Deliverable Report

Deliverable No:	D20.2	**Delivery Month:**	54
Deliverable Title	Definition of the optimum conditions for cage rearing of meagre		
WP No:	20	**WP Lead beneficiary:**	P3. IRTA
WP Title:	Grow out husbandry - meagre		
Task No:	20.2	**Task Lead beneficiary:**	P1. HCMR
Task Title:	Effect of rearing environment		
Other beneficiaries:	P 23. ARGO		
Status:	Delivered	**Expected month:**	36
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Objective Adaptations of the existing methodology for grow out in sea cages related to the rearing environment (depth and light conditions).

Description: Effect of rearing environment

Effect of cage depth. Trials in 180 (6x6x5) and 290 (6x6x8) m^3 cages will be performed in the HCMR pilot farm using two groups of sizes (200-600 g) and (800- 1.5 kg). In both cases the final stocking density will be 15 kg m^3. The duration of each trial will be 8 months. Growth performance will be estimated with monthly samples while every second month hematological (hematocrit, hemoglobin), biochemical (osmotic pressure, glucose, lactic acid, free fatty acids), immunological (lysozyme, myeloperoxidase serum), hormonal (cortisol) evaluation will be performed. Also, the vertical distribution in cages will be monitored using an echo integrator.

Effect of light intensity in the cage. Test cage rearing with and without shading at an SME farm (ARGO) applying standard commercial procedures for 2 rearing periods (each with 2 cages) with groups of different ages (200-600 g) and (800- 1.5 kg). In both cases the final stocking density will be 15 kg m^3. The duration of each trial will be 8 months. Growth performance will be estimated on monthly basis, while the vertical distribution in cages will be monitored for a specific period of time (2 weeks per trial) using an echo integrator.

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Summary

The purpose of this work was to determine the best conditions for the cage rearing of meagre (*Argyrosomus regius*). Two experiments were implemented. The first experiment investigated the effect of different depths during two developmental phases of rearing, *i.e.* for fish size of 200 to 800 g and for 800 to 1500 g. The duration of each trial was approximately a year during which the biological performance together with various physiological and biochemical indicators were monitored. The second experiment focused on the effect of light during the rearing, by applying shading on the cages. This second trial, implemented in a commercial farm, was repeated in two successive production cycles.

In the first experiment the only difference observed was during the 1st phase of the rearing (200-800 g) when the fish in deep cages exhibited an improved performance taking into account mortality and feed utilization (feed conversion ratio, FCR). For all the other parameters tested, no significant differences were observed, apart from the seasonal differences (see below). For the 2nd phase of the rearing no differences were observed between the tested conditions.

Hematocrit, hemoglobin and osmotic pressure exhibited seasonal differences and showed the highest concentrations in November. Cholesterol and total protein also showed only seasonal differences, whereas free fatty acids were influenced by both factors examined, presenting lower concentrations at lower temperatures. Lactate, glucose and lysozyme appeared to be affected by both factors examined, while for the latter, differences were shown between the two cage depths only in warm months. The thyroid hormone triiodothyronine (T_3) did not show differences neither among the seasons nor between the two tested depths, while thyroxine (T_4) showed seasonal differences probably related to photoperiod. Finally, cortisol showed only seasonal differences showing the highest concentration in March, the month with the lowest temperature.

The depth of the cages affected significantly the lysozyme and complement antibacterial activity of the plasma in small fish in an opposite manner, so it was impossible to give a recommendation about optimal cage depth for the health of small meager. Cage depth does not seem to be significantly implicated in the health status of large fish. Seasonal variations such as water temperature and photoperiod had a stronger effect on the fish immune parameters tested than the depth of the cages.

No differences were found in the parameters monitored during the shading experiment.

The behavior observed in all described trials was different from what was detected in the cage farming of other species. In the echograms the feeding periods are clearly marked with the vertical distribution of meagre being mostly in the lower half of the cage for a period of approximately 12 hours, while for the rest of the period the groups were distributed almost homogeneously in the whole available volume of the cage. This is a behavior clearly correlated with the light and dark periods of the day. Meagre seems to have a high tolerance to variable conditions, with a very conservative behavioral pattern.

1. INTRODUCTION

Meagre is a sciaenid fish found in the Mediterranean and Black Sea, and along the eastern Atlantic coast (Haffray et al., 2012). This fish has attractive attributes for the market that include large size, good processing yield, low fat content, excellent taste and firm texture (Monfort, 2010). The species also has the biological characteristics required for commercial aquaculture using well-established gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) culture technologies (Duncan et al., 2013). These characteristics include a fast growth of ~1 kg per year (Duncan et al., 2013), a low feed conversion ratio of 0.9-1.2 (Monfort, 2010; Duncan et al., 2013), relatively easy larval rearing (Roo et al., 2010; Vallés and Estévez, 2013) and established induced spawning protocols for the production of viable eggs (Duncan et al., 2012, 2013; Mylonas et al., 2011; Fernandez-Palacios et al., 2014). Meagre was first produced in 1997 in a commercial hatchery in France and since then it has exhibited annual production increases as high as 7fold (FAO). In 2010, European meagre aquaculture production was 2,387 t, produced mainly in Spain, with smaller quantities produced in France, Portugal, Italy, Greece, Cyprus and Croatia (FAO).

The technologies and practices used currently for **meagre** grow out are the same as those used for gilthead sea bream and European sea bass, although this fish presents significant differences in growth rates, feeding and spatial behaviour in the cage. Meagre presents a distinct feeding behavior and has a tendency to stay in the bottom of the cage, feed low in the water column and take time to rise towards the surface to feed.

This behavior results in high stocking densities as fish utilize only part of the available volume. This in turn may result in stress (Ashley, 2007; Branson, 2008) and also altered social interactions. Both potentially can affect feeding, growth performance and immune status of the fish (Ellis et al 2002). For meagre the available information on its response to stress is limited (Fanouraki et al 2011; Samaras et al 2015) and therefore further studies are required. This represent **the first objective of this study, *i.e.* the effect of rearing volume on the performance of the meagre**.

Conditions during fish rearing in aquaculture facilities differ from their natural habitats, and this artificial environment may influence the fish physiology and behaviour. Among others, one major issue of interest is the effect of light in the rearing environment on the overall performance of fish. Fish have color vision and their ability to recognize feed, and consequently their food intake, could be influenced by background light. Studies in juvenile fish have shown that different tank colors during rearing affects not only growth and survival (Papoutsoglou et al, 2005; Karakatsouli et al., 2007; Strand et al., 2007) but also their social interactions (Hoglund et al., 2002; Merighe et al., 2004), their response to stress (Rotllant et al., 2003; Van der Salm et al., 2006), and their immune response (Eslamloo et al., 2015). The effect of light conditions has been tested in experiments with tanks having different background colours and it showed to be species specific depending also on the life stage of fish (Papoutsoglou et al. 2005). In goldfish (*Carassius auratus*), barfin flounder (*Verasper moseri*), Eurasian perch (*Perca fluviatilis*) and white Sea bream (*Diplodus sargus*) the growth performance was enhanced when fish were reared in white tanks (Eslamloo et al., 2015; Amiya et al. 2005; Karakatsouli et al. 2007; Strand et al. 2007). In addition, black background showed to have a negative impact on the growth rate of rainbow trout (*Oncorrhynchus mykiss*) (Papoutsoglou et al. 2005) whereas green background proved to enhance fish growth in the same species (Luchiari & Pirhonen 2008). Therefore, **the study of the light conditions during rearing** is of paramount importance to decide on the optimum rearing environment for the meagre and this **represents the second objective of this study**.

2. MATERIALS AND METHODS

2.1 Experiment 1 Effect of cage depth.

The specific objective of the experiment was to test the performance of meagre in cages of different depth. Cages of 180 (6x6x5) and 290 (6x6x8) m^3 at the HCMR pilot farm in duplicates indicated as Shallow and Deep were used.
Fish were produced in the hatchery of HCMR. Eggs were obtained from a single spawning and larval rearing was performed at the Mesocosm hatchery of the institute. Juveniles of 2 gr were transferred to the cage facility and they were reared under similar conditions until the beginning of the trial.

Two trials were performed.
The **1st trial** started in May 2014. Four groups were created, two of ~5,150 for the 180 m^3 cages and two of ~8,240 for the 290 m^3 cages in order to keep similar stocking densities for the two conditions. The wet weight at the beginning of the trial was **200 ±20 g**. The duration of the trial was from May 2014 to February 2015.
The **2nd trial** started on March 2015. Four groups were created, two of ~2,000 individuals for the 180 m^3 cages and two with ~3,200 for the 290 m^3 ones. The initial weight at the beginning of the trial was **867 ± 18 g**. The trial was completed on January 2016.

The temperature profile during the two trials was natural seawater temperatures (**Figure 2.1.1**)

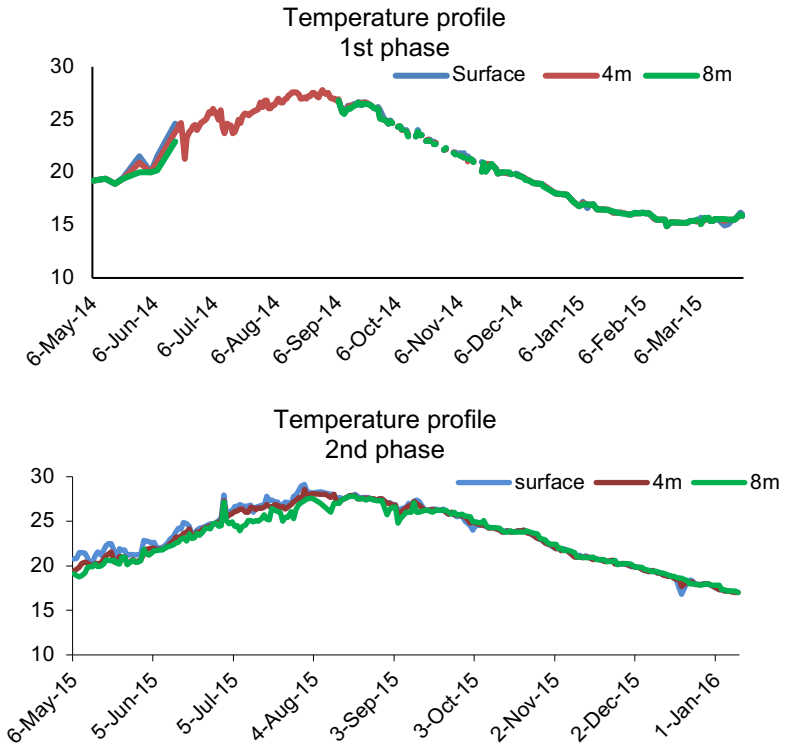

Figure 2.1.1 Temperature profile (surface, 4m and 8m depth) during the two experimental trials

During the experimental period, growth performance of the fish was estimated from monthly samples in both trials

Every second month blood samples were taken for hematological (hematocrit, hemoglobin), biochemical (osmotic pressure, glucose, lactic acid, free fatty acids), immunological (lysozyme, myeloperoxidase serum) and hormonal (cortisol) evaluation.

