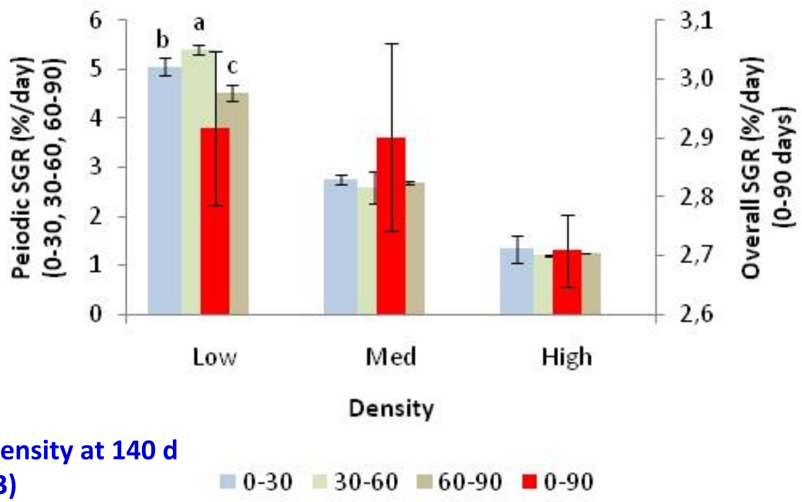
- Different stocking densities tested
  - Three different densities: low, med, and high
  - Triplicate tanks for a period of 140 days

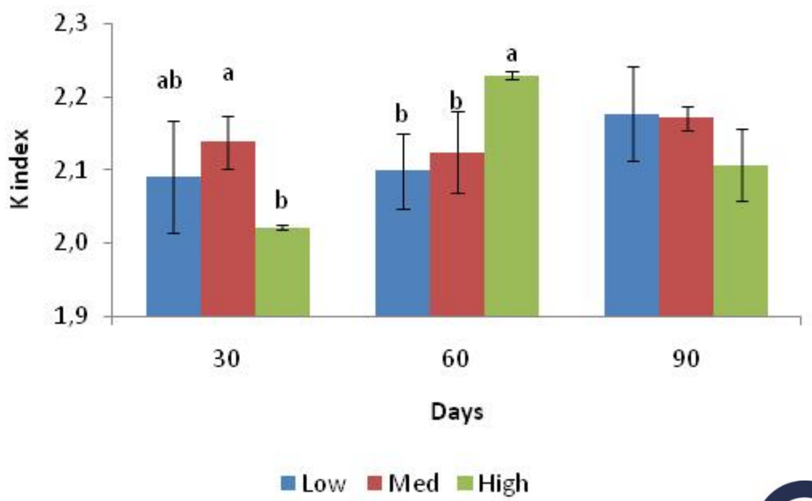
- Growth, condition and welfare of fish studied
  - Specific growth rate and fish condition (K)
  - Feed intake
  - Hematological and biochemical parameters
  - Immunological and oxidative stress studies

- Implementation: IEO, ULL

Task 21.3.2 **Results for 5 g juveniles (so far)**

- Significantly lower SGR in high density during the first 30 days and slightly lower in overall period (0-90 days)
- Lower Condition index (K) in high density during the first 30 days and higher at 60 days





Final density at 140 d (kg/m3)
Low: 3.66±0.46
Med: 5.75±1.69
High: 7.41±0.24

WP 22. Pike perch

- Bottlenecks (by SMEs)
  - unpredictable depression of growth
  - management manipulations are followed by high mortalities

- Reasons
  - high stress responsiveness to intensive culture conditions
  - use of pikeperch broodstock of various domestication levels, including wild populations

- Studies are required
  - effect of husbandry practices and environment
    - farm conditions
  - effect of domestication level and geographical origin

Task 22.1 **Effect of husbandry practices and environmental factors on pikeperch immune and physiological status**

Which are the main stressful factors for pikeperch?

How the fish respond to stress?