Blood sampling
Fish were netted (10 fish per group), anaesthetized (Phenoxy-ethanol), total length and body weight were measured and blood was drawn from the caudal vessel, using a sterile syringe, and placed in tubes containing heparin. After the determination of hematocrit and hemoglobin, blood was centrifuged (2000× *g*, 4◦C for 10 minutes) and plasma aliquots were stored at −20°C for further analysis of cortisol, glucose and lactate.

Statistical analysis
All statistical analyses were performed with SigmaPlot 11.0 (Jandel Scientific). Data are presented as means ± standard deviation (SD) (or S.E.M. for immunological parameters). Statistical comparisons of total length and bodyweight and also of the hematological, hormonal and biochemical parameters between the different groups and between the different sampling months were made using two-way ANOVA to assess differences among groups and Tukey's or Dunn's post-hoc tests to assess the level of significance. The significance level used was $P < 0.05$.

Hematological and biochemical analyses
Hematocrit measurements were based on the use of special capillary tubes where blood samples were transferred and centrifuged for 10 min in a capillary centrifuge at 2000× *g*). Hemoglobin and lactate determinations were carried out using the corresponding commercial kits (SPINREACT). Glucose measurements were carried out using a commercial kit (Biosis).

*Hormonal analysis***.**
For the determination of cortisol, plasma samples were extracted with diethyl ether and water samples were extracted with ethyl acetate according to Ellis et al. (2004). Briefly, 1 ml of diethyl ether was mixed with 100 μl of plasma and after allowing the phases to separate, the organic phase was transferred and evaporated under nitrogen gas. Residue was re-dissolved in 100 μl extraction buffer. **Cortisol** determinations were performed at the University of Crete (Lab of Fish Physiology). Plasma cortisol concentrations were measured using commercial cortisol enzyme immunoassay kit (Cayman).

Immunological analysis
Serum samples were obtained from 10 fish per cage every 2 months and kept at -80ºC until analysed. The measurements of immunological parameters in meagre, were adapted from methods routinely used in the plasma of Gilthead seabream or European sea bass.
The myeloperoxidase activity of serum was determined as described before (Kokou *et al.*, 2012) but using 50μl of the stopping solution (Henry *et al.*, 2015). Briefly, 15μl of serum were diluted with 135μl HBSS and 50μl of the TMB-H_2O_2 solution were incubated for 2 minutes before 1N H_2SO_4 was added to stop the reaction. OD was measured at 450nm.
The antibacterial activity of the serum was measured against a Gram positive bacterium (lysozyme activity) following a method previously described for gilthead seabream, *Sparus auratus* (Kokou *et al.*, 2012) and optimized for meagre. Briefly, lysozyme activity was measured using the turbidimetric method following the kinetic of lysis of the membrane of *Micrococcus luteus* (0.2mg/ml) by 10μl of serum at 450nm for 20 min.

Despite not being included in the original DOW of WP20, 2 supplementary parameters were investigated:
The kinetic of antibacterial activity of serum against a strain of the Gram-negative bacterium *E.coli* transformed with a luminescent gene (luciferase) followed the antibacterial activity of the complement. Serum volume added to the bacteria was 70µl in each well. The percentage of bacterial inhibition was obtained by comparing the peak luminescence of the serum sample with the peak luminescence obtained in the negative control without serum where bacterial growth was not inhibited (Henry *et al.*, 2015).
The serum anti-protease activity was measured as described before for shi drum, *Umbrina cirrosa* (Henry and Fountoulaki, 2014).

Behavioral monitoring
The vertical distribution of the populations in cages was monitored using an echo integrator. The system used is the CageEye 1.3, (Lindem Data Acquisition AS, Norway).

2.2 Experiment 2. Effect of light intensity in the cage

The objective of the trial was to test the rearing of meagre in cages with and without shading, at the installations of our commercial partner P23. ARGO, applying standard commercial procedures for 2 rearing periods.
In both trials juveniles from a commercial hatchery (Poisson de Soleil, France) were used. Before being introduced into the cages, the juveniles were reared (pre-growing) at the land-based facilities of the company under similar conditions. Homogenized groups were created and transferred to the cages at the beginning of the trial.

The first trial started on November 20, 2014 and lasted until April 29, 2016. The second trial started on October 20, 2016 and lasted until August 20, 2017.
Two rectangular cages of 10x10x8 m (V= 800 m^3) were used for each trial. One of them was covered with a net of 90-95% shading (**Figure 2.2.1**) while the second was covered only with a bird protecting net.

(a)  (b) 

Figure 2.2.1 Experimental cages at ARGOSARONIKOS SA. Shaded (a) and not shaded (b).

The temperature of the area during both the 1st and the 2nd experimental periods is presented in **Figure 2.2.2**

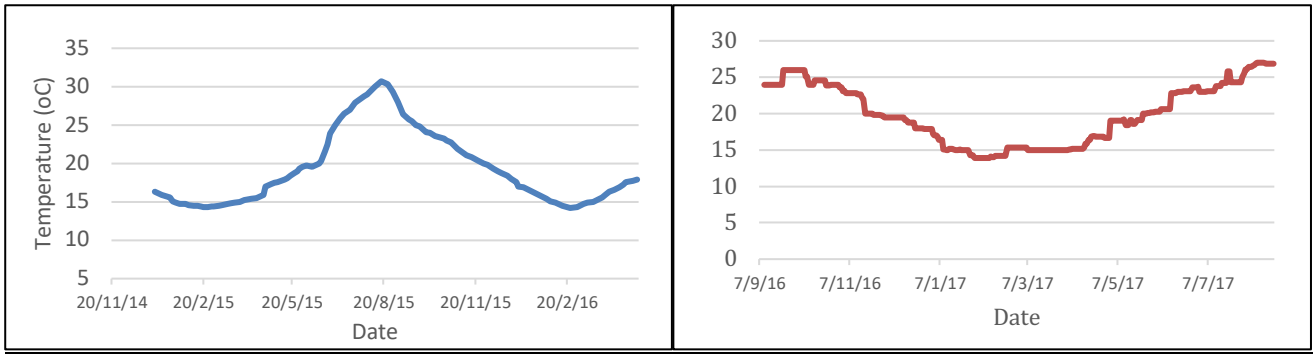

Figure 2.2.2 Temperature at 3 m depth at ARGOSARONICOS SA during the 1st (a) and the 2nd (b) experimental periods

The first trial started with groups of 11.000 individuals each with an individual weight of 135±25 g.

For the second trial the initial fish groups were 10,940 and 10,200 in each cage. The initial weight was 270 and 240 g respectively. One cage was covered with shading net as in the first trial while both were protected with net against birds.
Groups were fed manually, 3 times per day, with standard commercial diets. Samples to estimate growth rate were regularly taken.

Behavioral monitoring
The vertical distribution of the fish populations in the cages was monitored using an echo integrator at regular intervals during both trials. The system used is the CageEye 1.3, (Lindem Data Acquisition AS, Norway).

3. RESULTS

3.1 Experiment 1 Effect of cage depth.

3.1.1 Biological performance

During the experimental period, rearing was implemented without any particular problem such as pathologies that could have resulted in significant changes in the experimental conditions.
Figure 3.1.1.1 shows the growth performance observed in the two experimental phases (200 g and 800 g fish).

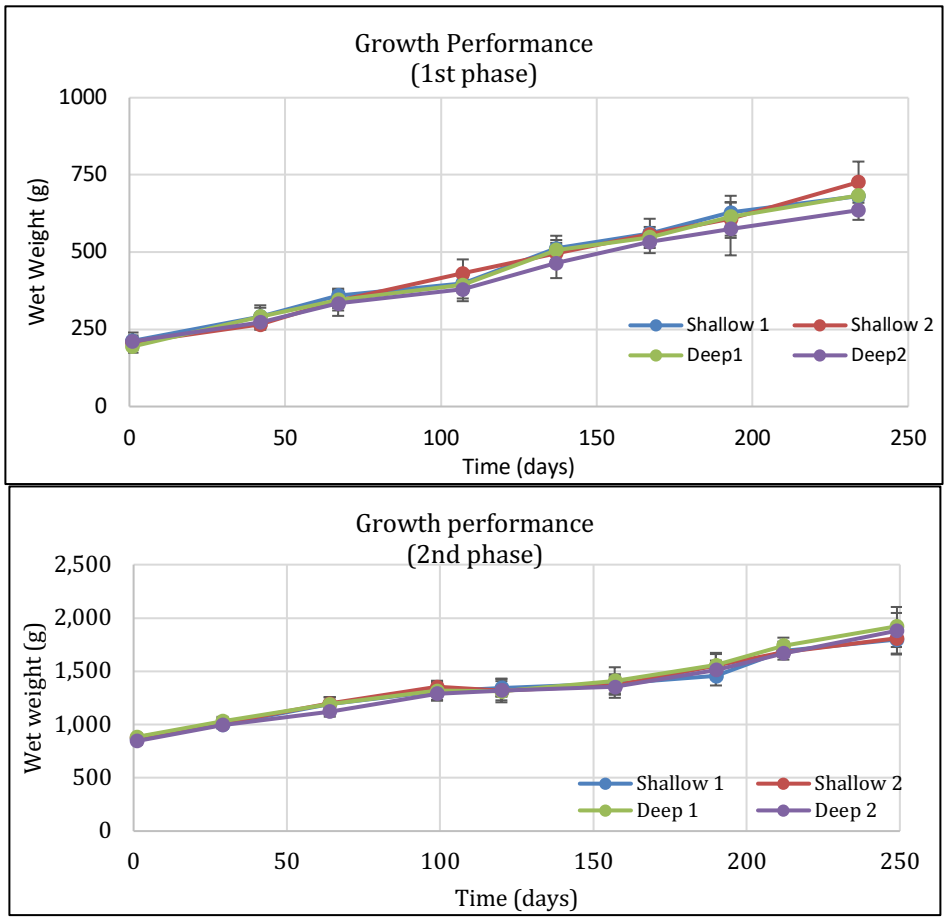

Figure 3.1.1.1 Growth performance in terms of averageweight, of meagre. Vertical bars showthe standard deviation (n=10)

During the 1st phase the growth rate was ~2 g d^{-1} while for the second phase it was increased to 3.5 g d^{-1}. In both phases no significant differences were observed between the tested conditions.

Regarding other performance indicators, **Table 3.1.1.1** shows the mortality (%) and the food conversion ratio (FCR). Significant differences were obtained only during the first period with the groups reared in the deep cages (D1 and D2) cage showing almost half the mortality rate and also ~25% lower FCR compared to the shallow groups (S1 and S2).

Table 3.1.1.1 Performance indicators during the two experimental phases

		S1	S2	D1	D2
1st phase	**Mortality (%)**	23,5	24,2	12,1	13,9
	FCR	1,92	1,92	1,58	1,60
2nd phase	**Mortality (%)**	10.8	9.7	7.9	8.1
	FCR	1.67	1.70	1.50	1.47

3.1.2 Hematological, Biochemical and Hormonal parameters

1st phase (200 g fish initial weight)

Hematocrit

Hematocrit levels appear to be affected by the sampling period ($P < 0.001$), but not by the depth of the cage, with no statistically significant interaction between the two factors (**Figure 3.1.2.1**). There was a statistically significant difference in the hematocrit levels between November (NOV S = 40.6 ± 4.4 % ; NOV D = 37.4 ± 4.6 %; $P < 0.001$) and the rest of the months with the lowest levels observed in March (MAR S = 77.9 ± 2.1 % ; MAR D = 27.1 ± 2.8 %).

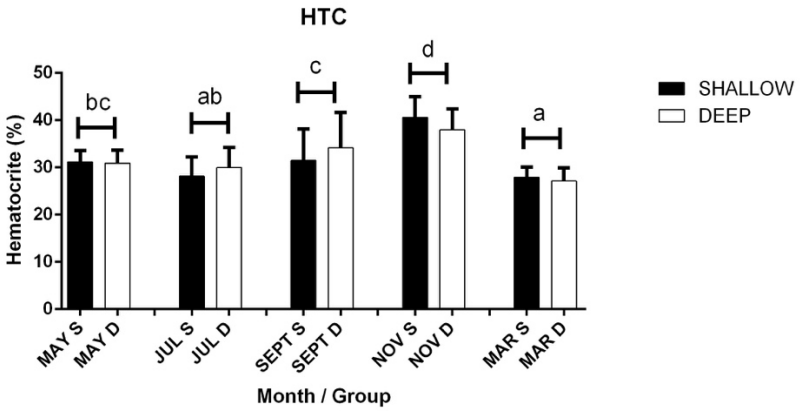

Figure. 3.1.2.1. Hematocrit levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate significant differences between the different samplings (months), $P < 0.05$.

Hemoglobin

The cage depth didn't have a statistically significant effect on hemoglobin levels. However, there was a statistically significant difference on hemoglobin levels between the different months ($P < 0.001$). The pattern is characterized by low hemoglobin levels from May to September and peak values ($P < 0.05$) in November (NOV S = 9.5 ± 2.9 g dl^{-1} ; NOV D = 9.2 ± 2.1 g dl^{-1}), which dropped significantly in March (MAR S = 7.8 ± 2.4 g dl^{-1} ; MAR D = 7.5 ± 1.5 g dl^{-1})(**Figure 3.1.2.2**.)

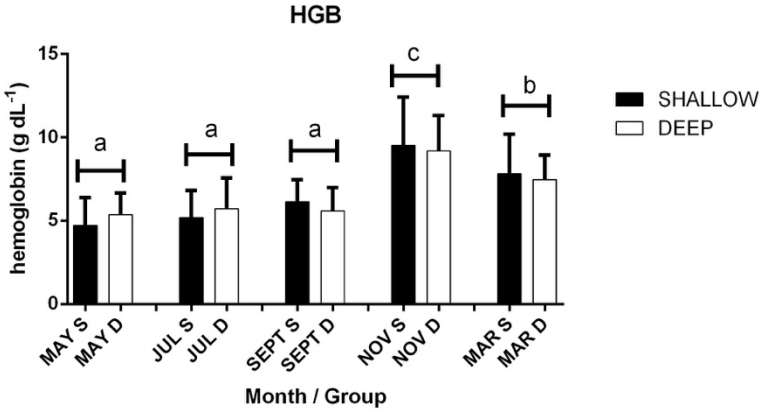

Figure. 3.1.2.2 Hemoglobin levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Plasma osmotic pressure

The cage depth did not have any statistically significant effect on plasma osmotic pressure levels. However, there was a statistically significant gradual increase of osmotic pressure levels from May (MAY S = 313.0 ± 14.3 mOsm kg^{-1}; MAY D = 320.3 ± 19.3 mOsm kg^{-1}) till November (NOV S = 385.7 ± 52.0 mOsm kg^{-1}; NOV D = 388.3 ± 33.5 mOsm kg^{-1}), and then in March, plasma osmotic pressure levels started to decrease (MAR S = 355.6 ± 6.4 mOsm kg^{-1}; MAR D = 357.1 ± 15.7 mOsm kg^{-1}) (**Figure 3.1.2.3**).

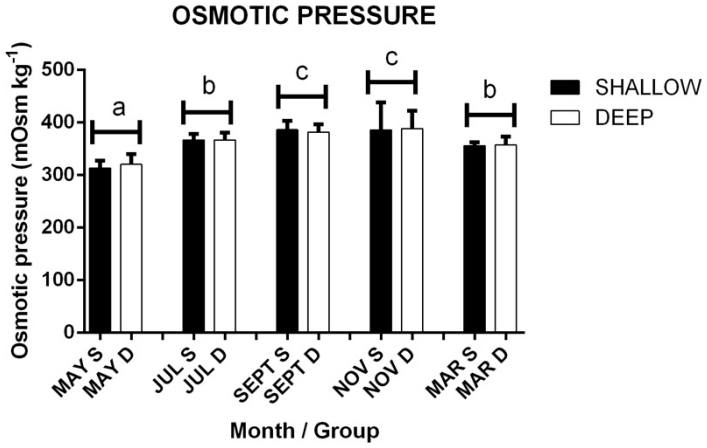

Figure 3.1.2.3. Plasma osmotic pressure during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Glucose

There is a statistically significant interaction between the cage depth and the sampling month ($P < 0.001$). In the Shallow cage there was a statistically significant difference between months May (MAY S = 9.7 ± 2.3 mmol dl^{-1}) and September (SEPT S = 7.3 ± 1.6 mmol dl^{-1}) when the highest values in glucose levels were observed and March (MAR S = 6.7 ± 1.7 mmol dl^{-1}) when the lowest

levels were observed. In fish from the Deep cage, highest glucose levels were observed in July (JULY D = 10.0 ± 2.3 mmol dl^{-1}) which gradually dropped to reach lowest levels in March (MAR D = 5.7 ± 1.6 mmol dl^{-1}). Additionally, the depth of the cage had an effect on plasma glucose levels in all samplings apart from March (**Figure 3.1.2.4**).

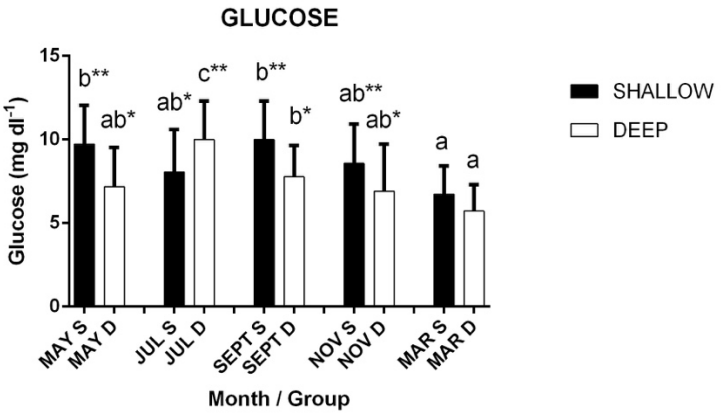

Figure. 3.1.2.4. Plasma glucose levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), while asterisks differences between the different cages, $P < 0.05$.

Lactate

There was a statistically significant interaction between month and cage ($P < 0.001$). In any case, highest lactate values were observed in July (JULY S = 4.7 ± 1.1 mmol dl^{-1} ; JULY D = 3.7 ± 0.9 mmol dl^{-1}) and lowest in March (MAR S = 2.2 ± 0.4 mmol dl^{-1} ; MAR D = 2.1 ± 0.4 mmol dl^{-1}), regardless the depth of the cage. The depth of the cage affected the lactate levels of the plasma, as in fish from the shallow cage lower levels were observed in May and November compared to the fish from the deep cage but in months July and September fish from the shallow cage exhibited higher lactate levels than fish from the deep cage (**Figure 3.1.2.5**).

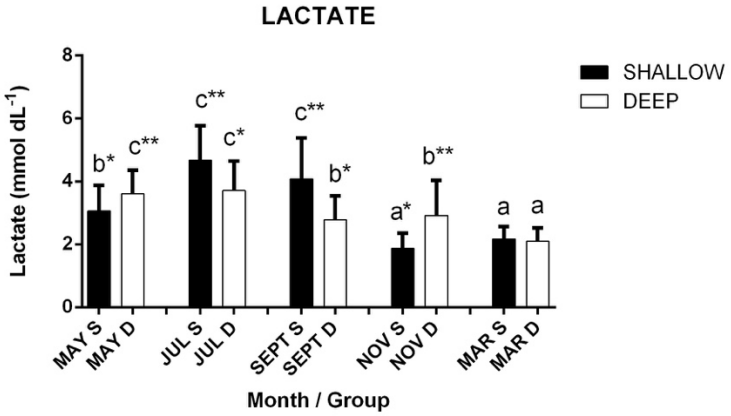

Figure. 3.1.2.5. Plasma lactate levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), while asterisks differences between the different cages, $P < 0.05$.

Cholesterol

The depth of the cage had no effect on the plasma cholesterol levels. However, there was an effect of the sampling month ($P < 0.001$) on the cholesterol levels with highest values observed in May (MAY S = 4.9 ± 1.2 mmol dl^{-1} ; MAY D = 4.7 ± 0.7 mmol dl^{-1}), dropped in July (JULY S = 4.1 ± 0.9 mmol dl^{-1} ; JULY D = 4.0 ± 0.3 mmol dl^{-1}), to gradually reach a minimum in November (NOV S = 1.0 ± 0.8 mmol dl^{-1} ; NOV D = 1.4 ± 0.6 mmol dl^{-1}) (**Figure 3.1.2.6**).

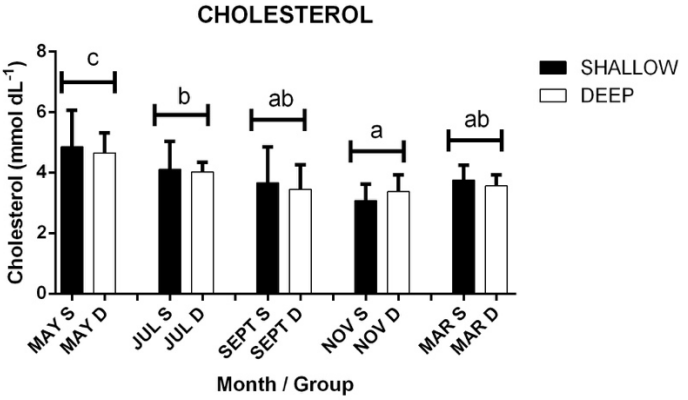

Figure. 3.1.2.6. Plasma cholesterol levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Total Proteins

There was no effect of the cage depth on the total protein levels in the plasma of the fish. However, the sampling month affected the levels of total proteins in the plasma of the fish ($P < 0.001$) regardless of the cage depth, with the highest values observed in July (JULY S = 4.1 ± 0.9 gr dl^{-1} ; JULY D = 4.0 ± 0.3 gr dl^{-1}) and the lowest observed in September (SEPT S = 4.9 ± 1.2 gr dl^{-1} ; SEPT D = 4.7 ± 0.7 gr dl^{-1}) (**Figure 3.1.2.7**).