- Trial for 8-12 months with juveniles (80-100 g)
  - Expose to various husbandry practices and environmental conditions

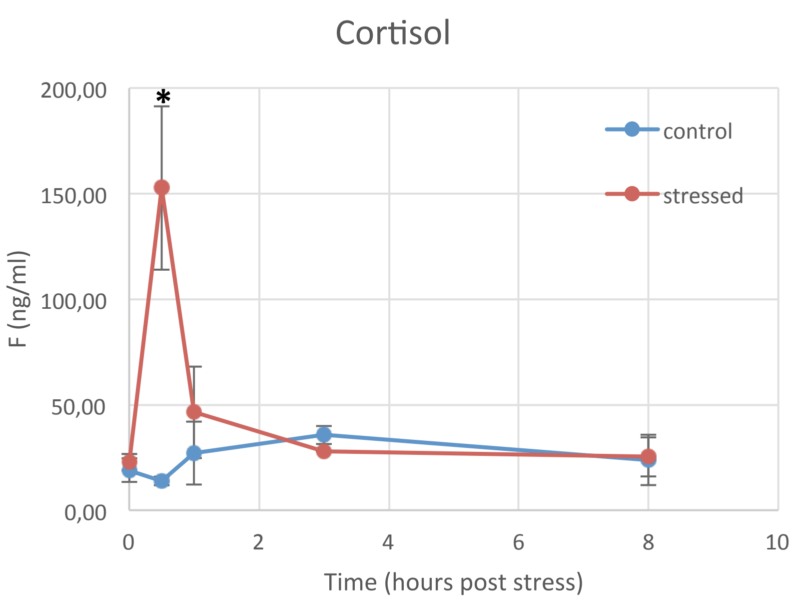
- Result: identify an optimal combination of environmental and husbandry factors

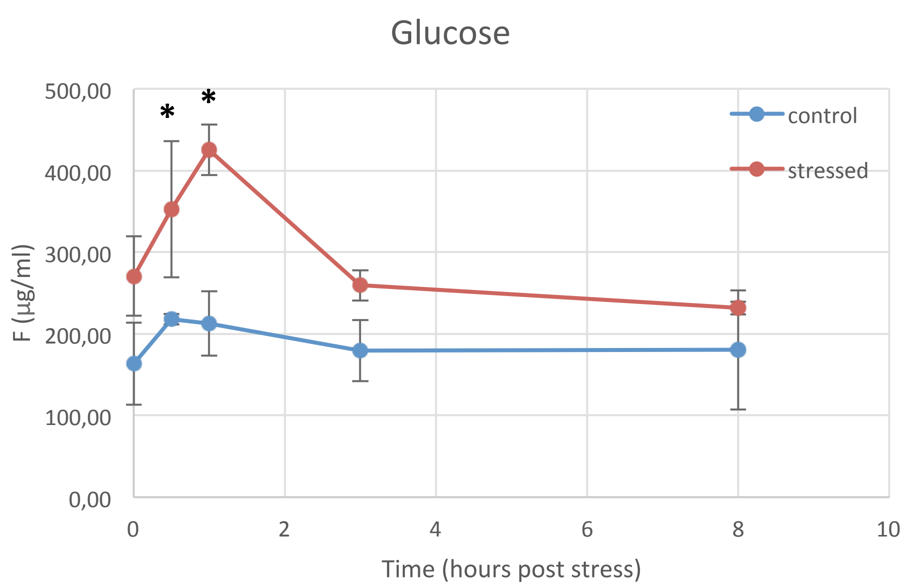
- Implementation: FUNDP, DTU, UL, ASIALOR

Preliminary refinement

- To determine the amplitude physiological response to repeatedly handling-emersion stress (1x/2weeks) in pikeperch

- To determine the optimal time for samplings in the multifactorial experiment





Plasma cortisol and glucose peaked after 30-60 min after handling-emersion

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WP 22.1a. multifactorial experiment

- To find the optimal combinations of environment and husbandry practices for improving growth rate and welfare
- To determine the effects of stressors on physiology, immune condition and husbandry

Experimental design

Exp. condition (n°)	Light intensity (Lux; LI)	Density (kg/m³; dens)	Light spectrum (spec)	Photoperiod (hours of light; photo)	Water temperature (C°; temp)	Type of feed(alim)	Handling (1x/2weeks; Y or N)	Oxygen saturation (%; oxy)
1	10	30	white	24	21	sinking	Y	90
2	100	15	red	10	26	floating	N	60
3	100	15	white	24	21	sinking	N	60
4	100	30	red	10	21	sinking	N	90
5	10	15	red	10	21	sinking	Y	60
6	10	15	white	10	21	floating	N	90
7	100	15	red	24	21	floating	Y	90
8	10	15	white	24	26	floating	Y	60
9	100	15	white	10	26	sinking	Y	90
10	100	30	white	10	21	floating	Y	60
11	100	30	white	24	26	floating	N	90
12	10	30	red	10	26	floating	Y	90
13	100	30	red	24	26	sinking	Y	60
14	10	30	red	24	21	floating	N	60
15	10	30	white	10	26	sinking	N	60
16	10	15	red	24	26	sinking	N	90