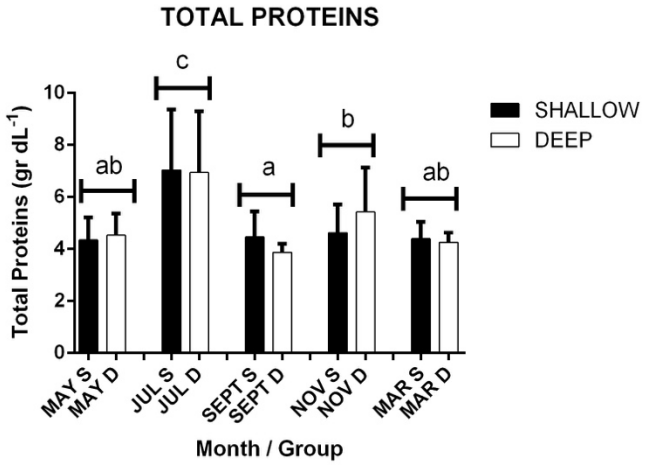

Figure. 3.1.2.7. Total proteins levels in the plasma during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Lysozyme

There is a statistically significant interaction between the cage depth and the sampling month. In fish reared in the deep cage the activity of lysozyme was not affected by the sampling period. However, in fish reared in the shallow cage lysozyme activity levels are low in May (MAY S = 306.3 ± 134.2 k units l^{-1}) and rise during July (JULY S = 10.0 ± 2.3 k units l^{-1}) and September (SEPT S = 646.1 ± 296.0 k units l^{-1}) in a statistically significant manner ($P < 0.05$) to fall to the initial values during the following months. Additionally, the depth of the cage had an effect on lysozyme activity levels in July and September with fish reared in the shallow cage exhibiting statistically significant higher activity levels ($P < 0.001$) than fish reared in the deep cage (**Figure 3.1.2.8**).

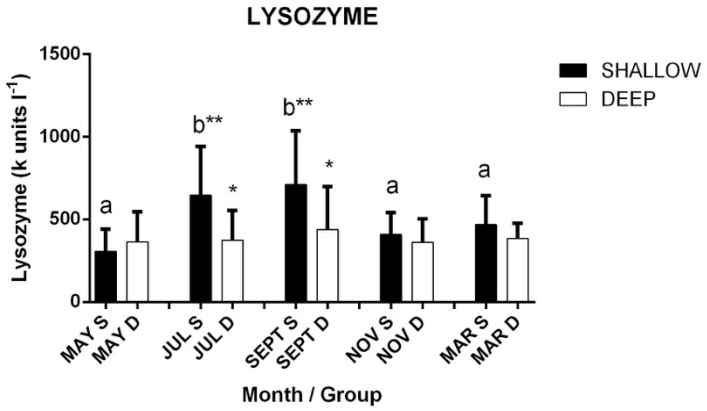

Figure. 3.1.2.8. Lysozyme activity levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), while asterisks differences between the different cages, $P < 0.05$.

Free Fatty Acids

There was a statistically significant interaction between the cage depth and the sampling month ($P < 0.001$). Statistically significant higher free fatty acids levels were observed for both cage depths in July (JULY S = 4.1 ± 0.9 mM; JULY D = 4.0 ± 0.3 mM) which gradually dropped to minimum levels in March SEPT S = 4.9 ± 1.2 mM; SEPT D = 4.7 ± 0.7 mM) (**Figure 3.1.2.9**).

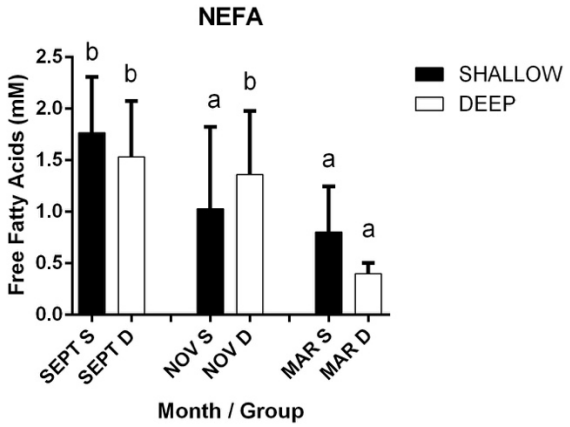

Figure. 3.1.2.9. Free Fatty acids levels during the period from September 2014 to March 2015 (September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Cortisol

The depth of the cage had no effect on cortisol levels and there was no statistical interaction between cage depth and month sampling. In general, cortisol levels remained at lower values that did not differ statistically for the period between May to November [(MAY S = 0.18 ± 0.1 ng ml^{-1} ; MAY D = 0.32 ± 0.4 ng ml^{-1}) ; (JULY S = 0.58 ± 0.5 ng ml^{-1}; JULY D = 0.31 ± 0.3 ng ml^{-1}) ; (SEPT S = 0.34 ± 0.1 ng ml^{-1}; SEPT D = 0.42 ± 0.3 ng ml^{-1}) ; (NOV S = 0.22 ± 0.1 ng ml^{-1}; NOV D = 0.21 ± 0.2 ng ml^{-1})] but there was a statistically significant increase on cortisol levels in March (MAR S = 1.2 ± 0.6 ng ml^{-1}; MAR D = 1.4 ± 0.6 ng ml^{-1}), (**Figure 3.1.2.10**).

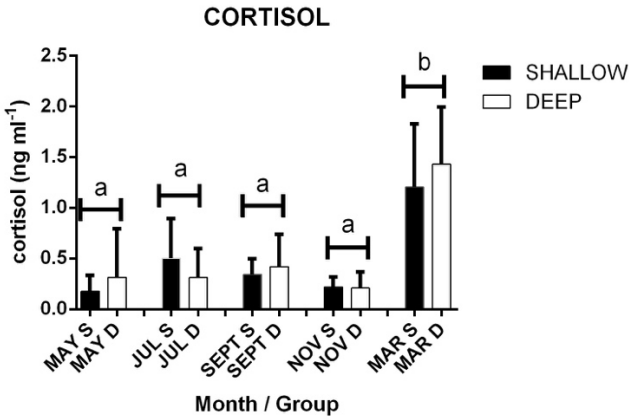

Figure. 3.1.2.10. Plasma cortisol levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), while asterisks differences between the different cages, $P < 0.05$.

Triiodothyronine T3

Neither the depth of the cage nor the sampling month had any statistically significant effect on T3 levels (**Figure 3.1.2.11**).

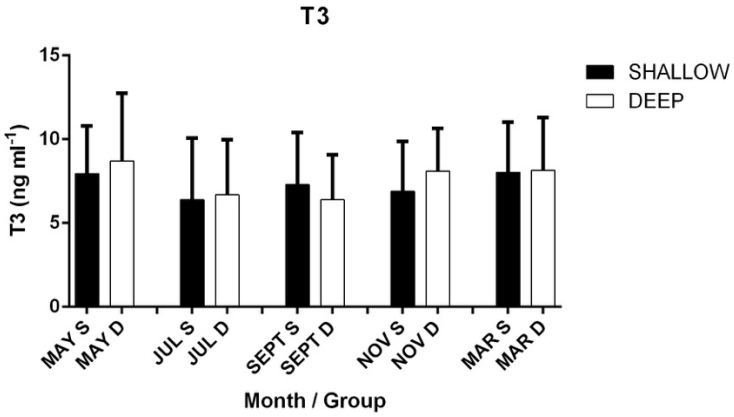

Figure. 3.1.2.11. Triiodothyronine (T3) levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month).

Thyroxine T4

The cage depth had no effect on T4 levels. However, the month of sampling had a statistically significant effect ($P < 0.001$) on T4 levels with minimum levels observed in July (JULY S = 5.31 ± 1.1 ng ml^{-1}; JULY D = 1.1 ± 0.3 ng ml^{-1}) which increased gradually to reach maximum values in November (NOV S = 11.4 ± 1.9 ng ml^{-1}; NOV D = 11.4 ± 2.6 ng ml^{-1}) and March (MAR S = 10.6 ± 2.1 ng ml^{-1}; MAR D = 10.7 ± 1.7 ng ml^{-1}) (**Figure 3.1.2.12**).

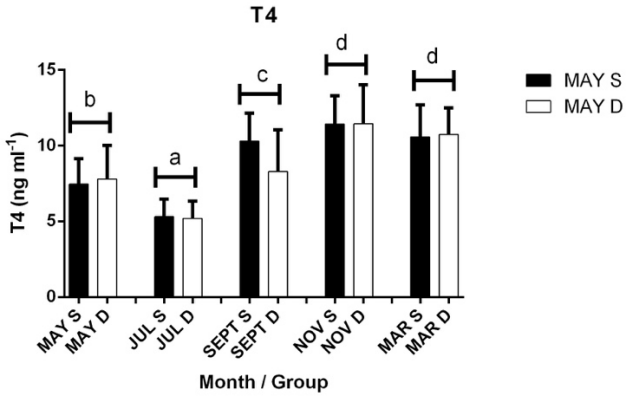

Figure. 3.1.2.12. Thyroxine (T4) levels during the period from May 2014 to March 2015 (May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; March/ Shallow cage: MAR S; March/ Deep cage: MAR D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), while asterisks differences between the different cages, $P < 0.05$.

2nd phase (800 g fish initial weight)

Hematocrit

There was a statistically significant difference in the hematocrit levels between November when peak levels were observed (NOV S = 39.93 ± 8.0; NOV D = 38.0 ± 5.5; $P < 0.001$) and the rest of the months when the hematocrit values fluctuated between similar levels. Moreover, the depth of the cage appeared to affect the hematocrit levels in July (JUL S = 30.45 ± 2.6; JUL D = 27.3 ± 6.6) and September (SEPT S = 27.65 ± 3.5; SEPT D = 30.45 ± 4.2) samplings ($P < 0.05$) (**Figure 3.1.2.13**).

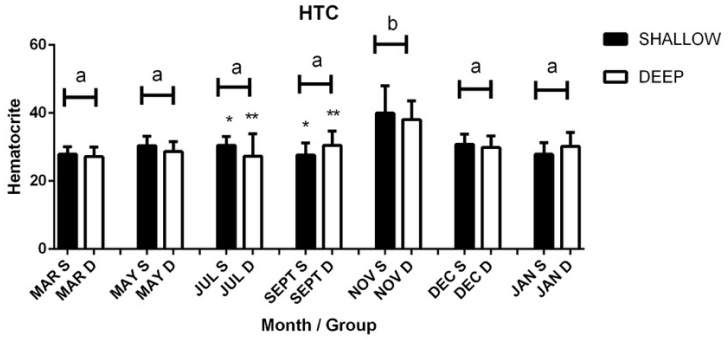

Figure. 3.1.2.13. Hematocrit levels during the period from March 2015 to January 2016 (March/ Shallow cage: MAR S; March/ Deep cage: MAR D; May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; December/ Shallow cage: DEC S; December/ Deep cage: DEC D; January/ Shallow cage: JAN S; January/ Deep cage: JAN D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months) while asterisks differences between the different cages, $P < 0.05$.