WP 22.1a. Multifactorial experiment

Results

Experimental Cond	Biomass gain (g)	Growth (%)	Mortality (%)	Cortisol D35 (ng/ml)	Cortisol D63 (ng/ml)	Glucose D35 (µg/ml)	Glucose D63 (µg/ml)
1	7238	81	4,32	84,05	31,38	456,9	317,7
2	2888	56,9	2,86	17,25	12,82	377,5	426,9
3	3059	84,9	12,86	20,29	14,78	355,1	371,7
4	17	53,7	30,94	18,76	14,39	263,5	340,2
5	1494	41	7,14	15,34	13,91	424,2	378,9
6	-609	0	7,14	16,24	14,01	342,0	365,2
7	-1210	21	24,29	17,06	13,42	272,8	342,7
8	1996	57,4	10	59,64	13,14	321,9	359,2
9	3216	102,8	12,86	25,73	13,18	371,2	411,3
10	-2056	57,4	41,01	17,00	14,26	339,5	317,5
11	1770	63,4	17,99	89,12	28,71	327,9	405,3
12	-1764	22,8	23,74	23,20	12,83	304,1	324,6
13	1534	62,4	31,65	47,18	14,81	466,5	411,8
14	3007	26,5	4,32	16,73	13,01	271,5	337,5
15	9042	80	2,88	27,22	14,95	419,9	373,1
16	3511	81,9	7,14	18,01	14,88	374,7	368,6

3 combinations look promising for pikeperch aquaculture !!!

WP 22.1b. Multifactorial stress and disease resistance

❖ *Objectives:*

- To emphasize the effects of selected mild-stressful conditions on disease resistance
- To confirm the optimal husbandry and environmental conditions for improving growth and welfare status of pikeperch

❖ *Experimental design:*

Exp. Condition (n°)	Light intensity	Density	Light spectrum	Photoperiod	Water temperature	Type of feed	Handling	Oxygen saturation
1	10	30	white	24	21	sinking	Y	90
15	10	30	white	10	26	sinking	N	60
16	10	15	red	24	26	sinking	N	90

Confirmation experiment

→ **Husbandry parameters**

→ **Physiological status**

→ **Immune status (*in progress*)**

Bacterial challenge

→ **Cumulative mortality curve**

→ **Physiological status**

→ **Immune status (*in progress*)**

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Task 22.2 **Characterization of pikeperch immune and physiological status in farm conditions**

Are the results applicable in farm conditions?

- rearing in farm conditions for 2 years to define best practice for pikeperch
  - Implementation UL, FUNDP, ASIALOR

To start in 2016

Task 22.3 **Effect of pikeperch domestication level and geographical origin on stress sensitivity**

How the level of domestication affect the stress response?

- Investigate the effects of domestication (wild vs domesticated strains) and geographical origin (freshwater vs brackish water strains)
  - Implementation: UL, FUNDP, DTU, ASIALOR

WP 23. Grey mullet

Evaluating the grow-out strategy of mullet in the Mediterranean basin

- Most **grey mullet** are reared extensively in polyculture systems
- Exists an established market (North Africa) and a growing one in the Med
- Intensive monoculture has to be developed

  - Best grow-out parameters of grey mullet in the Mediterranean basin
  - Determine appropriate weaning diets for juvenile grey mullet

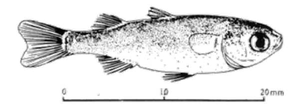
Task 23.1 **Determine the cost-benefit of different weaning diets on the performance and health status of wild juveniles**

Which is the optimum weaning methodology?

- Test in tanks with wild juveniles following an adaptation period
  - a weaning protocol for fry (24.2 ± 0.8 mm SL) with diets of different levels of fish meal (FM) and a blend of plant protein sources (PP)
    - days 0-5: 100% live 6 days-old *Artemia* metanauplii (15-20 metanauplii/mL);
    - days 6-10: 75% *Artemia* metanauplii + 25% inert feed (FM, PP50, PP75);
    - days 11-15: 50% *Artemia* metanauplii + 50% inert feed (FM, PP50, PP75);
    - days 16-20: 25% *Artemia* metanauplii + 75% inert feed (FM, PP50, PP75);
    - days 21-60: 100% inert feed (FM, PP50, PP75).
- Monitoring
  - fish growth, survival maturation of digestive system, health status
  - economic efficiency
- Result: recommendation of best weaning diet
- Implementation: IRTA Research & Technology Food & Agriculture