Hemoglobin

The cage depth didn't have any statistically significant effect on hemoglobin levels. However, there was a statistically significant difference on hemoglobin levels between the different months ($P < 0.001$). During March hemoglobin levels showed peak values (MAR S = 7.82 ± 2.4 g dl^{-1} ; MAR D = 7.46 ± 1.5 g dl^{-1}) which remained high during the following months of May (MAY S = 5.14 ± 1.8 g dl^{-1} ; MAY D = 6.29 ± 0.9 g dl^{-1}) and July (JULY S = 6.62 ± 1.5 g dl^{-1} ; JULY D = 6.26 ± 1.9 g dl^{-1}), to drop to lower levels during the following period of September to January [(SEPT S = 5.76 ± 0.6 g dl^{-1} ; SEPT D = 5.7 ± 0.7 g dl^{-1}) ; (NOV S = 4.55 ± 1.3 g dl^{-1} ; NOV D = 4.48 ± 1.3 g dl^{-1}) ; (DEC S = 4.49 ± 1.3 g dl^{-1} ; DEC D = 4.2 ± 1.2 g dl^{-1}) ; (JAN S = 4.75 ± 0.8 g dl^{-1} ; JAN D = 4.72 ± 0.8 g dl^{-1})] (**Figure 3.1.2.14**).

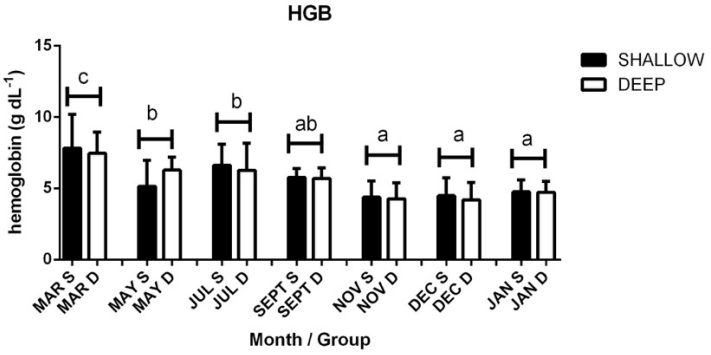

Figure. 3.1.2.14. Hemoglobin levels during the period from March 2015 to January 2016 (March/ Shallow cage: MAR S; March/ Deep cage: MAR D; May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; December/ Shallow cage: DEC S; December/ Deep cage: DEC D; January/ Shallow cage: JAN S; January/ Deep cage: JAN D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Glucose

The cage depth did not have a statistically significant effect on glucose levels. However, there was a statistically significant difference in glucose levels between the different months. Glucose levels remained low during March to September [(MAR S = 67.22 ± 16.9 mg dl^{-1} ; MAR D = 57.11 ± 15.6 mg dl^{-1}); (MAY S = 56.05 ± 36.5 mg dl^{-1}; MAY D = 89.21 ± 38.5 mg dl^{-1}); (SEPT S = 81.99 ± 26.4 mg dl^{-1}; SEPT D = 92.62 ± 34.9 mg dl^{-1})] with higher values for this period observed in July ((JULY S = 108.1 ± 35.3 mg dl^{-1}; JULY D = 103.7 ± 35.8 mg dl^{-1})) and appeared statistically higher ($P < 0.001$) during the period from November to January [(NOV S = 149.13 ± 44.4 mg dl^{-1}; NOV D = 172.43 ± 67.9 mg dl^{-1}) ; (DEC S = 158.21 ± 56.3 mg dl^{-1}; DEC D = 159.71 ± 52.2 mg dl^{-1}) ; (JAN S = 123.67 ± 40.8 mg dl^{-1}; JAN D = 137.28 ± 50.9 mg dl^{-1})] (**Figure 3.1.2.15**).

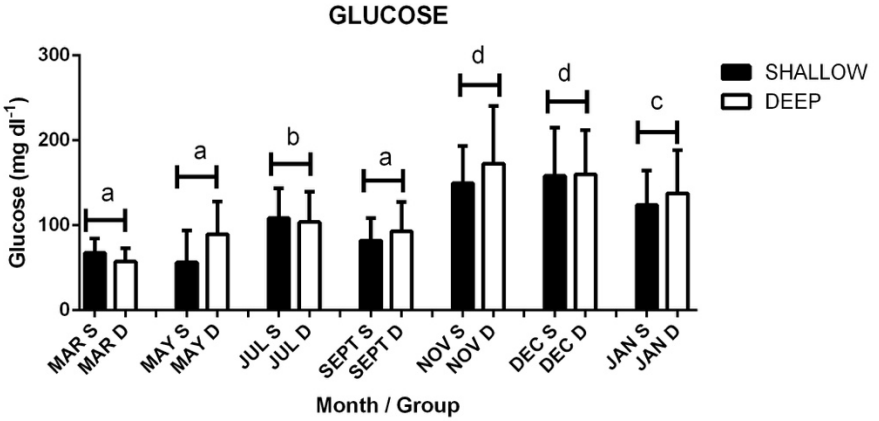

Figure. 3.1.2.15. Glucose levels during the period from March 2015 to January 2016 (March/ Shallow cage: MAR S; March/ Deep cage: MAR D; May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; December/ Shallow cage: DEC S; December/ Deep cage: DEC D; January/ Shallow cage: JAN S; January/ Deep cage: JAN D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Lactate

There was a statistically significant interaction between month and cage ($P < 0.001$) and the effect of month on lactate levels depends on the depth of the cage. The sampling month did not have any statistically significant effect on lactate levels in the case where the deep cage was used. However, there was a statistically significant difference in lactate levels between the different months when the shallow cage was used ($P < 0.001$), lactate levels were statistically higher for months May (MAY S = 4.6 ± 1.6 mg dl^{-1} ; MAY D = 2.4 ± 0.6 mg dl^{-1}), July (JULY S = 3.58 ± 0.6 mg dl^{-1} ; JULY D = 3.05 ± 1.0 mg dl^{-1}) and January (JAN S = 4.73 ± 2.9 mg dl^{-1} ; JAN D = 2.48 ± 1.1 mg dl^{-1}) compared to March (MAR S = 2.17 ± 0.4 mg dl^{-1} ; MAR D = 2.1 ± 0.4 mg dl^{-1}), September (SEPT S = 1.93 ± 0.5 mg dl^{-1} ; SEPT D = 1.97 ± 0.5 mg dl^{-1}), November (NOV S = 2.47 ± 2.4 mg dl^{-1} ; NOV D = 2.47 ± 1.6 mg dl^{-1}) and December (DEC S = 2.2 ± 0.7 mg dl^{-1} ; DEC D = 2.17 ± 0.8 mg dl^{-1}) (**Figure 3.1.2.16**).

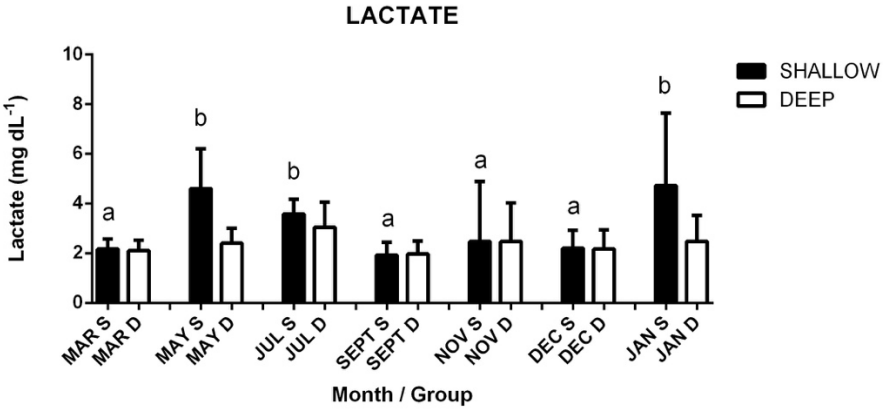

Figure. 3.1.2.16. Lactate levels during the period from March 2015 to January 2016 (March/ Shallow cage: MAR S; March/ Deep cage: MAR D; May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; December/ Shallow cage: DEC S; December/ Deep cage: DEC D; January/ Shallow cage: JAN S; January/ Deep cage: JAN D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

Cortisol

The depth of the cage had no effect on cortisol levels and there was no statistical interaction between cage depth and month of sampling. In general, cortisol levels remained at lower values that did not differ statistically for the period between March to November [(MAR S = 0.12 ± 0.1 ng ml^{-1} ; MAR D = 0.14 ± 0.1 ng ml^{-1}) ; (MAY S = 0.8 ± 0.04 ng ml^{-1}; MAY D = 0.9 ± 0.1 ng ml^{-1}); (JULY S = 0.27 ± 0.4 ng ml^{-1}; JULY D = 0.26 ± 0.4 ng ml^{-1}); (SEPT S = 0.13 ± 0.1 ng ml^{-1}; SEPT D = 0.15 ± 0.1 ng ml^{-1}); (NOV S = 0.25 ± 0.4 ng ml^{-1}; NOV D = 0.33 ± 0.3 ng ml^{-1})] but there was a statistically significant increase on cortisol levels in December (DEC S = 0.28 ± 0.8 ng ml^{-1}; DEC D = 0.53 ± 0.8 ng ml^{-1}) and January (JAN S = 0.68 ± 1.0 ng ml^{-1}; JAN D = 0.17 ± 0.2 ng ml^{-1}) (**Figure 3.1.2.17**).

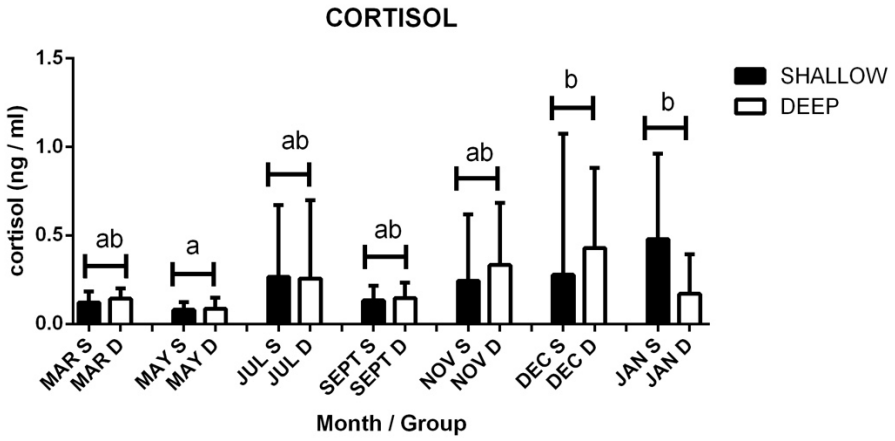

Figure. 3.1.2.17. Plasma cortisol levels during the period from March 2015 to January 2016 (March/ Shallow cage: MAR S; March/ Deep cage: MAR D; May/ Shallow cage: MAY S; May/ Deep cage: MAY D; July/ Shallow cage: JULY S; July/ Deep cage: JULY D; September/ Shallow cage: SEPT S; September/ Deep cage: SEPT D; November/ Shallow cage: NOV S; November/ Deep cage: NOV D; December/ Shallow cage: DEC S; December/ Deep cage: DEC D; January/ Shallow cage: JAN S; January/ Deep cage: JAN D). Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months), $P < 0.05$.

3.1.3 Immunological parameters

Myeloperoxidase activity in the serum was significantly higher in the spring/summer period than in autumn suggesting a stronger respiratory burst activity at high water temperatures (**Figure 3.1.3.1**). However, Myeloperoxidase activity was not reduced at low temperatures in fish kept in deep cage compared to what was observed in fish reared in the shallow cage. This was particularly visible in smaller fish.

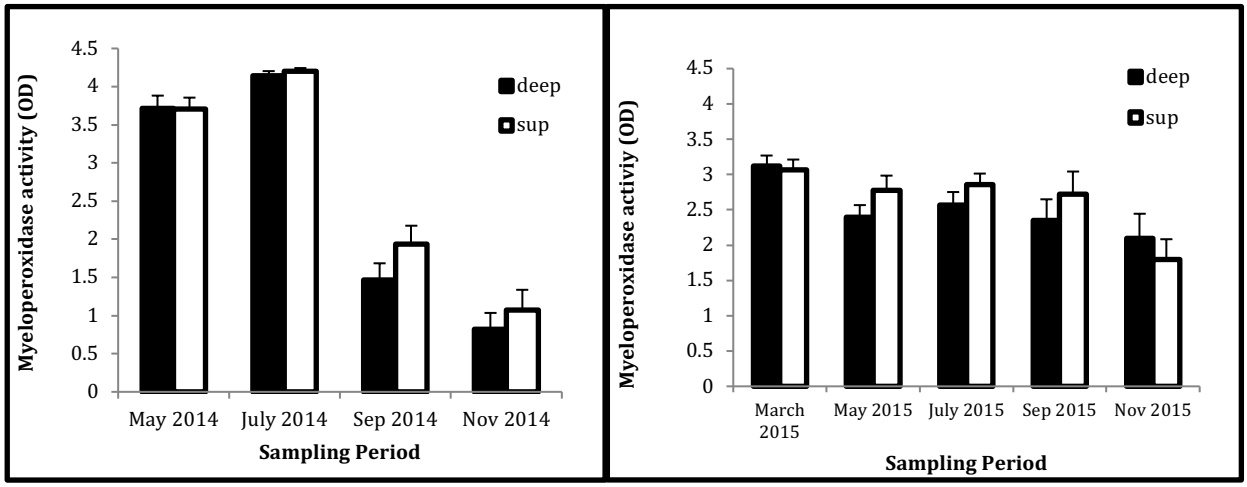

Figure 3.1.3.1: Serum myeloperoxidase activity of small (200-800g) and large (1-2kg) fish kept in cages at different depths (deep or shallow). Asterisks * show significant differences between different sampling times. Hashtag # shows a significant difference between the 2 depths. Different letters show significant differences between depths at different sampling times (One-Way ANOVA, P<0.05, Student-Newman-Keuls't-test). n=10.

The results of Lysozyme antibacterial activity of serum are shown in **Figures 3.1.3.2**. In small fish, lysozyme significantly increased during the summer of 2014 in deep cages compared to shallow cages. Lysozyme activity return to 300-400 units/ml during the autumn in fish kept at both depths. Concerning larger fish, lysozyme activity also increased during the summer in both fish kept in deep and in shallow cages. Lysozyme activity of large fish did not reduce as quickly as in small fish with the decreasing temperatures in autumn.

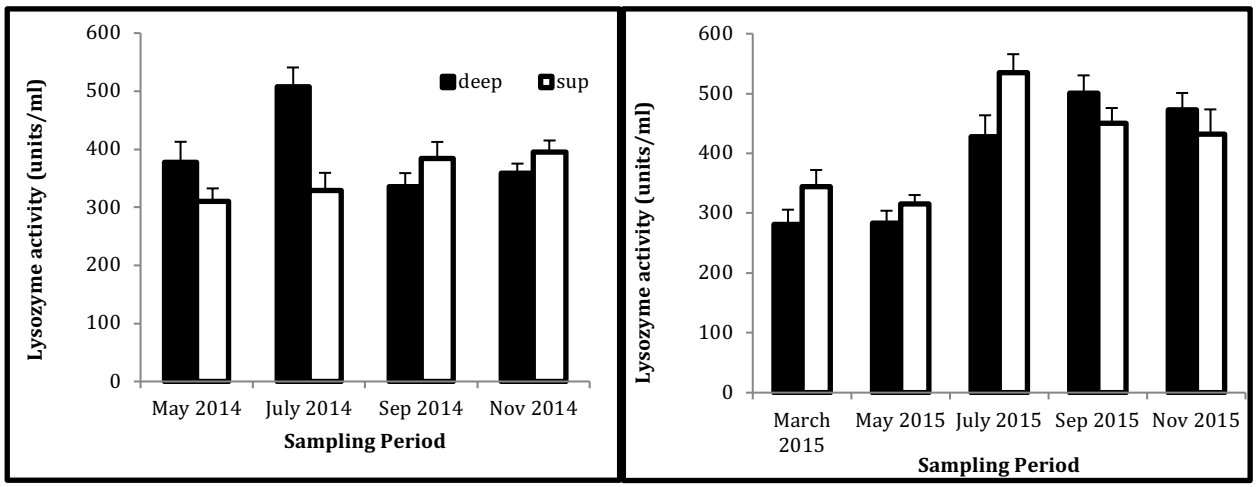

Figure 3.1.3.2: Serum lysozyme antibacterial activity of small (200-800g) and large (1-2kg) fish kept in cages at different depths (deep or superficial). Asterisks * show significant differences between different sampling times. Hashtag # shows a significant difference between the 2 depths. Different letters show significant differences between depths at different sampling times (One-Way ANOVA, P<0.05, Student-Newman-Keuls't-test). n=10.

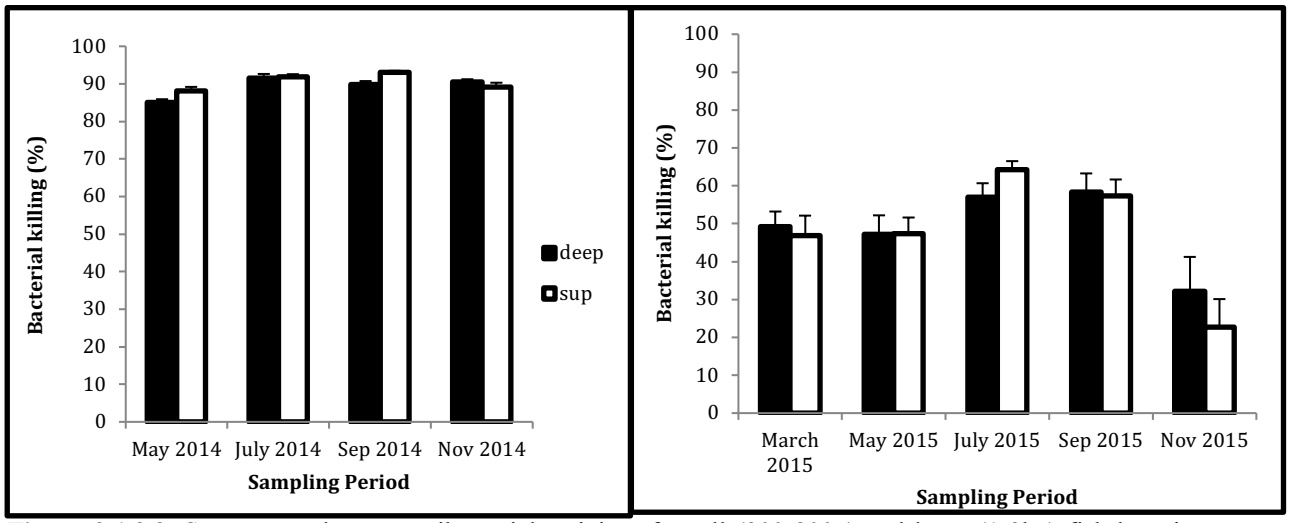

Figure 3.1.3.3: Serum complement antibacterial activity of small (200-800g) and large (1-2kg) fish kept in cages at different depths (deep or superficial). Asterisks * show significant differences between different sampling times. Hashtag # shows a significant difference between the 2 depths. Different letters show significant differences between depths at different sampling times (One-Way ANOVA, P<0.05, Student-Newman-Keuls't-test). n=10.

The bacterial killing activity of the fish serum (**Figure 3.1.3.3**) was reduced at first particularly in small fish kept in deep cages, suggesting a response to environmental stress, but fish later adapted and killing activity increased in both fish kept in deep and shallow cages. In larger fish, the bacterial killing capacity of the serum was much lower, increased slightly during the summer months but decreased further at the beginning of the winter. This suggested that small meagre relies more on the antibacterial activity of the complement than large fish, possibly linked to different sensibilities to diseases of small and large fish. Large fish or broodstock meagre seem to be more affected by parasitic infections (Toksen *et al.*, 2007, Andree *et al.*, 2015, Hayward *et al.*, 2007, Soares *et al.*, 2012). This was confirmed by the results of the anti-protease activity.

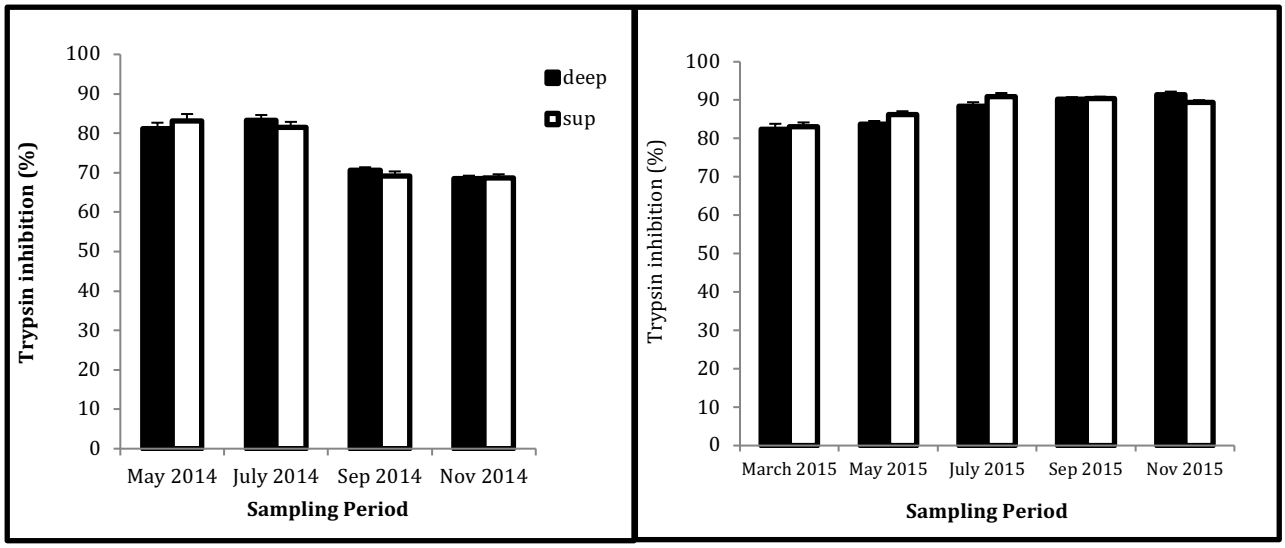

Figure 3.1.3.4: Serum anti-protease activity of small (200-800g) and large (1-2kg) fish kept in cages at different depths (deep or superficial). Asterisks * show significant differences between different sampling times. Hashtag # shows a significant difference between the 2 depths. Different letters show significant differences between depths at different sampling times (One-Way ANOVA, P<0.05, Student-Newman-Keuls't-test). n=10.