- Growth performance, condition and survival
- Proximate composition

} No differences



- FM substitution affected the n-6 PUFA levels in fish fed PP50 and PP75 diets
- FM substitution did not affect the {
  - AA composition of weaned fry
  - activity of pancreatic and intestinal enzymes assayed
  - peroxidation levels - activity of antioxidative enzymes
  - histological organization of the liver and intestine

Conclusions:

- FM substitution did not affect any of the performance and condition parameters analyzed
- Weaning wild grey mullet fry (zooplanktivorous) may be conducted using diets with a high level of FM substitution (complete substitution seems possible)

Wheat gluten	1,710	0.0	6.9	10.5	0	11,799	17,955
Soy protein concentrate	1,340	0.0	5.0	7.0	0	6,700	9,380
Corn gluten	720	0.0	5.0	7.0	0	3,600	5,040
Wheat meal	270	16.5	12.6	11.0	4,455	3,402	2,970
Fish oil	1,250	11.3	11.5	13.1	14,125	15,625	16,375
L-lysine	1,950	0.0	0.4	0.7	0	780	1,365
DL-methionine	3,550	0.2	0.3	0.4	710	1,065	1,420
Total	-	-	-	-	96,730	81,691	73,865
Δ feed price (%)					**0**	**-15.5**	**-23.6**

Task 23.2,3,4 **Feeding an improved diet in monoculture**

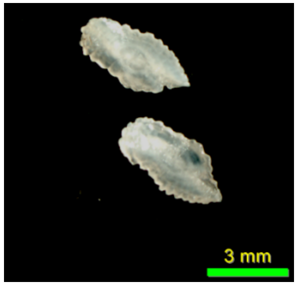
Which are the appropriate conditions of rearing?

- Test in cement (IL, GR), and earthen (IS, SP) ponds
  - Two stocking densities
  - Using wild (GR, SP) or F1 (IL) juveniles
  - Monitoring
- Result: best grow out management practice
- Implementation: IOLR, IRTA, HCMR, CTAQUA, DOR, GEI, IRIDA

Recruitment and maintenance of wild-caught animals under farm conditions – Acclimatization period

Fish recruitment

- Fry collection
  - Greece: 5.000 individuals, BW=0.28g during the period of September to October 2014
  - Spain: 1.500 individuals, BW=0.10g during the period of April 2015



Adaptation period

- Common conditions for 8 months
  - fed a commercial diet (Elite 1, 50% protein, 21% fat).
  - species identification based on body shape and the otoliths (Tuset et al., 2008)

ctaqua
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IRIDA
V. GEITONAS
hcmr
ΕΛΚΕΘΕ

Feeding wild caught juveniles an improved diet at two different densities in cement and earthen ponds

- Fish weighed and sorted on July, 2015
  - Greece: ~21(±1.4)g
  - Spain : 4g
- Distributed in
  - Greece: 2x3 cement tanks at densities 4 and 6 ind m^{-2}
    - artesian bore water, DO ~8 ppm at 22°C , natural photoperiod
  - Spain: 2 earthen ponds at densities of 0,5 and 1 ind m^{-2}
    - natural thermo- and photoperiod

- Experimental feed (IRIDA mullet 1.5mm)
  - formulation IOLR including Ulva sps
- Feeding procedure
  - Greece: 2 times d^{-1} to satiation (09:00 & 15:00 h) six days a week
  - Spain: automatic feeders till size allow use of demand feeders (farm standard procedure)
- Fish performance will be evaluated in terms of FCR, SGR, weight gain and survival, …

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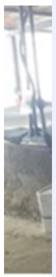
The trial is in progress

■ Feeding an improved diet in monoculture of F1 juveniles (IOLR)

- Grey mullet (from F1 eggs) juveniles (6 g at 142 dph) stocked In two cement tanks and two polypropylene tanks



New experiment

Stock homogenously sized fish from the first mullet trial into tanks for new experiment

- Four polypropylene tanks (diameter of 3m) will be stocked with 6 fish/m2 and 4 fish/m^2
- Two cement tanks will be stocked with 10 and 25 F2 fish /m^2
- Two cement tanks will be stocked with 10 and 25 Wild fish/m^2

Polypropylene tanks A

Polypropylene tanks B

Cement tanks

immediately started.

- A density effect on growth is visually apparent.

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Thank you for your attention!