Antiprotease activity (**Figure 3.1.3.4**) was significantly lower in autumn than in spring/summer in smaller fish at both deep and shallow depths suggesting a higher anti-parasitic activity of fish at higher water temperatures. In larger fish, the trypsin inhibition had returned to high values and continued to increase during the autumn. This was probably linked to the fact that large fish are more affected by parasitical infections, and large fish may have developed stronger immune parameters to fight these parasites, or to avoid the mechanisms put in place by the parasites to evade the fish immune system. This is the case for the trypsin inhibition which fights proteases produced by the parasites to inhibit the fish produced proteic parameters such as the complement complex.

This study provides a first estimation of the innate immune status of meagre, *Argyrosomus regius*. When compared to other Mediterranean fish species, it relied highly on antibacterial parameters such as lysozyme and on respiratory burst activity linked to reactive oxygen species such as hydrogen peroxide as measured through the myeloperoxidase activity. Lysozyme activity was stronger (double) in meagre than in European sea bass (Henry *et al.*, 2009) and much stronger (6 times) than that of Gilthead seabream (Henry *et al.*, 2015) but 5 times lower than that of shi drum(*Umbrina cirrosa*) (Henry and Fountoulaki, 2014). Myeloperoxidase activity was also very strong in meagre. Whereas complement activity was very strong in small meagre, it was much lower in large fish. The opposite was true for the trypsin inhibition and lysozyme activity, which was stronger in larger fish than in small meagre. This pattern may imply that small fish have a strong arsenal against bacterial infections (both Gram positive and negative) which evolves during fish growth to provide an additional protection against parasitical infections, known to affect large fish and assessed here through the anti-protease activity in fish sera.

3.1.4 Behavioral monitoring

All data collected were analyzed in terms of differences between the tested conditions. No significant alteration was observed neither within nor between the experimental groups. The expected behavior during the day light was apparent in all the cases with the majority of the individuals concentrated at the lower layers of the cage and with observed movements towards the surface when feeding occurred.

Hence, the vertical distribution of meagre was mostly in the lower half of the cage for a period of approximately 12 hours while the rest of the period the meagre were distributed almost homogeneous in the whole available volume of the cage (**Figure 3.1.4.1**).

This observation is independent of the cage depth and it is correlated with the light and dark periods of the day. The pattern was repeated during the implementation period.

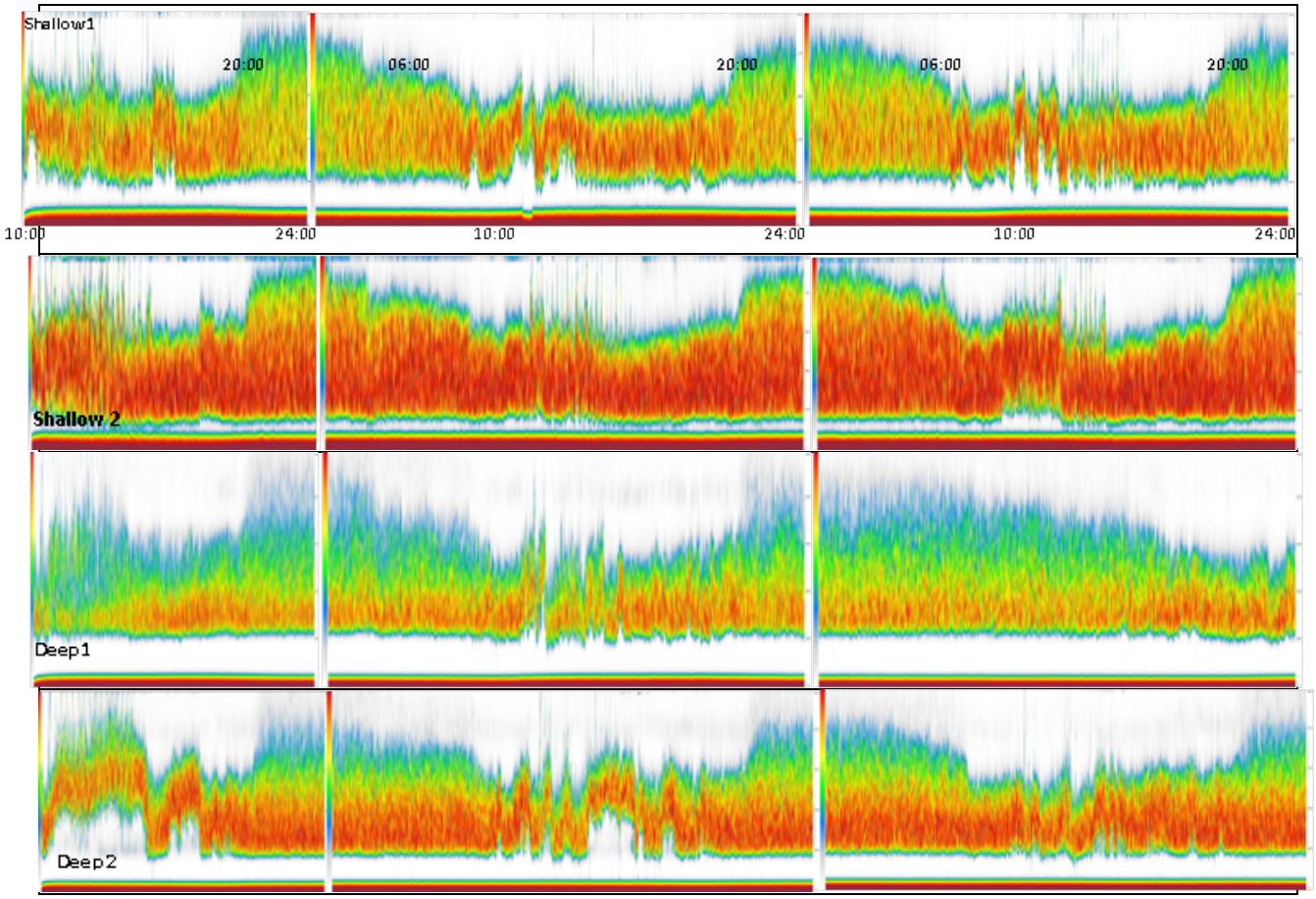

Figure 3.1.4.1. Vertical distribution of meagre in the experimental cages for a period of 3 days.

In Appendix 1 and 2, the results of all the observations during both experiments are presented. The observations were made for periods between 2 up to 7 days and during all 4 seasons. Also, different conditions relative to weather, currents, human presence, feeding were observed. All groups observed presented similar behavior, with no significant variations neither between the experimental conditions tested nor between the different seasons or rearing environment.

In the echograms the feeding periods are clearly marked with the vertical movements of the fish towards the feed, followed by a return to the lower layers of the cage. The only period that the fish appeared to be in stress was during the high temperature period of late August – September when the

individuals with body weight ~1,5 Kg were sluggish and with limited appetite. This was not the case for the younger groups (weight of apx 500g).

This behavior is in general different from what was observed in salmon (Oppedal et al 2011) or European seabass (Papandroulakis et al 2012) reared in cages, where fish express a different species-specific pattern. Meagre in general appears as a species with high tolerance to variable conditions and with a very conservative behavioral pattern. The observed nocturnal behavioral pattern that, to our knowledge, was observed for first time, may represent a potential alternative husbandry period for the species, i.e. a period that the species can be fed. This hypothesis was investigated during the course of the project and the findings are presented in ***D20.3 Methodology for meagre feeding***.

3.2 Experiment .2 Effect of light intensity in the cage

3.2.1 Biological performance

The growth performance during both trials is presented in the **Figure 3.2.1.1**.

During the first trial, growth rate was 1.2 g d^{-1} for the fish in the shaded cage while it was 1.3 for the second group. There was no significant difference between the populations. Both groups presented a poor performance compared to other groups reared in the farm before.
During the second trial, both groups performed significantly better than the first trial but again no difference was observed between the experimental conditions. Growth rates were 1.64 g d^{-1} and 1.68 g d^{-1} for the fish in the shaded cage and the non-shaded cage respectively.

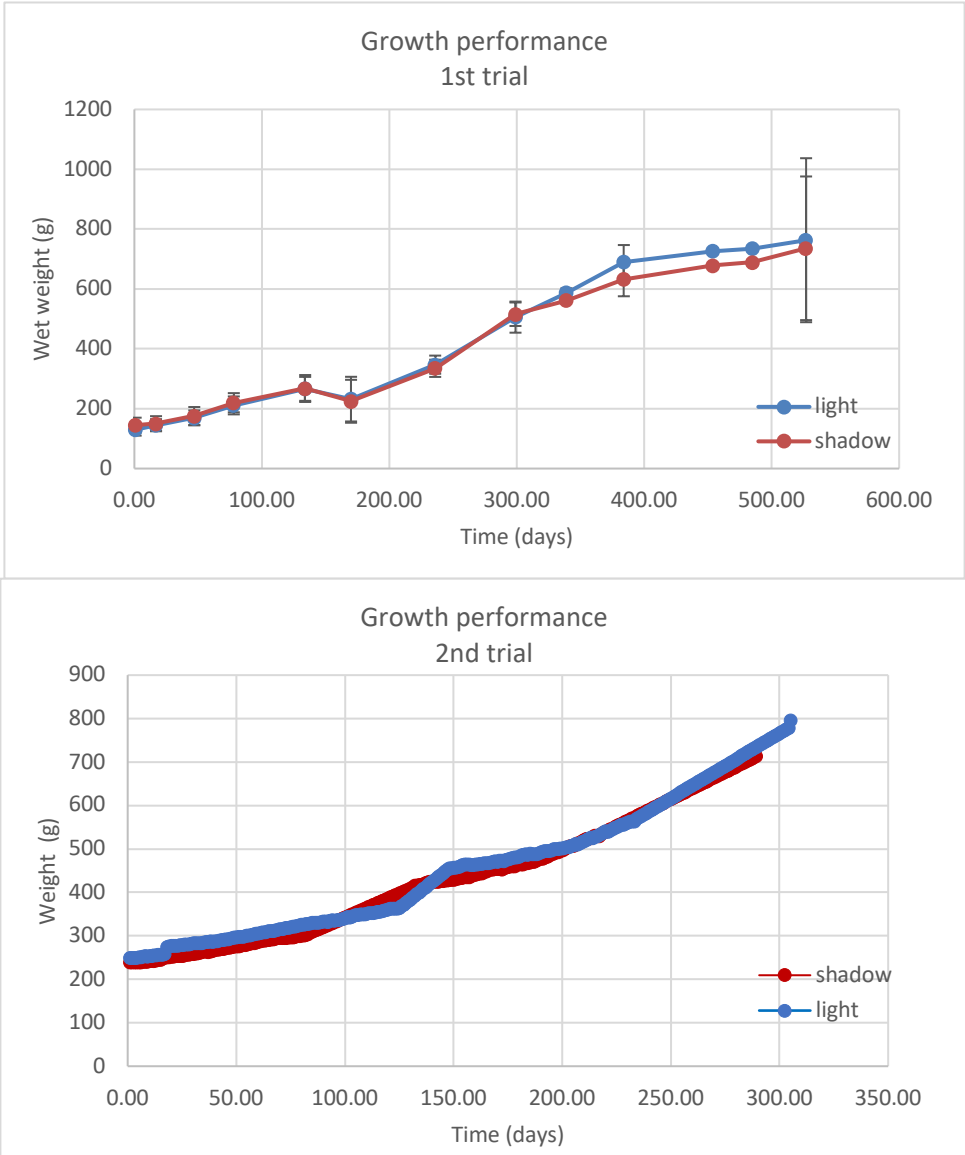

Figure 3.2.1.1. Growth performance of the experimental groups. Vertical bars show the standard deviation of the mean

At the end of each trial a large sample of 100 individuals was taken from each group in order to estimate both mean weight but also the growth variance of the weight distribution. The results are presented in **Table 3.2.1.1** and in **Figure 3.2.1.2**.

Table 3.2.1.1 Final body weights of the experimental groups

Final Body Weight	Trial 1		Trial 2	
	Shadow	Light	Shadow	Light
Average (g)	825.8	910.6	714.3	796.5
Standard Deviation (g)	240.1	274.0	169.8	181.9
Coefficient of variation	29.1%	30.1%	23.8%	22.8%

The size variation was higher in the first trial while there was an almost similar variability between the experimental conditions.

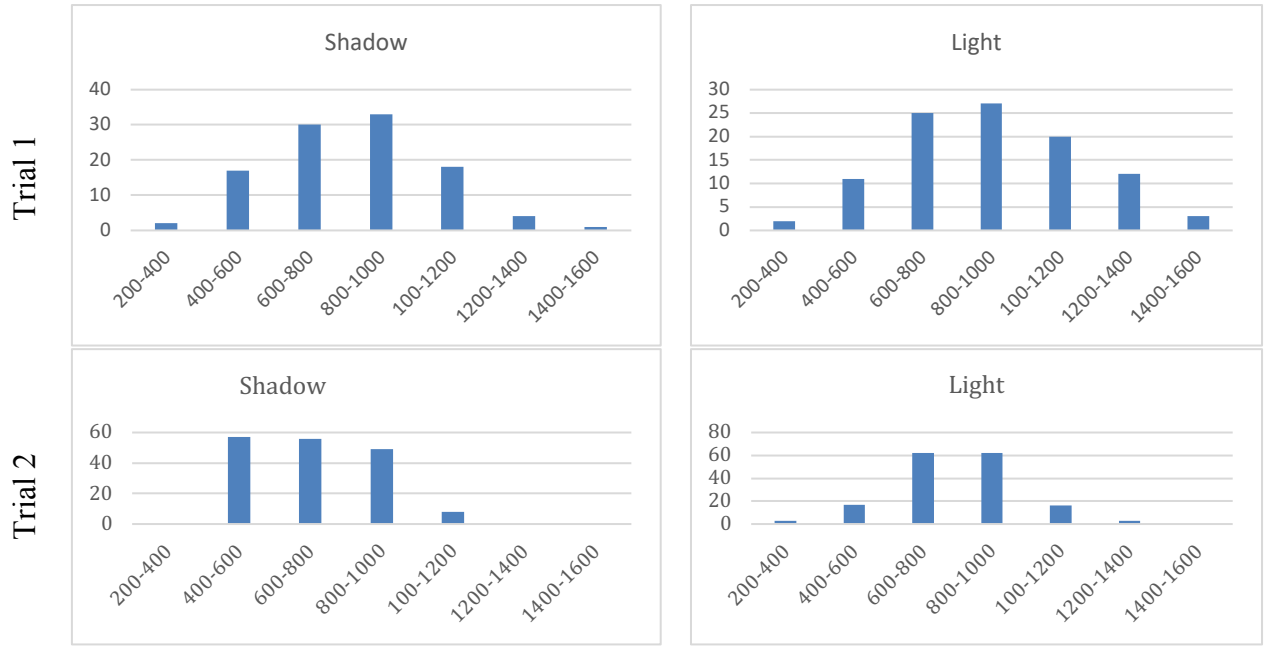

Figure 3.2.1.2. Final weight distribution of the experimental groups

Survival rate was 91.4% and 92.7% for the shaded and the non-shaded cage respectively, whereas for the second trial the relative values were 98.3% and 93.3%.

Finally, the results concerning feed consumption and feeding efficiency are summarized in **Table 3.2.1.2**.

Table 3.2.1.2 Performance parameters of the experimental groups

	Trial 1		Trial 2	
	Shadow	Light	Shadow	Light
Biomass gained (Kg)	5,804	6,353	4,712	5,677
Feed delivered (Kg)	17,675	18,318	9,195	9,998
FCR_{econ}	**3.0**	**2.9**	**2.0**	**1.8**

Although there was a significant difference between the first and the second trial,no difference between the tested conditions was observed.

3.2.2 Behavioral monitoring

Regarding the behaviour of the groups, in terms of their vertical distribution in cages, the pattern was similar to that observed during the experiment 1. In Appendix 3 all the echograms taken from the Argosaronikos farm are presented. A typical example is presented in (**Figure 3.2.2.1**).

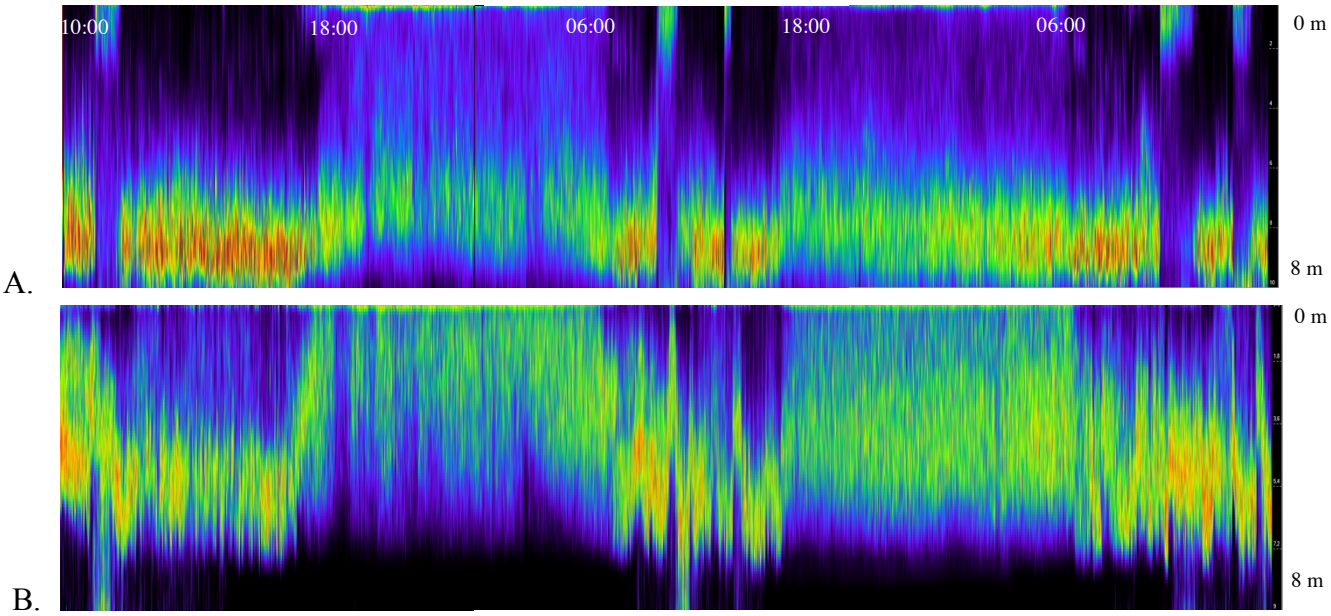

Figure 3.2.2.1. Echograms with the vertical distribution of the reared groups in (a) Not shaded and (b) shaded) cages

Although some problems were anticipated during the sampling because of the shallow location of the cages that disturb the proper installation of the equipment below the cage, the observations resulted in a similar pattern that that observed in the trials assessing the effect of cage depth, with fish mostly located in the bottom of the cage during the light hours of the day and spread in the whole available volume during the dark. This pattern is independent of the parameter tested.

4. CONCLUSIONS

The purpose of the experiments was to determine the best conditions for the rearing of meagre in sea cages. The first experiment investigated the effect of different depths for two phases of the on growing stage, *i.e.* for fish size of 200 to 800 gr and for 800 to 1500 g. Each trial lasted about a year during which the biological performance of the fish, together with various physiological and biochemical indicators, were monitored. The second experiment focused on the effect of light during the rearing by applying shading on the cages. The trial was repeated in two successive production cycles at a commercial scale at the facilities of P23. ARGO.

The results showed that in the first experiment, and only for the 1st phase of the rearing, the performance of the fish in deep cages was better, mostly based on the results of survival and Food Conversion Ratio (FCR), while for the other parameters tested, no statistically significant difference between the depths was observed, and only seasonal differences were detected. Specifically, both the FCR and the mortality rate had better values in cages with deep net. For the 2nd phase of the rearing no difference was observed between the tested conditions. The same holds also for the shading experiment.

Hematocrit, hemoglobin and osmotic pressure presented seasonal differences and showed the highest concentrations in November. Cholesterol, as well as total protein, showed only seasonal differences, whereas free fatty acids were influenced by both factors examined, but appeared to have lower concentrations at lower temperatures. Lactate, glucose and lysozyme appeared to be affected by both factors examined, while for the latter, differences were shown between the depths only during the warm months. The thyroid hormone triiodothyronine (T_3) did not show differences neither due to the rearing season nor between the two depths, while the thyroxine (T_3) showed seasonal differences probably related with photoperiod. Finally, cortisol showed only seasonal differences with the highest concentrations in March, the month with the lowest temperature.

The depth of the cages affected significantly the lysozyme and complement antibacterial activity of small fish in an opposite manner so it was impossible to give a recommendation about optimal cage depth for the health of small meager. Cage depth does not seem to be significantly involved in the health status of large fish.

Seasonal variations such as water temperature and photoperiod had a stronger effect on the fish immune parameters tested than the depth of the cages.

The fish behavior recorded in all described trials is different from what was observed in the cage farming of other species that, to our knowledge, was observed for the first time. Meagre appears as a species with high tolerance to variable conditions and with a very conservative behavioral pattern.

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